Critical Reviews in Biochemistry and Molecular Biology, 30(2):121–164 (1995)

Nucleo-Mitochondrial Interactions in Mitochondrial Gene Expression

L. A. Grivell

Section for Molecular Biology, Department of Molecular Cell Biology, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, Netherlands

ABSTRACT: All proteins encoded by mitochondrial DNA (mtDNA) are dependent on proteins encoded by nuclear genes for their synthesis and function. Recent developments in the identification of these genes and the elucidation of the roles their products play at various stages of mitochondrial gene expression are covered in this review, which focuses mainly on work with the yeast *Saccharomyces cerevisiae*. The high degree of evolutionary conservation of many cellular processes between this yeast and higher eukaryotes, the ease with which mitochondrial biogenesis can be manipulated both genetically and physiologically, and the fact that it will be the first organism for which a complete genomic sequence will be available within the next 2 to 3 years makes it the organism of choice for drawing up an inventory of all nuclear genes involved in mitochondrial biogenesis and for the identification of their counterparts in other organisms.

KEY WORDS: yeast, *Saccharomyces cerevisiae*, mtDNA, mitochondria, regulation of gene expression, transcription, RNA splicing, translation, membrane assembly, molecular sequence data.

I. INTRODUCTION

Numerous reviews on various aspects of mitochondrial biogenesis have appeared in recent years (Tzagoloff and Myers, 1986; Attardi and Schatz, 1988; Costanzo and Fox, 1990; Glick and Schatz, 1991; Bolotin-Fukuhara and Grivell, 1992; Gray et al., 1992; Wallace, 1992; de Winde and Grivell, 1993; Dieckmann and Staples, 1994; Pel and Grivell, 1994), so why yet another on the subject? Apart from its intrinsic interest, there are several reasons why this process deserves continuing study and review: mitochondria make a major contribution to energy production and conservation in all eukaryotic cells that use oxygen; they house key enzymes of major metabolic routes; they contain a genetic system that displays principles of organization and control not encountered elsewhere. Finally, like any other intricate biological process, mitochondrial biogenesis can go wrong. In man, mutations in mtDNA that result in an inability to assemble a fully functional mitochondrion are the basis of a wide spectrum of degenerative neurological and muscular disorders (Luft, 1994; Wallace, 1994), and this has resulted in a dramatic upswing in interest in the repair, replication, and expression of this compactly organized genome. In higher plants also, disturbance in mitochondrial function, resulting from rearrangements in mtDNA, or incompatibility between mitochondrial and nuclear genetic backgrounds can lead to aberrant patterns of mitochondrial gene expression, which often result in cytoplasmic male sterility. This phenomenon is of great importance to plant breeders both for its value in the commercial production of hybrid strains and for associated, undesirable side effects, such as increased susceptibility to fungal toxins (Hanson, 1991).

The information content of most mtDNAs is low and the number of encoded proteins contributed to the organelle is small. Nevertheless, all are essential for the assembly of a functional respiratory chain and all are dependent on proteins encoded by nuclear genes for their synthesis and function. Recent developments in the identification of these genes and the elucidation of the roles their products play at various stages of mitochon-

1040-9238/95/$.50

drial gene expression are covered in this review, which like an earlier review on these topics (Grivell, 1989), focuses mainly on work with the yeast *Saccharomyces cerevisiae*, with occasional excursions to other organisms.

II. THE YEAST MITOCHONDRIAL GENOME

S. cerevisiae remains the experimental organism of choice for the study of mitochondrial biogenesis, because, unlike most other organisms, respiratory functions are dispensable as long as the organism can fall back on glycolysis as a source of energy and intermediates. In practice, 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 (Bolotin-Fukuhara and Grivell, 1992).

Major features of the *S. cerevisiae* mitochondrial genome were established already a number of years ago. Dependent on strain, this mtDNA varies in complexity from about 74 to 85 kb (Zamaroczy and Bernardi, 1986; Wolf and Del Giudice, 1988; Grivell, 1989; Costanzo and Fox, 1990; Bolotin-Fukuhara and Grivell, 1992). The circular map presented in Figure 1 is the result of both genetic and restriction analysis, but circular molecules constitute only a small fraction of isolated mtDNA and the population may in fact consist *in vivo* of long linear concatemers (Maleszka et al., 1991; Bendich, 1993). In common with the much smaller mtDNAs from metazoa, yeast mtDNA contains genes coding for the two ribosomal RNAs, a set of tRNAs and some components of the respiratory and energy-conserving enzymes of the inner membrane. In addition, there are genes without counterparts in metazoan mtDNAs. These code for endonucleases involved in intron spreading (homing endonucleases); proteins required for RNA splicing (RNA maturases); a protein associated with the small subunit of the mitochondrial ribosome (*VAR1*) and an RNA that forms part of an RNAse P-like tRNA processing enzyme (*RPM2*). In addition, three reading frames identified by DNA sequence analysis bear some resemblance to the LAGLI-DADG homing endonuclease/RNA maturase family (see Section VII). In some strains, the product of one of them (*ENS2*) acts as one subunit of a site-specific endonuclease involved in general recombination of yeast mtDNA, the other subunit being related to the mitochondrial hsp70 protein SSC1 (Morishima et al., 1990; Nakagawa et al., 1991). Interestingly, the sequence selectivity of this enzyme is much more relaxed than that of other enzymes of the family (Kawasaki et al., 1991), a feature that may derive from influence exerted by the hsp70 subunit.

Genes notably absent from *S. cerevisiae*, but present in mtDNAs from mammals, plants, fungi, trypanosomes, and certain other yeasts, are those for the membrane-bound subunits of the complex I type of mitochondrial NADH:Q reductase (Weiss et al., 1991). This absence simply reflects the fact that *S. cerevisiae* lacks this type of NADH dehydrogenase, having instead two simpler enzymes whose subunits are encoded by nuclear genes (de Vries and Grivell, 1988; de Vries et al., 1992).

A compilation of all published DNA sequence data for *S. cerevisiae* covers an estimated 92% of the genome, with a few large gaps remaining (Zamaroczy and Bernardi, 1986). Unfortunately, results of early sequencing efforts revealed that the intergenic regions of yeast mtDNA are almost pure poly-d(AT) and this fact has effectively deterred subsequent generations of sequencers from undertaking efforts to complete the sequence. Although information now available from other mitochondrial genomes makes it unlikely that other genes remain to be discovered, this still has to be established formally.

Comparison of gene order in the mitochondrial genomes in different yeasts shows that it is not conserved, with the larger genomes displaying greater variability than the smaller (Hoeben et al., 1993). As in other mtDNAs, subgenomic molecules formed by deletions across repeated sequences are a likely source of such rearrangements. The *S. cerevisiae* mitochondrial genome abounds in such sequences, with both the extensive AT-rich regions and short (50 to 100 bp), highly homologous GC-rich sequence elements being implicated in formation of deletions (Bernardi, 1982). Additionally, large-scale deletions resulting from intron duplication and transposition may be a major source of genomic variation (see Section IX).

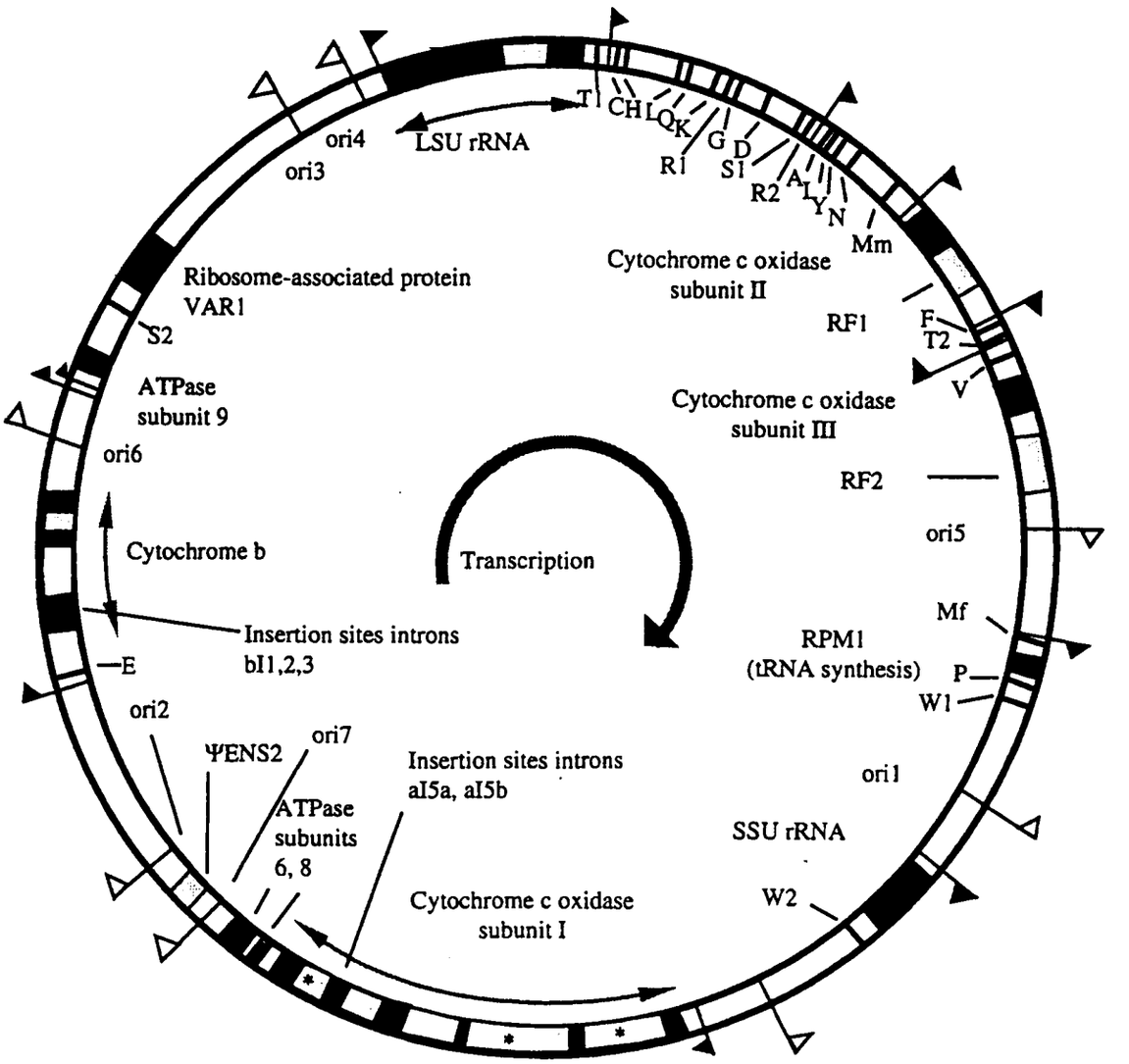

FIGURE 1. MtDNA in *S. cerevisiae* strain D273-10B. Gene positions are based on coordinates listed by Grivell (1993). Genes for rRNAs and known proteins are represented by black bars, those for ORFs by light-gray shaded areas. Note that *RF2* and *ΨENS2* consist of a series of overlapping reading frames linked by +1 and −1 frameshifts. For ease of presentation, these sequences are shown as continuous areas on the map. In some strains, the *ENS2* reading frame is continuous and encodes the catalytic subunit of a site-specific endonuclease involved in recombination of mtDNA (Nakagawa et al., 1991). tRNA genes are identified by means of the cognate amino acid, using the one-letter code. Subscripts indicate isoacceptors. Introns, where present, are indicated by gray bars and those of the group II type are additionally indicated by an asterisk. D273-10B contains a medium-sized mtDNA, which lacks a number of introns present in the genes for apocytochrome *b* and COX1 in so-called long strains. Insertion sites of the missing introns are indicated. Sites of transcriptional initiation are designated by (▷) and ori sites by (▶). For all genes but one (T2 $tRNA_2^{Trp}$), transcription is in a clockwise direction. For the ori sequences, the flags point in the direction of cluster A to cluster C (Zamaroczy and Bernardi, 1986).

III. REPLICATION, RECOMBINATION, AND REPAIR

In marked contrast to the situation for many other mtDNAs, remarkably little is known about replication of mtDNA in yeast. A high rate of recombination, together with the large size of the molecule have undoubtedly contributed to this state of affairs by compounding difficulties in identification and isolation of replication inter-

mediates. Additionally, despite the availability of the yeast mitochondrial DNA polymerase and other proteins likely to be involved in replication (Foury and Lahaye, 1987; van Dyck et al., 1992; Lahaye et al., 1993), there is as yet no *in vitro* system for study of replication. For the yeast *Torulopsis glabrata*, a rolling circle mechanism of replication of its 19-kb mitochondrial genome has been proposed, based on the detection by electron microscopy of lariats with double- or single-stranded tails alongside linear molecules of 2 to 7 genome units in length (Maleszka et al., 1991). A similar mode could also hold for *S. cerevisiae* (Maleszka et al., 1991; Bendich, 1993), but whether replication occurs exclusively by this mechanism is still not known.

A. Replication Origins

Depending on strain, yeast mtDNA contains 7 to 8 ori-like elements that resemble replication origins in other organisms in terms of both sequence motifs and predicted secondary structure (Faugeron-Fonty et al., 1984; Zamarocny et al., 1984). Of these, four (ori 1, 2, 3, and 5) could be functional *in vivo*, because they are able to initiate DNA synthesis through RNA priming (Baldacci and Bernardi, 1982; see below). Ori 1, 2, and 7 are apparently optional, because they can be deleted without obvious deleterious effects (Piskur, 1988a, 1988b), thus leaving ori 3 and 5 as serious candidates as replication origins. For ori 3, both functionality and importance has been established by the finding of a point mutation conferring temperature sensitivity on replication of mtDNA (Zelikson, R. and Bolotin-Fukuhara, M., unpublished observations).

As in other systems, DNA replication and transcription in yeast mitochondria are tightly linked processes. Ori sequences and promoters share the same highly conserved block of 11 bp and both are transcribed by the mitochondrial RNA polymerase, the product of the *RPO41* gene. Deletion of *RPO41* results in loss of mtDNA. Analysis of nascent DNA chains and RNA primers originating from ori1 and 5 shows that both are used as sites of RNA-primed, bidirectional DNA replication. On the basis of the positions of the RNA primers, it seems likely that in contrast to vertebrate mtDNAs, RNA priming of the complementary strand takes place in the immediate vicinity of the initial priming event, probably by an as yet unidentified primase (Baldacci et al., 1984). This situation resembles that observed with other DNAs (Staudenbauer, 1979), including that of bacteriophage T7, whose RNA polymerase is highly homologous to the mitochondrial core RNA polymerase (Masters et al., 1987). Interestingly, the T7 RNA primase initiates replication in lagging DNA strands by synthesizing short primers with the sequences ACCC or ACCA in a preexisting replication fork. Both sequences occur in the conserved region of the secondary origins of yeast mtDNA, and RNA primers have been mapped to these regions (Baldacci et al., 1984; Schinkel and Tabak, 1989).

B. Enzymology of DNA Replication

Of interest for the enzymology of mtDNA replication is the fact that the yeast mitochondrial DNA polymerase, encoded by the nuclear *MIP1* gene is an enzyme that displays homology to *Escherichia coli* type-I polymerases, bacteriophage and eukaryotic DNA polymerases, and reverse transcriptase (Blanco et al., 1991). In addition to recognizable similarity to the 3′-5′ exonuclease and polymerase domains of the *E. coli* Klenow polymerase, MIP1 contains a C-terminal region of approximately 300-aa not present in other DNA polymerases.

Another protein displaying high homology to its *E. coli* counterpart is MSH1, which is presumably part of the mitochondrial mismatch-proofreading system. Isolated by virtue of its sequence homology to the *E. coli* MutS gene, MSH1 is essential for maintenance of wild type mtDNA, with disruption leading to mutagenesis and large-scale rearrangements (Reenan and Kolodner, 1992).

C. Shared Proteins

At least one protein involved in recombination/repair of yeast mtDNA is also required for

DNA transactions in the nucleus. *PIF1 (TST1)* is an ATP-dependent 5′-3′ ss-DNA helicase and shares additional sequence motifs with the *E. coli* RecC and D polypeptides. The protein is required for recombination, repair, and maintenance of mtDNA, with *pif1* cells being respiratory deficient, deficient in certain recombination activities, and strongly inclined to degrade their mtDNA at 36° (Foury and Van Dyck, 1985; Lahaye et al., 1991; Lahaye et al., 1993). Like a number of other dual-location proteins (see Section X.B), the coding sequence of *PIF1* contains two in-frame initiation codons the use of which results in the production of proteins that differ in length at their amino-terminus. The longer product is imported into mitochondria. The shorter form is found in the nucleus, where it affects telomere length and the frequency of *de novo* telomere formation (Schulz and Zakian, 1994).

A second possible dual-function DNA-binding protein is ABF2 (HIM1) (Diffley and Stillman, 1991; Kao et al., 1993), closely related to the vertebrate high-mobility group protein HMG-1 and other HMG-box proteins, among which is the nuclear-encoded human mitochondrial transcriptional activator mtTF1 (Parisi et al., 1993). ABF2 is an abundant, nuclear-encoded mitochondrial protein and is required for maintenance of the yeast mitochondrial genome, to which it binds at many sites, including at least one replication origin (Diffley and Stillman, 1991). *In vitro,* ABF2 also binds to replication origins in nuclear DNA and at least half of the intracellular protein has been reported to be recoverable from the nucleus in subcellular fractionations (Diffley and Stillman, 1992). This distribution is, however, not observed in immunofluorescence studies and the possibility of some kind of redistribution during fractionation cannot fully be ruled out. It should also be noted that while ABF2 and mtTF1 share similarity in sequence and mode of binding to DNA and mtTF1 can even rescue the loss of mtDNA phenotype of *abf2*^{0}-mutant cells, there is no indication that the proteins fulfill similar roles in transcription in their respective mitochondria. In contrast to mtTF1, ABF2 is not required for selective initiation, nor does it stimulate transcription by mitochondrial RNA polymerase *in vitro* (Xu and Clayton, 1992).

D. Maintenance of mtDNA and Mitochondrial Translation

A puzzling aspect of mtDNA maintenance is the dependence of this process on mitochondrial translation, at least in wild type cells. A block in translation caused either by mutations in general translational components, or by growth of cells in the presence of inhibitors of mitochondrial translation leads to appearance of rho$^-$, or rho^0 cells, which, respectively, contain large deletions in, or lack mtDNA entirely. Nevertheless, the rho$^-$ petite cells, which also lack a functional translation system, are capable of the faithful propagation of their highly amplified mitochondrial genomes (Weislogel and Butow, 1970; Williamson et al., 1971; Myers et al., 1985). The explanation usually put forward for this phenomenon is that replication of wild type, but not petite mtDNA, requires an as yet unidentified mitochondrial translation product (Myers et al., 1985). It implies that there are important mechanistic differences in the replication or transmission of wild-type and petite mtDNAs. This is conceivable. Cells can partition single mitochondrial genomes and a single molecule partitioned at cell division is apparently sufficient to permit restoration of the normal complement of multiple copies (Jones and Fangman, 1992). Transmission of wild-type mtDNA could thus be much more sensitive to compromised mitochondrial function caused by lack of mitochondrial translation products than that of highly amplified petite mtDNAs. Puzzling aspects remain, however. First, all major products of mitochondrial translation have been identified, so this "replication factor" would have to be a minor product. Second, stringent mutations in the mitochondrial gene for $tRNA_{Asp}$ do not lead to loss of mtDNA (Myers et al., 1985), so this putative product would have to lack aspartate residues.

Maintenance of wild type yeast mtDNA also requires the product of the *PIM1* gene. *PIM1* encodes an La-like protease, which is also found in mitochondria of several other organisms (see Goldberg, 1992 for review). Disruptants of *PIM1* are respiratory deficient, display a marked tendency to lose their mtDNA (Suzuki et al., 1994; van Dyck et al., 1994), and are impaired in their

ability to degrade misfolded imported mitochondrial proteins (Wagner et al., 1994). Loss of mtDNA could, of course, simply be an indirect consequence of one of the other two defects, perhaps again reflecting an inability to maintain an electrochemical gradient across the inner membrane. On the other hand, it should be remembered that the *E. coli* La-protease has DNA-binding activity and that the enzyme was originally isolated on the basis of this ability (Zehnbauer et al., 1981). There may therefore be a more interesting role for PIM1 still to be discovered (see also Section XI.C).

E. Mitochondrial Inheritance

Finally, maintenance and transmission of yeast mtDNA is additionally dependent on a number of nuclear genes, whose functions are not yet clearly defined, but which are likely to exert their effects by way of involvement in the inheritance of the organelle itself. Mutations in these genes typically result in abnormal mitochondrial morphology and failure, or reduced ability to transfer their mitochondria to daughter cells during cell division. As might be expected, a number of these genes are essential to viability, because besides their role in respiration and energy conservation, mitochondria supply other functions essential for cell proliferation and survival (Gbelska et al., 1983; Yaffe and Schatz, 1984). Genes with a possible role in organelle inheritance are listed in Table 1. *MDM1* encodes an essential protein, displaying sequence similarity to mammalian vimentin and cytokeratin, which are both components of the intermediate filament network. Antibodies directed against MDM1 recognize novel cytoplasmic structures in yeast and the intermediate filament network in animal cells, suggesting that cytoskeletal structures mediate mitochondrial inheritance. Additional support for the interaction of mitochondria with the cytoskeleton comes from the characterization of the *MGM1* gene, encoding a protein highly related to a family of GTP-binding proteins that includes the microtubule-binding protein dynamin (Jones and Fangman, 1992; Guan et al., 1993). Mutation in or deletion of the *MGM1* gene results in temperature-sensitive loss of mtDNA and, as in the other mutants in this group, the appearance of abnormal mitochondrial structures.

MDM2, MDM10, MMM1, and YME1 are mitochondrial proteins with an involvement in organelle inheritance. MDM10 and MMM1 are integral components of the outer membrane. YME1 appears to be membrane attached, but further details of its location are not known. Mutations in all four genes result in abnormal mitochondrial morphology, impaired mitochondrial movement and distribution, and disturbances in function. For MDM2, a Δ9-fatty acid desaturase, this is presumably the consequence of a requirement for unsaturated fatty acids in one or more of these processes. For the other proteins, the basis of their involvement is less obvious. A function in mediating interactions with the cytoskeleton is one possibility and indeed has been proposed for MDM10 and MMM1 (Burgess et al., 1994; Sogo and Yaffe, 1994). For YME1, results of second-site suppressor analysis are consistent with a similar function (see Section XI.C for discussion).

