Protein Transfer and Organelle Biogenesis

Edited by
RATHINDRA C. DAS
Research & Development
Biotechnology Products Division
Miles Inc.
Elkhart, Indiana

PHILLIPS W. ROBBINS
Center for Cancer Research
Massachusetts Institute of Technology
Cambridge, Massachusetts
Contents

Contributors xi
Preface xiii

PART I. TRANSLOCATION

1. Transport of Proteins into and across the Endoplasmic Reticulum Membrane
   *Eve Perara and Vishwanath R. Lingappa*

   I. Introduction 3
   II. Historical Background 4
   III. Targeting 8
   IV. Mechanism of Translocation 14
   V. Membrane Assembly of Integral Transmembrane Proteins 22
   VI. Overview 37
   References 39

PART II. MODIFICATION, MATURATION, AND TRANSPORT

2. Role of Carbohydrate in Glycoprotein Traffic and Secretion
   *James B. Parent*

   I. Introduction 51
   II. Evidence for Intracellular Transport Signals 52
   III. Oligosaccharide Biosynthesis 55
   IV. Role of Carbohydrate in Protein Solubility, Structure, and Stability 62
   V. Evidence for Carbohydrate Transport Signals Using Site-Directed Mutagenesis 65
   VI. Mutations That Alter Glycosylation and Glycoprotein Traffic 66
VII. Tunicamycin and Glycoprotein Traffic 70
VIII. Inhibitors of Oligosaccharide Processing and Glycoprotein Traffic 76
IX. Vertebrate Lectins and Glycoprotein Traffic 82
X. Concluding Remarks 89
References 90

3. Membrane Insertion and Transport of Viral Glycoproteins: A Mutational Analysis
Eric Hunter

I. Introduction 109
II. Genetic Approaches to Viral Glycoprotein Transport 117
III. Conclusions 145
References 148

4. Posttranslational Modification during Protein Secretion
Kurt W. Runge

I. Introduction 159
II. Reactions in the Cytoplasm 160
III. Reactions in the Endoplasmic Reticulum 163
IV. Protein Modification in the Golgi Apparatus 183
V. Conclusion 198
References 199

5. Mannosidases in Mammalian Glycoprotein Processing
Kelley W. Moremen and Oscar Touster

I. Introduction 209
II. Processing Mannosidases and Multiple Routes 212
III. Conclusions 233
References 233

PART III. SORTING AND ORGANELLE ASSEMBLY

6. Biosynthesis and Sorting of Proteins of the Endoplasmic Reticulum
Michael Green and Richard A. Mazzarella

I. Introduction 243
II. Structure and Sorting of ERp99, an Abundant, Conserved ER Glycoprotein 253
III. Conclusions and Future Directions 274
References 280
7. Functional Topology of Golgi Membranes  
Becca Fleischer  
I. Introduction 289  
II. General Properties of the Golgi Apparatus 290  
III. Topology of Membrane-Bound Functions 293  
IV. Transport Properties of Golgi Membranes 300  
V. Concluding Remarks 311  
References 312  

8. Protein Sorting and Biogenesis of the Lysosome-like Vacuole in Yeast  
Joel H. Rothman and Tom H. Stevens  
I. Introduction 318  
II. Biosynthesis of Vacuolar Proteins 319  
III. Sorting of Vacuolar Proteins 330  
IV. Endocytosis in Yeast and Its Relationship to Vacuolar Protein Delivery 346  
V. Summary and Conclusions 352  
References 354  

9. Transport and Targeting of Lysosomal Enzymes in Dictyostelium discoideum  
James A. Cardelli and Randall L. Dimond  
I. Introduction 364  
II. Life History of a Cellular Slime Mold Lysosomal Enzyme 365  
III. Dual Pathways for the Secretion of Lysosomal Enzymes 375  
IV. Genetic Approaches to Dissect Intracellular Transport Pathways 381  
V. Developmental Regulation of the Synthesis, Modification, and Localization of Lysosomal Enzymes 387  
VI. Recombinant DNA Approaches 389  
VII. Summary and Future Perspectives 390  
References 391  

10. Organelles of Endocytosis and Exocytosis  
John A. Hanover and Robert B. Dickson  
I. Introduction 401  
II. Endocytosis 403  
III. Exocytosis 431  
IV. Summary and Future Prospects 446  
References 446
11. Endocytosis and Compartmentalization of Lysosomal Enzymes in Normal and Mutant Mammalian Cells: Mannose 6-Phosphate-Dependent Pathways
April R. Robbins

I. In the Beginning 464
II. The Recognition Marker 465
III. The Receptors 476
IV. Endocytosis 481
V. The Intracellular Pathway 490
VI. Junction of the Pathways 504
References 509

12. Biogenesis of Secretory Vesicles
Hsiao-ping Hsu Moore, Lelio Orci, and George F. Oster

I. Introduction 521
II. Structure and Function of Secretory Organelles 524
III. Formation of Secretory Vesicles 532
IV. Conclusion and Perspectives 554
References 555

13. Expression of Extracellular Matrixlike Glycoproteins by Macrophages and Other Leukocytes
Arthur M. Mercurio

I. Introduction 563
II. Biosynthesis of Extracellular Matrixlike Glycoproteins by Macrophages 566
III. Functions of Extracellular Matrixlike Glycoproteins in Macrophages and Related Cells 574
IV. Perspectives 579
References 580

PART IV. TRANSFER ACROSS BACTERIAL MEMBRANES

14. Synthesis and Export of Lipoproteins in Bacteria
Miguel Regue and Henry C. Wu

I. Introduction 587
II. Lipoprotein Structure 588
III. Biosynthesis 589
IV. Distributions of Lipoproteins among Bacteria 590
V. Signal Peptidases 592
VI. Genomic Organization of the lsp Gene 597
15. **Protein Secretion across the Outer Membrane of Gram-Negative Bacteria**

*Anthony P. Pugsley*

I. Introduction 607
II. Lysis or Secretion 609
III. Early Stages in the Secretion Pathway 611
IV. Later Stages in the Secretion Pathway: Secretion Proteins and Targeting Signals 620
V. Release of Proteins in Vesicles 634
VI. Activators and Inhibitors of Secreted Proteins 636
VII. Special Cases 638
VIII. Concluding Remarks and Perspectives 641
References 642

PART V. **MITOCHONDRIAL ASSEMBLY**

16. **Genetic Approaches to the Study of Mitochondrial Protein Import**

*Marjorie C. Brandriss*

I. Introduction 655
II. Isolation of Mutations affecting Mitochondrial Import 659
III. Conclusions 669
References 672

17. **Synthesis and Assembly of Mitochondrial Proteins**

*Donald W. Nicholson and Walter Neupert*

I. Introduction 677
II. Precursor Proteins 678
III. Targeting and Sorting Sequences 684
IV. Receptors 695
V. Energy Requirements 699
VI. Cytosolic Cofactors 703
VII. Translocation Contact Sites 705
VIII. Proteolytic Processing 709
IX. Mitochondrial Gene Products 716
X. Assembly and Coordination 718
XI. Overview 721
References 729
PART VI. NUCLEAR TRANSPORT

18. Transport of Proteins into the Nucleus
   Pamela A. Silver and Michael N. Hall

I. Introduction 747
II. Nuclear Pore 748
III. Possible Mechanisms for Specific Nuclear Protein Localization 749
IV. Nuclear Protein Localization in the Yeast Saccharomyces cerevisiae 753
V. Conclusion 764
     References 764

PART VII. APPLIED RESEARCH

19. Secretion Research in Industrial Mycology
   Ramunas Bigelis and Rathindra C. Das

I. Introduction 771
II. Commercially Important Enzymes of Fungal Origin 772
III. Secretion of Therapeutic Mammalian Polypeptides in Saccharomyces cerevisiae 792
IV. Applied Secretion Research: Prospects for the Future 796
     References 798

Index 809
Contributors

Numbers in parentheses indicate the pages on which the authors’ contributions begin.

Ramunas Bigelis (771), Biotechnology Products Division, Miles Inc., Elkhart, Indiana 46515

Marjorie C. Brandriss (655), Department of Microbiology and Molecular Genetics, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey 07103

James A. Cardelli (364), Department of Microbiology and Immunology, Louisiana State University Medical Center, Shreveport, Louisiana 71130

Rathindra C. Das (771), Biotechnology Products Division, Miles Inc., Elkhart, Indiana 46515

Robert B. Dickson (401), Medical Breast Cancer Section, NCI, National Institutes of Health, Bethesda, Maryland 20852

Randall L. Dimond (364), Promega-Biotec, Madison, Wisconsin 53711

Becca Fleischer (289), Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235

Michael Green (243), Department of Microbiology, St. Louis University School of Medicine, St. Louis, Missouri 63104

Michael N. Hall (747), Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143

John A. Hanover (401), Laboratory of Biochemistry and Metabolism, NIDDK, National Institutes of Health, Bethesda, Maryland 20892

Eric Hunter (109), Department of Microbiology, The University of Alabama at Birmingham, Birmingham, Alabama 35294

Vishwanath R. Lingappa (3), Departments of Physiology & Medicine, University of California, San Francisco, California 94143-0444

Richard A. Mazzarella (243), Department of Microbiology, St. Louis University School of Medicine, St. Louis, Missouri 63104
Arthur M. Mercurio (563), Laboratory of Cancer Biology, New England Deaconess Hospital, Harvard Medical School, Boston, Massachusetts 02115

Hsiao-ping Hsu Moore (521), Department of Physiology-Anatomy, University of California, Berkeley, California 94720

Kelley W. Moremen (209), Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Walter Neupert (677), Institut für Physiologische Chemie der Universität München, 8000 München 2, Federal Republic of Germany

Donald W. Nicholson (677), Institut für Physiologische Chemie der Universität München, 8000 München 2, Federal Republic of Germany

Lelio Orci (521), Institute of Histology and Embryology, University of Geneva Medical School, 1211 Geneva 4, Switzerland

George F. Oster (521), Departments of Biophysics, Entomology and Zoology, University of California, Berkeley, California 94720

James B. Parent (51), Metabolic Research Branch, Naval Medical Research Institute, Bethesda, Maryland 20814

Eve Perara (3), Departments of Physiology and Medicine, University of California, San Francisco, California 94143

Anthony P. Pugsley (607), Unité de Génétique Moléculaire, Institut Pasteur, Paris 75724, France

Miguel Regue (587), Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814

April R. Robbins (464), Genetics and Biochemistry Branch, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

Joel H. Rothman (318), Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene, Oregon 97403

Kurt W. Runge (159), Department of Genetics, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104

Pamela A. Silver (747), Department of Biology, Princeton University, Princeton, New Jersey 08544

Tom H. Stevens (318), Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, Oregon 97403

Oscar Touster (209), Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235

Henry C. Wu (587), Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799

1Present address: Department of Microbiology, Facultad de Farmacia, Universidad de Barcelona, Barcelona, Spain.
I. INTRODUCTION

Eukaryotic cells can perform a variety of metabolic tasks with high efficiency owing to the compartmentalization of functions within cellular organelles. The organelles are delineated by membrane structures and contain specific subsets of proteins related to their role within the cell. The uniqueness of each type of organelle is maintained by processes in
the cell which sort and deliver the individual components in a highly specific manner. In this chapter, we discuss the biogenesis of mitochondria and specifically the processes which are involved in the import of proteins into these organelles.

Mitochondria are not synthesized *de novo*. Instead, control of the number of mitochondria within a cell occurs by division and fusion events. In the steady state there is a constant synthesis and turnover of mitochondrial proteins. Mitochondria grow and are maintained by incorporating newly synthesized material into preexisting organelles. The mitochondrion itself has a genetic apparatus which is discrete from that of the rest of the cell, but because of its relatively small size it can only account for a small percentage of the hundreds of mitochondrial proteins. The remainder are imported from the cytoplasm.

Because mitochondria contain two membranes, the organelle can be spatially divided into four distinct compartments: the outer membrane, the inner membrane, the matrix, and the intermembrane space. These too are specialized in function so that imported proteins must be correctly and specifically sorted within the mitochondrion as well. The pathways by which this occurs can be resolved into a number of discrete steps. In general, most imported mitochondrial proteins are synthesized on free ribosomes in the cytosol as precursors containing amino-terminal extensions. They are released into a cytosolic pool and are rapidly taken up by mitochondria. Receptors on the outer surface of the outer mitochondrial membrane seem to be involved in the initial recognition process. Proteins which must be transported to or through the inner membrane do so via translocation contact sites where the inner and outer membranes come close enough together to be spanned simultaneously. This step is energy dependent and specifically requires an electrochemical potential across the inner membrane. Precursor proteins are proteolytically processed to their mature size by a metal ion-dependent matrix peptidase, in some cases in combination with a second proteolytic event. The imported proteins are sometimes modified further by covalent or noncovalent attachment of cofactors. Many proteins are also assembled into larger complexes composed of several different subunits. In this chapter, we shall examine, in chronological order, each individual step which occurs during the import of mitochondrial proteins.

II. PRECURSOR PROTEINS

A. Properties of Precursor Proteins

Proteins which are imported into mitochondria are synthesized as precursors that differ from their mature counterparts in a number of ways: (1)
most, but not all, are synthesized as higher molecular weight proteins with amino-terminal peptide extensions which are proteolytically removed during or following import; (ii) a number of precursor proteins are covalently modified when imported while some acquire cofactors which are associated with the protein in a tight but noncovalent manner; (iii) precursor proteins differ in conformation from their mature form; (iv) precursor proteins often aggregate in the cytosol, whereas mature proteins in mitochondria are more likely to be found as monomers, dimers, or as subunits in heterologous complexes; (v) precursor proteins accumulated in the cytosol are far less stable than their correctly imported mature equivalent. Changes which accompany the maturation of imported proteins occur for one of two reasons. Some (i.e., removal of amino-terminal extensions) are connected to the import process, while others (i.e., acquisition of cofactors) are required for functional properties once inside the mitochondrion.

Of the more than 60 different proteins examined to date which must be imported into either the matrix or the inner membrane, almost all contain amino-terminal presequences (for an extensive list see Hay et al., 1984, or Harmey and Neupert, 1985). Possible exceptions of the matrix proteins include 2-isopropylmalate synthase (Gasser et al., 1982a; Hampsey et al., 1983; Beltzer et al., 1986), extramitochondrially expressed yeast b14 matu­rerase (Banroques et al., 1987), and 3-oxoacyl-CoA thiolase from rat (Arakawa et al., 1987), none of which appear to contain an amino-termi­nal extension. Some proteins of the inner membrane are known to be imported in their mature size, namely, the ADP/ATP carrier (Zimmerman et al., 1979b; Hatalová and Kolarov, 1983), the uncoupling protein of brown adipose tissue (Freeman et al., 1983; Ricquier et al., 1983; Bouillaud et al., 1986; Ridley et al., 1986), two subunits (14 and 11 kDa) of the bc1 complex (Teintze et al., 1982; van Loon et al., 1983c; Maarse and Grivell, 1987), the ubiquinone-binding protein of the bovine bc1 complex (Nishikimi et al., 1986), and sweet potato cytochrome oxidase subunit Vc (Nakagawa et al., 1987). Proteins which are imported into the intermem­brane space seem to be divided in this regard. Some, like precursors to cytochrome c peroxidase (Macccechini et al., 1979b; Reid et al., 1982), cytochrome b2 (Reid et al., 1982; Daum et al., 1982b; Gasser et al., 1982b), and sulfite oxidase (Mihara et al., 1982b) carry amino-terminal extensions, while others, like cytochrome c (Korb and Neupert, 1978; Zimmermann et al., 1979a; Matsuura et al., 1981) and adenylate kinase (Watanabe and Kubo, 1982), do not.

Although only a few of the proteins which are imported to the outer membrane have been examined, none appears to be synthesized as a higher molecular weight precursor. These include the channel-forming porin protein (Freitag et al., 1982; Mihara et al., 1982a; Gasser & Schatz,
1983) and three outer membrane proteins of unknown function (70, 45, and 14 kDa; Gasser and Schatz, 1983). One possible exception is a 35 kDa protein from rat liver mitochondria (Shore \textit{et al.}, 1981) which appears to migrate on sodium dodecyl sulfate–polyacrylamide gels as a precursor of slightly greater molecular size. Whether this represents a precursor protein containing a peptide extension or is a gel artifact is not entirely clear and will require sequencing data to verify.

The trend that emerges is that the deeper into the mitochondrion a protein must be imported, the more likely it is to be synthesized as a precursor of higher molecular weight. As will be discussed in the following section, the amino-terminal prepiece carries targeting information which is necessary to direct imported proteins to their correct intramitochondrial location. In addition, the prepieces, which are generally hydrophilic, confer different solubility properties on the precursor proteins that may allow for their transfer through the cytosol and are important for subsequent import.

While proteolytic processing is the most prevalent covalent modification of imported mitochondrial proteins, there are a number of other changes which occur in many proteins during or following import. For example, protoheme is covalently attached to cytochromes \(c\) and \(c_1\) via cysteine residues in the corresponding apoproteins. Iron–sulfur centers (nonheme iron) are constructed in subunits of complexes I, II, and III of the respiratory chain, again via cysteine residues. In addition, many mitochondrial proteins are modified by tight but noncovalent bonding of coenzymes and cofactors (i.e., NAD, FAD, heme \(a\) and \(b\) and metal ions such as Cu). These proteins are predominantly constituents of the respiratory chain, and the cofactors which are associated with them are primarily involved in their unique roles as electron carriers. In some cases, however, such modifications also appear to be important for events in the import pathway. Covalent attachment of heme to apocytochrome \(c\) (holocytochrome \(c\) lacking heme), for instance, initiates conformational changes along the polypeptide chain which pull the protein through the outer membrane to its functional location in the intermembrane space (Hennig and Neupert, 1981; Nicholson \textit{et al.}, 1987). Cytochrome \(c_1\) is proteolytically processed in two distinct steps, the second of which has been suggested to be preceded by covalent attachment of the heme to the intermediate size apoprotein (Gasser \textit{et al.}, 1982b; Ohashi \textit{et al.}, 1982). Likewise, the second processing step of the Fe/S protein of the \(bc_1\) complex may be dependent on formation of the Fe/S cluster (F.-U. Hartl and W. Neupert, unpublished).

Precursor proteins are also distinguishable from their mature counterparts in conformational arrangement. Apocytochrome \(c\) (prepared by
chemical removal of the heme group from holocytochrome c, then rena-
tured to an import-competent form) shows a nearly featureless circular
dichroism (CD) pattern. Following interaction with negatively charged
lipids in model membranes, which is believed to represent the first event
in its import into mitochondria, up to 35% α-helical structure is expressed
(Rietveld et al., 1985). The disordered structure of the precursor cyto-
chrome c is folded into a highly ordered stable conformation when heme
is attached to form holocytochrome c (Fisher et al., 1973). The difference
between precursor and mature forms of cytochrome c is thus dependent
on events occurring during its import, namely, interactions with phospho-
lipids and covalent attachment of heme. These conformational changes
can be detected in vitro with differential antibodies raised against apo- and
holocytochrome c which do not cross-react (Korb and Neupert, 1978) or
by differential proteolytic sensitivity in solution (Basile et al., 1980).

The precursor to the ADP/ATP carrier does not bind carboxyatractylo-
side whereas the mature form does. This reflects conformational dif-
fferences in the proteins which can be distinguished by their binding
properties to columns of hydroxylapatite in the presence of carboxyatra-
tyloside. In vitro imported and correctly assembled ADP/ATP carrier,
when solubilized from mitochondria with detergent, behaves like authen-
tic mature ADP/ATP carrier and passes through these columns while the
precursor protein does not (Zimmermann and Neupert, 1980; Schleyer
and Neupert, 1984). Since ADP/ATP carrier is not proteolytically pro-
cessed during import, the binding properties to hydroxylapatite serve as
useful criteria for establishing whether correct import and assembly has
occurred. Such criteria are important for determining whether a protein
imported in vitro acquires the properties of the mature protein in vivo. In
some cases, for example, for proteins which are not proteolytically pro-
cessed, import can only be studied by following these changes.

Precursor conformation is important for import. For example, although
CD spectra for apocytochrome c show no detectable secondary structure
(Rietveld et al., 1985), binding of the protein to mitochondria is sensitive
to denaturation by a single freeze–thaw cycle (H. Köhler and W.
Neupert, unpublished). Similarly, the apocytochrome c produced by a
mutant of Neurospora crassa, in which the carboxy-terminus is 19 amino
acids longer than wild type apocytochrome c (the final 27 amino acids
being of an unrelated sequence), cannot bind or be imported into mito-
chondria, most likely because of conformational perturbation (Stuart et
al., 1987). On the other hand, the import of a fusion protein containing
mouse dihydrofolate reductase linked to the presequence of cytochrome
oxidase IV can be blocked by methotrexate, which stabilizes the folding
of the dihydrofolate reductase moiety. In this case, the protein must be at
least partially unfolded to be imported into mitochondria (Eilers and Schatz, 1986). Recent evidence indicates that most precursor proteins are maintained in, or "defolded" into, an import-competent conformation by the hydrolysis of nucleoside triphosphates (Pfanner and Neupert, 1986; Pfanner et al., 1987; see Section V).

Most newly synthesized precursor proteins tend to aggregate. Though this has not been well characterized in vivo, it is frequently observed in vitro in either homologous or heterologous (i.e., reticulocyte lysate) translation systems. The ADP/ATP carrier, for example, is present in soluble complexes with apparent molecular weights of 120K and 500K (Zimmermann and Neupert, 1980). Aggregation has also been demonstrated for ATPase IX (Schmidt et al., 1983b) and for cytochrome oxidase V (Neupert and Schatz, 1981). In all of these cases it is not clear whether aggregation occurs as homo- or heterooligomers. The precursor to rat ornithine carbamoyltransferase is transported to mitochondria as a 5 S complex (approximately 90 kDa) containing an unidentified import factor (Argan and Shore, 1985). Similarly, the import of the F1 ATPase β subunit into yeast mitochondria is dependent on a cytosolic factor believed to be a 40 kDa protein which binds to the precursor proteins and enables their correct association with mitochondria (Ohta and Schatz, 1984). These last two examples suggest that aggregation may occur in a heterooligomeric fashion for some imported proteins. On the other hand, the precursor to rat mitochondrial fumarase (fumarate hydratase) was reported to form homooligomeric aggregates containing six to eight molecules (Ono et al., 1985).

Besides the nonspecific interaction of proteins in solution, aggregation of precursor proteins may occur for specific reasons as well. Since many imported mitochondrial proteins have a membrane localization and are therefore at least partially hydrophobic, there probably exists some means for disguising these parts of the molecule and allowing their solubility in the cytosol. The amino-terminal prepiece may confer some solubility in aqueous environments, while aggregation of precursor proteins, presumably via their hydrophobic domains, may also contribute in this respect. The best example of this is ATPase subunit IX [proteolipid or dicyclohexylcarbodiimide (DCCD)-binding protein], one of the most hydrophobic proteins known. It contains a long polar prepiece (66 amino acids in Neurospora crassa) that accounts for the solubility of the highly hydrophobic mature sequence (81 amino acids) in aqueous environments (Viebrock et al., 1982). The protein also forms aggregates, perhaps to further aid its solubility in the cytosol. Cytochrome c, on the other hand, is a soluble protein of the intermembrane space which is not imported.
17. Synthesis and Assembly of Mitochondrial Proteins

with a prepiece and behaves as a monomer or dimer in solution (H. Köhler and W. Neupert, unpublished). Another possible role of aggregation is that it is required for import-competent transport and binding of precursors to mitochondria (Section VI).

Once precursor proteins are synthesized, they are rapidly cleared from the cytosol and imported into mitochondria. Precursors can be accumulated in vivo by growing cells in the presence of uncouplers of oxidative phosphorylation. Under these conditions they cannot be imported and are pooled in the cytosol. In contrast to the relative stability of mature mitochondrial proteins, the accumulated precursors (in general) are rapidly degraded. For example, yeast cells grown to early exponential phase and then treated with the uncoupler carbonyl cyanide N-chlorophenylhydrazone (CCCP) accumulated large amounts of some mitochondrial precursor proteins (Reid and Schatz, 1982a). In pulse-labeling experiments, the proteins were degraded at different rates. The precursor of cytochrome c₁ was unstable and was degraded with a half-life of about 10 min. On the other hand, the precursor to F₁ ATPase β subunit was more stable and was degraded with a half-life of 50 min. In similar experiments, the precursor of aspartate aminotransferase, accumulated in chick embryo fibroblast cultures treated with CCCP, was degraded with a half-life of about 5 min (Jaussi et al., 1982), and the precursor of carbamoyl-phosphate synthase in rat liver explants, in which proteolytic processing was blocked, was degraded with a half-life of 2–3 min (Raymond and Shore, 1981). The apparent relative stability comparing precursor with mature proteins is probably also related to the normal subcellular location of the proteins (i.e., cytosol versus mitochondria) and the degradative processes that exist there.

Maturation of imported mitochondrial proteins occurs within the mitochondrion itself and not in the cytosol immediately following synthesis. This occurs in this sequence for two reasons: (1) many maturation events are involved in the import pathway itself and/or can only take place after certain import steps have occurred, and (2) development of functional characteristics within the mitochondrion is consistent with the theme of compartmentation of processes in eukaryotic cells.

B. Cotranslational versus Posttranslational Transport

Two mechanisms exist in eukaryotic cells by which proteins may be synthesized and transported across biological membranes. In cotranslational transport, synthesis begins on soluble cytoplasmic polysomes. As the nascent polypeptide chain appears from the ribosome it is directed,
with the accompanying polysome, to the target membrane and is simultaneously inserted into or through the membrane as chain elongation continues. Cotranslational import is the primary means by which proteins are transported across the membranes of the endoplasmic reticulum. It appears that in this case the cotranslational nature, probably with a few exceptions, is obligatory (i.e., a completed polypeptide chain can never enter the transport pathway). It is likely that the polypeptide chain otherwise folds in such a way that it is transport incompetent. The function of the signal recognition particle and docking protein is in arrest and dearest of the elongation process to guarantee that the nascent polypeptide is kept in a translocation competent state for a sufficient period of time. In posttranslational transport, protein synthesis also begins on soluble cytoplasmic polysomes; however, complete polypeptide synthesis and release into the cytosol occurs before transport into or across membranes begins. Posttranslational import is the mechanism predominant in mitochondria, chloroplasts, microbodies, and probably nuclei. In mitochondria, import may occur with some cotranslational characteristics, though it is clearly not an obligatory process.

In cotranslational compared to posttranslational import, the way in which organelle targeting information is processed is different. For example, in the mechanism for microsomal targeting, the signal recognition particle and docking protein serve as common components for most proteins destined for the endoplasmic reticulum (ER). On the other hand, targeting information for proteins which are posttranslationally transported must be self-contained.

Import of mitochondrial proteins can occur posttranslationally, though early evidence pointed toward a cotranslational mechanism. Cytoplasmic 80 S ribosomes were coisolated with purified yeast mitochondria (Kellems and Butow, 1972). These ribosomes were tightly bound to mitochondria, and only one-third could be released by incubation at high ionic strength. The remaining two-thirds could only be released when their nascent polypeptide chains were dissociated from the ribosome with puromycin (Kellems et al., 1974), suggesting that the ribosomes were anchored via the newly synthesized nascent polypeptide chain which was presumably undergoing cotranslational (vectorial) transport. Furthermore, the mitochondria-associated ribosomes were enriched in mRNAs coding for mitochondrial proteins. When yeast mitochondria were isolated with their accompanying cytoplasmic ribosomes and placed in a readout system to complete polypeptide chain synthesis, over 80% of the products remained associated with the mitochondria and were imported to a location not accessible to externally added proteases (Ades and Butow, 1980a,b). The
distribution of total message for mitochondrial proteins, however, was not exclusively associated with these mitochondria-bound ribosomes. Although the mRNA from mitochondria-bound ribosomes was enriched for mitochondrial proteins, indicating that the association was specific, not all mitochondrial proteins were preferentially synthesized on them (Suissa and Schatz, 1982). For example, while 60% of the translatable mRNA for F1 ATPase β subunit or cytochrome c peroxidase was associated with mitochondria-bound polysomes, more than 95% of the message for cytochrome oxidase V or VI and porin was associated with free polysomes. In no case was the translatable message exclusively associated with mitochondria-bound polysomes. This suggested that cotranslational import was not necessarily the transport mechanism used by all imported mitochondrial proteins.

Evidence for posttranslational import of mitochondrial proteins came from studies both in vivo and in vitro:

1. Extramitochondrial pools of a number of mitochondrial proteins have been detected in pulse-labeling experiments in vivo. The appearance of label in mitochondrial proteins showed a lag compared to total cellular protein in Neurospora crassa (Hallermayer et al., 1977). The labeled mitochondrial proteins first appeared in a cytosolic pool and then in mitochondria. Similarly, in yeast, mitochondrial precursor proteins first appeared in a cytosolic pool and were then subsequently imported into mitochondria and converted to their mature forms (Reid and Schatz, 1982b; Schatz, 1979).

2. Posttranslational import in the absence of protein synthesis has been demonstrated both in vivo and in vitro. When protein synthesis was blocked with cycloheximide immediately following pulse-labeling in vivo, the precursor pool of mitochondrial proteins in the cytosol decreased with concomitant posttranslational uptake by mitochondria and conversion to the mature forms during the subsequent chase (Hallermayer et al., 1977; Schatz, 1979; Reid and Schatz, 1982b; Teintze et al., 1982). Similarly, mitochondrial precursor proteins which were accumulated in vivo by growing cells in the presence of CCCP could be subsequently chased into mitochondria by removing the inhibitory effects of CCCP with 2-mercaptoethanol (Reid and Schatz, 1982a,b) or cysteamine (Jaussi et al., 1982). This too was unaffected by the presence of cycloheximide and therefore occurred posttranslationally.

3. Perhaps the most convincing evidence that transport is possible in the absence of protein synthesis comes from in vitro studies where translation systems were programmed with poly(A)-containing RNA to synthe-
size precursor proteins. If further translation following synthesis was blocked by the addition of cycloheximide or if ribosomes were removed by centrifugation and the resulting supernatants incubated with isolated mitochondria, the precursor proteins were rapidly imported into mitochondria and processed to their mature form (Harmey et al., 1977; Korb and Neupert, 1978; Maccecchini et al., 1979a; Zimmermann and Neupert, 1980). The same effect is observed regardless of whether a heterologous (i.e., reticulocyte lysate) or homologous (from cell cytosol) system is used. Posttranslational transport has been demonstrated in vitro for all imported mitochondrial proteins studied so far and in many cases in vivo as well.

In mitochondria, protein transport normally occurs posttranslationally although some cotranslational import (which is clearly not an obligatory process) may occur as a consequence of the amino-terminal targeting prepiece being exposed before complete polypeptide synthesis is finished. The prepiece might then initiate the import process before chain elongation is complete. Whether this actually occurs in vivo is not clear since the early experiments with mitochondria-bound ribosomes did not distinguish whether the nascent polypeptide chains were concomitantly transported with translation or transported only after complete synthesis. In any case, the enrichment of mRNAs for mitochondrial proteins in mitochondria-bound ribosomes has provided a useful means for isolating and screening for genes for imported proteins. Clones for 16 different genes of imported yeast mitochondrial proteins were identified by selective hybridization to these enriched mRNAs (Suissa et al., 1984).

III. TARGETING AND SORTING SEQUENCES

The intracellular sorting of proteins synthesized on cytoplasmic polyosomes is a fairly specific process. The signals which direct these events are contained within the newly synthesized protein itself. In addition, components of the target organelle, such as receptors, recognize these signals and facilitate import and sorting.

A. Fusion Proteins

Delineation of the exact regions in precursor proteins which are responsible for mitochondrial targeting and intramitochondrial sorting has been demonstrated by gene fusion experiments in which parts of a mitochondri-
drial precursor protein can be attached to a nonmitochondrial "passenger" protein. Generally, three observations have been made: (i) the cleavable amino-terminal prepiece of imported mitochondrial proteins fused to nonmitochondrial proteins [such as mouse dihydrofolate reductase (DHFR) or Escherichia coli β-galactosidase] can correctly mediate mitochondrial targeting and frequently intramitochondrial sorting of the passenger protein; (ii) mitochondrial proteins in which the prepiece has been removed cannot be imported into mitochondria; and (iii) distinct regions of the polypeptide chain of proteins which are not proteolytically processed contain the information necessary for targeting and sorting. It has been suggested that some mitochondrial precursor proteins also have carboxy-terminal extensions that are removed during import and maturation, though the significance of these findings is as yet unclear (Okamura et al., 1985; Power et al., 1986; Patterson and Poyton, 1986).

