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Maintenance and Integrity of the Mitochondrial Genome: a Plethora of Nuclear Genes in the Budding Yeast

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INTRODUCTION

Mitochondrial DNA (mtDNA) is essential for most eucaryotic (obligate aerobe) species. Evidently, maintenance of the integrity of mtDNA during somatic divisions and sexual reproduction is of extreme importance for their survival. In particular, deletions, duplications, and point mutations in mtDNA have been shown to cause human diseases, either hereditary or sporadic (see references 115, 138, 174, 318, and 337 for reviews). Cases where mutant and wild-type mtDNA molecules are found in the same tissue define a situation termed heteroplasmy. However, the ratio of the two mitochondrial haplotypes often changes over the life of an individual and can be very different between cell types. Despite their fundamental and clinical importance, the factors which act upon the relative distribution of normal and mutant mtDNAs over individual development remain poorly understood. Recently, experimental studies have attempted to address this question (reviewed in reference 22): one of these stresses the roles of the nuclear background (77). In this context, it is noteworthy that several nuclear genes have been implicated in the concomitant accumulation of several classes of deleted mtDNAs in the same individual. Of these, only one has been recently cloned and characterized (219). It encodes thymidine phosphorylase, which is involved in thymidine catabolism and may be essential for mtDNA maintenance.

While very little information is available on nuclear genes which might (directly or indirectly) control mtDNA maintenance in higher eucaryotes, there is an overabundance of data concerning the budding yeast *Saccharomyces cerevisiae*. In this species, mitochondrial genetics and studies of nuclear control

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of integrity and transmission of mtDNA are simplified because of two features: (i) this yeast is a facultative aerobe, and (ii) it is very accessible in terms of classical and molecular genetics. However, in spite of its status as a model system, *S. cerevisiae* exhibits intrinsic weaknesses with respect to these questions: it is a facultative aerobe, it is unicellular and does not stably maintain a heteroplasmic state, and the structure of its mtDNA differs significantly from that of higher eucaryotes. Nonetheless, one can certainly postulate that at least some of the nuclear factors which control mtDNA integrity and transmission have been conserved through evolution. Thus, an understanding of this nucleomitochondrial problem in yeast may shed light on the relevant nuclear genes in higher eucaryotes. Alternate models are rare and have received very little study (see "Conclusions and prospects" below).

This review examines the field of yeast genes involved in mtDNA maintenance. Due to the large number of articles in this area, we may have missed some significant data and apologize for possible omissions. The bibliography was compiled in May 1999.

The symbols used in this review are those currently used by the yeast community: wild-type genes are in capitals and italics, mutant alleles are in lowercase italics, and proteins are in nonitalics and capitalized only on the first letter (e.g., Mip1 or Mip1p).

THE PETITE SYNDROME: PHENOMENOLOGY AND METHODOLOGY

The cytoplasmic petite mutants (petite colony, vegetative petites, or simply petites) were discovered 50 years ago (84, 85, 278; see reference 87 for a review). Numerous studies then showed that petite mutants lacked a functional mitochondrial (rho^+) genome but instead showed extensive deletion of mtDNA (rho^-) or no mtDNA at all (rho^0) (reviewed in reference 74; see also reference 238). The petite mutations are very pleiotropic because the mutants cannot perform mitochondrial protein synthesis. These mutations differ in this from *mit*$^-$ mutations, which are point mutations in mtDNA causing respiratory deficiency through impairment of only one (or a few) specific function (see reference 281 for a definition of *mit*$^-$ mutations and the operational differentiation between *mit*$^-$ and rho^-/rho^0 mutations). The petite mutations are also characterized by their high rate of spontaneous occurrence: in the majority of yeast strains, one finds a small percentage of spontaneously occurring petite clones. However, it should be stressed that the proportion of petite clones with respect to the wild-type clones (often referred to as frequency, percentage, or ratio of petites) is a classical problem of population genetics. The proportion one establishes experimentally results from both the frequency of mutation of rho^+ cells to rho^-/rho^0 cells (measured per cell per generation or per cell per hour) and the ratio of the growth rates of rho^+ cells to rho^-/rho^0 ones. L'Héritier and coworkers (86) have shown that under stationary conditions, when the proportion of petite clones is of the order of 1%, the frequency of mutation is 10 times lower, on the order of 0.1%. Conversely, Ogur and John (223) have shown that the proportion of petite clones, in a population that has multiplied on nonfermentable medium (i.e., ethanol or glycerol) where the petite cells are unable to multiply (but do not die), is equal to the frequency of mutation multiplied by 2. This frequency can be greatly increased (to complete transformation of the cell population into petites) by drugs such as ethidium bromide (280).

In rho^- mutants, the remaining fragment of rho^+ mtDNA is regularly repeated along the mtDNA molecules. There are two major types of repeat arrangements: either the sequence is directly repeated with head-to-tail junctions or the arrangement is of the inverted or palindromic type and the repeat unit is an inverted duplication of the retained sequence (91; reviewed in reference 74).

Numerous studies have shown, by crosses between strains with different mitochondrial genotypes, that the heteroplasmic state of the zygotes is very transient. In fact, their vegetative multiplication is coupled with a rapid segregation of mtDNA molecules, leading to homoplasmic clones. After a cross to the wild type, the petite mutants can be classified according to their suppressiveness, i.e., by the relative proportion of petite zygotic clones. Low or moderate suppressiveness probably results from random segregation of mtDNA molecules over cell divisions, while hypersuppressivity implies that rho^- molecules are preferentially transmitted to the progeny at the expense of the rho^+ genome. Numerous data have led to the proposal of a replicative advantage for these rho^- molecules, since they contain several copies of short sequences called *ori* (*rep*), which may represent replication origins of mtDNA (reviewed in reference 74). However, more recent data tend to favor the idea of a segregation (transmission) advantage in hypersuppressiveness (see "Nuclear control of biased transmission" below).

This review focuses preferentially on mutations which increase the level of petite production compared to the relevant wild-type background. However, it also concerns the few available mutations which either modify the biased transmission of some rho^- genomes (see "Nuclear control of biased transmission" below and Table 2) or slow (even abolish) petite production (see "No compromise" below and Tables 10 and 13). It would have been incomplete without a short summary (see "Petite positivity versus petite negativity" below) of recent data concerning the problem of petite positivity versus petite negativity, i.e., the ability or inability of yeast species to produce petite cells. The genes involved in mtDNA maintenance have been roughly classified by their known functions or effects on the mitochondrial genome (see the tables). However, at the beginning of the review, we have focused on the scientific projects which have helped to identify some genes.

Throughout this review, as far as possible, we have attempted to identify the type of petites (rho^0 and/or rho^-) produced in a given genetic background. We are in fact convinced that this is an important point for the understanding of two main problems: (i) why some genetic backgrounds lead to a complete loss of mtDNA (rho^0 petites) if the relevant genes are not (directly or indirectly) required for mtDNA replication, and (ii) why some genes are essential for the maintenance of a complete (rho^+) genome but dispensable for the maintenance of a truncated version (rho^-) of this genome. Several criteria are available: (i) ensuring that the petite colonies which appear at high levels in a given strain are respiration deficient; (ii) ascertaining a defect in mtDNA; and (iii) distinguishing between rho^0 and rho^- petites (see Table 1 footnotes). "Physiological" methods can address only the first goal. Genetic and molecular methods clarify the second. The third problem, however, can be solved by 4′,6-diamidino-2-phenylindole (DAPI) staining or extensive genetic tests and/or clear-cut molecular procedures. Unfortunately, in some cases mtDNA stability was clearly not a focus of the authors and in other cases the methods which distinguish between rho^0 and rho^- petites were not rigorously applied. These facts have prompted us to be (sometimes) more cautious about conclusions than were the authors. For example, in our opinion, lack of DNA hybridization with a probe containing a limited mtDNA fragment does not justify the conclusion that the relevant clone is of the rho^0 type: it may very well contain a rho^- genome with this fragment deleted. The same is true for genetic tests using too few mtDNA mark-

TABLE 1. Nuclear genes involved in mtDNA metabolism: replication, recombination and repair

Gene[a]	Protein function[e]	Type of mutation	Petite production	Reference(s)[j]
MIP1[b] (NE)	**mtDNA polymerase**	Point mutations	rho^{0f,g,h}	103, 139
		Deletion	rho^{0f,g,h}	94
		Heterozygotes	rho$^-$ and/or rho^{0i} (at 36°C)	139, 177
MGM101[b] (NE)	mtDNA repair?	Disruption	rho^{0h}	38
		Missense	rho^{0h}	205
PPA2[d] (NE)	**Mitochondrial pyrophosphatase**	Deletion	rho^{0h}	197
PIF1[b] (NE)	**mtDNA helicase**	Disruption	rho^{0i} (at 37°C)	169
ABF2/*HIM1*[c] (NE)	**Mitochondrial HMG**	Deletion	rho^{0f,g,h} (on glucose + amino acids)	69, 206, 335
		Overexpression	rho^{0h}	336
RIM1[b] (NE)	**Mitochondrial SSB protein**	Deletion	rho^{0g,h}	310
MHR1[b] (?)	mtDNA repair, recombination	Point mutation	rho$^-$ and rho^{0f,h} (at 37°C)	183
MSH1[d] (NE)	**Mitochondrial *E. coli* MutS homologue**	Disruptions	rho$^{-f,h,i}$	42, 246
CDC8[b] (E)	**Thymidylate kinase**	Point mutation	rho$^-$ and/or rho^{0f,h}	150, 217
CDC21[b] (E)	**Thymidylate synthetase**	Point mutation	rho$^-$ and/or rho^{0f,h}	217
RNR2[c,d] (E)	**Ribonucleotide reductase (small subunit)**	3′ disruption	rho$^-$ and/or rho^{0i}	80
RNR4[b] (E or NE)[k]	**Ribonucleotide reductase** (structural component)	Point mutation and deletion	rho$^-$ and/or rho^{0g}	319

[a] NE, nonessential gene; E, essential gene; (?), unknown.
[b] Gene identified by genetics.
[c] Gene identified by biochemistry.
[d] Gene identified by molecular biology.
[e] Bold type indicates that the protein function is well documented; while light type indicates a still putative function.
[f] Physiological criteria for increased production of rho$^-$ and/or rho^0. Observation of respiration deficiency through growth on differential medium, replica plating from glucose to glycerol, and tetrazolium staining.
[g] Genetic criteria for increased production of rho$^-$ and rho^0. Inability to give wild-type (respiration-competent) recombinants through crosses with known mtDNA mutants (*mit*$^-$, rho$^-$, or rho^0), colethality with the *op1* mutation.
[h] Molecular criteria for increased production of rho$^-$ and rho^0. Search for partial or total loss of mtDNA through DAPI staining, hybridization with mtDNA probes or whole mtDNA, incorporation of radioactive precursors into mtDNA, CsCl ultracentrifugation, or pulsed-field gel electrophoresis.
[i] Criteria for increased production of rho$^-$ and rho^0 not described.
[j] The references concern only the effects of the relevant mutants upon mtDNA stability. References related to the gene and its product (first two columns) are given in the text.
[k] *RNR4* appears essential or nonessential according to the genetic background.

ers. It is indeed easier to show that a petite is rho$^-$ than to demonstrate a rho^0 state. In addition to these methodological problems, other parameters require that caution be used in drawing definitive conclusions: the overall genetic background and the physiological state of the cells can strikingly modify the amount and nature of petites. As emphasized in some cases, these parameters may explain discrepancies between results obtained by different groups. To underline the influence of the genetic context, it seems that a mitochondrial genome lacking introns is more stable than an intron-containing genome (see below). To place the physiological parameters in a more concrete form, three observations can be reported. First, environmental parameters can strikingly influence petite production in mutant strains. A remarkable example concerns the effects of both temperature and culture medium on the *abf2* mutants (see "The *ABF2* paradox" below). Second, most rho$^-$ mutants are not very stable and may evolve to the rho^0 state (see, as an example, the discussion of overexpression of *ADR1* in "Gene overexpression can increase mtDNA instability" below). Thus, a conclusion about the nature of the petites produced by a given mutant may depend on the age of the culture (see, for instance, the discussion of *MGM101* in the following section). Third, petite production due to disruption of a nuclear gene can be significantly different depending on the procedures used. Disruption can be achieved in a haploid vegetative cell, and its effect upon petite production can be immediately observed; disruption can be achieved in a diploid cell, and its effect is observed in haploid cells resulting from sporulation of the heterozygous diploids. These two procedures can lead to different observations (as, for instance, in *rpo41* mutants; see "Does transcription play a direct role in mtDNA maintenance?" below and Table 3).

WIDE-SCALE SCREENING FOR rho^0 PRODUCTION

Nuclear genes involved in the maintenance of mtDNA can be identified by a systematic search for mutations increasing rho^0 production at elevated temperature. Screening for heat-sensitive mutations helps to recover mutations in genes essential for cell survival. This procedure was used by Genga et al. (103): among 360 clones able to grow on glycerol at 25°C but not at 35°C, 31 clones were heat sensitive due to massive production of petites, of which 12 completely lacked mtDNA. These 12 mutants belonged to 11 complementation groups. Only one mutant displayed a deficiency in mtDNA synthesis. This identified the *MIP1* gene (Table 1), encoding the catalytic subunit of mtDNA polymerase (94). In addition to the initial mutations and deletion, other *mip1* mutations impairing replication fidelity have been obtained. Some yield a high level of petites either at 36°C or at both 36 and 23°C. Interestingly, these mutations show a gene dosage effect, since heterozygous diploids are strong producers of petites (139). This effect was also observed (at 36°C) for diploids containing a single copy of the *MIP1* gene (177). The data suggest that Mip1p is produced in limited amounts and that the rho$^+$ genome cannot be maintained below a particular threshold of that protein.

In 1992, Jones and Fangman (149) used a similar procedure to identify genes required for mitochondrial genome maintenance (*MGM* genes). This led to the identification of *MGM1*, encoding a protein whose GTP-binding domain is related to dynamin (117, 149) (see Table 11). Careful examination of DAPI-stained cells of the *mgm1-1* mutant (which lost mtDNA after transfer at high temperature) strongly suggests that the defect of the mutant is not due to an asymmetric distribution of mtDNA during cell divisions. Furthermore, deletion of *MGM1* causes not only loss of the rho$^+$ genome but also that of rho$^-$

genomes, even those containing a *rep* (*ori*) sequence (149). In addition, Guan et al. (117) showed that disruption of *MGM1* led to a structural alteration of mitochondria and a defect in mitochondrial Hsp58 protein import. Recently, Shepard and Yaffe (272) have demonstrated that Mgm1 is an integral protein of the outer mitochondrial membrane, which plays a key role in mitochondrial morphology and inheritance (see "Mitochondrial morphology, the cytoskeleton, and mtDNA" below for a further discussion of *MGM1*).

More recently, Guan (118) described a similar program which led to the recovery of 120 mutants exhibiting temperature-induced loss of mtDNA (rho^0). These mutants belong to 36 complementation groups. Thirteen mutants contained mutations that could be assigned to six previously described genes: *MIP1* (see above and Table 1), *CDC8* and *CDC21* (see "mtDNA and the cell cycle" below) (Table 1), *RPO41* and *MTF1* (see "Does transcription play a direct role in mtDNA maintenance" below and Table 3), and *MGM1* (see above, "Mitochondrial morphology, the cytoskeleton, and mtDNA" below, and Table 11). This genetic analysis thus offered 30 new genes involved in mtDNA maintenance, of which 2 have been analyzed to date: *MGM101* (Table 1) and *MGM104* (see Table 9).

The *MGM101* gene was sequenced and disrupted. The predicted amino acid sequence showed no similarity to sequences of known proteins. The disrupted gene led to loss of mtDNA (38). Recently, Meeusen et al. (205) found Mgm101 among proteins associated with mitochondrial nucleoids. These authors have also isolated a new heat-sensitive allele of *MGM101* (*mgm101-2*) and attempted, by extensive biochemical and genetic approaches, to determine the precise function of Mgm101p. The data have led to the following conclusions. The protein binds to DNA, which probably explains its specific association with the nucleoid. However, it is not involved in mtDNA packaging, segregation, or partitioning. It is not required for mtDNA replication. Its loss does not cause defects in morphology or transmission of the organelle. In fact, the only clear-cut phenotypic defect of *mgm101-2* is the sensitivity of mtDNA to damage induced by UV irradiation and the hypersensitivity of mtDNA to damage induced by gamma rays and H_2O_2. Therefore, the authors propose that *MGM101* performs an essential function in the repair of oxidatively damaged mtDNA required for mtDNA maintenance. Interestingly, using the heat sensitivity of *mgm101-2*, they observed that after 12 h at 37°C, when the cell population is 100% respiration deficient, only 50% of cells are rho^0, while after a longer exposure to nonpermissive temperature (16 h), the entire population consisted of rho^0 cells. Thus, at least in this case, this rho^0 producer exhibits a transition from the rho$^+$ to the rho$^-$ state before undergoing a complete loss of mtDNA (205).

The *mgm104-1* mutation causes a greatly increased production of petites at 25 and 37°C, with a stronger effect at high temperature. Attempts to clone the *MGM104* gene led to the identification of the *TTS1* gene, which encodes the cytosolic tyrosyl-tRNA synthetase (44). *TTS1* is able to rescue the *mgm104-1* phenotype, even when present on a low-copy-number vector, but is not thought to be the cognate *MGM104* gene. This conclusion was drawn since no specific mutations were found in the coding or promotor regions of the *TTS1* gene in the *mgm104-1* mutant (118). The author suggests two major hypotheses: either Tts1p has a dual function in cytosolic translation and mtDNA maintenance, or it may interact with the *mgm104-1*-encoded protein and enhance its activity, thus rescuing the phenotypic defect of the mutant. However, one cannot exclude a third hypothesis: a slight overexpression of the tyrosyl-tRNA synthetase results in mischarging of noncognate tRNAs and thus in informational (missense) suppression of the *mgm104-1* mutation. The author suggested that additional plasmids able to complement this mutant were required to identify the cognate gene. However, since the *TTS1* mRNA is about 60% less abundant in the *mgm104-1* strain than in wild type, one cannot exclude the possibility that the mutation lies in the 3′ noncoding sequence of *TTS1*: this would lead to instability of the messenger and cause the *mgm104-1* phenotype. In fact, alteration of cytosolic translation may affect mtDNA stability, as shown in the filamentous fungus *Podospora anserina* (see "Conclusions and prospects" below).

The main advantage of such a wide screening procedure for nuclear mutations leading to complete loss of mtDNA is that it does not assume any mechanism. This is stressed by the few examples cited above: this type of program has permitted the recovery of genes involved in mtDNA replication, genes involved in mtDNA transcription, and genes whose functions are still unknown. Guan's assessment (118) also shows that the screening procedure is far from saturated. This explains why several genes whose mutations lead to rho^0 production were not identified in his experiments. Among those involved in mtDNA metabolism, one finds, for instance, *PIF1*, *RIM1*, *ABF2* (discussed below), and *PPA2*, which encodes a mitochondrial pyrophosphatase (Table 1). Inorganic pyrophosphate (PP_i) is generated in a number of biosynthetic reactions, notably replication and transcription. Hydrolysis of PP_i is thus an essential process which ensures the forward direction of these reactions. Lundin et al. (197) cloned the gene (*PPA1*) encoding the cytosolic enzyme and used it as a probe for the gene (*PPA2*) encoding the mitochondrial enzyme. Cells with *PPA2* deleted are unable to grow on respiratory carbon sources. DAPI staining showed that these cells have lost their mtDNA (Table 1), which is in accordance with the evident requirement for this enzyme in DNA replication (197).

In addition to genes specifically involved in mtDNA replication discussed in this section, four genes, required for replication of the mitochondrial and the nuclear genomes (*CDC8*, *CDC21*, *RNR2*, and *RNR4*) were shown to control mtDNA stability (Table 1). Because of their dual cellular function, it seemed to us more convenient to discuss these genes below in "mtDNA and the cell cycle."

