Molecular Biology of Intracellular Protein Sorting and Organelle Assembly

Proceedings of a DuPont-UCLA Symposium Held in Taos, New Mexico
January 30–February 5, 1987

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Fuyuhiko Tamanoi (University of Chicago) discussed how studies of *RAS* genes in yeast may lead to an understanding of the function of fatty acylation of some proteins. He has isolated mutants of *Saccharomyces cerevisiae* defective in the processing of *RAS* proteins [19]. These mutants are known as *DPR1* and are characterized by the following: 1) accumulation of *RAS2* protein precursors in the cytoplasm; and 2) lower levels of *RAS2* proteins in the plasma membrane. However, the acylation reaction appears to take place in these cells, albeit at a reduced level. These results suggest that the major effect of the *DPR1* mutation is in a processing step that takes place prior to acylation. The exact nature of this processing event has not been defined. Although the nonacylated intermediate has an increased mobility upon SDS polyacrylamide gel electrophoresis, results of microsequence analysis of the precursor and its processing intermediate indicate that proteolysis of the amino terminus of the molecule does not occur [19].

Similar yeast mutants were described by Susan Michaelis (University of California at San Francisco) in her plenary lecture at the UCLA meeting [20]. These mutants, termed *RAM* [21], are believed to encode an enzyme responsible for modification and membrane localization of proteins containing the C-terminal Cys-Aaa-Aaa-Xxx sequence. The *RAM* and *DPR1* mutants have been shown by Michaelis to be the same [20]. The *RAM* protein may encode the fatty acid acyltransferase or possibly an enzyme required to modify *RAS* and other proteins including the yeast *a* factor pheromone precursor, so that they may be subsequently acylated. Further studies of these mutants will lead to a better understanding of the protein processing reactions that result in membrane localization as a result of acylation with fatty acids.

REFERENCES

1. Uy R, Wold F: Science 198:890, 1977.
2. Evans EA, Gilmore R, Blobel G: Proc Natl Acad Sci USA 83:581, 1986.
3. Cleveland DW, Fischer SG, Kirschner MW, Laemmli UK: J Biol Chem 252:1102, 1977.
4. Rosenfeld MG, Marcantonio EE, Hakimi J, Ort VM, Atkinson PH, Sabatini D, Kreibich G: J Cell Biol 99:1076, 1984.
5. Lively MO, Walsh KA: J Biol Chem 258:9488, 1983.
6. Baker RK, Bentivoglio GP, Lively MO: J Cell Biochem 32:75, 1986.
7. Julius D, Schekman R, Thorner J: Cell 36:309, 1984.
8. Waters MG, Blobel G: J Cell Biol 102:1543, 1986.
9. Hansen W, Garcia PD, Walter P: Cell 45:397, 1986.
10. Von Heijne G: Eur J Biochem 133:17, 1983.
11. Lee DC, Rose TM, Webb NR, Todaro GJ: Nature 313:489, 1985.
12. Teixido J, Gilmore R, Lee DC, Massague J: Nature 326:883, 1987.
13. Von Heijne G: J Mol Biol 173:243, 1984.
14. Sefton BM, Trowbridge IS, Cooper JA, Scolnick EM: Cell 31:465, 1982.
15. Weeks MO, Hager GL, Lowe R, Scolnick EM: J Virol 54:586, 1985.
16. Willumsen BM, Christensen A, Hubbert NL, Papageorge AG, Lowry DR: Nature 310:583, 1984.
17. Taparowsky E, Shimizu K, Goldfarb M, Wigler M: Cell 34:581, 1983.
18. Buss JE, Sefton BM: Mol Cell Biol 6:116, 1986.
19. Fujiyama A, Matsumoto K, Tamanoi F: EMBO J 6:226, 1987.
20. Michaelis S, Herskowitz I, Powers S, Wigler M: (in this volume).
21. Powers S, Michaelis S, Broek D, Anna-A. SS, Field J, Herskowitz I, Wigler M: Cell 47:413, 1986.