F. Petite-Negativity

An area that deserves further study is the exact relationship between cell survival, mitochondrial inheritance, and maintenance of mtDNA. As stated above, mitochondria fulfill functions additional to those in energy metabolism and the presence of a functional mitochondrial compartment is essential for cell proliferation. Assembly of a functional respiratory chain is dependent on expression of genes in mtDNA, while other functions are not. Both wild-type *S. cerevisiae* (Slonimski et al., 1968) and animal cells (Desjardins et al., 1985) are able to tolerate the loss of a functional mtDNA, provided certain nutritional or genetic criteria are met. It is therefore intriguing that many yeasts are naturally unable to tolerate loss of mtDNA (petite-negative) and that certain nuclear mutations can impair this tolerance in *S. cerevisiae* (Janitor and Subik, 1993), or create it in other yeasts (Maleszka and Clark-Walker, 1989; Haffter and Fox, 1992). Whereas little is known about the latter class of mutations, recent sequence analysis of the *PEL1*

TABLE 1
Proteins Involved in Mitochondrial Inheritance in Yeast

Gene	Location	Essential?	Sequence similarity/function	Ref.
MDM1	Cytoskeletal structures	Yes	Vimentin; cytokeratin	McConnell and Yaffe (1992)
MDM2	Endoplasmic reticulum	?	Allelic to OLE1; Δ9-fatty acid desaturase	Stewart and Yaffe (1991)
MDM10	Mitochondrial OM	Yes at 37°	Identical to FUN37	Sogo and Yaffe (1994)
MGM1	??	No	Dynamin gene family	Guan et al. (1993);Jones and Fangman (1992)
MMM1	Mitochondria (outer membrane)		N.D.	Burgess et al. (1994)
YME1	Mitochondria	Synthetic lethal with rho⁻ mutation	AAA-family protein	Thorsness et al. (1993)

petite-lethality gene in *S. cerevisiae* shows that it is likely to encode a phosphatidylserine synthetase (Janitor and Subik, 1993). By analogy with the role of the *MDM2* gene (see above), petite-lethality may in this situation derive from an impairment in mitochondrial inheritance. *yme1* mutations also effectively convert *S. cerevisiae* into a petite-negative yeast. A single-point mutation in the ATP3 gene encoding the γ-subunit of the F1-ATP synthase is able to suppress this effect (Weber et al., 1994). The basis of this suppression is as yet unknown.

IV. TRANSCRIPTION

A. Promoters, RNA Polymerase, and Accessory Factors

Transcription of metazoan mtDNAs is initiated from a single promoter region and attenuation, processing, and stability are used as mechanisms to control levels of individual transcripts. In contrast, *S. cerevisiae* mtDNA contains 19 to 20 transcriptional initiation sites scattered around the genome (Christianson and Rabinowitz, 1983; Edwards et al., 1983), thus offering considerably more scope for differential controls of gene expression at the transcriptional level. Transcription starts within the sequence (A/T)TATAAGTA, with the last A being the site of initiation. Sequences outside this motif probably also contribute to promoter activity, as the motif is found at several sites in the genome, which lack corresponding transcripts (Edwards et al., 1983; Bordonné et al., 1987). In addition, the various promoters differ markedly in strength as measured by their ability to compete *in vitro* for limiting amounts of RNA polymerase (Biswas and Getz, 1986; Wettstein-Edwards et al., 1986). Features of this enzyme, which bears a strong resemblance to the RNA polymerases of bacteriophages T3, T7, and SP6, have been covered elsewhere (Schinkel and Tabak, 1989). It is, however, remarkable that the 153-kDa mitochondrial enzyme, in contrast to its smaller bacteriophage counterparts (circa 100 kDa), requires an additional specificity factor for promoter recognition and stabilization of the polymerase-promoter complex (Schinkel et al., 1988). This factor is the product of the *MTF1* gene, a protein with a molecular weight of 43 kDa. *MTF1* can act as a multicopy suppressor of a *ts*-mutation in the catalytic subunit, while its inactivation leads to the rapid loss of mtDNA (Lisowsky and Michaelis, 1988; Lisowsky and Michaelis, 1989). Interestingly, sequence comparison of MTF1 with the family of bacterial sigma factors reveals similarity to domains involved in –10 promoter recognition, promoter melting, and holoenzyme stability (Jang and Jaehning, 1991).

A second gene thought initially to encode a transcription factor is *MTF2* (Lisowsky, 1990), which, rather surprisingly, turns out to be identical to *NAM1*, a gene cloned on the basis of its ability to act as a high copy number suppressor of splicing defects caused by certain mitochondrial intron mutations (Ben Asher et al., 1989; see below). The properties of cells carrying a disrupted *NAM1* sequence suggest that the gene encodes a factor that specifically functions in the translation of the *COX1* mRNA. Its involvement in transcription could be indicative of the existence of independent functional domains within the protein, but direct evidence for interaction of the *MTF2* product with the catalytic subunit of RNA polymerase has yet to be obtained.

B. Transcriptional Termination

Surprisingly little is known of the sequences that signal termination or attenuation of transcription, despite the fact that attenuation may play an important role in the regulation of expression of promoter-distal genes in multigenic transcripts (Mueller and Getz, 1986). Although the 3′-ends of most mRNAs have been found to lie in (Osinga et al., 1984), or a couple of nucleotides downstream of (Hofmann et al., 1993) the dodecamer motif AAUAA(U/C)AUUCUU, this sequence appears to be primarily a site for endonucleolytic cleavage (see below).

V. RNA PROCESSING

Apart from RNA splicing, which will be dealt with in later sections, expression of *S. cerevisiae*

mtDNA is heavily dependent on one or other form of RNA processing carried out by nuclear-encoded, imported enzymes. Many genes lack their own promoter and are transcribed as part of larger transcriptional units (Osinga et al., 1984), mature RNAs being generated by endonucleolytic cleavage and trimming. Sequences cotranscribed in this way form a motley collection, and as might be expected, additional controls operate to adjust levels of individual transcripts or their products to meet the needs of the organelle. In addition to transcriptional attenuation, processing serves to control the rate at which mature transcripts arise and, as discussed below (see Section VI), also plays a role in determining their stability.

In the case of tRNAs, modifications include 5′- and 3′-trimming, -CCA addition, and base modification (see Hopper and Martin, 1992 for review). 5′-Maturation is carried out by an RNAse P-type enzyme, whose RNA moiety is encoded by a mitochondrial gene, the tRNA synthesis locus, *RPM1* (Miller and Martin, 1983). The protein component of this enzyme is the product of the nuclear *RPM2* gene (Dang, 1993), which, somewhat surprisingly, lacks obvious homology to any other known protein. The nuclear counterpart of RPM2 has not yet been cloned, however. Less is known about processing events at the 3′-terminus of tRNAs, apart from the demonstration of an endonuclease activity in crude mitochondrial extracts (Chen and Martin, 1988). Interestingly, in view of the sequence differences of their substrates, many of the enzymes involved in base modifications in mitochondrial tRNAs have dual locations in the cell and carry out the same job in the nucleus on tRNAs destined for the cytoplasm (Wolfe et al., 1994 and references therein).

Unlike the situation in protozoan and plant mitochondria, there is no evidence that editing of mRNAs occurs in yeast mitochondria.

VI. RNA STABILITY

Sequences marking the sites of stable 5′-termini have not been defined, but as mentioned above, stable 3′-termini appear to be generated by cleavage at or downstream of the dodecamer motif. Besides marking the site for cleavage of pre-mRNAs, this sequence may play an important role in stabilization by binding a protein complex that protects transcripts from the action of a progressive 3′-exoribonuclease (Min et al., 1993; Min and Zassenhaus, 1993).

Several nuclear genes influence the stability of mitochondrial transcripts in a more or less specific fashion. Of the transcript-specific proteins, CBP1, required for the stabilization of the cytochrome *b* mRNA, is possibly the best characterized. *cbp1* mutant strains lack cytochrome *b* mRNA, while maintaining relatively normal levels of the $tRNA^{glu}$ that is cotranscribed with the cytochrome *b* gene and derived from the primary transcript by processing. Generation of the mature cytochrome *b* requires three processing steps — one at the 3′-end of the tRNA; the others 100 and 144/5 nucleotides downstream at positions –999 and –955/954, respectively, relative to the initiator AUG. Cleavage at the most downstream sites yields the 5′-end of the mature mRNA. Deletion analysis shows that sequences lying between –961 and –898 are sufficient to confer CBP1-mediated stability on both precursor and mature cytochrome *b* transcripts, with the region –948 to –938 being particularly important (Mittelmeier and Dieckmann, 1993) both in stabilization and the correct positioning of the –955/954 processing sites. More recent work (Chen and Dieckmann, 1994) has shown that deletion of the $tRNA^{glu}$-cytochrome *b* intergenic region still allows mature mRNA to be generated in a single cleavage reaction, but does not relieve the requirement for CBP1, thereby strongly implying that the protein is required for stabilization both before and after cleavage, perhaps by formation of a protective complex.

Two other nuclear genes (*AEP1/NCA1* and *AEP2/ATP13*) resemble *CBP1* in being required for the maturation and stability of a single mitochondrial mRNA (Ackerman et al., 1991; Finnegan et al., 1991; Payne et al., 1993; Ziaja et al., 1993). Absence of either one of the proteins results in specific destabilization of the mRNA for ATP synthase subunit 9, which is cotranscribed with $tRNA^{Ser}$ and *VAR1*. Both proteins contain putative mitochondrial targeting sequences, but lack any other obvious sequence motifs that would

give clues to their mode of action. *AEP2* may have an additional function in translational activation (see Section X.E).

Even less is known of the action of other genes influencing RNA stability, often because of the complex phenotypes that mutations within them produce. Good illustrations of the kinds of problems involved are given by the *NAM1* and *suv3* genes (Table 2). *NAM1/MTF2* was first isolated as a multicopy suppressor of mitochondrial splicing deficiencies resulting from mutations in introns aI1 and bI2 (Ben Asher et al., 1989) and later was reisolated as *MTF2*, a gene capable of complementing a *ts*-respiratory-deficient mutant and identified as a possible transcription factor (Lisowsky and Michaelis, 1989; Lisowsky, 1990). Inactivation of *NAM1* in different mitochondrial backgrounds creates different phenotypes superficially suggestive of involvement in RNA splicing. Thus, inactivation in intron-containing strains leads to a reduction in the overall synthesis of mitochondrial proteins and strong reduction or complete absence of cytochrome *b* and *cox1* mRNAs, as well as of *atp6/ens2* transcripts, which are cotranscribed with *cox1*. In intronless strains, mitochondrial protein synthesis is normal and only the *atp6/ens2* transcript is decreased in amount. Unlike typical splicing mutants, however, intron-containing strains do not accumulate unspliced transcripts (Groudinsky et al., 1993). Taken together, these observations suggest that the *NAM1* product is not directly involved in either transcription, splicing, or translation, thus prompting the speculation (Groudinsky et al., 1993) that the protein plays a role in the processing or stabilization of *atp6* and intron-containing transcripts of the *COX1* and *COB* genes.

The *suv3* gene encodes a putative "DEAD-box" RNA helicase, whose role in mitochondrial RNA metabolism remains far from clear. A Val-to-Leu substitution in the second of eight sequence blocks conserved between this family of helicases produces the dominant suppressor allele *SUV3-1* (Stepien et al., 1992). Originally isolated as a suppressor of a deletion of the dodecamer processing site close to the 3′-end of the *VAR1* (mitoribosomal protein) gene in mtDNA (Conrad-Webb et al., 1990), *SUV3-1* was proposed to act as a translational enhancer of the aberrant VAR1 mRNA arising from the mutant gene. In a wild-type background, however, *SUV3-1* has pleiotropic effects on many mitochondrial transcripts, including increased abundance of the excised intron in the LSU rRNA gene and of six of seven other group I introns tested. In addition, *SUV3-1* decreases steady-state levels of both cytochrome *b* and Cox1 mRNAs, the latter effect deriving from impaired splicing of intron aI5b. Despite the reduced mRNA levels, amounts of cytochrome *b* and Cox1 proteins are comparatively normal, suggesting that the mutant suv3 protein enhances translation. Disruption of suv3 results in petite formation. By analogy with other members of this helicase family, suggested roles for the suv3 helicase include recycling of components of splicing complexes and translational initiation. Interesting in this latter context is the fact that SPB4, another yeast member of the family, acts to suppress the block in translational initiation resulting from deletion of PAB1, the poly(A)-binding protein (Sachs and Davis, 1990). Because *SUV3-1* permits translation of a VAR1 mRNA with an aberrant 3′-end, translational initiation in both mitochondria and cytoplasm could depend on interactions between 5′- and 3′-ends of mRNAs, and a common feature could be the dependence of these interactions on "DEAD-box" helicases.

An important principle emerging from the case histories described above is that there is a strong linkage between RNA processing and stability. Such a linkage is not peculiar to yeast mitochondria. In chloroplasts, several examples of proteins resembling CBP1 in their action have been described (Nickelsen et al., 1994 and references therein), while in plant mitochondria, the phenomenon of nuclear restoration of fertility to a cytoplasmic male sterile line of sunflower is associated with tissue-specific destabilization of a single mRNA that is cotranscribed with the atpA gene (Moneger et al., 1994).

VII. RNA SPLICING: RIBOZYMES AND MATURASES

As shown in Figure 1, the *S. cerevisiae* mitochondrial genome contains three split genes, the number of introns in each gene being strain de-

TABLE 2
Nuclear Gene Products Involved in Mitochondrial RNA Splicing

Nuclear gene	Introns involved	Sequence similarity; (other) function	Ref.
Category 1 — Directly involved in Splicing			
NAM2	al4, bl4	mt leucyl tRNA synthetase	Labouesse (1990); Li et al. (1992); Zagorski et al. (1991)
CYT18	Group I in *N. crassa*	mt tyrosyl tRNA synthetase	Akins and Lambowitz, (1987); Kittle et al. (1991)
YTS1	Group I in *P. anserina*	mt tyrosyl tRNA synthetase	Kämper et al. (1992)
CBP2	bl5	None known	Gampel et al. (1989); Gampel and Cech (1991)
MRS1/PET157	al5b, bl3	Homologous to CCE1 (cruciform-cutting endonuclease)	Bousquet et al. (1990)
PET54[a]	al5b	COX3 translation	Valencik and McEwen (1991)
MRS2	Group II	Membrane assembly	Wiesenberger et al. (1992)
Category 2 — Required for Maturase Synthesis			
MSS51[b]	al1, al2, al4, al5c	COX1 translation	Simon and Faye (1984); Decoster et al. (1990)
MSS18	al5b	None known	Séraphin et al. (1988)
MSS116	Several COX1, COB	Member of DEAD-box RNA helicase family	Séraphin et al. (1989)
MRF1	al1, al2	Translational release factor	Pel et al. (1992)
Category 3 — Multicopy Suppressors of Splicing Defects			
MRS3	bl1	Mitochondrial carrier	Wiesenberger et al. (1991)
MRS4	bl1	Mitochondrial carrier	Wiesenberger et al. (1991)
MRS6/MSI4	Group II	Component A of geranylgeranyl transferase	Waldherr et al. (1993); Ragnini et al. (1994); Fujimura et al. (1994); Jiang and Ferro-Novick (1994)
MRS5,7,11,12,13	Group II	None known	Schweyen (1994)
NAM1/MTF2	bl2, al1	Transcription/RNA stability	Wallis et al. (1994); Groudinsky et al. (1993); Lisowsky and Michaelis (1989); Lisowsky (1990); Lisowsky et al. (1990); Ben Asher et al. (1989)
NAM7/UPF1	bl2, al1	Zn-finger protein; RNA helicase? Related to *SEN1*-tRNA endonuclease	Ben Asher et al. (1989); Leeds et al. (1992); Altamura et al. (1992); Altamura et al. (1994)
NAM8	bl2, al1	Contains RNP RNA-binding motifs	Ben Asher et al. (1989); Ekwall et al. (1992)
Category 4 — Pleiotropic, with Effects on RNA Splicing			
CYT-4		Effects on 3′-end formation; RNA stability; similarity to SSD1/SRK1 protein phosphatases	Turcq et al. (1992); Dobinson et al. (1989)
SUV3	al5b, bl3	Member of DEAD-box RNA helicase family; dodecamer processing? Translation?	Conrad-Webb et al. (1990); Stepien et al. (1992)

[a] PET54 is also required for translation of the COX3 mRNA (see Table 3).
[b] MSS51 appears to be a specific translational activator for COX1 mRNA (see Table 3).

pendent. The introns can be divided into two groups (I and II) on the basis of differences in their predicted secondary structure and in the mechanism by which self-splicing occurs *in vitro* (Lambowitz and Belfort, 1993). The group I introns resemble those found in the nuclear DNAs of lower eukaryotes, in bacteriophage and eubacteria. Many possess self-splicing activity, which proceeds via coupled transesterification reactions triggered by binding of a guanine nucleotide in the neighborhood of the catalytic site. For many group I introns, a circular RNA arising from circularization of (part of) the excised intron is a byproduct of the splicing reaction. In the case of yeast mitochondria, the only group I intron giving rise to an abundant, stable circular transcript is that present in the LSU rRNA gene; other group I intron transcripts appear to be rapidly degraded.

Like other organellar and bacteriophage group I introns, most of the introns in yeast mitochondria contain an ORF, which encodes either a protein required for splicing (an RNA maturase), or a sequence-specific DNA endonuclease that acts in intron spreading via a recombinative process known as homing (see below; Dujon, 1989). Except aI5b and the LSU rRNA gene, these intronic ORFs are in frame and continuous with their upstream exons and are presumably translated by ribosomal readthrough into the intron. Several lines of evidence suggest that the resulting chimeric proteins are subsequently cleaved to release the intron-encoded sequence (de la Salle et al., 1982; Weiss-Brummer et al., 1982; Banroques et al., 1986). Whether this cleavage depends on a specific protease or is self-catalyzed (cf. Shub and Goodrich-Blair, 1992) is not known. In *S. cerevisiae*, both DNA endonucleases and RNA maturases belong to a single protein family characterized by two dodecapeptide sequences containing the motifs LAGLI-DADG and spaced some 100 amino acids apart. In other yeasts and bacteriophage, however, a second endonuclease type is found, distinguished by the presence of the short but characteristic sequence motifs GIY . . . YIG, which are separated by some 10 to 15 amino acids (Tian et al., 1991). LAGLI-DADG endonucleases have been encountered outside mitochondria, with the HO mating type switch endonuclease and the recently characterized VDE1 endonuclease being among the better-known representatives (Russell et al., 1986; Gimble and Thorner, 1992). Mitochondrial endonuclease and maturase activities are distinct manifestations of common features. Thus, the bI2-encoded proteins of *S. cerevisiae* and *S. capensis*, which are thought to possess maturase and endonuclease activities, respectively (Lazowska et al., 1992), differ in only 5 out of 280 residues. In the case of the aI4 (endonuclease) and bI4 (maturase) ORFs, which display some 70% sequence identity, both proteins are, when produced in *E. coli*, capable of binding to DNA with high affinity (Goguel et al., 1989; Perea et al., 1990). Only single-amino acid changes are required to convert the aI4 endonuclease into a protein capable of supporting RNA splicing. These changes may be in the aI4 protein itself (as in *mim2*-1 a glu to lys substitution at position 64; Dujardin et al., 1982), or in the mitochondrial leucine aminoacyl-tRNA synthetase, product of the nuclear *NAM2* gene, which normally cooperates with the bI4 maturase in the splicing of introns of aI4 and bI4 (Herbert et al., 1988). However, after mutation of a glycine residue at position 240 to either a serine or cysteine, *NAM2* can cooperate with the aI4 endonuclease to do the same job (Labouesse et al., 1987). In the *S. cerevisiae* aI4 and bI4 maturases, endonuclease and maturase activities appear to be mutually exclusive functions, because in bI4/aI4 domain-swapping experiments (Goguel et al., 1992), not one of 40 chimeric forms with RNA maturase activity was active as a DNA endonuclease. In *S. pombe*, however, both mobility and splicing of the first intron in the gene for cox1 are dependent on the same intron-encoded ORF, a member of the LAGLI-DADG family (Schäfer et al., 1994).

The group II introns in yeast mtDNA resemble those found in fungal and plant mitochondria, chloroplasts, and, more recently, cyano- and proteobacteria (Ferat and Michel, 1993). They share a structure distinct from that of group I introns. Some members of the group are capable of self-splicing in a reaction that displays mechanistic similarities to that catalyzed by spliceosomes

in the cell nucleus. As with group I introns, splicing occurs by way of two coupled transesterifications, but in this case the reaction is initiated by nucleophilic attack of an intron-internal nucleotide on the 5′-exon/intron junction (Saldanha et al., 1993 and references therein). The excised intron therefore accumulates in the form of a lariat containing a 5′–3′, 2′ branched nucleotide. Splicing is reversible and, given appropriate conditions, can be demonstrated *in vitro*. Interestingly, a group II intron can undergo a reverse splicing reaction with a foreign RNA species, provided that this RNA contains a short sequence motif complementary to the intronic sequence UCUGUC (exon-binding site EBS1) (Mörl and Schmelzer, 1990). During the forward reaction, EBS1 undergoes base pairing with a motif of the 5′-exon known as the intron-binding site (IBS1).

Reading frames, when present in group II introns, also appear to encode maturases involved in RNA splicing, as demonstrated genetically for the introns aI1 and aI2 (Carignani et al., 1983; Moran et al., 1994). These proteins show little resemblance to group I intron reading frames, but do in several cases exhibit significant sequence similarity to reverse transcriptases, in particular to those encoded by the LINE1-like, or non-LTR class of retroid elements (Michel and Lang, 1985). The sequence similarity extends across the seven conserved sequence domains present in all functional reverse transcriptases (Xiong and Eickbush, 1990). In yeast mitochondria, reverse transcriptase-like proteins are encoded by the group II introns aI1 and aI2, and reverse transcriptase activity that is dependent on the presence of a functional copy of one or other of the introns has recently been demonstrated biochemically (Kennell et al., 1993). The intron ORFs additionally display weak sequence similarity to a retroviral protease domain and contain a Zn^{2+}-fingerlike motif close to their C-terminus (Mohr et al., 1993). Part of this latter element contains a conserved H-N-H motif, which is also found in a wide range of group I endonucleases, leading to the suggestion that group II ORFs may also possess endonuclease activity (Shub et al., 1994). Between the reverse transcriptase and Zn^{2+}-finger domains lies a conserved region (X), which is sometimes the sole motif present. The conserved nature of the region and the finding of missense mutations that prevent splicing suggest that it has an essential function in maturase activity (Moran et al., 1994).

VIII. RNA SPLICING: NUCLEAR-ENCODED ACCESSORY FACTORS

Nuclear-encoded proteins are also required for RNA splicing *in vivo*. These can exert their influence on the splicing of only a single intron, on several introns belonging to the same group, or even on different groups of intron. Proteins so far identified are listed in Table 2, following lines laid down in earlier reviews (Bolotin-Fukuhara and Grivell, 1992; Pel and Grivell, 1993). Three main groups can be distinguished, with a fourth containing proteins exerting pleiotropic effects on several aspects of RNA metabolism, including splicing.

