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FOR THE RECORD

Saccharomyces cerevisiae mitochondria lack a bacterial-type Sec machinery

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Abstract: The bacterial Sec genes encode a generalized protein export machinery. Although the mitochondria present in eukaryotic cells are derived from bacterial ancestors, a comprehensive search of the complete genomic sequence for the eukaryotic yeast *Saccharomyces cerevisiae* did not reveal any close homologs of the bacterial Sec genes, strongly suggesting that yeast mitochondria lack a generalized bacterial-type export system. This finding has implications for the sorting of imported mitochondrial proteins to the intermembrane space compartment, and also for the insertion of mitochondrially encoded proteins into the inner membrane.

Keywords: conservative sorting; cytochrome b_2; cytochrome c_1; mitochondrial biogenesis; protein import; protein translocation

Mitochondria and chloroplasts are thought to be descended from bacterial endosymbionts (Gillham, 1994). Most genes that encode bacterial-type proteins have moved to the nucleus, but the sorting pathways of many such proteins have been conserved during evolution (Hartl & Neupert, 1990). It was proposed that this "conservative sorting" idea could be extended to include proteins such as cytochromes c_1 and b_2, which are imported to the mitochondrial intermembrane space in the yeast *Saccharomyces cerevisiae*. The presequences of cytochromes c_1 and b_2 contain sorting signals that resemble bacterial signal peptides. According to the conservative sorting model, these cytochromes would initially be imported into the mitochondrial matrix, where a bacterial-type Sec machinery would recognize the sorting signals and direct reexport of the proteins across the inner membrane (Hartl & Neupert, 1990). An analogous sorting pathway has been documented for protein import into chloroplast thylakoids, and chloroplast homologs of bacterial Sec proteins have been described (Robinson & Klösgen, 1994). However, no bacterial-type Sec proteins have been found in *S. cerevisiae* mitochondria. Three explanations are possible: (1) Such proteins exist but have not yet been detected; (2) yeast mitochondria have evolved a novel reexport mechanism that does not employ Sec proteins; or (3) during sorting of cytochromes c_1 and b_2 to the intermembrane space, the mature domains cross only the outer membrane. Such a stop-transfer pathway (van Loon & Schatz, 1987; Glick et al., 1992) would also be new in evolution.

The recent sequencing of the *S. cerevisiae* genome provides an unprecedented way to test whether the sorting of intermembrane space proteins has been evolutionarily conserved. We searched the Saccharomyces Genome Database (http://genome-www.stanford.edu/Saccharomyces/) for bacterial-type Sec genes. Initial searches focused on SecY and SecA, homologs of which have been identified in all eubacteria and plastids examined to date (Ito, 1995). TBLASTN searches (Altschul et al., 1994) were carried out using several SecY and SecA sequences (SecY: *Escherichia coli, Streptomyces lividans, Bacillus subtilis, Mycoplasma pneumoniae, Cyanophora paradoxa, Pyrenomonas salina*; SecA: *E. coli, B. subtilis, Mycoplasma genitalium, Odontella sinensis, Pisum sativum*). Each search was performed with all available scoring matrices (Altschul et al., 1994): BLOSUM30, 62, 100; PAM40, 120, 250; GONNET. Of the few sequences that yielded p-values ≤ 0.2, none were candidates for a gene encoding a SecY or SecA homolog. As a positive control, we used the same input sequences to search the entire nonredundant nucleotide database (http://www.ncbi.nlm.nih.gov/BLAST/). Twenty-nine bacterial-type SecY and 18 SecA sequences were available in this database, and each of the searches identified all of the corresponding homologs at a high level of significance (p-values $< 10^{-20}$ for SecY and $< 10^{-141}$ for SecA). We also searched the *S. cerevisiae* genome using the sequences of two proteins distantly related to bacterial SecY (Rensing & Maier, 1994): an archaebacterial SecY (from *Methanococcus vannielii*), and yeast Sec61p. Both searches identified the genes encoding Sec61p and the related protein Ssh1p (Finke et al., 1996), but did not reveal any additional related genes.