Molecular Biology of Intracellular Protein
Sorting and Organelle Assembly 127–132 (1988)

Resolution of Distinct Steps in Mitochondrial Protein Import

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The transport of proteins from the cytosol into mitochondria involves several distinct steps. Precursor proteins are synthesized on cytosolic polysomes; most carry aminoterminal presequences which contain sufficient information for the targeting of proteins to mitochondria. Hydrolysis of nucleoside triphosphates is necessary to keep the precursors in an import-competent conformation. The precursors bind to specific receptors on the mitochondrial surface and are then inserted into the outer membrane. Transport of precursors into or across the inner membrane takes place at translocation contact sites and requires the mitochondrial membrane potential. The presequences are cleaved off by the processing peptidase in the mitochondrial matrix. With several precursors, a second proteolytic processing step is also performed. Finally, proteins are assembled into multi-subunit complexes.

Key words: mitochondria, precursor proteins, receptors, contact sites

Two protein synthesizing systems are required for the biogenesis of mitochondria [for review, see 1–3]. A small number of proteins are coded for by the mitochondrial genome and are translated within mitochondria. The majority of mitochondrial proteins, however, are coded by nuclear genes and synthesized on cytosolic polysomes. The transport of proteins from the cytosol into mitochondria involves four essential features: 1) specific recognition of precursors by mitochondria; 2) translocation of proteins across the mitochondrial membranes; 3) sorting of proteins to one of the four mitochondrial compartments (outer membrane, intermembrane space, inner membrane, or matrix); and 4) functional assembly.

Several steps in the complex process of mitochondrial protein import have been resolved in the past 12 years. Our present knowledge is summarized in Table I. The details will be discussed in the following sections.

CYTOSOLIC PRECURSORS FOR MITOCHONDRIAL PROTEINS

In vivo and in vitro studies have shown that mitochondrial proteins, which are synthesized on cytosolic polysomes, can be released as water-soluble precursors into the

Received April 9, 1987.

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TABLE I. Proposed Steps for the Import of Mitochondrial Precursor Proteins

1. Synthesis on free cytosolic polysomes.
2. Nucleoside triphosphate-dependent folding of precursors into an import-competent conformation.
3. Amino terminal presequences (or positively charged domains in precursors without presequences) serve as targeting signals.
4. Binding to receptor proteins on the mitochondrial surface.
5. Insertion into the outer membrane.
6. Import via translocation contact sites.
7. Membrane potential-dependent insertion into the inner membrane.
8. Proteolytic processing by the processing peptidase located in the matrix; in some cases a second proteolytic processing step is performed (either by the processing peptidase or by so far uncharacterized processing activities). Attachment of prosthetic groups.
9. Intermembrane space and inner membrane proteins: either complete import into the matrix and sorting from the matrix space, or lateral diffusion from contact sites to their functional destination.
10. Assembly into multisubunit complexes.
11. Exceptions: import independent of contact sites and membrane potential.
    a) Outer membrane proteins (which have no cleavable presequence) are directly inserted into the outer membrane. b) Apocytochrome *c* (no cleavable presequence) is translocated across the outer membrane; holocytochrome *c* is formed following covalent heme attachment.

cytosol. The precursors are then post-translationally imported into mitochondria [4–6]. Their transport in the cytosol occurs mainly in the form of higher molecular weight aggregates [7]. Many precursors are synthesized with amino terminal presequences that are about 20 to 70 amino acid residues in length and that are positively charged [1,2]. The presequences seem to carry sufficient information for the targeting of proteins to mitochondria [8–10]. This was first shown by studies with fusion proteins that consisted of a mitochondrial presequence joined to a nonmitochondrial protein.

PROTEINACEOUS RECEPTORS ON THE MITOCHONDRIAL SURFACE

The first step in the interaction of precursors with mitochondria involves binding to specific receptor proteins on the mitochondrial surface. This is shown by the following lines of evidence.

1. Pretreatment of mitochondria with low concentrations of proteases inhibits the import of precursors [11–14]. This treatment with proteases does not inhibit the mitochondrial membrane potential, or the transport of precursors from the outer into the inner membrane, or the intramitochondrial sorting of precursors [15,16].

2. The number of binding sites and their affinity constants have been determined for the binding of three different precursors to mitochondria: porin of the outer membrane [14,17]; cytochrome *c* of the intermembrane space [18]; and the ADP/ATP carrier of the inner membrane [19].

3. Precursors can be bound to mitochondria in the absence of a membrane potential ($\Delta\Psi$). After re-establishing $\Delta\Psi$, they are imported from the bound state [20,21].