The first group contains proteins likely to function directly in RNA splicing, although in most cases an interaction with intron RNA remains to be directly demonstrated. The mechanism of action of CBP2, which acts exclusively on bI5, the terminal intron in the cytochrome *b* gene is relatively clear: the protein enhances splicing of the group I intron bI5 *in vitro* by reducing the dependence of catalytic activity of the RNA on GTP and Mg^{2+} ions, while leaving the basic transesterification mechanism of self-splicing unaltered (Gampel et al., 1989). It does this by binding either directly to the catalytic core of the intron or to sequences that depend for their exposure on the core's integrity (Gampel and Cech, 1991). A similar mode of action may hold for the amino-acyl-tRNA synthetases encoded by *CYT-18* in *Neurospora*, by *yts1* in *Podospora* and by *NAM2* in yeast. The tyrosyl-tRNA synthetase encoded by *CYT-18* is possibly the best studied of the group. In addition to its involvement in the splicing of group I introns in *Neurospora* mitochondria, the enzyme can promote splicing of mutant versions of the distantly related td group I intron in bacteriophage T4. It does this by binding to and stabilizing the catalytic core (Mohr et al., 1992), perhaps by recognizing features with struc-

tural similarities to its natural tRNA substrate (Guo and Lambowitz, 1992).

Among these relatively clear-cut cases, *MRS2* is something of an oddity. The gene is a multicopy suppressor of splicing deficiency caused by the insertion of a single base pair in a conserved stem in domain III of the group II intron bI1 and can suppress a number of other intron mutations (Wiesenberger et al., 1992). Disruption of *MRS2* results in a block of the excision of all four mitochondrial group II introns and respiratory deficiency. Curiously, however, intronless *mrs2* disruptants also display a respiratory and cytochrome-deficient phenotype, even though the standard pattern of mitochondrial translation products is observed (Wiesenberger et al., 1992). The protein's role in splicing may therefore be either dependent on, or distinct from a second function in mitochondrial membrane assembly.

The second and third groups contain proteins whose involvement in splicing is indirect. For the second group, which in the main appears to consist of proteins required for translation of Cox1 mRNA, involvement in splicing can be rationalized in terms of a need for translation as a necessary step in the synthesis of maturases.

In contrast, the third group is a heterogeneous one, consisting of proteins that when overproduced are capable of extragenic suppression of splicing defects. Within this group *MRS2, 3,* and *4* share the ability to restore splicing to the same defective group II intron as used in the identification of *MRS2* (Söllner et al., 1987). *MRS3, 4,* and others in the series were later identified on the basis of their ability to restore respiratory competency to an *mrs2* disruptant (Waldherr et al., 1993). These suppressors can be broadly divided into two groups, depending on whether they restore respiratory function to strains containing introns (*MRS3, 4, 5, 7, 11, 12, and 13*) or lacking introns (*MRS6, 14, 15, 16, and 17*). Three of the genes within the former group (*MRS3, 4,* and *12* [=*RIM2*]) have sequence homology to mitochondrial carrier proteins (Wiesenberger et al., 1991; van Dyck et al., 1994) and may function in the transport of as yet unidentified solutes. It has been speculated that the involvement of these proteins in splicing is indirect, perhaps attributable to changes in RNA structure induced by changes in the ionic balance within mitochondria. *MRS5* and *11* are related in sequence, but *MRS11*, which is considerably shorter than *MRS5*, does not cross-complement lethality caused by *MRS5* disruption (Schweyen, 1994). Of the genes within the second group that are presumably capable of substituting for the membrane assembly function of *MRS2* or compensating for its loss, *MRS6/MSI4* is the best characterized, turning out to be the yeast equivalent of the human choroideraemia gene (Ragnini et al., 1994). This gene encodes the component A escort protein of the geranylgeranyl transferase (Fujimura et al., 1994; Jiang and Ferro-Novick, 1994). It is required for the modification and consequent membrane attachment of members of the family of small GTP-binding proteins (Rabs in mammalian cells; Ypt and Sec4 in yeast), which regulate vesicular traffic in all eukaryotic cells. As *MSI4, MRS6* has been isolated as a multicopy suppressor of the heat shock sensitivity of an *ira1-1* mutant and can suppress the heat shock phenotype of either $RAS2^{Val-19}$ or *bcy1* mutants. Whether the *mrs2*-suppressor activity of this protein derives from a function in or outside the mitochondrion still remains to be determined.

Like *MRS6*, the role of *NAM7* in mitochondrial splicing is difficult to explain. First isolated as a multicopy suppressor of a mutant defective in the splicing of intron bI2 (Ben Asher et al., 1989), *NAM7* is identical to *UPF1*, a gene whose product mediates selective destabilization of cytosolic unspliced pre-mRNAs and mRNAs containing nonsense mutations (Altamura et al., 1992; He et al., 1993). NAM7 displays extensive similarity to SEN1 an essential protein required for endonucleolytic cleavage of introns from precursor tRNAs (Leeds et al., 1992) and contains a number of motifs diagnostic for members of a superfamily of RNA helicases (Altamura et al., 1992). Like MRS6, it remains to be seen whether the effects of *NAM7/UPF1* overexpression on mitochondrial splicing result from mislocalization of the protein to the organelle, or from action on specific cytosolic mRNAs encoding mitochondrial (regulatory) proteins. The results of a subcellular localization are eagerly awaited.

Although it may seem paradoxical that despite the ability to self-splice *in vitro* so many other proteins should be required to help splicing along, this dependency is by no means exceptional: even the ribozyme from *Tetrahymena*

splices some 50-fold more efficiently *in vivo* than *in vitro* (Brehm and Cech, 1983), while the splicing reactions catalyzed by group I introns in bacteriophage T4 exhibit various features strongly suggestive of an involvement of facilitatory factors (Coetzee et al., 1994). In the case of fungal mitochondrial introns, speculations on the role of the accessory proteins have included (1) stabilization of correct intron folding (Davies et al., 1982), (2) prevention of aberrant side reactions (Tabak and Grivell, 1986), and (3) enhancement of the efficiency of RNA catalysis by electrostatic shielding and/or activation of reactive groups (Van der Veen et al., 1987). For the *Neurospora* CYT-18 protein, the mechanism of action seems clear-cut: the protein acts as a kind of RNA chaperone by binding specifically to the catalytic core, thereby thermodynamically stabilizing correct folding (Guo and Lambowitz, 1992). Assuming a close relationship between maturase and endonuclease activities, the DNA-binding properties of various LAGLI-DADG proteins suggest that a similar function could hold for this group of maturases. For example, when produced in *E. coli*, maturase bI4 stimulates rec A-D-dependent recombination, probably promoting initial strand exchange (Goguel et al., 1989). Additionally, the aI3 encoded endonuclease I-Sce III does not bind symmetrically to its target site in DNA, but has a higher affinity for a region in the upstream exon, which in RNA is involved in formation of a base-paired stem required for splicing (Schapira et al., 1993).

One of the striking features of mitochondrial RNA splicing is the diversity of cellular proteins involved in excision of the various introns in different organisms, suggesting that these proteins may have been recruited or press-ganged into this process fairly late in evolution (Lambowitz and Perlman, 1990). How this may have worked is nicely illustrated by recent results with the phage T4 group I introns, which suggest that any of a number of *E. coli* RNA-binding proteins, among them the ribosomal protein S12, can promote splicing *in vitro* by binding aspecifically to the intron and acting as RNA chaperones (Coetzee et al., 1994). The proteins may prevent formation of a collapsed conformation unfavorable to splicing (cf. Franzen et al., 1994) or promote conformational rearrangements once such structures have formed. The fact that in the experiments of Coetzee et al. (1994) other basic RNA-binding proteins do not stimulate splicing suggests that their role is not simply that of electrostatic shields. Interestingly, these experiments also demonstrate that S12 can reverse the inhibitory effect on splicing of the *sun*Y intron ORF and that splicing efficiency is inversely proportional to the length of the ORF coding sequence contained within the intron. These findings thus provide the rationale for an evolutionary development in which an initially self-splicing intron acquires mobility by virtue of the insertion of sequences coding for a homing endonuclease (see Section IX), but is thereby forced to compensate for the resultant reduced splicing efficiency by recruitment of cellular proteins.

IX. INTRON MOBILITY: HOMING AND TRANSPOSITION

Like the group I/II introns in many other organisms, those in yeast mtDNA are descended from, or still are mobile genetic elements. Both in yeast and other fungal mtDNAs, first suspicions of intron mobility were aroused by the discovery of relationships in sequence (Hensgens et al., 1983) that implied the existence of families whose members had arisen from common ancestors by a process of duplication and dispersal. Since then, other evidence for mobility involving acquisition and loss of mitochondrial introns has accumulated. For example, loss by "clean excision" of one or more introns from *S. cerevisiae* and *S. pombe* mtDNAs is a relatively frequent event, thought to be brought about by recombination with cDNAs produced by the action of a reverse transcriptase on spliced mRNAs (Gargouri et al., 1983; Schäfer et al., 1991). In *Podospora*, the phenomenon of senescence is associated with the massive accumulation of closed circular DNAs (senDNAs), which turn out to be cleanly excised, tandemly duplicated, and amplified group II introns (Osiewacz and Esser, 1984; Sainsard-Chanet et al., 1993). In *Euglena*, the acquisition of twintrons — elements consisting of at least two introns, with one or more copies of one type being inserted piggyback style into another — has also been attributed to a sequence of events involving re-

verse splicing, reverse transcription, and homologous recombination (Copertino and Hallick, 1991; Hong and Hallick, 1994; see below).

It is now clear that different mechanisms are operative in intron mobility, depending on intron type and on whether movement is to a cognate site in an intronless gene (homing) or to a new location (transposition). For yeast mitochondrial group I introns, it is ironic that the first experimental evidence for homing came from genetic analysis in 1971, long before the existence of the introns themselves had been recognized. In early genetic crosses (see Dujon, 1989 and references therein), markers subsequently mapped to the organellar large rRNA gene were found to display nonreciprocal recombination and highly biased transmission, with the alleles of one parent (ω^+) being overrepresented in the progeny at the expense of the other (ω^-). The explanation for this behavior came with the discovery of an optional intron in the rRNA gene of parents displaying high transmission (Bos et al., 1978; Faye et al., 1979). Analysis of mtDNA in progeny arising from such crosses showed that in all cases high transmission of parental markers was associated with a gene conversion event that resulted in acquisition of the intron by the other, initially intronless parent and extensive coconversion of flanking sequences (Jacquier and Dujon, 1985). The trigger for intron transfer turned out to be a double-strand break at the exon-exon junction of the intronless gene, produced by the action of the endonuclease encoded by the intron, a member of the LAGLI-DADG family mentioned above (Dujon et al., 1985). In the meantime, several other examples of intron homing have come to light. In each case, sequence-specific cleavage by an intron-encoded endonuclease is the initial event leading to integration of the intron at the corresponding site in an intronless gene (Strausberg and Perlman, 1978; see Belfort, 1990 for review).

Although group II introns also exhibit homing behavior, in some cases with efficiencies approaching 100% (Meunier et al., 1990), the DNA endonuclease-based mechanism described above for group I introns seems unlikely to apply. Homing of group I introns is insensitive to *cis*-acting mutations that block splicing (Wenzlau et al., 1989), whereas homing of group II introns requires splicing proficiency (Meunier et al., 1990). Homing of group I introns is accompanied by bidirectional coconversion of sequences in flanking exons (Jacquier and Dujon, 1985). In contrast, homing of group II introns is accompanied by a unidirectional coconversion that proceeds in a 3′–5′ direction toward the upstream exon (Lazowska et al., 1994). Both features strongly suggest that group II homing is an RNA-mediated process, but do not yet make clear what principles are involved. Thus, a simple model assuming recombination between intronless DNA and a cDNA derived from reverse transcription of an unspliced RNA accounts neither for the dependence of the process on splicing proficiency nor for the evident sequence specificity observed during intron transfer in interspecific crosses (Lazowska et al., 1994). The latter implicitly suggests involvement of a site-specific endonuclease and in this context, the suggestion (Shub et al., 1994; see above) that group II ORFs may also possess endonuclease activity assumes increased significance.

For both groups of introns, cleavage by a DNA endonuclease fails to account for efficient movement to new locations, because, depending on endonuclease, the target sequence recognized can be anything from 14 to 40 bp long, and the number of potential new sites in the mitochondrial genome must thus be limited. On the other hand, a mechanism involving reversal of the splicing reaction with insertion of intron sequences at either cognate or new sites, followed by reverse transcription and recombinative integration of the resulting cDNA into the genome, offers an effective mechanism of dispersal, as has been directly confirmed in both yeast and *Podospora anserina* (Mueller et al., 1993; Sellem et al., 1993; Grivell, 1994).

In both instances of intron transposition a number of issues remain unresolved. First, there is the question of what drives reverse splicing. *In vitro,* combination of a high concentration of intron lariats with conditions that slow the forward reaction will yield small amounts of intron-containing RNAs. Such circumstances may be approached in *Podospora,* in which high concentrations of both excised intron and transcripts of the targeted tRNA region are present. However, they are unlikely to hold for yeast, in which the insertion sites so far characterized turn out to lie in group I introns. Targets for reverse splicing are

thus partially spliced precursor RNAs, present in only low amounts in wild-type strains. One possible solution to this paradox is that intron insertion may also occur at DNA replication forks: either into RNA primers, or single-stranded DNA (Mueller et al., 1993), because for both groups of intron, the first step in splicing reversal has been observed with a DNA substrate containing the splice-junction sequence (Beaudry and Joyce, 1992; Mörl et al., 1992). Additionally, proteins involved in splicing may also facilitate the reverse reaction (Lambowitz and Belfort, 1993).

A second question concerns the mechanism whereby reverse transcription of intron sequences is initiated. Sequence analysis carried out in both yeast and *Podospora* reveals invasion by a complete intron, implying that reverse transcription is being initiated downstream of the insertion site in regions lacking any obvious similarity to the downstream exons. This would seem remarkably promiscuous behavior, even for an enzyme that may have a wider than normal priming specificity (Kennell et al., 1993).

X. TRANSLATION

Because general features of mitochondrial translation systems have been reviewed recently (Pel and Grivell, 1994), the following sections are restricted to a summary of the more unusual features of the translational machinery in yeast mitochondria and the implications these features have for the mechanism of protein synthesis and its control.

A. Ribosomes

Like the ribosomes of animal mitochondria, the 74S ribosomes in yeast mitochondria are richer in protein than their prokaryotic or cytosolic counterparts. They are dissociable into 54S and 37S subunits, which each yield more than 40 proteins in 2D-electrophoretic separations. The structural RNAs, with lengths of 1686 and 3273 nt, respectively, are intermediate in size between typical prokaryotic and eukaryotic rRNAs and conserve many of the features characteristic of the former (Sor and Fukuhara, 1983; Neefs et al., 1991). A typical 5S rRNA species is absent. In common with other mitochondrial rRNAs, those of yeast mitochondria display minimal posttranscriptional modification. Both rRNAs lack methylated bases and neither contains more than one pseudouridine residue. Ribose methylation is restricted to only two groups in the LSU rRNA, while the SSU rRNA appears to be completely unmodified (Klootwijk et al., 1975). For the LSU rRNA, this type of modification probably occurs on a universally conserved G residue that corresponds to G_{2251} in the peptidyl transferase center of *E. coli* 23S rRNA. The modification is introduced by the *PET56* gene product, a guanosine methyltransferase, and is essential for assembly of the 50S ribosomal subunit (Sirum-Connelly and Mason, 1993).

Of the 23 yeast mitoribosomal proteins whose primary sequence has been elucidated to date, all but a couple are essential for mitochondrial function, yet only 10 contain stretches of amino acids that display similarity to known *E. coli* ribosomal proteins (Kitakawa and Isono, 1991; Matsushita and Isono, 1993 and references therein). This lack of homology and the relatively large number of proteins compared to bacterial or cytoplasmic ribosomes could mean that mitoribosomes possess functions absent from ribosomes of free-living organisms. Among these could be the ability to interact with specific translational activators and the mitochondrial membrane (see below), and in this respect it is worth noting that of three ribosomal proteins thought to interact with one such activator, none displays similarity to any other known ribosomal protein (McMullin et al., 1990; Haffter et al., 1991; Haffter and Fox, 1992).

B. tRNAs, Modification Enzymes, and RNA Synthetases

Yeast mtDNA contains genes for 24 tRNAs that, despite fairly conventional structures, operate with a simplified set of decoding rules, thus allowing recognition of all codons (Bonitz et al., 1980). As in other mitochondria, AUA and UGA are recognized as methionine and tryptophan, respectively. The idiosyncratic recognition of the rarely used CUN family as threonine derives from the presence of a 3′-GAU-5′ leucine anticodon in

one of the two $tRNA^{Thr}$ genes. This gene contains an unusual anticodon loop and an off-center, presumably nonfunctional GGU threonine anticodon (Li and Tzagoloff, 1979). Interestingly, this tRNA gene is transcribed from the strand opposite to that containing all other genes and is to date the only coding sequence found on this strand.

As in Gram-positive bacteria and chloroplasts, Gln-tRNA is produced by transamidation of a missense Glu-$tRNA^{Gln}$ intermediate (Martin and Rabinowitz, 1976; Schön et al., 1988). In contrast to plant or protozoan mitochondria (Dietrich et al., 1992), there is no need for tRNAs to be imported from the cytoplasm. Nevertheless, import of a single tRNA species, a cytoplasmic $tRNA^{lys}$(CUU), has been observed (Martin et al., 1979). Its function is apparently not in translation and is so far unknown.

Products of at least four nuclear genes involved in posttranscriptional modification of mitochondrial tRNAs, *CCA1*, *TRM1*, *TRM2*, and *MOD5*, are also responsible for the modification of cytosolic tRNAs. Both the *CCA1* and *MOD5* products are localized to three sites in the cell, namely, mitochondria, nucleus, and cytosol (Boguta et al., 1994; Wolfe et al., 1994), while the *TRM1* product localizes to mitochondria and nucleus (Rose et al., 1992). The coding sequences of each of these genes contain multiple in-frame initiation codons the use of which results in the production of proteins that differ in length at their amino-terminus, the longer products being more efficiently imported into mitochondria. A dual location has also been reported for the valyl- and histidyl-tRNA synthetases, two of the 10 *S. cerevisiae* mitochondrial tRNA synthetases identified so far (reviewed in Tzagoloff et al., 1990). The mitochondrial leucyl-tRNA synthetase in yeast and the tyrosyl-tRNA synthetases in both *Neurospora* and *Podospora* all appear to be bifunctional mitochondrial proteins, with roles in the splicing of one or more group I introns as well as in amino acylation (Labouesse, 1990; Kittle et al., 1991; Kämper et al., 1992).

C. Elongation and Termination Factors

To judge from factors so far characterized, both elongation and termination phases of yeast mitochondrial protein synthesis also appear fairly conventional. Genes encoding yeast mitochondrial homologues of prokaryotic elongation factors EF-Tu and EF-G and termination factor RF-1 have been cloned and sequenced (Nagata et al., 1983; Vambutas et al., 1991; Pel et al., 1992) and all display considerable levels of sequence identity with their bacterial counterparts. EF-G and EF-Tu both display activity in combination with *E. coli* ribosomes (Rosenthal and Bodley, 1987), EF-Tu having a relatively low affinity for guanine nucleosides. Curiously, in light of such strong conservation of both structure and function, efforts to detect EF-Ts activity in yeast mitochondrial extracts using *E. coli* ribosomes and EF-Tu have so far failed (Rosenthal and Bodley, 1987). Whether this means that a mitochondrial Ts equivalent does not exist or that it differs significantly from the bacterial protein has yet to be resolved.

D. mRNA Structure

Even by mitochondrial standards, the mRNAs in yeast mitochondria are unusual. They lack caps (Christianson and Rabinowitz, 1983) and conventional (i.e., posttranscriptionally added) poly(A) tails (Moorman et al., 1978). In most cases, the 5′-leaders are long, AU-rich, and are littered with short open reading frames likely to mislead any ribosome intent on scanning for initiation codons. Scanning, if it occurs, is moreover likely to be made more hazardous by the presence in these leaders of short, strain-dependent G+C-rich clusters capable of forming highly stable secondary structures. Other mechanisms, such as entry at an internal landing site may therefore be used to guide the ribosome to the initiation site. How this site is recognized remains unclear. Sequence and structure elements additional to the initiator AUG must be involved, as shown by the results of studies carried out with *COX2* and *COX3* mRNAs in which the initiator AUG has been mutated to AUA (Folley and Fox, 1991; Mulero and Fox, 1994). Such mRNAs appear to be recognized correctly, albeit with reduced efficiency, despite the presence, respectively, of 4 or 98 upstream AUA codons. The elements responsible are unlikely to be the sequences complementary to a region of the small subunit rRNA that are present in most mRNAs (Li et al., 1982), because these

are found at different sites relative to the AUG start codon in the various leaders and an mRNA lacking them is still active in protein synthesis (Costanzo and Fox, 1988). Unfortunately, biochemical analysis of initial stages in mRNA recognition has been severely hampered because of a singular lack of success in the development of an mRNA-dependent *in vitro* translation system using yeast mitochondrial ribosomes. Failure to correctly recognize the start codon (Dekker et al., 1993) may be responsible for this. Rigorous procedures are required to free ribosomes from membranes and nascent polypeptide chains and these could possibly lead to the loss of mRNA-specific activators.

E. Translational Activation

A major distinguishing feature of yeast mitochondrial protein synthesis is that translation of at least five of the eight major mRNAs requires the action of one or more nuclear-encoded gene-specific translational activator proteins (Table 3). Increasingly strong circumstantial evidence suggests that these proteins function by interacting with the mitochondrial ribosome, with specific regions of the 5′-leaders, and with the inner membrane. As discussed in detail below for individual mRNAs and cognate factors, the proteins may play important roles in mRNA selection, recognition of the correct starting point for translation, and/or interaction of translationally active ribosomes with the mitochondrial membrane, thus facilitating insertion of translation products into the appropriate respiratory complexes.

1. Cytochrome c Oxidase Subunit 3 (COX3) mRNA

As shown in Table 3, products of three nuclear genes, *PET494*, *PET54*, and *PET122*, specifically activate translation of *COX3* mRNA, most probably by interaction with a region of the 5′-leader lying between its 5′-end at position –610 and position –173 relative to the initiator AUG (Costanzo et al., 1986; Costanzo and Fox, 1988; Kloeckener-Gruissem et al., 1988). PET54 appears to be a dual function protein, being also required for the excision of the aI5b intron from precursors to the *COX1* mRNA (Valencik et al., 1989). Despite the suggestion that both activities derive from recognition of similar sequence motifs in the 5′-leader and intron, respectively, subsequent mutational analysis has indicated that the two activities are separate and located in different domains of the protein (Valencik and McEwen, 1991). Early ideas that the three factors act as a single complex have now been lent strong support by the finding that in the GAL4-based two-hybrid system physical interactions are detectable between PET54 and PET122, and between PET54 and PET494 (Brown et al., 1994). PET54 is a mitochondrial protein, about 50% being peripherally bound to the inner membrane, while the remainder is present in soluble form (McMullin and Fox, 1993). PET122 and PET494 are both low-abundance proteins, below detection levels in wild type cells, but are found firmly attached to mitochondrial membranes when overproduced. Although the possibility exists that overproduction is responsible for artifactual membrane association, the results also indicate that these factors may be naturally associated with the mitochondrial inner membrane.