Cytochrome oxidase IV (COX IV) from yeast, an inner membrane protein, is synthesized as a pre-sequence with a 25 amino acid amino-terminal prepiece (Maarse et al., 1984). When progressively truncated parts of the COX IV presequence were fused to DHFR, the first 12 amino acids, but no less, directed DHFR to the mitochondrial matrix both in vivo and in vitro (Hurt et al., 1984b, 1985a). When the entire 25 amino acid presequence or the first 22 amino acids were fused to DHFR, proteolytic processing by the matrix peptidase also took place, albeit at an alternative site in the latter case. Since the fusions were directed to the matrix instead of the inner membrane, even when the first 53 amino acids of the COX IV precursor were fused to DHFR (Hurt et al., 1984a), the intramitochondrial sorting information must be contained within the mature part of COX IV. Mature COX IV, prepared from either pre-COX IV in vitro or with fusions in vivo, was neither imported nor bound to mitochondria (Hurt et al., 1984a). In fact, removal of only the first 7 amino acids of the prepiece prevented COX IV import (Hurt et al., 1985b).

Similarly, the targeting information for yeast pre-ATPase F₁β, which contains about a 20 amino acid prepiece and is imported to the matrix, exists within the first 27 amino acids of the precursor protein (Douglas et al., 1984; Emr et al., 1986). In fusion studies, as few as 39 amino acids from the amino terminus of pre-F₁β (shorter fusions were not examined) could direct invertase to mitochondria in vivo, but 169 amino acids were required to direct β-galactosidase (presumably due to a folding artifact). Internal deletion studies of a fusion between pre-F₁β amino acids 1–380 and β-galactosidase narrowed down the portion with the targeting information to the first 27 amino acids of the F₁β. In all cases, however, the importable fusions were misdirected to the inner membrane. Deletion
proteins made from authentic F₁β in which amino acids were removed between residues 10 and 36 of the pre-F₁β were fully import competent, while deletions within the first 10 amino acids abolished import (Vas SARotti et al., 1987a).

The amino-terminal prepiece of both rat and human ornithine carbamoyltransferase (OTC), which is 32 amino acids long and directs the protein to the matrix, can also target nonmitochondrial passenger proteins to the matrix in vitro (Horwich et al., 1985b; Nguyen et al., 1986). In contrast to the case of COX IV, the targeting information does not appear to reside in the very amino terminus of the prepiece since deletion studies in which either the amino or carboxy terminus of the prepiece were removed produced proteins which were still import competent. Instead, the targeting information appears to reside between amino acids 8 and 22 of the 32 amino acid prepiece, while the flanking regions contribute to import efficiency (Horwich et al., 1986). The shortest targeting sequence identified to date is from the prepiece of 5-aminolevulinate synthase, in which the amino-terminal 9 amino acids fused to β-galactosidase correctly directs the fusion protein to the mitochondrial matrix in vivo, though with low efficiency (Keng et al., 1986).

Proteins which do not contain removable prepieces also contain specific targeting information. In yeast ADP/ATP carrier this is contained within the first 115 amino-terminal amino acids but has not been further resolved (Adrian et al., 1986). Since the ADP/ATP carrier is a tripartite protein having three segments (each of approximately 100 amino acids) which have a high degree of homology (Saraste and Walker, 1982), then similar targeting ability might also exist in the other two segments of the protein. [Interestingly, the bovine phosphate carrier protein, which has a high degree of structural homology with the ADP/ATP carrier and would presumably follow a similar import pathway, is synthesized as a precursor with a 49 amino acid amino-terminal extension (Runswick et al., 1987)]. In the 70 kDa outer membrane protein from yeast, the targeting and sorting functions are contained in the first 41 amino-terminal amino acids. Deletion and fusion studies have identified two critical regions: amino acids 1 through 21 are required for mitochondrial targeting while the overlapping amino acid sequence 10 through 37 is necessary for sorting via membrane anchoring (Riezman et al., 1983c; Hase et al., 1984, 1986). As for proteins containing removable prepieces, the information exists in the amino-terminal end of the protein. In fact, the amino-terminal 12 amino acids of the 70 kDa protein fused to mature COX IV could target the fusion protein to mitochondria and restore cytochrome oxidase function in COX IV-deficient mutants in vivo (Hurt et al., 1985b).
That the amino-terminal prepiece carries specific mitochondrial targeting information is confirmed by comparing differences in alcohol dehydrogenase (ADH) isozymes which have different subcellular locations. ADH I and II are located in the cytoplasm while ADH III is a mitochondrial protein. They are 80–90% identical in sequence except for a 27 amino acid prepiece in ADH III. If the presequence of ADH III was removed it could not be imported into mitochondria. If the ADH III prepiece was fused to cytosolic ADH II, then the fusion protein was imported into the mitochondrial matrix. Therefore, ADH II is a cytosolic protein since it lacks an amino-terminal targeting sequence while ADH III is a mitochondrial protein since it contains the targeting sequence (van Loon and Young, 1986).

The mitochondrial targeting function of amino-terminal extensions has also been suggested by studies of other proteins in which isozymes having different subcellular locations are encoded by the same gene. For example, the gene encoding yeast histidyl-tRNA synthase has two in-frame translation starts, both of which are expressed (Natsoulis et al., 1986). Mutations destroying the first start codon resulted in a respiratory deficient (Pet-) phenotype without affecting the cytoplasmic isozyme suggesting that the longer mRNA encodes the mitochondrial isozyme while the shorter message encodes the cytoplasmic form. Similarly, the yeast 2-isopropylmalate synthase gene contains multiple in-frame transcription start sites (Beltzer et al., 1986) which produce two related proteins of different sizes in cell-free translation mixtures (Hampsey et al., 1983). Only the larger of these two proteins was imported into mitochondria, suggesting that selection of the appropriate transcription and translation start sites controls the subcellular location of the protein and that mitochondrial targeting information is contained in the amino terminus of the larger precursor protein. This is also the case for a 20 kDa protein from Neurospora crassa (M. Tropschug, H. Köhler, R. A. Stuart, and W. Neupert, in preparation). A single gene encodes the mature-size cytosolic form and a larger precursor (24 kDa), which is imported into mitochondria. During import, the amino-terminal extension is removed in two proteolytic steps.

Mitochondrial prepieces appear to be able to direct almost any passenger protein into mitochondria. For example, in addition to fusions with DHFR and β-galactosidase, it has been demonstrated that a mitochondrial presequence (in this case from COX IV) can direct a chloroplast-encoded protein (ribulose-1,5-bisphosphate carboxylase, large subunit) into mitochondria (Hurt et al., 1986b) and that ATPase subunit VIII, normally a mitochondrial gene product, can also be imported back into
mitochondria when fused to a mitochondrial-targeting sequence (Gearing and Nagley, 1986). In the latter case, the 66 amino acid prepiece of ATPase subunit IX could mediate import of ATPase VIII, while the shorter 40 amino acid COX VI leader could not. It is clear then that targeting information itself resides in the amino-terminal extension of most precursor proteins. The efficiency of import with a given prepiece, however, is significantly affected by the passenger protein (van Steeg et al., 1986).

B. Properties of Prepiece Sequences

The prepiece sequences for a number of imported mitochondrial proteins have now been determined (Table I). They vary in length between 20 and 80 amino acids. Although the prepieces do not share extensive sequence homology (except perhaps between equivalent proteins in different organisms), they do have several similar characteristics including the following: (i) a high content of positively charged basic amino acids (particularly arginine) which are distributed somewhat randomly throughout the prepiece; (ii) an absence or near absence of negatively charged acidic amino acid residues; (iii) a high content of hydroxylated amino acids (particularly serine); and (iv) a propensity to form amphiphilic α-helical structures. Only a few exceptions exist, the most striking of which is the amino-terminal sequence of the 17 kDa subunit IV of yeast bc1 complex which contains an extremely high content of acidic amino acids (van Loon et al., 1984); however, import of this protein into mitochondria has not been examined. Another deviation from these general properties occurs in human OTC, which, unlike its counterpart in rat, contains no hydroxylated amino acids. The placement of positively charged amino acids is well conserved between human and rat OTC but the hydroxylated amino acids in rat OTC have been replaced in human OTC by asparagine (instead of threonine) and phenylalanine (instead of serine).

How these amino-terminal prepieces direct proteins to mitochondria and how they facilitate transport across membranes is not entirely clear. One possibility is that they are recognized by specific receptors on the mitochondrial surface. Another is that, because of their amphiphilic helical nature, they can penetrate lipid bilayers. Finally, because they are positively charged, they may be electrophoretically drawn toward the mitochondrial matrix by the potential across the inner membrane (inside negative). It is possible that all of these events are involved. For example, a membrane potential is necessary for the import of all proteins which must be directed to the inner membrane or matrix. Similarly, if positively charged amino acids in the prepiece are replaced by uncharged amino acids...
acids, then import is abolished (Horwich et al., 1985a). Specific amino acids, and not just net positive charge, also appear to be important. In human OTC, arginine 23 of the 32 amino acid prepiece is critical for both import and proteolytic processing; however, when it is replaced by an amino acid supporting α-helical structure, the import function is conserved (Horwich et al., 1986).

The ability to form amphiphilic helical structures may be a critical feature of presequences. In a theoretical analysis of many mitochondrial presequences, von Heijne identified regions which can be folded into helices with high hydrophobic moment (von Heijne, 1986a). The segments of highest hydrophobic moment correlated very well with the critical regions identified by fusion studies for yeast COX IV and 70 kDa protein, and human OTC (see Table I). In another study, removal of the basic amphipathic α-helix of the ATPase F₁β prepiece prevented import into mitochondria (Vassarotti et al., 1987b). When mutations were selected that restored the correct in vivo localization of ATPase F₁β, it was found that these modifications specifically replaced acidic amino acids at the amino-terminus with basic or neutral amino acids that support amphipathic helix formation.

Some of the physical properties of prepieces have been examined with synthetic prepiece peptides. Synthetic peptides of the COX IV prepiece are soluble in aqueous solutions but were able to penetrate phospholipid monolayers or disrupt liposomes having a diffusion potential negative inside but not of the opposite polarity (Roise et al., 1986). A synthetic peptide equivalent to the first 27 amino acids of pre-OTC assumed an amphiphilic helical conformation that was induced by the presence of anionic phospholipids and could perturb the bilayer of synthetic liposomes (Epand et al., 1986). Synthetic prepiece peptides dissipated the membrane potential in isolated mitochondria (Ito et al., 1985; Gillespie et al., 1985; Roise et al., 1986); however, in the presence of reticulocyte this did not appear to occur (Gillespie et al., 1985). In this case, a synthetic peptide of amino acids 1–27 of the rat OTC prepiece completely blocked the import of pre-OTC, while amino acids 16–27 did not. The peptide (1–27) also blocked the import of pre-malate dehydrogenase (a matrix protein) and pre-thermogenin (uncoupling protein of the inner membrane), suggesting common components are shared in the import pathway of these proteins.

Artificial presequences, encoded by synthetic oligonucleotides, that were fused to the mature part of COX IV were able to mediate import into mitochondria both in vitro and in vivo if the balance of basic, hydrophobic and hydroxylated amino acids was similar to that of authentic presequences (Allison and Schatz, 1986). In all cases, the import-competent extensions were surface active, indicating that targeting may not depend
<table>
<thead>
<tr>
<th>Sequence*</th>
<th>Notes†</th>
</tr>
</thead>
<tbody>
<tr>
<td>OUTER MEMBRANE</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>MKPTTRMKATVAPCTGLGQVSQKKK...</td>
</tr>
<tr>
<td>INTERMEMBRANE SPACE</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>NVAVMMKTCGTVSPELRTGLLH</td>
</tr>
<tr>
<td>3</td>
<td>INRTFCRTPLR</td>
</tr>
<tr>
<td>INNER MEMBRANE - C SIDE</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>MAPVSIVSTRAAMRAAAAPARAVR</td>
</tr>
<tr>
<td>5</td>
<td>MFSNLSK^WAQRTLSK^FYSTATG</td>
</tr>
<tr>
<td>6</td>
<td>MLRTPTVSALVRNVAVRRAKTPMVRAASTMPISNPT</td>
</tr>
<tr>
<td>7</td>
<td>MALTVPVSALVRNVAVRRAKTPMVRAASTMPISNPT</td>
</tr>
<tr>
<td>8</td>
<td>MLQRLPLRNK</td>
</tr>
<tr>
<td>9</td>
<td>MAFTRVFSL</td>
</tr>
<tr>
<td>10</td>
<td>MSTRVLASRLVASQMAASA</td>
</tr>
<tr>
<td>11</td>
<td>MQTTGALLISP</td>
</tr>
<tr>
<td>12</td>
<td>MTRTLTACKN/VKTLKSGFGLANVTSKRDWDFSRPGIRJLSVKAQTAHI</td>
</tr>
<tr>
<td>13</td>
<td>MVLPRLYTATSSRA'AFKAAQSAPLL'STSWKRCMASA</td>
</tr>
<tr>
<td>14</td>
<td>MSLRGKLYTTPA</td>
</tr>
<tr>
<td>MATRIX</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>MSLRTTS</td>
</tr>
<tr>
<td>16</td>
<td>MLSKASLYTAALRVRVSASHVSVATKTEQ</td>
</tr>
<tr>
<td>17</td>
<td>MALLQSRLLISAPRAAATARASSWSHVEMG</td>
</tr>
<tr>
<td>18</td>
<td>MALLHPVQYFASHPLGLEASYRASASHVSHVEMG</td>
</tr>
<tr>
<td>19</td>
<td>MFARKAA</td>
</tr>
<tr>
<td>20</td>
<td>MNSLIRARALVRPTAVRAPLRQPKGAEAVADKR</td>
</tr>
<tr>
<td>21</td>
<td>MALTQFRRNSSAA</td>
</tr>
<tr>
<td>22</td>
<td>MIFTSLLT</td>
</tr>
<tr>
<td>23</td>
<td>MLFNLRILLNAASRNGHIMVRFRCGKTVKVLARLRL</td>
</tr>
<tr>
<td>24</td>
<td>MSLNIRLLNAASRNGHIMVRFRCGKTVKVLARLRL</td>
</tr>
<tr>
<td>25</td>
<td>MSLAPF</td>
</tr>
<tr>
<td>26</td>
<td>MTRILACKNGVVLKQFG</td>
</tr>
<tr>
<td>27</td>
<td>MVLPRLYTATSRNFAQGAQSPISTEWSKRCMASA</td>
</tr>
<tr>
<td>28</td>
<td>MSLNVQYF</td>
</tr>
<tr>
<td>29</td>
<td>MSLWKGTL</td>
</tr>
<tr>
<td>30</td>
<td>MMALLEVSAALGOTACR</td>
</tr>
</tbody>
</table>
### Table I (continued)

<table>
<thead>
<tr>
<th>Sequence*</th>
<th>Notes†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OTHER SEQUENCES OF INTEREST</strong></td>
<td>(k)</td>
</tr>
<tr>
<td>3' MAAVIASSVSAAVARPARSVRPAALKPKAVVPARRPAAPVAAPQAQNNMVWTPVNN</td>
<td></td>
</tr>
<tr>
<td>3' DQRFKTYNCWPTVAAADDNFEHKEAKAAEKEKEEKE</td>
<td></td>
</tr>
<tr>
<td>3' KQSEISSYTVFRQPVLSKLCVPVANQFNLAGKGLKLFDDLIA</td>
<td></td>
</tr>
<tr>
<td>3' IMDMLELVGEYWEQLKLFVTVPVVAAAEDDDNEQHEEKAAEGEEKEEENGDE</td>
<td></td>
</tr>
<tr>
<td>3' IMPOSFYSI AR IGDV ILK^JPVLSKLCVPVANQFNLAGKGLKLFDDLIA</td>
<td></td>
</tr>
<tr>
<td>3' MKIQLVRWHCSRNALWNRAFYSTRKATKNASSATPATMTMSVSRQDFLM</td>
<td></td>
</tr>
<tr>
<td>3' MSNQAVLKLISFRWISTVQRADFILNSELHISATVFSM10PTGCFHGL</td>
<td></td>
</tr>
<tr>
<td>3' MSRLS1KVVTSSIKSSS1I1RMSSATAAT5SAPTANAALKASKRAKPKGK</td>
<td></td>
</tr>
<tr>
<td>3' NVKES1IALAEHAASRASV1PPKLYAAYNLMLDPSKYSKIPNPKLNRR</td>
<td></td>
</tr>
<tr>
<td>3' MVYWVGLMVSPRRGGSSPGYKETNMLSPNQMTKKQQFQKXHTNVL</td>
<td></td>
</tr>
<tr>
<td>3' MSSQVVRDSSAKKLWNLKYPDELR1HILVSFRDOMIQAFRRVAGNPMD</td>
<td></td>
</tr>
</tbody>
</table>

* The amino-terminal prepiece sequences of imported mitochondrial precursor proteins are listed (by the single letter amino acid code) in groups according to the intramitochondrial location of the mature protein. Above the primary sequence, basic (lysine and arginine), acidic (aspartate and glutamate), and hydroxylated amino acids (serine and threonine) are identified (+, −, and •, respectively). Proteolytic cleavage sites which yield intermediate or mature proteins are indicated by an arrow or by a bracket above the sequence where only the general region of proteolytic processing is known (i.e., based on apparent molecular weight differences between precursor and intermediate or mature proteins). The segment of the prepiece identified by von Heijne (1986a) as having the highest hydrophobic moment (18 residue window) is indicated by von Heijne (1986a) and Schiffer and Edmundson, 1967 is indicated by a box.

Proteins: (1) 70 kDa protein (yeast), Hase et al. (1983); (2) cytochrome c peroxidase (yeast), Kaput et al. (1982), Reid et al. (1982); (3) cytochrome b2 (yeast), Giaurdi (1985), Gasser et al. (1982b); (4) Rieske Fe/S of bc1 complex (N. crassa), Harmsisch et al. (1985). Hartl et al. (1986); (5) cytochrome c1 (yeast), Sadler et al. (1984), Gasser et al. (1982b); (6) cytochrome oxidase V (yeast), Koerner et al. (1985); (7) cytochrome oxidase V (N. crassa), Sachs et al. (1986); (8) cytochrome oxidase IV (bovine), Lomax et al. (1984); (9) cytochrome P-450 (SCC) (bovine), Morohashi et al. (1984); (10) cytochrome oxidase VI (yeast), Wright et al. (1984); (11) ATPase IX (N. crassa), Viebrock et al. (1982), Schmidt et al. (1984); (12) ATPase IX-P1 (bovine), Gay and Walker (1985); (13) ATPase IX-P2 (bovine), Gay and Walker (1985); (14) cytochrome oxidase (yeast), Maarse et al. (1984); (15) citrate synthase (yeast), Suzuki et al. (1984); (16) ornithine aminotransferase (rat), Mueckler and Pitot (1985). Simmons et al. (1986); (17) aspartate aminotransferase (chicken), Jaus et al. (1985); (18) aspartate aminotransferase (porcine), Joh et al. (1985); (19) Mn-superoxide dismutase (yeast), Marres et al. (1985); (20) ATPase F1β subunit (N. crassa), Kruse and Sebald (1984); (21) 5-aminolevulinate synthase (yeast), Keng et al. (1986), Urban-Grimal et al. (1986); (22) alcohol dehydrogenase iso-III (yeast), Young and Pilgrim (1985); (23) ornithine carbamoyltransferase (human), Horwich et al. (1984); (24) ornithine carbamoyltransferase (rat), McIntyre et al. (1984), Takiguchi et al. (1984), Kraus et al. (1985); (25) mEF-Tu (yeast), Nagata et al. (1983); (26) carbamoyl-phosphate synthase I (rat), Nyunoya et al. (1985); (27) ATPase F1β subunit (yeast), Takeda et al. (1985); (28) ATPase F1β (Nicotiana plumbaginifolia), Boutry and Chua (1985). Kobayashi et al. (1986); (29)
on specific amino acid sequences but rather on the overall composition of
the prepiece and its amphipathic nature. Sequences with these character-
istics may reside at the amino-terminus of mitochondrial precursor pro-
teins, where they are exposed and can be active, but they also appear to
exist within nonmitochondrial proteins, although they are apparently
masked such that they do not normally direct these proteins to mitochon-
dria (Hurt and Schatz, 1987).

C. The Stop Transport Model

Based on the sequence characteristics of mitochondrial precursor pro-
teins, Hurt and van Loon (1986) proposed a model for intracellular target-

---

ATPase F_{i}\beta (human), Ohta and Kagawa (1986); (30) adrenodoxin (bovine), Okamura et al. (1985); (31) ribulose-1,5-bisphosphate carboxylase small subunit (Chlamydomonas reinhardtii), Schmidt et al. (1979), Hurt et al. (1986a); (32) subunit VI of bc\textsubscript{1} complex (yeast), van Loon et al. (1984); (33) 14 kDa subunit of bc\textsubscript{1} complex (yeast), De Haan et al. (1984); (34) threonyl-tRNA synthase (yeast), Pape et al. (1985); (35) tryptophanyl-tRNA synthase (yeast), Myers and Tzagoloff (1985); (36) histidyl-tRNA synthase (yeast), Natsoulis et al. (1986); (37) 2-isopropylmalate synthase (yeast), Beltzer et al. (1986); (38) MSS51 (COX I pre-mRNA maturase, yeast), Faye and Simon (1983); (39) CBP2 (cyto-

Notes: (a) Not an actual prepiece (i.e., not proteolytically removed) but amino ter-
nus is able to specifically target \(\beta\)-galactosidase to mitochondria (Hase et al., 1984, 1986).
(b) Precursors are processed in two proteolytic steps. This has not yet been demonstrated
for bovine ATPase IX (P1 or P2) but it may occur in two steps like \textit{N. crassa} ATPase IX. (c)
Bovine COX IV is equivalent to COX V in yeast or \textit{N. crassa} (Gregor and Tsugita, 1982).
(d) Possible cleavage site based on homology with \textit{N. crassa} ATPase IX processing sites.
Grouped with matrix proteins since it is probably imported as a matrix protein and then
assembled into ATPase. (e) Possible cleavage site based on alignment with cytosolic ADH
II. (f) Possible cleavage site based on alignment with bacterial mature sequence. (g) An
alternate region with similar hydrophobic moment exists between residues 22 and 39. (h)
ATPase F_{i}\beta (a subunit of ATPase at the inner face of the inner membrane) is grouped
with matrix proteins since it is probably imported as a matrix protein and then assembled
into ATPase. (i) Possible cleavage site based on homology with amino terminus of mature
F_{i}\beta from Ipomoea batatas (Kobayashi et al., 1986). (j) Possible cleavage site based on
homology with bovine mature sequence. (k) The presequence of chloroplast RuBPCase
(31) is presented since the first 35 amino-terminal amino acids can direct DHFR or mature
COX IV to mitochondria (Hurt et al., 1986). The first 50 amino acids of the 17K subunit VI
of the bc\textsubscript{1} complex (32; determined from the nuclear gene sequence)—which contains an
unusually high content of acidic amino acids—are presented, though import has not
been examined. Sequences 33–40 are amino-terminal sequences (determined from the
nuclear gene sequence) for putative mitochondrial proteins or proteins for which import
into mitochondria has not yet been characterized. The first 50 amino acids from the pre-
sumed start site are presented. (I) Apparently imported into mitochondrial matrix with-
out proteolytic processing (Gasser et al., 1982a; Hampsey et al., 1983).
ing and intramitochondrial sorting of imported mitochondrial proteins. In this model, mitochondrial presequences (or amino-terminal sequences in noncleavable proteins) can be divided into distinct domains: (1) matrix targeting domains; (2) stop transport domains; and (3) proteolytic cleavage sites. The presence or absence and arrangement of these domains determines the targeting and sorting of the accompanying mature piece of the protein. The matrix targeting domain (the amino-terminal positively charged region of the prepiece) directs the attached protein to mitochondria and mediates its transfer across both mitochondrial membranes to the matrix. If this domain is followed by a stop transport domain (a long uninterrupted sequence of uncharged amino acids capable of forming a transmembrane anchor), then the transfer of the protein is halted at either the outer or inner membrane, depending on subtle differences in the size and strength of the stop transport region. Further sorting or simply removal of the prepiece can then occur by proteolytic processing.

For example, in this model ADH III is a matrix protein because it contains the amino-terminal matrix targeting domain but no subsequent stop transport sequence. Inner membrane proteins also contain the matrix targeting domain but are halted at the inner membrane because of stop transfer domains within the mature part of the sequence. The matrix targeting sequence is then removed by proteolytic processing to prevent further import. For intermembrane space proteins, such as cytochrome $b_2$, the stop transport domain is in the prepiece so that when transport is halted at the inner membrane and proteolytic cleavage occurs at the outer face of the inner membrane the mature part of the protein is released into the intermembrane space. Cytochrome $c_1$ is proposed to follow a similar mechanism but remains associated with the inner membrane because of a carboxy-terminal anchor. Indeed, when the first 64 amino acids of cytochrome $c_1$ (containing the entire 61 amino acid presequence) was fused to DHFR, the fusion protein was directed to the inner membrane and DHFR was released into the intermembrane space following processing (van Loon et al., 1986). Identical results were observed when the cytochrome $c_1$ prepiece was fused to the mature part of COX IV (van Loon et al., 1987). Outer membrane proteins are simply halted at the outer membrane by a stop transport domain with presumably different characteristics from the inner membrane stop transport region.

The stop transport model accounts for the intramitochondrial sorting of many imported proteins. For the most part, it assumes a relatively simple linear arrangement of targeting and sorting domains, which may not be the case for all proteins. It does not, however, account for the import mechanism of intermembrane space proteins which do not contain presequences, such as cytochrome $c$ or adenylate kinase. Other movements
may also be possible which the data supporting the stop-transport model cannot yet exclude. For example, the Fe/S protein of the \( bc_1 \) complex, which like cytochrome \( c_1 \) is also synthesized with a long prepiece (that is processed in two steps), is fully transported into the matrix, where it is processed by the matrix peptidase, and is then redirected back to the intermembrane space (Hartl et al., 1986; see Section XIB). In *Neurospora crassa*, cytochrome \( c_1 \) follows a similar transport pathway through the matrix (F.-U. Hartl, J. Ostermann, and W. Neupert, in preparation). In yeast, however, the imported cytochrome \( c_1 \) was never found on the matrix side of the inner membrane (van Loon and Schatz, 1987).

D. Heterologous Import

The precursor proteins and import machinery of mitochondria from different organisms share many common features. This can be demonstrated in heterologous import experiments in which the mitochondrial precursor protein from one organism can be imported into the mitochondria of another. For example, the Fe/S protein from *Neurospora crassa bc_1* complex could be imported into yeast mitochondria (Teintze et al., 1982), and, similarly, either ADP/ATP carrier or ATPase subunit IX could be imported into rat liver mitochondria (Schleyer et al., 1982). This is not at all surprising since conservation of complicated import machinery is likely to be high during evolution. What is interesting, however, is that the precursor to *N. crassa* ATPase subunit IX could be imported into yeast mitochondria and be correctly processed (Schmidt et al., 1983a; Fig. 1). In *Neurospora*, ATPase IX is a nuclear gene product which is synthesized on cytoplasmic polysomes (Jackl and Sebald, 1975; van den Boogaart et al., 1982b) while in yeast it is coded for by mitochondrial DNA and synthesized inside the mitochondrion (Tzagoloff and Meagher, 1972; Macino and Tzagoloff, 1979; Hensgens et al., 1979). The mature proteins are similar, and their sequences are 53% homologous in overlapping regions; however, the *Neurospora* pre-ATPase IX, which must be imported from the cytosol, contains a long 66 amino acid amino-terminal prepiece which presumably directs it to the mitochondrial inner membrane. A similar prepiece is absent in the yeast mitochondrial gene product.

Heterologous import has also been demonstrated between different subcellular organelles. When the prepiece (transit peptide) of chloroplast ribulose-1,5-bisphosphate carboxylase (small subunit) was fused to either DHFR or the mature part of cytochrome oxidase IV, the resulting fusion proteins were imported into mitochondria *in vivo*, albeit with lower efficiency than with authentic mitochondrial prepieces. In the latter case, the fusion protein could restore cytochrome oxidase activity *in vivo* when
used to transform a COX IV-deficient mutant of yeast (Hurt et al., 1986a,b). When compared to mitochondrial prepipee sequences, the chloroplast transit peptide shares many common features (e.g., high content of basic and hydroxylated amino acids with no acidic amino acids; Table I). What subtle differences exist in the targeting prepipees that correctly direct proteins to either chloroplasts or mitochondria in photosynthetic cells are unknown.

IV. RECEPTORS

While much information is obviously contained within specific regions of mitochondrial precursor proteins, there must exist complementary structures within mitochondria themselves to decode and process it. One obvious possibility is that receptors are involved in the initial recognition of proteins imported from the cytosol.

Proteinaceous components on the outer surface of mitochondrial membranes were first demonstrated by shaving isolated mitochondria with low concentrations of protease which did not penetrate or destroy the outer
membrane (Gasser et al., 1982a; Zwizinski et al., 1984; Pfaller and Neupert, 1987). Following this treatment, the specific binding of precursor proteins to the outer membrane was blocked and import was abolished.

The binding of precursor proteins to mitochondria is independent of import and precedes all other events in the import pathway. For example, mature outer membrane porin is in a location which is protected from externally added proteases. If the insertion of precursor porin into the lipid bilayer of the outer membrane was inhibited by importing at 0°C, the binding of the protein to mitochondria was unaffected and it remained in a protease-susceptible location at the outer face of the outer membrane. When mitochondria containing the bound porin were reisolated and warmed to 25°C, the protein was subsequently imported from its receptor sites to the protease-protected location (Freitag et al., 1982). Similarly, the import of cytochrome c could be blocked with the heme analog deuterohemin which prevents covalent attachment of heme to the precursor apocytochrome c and subsequent translocation across the outer membrane. Under these conditions, apocytochrome c could still bind to mitochondria independently of import. When the inhibition by deuterohemin was reversed by adding excess amounts of hemin, cytochrome c was subsequently imported from its receptor sites into the intermembrane space (Hennig and Neupert, 1981).

Proteins such as the ADP/ATP carrier, which require a membrane potential for import, could be stalled at their receptor sites by disrupting the potential with CCCP. On reestablishing the membrane potential, the bound precursor could be imported (Zwizinski et al., 1983; Pfanner and Neupert, 1985). In these cases, however, the binding to deenergized mitochondria was slow, though clearly independent of a membrane potential. Binding of pre-ATPase F\(_{\beta}\) to deenergized mitochondria did not occur at all (Zwizinski et al., 1984), though for unknown reasons. The binding of membrane potential-dependent proteins to deenergized mitochondria may be poor because of the low capacity of specific sites which may be occupied by precursors with higher affinity.

Receptors for imported mitochondrial proteins are specific in many regards as was demonstrated by the receptors for precursors to cytochrome \(b_2\) and citrate synthase in yeast (Riezman et al., 1983b). First, binding was specific to the outer mitochondrial membrane. These precursor proteins were able to bind to whole mitochondria or to isolated outer membrane vesicles (Riezman et al., 1983a) but not to inner membrane preparations. Binding was also specific for precursor proteins. When the partially processed intermediate form of the cytochrome \(b_2\) precursor was generated by treatment with an extract of the mitochondrial matrix (con-
taining the processing peptidase), the intermediate size cytochrome $b_2$ did not bind to mitochondria. Similarly, binding of the mature form did not occur either. The same observation has been made with cytochrome $c$ where mature holocytochrome $c$ did not recognize the binding sites in *Neurospora* mitochondria (Hennig *et al.*, 1983) and even large excesses could not displace prebound apocytochrome $c$ (H. Köhler and W. Neupert, unpublished). Last, binding was specific for mitochondrial proteins. For example, the binding of cytosolic hexokinase and glyceroldehyde-3-phosphate dehydrogenase to whole yeast mitochondria or isolated outer membrane vesicles was negligible (Riezman *et al.*, 1983b).

How many different receptors exist to mediate the recognition and binding of imported mitochondrial proteins? It is clear from the large number of mitochondrial proteins and the limited number associated with the outer membrane that it would be impossible, if not just simply impractical, for a different receptor to exist for each protein. Nevertheless, experiments have shown that many mitochondrial proteins do not share common binding sites so that a single receptor for all imported proteins is not the case either. For example, the binding of porin to yeast mitochondria was not affected when the mitochondria were pretreated with low concentrations of trypsin that were sufficient to abolish the binding of F$_1$ ATPase $\beta$ subunit and cytochrome $b_2$ (Gasser and Schatz, 1983). This suggests that they are bound by different receptors having different trypsin sensitivities. Similarly, shaving *Neurospora* mitochondria with elastase inhibited the binding and import of ADP/ATP carrier and porin but had no effect on ATPase F$_1\beta$ (Zwizinski *et al.*, 1984).

