Minireview

Mitochondrial assembly in yeast

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Abstract The yeast *Saccharomyces cerevisiae* is likely to be the first organism for which a complete inventory of mitochondrial proteins and their functions can be drawn up. A survey of the 340 or so proteins currently known to be localised in yeast mitochondria reveals the considerable investment required to maintain the organelle's own genetic system, which itself contributes seven key components of the electron transport chain. Translation and respiratory complex assembly are particularly expensive processes, together requiring around 150 of the proteins so far known. Recent developments in both areas are reviewed and approaches to the identification of novel mitochondrial proteins are discussed.

Key words: Respiratory chain; Translational control; Chaperone; Quality control; Mitochondrial dysfunction; Functional genomics

1. Introduction

Mitochondria are essential to all eukaryotic cells that use oxygen. For reasons that are still unclear, the organelle has retained its own genome, a remnant of the primitive endosymbiont that first colonised a predecessor of today's eukaryotes. Mitochondrial assembly depends on balanced synthesis of a handful of proteins encoded by mtDNA with several hundred others encoded by nuclear genes. The inability to assemble a functional mitochondrion underlies a wide spectrum of degenerative diseases in man. Despite an increasing realisation of the extent of involvement of mutations in mtDNA in both these diseases and aging, much has still to be done on the characterisation of those diseases for which dysfunction is the result of disturbances in the complex interactions between mitochondrial- and nuclear-encoded components of the respiratory chain. As an illustration, although more than 50 disease-producing mutations in mtDNA have been characterised, the nuclear genes involved still only run to single figures (see [1] for review).

Despite advances in other systems, the facultative anaerobic yeast *Saccharomyces cerevisiae* still remains a highly attractive experimental organism for the study of mitochondrial assembly. Respiratory functions are dispensable as long as cells can fall back on glycolysis as a source of energy and intermediates. This means that mutations affecting synthesis or function of respiratory chain components are viable and that levels of these components can be manipulated simply by changes in culture conditions. The availability of full genomic sequence data greatly facilitates the characterisation of mutations in genes involved in mitochondrial function or assembly, the identification of novel mitochondrial proteins and the genome-wide study of changes in gene expression. It is therefore highly likely that yeast will be the first organism for which a complete inventory of mitochondrial proteins and their functions can be drawn up.

Since the publication of the complete genomic sequence of *S. cerevisiae* in 1996, the number of genes known to encode mitochondrial proteins has increased from just under 200 to the current 340 (as of March 1999) out of a total gene complement of about 6100 (Table 1). Ongoing gene function analysis programmes have thrown light on the nature and role of these newly identified proteins and are opening novel routes to the identification of additional ones. Aims of this minireview are to survey this recent information in terms of the insight it provides into the synthesis and assembly of the enzymes of the respiratory chain and to assess the value of yeast as a test bed for ideas about mitochondrial dysfunction in man and other organisms.

2. The price of mitochondrial assembly

A rapid survey of information available in the Yeast Proteome Database (YPD, [2]) shows that about 100 of the 340 known mitochondrial proteins are located in the inner membrane, with 15 in the outer membrane. Major functional classes include proteins of the ribosome (63); transporters of the mitochondrial carrier family (MCF; up to 35); proteins involved in translational activation (11) and respiratory enzyme assembly (26, including five members of the triple-A family).

Altogether, some 127 of these 340 proteins are components of the mitochondrion's own genetic machinery, being directly involved in either maintenance of mtDNA or a subsequent step in gene expression. This figure forcibly emphasises the considerable investment required to maintain the organelle's own genetic system and the ability to synthesise the mere seven respiratory chain proteins encoded by it.

Translation and respiratory complex assembly are particularly expensive processes, together requiring around 150 proteins. Both are still poorly understood, with many steps, even those involving mRNA recognition or translational control, occurring with the help of membrane-associated proteins. The schematic overview presented in Fig. 1 indicates five broad areas for which new insights have recently become available and which will be discussed in more detail below.

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Table 1
The price of mitochondrial assembly (March 1999)

Category	Number
Known mitochondrial proteins	340
Involved in biogenesis and assembly	176
Mitochondrial gene expression	127
MtDNA maintenance	10
RNA processing	20
Translation	97

The table sums nuclear genes currently known to be involved in mitochondrial assembly and maintenance of the mitochondrial genetic system in yeast (based on information available in the YPD database (http://proteome.com/YPDhome.html).

