gag-pol products, effectively solubilizing the protein, and permitting the protease (and the C-terminal linked enzymes) to diffuse away from the plane of the membrane. This diffusion would prevent premature initiation of virus maturation. Once the nascent virus particles have budded from the cell membrane, however, autoproteolytically liberated protease could diffuse only within the isolated environment of the virus particle. A cascade of proteolytic events, liberating gag and pol structural proteins and enzymes (including additional copies of the protease itself) would be initiated. Such a proteolytic cascade in the confined space of the viral envelope would generate a burst of mature viral proteins and allow the rapid self-assembly necessary to achieve full infectivity. If this mechanism is correct, it implies that retroviral proteases, including HIV-1 protease, may be more than 'fossil' examples of an ancestral dimeric aspartyl protease[27].

Conclusions

Self-assembly of two identical monomers into a symmetric structure represents an elegant method for creating an active enzyme while encoding a minimal amount of genetic information. The proposed mechanisms of action of the aspartyl proteases[33-35] require the exact symmetry observed in the HIV-1 protease dimer to be broken at some point during the binding and catalysis of intrinsically asymmetric peptidyl substrates. Because of the symmetric nature of the active site, however, the initial orientation of substrate can be either right-to-left or left-to-right in the view of Fig. 2*a*.

HIV-1 protease is the first of the HIV-1 proteins to have its structure determined. The close resemblance between its active sites and those of the pepsin-like aspartyl proteases, where many inhibitor complexes have been investigated crystallographically suggests that it may be possible to carry over the insights derived from these studies to the design of specific HIV-1 protease inhibitors, for use as safe and effective agents in the control of AIDS.

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Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria

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A nuclear encoded mitochondrial heat-shock protein hsp60 is required for the assembly into oligomeric complexes of proteins imported into the mitochondrial matrix. hsp60 is a member of the 'chaperonin' class of protein factors, which include the Escherichia coli *groEL protein and the Rubisco subunit-binding protein of chloroplasts.*

MOST mitochondrial proteins are encoded by nuclear genes, synthesized in the cytosol as precursor proteins and subsequently imported into the organelles (for reviews see refs 1-3). Newly synthesized precursors have to assume an 'unfolded' conformation to be translocated across the mitochondrial membranes[4-6]. Cytosolic heat-shock proteins of the hsp70 family plus an additional activity which might be an ATP-dependent 'unfoldase' may have a role in maintaining this translocation-competent conformation[7-12]. Transport across the mitochondrial membranes occurs at translocation contact sites between outer and inner membranes[13,14]. Proteolytic cleavage of presequences is performed during or after translocation by the mitochondrial