Besides making contact with *COX3* mRNA and the mitochondrial membrane, the PET494/54/122 complex is likely to interact with the mitochondrial ribosome. Three of four nuclear suppressor mutations that partially compensate for the loss of the carboxy-terminus of PET122 turn out to be located in genes encoding proteins of the small mitoribosomal subunit (McMullin et al., 1990; Haffter et al., 1991; Haffter and Fox, 1992). A role for the complex in facilitating coupled synthesis and insertion of subunit 3 into the membrane thus seems extremely likely.

2. Cytochrome b (COB) mRNA

Facilitation of membrane insertion would also appear to hold for CBS1 and CBS2 (CBP7), translational activators of the cytochrome b mRNA (Rodel et al., 1986). As with *COX3*, activation also depends on sequences upstream of the initiator AUG (Rodel 1986), but as yet the targets within this 954-nt-long leader have not been further defined. CBS2 associates with mitochondrial

TABLE 3
Factors Involved in Translation of Specific Yeast Mitochondrial mRNAs

mRNA	Factor	Location	Region 5′-UTR required for action	Interaction with ribosome?	Phenotypic suppressor	Ref.
COX1	MSS51 PET309[a]		Not required		Par_{454}	Decoster et al. (1990) Manthey and McEwen (1994)
COX2	PET111[b]	Mitochondrial membrane	−30 to −1	No	Five uncharacterized nuclear genes	Mulero and Fox (1993); Mulero and Fox (1993); Strick and Fox (1987)
COX3	PET494	Mitochondrial membrane	5′-End to −173			Costanzo et al. (1986); Costanzo and Fox (1993); McMullin and Fox (1993); Brown et al. (1994)
	PET54[c]	Mitochondrial membrane (loose)				Costanzo et al. (1986); Costanzo and Fox (1993); McMullin and Fox (1993); Costanzo et al. (1989); Valencik et al. (1989)
	PET122	Mitochondrial membrane		Yes	PET123, MRP1, MRP17 PET127	Costanzo et al. (1986); Costanzo and Fox (1993); McMullin and Fox (1993); Haffter et al. (1990); Haffter et al. (1991); Haffter and Fox (1992); McMullin et al. (1990); Ohmen et al. (1988)
COB	CBS1	Mitochondrial membrane (tight)	N.d.			Rodel (1986); Michaelis and Rodel (1990)
	CBS2	Mitochondrial membrane (loose)	N.d.	Yes		Rodel (1986); Michaelis and Rodel (1990)
	CBP6		Not required			Dieckmann and Tzagoloff (1985)

ATP9	AEP1/NCA1[d]		Payne et al. (1993); Ziaja et al. (1993)
	AEP2/ATP13[e]	N.d.	Finnegan et al. (1991); Ackerman et al. (1991)

Note: N.d. = Not determined.

[a] PET309 is likely also to have a function in determining the stability or in the transcriptional elongation of intron-containing transcripts (Manthey and McEwen, 1994).
[b] PET111 displays sequence similarity to a recently sequenced orf (YHR160c) on chromosome VIII.
[c] PET54 also plays a role in the splicing of intron al5b (see Table 2).
[d] AEP1 and NCA1 appear to be identical. Published sequences differ, presumably due to sequence errors. AEP1 may also function in mRNA stabilization.
[e] AEP2 and ATP13 appear to be identical. Published sequences differ, presumably due to sequence errors.

ribosomes, as indicated by appearance of the protein in two-dimensional separations of small mitoribosomal subunit proteins (Michaelis et al., 1991), and in subfractionations both proteins display association with mitochondrial membranes, which is tight for CBS1 and loose in the case of CBS2. Consistent with a role in membrane attachment/insertion, neither CBS1 nor CBS2 appears to be necessary for the expression of the RNA maturase involved in the processing of intron bI4 in the cytochrome *b* gene (Muroff and Tzagoloff, 1990). It should be noted, however, that both the p55 and p27 maturase proteins produced by *cbs1* and *cbs2* mutant cells appear to be nonfunctional, as demonstrated by the accumulation of intron-containing pre-mRNAs. Michaelis et al. (1991) have speculated that this may reflect a dependence of maturase processing on a membrane-bound protease (see also Section XI.C).

3. Cytochrome c Oxidase Subunit 2 (COX2) mRNA

Translation of *COX2* mRNA is dependent on PET111 (Poutre and Fox, 1987), which, unlike the factors discussed so far, may play a role in mRNA and/or start-site selection. Similar to other translational activators, PET111 acts via the 5′-leader of the mRNA, which in this case is only 54-nt long (Mulero and Fox, 1993). Direct interaction of the protein with the mRNA is suggested by the fact that a translational defect resulting from a single-base deletion at position –24 can be efficiently suppressed by a second-site mutation in the *PET111* gene (Mulero and Fox, 1993). This same PET111 missense mutation ($A_{652}T$) partially suppresses the leaky respiratory-deficient growth phenotype of a mutant in which the *COX2* initiation codon has been changed to AUA. In addition, both leader and start-site mutations are to some extent alleviated by overproduction of PET111, suggesting that their effect is to weaken interaction of the RNA with the protein (see also Mulero and Fox, 1994). Just like the *COX3* activators PET122 and PET494, PET111 is detectable only after overproduction and is then recovered in mitochondria as a tightly bound inner-membrane protein (Strick and Fox, 1987). No functional link between PET111 and the mitochondrial ribosome has so far been demonstrated. This function may belong to as yet uncharacterized factors. Five of the six nuclear suppressors of the –24 single-base deletion in the *COX2* leader are genetically unlinked to PET111 (Mulero and Fox, 1993) and are thus potential candidates for components of a PET111 activator complex.

4. Miscellaneous Activators

As Table 3 shows, four other genes (*MSS51*, *CPB6*, *PET309*, and AEP2/ATP13) appear to function as activator proteins. Mutations in *MSS51* and *CPB6* result in absence of a specific translation product, without marked effects on the corresponding mRNA (at least in intronless strains). *AEP2/ATP13* mutants display a lower steady-state level of mature mRNA and increased levels of higher-molecular-weight precursors, suggestive of an involvement in both mRNA maturation and translation. Neither *mss51* nor *cbp6* mutations are suppressible by the kind of leader substitutions that overcome the effects of mutations in other translational activators, suggesting a quite different mode of action (Dieckmann and Tzagoloff, 1985; Decoster et al., 1990). Interestingly, the paromomycin-resistance mutation Par_{454}, which is a G-to-C substitution at position 1514 within the region of 15S rRNA corresponding to helix 47 in *E. coli* SSU rRNA (Li et al., 1982), interferes with the action of the *MSS51* product (Decoster et al., 1990).

A mutation in the *PET112* gene (*pet112-1*) also results in absence of a specific translation product (COX2), without effect on the corresponding mRNA (Mulero et al., 1994). Unlike the original mutation, however, disruption of the gene destabilizes mtDNA and causes accumulation of rho^- cells. The protein may therefore have a more general role in translation, or a second function in biogenesis.

F. Isocitrate Dehydrogenase: A Novel Translational Repressor?

Sequence and gene disruption analysis show conclusively that an abundant, nuclear-encoded 40-kDa protein that binds specifically and with

high affinity to the 5′-untranslated leaders for all major yeast mitochondrial mRNAs (Papadopoulou et al., 1990; Dekker et al., 1992) is in fact the mitochondrial NAD^+-dependent isocitrate dehydrogenase (Idh) (Elzinga et al., 1993). Both subunits of this Krebs cycle enzyme, encoded by the nuclear genes *IDH1* and *IDH2* (Cupp and McAlister-Henn, 1991, 1992), are required for enzyme and RNA-binding activities. Although it may come somewhat as a surprise that such a familiar enzyme should lead a double life, Idh is just one of a rapidly growing family of dual-function RNA-binding proteins. The canonical protein that combines functions in both translation and metabolism is the iron-responsive element-binding protein (IRE-BP), which also doubles as cytoplasmic aconitase. The RNA-binding activity of this protein is modulated in response to iron levels in the cell by changes in an Fe-S cluster and is reciprocally related to its enzymic activity as cytosolic aconitase (Basilion et al., 1994 and references therein).

RNA-binding activity has also been recently attributed to other "regular" enzymes, including thymidylate synthase, dihydrofolate reductase, catalase, and glyceraldehyde-3-phosphate dehydrogenase (see Hentze, 1994 for review). A common feature of all members of the family examined so far is the presence of a (di-)nucleotide-binding site, and in a number of cases nucleotide- and RNA-binding sites appear to overlap. Such overlap could have a structural basis and reflect the evolutionary pathway followed during acquisition of poly(ribo)nucleotide-binding activity. Additionally, it opens possibilities for redox sensor-mediated modes of genetic regulation, allowing translation to be coupled to the metabolic state of the cell. Besides Idh, another Krebs cycle enzyme with RNA-binding activity is the bovine NAD^+-linked glutamate dehydrogenase (Preiss et al., 1993). The identity of RNA(s) normally bound in this latter case is unknown, but for both enzymes, the ability to bind RNA could be part of a regulatory circuit that couples the rate of mitochondrial biogenesis to the need for mitochondrial function.

Action of the various translational activators should be considered in relation to that of Idh. Dekker et al. (1992) have previously speculated that the enzyme, which at a level of some 25,000 molecules per cell is in principle sufficient to complex all mitochondrial mRNAs, may act as a repressor of translational initiation in the mitochondrial matrix. Steric hindrance of initiation, caused by protein binding to the 5′-leader, is a common theme in eukaryotic translational regulation (Gray and Hentze, 1994; Stripecke et al., 1994) and for Idh, such a role is additionally supported by the observations that (1) chimeric mRNAs apparently lacking p40-binding sites are still translatable (cf. Costanzo and Fox, 1988) and (2) for COX1, COX2, and ATP9 leaders, Idh binding leads to the exposure of sequences potentially capable of interacting with the initiator AUG, thereby regulating ribosome access (Dekker et al., 1992). The fact that *IDH1/IDH2* disruption strains are still capable of slow nonfermentable growth is also consistent with a nonessential role of Idh in translation or mRNA stability (Elzinga et al., 1993; cf. also Cupp and McAlister-Henn, 1991, 1992), but it should be noted that disruption strains display increased rates of rho^--cell production, a property normally indicative of impaired or abnormal translational activity (see Section II.D).

Interestingly, the region of the COX2 mRNA that is bound by Idh contains the sequence UCUAA, a motif that is conserved between a number of yeasts between 18 and 37 bases upstream of the initiator codon of this mRNA. This region is suggested by mutational analysis to be a good candidate for the site of PET111 action (Mulero and Fox, 1993). Therefore, for this mRNA, binding of Idh and PET111 could be mutually exclusive so that translation is prevented until Idh is displaced by PET111 and other components of an initiation complex acting at the surface of the mitochondrial membrane.

G. Translation and the Mitochondrial Membrane

A functional association between mitochondrial protein synthesis and the mitochondrial membrane was suggested many years ago as an explanation for the firm binding of ribosomes to membranes in yeast mitochondria and the properties of a group of cytoplasmic antibiotic-resistant mutants (Bunn et al., 1970). At the time none of

the arguments were compelling and all were open to alternative explanations (see Borst and Grivell, 1971 for discussion). The evidence listed above, which indicates that the translational activators for *COX3* and *COB* mRNAs mediate interactions between mRNA and ribosome while themselves attached to the mitochondrial inner membrane, gives this idea a much firmer basis, as does also the finding that overproduction of ABC1, a protein implicated in the correct folding or assembly of apocytochrome *b*, leads to partial suppression of a mutation in *CBS2* (Bousquet et al., 1991). Additional observations in support of a close coupling between translational activity and membrane insertion of the nascent products are discussed in Section XI.

H. Regulation of Translation

Finally, as reviewed by Costanzo and Fox (1990) and Dieckmann and Staples (1994), the existence of mRNA-specific translational activators opens up additional possibilities for nuclear control at this stage of mitochondrial gene expression. Several of these activators are present in low amounts, which in the case of PET111, PET122, and PET494 may even be limiting for translation (Mulero and Fox, 1994; Pinkham et al., 1994), and expression of the corresponding genes is subject to regulation by the oxygen and/or carbon source. Taken together, these features open possibilities for subtle regulatory effects on individual translation products over and above more general controls exerted by environmentally induced changes in the activities of the mitochondrial transcription and translation machineries as a whole.

XI. POSTTRANSLATIONAL EVENTS: MODIFICATION, ASSEMBLY, AND DEGRADATION

With the exception of the ribosomal protein Var1 and intron-encoded proteins, all products of mitochondrial translation in yeast are fairly hydrophobic membrane proteins that ultimately assemble into the respiratory complexes of the inner membrane. Although association of mitochondrial ribosomes and the various translational activators with the inner surface of the inner membrane may indeed facilitate insertion of these proteins into the membrane, the appearance of a growing list of new gene products with proposed functions in posttranslational modification and assembly is sufficient to justify an increasingly uneasy feeling that we really have little idea of what is going on at this late stage of mitochondrial gene expression. The list is presented in Table 4. None of the proteins display sequence features typical of the heat shock type chaperones known to be involved in the translocation and folding of cytoplasmically synthesized, imported proteins (see Stuart et al., 1994 for review) and it thus seems reasonable to suppose that in many cases principles other than straightforward folding/refolding of translation products will be involved. This supposition is already supported by the finding that COX10 and COX11, two gene products originally reported to be essential for a posttranslational stage of cytochrome c oxidase synthesis (Nobrega et al., 1990), are in fact required for synthesis of heme A. *COX10* encodes a heme A:farnesyl transferase, while *COX11* is predicted to encode an enzyme involved in forming the formyl group at position 8 of the porphyrin ring (Tzagoloff et al., 1993; Glerum and Tzagoloff, 1994). Both COX10 and COX11 are evolutionarily conserved proteins, their considerable sequence similarity to their bacterial counterparts yielding important clues to their probable functions. ABC1, OXA1, and SCO1 are similarly evolutionarily conserved, with recently sequenced counterparts in both the pro- and eukaryotic kingdoms. The *ABC1* gene was isolated as a multicopy suppressor of the translational defect in a *cbs2* mutant, yet turns out to be essential for assembly of a functional UQ-cytochrome *c* oxidoreductase, being somehow involved at a stage subsequent to heme incorporation into holocytochrome *b* (Bousquet et al., 1991). The *OXA1* gene is also required for a late step in synthesis of cytochrome *c* oxidase (Bonnefoy et al., 1994). *oxa1* mutant cells exhibit somewhat decreased levels of the three mitochondrially synthesized subunits of cytochrome oxidase, yet a total absence of cytochrome *aa3*, together with a reduced amount of

TABLE 4
Proteins Required for Posttranslational Steps in Expression of Mitochondrial Genes

Protein	Location	Phenotype mutant/ disruptant	Sequence similarity	Function	Ref.
			ATPase		
ATP10	Mitochondrial membrane	Defect in assembly Fo-sector	None known	Chaperone?	Ackerman and Tzagoloff (1990)
ATP11	Mitochondrial matrix	Defect in F1-assembly; aggregation alpha/beta subunits	None known	Chaperone?	Ackerman et al. (1992)
ATP12	Mitochondrial matrix, or weakly bound to matrix side IM	Defect in F1-assembly; aggregation alpha/beta subunits	None known	Chaperone?	Bowman et al. (1991)
			UO-Cytochrome *c* Reductase		
ABC1	Mitochondrial? (potential mt targeting signal)	Absence of NADH-succinate dehydrogenase activities; all cytochromes spectrally detectable	See Figure 2A	Translation COB mRNA; assembly bc1 complex	Bousquet et al. (1991)
BCS1	Mitochondrial membrane-bound	Decreased amount of FeS protein	AAA-family (see Figure 3)	Incorporation of FeS group or chaperone?	Nobrega et al. (1992)
CPB3	Mitochondrial membrane-bound	Absence of spectrally detectable cytb	None known		Wu and Tzagoloff (1989)
CPB4	Mitochondrial membrane-bound	Absence of spectrally detectable cytb	None known		Crivellone (1994)
			Cytochrome *c* Oxidase		
COX10	Mitochondrial	Lack of oxidase activity and cytochromes a+a3	Farnesyl transferase	Conversion of protoheme to heme O	Nobrega et al. (1990); Tzagoloff et al. (1993)

TABLE 4 (continued)
Proteins Required for Posttranslational Steps in Expression of Mitochondrial Genes

Protein	Location	Phenotype mutant/ disruptant	Sequence similarity	Function	Ref.
COX11	Mitochondrial, tightly attached to inner membrane	Lack of oxidase activity and cytochromes a+a3	ORF3 *P. denitrificans*	Formation formyl group at position 8 of porphyrin ring	Tzagoloff et al. (1993); Tzagoloff et al. (1990)
PET117	Mitochondrial? (potential mt targeting signal)	Lack of oxidase activity and cytochromes a+a3; presence of all oxidase subunits	None known		McEwen et al. (1993)
PET191	Mitochondrial? (potential mt targeting signal)	Lack of oxidase activity; presence of all oxidase subunits	None known		McEwen et al. (1993)
SCO1	Mitochondrial inner membrane	Rapid turnover of COX1 and COX2	See Figure 2B		Schulze and Rödel (1989); Krummeck and Rödel (1990); Buchwald et al. (1991)
Cytochrome *c* Oxidase: UO-Cytochrome *c* Reductase					
IMP1/2	Mitochondrial inner membrane	Defect in processing COX2, $cytb_2$ and $cytc_1$	Leader peptidases	Cleavage IMS sorting signals	Nunnari et al. (1993)
MRS2	Mitochondrial? (potential mt targeting signal)	Cytochrome aa3 deficiency; decreased amount of cytb	None known		Wiesenberger et al. (1992)
OXA1	Mitochondrial?	Lack of oxidase activity; absence of cytochromes a+a3; decrease in amount of cytb	Several pro- and eukaryotic proteins		McEwen et al. (1986); Bonnefoy et al. (1994)
Pleiotropic					
AFG3	Mitochondrial inner membrane	Loss of COX1-3; and decrease in amount cytb	AAA-family (see Figure 3)	Chaperone? Protease?	Guelin et al. (1994); Pajic et al. (1994)
RCA1	Mitochondrial inner membrane	Loss of activity of all respiratory complexes	AAA-family (see Figure 3)	Chaperone? Protease?	Paul et al. (1994)
YME1	Mitochondrial	ts respiratory deficiency; complex phenotype (see text)	AAA-family (see Figure 3)	Chaperone? Protease?	Thorsness et al. (1993)

cytochrome *b*. The *SCO1* gene is also specifically needed for formation of a functional cytochrome *c* oxidase, possibly being required for stabilization of the mitochondrially synthesized COX1 and COX2 proteins during or immediately after their synthesis (Krummeck and Rödel, 1990). Interestingly, all three genes contain sequence motifs that have been strongly conserved in evolution. *ABC1* displays extensive similarity to ORFs in both *Clostridium* and *Mycobacterium* and a more limited similarity to the gene 55 family of proteins in herpes viruses (Figure 2A); *OXA1* to a series of ORFs in *E. coli*, *Bacillus*, *Pseudomonas*, *Oryza*, and *Caenorhabditis* (Bonnefoy et al., 1994) and SCO1 to an ORF in *Pseudomonas*, a major surface protein in *Anaplasma* and a bovine immunodominant protein (Figure 2B). Further mutagenic analysis of such conserved features should provide important clues to the function of all the proteins involved.

A. Mitochondrial Proteases

As has been reviewed extensively elsewhere (Hartl and Neupert, 1990), nuclear-coded mitochondrial proteins are in most cases divested of their targeting signals by the action of the highly specific matrix and inner-membrane proteases (MPP, MIP, IMP) during or shortly after import. MPP, or more rarely MIP* (Isaya et al., 1992; Isaya et al., 1994), cleave precursors of proteins with the mitochondrial matrix or inner membrane as their final destinations and carry out the initial

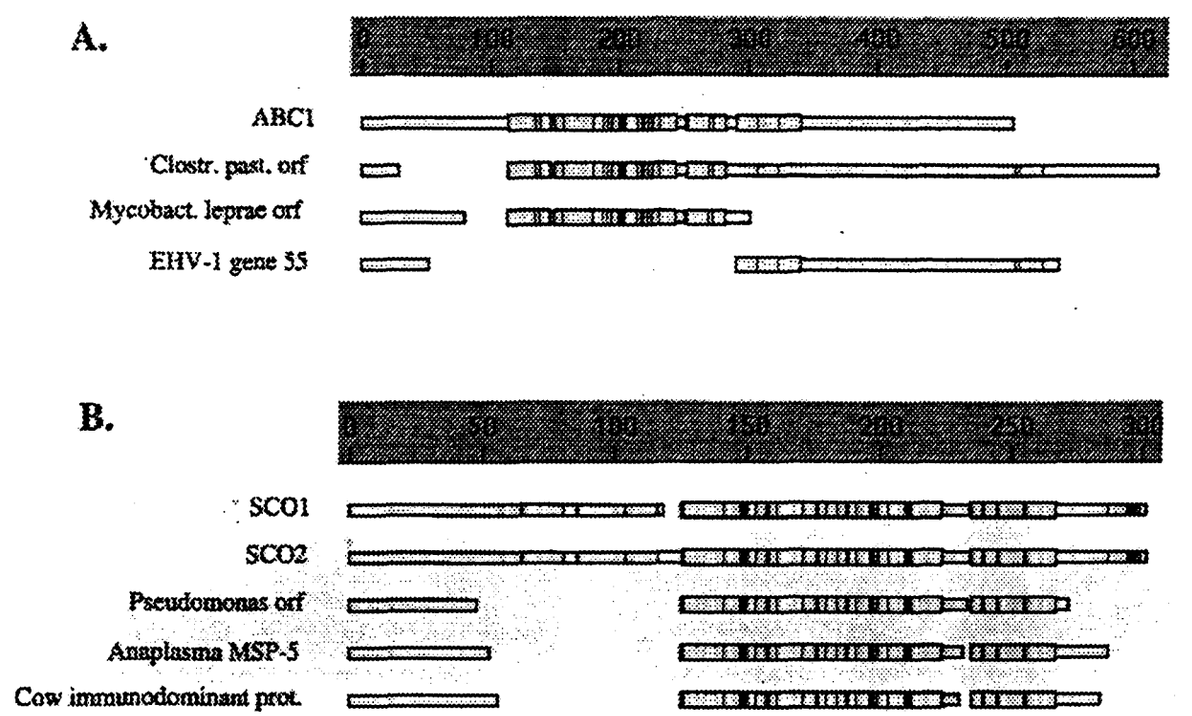

FIGURE 2. ABC1 and SCO1 are evolutionarily conserved proteins. Block alignments were made using the program MACAW (Schuler et al., 1991) on sequences available from the Swissprot and Genbank databases. Intensity of shading is a rough measure of the extent of sequence identity. (A) *S. cerevisiae* ABC1, acc. no. P27697; *Clostr. past.* ORF acc. no. Z28353; *Mycobact. leprae* ORF acc. no. U00016; EHV-1 gene 55 acc. no. P28945. (B) SCO1 acc. no. P23833; SCO2 acc. no. X76078; *Pseudomonas* ORF acc. no. Z26044; Anaplasma marginale MSP-5 acc. no. A49213; Cow immunodominant protein acc. no. L07385.