3. Processing, splicing and stabilisation of mRNAs

Both the presence of introns and the fact that many genes in yeast mtDNA are co-transcribed mean that processing is an important feature of gene expression. An important development in the past year has been the unraveling of the so far puzzling nature of the functional link between the *MRS2* gene and a number of other genes capable of suppression of the respiratory deficiency caused by loss of its function. *MRS2* is required both for splicing of group II introns and for an as yet undefined function in (mainly) cytochrome *c* oxidase assembly [3]. The gene encodes a membrane-associated protein that contains short, but distinctive sequence motifs characteristic of a family of bacterial divalent metal ion transporters. Recent work by Schweyen and colleagues (R.J. Schweyen, personal communication) now suggests that Mrs2p functions as a mitochondrial Mg^{2+} transporter. This neatly explaining its involvement in Mg^{2+}-dependent RNA self-splicing and assembly of at least cytochrome oxidase (each monomer of which contains a Mg^{2+} ion). It also accounts for the multi-copy suppression of $mrs2^0$ mutants by membrane carriers like Mrs3p and Mrs4p [4] and by Tim10p, Tim12p (Mrs5p, Mrs7p [5]. The latter are components of the protein translocation machinery that specifically interact with members of the mitochondrial carrier family during their import into the inner membrane.

Despite these successes, however, much has still to be resolved, with questions relating to mRNA stability heading the list. Still far too little is known about the mitochondrial degradosome – a complex responsible for the 3′-5′ degradation of almost all mRNAs and containing Suv3p and Dss1p together with the product of a third as yet unidentified gene [6]. The same can be said of a number of factors, which have undefined roles in mRNA-specific stabilisation, sometimes with additional (and undefined) roles in other processes like transcription (Nam1p/Mtf2p [7]), processing (Cbp1p, Pet127p [8,9]), or translation (Pet309p [10])

4. Components of the translational machinery

The most recent head count of ribosomal proteins (MRPs) in yeast mitochondria yields a grand total of 63 – modest in comparison with the 136 of the cytoplasmic ribosome, but still a large part of the high price paid for the luxury of an independent translation system. Whether this represents a complete set is not yet clear. These proteins were identified by either genetic means or direct protein sequencing and both are ongoing approaches with plenty of scope for new discoveries. One of the most surprising features of the current set is the relatively low degree of sequence conservation. Only a minority displays significant sequence similarity to ribosomal proteins from other sources [11]. Yeast and mammalian MRPs are as much divergent as the latter are from *Escherichia coli* ribosomal proteins [12] and only 10 MRPS in *Caenorhabditis elegans* could be identified by a direct sequence comparison with yeast [13]. What this means in functional terms is hard to say. The lack of a faithful reconstituted in vitro translation system in mitochondria of any kind still represents a barrier to further progress in this area.

5. Translational activation

Synthesis of the mitochondrially encoded components of the yeast respiratory chain is unusual in its requirement for subunit-specific translational activators. Those required for