NUCLEAR CONTROL OF BIASED TRANSMISSION: THE *MGT1*/*CCE1* GENE

As stressed in the Introduction and in "The petite syndrome" (above), mtDNA maintenance is dependent on two basic parameters: the frequency of mtDNA deletions and the management of heteroplasmic states, i.e., the relative transmission of wild-type and altered molecules during cell divisions. In 1991, Zweifel and Fangman (339) found a mutation which disrupted the biased inheritance of hypersuppressive rho$^-$ genomes (Table 2), thus identifying the first *MGT* (for "mitochondrial genome transmission") gene. Strikingly, the *MGT1* gene was rediscovered by a totally different procedure. Originally, Kleff et al. (158) were interested in a cruciform cutting endonuclease (Cce), which is required during recombination to resolve Holliday junctions. They developed an assay to detect this activity in crude yeast extracts and sought mutants lacking it. Approximately 150 heat-sensitive mutants were screened before a relevant (*cce1*) mutant was found. Cloning of the *CCE1* gene and assays for Cce1p activity yielded evidence that *CCE1* was the structural gene for the cruciform cutting endonuclease. It was shown that *cce1* mutants did not affect nuclear recombination but that *CCE1* and *MGT1* were allelic: the *mgt1* mutants showed no Cce1p activity and, in turn, the *cce1* mutant was resistant to hypersuppressive rho$^-$. It was then demonstrated that Cce1p was localized to the mitochon-

TABLE 2. Genes which influence biased transmission of rho$^-$ genomes

Gene[a]	Protein function[d]	Type of mutation	Phenotype	Reference(s)[e]
MGT1[b]/*CCE1*[c] (NE)	**Mitochondrial cruciform cutting endonuclease**	Deletion, point mutations	Reversal of hypersuppressivity	158, 339
		Overexpression	Increased transmission of neutral rho$^-$	194
PIF1[b] (NE)	**mtDNA helicase**	Point mutations	Increased suppressivity of all types of rho$^-$	96

[a] NE, nonessential gene.
[b] Gene identified by genetics.
[c] Gene identified by biochemistry.
[d] Bold type indicates that the protein function is well documented.
[e] The references concern only the effects of the relevant mutations upon biased transmission of rho$^-$ genomes. References related to the gene and its product (first two columns) are given in the text.

dria and associated with the inner membrane (89). Later, Lockshon et al. (194) demonstrated that the lack of Cce1p led to an increased ratio of mtDNA molecules linked by recombination junctions, resulting in aggregation of mtDNA molecules in a smaller number of cytological structures than in wild-type cells. This effect was more pronounced for rho$^-$ than for rho$^+$ genomes. In accordance with these results, overexpression of *MGT1*/*CCE1* reduced the frequency of branched structures and enhanced the transmission of nonsuppressive rho$^-$ genomes (Table 2). In a recent report (239), it was shown that the *mgt1*/*cce1* background also influences the transmission of respiration-competent mtDNAs lacking intergenic sequences involved in the biased transmission of hypersuppressive rho$^-$ mutants. Thus, the resolution of recombination junctions plays a key role in the segregation and transmission of yeast mtDNA. However, deletion of *MGT1*/*CCE1* causes only a slight increase in rho$^-$ production (158). It is noteworthy that this increase is more marked in the presence of a deletion of the *NUC1* gene, encoding the major mitochondrial exoendonuclease. This gene was cloned by reverse genetics, using an antibody against the protein. Unexpectedly, neither deletion nor overexpression of the gene resulted in phenotypic effects (334). However, at that time, the authors did not perform extensive analyses of rho$^-$ production or recombination rates in these two contexts. This should now be undertaken in the light of the interaction between *nuc1* and *mgt1*/*cce1* deletions.

Unfortunately, despite its evident interest, very few analyses have been devoted to the problem of biased transmission. However, in addition to the *MGT1*/*CCE1* gene, data concerning the *PIF1* gene are available. This gene (described in "The recombination/repair track" below) encodes an mtDNA helicase involved in recombination between rho$^+$ and tandemly organized rho$^-$ genomes. With respect to rho$^-$ transmission (in crosses with a rho$^+$ partner), a high level of suppressiveness was observed when diploids were homozygous for the *pif1* mutations. This occurred for all types of rho$^-$, regardless of structure (tandem or inverted organization), and was not dependent on the sequence or the size of the repeat (96) (Table 2). This general effect of the *pif1* mutants remains unclear. However, when secondary rho$^-$ clones were obtained from a tandem rho$^-$ insensitive to the recombinogenic effect of *PIF1*, those which showed *PIF1* sensitivity became hypersuppressive in a *pif1* context (98).

Thus, the *MGT1*/*CCE1* and *PIF1* data suggest a strong link between mtDNA recombination and transmission at least for rho$^-$ genomes. With respect to this remarkable point, a systematic analysis of other genes involved in recombination (see "The *ABF2* paradox" and "The recombination/repair track" below) should be undertaken.

THE *ABF2* PARADOX: HIGHLY PLEIOTROPIC BUT DISPENSABLE

The presence of a histone-like protein inside mitochondria (HM) was first reported 20 years ago (32). This 20-kDa protein was then rediscovered by Certa et al. (33) in the search for a histone H1 in yeast. It was lastly and serendipitously identified as an ARS binding factor, i.e., a protein binding an autonomously replicating sequence of the nucleus (68). A few years later, it was shown that Abf2 was a mitochondrial protein closely related to DNA-binding proteins of the HMG (high-mobility-group) family (69). These authors showed that *abf2* null mutants lose their mtDNA when grown on rich glucose medium (Table 1). However, when tetrads issued from diploids heterozygous for the *abf2* null mutation were dissected directly on a nonfermentable substrate, the mutant strains could grow and be maintained indefinitely.

Evidence that HM and Abf2 were the same protein was provided by Megraw and Chae (206). In addition to confirming the data of Diffley and Stillman (69), they added two interesting observations. First, disruption of *ABF2* results in a heat-sensitive phenotype on respiratory substrates due to mtDNA instability, as shown by the fact that the *abf2* mutant is lethal in a *op1* context (see "No compromise" below and Table 13 for the *op1* mutation and its lethality in a petite background). Second, overexpression of *ABF2* leads to a high frequency of rho^0 petites (Table 1). The same year, three papers dealt with the issue of the evolutionary relationship of Abf2 and DNA-binding proteins involved in DNA maintenance and/or expression. Abf2p is the functional homologue of the bacterial histone-like protein HU: despite the lack of sequence similarity between the two proteins, Abf2 and HU can substitute for each other (206). When directed toward the mitochondria, a yeast nuclear HMG protein is able to complement an *abf2* mutant (153). Functional similarities were discovered between a human mitochondrial transcriptional activator (mtTFA or mtTF1) and Abf2p: the human protein partly rescues the phenotypic defects of a *abf2* strain (227). However, the two proteins display structural differences. In particular, the tail region of mtTFA, which plays a critical role in its transcriptional activity, is absent in Abf2p (59a). The data as a whole (see also reference 309a) indicate that, unlike its human homologue, Abf2p plays only a small, if any, role in mtDNA transcription, a notion supported by the fact that *abf2* mutants are able to maintain and express mtDNA under specific growth conditions (69, 336).

In a recent report, Zelenaya-Troitskaya et al. (335) exemplified the pleiotropic role that *ABF2* may play with respect to mtDNA maintenance. These authors show (i) that the distribution and mixing of mtDNA from two *abf2* parental cells after zygote formation differ slightly from the patterns observed in

wild-type zygotes; (ii) that recombination between mitochondrial markers is reduced five- to sevenfold in the mutant context; (iii) that rho$^-$ genomes (introduced through cytoduction experiments) are stable in *abf2* mutants, in contrast to rho$^+$ genomes; (iv) that (in accordance with the data of Megraw and Chae [206]) hyperexpression of *ABF2* leads to a complete loss of mtDNA (Table 1); and (v) that a moderate (two- to threefold) increase in the level of the protein raises the content of mtDNA without affecting mtDNA stability. These data have been completed by Okamoto et al. (224a) and by MacAlpine et al. (198). In the first case, the authors showed that in a rho$^+$ $\times$ rho^0 zygote, mtDNA (stained by DAPI) is preferentially transmitted to the diploid bud while (GFP-tagged) proteins have equilibrated throughout the zygote and the bud. In homozygous Δ*abf2* crosses, mtDNA sorting is delayed and preferential sorting is reduced (224a). In the second case, the authors analyzed the effect of either a null allele or overexpression of *ABF2* upon levels of mtDNA recombination intermediates. In rho$^+$ genomes this level is reduced when Abf2p is absent and increased when Abf2p is overproduced. In contrast, the absence of Abf2p does not influence the level of recombination intermediates of rho$^-$ genomes (198).

Three multicopy suppressors of *abf2* phenotypes have been isolated to date. Two (*SHM1*/*YMH1* and *YMH2*) were identified by their ability to restore the growth of *abf2* null mutants at 37°C on a respiratory substrate. They could encode mitochondrial carrier proteins. Deletion of *SHM1*/*YMH1* has no effect on mtDNA in a *ABF2* context (152). Interestingly, *YMH2*, whose deletion leads to respiration deficiency, appears to be the first membrane-bound protein associated with the mitochondrial nucleoid in vivo (43). We discuss this important property of *YHM2* in "Is there a centromere-like structure for the mitochondrial genome" (below). The third suppressor is *ILV5*, isolated by its ability to restore mtDNA stability in an *abf2* null mutant grown on rich glucose medium. The gene encodes an enzyme of the biosynthesis of branched-chain amino acids (see "The secret life of well-known proteins", below, and Table 8) (336). This discovery and subsequent analyses revealed that mtDNA is highly stable in *abf2* mutants grown on glucose medium lacking leucine, isoleucine, and valine, a condition which enhances the expression of *ILV5*. The inevitable conclusion is therefore that *ABF2* is dispensable for mtDNA stability when *ILV5* is sufficiently expressed! The effect of *ILV5* on petite production by *abf2* mutants at high temperature has not yet been tested.

In any case, the data as a whole suggest that Abf2p plays a role in the recombination process. However, they raise more questions than they solve. Why (as observed in other cases) are rho$^-$ genomes stable in the *abf2* mutants while rho$^+$ genomes are not? Why do *abf2* mutants completely lose their mtDNA even though they are able to maintain rho$^-$ genomes? Why does hyperexpression of *ABF2* lead to loss of rho$^+$ and rho$^-$ genomes? Why are *abf2* null mutants unable to maintain a functional mtDNA on respiratory substrates at high temperature? Zelenaya-Troitskaya et al. (335) favor the idea that the main effects of *ABF2* result from its role in recombination. This hypothesis is consistent with many data concerning both the properties of HMG proteins and the role of recombination intermediates in mtDNA transmission (reference 335 and references therein) (see also the discussion of *MGT1*/*CCE1*, above). However, as pointed out by the authors (335), this hypothesis is difficult to reconcile with the data obtained from the *MGT1*/*CCE1* analysis: in this case, lack of the protein has a very slight effect on mtDNA stability (194). Another interpretation (198) suggests an indirect effect of Abf2p on replication through recombination structures. However, the naive point of view would be that *ABF2* is highly pleiotropic: in fact, as a DNA-packaging protein, which plays a role in mtDNA and nucleoid organization (218), Abf2p may be (directly or indirectly) involved in mtDNA replication, recombination, and distribution. The data would therefore be explained by the sum of discrete effects, more or less pronounced depending on the growth conditions and the structure (rho$^+$ or rho$^-$) of the mitochondrial genome.

THE RECOMBINATION/REPAIR TRACK

Yeast mitochondria are very active in recombination (76), and it has been suggested that mtDNA deletions which give rise to the rho$^-$ genomes can occur through homeologous recombination between imperfect repeats (102, 279). Although this mechanism does not apply to all petite deletions, mutations affecting recombination may aid in identifying genes involved in mtDNA maintenance.

A direct screen for recessive mutations altering mtDNA recombination is impossible since the experimental test requires the fusion of two cells carrying different mtDNAs: in the resultant diploid, the nuclear mutation cannot be expressed. However, Foury and Kolodynski (96) succeeded by using a genetic ruse, i.e., a mutation which delays nuclear fusion; thus, the daughter cells of the zygote contain a haploid nucleus along with a mixed mtDNA population. By using this procedure, they obtained three allelic mutants which identified the *PIF1* (for "petite integration frequency") gene. The *pif1* mutations affect the frequency of recombination between rho$^+$ and certain rho$^-$ genomes, which are tandemly organized. The rho$^-$ genomes with an inverted organization are not sensitive to these mutations. Similarly, recombination between rho$^+$ genomes is identical in the wild-type and mutant backgrounds. The *pif1* mutations are pleiotropic since, in addition to their specific effects on recombination, they increase the level of rho$^-$ induced by UV irradiation, cause a total loss of mtDNA at high temperature (Table 1), and increase the suppressiveness of all types of rho$^-$ genomes (Table 2). *PIF1* encodes an mtDNA helicase which is thus involved in recombination, repair, and mtDNA maintenance (169). Amazingly, it was later shown that the protein also acts in the nucleus by inhibiting telomere elongation and de novo telomere formation (268). In fact, *PIF1* encodes two forms of the protein through the alternative use of two AUG codons: the longer form goes to the mitochondria, and the shorter form goes to the nucleus.

Although its role in mtDNA repair and recombination remains to be elucidated, we must mention here the *RIM1* (for "replication in mitochondria") gene. *RIM1* was discovered as a partial suppressor of the heat-sensitive phenotype of the *pif1* null mutants (310). Interestingly, the suppressor effect was observed with a few additional copies of the *RIM1* gene (i.e., when carried on a centromeric vector). Unfortunately, it has not been reported if strong *RIM1* overexpression could lead to a complete suppression of *pif1* mutants. *RIM1* encodes a mitochondrial single-stranded DNA-binding (SSB) protein required for mtDNA maintenance; deletion of this gene causes complete mtDNA loss (310) (Table 1). Since the heat sensitivity of the *pif1* disruptants remains unclear, it is difficult to speculate on the precise role of Rim1p in mtDNA stability. However, SSB proteins and DNA helicases often perform complementary functions in DNA metabolism, involving unwinding of duplex DNA. Thus, Rim1p might be involved not only in mtDNA replication but also in mtDNA recombination, repair, and transcription (310). As suggested by the authors (310), it would be interesting to test the ability of *RIM1* to suppress the defects of the *pif1* mutants in mtDNA repair and

TABLE 3. Genes involved in mtDNA transcription and translation

Gene[a]	Protein functions[e]	Type of mutation	Petite production	Reference[j]
RPO41[c] (NE)	**mtRNA polymerase**	Disruption	$rho^{0/+}$	112
		Disruption	rho^- and $rho^{0/+}$	90
MTF1/*mtTFB*[b] (NE)	**Mitochondrial transcription factor**	Disruption	rho^- and/or $rho^{0/+}$	188
MST1[b] (NE)	**Mitochondrial $tRNA^{Thr}$ synthetase**	Disruption	$rho^{-f,g}$	213[k]
MSW[b] (NE)	**Mitochondrial $tRNA^{Trp}$ synthetase**	Disruption	$rho^{-f,g}$	213[k]
tufM[d] (NE)	**Mitochondrial elongation factor**	Disruption	$rho^{-f,g}$	213[k]
MRP1[b] (NE)	**Mitochondrial r-protein**	Disruption	$rho^{-f,g}$	213[k]

[a] to [j] See Table 1 footnotes.

[k] Many other reports (too numerous to be quoted) have confirmed the conclusions of Myers et al. (213) that stringent mutations in all (mitochondrial and nuclear) genes required for mitochondrial translation lead to high frequencies of rho^- production.

recombination. It could also be informative to know the effect of strong overexpression of *RIM1* in a *PIF1* (wild-type) background.

A genetic procedure linking mtDNA repair and recombination was used by Ling et al. (183). Of two mutants displaying an elevated UV induction of rho^- mutants, one also exhibited a deficiency in gene conversion. The *MHR1* (for "mitochondrial homologous recombination") gene is also involved in mtDNA maintenance at high temperature (Table 1). Genetic localization showed that *MHR1* was a previously unknown gene.

A direct search for genes involved in DNA repair was undertaken by Reenan and Kolodner (245) to identify the yeast homologue of the *mutS* gene, which encodes a component of the bacterial MutHLS system. This was performed by reverse genetics, using degenerate oligonucleotide primers and PCR. Two genes were identified, one of which encoded a protein targeted to the mitochondria. Disruption of the *MSH1* (for "MutS homologue") gene leads to a strikingly rapid accumulation of rho^- mutations, mostly hypersuppressive (246) (Table 1). DAPI staining of the mutant cells revealed large fluorescent patches instead of the small dispersed patches observed in wild-type cells. This phenotype might be the result of either altered mtDNA distribution or abnormal morphology and distribution of mitochondria (246). In addition, Chi and Kolodner (42) noted that strains heterozygous for a *MSH1* deletion accumulated mtDNA point mutations (mit^-) faster than wild-type strains did. The authors propose a role for Msh1p in the suppression of homeologous recombination, as supported by the in vitro binding specificity of the protein for DNA with multiple mismatches and loops of unpaired nucleotides. They also suggest that Msh1 could interact with other mitochondrial proteins, especially Pif1 and Rim1 (see above) (Table 1), which are, respectively, a DNA helicase and an SSB protein: they have similarities to components of the *Escherichia coli* MutHLS system, and both are required for mtDNA maintenance. This assumption could be tested by using the two-hybrid system in yeast or, even more clearly, by performing coimmunoprecipitation experiments, since the three proteins, Pif1, Rim1, and Msh1, have been purified.

Thus, the recombination/repair approach (which has been difficult to perform) appears very promising with respect to mtDNA maintenance, even though most of the genes required for these processes in mitochondria remain to be identified. Furthermore, as described in "Nuclear control of biased transmission" (above), this specific screen also identified the *CCE1* gene (allelic to *MGT1* [Table 2]), encoding a mitochondrial cruciform cutting endonuclease (158). Although discovered by other methods, *ABF2* (see "The *ABF2* paradox" above) must be added to the list of the genes involved in recombination. In fact, among the genes required for mtDNA maintenance, four are clearly involved in recombination processes: *MGT1*/*CCE1*, *PIF1*, *MHR1*, and *ABF2*. However, in the first three cases, recombination between markers in $rho^+ \times rho^+$ crosses is unaffected or only slightly affected in the relevant mutant backgrounds (96, 183, 339). Thus, *ABF2* is the only gene among these four to be implicated in intergenic recombination between rho^+ genomes (335). There is another striking point concerning *ABF2*: it stands apart from *MGT1*/*CCE1* with respect to recombination intermediates. In fact, their level is reduced in the absence of *ABF2* and increased in the absence of *MGT1*/*CCE1*; moreover, these effects are seen only on rho^+ genomes in the first case and preferentially on rho^- genomes in the second case (194, 198). Clearly, the field of recombination has been underinvestigated.

DOES TRANSCRIPTION PLAY A DIRECT ROLE IN mtDNA MAINTENANCE?

It has long been known that mitochondrial protein synthesis is required for maintenance of rho^+ genomes (reference 213 and references therein). This conclusion has been confirmed over time: all nuclear or mitochondrial mutations which completely block mitochondrial translation inescapably lead to rho^- genome production (see below). In accordance with this constraint, mutations which impair mtDNA transcription should lead to rho^- genome production. The transcriptional apparatus has also been thought to be directly involved in mtDNA replication. This assumption is based on indirect (mostly in vitro) data, suggesting that (at least in vertebrates) mtRNA polymerases are primases for mtDNA replication (reviewed in reference 49). Thus, transcription might play a dual role in mtDNA maintenance: directly through initiation of mtDNA synthesis and indirectly through translation. However, evidence for a direct role is still questionable.

The *RPO41* gene encoding the catalytic subunit of mtRNA polymerase in yeast was cloned by reverse genetics (155). Disruption experiments led to the conclusion that this enzyme was required for mtDNA maintenance (112) (Table 3). Since the authors provided evidence for a complete loss of mtDNA in a *rpo41* mutant background, it seemed of utmost importance to determine if Rpo41p was also required for maintenance of rho^- genomes. This experiment was performed by Fangman et al. (90). They used two types of rho^- strains. The first was hypersuppressive, containing an (amplified) mtDNA fragment with an *ori*/*rep* sequence, which is assumed to offer a replicative advantage in matings with rho^+ strains. The second was neutral, lacking such sequences and consisting solely of AT base pairs. Such rho^- genomes replicate by an unknown process and are most often lost after mating with a rho^+ strain. Both rho^- genomes were maintained in haploid strains carrying a disrupted *rpo41* gene. Thus, Rpo41p did not seem to be required for rho^- mtDNA replication. This observation

prompted the authors to reexamine the effects of *RPO41* disruption on rho$^+$ genomes. In contrast to the results of Greenleaf et al. (112), they observed that the *rpo41* mutation did not cause a complete loss of mtDNA but led mainly to the accumulation of rho$^-$ genomes (90) (Table 3). As emphasized by the latter authors, this discrepancy is probably due to the experimental procedures used in the two laboratories. In one case (112), the observations were performed on cultures obtained from haploid spores which inherited the *rpo41* mutation through meiosis, while in the other case (90), the observations were made on vegetative cells after inactivation of the *RPO41* gene. When these authors repeated the experiment by sporulating a diploid strain heterozygous for the *rpo41* mutation, they observed a lower recovery of mtDNA than in their previous experiment with vegetative cells. These data as a whole lead to three clear-cut conclusions. First, inactivation of *RPO41* does not cause a complete loss of mtDNA. Second, even if the mutant strain produces a large amount of rho^0 petites, cytoduction experiments provide evidence that it is able to maintain rho$^-$ genomes. This interesting feature has also been noticed for the *abf2* mutants (see "The *ABF2* paradox" above). Third, conclusions about the effect of a nuclear mutation upon the production of rho^0 versus rho$^-$ petites must be drawn cautiously, since they may depend on the experimental procedures used in different laboratories. In particular, this could explain why *rpo41* mutations were recovered by Guan (118) among rho^0 producers (see "Wide-scale screening for rho^0 production" above).

Another very interesting way to test the putative role of transcription for replication was to analyze the effects of a *rpo41* deletion on biased transmission of hypersuppressive rho$^-$, assumed to result from their replicative advantage. This experiment was also performed in Fangman's laboratory (196). Since rho$^+$ genomes are unstable in a *rpo41* background while rho$^-$ genomes are stable, the authors examined diploid clones issued from matings between hypersuppressive and neutral petites. The hypersuppressive rho$^-$ genomes were always preferentially transmitted regardless of the nuclear background, *RPO41* or *rpo41* (deletion). The authors proposed two hypotheses based on all available data. First, hypersuppressive petites indeed have a replication advantage, but transcription is not required for replication initiation (at least for rho$^-$ genomes). Second, the preferential transmission of rho$^-$ genomes containing a *ori*/*rep* sequence is not at the level of replication but at the level of segregation. This hypothesis is in agreement with the deletion and overexpression of *MGT1*/*CCE1* (see "Nuclear control of biased transmission" above and Table 2), which suggest that the resolution of recombination junctions could play a key role in the segregation and transmission of mtDNA. A similar hypothesis has also been proposed for *ABF2* (see "The *ABF2* paradox" above).