Apocytochrome *c*, the precursor to (holo)cytochrome *c*, does not compete for the binding or import of any other protein tested so far [22,23]. The precursor to porin, however, seems to compete for the specific binding of ADP/ATP carrier to mitochondria [17]. In summary, mitochondria apparently carry more than one class of import receptors.

On the other hand, even precursors destined for distinct submitochondrial locations may use the same receptor sites.

TRANSLOCATION CONTACT SITES

Sites of close contact between the outer and inner mitochondrial membranes were described in electron microscopic studies 20 years ago [24]. Recently, it could be shown that import of mitochondrial precursor proteins occurs via contact sites. The import of the β-subunit of the F_1-ATPase ($F_1\beta$) and subunits IV (cytochrome c_1) and V (FeS protein) of the bc_1-complex were performed at low temperatures [15,25]. This yielded translocation intermediates that spanned both mitochondrial membranes. The amino terminal presequence was cleaved off by the processing peptidase in the mitochondrial matrix, whereas a carboxy terminal part of the precursor was still accessible to externally added proteases. Only the first import step into translocation contact sites required $\Delta\Psi$. Raising the temperature led to complete import of the precursors, even in the absence of $\Delta\Psi$. Similar translocation intermediates were obtained when specific antibodies were prebound to the precursors before exposing them to mitochondria. The amino terminus was imported into mitochondria and proteolytically processed, while the carboxy terminal part was kept outside of the outer membrane by the bound antibody.

Precursors without amino terminal presequences, like the precursor to the ADP/ATP carrier, were also shown to be imported via translocation contact sites. Precursor to the ADP/ATP carrier was incubated with mitochondria at a low temperature in the presence of a membrane potential. The precursor, which associated with mitochondria, was still accessible to proteases from the outside. By raising the temperature, however, the completion of import into the inner membrane could be performed in the absence of $\Delta\Psi$. Thus the $\Delta\Psi$-dependent interaction of precursor with the inner membrane had already taken place when precursor was incubated with mitochondria at a low temperature. The precursor therefore extended from the outside of the outer membrane into the inner membrane, thereby spanning translocation contact sites [16].

The antibody-bound translocation intermediate of $F_1\beta$ in contact sites was labeled with protein-A gold particles. Morphometric and statistical evaluation of electron micrographs showed that most of the gold particles were located at sites of close contact between outer and inner membranes. Thus protein import seems to occur at the morphological contact sites [26].

When most of the outer membrane was removed by treating mitochondria with digitonin, the contact sites still retained a part of the outer membrane. These "mitoplasts" showed the normal rate in import of $F_1\beta$. Since the presence of additional import sites on the exposed inner membrane could be excluded, this suggests that practically all import of $F_1\beta$ occurs at translocation contact sites. Furthermore, by separating submitochondrial vesicles on a sucrose gradient, the translocation contact sites could be enriched 10-fold [26].

ENERGY REQUIREMENT OF IMPORT

It has been known for many years that mitochondrial protein import requires energy [4,27]. The transport of proteins into or across the inner membrane depends on an energized inner membrane [12,28,30]. The necessary energy form is the electrical component $\Delta\Psi$ of the total protonmotive force [42]. Only the initial interaction of positively

charged precursor domains with the inner membrane seems to require $\Delta\Psi$ [16,25]. It is suggested that the role of $\Delta\Psi$ involves an electrophoretic effect on positively charged precursor domains.

Recently, it was shown that the import of mitochondrial precursor proteins also needs nucleoside triphosphates (NTPs), independently of the requirement for a membrane potential. This was demonstrated for the import of $F_1\beta$ [31] ADP/ATP carrier, fusion proteins between F_0-ATPase subunit 9 and dihydrofolate reductase [32], and porin [33]. All import steps that are involved in the transport of precursors from the cytosol into the outer membrane required NTPs. The insertion of precursors into the outer membrane required higher levels of NTPs than the binding to receptor sites on the mitochondrial surface. Three precursors, each of which contained the presequence of F_0-ATPase subunit 9, but different carboxy terminal parts, required different levels of NTPs for import. The protease resistance of in vitro synthesized precursor proteins in reticulocyte lysate was decreased by the presence of NTPs. We conclude that NTPs affect the cytosolic conformation of precursor proteins. We propose that cytosolic proteins act together with NTPs to keep precursor proteins in an import-competent conformation [32].