• In yeast, the gene encoding MIP has rather unfortunately been designated MIP1. It should not be confused with the gene of the same name encoding the mitochondrial DNA polymerase (see Section III.B).

processing of the bipartite addressing signal of proteins destined for the intermembrane space. The MPP protease is a two-subunit enzyme, consisting of the catalytic subunit (encoded by the *MAS2* gene) and a second protein that enhances activity (protease-enhancing protein, PEP, product of the *MAS1* gene). Examination of the sequences of yeast and *Neurospora* subunits shows that they display a considerable degree of sequence similarity and belong to the same superfamily of metalloendoproteases. Quite unexpected, however, is the finding that in *Neurospora,* PEP is identical with subunit I of the ubiquinol-cytochrome *c* reductase (Schulte et al., 1989), leading to the conclusion that this rather unremarkable-looking protein combines a role in protein import with a function in the respiratory chain. At the time of this discovery, it looked as though *Neurospora* was an interesting exception, because the corresponding functions in yeast reside in separate (albeit distantly related) proteins. It has, however, become clear that this sharing of functions is a more widespread phenomenon, because in both potato and spinach mitochondria, the processing protease appears to be an integral component of the cytochrome *c* reductase (Braun et al., 1992; Eriksson and Glaser, 1992). In potato, subunit II is the processing peptidase and the complex is bifunctional: it transfers electrons from ubiquinol to cytochrome *c and* it cleaves presequences from imported precursor proteins (Emmermann et al., 1993). These findings raise some interesting questions as to the relationship between protein import and electron transport, including whether bioenergetic activity can influence the rate of biogenesis (Weiss et al., 1990).

The second processing step undergone by proteins of the intermembrane space is carried out by IMP, which in fact consists of two sequence-related, leader peptidase-like catalytic subunits (IMP1 and IMP2) with nonoverlapping specificities (Nunnari et al., 1993). Both enzymes sit on the intermembrane face of the inner membrane. IMP1 is responsible for processing of the intermembrane enzyme cytochrome b_2 and, curiously, also mitochondrially coded pre-COX2, one of the two regular mitochondrial translation products to be synthesized in precursor form (Behrens et al., 1991; Michon et al., 1988). IMP2 processes precytochrome c_1 and is further required for stabilization of IMP1 (Nunnari et al., 1993).

In addition to these specific enzymes, mitochondria of a variety of organisms contain proteases that possibly function in the ATP-dependent degradation of abnormal, misfolded, or incomplete polypeptides (see Goldberg, 1992 and references therein). These are homologues of the *E. coli* La and Ti (Clp) proteases, and in yeast are encoded by the *PIM1* and *HSP78* genes, respectively (Leonhardt et al., 1993; Suzuki et al., 1994; van Dyck et al., 1994). PIM1 is a versatile and intriguing protein. In collaboration with the mitochondrial hsp70, it is capable of degradation of misfolded imported proteins (Wagner et al., 1994). It is also somehow involved in the maintenance of wild type mtDNA (Section III.D) and, when overexpressed, can suppress the phenotype of null mutants in the mitochondrial FtsH homologue AFG3 (Section XI.C). In contrast, much less is known about *HSP78,* apart from the fact that disruption of the gene does not detectably impair mitochondrial function. It remains to be seen whether this heat shock protein is itself a protease or a chaperone that facilitates protease activity in another subunit, for example, a mitochondrial homologue of the *E. coli* ClpP protein (cf. Goldberg, 1992).

B. AAA-Family Proteins in Mitochondria

Finally, perhaps most intriguing are *BCS1, AFG3, RCA1,* and *YME1,* genes that cannot easily be assigned to any one class in terms of action, despite considerable similarity in sequence. These are all mitochondrial members of a large and growing family of ATPases associated with diverse cellular activities (AAA-proteins) and are related by virtue of the possession of one or two highly conserved domains of approximately 200-aa (Figure 3). These domains contain consensus sequences characteristic of ATPases and nucleotide-binding proteins, thus making it likely that the proteins are also ATPases, capable of ATP-hydrolysis for performance of their various functions (see Kunau et al., 1993 for review).

BCS1 is an inner-membrane protein, most closely related to Afg1/Sec18, and is required for the assembly of a functional Rieske FeS protein

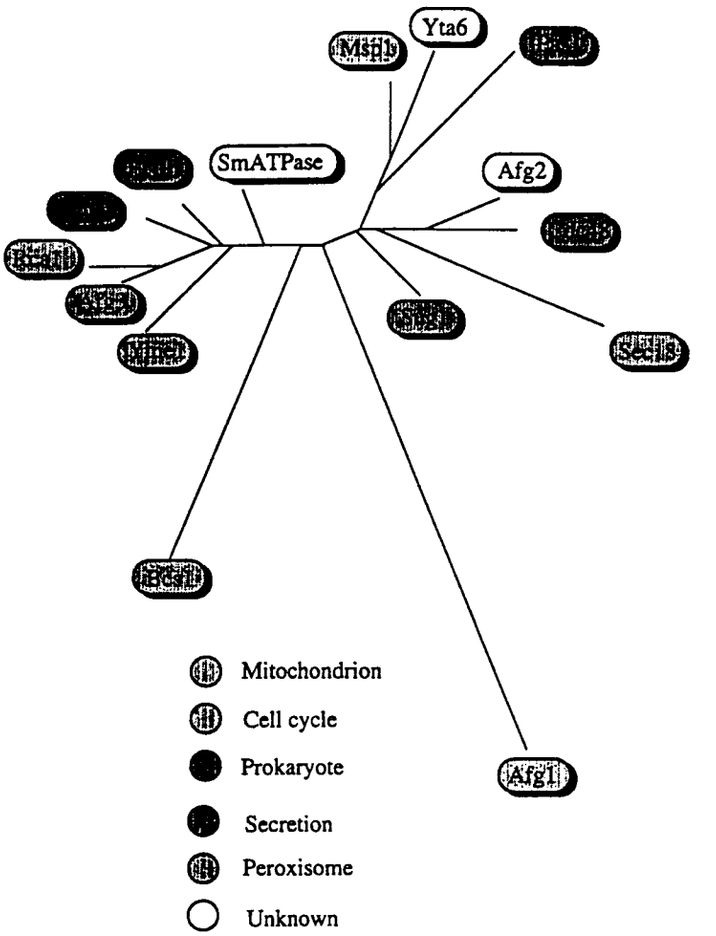

FIGURE 3. The yeast AAA-protein family and its closest prokaryotic relatives. The unrooted neighboring-joining tree is based on alignments made with the GCG program PILEUP and constructed using the Drawgram routine in the Phylogeny Inference Package Phylip. Sequences are available in Swissprot and Genbank databases.

into the UQ-cytochrome *c* oxidoreductase (Nobrega et al., 1992). YME1 and AFG3 form a subgroup together with the prokaryotic members of the AAA-family — *E. coli* FtsH and its homologues in other bacteria (Thorsness et al., 1993; Guelin et al., 1994; Schnall et al., 1994). Despite high sequence similarity, AFG3 and YME1 appear to be involved in quite distinct mitochondrial processes. YME1 is one of six genes, mutations in which have drastic effects on mitochondrial structure, greatly elevating the rate at which mtDNA escapes from the organelle (Thorsness and Fox, 1993). AFG3, in contrast, was isolated via its ability to restore respiratory function to a mutant that is temperature sensitive for growth on nonfermentable carbon sources (Guelin et al., 1994). Disruptions of the *AFG3* gene lead to respiratory deficiency, reduced assembly of a number of mitochondrial translation products, and increased rate of petite formation as a result of mtDNA loss (Rep, M. and Guelin, E., unpublished observations). Interestingly, the subgroup can be extended with additional members. The first of these is a putative mitochondrial ATPase present in the parasitic protozoan *Schistosoma mansonii* (Menrath and Kunz, 1994). The second is *RCA1*, a gene recently isolated on the basis of its requirement for assembly of mitochondrial

respiratory complexes (Tzagoloff et al., 1994). The N-terminal regions of both AFG3 and RCA1 display features characteristic of a typical mitochondrial targeting sequence and both have been localized to the mitochondrial inner membrane (Rep, M., unpublished observations; Paul et al., 1994). Like FtsH (Tomoyasu et al., 1993), AFG3 appears to be anchored in the inner membrane in such a way that a large part of the protein, including the C-terminus, extends into the matrix (Rep, M., unpublished observations; Pajic et al., 1994).

C. AFG3, RCA1, and YME1: Chaperones or Proteases?

Also like FtsH, AFG3, RCA1, and YME1 share a sequence motif (HEXXH) that is characteristic of the active site in a family of neutral, zinc metalloendopeptidases including neprilysin and the thimet oligopeptidase (Bode et al., 1993). This motif is located at a similar position and within a highly conserved sequence in each protein (Figure 4). Alias HflB, FtsH has been shown to stimulate degradation of the lambda cII-activator protein (Herman et al., 1993), thus leading to the suggestion that the protein is either a protease or a chaperone that induces a conformation susceptible to proteolysis. Action as protease is suggested by the fact that a mutant gene containing a substitution of lysine for glutamate in the HEXXH motif is no longer functional (Akiyama et al., 1994), and this strongly implies a similar function for the mitochondrial members of the family. The occurrence of three new, putative proteases in yeast mitochondria raises immediate questions concerning their role in mitochondrial biogenesis or function. In the case of AFG3, clues to such a role are given by recent genetic and biochemical analyses. First, the respiratory-deficient phenotype of *afg3* disruptants can be suppressed by overexpression of the PIM1 protease

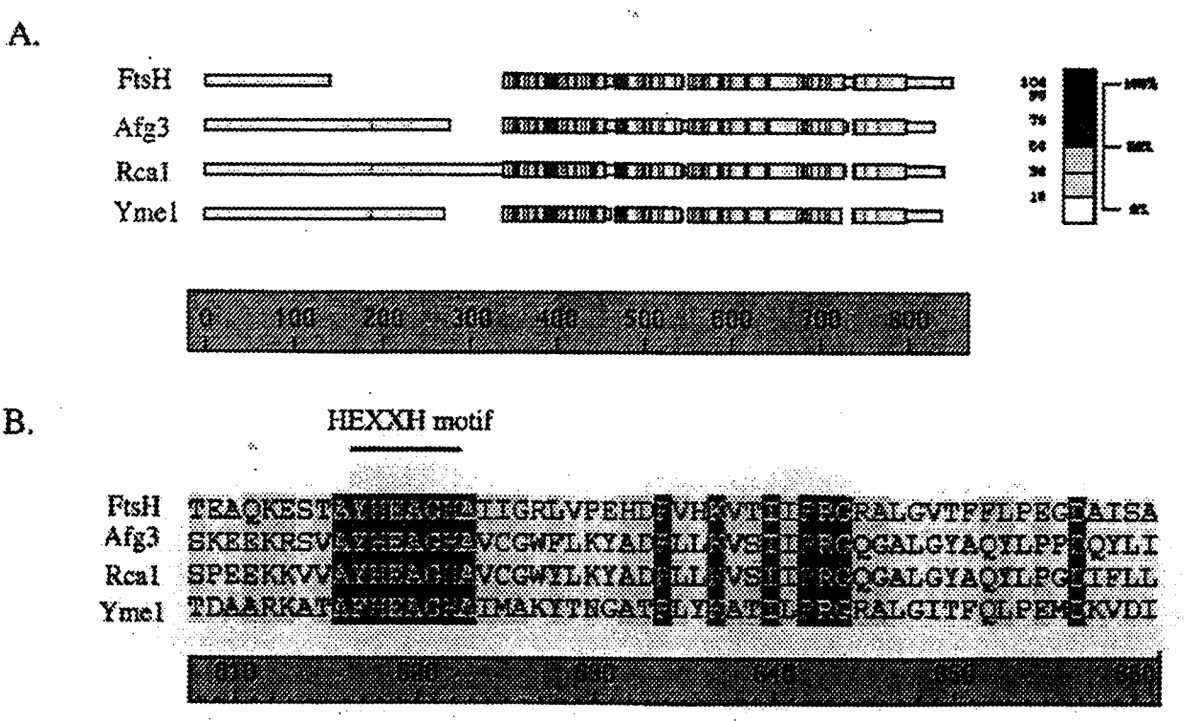

FIGURE 4. Conservation of a metallopeptidase sequence motif in mitochondrial FtsH-like proteins. Both block (A) and sequence (B) alignments were made using the program MACAW (Schuler et al., 1991) on sequences available from the Swissprot and Genbank databases. *FtsH* acc. no. M83138; *AFG3* acc. no. X76643; *RCA1* acc. no. U09358; *YME1* acc. no. P32795. Note that the latter three genes have also been sequenced under the names *YTA10, YTA12,* and *YTA11* (Schnall et al., 1994). The HEXXH metallopeptidase motif (Bode et al., 1993) is overlined.

(Rep, M., unpublished observations). Second, such disruptants are deficient in the metal ion- and ATP-dependent degradation of incompletely synthesized mitochondrial translation products (Pajic et al., 1994), an activity that has been suggested to reflect a physiological mechanism for regulation of membrane assembly (Wheeldon et al., 1974; Yasuhara et al., 1994). Both observations strongly support the idea that AFG3 either is itself a membrane-bound protease or is closely involved in the activation of the catalytic subunit of such a protease. Several considerations complicate this simple picture, however. First, if AFG3 has such a broad-spectrum degradative ability, it is not easy to see how this can be responsible for the clear-cut deficiency in cytochrome *b* and subunits of cytochrome *c* oxidase, which seems to reflect reduced synthesis rather than increased turnover (Rep, M., unpublished observations). Second, if AFG3 is indeed a broad-spectrum protease, why is the highly similar RCA1 protein unable to compensate for its loss? Finally, why should lack of such a degradative ability have such serious consequence for respiratory sufficiency and mtDNA stability? One explanation may be that AFG3 and RCA1 function both as chaperone and protease, promoting membrane insertion during translation, but capable of carrying out degradation of aberrant or incomplete translation products. Alternatively, protease action may be a specific and, by way of ATP hydrolysis, strictly controlled activity required for disruption of some kind of proteinaceous pore involved in membrane insertion. In *E. coli*, FtsH has been proposed to function in a similar capacity to assure efficient stop-transfer of some transmembrane proteins (Akiyama et al., 1994; Akiyama et al., 1994).

Although possibly differing in function from AFG3 and RCA1, a discussion on mitochondrial AAA-proteins would not be complete without coverage of YME1. The gene for this protein emerged from a screen for mutants that displayed an increased rate of escape of mitochondrial DNA to the nucleus. *yme1* mutants contain abnormal, distended mitochondrial structures, are temperature sensitive for growth on nonfermentable carbon sources, cold sensitive for growth on rich glucose medium, and lose mtDNA at an accelerated rate (Thorsness et al., 1993). The resultant mtDNA-less mutants display severely impaired growth and rapidly become unviable, a property that essentially converts *S. cerevisiae* into a petite-negative yeast (see Section III.F). Thorsness et al. (1993) have suggested that all these features could be symptomatic of a defect in the maintenance of mitochondrial structure, leading to improper organelle fusion or division. Six independent *yme1* mutant alleles were isolated in the original screen carried out by Thorsness and Fox (1993) and all display essentially identical phenotypes to that displayed by a *yme1* gene disruption. the *yme1-1* allele shows weak intragenic complementation with the *yme1-3* allele, suggesting that the functional unit contains more than one copy of the protein. The fact that increased dosage of the *YME1* gene does not increase cellular levels of YME1 could mean that it is part of a complex whose assembly limits the amount of the protein within the cell. Candidates for other components of such a complex could be proteins encoded by other *YME* genes.

Arguing that YME1 may also be a mitochondrial ATP-dependent metalloprotease, Campbell et al. (1994) have attempted to gather information on the protein's function by searching for interacting gene products. The search, directed at detection of mutations that bypass a requirement for YME1, has yielded an unexpected result in the shape of the *ynt1-1* mutation. *ynt1-1* cells display an intrinsic cold sensitivity for growth on nonfermentable substrates. Yet, in combination with a *yme1* null mutation, *ynt1-1* suppresses all four phenotypes associated with *yme1* dysfunction and partially reverses the morphological abnormalities displayed by mitochondria in *yme1* cells. The sequence of *YNT1* (also known as *YTA2*) shows it to be one of a family of genes encoding additional members of the AAA-extended family. In this case, the products are related to regulatory subunits of the 26S proteasome, whose activity is necessary for a variety of activities, including progression through the cell cycle (Campbell et al., 1994 and references therein).

At present, this finding raises more questions than it answers. If YME1 is a mitochondrial protease, the distinctive phenotype resulting from its dysfunction suggests that other proteases present in mitochondria are also unable to substitute its function. It is therefore especially surprising that YNT1, as a possible component of the cyto-

solically located 26S proteosome, should have such an ability. Campbell et al. (1994) speculate that both YME1 and YNT1 may in fact fulfill a similar role, acting in the processing of a subset of mitochondrially targeted proteins. Given a cytosolic location for the 26S proteosome, testable predictions of such a role are that processing may occur prior to import and that the mitochondrial location of Yme1 may be such that it has access to cytosolic proteins. An interesting extension to this speculation is that both YME1 and YNT1 are involved in interactions with the cytoplasmic structures that mediate organelle movement and inheritance (Section III.E), thereby playing an important role in mitochondrial proliferation at cell division.

XII. CONCLUSIONS AND PROSPECTS

Reviewing the field in 1990, Tzagoloff and Dieckmann (1990) listed some 215 yeast nuclear genes required for the synthesis of a functional mitochondrion and showed that the majority of these encoded components of the mitochondrial genetic system, proteins required for the maintenance of this system, or for various stages of gene expression. As documented in the previous sections, the last few years have led to an almost explosive increase in the number of new gene products characterized, and new insights have been gained into the mode of action of genes characterized previously. New genes have emerged (and are still emerging) from three main approaches:

1. The use of mutant selection strategies that take into account the essential nature of the mitochondrial compartment and of many of the metabolic reactions that occur there. The emergence of new genes involved in mitochondrial protein import (Pfanner et al., 1994) or mitochondrial inheritance and cytoskeletal interactions (Section III.E) nicely illustrates this point and provides sufficient justification for further searches, which, hopefully, will clarify the ways in which mitochondria contribute to decision making with respect to cell growth and division.
2. The application of novel multicopy suppressor or synthetic lethal screens that have resulted in the identification of gene products whose ability to bypass the mutational block may derive from unconventional and informative principles. The *MRS* series of suppressors of splicing and respiratory deficiency (Section VIII) and the *YNT1* suppressor of *YME1* deficiency (Section XI.C) are good examples of the puzzling yet challenging examples of nucleomitochondrial relationships still to be unravelled.
3. The rapid increase in availability of genomic sequence information. Indeed, given the high degree of evolutionary conservation of many cellular processes between yeast and higher eukaryotes and the fact that it will be the first organism for which a complete genomic sequence will be available within the next 2 to 3 years, *S. cerevisiae* currently offers unequalled advantages for drawing up an inventory of all nuclear genes involved in mitochondrial biogenesis and for identification of their counterparts in other organisms. Especially informative in this context will be the further characterization of newly discovered copies of previously identified genes. Recent sequencing efforts indicate that extensive regions of the *S. cerevisiae* genome have undergone duplications and subsequent divergence, leading to the appearance of gene families, whose members often differ in sequence or mode of regulation (Oliver, 1992; Dujon et al., 1994; Johnston et al., 1994).

Sequence analysis and comparison have in several instances provided new insights into functions of familiar genes. The identification of the evolutionarily conserved *COX10* and *COX11* gene products as enzymes involved in heme A biosynthesis, rather than as chaperones (Section XI), is a good case in point and by analogy, the discovery of sequence relationships in the *ABC1*, *OXA1*, and *SCO1* genes may provide new impetus in the search for their functions. However, sequence comparisons alone may fail to reveal key information. Without mutational and biochemical studies, the aminoacyl-tRNA synthetases encoded by the *CYT-18* and *NAM2* genes and the NAD^+-linked mitochondrial isocitrate dehydrogenase would not have disclosed the secrets of their double lives.

An area of particularly fast growth has been that involving posttranslational modification of mitochondrial translation products and their assembly into respiratory complexes. Yeast mitochondria appear to have chosen an entirely idiosyncratic system for targeting ribosomes, associated mRNAs, and nascent polypeptide chains to the membrane (Sections X.G and XI) instead of using evolutionarily tried and tested techniques for coaxing or driving such newly synthesized products into or across the membrane (cf. Schekman, 1994; Wickner, 1994). Finding out how this system works will be a task for the future, as will be the elucidation of the relevance for this process of protein turnover and of the exact roles played by the AAA-family proteins (Section XI.C).

Finally, better characterization of nuclear genes encoding components of the mitochondrial genetic system is a prerequisite for an understanding of the etiology of human mitochondrial diseases. Although the vast majority of such diseases can be attributed to more or less severe mutations in the compactly organized mitochondrial genome, it is becoming increasingly clear that nuclear gene products are important determinants in the occurrence of mutations, the manifestation of clinical symptoms, and their time of onset (Luft, 1994). In addition, the mutations in mtDNA are just one of the damaging consequences of reactive oxygen species produced as byproducts of aerobic respiration in mitochondria, and nuclear-encoded mitochondrial proteins play important roles in minimizing oxidative damage. Bcl-2 is a mammalian protooncogene capable of blocking apoptotic cell death in a variety of contexts and its product is thought to function in an antioxidant pathway that provides protection against such damage (Hockenbery et al., 1993). The discovery that a large fraction of the Bcl-2 localizes to the mitochondrion (Hockenbery et al., 1990), most likely anchored in the outer membrane (Nguyen et al., 1993), may therefore open a new chapter in nucleomitochondrial interactions.

ACKNOWLEDGMENTS

The author thanks Peter Dekker, Emmanuel Guelin, and Martijn Rep for discussion and their constructive comments on the manuscript; Françoise Foury, Joan McEwen, Rudolf Schweyen, and Peter Thorsness for advice and their permission to cite unpublished data; Philip Grivell for his help in automating the manipulation of the literature references on which this review is based. Work carried out in the author's laboratory was supported in part by NWO/SON and by the ECC under contracts.

REFERENCES

Ackerman, S. H., D. L. Gatti, P. Gellefors, M. G. Douglas, and A. Tzagoloff (1991). ATP13, a nuclear gene of *Saccharomyces cerevisiae* essential for the expression of subunit 9 of the mitochondrial ATPase. *FEBS Lett.*, **278:** 234–238.

Ackerman, S. H., J. Martin, and A. Tzagoloff (1992). Characterization of ATP11 and detection of the encoded protein in mitochondria of *Saccharomyces cerevisiae*. *J. Biol. Chem.*, **267:** 7386–7394.

Ackerman, S. H. and A. Tzagoloff (1990). ATP10, a yeast nuclear gene required for the assembly of the mitochondrial F1-F0 complex. *J. Biol. Chem.* **265:** 9952–9959.

Akins, R. A. and A. M. Lambowitz (1987). A protein required for splicing group I introns in *Neurospora* mitochondria is mitochondrial tyrosyl-tRNA synthetase or a derivative thereof. *Cell* **50:** 331–345.