The catalytic (Rpo41) subunit of mtRNA polymerase requires an additional factor for promoter recognition. This factor (Mtf1 or sc-mtTfb) was initially characterized biochemically (264), while the gene was first identified as a high-copy suppressor of an *rpo41* heat-sensitive mutant (188) (Table 3). The relationship between the protein and its structural gene was then established by Jang and Jaehning (144) using reverse genetics. The geneticists (188) claimed that disruption of *MTF1* led to a complete loss of mtDNA. However, since their experimental evidence was limited to a lack of hybridization with a single probe (21S rDNA), we must be more cautious (Table 3). Directed mutageneses of the *MTF1*/*mtTFB* gene were performed by Shadel and Clayton (271). The mutants showing respiration deficiency were not analyzed at the mtDNA level. This would have been especially interesting with respect to a small deletion (of 5 amino acids) resulting in a mutant phenotype, while the protein showed significant levels of transcription in vitro. This was the only mutant of the series exhibiting a contradictory (in vitro/in vivo) phenotype. This led the authors to propose that the Mtf1/mtTfb protein could have a mitochondrial function other than transcription.

Thus, to date, the data neither prove nor disprove a role of transcription in replication initiation of the rho$^+$ genome. However, they lead to two conclusions concerning petite mutants: (i) rho$^-$ genomes (either hypersuppressive or neutral) do not require transcription for their replication, and (ii) preferential transmission of hypersuppressive rho$^-$ genomes is maintained in the absence of transcription.

THE TRANSLATION-ATP SYNTHASE CONNECTION

Background

The idea that mitochondrial translation is required for mtDNA stability was first suggested by experiments demonstrating that growth of yeast in the presence of inhibitors of mitochondrial protein synthesis caused a high frequency of rho$^-$ genomes (references in reference 213). This idea has been reinforced by these authors, who showed that the inactivation of four genes involved in mitochondrial translation led to loss of wild-type mtDNA (213). They also showed that induction of rho$^-$ was observed only if the mutations were stringent and not if the relevant nuclear genes carried leaky mutations. Finally, they provided evidence that rho$^-$ genomes could be maintained in these contexts, which thus appeared deleterious only for the wild-type mitochondrial genome. In these experiments (213) (Table 3), the genes studied encoded two aminoacyl-tRNA synthetases, one elongation factor, and a putative ribosomal protein later shown to be a bona fide r-protein (60). To date, there has been no exception to the rule: rho$^-$ genomes are produced at high frequency as long as a stringent mutation occurs in a mitochondrial or nuclear gene encoding an essential component of the mitochondrial translational apparatus. It would be redundant to quote all reports on the subject published over the last 14 years.

One must keep in mind that the effect of a mutation may be indirect, if the product of the relevant gene acts upstream of translation. Among many examples, two illustrate this fact. The first case concerns the *RPM2* gene, encoding the protein component of a ribonucleoprotein required for 5′ maturation of mitochondrial tRNAs (154, 210). A less obvious example concerns the *NAM1* gene, first identified as a multicopy suppressor of mitochondrial splicing deficiencies caused by mitochondrial mutations (73). The effects of *NAM1* inactivation were carefully analyzed in two mitochondrial genetic backgrounds, i.e., in strains carrying either an intron-containing or an intronless mitochondrial genome. The data led to the conclusion that Nam1p was required for the processing and/or stability of *cytb* and *cox1* intron-containing pre-mRNAs and of the *atp6* transcript (which does not contain introns) (116). Interestingly, *NAM1* inactivation leads to 20 to 50% petite production in a strain with an intron-containing mitochondrial genome (16) while there is no increase in petite production (with respect to wild type) when the mitochondrial genome does not contain introns (116). According to the authors, the high level of petites observed in the first context may be explained by a defect in mitochondrial translation due to a starvation for tRNA^{Glu}, which is cotranscribed with the *cytb* gene, the intron-containing *cytb* transcript being unstable in the absence of Nam1p. Although this attractive hypothesis has yet to be experimentally tested, it strikingly illustrates the chain of events which can

TABLE 4. Structural genes of ATP synthase subunits

Gene[a]	Protein[b]	Mutation	% Petite production[c]	Reference[d]
OLI2, PHO1, ATP6 (mt)	Atp6 (F_0)	Point	20–80	97
OLI1, PHO2, ATP9 (mt)	Atp9 (F_0)	Point	35–49	54
AAP1, ATP8 (mt)	Atp8 (F_0)	Point	50–70	214
ATP4 (n)	b (F_0)	Disruption	70	229
ATP5 (n)	OSCP (F_0)	Disruption	40–80	242
ATP7 (n)	d (F_0)	Deletion	High	220
ATP14 (n)	h (F_0)	Deletion	90	8
ATP17 (n)	f (F_0)	Disruption	60	285
ATP18 (n)	j (F_0	Disruption	High	7[e]
ATP1 (n)	α (F_1)	Uncharacterized	1	306
		Deletion	<1	170
ATP2 (n)	β (F_1)	Uncharacterized	1	306
		Deletion	<1	170
ATP3 (n)	γ (F_1)	Disruption	20	228
		Deletion	100	170[f]
ATP16 (n)	δ (F_1)	Deletion	Near 100	106
		Deletion	100	170
ATP15 (n)	ε (F_1)	Deletion	25–50	119
		Deletion	60	170

[a] The genes are carried by either the mitochondrial (mt) or the nuclear (n) genomes.

[b] The proteins are parts of either the proton channel membrane (F_0) or the catalytic (F_1) sectors of ATP synthase.

[c] The methods used by the authors do not discriminate between rho^- and rho^0 petites. The values must be interpreted with caution since the basal level of petite production observed in the reference strains was not reported except in the study performed by Lai-Zhang et al. (170): here the wild-type reference strain produces 9% petites.

[d] References are those reporting about petite production. See the text for other relevant references.

[e] Vaillier et al. (309) reported contradictory data concerning *ATP18*.

[f] The slight discrepancy between these data might be explained, as suggested by Lai-Zhang et al. (170), by the differences in how the null mutants were constructed, giving rise to a complete or partial deletion.

indirectly link a mutation with mitochondrial translation and hence with petite production.

In 1985, Myers et al. (213) proposed two hypotheses concerning the role of mitochondrial translation in maintenance of the rho^+ genome. First, mtDNA would encode a protein required for replication or repair. Second, perturbation of the inner membrane (due to the lack of a component encoded by mtDNA) might specifically prevent the import of proteins required for replication or repair of the rho^+ genome. Fourteen years later, the question remains open. However, many data have meanwhile focused on the mitochondrial ATP synthase.

ATP Synthase and Petite Production

The yeast mitochondrial ATP synthase (ATPase) contains at least 14 subunits, of which 5 (α, β, γ, δ, and ε) are found in the catalytic F_1 sector (responsible for both ATP synthesis and hydrolysis) while the rest participate in the proton membrane channel F_0 sector (see reference 232 for a general review on ATPases and Table 4 for yeast mtATPase). Three of the F_0 subunits (Atp6, Atp8, and Atp9) are encoded by the mitochondrial genome, and the remainder of the complex is specified by nuclear genes (Table 4).

Two of the three mitochondrial genes, those encoding Atp9 (54) and Atp6 (254), were initially identified through mutations conferring resistance to oligomycin, an inhibitor of mitochondrial ATPase (*OLI1* and *OLI2*, respectively) (10). Later, mit^- mutations leading to the loss of ATPase activity were obtained, defining two genes, *PHO2* and *PHO1*, which mapped near the *OLI1* and *OLI2* loci, respectively (54, 97). These loss-of-function mutations produced high levels of petites (Table 4). To simplify the nomenclature, the genes are now called *ATP9* and *ATP6*. A final group of mit^- mutations helped to identify the third gene, *AAP1* (*ATP8*), encoding Atp8 (199). Again, the mutants were high producers of petites (214) (Table 4). At the time, it seemed clear that mutations in the ATPase mitochondrial genes so far identified were inherently unstable with respect to the mitochondrial genome. This conclusion was strengthened by the observation that very few mit^- mutations were found in these three genes compared to the mitochondrial genes encoding cytochrome oxidase subunits and cytochrome *b*. For instance, it was reported that mutants with deficiencies in cytochrome *b* and in cytochrome oxidase were, respectively, 50 and 300 times more frequent than mutations in the *ATP9* gene (54, 163, 307). As discussed by these authors, the rare occurrence of the *ATP9* mutants could have two explanations. First, one must take into account the relative sizes of the targets: *ATP9* is a small gene (this is also the case for *ATP6* and even more so for *ATP8*, which encodes a 48-amino-acid protein). Second, all loss-of-function mutations in the *ATP9* gene (and in *ATP6* and *ATP8* as well) are unstable. In fact, one can presume that all *ATP9* (and *ATP6* and *ATP8*) mutations able to produce nearly 100% petites could not have been recovered as mit^- mutations. This hypothesis implies that most mit^- mutations in the three relevant genes are leaky. Although frameshift and nonsense mutations have been obtained in these genes, this does not imply stringent mutations: phenotypic suppression occurs in mitochondria, due to its error-prone translational apparatus (see reference 284 for an example of a leaky frameshift mutation in the *ATP6* gene).

This high instability of the rho^+ genome, observed when the F_0 sector of ATPase is deficient, is not limited to the mitochondrial genes. In fact, mutations in nuclear genes encoding the other F_0 subunits also display this property (Table 4). Three F_0 subunits (e, g, and k) have been omitted in Table 4 since they are not required for the formation of enzymatically active ATP synthase (6).

In 1973, Ebner and Schatz (79) showed that a nuclear mutant (*pet936*) lacking F_1 produced a large number of petites. However, this observation was forgotten, and before 1992 it was believed that the F_1 ATPase sector was not involved in mtDNA stability (see, for instance, reference 2). In fact, at that time two *pet* mutations, later shown to lie in the structural genes for α and β subunits (260, 297), appeared genetically stable with respect to mtDNA (Table 4). In any case, the situation was strikingly modified when it appeared that deletions of the genes encoding the three other F_1 subunits (γ, δ, and ε) led to a more or less pronounced production of petites (Table 4). Very recently, Lai-Zhang et al. (170) showed that strains carrying a deletion of either *ATP1* (subunit α) or *ATP2* (subunit β) produce less than 1% petites. The authors did not discuss the fact that deletions of either one of these two genes give a petite level 10-fold below what they observed in the wild-type strain from which the deletions were obtained (Table 4). Our hypothesis is that these mutants cannot survive in a petite context (see "No compromise," below, for a more extensive discussion of nuclear mutants killed by mtDNA instability).

In addition to these structural genes, several nuclear genes whose products are involved either in ATPase subunit synthesis or in assembly of the complex have been identified. Three concern the expression of the mitochondrial *ATP9* gene: *AEP1* (for "ATPase expression protein"), *AEP2* (*ATP13*), and *NCA1* (for "nuclear control of ATPase"). The first is necessary for *ATP9* translation (231). Disruption of the *AEP1* gene leads to

a high frequency of petites (230). The second gene, isolated by two research groups, is required for *ATP9* transcript maturation or stabilization. The original *pet* mutant, which helped identify the *ATP13* gene (1), showed a low level (8%) of petite production (306). This can be explained by some leakiness of the mutant, since disruption of *AEP2* (*ATP13*) clearly shows an instability of mtDNA (93). The third gene (*NCA1*) involved in *ATP9* expression is required for transcript stability, but there are no data concerning the effects of either the original mutation or the *NCA1* disruption upon petite production (338).

Three other genes are required for stabilization (*NAM1*) or normal maturation (*NCA2* and *NCA3*) of the *ATP8-ATP6* cotranscripts. As mentioned above, inactivation of the *NAM1* gene leads to a high production of petites in a strain bearing an intron-containing mitochondrial genome (probably due to an indirect effect on translation) whereas there is only a basal level of petites when the mitochondrial genome contains no introns. In fact, in the latter condition, the only defect of the *nam1* mutant is a diminution of the *ATP6* transcripts. However, this is a leaky phenotype, as shown by the fact that the *nam1* strain is able to grow (although only slowly) on glycerol-containing medium at 28°C: this probably explains the mtDNA stability. It would have been interesting to observe petite production by the mutant at 36°C, a temperature at which it displays a complete respiratory growth defect (116). The two other genes so far identified, which affect the synthesis of *ATP6* and *ATP8* subunits, are part of a complex procedure that is not yet understood. It was demonstrated that the simultaneous presence of mutations in two genes resulted in a cold-sensitive phenotype (on a nonfermentable carbon source), associated with a small amount of one of the *ATP8-ATP6* cotranscripts (234). Isolation of the *NCA2* (29) and *NCA3* (233) genes shed no light on their functions. The petite levels were not reported, either for the original double mutant or for the double-disruption strain.

Three genes (*ATP10*, *ATP11*, and *ATP12*) specifically involved in ATPase assembly have so far been described. The Atp11 and Atp12 proteins are required for normal assembly of the F_1 sector. The *atp11* and *atp12* mutants display 10% of wild-type ATPase activity and seem to be defective in a late step of F_1 assembly, the incorporation of α and β subunits into an active oligomer (3). The two mutants display a basal (1 to 5%) level of petite production (2). The genes were cloned (2, 23), but the effects of null alleles on the fate of mtDNA have been reported only for *ATP11* by Lai-Zhang et al. (170): a strain carrying a deletion of this gene produces 1% petites. With respect to *ATP10*, biochemical data suggest that the mutants have an abnormal F_0 structure, which would impair the coupling of F_1 to F_0. Biochemical criteria, in accordance with growth phenotypes, also suggest that the mutants (the original *pet* as well as the disruptant) are leaky. One of the *pet* mutants has been analyzed with respect to mtDNA stability. According to the authors, it is genetically stable since it accumulates only 10 to 15% petites after long-term culture (4).

The final class of nuclear genes reported here are those whose products are required to facilitate the assembly of ATPase and other complexes in the mitochondrial inner membrane as well, namely, cytochrome oxidase. They are the *AFG3* (*YTA10*), *RCA1* (*YTA12*), and *OXA1* genes (reference 248 and references therein). Again, all the mutants (even the null mutants) display a leaky phenotype with respect to the ATPase complex. For instance, as reported by Rep et al. (249), *afg3 rca1* double mutants contain 56% of fully assembled ATPase relative to wild-type. The single- and double-mutant strains are genetically stable with respect to mtDNA (5). The same situation is encountered with the *oxa1* mutants (G. Dujardin, personal communication).

These data as a whole lead to the following conclusions and comments. First, null alleles of all genes encoding essential subunits of the F_0 sector cause a strong increase in petite production. Second, deletion (disruption) of the genes encoding subunits γ, δ, and ε of the F_1 sector also increase mtDNA instability. Third, the status of the α and β subunits (which are the catalytic subunits of ATP synthase) remains ambiguous. Ebner and Schatz (79) showed that absence of ATP synthase activity (due to an uncharacterized *pet* mutation) increases petite production. However, the petite colonies could be detected only after 6 to 10 days (instead of 2 to 3 days in the usual procedures). This raises a fundamental question concerning the conclusions of Lai-Zhang et al. (170). In fact, these authors observed that deletion of *ATP1* (α subunit) or *ATP2* (β subunit) resulted in less than 1% petites (compared to 9% in their reference strain). However, they did not state whether they found any petites and whether they examined the plates after long-term culturing. The last conclusion concerns the regulatory proteins. Clearly, some of them facilitate only ATPase assembly, since, in their absence, significant amounts of ATPase are assembled. In these cases, the mutant strains do not accumulate petites at high rates. In contrast, deletions of *AEP1* or *AEP2*, required for *ATP9* expression, increase mtDNA instability.

Is *ATP6* the Key to the Mystery?

We must now reformulate the problem of mitochondrial translation. If the lack of one (or several) protein encoded by mtDNA is responsible for the high rates of petites observed when translation is blocked, one can easily exclude cytochrome *b* and subunits I, II, and III of cytochrome oxidase: stringent mutations in the four genes do not increase the levels of petites. However, in direct contrast, each time a mutation abolishes the synthesis (or drastically alters the structure) of one of the three ATPase subunits encoded by mtDNA, an extreme level of petite production is observed (see above and Table 4). The question must be posed: what is the link between Atp6, Atp8, Atp9, and mtDNA stability?

To answer this question, one must keep in mind that mutations which abrogate the synthesis of any of the ATPase subunits, even those which are nuclearly encoded (with the possible exception of α and β), cause the same phenotype, i.e., high production of petites. A careful analysis of the data leads one to favor a special role for Atp6 (see references in Table 4 for most of the relevant data). First, Atp6 assembly depends on Atp9 and Atp8 (reviewed in reference 214). Second, the lack of either of the nuclearly encoded F_0 subunits is always associated with the absence of Atp6. Third, in a strain lacking the F_1 subunit γ, the concentrations of the F_0 subunits Atp6, Atp8, and Atp9 are reduced by more than 70% (although petite production is only 20% in the culture analyzed) (228). Fourth, when the F_1 subunit ε is absent, Atp6, Atp8, and Atp9 are present but there is a proton leak, suggesting a conformational change of F_0 (119). The relevant observations cannot be performed with subunit δ, since deletion of the gene leads to 100% petites. Finally, disruption of *ATP2* (subunit β) blocks the functional assembly of F_0 (298). Thus, the data as a whole show (i) that the F_0 sector is altered when one of the F_0 or F_1 subunits is absent and (ii) that Atp6 is the ultimate and most sensitive target of F_0 assembly.

These data do not argue against the proposals of Myers et al. (213) but suggest the following considerations: in the absence of mitochondrial translation, either the import of proteins re-

TABLE 5. Genes encoding mitochondrial proteases (peptidases) and chaperones

Gene[a]	Protein function[e]	Type of mutation	Petite production	Reference(s)[j]
"*MIP*"[d] (NE)	**Mitochondrial intermediate peptidase**	Disruption	rho$^-$ and/or rho^{0g}	24
PIM1/*LON*[d] (NE)	**Mitochondrial protease**	Disruption	rho$^{-g,h}$	295, 313
SSC1[d] (E)	**Mitochondrial Hsp70**	Point mutations in a Δ*hsp78* context	rho$^-$ and/or rho^{0g}	209
YGE1/*MGE1*[b,c,d] (E)	**Mitochondrial GrpE homologue**	Point mutation	rho$^-$ and/or rho^{0i}	171
MDJ1[d] (E at 37°C)	**Mitochondrial DnaJ family**	Deletion	rho^{0g,h}	258

[a to j] See Table 1 footnotes.

quired for replication/repair of the rho$^+$ genome would be specifically prevented by alteration of the F_0 sector or the putative mtDNA-encoded protein required for replication/repair of the rho$^+$ genome would be Atp6. In our opinion, these hypotheses now appear quite unlikely and we favor a structural and more direct role of Atp6 in the maintenance of the rho$^+$ genome: for instance, Atp6 might be involved in physical attachment of the rho$^+$ molecule to a centromere-like structure required for its faithful transmission (see "Is there a centromere-like structure for the mitochondrial genome?" below).

In any case, a critical test with respect to the translation-ATPase connection would be to supply the mitochondria with Atp6, Atp8, and Atp9 encoded by artificial nuclear genes specifying imported forms of the proteins (already performed with Atp8 [214]) and then examine the effect of a mitochondrial translational block on petite production. If in this context the level of petites remains stable, one may conclude that at least one of these subunits is indeed the target of mitochondrial translation with respect to rho$^+$ maintenance. However, if a high production of petites is observed, one must assume that either another (uncharacterized) protein encoded by the mitochondrial genome or translation per se of the relevant subunit is involved in the rho$^+$ maintenance.

The problem of ATPase does not end here: we will find it again as a possible key in the old problem of petite positivity versus petite negativity (see "No compromise" and "Petite positivity versus petite negativity" below).

POSTTRANSLATIONAL EVENTS

Expected and Surprising Roles for Proteases (Peptidases) in mtDNA Stability

The previous sections were devoted to mtDNA metabolism (replication, repair, and recombination) and its expression (transcription and translation) with regard to its maintenance. In this section, we discuss posttranslational processing of proteins encoded by nuclear or mitochondrial genes, defects of which cause mtDNA instability.

As reviewed in reference 247, mitochondrial proteases can be divided into two classes: processing of precursor proteins and involvement in protein degradation. Most mitochondrial proteins encoded by nuclear genes are translated as precursors whose N-terminal presequences are required for mitochondrial targeting. These presequences are eliminated by proteolytic cleavage during their transport across the mitochondrial membranes. This is achieved by the mitochondrial processing peptidase (Mpp). One may assume that Mpp is indirectly required for mtDNA stability through processing of numerous proteins directly (or indirectly) involved in this process. However, this prediction cannot be tested, because the genes encoding the two subunits of Mpp are essential for cell viability (147, 329). This is not surprising if Mpp is a global mitochondrial processing enzyme: mitochondria are indeed required for many functions besides respiration (reviewed in reference 305). A few proteins, encoded by nuclear genes and delivered to the intermembrane space or encoded by the mitochondrial genome and devoted to the inner membrane, are processed by Imp, a inner membrane peptidase (reference 221 and references therein). This protein is beyond the scope of this review, since mutations of the two genes encoding its catalytic subunits (though leading to respiration deficiency) do not cause mtDNA instability.