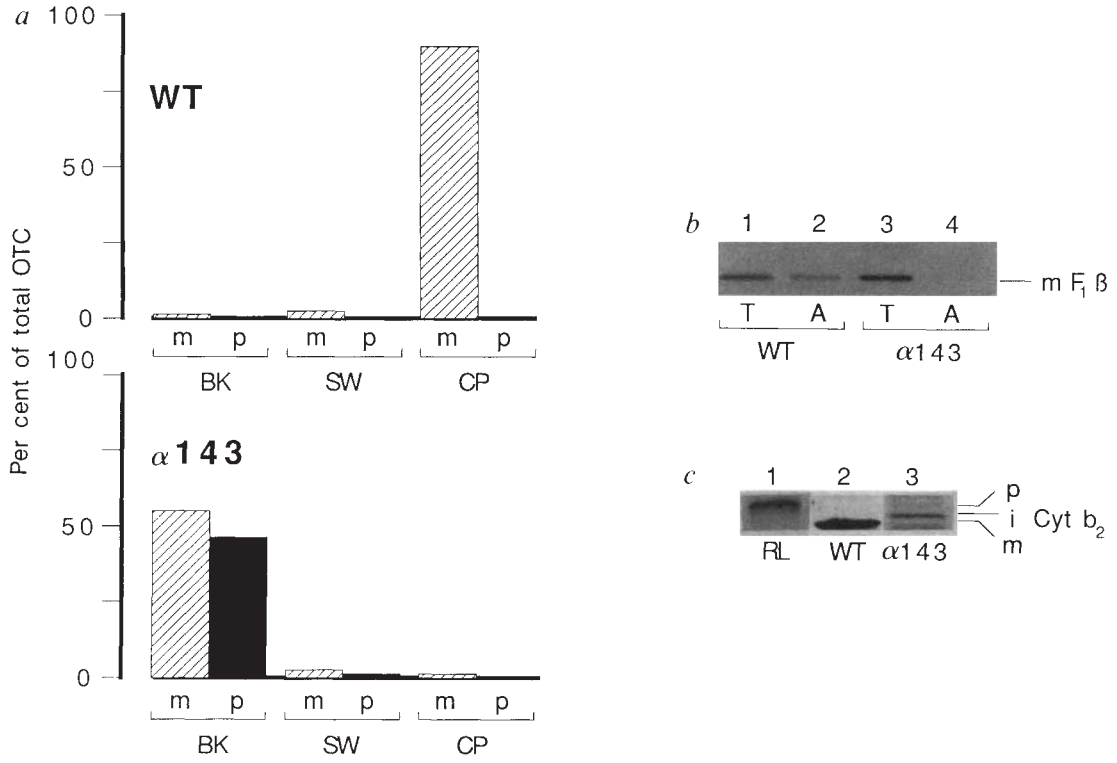

Fig. 1 Analysis of mitochondrial proteins in α143 cells. *a*, Defective assembly of OTC. Amounts of OTC contained in breakthrough (BK), salt wash (SW), and carbamyl phosphate elution (CP) of PALO columns are expressed as percentages of total OTC in cell extracts. p, Precursor; m, mature-sized OTC. *b*, Defective assembly of $F_1\beta$-ATPase. Lanes 1 and 3, immunoprecipitation of $F_1\beta$-ATPase from total amount (T) of mitochondrial fractions equivalent to the amounts analysed by chloroform extraction; lanes 2 and 4, immunoprecipitation of aqueous phase (A) of chloroform extracts. *c*, Defective maturation of cytochrome b_2. Lane 1, precursor of cytochrome b_2 immunoprecipitated after synthesis in a reticulocyte lysate (RL) directed by mRNA transcribed from the cytochrome b_2 gene cloned into pGEM3 (refs 20, 41); lane 2, mature-sized cytochrome b_2 immunoprecipitated from [^{35}S]methionine-labelled wild-type cells (WT); lane 3, intermediate-sized cytochrome b_2 immunoprecipitated from α143 cells.

Methods. *a*, Yeast cells were grown at 23 °C in YPEG medium until $A_{600} = 1$. Five A_{600} units of cells were resuspended in 1 ml fresh YPEG medium. After 30 min at 37 °C, galactose was added and incubation carried on for 2 h. Spheroplasts were then prepared[18], extracted with 20 mM Hepes, *p*H 7.4/1% Lubrol, and analysed on PALO columns[30]. Eluted fractions were precipitated with trichloroacetic acid (TCA) and analysed by SDS gel electrophoresis[42] and immunoblotting using anti-OTC antiserum and ^{125}I-protein A. Fluorographs were quantified by densitometry[20]. *b*, For *in vivo* labelling cells were grown at 23 °C in minimal galactose medium to an OD_{600} of 0.5. Four A_{600} units of cells were resuspended in 2 ml fresh minimal galactose medium and shifted to 37 °C. After 2 h, 400 μCi [^{35}S]methionine was added and incubation carried on for an additional 30 min. The reaction was stopped by adding NaN_3 to 10 mM and 25 OD_{600} units of nonlabelled cells as carrier. Spheroplasts were prepared and a crude mitochondrial fraction was isolated[18,20]. 5×10^6 c.p.m. of wild-type and 10×10^6 c.p.m. of α143 mitochondrial fractions were adjusted to 100 μl with 0.6 M sorbitol buffer and extracted with 50 μl chloroform[31] by vortexing for 1 min. Approximately 7.5% of total counts in wild-type and 4.5% in α143 were recovered in the aqueous phase. The aqueous phases were boiled in SDS-containing buffer and diluted 20-fold with TNTE (20 mM Tris *p*H 7.8, 150 mM NaCl, 1% Triton X-100 and 5 mM EDTA). $F_1\beta$-ATPase was immunoprecipitated[18] and the precipitates were analysed by SDS gel electrophoresis and fluorography.

processing peptidase in the matrix[15-18]. Some precursors of intermembrane-space proteins are re-translocated from the matrix back across the inner membrane by a mechanism similar to bacterial protein export[19,20]. Finally, all imported proteins have to (re)fold and in most cases assemble into supramolecular complexes to become functionally active. Very little is known about these last stages of the import pathway.

The complex processes involved in folding and assembly of proteins imported into mitochondria might not occur spontaneously but rather might require one or more gene products. To detect such components, a bank of temperature-sensitive lethal yeast mutants was screened for a phenotype which results from deficiency in assembly of mitochondrial proteins. We describe here a nuclear mutation, *mif4*, in which subunits of mitochondrial enzymes such as subunit β of F_1-ATPase are completely translocated into the organelles, are processed to their mature-sized forms but fail to assemble into their respective supramolecular complexes. Proteins with complex presequences like cytochrome b_2 and the Rieske Fe/S protein of complex III are also affected. These proteins normally pass through the matrix on their way to the intermembrane space and they probably require interaction with protein factors during their assembly process. In the *mif4* mutant they accumulate as incompletely processed import intermediates.