By this criterion it is difficult to assign possible common binding sites for the few mitochondrial proteins that have been studied to date. In addition, proper competition studies are limited by the difficulties of preparing sufficient amounts of mitochondrial precursor proteins. In three cases this has been overcome: (1) apocytochrome $c$, the precursor to holocytochrome $c$, can be prepared by chemical cleavage of the heme group and renaturation of the protein by dialysis from urea (Ambler and Wynn, 1973); (2) a water-soluble form of porin can be made by subjecting isolated porin to acid precipitation and then resolubilizing the protein at alkaline pH in the absence of detergent (Pfaller *et al.*, 1985); and (3) the precursor to F$_1$ ATPase $\beta$ subunit can be accumulated then purified from yeast rho$^-$ mutants grown in the presence of CCCP (Ohta and Schatz, 1984). In all three cases, the resulting precursor proteins were import competent and retained characteristics of their precursors synthesized in cell-free systems. Apocytochrome $c$ does not compete for the binding of any mitochondrial proteins tested so far, including cytochrome $c_1$, the Fe/S protein of $bc_1$ complex, ATPase F$_1\beta$, ATPase IX, or ADP/ATP carrier
(Zimmermann et al., 1981; Teintze et al., 1982). It appears to have a unique receptor. Porin, however, is able to compete for the binding and import of the ADP/ATP carrier, suggesting a common receptor (Pfaller and Neupert, 1987). Binding competition has not yet been examined using the ATPase $F_1\beta$ precursor purified from yeast.

The binding of cytochrome $c$ to Neurospora mitochondria is the best characterized of imported mitochondrial proteins so far. When radiolabeled apocytochrome $c$ was bound to mitochondria in the presence of deuterohemin (to prevent subsequent import), it could be completely displaced by adding unlabeled apocytochrome $c$ but not holocytochrome $c$ (Hennig et al., 1983). Apocytochrome $c$ from different species could also displace the precursor protein to varying degrees. Titration of the binding sites on mitochondria by Scatchard analysis indicated that there were 90 pmol of high affinity binding sites for apocytochrome $c$ per milligram of mitochondrial protein. These sites had an association constant ($K_a$) of $2.2 \times 10^7$ M$^{-1}$ (Table II).

When mitochondria were solubilized with octyl glucoside and the resulting extract was reconstituted into liposomes, the high affinity binding sites were also reconstituted (Köhler et al., 1987). Using this procedure to identify high affinity binding for apocytochrome $c$, a putative receptor protein was identified and purified to homogeneity. The protein, when reconstituted into liposomes, bound apocytochrome $c$ with similar affinity compared to whole mitochondria. In addition, the purified protein could compete with mitochondria for the binding of apocytochrome $c$. The unusual feature of the apocytochrome $c$ binding protein, however, is that it is a soluble protein of the intermembrane space. This explains why more than a 10-fold higher concentration of proteases are required to abolish apocytochrome $c$ binding to mitochondria compared to other imported proteins. Although the binding of apocytochrome $c$ to the protein meets all criteria for specific interaction of a protein with a receptor—namely, (i)
17. Synthesis and Assembly of Mitochondrial Proteins

rapid and reversible binding, (ii) saturable and limited in number, and (iii) specificity—the location in the intermembrane space is unexpected. Cytochrome c appears to have an unusual import pathway which is not representative of the mechanism for other imported proteins. Apocytochrome c is able to spontaneously insert into lipid bilayers in a nonspecific manner with low affinity (Rietveld et al., 1983, 1985, 1986a,b; Rietveld and Kruijff, 1984; Dumont and Richards, 1984). A possible explanation of the binding protein's location is that it recognizes the partially inserted apocytochrome c and then binds it from the inner face of the outer membrane, perhaps to mediate exposure of the cysteine sulfhydryl groups to the heme attaching enzyme. The apocytochrome c binding protein and the heme attaching enzyme are distinct proteins (Nicholson et al., 1987).

The high affinity binding of porin to mitochondria was sensitive to treatment of whole mitochondria with very low concentrations of trypsin, indicating that the receptor is exposed to the outer surface of the outer membrane (Pfaller and Neupert, 1987). As for cytochrome c, the high affinity binding sites for porin could be solubilized with detergent and reconstituted into liposomes. Using this approach, it should be possible to purify the porin receptor.

The identification of putative receptor proteins mediating import into mitochondria has also been successful using other methods. For example, antibodies raised against the total outer mitochondrial membrane of yeast blocked the import of the preCOX IV-DHFR fusion protein (Ohba and Schatz, 1987a). Antibodies raised against a 45 kDa outer membrane protein also blocked import while sera against other outer membrane proteins did not. The Fab fragments of the anti-45 kDa antibodies alone also blocked import. In another study, a 30 kDa protein from the outer membrane of rat mitochondria was specifically cross-linked to the synthetic prepiece (amino acids 1–27) of pre-OTC (Gillespie, 1987). Mild pretreatment of mitochondria with trypsin reduced both cross-linking of the prepiece peptide to the 30 kDa protein and the import of pre-OTC into mitochondria.

V. ENERGY REQUIREMENTS

Precursor proteins bind to mitochondria independently of other events in the import pathway; however, subsequent translocation into the mitochondrion is dependent on energy in most cases. Generally, energy is required for (i) all proteins having an amino-terminal extension, (ii) all proteins which are inserted into or translocated across the inner membrane, and (iii) only for import to the first processing stage for those
proteins which are proteolytically processed in two steps. Where import requires energy, it is needed in the form of an energized inner membrane, specifically, the membrane potential component ($\Delta \psi$) of the total proton-motive force ($\Delta \rho$).

The primary energy source required for import was first thought to be ATP (Nelson and Schatz, 1979). It was later shown, however, that an energized inner membrane was the necessary energy form. Even a low potential of 20–40 mV, about one-tenth the normal value of 230 mV (Mitchell and Moyle, 1969), can drive the import of mitochondrial proteins (Pfanner and Neupert, 1985).

The requirement for an electrochemical potential and not ATP as the immediate energy source was clearly demonstrated in vitro in a series of experiments by Schleyer et al., (1982) in which high intramitochondrial ATP plus a dissipated membrane potential versus low ATP plus a normal membrane potential were compared in Neurospora crassa mitochondria. In the first case (high ATP/no membrane potential), the membrane potential was dissipated by the protonophore CCCP, and intramitochondrial ATP was elevated via the ADP/ATP carrier by adding ATP externally. Oligomycin was added to inhibit the $F_0F_1$ ATPase and prevent the hydrolysis of ATP which would generate a small potential. Under these conditions, high internal concentrations of ATP in the absence of an energized inner membrane could not drive import. In the opposite case (low ATP/normal membrane potential), intramitochondrial ATP was depleted by preincubating mitochondria in the presence of oligomycin (to inhibit the membrane potential-driven synthesis of ATP) and carboxyatractyloside (to block the ADP/ATP carrier and prevent the translocation of external ATP into mitochondria). Under these circumstances, the electrochemical potential in the absence of ATP was sufficient to power import. In yeast mitochondria, import was blocked when the membrane potential was dissipated with CCCP but not when ATP synthesis was inhibited by oligomycin (Gasser et al., 1982a). Furthermore, ATP-supported import in cyanide-inhibited mitochondria was blocked by either carboxyatractyloside (so ATP could not enter the matrix) or oligomycin (which would prevent ATP hydrolysis), demonstrating that ATP-stimulated import was a consequence of the small electrochemical gradient generated by the hydrolysis of ATP by the $F_0F_1$ ATPase.

The electrochemical potential (or total protonmotive force) $\Delta \rho$ is the sum of the membrane potential ($\Delta \psi$) from the separation of charged species across the inner membrane and the force exerted by the pH gradient ($\Delta \rho$) ($\Delta \rho = \Delta \psi - Z \Delta \rho$). It is the membrane potential component of the total protonmotive force which is responsible for powering import. This was first suggested by experiments where the ionophore nigericin, which
exchanges $K^+$ for $H^+$ in a stoichiometric way across the inner membrane, did not affect the import of ADP/ATP carrier or ATPase IX into *Neurospora* mitochondria (Schleyer et al., 1982). Since nigericin leads to the breakdown of the proton gradient without affecting the membrane potential, it is the latter which drives import of precursor proteins. This was substantiated in experiments where the physiological membrane potential was inhibited by antimycin A/oligomycin and then a valinomycin-induced potassium diffusion potential could drive the import of the ADP/ATP carrier into *Neurospora* mitochondria (Pfanner and Neupert, 1985). This import was not abolished by protonophores like CCCP which dissipate the proton gradient and, in the absence of a $K^+$ diffusion potential, would normally dissipate the accompanying membrane potential. Furthermore, the establishment of a $\Delta p$H did not power import. The importance of the membrane potential component of the total protonmotive force is not surprising since $\Delta p$H makes only a relatively small contribution to $\Delta p$ under physiological conditions.

The energy necessary for import is not needed for proteolytic processing itself. For example, proteins that are transported to the inner membrane or matrix, but which are not proteolytically processed, require an energized inner membrane. This has been demonstrated for the ADP/ATP carrier (Schleyer et al., 1982; Pfanner and Neupert, 1985) and for the matrix-localized 2-isopropylmalate synthase (Hampsey et al., 1983). In addition, the purified matrix processing peptidase does not require any form of energy to process protein outside of whole mitochondria (G. Hawlitschek and W. Neupert, unpublished). Instead, energy is required for interaction with the mitochondrial inner membrane. Evidence for this is 3-fold. First, all precursor proteins which must be translocated into or across the inner membrane require a membrane potential for import. This is also true for intermembrane space proteins which require proteolytic processing (e.g., cytochrome $b_2$, cytochrome $c_1$, and cytochrome $c$ peroxidase). In these cases, the precursor protein must at least partially penetrate the inner membrane to reach the matrix peptidase for the first processing event (Daum et al., 1982b; Gasser et al., 1982b; Reid et al., 1982; Teintze et al., 1982). Second, proteins that are imported into the intermembrane space but do not come in contact with the inner membrane, such as cytochrome $c$ (Zimmermann et al., 1981), and those imported into the outer membrane, such as porin (Freitag et al., 1982; Mihara et al., 1982a) and the major outer membrane polypeptides (Gasser and Schatz, 1983), do not require an energized inner membrane for import. Finally, when import of cytochrome $c_1$ and $F_1$ ATPase $\beta$ subunit was performed at 7°C, an intermediate could be trapped in which the amino-terminal prepiece could be processed by the matrix peptidase, but the
major portion of the precursor was still outside the mitochondrion and could be digested by externally added proteases (Schleyer and Neupert, 1985). Import to this stage was dependent on energy; however, subsequent translocation of the proteins completely into mitochondria was independent of a membrane potential when chased at 25°C. Therefore, only import of the amino terminus through the inner membrane required a membrane potential while transport of the rest of the protein did not.

Proteolytic processing, however, is not obligatory for import past the energy-dependent step. Precursors to F\textsubscript{1} ATPase $\beta$ and IX subunits could be partially imported into mitochondria without processing in the presence of $\sigma$-phenanthroline, which blocks the matrix processing peptidase. The precursors could then be chased to the mature size by adding Mn$^{2+}$ in a step that did not require a membrane potential (Zwizinski and Neupert, 1983). The energy-dependent step precedes and is independent of proteolytic processing.

Exactly why the membrane potential is required for import is not clear. One possibility is that it produces an electrophoretic driving force (negative inside) on the positively charged prepieces which mediates penetration into or through the inner membrane (Fig. 2). Another is that it induces transient conformational changes in lipid and protein organization which allow the initial entry of the precursor protein into or through the membrane barrier. Both effects probably contribute to energy-dependent import.

Not all energy requirements are strictly for an energized inner mem-

---

**Fig. 2.** A membrane potential ($\Delta\psi$) is required for the import of proteins which must go to or through the inner membrane. Energy is not required for binding to receptors but is necessary for penetration of the positively charged amino-terminal prepiece through the inner membrane. Subsequent translocation of the remainder of the protein through the inner membrane, or movement away from contact sites (i.e., inner membrane proteins), does not require the continued presence of $\Delta\psi$. Proteolytic processing is also independent of energy. OM, Outer membrane; IM, inner membrane; R, receptor.
brane. For example, degradation of endogenous ATP, by the enzyme apyrase, reduced the import of ATPase F₁β into Neurospora crassa mitochondria even in the presence of a membrane potential, whereas subsequent addition of ATP or GTP restored import (Pfanner and Neupert, 1986). Nonhydrolyzable ATP analogues also blocked the import of mitochondrial precursor proteins (Pfanner and Neupert, 1986; Eilers et al., 1987; Chen and Douglas, 1987), indicating that cleavage of the nucleotide triphosphate (NTP) phosphodiester bond is necessary. It has been suggested that NTPs maintain or confer an import-competent conformation in mitochondrial precursor proteins. This is supported by experiments in which the proteolytic sensitivity of precursor proteins is greater in the presence of NTPs (Pfanner et al., 1987; Verner and Schatz, 1987), indicating that a less folded conformation is sustained by NTP hydrolysis and that such a conformation is necessary for import. The requirement for NTPs depends primarily on the mature part of the precursor protein. For example, precursors having identical presequences but different mature polypeptides required different concentrations of NTPs for optimal import (Pfanner et al., 1987). It appears that NTPs are necessary for conferring import-competence during all steps that precede and include the interaction of the precursor with the outer membrane (Pfanner et al., 1987; Eilers et al., 1987). In addition, maturation steps for some imported proteins require NADH. For example, the covalent attachment of heme to apocytochrome c and concomitant translocation across the outer membrane is dependent on NADPH in yeast (Basile et al., 1980) and NADH in N. crassa (Nicholson et al., 1987). Similarly, the second processing step of cytochrome c₁, which is accompanied by covalent heme attachment, is also dependent on NADH (Teintze et al., 1982; Schleyer and Neupert, 1985). The second processing step of the Fe/S protein of the bc₁ complex requires NADH (F.-U. Hartl and W. Neupert, unpublished), presumably for the Fe/S cluster formation. Maturation of cytochrome oxidase II, a mitochondrial translation product, also requires NADH (W. Driever, R. Cook, and W. Neupert, unpublished). These proteins all have the common feature of having iron, either in heme or as nonheme Fe/S clusters.

VI. CYTOSOLIC COFACTORS

By virtue of the elaborate mixtures which are required for the cell-free synthesis of precursor proteins, import reactions in vitro contain many components. In the few cases where mitochondrial precursor proteins could be purified or where minimal amounts of cell-free translation mixtures have been used, a number of potential "cytosolic" cofactors which are required for import have been identified. These cofactors are present
both in reticulocyte lysates and in homologous postribosomal cytosol preparations. They fall into three broad classes: (i) soluble low molecular weight components; (ii) proteins; and (iii) RNA.

When a reticulocyte lysate translation mixture containing the newly synthesized precursor to rat ornithine carbamoyltransferase (OTC) was passed over a Sephadex G-25 column, the precursor, recovered in the excluded fraction, could no longer by imported into mitochondria. Import of the precursor could be restored by fresh unlabeled reticulocyte lysate mixture but not by its individual components, including Mg\(^{2+}\), K\(^{+}\), or ATP (Argan et al., 1983). The reticulocyte lysate itself, without additives for translation, completely restored import. In an independent report, the postribosomal supernatant of the reticulocyte lysate, which had been dialyzed, stimulated the import of pre-OTC severalfold (Miura et al., 1983). The stimulating activity of the added dialyzed lysate was inactivated by either pretreatment with trypsin or heat denaturation, suggesting that the cofactor is a protein. The cofactor was further characterized by examining requirements for the import of pre-OTC, which had been purified by immunoaffinity chromatography, into mitochondria (Argan and Shore, 1985). When pre-OTC was mixed with the untreated lysate, the cofactor bound the precursor to form a 5S complex. The pre-OTC in the isolated 5S complex was imported into mitochondria without a requirement for extra lysate. When mitochondria were pretreated with reticulocyte lysate and then reisolated, import of purified pre-OTC did not occur. However, when pre-OTC plus reticulocyte lysate was mixed with mitochondria at 4°C, 50% of the pre-OTC bound to the mitochondrial surface and could be imported when the mitochondria were isolated and incubated at 30°C. The import factor appears to be necessary for the import-competent delivery and binding of pre-OTC to mitochondria.

The purified precursor to the F\(_{1}\) ATPase β subunit is poorly imported into yeast mitochondria. In the presence of the cytosolic fraction from yeast or reticulocytes, however, import and processing was stimulated 4- to 8-fold (Ohta and Schatz, 1984). The cofactor was nondialyzable, protease sensitive, and had an apparent molecular mass of 40 kDa. Similar stimulation of import of the in vitro synthesized cytochrome b\(_{2}\) precursor by unlabeled reticulocyte lysate indicated that cofactors may be necessary for other proteins as well. Again, the cofactor appeared to aid the correct association of the precursor with the mitochondrial surface.

A cytoplasmic RNA component has also been implicated in the import of several proteins into rat liver mitochondria. Posttranslational treatment with RNase of reticulocyte lysates containing newly synthesized precursor proteins inhibited subsequent import into mitochondria (Firgaira et al., 1984). The cofactor and OTC precursor fractionated as a 400 kDa
complex with characteristics of a ribonucleoprotein. On the other hand, the cofactor which stimulated the import of purified OTC precursors was not sensitive to RNase (Argan and Shore, 1985). The import of $F_1$ ATPase $\beta$ subunit and citrate synthase precursors into yeast mitochondria was also inhibited by pretreatment of the reticulocyte lysate (in which they were synthesized) with RNase; however, removal of the ribosomes by centrifugation abolished the sensitivity of import to RNase, suggesting that the RNA cofactor is not a specific component necessary for import but that degraded ribosomes inhibit import (Burns and Lewin, 1986). In contrast, RNase treatment of reticulocyte lysate postribosomal supernatants containing newly synthesized ATPase $F_1\beta$ inhibited binding and import into *Neurospora crassa* mitochondria (N. Pfanner and W. Neupert, unpublished). The role of putative RNA cofactors requires further investigation.

Protein and RNA cofactors present in cytosolic fractions may serve a number of possible functions during import. For example, they may stabilize precursor proteins against premature proteolytic digestion in the cytosol or mediate conformational arrangements which are necessary for import competence. These cofactors appear to be necessary for the specific binding of precursors to mitochondria but not for subsequent stages of import. Specific cofactors have not yet been isolated so that it cannot be ruled out that they may act in a nonspecific manner by, for instance, preventing the small amounts of precursor proteins from aggregating. Whether such cofactors are important *in vivo* is unknown.

The import of apocytochrome $c$ into mitochondria is also dependent on a cytosolic or reticulocyte lysate cofactor. In this case, however, the stimulatory component is a low molecular weight, heat-stable factor which is not sensitive to proteases. In yeast, the cofactor can be substituted by an NADPH-regenerating system (Taniuchi *et al.*, 1982), but in *N. crassa* the cofactor appears to serve some other function (Nicholson *et al.*, 1987). It is not involved in the binding of apocytochrome $c$ to mitochondria, but is necessary for enzymatic attachment of heme and subsequent translocation across the outer membrane.

VII. TRANSLOCATION CONTACT SITES

Proteins which are imported into the mitochondrial matrix or inner membrane must cross two membrane barriers to reach their final location. The question arises as to whether transport across the outer and inner membrane occurs in two distinct steps, with a soluble intermediate in the intermembrane space, or whether the inner and outer membranes come
close enough together to be spanned and crossed in a single event. For proteins imported into the inner membrane or matrix of *Neurospora crassa* mitochondria, the latter mechanism appears to be the case. These translocation contact sites have been demonstrated for the import of the F1 ATPase β subunit and cytochrome c1 (Schleyer and Neupert, 1985), the Fe/S protein of bc1 complex (Hartl *et al.*, 1986), and the ADP/ATP carrier (Pfanner and Neupert, 1987).

Membrane-spanning intermediates could be detected when translocation was impeded by importing the precursors of ATPase F1β or cytochrome c1 at low temperatures (4–12°C). Under these conditions, translocation intermediates were accumulated which had penetrated far enough through the inner membrane to be processed by the matrix-localized processing peptidase but which still had the major portion of the polypeptide exposed to the outer surface of the outer membrane since they could be digested with externally added proteases (Schleyer and Neupert, 1985). Import to this stage was dependent on a membrane potential. When translocation intermediates spanning contact sites were accumulated this way, they could be subsequently chased into a protease-insensitive location by raising the temperature to 25°C in a step that was independent of the membrane potential. Similarly, complete transport across the mitochondrial membranes could be blocked by prebinding the precursor proteins to specific antibodies generated against the mature size proteins. When incubated with mitochondria, the amino terminus of the antibody-bound precursors penetrated far enough into the matrix to be proteolytically processed while the major part of the precursor was retained by the antibodies outside the mitochondria in a location susceptible to externally added proteases. These experiments indicate that (i) the ATPase F1β and cytochrome c1 precursors first entered the matrix by their amino termini in a step that is dependent on a membrane potential; (ii) the membrane potential is required only for import of the amino-terminal prepiece which is then sufficient to trigger the transmembrane movement of the remainder of the polypeptide chain; and (iii) transport across the mitochondrial membranes occurs via translocation contact sites in which the outer and inner membranes come close enough together to be simultaneously spanned by the imported polypeptide.

Sites of contact between the outer and inner mitochondrial membranes have been visualized by transmission electron microscopy (Hackenbrock, 1968). They appear to be stable structures since they are visible in mitochondria in both the condensed and orthodox conformations. It was estimated that there were 115 of these sites in a 1 μm diameter mitochondrion from rat liver. Similar regions of contact have been observed in mitochondria that were prepared for electron microscopy by freeze-etching (van
Venetie and Verkleij, 1982). Interpretation of the fracture plane, which jumped back and forth between the outer and inner limiting membranes, suggested that semifusion of the membranes had occurred in which nonbilayer lipids (specifically hexagonal II phase lipids) were involved.

Recently, the sites where mitochondrial precursor proteins form translocation intermediates spanning both membranes were correlated to the morphological contact regions (Schwaiger et al., 1987). The precursor of the F$_1$ ATPase $\beta$ subunit, synthesized in reticulocyte lysate, was pre-bound to a specific antibody directed against the mature protein and then incubated with mitochondria. The antibody prevented the protein from being completely imported into mitochondria, but the amino-terminal pre-piece was removed by the matrix-localized processing peptidase. When the antibody-bound pre-F$_1$$\beta$ was tagged by protein A–gold and visualized by electron microscopy, the gold particles were exclusively localized in regions of contact between inner and outer membranes. These contact sites had three distinct characteristics. First, they appeared to be stable structures. Mitochondria which had been treated with low concentrations of digitonin to disrupt the outer membrane still contained regions of contact between the inner membrane and outer membrane fragments. This agrees with the persistent occurrence of contact sites in condensed versus orthodox states. Second, contact site formation did not require precursor proteins. Mitochondria which had been pretreated with digitonin were able to mediate protein import. Therefore, contact sites seem to be preformed to facilitate import as opposed to being formed as a consequence of the presence of precursor proteins. And third, the outer membrane was necessary for contact site-mediated import. When mitochondria were pretreated with digitonin (to create mitoplasts), then incubated with antibody-bound F$_1$$\beta$, none of the protein A–gold particles was associated with the inner membrane, but they were associated with the remaining contact regions. Similarly, intact mitochondria which were shaved with trypsin then treated with digitonin did not import pre-F$_1$$\beta$ while the nonprotease-treated mitochondria (also treated with digitonin) did. While this indicates that second sites on the inner membrane that recognize precursor proteins do not exist, it has been demonstrated in yeast that import into trypsin-inactivated mitochondria can be restored if the outer membrane is disrupted by osmotic shock (Ohba and Schatz, 1987b).

How contact sites mediate transmembrane transport of proteins is unclear, particularly for proteins directed to the inner membrane. One possibility is that the formation and dissipation of contact sites is in constant flux so that proteins are translocated into the sites and then pulled into the mitochondrion with the inner membrane as they are dissipated. Another
possibility is that the imported proteins migrate laterally from the contact regions. Finally, inner membrane proteins may be completely transported through the contact sites to the matrix and then redirected back to the inner membrane. Whether physical contact between the two membranes is necessary for import is unknown. The shortest precursor protein shown to be imported via contact sites so far is the Fe/S protein of the bc₁ complex (231 amino acids) (Hartl et al., 1986). Calculation of the minimal distance which the membranes must come together to be spanned by pre-Fe/S indicates that they do not necessarily have to make contact, but they must come very close together (i.e., 10–20 nm, Fig. 3). The constituents and events occurring at contact sites remain unclear.

VIII. PROTEOLYTIC PROCESSING

During or shortly following the translocation step, the amino-terminal prepiece of many proteins directed to the inner membrane or matrix is removed by a specific protease which is located in the matrix (Böhni et al., 1980; Mori et al., 1980; Schmidt et al., 1984). This occurs very rapidly in vivo. For example, in pulse–chase experiments in rat liver explants, the precursor for carbamoyl-phosphate synthase was imported into mitochondria and processed with a half-life of 2 min (Raymond and Shore, 1981). Similarly, in yeast, the F₁ ATPase β subunit precursor was imported and processed with a half-life of 0.5 min (Reid and Schatz, 1982b). Proteolytic processing must normally occur immediately on exposure of the precursor proteins to the matrix protease since precursor proteins cannot normally be detected in mitochondria. Processing, however, is not obligatory for import since the precursors to F₁ ATPase β and IX subunits could be imported into mitochondria when proteolytic processing was blocked with o-phenanthroline (Zwizinski and Neupert, 1983). Similarly, the precursor of the bc₁ complex Fe/S protein could be imported and did accumulate in the mitochondrial matrix when processing was blocked (Hartl et al., 1986).

The matrix proteases identified and characterized in yeast, rat, and Neurospora mitochondria share similar properties: (1) the protein is a soluble component of the mitochondrial matrix; (2) it has a neutral pH optimum; (3) it is not affected by inhibitors of serine proteases [i.e., 4-nitrophenyl phosphate (PMSF)]; (4) it is sensitive to divalent cation chelators (i.e., o-phenanthroline); and (5) activity is stimulated by divalent cations such as Co²⁺, Mn²⁺, and Zn²⁺ (which can also reverse chelator inhibition; Böhni et al., 1980, 1983; McAda and Douglas, 1982; Mori et al., 1980; Miura et al., 1982; Conboy et al., 1982; Schmidt et al., 1984). In
whole mitochondria, only o-phenanthroline inhibits processing activity, whereas chelators such as ethylenediaminetetraacetic acid (EDTA) or bathophenanthroline, which cannot penetrate the inner membrane, do not. Partially purified preparations of the protease are inhibited by all these chelators and also by nucleoside triphosphates (Böhni et al., 1983). In addition, activity was reported to be specifically inhibited in whole cells (isolated hepatocytes) by rhodamine 123 and 6G, and it was described to occur without affecting the membrane potential (Morita et al., 1982; Kolarov and Nelson, 1984; Kuzela et al., 1986). The underlying mechanism is not understood, but inhibition could not be overcome with excess divalent cations.

The matrix-located peptidase has been partially purified from yeast mitochondria. In one report, activity was purified 200-fold over whole mitochondria (McAda and Douglas, 1982). When analyzed by gel filtration, the protease had an apparent molecular weight of 150,000, and activity correlated best with a 59 kDa protein subunit identified by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). In another study where the protease activity was enriched 100-fold over whole mitochondria, the partially purified enzyme migrated with an apparent molecular weight of 115,000 but displayed 10 protein bands on SDS–PAGE, none of which corresponded to the 59 kDa band identified in the former study (Böhni et al., 1983). The matrix protease was found to be a nuclear gene product which itself must be imported into mitochondria.

The matrix-localized peptidase has been purified to homogeneity from *N. crassa* mitochondria. Activity was enriched 5000-fold over whole mitochondria by a series of purification steps and displayed two bands on SDS–PAGE (52 kDa and 57 kDa; Hawlitschek et al., 1988). When the two subunits were separated, neither was able to process precursor proteins. Activity was fully restored, however, when they were recombined. Activity of the matrix-localized processing peptidase depends on the presence of both proteins, though the contribution of each is as yet unclear.

Some imported mitochondrial proteins are proteolytically processed in two distinct steps. This has been demonstrated in yeast cytochrome b\(_2\) (Gasser et al., 1982b; Reid et al., 1982; Daum et al., 1982b), yeast and *Neurospora* cytochrome c\(_1\) (Gasser et al., 1982b; Ohashi et al., 1982; Teintze et al., 1982; Schleyer and Neupert, 1985), yeast cytochrome c peroxidase (Reid et al., 1982), *Neurospora* ATPase subunit IX (Schmidt et al., 1984), and the Fe/S protein of bc\(_1\) complex in yeast (Sidhu and Beattie, 1983) and *Neurospora* (Hartl et al., 1986). The precursor for rat liver OTC was originally thought to be processed in two steps as well, with the transient formation of an intermediate size protein (Mori et al., 1980; Kraus et al., 1981; Miura et al., 1982; Conboy et al., 1982); how-
Fig. 3. Proximity of inner and outer membranes is required for simultaneous crossing by imported precursor proteins. Components are drawn approximately to scale. (A) The shortest protein demonstrated to be imported via translocation contact sites to date is the Fe/S protein of the bc$_1$ complex (231 amino acids). The approximate linear length (at 0.36 nm/residue) is 83.1 nm. When compression owing to a helical structure is included (yielding 0.15 nm/residue), as predicted from the primary sequence (Chou and Fasman, 1974; Argos et al., 1978), the contour length is 74.6 nm. Helical regions are indicated by shaded boxes. Sites of proteolytic processing are indicated by arrowheads. (B) Translocation intermediates are shown where the amino terminus protrudes far enough into the matrix to be processed by the chelator-sensitive matrix peptidase but the protein still has a major part outside the mitochondrion where it is accessible to externally added proteases. Three contact proximities are illustrated: (I) membranes in contact, (II) a 5 nm space between membranes as observed by electron microscopy (Schwaiger et al., 1987), and (III) estimated maximum
ever, evidence now suggests otherwise since: (i) "intermediate" OTC can be found outside of mitochondria while the processing peptidase is located in the mitochondrial matrix (Kolansky et al., 1982); (ii) intermediate size OTC cannot be detected in vivo (Mori et al., 1981; Morita et al., 1982); and (iii) apparent "intermediate" OTC is found in the absence of mitochondria and does not associate with mitochondria in vitro (Argan et al., 1983).

The first and second processing events appear to be performed by distinct proteases in different submitochondrial locations, with the possible exception of N. crassa ATPase IX processing. The ATPase IX precursor is imported to the inner membrane where its amino-terminal extension protrudes into the matrix and is processed in two steps by the chelator-sensitive matrix peptidase (Schmidt et al., 1984). Evidence that the same enzyme is responsible for both cleavage steps includes the following: (1) both processing steps take place in the matrix, (2) both steps have the same sensitivity to chelating agents, (3) the two cleavage sites share amino acid sequence homology (hydrophobic-polar-Lys-Arg-small/bulky hydrophobic), and (5) the purified processing peptidase performs both cleavage steps (G. Hawlitschek and W. Neupert, unpublished).

Other than ATPase IX, precursor proteins which are processed in two steps share common features (Table III): (i) their respective mature forms are exposed to the intermembrane space (cytochrome b$_2$ and cytochrome c peroxidase are soluble components of the intermembrane space, whereas cytochrome c$_1$ and Fe/S of bc$_1$ complex are attached to the inner membrane but with their bulk protruding into the intermembrane space); (ii) they all make membrane-potential dependent contact with the inner membrane; (iii) the first proteolytic processing event occurs by the chelator-sensitive matrix peptidase; and (iv) the second processing event is catalyzed by a different protease.