A subset of nuclearly encoded proteins are processed to the mature form in two steps. The precursors are first cleaved by Mpp and then cleaved by the mitochondrial intermediate peptidase "Mip" (we put this acronym in quotation marks to avoid confusion with the acronym used above for the mitochondrial DNA polymerase [Table 1]). The yeast "*MIP1*" gene (Table 5) was cloned by hybridization with the cDNA encoding this enzyme in the rat. Disruption of the gene leads to respiration deficiency (142). Among the precursors which require "Mip" in order to be processed in the mature form, one r-protein, one translational elongation factor, and the Rim1 precursor were found (24). Because mitochondrial translation (see the previous section) (Table 3) and Rim1p (see "The recombination/repair track" above) (Table 1) are involved in mtDNA stability, the petite production observed when "*MIP1*" is inactivated is an indirect but expected effect (24).

The problem of the Pim1/Lon protease is much more puzzling. It belongs to the second group of mitochondrial proteases involved in protein degradation (247). The gene encoding this ATP-dependent protease was cloned independently by two groups. In one case (313), it was discovered during the systematic sequencing project of chromosome II and identified due to similarities of the deduced protein to bacterial Lon proteases. In the other case (295), the gene was obtained through PCR experiments, using the sequence conservation between these bacterial proteases and their human homologues. Evidence for proteolytic activity of the protein in vivo was obtained. The data also showed its role both in selective protein turnover (295) and in degradation of misfolded proteins (316). The *PIM1*/*LON* gene was rediscovered as a multicopy suppressor of mutations in two nuclear genes encoding proteins of the mitochondrial inner membrane, *AGF3*/*YTA10* and *RCA1*/*YTA12* (248). Mutations in these two genes lead to defects in degradation of mitochondrially synthesized proteins and in assembly of inner-membrane complexes. Several arguments led to the conclusion that these proteins may play a dual role, with both degradation and chaperone activities (reviewed in reference 247). Interestingly, overproduction of the Pim1/Lon protein suppressed respiration-dependent growth defects and protein assembly defects of the *agf3*/*yta10* and *rca1*/*yta12* mutants but not defects in protein degradation. Furthermore, suppression was maintained and even enhanced when the proteolytic activity of the Pim1/Lon protein was destroyed (249). This suggests that this protein (at least when overproduced) also displays a chaperone-like function in the assembly of mitochondrial complexes.

Disruption of the *PIM1*/*LON* gene is not lethal but leads to

respiration deficiency and rho$^-$ genome production (295, 313) (Table 5). The stability of mtDNA depends on the proteolytic activity of the Pim1/Lon protein. Two types of data lead to this conclusion. First, a missense mutation which impairs this activity results in mtDNA aberrations. Second, a strain carrying a deletion of the *PIM1* gene is complemented (at 30 but not 37°C) by the *E. coli LON* gene with respect to mtDNA stability, provided that the bacterial protein was proteolytically active (299). These observations prompted the authors to propose that the Pim1/Lon protein could be involved either in the proteolytic activation of a protein required for mtDNA integrity or in the turnover of a protein which would negatively affect mtDNA stability. Notably, van Dyck et al. (312) succeeded in dissociating the role of Pim1/Lon on mtDNA stability from another function of its protease activity in biogenesis of the respiratory chain. In this fascinating problem, elucidation of the specific target(s) of the mitochondrial Pim1/Lon protease with respect to maintenance of the rho$^+$ genome is now necessary. New insights into the functions of its bacterial homologues will undoubtably help in this research. In fact, it has been recently found that the *Caulobacter* Lon protease plays a crucial role in cell cycle control of DNA methylation (330) and that the *E. coli* Lon protease is a site-specific DNA-binding protein (101). This property has also been reported for the mitochondrial human Lon protease (100).

Conventional Mitochondrial Chaperones

In addition to proteins bifunctional as proteases and chaperones (see above) (Table 5), mitochondria contain more conventional chaperones. Most of the nuclear genes encoding these chaperones are necessary for cell survival. This might be expected if proteins which perform essential functions in mitochondria (see reference 305 for a review) require these chaperones for translocation across the membranes and/or proper folding within the organelle. Since these genes are essential, it has often been impossible to address the specific question of their role with respect to mtDNA stability. However, a few points can be made.

The yeast mtHsp70 is encoded by the nuclear gene *SSC1* (for "stress seventy subfamily C"). This gene was isolated by hybridization with the *SSA2* gene, which encodes another member of the Hsp70 family in yeast. *SSC1* is essential (55) and encodes a mitochondrial protein (56). Further studies have shown that mtHsp70 is required for the translocation of proteins into mitochondria, folding of these proteins in the matrix, and folding of proteins synthesized in the organelle (reviewed in reference 215). Although two heat-sensitive mutants have been used in these studies, no effects were reported on mtDNA stability at subrestrictive temperatures. However, Moczko et al. (209) found that double-mutant strains bearing either of the two heat-sensitive *ssc1* mutations and a deletion of the gene encoding mtHsp78 were unable to grow on nonfermentable carbon sources at 30°C, conditions which allow growth of the two single mutants. The double mutant is in fact in a rho$^-$ (or rho^0) state (Table 5). Thus, the absence of mtHsp78 leads to mtDNA instability when the activity of mtHsp70 is limiting. The exact role of mtHsp78 (179) remains unclear, although some data suggest that it can partially substitute for mtHsp70 (265).

In *E. coli*, Hsp70 (DnaK) is assisted by two proteins, DnaJ and GrpE. DnaJ is a chaperone in its own right; however, it also interacts with DnaK and stimulates its ATPase activity. GrpE acts as a nucleotide exchange factor for DnaK (see reference 104 for a review). Although the mitochondrial Hsp70 does not seem to be the functional homologue of DnaK (62), the functional homologues of DnaJ and GrpE were found in yeast mitochondria. The nuclear gene encoding the mitochondrial GrpE was independently discovered by three groups during the same year. Named *YGE1* by Ikeda et al. (140), it was a serendipitous discovery, since these authors identified the gene as responsible for resistance to staurosporine (an inhibitor of protein kinases) when present on a multicopy plasmid! The article also reports that the gene is essential and able to complement a *grpE* mutant of *E. coli* and that a strain with *YGE1* disrupted has mitochondria stacked in the mother cell and thus unable to enter the buds. Bolliger et al. (21) purified the protein by using its interaction with mtHsp70 and then cloned the gene, which was shown to be essential for cell survival. Laloraya et al. (172) discovered the gene (named *MGE1* in this article) by DNA sequence analysis. They showed that *MGE1* is essential and encodes a mitochondrial protein that is probably required for protein translocation into mitochondria. This assumption was fully confirmed by later studies using heat-sensitive mutants of *YGE1*/*MGE1*, and it was shown that Mge1p acts in concert with mtHsp70 (see reference 215 for a review). Laloraya et al. (171) reported an increased frequency of petites in a heat-sensitive *mge1* mutant (Table 5).

In bacteria, DnaJ is (like GrpE) an essential partner of Hsp70 (see above). Its yeast mitochondrial homologue was identified during a DNA sequencing project (258). Deletion of *MDJ1* (for "mitochondrial DnaJ") causes death at 37°C and an inability to grow on nonfermentable carbon sources at 24°C. This second phenotype cannot be rescued by the wild-type gene: evidence was obtained that these petites displayed a rho^0 state (Table 5). Mdj1p is not required for protein import but is required in later steps of protein folding. However, Mdj1p cooperates with mtHsp70 to prevent aggregation of misfolded proteins, thus allowing their degradation by Pim1/Lon (see above). Finally, again in association with mtHsp70, Mdj1 can act as a chaperone for nascent proteins synthesized in mitochondria (258, 316, 323). Thus, the role of Mdj1 with respect to mtDNA stability is probably indirect, through its effect upon proteins that are directly or indirectly involved in mtDNA metabolism.

Another canonical chaperonin system, consisting of Hsp60 and Hsp10, is also required in the mitochondrial matrix. The genes encoding these two proteins are essential for viability (reviewed in reference 215). There is, to our knowledge, no report concerning their effect on mtDNA stability under subrestrictive conditions.

This section terminates the first part of this review, which tentatively concerned genes clearly involved in mtDNA metabolism and in gene expression including posttranslational events. The genes discussed in the following sections have been grouped (more or less artificially) according to a few governing ideas.

THE WORLD OF CARRIERS AND TRANSPORTERS

Mitochondrial carriers ensure the traffic of a number of small molecules (adenine nucleotides, inorganic ions, amino acids, fatty acids) across the inner membrane (see reference 168 for a review). It is thus not surprising that some of them are indirectly involved in mtDNA metabolism and consequently mtDNA stability.

The *MIR1* (for "mitochondrial import receptor") gene was initially identified as encoding an integral membrane protein (p32) which was proposed to act as a receptor for protein import into mitochondria (211, 225). Indirect (in vitro) data suggested that p32 contained a binding site specific for the signal sequence of preproteins directed toward mitochondria

TABLE 6. Genes encoding mitochondrial carriers/transporters and genes involved directly or indirectly in oxidative stress

Gene[a]	Protein function[e]	Type of mutation	Petite production	Reference[j]
MIR1[c] (NE)	**Mitochondrial phosphate carrier**	Disruption?	rho$^-$ and/or rho^{0i}	333[k]
RIM2/*MRS12*[b] (NE)	**Mitochondrial carrier family**	Disruption	rho^{0g,h}	311
ATM1[b,d] (NE)	**Mitochondrial ABC transporter** (iron homeostasis)	Disruption	rho$^{-g,h}$	178
GSH1[b] (E)	**γ-Glutamylcysteine synthetase**	Point mutations	rho^{0h}	157
YFH1[b,d] (NE)	Mitochondrial iron homeostasis	Disruption	rho$^-$ and/or rho^{0f,g}	11
		Deletion	rho^{0f,g,h}	328
		Disruption	rho$^-$ and/or rho^{0g,h} (in some genetic backgrounds)	95
SSH1[d]/*SSC2*[b]/*SSQ1* (NE)	**Mitochondrial Hsp70 homologue** (iron homeostasis)	Point mutation	rho$^-$ and/or rho^{0g}	159[l]

[a to j] See Table 1 footnotes.
[k] Murakami et al. (211) reported contradictory data (see "The world of carriers and transporters").
[l] Schilke et al. (263) reported contradictory data (see "Oxidative stress, iron homeostasis, and mtDNA and "No compromise" and Table 13).

(212). However, it appeared that the *MIR1* gene encodes a protein displaying 40% identity to mammalian mitochondrial membrane proteins which could act as phosphate carriers (211). Furthermore, a protein of the mitochondrial inner membrane which could function as a phosphate translocator was purified and sequenced (120): it is 100% identical to p32. Sequencing of the relevant gene led to the same conclusion (237). Data from Zara et al. (333) gave new insights with regard to the controversy about the function(s) of p32. In fact, these authors provided evidence that lack of p32 causes both a block in the import of phosphate and a pronounced reduction in the import of proteins devoted to the mitochondrial inner membrane and matrix. The second defect would be indirect, due to a strong reduction of the membrane potential in the mutant mitochondria. It was shown that (in vitro) restoration of a membrane potential restored protein import without relieving the phosphate transport deficiency. The viability of the mutant implies that, in vivo, protein import is sufficient for mitochondria biogenesis. However, this rate would be limiting with respect to mtDNA stability because the mutant accumulates petites at a higher frequency than the wild type does (333) (Table 6). Another explanation would be that a lower potential directly affects mtDNA stability. Nonetheless, Murakami et al. (211) demonstrated that the inability of a *mir1* mutant (partial deletion) to grow on glycerol was not due to the loss of the rho$^+$ genome. This contradictory result can be explained either by the genetic background or by the fact that the *MIR1* gene was only partially deleted. In addition, the high production of petites observed in a *mir1* disruptant whose mtDNA contains introns is abolished when the mitochondrial genome lacks introns (P. Hamel and G. Dujardin, personal communication). This is one of several examples, as reported in this review, of the strain influence in yeast due to variations in the nuclear and/or mitochondrial genomes.

The *RIM2*/*MRS12* gene was identified as a multicopy suppressor of respiration deficiency both of a *pif1* null strain (at 36°C) and of a *mrs2* disruptant. As mentioned above, *PIF1* encodes a DNA helicase essential for mtDNA stability at high temperature (Table 1). Mrs2p (for "mitochondrial RNA splicing") is bifunctional: it is required for group II intron splicing and for assembly of the respiratory chain (325). Multicopy suppressors of the respiratory defect of either *pif1* or *mrs2* mutants were independently sought. This allowed, in the first case, identification of the *RIM1* gene required for mtDNA replication (see "The recombination/repair track" above) (Table 1). In the second, a multitude of suppressors was obtained (317). It appeared that one (*MRS12*) was also discovered as a suppressor of *pif1* and was named *RIM2* (311). Deletion of *RIM2*/*MRS12* causes a slow-growth phenotype on glucose medium and an inability to grow on glycerol. This respiration deficiency is in fact due to a massive loss of mtDNA (Table 6). This rho^0 phenotype is seen as early as spore germination (issued from a diploid heterozygous for the deletion). The *RIM2*/*MRS12* gene encodes a member of the mitochondrial carrier family (311) whose substrates are as yet unknown. However, the authors suggest that it could be involved either in the transport of mtDNA precursors (to explain the suppression of *pif1*) or, more likely, in the transport of Mg^{2+}, which could explain its pleiotropic suppression effect on both *pif1* and *mrs2* mutations. It is noteworthy that, in the latter case, suppression acts on both defects: splicing and respiratory chain assembly (311).

ATP-binding cassette (ABC) transporters also assure transport of substrates across membranes. However, their substrates are extremely diverse, ranging from small molecules to large proteins. Each transporter is substrate-specific (reviewed in reference 132; see also reference 296 for a review of yeast ABC proteins). *ATM1* (ABC transporter of mitochondria) encodes a protein located in the mitochondrial inner membrane (178). Disruption of *ATM1* leads to a dramatic growth defect in rich glucose medium and an inability to grow at all on minimal medium. Although it grows (very slowly) on rich medium containing nonfermentable carbon sources, the disruptant loses this ability because it becomes rho$^-$ (Table 6). The mutant lacks all major cytochromes, including cytochromes *c* and c_1, which are encoded by nuclear genes. However, the authors provided evidence that this defect is not due to a failure to export heme from the mitochondria. Thus, the true function of *ATM1* remains to be characterized, as do the reasons for which this gene is essential not only for mtDNA stability but also for optimal growth. These two properties appear related, since some *atm1* disruptants show a moderate growth defect and do not become rho$^-$. This variation in the phenotype strength of the mutants is probably due to the spontaneous appearance of nuclear suppressors. Analysis of these suppressors will certainly aid in the elucidation of the (dual?) function of *ATM1* (178). Interestingly, *ATM1* was rediscovered as a gene able to complement a mutant defective in mitochondrial cytochromes (156). The data reported suggest that Atm1p could play a key role in mitochondrial iron homeostatis; this aspect is discussed in the following section.

With respect to the purpose of this review, it is interesting that mitochondrial carriers and transporters comprise three classes. In the first class, absence of the protein has no effect on mtDNA stability, even though in some cases the relevant genes are multicopy suppressors of *abf2* (see "The *ABF2* paradox"

above) (43, 152). In the second class, one finds the *AAC2* gene, encoding the major mitochondrial ADP/ATP translocator, required for viability of cytoplasmic petites (see "No compromise" below). The third class regroups the *RIM2*/*MRS12* and *ATM1* genes (Table 6), in which mutations lead to a high production of petites. Depending on the genetic background, *MIR1*, which encodes a phosphate carrier, can enter in either the first or the third class.

OXIDATIVE STRESS, IRON HOMEOSTASIS, AND mtDNA: A CONFUSING BUT EXCITING AREA

Oxygen, although essential for life, is deleterious when present in one of its reactive species (ROS). While some cytosolic oxidative processes produce ROS, it seems that mitochondrial respiration is the main source of production of superoxide ($O_2^{\bullet -}$) and other ROS (H_2O_2 and the hydroxyl radical $^{\bullet}OH$). Most superoxides are probably produced by complex III (reference 195 and references therein). This radical is normally converted into H_2O_2 by superoxide dismutase, while H_2O_2 is destroyed by catalases and peroxidases. The hydroxyl radical, assumed to be the most deleterious, is formed by iron-catalyzed reactions (Fenton reactions) of $O_2^{\bullet -}$ and H_2O_2. Generally, these reactions are minimized by compartmentalization of free iron and superoxide (reviewed in reference 128).

Superoxide ($O_2^{\bullet -}$) is removed by superoxide dismutase, which catalyzes the reaction $2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$. Like most eucaryotic cells, *S. cerevisiae* contains a cytosolic and a mitochondrial SOD, respectively the products of the *SOD1* and *SOD2* genes (reviewed in reference 109).

Disruption of the gene encoding the mitochondrial Sod (Sod2p) was first performed by van Loon et al. (314), who showed that the mutant was oxygen sensitive, especially when grown in 100% oxygen. However, it was sensitive even to normal air when grown in liquid medium with nonfermentable carbon sources. These authors also noted that there was no increase in the production of petites in this mutant context. This search was performed in air (G. Schatz, personal communication). Seven years later, Guidot et al. (121) showed that growth in hyperoxia of a strain with *SOD2* disrupted is fully restored in a petite (rho^0) background. This is explained by the fact that petites produce less $O_2^{\bullet -}$ and thus overcome the absence of Sod2p. In any case, the status of *sod2* mutants with respect to petite production still requires careful examination.

In contrast to the possible innocuity of Sod2p with respect to mtDNA stability, very low levels of glutathione (GSH) lead to a loss of mtDNA (157) (Table 6). GSH performs many functions, among them protection against ROS, especially H_2O_2 (see reference 207 for a review). The *GSH1* gene, encoding the first enzyme of the two-step biosynthesis of GSH, was cloned by complementation of a *gsh1* mutant (224) and rediscovered as a multicopy suppressor of a *pet* mutation impairing mitochondrial translation (185). Strains carrying a deletion of *GSH1* are unable to grow in the absence of exogenous GSH (110, 331). It was also demonstrated that these strains are hypersensitive to H_2O_2, and that reducing agents (such as dithiothreitol or β-mercaptoethanol) can restore the growth of the mutants, at least under nonstress conditions (110). Thus, as emphasized by the authors, GSH is essential only as a reductant. However, the effect of low levels of glutathione on mtDNA remains to be clarified. An effect of oxidative stress cannot be excluded. However, one might imagine an indirect effect which could involve ribonucleotide reductase. This enzyme, which catalyzes the conversion of ribonucleotides into deoxyribonucleotides, requires GSH for its recycling. As discussed below (see "mtDNA and the cell cycle"), mtDNA replication is highly sensitive to limiting amounts of DNA precursors. In contrast to *gsh1* mutants, strains lacking the second enzyme of GSH biosynthesis appear unaffected in mitochondrial function (111). As shown by the authors, *gsh2* mutants accumulate γ-glutamylcysteine, the intermediate in GSH synthesis, which would partly substitute for GSH.

Friedreich's ataxia is an autosomal recessive degenerative disease which primarily affects the nervous system and the heart. The *FDRA* gene was identified and shown to encode a protein (frataxin) whose function could not be inferred from its amino acid sequence (31). The yeast homologue was independently found through protein sequence similarity (31) and as a multicopy suppressor of a mutant unable to grow on iron-limited media (11). Disruption of *YFH1* (for "yeast frataxin homologue") leads to the inability to grow on respiratory substrates, due to instability of mtDNA (11). The petites are in fact rho^0 (328) (Table 6). However, this production of petites seems highly strain dependent (95). Similarly, growth defects of the *yfh1* mutants on fermentable carbon sources can be either severe or not evident, depending on the strain. Disruption of *YFH1* results in selective accumulation of iron in mitochondria, at the expense of the cytosol: the amount of iron is approximately 10-fold that in wild-type mitochondria in mutants (11, 95). Yfh1p localizes to the mitochondria (11, 328). This was also demonstrated for the human protein (164). Notably, Rötig et al. (256) did not detect any quantitatively significant rearrangements of the mitochondrial genome in Friedreich's ataxia patients. However, they observed a deficiency in the activity of the iron-sulfur (Fe-S) proteins of mitochondrial respiratory complexes I, II, and III. This could be explained as being due to oxidative stress (Fenton reactions) caused by the increased mitochondrial iron uptake, if one takes into account the fact that Fe-S proteins are critical targets for oxygen free radicals (reference 256 and references therein). Yeast strains carrying a *YFH1* disruption appear hypersensitive to oxidative stress (11). These authors, as well as Koutnikova et al. (164), suggested that yeast mtDNA instability might be a secondary effect of frataxin absence: inhibition of oxidative phosphorylation would reduce the production of ROS and thus buffer the mitochondrial iron accumulation. This implies that the *yfh1* mutant context would cause a selection of the petite state and not a real increase in the petite frequency. While very attractive, this hypothesis neglects the point that the petites are rho^0 (328). One would in fact expect that partial deletions of mtDNA (rho$^-$ petites) could equally counter the lack of frataxin.