PROTEOLYTIC PROCESSING

The amino terminal presequences are proteolytically removed by the processing peptidase, which is located in the mitochondrial matrix [34–36]. The processing peptidase from *Neurospora crassa* has been enriched 2,000-fold over mitochondria [37]. Proteolytic processing is not a prerequisite for transport across the mitochondrial membranes, since precursors can be imported into the matrix in the absence of processing [15,38].

For precursors that are proteolytically processed in two steps, the first processing step is always performed by the matrix-located processing peptidase. The second processing step is either done by the processing peptidase as well [36] or by different processing activities that are assumed to be located in the intermembrane space [29].

INTRAMITOCHONDRIAL SORTING OF PRECURSORS

Whereas the mature FeS protein of the bc_1-complex is located on the outer face of the inner membrane, it has been shown that the precursor is first completely transported into the matrix via translocation contact sites. After proteolytic processing by the processing peptidase, the FeS protein is redirected back across the inner membrane [15]. Proteolytic processing takes place in two steps, but it is not known so far whether the second processing event occurs before, during, or after retranslocation. The insertion of the FeS cluster into the protein seems to occur in the matrix space [Hartl and Neupert, unpublished]. In agreement with the endosymbiotic hypothesis of mitochondrial origin, it is proposed that folding and assembly pathways have been conserved during evolution. After transfer of mitochondrial genes to the nucleus, presequences and import via contact sites have been introduced to transport precursors back into the mitochondrial matrix—the equivalent of the procaryotic cytoplasm. From there, the conserved folding and assembly pathways, which were already present in the procaryotic ancestor, are used.

A different import pathway for intermembrane space proteins is proposed in the following model. Precursors to intermembrane space proteins are kept in the inner membrane by the second part of the presequence, called the "stop transfer signal" [39]. The first part of the presequence could then be cleaved off by the processing peptidase,

whereas the second cleavage step is performed by a protease in the intermembrane space. In this model, the precursors should diffuse laterally from contact sites into the intermembrane space.

The transport of cytochrome *c* into the intermembrane space is an exception in many respects. The import is independent of translocation contact sites and of the membrane potential. Apocytochrome *c*, which does not contain a cleavable presequence, is translocated across the outer membrane. Holocytochrome *c* is formed by covalent attachment of heme in the intermembrane space [40]. Apocytochrome *c* from a *Neurospora* mutant in which only the carboxyterminus is altered is imported into mitochondria at a very low efficiency [41]. Cytochrome *c* seems to circumvent the "complicated" import pathway of other intermembrane space proteins that carry cleavable amino terminal signal sequences and are imported via translocation contact sites in a $\Delta\Psi$-dependent manner.

In summary, it might well be that several (at least three) different pathways exist for the import of proteins into the mitochondrial intermembrane space.

PERSPECTIVES

During the last few years, functional studies have resolved the pathways of mitochondrial protein import into several distinct steps. Now the most important aims are the isolation and characterization of the components involved in protein import. These include the precursor proteins, cytosolic cofactors, receptors, components of contact sites, and other components of the translocation apparatus, the processing peptidase of the matrix and other processing enzymes, putative cofactors inside mitochondria, components of the transport apparatus for retranslocation of proteins across the inner membrane, and components required for assembly of subunits into protein complexes. Thus it should be possible to understand mitochondrial protein import at a molecular level.

ACKNOWLEDGMENTS

We thank Dr. D.W. Nicholson for critically reading the manuscript.