The *Mif4* gene codes for a constitutively expressed stress protein, hsp60, which is structurally related to the α-component of the chloroplast Rubisco subunit-binding protein and the *E. coli* heat-shock protein groEL[21,22]. These two proteins have been named 'chaperonins'[21,23] because they were proposed to chaperone oligomeric protein assembly, such as bacteriophage head assembly in *E. coli*[24,25], and Rubisco assembly in chloroplasts[26,27]. Yeast hsp60 is localized in the mitochondrial matrix as a high molecular weight complex which has a distinct sedimentation behaviour on sucrose gradients. We suggest that interaction of imported proteins with this hsp60 scaffold is an essential step in conferring a conformation to imported precursor proteins which renders them competent for assembly with other subunits or for further intramitochondrial sorting.

Mutants

Mutants lacking enzymatic activity of imported mitochondrial proteins. Recently, we described a procedure for the selection of so-called *mif*-mutants (*mif*, for mitochondrial import functions), conditional yeast mutants affecting the mitochondrial

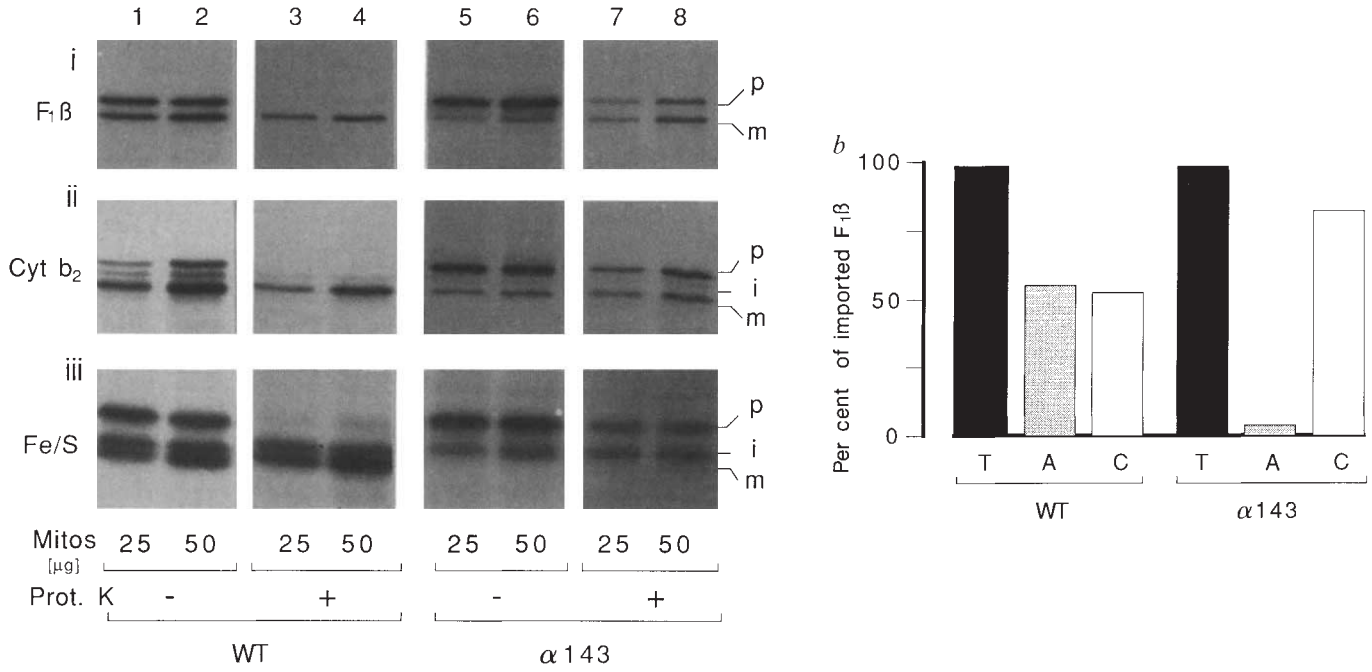

Fig. 2 Import of precursor proteins into isolated α143 mitochondria. *a*, Import of precursors of $F_1\beta$ (i), cytochrome b_2 (ii) and Rieske Fe/S protein (iii) into isolated mitochondria of wild-type (WT) and α143 mutant. Prot. K, proteinase K; p, precursor; i, intermediate-sized form; m, mature-sized form. *b*, Assembly of $F_1\beta$ imported *in vitro* into F_1-ATPase complex. Amounts of mature-sized $F_1\beta$ contained in chloroform (C) and aqueous phases (A) are given as percentages of total $F_1\beta$ contained in 50 µg of mitochondria (T) subjected to chloroform extraction. **Methods.** Wild-type and α143 yeast were grown overnight in YPEG at 23 °C. Then the temperature was shifted to 37 °C for 1 h. Spheroplasts were prepared and mitochondria were isolated[20]. Precursor proteins were synthesized in reticulocyte lysates in the presence of [^{35}S]methionine by coupled transcription/translation of the cDNAs ($F_1\beta$, Rieske Fe/S protein) or the genomic DNA (cytochrome b_2) cloned into pGEM3 using SP6 polymerase[20,41,43]. *a*, For import *in vitro* mitochondria were incubated for 30 min at 25 °C in reticulocyte lysates diluted 5-fold with BSA buffer (3% bovine serum albumin, 80 mM KCl, 10 mM MOPS (3-[*N*-morpholino]propanesulphonic acid) *p*H 7.2) containing 1 mM NADH and 2 mM ATP. The final concentration of mitochondria in the import reactions was 25 µg (lanes 1,3,5,7) and 50 µg (lanes 2,4,6,8) in a reaction volume of 100 µl. The reactions were placed on ice and one half of each sample was treated with proteinase K (Prot. K) (20 µg ml^{-1}, final concentration) for 30 min at 0 °C (lanes 3,4 and 7,8) (ref. 20). The other halves remained on ice for the same period. Finally, mitochondria were re-isolated, solubilized in SDS-containing buffer and analysed by SDS polyacrylamide gel electrophoresis and fluorography[20,42]. *b*, Mitochondria of wild-type and α143 cells (50 µg each) which had imported the radiolabelled precursor of $F_1\beta$ from a reticulocyte lysate were treated with protease as described above and subjected to chloroform extraction (see legend to Fig. 1*b*)[31]. Chloroform phases were dried under vacuum and aqueous phases precipitated with trichloroacetic acid (TCA) were analysed by SDS electrophoresis and fluorography. Fluorographs were quantified by densitometry.