Evidence indicates that different proteases are involved in two-step processing. First, whereas the first processing step is clearly performed by the matrix peptidase, the second step of cytochrome b$_2$ maturation is not sensitive to chelators (Daum et al., 1982b). The sensitivity of the second step of bc$_1$ complex Fe/S protein processing to o-phenanthroline is believed to be due to inhibition of the Fe/S cluster formation (Hartl et al., 1986), which, like attachment of heme to intermediate cytochrome c$_1$ separation which could still account for experimental observations (Schleyer and Neupert, 1985). Distances greater than 10–20 nm would probably produce detectable fragments from the imported part of the protein following external protease digestion. Mitochondrial membranes are assumed to be 5 nm thick. Insertion of the first processing site 10 nm past the inner face of the inner membrane is allowed for accessibility by the matrix peptidase.
TABLE III

Topology and Requirements for Mitochondrial Precursor Proteins Processed in Two Steps

<table>
<thead>
<tr>
<th>Precursor protein</th>
<th>Final location&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Two steps in vivo?</th>
<th>First step</th>
<th>Second step</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Energy&lt;sup&gt;c&lt;/sup&gt;</td>
<td>o-Phe-sensitive</td>
<td>Intermediate location&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cytochrome b&lt;sub&gt;2&lt;/sub&gt; (yeast)</td>
<td>IMS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O-IM</td>
</tr>
<tr>
<td>Cytochrome c Peroxidase (yeast)</td>
<td>IMS</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>O-IM</td>
</tr>
<tr>
<td>Cytochrome c&lt;sub&gt;1&lt;/sub&gt; (yeast)</td>
<td>O-IM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O-IM</td>
</tr>
<tr>
<td>Cytochrome c&lt;sub&gt;1&lt;/sub&gt; (Neurospora)</td>
<td>O-IM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O-IM</td>
</tr>
<tr>
<td>ATPase IX (Neurospora)</td>
<td>IM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> Features of import for proteins which are processed in two steps are summarized. The energy for the first processing step is required for transport of the precursor to or through the inner membrane and not for proteolytic activity itself. Features of the second processing step are described only where conversion of the intermediate to the mature form has been examined independently of the first processing step. Areas left blank were not determined.

<sup>b</sup> IMS, intermembrane space; O-IM, outer surface of inner membrane; M, matrix.

<sup>c</sup> Energy required is a membrane potential unless otherwise indicated.

<sup>d</sup> o-Phe, o-phenanthroline.
17. Synthesis and Assembly of Mitochondrial Proteins

(Ohashi et al., 1982), might precede the second proteolytic processing step. Second, the topological arrangement of the intermembrane cytochromes $b_2$ and $c_1$ (and probably cytochrome $c$ peroxidase) presumably exposes the second processing site to the intermembrane space (Daum et al., 1982b; Ohashi et al., 1982; Reid et al., 1982). Third, the partially purified matrix peptidase cleaves cytochrome $b_2$, $c_1$, and cytochrome $c$ peroxidase precursors only to their intermediate size (Gasser et al., 1982b; Reid et al., 1982). Finally, a mutation in yeast blocks the second processing step of cytochrome $b_2$ without affecting the first step (Pratje and Guiard, 1986).

Two-step processing of these proteins has been demonstrated in vivo, with the exception of cytochrome $c$ peroxidase (Reid et al., 1982; Maccecchini et al., 1979b) in which an intermediate size protein cannot be detected (presumably for kinetic reasons). For some proteins, NADH is required for the second processing step. As discussed in Section V, this may be necessary for heme attachment or Fe/S cluster formation and not for proteolytic processing itself. In these cases, such modifications appear to be obligatory and precede the second processing step.

Mutants have been isolated in yeast which are defective in proteolytic processing activity. In an attempt to identify essential components in the pathway of protein import, two complementation groups of temperature-sensitive mutants, defective in the import of mitochondrial proteins, were identified (Yaffe and Schatz, 1984). Termed mas1 and mas2 (mitochondrial assembly), the mutants were normal at the permissive temperature (23°C) but accumulated $F_1$ ATPase $\beta$ subunit precursor at 37°C and stopped growing after 2–3 generations. The mas1 mutants were deficient in the matrix-localized protease activity and could not process a number of mitochondrial precursor proteins (Yaffe et al., 1985). The phenotype suggested that processing of imported proteins is essential for mitochondrial function (though processing is not necessary for import). Another temperature-sensitive mutation in yeast has also been identified which was defective in the second processing step of cytochrome $b_2$ but not cytochrome $c$ peroxidase (Pratje et al., 1983; Pratje and Guiard, 1986). In addition, the mutant was unable to process the precursor of cytochrome oxidase subunit II, a mitochondrial gene product. Assuming that the mutation is in a structural gene coding for a processing enzyme, this suggests that (i) the second processing protease differs from the first step protease; (ii) the processing of the mitochondrial gene product COX II is catalyzed by the same protease, and probably in the same location, as a second step protease; and (iii) the second processing step for cytochrome $b_2$ and cytochrome $c$ peroxidase occurs by different proteases. All evidence taken together, the minimum number of distinct processing peptidases in mitochondria now appears to be three.
<table>
<thead>
<tr>
<th>One-step Processing</th>
<th>-10</th>
<th>+10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cytochrome oxidase IV (N. crassa)</td>
<td>RSIATTVVRNAGTVKPVP</td>
<td>...</td>
</tr>
<tr>
<td>2. Cytochrome oxidase V (N. crassa)</td>
<td>AKTPAVRSTMPISNPT</td>
<td>...</td>
</tr>
<tr>
<td>3. Cytochrome oxidase IV (yeast)</td>
<td>RTLCSSYRQKPVVTAQ</td>
<td>...</td>
</tr>
<tr>
<td>4. Cytochrome oxidase V (yeast)</td>
<td>GLSRTSVMFAOTHALSNA</td>
<td>...</td>
</tr>
<tr>
<td>5. Cytochrome oxidase VI (yeast)</td>
<td>KNTFQSKDAAIDEETE</td>
<td>...</td>
</tr>
<tr>
<td>6. Mn-superoxide dismutase (yeast)</td>
<td>SLLSTPPNTTVLPWLKD</td>
<td>...</td>
</tr>
<tr>
<td>7. Aspartate aminotransferase (chicken)</td>
<td>PRRAATARASSWHSKVENG</td>
<td>...</td>
</tr>
<tr>
<td>8. Ornithine aminotransferase (rat)</td>
<td>RGLRTSVASATSVATKTEQ</td>
<td>...</td>
</tr>
<tr>
<td>9. Ornithine carbamoyltransferase (rat)</td>
<td>RNFRCYKPSVGQKLGRDL</td>
<td>...</td>
</tr>
<tr>
<td>10. Carbamoyl-phosphate synthase I (rat)</td>
<td>WDFSPPPGLLLSVAATAMI</td>
<td>...</td>
</tr>
<tr>
<td>11. Aspartate aminotransferase (porcine)</td>
<td>GLAASSARASWSAHVEMCP</td>
<td>...</td>
</tr>
<tr>
<td>12. Adrenodoxin (bovine)</td>
<td>RTLSVEMASSEEDTIV</td>
<td>...</td>
</tr>
<tr>
<td>13. Cytochrome oxidase IV (bovine)</td>
<td>RAiSTSVCVRLHGSVVKSED</td>
<td>...</td>
</tr>
<tr>
<td>14. Cytochrome P-450 (SCC) (bovine)</td>
<td>HRCYCTGCAAGSTTPPYS</td>
<td>...</td>
</tr>
<tr>
<td>15. Ornithine carbamoyltransferase (Human)</td>
<td>RNFRCQGPLQHKVLGRLDL</td>
<td>...</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Two-step processing</th>
<th>First Site</th>
<th>Second Site</th>
</tr>
</thead>
</table>
| 16. ATPase IX (N. crassa) | VRVAUVSRRQGTCFLQTL | TTPQAYKPRAYSESSEIAQAMV | b
| 17. Rieske Fe/S of bc1 complex (N. crassa) | AAAPARAVTTSTALQG | LTSTALQOSSSTFES | c
| 18. Cytochrome c peroxidase (yeast) | ? | NWGAAALASTTPLTVHVASV | |
| 19. Cytochrome b2 (yeast) | ? | LNHSNQIDNEPCLDM}| |
| 20. Cytochrome c1 (yeast) | ? | IYADSSTAEATAAEHGLHA | |
| 21. ATPase IX P1 (bovine) | ? | REPQTSVSKQIDOTAAYKFG | d
| 22. ATPase IX P2 (bovine) | ? | RSPQTSVSKQIDOTAAYKFG | d

The amino acid sequences flanking the sites of proteolytic processing of imported mitochondrial proteins are listed by the single letter amino acid code (left to right, amino to carboxy terminus). The vertical lines indicate the sites of cleavage. Basic, acidic, and hydroxylated amino acids are indicated by the symbols +, −, and •, respectively, above the primary sequence. The proteins are grouped by the organism in which they occur and then according to rough evolutionary order. Only sequences in which the cleavage sites have been positively identified are presented. References are the same as those indicated in Table I. In addition, the processing site for cytochrome oxidase IV from Neurospora crassa was determined by Sachs et al. (1986).

b The second processing step of N. crassa ATPase IX is catalyzed by the same protease as the first step (Schmidt et al., 1984). Comparison of the second processing site with first site sequences might be more appropriate.

c Only eight amino acids preceding the second processing site are presented since the first processing step occurs at this position.

d Homology of bovine ATPase IX presequences (P1 and P2) with ATPase IX from N. crassa suggests that the bovine prepieces might also be processed in two steps. This has not been demonstrated yet.
What are the recognition sites for proteolytic processing? A comparison of the amino acid sequences flanking the cleavage sites (Table IV) does not indicate a clear consensus sequence. There may be a number of reasons why very few trends are apparent. (1) the site specificity may vary between different organisms; however, a certain degree of conservation must exist since heterologous import and processing can occur. (2) Processing could be catalyzed by more than one chelator-sensitive matrix protease with different specificities. First site processing and processing of proteins in which the prepiece is removed in a single step, however, all appear to be catalyzed by the same chelator-sensitive matrix peptidase. The partially purified matrix peptidase from yeast processed precursors to F$_1$ ATPase $\beta$ and $\alpha$ subunits, cytochrome oxidase IV, citrate synthase (Böhni et al., 1983), and cytochrome oxidase V (Cerletti et al., 1983). Similarly, the purified Neurospora matrix peptidase processed all precursor proteins tested to date (G. Hawlitschek and W. Neupert, unpublished). (3) The specificity of the matrix peptidase may depend on regions within the prepiece or mature part of the protein distal from the actual cleavage site. For example, cytochrome oxidase IV is normally processed between amino acids 25 and 26 to remove the 25 amino acid prepiece. When only the first 22 amino acids of the COX IV prepiece were fused to DHFR, thus removing the normal cleavage site, the fusion protein was still processed, but between amino acids 17 and 18 instead (Hurt et al., 1985a). The sequence of amino acids flanking the new cleavage site show no homology to the authentic site, suggesting that other elements in the prepiece may contribute to the signal for processing. Processing of a fusion protein consisting of the presequence of pre-COX IV and DHFR by the solubilized matrix peptidase was blocked when even small deletions were made at the very amino terminus of the prepiece (Hurt et al., 1987).

In some cases, the specificity of processing has requirements in the mature sequence. Correct processing of the rat OTC prepiece occurred when the first 60 amino-terminal amino acids of the pre-OTC, containing the 32 amino acid prepiece, were fused to asparagine synthase; however, incorrect processing at a site 14 amino acids closer to the amino terminus occurred when only the first 37 amino acids (still containing the full 32 amino acid prepiece) were fused (Nguyen et al., 1986). The processing of human pre-OTC, also containing a 32 amino acid prepiece, was inhibited when deletions or substitutions were made between amino acids 8 and 22 of the prepiece. In addition, the glutamine at position −1 was also critical for proteolytic cleavage (Horwich et al., 1986). Similarly, small deletions up to 17 amino acids away from the ATPase F$_1$β cleavage site also prevented processing (Vassarotti et al., 1987a).
How processing peptidases recognize the correct sites of cleavage is unclear. Undoubtedly, conformation plays an important role in the recognition of cleavage sites. The alkali-denatured OTC precursor, for example, was not processed by a partially purified form of the matrix peptidase (Miura et al., 1986). Sequences around the cleavage sites show only minor similarities (Table IV, sequences 1–17). (1) Position -1 rarely has a charged amino acid. In lower eukaryotes it is usually a hydrophobic amino acid while in higher eukaryotes it is more often an uncharged polar amino acid. (2) In position -2, 10 of 17 amino acids are positively charged, mostly in lower eukaryotes. When not positively charged, the -2 amino acid is usually hydrophobic with an aliphatic side chain. (3) Charged amino acids are also rare in the +1 and +2 positions, but hydroxylated amino acids (particularly serine) frequently occur. Despite no obvious consensus sequence, however, proteolytic processing is highly specific. Neither mature mitochondrial proteins nor nonmitochondrial proteins are cleaved by the matrix-located peptidase (Böhni et al., 1983).

IX. MITOCHONDRIAL GENE PRODUCTS

Not all mitochondrial proteins are nuclear gene products which are imported from their site of synthesis in the cytosol. A small number (<10%) are coded for by the mitochondrial genome and are synthesized on 70 S mitochondrial ribosomes which are associated with the inner face of the inner membrane. The mitochondrial genomes in a number of species have been completely or partially sequenced. They vary in size from 17 kilobases in humans (Anderson et al., 1981) to over 200 kilobases in plants (Palmer and Shields, 1984). Despite this variation, however, they code for a similar complement of proteins. These usually include apocytochrome \( b \), cytochrome oxidase subunits I, II, and III, ATPase subunits VI and VIII, and several subunits from the NADH dehydrogenase complex (for review, see Breitenberger and RajBhandary, 1985). Yeast, fungi, and plant mitochondrial genomes also code for other proteins. For example, yeast and fungi mitochondria contain genes for the S5 protein of the small ribosomal subunit, ATPase subunit IX (which is dormant in Neurospora crassa), and intron-coded proteins involved in RNA maturation. Plant mitochondrial genomes also encode ATPase \( F_{\alpha} \). Finally, information coding for mitochondrial transfer RNAs and for ribosomal RNAs are contained within the genome.

Why mitochondria need a distinct genome at all and why the specific proteins that they encode are so highly conserved is unknown. One theory is that the hydrophobic nature of the mitochondrial gene products
necessitates their synthesis within the organelle. This, however, seems unlikely to be the only reason since ATPase IX, a very hydrophobic protein, is synthesized in the cytosol of *N. crassa* containing a hydrophilic amino-terminal prepiece to mask the hydrophobic mature part (Jackl and Sebald, 1975; van den Boogaart *et al.*, 1982b). In fact, the ATPase IX prepiece has been shown to be able to mediate the import of ATPase VIII, an authentic mitochondrial gene product, back into mitochondria (Gearing and Nagley, 1986), although the shorter COX VI prepiece did not. On the other hand, the S5 ribosomal protein is water soluble but is still a mitochondrial gene product in some species. A second possibility is that the mitochondrial genome is an evolutionary remnant and that most of the genes from the bacterial endosymbiont were transferred to the nucleus of the host cell, but the process was incomplete. A third possibility is that the mitochondrial gene products form nucleating points around which the remainder of respiratory complexes are built. This cannot be absolutely critical though, since ATPase, for example, is assembled into a functional, albeit somewhat less efficient, complex when the subunits encoded by the mitochondrial genome are absent (Schatz, 1968; De Jong *et al.*, 1979; Marzuki and Linnane, 1985).

A final hypothesis is that the cotranslational protein export mechanism existed prior to the evolutionary endosymbiotic event. Accordingly, the genes for proteins which had amino acid sequences within them that resembled the export signal sequence were retained in the mitochondrial genome so that they would not be mistakenly exported. Indeed, it has been determined that most mitochondrial gene products from *Xenopus* and yeast seem to contain signal sequence-like segments near the amino terminus (von Heijne, 1986b). Eukaryotic cells maintain a distinct genome in the mitochondrion at great expense, since a large number of proteins must be imported simply to accommodate a separate protein synthetic system.

Mitochondrial protein synthesis occurs on ribosomes which are associated with the inner mitochondrial membrane, suggesting that translation is coupled to the insertion of the newly synthesized proteins into the membrane. Even the soluble S5 ribosomal protein, however, is synthesized on membrane-bound ribosomes (Marzuki and Hibbs, 1986). Cytochrome oxidase II is synthesized as a larger precursor in *N. crassa* (Machleidt and Werner, 1979; van den Boogaart *et al.*, 1982a) and in yeast (Sevarino and Poyton, 1980). In both cases, the higher molecular weight precursor can be chased to the mature size protein in the absence of protein synthesis (Sevarino and Poyton, 1980; Driever *et al.*, 1987). Furthermore, the newly synthesized COX II precursor from *Neurospora* was not integrated into the inner membrane, demonstrating that assembly can
occur posttranslationally. Processing of the COX II precursor was catalyzed by a protease which is located in the intermembrane space, and formation of the mature size protein is dependent on NADH but not on a membrane potential. Cytochrome oxidase II is probably not representative of the other mitochondrial gene products since it is synthesized with an amino-terminal prepiece while others from the mitochondrial genome are not (with the possible exception of COX I; Burger et al., 1982).

X. ASSEMBLY AND COORDINATION

Many of the proteins which are imported into mitochondria are subunits of respiratory complexes. Therefore, assembly into functional complexes represents the final step in the import pathway. What sequence of events occurs during assembly, and how is the supply of subunits coordinated and regulated?

Since two genetic systems are responsible for the synthesis of proteins for most respiratory complexes, it would seem logical that they would be synchronized in some way. No doubt there is some long-term regulation of the mitochondrial genome by the nucleus since most of the proteins comprising the mitochondrial transcription/translation system are nuclear gene products. In the short term, however, the two systems do not appear to be tightly coupled. For example, when synthesis of mitochondrial gene products was blocked in vivo by growth of *Neurospora crassa* on chloramphenicol, thereby inhibiting the synthesis of cytochrome *b* of the *bc* complex, normal amounts of cytochrome *c* were synthesized in the cytosol (Weiss and Kolb, 1979). Furthermore, cytochrome *c* was imported normally and assembled into a cytochrome *b*-deficient complex. Similarly, when overexpression of the 11 kDa subunit (a nuclear gene product) of the *bc* complex was induced in yeast cells by transformation, it did not affect the rate of synthesis or degradation of the other subunits of the complex (van Loon et al., 1983a). The same was observed when individual subunits of the *bc* complex (also nuclear gene products) were overexpressed, suggesting that stringent coupling does not exist (van Loon et al., 1983b).

Stringent coordination may not occur at the level of translation for the nuclear gene products either. Cytoplasmically made subunits for the ATPase, the *bc* complex, and cytochrome oxidase are synthesized as individual subunits, not as polyproteins (Lewin et al., 1980; Mihara and Blobel, 1980; van Loon et al., 1983c). Notwithstanding this apparent lack of coordination, excess unincorporated subunits do not accumulate in mitochondria. One suggestion has been that surplus amounts of unas-
sembled subunits are simply eliminated by proteolytic digestion (Luzikov, 1986) regardless of the waste of cellular resources by such a process.

Despite the apparent lack of coordination of synthesis of subunits in these experiments, nuclear genes are involved in regulating the expression of the mitochondrial genome. This appears to occur at three levels: (i) control of mRNA processing, (ii) control of translation, and (iii) post-translational modification (e.g., proteolytic processing of pre-COX II by a nuclear-coded protease; Pratje et al., 1983; Pratje and Guiard, 1986). These systems have been studied best in *Saccharomyces cerevisiae*. Yeast mitochondrial genes contain introns, separating the exons of a gene, which must be spliced out prior to translation. These intervening sequences are removed from pre-mRNAs by self-splicing mechanisms and by protein-assisted splicing. In the latter case, these proteins (termed mRNA maturases) are encoded by either mitochondrial intron open reading frames (Weiss-Brummer et al., 1982; Carignani et al., 1983; Guiso et al., 1984; Jacq et al., 1984) or are nuclear gene products (Faye and Simon, 1983; Pillar et al., 1983; McGraw and Tzagoloff, 1983; Dieckmann et al., 1984) and have been shown to be necessary for processing of COX I and apocytochrome *b* pre-mRNAs. The interesting feature of these studies is that the maturation proteins appear to be specific for individual mitochondrial gene transcripts so that the expression of mitochondrial gene products can be selectively controlled by the activity of the different nuclear-encoded mRNA maturases.

Nuclear gene products are also required for the specific translation of mitochondrial mRNAs (Fox, 1986). For example, the yeast pet494 mutant has normal levels of fully processed COX III mRNA. A nuclear gene product was found to be required to promote the translation of the COX III mRNA that appeared to interact with the 5'-untranslated leader (Müller et al., 1984). Similar nuclear gene products were also required for the specific translation of apocytochrome *b* (CPB6, Dieckmann and Tzagoloff, 1985; and MK2, Rödel et al., 1985) and for COX II (PET111; Fox, 1986). Though they serve similar functions, there seems to be little homology between the PET494, CBP6, and PET111 gene products. They all appear, however, to act on the 5'-untranslated mRNA leader sequence in a specific way so that translation of mitochondrial-encoded proteins can be independently controlled.

Compared to the loose coordination between nuclear and mitochondrial genomes, the assembly of individual subunits into functional complexes is a more ordered process. The best studied examples of complex assembly to date are the F$_{0}$F$_{1}$ ATPase and cytochrome oxidase. When the synthesis of yeast mitochondrial gene products was blocked by growth in the presence of chloramphenicol (De Jong et al., 1979) or as in rho$^{-}$ mutants
(Schatz, 1968), a correctly assembled functional ATPase was still produced which was loosely associated with the inner membrane but no longer sensitive to oligomycin. This suggests that the mitochondrial gene products (subunits VI, VIII, and IX) are not essential for either assembly or function but are required for stability of the complex and confer oligomycin sensitivity. Sequential assembly of the ATPase subunits was demonstrated in yeast mutants lacking each of the mitochondrially encoded proteins (Marzuki and Linnane, 1985). The mutant lacking subunit IX was deficient in the assembly of both VI and VIII, while the mutant lacking subunit VIII could assemble IX but not VI. This indicated that the relative order of assembly of the mitochondrial gene products was IX then VIII then VI and that sequential assembly was necessary. Each of these mutants had a functional but unstable ATPase.

A large number of components are required for the functional assembly of cytochrome oxidase. In a *N. crassa* mutant lacking COX I (COX I, II, and III are mitochondrial gene products) only the assembly of subunits V and VI occurred (Nargang *et al.*, 1978). In pulse-chase experiments in rats, COX II and III were immediately assembled while COX I arrived only after a long chase (Wielburski *et al.*, 1982). Taken together, this suggests that COX II and III are assembled first, followed by COX I, which in turn is necessary for the subsequent assembly of COX V and VI. In a yeast mutant lacking COX IV, no cytochrome oxidase activity was observed, although the mutant still contained the other mitochondrial COX subunits. This suggested that COX IV is necessary for the proper assembly of cytochrome oxidase (Dowhan *et al.*, 1985).

Cofactors such as heme, oxygen, and copper are also necessary for cytochrome oxidase assembly. In yeast, a mutant lacking heme contained no cytochrome oxidase activity. Although the mitochondria still contained subunits II, III, and IV, it had only low amounts of COX I and IV and no V or VI. The residual subunits were not assembled (Saltzgaber-Müller and Schatz, 1978). In rats, heme was shown to be necessary for the assembly of subunit I with the preassembled COX II and III (Wielburski and Nelson, 1984). COX I was predominantly associated with COX III. Yeast cells grown anaerobically did not contain assembled cytochrome oxidase. When shifted to an oxygen environment, however, subunits I and II were immediately assembled with VI and VII (Woodrow and Schatz, 1979). Cytochrome oxidase is not assembled in copper-depleted *N. crassa*, although both nuclear and mitochondrial gene products are synthesized normally (Werner *et al.*, 1974).

The coordination and assembly of functional mitochondrial complexes occurs by a series of events which are dependent on components other than the mitochondrial subunits alone. Whether assembly is controlled by
factors other than the simple stoichiometric availability of components is unknown.

XI. OVERVIEW

A. Import Pathway Models

Owing to the diversity of mitochondrial proteins and their topological locations, a variety of different pathways exist to facilitate their import. We summarize what is known about the import pathways of representative proteins and what can be hypothesized.

Porin: Outer Membrane (Fig. 4). The precursor to porin differs from the mature form only in conformational arrangement, thereby allowing its solubility in the cytosol. It does not contain an amino-terminal prepiece and does not require an energized inner membrane for import. Preporin binds to its receptor at the outer face of the outer membrane and is inserted into the outer membrane where it is protected from externally added proteases. It then forms dimers and trimers. The porin pathway is probably representative of most outer membrane proteins (Pfaller et al., 1985; Pfaller and Neupert, 1987, and references therein).

Cytochrome c: Intermembrane Space (Fig. 5). Cytochrome c is synthesized as apocytochrome c which does not contain covalently attached heme and has a loosely ordered conformation. Apocytochrome c spontaneously inserts partway through the lipid bilayer of the outer membrane. It is then sequestered at the inner face of the outer membrane by an apocytochrome c binding protein which in turn exposes the cysteine

![Diagram of porin import pathway into mitochondria.](Fig. 4. Import pathway of porin into mitochondria. OM, Outer membrane; IM, inner membrane; R, receptor; p, precursor; m, mature.)
Fig. 5. Import pathway of cytochrome \( c \) into mitochondria. OM, Outer membrane; IM, inner membrane; Apo \( c \), apocytochrome \( c \); Holo \( c \), holocytochrome \( c \); BP, apocytochrome \( c \) binding protein; H, heme; HL, cytochrome \( c \) heme lyase.

Fig. 6. Import pathway of cytochrome \( c_1 \) into mitochondria (according to Hurt and van Loon, 1986). OM, Outer membrane; IM, inner membrane; p, precursor; i, intermediate; m, mature; R, receptor; \( \Delta \phi \), membrane potential; PP, processing peptidase; H, heme. Boxes represent prepiece segments.

Fig. 7. Import pathway of the Rieske Fe/S protein of \( bc_1 \) complex into mitochondria. Abbreviations are as in Fig. 6.
thiols to the intermembrane space. Heme is enzymatically attached by cytochrome c heme lyase, and the resulting conformational change pulls the protein through the outer membrane. Holocytochrome c then migrates to its functional location in association with cytochrome c reductase and cytochrome c oxidase at the outer face of the inner membrane (Hennig and Neupert, 1981; Nicholson et al., 1987, and references therein).

**Cytochrome c**: Intermembrane Space, Inner Membrane (Fig. 6). The cytochrome c precursor is synthesized with a two-domain prepiece. Pre-c binds to its receptor at the outer face of the outer membrane. The amino terminus is then translocated through the inner membrane via contact sites in a step which is dependent on a membrane potential. The first part of the prepiece is removed by the chelator-sensitive matrix peptidase to generate intermediate c*. The carboxy terminus, which eventually anchors the mature protein to the inner membrane, is embedded into the inner membrane at some point following translocation across the outer membrane. Heme is covalently attached to intermediate c*, and the second part of the prepiece is then removed yielding the mature c which is then assembled into the bc1 complex (Ohashi et al., 1982; Schleyer and Neupert, 1985; Hurt and van Loon, 1986, and references therein.) The same basic pathway may be followed by cytochrome c peroxidase, which is released as a soluble protein into the intermembrane space following the second proteolytic step because it does not contain a carboxy-terminal anchoring segment. This pathway demonstrates the principles of the stop transfer model suggested by Hurt and van Loon (1986). Another possibility is that pre-c is completely translocated into the matrix, then redirected back to the inner membrane like the Fe/S protein of the bc1 complex (Fig. 7). This has been demonstrated to be the case in Neurospora crassa and also for the import of cytochrome b2 into yeast mitochondria (Hartl et al., 1987).

**Fe/S Protein of bc1 Complex**: Inner Membrane (Fig. 7). The precursor to the Fe/S protein is synthesized with a two-part prepiece. Pre-Fe/S binds to its receptor on the outer membrane and is then completely translocated into the mitochondrial matrix via translocation contact sites in a step which is dependent on the membrane potential. The matrix-located pre-Fe/S is then processed to the intermediate size protein by the chelator-sensitive processing peptidase. The Fe/S protein is then directed back across the inner membrane to the outer face of the inner membrane. The protein is processed to its mature form by removal of the second half of the prepiece and formation of the Fe/S cluster, but when and where this occurs are unknown (Hartl et al., 1986).

**ADP/ATP Carrier**: Inner Membrane (Fig. 8). The ADP/ATP carrier is synthesized without a prepiece but contains stretches of positively
Fig. 8. Import pathway of ADP/ATP carrier into mitochondria. Abbreviations are as in Fig. 6.

Fig. 9. Import pathway of cytochrome oxidase subunit IV into mitochondria. Abbreviations are as in Fig. 6.

Fig. 10. Import pathway of F$_1$ ATPase $\beta$ subunit into mitochondria. Abbreviations are as in Fig. 6.
charged amino acids which resemble mitochondrial targeting sequences. The precursor binds to its receptor and is inserted into the mitochondrial membrane where it is protected from externally added proteases. The first intermediate \((i_1)\) is past the receptor stage but before the membrane potential-dependent stage of import. The second intermediate \((i_2)\) is found in the presence of a membrane potential and is transported to the inner membrane via translocation contact sites. The imported ADP/ATP carrier then undergoes a conformational change, in which it acquires properties of the mature protein, followed by the formation of dimers (Schleyer and Neupert, 1984; Pfanner and Neupert, 1987). The uncoupling protein from brown fat mitochondria has similar sequence and folding characteristics (Aquila et al., 1985) and is probably imported in a similar manner.

**Cytochrome Oxidase IV:** Inner Membrane (Fig. 9). Pre-COX IV is synthesized with an amino-terminal prepiece in the cytosol. It binds to its receptor on the outer membrane and is subsequently transported to the inner membrane by translocation contact sites in a membrane potential-dependent step. The amino-terminal prepiece, which protrudes into the matrix, is removed by the chelator-sensitive peptidase, and the mature part of the protein remains integrated in the inner membrane where it is assembled into cytochrome oxidase (Hurt and van Loon, 1986, and references therein). This mechanism is probably representative of most imported inner membrane proteins which are processed in a single step.

**F_0F_1 ATPase F_{i\beta}:** Matrix (Fig. 10). Pre-F_{i\beta} binds to its receptor and is transported completely into the matrix. This occurs via translocation contact sites and is dependent on a membrane potential. During or shortly following translocation, the mature F_{i\beta} is generated by removal of the prepiece by the matrix peptidase. Mature F_{i\beta} is then assembled with the other F_1 ATPase subunits at the inner face of the inner membrane. Most imported matrix proteins probably follow a similar pathway (Schleyer and Neupert, 1985, and references therein).

**Cytochrome Oxidase II:** A Mitochondrial Gene Product (Fig. 11). Most mitochondrial gene products are probably inserted directly into the inner membrane as they are synthesized on membrane-bound ribosomes (except the S5 ribosomal protein which is released into the matrix). COX II, on the other hand, is synthesized with a prepiece (in lower eukaryotes) and can be posttranslationally inserted into the inner membrane (B). Cotranslational insertion may occur *in vivo* (A) but is not obligatory. Pre-COX II is converted to mature COX II by removal of the prepiece by a protease located in the intermembrane space and noncovalent binding of heme \(a\) and copper. The sequence in which these changes occur is unknown, but is dependent on NADH. Mature COX II is then assembled into cytochrome oxidase (Driever et al., 1987).
B. Evolutionary Considerations

A particularly interesting finding which emerged while examining the import of the Fe/S protein of bc₁ complex into Neurospora crassa mitochondria is that the precursor protein was completely translocated into the matrix where it was partially processed and then redirected back across the inner membrane to its functional location on the other side facing the intermembrane space. (Hartl et al., 1986; see Fig. 7). This seemingly complex assembly pathway was explained in terms of a rerouting mechanism by which the Fe/S protein is returned to remnants of its “ancestral” assembly pathway. Following evolutionary gene transfer from the bacterial endosymbiont to the host cell nucleus, mechanisms had to evolve to return the gene product to its functional location. Rather than completely rebuild the means by which the protein was folded, assembled, and acquired the correct topology, the import pathway of the Fe/S protein has evolved to make use of preexisting mechanisms. To do this, the Fe/S protein had to be completely transported across both mitochondrial membranes back into the matrix. This was accommodated by adding a matrix targeting prepiece to the Fe/S protein (i.e., the first part of the pre-Fe/S prepiece) which allows it to enter the matrix by components which had evolved to mediate the import of other mitochondrial proteins. At this point, “ancestral” transport pathways take over to correctly insert the Fe/S protein into the inner membrane.