Akiyama, Y., T. Ogura, and K. Ito (1994). Involvement of FtsH in protein assembly into and through the membrane. 1. Mutations that reduce retention efficiency of a cytoplasmic reporter. *J. Biol. Chem.* **269:** 5218–5224.

Akiyama, Y., Y. Shirai, and K. Ito (1994). Involvement of FtsH in protein assembly into and through the membrane. 2. Dominant mutations affecting FtsH functions. *J. Biol. Chem.* **269:** 5225–5229.

Altamura, N., G. Dujardin, O. Groudinsky, and P. P. Slonimski (1994). Two adjacent nuclear genes, ISF1 and NAM7/UPF1, cooperatively participate in mitochondrial functions in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **242:** 49–56.

Altamura, N., O. Groudinsky, G. Dujardin, and P. P. Slonimski (1992). NAM7 nuclear gene encodes a novel member of a family of helicases with a Zn-ligand motif and is involved in mitochondrial functions in *Saccharomyces cerevisiae*. *J. Mol. Biol.* **224:** 575–587.

Attardi, G. and G. Schatz (1988). Biogenesis of mitochondria. *Annu. Rev. Cell Biol.* **4:** 289–333.

Baldacci, G. and G. Bernardi (1982). Replication origins are associated with transcription initiation sequences in the mitochondrial genome of yeast. *EMBO J.* **1:** 987–994.

Baldacci, G., B. Chérif-Zahar, and G. Bernardi (1984). The initiation of DNA replication in the mitochondrial genome of yeast. *EMBO J.* **3:** 2115–2120.

Banroques, J., A. Delahodde, and C. Jacq (1986). A mitochondrial RNA maturase gene transferred to the yeast

nucleus can control mitochondrial mRNA splicing. *Cell* **46:** 837–844.

Basilion, J. P., T. A. Rouault, C. M. Massinople, R. D. Klausner, and W. H. Burges (1994). The iron-responsive element-binding protein: localization of the RNA binding site to the aconitase active-site cleft. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 574–578.

Beaudry, A. A. and G. F. Joyce (1992). Directed evolution of an RNA enzyme. *Science* **257:** 635–641.

Behrens, M., G. Michaelis, and E. Pratje (1991). Mitochondrial inner membrane protease 1 of Saccharomyces cerevisiae shows sequence similarity to the *Escherichia coli* leader peptidase. *Mol. Gen. Genet.* **228:** 167–176.

Belfort, M. (1990). Phage T4 introns: self-splicing and mobility. *Annu. Rev. Genet.* **24:** 363–385.

Ben Asher, E., O. Groudinsky, G. Dujardin, N. Altamura, M. Kermorgant, and P. P. Slonimski (1989). Novel class of nuclear genes involved in both mRNA splicing and protein synthesis in *Saccharomyces cerevisiae* mitochondria. *Mol. Gen. Genet.* **215:** 517–528.

Bendich, A. J. (1993). Reaching for the ring — the study of mitochondrial genome structure. *Curr. Genet.* **24:** 279–290.

Bernardi, G. (1982). The origins of replication of the mitochondrial genome of yeast. *Trends Biochem. Sci.* **7:** 404–408.

Biswas, T. K. and G. S. Getz (1986). Nucleotides flanking the promoter sequence influence the transcription of the yeast mitochondrial gene coding for ATPase subunit 9. *Proc. Natl. Acad. Sci. U.S.A.* **83:** 270–274.

Blanco, L., A. Bernad, and M. Salas (1991). MIP1 DNA polymerase of *S. cerevisiae:* structural similarity with the *E. coli* DNA polymerase I-type enzymes. *Nucl. Acids Res.* **19:** 955.

Bode, W., F. X. Gomis-Ruth, and W. Stockler (1993). Astacins, serralysins, snake venom and matrix metalloproteinases exhibit identical zinc-binding environments (HEXXHXXGXXH and Met-turn) and topologies and should be grouped into a common family, the "metzincins". *FEBS Lett.* **331:** 134–140.

Boguta, M., L. A. Hunter, W. C. Shen, E. C. Gillman, N. C. Martin, and A. K. Hopper (1994). Subcellular locations of MOD5 proteins: mapping of sequences sufficient for targeting to mitochondria and demonstration that mitochondrial and nuclear isoforms commingle in the cytosol. *Mol. Cell. Biol.* **14:** 2298–2306.

Bolotin-Fukuhara, M. and L. A. Grivell (1992). Genetic approaches to the study of mitochondrial biogenesis in yeast. *Antonie van Leeuwenhoek; J. Gen. Microbiol.* **62:** 131–153.

Bonitz, S. G., R. Berlani, G. Coruzzi, M. Li, G. Macino, F. G. Nobrega, M. P. Nobrega, B. E. Thalenfeld, and A. Tzagoloff (1980). Codon recognition rules in yeast mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* **77:** 3167–3170.

Bonnefoy, N., F. Chalvet, P. Hamel, P. P. Slonimski, and G. Dujardin (1994). OXA1, a *Saccharomyces cerevisiae* nuclear gene whose sequence is conserved from prokaryotes to eukaryotes controls cytochrome oxidase biogenesis. *J. Mol. Biol.* **239:** 201–212.

Bordonné, R., G. Dirheimer, and R. P. Martin (1987). Transcription initiation and RNA processing of a yeast mitochondrial tRNA gene cluster. *Nucl. Acids Res.* **15:** 7381–7394.

Borst, P. and L. A. Grivell (1971). Mitochondrial ribosomes. *FEBS Lett.* **13:** 73–88.

Bos, J. L., C. Heyting, P. Borst, A. C. Arnberg, and E. F. J. van Bruggen (1978). An insert in the single gene for the large ribosomal RNA in yeast mitochondrial DNA. *Nature* **275:** 336–338.

Bousquet, I., G. Dujardin, R. O. Poyton, and P. P. Slonimski (1990). Two group I mitochondrial introns in the cob-box and coxI genes require the same MRS1/PET157 nuclear gene product for splicing. *Curr. Genet.* **18:** 117–124.

Bousquet, I., G. Dujardin, and P. P. Slonimski (1991). ABC1, a novel yeast nuclear gene has a dual function in mitochondria: it suppresses a cytochrome b mRNA translation defect and is essential for the electron transfer in the bc1 complex. *EMBO J.* **10:** 2023–2031.

Bowman, S., S. H. Ackerman, D. E. Griffiths, and A. Tzagoloff (1991). Characterization of ATP12, a yeast nuclear gene required for the assembly of the mitochondrial F1-ATPase. *J. Biol. Chem.* **266:** 7517–7523.

Braun, H.-P., M. Emmermann, V. Kruft, and U. K. Schmitz (1992). The general mitochondrial processing peptidase from potato is an integral part of cytochrome c reductase of the respiratory chain. *EMBO J.* **11:** 3219–3227.

Brehm, S. L. and T. R. Cech (1983). Fate of an intervening sequence ribonucleic acid: excision and cyclization of the *Tetrahymena* ribosomal ribonucleic acid intervening sequence *in vivo*. *Biochemistry* **22:** 2390–2397.

Brown, N. G., M. C. Costanzo, and T. D. Fox (1994). Interactions among three proteins that specifically activate translation of the mitochondrial COX3 mRNA in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **14:** 1045–1053.

Buchwald, P., G. Krummeck, and G. Rödel (1991). Immunological identification of yeast SCO1 protein as a component of the inner mitochondrial membrane. *Mol. Gen. Genet.* **230:** 413–420.

Bunn, C. L., C. H. Mitchell, H. B. Lukins, and A. W. Linnane (1970). Biogenesis of mitochondria, XVIII. A new class of cytoplasmically determined antibiotic resistant mutants in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* **67:** 1233–1240.

Burgess, S. M., M. Delannoy, and R. E. Jensen (1994). MMM1 encodes a mitochondrial outer membrane protein essential for establishing and maintaining the structure of yeast mitochondria. *J. Cell Biol.* **126:** 1375–1391.

Campbell, C. L., N. Tanaka, K. H. White, and P. E. Thorsness (1994). Mitochondrial morphological and functional defects in yeast caused by yme1 are suppressed by mutation of a 26S protease subunit homologue. *Mol. Biol. Cell* **5:** 899–905.

Carignani, G., O. Groudinsky, D. Frezza, E. Schiavon, E. Bergantino, and P. P. Slonimski (1983). An mRNA maturase is encoded by the first intron of the mitochondrial gene for the subunit I of cytochrome oxidase in *S. cerevisiae*. *Cell* **35:** 733–742.

Chen, Y.-Y. and N. C. Martin (1988). Biosynthesis of tRNA in yeast mitochondria. An endonuclease is responsible for the 3′-processing of tRNA precursors. *J. Biol. Chem.* **263:** 13677–13682.

Chen, W. and C. L. Dieckmann (1994). Cbp1p is required for message stability following 5′-processing of COB mRNA. *J. Biol. Chem.* **269:** 16574–16578.

Christianson, T. and M. Rabinowitz (1983). Identification of multiple transcriptional initiation sites on the yeast mitochondrial genome by *in vitro* capping with guanylyltransferase. *J. Biol. Chem.* **258:** 14025–14033.

Coetzee, T., D. Herschlag, and M. Belfort (1994). *Escherichia coli* proteins, including ribosomal protein S12, facilitate *in vitro* splicing of phage T4 introns by acting as RNA chaperones. *Genes Dev.* **8:** 1575–1588.

Conrad-Webb, H., P. S. Perlman, H. Zhu, and R. A. Butow (1990). The nuclear SUV3-1 mutation affects a variety of post-transcriptional processes in yeast mitochondria. *Nucl. Acids Res.* **18:** 1369–1376.

Copertino, D. W. and R. B. Hallick (1991). Group II twintron: an intron within an intron in a chloroplast cytochrome b-559 gene. *EMBO J.* **10:** 433–442.

Costanzo, M., E. C. Seaver, and T. D. Fox (1986). At least two nuclear gene products are specifically required for translation of a single yeast mitochondrial mRNA. *EMBO J.* **5:** 3637–3641.

Costanzo, M. C. and T. D. Fox (1988). Specific translational activation by nuclear gene products occurs in the 5′ untranslated leader of a yeast mitochondrial mRNA. *Proc. Natl. Acad. Sci. U.S.A.* **85:** 2677–2681.

Costanzo, M. C. and T. D. Fox (1990). Control of mitochondrial gene expression in *Saccharomyces cerevisiae*. *Annu. Rev. Genet.* **24:** 91–113.

Costanzo, M. C. and T. D. Fox (1993). Suppression of a defect in the 5′ untranslated leader of mitochondrial COX3 messenger RNA by a mutation affecting a messenger RNA-specific translational activator protein. *Mol. Cell Biol.* **13:** 4806–4813.

Costanzo, M. C., P. P. Mueller, C. A. Strick, and T. D. Fox (1986). Primary structure of wild-type and mutant alleles of the PET494 gene of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **202:** 294–301.

Costanzo, M. C., E. C. Seaver, and T. D. Fox (1989). The PET54 gene of *Saccharomyces cerevisiae:* characterization of a nuclear gene encoding a mitochondrial translational activator and subcellular localization of its product. *Genetics* **122:** 297–305.

Crivellone, M. D. (1994). Characterization of CBP4, a new gene essential for the expression of ubiquinol-cytochrome c reductase in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **269:** 21284–21292.

Cupp, J. R. and L. McAlister-Henn (1991). NAD(+)-dependent isocitrate dehydrogenase. Cloning, nucleotide sequence and disruption of the IDH2 gene from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **266:** 22199–22205.

Cupp, J. R. and L. McAlister-Henn (1992). Cloning and characterization of the gene coding the IDH1 subunit of NAD(+)-dependent isocitrate dehydrogenase from *Saccharomyces cerevisiae*, *J. Biol. Chem.* **267:** 16417–16423.

Dang, Y. L. and N. C. Martin (1993). Yeast mitochondrial RNase-P — sequence of the RPM2 gene and demonstration that its product is a protein subunit of the enzyme. *J. Biol. Chem.* **268:** 19791–19796.

Davies, R. W., R. B. Waring, J. A. Ray, T. A. Brown, and C. Scazzocchio (1982). Making ends meet: a model for RNA splicing in fungal mitochondria. *Nature* **300:** 719–724.

de la Salle, H., C. Jacq, and P. P. Slonimski (1982). Critical sequences within mitochondrial introns: pleiotropic mRNA maturase and cis-dominant signals of the box intron controlling reductase and oxidase. *Cell* **28:** 721–732.

de Vries, S. and L. A. Grivell (1988). Purification and characterization of a rotenone-insensitive NADH:Q6 oxidoreductase from mitochondria of *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **176:** 377–384.

de Vries, S., R. van Witzenburg, L. A. Grivell, and C. A. M. Marres (1992). Primary structure and import pathway of the rotenone-insensitive NADH-ubiquinone oxidoreductase of mitochondria from *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **203:** 587–592.

de Winde, J. H. and L. A. Grivell (1993). Global regulation of mitochondrial biogenesis in *Saccharomyces cerevisiae*. *Prog. Nucl. Acid Res. Mol. Biol.* **46:** 51–91.

Decoster, E., M. Simon, D. Hatat, and G. Faye (1990). The MSS51 gene product is required for translation of the COX1 mRNA in yeast mitochondria. *Mol. Gen. Genet.* **224:** 111–118.

Dekker, P. J., J. Stuurman, K. van Oosterum, and L. A. Grivell (1992). Determinants for binding of a 40 kDa protein to the leaders of yeast mitochondrial mRNAs. *Nucl. Acids Res.* **20:** 2647–2655.

Dekker, P. J. T., B. Papadopoulou, and L. A. Grivell (1993). In-vitro translation of mitochondrial mRNAs by yeast mitochondrial ribosomes is hampered by the lack of start-codon recognition. *Curr. Genet.* **23:** 22–27.

Desjardins, P., E. Frost, and R. Morais (1985). Ethidium bromide-induced loss of mitochondrial DNA from primary chicken embryo fibroblasts. *Mol. Cell. Biol.* **5:** 1163–1169.

Dieckmann, C. L. and R. R. Staples (1994). Regulation of mitochondrial gene expression in *Saccharomyces cerevisiae*. *Int. Rev. Cytol.* **32:** 145–181.

Dieckmann, C. L. and A. Tzagoloff (1985). Assembly of the mitochondrial membrane system. CBP6, a yeast nuclear gene necessary for the synthesis of cytochrome b. *J. Biol. Chem.* **260:** 1513–1520.

Dietrich, A., J. H. Weil, and L. Marechal-Drouard (1992). Nuclear-encoded transfer RNAs in plant mitochondria. *Ann. Rev. Cell Biol.* **8:** 115–131.

Diffley, J. F. X. and B. Stillman (1991). A close relative of the nuclear, chromosomal high-mobility group protein HMG1 in yeast mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* **88:** 7864–7868.

Diffley, J. F. X. and B. Stillman (1992). DNA binding properties of an HMG1-related protein from yeast mitochondria. *J. Biol. Chem.* **267:** 3368–3374.

Dobinson, K. F., M. Henderson, R. L., Kelley, R. A. Collins, and A. M. Lambowitz (1989). Mutations in nuclear gene cyt-4 of *Neurospora crassa* result in pleiotropic defects in processing and splicing of mitochondrial RNAs. *Genetics* **123:** 97–108.

Dujardin, G., C. Jacq, and P. P. Slonimski (1982). Single base substitution in an intron oxidase gene compensates splicing defects of the cytochrome b gene. *Nature* **298:** 628–632.

Dujon, B. (1989). Group I introns as mobile genetic elements: facts and mechanistic speculations — a review. *Gene* **82:** 91–114.

Dujon, B., D. Alexandraki, B. Andre, W. Ansorge, V. Baladron, J. P. G. Ballesta, A. Banrevi, P. A. Bolle, M. Bolotin-Fukuhara, P. Bossier, et al. (1994). Complete DNA sequence of yeast chromosome XI. *Nature* **369:** 371–378.

Dujon, B., G. Cottarel, L., Colleaux, M. Betermier, A. Jacquier, L. d'Auriol, and F. Galibert (1985). Mechanism of integration of an intron within a mitochondrial gene: a double strand break and the transposase function of an intron encoded protein as revealed by *in vivo* and *in vitro* assays. *Achievements and Perspectives of Mitochondrial Research.* (eds. E. Quagliariello, E. C. Slater, F. Palmieri, C. Saccone, and A. M. Kroon.) Amsterdam, Elsevier. 215–225.

Edwards, J. C., D. Levens, and M. Rabinowitz (1983). Analysis of transcriptional initiation of yeast mitochondrial DNA in a homologous *in vitro* transcription system. *Cell* **31:** 337–346.

Ekwall, K., M. Kermorgant, G. Dujardin, O. Groudinsky, and P. P. Slonimski (1992). The NAM8 gene in *Saccharomyces cerevisiae* encodes a protein with putative RNA binding motifs and acts as a suppressor of mitochondrial splicing deficiencies when overexpressed. *Mol. Gen. Genet.* **233:** 136–144.

Elzinga, S. D. J., A. L. Bednarz, K. van Oosterum, P. J. T. Dekker, and L. A. Grivell (1993). Yeast mitochondrial NAD+-dependent isocitrate dehydrogenase is an RNA-binding protein. *Nucl. Acids Res.* **21:** 5328–5331.

Emmermann, M., H. P. Braun, M. Arretz, and U. K. Schmitz (1993). Characterization of the bifunctional cytochrome-c reductase-processing peptidase complex from potato mitochondria. *J. Biol. Chem.* **268:** 18936–18942.

Eriksson, A. C. and E. Glaser (1992). Mitochondrial processing proteinase — a general processing proteinase of spinach leaf mitochondria is a membrane-bound enzyme. *Biochim. Biophys. Acta* **1140:** 208–214.

Faugeron-Fonty, G., C. Le Van Kim, de Zamaroczy, R. Goursot, and G. Bernardi (1984). A comparative study of the ori sequences from the mitochondrial genomes of twenty wild-type yeast strains. *Gene* **32:** 459–473.

Faye, G., N. Dennebouy, C. Kujawa, and C. Jacq (1979). Inserted sequence in the mitochondrial 23S ribosomal RNA gene of the yeast *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **168:** 101–109.

Ferat, J. L. and F. Michel (1993). Group-II self-splicing introns in bacteria. *Nature* **364:** 358–361.

Finnegan, P. M., M. J. Payne, E. Keramidaris, and H. B. Lukins (1991). Characterization of a yeast nuclear gene, AEP2, required for accumulation of mitochondrial mRNA encoding subunit 9 of the ATP synthese, *Curr. Genet.* **20:** 53–61.

Folley, L. S. and T. D. Fox (1991). Site-directed mutagenesis of a *Saccharomyces cerevisiae* mitochondrial translation initiation codon. *Genetics* **129:** 659–668.

Foury, F. and A. Lahaye (1987). Cloning and sequencing of the PIF gene involved in repair and recombination of yeast mitochondrial DNA. *EMBO J.* **6:** 1441–1449.

Foury, F. and E. Van Dyck (1985). A PIF-dependent recombinogenic signal in the mitochondrial DNA of yeast. *EMBO J.* **4:** 3525–3530.

Franzen, J. S., M. C. Zhang, T. R. Chay, and C. L. Peebles (1994). Thermal activation of a group II intron ribozyme reveals multiple conformational states. *Biochemistry* **33:** 11315–11326.

Fujimura, K., K. Tanaka, A. Nakano, and A. Toh-e (1994). The *Saccharomyces cerevisiae* MSI4 gene encodes the yeast counterpart of component A of Rab geranylgeranyltransferase. *J. Biol. Chem.* **269:** 9205–9212.

Gampel, A. and T. R. Cech (1991). Binding of the CBP2 protein to a yeast mitochondrial group I intron requires the catalytic core of the RNA. *Genes Dev.* **5:** 1870–1880.

Gampel, A., M. Nishikimi, and A. Tzagoloff (1989). CBP2 protein promotes *in vitro* excision of a yeast mitochondrial group I Intron. *Mol. Cell. Biol.* **9:** 5424–5433.

Gargouri, A., J. Lazowska, and P. P. Slonimski (1983). DNA-splicing of introns in the gene: a general way of reverting intron mutations. **In:** *Mitochondrial Interactions,* (eds. R. J. Schweyen, K. Wolf, and F. Kaudewitz) Berlin, de Gruyter. 259–268.

Gbelska, Y., J. Subik, A. Goffeau, and L. Kovac (1983). Intramitochondrial ATP and cell functions: yeast cells depleted of intramitochondrial ATP lose the ability to grow and multiply. *Eur. J. Biochem.* **130:** 281–286.

Gimble, F. S. and J. Thorner (1992). Homing of a DNA endonuclease gene by meiotic gene conversion in *Saccharomyces cerevisiae*. *Nature* **357:** 301–306.

Glerum, D. M. and A. Tzagoloff (1994). Isolation of a human cDNA for heme A:farnesyltransferase by functional complementation of a yeast cox10 mutant. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 8452–8456.

Glick, B. and G. Schatz (1991). Import of proteins into mitochondria. *Annu. Rev. Genet.* **25:** 21–44.

Goguel, V., A. Bailone, R. Devoret, and C. Jacq (1989). The b14 RNA mitochondrial maturase of *Saccharomyces cerevisiae* can stimulate intra-chromosomal recombi-

nation in *Escherichia coli*. *Mol. Gen. Genet.* **216:** 70–74.

Goguel, V., A Delahodde, and C. Jacq (1992). Connections between RNA splicing and DNA intron mobility in yeast mitochondria: RNA maturase and DNA endonuclease switching experiments. *Mol. Cell. Biol.* **12:** 696–705.

Goldberg, A. L. (1992). The mechanism and functions of ATP-dependent proteases in bacterial and animal cells. *Eur. J. Biochem.* **203:** 9–23.

Gray, M. W., P. J. Hanic-Joyce, and P. S. Costello (1992). Transcription, processing and editing in plant mitochondria. *Annu. Rev. Plant Physiol.* **43:** 145–175.

Gray, N. K. and M. W. Hentze (1994). Iron regulatory protein prevents binding of the 43S translation pre-initiation complex to ferritin and eALAS mRNAs. *EMBO J.* **13:** 3882–3891.

Grivell, L. A. (1993). *Mitochondrial DNA in the Yeast Saccharomyces cerevisiae. Genetic Maps. Locus Maps of Complex Genomes.* (ed. S. J. O'Brien) Cold Spring Harbor, Cold Spring Harbor Laboratory Press. 2.57–3.65.

Grivell, L. A. (1994). Invasive introns. *Curr. Biol.* **4:** 161–164.

Groudinsky, O., I. Bousquet, M. G. Wallis, P. P. Slonimski, and G. Dujardin (1993). The NAM1/MTF2 nuclear gene product is selectively required for the stability and/or processing of mitochondrial transcripts of the atp6 and of the mosaic, cox1 and cytb genes in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **240:** 419–427.

Guan, K. L., L. Farh, T. K. Marshall, and R. J. Deschenes (1993). Normal mitochondrial structure and genome maintenance in yeast requires the dynamin-like product of MGM1 gene. *Curr. Genet.* **24:** 141–148.