Knight et al. (159) sought mutants with abnormal iron metabolism and analyzed one which showed a respiratory defect. It appeared that this strain carried a nonsense mutation in the *SSC2* gene encoding a minor mitochondrial Hsp70, previously described and named *SSH1* (263). Knight et al. (159) showed that their *ssc2* mutant exhibited an increased total mitochondrial iron content, twofold higher than that observed in *yfh1* mutants. These authors also demonstrated that the *ssc2* mutant displayed a partial defect in Yfh1p maturation, and they consequently favored the idea that Ssc2p might regulate mitochondrial iron accumulation through its effect upon Yfh1 and other proteins. The nonsense *ssc2* mutant exhibits a high production of petites (Table 6) and slow growth at 30°C, which is exacerbated at both 23 and 37°C (159). These data contradict those of Schilke et al. (263), who showed that a strain with *SSH1* (*SSC2*) deleted grew quite normally at 37°C but very slowly at 30°C and below and that this mutant background does not induce petite production at the permissive temperature (see "No compromise" below and Table 13).

The mitochondrial ABC transporter Atm1 previously iden-

TABLE 7. Genes involved in fatty acid and phospholipid metabolism

Gene[a]	Protein function[e]	Type of mutation	Petite production	Reference(s)[j]
OLE1[b] (NE)	**Δ9 fatty acid desaturase**	Uncharacterized	rho$^-$ and/or rho^{0f}	201, 108
CHO1[b] (NE)	**PS synthase**	Uncharacterized	rho$^-$ and/or rho^{0f}	9[k]
PSD1[b] (NE)	**PS decarboxylase**	Disruption	rho$^-$ and/or rho^{0i}	303
CHO2[b] (NE)	**PE *N*-methyltransferase**	Triple mutant	rho$^-$ and/or rho^{0f}	113
CPT1[b] (NE)	***sn*-1,2-Diacylglycerol choline phosphotransferase**			
EPT1[b] (NE)	***sn*-1,2-Diacylglycerol ethanolamine phosphotransferase**			
ACP1[d] (NE)	**Mitochondrial ACP**	Disruption	rho$^-$ and/or rho^{0g}	267, 25
OAR1[d] (NE)	**Mitochondrial 3-oxoacyl-ACP reductase**	Disruption	rho$^-$ and/or rho^{0g}	266
MCT1[d] (NE)	**Mitochondrial malonyl-CoA:ACP transferase**	Disruption	rho$^-$ and/or rho^{0g}	266
PPT2[d] (NE)	**Mitochondrial phosphopantetheine: protein transferase**	Disruption	rho$^-$ and/or rho^{0g}	288
ACC1[d]/*FAS3*[c]	**Acetyl-CoA carboxylase**	Uncharacterized	rho$^-$ and/or rho^{0g}	137

[a] to [j] See Table 1 footnotes.
[k] Another group (165) reported no increase in the petite level of their *cho1* mutant.

tified by Leighton and Schatz (178) (see "The world of carriers and transporters" above) (Table 6) was further analyzed by Kispal et al. (156). They demonstrated that a disrupted *atm1* mutant exhibits an oxidative stress phenotype and that the mutant mitochondria accumulate free iron at levels 30-fold higher than do wild-type organelles and thus threefold more than that observed in *yfh1* mitochondria. This observation may explain the oxidative damage of heme-containing proteins in the *atm1* mutant (156). These authors gave no information about petite production in their *atm1* background.

It is well known that yeast reference strains used in different laboratories are not isogenic. Thus, it is not surprising to observe that mutant phenotypes can differ more or less depending on the genetic background. Furthermore, transformation procedures (used to perform disruption experiments) are highly mutagenic and can induce suppressor mutations able to alleviate the mutant phenotype. Such effects are particularly striking in the case of *atm1* and *yfh1* mutants. The *atm1* mutant shows a strong or slight growth defect depending on the disruptant, and this effect is correlated with a high or low level of petite production (178). Tetrad analysis aided these authors in the identification of a suppressor of the *atm1* disruptant. A similar correlation between growth defect and petite production was observed, depending on the strain in which the *YFH1* disruption was achieved. However, in this case, the two disruptants display a similar mitochondrial iron content, about 10-fold more than in the two *YFH1* reference strains (95). This latter observation is really provocative and leads one to question the exact linkage between mitochondrial iron content, oxidative stress, and mtDNA instability. This observation is strengthened by the fact that disruption of *YFH1*, in the most permissive background, causes hypersensitivity to oxidant reagents (also reported by Babcock et al. [11]) and shows 100% petite production in the presence of 1 mM $FeSO_4$ (95).

In this complex problem, it is important to note that two homologous genes encoding mitochondrial iron (Fe) transporters (*MTF1* and *MTF2*) have been characterized (182). A double-deletion strain grows well on both respiratory and nonrespiratory substrates. This observation was valid when either of the two genes is overexpressed. In that case, total mitochondrial iron levels are increased about fivefold, which is less than in *yfh1* mitochondria. The fact that strains overexpressing *MTF1* or *MTF2* do not exhibit respiratory defects led the authors to suggest two hypotheses: (i) iron is not toxic for mitochondria below a precise threshold, and (ii) iron accumulates in different mitochondrial subcompartments in the two mutant backgrounds involving lack of *YFH1* and overexpression of *MTF1*/*MTF2* (182).

In any case, a high mitochondrial iron content is associated with mtDNA instability. It remains to be shown if there is a direct relationship between these two parameters, how this effect is manifested, and why the *atm1* mutant accumulates rho$^-$ genomes while the *yfh1* mutants accumulate rho^0 petites (Table 6). The data reported above clearly show that further experiments are required to understand the ways that mitochondria use to combat the ROS they produce and to understand the relationship between iron homeostasis, oxidative stress, and mtDNA instability.

FATTY ACIDS AND PHOSPHOLIPIDS: THE CHALLENGING DISCOVERY OF A MITOCHONDRIAL FAS COMPLEX

The major phospholipids found in yeast membranes are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol, and phosphatidylserine (PS). In addition, mitochondrial membranes contain cardiolipin, which is specific to this compartment. The status of cardiolipin with respect to mtDNA stability is discussed in "No compromise" (below), since mutations in the genes involved in its biosynthesis have either no effect on petite levels (*CLS1*/*CRD1*) or are lethal in a petite background (*PEL1*/*PGS1*).

Most genes involved in the synthesis of either long-chain fatty acids, which form the water-insoluble part of these lipids, or their polar head segments have been cloned and characterized (see references 61 and 129 for recent reviews). In four cases, mutations have been reported to increase mtDNA instability. These are single mutations in the *OLE1*, *CHO1*, and *PSD1* genes and the mutations in the *CHO2 CPT1 EPT1* triple-mutant strain (Table 7) (see below).

Although the data are incomplete, they focus on the fact that mtDNA instability can be increased by changes in the lipid composition of the mitochondrial membranes. This idea is strengthened by the recent discovery of a mitochondrial fatty acid synthase (FAS) whose defects can lead to a high production of petites. However, with the exception of lipoic acid, the compounds synthesized by this complex remain to be identified.

Fatty Acids: the Case of *OLE1*

The *KD115* (*ole1*) strain was obtained as a mutant auxotrophic for an unsaturated fatty acid, oleic or linoleic acid (250). An increased frequency of petites was observed when the mutant was grown in limited amounts of Tween 80 (a source of oleic acid), with the level of petites (from 0.4 to 80%) being determined by the degree of fatty acid depletion (201). In subsequent studies, Graff et al. (108) showed that mtDNA

stability depends not only on an adequate supply of cognate fatty acids but also on the quality (i.e., the structural features) of the relevant fatty acids, even though these "inadequate" compounds are incorporated into cellular phospholipids. The *OLE1* gene was cloned by complementation of the *KD115* (*ole1*) mutant (289) and shown to encode the Δ9 fatty acid desaturase (290). Amazingly, the *OLE1* gene was rediscovered as the gene complementing the heat-sensitive *mdm2* mutant, which exhibits an altered mitochondrial morphology and a general defect in mitochondrial movement at high temperatures (287). The authors show that addition of unsaturated fatty acid (oleic acid) to the medium restores *mdm2* growth at 37°C and corrects the mutant's defects in mitochondrial movement and morphology.

As proposed by Graff et al. (108), the data suggest that mitochondrial lipids containing unsaturated fatty acids may exhibit specific quantitative and qualitative requirements with respect to these compounds in order to ensure the correct insertion of proteins into the membrane. As an extension of this idea, one could imagine that mtDNA stability depends on its own attachment to one of these proteins (see the following sections and "Is there a centromere-like structure for the mitochondrial genome," below, for further discussion).

Requirements for Glycerophospholipids

PC can be synthesized via two pathways. One involves three successive methylations of PE, while the other uses free choline in a salvage pathway. PE can be obtained from either PS or free ethanolamine.

PS synthase is encoded by the *CHO1* gene, cloned by complementation of a mutant auxotrophic for choline or ethanolamine (180). Two groups provided contradictory data on mtDNA stability in the mutant strain. In the first case (165), the proportion of petites in the mutant cultures was in the range of 5%. In the second (9), a high proportion of petites was found, markedly affected by temperature: at 25°C, almost 100% of the cells became respiration deficient after a few days of growth, while at 30°C, the petite level did not exceed 50%. These properties cosegregated with the *cho1* mutation (9). It would be interesting to examine the effects of temperature in a null *cho1* mutant (this strain is now available [12]). Atkinson et al. (9) suggest that the observed defect could be due to mitochondrial membrane alterations, caused either by the lack of PS or by more subtle changes, since the mutants can synthesize PC only through the salvage pathway: in particular, the balance between PC and PE appears significantly disturbed in the mutants.

Transformation of PS into PE requires PS decarboxylases, encoded by two genes (*PSD1* and *PSD2*). The major form of the enzyme is encoded by *PSD1* and is located at the inner mitochondrial membrane, which provides PE directly to the mitochondria. This gene was cloned and disrupted by two laboratories (47, 303). The latter authors provided evidence that the disrupted strains (although able to grow on nonfermentable carbon sources) show an increased production of petites on glucose medium. The Psd2 enzyme is not localized to the mitochondria (304). The observation that the *psd1* mutants have significantly less PE in the mitochondria than the wild-type strain does prompted the authors to propose that PE formed by Psd2p is not efficiently transported into the organelle: this depletion would cause the phenotype observed in the *psd1* mutants.

The three final steps required to synthesize PC from PE involve two phospholipid methyltransferases, encoded by the *CHO2*/*PEM1* gene for conversion of PE into PMME (phosphatidylmonomethylethanolamine) and by the *OPI3*/*PEM2* gene for conversion of PMME into PDME (phosphatidyldimethylethanolamine) and PDME into PC. The two genes were cloned by complementation of the relevant mutants (160, 293). The fact that mutations in either gene cause (160) or do not cause (204, 293) choline auxotrophy is explained by different strain backgrounds (61). Prototrophy for choline of the *cho2* mutants is probably due to partial substitution by the Opi3 enzyme. With respect to the *opi3* mutants, it is postulated that accumulation of PMME can partially replace PC. The sole report on petite production concerns a *cho2* mutant which does not accumulate more petites than the reference strain (113). With respect to mtDNA, we would like to mention two of the five genes required for the salvage pathways towards either PE or PC (61). *CPT1* encodes *sn*-1,2-diacylglycerol cholinephosphotransferase (133), while *EPT1* encodes *sn*-1,2-diacylglycerol ethanolaminephosphotransferase (134). Griac and Henry (113) reported that a *cpt1 ept1* double mutant, in which the salvage pathways through both free choline and free ethanolamine are blocked, does not produce more petites than the reference strain but that a *cho2 cpt1 ept1* triple mutant exhibits a high level of petites (113). In this case, cell survival would be assured by the presence of PE (from the de novo pathway) and small amount of PC (due to the Opi3 activity and its ability to partly substitute for the Cho2 enzyme).

In all cases reported above (Table 7), the authors suppose that mitochondria are more sensitive to phospholipid starvation (or to desequilibrium in phospholipid composition of their membrane) than is the cell per se. Here again, the reason why these changes in membrane structure cause mtDNA instability relies on a putative link between mtDNA and the inner membrane (see "Is there a centromere-like structure for the mitochondrial genome" below). However, the recent discoveries reported below (and given in Table 7) open new windows on this major problem.

Mitochondrial FAS Complex

Until recently, it was assumed that mitochondrial needs for fatty acids were satisfied by import of molecules synthesized in the cytosol. However, several genes encoding subunits of a mitochondrial fatty acid synthase (FAS) complex similar to the bacterial (and chloroplast) complex (FAS type II) have now been characterized. Inactivation of these genes leads to a pet (respiration-deficient) phenotype and in some cases, to an increased production of petites (Table 7). The first gene discovered was *CEM1* (for "condensing enzyme with mitochondrial function"), encoding a protein with strong similarities to β-keto-acyl synthases (also called condensing enzymes). This gene was cloned by complementation of a *pet* mutant. Inactivation of *CEM1* does not lead to a high production of petites (126). Two years later, Schneider et al. (267) disrupted the *ACP1* gene encoding the mitochondrial acyl carrier protein (ACP), previously identified by systematic sequencing (40). In this case, inactivation of the gene causes loss of the wild-type mtDNA (Table 7). Schneider et al. (266) then characterized two other genes of the mitochondrial FAS on the basis of similarities between two open reading frames of the yeast genome and bacterial FAS subunits. These genes were named *OAR1* (for "3-oxo-acyl-ACP reductase") and *MCT1* (for "malonyl coenzyme A:ACP transferase"). Disrupted *oar1* and *mct1* strains exhibit an increased level of petites (266) (Table 7).

The role of mitochondrial FAS (excluding that of the *CEM1*-encoded subunit) in mtDNA stability has been recently supported by data from Stuible et al. (288). These authors identified (by sequence similarity) the *PPT2* gene, encoding a

mitochondrial phosphopantheine:protein transferase which specifically activates the mitochondrial ACP protein. Inactivation of *PPT2* causes loss of the wild-type mtDNA, as previously shown for the *acp1* disruptants (25, 267) (Table 7).

Harington et al. (126, 127), on one hand, and Schneider et al. (266, 267), on the other, propose two (nonexclusive) hypotheses to explain the pet phenotype of the four mitochondrial FAS mutants presently available. In the first case, the authors favor the hypothesis that the defect is situated at the level of the membrane insertion of proteins, a process which can require the covalent addition of fatty acid-like molecules to these proteins. This assumption is based on the fact that in the *cem1* strain, mitochondrial apo-cytochromes are synthesized while holo-cytochromes are absent. Since neither the global synthesis of fatty acids nor their overall content and composition in the mitochondrial membranes appear altered in the *cem1* disruptant, the authors suppose that Cem1p is involved in the synthesis of a specialized fatty acid required for proper anchorage of some proteins in the membrane (126, 127). Because the mutant does not produce a high level of petites, one must assume that this putative fatty acid-like component is dispensable for correct positioning into the membrane of all proteins required for mtDNA stability. The second hypothesis, proposed by Schneider et al. (266, 267), proposes that the mitochondrial FAS provides fatty acids required for remodeling and/or repair of phospholipids damaged by oxidation. This hypothesis may explain why the lack of any one of the four FAS subunits causes a respiration-deficient phenotype. There was no discussion of the increase in petite production which the authors observed in their mutants (266, 267). Invoking oxidative damage appears unsatisfactory, since the role of such processes in the maintenance of mtDNA is far from clear, as shown in the above section.

We would prefer to explore and broaden the ideas of Harington et al. (127). Specialized fatty acid-like molecules (as yet uncharacterized) may be required for the insertion of proteins into the mitochondrial membrane. Some might involve cytochromes: their absence would cause a pet phenotype, and their synthesis would depend on the entire FAS complex (with the four subunits so far identified). Others would concern the membrane insertion of (at least) one protein involved in mtDNA stability: their absence would lead to an increased production of petites, and their synthesis would not require Cem1p activity. The main virtue of this hypothesis is to offer a unifying view, including phospholipids (see above), of the relationship between fatty acid depletion and instability of mtDNA.

The Ambiguous Position of Lipoic Acid

The identification of the molecules synthesized by the mitochondrial FAS should shed light on the problem. With respect to the pet phenotype of the *cem1* mutants, Harington et al. (127) discussed the possible involvement of lipoic acid (which is a cofactor of the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes) but rejected this candidate for two reasons. First, although lipoic acid-deficient mutants display a pet phenotype, they differ from the *cem1* mutants because they have spectrally detectable cytochromes (308). Second, the addition of lipoic acid to the medium does not restore respiratory growth to the *cem1* strains. In contrast, Brody et al. (25) showed that strains carrying a disrupted *acp1* gene have only 5 to 10% of the wild-type lipoic acid content. The mutants are obviously respiration defective since they are massively converted into petites, as previously shown by Schneider et al. (267). The role of Acp1p in lipoic acid synthesis is well supported in that a similar deficiency in this compound is observed when the *PPT2* gene is disrupted, a situation in which Acp1p is not activated (288).

The fact that mtAcp1p is clearly involved in lipoic acid synthesis does not disprove the idea that the mitochondrial FAS complex (with the exception of its Cem1 subunit) could be involved in the synthesis of another fatty acid-like molecule required for mtDNA stability. That *acp1* mutants are not supplementable by addition of lipoic acid to growth medium (25) is not surprising, because the massive production of petites occurs as early as spore germination. This experiment should be performed on strains carrying a disrupted *acp1* gene complemented by the wild-type gene present on a plasmid, such as those constructed by Schneider et al. (266) and Stuible et al. (288): loss of the plasmid in the presence of lipoic acid would help to clarify whether the massive production of petites characteristic of the mutants defective for Acp1p is due to the lack of lipoic acid or to another function of Acp1p. For the moment, a possible role of lipoic acid in mtDNA stability cannot be excluded. However, data from Sulo and Martin (292) concerning the *LIP5* gene (see below) suggest that the absence of lipoic acid per se is not responsible for a high production of petites. Thus, the reason why three of the four subunits of the mitochondrial FAS are required for mtDNA stability remains unclear.

In fact, the idea that other mitochondrial fatty acids in addition to lipoic acid are required for respiratory competence and/or mtDNA stability is strongly supported by data obtained by Hoja et al. (137) on *acc1* and *bpl1*/*acc2* mutants. *BPL1* encodes a biotin-protein ligase which is required for acetyl coenzyme A (acetyl-CoA) carboxylase holoenzyme formation. This enzyme (whose apoform is encoded by *ACC1*/*FAS3*) catalyzes key steps of fatty acid synthesis. Viable mutants of the two genes require long-chain fatty acids for growth and are respiration deficient, even though lipoic acid is reduced only to about half its normal level. Furthermore, the *acc1* mutant studied was a petite, because its respiration defect could not be cured by transformation with the wild-type *ACC1* gene (137) (Table 7).

In addition to the involvement of lipids, the role of the mitochondrial membrane for mtDNA maintenance will be further discussed (see "Mitochondrial morphology, the cytoskeleton, and mtDNA" and "Is there a centromere-like structure for the mitochondrial genome" below).

THE SECRET LIFE OF WELL-KNOWN PROTEINS

A growing number of enzymes whose functions in metabolism have been well documented appear to play an unexpected role in mtDNA stability. To our knowledge, the first case reported was the E1α subunit of the pyruvate dehydrogenase complex (322). This complex links glycolysis to the citric acid (Krebs) cycle by conversion of pyruvate to acetyl-CoA. Deletion of the *PDA1* gene encoding the E1α subunit leads to two unexpected phenotypes: a partial auxotrophy for leucine and an increased production of rho^0 petites (Table 8). The first defect could be explained by a high concentration of CoA in the mitochondria, inhibiting a reaction specific for the leucine pathway, while the effect of *PDA1* deletion on rho^0 production remains unexplained (322). It would be interesting to know if petite production is also increased in mutants with mutations of the genes encoding the other subunits of the pyruvate dehydrogenase complex (reference 243 and references therein).

Sulo and Martin (292) were interested in mitochondrial tRNA processing. For this reason, they cloned a gene able to complement a respiratory mutant which showed an accumula-

TABLE 8. Genes encoding proteins which might be bifunctional

Gene[a]	Protein function[e]	Type of mutation	Petite production	Reference[j]
PAD1[c] (NE)	**Pyruvate dehydrogenase E1α subunit**	Deletion	rho^{0}[f, h]	322
LIP5[b] (NE)	**Lipoic acid metabolism**	Disruption	rho^{-}[g]	292
IDH1[c] (NE)	**NAD$^+$-dependent isocitrate dehydrogenase**	Deletion	rho$^-$ and/or rho^{0}[i]	82
IDH2[b,c] (NE)	**NAD$^+$-dependent isocitrate dehydrogenase**	Disruption	rho$^-$ and/or rho^{0}[i]	82
ILV5[b] (NE)	**Acetohydroxy acid reductoisomerase**	Deletion	rho^{-}[f,g]	336

[a] to [j] See Table 1 footnotes.

tion of mitochondrial tRNA precursors. The gene in question was involved in lipoic acid synthesis. Disruption of this gene (*LIP5*) leads to the production of rho$^-$ mutants (Table 8). Addition of lipoic acid to the culture medium permits the recovery of protein-bound lipoate but does not prevent the destabilization of mtDNA. In contrast to *acp1* mutants, which massively lose their rho$^+$ genome (25) (see the previous section), the kinetics of rho$^-$ accumulation in *lip5* disruptants are slow (292). Thus, in this case, the attempt to prevent petite production with lipoic acid makes sense. Because other genes involved in lipoate metabolism do not show this phenotype, the authors propose that Lip5p may have dual functions, with the second function being required for mitochondrial translation. However, this second role might be dispensable, because disruption of *LIP5* in a *op1* mutant background is viable (292). Notably, in *Kluyveromyces lactis*, a mutation in the *LIPB* gene (which encodes a lipoyl-protein ligase) causes a destabilization of the mitochondrial genome (36).