Indeed, the equivalent Fe/S protein from the photosynthetic bacteria Rhodopseudomonas sphaeroides is synthesized in the bacterial cytoplasm (comparable to the mitochondrial matrix) and transferred across the photosynthetic membrane to the side opposite the cytoplasm where, as in mitochondria, it is topologically opposed to the F₁ part of ATPase. This transfer is accompanied by a reduction in molecular weight (Gabellini et al., 1985) which may be equivalent to processing of the mitochon-
Table V

Comparison of Amino-Terminal Sequences of Mitochondrial and Bacterial Proteins

<table>
<thead>
<tr>
<th>A. Rieske Fe/S protein of bc\textsubscript{1} complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. crassa</td>
</tr>
<tr>
<td>MAPVSIVSRAAARAAAPARAARVRLTTSTALQGSSTTSFESPKGETAK</td>
</tr>
<tr>
<td>R. sphaeroides</td>
</tr>
<tr>
<td>MSHAEDNAGTRELHYYATANGGVVTGAA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Cytochrome c\textsubscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast</td>
</tr>
<tr>
<td>NFSNLKSRWQRTLSKYSTATGAEKSKGRLTQKLYTVAAGITASTLVPESAEEKACGHLHAPAVLYS...</td>
</tr>
<tr>
<td>R. sphaeroides</td>
</tr>
<tr>
<td>MSLIEHNALVLOGCHANSNVQDHUES...</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Cytochrome c (N. crassa) and c\textsubscript{2} (R. capsulata)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. crassa</td>
</tr>
<tr>
<td>MGFLFNSNLIMNFDAPSPAGYF...</td>
</tr>
<tr>
<td>R. capsulata</td>
</tr>
<tr>
<td>MSLIEHNALVLOGCHANSNVQDHUES...</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D. Cytochrome oxidase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. crassa</td>
</tr>
<tr>
<td>MGLLFNNLIMNFDAPSPAGYF...</td>
</tr>
<tr>
<td>Maize</td>
</tr>
<tr>
<td>MRLSLFCHTLICDDAEHPWQG...</td>
</tr>
<tr>
<td>Yeast</td>
</tr>
<tr>
<td>MLDLLRLTTTFINN-DYTPYACYF...</td>
</tr>
</tbody>
</table>

\(A - C)\) Amino acid sequences of the amino terminus of mitochondrial precursor proteins are compared to their equivalent in bacteria. Arrows indicate the sites of proteolytic processing. The estimated region containing the first processing site of yeast cytochrome c\textsubscript{1} is indicated by a bracket. The processing site for R. sphaeroides Fe/S protein is not known. Segments having identical amino acid sequences are marked by a box. Alignment inserts are indicated by (\(\_\_\_\_\_\)). (D) The prepiece sequences of mitochondrial cytochrome oxidase subunit II. Sequences were taken from Harnisch et al. (1985) (N. crassa Fe/S protein of bc\textsubscript{1} complex), Gabellini and Sebald (1986) (R. sphaeroides Fe/S protein of bc\textsubscript{1} complex and cytochrome c\textsubscript{1}), Sadler et al. (1984) (yeast cytochrome c\textsubscript{1}), Lederer and Simon (1974) (N. crassa cytochrome c), Daldal et al. (1986) (R. capsulata cytochrome c\textsubscript{2}), van den Boogaart et al. (1982a) (N. crassa COX II), Fox and Leaver (1981) (maize COX II), and Coruzzi and Tzagoloff (1979) (yeast COX II).
semblance to the export leader sequence of the equivalent bacterial protein (Table VB).

The Fe/S protein of the $bc_1$ complex may have retained this elaborate import pathway because of stringent assembly requirements which necessitate its insertion into the inner membrane from the matrix side. On the other hand, cytochrome $c$ is a soluble protein of the intermembrane space and is less likely to be so demanding. This is reflected in the way in which import pathway for cytochrome $c$ has evolved. In the bacterium *Rhodopseudomonas capsulata*, the equivalent protein (cytochrome $c_2$) is synthesized as a precursor protein in the bacterial cytosol and is processed as it is secreted into the periplasmic space (comparable to the mitochondrial intermembrane space). Rather than via a redirected import pathway, mitochondrial cytochrome $c$ is transported across just the outer membrane (Fig. 5). On evolutionary gene transfer to the host cell nucleus, the bacterial leader prepiece was removed (Table VC).

Some bacterial-like leader sequences remain in mitochondria. In lower eukaryotes such as *Neurospora*, yeast, and maize, for example, cytochrome oxidase II (a mitochondrial gene product) is synthesized with a prepiece having some characteristics of bacterial leader sequences (e.g., a stretch of hydrophobic amino acids following a charged amino terminus; Table VD). On the other hand, proteins which have no equivalent in bacteria, and therefore presumably did not evolve from the bacterial endosymbiont, may be imported by novel mechanisms which differ from most mitochondrial proteins. For example, the ADP/ATP carrier does not have an amino-terminal prepiece, but specific targeting information is contained within internal sequences that have accompanied the structural part of the protein during evolutionary formation.

Evolutionary remnants of the bacterial endosymbiont may still be present in the import pathways of mitochondrial proteins. The differing pathways could reflect specific assembly requirements of the various proteins and, in some cases, the relative time at which they were introduced as mitochondrial constituents.

C. Summary

Nearly the entire complement of mitochondrial proteins must be imported from the cytosol. To do this correctly, mitochondrial precursor proteins must be specifically targeted to mitochondria and then properly sorted to their functional submitochondrial location. In addition, the proper topological arrangement and assembly with other subunits must occur. Information to enable these processes is embodied within the precursor proteins themselves, and components required to facilitate these
events are molecules and structures within the mitochondrion. The import pathways for a variety of mitochondrial proteins have been resolved into a sequence of discrete but contiguous steps. In doing so, the components necessary for each step have frequently been identified at a molecular level. These can be divided into three broad classes, namely, components necessary for (i) specific targeting to mitochondria, (ii) sorting and translocation processes, and (iii) processing events. In the first group, targeting signals, usually contained within the amino-terminal pre-piece of the precursor protein, mediate recognition of the remainder of the protein by mitochondria. Receptors on the surface of the mitochondrion bind the precursor protein and initiate its entry into mitochondria. In the second group, translocation contact sites facilitate transmembrane movement of most imported proteins. The constituents of these contact regions are unknown but probably contain specific proteins which are necessary for import. The membrane potential supplies the energy or the circumstances to initiate translocation. Features within the precursor proteins and perhaps processes which exist at the site of translocation guide the imported protein to its correct sub mitochondrial location. Finally, processing occurs in many forms. Prepieces are proteolytically removed by specific proteases, and some proteins are covalently or noncovalently modified. Refolding into an active conformation often occurs, and then assembly into a functional location marks the end of the import process.

The general sequence of events by which import occurs has been well-characterized for a number of proteins having different sub mitochondrial locations and different roles in mitochondrial function. The molecular mechanisms are now of greater interest and are the focus of current research.

ACKNOWLEDGMENTS

We would like to thank M. E. Clement for typing and Dr. M. Schwaiger for critically reviewing the manuscript. We would also like to thank our colleagues for their many contributions to this review.

REFERENCES


17. Synthesis and Assembly of Mitochondrial Proteins


17. Synthesis and Assembly of Mitochondrial Proteins


Saltzgaber-Müller, J., and Schatz, G. (1978). Heme is necessary for the accumulation and
17. Synthesis and Assembly of Mitochondrial Proteins


cytochrome c mutant of Neurospora crassa: Importance of carboxy terminus for import of apocytochrome c into mitochondria. EMBO J. 6, 2131–2137.


Index

A

Acetolysis, mannosidases and, 224
Acetylcholine, secretory vesicles and, 528
Acetylcholine receptor
  carbohydrate and, 74
  translocation and, 33
N-Acetylgalactosamine
  carbohydrate and, 55
  Golgi membranes and, 291, 310
N-Acetylglucosamine
  carbohydrate and, 55, 57
  Dictyostelium and, 371, 380
  endoplasmic reticulum proteins and, 261
  exocytosis and, 438, 439, 444
  Golgi membranes and, 310
  lysosomal enzymes and, 467, 470, 473, 492-494
  mannosidases and, 210, 212, 227, 228
  endoplasmic reticulum, 219-221, 225
  mycology and, 785
  posttranslational modification and, 171, 188
α-N-Acetylgalactosaminidase, lysosomal
  enzymes and, 466, 468, 470
β-N-Acetylgalactosaminidase, lysosomal
  enzymes and, 466
N-Acetylglucosaminyltransferase, carbo-
  hydrate and, 56, 58
Acid hydrolases
  carbohydrate and, 51, 90
  lectins, 82, 83, 89
  oligosaccharide biosynthesis, 60
  solubility, 65
  tunicamycin, 71
  Dictyostelium and, 364, 365, 375, 377, 380, 381
  endocytosis and, 407
  Golgi membranes and, 290
  lysosomal enzymes and, 465, 480, 503
Acid phosphatase
  carbohydrate and, 73
  Dictyostelium and, 365, 377, 381, 383, 388
  Golgi membranes and, 294, 299
  lysosomal like vacuole in yeast and, 330, 333, 335
  mycology and, 787, 792
  posttranslational modification and
    endoplasmic reticulum, 164, 167, 169, 170, 180
  Golgi apparatus, 190, 194, 196
Acidification
  endocytosis and, 413, 417
  lysosomal like vacuole in yeast and, 341, 342, 350
Acidity
  Dictyostelium and, 373, 374, 384, 388
  endocytosis and, 403, 408, 412, 420, 427
  Golgi membranes and, 302, 309
  lysosomal enzymes and
    endocytosis, 483, 485, 488, 489
     intracellular pathway, 498-500, 503, 504
     junction of pathways, 505-508
     recognition marker, 466, 467, 473
     mycology and, 779, 780, 782, 783, 788
     secretory vesicles and, 528, 543
Actin
  lysosomal like vacuole in yeast and, 318
  posttranslational modification and, 198
  secretory vesicles and, 528, 530
Activation, translocation and, 8, 19
Acyclation, posttranslational modification
  and, 160, 182, 183
Adenosine 5′-monophosphate, Golgi
  membranes and, 299, 305, 307, 311
Adenovirus, endoplasmic reticulum proteins
  and, 251, 252, 261
Adenylate cyclase, lysosomal like vacuole in
  yeast and, 350
Adenylate kinase, mitochondrial proteins
  and, 693
Adhesion
  carbohydrate and, 88
  extracellular matrix-like glycoproteins and,
    568, 569, 574, 576-578
  β-Adrenergic receptors, carbohydrate and,
    74, 81
Adrenocorticotropin hormone
  carbohydrate and, 65
  *Dictyostelium* and, 378
  secretory vesicles and, 524, 537, 545
Aerolysin, secretion, gram negative bacteria
  and, 636, 637
Agammaglobulinemia, carbohydrate and, 72
Agglutination, posttranslational modification and, 185
Aggregation
  carbohydrate and, 64, 68, 77
  *Dictyostelium* and, 383, 387, 388
  mitochondrial proteins and, 679–681, 705
  posttranslational modification and, 168
  viral glycoproteins and, 139
Alanine, viral glycoproteins and, 129
Albumin
  carbohydrate and, 53, 55, 77
  lysosomal enzymes and, 482, 495, 499
  mannosidases and, 230
  secretory vesicles and, 527, 536
Alcohol dehydrogenase
  mitochondrial proteins and, 687
  mycology and, 796
Alcohol dehydrogenase III, mitochondrial protein import and, 669
Alkaline phosphatase
  lysosomelike vacuole in yeast and, 320, 324, 326, 332, 345
  posttranslational modification and, 180, 182, 188
  secretion, gram negative bacteria and, 609, 633
Alkalinity, translocation and, 21
Alkylation, translocation and, 9
Alleles
  carbohydrate and, 70
  lysosomelike vacuole in yeast and, 327, 340, 343
Alphaviruses, viral glycoproteins and, 115
Amantadine, viral glycoproteins and, 116
Amebas, *Dictyostelium* and, 370, 387, 388
Amides, lipoproteins and, 588–590
Amines
  endocytosis and, 412
  secretory vesicles and, 532
Amino acids
  carbohydrate and
    intracellular transport signals, 55
    lectins, 88
    mutations, 70
  oligosaccharide biosynthesis, 56, 61
  solubility, 63, 64
  *Dictyostelium* and, 386, 389
    life history, 365, 388
    secretion, 377, 379, 380
    endocytosis and, 403, 405
    endoplasmic reticulum proteins and, 244, 245, 277–279
    cloning, 264, 269–275
    ERp99, 254
    signals, 248, 252
    exocytosis and, 433, 436, 437
    lipoproteins and, 589, 590, 592–595, 597–600
    lysosomal enzymes and, 490, 503
    lysosomelike vacuole in yeast and
      biosynthesis, 320–322, 324–326, 328
      sorting, 335–337, 343
    mannosidases and, 231
    mitochondrial protein import and, 657–659, 671
      mutation isolation, 661–663, 665, 667–669
    mitochondrial proteins and, 680, 717, 725, 727, 728
    proteolysis, 711, 715, 716
    sequences, 685–689, 692–694
    mycology and
      enzymes, 772, 774–780, 785, 788
      *Saccharomyces cerevisiae*, 790, 793
    nuclear transport and, 764
      mechanisms, 749–753
    yeast, 755–759, 761–763
    posttranslational modification and
      endoplasmic reticulum, 165, 167–171, 178
    Golgi apparatus, 192, 193, 195, 196
    secretion, gram negative bacteria and, 639
      early stages, 615, 619, 620
      later stages, 628, 629, 633
    translocation and, 5
      mechanism, 17
    membrane assembly, 26, 28, 30–32, 35, 37
    targeting, 8, 11, 13
    viral glycoproteins and, 145, 146
      cytoplasmic domain, 132–134
      features, 111, 112
      genetic approach, 117, 120, 121
      probes, 114
      signal peptide region, 122, 125–130, 132
transmembrane anchor domain, 136–138, 140–142

Aminopeptidases
carbohydrate and, 81
lysosomes in yeast and, 326
posttranslational modification and, 197

Amylases, mycology and, 772
\(\alpha\)-Amylases, mycology and, 772, 774, 778, 787

Antibodies
carbohydrate and, 73, 86
\textit{Dictyostelium} and, 365, 388
endocytosis and, 404, 411, 416
endoplasmic reticulum proteins and, 253–256, 259, 260, 277
exocytosis and, 435
extracellular matrixlike glycoproteins and, 565, 575, 577
Golgi membranes and, 295
lysosomal enzymes and, 481, 482, 484, 500, 501, 505, 507
lysosomal vacuole in yeast and, 331, 342
mitochondrial protein import and, 660
mitochondrial proteins and, 699, 706, 707
mycology and, 775
nuclear transport and, 756
posttranslational modification and, 185, 186, 192
secretory vesicles and, 532, 537
translocation and, 33
viral glycoproteins and, 114, 116

Antichymotrypsin, carbohydrate and, 77

Antigens
carbohydrate and, 78, 80
mutations, 66
oligosaccharide biosynthesis, 61
tunicamycin, 76
\textit{Dictyostelium} and, 379
endoplasmic reticulum proteins and, 244, 254
exocytosis and, 445
extracellular matrixlike glycoproteins and, 569
lysosomal enzymes and, 482, 500
lysosomal vacuole in yeast and, 327, 335
mannosidases and, 225, 226
nuclear transport and, 751, 752, 756, 759, 761, 763
posttranslational modification and, 182, 193
secretion, gram negative bacteria and, 630
secretory vesicles and, 552
translocation and, 35
viral glycoproteins and, 133

Antipain, \textit{Dictyostelium} and, 373, 374
\(\alpha\)-Antitrypsin, mannosidases and, 218, 230
\(\alpha_1\)-Antitrypsin
carbohydrate and inhibitors, 77–80
mutations, 69, 70
solubility, 63
tunicamycin, 72
\textit{Dictyostelium} and, 379

Apoctochrome \(c\)
mitochondrial protein import and, 661
mitochondrial proteins and, 703, 705, 716, 719, 721
precursor proteins, 679, 680
receptors, 696–699

Apoprotein, carbohydrate and, 75

Arginine
exocytosis and, 436
mitochondrial protein import and, 667–669
mitochondrial proteins and, 689
nuclear transport and, 751
posttranslational modification and, 164, 193, 195
viral glycoproteins and cytoplasmic domain, 133
transmembrane anchor domain, 137–140, 143

Arylsulfatase, lysosomal enzymes and, 466

Asialglycoprotein
carbohydrate and, 76, 81, 83, 86
endocytosis and, 406, 408, 409, 419, 425
lysosomal enzymes and, 481, 482, 486, 506

Asparagine
carbohydrate and, 52, 55, 56, 64
\textit{Dictyostelium} and, 364, 369
endoplasmic reticulum proteins and, 247, 261
extracellular matrixlike glycoproteins and, 578
Golgi membranes and, 289, 291, 293
lysosomal enzymes and, 467, 472, 492

Asparagine
endocytosis and, 404, 411, 416
extracellular matrixlike glycoproteins and, 578
lymphocyte vacuole in yeast and, 327, 335
mannosidases and, 225, 226
Asparagine (cont.)
lysosomalike vacuole in yeast and, 319, 322, 324
mannosidases and, 209–212
mitochondrial protein import and, 662
mycology and, 775, 780
nuclear transport and, 751
posttranslational modification and, 162, 163, 195
endoplasmic reticulum, 171–179
translocation and, 5
Aspartic acid, mitochondrial protein import and, 669
Aspergillus
mycology and, 772, 774–780, 782, 784, 786, 788–791, 796
posttranslational modification and, 169, 195
Astrocytoma cells, carbohydrate and, 74
ATP
endocytosis and, 410, 413, 417, 422, 427
exocytosis and, 438
Golgi membranes and, 301, 306
lysosomal enzymes and, 485, 488, 497, 503
mitochondrial proteins and, 723–725, 728
cytosolic cofactors, 704
energy requirements, 700, 701, 703
precursor proteins, 679, 680
receptors, 696–698
sequences, 685, 694
translocation, 706
nuclear transport and, 749, 750
secretory vesicles and, 545
translocation and, 17, 19
ATPase
endocytosis and, 416, 418
lysosomalike vacuole in yeast and, 319, 327, 342, 346
mitochondrial protein import and, 657, 671, 672
mutation isolation, 660, 664, 666, 668, 669
mitochondrial proteins and, 724–726
assembly, 718, 720
cytosolic cofactors, 705
energy requirements, 700–703
gene products, 716, 717
precursor proteins, 680, 681, 683, 684
proteolysis, 709, 711, 713, 716
receptors, 696–698
sequences, 688, 689, 694
translocation, 706, 707
secretory vesicles and, 527
translocation and, 3
Autophosphorylation, carbohydrate and, 81
Autoradiography
endocytosis and, 422
Golgi membranes and, 294
B
B cell lymphoma, carbohydrate and, 61, 72
Bacillus licheniformis, lipoproteins and, 589, 590, 592, 598, 599
Bacteria
Dictyostelium and, 387
extracellular matrixlike glycoproteins and, 576
gram negative, secretion and, see
Secretion, gram negative bacteria and lipoproteins and, 587, 588, 590, 592, 601
mitochondrial protein import and, 663, 665
mitochondrial proteins and, 726–728
mycology and, 774, 785, 787
translocation and, 5, 8, 9, 21, 28
viral glycoproteins and, 118, 128
Basement membranes
Dictyostelium and, 370
extracellular matrixlike glycoproteins and, 564, 565, 570, 572, 573
Bovine rhodopsin, translocation and, 29, 30
Brain microsomal α-mannosidase,
glycoprotein processing and, 232, 233
Bromoconduritol, carbohydrate and, 78
Bunyaviruses, viral glycoproteins and, 115
C
Calcium
carbohydrate and, 88
lysosomal enzymes and, 476, 480, 486
secretion, gram negative bacteria and, 625
secretory vesicles and, 530–532
Calmodulin
endocytosis and, 411, 416
secretory vesicles and, 532
cAMP
lysosomalike vacuole in yeast and, 350
secretory vesicles and, 530
Carbohydrate

Dictyostelium and, 371, 380, 390
intracellular transport pathways, 382, 384, 386, 387
endocytosis and, 404, 413
diendoplasmic reticulum proteins and, 245, 246, 252, 260, 261
exocytosis and, 437, 439
extracellular matrixlike glycoproteins and, 566
Golgi membranes and, 290–292
lysosomal enzymes and, 465, 466, 476
lysosomal vacuole in yeast and biosynthesis, 319, 322–325, 328
sorting, 331, 332, 337, 340
mannosidases and, 209, 227, 231
mycology and, 775, 781, 785, 787, 788, 791
posttranslational modification and, 152, 178, 179, 182
asparagine-linked, 171–176
Golgi apparatus, 183, 184, 187–190, 194
secretion, gram negative bacteria and, 640
viral glycoproteins and, 112, 113, 129
Carbohydrate, glycoprotein traffic and, 51, 52, 89, 90
inhibitors, 76–81
intracellular transport signals, 52–55
lectins, 81–89
mutations, 66–70
oligosaccharide biosynthesis, 55–62
site-directed mutagenesis, 65, 66
solubility, 62–65
tunicamycin, 70–76
Carbonyl cyanide N-chlorophenylhydrazone
mannosidases and, 217, 218
mitochondrial proteins and, 681, 683, 684, 686, 697, 700
Carboxypeptidase, posttranslational modification and, 193, 196
Carboxypeptidase Y
carbohydrate and, 62, 63, 65, 71
Dictyostelium and, 367, 386
lysosomal enzymes and, 497
lysosomal vacuole in yeast and biosynthesis, 319–326, 330
endocytosis, 350, 352
sorting, 330–333, 335–342, 345, 346
mannosidases and, 219
posttranslational modification and endoplasmic reticulum, 170, 175, 180–182
Golgi apparatus, 186–191, 196, 197
Carcinoma cells, endocytosis and, 407, 408
Caseinolysis, mycology and, 782
Castanospermine
carbohydrate and, 76, 78
mannosidases and, 226
Catalysis
Golgi membranes and, 294–296
lysosomal enzymes and, 465, 469
lysosomal vacuole in yeast and, 327, 329, 350
mannosidases and, 211, 223, 231, 232
mitochondrial protein import and, 657
mitochondrial proteins and, 711, 713, 715, 718
secretion, gram negative bacteria and, 611, 620, 626, 630, 637, 639
viral glycoproteins and, 116
Cathepsins
carbohydrate and, 65, 78
Dictyostelium and, 367, 371–373, 375, 389
lysosomal enzymes and, 467, 476, 490, 491, 493, 498–502
lysosomal vacuole in yeast and, 328
mannosidases and, 226
cDNA
carbohydrate and, 65
endoplasmic reticulum proteins and, 251
cloning, 264–274
ERp99, 256, 260
exocytosis and, 432, 433, 442
extracellular matrixlike glycoproteins and, 567, 568, 571, 572
lysosomal enzymes and, 490
mycology and, 774, 776, 785
posttranslational modification and, 193
translocation and, 13, 15
viral glycoproteins and, 122, 132
Celllobiohydrolases, mycology and, 785, 786
Celllobiose, mycology and, 783, 784, 786, 787
Cellulases, mycology and, 783–789
Ceramide, Golgi membranes and, 291, 297
Cerebrosides, Golgi membranes and, 291
Ceruloplasmin
carbohydrate and, 72, 79
mannosidases and, 230
Chinese hamster ovary cells
carbohydrate and, 58, 67, 68, 79
endocytosis and, 404, 408
exocytosis and, 441, 445
Chinese hamster ovary cells (cont.)
extracellular matrixlike glycoproteins and, 569
Golgi membranes and, 297, 309
lysosomal enzymes and
endocytosis, 481–484, 486, 488
intracellular pathway, 497, 499, 502, 503
junction of pathways, 505, 506
receptors, 477–479
recognition marker, 467, 473, 474
mannosidases and, 215, 222, 227
Chloramphenicol, mitochondrial proteins
and, 719
Chlorides, Golgi membranes and, 301
Chloroplasts
Dictyostelium and, 364, 389
endoplasmic reticulum proteins and, 244
lysosomelike vacuole in yeast and, 338
mitochondrial protein import and, 665
mitochondrial proteins and, 682, 687, 694, 695
secretion, gram negative bacteria and, 620
Chloroquine
Dictyostelium and, 374
endocytosis and, 412
lysosomal enzymes and, 482, 484, 485, 500, 505, 506
viral glycoproteins and, 139–141
Cholesterol
endocytosis and, 403–405, 416
endoplasmic reticulum proteins and, 253
Golgi membranes and, 292, 293, 297
lysosomal enzymes and, 492
Choline, Golgi membranes and, 301
Chromaffin cells
exocytosis and, 445
secretory vesicles and, 530, 532, 552
Chromosomes
lipoproteins and, 594
mitochondrial protein import and, 664, 666
mycology and, 777
secretion, gram negative bacteria and, 623
Chymosin, mycology and, 779, 780, 789
Chymotrypsin
Dictyostelium and, 373
mannosidases and, 231
posttranslational modification and, 193, 194, 196
Clathrin
exocytosis and, 443, 444
lysosomal enzymes and, 497, 500
lysosomelike vacuole in yeast and, 318, 352, 353
secretory vesicles and, 543–545, 554
viral glycoproteins and, 116
Cleavage, see also Signal sequence cleavage
Dictyostelium and, 365, 369, 371
endoplasmic reticulum proteins and, 251, 262, 272, 276
exocytosis and, 433, 435
Golgi membranes and, 299
lipoproteins and, 593–595, 598, 599
lysosomal enzymes and, 470, 490, 494
lysosomelike vacuole in yeast and, 328, 330, 335, 338, 344
mannosidase I and, 221, 224, 225
mannosidase II and, 221, 224, 225
mannosidases and, 212, 216, 217, 219, 233
mitochondrial protein import and, 659, 662, 667–669
mitochondrial proteins and, 685, 693, 697, 703, 717
proteolysis, 711, 713, 715, 716
mycology and, 772, 776, 777, 784, 792, 793
posttranslational modification and, 187, 191, 193, 195–198
secretion, gram negative bacteria and, 636, 637, 639–641
early stages, 611, 612, 615
later stages, 624, 627, 628
secretory vesicles and, 542
Cloning
carbohydrate and, 65
Dictyostelium and, 389
endocytosis and, 402, 429
endoplasmic reticulum proteins and, 251, 256, 260
cDNA, 264–274
exocytosis and, 432
extracellular matrixlike glycoproteins and, 567, 568, 572, 573
Golgi membranes and, 309
lipoproteins and, 594, 597
lysosomelike vacuole in yeast and, 320, 327, 342–345, 352
mannosidases and, 231, 233
mitochondrial protein import and, 659, 661–664, 671
mitochondrial proteins and, 684
mycology and enzymes, 774, 776-779, 782, 785, 786, 788, 789, 791
Saccharomyces cerevisiae, 793
nuclear transport and, 755
posttranslational modification and, 163, 169, 183, 193, 195
secretion, gram negative bacteria and, 618, 620, 621, 623, 626, 628, 639
translocation and, 13, 15
viral glycoproteins and, 145
features, 110
geneic approach, 122
polarized cells, 144
probes, 113
signal peptide region, 128
Colchicine
endocytosis and, 412
Golgi membranes and, 297
Colicins
mitochondrial protein import and, 665
secretion, gram negative bacteria and, 636, 638
early stages, 618-620
later stages, 622-625, 627-633
Collagens, extracellular matrixlike glycoproteins and, 564, 570, 571
Colostrum, mannosidases and, 211
Common antigen-1, Dictyostelium and, 369, 382
Concanavalin A
carbohydrate and, 68, 79
mannosidases and, 214, 224, 227, 232
posttranslational modification and, 176
Coronavirus
endoplasmic reticulum proteins and, 251
viral glycoproteins and, 114, 115
Cross-linkage, translocation and, 11
CURLEndocytosis and, 406, 408, 425
lysosomal enzymes and, 481, 482, 486, 495
Cycloheximide
carbohydrate and, 73
lysosomal enzymes and, 483, 497
mitochondrial proteins and, 683, 684
viral glycoproteins and, 115
Cysteine
Dictyostelium and, 371-374, 389
endocytosis and, 403-405
exocytosis and, 433, 435, 437
lipoproteins and, 588, 590, 599, 600
mitochondrial protein import and, 661, 669
mitochondrial proteins and, 699, 721
posttranslational modification and, 182
secretion, gram negative bacteria and, 612, 627, 636
viral glycoproteins and, 127, 128, 138
Cytidine 5'-monophosphate, Golgi membranes and, 299, 305, 307, 309
Cytochemistry, Golgi membranes and, 293, 294
Cytochrome oxidase, translocation and, 3
Cytochromes
endoplasmic reticulum proteins and, 248, 251
Golgi membranes and, 292
mitochondrial protein import and, 657, 658, 660-662, 665, 666
mitochondrial proteins and, 721-725, 727, 728
assembly, 718-720
cytosolic cofactors, 704
energy requirements, 701, 703
gene products, 716-718
precursor proteins, 679-681, 683
proteolysis, 710, 711, 713, 715
receptors, 696-699
sequences, 685, 687-689, 693-695
translocation, 706
Cytoplasm
carbohydrate and, 65, 67
endocytosis and, 403, 405, 411, 413, 416, 431
endoplasmic reticulum proteins and, 243, 245, 274, 278, 279
ERp99, 262
signals, 248, 251-253
exocytosis and, 433, 435, 436, 439, 442, 444
Golgi membranes and, 291, 294, 295, 298-300, 312
lipoproteins and, 588, 590, 593-595, 597
lysosomelike vacuole in yeast and, 318
biosynthesis, 324
sorting, 336, 344-346
mitochondrial protein import and, 656, 670, 671
mutation isolation, 659, 661, 664, 665
signal sequences, 657, 658
mitochondrial proteins and, 678, 726, 727
assembly, 718
cytosolic cofactors, 704
sequences, 684, 687
Cytoplasm (cont.)
mycology and, 780
nuclear transport and, 747, 748, 764
mechanisms, 749–752
yeast, 754, 755, 760–762
posttranslational modification and,
160–162
endoplasmic reticulum, 164, 167, 172,
180, 183
yeast mutation, 162, 163
secretion, gram negative bacteria and,
607, 608, 637–641
early stages, 611, 613, 615, 619, 620
later stages, 624–626, 630–634
secretory vesicles and, 521, 523, 526, 528,
532, 536, 550
translocation and, 4, 8
mechanism, 15, 19, 20
membrane assembly, 22, 25, 26, 29–31,
33, 35, 36
targeting, 9, 10, 12, 13
viral glycoproteins and
endocytic pathway, 116
features, 109
genetic approach, 118
mutation, 132–135
polarized cells, 144
probes, 114
signal peptide region, 123, 127, 128,
130
transmembrane anchor domain, 136,
137, 142

Cytosol
endocytosis and, 420
exocytosis and, 438
lysosomal vacuole in yeast and, 343
mannosidases and, 212–221, 232
mitochondrial protein import and, 671
mitochondrial proteins and, 678,
703–705, 721, 725, 728
assembly, 718
gene products, 716
precursor proteins, 679–681, 683
receptors, 697
sequences, 687, 694

Cytotoxicity, carbohydrate and, 68

D
mutations, 68
site-directed mutagenesis, 65
solubility, 65
tunicamycin, 72, 74
Dictyostelium and, 372
endocytosis and, 403, 404, 407, 413, 428,
429, 431
endoplasmic reticulum proteins and, 262,
277
Golgi membranes and, 298
lipoproteins and, 595
lysosomal enzymes and, 464, 479
lysosomelike vacuole in yeast and, 327,
348, 349
mannosidases and, 214, 224
mitochondrial proteins and, 681, 703, 718
mycology and, 772, 783, 789, 791
nuclear transport and, 749, 756, 762
posttranslational modification and, 195,
197
translocation and, 4
viral glycoproteins and
signal peptide region, 125, 127
transmembrane anchor domain,
139–141, 143