Guelin, E., M. Rep, and L. A. Grivell (1994). Sequence of the AFG3 gene encoding a new member of the FtsH/Yme1/Tma subfamily of the AAA-protein family. *Yeast* **10:** 1389–1394.

Guo, Q. and A. M. Lambowitz (1992). A tyrosyl-tRNA synthetase binds specifically to the group I intron catalytic core. *Genes Dev.* **6:** 1357–1372.

Haffter, P. and T. D. Fox (1992). Nuclear mutations in the petite-negative yeast Schizosaccharomyces pombe allow growth of cells lacking mitochondrial DNA. *Genetics* **131:** 255–260.

Haffter, P. and T. D. Fox (1992). Suppression of carboxy-terminal truncations of the yeast mitochondrial mRNA-specific translational activator PET122 by mutations in two new genes, MRP17 and PET127. *Mol. Gen. Genet.* **235:** 64–73.

Haffter, P., T. W. McMullin, and T. D. Fox (1990). A genetic link between a messenger RNA-specific translational activator and the translation system in yeast mitochondria. *Genetics*, **125:** 495–503.

Haffter, P., T. W. McMullin, and T. D. Fox (1991). Functional interactions among two yeast mitochondrial ribosomal proteins and an mRNA-specific translational activator. *Genetics* **127:** 319–326.

Hanson, M. R. (1991). Plant mitochondrial mutations and male sterility. *Annu. Rev. Genet.* **25:** 461–486.

Hartl, F.-U. and W. Neupert (1990). Protein sorting to mitochondria: evolutionary conservations of folding and assembly. *Science* **247:** 930–938.

He, F., S. W. Peltz, J. L. Donahue, M. Rosbash, and A. Jacobson (1993). Stabilization and ribosome association of unspliced pre-mRNAs in a yeast upf1-mutant. *Proc. Natl. Acad. Sci. U.S.A.* **90:** 7034–7038.

Hensgens, L. A. M., L. Bonen, M. De Haan, G. Van der Horst, and L. A. Grivell (1983). Two intron sequences in yeast mitochondrial COX1 gene: homology among URF-containing introns and strain-dependent variation in flanking exons. *Cell* **32:** 379–389.

Hentze, M. W. (1994). Enzymes as RNA-binding proteins: a role for (di)nucleotide-binding domains? *Trends Biochem. Sci.* **19:** 101–103.

Herbert, C. J., M. Labouesse, G. Dujardin, and P. P. Slonimski (1988). The NAM2 proteins from *S. cerevisiae* and *S. douglasii* are mitochondrial leucyl-tRNA synthetases, and are involved in mRNA splicing. *EMBO J.* **7:** 473–483.

Herman, C., T. Ogura, T. Tomoyasu, S. Hiraga, Y. Akiyama, K. Ito, R. Thomas, R. D'Ari, and P. Bouloc (1993). Cell growth and lambda phage development controlled by the same essential *Escherichia coli* gene, ftsH/hflB. *Proc. Natl. Acad. Sci. U.S.A.* **90:** 10861–10865.

Hockenbery, D. M., G. Nuñez, C., Milliman, R. D. Schreiber, and S. J. Korsmeyer (1990). Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* **348:** 334–336.

Hockenbery, D. M., Z. N. Oltvai, X. M. Yin, C. L. Milliman, and S. J. Korsmeyer (1993). Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* **75:** 241–251.

Hoeben, P., G. Weiller, and G. D. Clark Walker (1993). Larger rearranged mitochondrial genomes in Dekkera/Brettanomyces yeasts are more closely related than smaller genomes with a conserved gene order. *J. Mol. Evol.* **36:** 263–269.

Hofmann, T. J., J. J. Min, and H. P. Zassenhaus (1993). Formation of the 3′-end of yeast mitochondrial mRNAs occurs by site-specific cleavage two bases downstream of a conserved dodecamer sequence. *Yeast* **9:** 1319–1330.

Hong, L. and R. B. Hallick (1994). A group III intron is formed from domains of two individual group II introns. *Genes Dev.* **8:** 1589–1599.

Hopper, A. K. and N. C. Martin (1992). Processing of yeast cytoplasmic and mitochondrial precursor tRNAs. *The Molecular and Cellular Biology of the Yeast* Saccharomyces. (eds. E. W. Jones, J. R. Pring, and J. R. Broass) (Cold Spring Harbor, Cold Spring Harbor Laboratory Press. 99–141.

Isaya, G., F. Kalousek, and L. E. Rosenberg (1992). Sequence analysis of rat mitochondrial intermediate peptidase: similarity to zinc metallopeptidases and to a putative yeast homologue. *Proc. Natl. Acad. Sci. U.S.A.* **89:** 8317–8321.

Isaya, G., D. Miklos, and R. A. Rollins (1994). MIP1, a new yeast gene homologous to the rat mitochondrial intermediate peptidase gene, is required for oxidative metabolism in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **14:** 5603–5616.

Jacquier, A. and B. Dujon (1985). An intron-encoded protein is active in a gene conversion process that spreads an intron into a mitochondrial gene. *Cell* **41:** 383–394.

Jang, S. H. and J. A. Jaehning (1991). The yeast mitochondrial RNA polymerase specificity factor, MTF1, is similar to bacterial sigma factors. *J. Biol. Chem.* **266:** 22671–22677.

Janitor, M. and J. Subik (1993). Molecular cloning of the PEL1 gene of *Saccharomyces cerevisiae* that is essential for the viability of petite mutants. *Curr. Genet.* **24:** 307–312.

Jiang, Y. and S. Ferro-Novick (1994). Identification of yeast component A: reconstitution of the geranylgeranyl transferase that modifies Ypt1p and Sec4p. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 4377–4381.

Johnston, M., S. Andrews, R. Brinkman, J. Cooper, H. Ding, J. Dover, Z. Du, A. Favello, L. Fulton, S. Gattung, et al. (1994). Complete nucleotide sequence of *Saccharomyces cerevisiae* chromosome VIII. *Science* **265:** 2077–2082.

Jones, B. A. and W. L. Fangman (1992). Mitochondrial DNA maintenance in yeast requires a protein containing a region related to the GTP-binding domain of dynamin. *Genes Dev.* **6:** 380–389.

Kämper, U., U. Kück, A. D. Cherniack, and A. M. Lambowitz (1992). The mitochondrial tyrosyl-tRNA synthetase of *Podospora anserina* is a bifunctional enzyme active in protein synthesis and RNA splicing. *Mol. Cell. Biol.* **12:** 499–511.

Kao, L. R., T. L. Megraw, and C. B. Chae (1993). Essential role of the HMG domain in the function of yeast mitochondrial histone HM — functional complementation of HM by the nuclear nonhistone protein NHP6A. *Proc. Natl. Acad. Sci. U.S.A.* **90:** 5598–5602.

Kawasaki, K., M. Takahashi, M. Natori, and T. Shibata (1991). DNA sequence recognition by a eukaryotic sequence-specific endonuclease, Endo. SceI, from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **266:** 5342–5347.

Kennell, J. C., J. V. Moran, P. S. Perlman, R. A. Butow, and A. M. Lambowitz (1993). Reverse transcriptase activity associated with maturase-encoding group-II introns in yeast mitochondria. *Cell* **73:** 133–146.

Kitakawa, M. and K. Isono (1991). The mitochondrial ribosomes. *Biochimie* **73:** 813–825.

Kittle, J. D., Jr., G. Mohr, J. A. Gianelos, H. Wang, and A. M. Lambowitz (1991). The *Neurospora* mitochondrial tyrosyl-tRNA synthetase is sufficient for group I intron splicing *in vitro* and uses the carboxy-terminal tRNA-binding domain along with other regions. *Genes Dev.* **5:** 1009–1021.

Kloeckener-Gruissem, B., J. E. McEwen, and R. O. Poyton (1988). Identification of a third nuclear protein-coding gene required specifically for post-transcriptional expression of the COX3 gene in *Saccharomyces cerevisiae*. *J. Bacteriol.* **170:** 1399–1402.

Klootwijk, J., I. Klein, and L. A. Grivell (1975). Minimal post-transcriptional modification of yeast mitochondrial ribosomal RNA. *J. Mol. Biol.* **97:** 337–350.

Krummeck, G. and G. Rödel (1990). Yeast SCO1 protein is required for a post-translational step in the accumulation of mitochondrial cytochrome c oxidase subunit I and II. *Curr. Genet.* **18:** 13–15.

Kunau, W. H., A. Beyer, T. Franken, K. Gotte, M. Marzioch, J. Saidowsky, A. Skaletz-Rorowski, and F. F. Wiebel (1993). Two complementary approaches to study peroxisome biogenesis in *Saccharomyces cerevisiae:* forward and reversed genetics. *Biochimie* **75:** 209–224.

Labouesse, M. (1990). The yeast mitochondrial leucyl-tRNA synthetase is a splicing factor for the excision of several group I introns. *Mol. Gen. Genet.* **224:** 209–221.

Labouesse, M., C. J. Herbert, G. Dujardin, and P. P. Slonimski (1987). Three suppressor mutations which cure a mitochondrial RNA maturase deficiency occur at the same codon in the open reading frame of the nuclear NAM2 gene. *EMBO J.* **6:** 713–721.

Lahaye, A., S. Leterme, and F. Foury (1993). PIF1 DNA helicase from *Saccharomyces cerevisiae* — biochemical characterization of the enzyme. *J. Biol. Chem.* **268:** 26155–26161.

Lahaye, A., H. Stahl, D. Thines-Sempoux, and F. Foury (1991). PIF1: a DNA helicase in yeast mitochondria. *EMBO J.* **10:** 997–1007.

Lambowitz, A. M. and M. Belfort (1993). Introns as mobile genetic elements. *Annu. Rev. Biochem.* **62:** 587–622.

Lambowitz, A. M. and P. S. Perlman (1990). Involvement of aminoacyl-tRNA synthetases and other proteins in group I and group II intron splicing. *Trends Biochem. Sci.* **15:** 440–444.

Lazowska, J., B. Meunier, and C. Macadre (1994). Homing of a group II intron in yeast mitochondrial DNA is accompanied by unidirectional co-conversion of upstream-located markers. *EMBO J.* **13:** 4963–4972.

Lazowska, J., T. Szczpanek, C. Macadre, and M. Dokova (1992). Two homologous mitochondrial introns from closely related *Saccharomyces* species differ by only a few amino acid replacements in their open reading frames: one is mobile, the other is not. *C.R. Acad. Sci. Paris* **315:** 37–41.

Leeds, P., J. M. Wood, B. S. Lee, and M. R. Culbertson (1992). Gene products that promote mRNA turnover in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12:** 2165–2177.

Leonhardt, S. A., K. Fearson, P. N. Danese, and T. L. Mason (1993). HSP78 encodes a yeast mitochondrial heat shock protein in the Clp family of ATP-dependent proteases. *Mol. Cell. Biol.* **13:** 6304–6313.

Li, G.-Y., C. J. Herbert, M. Labouesse, and P. P. Slonimski (1992). *In vitro* mutagenesis of the mitochondrial leucyl-tRNA synthetase of *S. cerevisiae* reveals residues critical for its *in vivo* activities. *Curr. Genet.* **22:** 69–74.

Li, M. and A. Tzagoloff (1979). Assembly of the mitochondrial membrane system: sequences of yeast mitochondrial valine and an unusual threonine tRNA gene. *Cell* **18:** 47–53.

Li, M., A. Tzagoloff, K. Underbrink-Lyon, and N. C. Martin (1982). Identification of the paromomycin-resistance mutation in the 15S rRNA gene of yeast mitochondria. *J. Biol. Chem.* **257:** 5921–5928.

Lisowsky, T. (1990). Molecular analysis of the mitochondrial transcription factor MTF2 of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **220:** 186–190.

Lisowsky, T. and G. Michaelis (1988). A nuclear gene essential for mitochondrial replication suppresses a defect of mitochondrial transcription in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **214:** 218–223.

Lisowsky, T. and G. Michaelis (1989). Mutations in the genes for mitochondrial RNA polymerase and a second mitochondrial transcription factor of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **219:** 125–128.

Lisowsky, T., G. Riemen, and G. Michaelis (1990). Change of serine309 into proline causes temperature sensitivity of the nuclearNAM1/MTF2 gene product for yeast mitochondria. *Nucl. Acids Res.* **18:** 7163.

Luft, R. (1994). The development of mitochondrial medicine. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 8731–8738.

Maleszka, R. and G. D. Clark-Walker (1989). A petite positive strain of *Kluyveromyces lactis* has a 300 kb deletion in the rDNA cluster. *Curr. Genet.* **16:** 429–432.

Maleszka, R., P. J. Skelly, and G. D. Clark-Walker (1991). Rolling circle replication of DNA in yeast mitochondria. *EMBO J.* **10:** 3923–3929.

Manthey, G. M. and J. E. McEwen (1994). The product of the nuclear gene PET309 is required for the translation of mature mRNA and stability or elongation of intron-containing RNAs derived from the mitochondrial COX1 locus of *Saccharomyces cerevisiae*. Submitted for publication.

Martin, N. C. and M. Rabinowitz (1976). Transfer RNAs of yeast mitochondria. *Genetics and Biogenesis of Chloroplasts and Mitochondria*. Amsterdam, Elsevier, 749–754.

Martin, R. P., J.-M. Schneller, A. J. C. Stahl, and G. Dirheimer (1979). Import of nuclear deoxynucleic acid coded lysine-accepting transfer ribonucleic acid (anticodon C-U-U) into yeast mitochondria. *Biochemistry*, **18:** 4600–4605.

Masters, B. S., L. L. Stohl, and D. A. Clayton (1987). Yeast mitochondrial RNA polymerase is homologous to those encoded by bacteriophages T3 and T7. *Cell* **51:** 89–99.

Matsushita, Y. and K. Isono (1993). Mitochondrial transport of mitoribosomal proteins, YmL8 and YmL20, in *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **214:** 577–585.

McConnell, S. J. and M. P. Yaffe (1992). Nuclear and mitochondrial inheritance in yeast depends on novel cytoplasmic structures defined by the MDM1 protein. *J. Cell Biol.* **118:** 385–395.

McEwen, J. E., K. H. Hong, S. Park, and G. T. Preciado (1993). Sequence and chromosomal localization of two PET genes required for cytochrome c oxidase assembly in *Saccharomyces cerevisiae*. *Curr. Genet.* **23:** 9–14.

McEwen, J. E., C. Ko, B. Kloeckner-Gruissem, and R. O. Poyton (1986). Nuclear functions required for cytochrome c oxidase biogenesis in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **261:** 11872–11879.

McMullin, T. W. and T. D. Fox (1993). COX3 messenger RNA-specific translational activator proteins are associated with the inner mitochondrial membrane in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **268:** 11737–11741.

McMullin, T. W., P. Haffter, and T. D. Fox (1990). A novel small-subunit ribosomal protein of yeast mitochondria that interacts functionally with an mRNA-specific translational activator. *Mol. Cell. Biol.* **10:** 4590–4595.

Menrath, M. and W. Kunz (1994). EMBL Acc. No. Z29947.

Meunier, B., G.-L. Tian, C. Macadre, P. P. Slonimski, and J. Lazowska (1990). Group II introns transpose in yeast mitochondria. *Structure, Function and Biogenesis of Energy Transfer Systems*. (eds. E. Quagliariello, S. Papa, F. Palmieri, and C. Saccone.) Amsterdam, Elsevier. 169–174.

Michaelis, U., A. Körte, and G. Rödel (1991). Association of cytochrome b translational activator proteins with the mitochondrial membrane: implications for cytochrome b expression in yeast. *Mol. Gen. Genet.* **230:** 177–185.

Michaelis, U. and G. Rödel (1990). Identification of CBS2 as a mitochondrial protein in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **223:** 394–400.

Michel, F. and B. F. Lang (1985). Mitochondrial class II introns encode proteins related to the reverse transcriptases of retroviruses. *Nature* **316:** 641–643.

Michon, T., M. Galante and J. Velours (1988). NH_2-terminal sequence of the isolated yeast ATP synthase subunit 6 reveals posttranslational cleavage. *Eur. J. Biochem.* **172:** 621–625.

Miller, D. L. and N. C. Martin (1983). Characterization of the yeast mitochondrial locus necessary for tRNA biosynthesis: DNA sequence analysis and identification of a new transcript. *Cell* **34:** 911–917.

Min, J. J., R. M. Heuertz, and H. P. Zassenhaus (1993). Isolation and characterization of an NTP-dependent 3′-exoribonuclease from mitochondria of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **268:** 7350–7357.

Min, J. J. and H. P. Zassenhaus (1993). Identification of a protein complex that binds to a dodecamer sequence found at the 3′ ends of yeast mitochondrial messenger RNAs. *Mol. Cell. Biol.* **13:** 4167–4173.

Mittelmeier, T. M. and C. L. Dieckmann (1993). *In vivo* analysis of sequences necessary for CBP1-dependent accumulation of cytochrome-b transcripts in yeast mitochondria. *Mol. Cell. Biol.* **13:** 4203–4213.

Mohr, G., P. S. Perlman, and A. M. Lambowitz (1993). Evolutionary relationships among group-II intron-encoded proteins and identification of a conserved domain that may be related to maturase function. *Nucl. Acids Res.* **21:** 4991–4997.

Mohr, G., A. Zhang, J. A. Gianelos, M. Belfort, and A. M. Lambowitz (1992). The *Neurospora* CYT-18 protein suppresses defects in the phage T4 td intron by stabilizing the catalytically active structure of the intron core. *Cell* **69:** 483–494.

Moneger, F., C. J. Smart, and C. J. Leaver (1994). Nuclear restoration of cytoplasmic male sterility in sunflower is associated with the tissue-specific regulation of a novel mitochondrial gene. *EMBO J.* **13:** 8–17.

Moorman, A. F. M., G. J. B. van Ommen, and L. A. Grivell (1978). Transcription in yeast mitochondria: isolation and physical mapping of messenger RNAs for subunits of cytochrome c oxidase and ATPase. *Mol. Gen. Genet.* **160:** 13–24.

Moran, J. V., K. L. Mecklenburg, P. Sass, S. M. Belcher, D. Mahnke, A. Lewin, and P. Perlman (1994). Splicing defective mutants of the COXI gene of yeast mitochondrial DNA: initial definition of the maturase domain of the group II intron AI2. *Nucl. Acids Res.* **22:** 2057–2064.

Morishima, N., K. Hakagawa, E. Yamamoto, and T. Shibata (1990). A subunit of yeast site-specific endonuclease SceI is a mitochondrial version of the 70-kDa heat shock protein. *J. Biol. Chem.* **265:** 15189–15197.

Mörl, M., I. Niemer, and C. Schmelzer (1992). New reactions catalyzed by a group II intron ribozyme with RNA and DNA substrates. *Cell* **70:** 803–810.

Mörl, M. and C. Schmelzer (1990). Integration of group II intron BI1 into a foreign RNA by reversal of the self-splicing reaction *in vitro*. *Cell* **60:** 629–636.

Mueller, D. M. and G. S. Getz (1986). Transcriptional regulation of the mitochondrial genome of yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* **261:** 11756–11764.

Mueller, M., M. Allmaier, R. Eskes, and R. J. Schweyen (1993). Transposition of group II intron aI1 in yeast and invasion of mitochondrial genes at new locations. *Nature* 366: 174–176.

Mulero, J. J. and T. D. Fox (1993). Alteration of the *Saccharomyces cerevisiae* cox2 mRNA 5′-untranslated leader by mitochondrial gene replacement and functional interaction with the translational activator protein PET111. *Mol. Biol. Cell* **4:** 1327–1335.

Mulero, J. J. and T. D. Fox (1993). PET111 acts in the 5′-leader of the *Saccharomyces cerevisiae* mitochondrial COX2 messenger RNA to promote its translation. *Genetics* **133:** 509–516.

Mulero, J. J. and T. D. Fox (1994). Reduced but accurate translation from a mutant AUA initiation codon in the mitochondrial COX2 mRNA of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **242:** 383–390.

Mulero, J. J., J. K. Rosenthal, and T. D. Fox (1994). PET112, a *Saccharomyces cerevisiae* nuclear gene required to maintain rho(+) mitochondrial DNA. *Curr. Genet.* **25:** 299–304.

Muroff, I. and A. Tzagoloff (1990). CBP7 codes for a cofactor required in conjunction with a mitochondrial maturase for splicing of its cognate intervening sequence. *EMBO J.* **9:** 2765–2773.

Myers, A. M., L. K. Pape, and A. Tzagoloff (1985). Mitochondrial protein synthesis is required for maintenance of intact mitochondrial genomes in *Saccharomyces cerevisiae*. *EMBO J.* **4:** 2087–2092.

Nagata, S., Y. Tsunetsugu-Yokota, A. Naito, and Y. Kaziro (1983). Molecular cloning and sequence determination of the nuclear gene coding for mitochondrial elongation factor Tu of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* **80:** 6192–6196.

Nakagawa, K., N. Morishima, and T. Shibata (1991). A maturase-like subunit of the sequence-specific endonuclease Endo.SceI from yeast mitochondria. *J. Biol. Chem.* **266:** 1977–1984.

Neefs, J.-M., Y. van de Peer, P. de Rijk, A. Goris, and R. de Wachter (1991). Compilation of small ribosomal subunit RNA sequences. *Nucl. Acids Res.* **19:** 1987–2015.

Nguyen, M., D. G. Millar, V. W. Yong, S. J. Korsmeyer, and G. C. Shore (1993). Targeting of Bcl-2 to the mitochondrial outer membrane by a COOH-terminal signal anchor sequence. *J. Biol. Chem.* **268:** 25265–25268.

Nickelsen, J., J. Vandillewijn, M. Rahire, and J. D. Rochaix (1994). Determinants for stability of the chloroplast psbD RNA are located within its short leader region in *Chlamydomonas reinhardtii*. *EMBO J.* **13:** 3182–3191.

Nobrega, F. G., M. P. Nobrega, and A. Tzagoloff (1992). BCS1, a novel gene required for the expression of functional Rieske iron-sulfur protein in *Saccharomyces cerevisiae*. *EMBO J.* **11:** 3821–3829.

Nobrega, M. P., F. G. Nobrega, and A. Tzagoloff (1990). COX10 codes for a protein homologous to the orf1 product of *Paracococcus denitrificans* and is required for the synthesis of yeast cytochrome oxidase. *J. Biol. Chem.* **265:** 14220–14226.

Nunnari, J., T. D. Fox, and P. Walter (1993). A mitochondrial protease with 2 catalytic subunits of nonoverlapping specificities. *Science* **262:** 1997–2004.

Ohmen, J. D., B. Kloeckener-Gruissem, and J. E. McEwen (1988). Molecular cloning and nucleotide sequences of the nuclear PET122 gene required for expression of the mitochondrial COX3 gene in *S. cerevisiae*. *Nucl. Acids Res.* **16:** 10783–10802.

Oliver, S. G. et al. (1992). The complete DNA sequence of yeast chromosome III. *Nature* **357:** 38–46.

Osiewacz, H. D. and K. Esser (1984). The mitochondrial plasmid of *Podospora anserina:* a mobile intron of a mitochondrial gene. *Curr. Genet.* **8:** 299–305.