NAD$^+$-dependent isocitrate dehydrogenase catalyzes an essential step in the Krebs cycle, the conversion of isocitrate to α-ketoglutarate. The genes encoding the two subunits of this enzyme (*IDH1* and *IDH2*) have been cloned (58, 59). The complex was rediscovered through its RNA-binding property. In fact, it binds specifically to the 5′ untranslated leaders of mitochondrial mRNAs (reference 82 and references therein). The authors proposed that the protein plays a role in mitochondrial translation. This hypothesis was strengthened by the observation that strains with either of the *IDH1* and *IDH2* genes disrupted displayed increased rates of petite production (Table 8). However, as reported to us by L. Grivell, the problem is more complicated than it appears. First, the increase in petite frequencies is not as high as that observed with mutations in genes encoding components of the mitochondrial translational apparatus. Second, a wild-type level of petites can be observed in a Δ*idh1* mutant complemented by a mutant form, nonbinding to RNA, of Idh1p. Third, there is no significant difference (observed by several tests) between the wild type and the nonbinding mutant in mitochondrial translation. The slight but significant effect of the *idh* disruptions on mtDNA stability might be explained in two ways, as suggested by Grivell. First, RNA binding may not be important to this process. This hypothesis suggests that the Idh subunits may have three functions: one in the Krebs cycle (which is probably not related to mtDNA stability), one in mtRNA binding (with a nonessential function with respect to translation), and an unknown role distantly related to mtDNA stability. In the second hypothesis, the RNA-binding property of Idh subunits would be related to mtDNA stability but the low level of RNA binding activity remaining in the *idh* (non binding) mutants may be sufficient to fulfill the needs of mitochondria to maintain their genome (L. Grivell, personal communication).

The *ILV5* gene encodes the mitochondrial protein acetohydroxy acid reductoisomerase, catalyzing a step in the biosynthesis of the amino acids isoleucine, leucine, and valine (235). This gene was rediscovered as a multicopy suppressor of the mtDNA instability phenotype of *abf2* mutants (see "*ABF2* paradox" above) (Table 1). Interestingly, suppression is also seen when *ILV5* is carried by a low-copy-number plasmid. Furthermore, it was shown that deletion of *ILV5* leads to a high frequency of petites (336) (Table 8). This mtDNA instability is clearly not the consequence of a block in biosynthesis of branched-chain amino acids. The authors propose a bifunctional role for acetohydroxy acid reductoisomerase: in addition to its catalytic activity, the protein could also function as do both Abf2 (a DNA-packaging protein) and Idh (an mRNA-binding protein). Interestingly, while Δ*abf2* petites are rho^0, those produced in the Δ*ilv5* context are rho$^-$. Furthermore, there is a synergistic effect of the two deletions, with a more extreme mtDNA instability than that seen in either of the two single deletions: in contrast to the single mutants, the double mutant loses its rho$^+$ genome as early as spore germination. This suggests a synergistic effect of *ABF2* and *ILV5* in mtDNA maintenance, which may explain the suppression effect of *ILV5* on Δ*abf2* (336). It would be interesting to see the phenotype caused by overexpression of *ABF2* in a Δ*ilv5* context.

SERENDIPITOUS DISCOVERIES

Throughout the history of genetics, different screening procedures have sometimes led to the discovery of different genes, even though all are involved in the same function. Also, different screens can lead to the discovery of the same gene: the reader will find several examples in this review. It is therefore not surprising that some genes required for mtDNA stability have been fortuitously identified. In most cases covered in this review, the actual mechanisms of the relevant proteins responsible for their involvement in mtDNA stability seem plausible. However, there are (at least) seven serendipitous discoveries which remain unexplained. They concern the four enzymes discussed in the above section and the proteins encoded by the *ADR1*, *SLS1*, and *SMP1* genes. The story of *ADR1* is presented below in "Gene overexpression can increase mtDNA instability" (see Table 12).

The *SLS1* gene (for "synthetic lethal of Δ*ssm4*") was identified by a mutation colethal with a deletion of the *SSM4* gene. The latter, when inactivated, is able to suppress some (but not all) mutations of *RNA14*, which encodes a protein localized in the nucleus and required for mRNA polyadenylation. Ssm4 is an integral nuclear membrane protein (reference 257 and references therein). To better understand the function of Ssm4p, these authors sought mutations colethal with an *SSM4* deletion (257). The *sls1-1* mutation was one of these. It was shown to display a pet phenotype, as did a complete deletion of the gene. Furthermore, the data strongly suggest that Sls1 is a mitochondrial integral membrane protein. The protein does not seem to be involved in mtDNA replication or transcription or in mtRNA splicing, maturation, or stability. However, the mu-

TABLE 9. Miscellaneous genes

Gene[a]	Protein function[e]	Type of mutation	Petite production	Reference[j]
MGM104[b] (?)	Unknown	Point mutation	rho$^-$ and/or rho^{0h}	118
SLS1[b] (NE)	**Mitochondrial integral membrane protein**	Deletion	rho$^-$ and/or rho^{0g} (in intron-containing mtDNA context)	257
SMP1[b] (?)	Unknown	Uncharacterized	rho^{0g,h}	141
GCS1[b] (?)	Unknown	Double mutant	rho^{0h}	92
SED1[b] (?)	Unknown	Double mutant	rho^{0h}	92
ERV1[b] (E)	Unknown	Disruption and *Gal1-10* promoter-*ERV1*	rho^{0h}	186
TPM1[c] (NE)	**Tropomyosin**	Disruption	rho$^-$ and/or rho^{0i}	193

[a to j] See Table 1 footnotes.

tants show a defect in the assembly of complexes III and IV (lack of cytochromes c_1, b, and aa_3). The original mutant, as well as the deletion, leads to a high production of petites (Table 9) when the strains harbor a mitochondrial genome containing introns. This production is reduced when the cells contain an intronless mitochondrial genome (257), but, this value was not compared to the level of petites produced in the isogenic wild-type context. Although the increased stability of intronless mitochondrial genomes has been found in other nuclear mutant contexts (for instance, the *nam1* mutants [see "The translation-ATP synthase connection" above]), the problem remains unsolved. Concerning the *SLS1* gene, its requirement for the assembly of two respiratory complexes does not shed light on its role in mtDNA stability. However, the authors did not report on its effects on mitochondrial translation or in the assembly of ATP synthase, two processes clearly linked to mtDNA stability (see "The translation-ATP synthase connection" above). Even more mysterious is the reason why mutations of the *SLS1* gene, encoding a mitochondrial membrane protein, can be colethal with deletion of the *SSM4* gene, encoding a nuclear membrane protein (257).

The *smp1* (for "stable maintenance of pSR1") mutation was identified among mutations increasing the stability of pSR1, a *Zygosaccharomyces rouxii* plasmid which is maintained in *S. cerevisiae* less stably than in *Z. rouxii*. The pSR1 molecule shows three open reading frames: one encodes a protein essential for intramolecular recombination of the plasmid, while the two others encode proteins involved in its maintenance, which would also require a specific host factor (reference 141 and references therein). The *smp1* mutation enhances the stability not only of this foreign plasmid but also of *S. cerevisiae* plasmids, if they contain an ARS sequence but no centromere. For an as yet unexplained reason, the *smp1* cells are rho^0 petites (Table 9). Amazingly, the stability of the pSR1 plasmid is also increased (by a factor of 20, as in the *smp1* context) in *SMP1* cells which are rho$^-$ or rho^0 (141). This effect is not due to respiration deficiency per se, because *pet* mutants (which maintain their rho$^+$ genome) do not show this phenotype. These data pose two (probably related) questions. First, how can mtDNA loss enhance the stability of a nonmitochondrial plasmid? Second, how can a nuclear mutation lead to both increased stability of a plasmid and loss of mtDNA? With respect to the first question, "incompatibility" between mtDNA and a foreign plasmid was previously reported. For instance, Gunge and Yamane (122) reported two linear DNA killer plasmids from *K. lactis* which were stably transmitted in *S. cerevisiae* if the recipient strain was a neutral petite. In contrast, *mit*$^-$ mutations exerted an "incompatibility" effect on plasmid transmission, as observed in a rho$^+$ context. An ad hoc hypothesis to explain this effect of a petite background relies on established observations that the status of the mitochondrial genome can modify the expression of some nuclear genes (151, 226). This phenomenon, called retrograde regulation, has been extensively studied in yeast (reviewed in references 35 and 240). New technologies, which can describe the transcriptional status of the entire yeast nuclear genome in a given genetic background, will aid in obtaining a catalogue of genes whose expression is altered in a petite context. However, we believe that a simple titration idea could account for the observed situations. If a protein X is titrated both by mtDNA and by some specific plasmid DNAs, loss of one of the partners could increase the availability of X for the other partners. With respect to the second question, one should note that nuclear mutations stabilizing a plasmid do not systematically cause mtDNA instability. For example, Thrash-Bingham and Fangman (302) reported that the *amm1* (for "altered minichromosome maintenance") mutation, although leading to a reduced growth in glycerol medium at 34°C, did not increase petite production. Clearly, further experiments (especially cloning of *SMP1*) are required to understand why the *smp1* mutation leads to loss of mtDNA.

mtDNA AND THE CELL CYCLE: A BLACK BOX

In contrast to nuclear DNA replication, which occurs at a specific step of the cell cycle (the S phase), mtDNA replication occurs throughout the cell cycle (270, 326). However, the localization of mtDNA molecules within the organelle and the organization of the mitochondrial network inside the cell must be tightly controlled so that mitochondria and the mitochondrial genomes are precisely distributed between mother and daughter cells, both during the mitotic cycle and among the spores after meiosis. In both cases, careful cytological analyses have been performed. With respect to mitotic cell divisions, mitochondria can be seen at the mother cell/bud neck as early as bud emergence. The growing bud is then colonized by mitochondria (reviewed in reference 286). In meiosis, mitochondria are arranged around the meiotic nucleus in a very specific pattern (208). Other studies have carefully described the fate of mitochondria and their genomes during and after mating (reference 222 and references therein). However, the precise mechanisms which orchestrate mitochondrial shape, fusion, fission, and movement, as well as the mechanisms by which the pool of mtDNA molecules is distributed in mitotic and meiotic progeny, remain unclear. These problems are discussed below (see "Mitochondrial morphology, the cytoskeleton, and mtDNA" and "Is there a centromere-like structure for the mitochondrial genome?"). To date, studies of the cell cycle have provided very little information on that topic.

In fact, very few *CDC* (for "cell division cycle") genes have been reported to influence mtDNA stability. One can distinguish two groups, whose effects on mtDNA are either quite

TABLE 10. Nuclear mutations which decrease petite production

Mutation	% of petites in cultures		Reference
	Wild type	Mutant	
srm1[a] (*mmg1*[b])	51.2 ± 5.6	1.1 ± 0.3	66
srm2 (*mmg2*)	51.2 ± 5.6	0.8 ± 0.3	66
srm3 (*mmg3*)	51.2 ± 5.6	0.9 ± 0.3	66
srm4 (*mmg4*)	51.2 ± 5.6	9.3 ± 1.2	66
srm5 = *cdc28-srm*	30.3 ± 6.1	3.1 ± 0.7	67
srm1	30.3 ± 6.1	1.1 ± 0.2	67
cdc28-srm + *srm1*	30.3 ± 6.1	0.06 ± 0.03	67
cdc28-1	79.9 ± 12.2	5.1 ± 2.9	162

[a] *srm* means spontaneous rho^0 mutations. The *srm1* to *srm4* mutations define four complementation groups. The relevant genes have not been cloned.
[b] *mmg* means mutability of mitochondrial genome.

trivial or still very puzzling. The first includes the *CDC8* and *CDC21* genes (Table 1), which encode thymidylate kinase (150) and thymidylate synthase (19), respectively. It was initially reported that the *cdc8* and *cdc21* mutants not only stop nuclear replication at high temperature but also arrest mtDNA replication, which is not a general feature of *cdc* mutants impaired in the initiation or achievement of the S phase (216). Later, a high frequency of petites was observed in these mutants at permissive temperature (217). It is not surprising that blocks in the pathway of deoxyribonucleotides lead to an arrest of both nuclear and mitochondrial DNA replication. The fact that the fate of mtDNA is altered at temperatures still permissive for nuclear replication is in accordance with other data and suggests that mtDNA synthesis is highly sensitive to a decrease in the supply of DNA precursors. This is exemplified by the effects of low-activity mutants or overexpression of the *RNR* genes encoding subunits of ribonucleotide reductase, which catalyzes the conversion of ribonucleotides into deoxyribonucleotides. A partial deletion of *RNR2* causes a 10-fold increase in petite formation (Table 1) (80), while a strain carrying a deletion of the *RNR4* gene displays a petite phenotype due to the loss of functional mtDNA (Table 1) (319). Reciprocally, the *RNR1* gene (81) acts as a multicopy suppressor of mutations in the *MIP1* gene, which encodes the mtDNA polymerase (see "Wide-scale screening for rho^0 production" above) (Table 1) (177). The suppressor effect was observed not only in several heat-sensitive *mip1* mutants but also in diploid strains harboring a single wild-type copy of the *MIP1* gene which accumulate petites at high temperature: overexpression of *RNR1* decreased the level of petite production. The data as a whole strongly suggest that the deoxyribonucleotide concentration is rate limiting for mtDNA replication. Although it is unclear if the relevant petites are rho$^-$ and/or rho^0, it should be noted that Guan (118) found mutations in the *CDC8* and *CDC21* genes during his systematic search for rho^0 producers (see "Wide-scale screening for rho^0 production" above).

The second class of mutants impaired in both the cell cycle and mtDNA stability can be illustrated by three examples: the *cdc28-srm* allele, the *gcs1 sed1* double-mutant strain, and the *erv1* mutant. Devin and Koltovaya (66) directly sought nuclear mutations decreasing petite production. They began with a strain producing 50% petites and selected four mutations giving rise to a lower level of petites (Table 10) (see "No compromise" below). In further studies, Devin et al. (67) found a fifth mutation, shown to be allelic to *CDC28*. This gene encodes the protein kinase called p34^{cdc28} (p34^{cdc2} in *Schizosaccharomyces pombe* and other eucaryotes) which, in yeasts, controls both the G_1/S and G_2/M transitions of the cell cycle by interactions with specific cyclins (reviewed in reference 181). Mutations in *CDC28* which impair either one of these key steps lead to heat-sensitive mutants. The *cdc28-srm* allele described by Devin et al. (67) is not conditional. However, the mutation is pleiotropic: it leads to alterations in cell morphology, loss of extra chromosomes in disomic strains, and loss of circular centromeric plasmids and decreases the spontaneous and ethidium bromide-induced rates of petites (Table 10). This effect on petite frequency was also found for one of two conditional *cdc28* mutants at permissive or semipermissive temperatures (162) (Table 10). The reason why some *CDC28* alleles have this striking effect on mtDNA stability remains unknown.

Drebot et al. (70) described a strain (carrying two mutations) defective for reentry into the cell cycle from the stationary phase at low temperature. One of the mutations causes a general cold sensitivity (*gcs1*), while the other (*sed1*) suppresses this defect in proliferating cells. The double mutant (but not the single mutants) accumulates petites of the rho^0 type at high rates (92) (Table 9). Here again, the observation remains unexplained.

The *ERV1* gene (for "essential for respiration and viability") was identified by a *pet* mutation. The mutant was respiration deficient at high temperature, showing strongly reduced levels of mitochondrial transcripts. Disruption of the complementing gene demonstrated that it was essential for viability (184). Complementation of the *erv1* disruption by the wild-type gene under the control of an inducible promoter permitted the author to monitor the fate of cells transferred to culture conditions which no longer allow *ERV1* expression (184). It appeared (by DAPI staining) that the cells first lose their mtDNA (Table 9) and then arrest, after a few divisions, with a cdc phenotype in a budded state, after migration of the nucleus into the neck between the mother cell and the bud (186). This phenotypic trait does not clearly determine if the cells are arrested in the G_1 or G_2 state. The protein was later shown to be associated with the mitochondria (187). The identification of a human homologue of the *ERV1* gene (105, 189) has not shed light on the function of the protein.

With respect to cell cycle topics, it is interesting that some mutations which impair mitochondrial morphology (see "Mitochondrial morphology, the cytoskeleton, and mtDNA" below) also lead to a cdc phenotype. For instance, the *mmm1* (for "maintenance of mitochondrial morphology") mutants not only display a defect in organelle morphology and inheritance but also are defective in the completion of cytokinesis at restrictive temperature when grown on a nonfermentable carbon source (28). At the time, the authors could propose the attractive hypothesis of a cell cycle checkpoint associated with mitochondria: when the bud does not receive mitochondria, cell division is forbidden. However, the situation is no longer so clear. Among the *mdm* (for "mitochondrial distribution and morphology") mutants selected by Yaffe's laboratory (202), some, but not all, display specific cell cycle defects. For instance, the *mdm1* (203) and *mdm2* (287) mutants are unable to achieve cytokinesis. In contrast, mutations in the *MDM10* and *MDM12* genes (see the next section and Table 11) do not exhibit this feature. Furthermore, there is no correlation between the cell cycle defect and the ability of the mutants to maintain their mitochondrial genome: the *mmm1* mutants do not accumulate petites (28), while at least some *mdm2*/*ole1* mutants exhibit this property (see "Fatty acids and phospholipids," above, and Table 7). Clearly, elucidation of the relationship between the cell cycle and mtDNA transmission remains a formidable challenge.

TABLE 11. Genes involved in mitochondrial morphology and inheritance

Gene[a]	Protein function[e]	Type of mutation	Petite production	Reference(s)[j]
MGM1[b] (NE)	**GTP-binding protein (dynamin family), mitochondrial outer membrane**	Deletion	$rho^{0f,h}$	117, 149, 272
MDM10[b] (NE)	**Mitochondrial outer membrane**	Disruption	rho^- and/or rho^{0f}	17
MDM12[b] (NE)	**Mitochondrial outer membrane**	Deletion	rho^- and/or rho^{0f}	17
MDM28/*PTC1*[b] (NE)	**Serine/threonine phosphatase**	Disruption?	rho^- and/or rho^{0h}	255
FZO1[d] (NE)	**Mitochondrial membrane GTPase**	Deletion	rho^- and/or rho^{0g}	244
		Deletion	$rho^{0g,h}$	131

[a to j] See Table 1 footnotes.

MITOCHONDRIAL MORPHOLOGY, THE CYTOSKELETON, AND mtDNA: THE MISSING LINKS

In growing yeast cells, mitochondria form reticular structures localized at the cell cortex (reviewed in reference 286). The continuity of this network depends on a balance between the fusion and fission events required, along with mitochondrial movements, for correct transmission of the organelles at cell division, mating, and meiosis (references 222 and 282 and references therein). Genes involved in mitochondrial movements and morphology have been identified either by a specific search for mutants altered for these processes or by a posteriori inspection of mutants screened for other purposes. Some encode components of either the cytoskeleton or the outer membrane of mitochondria, while others could play regulatory roles in these processes (reviewed in reference 18). However, the link between the fate of the organelle and its genome remains elusive, because only a subset of these genes also control mtDNA stability.

McConnell et al. (202) selected, by visual inspection, temperature-sensitive mutants exhibiting defects in mitochondrial distribution and morphology (*mdm* mutants). The *MDM10* (283) and *MDM12* (17) genes encode proteins of the mitochondrial outer membrane. In either case, loss of the proteins results in giant mitochondria defective for transfer into daughter buds. The authors suggest a role for these proteins in the mitochondrion-cytoskeleton linkage. This attachment would be required for maintenance of the normal (tubular) mitochondrial morphology, with the defects in organelle inheritance being a secondary consequence of abnormal morphology. The *mdm10* and *mdm12* mutants produce petites at rates much higher than the wild type does (17) (Table 11). Two other proteins of the outer mitochondrial membrane are also involved in morphology and mtDNA stability: Fzo1 and Mgm1. They share a GTP-binding property, as discussed below. Interestingly, there is (at least) a fifth outer membrane protein of the mitochondria essential for maintenance of organelle morphology; it is encoded by the *MMM1* gene, whose mutations also lead to giant mitochondria unable to enter buds. It differs from the four other genes in that *mmm1* mutants contain functional mtDNA (28). This suggests that abnormal mitochondrial morphology does not inescapably lead to petite production.