Deletion
endoplasmic reticulum proteins and, 245,
246
lipoproteins and, 597, 599
lysosomal vacuole in yeast and, 321,
335, 342, 352
mitochondrial protein import and, 658,
667, 668, 671
mitochondrial proteins and, 686, 715
mycology and, 790
nuclear transport and, 752, 755, 759
posttranslational modification and,
167–169, 180
secretion, gram negative bacteria and, 630
translocation and, 9, 30
viral glycoproteins and
signal peptide region, 123–125
transmembrane anchor domain, 137,
140, 141
Deoxymannojirimycin, mannosidases and,
232
endoplasmic reticulum, 215, 216, 218
mannosidase I, 222, 224–226
Deoxynorjirimycin
carbohydrate and, 70
Dictyostelium and, 386
exocytosis and, 437, 440
lysosomal enzymes and, 474
mannosidases and, 226
1-Deoxynojirimycin, carbohydrate and, 76–81, 90
Diacylglycerol, secretory vesicles and, 530, 532
Diacytosis, endocytosis and, 409
Dictyostelium, lysosomal enzymes in, 364, 365, 390, 479, 480, 497
intracellular transport pathways, 381
enzyme localization, 384, 386, 387
α-mannosidase, 384, 385
modification mutants, 380, 383
secretion defects, 381, 382
structural mutants, 383, 384
life history, 365
maturation of precursors, 371–375
posttranslational modifications, 369–371
in vitro studies, 367–369
in vivo studies, 365–367
regulation, 387
posttranslational modification, 388, 389
synthesis, 387, 388
secretion, 375
constitutive secretory pathways, 376, 377
receptors, 378–380
regulation, 377, 378
yeast, 336
Differentiation
Dictyostelium and, 387
extracellular matrixlike glycoproteins and, 563, 570, 580
Dihydrofolate reductase
mitochondrial protein import and, 658, 659
mitochondrial proteins and, 680, 699, 715
sequences, 685, 687, 693, 694
Dipeptidyl aminopeptidase A, mycology and, 793
Dipeptidyl aminopeptidases
lysosomal vacuole in yeast and, 319, 320, 327, 343–345
posttranslational modification and, 193, 195, 196
Diptheria toxin, lysosomal enzymes and, 486, 488, 489
Disaccharides
carbohydrate and, 57, 80
Golgi membranes and, 302, 304
Disulfides
endoplasmic reticulum proteins and, 248, 278
extracellular matrixlike glycoproteins and, 566, 569, 570
mycology and, 792
translocation and, 5
viral glycoproteins and, 11, 143
DNA
carbohydrate and, 62
Dictyostelium and, 364, 386, 388
endoplasmic reticulum proteins and, 246, 256, 265, 269, 277, 280
lipoproteins and, 590, 592, 597
lysosomal vacuole in yeast and, 320, 344
mitochondrial protein import and, 671
mitochondrial proteins and, 694
mycology and, 773, 778, 785
nuclear transport and mechanisms, 749, 751
yeast, 755, 757, 759, 760, 762, 763
posttranslational modification and, 180, 181
secretion, gram negative bacteria and, 621, 622, 628
secretory vesicles and, 538
translocation and, 15, 30
viral glycoproteins and, 145, 146
cytoplasmic domain, 132, 133
features, 110
genetic approach, 120, 122
polarized cells, 144
probes, 113, 115
signal peptide region, 127
Docking protein
mitochondrial proteins and, 682
posttranslational modification and, 161, 163
viral glycoproteins and, 117
Dolichol phosphate
carbohydrate and, 56, 67, 71
exocytosis and, 439
posttranslational modification and, 171, 172, 174, 175, 179
Dolichol pyrophosphate
carbohydrate and, 59
Golgi membranes and, 291
lysosomal vacuole in yeast and, 322
Dopamine, secretory vesicles and, 528
Drosophila, extracellular matrixlike glycoproteins and, 573
Drosophila melanogaster, endoplasmic reticulum proteins and, 272, 274, 277

E

EDTA
endoplasmic reticulum proteins and, 253, 254
lipoproteins and, 588
mitochondrial proteins and, 710

Egasyn
Dictyostelium and, 380
endoplasmic reticulum proteins and, 251, 252

Electron microscopy
carbohydrate and, 86
Dictyostelium and, 371, 388
endocytosis and, 402, 406-408, 421, 424, 426
endoplasmic reticulum proteins and, 253
exocytosis and, 440, 442, 444
Golgi membranes and, 290
lysosomal enzymes and, 481, 483, 492, 495, 500
mannosidases and, 217
mitochondrial proteins and, 706, 707
nuclear transport and, 748, 753
posttranslational modification and, 178
secretory vesicles and, 543

Electrophoresis
Dictyostelium and, 365, 382
endocytosis and, 416, 422, 427
extracellular matrixlike glycoproteins and, 569
lysosomal enzymes and, 464, 466, 467, 477-479, 484
lysosomelike vacuole in yeast and, 323
mannosidases and, 225
mycology and, 776, 780
posttranslational modification and, 183

Elongation
mannosidases and, 211
mitochondrial proteins and, 682, 684
posttranslational modification and, 179, 183, 188
translocation and, 5
mechanism, 19
membrane assembly, 30, 35
targeting, 11-13


Endoglycosidasases, posttranslational modification and, 187
Endomannosidases, carbohydrate and, 80
Endo-β-N-acetylglucosaminidase H carbohydrate and, 54, 63, 79 Dictyostelium and, 368, 369 endocytosis and, 404 endoplasmic reticulum proteins and, 247, 259-262, 265 lysosomal enzymes and, 477, 478, 490 intracellular pathway, 493-495 recognition marker, 467, 471-474 mannosidases and, 219, 220, 230 mycology and, 776, 777, 780
posttranslational modification and, 175, 176, 183, 187, 191
viral glycoproteins and, 112, 113
Endopeptidase, posttranslational modification and, 194
Endoplasmic reticulum, see also Rough endoplasmic reticulum
carbohydrate and, 51
inhibitors, 78, 80, 81
intracellular transport signals, 54, 55
lectins, 86, 88
mutations, 67, 70
oligosaccharide biosynthesis, 58
solubility, 63
tunicamycin, 74

*Dictyostelium* and, 364, 389, 390
life history, 367, 369, 371, 372, 375
secretion, 378–380, 383, 386
deyocytosis and, 404
exocytosis and, 432, 435, 440, 443
Golgi membranes and, 290–295, 297
lysosomal enzymes and, 474, 490
lysosomelike vacuole in yeast and, 318, 346, 353
biosynthesis, 320–322
sorting, 330, 331, 338
mannosidases and, 211–223
mitochondrial protein import and, 665
mitochondrial proteins and, 682
mycology and, 781, 787, 789, 790
nuclear transport and, 748
posttranslational modification and, 160–163, 198
asparagine-linked carbohydrates, 171–175
asparagine-linked oligosaccharides, 175–178
core oligosaccharides, 180, 181
fatty acid, 182, 183
Golgi apparatus, 183, 184, 186–189, 194
mutation, 170, 171
O-linked oligosaccharides, 178, 179
phosphoryl groups, 181, 182
signal sequence cleavage, 163–170
secretory vesicles and, 533, 536, 539, 549–552
translocation and, see Translocation
viral glycoproteins and, 145, 146
genetic approach, 119, 121
signal peptide region, 128, 130
Endoplasmic reticulum proteins, sorting of, 243–246, 274, 276–280
cloning, 264–275
ERp99
membrane topography, 262–264
metabolic fate, 260–262
preparation, 253–256
subcellular distribution, 256–258
tissue distribution, 258–260
signals, 247–253
site, 246, 247

Endosomes
endocytosis and, 406, 418–428
Golgi membranes and, 301, 302
lysosomal enzymes and, 500, 503, 505, 506
decytosis, 482, 488, 489
lysosomelike vacuole in yeast and, 341, 347, 350
viral glycoproteins and, 116, 117

Endothelial cells
carbohydrate and, 73
*Dictyostelium* and, 377
deyocytosis and, 410
extracellular matrixlike glycoproteins and, 564

Endotoxin, extracellular matrixlike glycoproteins and, 565

Enzymes
carbohydrate and
inhibitors, 77, 78, 80
lectins, 83, 88, 89
mutations, 68
oligosaccharide biosynthesis, 57–61
solubility, 62, 63
tunicamycin, 71, 73

*Dictyostelium* and, 364
deyocytosis and, 406, 413, 416, 427
endoplasmic reticulum proteins and, 248, 256, 277, 278
deyocytosis and, 441–444
extracellular matrixlike glycoproteins and, 572
Golgi membranes and, 289, 292, 293, 311
methods, 294, 295
orientation, 298, 299
transport, 301
lipoproteins and, 592–595, 598, 600, 601
lysosomal, see Lysoosomal enzymes
lysosomelike vacuole in yeast and
biosynthesis, 319, 324–328
sorting, 333, 336, 338, 342
mannosidase I and, 222–226
Enzymes (cont.)
mannosidase II and, 227, 230–232
mannosidases and, 211, 212, 232, 233
endoplasmic reticulum, 212, 214–216, 219, 221
mitochondrial protein import and, 659, 662, 663, 665, 666, 668–671
mitochondrial proteins and, 699, 703, 705, 710, 713
mycology and, see Mycology, secretion research and
posttranslational modification and, 160, 162, 198
dendoplasmic reticulum, 167–169, 171, 174–176, 179, 180
Golgi apparatus, 186–190, 193–197
secretion, gram negative bacteria and, 608, 613, 618, 636, 637
secretory vesicles and, 527, 528, 532, 545
translocation and, 21, 38
viral glycoproteins and, 146
features, 109
probes, 113
signal peptide region, 125, 127, 128
Epidermal growth factor
carbohydrate and, 74, 81
endocytosis and, 405, 407, 408, 414, 418, 419, 425, 428, 429, 431
Epidermal growth factor receptor, translocation and, 35
Epithelial cells
carbohydrate and, 73, 76, 81
Dictyostelium and, 389
endocytosis and, 410
extracellular matrixlike glycoproteins and, 564
secretory vesicles and, 523–525, 529
viral glycoproteins and, 115, 144, 147
Epitopes, Dictyostelium and, 369
Epoxide hydrolase, endoplasmic reticulum proteins and, 251, 252
Erythrocytes, Golgi membranes and, 295
Escherichia coli
carbohydrate and, 62
lipoproteins and, 588–590, 592–595, 598, 599, 601
lysosomelike vacuole in yeast and, 321, 342
mitochondrial protein import and, 656, 657, 663
mycology and, 779, 785
nuclear transport and, 752, 754, 755, 762
secretion, gram negative bacteria and, 607, 609, 636, 637, 639, 641
early stages, 615–620
later stages, 622, 623, 626–629, 631, 633, 634
translocation and, 9, 33
viral glycoproteins and, 128
Ethylenebis, carbohydrate and, 88
N-Ethylmaleimide, translocation and, 19
Eukaryotes
carbohydrate and, 62
Dictyostelium and, 390
endocytosis and, 401, 446
endoplasmic reticulum proteins and, 259, 260, 269, 280
exocytosis and, 431
extracellular matrixlike glycoproteins and, 580
Golgi membranes and, 290
lysosomelike vacuole in yeast and
biosynthesis, 322
endocytosis, 318, 319, 352, 353
sorting, 337, 341
mitochondrial protein import and, 663
mitochondrial proteins and, 677, 682, 716, 728
nuclear transport and, 748, 754
posttranslational modification and, 160, 198
cyttoplasm, 162
endoplasmic reticulum, 170–172, 178, 182
Golgi apparatus, 183, 186, 187, 191
secretion, gram negative bacteria and, 618
translocation and, 3, 5
mechanism, 19, 21
membrane assembly, 28
targeting, 9
viral glycoproteins and, 146
features, 109
 genetic approach, 119, 121
signal peptide region, 126, 128, 130
transmembrane anchor domain, 143
Exocytosis, 401, 402, 409, 425, 431, 432
biochemical characterization
Golgi, 444
nuclear envelope, 442, 443
RER, 442, 443
secretory vesicles, 444, 445
carbohydrate and, 55
**Index**

*Dictyostelium* and, 376, 377

- genetic analysis, 440, 441
- inhibitors, 439, 440
- lysosomal enzymes and, 497
- pathways, 438, 439
- secretory vesicles and, 527, 532, 539, 552

VSV G protein, 432

- domain structure, 433
- morphology, 434, 435
- posttranslational modification, 435
- reconstitution, 438
- structure, 435–438

Extracellular matrixlike glycoproteins, macrophages and, 563–566, 579, 580

biosynthesis

- fibronectin, 566–570
- laminin, 570–573
- proteoglycans, 573, 574
- thrombospondin, 570

functions

- cell interactions, 577–579
- phagocytosis, 574–576

**F**

α-Factor

- endocytosis and, 413

lysosomal enzymes and, 321, 322

- endocytosis, 348, 349
- sorting, 330, 344, 345

mycology and, 774, 780, 789, 793, 794

- posttranslational modification and, 162, 163

endoplasmic reticulum, 180, 181

Golgi apparatus, 188, 190–197

- secretory vesicles and, 527

Fatty acids

- endocytosis and, 403

lipoproteins and, 588–590, 592

- posttranslational modification and, 182, 183

secretion, gram negative bacteria and, 625

Fatty acyl chains, secretion, gram negative bacteria and, 627, 632, 636

Ferritin

- endocytosis and, 422, 424, 425

lysosomal enzymes and, 506, 507

- nuclear transport and, 753

- secretory vesicles and, 552

α-Fetoprotein, carbohydrate and, 72, 79

Fibrin, extracellular matrixlike glycoproteins and, 570

Fibrinogen, carbohydrate and, 72

Fibroblasts

- carbohydrate and, 81

- intracellular transport signals, 55

- lectins, 83, 86

- solubility, 63, 65

*Dictyostelium* and, 364, 370–375, 377, 378

endocytosis and, 407, 411, 413, 419

endoplasmic reticulum proteins and, 244, 265

Golgi membranes and, 297, 302

lysosomal enzymes and, 464, 465

- endocytosis, 482, 483, 485, 489

- intracellular pathway, 491, 493, 495, 496, 498–502

- junction of pathways, 504, 505

- receptors, 476–478, 480

- recognition marker, 465, 467, 468, 471, 473, 475, 476

lysosomal vacuole in yeast and, 322–324, 328, 331

mannosidases and, 219, 226

mitochondrial proteins and, 681

- secretory vesicles and, 528, 542

viral glycoproteins and, 139

Fibronectin

- carbohydrate and, 65, 72, 79, 81

extracellular matrixlike glycoproteins and, 564, 565, 579, 580

biosynthesis, 556–570

- functions, 575–577

Filipin, Golgi membranes and, 298, 301, 307

Flaviviruses, viral glycoproteins and, 114

Fluorescence

- endocytosis and, 406, 408, 419, 421, 427

- exocytosis and, 435

Golgi membranes and, 297

lysosomal vacuole in yeast and, 346, 347

Fluorescence-activated cell sorting, posttranslational modification and, 186

Fractionation

- carbohydrate and, 54

*Dictyostelium* and, 369, 370, 372

endocytosis and, 405, 425, 428

endoplasmic reticulum proteins and, 247, 256, 257, 262
Fractionation (cont.)
exocytosis and, 432
Golgi membranes and, 293
lysosomal enzymes and, 470, 481–483, 492, 493
lysosomelike vacuole in yeast and, 318
mannosidases and, 214, 222, 223
mitochondrial protein import and, 667
mitochondrial proteins and, 704
nuclear transport and, 756
posttranslational modification and, 163, 178, 183
secretion, gram negative bacteria and, 620
translocation and, 10
Fragmentation, Golgi membranes and, 292
Friend erythroleukemia virus, carbohydrate and, 70
Fructose 1-phosphate, lysosomal enzymes and, 466
Fucose
  carbohydrate and, 55, 57, 59, 86
  Dictyostelium and, 371, 388
  mannosidases and, 210, 211
  viral glycoproteins and, 112, 120
Fucosyltransferase
carbohydrate and, 56
mannosidases and, 222
Fungi, mycology and, 771, 772, 796, 797
  enzymes, 774, 778, 782–785, 787, 789, 791
  Saccharomyces cerevisiae, 795, 796
Fusion
  endocytosis and, 414, 422
  exocytosis and, 432, 436–440, 445
  extracellular matrixlike glycoproteins and, 574
  lysosomal enzymes and, 469
  lysosomelike vacuole in yeast and biosynthesis, 324, 325
  endocytosis, 346, 350
  sorting, 331, 337, 340
  mitochondrial protein import and, 656, 657, 670
  mutation isolation, 660, 662–669
  mitochondrial proteins and, 678, 699, 715
  precursor proteins, 682
  sequences, 685–689, 693, 694
  mycology and, 774, 779–781
  nuclear transport and, 752, 754–756, 758, 761
  posttranslational modification and, 189, 190, 195, 196
secretion, gram negative bacteria and, 631–634
secretory vesicles and
  formation, 538, 549–551, 554
  structure, 525, 526, 530–532
translocation and, 15, 31
viral glycoproteins and
  endocytic pathway, 117, 118
  features, 111
  polarized cells, 144
  signal peptide region, 125

G

G proteins
  carbohydrate and
  inhibitors, 78, 80
  site-directed mutagenesis, 65, 66
  solubility, 63, 64
  Dictyostelium and, 379
  endoplasmic reticulum proteins and, 24
  246, 274
  exocytosis and, 432–438, 441, 444, 446
  mannosidases and, 217
  posttranslational modification and, 180
  viral glycoproteins and
    cytoplasmic domain, 132–134
    features, 112
    genetic approach, 118–121
    polarized cells, 144, 145
    transmembrane anchor domain, 136,
    137, 140, 142
Galactose
  carbohydrate and, 55, 57, 59
  endocytosis and, 409
  exocytosis and, 444
  Golgi membranes and, 299, 307, 309, 3...
  lysosomal enzymes and, 493
  mannosidases and, 210, 211, 226, 227
  mitochondrial protein import and, 666
  nuclear transport and, 755
  posttranslational modification and, 188
Galactosidase, mannosidases and, 226
β-Galactosidase
  Dictyostelium and, 372, 377
  lysosomal enzymes and, 491, 502, 508
  endocytosis, 481, 482, 484, 486
  receptors, 77, 80, 488
  recognition marker, 466
  mitochondrial protein import and, 657,
  658, 664, 666–668
mitochondrial proteins and, 685–687
mycology and, 788, 789, 795
nuclear transport and, 752, 754–756, 758, 761, 763
secretion, gram negative bacteria and, 619, 631, 632
Galactosylation, Golgi membranes and, 294, 307
Galactosyltransferase carbohydrate and, 56
Dictyostelium and, 388
Golgi membranes and, 293, 297–302, 310
lysosomal enzymes and, 470, 492, 493
mannosidases and, 221, 222, 228
viral glycoproteins and, 112
Gangliosides, Golgi membranes and, 291, 307
Gene dosage, lysosomalike vacuole in yeast and, 333
GERL, lysosomal enzymes and, 482, 506, 507
intracellular pathway, 492, 494, 497, 499, 500, 502, 504
Globin, translocation and, 15
α-Globin, translocation and, 17
Globomycin lipoproteins and, 588–590, 594, 600
secretion, gram negative bacteria and, 619
Glucanases, mycology and, 791, 795
Glucoamylase
mycology and, 772, 774–778, 782
posttranslational modification and, 169, 170, 195
Glucocerebrosidase, Dictyostelium and, 389
Glucocerebroside, Golgi membranes and, 298
β-Glucoronidase
Dictyostelium and, 367, 371, 372, 380, 389
lysosomal enzymes and, 476, 483, 484, 505
intracellular pathway, 450–452, 490, 491
recognition marker, 465–467, 471, 473, 474, 476
Glucosamine carbohydrate and, 72
lysosomal enzymes and, 467
viral glycoproteins and, 112
Glucose carbohydrate and, 90 inhibitors, 76–80
lectins, 88, 89
mutations, 70
oligosaccharide biosynthesis, 56–60
Dictyostelium and, 382, 384, 386
endoplasmic reticulum proteins and, 278, 279
exocytosis and, 433, 435, 437, 442, 444
Golgi membranes and, 291, 302
lysosomal enzymes and, 467, 471, 474, 475
lysosomalike vacuole in yeast and, 321
mannosidases and, 210, 217, 226
mycology and, 784, 789
posttranslational modification and, 162, 167, 169, 175
translocation and, 34
viral glycoproteins and, 112
Glucose 6-phosphatase, Golgi membranes and, 292, 294
Glucose 6-phosphate endoplasmic reticulum proteins and, 257
lysosomal enzymes and, 480
Glucosidases carbohydrate and, 78, 79
endoplasmic reticulum proteins and, 247, 248, 251, 261
Golgi membranes and, 291
lysosomal enzymes and, 474
mannosidases and, 210, 212, 214, 215, 224, 226
mycology and, 786
posttranslational modification and, 176, 177
α-Glucosidases carbohydrate and inhibitors, 77, 79, 80
oligosaccharide biosynthesis, 56, 58, 59
Dictyostelium and, 372, 377, 382, 386, 388, 389
lysosomal enzymes and, 467, 476, 501
β-Glucosidases
Dictyostelium and intracellular transport pathways, 383, 384, 386
life history, 365, 367–369, 372–376
regulation, 387, 388
secretion, 377, 378, 380
mycology and, 784, 786, 787
Glucosylation carbohydrate and, 60, 79
lysosomal enzymes and, 473
Glucosylmannose disaccharide, carbohydrate and, 59
Glutamate, mitochondrial protein import and, 668
Glutamic acid
  carbohydrate and, 70
  mycology and, 786
  viral glycoproteins and, 129, 140
Glutamine, mitochondrial proteins and, 715
Glycan chains, carbohydrate and, 52, 89, 90
  inhibitors, 77, 79, 80
  intracellular transport signals, 54, 55
  lectins, 86–88
  mutations, 68, 70
  oligosaccharide biosynthesis, 56, 59–61
  solubility, 62–64
  tunicamycin, 75
Glycans, posttranslational modification and, 179
Glycerides, viral glycoproteins and, 128
Glycerol
  lipoproteins and, 588, 589, 592
  mitochondrial protein import and, 664, 668, 669
Glycerylcysteine, lipoproteins and, 588
Glycine
  mitochondrial protein import and, 667
  secretion, gram negative bacteria and, 629, 630
  viral glycoproteins and, 129
Glycolipids
  carbohydrate and, 56
  Dictyostelium and, 380
  extracellular matrixlike glycoproteins and, 572
  Golgi membranes and, 290, 297–299, 309
Glycopeptides
  carbohydrate and, 79
  endoplasmic reticulum proteins and, 247
  lysosomal enzymes and, 467, 468, 471
Glycophorin A, carbohydrate and, 75
Glycoprotein
  Dictyostelium and, 364, 370, 382, 384
  endocytosis and, 413
  endoplasmic reticulum proteins and, 245, 276
  cloning, 274
  ERp99, 254, 256, 261
  signals, 251
  exocytosis and, 432, 439, 441–443, 445
  extracellular matrixlike, see Extracellular matrixlike glycoproteins
Golgi membranes and, 289, 290, 292, 293, 299, 309
  lysosomal enzymes and, 466, 468, 474, 475, 491
  lysosomelike vacuole in yeast and, 331, 345
  mannosidases and, see Mannosidases, glycoprotein processing and
  mycology and, 775, 785, 788, 797
  posttranslational modification and, 163, 180–183, 188
  secretory vesicles and, 527, 531, 536
  traffic, carbohydrate and, see Carbohydrate, glycoprotein traffic and
  translocation and, 20, 21, 25
  viral, see Viral glycoproteins, membrane insertion and
Glycosaminoglycans, Golgi membranes and, 290, 291
Glycosidases
  carbohydrate and
    inhibitors, 76, 79, 80
    mutations, 70
  oligosaccharide biosynthesis, 60
  Dictyostelium and, 377, 381
  lysosomal enzymes and, 470, 493
  mannosidases and, 226
Glycosphingolipids, Golgi membranes and, 290–292, 297
Glycosylation
  carbohydrate and, 52, 89
    inhibitors, 79
    mutations, 66–70
    oligosaccharide biosynthesis, 56, 58, 59, 61
    site-directed mutagenesis, 65, 66
    solubility, 62–65
    tunicamycin, 71–76
  Dictyostelium and, 367–369, 380, 382, 386, 390
  endocytosis and, 403–405, 413
  endoplasmic reticulum proteins and, 247, 248, 276, 277
  exocytosis and, 433, 435, 437, 440
  extracellular matrixlike glycoproteins and, 572, 579
  Golgi membranes and, 289–291, 293, 298, 302, 305
  lysosomal enzymes and, 471, 475, 488, 490, 492, 494, 499
  lysosomelike vacuole in yeast and
  biosynthesis, 320–326, 328
Index

827

sorting, 332, 344

mannosidases and, 209, 219-221

mycology and, 797

enzymes, 774-778, 780, 781, 785-790

Saccharomyces cerevisiae, 793

posttranslational modification and, 160, 163, 198

endoplasmic reticulum, 167-169, 176, 180-182

Golgi apparatus, 187-191, 193

viral glycoproteins and

features, 111

genetic approach, 117, 118

probes, 112, 114

signal peptide region, 122, 123, 125, 127-129

transmembrane anchor domain, 138-140

Glycosyltransferase

carbohydrate and, 57, 60

Golgi membranes and, 291, 293, 298, 299, 309

mannosidases and, 233

Golgi apparatus

carbohydrate and, 89, 90

inhibitors, 76-81

intracellular transport signals, 52, 54, 55

lectins, 82, 83, 86-88

mutations, 67, 70

oligosaccharide biosynthesis, 57-59

site-directed mutagenesis, 65, 66

tunicamycin, 71, 74

Dictyostelium and, 364, 388, 390

life history, 369-372, 374

secretion, 378-380

endocytosis and, 404, 407, 409, 411, 413, 414, 429

biochemical characterization, 414, 416, 419, 424, 425, 427, 428

endoplasmic reticulum proteins and, 245-247

ERp99, 257, 258, 261, 262

signals, 248, 252

exocytosis and, 432, 434-440, 442-444, 447

lysosomal enzymes and, 470, 483

intracellular pathway, 492-494, 496, 497, 500, 503

junction of pathways, 506, 507

lysosomal vacuole in yeast and, 353

biosynthesis, 322-324

dendocytosis, 346, 350, 352

sorting, 330-332, 341, 343

mannosidase I and, 222-224

mannosidase II and, 227, 228, 230-232

mannosidases and, 211, 212

endoplasmic reticulum, 214, 217, 218, 220, 221

membranes, functional topology of, see Golgi membranes, functional topology of

mycology and, 781, 787, 790

posttranslational modification and, 161, 183, 184, 198

endoplasmic reticulum, 168, 170, 171, 175, 179, 181, 182

oligosaccharides, 184-188

proteolysis, 191-197

sorting, 188-191

secretory vesicles and, 555

formation, 533, 539, 542-545, 549-552

structure, 524, 525, 528, 529

translocation and, 4

viral glycoproteins and, 147

cytoplasmic domain, 132-134

genetic approach, 117, 120, 121

polarized cells, 144

probes, 112, 114, 115

signal peptide region, 122, 123, 130, 132

transmembrane anchor domain, 139, 140, 142

Golgi membranes, functional topology of, 289, 290, 311, 312

methods

catalysis, 294, 295

cytochemistry, 293, 294

enzymatic digestion, 295

orientation

enzymes, 298, 299

function, 299, 300

lipids, 295-298

properties

characterization, 292, 293

function, 290-292

polarity, 293

structure, 290

transport

disaccharides, 302, 304

measuring, 300, 301

monosaccharides, 302, 303

nucleotides, 304-309

permeability, 301, 302

sugar uptake, 309-311
Gram negative bacteria, see Secretion, gram negative bacteria and
Growth factors
endocytosis and, 403, 404
extracellular matrixlike glycoproteins and, 564
Growth hormone
endoplasmic reticulum proteins and, 245, 246
exocytosis and, 436, 437, 443
secretory vesicles and, 527, 538, 545

H

Haptoglobin, carbohydrate and, 73
Hemagglutinin
carbohydrate and, 65, 78, 81, 88
Dictyostelium and, 379
endoplasmic reticulum proteins and, 245
exocytosis and, 436, 443
secretion, gram negative bacteria and, 637
secretory vesicles and, 531, 539
viral glycoproteins and
cytoplasmic domain, 133, 134
features, 111, 112
genetic approach, 118, 121
polarized cells, 144, 145
signal peptide region, 123, 125, 132
transmembrane anchor domain, 136, 142
Hematopoietic cells, extracellular matrixlike
glycoproteins and, 563
Hemin, mitochondrial proteins and, 696
Hemolysin, secretion, gram negative
bacteria and, 619, 620, 636, 637
later stages, 623, 626, 627, 630, 632
α-Hemolysin, secretion, gram negative
bacteria and
eyearly stages, 619, 620
later stages, 622, 626, 627, 630–632, 634
Hepatitis B surface antigen, translocation
and, 31, 32
Hepatitis virus, carbohydrate and, 78
Hepatocytes
Dictyostelium and, 371, 377
endocytosis and, 408
Golgi membranes and, 291, 300
lysosomal enzymes and, 506
endocytosis, 481, 482, 486
intracellular pathway, 490, 492, 496, 497, 499, 502
mannosidases and, 218, 224
secretory vesicles and, 527, 536
Hepatoma cells
carbohydrate and
inhibitors, 77–81
intracellular transport signals, 53–55
lectins, 87
mutations, 70
tunicamycin, 72, 74–76
exocytosis and, 440
mannosidases and, 215
Herpes simplex virus, viral glycoproteins
and, 113
Heterogeneity
carbohydrate and, 54, 61
Dictyostelium and, 378, 381, 382
Golgi membranes and, 293, 302
lysosomal enzymes and, 494
mannosidases and, 222, 230
posttranslational modification and, 163
endoplasmic reticulum, 176, 178, 183
Golgi apparatus, 185, 191
Hexosaccharides, carbohydrate and, 87
β-Hexosaminidase
carbohydrate and, 78
Dictyostelium and, 367, 371–373, 389
lysosomal enzymes and
intracellular pathway, 490, 491, 493, 494, 498, 500–502
receptors, 478
recognition marker, 465–469, 473
Hexose 6-phosphate dehydrogenase,
endoplasmic reticulum proteins and, 247, 261
Hexoses, Dictyostelium and, 382
Histidine
mitochondrial protein import and, 665, 670, 671
mitochondrial proteins and, 687
Homogeneity
endocytosis and, 422
mannosidases and, 210, 212
endoplasmic reticulum, 214, 215
mannosidase I, 224, 225
mannosidase II, 230
translocation and, 10
Homogenization, Golgi membranes and, 292, 299
Homology
carbohydrate and, 61
endocytosis and, 431
endoplasmic reticulum proteins and, 260, 272, 277, 279
extracellular matrixlike glycoproteins and, 569, 570
lipoproteins and, 590
lysosomal enzymes and, 464
lysosomelike vacuole in yeast and, 320, 328, 337, 345
mitochondrial protein import and, 671
mitochondrial proteins and, 704, 711, 715, 719, 727
precursor proteins, 680, 684
sequences, 686, 688, 694
mycology and, 775, 780, 785, 788
nuclear transport and, 752, 759, 762
secretion, gram negative bacteria and, 626, 628, 630
secretory vesicles and, 530
translocation and
mechanism, 18
membrane assembly, 26, 33
targeting, 8, 11
viral glycoproteins and, 121, 127
Hormones
carbohydrate and, 66, 81
Dictyostelium and, 370
endocytosis and, 403, 405, 408, 429
endoplasmic reticulum proteins and, 278
lysosomal enzymes and, 506
Human chorionic gonadotropin,
endocytosis and, 405, 406
Hybridization
carbohydrate and, 61, 66, 76, 77
endoplasmic reticulum proteins and, 246, 264
exocytosis and, 436, 437, 443
lipoproteins and, 594, 595, 597, 600, 601
lysosomal enzymes and, 467, 473, 487, 493
lysosomelike vacuole in yeast and,
324–326, 340
mannosidases and, 211, 221, 228, 230, 233
mitochondrial protein import and,
656–658, 670, 671
mutation isolation, 659, 661, 663–668
mitochondrial proteins and, 684
mycology and, 793, 794
nuclear transport and, 755, 756, 759–763
secretion, gram negative bacteria and,
608, 609, 619, 631–634, 642
secretory vesicles and, 538, 542
translocation and, 20
viral glycoproteins and, 121, 125, 133, 136
Hybridoma cells, carbohydrate and, 78
Hydrogen, viral glycoproteins and, 139, 140
Hydrolysis
Dictyostelium and, 386
endocytosis and, 427
Golgi membranes and, 301
lipoproteins and, 595
lysosomal enzymes and, 466, 467, 493, 494
lysosomelike vacuole in yeast and, 319
mannosidases and, 214–216
mitochondrial proteins and, 680, 700, 703
mycology and, 772, 775, 783, 784
posttranslational modification and, 167, 169
secretion, gram negative bacteria and, 636
secretory vesicles and, 530
translocation and, 38
mechanism, 17–19
membrane assembly, 22
Hydropathy, endoplasmic reticulum proteins
and, 272
Hydrophilicity
Hydrogen, viral glycoproteins and, 139, 140
Hydrophobicity
Hydrogen, viral glycoproteins and, 139, 140
Hydrophobicity (cont.)
lipoproteins and, 593–595, 598
lysosomelike vacuole in yeast and, 320, 321, 345
mannosidases and, 231
mitochondrial protein import and, 658
mitochondrial proteins and, 680, 681, 689, 716, 717, 728
mycology and, 778, 785, 790, 792, 793
nuclear transport and, 754, 759
posttranslational modification and, 163–165, 167, 168, 170, 196
secretion, gram negative bacteria and, 611, 615, 618, 627, 630, 640
secretory vesicles and, 531, 547
translocation and, 7
membrane assembly, 27, 28, 30, 32, 33, 37
targeting, 8, 9
viral glycoproteins and, 147
cytoplasmic domain, 132, 134, 135
features, 111
genetic approach, 118, 119, 121
probes, 114
signal peptide region, 123, 126–128
transmembrane anchor domain, 135–143
β-Hydroxyleucine, translocation and, 11
Hydroxymethylglutaryl-coenzyme A reductase
doplasmic reticulum proteins and, 251–253, 261, 277
mannosidases and, 214, 218