protein import machinery[18]. Temperature-sensitive (ts) lethal mutants were derived by EMS-mutagenesis from a yeast strain (Gal1OTCRP/11) that is defective at the (cytosolic) ornithine transcarbamylase (OTC) locus (*arg3*) but contains the sequence coding for the human mitochondrial OTC (joined to an inducible yeast Gal1 promoter) integrated in its *URA3* locus. Upon expression in wild-type yeast cells, human OTC was imported into the mitochondrial matrix, processed and assembled to the enzymatically active trimer[28]. Mutants defective in expression of OTC activity at the non-permissive temperature were analysed. This led to the identification of mutants which accumulated at non-permissive temperature the precursor of human OTC but also the precursors of endogenous yeast mitochondrial proteins. This class of mutants included the complementation groups *mif1* and *mif2* which affect the processing enhancing protein (PEP) and the mitochondrial processing peptidase (MPP), respectively, components required for proteolytic processing of precursor proteins[16-18,29].

In a second class of ts-lethal mutants mature-sized OTC subunits accumulated. One mutant, α143, was further characterized. At non-permissive temperature the cells produced mature-sized OTC subunits and OTC precursor. But, no OTC activity was detectable. When examined at the permissive temperature, α143 cells behaved like wild-type. A single ts mutation which did not reside in the *OTC* gene itself was responsible for this phenotype.

Mutation affects assembly of several mitochondrial proteins. To test whether OTC subunits in the α143 cells had assembled into the homotrimeric enzyme an assay was carried out using the OTC substrate analogue δ-*N*-(phosphono-acetyl)-L-ornithine (PALO)[30] (Fig. 1*a*). Total extracts were prepared from cells grown for 2 h at 37 °C in galactose medium and were applied to columns containing PALO linked to epoxy-Sepharose. Extracts of wild-type cells contained only mature-sized OTC. Practically none of this was detected in the flow-through and wash fraction of the column, but OTC could be completely eluted with the OTC substrate carbamyl phosphate (Fig. 1*a*, upper panel). In contrast, with extracts of α143 cells both precursor and mature-sized OTC subunits were observed. They were quantitatively recovered in the flow-through (Fig. 1*a*, lower panel). The total contents of OTC polypeptides in wild-type and α143 cells were very similar. At non-permissive temperature mature-sized human OTC subunits were produced in α143 cells but failed to assemble into the homotrimeric, catalytically active, enzyme.

To find out whether the α143 mutation affects the assembly of an endogenous yeast mitochondrial protein we investigated the assembly of the β-subunit of F_1-ATPase ($F_1\beta$) into the F_1-ATPase complex (Fig. 1*b*). Our assay took advantage of the previous observation that upon treatment of a mitochondrial suspension with chloroform, assembled F_1-ATPase complex partitions into the aqueous phase, whereas non-assembled subunits of the ATPase partition into the organic phase[31]. Cultures of α143 and of wild-type cells were grown at 23 °C, then shifted to 37 °C and radio-labelled with [^{35}S]methionine. Mitochondria were isolated and were subjected to chloroform extraction.