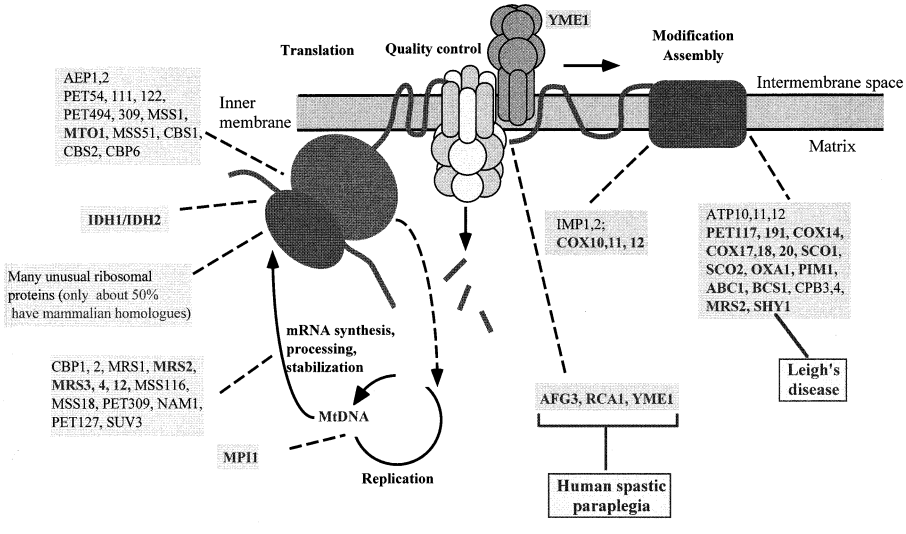

Fig. 1. Factors required for respiratory complex assembly in yeast. The figure presents a non-exhaustive overview of proteins involved at various stages of the synthesis and assembly of mitochondrial gene products into a functional respiratory chain. Genes listed in bold print display evolutionary conservation of sequence. Additional information on individual proteins can be obtained by consultation of the YPD database (http:// proteome.com/YPDhome.html). See text for discussion.

Cox1, Cox2, Cox3 and Cytb synthesis are genetically the best characterised [14]. Their action is dependent on the presence of specific sequences in the 5′-untranslated leaders of the respective mRNA. Several display additional interactions with the mitochondrial ribosome; some are integral membrane proteins. In one case (*COX3*), the amount of at least two activators (Pet122p and Pet494p) appears to be limiting and thus could exert control on synthesis [15,16]. Recent work [17] suggests that these properties usefully combine to effectively tether ribosomes and translated mRNAs to the inner membrane, thereby plausibly facilitating the co-translational insertion of newly synthesised polypeptides into the membrane. In support of this idea, the same authors also show that inappropriate synthesis in the mitochondrial matrix results in Cox polypeptides that turn over rapidly. This model blurs somewhat the distinction between a translational activator and assembly factor. It invites questions such as (1) why should different subunits of a single enzyme require different activators; (2) do factors with corresponding functions exist in the metazoa whose mitochondria contain mRNAs mostly lacking in 5′-UTRs? None of the yeast proteins displays a particularly strong conservation of sequence, making identification of counterparts in even quite closely related yeasts and fungi difficult [18] and in other organisms almost impossible.

Not all translational activators obviously depend on a 5′-UTR for their action. So far, unlike other translational activators, no mitochondrial 5′-leader rearrangements have been found to suppress mutations in *MSS51*, a gene encoding a membrane-bound putative translational activator for Cox1 mRNA ([19]; M. Siep and H. van der Spek, unpublished observation). This may mean that Mss51p does not act via direct interaction with the 5′-UTR, or simply that the right rearrangements have yet to be found. Unlike other translational activators, Mss51p may be present in appreciable excess. The *mss51-3* mutant [19], which produces a wild type protein at around 1% or less of wild type levels is capable of restoring 10–20% of wild type CoxI synthesis to an *mss51°* mutant (M. Siep, H. van der Spek and L.A. Grivell, in preparation).

6. Quality control

Somewhat speculatively shown in Fig. 1 as intervening between membrane insertion and respiratory complex assembly are two complexes constituted of members of the mitochondrial triple-A family. The first complex contains Afg3p and Rca1p, the second Yme1p. All three proteins belong to the metalloprotease sub-group of this family of ATPases and are capable of the degradation of incomplete or unassembled newly synthesised mitochondrial translation products [20,21]. Additionally, at least the Afg3/Rca1 complex (which is formed in the presence of ATP and has a molecular mass around 850–1000 kDa [22,23]) plays a chaperone-like role in respiratory complex assembly. It may possibly achieve this by putting newly synthesised proteins on hold until partner proteins become available, i.e. by acting as a 'holdase' rather than a 'foldase' (cf. [24]).

The importance of the metalloprotease sub-group of triple-A proteins for mitochondrial assembly is emphasised by recent studies, which have resulted in the identification of the gene responsible for an autosomal recessive form of hereditary spastic paraplegia (HSP [25]). This gene encodes paraplegin, a triple-A protein only distantly related to other members of the metalloprotease sub-group. HSP is characterised by progressive weakness and spasticity of the lower limbs due to degeneration of the corticospinal axons, with patients showing wide variation in the severity of clinical defects. Why the loss of an apparently ubiquitous protein like paraplegin should primarily affect specific axonal populations is as yet unclear. It may be that these cells, which are among the longest in the human body, are extremely sensitive to loss of either assembly or protease functions provided by paraplegin.