I
α-L-Iduronidase, lysosomal enzymes and, 466, 474, 476, 491, 502
Immunoelectron microscopy
lysosomelike vacuole in yeast and, 318, 333, 339, 350
secretory vesicles and, 539
viral glycoproteins and, 119, 120
Immunofluorescence
carbohydrate and, 65
endocytosis and, 434
lysosomelike vacuole in yeast and, 318
viral glycoproteins and, 130, 131, 137
Immunofluorescence microscopy, viral
viral glycoproteins and, 115
Immunoglobulins
carbohydrate and inhibitors, 78, 80
oligosaccharide biosynthesis, 59
tunicamycin, 71, 72, 74
endocytosis and, 408, 409, 424
doplasmic reticulum proteins and, 245, 246, 252, 277
extracellular matrixlike glycoproteins and, 575, 576
lysosomal enzymes and, 504
mannosidases and, 217, 220, 225
nuclear transport and, 753
posttranslational modification and, 162
secretion, gram negative bacteria and, 628
translocation and, 4, 26, 35
Immunoprecipitation
Dictyostelium and, 365–368, 384, 388
endocytosis and, 416
doplasmic reticulum proteins and, 254, 256, 258, 262, 265
extracellular matrixlike glycoproteins and, 571
lysosomal enzymes and endocytosis, 482–484, 488
receptors, 477, 479
recognition marker, 467, 469, 471, 473
mannosidases and, 235
mycology and, 775
Inflammation, extracellular matrixlike
glycoproteins and, 564, 565, 575, 578
Influenza virus
carbohydrate and, 61, 65, 78, 81
Dictyostelium and, 379
doplasmic reticulum proteins and, 245
endocytosis and, 436, 443
mannosidases and, 225
nuclear transport and, 759, 761
secretory vesicles and, 531
viral glycoproteins and cytoplasmic domain, 123, 134
endocytic pathway, 117
features, 111, 112
genetic approach, 118, 119, 121
polarized cells, 144
signal peptide region, 123, 125
transmembrane anchor domain, 136, 141
Inhibitors
Dictyostelium and, 374, 375
endocytosis and, 410–412
dendocytosis and, 439, 440
lysosomal enzymes and, 499, 500
secretion, gram negative bacteria and, 638
Index

Inositol triphosphate, secretory vesicles and, 530
Insulin
carbohydrate and, 73, 81
Dictyostelium and, 375
dendrimerization and, 405, 407, 425, 431
secretory vesicles and, 527, 528, 538, 542, 545, 552
Integral membrane proteins
da endoplasmic reticulum proteins and, 243
lysosomal enzymes and, 344, 345
Integral transmembrane proteins,
translocation and membrane assembly, 22–25, 37
topoliteic, 30–34
stop transfer sequences, 25–29
topogenic sequences, 34–37
Integrin, extracellular matrixlike
glycoproteins and, 568, 570
Interferon
carbohydrate and, 63
extracellular matrixlike glycoproteins and, 564, 565
biosynthesis, 567–569, 571, 572
functions, 577, 578
mycology and, 789, 792, 793
Interleukin-2
carbohydrate and, 63
extracellular matrixlike glycoproteins and, 578
Intracellular transport signals, carbohydrate and, 52–55
Invertase
Dictyostelium and, 379
eocytosis and, 440, 443
lysosomal enzymes and, 347
biotranscriptase, 319, 321, 322, 324, 325
sorting, 332, 333, 335–337, 339–341
mannosidases and, 219
mycology and enzymes, 779–781, 788–790
Saccharomyces cerevisiae, 792, 793, 795
posttranslational modifications and, 162
endoplasmic reticulum, 164, 167–170, 176, 178, 180, 181
Golgi apparatus, 183, 185, 187, 189, 190
Ionophores
eocytosis and, 411
eocytosis and, 439
Iron
dendrimerization and, 403, 413
lysosomal enzymes and, 487, 489
mitochondrial proteins and, 703
Isoleucine
Dictyostelium and, 379
eocytosis and, 436
viral glycoproteins and, 127
K
Kidney
Dictyostelium and, 377, 378, 380
Golgi membranes and, 292, 293
lysosomal enzymes and, 492, 499
Kinensin, secretory vesicles and, 528
L
β-Lactamase, translocation and, 15, 17
Lactase, mycology and, 788, 789
Lactate, mitochondrial protein import and, 669
Lactogen, translocation and, 17
Lactose
carbohydrate and, 88
Golgi membranes and, 300, 302
Laminin, extracellular matrixlike
glycoproteins and, 564, 565, 579, 580
biosynthesis, 568, 579–573
functions, 575–578
Lecithin, Golgi membranes and, 292
Lectins
carbohydrate and, 52, 81–89
intracellular transport signals, 54
mutations, 67, 68
Golgi membranes and, 295
lysosomal enzymes and, 493
posttranslational modifications and, 176
Leucine
lysosomal enzymes and, 338, 339
translocation and, 11
viral glycoproteins and
signal peptide region, 125, 127, 129
transmembrane anchor domain, 137, 138
Leukemia cells, carbohydrate and, 74
Leukocytes
carbohydrate and, 373, 374
Dictyostelium and, 372

Leukodystrophy, Dictyostelium and, 372

Leupeptin
carbohydrate and, 74
Dictyostelium and, 370, 380
endocytosis and, 403–405, 407–412, 414
biochemical characterization, 418–422
lysosomal enzymes and, 465, 475–477, 495
endocytosis, 481, 484–486, 489
lysosomelike vacuole in yeast and, 349
mannosidases and, 230
secretory vesicles and, 530, 543
viral glycoproteins and, 116

Ligandin, carbohydrate and, 88, 89

Lipids
carbohydrate and
inhibitors, 79
mutations, 67, 68
oligosaccharide biosynthesis, 56, 59
tunicamycin, 71, 73
Dictyostelium and, 364
endocytosis and, 406, 411
exocytosis and, 442
Golgi membranes and, 290–293, 295–299
lipoproteins and, 588, 590
lysosomal enzymes and, 467, 473
mannosidases and, 209, 216
mitochondrial proteins and, 687, 696, 707
mycology and, 790, 791
posttranslational modification and, 163
endoplasmic reticulum, 164, 171, 172, 174, 175, 178, 179
Golgi apparatus, 185
secretion, gram negative bacteria and, 611, 627, 633, 635, 641
secretory vesicles and, 531, 545, 547–550
translocation and, 4, 5
mechanism, 14, 15
membrane assembly, 22, 25–28, 30, 31, 34
viral glycoproteins and
cytoplasmic domain, 135
features, 110
polarized cells, 114
transmembrane anchor domain, 135, 139

Lipopoly saccharides, secretion, gram
negative bacteria and, 634, 635, 640

Lipoproteins, 587, 588, 601
in bacteria, 590, 592
biosynthesis, 589–591
carbohydrate and, 55
endocytosis and, 402
lsp gene, 596, 597
mutation, 598–600
secretion, 600, 601
secretion, gram negative bacteria and, 612, 619, 624
signal peptidases, 592–595
structure, 588, 589
viral glycoproteins and, 116

Liver
endocytosis and, 409, 419
Golgi membranes and, 292, 293
methods, 294, 295
orientation, 296–299
transport, 301, 302, 305
lysosomal enzymes and
endocytosis, 482, 486
intracellular pathway, 492, 493, 495, 496, 498
junction of pathways, 506
receptors, 477, 478, 480
recognition marker, 465, 467, 468, 470
mannosidases and, 211, 232
endoplasmic reticulum, 212, 214, 216, 220
mannosidase I, 223, 225, 226
mannosidase II, 227
mitochondrial proteins and, 681, 694, 704, 706, 709
posttranslational modification and, 172, 179

Localization
carbohydrate and
lectins, 83, 87, 89
oligosaccharide biosynthesis, 58
tunicamycin, 71, 75
Dictyostelium and, 364, 365, 389, 390
intracellular transport pathways, 381, 383, 384, 386, 387
life history, 369–372, 374, 375
secretion, 377, 378, 380
endocytosis and, 404, 409, 410, 425, 431
endoplasmic reticulum proteins and, 244,
245, 247, 274, 276, 278–280
cloning, 264
ERp99, 257, 258, 262
signals, 253–255
exocytosis and, 435, 444, 446
extracellular matrixlike glycoproteins and, 564
Golgi membranes and, 293, 294, 299
lipoproteins and, 587, 593, 594
lysosomal enzymes and, 470, 482, 492, 496, 497, 504
lysosomelike vacuole in yeast and, 318, 347, 352, 353
biosynthesis, 320, 324, 325, 328
sorting, 333–339, 344
mannosidases and, 210, 217, 233
mannosidase I, 222, 223
mannosidase II, 228, 230
mitochondrial protein import and, 656–658, 671
mutation isolation, 662–668
mitochondrial proteins and, 680, 689, 706, 707, 710, 713
mycology and, 787, 792
nuclear transport and, see Nuclear transport
posttranslational modification and, 160, 170, 180, 182, 187–189
secretion, gram negative bacteria and, 620, 626, 627, 630, 632, 634
secretory vesicles and, 542, 543
translocation and, 3, 33, 37
viral glycoproteins and cytoplasmic domain, 132
genetic approach, 122
probes, 113
signal peptide region, 130
transmembrane anchor domain, 140
Low density lipoprotein carbohydrate and, 90
mutations, 68, 69
tunicamycin, 73, 74
endocytosis and, 402–406, 408, 413, 431
Lucifer yellow, lysosomelike vacuole in yeast and, 346, 347, 350
Lung carbohydrate and, 81
extracellular matrixlike glycoproteins and, 567
Golgi membranes and, 297
Lymphoblasts, endoplasmic reticulum proteins and, 256
Lymphocytes endoplasmic reticulum proteins and, 256
extracellular matrixlike glycoproteins and, 565, 569, 574
mannosidases and, 230
Lymphoid cells, extracellular matrixlike glycoproteins and, 569
Lymphokines, extracellular matrixlike glycoproteins and, 564, 565, 575, 578
Lymphoma cells carbohydrate and inhibitors, 79
intracellular transport signals, 54
mutations, 66–68
oligosaccharide biosynthesis, 58
lysosomal enzymes and, 467
Lysine carbohydrate and, 70
lipoproteins and, 588
mycology and, 777, 778
nuclear transport and, 751, 753, 756
viral glycoproteins and, 137, 139, 140, 143
Lysis extracellular matrixlike glycoproteins and, 564, 565, 573, 577
Golgi membranes and, 300, 301
secretion, gram negative bacteria and, 608–610, 640, 641
early stages, 619
later stages, 623–625, 628, 629, 632
Lysolipids, secretion, gram negative bacteria and, 624, 625
Lysosomal enzymes, 464, 465
Dictyostelium and, see Dictyostelium, lysosomal enzymes in endocytosis
disruption, 483–490
pathway, 481–483
intracellular pathway
biosynthesis, 490, 491
disruption, 499–504
secretory, 491–499
junction of pathways components, 504–507
disruption, 507, 508
receptors binding-deficient cells, 478, 479
cation-dependent, 480, 481
cation-independent, 476–478
Lysosomal enzymes (cont.)
recognition marker
biosynthesis, 467–472
mannose 6-phosphate, 465–467

Lysosomal hydrolases
endocytosis and, 406, 425
lysosomal enzymes and, 464, 466, 478, 479, 503
secretory vesicles and, 536, 542

Lysosomelike vacuole in yeast, see Yeast,
lysosomelike vacuole in

Lysosomes
carbohydrate and, 51, 90
inhibitors, 77, 78
lectins, 82, 83, 88, 89
mutations, 67, 68
oligosaccharide biosynthesis, 58, 60, 61
solubility, 65
tunicamycin, 71
disaccharide biosynthesis, 403, 405–414, 431
biochemical characterization, 418, 424, 425, 427, 428
endoplasmic reticulum proteins and, 244, 248
Golgi membranes and, 290–292, 302
mannosidases and, 212, 214, 225, 227, 230, 232
posttranslational modification and, 181
secretory vesicles and, 533, 534, 536, 542, 545, 552
translocation and, 4
viral glycoproteins and, 116, 118, 138–140

M

α-Macroglobulin, endocytosis and, 403, 406, 408, 425
α2-Macroglobulin
carbohydrate and, 72, 79
mannosidases and, 230

Macrophages
carbohydrate and, 75, 82, 83, 88
Dictyostelium and, 377–379
extracellular matrixlike glycoproteins and,
see Extracellular matrixlike
glycoproteins
lysosomal enzymes and
endocytosis, 483, 485
intracellular pathway, 490, 491, 501, 502
receptors, 479, 480
recognition marker, 471, 473

Madin-Darby canine kidney cells
carbohydrate and, 75, 76
secretory vesicles and, 529
viral glycoproteins and, 117, 144

Magnesium
lysosomal enzymes and, 476, 480
secretion, gram negative bacteria and, 625

Major histocompatibility antigens, carbohydrate and, 61

Major histocompatibility molecules,
extracellular matrixlike glycoproteins and, 573, 577

Maltose
lipoproteins and, 595
lysosomelike vacuole in yeast and, 321
mycology and, 774

Mammary gland, Golgi membranes and,
299, 300

Mannoprotein, posttranslational modification and, 184, 185

Mannose
carbohydrate and
inhibitors, 77–80
lectins, 82, 87, 88
mutations, 67, 68, 70
oligosaccharide biosynthesis, 55–57, 59–61
tunicamycin, 72, 74, 75

Dictyostelium and, 364, 369, 371, 382, 386
endoplasmic reticulum proteins and, 247, 260, 261
exocytosis and, 435, 437, 442, 444
Golgi membranes and, 291
lysosomal enzymes and, 479, 494, 507
recognition marker, 467, 468, 470, 472–476
lysosomelike vacuole in yeast and, 322, 331, 337
mannosidases and, 211, 233
endoplasmic reticulum, 215–218, 220, 221
mannosidase I, 222–225
mannosidase II, 226, 227, 230
mycology and, 775, 785
posttranslational modification and
endoplasmic reticulum, 171, 172, 174–177, 179, 183
Golgi apparatus, 184–189
viral glycoproteins and, 112, 119

Mannose 6-phosphate
Index

carbohydrate and, 51, 90
lectins, 82, 83, 86, 87, 89
mutations, 68, 69
oligosaccharide biosynthesis, 60, 61
solubility, 65
tunicamycin, 71

*Dictyostelium* and, 364, 369, 370, 375
endocytosis and, 407, 427
endoplasmic reticulum proteins and, 244
Golgi membranes and, 292, 294
lysosomal enzymes and
endocytosis, 481–489
intracellular pathway, 491, 493–495, 497–504
junction of pathways, 505–508
receptors, 476–480
recognition marker, 466, 467, 470, 473–475
lysosomelike vacuole in yeast and, 331, 337, 341, 343
mannosidases and, 222
posttranslational modification and, 181
secretory vesicles and, 536, 542, 543

*Mannose 6-sulfate, Dictyostelium* and, 382, 388

Mannosidase I, glycoprotein processing and, 221–223
inhibitors, 225, 226
purification, 223–225

Mannosidase II, glycoprotein processing and
biosynthesis, 230–232
inhibitors, 228–230
purification, 227, 228

Mannosidases
*Dictyostelium* and, 371, 389
endocytosis and, 409
exoxytosynthesis and, 440
glycoprotein processing and, 233
asparagine-linked oligosaccharides, 209–212
endoplasmic reticulum, 212–218
oligosaccharide processing, 218–221
Golgi membranes and, 291, 293
lysosomal enzymes and, 492, 493
lysosomelike vacuole in yeast and, 327
posttranslational modification and, 176, 191

α-Mannosidases
carbohydrate and
inhibitors, 76, 78, 80, 81

intracellular transport signals, 54
oligosaccharide biosynthesis, 56, 59

*Dictyostelium* and
intracellular transport pathways, 383–386
life history, 365, 367–369, 372–375
regulation, 387, 388
secretion, 376–380
endoplasmic reticulum proteins and, 248, 261
exocytosis and, 435
lysosomal enzymes and, 466, 474, 476, 479, 493, 497, 502
lysosomelike vacuole in yeast and, 319, 320, 336, 343

Mannosyl
mannosidases and, 211, 212, 216, 221, 224, 227
posttranslational modification and, 179, 186

Mannosylphosphotransferase, lysosomelike vacuole in yeast and, 323, 331

Mannosyltransferases, posttranslational modification and, 179, 186, 191

Mast cells, *Dictyostelium* and, 377

Membrane assembly, translocation and,
22–25, 37
polytopic ITMPs, 30–34
stop transfer sequences, 25–29
topogenic sequences, 34–37

Membrane insertion, viral glycoproteins and, see Viral glycoproteins, membrane insertion and

Methionine
carbohydrate and, 53
*Dictyostelium* and, 372
endocytosis and, 422
dendoplasmic reticulum proteins and, 259
extracellular matrixlike glycoproteins and, 571
lysosomal enzymes and, 479, 491
mitochondrial protein import and, 669

*N-Methyldeoxynojirimycin, carbohydrate* and, 76, 78

α-Methylmannoside, lysosomal enzymes and, 468, 469

Methyl-p-nitrophenyltriazene, mannosidase and, 226

Microfilaments
endocytosis and, 412
secretory vesicles and, 530
Microsomes
carbohydrate and, 87, 88
*Dictyostelium* and, 367, 368
endoplasmic reticulum proteins and, 248,
258, 262, 277
lysosomal enzymes and, 467, 490
lysosomal-like vacuole in yeast and, 321
mannosidases and, 215, 225, 232, 233
mitochondrial proteins and, 681
posttranslational modification and, 161,
162, 172

Microtubules
endocytosis and, 412, 416, 419, 425, 429
secretory vesicles and, 528–530, 550

Mitochondria
*Dictyostelium* and, 364, 389
endoplasmic reticulum proteins and, 244,
248
Golgi membranes and, 293, 295, 297, 306
lysosomal-like vacuole in yeast and, 318,
338
mitochondrial protein import and, 657,
658, 669–672
mutation isolation, 659–663, 665–667,
679
nuclear transport and, 753
posttranslational modification and, 160
secretion, gram negative bacteria and, 620
secretory vesicles and, 533, 534
translocation and, 3, 5, 8
mitochondrial protein import, 655, 669–672
biochemistry, 656
mutation isolation, 659
classical approaches, 660–662
molecular approaches, 662–669
signal sequences, 656–659, 671, 672
mitochondrial proteins, 677, 678, 728, 729
assembly, 718–721
cytosolic cofactors, 703–705
energy requirements, 699–703
evolution, 726–728
gene products, 716–718
import pathway models, 721–725
precursor proteins
properties, 678–682
transport, 682–684
proteolysis, 709–716
receptors, 695–699
sequences, 684
fusion, 685–688
heterologous import, 694, 695
prepiece, 688–692
stop transport model, 692–694
translocation, 705–709
mitosis, nuclear transport and, 754
Monensin
*Dictyostelium* and, 375
dendocytosis and, 412
exocytosis and, 439
Golgi membranes and, 297, 302
lysosomal enzymes and, 504
Monoclonal antibodies
*Dictyostelium* and, 365, 369, 382, 389
dendocytosis and, 411
extracellular matrix-like glycoproteins and,
569
secretory vesicles and, 552
Monocytes, extracellular matrix-like
glycoproteins and, 564, 576
biosynthesis, 567, 569, 570, 572, 574
Morphology
carbohydrate and, 88
*Dictyostelium* and, 371, 387
dendocytosis and, 412, 414, 418, 422, 424,
425, 427–429
exocytosis and, 434, 435, 441–444
Golgi membranes and, 293
lysosomal enzymes and, 500
lysosomal-like vacuole in yeast and, 339,
347, 349
mitochondrial proteins and, 707
secretory vesicles and, 521, 525, 529
Mouse mammary tumor virus, carbohydrate
and, 74
mRNA
*Dictyostelium* and, 367, 368, 387
endoplasmic reticulum proteins and, 260,
262, 264–269, 274
extracellular matrix-like glycoproteins and,
571
lipoproteins and, 597
lysosomal enzymes and, 490
mitochondrial protein import and, 664
mitochondrial proteins and, 683, 684,
687, 719
mycology and, 775, 780, 789
posttranslational modification and, 167,
193
translocation and, 4, 17
viral glycoproteins and, 122
Index

Mucin, carbohydrate and, 88
Mucolipidosis, lysosomal enzymes and, 468, 469, 471, 493
Mucopolysaccharides, lysosomal enzymes and, 464
Mucor miehei, mycology and, 782
Multivesicular bodies, lysosomal enzymes and, 506, 507
Mumps, carbohydrate and, 74
Murine leukemia virus carbohydrate and, 53, 55, 61
viral glycoproteins and, 144, 145
Mutagenesis
carbohydrate and, 52, 65, 66
endocytosis and, 429, 431, 446
endoplasmic reticulum proteins and, 244, 280
exocytosis and, 435–437
lipoproteins and, 598
lysosomal vacuole in yeast and, 333, 335
mitochondrial protein import and, 665, 667–669
mycology and, 781
posttranslational modification and, 186
secretory vesicles and, 542
viral glycoproteins and, 133
Mutation
carbohydrate and, 52
glycosylation, 66–70
inhibitors, 79
intracellular transport signals, 54, 55
oligosaccharide biosynthesis, 58, 59
site-directed mutagenesis, 66
Dictyostelium and, 388–390
life history, 375, 379
secretion, 381–386
endocytosis and, 404, 405, 412–414, 431
endoplasmic reticulum proteins and, 246, 252, 254, 278, 279
exocytosis and, 436, 440, 441, 445
Golgi membranes and, 309
lipoproteins and, 588–590, 594–596, 598–601
lysosomal enzymes and, 464, 465, 478
endocytosis, 485–490
intracellular pathway, 501–504
junction of pathways, 505–508
recognition marker, 469, 473–475
lysosomal vacuole in yeast and, 353
bioynthesis, 321, 325, 327, 330
endocytosis, 346, 348–350, 352
sorting, 330, 331, 333, 335–345
mannosidases and, 215, 217, 219
mitochondrial protein import and, 656, 657, 669–672
isolation, 659–669
mitochondrial proteins and, 689, 695, 697, 713, 719, 720
mycology and, 776, 781, 782, 787–790, 792
nuclear transport and, 764
mechanisms, 751, 752
yeast, 753, 755–757, 763
posttranslational modification and, 162, 163, 198
endoplasmic reticulum, 164, 166–171, 174–183
Golgi apparatus, 184, 185, 188, 189, 191, 194, 195, 197
outer chain biosynthesis, 185–187
secretion, gram negative bacteria and, 637, 640, 641
early stages, 618, 619
later stages, 620–623, 625, 628, 630, 631
secretory vesicles and, 527, 536
translocation and, 9
viral glycoproteins and, see Viral glycoproteins, membrane insertion and
Mycology, secretion research and, 771, 772, 796, 797
enzymes
amyloses, 772–774
cellulases, 783–788
glucanases, 791
glucoamylases, 774–778
invertases, 789, 790
lactases, 788, 789
lipases, 790, 791
pectic, 791
prochymosin gene, 779–782
proteases, 778, 782, 783
Saccharomyces cerevisiae, 792–796
Myeloid cells, extracellular matrixlike glycoproteins and, 569
Myeloma cells
Dictyostelium and, 375
endocytosis and, 409, 424
mannosidases and, 220
Natural killer cells, extracellular matrixlike glycoproteins and, 565, 568, 573, 574, 577, 578

Neuraminidase
  *Dictyostelium* and, 372
  lysosomal enzymes and, 493, 507
  mannosidases and, 226
  viral glycoproteins and, 144

Neurites, extracellular matrixlike glycoproteins and, 571

Neuroblastoma cells, carbohydrate and, 74

Neurotoxin, carbohydrate and, 74

Neurotransmitters, secretory vesicles and, 525, 528, 530

Neutrophils
  *Dictyostelium* and, 377
  extracellular matrixlike glycoproteins and, 576

Nigericin, mitochondrial proteins and, 700, 701


Nuclear envelope, exocytosis and, 442, 443

Nuclear magnetic resonance spectroscopy, mannosidases and, 220

Nuclear transport, 747, 764
  mechanisms, 749–753
  nuclear pore, 748, 749
  in yeast, 753–755
  α2 protein, 757–761
  DNA binding, 762, 763
  *GAL4* gene, 755–757
  ribosomal proteins, 761, 762

Nucleic acid, translocation and, 13

Nucleocapsids
  carbohydrate and, 74
  viral glycoproteins and, 114, 115

Nucleoplasmin, nuclear transport and, 748–751, 760

Nucleoside triphosphates
  mitochondrial proteins and, 703, 710
  translocation and, 38
  mechanism, 17, 18
  membrane assembly, 22

Nucleosides
  carbohydrate and, 70
  exocytosis and, 439
  Golgi membranes and

Nucleotides
  carbohydrate and, 56
  endoplasmic reticulum proteins and, 265, 269, 274
  exocytosis and, 439
  Golgi membranes and, 291, 312
    orientation, 298–300
    transport, 301, 302, 305–311
  lipoproteins and, 592, 597
  mycology and, 774, 775, 778, 785, 789, 796
  posttranslational modification and, 172, 179
  translocation and, 10
  viral glycoproteins and, 120–123

Oligomerization, *Dictyostelium* and, 380

Oligonucleotides
  lipoproteins and, 598
  translocation and, 20
  viral glycoproteins and, 130, 133, 137

Oligosaccharides
  carbohydrate and, 51, 90
    biosynthesis, 55–62
    inhibitors, 76, 77, 79–81
    intracellular transport signals, 54
    lectins, 82, 86–89
    mutations, 67–70
    site-directed mutagenesis, 65, 66
    solubility, 62, 64
    tunicamycin, 70, 73–75
  *Dictyostelium* and, 364, 388, 390
    intracellular transport pathways, 382, 386
    life history, 369–371
    secretion, 380
    endoplasmic reticulum proteins and, 247, 265, 276
    ERp99, 260–262, 264
    signals, 248–251, 253
    exocytosis and, 433, 437–442, 444
    extracellular matrixlike glycoproteins and, 566, 571, 578