About 40–50% of total mature-sized $F_1\beta$ of wild-type mitochondria was recovered in the aqueous phase of the chloroform extraction, reflecting assembly of mature subunits into the ATPase complex (Fig. 1*b*, lane 2 versus 1). In contrast, virtually no mature $F_1\beta$ subunit of α143 mitochondria was detectable in the aqueous phase (Fig. 1*b*, lane 4 versus 3). We conclude that mature $F_1\beta$ subunits produced in the α143 mutant at the non-permissive temperature failed to assemble into the F_1-ATPase complex.

This observed deficiency in assembly of two matrix-located proteins in the α143 cells led us to ask whether the import pathway of proteins passing through the matrix compartment *en route* to the intermembrane space was also affected. One example of such a protein is cytochrome b_2. The precursor containing a bipartite targeting sequence is first completely translocated into the matrix and cleaved by the mitochondrial processing peptidase to an intermediate-sized form. This intermediate is directed back across the inner membrane by the second part of the presequence and cleaved to the mature-sized form at the outer surface of the inner membrane[20]. When extracts of α143 cells grown at non-permissive temperature were analysed, essentially only the intermediate-sized form was detected (Fig. 1*c*). These observations could be explained either by a defect in the translocation of the precursor into the matrix space or by a conformational alteration conferred to the precursor within the matrix compartment.

Block is after membrane translocation. To decide which step of the import pathway was affected by the α143 mutation the same proteins whose assembly had been examined in intact cells were synthesized as radiolabelled precursors in a reticulocyte lysate and added to isolated mitochondria. Upon incubation with wild-type mitochondria prepared from cells grown at 37 °C, precursor of $F_1\beta$ was converted to its mature-sized form which was protected against externally-added protease (Fig. 2*a*, panel *i*). When import into mitochondria from α143 cells grown for 1 h at 37 °C was examined, again the mature-sized subunit (and the precursor) was observed to be protected from digestion by added protease. This excluded a defect in translocation of precursor proteins. To determine the state of assembly of the imported mature $F_1\beta$ subunits, the organelles were re-isolated from the import reactions and subjected to chloroform extraction (Fig. 2*b*). With wild-type mitochondria, more than 50% of the imported mature-sized $F_1\beta$ partitioned into the aqueous phase. In contrast, after extraction of α143 mitochondria virtually no mature-sized $F_1\beta$ was detected in the aqueous phase, indicating that the imported subunit had failed to assemble.

Precursor of cytochrome b_2 was also imported by α143 mitochondria into a protease-protected position (Fig. 2*a*, panel ii). In contrast to intact cells, isolated mitochondria from α143 cells accumulated the precursor rather than the intermediate-sized form. This might reflect kinetic differences between import reactions *in vivo* and *in vitro*. The activity of the mitochondrial processing enzyme measured in detergent extracts of α143 mitochondria was not reduced compared to extracts prepared from wild-type organelles (data not shown).

In a similar way, the mutation affected assembly of the Rieske Fe/S protein of complex III. After translocation into the matrix, this protein, like cytochrome b_2, reaches a location at the outer surface of the inner membrane by re-translocation across the inner membrane. Fe/S precursor is proteolytically processed in two steps. In contrast to cytochrome b_2, however, the second processing event takes place in the matrix before re-transport to the intermembrane space. The second processing event which removes the carboxy-terminal eight residues of the presequence is probably dependent on a conformational change of the protein induced by the formation of the iron-sulphur cluster[19]. Isolated α143 mitochondria imported the precursor of Fe/S protein into a protease protected position but were unable to process it to the mature-sized form (Fig. 2*a*, panel iii). Precursor and intermediate-sized Fe/S protein accumulated. In view of the results