REFERENCES

1. Hay R, Böhni P, Gasser S: Biochim Biophys Acta 779:65–87, 1984.
2. Harmey MA, Neupert W: In Martonosi A (ed): "The Enzymes of Biological Membranes," Vol. 4. New York: Plenum Publishing Co., 1985, pp 431–464.
3. Pfanner N, Neupert W: In Lee CP (ed): "Current Topics in Bioenergetics," Vol. 15. New York: Academic Press, 1987, pp 177–219.
4. Hallermayer G, Neupert W: In Bücher T, Neupert W, Sebald W, Werner S (eds): "Genetics and Biogenesis of Chloroplasts and Mitochondria." Amsterdam: North-Holland, 1976, pp 807–812.
5. Hallermayer G, Zimmermann R, Neupert W: Eur J Biochem 81:523–532, 1977.
6. Harmey MA, Hallermayer G, Korb H, Neupert W: Eur J Biochem 81:533–544, 1977.
7. Zimmermann R, Neupert W: Eur J Biochem 109:217–229, 1980.
8. Hurt EC, Pesold-Hurt B, Schatz G: FEBS Lett 178:306–310, 1984.
9. Horwich AL, Kalousek F, Mellman I, Rosenberg LE: EMBO J 4:1129–1135, 1985.
10. van Loon APGM, Young ET: EMBO J 5:161–165, 1986.
11. Hennig B, Neupert W: Eur J Biochem 121:203–212, 1981.
12. Gasser SM, Daum G, Schatz G: J Biol Chem 257:13034–13041, 1982.
13. Zwizinski C, Schleyer M, Neupert W: J Biol Chem 259:7850–7856, 1984.
14. Pfaller R, Freitag H, Harmey MA, Benz R, Neupert W: J Biol Chem 260:8188–8193, 1985.

15. Hartl F-U, Schmidt B, Wachter E, Weiss H, Neupert W: Cell 47:939–951, 1986.
16. Pfanner N, Neupert W: J Biol Chem 262:7528–7536, 1987.
17. Pfaller R, Neupert W: EMBO J 6:2635–2642, 1987.
18. Hennig B, Köhler H, Neupert W: Proc Natl Acad Sci USA 80:4963–4967, 1983.
19. Schmidt B, Pfaller R, Pfanner N, Schleyer M, Neupert W: In Quagliariello E, et al (eds): "Achievements and Perspectives of Mitochondrial Research," Vol II. Amsterdam: Elsevier Science Publishers B.V., 1985, pp 389–396.
20. Zwizinski C, Schleyer M, Neupert W: J Biol Chem 258:4071–4074, 1983.
21. Riezman H, Hay R, Witte C, Nelson N, Schatz G: EMBO J 2:1113–1118, 1983.
22. Zimmermann R, Hennig B, Neupert W: Eur J Biochem 116:455–460, 1981.
23. Teintze M, Slaughter M, Weiss H, Neupert W: J Biol Chem 257:10364–10371, 1982.
24. Hackenbrock CR: Proc Natl Acad Sci USA 61:589–602, 1968.
25. Schleyer M, Neupert W: Cell 43:339–350, 1985.
26. Schwaiger M, Herzog V, Neupert W: J Cell Biol 105:235–246, 1987.
27. Nelson N, Schatz G: Proc Natl Acad Sci USA 76:4365–4369, 1979.
28. Schleyer M, Schmidt B, Neupert W: Eur J Biochem 125:109–116, 1982.
29. Gasser SM, Ohashi A, Daum G, Böhni PC, Gibson J, Reid GA, Yonetani T, Schatz G: Proc Natl Acad Sci USA 79:267–271, 1982.
30. Kolanski DM, Conboy JG, Fenton WA, Rosenberg LE: J Biol Chem 257:8467–8471, 1982.
31. Pfanner N, Neupert W: FEBS Lett 209:152–156, 1986.
32. Pfanner N, Tropschug M, Neupert W: Cell 49:815–823, 1987.
33. Kleene R, Pfanner N, Pfaller R, Link TA, Sebald W, Neupert W, Tropschug M: EMBO J 6:2627–2633, 1987.
34. Böhni P, Gasser S, Leaver C, Schatz G: In Kroon AM, Saccone C (eds): "The Organization and Expression of the Mitochondrial Genome." Amsterdam: Elsevier/North-Holland, 1980, pp 423–433.
35. McAda PC, Douglas MG: J Biol Chem 257:3177–3182, 1982.
36. Schmidt B, Wachter E, Sebald W, Neupert W: Eur J Biochem 144:581–588, 1984.
37. Hawlitschek G, Schneider H, Schmidt B, Tropschug M, Hartl F-U, Neupert W: 1988, submitted.
38. Zwizinski C, Neupert W: J Biol Chem 258:13340–13346, 1983.
39. Hurt EC, van Loon APGM: Trends Biochem Sci 11:204–207, 1986.
40. Nicholson DW, Köhler H, Neupert W: Eur J Biochem 164:147–157, 1987.
41. Stuart RA, Neupert W, Tropschug M: EMBO J 6:2131–2137, 1987.
42. Pfanner N, Neupert W: EMBO J 4:2819–2825, 1985.