Oigitonin, carbohydrate and, 83
Index

Golgi membranes and, 291, 293
lyosomelike enzymes and
  intracellular pathway, 490, 492-495
receptors, 477, 478, 481
recognition marker, 466-468, 470-476
lyosomelike vacuole in yeast and, 322-325, 331, 344
mannosidases and
  asparagine-linked, 209-212
  brain microsomal α-mannosidase, 232, 233
endoplasmic reticulum, 215-221
mannosidase I, 222, 224, 225
mannosidase II, 226-228, 230, 231
mycology and, 779-781, 784, 786, 788, 792
posttranslational modification and, 162, 163
endoplasmic reticulum, 168, 171-173, 175-181, 183
Golgi apparatus, 184-191
translocation and, 5, 20
viral glycoproteins and, 119, 120, 133, 137
Oligosaccharyltransferase, endoplasmic reticulum proteins and, 262, 264
Oncogenes, translocation and, 35
Opsin, carbohydrate and, 75
Opsonin, extracellular matrixlike glycoproteins and, 574-576, 578
Ornithine carbamoyltransferase, endoplasmic reticulum proteins and, 262, 264
Pancreas
  carbohydrate and, 53, 63
  Dictyostelium and, 367, 368, 374
  lysosomal enzymes and, 497
  mannosidases and, 220
  mycology and, 774
  posttranslational modification and, 161
  secretory vesicles and, 527, 537, 543, 545
  translocation and
    mechanism, 21
    membrane assembly, 33
    targeting, 10, 13
  viral glycoproteins and, 128
Papain, endoplasmic reticulum proteins and, 262, 263, 277
Paramyxovirus, viral glycoproteins and, 115, 116, 144
Pectic enzymes, mycology and, 791
Penicillin, secretion, gram negative bacteria and, 632
Penicillinase
  lipoproteins and, 588-590, 592, 598, 600
  secretion, gram negative bacteria and, 612, 613
  viral glycoproteins and, 128
Pepsinogen, lysosomal enzymes in yeast and, 329
Peptide, see also Signal peptide
  lysosomal enzymes in yeast and, 338
  mitochondrial proteins and, 678, 723, 725
  energy requirements, 701, 702
  sequences, 685, 694
  translocation, 710, 711, 713, 715, 716
Peptides, see also Signal peptide
  carbohydrate and, 61, 73, 87, 89
  exocytosis and, 435
  extracellular matrixlike glycoproteins and, 569
  lysosomal enzymes and, 470
Peptides (cont.)
mitochondrial proteins and, 679, 689, 695
mycology and, 775, 776, 786, 793, 796, 797
nuclear transport and, 750, 752, 753, 755
posttranslational modification and, 160-162
endoplasmic reticulum, 164, 168, 172, 176
Golgi apparatus, 192-195
secretion, gram negative bacteria and, 620, 630, 631, 639
secretory vesicles and, 525, 527, 528, 531, 532, 542, 543
translocation and, 4, 34
viral glycoproteins and
features, 111
genetic approach, 122
probes, 112
signal peptide region, 123
transmembrane anchor domain, 135, 136, 141, 142
Periplasm
lipoproteins and, 588, 593, 594, 600
lysosomelike vacuole in yeast and, 331, 336, 338
mitochondrial proteins and, 728
mycology and, 787, 789, 790, 792
posttranslational modification and, 186, 188, 191-197
secretion, gram negative bacteria and, 608, 635, 638
eyearly stages, 611, 613-615
later stages, 620, 623, 624, 626, 633
Permeability
Golgi membranes and, 301, 302
secretion, gram negative bacteria and, 625
Peroxisomes, endoplasmic reticulum
proteins and, 248
pH
carbohydrate and, 62
Dictyostelium and, 373, 374, 384
endocytosis and, 408, 412, 420, 421, 427
exocytosis and, 445
Golgi membranes and, 310
lysosomal enzymes and, 468, 505
dendocytosis, 483, 485, 488, 489
intracellular pathway, 500, 504
receptors, 476, 477
lysosomelike vacuole in yeast and, 328, 329, 342, 344, 347
mannosidases and, 215, 227, 232
mitochondrial proteins and, 697, 700, 701, 709
mycology and, 778, 783, 788
secretory vesicles and, 527, 528, 531, 543, 551, 552, 554
translocation and, 21
viral glycoproteins and, 116, 117
Phagocytosis
Dictyostelium and, 365, 388, 390
endocytosis and, 401
extracellular matrixlike glycoproteins and, 567, 574-576, 579
Phenothiazines, endocytosis and, 411
Phenotype
carbohydrate and, 69
Dictyostelium and, 384
endocytosis and, 404, 405
exocytosis and, 433
extracellular matrixlike glycoproteins and, 564, 565
lysosomal enzymes and, 470, 471, 488
lysosomelike vacuole in yeast and, 349, 352, 353
biosynthesis, 327
sorting, 332, 340, 342, 343
mitochondrial protein import and, 659, 660, 663, 664, 666, 669, 670
nuclear transport and, 751, 759
posttranslational modification and, 163, 167, 195
viral glycoproteins and, 146
genetic approach, 121
signal peptide region, 127
transmembrane anchor domain, 140, 142
Phenylalanine, viral glycoproteins and, 121
Pheromone
endocytosis and, 413
lysosomal enzymes and, 344, 348, 349
mycology and, 793
posttranslational modification and, 191, 192, 196
Phosphatases, Golgi membranes and, 294, 298
Phosphates
Dictyostelium and, 369, 371, 386, 387
Golgi membranes and, 294, 307
lysosomal enzymes and, 477, 480, 490
recognition marker, 465-467, 470-474, 476
lysosomal enzymes and, 322, 326, 328, 331
mannosidases and, 217, 219
mitochondrial proteins and, 681
phospholipase
lysosomal enzymes and, 465, 505
lysosomal enzymes and, 481, 483,
lysosomal enzymes and, 476, 478, 480,
lysosomal enzymes and, 477, 478, 480,
lysosomal enzymes and, 477, 478, 480,
lysosomal enzymes and, 322-326, 328, 331
mannosidases and, 217, 219
mitochondrial proteins and, 681
posttranslational modification and, 160, 181, 182, 186, 191
translocation and, 3
Phosphatidycholine, Golgi membranes and, 296
Phosphatidylglycerol, Golgi membranes and, 298
3′-Phosphoadenosine 5′-phosphosulfate, Golgi membranes and, 299, 300, 306, 310, 311
Phosphodiesterase, lysosomal enzymes and, 470, 471, 475, 492-494
Phospholipids
endocytosis and, 405
Golgi membranes and, 292, 295, 296
mannosidases and, 225
mitochondrial proteins and, 689
secretion, gram negative bacteria and, 620, 634, 636, 641
translocation and, 26
Phosphomannan
lysosomal enzymes and, 477, 478, 480, 481, 484
mannosidases and, 216
Phosphomannose
endocytosis and, 407, 413
posttranslational modification and, 186
Phosphomannosyl
Dictyostelium and, 364, 369, 370, 378, 380
endocytosis and, 407, 408
Phosphoprotein, endoplasmic reticulum proteins and, 278
Phosphoryl groups, posttranslational modification and, 181, 182, 186, 187
Phosphorylation
endocytosis and, 405, 410, 429–431
exocytosis and, 439, 444
lysosomal enzymes and, 476, 477
intracellular pathway, 490, 493–495, 501
lysosomal enzymes and, 322–326, 328, 331
mannosidases and, 217, 219
mitochondrial proteins and, 681
posttranslational modification and, 160, 181, 182, 186, 191
translocation and, 3
Phosphotransferase
lysosomal enzymes and, 467–469
lysosomal enzymes and, 337
Pinocytosis
Dictyostelium and, 365, 390
endocytosis and, 401
Pituitary
endocytosis and, 422
exocytosis and, 445
lysosomal enzymes and, 497
secretory vesicles and, 524, 527, 537, 538, 545
Placenta, lysosomal enzymes and, 465, 470, 498
Plasma
endoplasmic reticulum proteins and, 254
extracellular matrixlike glycoproteins and, 565, 566, 568, 576
lysosomal enzymes and, 471
secretory vesicles and, 523
Plasma membrane
endocytosis and, 58, 60, 82, 88
Dictyostelium and, 364, 370, 375, 380, 388, 390
endocytosis and, 405, 410
exocytosis and, 439, 444
lysosomal enzymes and, 476, 477
intracellular pathway, 490, 493–495, 501
lysosomal enzymes and, 467, 469, 471–476
Plasma membrane (cont.)
lysosomelike vacuole in yeast and, 331, 346
posttranslational modification and, 170, 183, 190, 197
secretory vesicles and, 521
formation, 532, 533, 536, 537, 545, 549, 552
structure, 524, 525, 527, 529–531
translocation and, 4, 23
viral glycoproteins and
cytoplasmic domain, 134, 135
endocytic pathway, 116
features, 110
genetic approach, 121
probes, 112, 114, 115
signal peptide region, 125
Plasmacytoma cells
carbohydrate and, 72
endoplasmic reticulum proteins and, 247, 265, 277
ERp99, 253, 254, 256, 259
mannosidases and, 220
Plasmids
carbohydrate and, 71
Dictyostelium and, 386
endoplasmic reticulum proteins and, 265
lipoproteins and, 592, 597
lysosomelike vacuole in yeast and, 332, 336
mitochondrial protein import and, 657, 666
mycology and, 774, 775, 777, 785–787, 791
posttranslational modification and, 188, 190, 193, 196
secretion, gram negative bacteria and,
627, 628, 638, 639
viral glycoproteins and, 127
Plasminogen, carbohydrate and, 72
Plasminogen activator, carbohydrate and,
63
Plasmolysis, secretion, gram negative bacteria and, 625
Platelets, secretory vesicles and, 532
Polarity
carbohydrate and, 75, 76
Dictyostelium and, 389
exocytosis and, 444
Golgi membranes and, 290, 293
lysosomelike vacuole in yeast and, 345
mannosidases and, 209, 210
mitochondrial proteins and, 689
secretory vesicles and, 524, 528
Polarization, viral glycoproteins and, 115
Polarized cells
endocytosis and, 409, 410
viral glycoproteins and, 144, 145, 147
Polyethylene glycol, carbohydrate and, 62
Polymerization, endocytosis and, 411
Polypeptides
carbohydrate and
mutations, 67, 69, 70
oligosaccharide biosynthesis, 55, 56, 61
site-directed mutagenesis, 66
solubility, 64
tunicamycin, 73–75
Dictyostelium and, 383, 386, 389
life history, 365–375
secretion, 376, 380
endocytosis and, 416, 420, 422, 425
endoplasmic reticulum proteins and, 248, 272
exocytosis and, 436, 437, 442, 443, 445
extracellular matrixlike glycoproteins and,
569, 570
Golg membranes and, 291
lysosomal enzymes and, 478
lysosomelike vacuole in yeast and, 348
biosynthesis, 319–321, 325–327
sorting, 333, 336, 337, 340
mannosidases and, 209, 230, 231
endoplasmic reticulum, 214, 217–221
mitochondrial protein import and, 655, 658, 670, 671
mitochondrial proteins and, 682–685, 701, 703, 706
mycology and, 775, 779, 783, 792–796
posttranslational modification and,
161–164
secretion, gram negative bacteria and,
609, 637–639
early stages, 611–613, 615, 619, 620
later stages, 623, 626, 629–634
secretory vesicles and, 542, 545
translocation and, 4, 5, 7, 38
mechanism, 17, 19–21
membrane assembly, 22, 25–28, 30, 33, 35, 37
targeting, 8, 10–12
viral glycoproteins and, 146, 147
cytoplasmic domain, 133–135
Index

features, 109-111
  genetic approach, 117-120
  polarized cells, 144
  probes, 112-115
  signal peptide region, 122, 125, 127, 129
  transmembrane anchor domain, 135-137, 139, 140, 142, 143
Polysaccharides, mycology and, 772, 774
Polysomes, translocation and, 4, 5, 38
  mechanism, 20
  membrane assembly, 22
  targeting, 10, 12
Porin, mitochondrial proteins and, 683, 701, 721
  receptors, 696-699
Posttranslational modification
  Dictyostelium and, 369-371, 388, 389
  exocytosis and, 435, 437
  extracellular matrixlike glycoproteins and, 572, 579
  lysosomal enzymes and, 477
  mitochondrial proteins and, 719
Posttranslational modification, protein secretion and, 159, 160, 198
  asparagine-linked carbohydrates, 171-175
  asparagine-linked oligosaccharides, 175-178
  core oligosaccharides, 180, 181
  cytoplasm
    endoplasmic reticulum, 160-162
    yeast mutation, 162, 163
  fatty acid, 182, 183
  Golgi apparatus, 183, 184
  oligosaccharides, 184-188
  proteolysis, 191-197
  sorting, 188-191
  mutation, 170, 171
  O-linked oligosaccharides, 178, 179
  phosphoryl groups, 181, 182
  signal sequence cleavage, 163-170
Potassium
  endocytosis and, 410, 411
  mitochondrial proteins and, 701
Precursor protein, mitochondrial proteins and, 678-684
Preprolactin, translocation and, 15
Preproteins
  endoplasmic reticulum proteins and, 244
  posttranslational modification and, 160
Preprotoxin, lysosomelike vacuole in yeast and, 321
Primaquine, lysosomal enzymes and, 484, 499
Prochymosin, mycology and, 779-782
Procollagen, carbohydrate and, 73
Proinsulin
  Dictyostelium and, 374
  secretory vesicles and, 527, 539
Prokaryotes, translocation and, 9
Prolactin
  endocytosis and, 405, 407, 425
  secretory vesicles and, 543
  translocation and, 15
Proline
  carbohydrate and, 56
  mitochondrial protein import and, 667
  nuclear transport and, 759, 762
  viral glycoproteins and, 129
Prolipoprotein, 588-590, 592, 594, 598-600
  viral glycoproteins and, 128
Prolyl hydroxylase, endoplasmic reticulum proteins and, 248, 251, 252, 261
Pronase, mannosidases and, 231
Proopiomelanocortin, carbohydrate and, 73
Propeptides, lysosomelike vacuole in yeast and, 321, 322, 327, 333, 336, 337
Proteases
  carbohydrate and, 52
    mutations, 69, 70
    solubility, 64, 65
    tunicamycin, 74
  Dictyostelium and, 373, 379
    endocytosis and, 403
    endoplasmic reticulum proteins and, 277
    extracellular matrixlike glycoproteins and, 564
  lipoproteins and, 595
    lysosomal enzymes and, 490, 497
    lysosomelike vacuole in yeast and, 318, 327-329, 348
    mannosidases and, 214, 215, 224, 231
    mitochondrial protein import and, 656, 660, 662, 665, 668-670
    mitochondrial proteins and, 683, 718, 721, 725, 729
    energy requirements, 702
    proteolysis, 709-711, 713, 715
    receptors, 695, 696
    translocation, 706
    mycology and, 778-783, 787, 791
    posttranslational modification and, 182, 189, 191, 194-198
Proteases (cont.)
secretion, gram negative bacteria and, 627, 628, 637, 638
translocation and, 5, 20, 21
Protein kinase C
endocytosis and, 429
secretory vesicles and, 532
Protein kinases, endocytosis and, 404
Proteinase K, mannosidases and, 231
Proteinases
Dictyostelium and, 371, 372, 374
lysosomal-like vacuole in yeast and
biosynthesis, 319–321, 324, 326–328, 330
sorting, 332, 333, 337, 339, 340, 344, 345
mycology and, 782
posttranslational modification and, 175, 188–190, 196
Proteoglycans
exocytosis and, 445
extracellular matrix-like glycoproteins and, 573, 574, 580
Golgi membranes and, 302
Proteolysis
carbohydrate and, 52
inhibitors, 77, 78, 81
oligosaccharide biosynthesis, 60
site-directed mutagenesis, 65
solubility, 65
tunicamycin, 73, 74
Dictyostelium and, 388, 390
life history, 365, 371–374
secretion, 378, 380
endocytosis and, 407
endoplasmic reticulum proteins and, 262
extracellular matrix-like glycoproteins and, 566
Golgi membranes and, 295
lipoproteins and, 593
lysosomal enzymes and, 491, 499, 501
lysosomal-like vacuole in yeast and, 322, 326–330, 344
mannosidases and, 214, 226, 231
mitochondrial protein import and, 656, 667
mitochondrial proteins and, 678, 709–716, 723, 729
assembly, 719
cytosolic cofactors, 705
energy requirements, 700–703
precursor proteins, 679, 681
sequences, 685, 687, 689, 693
translocation, 706
mycology and, 775, 778, 782, 783, 785, 788
nuclear transport and, 750
posttranslational modification and, 160, 169, 191–197
secretion, gram negative bacteria and, 612, 613, 627, 628, 634, 637
secretory vesicles and, 542
translocation and, 20
viral glycoproteins and, 122
Pseudomonas
lysosomal enzymes and, 486, 488
secretion, gram negative bacteria and, 632, 639
Pullulanase, secretion, gram negative bacteria and, 623, 626, 627, 632–636
Purification, translocation and, 4
mechanism, 21
targeting, 10–12
Puromycin
mitochondrial proteins and, 682
translocation and, 5, 21
Pyruvate kinase, nuclear transport and, 752
R
Radioactivity
Dictyostelium and, 365, 369
endoplasmic reticulum proteins and, 254
Golgi membranes and, 296, 300, 301
lysosomal enzymes and, 484, 489
intracellular pathway, 494, 495, 499, 504
recognition marker, 471, 474, 475
Receptor-mediated endocytosis, 402, 405–410
biochemical characterization, 414, 416, 418
Dictyostelium and, 378
lysosomal-like vacuole in yeast and, 348, 349
mutation, 412, 413
Retina, carbohydrate and, 75, 88, 89
Retinal rod outer segment membranes, carbohydrate and, 75
Retinol, Dictyostelium and, 379
Retrovirus, viral glycoproteins and cytoplasmic domain, 133, 135
Index

features, 110
polarized cells, 144
probes, 115
Rhabdovirus, viral glycoproteins and, 115, 116, 144
*Rhizopus*, mycology and, 772, 776–778, 790

Ribonucleases
carbohydrate and, 62, 63
lysosomal vacuole in yeast and, 326
mannosidases and, 220

Ribophorins
endoplasmic reticulum proteins and, 247, 251, 253, 261
exocytosis and, 442
mannosidases and, 217
translocation and, 20, 21

Ribosomes
carbohydrate and, 52
exocytosis and, 442
Golgi membranes and, 295
lysosomal vacuole in yeast and, 346
mitochondrial proteins and, 682, 684, 716, 717, 725
nuclear transport and, 748, 761, 762
posttranslational modification and, 161, 180
secretory vesicles and, 533, 536
translocation and, 4, 38
mechanism, 17–20, 22
membrane assembly, 36, 37
receptor, 20, 21
targeting, 11–13

Ricin
lysosomal enzymes and, 478, 486, 487
mannosidases and, 224, 227

RNA
depolymerase I and, 252, 254, 257
viral glycoproteins and, 112

RNA carboxypeptidase
viral glycoproteins and, 110

RNA endonuclease
viral glycoproteins and, 112

RNAse
viral glycoproteins and, 112

Rotavirus
endoplasmic reticulum proteins and, 251, 252, 261, 274
mannosidases and, 217, 218
viral glycoproteins and, 114, 140, 143, 146

Rough endoplasmic reticulum
carbohydrate and, 89, 90
inhibitors, 76–80
intracellular transport signals, 52–54
lectins, 82, 87
mutations, 70
oligosaccharide biosynthesis, 56–58, 60
solubility, 62
tunicamycin, 71–75
exocytosis and, 432–436, 439, 441–443
endoplasmic reticulum proteins and, 253, 254, 257, 261, 262, 279
Golgi membranes and, 290, 291, 294, 295, 302, 305, 307
mannosidases and, 209–212
posttranslational modification and, 161–163
secretory vesicles and, 533, 539, 542
viral glycoproteins and
cytoplasmic domain, 133, 134
features, 112
genetic approach, 117, 120
probes, 112–114
signal peptide region, 123, 125, 126, 128, 130
transmembrane anchor domain, 136, 137, 141–143

Rous sarcoma virus
exocytosis and, 440, 441, 443
mannosidases and, 225
viral glycoproteins and
cytoplasmic domain, 132, 133, 135
features, 110–112
genetic approach, 118, 122
probes, 112
signal peptide region, 122, 123, 125, 126, 129, 130
transmembrane anchor domain, 136, 137, 140, 142

S

Saccharomyces cerevisiae
depolymerase I and, 252, 254, 257
mitochondrial protein import and, 655, 666
mitochondrial proteins and, 719
mycology and, 772, 792–796
enzymes, 774–782, 785–790
Saccharomyces cerevisiae (cont.)
nuclear transport and, 747, 753–755, 764
α2 protein, 757–761
DNA binding, 762, 763
GAL4 gene, 755–757
ribosomal proteins, 761, 762
posttranslational modification and, 179, 185
Saponin
  carbohydrate and, 86
  lysosomal enzymes and, 494
  mannosidases and, 231
Saxitoxin, carbohydrate and, 74
Secretion
  mycology and, see Mycology, secretion research and
  posttranslational modification and, see Posttranslational modification
Secretion, gram negative bacteria and, 607, 608, 641, 642
  activators, 636, 637
  early stages, 611
    signal peptide route, 611–618
    signal sequences, 618–620
    filamentous bacteriophages, 640, 641
  inhibitors, 636, 638
  later stages
    identification, 620–623
    mode of action, 623–628
    targeting signals, 628–634
  lysis, 609, 610
  membrane appendages, 638–640
  vesicles, 628–634–636
Secretory vesicles, 521–524, 554, 555
  formation, 532, 533
    Golgi, 545–552
    molecular sorting, 533–536
    recycling, 552–555
    sorting in storage cells, 536–545
  structure, 524
    composition, 527–532
    types, 524–527
Sendai virus, viral glycoproteins and, 116
Sepharose
  carbohydrate and, 87
  mannosidases and, 225
Serine
  carbohydrate and, 64, 69
    Dictyostelium and, 370, 373, 374
  endocytosis and, 429, 431
  Golgi membranes and, 291
  lipoproteins and, 599
  lysosomal enzymes and, 477
  mannosidases and, 231
  mitochondrial proteins and, 710
  mycology and, 775–777, 786
  posttranslational modification and, 178, 179, 197
  viral glycoproteins and, 121, 127
Serine proteases, secretion, gram negative bacteria and, 618, 628
Serotonin
  mannosidases and, 224
  secretory vesicles and, 532
Sialic acid
  carbohydrate and
    oligosaccharide biosynthesis, 55, 57, 58, 61
    solubility, 62
  endocytosis and, 409
  exocytosis and, 444
  Golgi membranes and, 299, 307
  mannosidases and, 210, 211
  posttranslational modification and, 188
  viral glycoproteins and, 120
Sialylation, Golgi membranes and, 294
Sialyltransferases
  carbohydrate and, 56
    Dictyostelium and, 371
  Golgi membranes and, 293, 298, 299
  lysosomal enzymes and, 492, 493, 504
  mannosidases and, 222
Signal peptidase
  endoplasmic reticulum proteins and, 248
  lipoproteins and, 589, 590, 592–595, 600, 601
  lysosomialike vacuole in yeast and, 322
  mycology and, 776, 777, 792
  posttranslational modification and, 160, 164, 165, 167–179, 198
  secretion, gram negative bacteria and, 611, 640
  secretory vesicles and, 542
  translocation and, 5, 7
    mechanism, 20–22
    membrane assembly, 34
    targeting, 9
Signal peptidase cleavage, viral glycoproteins and, 125–132
Signal peptide
  endoplasmic reticulum proteins and, 251, 262, 272, 276, 277
Index

exocytosis and, 433, 435
lipoproteins and, 590, 592, 593, 598, 599, 601
mycology and, 774, 777, 785, 786, 789, 792
posttranslational modification and, 164
secretion, gram negative bacteria and, 640
early stages, 611-618
later stages, 621, 626, 630, 632, 633
secretory vesicles and, 536
Signal peptide cleavage, endoplasmic reticulum proteins and, 272
Signal peptide region, viral glycoproteins and, 122, 123, 146
deletion, 123-125
mutation, 126-132
Signal recognition particle
Dictyostelium and, 367
endoplasmic reticulum proteins and, 252, 277
exocytosis and, 442
nuclear transport and, 749
posttranslational modification and, 161-163
secretory vesicles and, 536
translocation and, 10, 38
elongation arrest, 11, 12
mechanism, 19, 21
membrane assembly, 22, 29-31, 33-37
receptor, 12, 13
sequence, 11
structure, 10, 11
targeting, 13, 14
viral glycoproteins and, 117
Signal sequence
mitochondrial protein import and, 656-659, 666-668, 670-672
mycology and enzymes, 774, 778, 780, 788, 790
Saccharomyces cerevisiae, 792, 793
translocation and, 8-10
Signal sequence cleavage
lysosomelike vacuole in yeast and, 322, 335
posttranslational modification and, 163-170
Sindbis virus
carbohydrate and, 78
exocytosis and, 441
lysosomal enzymes and, 487, 504
mannosidases and, 217, 219
posttranslational modification and, 180
Sodium channel, carbohydrate and, 74
Sodium dodecyl sulfate
  carbohydrate and, 63
endoplasmic reticulum proteins and, 254, 256, 259, 262, 278
extracellular matrixlike glycoproteins and,
  571, 572, 579
lysosomal enzymes and, 477, 479, 484
mannosidases and, 225
mitochondrial proteins and, 710
Sorting, endoplasmic reticulum proteins and, see Endoplasmic reticulum proteins, sorting of
Spectrin, secretory vesicles and, 530
Spheroplasts
lipoproteins and, 594
lysosomal enzymes and, 436
Sphingolipids, Golgi membranes and, 297
Sphingomyelin, Golgi membranes and, 292, 297, 298
Spleen
Dictyostelium and, 372
lysosomal enzymes and, 472
Starch, mycology and, 772, 774-777, 782
Steroids
endoplasmic reticulum proteins and, 278
Golgi membranes and, 291
Stop transfer sequences, translocation and,
  25, 26, 34-37
combination signal, 29, 31
hydrophobicity, 28
structure, 26-28
Stop transport region, mitochondrial proteins and, 692-694, 723
Streptomyces lysosuperificus, carbohydrate and, 70
Sucrose
  carbohydrate and, 77
Dictyostelium and, 372
endocytosis and, 422
endoplasmic reticulum proteins and, 247, 257, 264
Golgi membranes and, 292, 297, 298, 302, 304, 305
lysosomal enzymes and, 492, 493, 506
lysosomal vacuole in yeast and, 339
mycology and, 786
posttranslational modification and, 167
secretion, gram negative bacteria and, 625
Sugar
  carbohydrate and
    inhibitors, 76
    lectins, 86-89
Sugar, carbohydrate and (cont.)
mutations, 69
oligosaccharide biosynthesis, 56–58
solubility, 62
exocytosis and, 439, 442
Golgi membranes and, 291, 299, 300
transport, 301, 305–311
lysosomal enzymes and, 466, 467, 472, 480, 490
mannosidases and, 224, 226
mycology and, 772, 775, 783, 786, 797
posttranslational modification and, 172, 178, 179, 188
viral glycoproteins and, 130, 139
Sulfate
*Dictyostelium* and, 371, 386, 388
lysosomal enzymes and, 464
Sulfatides, Golgi membranes and, 291
Sulfation
*Dictyostelium* and, 369, 382, 386, 390
exocytosis and, 439, 445
extracellular matrixlike glycoproteins and, 572, 573
Golgi membranes and, 291, 294, 299, 302, 305
Sulfotransferase, *Dictyostelium* and, 370
Swainsonine
carbohydrate and, 76, 81
exocytosis and, 440
mannosidases and, 215, 226, 227, 229–233

T

T lymphocytes, carbohydrate and, 67
Targeting
*Dictyostelium* and, 364, 365, 369, 374, 384, 387, 389
extracellular matrixlike glycoproteins and, 578
mitochondrial protein import and, 657, 667
mitochondrial proteins and, 725–727
precursor proteins, 682, 684
sequences, 685–689, 693, 695
nuclear transport and, 764
mechanisms, 751–753
yeast, 755, 756, 759, 762
posttranslational modification and, 160–162, 191
secretion, gram negative bacteria and, 611, 628–634, 638
secretory vesicles and, 530, 532
translocation and, 8, 13, 14
signal recognition particle, 10–13
signal sequences, 8–10
Temperature
carbohydrate and, 62–64
decoytosis and, 410, 411, 413
exocytosis and, 434–437, 440, 441, 443
lipoproteins and, 594
lysosomal enzymes and, 486, 488–490, 503, 504, 507
lysosomalike vacuole in yeast and, 330, 343, 344, 346–348
mitochondrial protein import and, 660, 661, 664–666, 669
mitochondrial proteins and, 706, 713
mycology and, 783, 787
posttranslational modification and, 163, 170, 175, 180, 198
viral glycoproteins and, 115, 119–121
*Tetrahymena*, *Dictyostelium* and, 377
Tetrapeptides, carbohydrate and, 64
Thermodynamics, translocation and, 5, 7, 25
Thermotolerance, lysosomalike vacuole in yeast and, 339
Thiamin pyrophosphatase, Golgi membranes and, 294, 298
Thioglycolate broth, extracellular matrixlike glycoproteins and, 565, 567, 568, 571, 575, 577
Threonine
carbohydrate and, 64, 69
*Dictyostelium* and, 378
decoytosis and, 429, 431
Golgi membranes and, 291
mycology and, 775–778, 786
nuclear transport and, 751, 753
posttranslational modification and, 178, 179
Thrombospondin, extracellular matrixlike glycoproteins and, 564, 570, 579, 580
Thy-1 antigen, carbohydrate and, 54, 66, 69, 90
Thyroglobulin
carbohydrate and, 73, 81
lysosomal enzymes and, 467, 468
mannosidases and, 217
Thyroid
carbohydrate and, 60, 81
mannosidases and, 217
Thyroxine-binding globulin, carbohydrate and, 72
Togaviruses, viral glycoproteins and, 116
Tosyl-L-phenylalanylchloromethyl ketone, posttranslational modification and, 194, 196
Transcription
mitochondrial protein import and, 666, 670
mitochondrial proteins and, 687, 718, 719
mycology and, 775, 779, 780
nuclear transport and, 755, 758, 762, 763
Transcytosis
endocytosis and, 408–410
secretory vesicles and, 526
Transferrin
carbohydrate and inhibitors, 77, 79
intracellular transport signals, 53, 55
tunicamycin, 72, 74
endocytosis and, 403, 405, 408, 409, 413, 446
biochemical characterization, 418, 420, 425, 427–429, 431
lysosomal enzymes and, 487–489, 507
mannosidases and, 230
secretory vesicles and, 527, 536
Trans-Golgi reticulum, secretory vesicles and, 542
Translation
Dictyostelium and, 368
lysosomal like vacuole in yeast and, 344
mitochondrial protein import and, 659, 670
mycology and, 775
posttranslational modification and, 161, 162, 165
translocation and, 4, 38
mechanism, 14, 15, 17–19
targeting, 10, 11, 13
viral glycoproteins and, 146
Translocation
carbohydrate and, 73, 77
Dictyostelium and, 367, 369, 371
endocytosis and, 409
endoplasmic reticulum proteins and, 248, 279
exocytosis and, 433, 435, 442
lipoproteins and, 593
lysosomal like vacuole in yeast and, 320–322, 337, 353
mannosidases and, 217
mitochondrial protein import and, 665, 666
mitochondrial proteins and, 678, 684, 705–709, 717–719, 723, 725, 726
cytosolic cofactors, 703, 704
energy requirements, 699, 701–703
precursor proteins, 682
receptors, 696
sequences, 687
nuclear transport and, 749, 756
posttranslational modification and, 160, 162, 163, 172, 174, 188
secretion, gram negative bacteria and, 607, 611, 619, 638, 642
later stages, 620, 626, 628, 630, 631
secretory vesicles and, 542
viral glycoproteins and, 145, 146
probes, 112
signal peptide region, 123, 125, 127, 128, 130
transmembrane anchor domain, 136–143
Translocation, ER membrane and, 3–8, 37, 38
mechanism, 14
altered substrates, 15–19
components, 19–22
membrane assembly, 22–25, 37
polytropic ITMPs, 30–34
stop transfer sequences, 25–29
topogenic sequences, 34–37
targeting, 8, 13, 14
signal recognition particle, 10–13
signal sequences, 8–10
Transposon, secretion, gram negative bacteria and, 631
Transreticular Golgi, endocytosis and, 406
Trehalase, lysosomal like vacuole in yeast and, 326
Trichoderma, mycology and, 784–789
Trypanosoma cruzi, carbohydrate and, 59, 79
Trypsin
carbohydrate and, 86
Dictyostelium and, 368, 373
endoplasmic reticulum proteins and, 262, 263, 277
Golgi membranes and, 297, 298
lipoproteins and, 594
mannosidases and, 231
Trypsin (cont.)
mitochondrial proteins and, 697, 699, 704, 707
Trypsinogen, endocytosis and, 405
Tubulin
endocytosis and, 416
lysosomelike vacuole in yeast and, 318
Tumors
carbohydrate and, 55
endoplasmic reticulum proteins and, 262
extracellular matrixlike glycoproteins and, 565, 569, 577, 578
biosynthesis, 570, 572–574
mannosidases and, 230
secretory vesicles and, 538
Tunicamycin
carbohydrate and, 52, 70–76, 90
inhibitors, 76, 78, 79
solubility, 63, 65
Dictyostelium and, 384
exocytosis and, 439
Golgi membranes and, 299, 307, 308
lysosomal enzymes and, 497, 499
mycology and, 780, 788
posttranslational modification and, 174, 180, 182
viral glycoproteins and, 118, 123, 129, 144
Tyrosine
carbohydrate and, 63
endocytosis and, 405, 429, 431
endoplasmic reticulum proteins and, 278
posttranslational modification and, 193
viral glycoproteins and, 139, 140
Tyrosine kinase, endocytosis and, 405, 431
U
Ubiquinone, Golgi membranes and, 293
Ubiquitin, endocytosis and, 404
Urea, translocation and, 21
Uridine, Golgi membranes and, 302, 303, 307, 308
Uridine diphosphate, Golgi membranes and, 299, 305
Uridine 5'-monophosphate, Golgi membranes and, 299, 304–307, 311
Urokinase, carbohydrate and, 63
V
Valinomycin, Golgi membranes and, 310
Vesicular stomatitis virus
   carbohydrate and
   inhibitors, 78, 80
   oligosaccharide biosynthesis, 61
   site-directed mutagenesis, 65, 66
   solubility, 63, 64
Dictyostelium and, 379
endoplasmic reticulum proteins and, 245, 246, 274, 280
exocytosis and, 432–438, 441, 444
lysosomal enzymes and, 497, 500
mannosidases and, 217, 225
posttranslational modification and, 180
secretory vesicles and, 527, 542
translocation and, 25
viral glycoproteins and
   cytoplasmic domain, 132, 134
   features, 112
   genetic approach, 118–121
   polarized cells, 144
   transmembrane anchor domain, 136, 137, 140, 142
Viral envelope glycoproteins,
   posttranslational modification and, 182
Viral glycoproteins, mannosidases and, 219
Viral glycoproteins, membrane insertion and, 145–147
cytoplasmic domain, 132–135
endocytic pathway, 116, 117
features, 109–112
 genetic approach, 117–119
   classic, 119–122
   recombinant DNA, 122
   polarized cells, 144, 145
   probes, 112–115
   signal peptide region, 122–132
   transmembrane anchor domain, 135–143
Viroplastm, viral glycoproteins and, 114
Viruses
   carbohydrate and
   inhibitors, 53, 54
   solubility, 63, 64
   tunicamycin, 74
endocytosis and, 403, 412, 413, 420
exocytosis and, 432, 437, 440, 441
lysosomelike vacuole in yeast and, 347
viral glycoproteins and, 112, 135, 147
X
Xenopus
mitochondrial proteins and, 717
nuclear transport and, 748, 750, 753, 760, 762
translocation and, 33
Y

Yeast

- carbohydrate and oligosaccharide biosynthesis, 59
- solubility, 63, 65
- tunicamycin, 71, 73, 75

*Dictyostelium* and, 364, 380, 381, 386

endocytosis and, 413, 414

endoplasmic reticulum proteins and, 260, 280

exocytosis and, 432, 440, 443, 445

lysosomal enzymes and, 477

mannosidases and, 214-216

mitochondrial protein import and, 660, 664, 668, 669

mitochondrial proteins and, 723, 728

- assembly, 718-720
- energy requirements, 700, 703
- gene products, 716
- precursor proteins, 682
- proteolysis, 710, 711, 713
- receptors, 696-699
- sequences, 685-689, 694, 695

mycology and, 796

- enzymes, 772, 774-783, 785-791
- *Saccharomyces cerevisiae*, 792-795

nuclear transport and, 747, 753-755, 764

- α2 protein, 757-761
- DNA binding, 762, 763
- *GAL4* gene, 755-757
- ribosomal proteins, 761, 762

posttranslational modification and, 160, 162, 163, 198

endoplasmic reticulum, 164, 167, 169, 170, 172, 174-77, 179-183

Golgi apparatus, 183-189, 195, 197

- secretion, gram negative bacteria and, 634
- secretory vesicles and, 527, 536, 544, 545
- translocation and, 18, 19

viral glycoproteins and, 146

Yeast, lysosomelike vacuole in, 318, 319, 352, 353

biosynthesis, 319

- endoplasmic reticulum, 320-322
- glycosyl modifications, 322-326
- phosphoryl modifications, 322-326
- polypeptides, 319, 320
- proteolytic activation, 326-330

endocytosis

- fluid phase, 346, 347
- mating response, 349, 350
- pathway coupling, 350-352
- receptor-mediated, 348, 349

sorting

- gene requirement, 338-343
- genetic analysis, 343-346
- Golgi apparatus, 330-332
- localization, 333-338
- overproduction, 332, 333

Z

Zinc, mannosidases and, 232

Zymogen

- exocytosis and, 445
- lysosomelike vacuole in yeast and, 319, 322, 327-330, 337
- posttranslational modification and, 197