Similar TBLASTN searches were performed using sequences of SecB (*E. coli*), SecD (*E. coli, Mycobacterium leprae*), SecE (*E. coli, B. subtilis, Streptomyces griseus*), SecF (*E. coli, M. leprae*) and SecG (*E.coli, M. leprae*). No yeast homologs were detected, although the results with SecB, SecE, and SecG are not definitive because of the low or uncertain sequence conservation of these proteins. In addition to the classical Sec machinery, bacteria contain an SRP-type system (Ito, 1995) that includes FtsY (a homolog of SRα, the α-subunit of the SRP receptor) and Ffh (a homolog of SRP54); an Ffh protein has also been identified in chloroplasts (Robinson & Klösgen, 1994). Searches of the *S. cerevisiae* genome with sequences of FtsY (*E. coli, M. genitalium*) and Ffh

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(*E. coli, M. genitalium, Arabidopsis thaliana*) revealed only the genes encoding SRα and SRP54.

More than 90% of the *S. cerevisiae* mitochondrial genome has been sequenced, with the gaps representing mostly A+T-rich intergenic DNA (Grivell, 1995). Neither sequence data nor biochemical experiments have yielded evidence for the synthesis of Sec-type proteins within *S. cerevisiae* mitochondria (Gillham, 1994; Grivell, 1995). Moreover, Sec genes are absent from other fungal mitochondrial genomes that have been completely sequenced (Gillham, 1994). Interestingly, in the protist *Reclinomonas americana* the mitochondrial genome encodes an unusually large number of bacterial-type proteins, including a SecY homolog (B.F. Lang, M. Gray, & G. Burger, pers. comm.; http://megasun.bch.umontreal.ca/). Apparently the corresponding gene was lost during fungal evolution.

The combined data indicate that *S. cerevisiae* mitochondria lack close homologs of bacterial Sec proteins. Yeast cells do contain the Sec61p and Ssh1p complexes, which are distantly related to the bacterial translocase (Ito, 1995; Finke et al., 1996), but immunological studies showed that these complexes are restricted to the endoplasmic reticulum (Esnault et al., 1993; Glick & Pon, 1995; Finke et al., 1996). Thus, while the final step in the sorting of cytochromes c_1 and b_2 is cleavage by an evolutionarily conserved peptidase (Nunnari et al., 1993), the earlier steps in this sorting pathway have diverged during evolution. One possible interpretation is that sorting to the intermembrane space in *S. cerevisiae* still involves bacterial-type Sec proteins, but that the sequences of these proteins have diverged strongly. This possibility seems unlikely because mitochondrial and chloroplast proteins with conserved functions generally show clear homology to their bacterial counterparts (Gillham, 1994). The more plausible interpretation is that a novel mechanism has arisen for the sorting of cytochromes c_1 and b_2. This hypothesis can be tested by identifying components of the sorting machinery (Tokatlidis et al., 1996).

Even in the absence of mitochondrial Sec homologs, the conservative sorting concept remains valid for many mitochondrial inner membrane proteins that are either synthesized within mitochondria or else initially imported into the matrix (Hartl & Neupert, 1990; Glick et al., 1992; Herrmann et al., 1995; Rojo et al., 1995). We suggest that membrane insertion of these proteins is analogous to the so-called Sec-independent assembly that has been described for some *E. coli* inner membrane proteins (von Heijne, 1994). For technical reasons it has not been possible to obtain definitive proof of Sec-independent assembly in bacteria, and in this sense *S. cerevisiae* mitochondria represent a perfect "knockout" experiment.

A salient feature of Sec-independent *E. coli* inner membrane proteins is that their periplasmic segments are short and contain only few positively charged amino acids (von Heijne, 1994). The same pattern generally holds for mitochondrially encoded (but not nuclear-encoded) mitochondrial inner membrane proteins (Gavel & von Heijne, 1993), as demonstrated by the recent crystallographic analysis of cytochrome *c* oxidase (Tsukihara et al., 1996). Mitochondrially encoded COXII is particularly interesting because it contains two transmembrane helices, with the N- and C-terminal tails both projecting into the intermembrane space (Herrmann et al., 1995). The C-terminal tail of *S. cerevisiae* COXII is unusually long (144 residues) and contains 23 acidic but only nine basic residues; translocation of this tail requires a membrane potential (Herrmann et al., 1995). Similarly, the 100-residue N-terminal tail of the *E. coli* inner membrane protein ProW is strongly acidic and is translocated in a Sec-independent but membrane potential-dependent manner (Whitley et al., 1994). Thus, it appears that acidic sequences of various lengths can be exported across the inner membranes of bacteria and of yeast mitochondria in a process that is independent of Sec proteins, but probably conserved in evolution.

In summary, the apparent absence of Sec components in *S. cerevisiae* mitochondria suggests the evolution of a novel sorting mechanism for certain intermembrane space proteins, and also supports the existence of a truly Sec-independent membrane protein assembly mechanism in bacteria.

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