Osinga, K. A., E. De Vries, G. Van der Horst, and H. F. Tabak (1984). Processing of yeast mitochondrial messenger RNAs at a conserved dodecamer sequence. *EMBO J.* **3:** 829–834.

Pajic, A., R. Tauer, H. Feldmann, W. Neupert, and T. Langer (1994). Yta10p is required for the ATP-dependent degradation of polypeptides in the inner membrane of mitochondria. *FEBS Lett.* **353:** 201–206.

Papadopoulou, B., P. Dekker, J. Blom, and L. A. Grivell (1990). A 40 kd protein binds specifically to the 5′-untranslated regions of yeast mitochondrial mRNAs. *EMBO J.* **9:** 4135–4143.

Parisi, M. A., B. J. Xu, and D. A. Clayton (1993). A human mitochondrial transcriptional activator can functionally replace a yeast mitochondrial HMG-box protein both *in vivo* and *in vitro*. *Mol. Cell. Biol.* **13:** 1951–1961.

Payne, M. J., P. M. Finnegan, P. M. Smooker, and H. B. Lukins (1993). Characterization of a second nuclear gene, AEP1, required for expression of the mitochondrial OLI1 gene in *Saccharomyces cerevisiae*. *Curr. Genet.* **24:** 126–135.

Pel, H. J. and L. A. Grivell (1993). The biology of yeast mitochondrial introns. *Mol. Biol. Rep.* **18:** 1–13.

Pel, H. J. and L. A. Grivell (1994). Protein synthesis in mitochondria. *Mol. Biol. Rep.* **19:** 183–194.

Pel, H. J., C. Maat, M. Rep, and L. A. Grivell (1992). The yeast nuclear gene MRF1 encodes a mitochondrial peptide chain release factor and cures several mitochondrial RNA splicing defects. *Nucl. Acids Res.* **20:** 6339–6346.

Perea, J., A. Delahodde, V. Goguel, D. Hatat, B. Sargueil, and C. Jacq (1990). Functions of intron-encoded proteins from yeast mitochondrial genome. RNA maturase and DNA endonuclease. *Structure, Function and Biogenesis of Energy Transfer Systems,* (eds. E. Quagliariello, S. Papa, F. Palmieri, and C. Saccone). Amsterdam, Elsevier, 205–208

Pfanner, N., E. A. Craig, and M. Meijer (1994). The protein import machinery of the mitochondrial inner membrane. *Trends Biochem. Sci.* **19:** 368–372.

Pinkham, J. L., A. M. Dudley, and T. L. Mason (1994). T7 RNA polymerase-dependent expression of COXII in yeast mitochondria. *Mol. Cell. Biol.* **14:** 4643–4652.

Piskur, J. (1988a). A 5 kb intergenic region containing ori1 in the mitochondrial DNA of *Saccharomyces cerevisiae* is dispensable for expression of the respiratory phenotype. *FEBS Lett.* **229:** 145–149.

Piskur, J. (1988b). Transmission of yeast mitochondrial loci to progeny is reduced when nearby intergenic regions containing ori sequences are deleted. *Mol. Gen. Genet.* **214:** 425–432.

Poutre, C. and T. D. Fox (1987). PET111, a *Saccharomyces cerevisiae* nuclear gene required for translation of the mitochondrial mRNA encoding cytochrome c oxidase subunit II. *Genetics* **115:** 637–647.

Preiss, T., A. G. Hall, and R. N. Lightowlers (1993). Identification of bovine glutamate dehydrogenase as an RNA-binding protein. *J. Biol. Chem.* **268:** 24523–24526.

Ragnini, A., R. Teply, M. Waldherr, A. Voskova, and R. J. Schweyen (1994). The yeast protein Mrs6p, a homologue of the rabGDI and human choroideraemia proteins, affects cytoplasmic and mitochondrial functions. *Curr. Genet.* **26:** 308–314.

Reenan, R. A. G. and R. D. Kolodner (1992). Characterization of insertion mutations in the *Saccharomyces cerevisiae* MSH1 and MSH2 genes — evidence for separate mitochondrial and nuclear functions. *Genetics,* **132:** 975–985.

Rodel, G. (1986). Two yeast nuclear genes, CBS1 and CBS2, are required for translation of mitochondrial transcripts bearing the 5′-untranslated COB leader. *Curr. Genet.* **11:** 41–45.

Rodel, G., U. Michaelis, V. Forsbach, J. Kreike, and F. Kaudewitz. (1986). Molecular cloning of the yeast nuclear genes CBS1 and CBS2. *Curr. Genet.* **11:** 47–53.

Rose, A. M., P. B. Joyce, A. K. Hopper, and N. C. Martin (1992). Separate information required for nuclear and subnuclear localization: additional complexity in localizing an enzyme shared by mitochondria and nuclei. *Mol. Cell. Biol.* **12:** 5652–5658.

Rosenthal, L. P. and J. W. Bodley (1987). Purification and characterization of *Saccharomyces cerevisiae* mitochondrial elongation factor Tu. *J. Biol. Chem.* **262:** 10955–10959.

Russell, D. W., R. Jensen, M. J. Zoller, J. Burke, B. Errede, M. Smith, and I. Herskowitz (1986). Structure of the *Saccharomyces cerevisiae* HO gene and analysis of its upstream regulatory region. *Mol. Cell. Biol.* **6:** 4281–4294.

Sachs, A. B. and R. W. Davis (1990). Translation initiation and ribosomal biogenesis: involvement of a putative rRNA helicase and RPL46. *Science* **247:** 1077–1079.

Sainsard-Chanet, A., O. Begel, and L. Belcour (1993). DNA deletion of mitochondrial introns is correlated with the process of senescence in *Podospora anserina*. *J. Mol. Biol.* **234:** 1–7.

Saldanha, R., G. Mohr, M. Belfort, and A. M. Lambowitz (1993). Group I and group II introns. *FASEB J.* **7:** 15–24.

Schäfer, B., A. M. Merlos-Lange, C. Anderl, F. Welser, M. Zimmer, and K. Wolf (1991). The mitochondrial genome of fission yeast: inability of all introns to splice autocatalytically, and construction and characterization of an intronless genome. *Mol. Gen. Genet.* **255:** 158–167.

Schäfer, B., B. Wilde, D. R. Massardo, F. Manna, L. DelGiudice, and K. Wolf (1994). A mitochondrial group-I intron in fission yeast encodes a maturase and is mobile in crosses. *Curr. Genet.* **25:** 336–341.

Schapira, M., C. Desdouets, C. Jacq, and J. Perea (1993). I-Sce-III an intron-encoded DNA endonuclease from yeast mitochondria — asymmetrical DNA binding properties and cleavage reaction. *Nucl. Acids Res.* **21:** 3683–3689.

Schekman, R. (1994). Translocation gets a push. *Cell* **78:** 911–913.

Schinkel, A. H., M. J. A. Groot-Koerkamp, and H. F. Tabak (1988). Mitochondrial RNA polymerase of *Saccharomyces cerevisiae:* subunit composition and mechanism of promoter recognition. *EMBO J.* **7:** 3255–3262.

Schinkel, A. H. and H. F. Tabak (1989). Mitochondrial RNA polymerase: dual role in transcription and replication. *Trends Genet.* **5:** 149–154.

Schnall, R., G. Mannhaupt, R. Stucka, R. Tauer, S. Ehnle, C. Schwarzlose, I. Vetter, and H. Feldmann (1994). Identification of a set of yeast genes coding for a novel family of putative ATPases with high similarity to

constituents of the 26S protease complex. *Yeast* **10:** 1141–1155.

Schön, A., C. G. Kannangara, S. Gough, and D. Söll (1988). Protein biosynthesis in organelles requires misacylation of tRNA. *Nature* **331:** 187–190.

Schuler, G. D., S. F. Altschul, and D. J. Lipman (1991). A workbench for multiple alignment construction and analysis. *Proteins Struct. Funct. Genet.* **9:** 180–190.

Schulte, U., M. Arretz, H., Schneider, M. Tropschug, E. Wachter, W. Neupert, and H. Weiss (1989). A family of mitochondrial proteins involved in bioenergetics and biogenesis. *Nature* **339:** 147–149.

Schulz, V. P. and V. A. Zakian (1994). The *Saccharomyces* PIF1 DNA helicase inhibits telomere elongation and *de novo* telomere formation. *Cell* **76:** 145–155.

Schulze, M. and G. Rödel (1989). Accumulation of the cytochrome c oxidase subunits I and II in yeast requires a mitochondrial membrane-associated protein, encoded by the nuclear SCO1 gene. *Mol. Gen. Genet.* **216:** 37–43.

Schweyen, R. J. (1994). Personal communication.

Sellem, C. H., G. Lecellier, and L. Belcour (1993). Transposition of a group II intron. *Nature* **366:** 176–178.

Séraphin, B., M. Simon, A. Boulet, and G. Faye (1989). Mitochondrial splicing requires a protein from a novel helicase family. *Nature* **337:** 84–87.

Séraphin, S., M. Simon, and G. Faye (1988). MSS18, a yeast nuclear gene involved in the splicing of intron a15beta of the mitochondrial cox1 transcript. *EMBO J.* **7:** 1455–1464.

Shub, D. A. and H. Goodrich-Blair (1992). Protein introns: a new home for endonucleases. *Cell* **71:** 183–186.

Shub, D. A., H. Goodrich-Blair, and S. R. Eddy (1994). Amino acid sequence motif of group I intron endonucleases is conserved in open reading frames of group II introns. *Trends Biochem. Sci.* **19:** 402–404.

Simon, M. and G. Faye (1984). Organization and processing of the mitochondrial oxi3/oli2 multigenic transcript in yeast. *Mol. Gen. Genet.* **196:** 266–274.

Sirum-Connelly, K. and T. L. Mason (1993). Functional requirement of a site-specific ribose methylation in ribosomal RNA. *Science* **262:** 1886–1889.

Slonimski, P. P., G. Perrodin, and J. H. Croft (1968). Ethidium bromide induced mutation of yeast mitochondria: complete transformation of cells into respiratory-deficient non-chromosomal petites. *Biochem. Biophys. Res. Commun.* **30:** 232–239.

Sogo, L. F. and M. P. Yaffe (1994). Regulation of mitochondrial morphology and inheritance by Mdm10p, a protein of the mitochondrial outer membrane. *J. Cell Biol.* **126:** 1361–1373.

Söllner, T., C. Schmidt, and C. Schmelzer (1987). Amplification of the yeast nuclear gene MRS3 confers suppression of a mitochondrial RNA splice defect. *Curr. Genet.* **12:** 497–501.

Sor, F. and H. Fukuhara (1983). Complete DNA sequence coding for the large ribosomal RNA of yeast mitochondria. *Nucl. Acids Res.* **11:** 339–348.

Staudenbauer, W. L. (1979). Replication of the colicin E1 plasmid in extracts of *Escherichia coli:* uncoupling of leading strand from lagging strand synthesis. *Mol. Gen. Genet.* **177:** 113–120.

Stepien, P. P., S. P. Margossian, D. Landsman, and R. A. Butow (1992). The yeast nuclear gene suv3 affecting mitochondrial post-transcriptional processes encodes a putative ATP-dependent RNA helicase. *Proc. Natl. Acad. Sci. U.S.A.* **89:** 6813–6817.

Stewart, L. C. and M. P. Yaffe (1991). A role for unsaturated fatty acids in mitochondrial movement and inheritance. *J. Cell. Biol.* **115:** 1249–1257.

Strausberg, R. L. and P. S. Perlman (1978). The effect of zygotic bud position on the transmission of mitochondrial genes in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **163:** 131–144.

Strick, C. A. and T. D. Fox (1987). *Saccharomyces cerevisiae* positive regulatory gene PET111 encodes a mitochondrial protein that is translated from an mRNA with a long 5′ leader. *Mol. Cell. Biol.* **7:** 2728–2734.

Stripecke, R., C. C. Oliveira, J. E. G. McCarthy, and M. W. Hentze (1994). Proteins binding to 5′ untranslated region sites: a general mechanism for translational regulation of mRNAs in human and yeast cells. *Mol. Cell. Biol.* **14:** 5898–5909.

Stuart, R. A., D. M. Cyr, E. A. Craig, and W. Neupert (1994). Mitochondrial molecular chaperones — their role in protein translocation. *Trends Biochem. Sci.* **19:** 87–92.

Suzuki, C. K., K. Suda, N. Wang, and G. Schatz (1994). Requirement for the yeast gene LON in intramitochondrial proteolysis and maintenance of respiration. *Science* **264:** 273–276.

Tabak, H. F. and L. A. Grivell (1986). RNA catalysis in the excision of mitochondrial introns. *Trends Genet.* **2:** 51–55.

Thorsness, P. E. and T. D. Fox (1993). Nuclear mutations in *Saccharomyces cerevisiae* that affect the escape of DNA from mitochondria to the nucleus. *Genetics*, **134:** 21–28.

Thorsness, P. E., K. H. White, and T. D. Fox (1993). Inactivation of YME1, a member of the FtsH-SEC18-PAS1-CDC48 family of putative ATPase-encoding genes, causes increased escape of DNA from mitochondria in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13:** 5418–5426.

Tian, G.-L., F. Michel, C. Macadre, P. P. Slonimski, and J. Lazowska (1991). Incipient mitochondrial evolution in yeasts. II. The complete sequence of the gene for cytochrome b in *Saccharomyces douglasii* reveals the presence of both new and conserved introns and discloses major differences in the fixation of mutations in evolution. *J. Mol. Biol.* **218:** 747–760.

Tomoyasu, T., K. Yamanaka, K. Murata, T. Suzaki, P. Bouloc, A. Kato, H. Niki, S., Hiraga, and T. Ogura (1993). Topology and subcellular localization of FtsH protein in *Escherichia coli*. *J. Bacteriol.*, **175:** 1352–1357.

Turcq, B., K. F. Dobinson, N. Serizawa, and A. M. Lambowitz (1992). A protein required for RNA processing and splicing in *Neurospora* mitochondria is related to gene

products involved in cell cycle protein phosphatase functions. *Proc. Natl. Acad. Sci. U.S.A.* **89:** 1676–1680.

Tzagoloff, A., N. Capitanio, M. P. Nobrega, and D. Gatti (1990). Cytochrome oxidase assembly in yeast requires the product of COX11, a homolog of the *P. denitrificans* protein encoded by ORF3. *EMBO J.* **9:** 2759–2764.

Tzagoloff, A. and C. L. Dieckmann (1990). Pet genes of *Saccharomyces cerevisiae*. *Microbiol. Rev.* **54:** 211–225.

Tzagoloff, A., D. Gatti, and A. Gampel (1990). Mitochondrial aminoacyl-tRNA synthetases. *Prog. Nucl. Acid Res. Mol. Biol.* **39:** 129–158.

Tzagoloff, A. and A. M. Myers (1986). Genetics of mitochondrial biogenesis. *Annu. Rev. Biochem.* **55:** 249–285.

Tzagoloff, A., M. Nobrega, N. Gorman, and P. Sinclair (1993). On the functions of the yeast COX10 and COX11 gene products. *Biochem. Mol. Biol. Int.* **31:** 593–598.

Valencik, M. L., B. Kloeckener-Gruissem, R. O. Poyton, and J. E. McEwen (1989). Disruption of the yeast nuclear PET54 gene blocks excision of mitochondrial intron a15β from pre-mRNA for cytochrome c oxidase subunit I. *EMBO J.* **8:** 3899–3904.

Valencik, M. L. and J. E. McEwen (1991). Genetic evidence that different functional domains of the PET54 gene product facilitate expression of the mitochondrial genes COX1 and COX3 in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **11:** 2399–2405.

Vambutas, A., S. H. Ackerman, and A. Tzagoloff (1991). Mitochondrial translational-initiation and elongation factors in *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **201:** 643–652.

Van der Veen, R., J. H. J. M. Kwakman, and L. A. Grivell (1987). Mutations at the lariat acceptor site allow self-splicing of a group II intron without lariat formation. *EMBO J.* **6:** 3827–3831.

van Dyck, E., F. Foury, B. Stillman, and S. J. Brill (1992). A single-stranded DNA binding protein required for mitochondrial DNA replication in *S. cerevisiae* is homologous to *E. coli* SSB. *EMBO J.* **11:** 3421–3430.

van Dyck, E., B. Jank, A. Ragnini, R. J. Schweyen, C. Duyckaerts, F. Sluse, and F. Foury (1994). Overexpression of a novel member of the mitochondrial carrier family rescues both DNA and RNA metabolism defects in yeast mitochondria. *Mol. Gen. Genet.* In press.

van Dyck, L., D. A. Pearce, and F. Sherman (1994). PIM1 encodes a mitochondrial ATP-dependent protease that is required for mitochondrial function in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* **269:** 238–242.

Wagner, I., H. Arlt, L. van Dyck, T. Langer, and W. Neupert (1994). Molecular chaperones cooperate with PIM1 protease in the degradation of misfolded proteins in mitochondria. *EMBO J.* **13:** 5135–5145.

Waldherr, M., A. Ragnini, B. Jank, R. Teply, G. Wiesenberger, and R. J. Schweyen (1993). A multitude of suppressors of group-II intron-splicing defects in yeast. *Curr. Genet.* **24:** 301–306.

Wallace, D. C. (1992). Diseases of the mitochondrial DNA. *Annu. Rev. Biochem.* **61:** 1175–1212.

Wallace, D. C. (1994). Mitochondrial DNA sequence variation in human evolution and disease. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 8739–8746.

Wallis, M. G., O. Groudinsky, P. P. Slonimski, and G. Dujardin (1994). The NAM1 protein (NAM1p), which is selectively required for cox1, cytb and atp6 transcript processing/stabilisation, is located in the yeast mitochondrial matrix. *Eur. J. Biochem.* **222:** 27–32.

Weber, E. R., R. S. Rooks, K. S. Schafer, and P. E. Thorsness (1994). Mutations in the mitochondrial ATP synthase gamma subunit suppress a slow-growth phenotype of yme1 yeast lacking mitochondrial DNA. In press.

Weislogel, P. O. and R. A. Butow (1970). Low temperature and chloramphenicol induction of respiratory deficiency in a cold-sensitive mutant of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* **67:** 52–58.

Weiss, H., T. Friedrich, G. Hofhaus, and D. Preis (1991). The respiratory-chain NADH dehydrogenase (complex I) of mitochondria. *Eur. J. Biochem.* **197:** 563–576.

Weiss, H., K. Leonard, and W. Neupert (1990). Puzzling subunits of mitochondrial cytochrome reductase. *Trends Biochem. Sci.* **15:** 178–180.

Weiss-Brummer, B., G. Rödel, R. J. Schweyen, and F. Kaudewitz (1982). Expression of the split gene cob in yeast: evidence for a precursor of a 'maturase' protein translated from intron 4 and preceding exons. *Cell* **29:** 527–536.

Wenzlau, J. M., R. J. Saldanha, R. A. Butow, and P. S. Perlman (1989). A latent intron-encoded maturase is also an endonuclease needed for intron mobility. *Cell* **56:** 421–430.

Wettstein-Edwards, J., B. S. Ticho, N. C. Martin, D. Najarian, and G. S. Getz (1986). *In vitro* transcription and promoter strength analysis of five mitochondrial tRNA promoters in yeast. *J. Biol. Chem.* **261:** 2905–2911.

Wheeldon, L. W., A.-C. Dianoux, M. Bof, and P. V. Vignais (1974). Stable and labile products of mitochondrial protein synthesis *in vitro*. *Eur. J. Biochem.* **46:** 189–199.

Wickner, W. (1994). How ATP drives proteins across membranes. *Science.* **266:** 1197–1198.

Wiesenberger, G., T. A. Link, U. V. Ahsen, M. Waldherr, and R. J. Schweyen (1991). MRS3 and MRS4, two suppressors of mtRNA splicing defects in yeast, are new members of the mitochondrial carrier family. *J. Mol. Biol.* **217:** 23–37.

Wiesenberger, G., M. Waldherr, and R. J. Schweyen (1992). The nuclear gene MRS2 is essential for the excision of group II introns from yeast mitochondrial transcripts *in vivo*. *J. Biol. Chem.* **267:** 6963–6969.

Williamson, D. H., N. G. Maroudas, and D. Wilkie (1971). Induction of the cytoplasmic petite mutation in *Saccharomyces cerevisiae* by the antibacterial antibiotics erythromycin and chloramphenicol. *Mol. Gen. Genet.* **111:** 209–223.

Wolf, K. and L. Del Giudice (1988). The variable mitochondrial genome of *Ascomycetes:* organization, mutational alterations, and expression. *Adv. Genet.* **25:** 185–308.

Wolfe, C. L., Y. C. Lou, A. K. Hopper, and N. C. Martin (1994). Interplay of heterogeneous transcriptional start

sites and translational selection of AUG's dictate the production of mitochondrial and cytosolic nuclear tRNA nucleotidyltransferase from the same gene in yeast. *J. Biol. Chem.* **269:** 13361–13366.

Wu, M. and A. Tzagoloff (1989). Identification and characterization of a new gene (CBP3) required for the expression of yeast coenzyme QH2-cytochrome c reductase. *J. Biol. Chem.* **264:** 11122–11130.

Xiong, Y. and T. H. Eickbush (1990). Origin and evolution of retroelements based upon their reverse transcriptase sequences. *EMBO J.* **9:** 3353–3362.

Xu, B. and D. A. Clayton (1992). Assignment of a yeast protein necessary for mitochondrial transcription initiation. *Nucl. Acids Res.* **20:** 1053–1059.

Yaffe, M. P. and G. Schatz (1984). Two nuclear mutations that block mitochondrial protein import in yeast. *Proc. Natl. Acad. Sci. U.S.A.* **81:** 4819–4823.

Yasuhara, T., Y. Mera, T. Nakai, and A. Ohashi (1994). ATP-dependent proteolysis in yeast mitochondria. *J. Biochem. Tokyo* **115:** 1166–1171.

Zagorski, W., B. Castaing, C. J. Herbert, M. Labouesse, R. Martin, and P. P. Slonimski (1991). Purification and characterization of the *Saccharomyces cerevisiae* mitochondrial leucyl-tRNA synthetase. *J. Biol. Chem.* **266:** 2537–2541.

Zamarocny, M. de G. Faugeron-Fonty, G. Baldacci, R. Coursot, and G. Bernardi (1984). The ori sequences of the wild type genome of a wild-type yeast strain: number, location, orientation and structure. *Gene* **32:** 439–457.

Zamaroczy, M. de and G. Bernardi (1986). The primary structure of the mitochondrial genome of *Saccharomyces cerevisiae* — a review. *Gene* **47:** 155–177.

Zehnbauer, B. A., E. C. Foley, G. W. Henderson, and A. Markovitz (1981). Identification and purification of the Lon+ (capR+) gene product, a DNA-binding protein. *Proc. Natl. Acad. Sci. U.S.A.* **78:** 2043–2047.

Ziaja, K., G. Michaelis, and T. Lisowsky (1993). Nuclear control of the messenger RNA expression for mitochondrial ATPase subunit-9 in a new yeast mutant. *J. Mol. Biol.* **229:** 909–916.