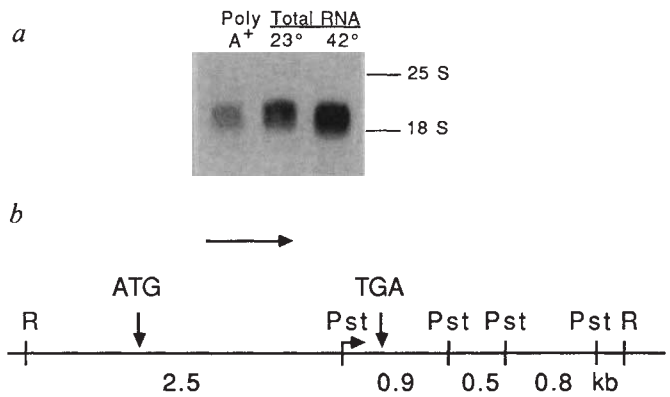

Fig. 3 Analysis of the rescuing plasmid p8. *a*, The insert of plasmid p8 recognizes a heat-inducible mRNA. 25 S and 18 S indicate migration positions of the respective yeast ribosomal RNAs. *b*, The restriction map of the p8 plasmid insert is identical to that of a λ-phage bearing the *Hsp60* gene. A 5 kilobase (kb) EcoRI fragment derived from plasmid p8 was analysed by *Pst*I digestion, and the fragment sizes compared with those of λ-Hsp60 (ref. 32). This latter clone had been isolated from a λgt11 yeast genomic library using an antiserum directed against the 58K mitochondrial heat-shock protein of *Tetrahymena thermophila*[33]. The fragment order is that indicated from sequence analysis of λ-Hsp60. A 0.9-kb *Pst*I fragment derived from p8 was sequenced in the region indicated by the horizontal arrow, and found to be identical to the one reported for the *Hsp60* gene[32]. The translational start and stop codons and direction of translation (long horizontal arrow) of *Hsp60* as determined from the clone are shown.
Methods. Wild-type cells were grown in YPEG medium at 23 °C to mid-log phase. Half of the culture was further incubated for 1 h at 23 °C and the other half at 42 °C. Total RNA was prepared[44]. Poly(A)$^+$ RNA was prepared from wild-type cells grown at 23 °C (ref. 45). RNA (10 μg of total, 2 μg of poly(A)$^+$) was electrophoresed in a 1% agarose gel, transferred to nitrocellulose[46], and hybridized with a nick-translated 0.9-kb *Pst*I fragment derived from the p8 insert (shown in Fig. 3*b*).

with import of the other mitochondrial proteins analysed, it is unlikely that the α143 mutation affects a mitochondrial component responsible for iron-sulphur cluster formation or the second proteolytic processing of Fe/S protein.

Altogether, the data suggest that α143 mitochondria are defective in a component which has a role in interacting with imported precursors, conferring conformational competence required for further steps of the import pathway. Such steps may include proteolytic processing, perhaps other covalent modifications (such as iron-sulphur cluster formation), assembly into supramolecular complexes, and further membrane translocation events.

Mutation is in the gene for hsp60. The α143 strain was mated at 23 °C with a wild-type strain of the opposite mating type and the diploids were shifted to 37 °C. They exhibited a wild-type phenotype indicating that the mutation in α143 is recessive. The α143 strain was next crossed with other recessive lethal mutant strains which are defective in mitochondrial import, belonging to the complementation groups of *mif1*, *mif2*, and *mif3* (ref. 18). Diploid cells from all three crosses grew normally at 37 °C, indicating that α143 belongs to a different complementation group, which we designated *mif4*.

To isolate a wild-type copy of the *Mif4* gene, the haploid α143 strain was transformed with a library containing yeast genomic DNA fragments inserted into a centromere-bearing plasmid. DNA was prepared from colony-purified transformants growing at 37 °C and used to transform *E. coli* to ampicillin-resistance[18]. Plasmids were isolated and found to share several restriction fragments of inserted yeast DNA.

To test whether the rescuing plasmids might encode a heat-shock protein, an insert from one plasmid (p8) was used to probe blots containing RNA prepared from wild-type yeast