Hermann et al. (130) also screened a collection of mutants for strains unable to transport mitochondria into the buds at high temperature. Isolation of the *mdm28* mutant has contributed to the comprehension of the timing of mitochondrial movement at this stage of the cell cycle (255). In fact, the mutant shows a delay (but not a block) in mitochondrial inheritance: while in wild-type cells mitochondria can be seen in the buds as early as their emergence, in the mutant most small buds are devoid of mitochondria while most large buds receive a mitochondrial network. Cloning and sequencing of the *MDM28* gene revealed that it corresponds to the previously identified gene *PTC1*, encoding a type 2C serine/threonine phosphatase (200) and initially identified in a screen for mutants deficient in tRNA biosynthesis (*TPD1* [315]). In their study of *MDM28*/*PTC1*, Roeder et al. (255) showed that Δ*ptc1* mutants generate petites at a higher frequency than the wild type does (24.4 and 1.8%, respectively, at 30°C) (Table 11). Although Ptc1p is assumed to negatively regulate mitogen-activated protein kinases in the high-osmolarity glycerol response (HOG) pathway (which plays a critical role in the osmostress response in yeast), the authors provide evidence that Ptc1p does not act by this pathway with respect to mitochondrial transport. Ptc1p may act directly on proteins required to move mitochondria into buds, or it may affect mitochondrial inheritance indirectly, by a pathway different from HOG. Since the actin cytoskeleton is known to play important roles in mitochondrial distribution, morphology, and movement (71, 176, 277), Roeder et al. (255) compared actin structures in *ptc1* and *PTC1* cells but observed no differences. Thus, the reasons why *ptc1* mutants exhibit this delay in mitochondrial transmission and the possible existence of a link between this defect and mtDNA instability remain unexplained.

The *fuzzy onion* gene (*fzo*) was first identified in *Drosophila melanogaster* as essential for a developmentally regulated step in mitochondrial fusion (124). Mitochondrial aggregation, fusion, and elongation alongside the growing flagellar axoneme are successive events during *Drosophila* spermatogenesis and are necessary for sperm motility. The mitochondrial structure then resembles a slice of onion, as viewed in cross-section by electron microscopy. Mutations in *fzo* cause a specific defect in the fusion step, leading to aberrant structures ("fuzzy onions") and male sterility. The gene encodes a transmembrane GTPase targeted to the mitochondria. Related proteins have been found in several organisms, including *S. cerevisiae* (124). The yeast gene has been studied by two groups (131, 244). In both cases, the data lead to the conclusions that Fzo1 is an integral protein of the mitochondrial outer membrane and Δ*fzo1* mutants display fragmented mitochondria and mtDNA instability (Table 11). However, one of the two groups supplied several important observations (131). First, in conditional mutants transferred to restrictive conditions, the mitochondrial network initially fragments into very small mitochondria which then aggregate into a few clusters, a profile also observed in the Δ*fzo1* mutants. Second, the absence of Fzo1p does not affect mitochondrial inheritance but blocks mitochondrial fusion during mating. Third, the *fzo1* petites are rho^0 (Table 11). Fourth, loss of mtDNA is observed many hours after changes in mitochondrial morphology (131). Although further experiments are required to understand why *fzo1* mutants lose their mtDNA, one cannot exclude a trivial possibility: some of the small mitochondria might not contain DNA. Because the mutant mitochondria never fuse, the level of organelles devoid of DNA would continuously increase in the population. If this

hypothesis was confirmed, the implication would be that loss of mtDNA in the *fzo1* mutants is a passive consequence of mitochondrial fragmentation which, in this case, cannot be balanced by fusion events. However, a fragmented mitochondrial pattern does not seem systematically linked to mtDNA instability (see reference 252, for example).

In addition to *FZO1*, *MGM1* encodes a mitochondrial GTP-binding protein, as mentioned above (see "Wide-scale screening for rho^0 production"). In this case, we know not only that the mutant produces rho^0 petites (Table 11) but also that it is unable to maintain rho$^-$ genomes, even those which are otherwise very stable (149). The *MGM1* gene encodes a protein related to dynamin, whose protein family may have different functions. In several cases, a role in intracellular vesicle movement has been suggested. Interestingly, Guan et al. (117) observed that mitochondria of an *mgm1*-deleted strain are present in condensed structures rather than in the dispersed pattern seen in *MGM1* strains (either rho$^+$ or rho^0). Although reminiscent of the giant mitochondria found in *mdm10*, *mdm12*, and *mmm1* mutants, the aberrant structures in *mgm1* partition at cell divisions, at least in the strain background used by these authors. The data also suggest a defect in protein import. Mgm1p has been recently rediscovered by Shepard and Yaffe (272) by using the heat-sensitive mutation *mdm17*. Studies of *mdm17* and a null allele of *MGM1* confirm previous observations concerning mitochondrial aggregation and loss of mtDNA (Table 11) and add important information. First, Mgm1p is located to the mitochondrial outer membrane. Second, the GTP-binding site is required for Mgm1p functions. Third, in contrast to the observations of Guan et al. (117), Shepard and Yaffe (272) observed altered mitochondrial distribution to the buds (suggesting that this defect may be strain dependent). Fourth, after a shift to the nonpermissive temperature, loss of mtDNA occurs after alterations in mitochondrial morphology and distribution. This delay in mtDNA loss was also reported for the *fzo1* mutants (131). Another nucleotide-binding protein must be mentioned here. It is encoded by the *YME1* (for "yeast mitochondrial DNA escape") gene, which is discussed below (see "No compromise"). Yme1p is an ATP- and zinc-dependent protease, localized to the inner mitochondrial membrane, whose substrates are not yet clearly defined (320). In any case, *yme1* mutants display abnormal mitochondrial morphology: rather than being reticular, mutant mitochondria form small globular compartments (30). In contrast to the above-mentioned situations, *yme1* mutations do not cause a high production of petites but are nearly lethal in a petite background (see "No compromise" below and Table 13).

The putative link between mtDNA stability and the cytoskeleton is emphasized by the observation that deletion of the *TPM1* gene encoding tropomyosin (192), an actin-binding protein, leads to increased petite production (193) (Table 9). This phenotypic property of the *tpm1* mutant cannot be generalized to all components of the cytoskeleton. For instance, some actin mutants display aberrant mitochondrial organization while maintaining normal rates of petite formation (71). It is noteworthy that sporulation conditions (which require respiration) enhance defects in mitochondrial organization: under these conditions, some actin mutants exhibit giant mitochondria similar to those observed in the *mdm* and *mmm* mutants (282).

Where is Ariadne's thread in this complex network of probably indirect effects in the cytoskeleton, mitochondrial morphology, and mtDNA stability? One can follow two (nonexclusive) trails. The first relies on a link between the frameworks required for mitochondrial morphology and mtDNA maintenance, and the second relies on a link between organelle morphology and the cytoskeleton, and/or an intact import process required for mtDNA stability.

The first proposal is evoked by Burgess et al. (reference 28 and references therein). These authors posit an internal framework (mitoskeleton) and an external framework (attachment of mitochondria to the cytoskeleton) which could be involved in mitochondrial morphology. The external framework was also proposed for the *MDM10* and *MDM12* genes (17, 283). It has recently been shown that Mmm1p and Mdm10p play a key role in linking mitochondria to the actin cytoskeleton (20). However, the three mutants clearly differ with respect to mtDNA stability, which is not affected in the *mmm1* mutant. These are cellular nuances we have not yet grasped, although the idea that mtDNA transmission could be influenced by organelle morphology appears sensible. There is in fact an enormous gap in our understanding of the possible interactions between mtDNA and the inner membrane, which certainly reacts to modifications of the outer membrane and its attachment to the cytoskeleton (see "Is there a centromere-like structure for the mitochondrial genome?" below). The *TPM1* problem may also be explained in the context of a putative external framework. Similarly, Mgm1p and Fzo1p might be involved in one of these frameworks. Here again, the suppressor method may help to distinguish between this hypothesis and that proposed by Jones and Fangman (149), who favor the idea of a signal transmission (by GTP binding and hydrolysis) required for mtDNA replication.

The second proposal is based on the still unproven but attractive hypothesis that efficient import of proteins into the mitochondria involves a selective transport of the relevant mRNAs to the vicinity of the organelle (reference 190 and references therein). Particular defects in the cytoskeleton (*TPM1*) or in the attachment of mitochondria to the cytoskeleton (*MMM1*, *MDM10*, and *MDM12*) may disturb the delivery of proteins by causing a defect in the routing of mRNAs. On the other hand, and without a cytoskeletal implication, the abnormal organelle morphology might alter the import process, as shown for the mitochondrial Hsp58 protein in *mgm1* mutants (117), even though the relevant proteins (in their wild-type form) are not directly involved in the process. It is noteworthy that a null allele of *YGE1*/*MGE1* (encoding a protein required for protein translocation into mitochondria [see "Conventional mitochondrial chaperones" above]) results in mitochondria stacked in the mother cell and unable to enter the bud while a heat-sensitive allele results in a high production of petites (Table 5). Depending on these more or less specific defects, mtDNA stability might be either impaired (*MGM1*, *MDM10*, *MDM12*, or *FZO1*) or unaffected (*MMM1*). Careful analysis of the import process in the relevant mutants would be very informative.

GENE OVEREXPRESSION CAN INCREASE mtDNA INSTABILITY

Although gene overexpression can give insight into gene functions, this approach has been little used on studies of mtDNA stability. We have previously noted that overexpression of *MGT1*/*CCE1* enhances the transmission of nonsuppressive rho$^-$ genomes (see "Nuclear control of biased transmission" above and Table 2) and that overexpression of *ABF2* leads to rho^0 petites while deletion of the gene increases the production of rho$^-$ genomes (see "The *ABF2* paradox" above and Table 1). We would like to discuss two genes at this point, *PET127* and *ADR1* (Table 12), whose overexpression increases mtDNA instability while their absence does not.

PET127 was identified by two different screening procedures

TABLE 12. Genes whose overexpression increases petite production[k]

Gene[a]	Protein function[e]	Type of mutation	Petite production	Reference[j]
PET127[b] (NE)	mtRNA turnover and 5′ processing	Overexpression	rho$^-$ and/or rho^{0g}	324
ADR1[b] (NE)	**Transcriptional activator of nuclear genes**	Overexpression	rho$^-$ and rho^{0f,g}	41

[a to j] See legend of Table 1.
[k] See Table 1 and "The *ABF2* paradox" for overexpression of *ABF2*.

(123, 324). In the first, the *pet127* mutation acted as a suppressor of mutations in *PET122*, a gene encoding a translational activator of the mitochondrially encoded *COX3* mRNA. In the second, *pet127* suppressed *cox3* mutations with decreased mRNA stability. Deletion of *PET127* causes only a slight respiratory defect at 37°C. However, the data suggest that Pet127, which is a membrane-associated protein, plays important roles in mtRNA turnover and 5′-processing cleavages. In contrast, overexpression of *PET127* shifts cells to the petite phenotype (324). The authors suggest that excess of Pet127p results in the destruction of an mtRNA required for mtDNA replication or translation.

Adr1p is the primary transcriptional activator of the *ADH2* gene, encoding the glucose-repressible alcohol dehydrogenase in *S. cerevisiae* (45, 63). Since Adr1p has no obvious mitochondrial function, it was quite surprising to find that overexpression of the *ADR1* gene led to a high production of petites (41) (Table 12). The authors provided evidence that this phenomenon was not due to increased expression of *ADH2* but to the Adr1 activity per se. Furthermore, they performed a careful analysis of petites which extended far beyond that of most of the studies discussed in this review. Using genetic criteria, they made several observations. First, 25% of these petites were rho^0 (or contained severely deleted mtDNA) while the others were rho$^-$ with nonspecific deletions and a bias in marker loss similar to what is observed in wild-type strains. Second, the rho$^-$ petites were unstable and progressed to the rho^0 state. Third, only daughter cells became petites whereas a mother cell was still able to give rise to normal progeny after producing a petite bud. Fourth, resistance to Adr1 hyperactivity (in a certain genetic context) correlated with resistance to euflavine and high temperature effects for petite production; these two conditions also cause only buds to become petites (83, 273). Suppressors of the overproduction of petites were sought in order to clarify the Adr1p effect. Unfortunately, all (about 100) revertants displayed a reduced activity of Adr1p. The authors suggest that, when overproduced or hyperactive, Adr1p may bind (specifically or nonspecifically) to regulatory sequences of nuclear genes required for mtDNA stability and decrease their expression (41). In fact, more recent data have shown that the regulatory functions of Adr1p are much broader than previously assumed (references 53 and 276 and references therein). However, the function of Adr1p with respect to mtDNA stability remains unclear.

NO COMPROMISE: NUCLEAR MUTANTS KILLED BY mtDNA INSTABILITY AND NUCLEAR MUTANTS CURED BY LOSS OF mtDNA

S. cerevisiae is a petite-positive yeast, which means that it can survive without mtDNA (27). However, mutations in several nuclear genes transform *S. cerevisiae* into a petite-negative yeast which no longer (or barely) tolerates the cytoplasmic petite state (Table 13). In one case (*adc1* mutants), the explanation relies on energy production. In the others, there is a common problem which concerns the membrane potential ($\Delta\psi$) required for mitochondrial biogenesis and thus for cell survival.

S. cerevisiae contains two cytosolic alcohol dehydrogenases: AdhI is constitutively expressed, while AdhII is glucose repressed and can be induced only under respiratory conditions. They are responsible for alcoholic fermentation, by driving the glycolytic pathway to ethanol, which leads to the production of ATP. Under anaerobic conditions the entire energy supply of the cell depends on AdhI, because AdhII cannot be induced. AdhII activity is also absent in respiration-deficient mutants. Therefore, under nonrespiratory conditions, energy production is absolutely dependent on AdhI. This easily explains why cells lacking AdhI (*adc1* mutants) cannot survive as petites: both their energy sources, fermentation by constitutive AdhI and respiration, are blocked (references 45, 46, and 327 and references therein).

To fully understand the other situations, one must keep in mind the following proposal: it is currently assumed that the

TABLE 13. Genes which are (almost) essential in a petite background

Gene[a]	Protein function[e]	Type of mutation	Phenotype in a petite background	Reference(s)[f]
ADH1[b] (NE)	**Constitutive alcohol dehydrogenase**	*adc1*	Lethal	45, 46, 327
AAC2[b] (NE)	**Mitochondrial ADP/ATP carrier**	*op1* (missense)	Lethal	166, 167
PEL1/PGS1[b] (NE)	**Mitochondrial phosphatidyl** glycerophosphate synthase	Point mutation	Lethal	291
		Disruption	Lethal	146
PET 936[b] (?)	F_1-ATPase assembly?	Uncharacterized	Growth decreased by 20-fold	79
ATP3[b] (NE)	**F_1-ATPase subunit γ**	Disruption	Slow growth	321
		Deletion	Slow growth	170
ATP16[c] (NE)	**F_1-ATPase subunit δ**	Deletion	Low growth rate	107, 170
ATP15[c] (NE)	**F_1-ATPase subunit ε**	Deletion	Low growth rate	107
YME1[b] (NE)	**Mitochondrial ATP- and zinc-dependent protease**	Disruption	Very slow growth	300, 321
SSH1[d] (NE)	**Mitochondrial Hsp70 family**	Deletion	Lethal at high (permissive) temperature?	263[g]
			Cold resistance (in a rho^0 context)	263[g]

[a to e] See Table 1 footnotes.
[f] The references concern only the effects of the petite mutations on the survival of the relevant mutants. References related to the gene and its product (first two columns) are given in the text.
[g] Knight et al. (159) reported contradictory data (see "Oxidative stress, iron homeostasis, and mtDNA" and Table 6).

membrane potential of respiration-deficient mutants relies on the entry of ATP (via the ADP-ATP carriers) in exchange for ADP produced by the F_1 sector of ATPase, which, in this context, acts as a ATP hydrolase (reference 107 and references therein).

The first case reported was that of the *op1* mutation, which prevents growth on nonfermentable media (166, 167) due to a missense mutation in the *AAC2* gene (175), the most important of three genes encoding the mitochondrial ADP-ATP carriers (reference 161 and references therein). The colethality of the *op1* and rho^-/rho^0 mutations was demonstrated as follows. Mutants bearing the *op1* mutation lose the ability to multiply even on fermentable media after growth in the presence of acriflavine, which converts wild-type cells into petites. However, rho^-/rho^0 mutations indeed occur in the *op1* context, since the acriflavine-treated cells can give rise to viable respiration-deficient diploids when crossed with *OP1* petite cells. Furthermore, petite production is restored in *op1* revertants (167). The reason why the *op1* mutation is lethal in a petite context is still unclear. However, ATP certainly is at the heart of the problem: in petites, ATP is provided to mitochondria via the AAC translocators (this import is probably reduced when the major [Aac2p] translocator is nonfunctional [*op1* mutant]). However, the fact that *op1* is unable to withstand the petite state cannot be explained simply by the limited amount of ATP provided to the mitochondria by the minor translocators Aac1 and Aac3: *op1* is viable in presence of *mit*$^-$ mutations. This mutant has even been used as a powerful tool in isolating *mit*$^-$ mutations, without the background noise of rho^-/rho^0 petites (163). Interestingly, not a single stringent *mit*$^-$ mutation has been found, in the *op1* context, in the genes encoding Atp6, Atp8, and Atp9 subunits of ATPase, among hundreds of *mit*$^-$ mutations located in *COX1*, *COX2*, *COX3*, and *cytb* genes (see references 163 and 241 for examples). This observation can be explained by the fact that *atp6*, *atp8*, and *atp9* stringent mutants would be quickly converted into petites. In fact, one major difference between *mit*$^-$ mutants (mutated in genes encoding a component of the electron transport chain) and petites is that the F_0 sector of ATPase is present in the first case and absent in the second. The F_1 complex might be less stable (or less active) when it is not anchored (petite mutants) than when it is anchored to the F_0 sector (*mit*$^-$ mutants): differences between these two forms of F_1 were suggested by Schatz (262). Thus, in *op1*, limited entry of ATP (via the minor Aac1/Aac3 translocators) in addition to insufficient ATP hydrolysis could cause a dramatic decrease in the ATP-ADP exchange and thus in the potential $\Delta\psi$, below the threshold required for viability. A search for extragenic suppressors of the Δ*aac2* petite lethality could be very useful in clarifying this problem. The above hypothesis predicts that mutations increasing the stability (activity) of the "soluble" petite F_1 would be found among these suppressors.

The second mutation demonstrated as intolerant of the petite state lies in the *PEL1* (for "petite lethal") gene (146, 291). It has been recently demonstrated that *PEL1* (renamed *PGS1*) encodes phosphatidylglycerophosphate synthase, the first enzyme in the cardiolipin biosynthesis pathway (34). The Pel1/Pgs1 protein is localized to mitochondria (78). Cardiolipin is a lipid found predominantly in the inner mitochondrial membrane (reviewed by Daum et al. [61]). The biosynthesis of this dimeric phospholipid is a three-step pathway, with the final step being catalyzed by cardiolipin synthase. The gene (*CLS1*) encoding this enzyme was identified in the yeast genome by similarities to eucaryotic cardiolipin synthases (148). It appeared that *CLS1* inactivation was not lethal and did not cause a pet phenotype (below 37°C), although growth on respiratory substrates was lowered. To explain this surprising result, the authors suggested that PG, the precursor of cardiolipin which accumulates in *cls1* mutants, might adequately substitute for cardiolipin, at least below 37°C. This hypothesis was confirmed a year later, when the gene (*PEL1*/*PGS1*) encoding the first enzyme of the cardiolipin biosynthesis pathway was clearly identified (34). Mutants of this gene lack not only cardiolipin (145) but also PG (34). Disruption of *PEL1*/*PGS1* leads to the same phenotypic defects reported for the original *pel1* mutants: respiration deficiency, heat sensitivity for growth on fermentable conditions, and lethality in a petite background (34). Many data have focused on the pleiotropic role that cardiolipin (and its precursor) can play in mitochondria (reviewed in reference 135). This situation makes it difficult to speculate on the petite-negative phenotype of the *pel1*/*pgs1* mutants. However, an interesting approach was suggested by Hoffmann et al. (136). These authors showed that in vitro activity of a reconstituted *aac2* mutant carrier specifically and absolutely requires cardiolipin: they proposed that this compound would be required in vivo for activity of the wild-type ADP-ATP carrier. If this idea was confirmed, it would provide a single and unified explanation for both the *op1* and *pel1* mysteries: a decrease in $\Delta\psi$ potential below the threshold required for cell viability in a petite background, due to either the absence or the inactivity of the major mitochondrial ADP-ATP translocator.

Besides *ADH1*, *AAC2*, and *PEL1*/*PGS1*, which appear totally essential in a petite background, other genes are almost essential when the rho^+ genome is absent: mutations in these genes cause severely impaired growth in a petite context (Table 13). The most heavily documented situation concerns *YME1* (for "yeast mitochondrial DNA escape"), but there are also a few reports concerning the F_1 sector of ATPase.

It was first reported by Ebner and Schatz (79) that a mutant strain lacking F_1 barely grew in a petite background. This observation has been strengthened by the following reports. A strain carrying a deletion of *ATP3* (subunit γ) grows very slowly when mtDNA is lacking (321). Strains with deletions of either *ATP16* or *ATP15*, encoding subunits δ and ε, respectively, also exhibit slow growth (107). The growth defect of *atp3* and *atp16* mutants was also noticed by Lai-Zang et al. (170). As discussed above (see "The translation-ATP synthase connection"), the data reported by these authors with respect to *ATP1* (subunit α) and *ATP2* (subunit β) deletions might be explained if these mutations were (almost) lethal in a petite background: the petite levels of Δ*atp1* and Δ*atp2* strains are below 1%, while the reference strain produces 9% petites (170). As a whole, the data strongly suggest that viability of the petites can be ensured only if enough ATP is hydrolyzed in mitochondria to provide the threshold of ATP import needed to generate the minimum membrane potential, which is in turn required for mitochondrial biogenesis and thus for cell viability. This idea links the tales of the *op1* and *pel1* mutants and the mutants impaired in F_1 ATPase.