Of great interest is the question how degradative and assembly functions of this 'quality control' machinery are balanced. Some useful hints on this point may come from a comparison of the functionally homologous FtsH complex in *E. coli*, where a complex of the related proteins HflC and HflK exerts a negative effect on the degradative activity of FtsH [26]. Yeast contains two proteins with clear sequence similarity to HflC and a lower, but significant similarity to HflK. These two proteins (Phb1 and Phb2) show extensive homology to mammalian prohibitins. Like the mammalian proteins, they have a mitochondrial location [27,28], displaying a firm association with the inner membrane, where they together form large complexes with molecular mass of 900–1100 kDa (L.G.J. Nijtmans and M. Artal-Sanz, unpublished observation). Unlike the HflC/K complex and FtsH, there is as yet no evidence for a direct interaction between Phb1/2 and Afg3/Rca1 (L.G.J. Nijtmans and M. Artal-Sanz, unpublished observation). However, careful in vitro pulse-chase labelling studies on mitochondrial translation products (L. de Jong and H. van der Spek, unpublished observation) suggest some kind of functional interaction does exist, which serves to stabilise unassembled polypeptides.

7. Complex assembly

Compared with the intricacy of detail provided by the complete 3D structures of the bovine mitochondrial F1-ATP synthase, cytochrome *c* oxidase and bc_1 complexes [29–31], very little is known in any organism of the processes involved in their assembly. In yeast, analysis of mutants disturbed in assembly has led to the identification of a number of nuclear gene products, many of which are indicated in Fig. 1. In contrast to factors involved in translational controls, a wide-ranging evolutionary conservation is often observed, suggesting a dependence on basic mechanisms for achieving protein folding, stabilisation, membrane translocation and co-factor addition. For some of these factors (like (Cox10p/Cox11p (haem farnesylation [32]), Sco1p/Cox17p (Cu-addition [33]), Oxa1p (protein flippase [34,35]), the involvement can be defined in these broad terms; exact mechanisms are still obscure. For others (the majority), little can be said beyond that they play roles at some stage after synthesis of the mitochondrially encoded subunits. More detailed demarcation has so far been effectively hampered by the remarkable ability of the quality control system to recognise and initiate degradation of partially or incorrectly assembled complexes. Progress in this area may be achievable by study of double mutants in which, in addition to the loss of a specific assembly factor, the degradative activities of the Afg3p, Rca1p and Yme1p metalloproteases have been eliminated by mutation. This is unlikely to eliminate all turnover of unassembled subunits, since other proteolytic activities have been described in yeast mitochon-

dria [36,37], but it may be enough to allow accumulation of distinctive assembly intermediates to detectable levels.

8. Genomic aspects

How many proteins does it take to make a working mitochondrion? In yeast, most of the 340 or so known mitochondrial proteins were identified by biochemical and/or genetic approaches that were based on prior notions of activity or (respiratory-deficient) phenotype. With still somewhere around 2100 orfs of unknown function in the yeast genome, there is still scope for the discovery of new genes involved in mitochondrial assembly or function. Disruptants for each of 6100 genes are gradually emerging from ongoing systematic gene function programmes and a screen for those displaying respiratory deficiency will undoubtedly turn up additional candidates. However, working on the assumption that a number of genes may have so far escaped attention because their mutation or loss of function results in either too mild, too severe or complex a phenotype, alternatives should also be considered.

Within EUROFAN, the EU consortium engaged in the study of gene function on a genome-wide scale in yeast, use has been made of the PSORTII programme [38] to predict sub-cellular location. Of some 50 orfs examined by this approach, just over 30 have been assigned to mitochondria by (immuno-)fluorescence, or fractionation and Western blotting of the GFP- or HA-tagged fusion protein (G.-J. Hakkaart, C.J. Herbert and R.J. Schweyen, unpublished observatopm). However, as the perfect algorithm for prediction of all classes of mitochondrial protein does not and may never exist, another in silico approach worth consideration is one based on patterns of co-expression as revealed by a combination of micro-array hybridisation with statistical cluster analysis [39].

Such an analysis, although primarily of value in the identification of groups of co-regulated genes, of shared promoter elements and thereby also of common transcription control proteins, has potential for the assignment of novel proteins to functional groups. Studies carried out so far are based on limited amounts of data and on relatively unsophisticated clustering algorithms. Nevertheless, it is striking how much information on gene function and even physical interaction between gene products can be gleaned from them [40], thereby opening up new and exciting avenues for further experimentation.

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