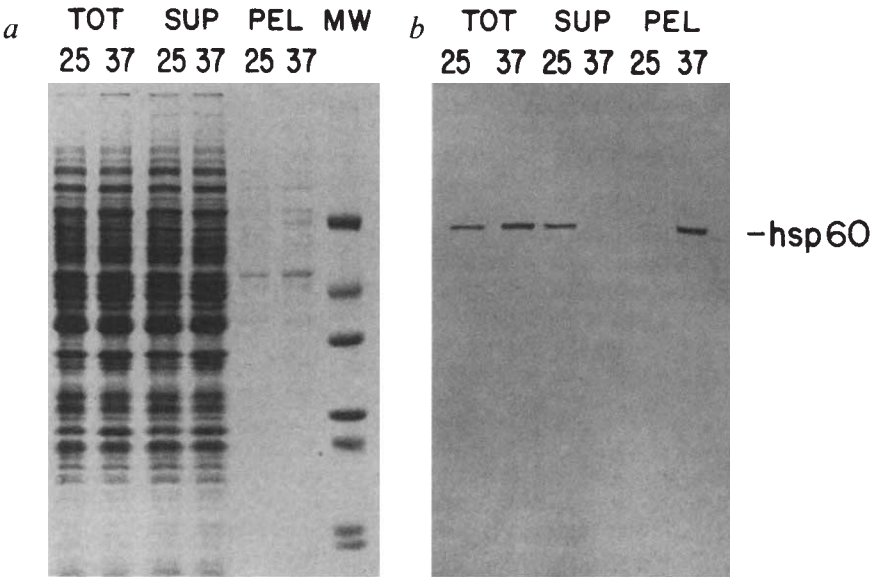

Fig. 4 Altered sedimentation of hsp60 protein in α143 cells. Fractionation by centrifugation of detergent extracts of α143 cells grown at 25 °C (25) or exposed to 37 °C for 2 h (37). *a*, Coomassie stained total proteins. *b*, Hsp60 as shown by immunoblotting using anti-hsp58 antiserum[33]. TOT, total extracts; SUP, supernatants; PEL; pellets.
Methods. An overnight culture of α143 cells grown at 25 °C was divided into halves. One half remained at 25 °C, whereas the other was transferred to 37 °C for 2 h. Then the cells were washed, broken by vortexing with glass beads and solubilized with 1% Triton X-100 and 0.5% deoxycholate[22,32]. After a clarifying centrifugation at 1,000*g* for 5 min, the extracts were centrifuged at 15,000*g* for 15 min. Equivalent amounts of total extracts, 15,000*g* supernatants and pellets were analysed on 12.5% SDS polyacrylamide gels.

maintained at 23 °C or exposed to 42 °C for 1 h. The insert identified a poly(A)$^+$ mRNA of ~1,800 nucleotides whose content in a preparation of total RNA was increased 2- to 3-fold by incubation of cells at 42 °C (Fig. 3*a*). It seemed possible that the *Mif4* gene encodes the heat shock protein (relative molecular mass, M_r, 60,000 (60K)) recently identified in mitochondria of yeast[22]. The restriction map of the insert p8 was compared with the restriction map of a λgt11 clone containing the *Hsp60* gene[32]. The maps of the two cloned yeast segments overlapped precisely (Fig. 3*b*). A region of p8 extending over 150 base pairs (bp) was subjected to DNA sequence analysis. The sequence was identical with that from the corresponding region in *Hsp60*, which lies within the protein-coding region[32] (data not shown).

To confirm that the *Hsp60* gene is the one affected by the *mif4* mutation a wild-type copy of *Hsp60* was integrated into the *Hsp60* locus in α143 cells and mating was carried out with a wild-type strain. Following sporulation, tetrad analysis revealed that all spores were viable at 37 °C. Thus, the *mif4* mutation responsible for the observed defect of assembly of mitochondrial proteins, is in the structural gene for hsp60.

Mutant contains an altered hsp60 complex. The *Hsp60* gene is essential for the growth of yeast and encodes a precursor (~61K) that is targeted to the mitochondrial matrix, probably by an amino-terminal cleavable targeting sequence[32]. The mature protein bears a distinct resemblance to the 58K product of the *groEL* gene of *E. coli*, a product required for bacteriophage head assembly and essential for cell viability[21,24]. Like groEL, hsp60 resides in a macromolecular complex[22]. Following extraction in its native state it sediments in sucrose gradients to a size position corresponding to a multimer of 12–16 identical subunits. In electron microscopic analysis of fractions containing the protein, two 7-fold symmetric 'doughnuts' are observed, placed one on top of the other[22].

If the macromolecular complex containing hsp60 is the functionally active form, then alteration of the hsp60 component by mutation might affect the integrity of the complex. Total extracts were prepared from α143 cells that had been either maintained at permissive temperature or shifted to 37 °C. Supernatants obtained by centrifugation at 15,000*g* were fractionated on sucrose gradients (data not shown). Hsp60 extracted from cells grown at 25 °C migrated as an approximately 20S particle. Analysis of extracts from α143 cells incubated at 37 °C for 30 min revealed a much smaller portion of the total hsp60 protein at this position of the gradient. Instead, most of the product was found in the pellet of the 15,000*g* centrifugation. When an extract from α143 cells which had been kept at 37 °C for 2 h was analysed, the total amount of hsp60 present increased about 2-fold but all of it was detected in the 15,000*g* pellet fraction (Fig. 4). In a control experiment with wild-type yeast, all of the hsp60 remained in the supernatant of the 15,000*g* centrifugation and migrated as 20S particles, irrespective of whether the cells were exposed to 37 °C. It is not known why hsp60 from α143 cells exposed to 37 °C sediments into a 15,000*g* pellet. The hsp60 complex may have become denatured and thus precipitated. Alternatively the complex might somehow have become associated with the mitochondrial membranes, although the presence of detergents during extraction makes this unlikely. Whatever the precise alteration, we conclude that the physical properties of the protein complex containing hsp60 are affected by the mutation present in the α143 strain.

Conclusions

Polypeptides entering the mitochondrial matrix space from the cytosol require a matrix protein, the *Mif4* gene product hsp60, for assembly and/or for further processing and sorting events. Consistent with this essential function, hsp60 is constitutively expressed. Its classification as a stress protein refers to the 2- to 3-fold induction observed in response to incubation of yeast cells at 42 °C. This response may reflect a cellular mechanism for maintaining functional conformation of mitochondrial proteins during and following heat stress.

The mechanism by which hsp60 affects the conformation of polypeptides entering the matrix space is undefined so far. The presence, however, of hsp60 in a macromolecular complex whose sedimentation properties are affected by mutation, could suggest that a 'machinery' is involved. Whether hsp60 is the only component of such a machinery is unknown. If the resemblance of hsp60 to the *E. coli* groEL protein[21] is any indicator, however, then other proteins are likely to play a role in addition, as in *E. coli* at least one other protein, groES, is required for assembly of phage heads[34,35]. Recently, the association of groEL with newly-synthesized, unfolded proteins has been demonstrated[36], suggesting that groEL might indeed have a function equivalent to its mitochondrial counterpart. Considering the double doughnut structure in which hsp60 apparently resides and the similar 14-subunit scaffold structure of groEL, we favour the idea that hsp60 may play mainly a structural role, acting as a 'workbench' on which folding and oligomerization is carried out by other proteins with catalytic activity. Putative catalytic components might reside either within the complex or within the soluble matrix. Included among such components could be another stress protein recently found to reside in the matrix space, belonging to the *Hsp70* gene family[37].

Based on the endosymbiont theory for the origin of mitochondria the utilization of hsp60 in the process of folding and assembly of imported proteins adds to the recently described evolutionarily conserved steps involved in the intramitochondrial sorting pathways of proteins of the intermembrane space[19,20]. Although the gene for hsp60 would have emigrated to the nuclear compartment at some point after the endosymbiotic event, the acquisition of a leader peptide permitted the gene product to remain in the mitochondria. An hsp60-related function may also be present in other cellular compartments of probable endosymbiotic origin. In chloroplasts, such a function is obviously present as indicated by structural similarity of the Rubisco subunit binding-protein α-subunit to both groEL and hsp60 and by its role in the assembly of the oligomeric Rubisco complex[21,26,27]. As defined by Ellis and colleagues[21,23] the groEL and Rubisco subunit-binding protein belong to one class of so-called 'molecular chaperones' termed 'chaperonins'. Our findings qualify the hsp60 protein of the mitochondrial matrix as a member of the 'chaperonin' class in a functional context. The involvement of protein factors, in particular heat-shock proteins, in folding and assembly of polypeptides has been suggested previously[10,38]. Some of these factors, such as cytosolic hsp70, may help in maintaining precursor proteins in a translocation-competent conformation. Other factors may control correct folding of newly synthesized polypeptides. The immunoglobulin heavy-chain binding protein (BiP), also known as GRP78 (refs 39, 40), might belong to this class of factors. The special function of the groEL/hsp60 family appears to be in actively catalysing the acquisition of the native conformation of oligomeric proteins. Our data strongly support the concept of protein-catalysed assembly of proteins entering the mitochondrial matrix space.

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LETTERS TO NATURE

Ultra-luminous OH maser emission from an IRAS galaxy

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Since the discovery[1] in 1982 of intense hydroxyl (OH) maser emission from the infrared-luminous galaxy Arp 220, twenty similar sources have been found in single-dish radio searches[2–7]. These so-called OH megamasers are associated with infrared-luminous galaxies with far-infrared colour temperatures of about 50 K. Here we report the detection of luminous OH maser emission in the galaxy IRAS20100–4156. This galaxy, at redshift $Z = 0.129$, displays similar optical properties to Arp 220, but exceeds it in OH luminosity by well over an order of magnitude, making it the most luminous OH maser known. The existence of such luminous megamasers increases the volume of space accessible for their study by a factor of about 50.

The generally accepted physical mechanism for OH emission in megamasers is the amplification of a centrally located continuum source by OH molecules in galactic molecular clouds[8]. The OH lines are most prominent at the main-line frequencies of 1,665 and 1,667 MHz and have velocity widths in excess of 100 km s^{-1}. Isotropic OH luminosities in the range 10^2–$10^3 L_\odot$ are typical, although a class of lower-luminosity sources (1–10 $L_\odot$) of somewhat smaller pumping efficiency is also known to exist (for example, NGC3690, NGC4418, IC5298).

Most searches for OH megamasers so far have been conducted using telescopes in the Northern Hemisphere. The only major search for sources in the Southern Hemisphere[9] was limited to galaxies with 60-μm-flux densities >10 Jy. We are now extending that survey to include IRAS galaxies with fluxes <10 Jy. The candidate objects have been selected by means of the distinctive far-infrared colours[10] of megamasers. Those which appear to be extragalactic, on the basis of identification from SERC sky survey films, are being searched for maser emission. Redshifts are being obtained with the Mt Stromlo 1.9-m reflector. A full description of this survey will be given on completion.