Thorsness and Fox (300) were interested in the transfer of DNA from mitochondria to the nucleus, a phenomenon which most probably occurred during eucaryotic evolution. They therefore developed a genetic screen for mutations increasing the frequency of such events and obtained mutations in six genes. Mutations in *YME1* are pleiotropic: they cause heat sensitivity under respiratory conditions, cold sensitivity on rich glucose medium (300, 301), altered morphology of the mitochondrial compartment (30) (see "Mitochondrial morphology, the cytoskeleton, and mtDNA" above), and severely impaired growth in a petite background (321). With respect to the latter phenotype, it was initially reported that *yme1* cells were inviable in the absence of a rho^+ genome (301). This was ascer-

tained by several methods, including careful genetic tests. Spontaneous petite mutants (which appear at a frequency of 1% in the wild-type strain) were not found in the *yme1* strains. These strains did not grow on medium containing ethidium bromide (which induces rho$^-$ very efficiently). The *yme1* mutations were colethal with a nuclear mutation which causes destabilization of mtDNA and viable in combination with another *pet* mutation which prevents respiration but does not destabilize mtDNA (301). It was later shown that *yme1* rho$^-$ (rho^0) cells were able to form colonies if they were incubated at 30°C for 7 to 10 days instead of 3 to 4 days as in the original experiments (321). *YME1* encodes an ATP- and zinc-dependent protease tightly associated with the mitochondrial inner membrane (320). Unfortunately, the precise functions of Yme1 are not yet well established, which does not aid us in understanding why *yme1* petite cells are so sick. However, the search for specific suppressors of the slow-growth phenotype of these strains should shed light on this problem. This has been undertaken by Weber et al. (321), who identified six dominant suppressor mutations located in two genes. It is noteworthy that the major gene (five of six mutations) is *ATP3*, which encodes the γ subunit of ATP synthase (see "The translation-ATP synthase connection" above) (Table 4) and that the suppressor mutations are missense mutations. This is a major discovery with respect to the general problem of petite negativity and its connection to ATPase, as emphasized in the next section.

In contrast to these mutants, others might survive only in a petite context. We describe here a mutant cured by a complete loss of mtDNA. The *SSH1* (for "stress seventy subfamily H") gene encodes a protein of the Hsp70 family which is localized in the mitochondria (263). A strain bearing a deletion of *SSH1* grows quite normally at 37°C but very slowly at 30°C and below. Overexpression of the *SSC1* gene (encoding the major mitochondrial Hsp70) (see "Conventional mitochondrial chaperones" above) (Table 5) leads to partial suppression of *ssh1* cold sensitivity. In contrast, overexpression of *SSH1* cannot rescue the heat sensitive phenotype of a *ssc1* mutant. Furthermore, and unlike Ssc1p, Ssh1p does not seem to be required for translocation of proteins into mitochondria. Spontaneous revertants of the *ssh1* mutant, growth competent below 30°C, were obtained at high frequency (in the range of 1%). Convincing criteria demonstrated the presence of rho^0 mutants. Among 15 revertants analyzed, only 1 showed mtDNA, in an amount fourfold below the wild-type level. These data were satisfyingly completed by a screening of the *ssh1* mutant at 37°C (the permissive temperature) for clones which had become respiration deficient. Among 4,500 colonies, 6 showed this phenotype but only 1 was a suppressor of *ssh1* at low temperature, and it was a petite of the rho^0 type; the others, which could be siblings (not independent isolates), were nuclear *pet* mutants. Thus, it was impossible to obtain rho$^-$ mutations in the *ssh1* context. Does this mean that these petites are lethal in an *ssh1* context at high (permissive) temperature? In this case, why does this nuclear background perform a selection of the rho^0 state, especially at restrictive temperatures (263)? The authors propose two hypotheses. First, *SSH1* might be involved in mtDNA replication, so that its absence (at low temperatures) would lead to a replication mode that is deleterious for the organelle and thus for the cell. Second, lack of mtDNA might affect the expression of nuclear genes able to suppress the cold sensitivity of the mutant. This hypothesis is reminiscent of the retrograde regulation already evoked above (see "Serependipitous discoveries") for the *smp1* mutants (see reference 240 for a review). However, in the retrograde regulation system, both the rho$^-$ and rho^0 states lead to modifications in nuclear gene expression. With respect to the first hypothesis, it remains to be seen if the *ssh1* context cannot tolerate a rho$^-$ state at low temperature. This test could be performed through cytoduction experiments (at high temperature) between a *ssh1* rho^0 strain and a strain carrying a very stable rho$^-$ genome, as in the procedure used by Jones and Fangman (149) in their analysis of the *MGM1* gene (see "Mitochondrial morphology, the cytoskeleton, and mtDNA" above). There are in fact two questions. Is the *ssh1* mutant able to maintain a rho$^-$ genome at high temperature? Does the transfer at low temperature of the *ssh1* cytoductants carrying the rho$^-$ genome lead to the loss of mtDNA? In any case, the mutation causes a strong decrease in the petite level. In fact, in the *ssh1* background, only 1 petite (rho^0) was found among 4,500 colonies at 37°C. This is far below the spontaneous rate of petites normally observed in a wild-type context.

As to this decrease in petite production, there is to our knowledge only one report of a direct search for nuclear mutations producing this effect (Table 10). Devin and Koltovaya (66) began with a strain which produced 50% petite mutants and selected four nuclear mutations giving rise to a low level of petites (1% in three cases and 9% in the fourth). The four mutations are recessive and map to different complementation groups. As reported above (see "mtDNA and the cell cycle") (Table 10), a fifth mutation was shown to be allelic to *CDC28* (67), while Koltovaya et al. (162) observed the same phenomenon with one of the "classical" *cdc28* alleles. It is quite surprising that these mutations have not yet been introduced into a more "classical" background (with 1% spontaneous petites) to determine whether they are able to reduce the petite production and identify the relevant genes.

PETITE POSITIVITY VERSUS PETITE NEGATIVITY: ATPase IN THE FOOTLIGHTS

In contrast to *S. cerevisiae*, which spontaneously produces petites at a high rate, most yeast species are petite negatives: they do not give rise to petite cells even after treatment with ethidium bromide (which massively converts *S. cerevisiae* rho$^+$ cells into rho^0). This inability to lose mtDNA could easily be explained if these yeasts were strict aerobes. This is not the case, at least for *S. pombe* (reference 123 and references therein) and *K. lactis* (38), in which nonrespiratory mutants have been obtained. In fact, recent data on these petite-negative yeasts have provided a novel viewpoint on this problem. We will focus on *K. lactis*, for which the most documented information is so far available. It has been shown that large deletions (and even total loss) of mtDNA do occur but are lethal in the wild-type nuclear background (48). However, in strains carrying mutations in either one of several nuclear genes, named *MGI* (for "mitochondrial genome integrity"), petite production is observed spontaneously (in the range of 1 to 5%) and at high frequencies after treatment with ethidium bromide (38). Thus, *mgi* mutations allow petites to survive. It has been shown that *MGI2*, *MGI1*, and *MGI5* encode, respectively, the α, β, and γ subunits of mitochondrial ATPase. The *mgi* mutations are missense mutations in highly conserved positions. Null mutations in these genes abolish the petite-forming capacity, strongly suggesting that integrity of the F_1 complex is required for expression of the mgi phenotype. These authors propose the following model: the absence of F_0 subunits (encoded by the mitochondrial genome) in rho$^-$ (rho^0) cells in the presence of a wild-type F_1 would create a proton-permeable channel abrogating the membrane potential, thus killing the mitochondria and consequently the cell. Subtle and specific changes in α, β, or γ subunits would cause the forma-

tion of an altered F_1 complex that was able to prevent the leakage of protons, allowing the survival of petites (37). This hypothesis has been rather complicated by recent data which show that cells lacking the mitochondrial *ATP9* gene or disrupted for three nuclear genes (*ATP4*, *ATP5*, *ATP7*) encoding F_0 subunits are viable (39). The obvious conclusion is that lack of F_0 per se is not responsible for petite death. As emphasized by the authors, it remains to be determined if loss of *ATP6* and/or *ATP8* leads to cell death (39). If not, the mitochondrial component for the vital function of mtDNA will have to be identified, along with the role played by F_1.

As suggested by Chen and Clark-Walker (37), one can postulate that petite-positive yeasts are natural *mgi* mutants. For *S. cerevisiae*, the status of F_1 remains ambiguous: on one hand, loss of F_1 subunits strongly increases petite levels (see "The translation-ATP synthase connection" above and Table 4); on the other, the petites appear very ill when F_1 is lacking (see the previous section and Table 13). The most promising *MGI* candidate in *S. cerevisiae* remains *YME1*. As mentioned in the previous section, *yme1* mutations are sublethal in the presence of rho^- (rho^0) mutations. Extragenic suppressors of this defect have been obtained. Interestingly, five of the six correspond to mutations occurring in the *ATP3* gene, encoding the ATPase γ subunit. Two were sequenced and show missense mutations in strictly conserved positions (321). The remarkable fact is that one corresponds to the same substitution event as that observed in the *mgi5-1* mutant of *K. lactis*. Deletion of the *ATP3* gene in *S. cerevisiae* does not restore petite viability in the *yme1* context: on the contrary, the double-mutant strains show a very slow growth on any media, probably because inactivation of *ATP3* causes a high level of petites. It is too early to speculate on the role Yme1p plays in the viability of *S. cerevisiae* petites. What we presently know is that it is involved in a complex located in the mitochondrial inner membrane and that it displays a protease activity involved in the degradation of unassembled or abnormal mitochondrially synthesized proteins of the inner membrane (see reference 294 for a review). However, Atp3 is probably not a substrate for Yme1p (320). In any case, the near-essential role of *YME1* in rho^0 (rho^-) contexts and the fact that its absence is suppressed by a subtle change in ATPase γ subunit are provocative. We are really not so far from the situation observed in *K. lactis*. It will be interesting to obtain more data on the status of the *YME1* gene in *K. lactis* and the effects of the *mgi2* (α subunit) and *mgi1* (β subunit) missense mutations in *S. cerevisiae* as putative suppressors of the *yme1* mutants.

Although these data have made a significant breach in the wall of an old problem, further interdisciplinary studies are still necessary to find a satisfactory answer. However, a unitary view of the problem in both *S. cerevisiae* (see the previous section) and *K. lactis* (this section) relies on the essential role of F_1 as an ATP hydrolase in petite cells. It is noteworthy that, beyond yeasts, functional F_1 is essential in maintaining the mitochondrial membrane potential and the growth of human cells devoid of mtDNA (26).

IS THERE A CENTROMERE-LIKE STRUCTURE FOR THE MITOCHONDRIAL GENOME?

At this point in the review, we pose three questions. First, why is the mitochondrial genome of *S. cerevisiae* so unstable (with a baseline of 1% petites)? Second, why do particular genetic backgrounds lead to a complete loss of mtDNA (rho^0 petites)? Third, how can some genetic contexts be unable to maintain a complete (rho^+) mitochondrial genome while maintaining truncated (rho^-) versions of this genome?

To our knowledge, there is as yet no satisfactory explanation for the high intrinsic instability of the mitochondrial genome of *S. cerevisiae* (and other yeasts as well; as reported in the previous section, some petite-negative yeasts can be converted into petite-positive forms which spontaneously produce 1 to 5% petite cells). There are many parameters which can influence the fate of mtDNA. Among the most obvious are the structure of the genome per se (for instance, mtDNA without introns appears more stable than intron-containing molecules), the high rate of recombination observed in mitochondria (and, consequently, a possible high probability of recombination between direct repeats), the selective advantages the defective mtDNA molecules may exhibit (in terms of replication and/or transmission), and the rapid loss of heteroplasmy due to the transmission of a small number of molecules (or a preferential transmission of related molecules) to the buds. One way to clarify this problem may be to study mutations which decrease (without abolishing) petite production (Table 10) (see the end of "No compromise" above). Another method is to screen for genes whose overexpression could decrease petite levels: this might identify genes whose products (normally synthesized in limited amounts) are required for mtDNA stability. One possible candidate is *MSH1*, which is assumed to protect mtDNA against recombination events between imperfect repeats (see "The recombination/repair track" above). This path will certainly be difficult to follow if several genes are normally expressed under the threshold required for an efficient mtDNA stability. Moreover, it is obviously easier to observe an increase than a decrease in petite production. However, even with respect to an increase in petite levels, a systematic study of gene overexpression is still lacking (Table 12) (see "Gene overexpression can increase mtDNA instability" above).

There are at least two possible answers to the second question. First, rho^0 production may simply demonstrate that the mitochondrial compartment is unable to replicate any type of DNA. Thus, in the relevant genetic contexts, mtDNA replication is (directly or indirectly) abolished. The second possibility is based on the fact that some genetic backgrounds may select for a complete loss of mtDNA, as if any type of mtDNA molecules were deleterious to the cell in these contexts. The reasons why rho^0 petites would be the only survivors in these situations remain unclear. This hypothesis has been proposed, for instance, to explain why the *ssh1* mutants are able to grow at low temperature only if they are in the rho^0 state (Table 13) (see "No compromise" above) and why an increased iron concentration might lead to rho^0 production in the *yfh1* mutants (Table 6) (see "Oxidative stress, iron homeostasis, and mtDNA above). However, the situation can be envisaged as more complex and very puzzling. In fact, some mutants which exhibit a complete loss of mtDNA (rho^0 producers) are nonetheless able to maintain rho^- genomes. The relevant experimental tests have been performed in only a few cases (see "The *ABF2* paradox" and "Does transcription play a direct role in mtDNA maintenance," above, for *abf2* and *rpo41* mutants, respectively).

The same sort of assumption can also explain the high production of petites (here rho^- and rho^0) in a few situations. However, most situations would be more easily accounted for if one assumes that the wild-type (rho^+) genome requires a specific structure to be consistently transmitted to the bud. Analysis of the sorting of mtDNA in zygotes also led Okamoto et al. (224a) to propose the existence of a segregation apparatus for the mitochondrial genome. Such a centromere-like structure has also been recently proposed for the bacterial chromosome (88). Concerning yeast mitochondria, the data suggest that this putative structure would be dispensable for rho^- molecules due to their abilities to use other routes of

transmission. Presently, one can only emphasize some observations which focus on a possible attachment of the mitochondrial genome to the inner membrane. In fact, very few biochemical and cytological studies have been devoted to this problem. We would like to review two of these.

The first concerns specific structures of the mitochondrial membrane, seen in close association with the mitochondrial nucleoid. These myelin-like structures were clearly observed under nonrespiratory conditions (in the wild type or *pet* mutants grown on glucose-rich medium) but disappeared under respiratory conditions (332). It was not reported if these structures were present in petite mitochondria. However, the data led the author to suggest that these structures would be similar to those assumed, at that time, to link the bacterial chromosome to the membrane and be responsible for the distribution of daughter molecules at division (258a). Such ultrastructural analyses would be profitable in terms of the techniques available 30 years later. The second study concerns the isolation of mtDNA-protein complexes under different experimental conditions. The most interesting fact is the effect of RNase on the sedimentation pattern and composition of the nucleoids. The authors suggest an attachment of mtDNA to the inner membrane via nascent mRNAs in the course of their translation by membrane-bound ribosomes (251). In the intervening period, it has been shown that proteins encoded by mtDNA and devoted to the inner membrane are indeed synthesized on membrane-bound ribosomes. Specific proteins might ensure the link between mRNAs and the membrane (see reference 99 for a review). Since blocks in mitochondrial translation lead to conversion of rho^+ cells to rho^- (Table 3) (see "The translation-ATP synthase connection" above), one could assume that translation per se is required for rho^+ maintenance. However, the data suggest a more specific relationship between these two parameters, which focus on the Atp6 subunit. We have previously suggested experiments which might clarify whether Atp6 is required per se or must be translated in mitochondria (see "The translation-ATP synthase connection" above). In either case, it is clear that morphological alterations of mitochondria (Table 11) (see "Mitochondrial morphology, the cytoskeleton, and mtRNA" above) may influence the structure of the inner membrane and thus weaken mtDNA attachment. However, one cannot exclude a more indirect effect on mtDNA stability through specific impairments of the import process. Thus, analysis of this process and of the inner membrane structure in *mdm*/*mgm* conditional mutants (at subrestrictive temperatures) would be very informative. Notably, mtDNA instability is a postponed effect of morphological alterations, at least in cases where the relationship between the two defects has been examined (see "Mitochondrial morphology, the cytoskeleton, and mtDNA" above).

In the frame of the hypothesis of a centromere-like structure (by attachment of mtDNA to the inner membrane), one must keep in mind that some fatty acids might play a key role in anchoring the relevant proteins to the membrane, although we cannot exclude an indirect role for the lipid composition of the membranes on the import process (see "Fatty acids and phospholipids" above). Last but not least, there is the provocative discovery of Ymh2p. The *YHM2* gene was discovered as a multicopy suppressor of *abf2* null mutants. As reported above (see "The *ABF2* paradox"), Ymh2p is localized to the inner membrane and associated with mtDNA in vivo. This is, in fact, the first case of a membrane-bound protein associated with the nucleoid (43). The confusing point of this problem is that disruption (with a partial deletion) of *YMH2* does not increase petite production. However, the authors mention that *ymh2* cells lose significant amounts of their mtDNA even when they are grown under respiratory conditions (this property was also reported for Δ*abf2* cells [335]). Clearly, further studies performed on strains bearing a complete deletion of the gene are required to fully understand the role played by Ymh2p.

The situation as a whole resembles a maze, whose branches include mitochondrial translation, the Atp6 subunit, fatty acids, mitochondrial morphology, and so on. Multidisciplinary approaches are necessary to know if this labyrinth is to emerge into either a centromere-like structure or another form liable to explain the requirements for rho^+ maintenance.

CONCLUSIONS AND PROSPECTS: YEAST AND BEYOND YEAST

In addition to the nuclear genes required for general mitochondrial translation (see "The translation-ATP synthase connection" above), which number more than 100, at least 70 nuclear genes have been identified to date as being involved in the fate of the rho^+ mitochondrial genome of *S. cerevisiae*. This inventory is obviously underestimated, for two reasons. First, not all genes so far analyzed have been investigated with regard to mtDNA stability. Second, functional analysis of "new" genes discovered through the systematic sequencing of the yeast genome is far from complete. When the dust settles, we would not be surprised to learn that several hundred genes are (directly or indirectly) involved in faithful maintenance of mtDNA. This involvement, so highly multigenic, may reflect the long history shared by the two original partners which gave rise to the eucaryotic cell, with its two informative compartments, the nucleus and the mitochondrion. Thus, a multigenic control of mtDNA stability is also expected to be found in obligate aerobes.

However, one cannot avoid the fundamental questions of the general and specific controls of mtDNA maintenance. In other words, which are the genes conserved through evolution that influence the fate of mtDNA from *S. cerevisiae* to humans? Which are the genes which fulfill this function in a more specific way, depending on the structure of the mitochondrial genome, the physiology (obligate versus facultative aerobe) of the organism, its unicellular or multicellular organization, and so on? Although one can expect that genes encoding proteins required for mtDNA metabolism (replication, repair, and recombination) will be found in the first class, definitive answers to these questions will be obtained only by a systematic functional analysis performed on several genomes completely sequenced. However, even if the problem of mtDNA maintenance were considered a priority, this will be a long road to walk.

Meanwhile, more classical approaches may give some insight into these questions. Unfortunately, there are very few model systems in this field. One must briefly mention the filamentous fungus *Podospora anserina*, which, although far behind *S. cerevisiae*, appears (in our opinion) the most advanced system with respect to the nuclear control of mtDNA stability. Its mitochondrial genome is nearly 100 kb and contains many introns (57). Thus, in this aspect, it resembles *S. cerevisiae*. However, it is an obligate aerobe. Furthermore, as a filamentous fungus, it is on the borderline between unicellular and multicellular eucaryotes. *P. anserina* displays a spontaneous degenerative process, called senescence (253). Short mtDNA sequences (senDNAs) are found amplified as circular multimeric molecules in senescent cultures (see references 15 and 75 for reviews). This phenomenon is under multigenic control (reference 15 and references therein). With respect to this control, there are two major differences between *P. anserina* and *S. cerevisiae*. First, while in *P. anserina* numerous muta-

tions altering the cytosolic translational apparatus can modify the timing of cellular death and/or the nature of the relevant mtDNA rearrangements (14, 274, 275), there is no report of this type for *S. cerevisiae* petite production (with the possible exception of the *mgm104-1* mutation [see comments in "Wide-scale screening for rho^0 production" above]). Second, while blocks in mitochondrial translation increase mtDNA instability in yeast (see "The translation-ATP synthase connection" above), the opposite effect is observed in *P. anserina*: a slowing in mitochondrial translation delays senescence (13).

With respect to mtDNA maintenance, another degenerative process, called premature death, has been more recently discovered in *P. anserina*. It is linked to the accumulation of mtDNA molecules in which a specific part of the genome is deleted (14, 259, 269). It occurs only in the presence of either one of two (among five) mutations of the *AS1* gene encoding a cytosolic ribosomal protein (14, 65). In some ways, premature death exhibits similarities to human diseases characterized by the accumulation of the so-called "common" deletion (174). This situation should also be observed in yeast. However, searches for a mutation leading to the accumulation of a specific rho$^-$ deletion have so far probably been hampered by the high spontaneous rate of petites. In fact, the approaches used in yeast and in *Podospora* (with respect to premature death) have been quite different, while complementary. With a few exceptions (see, in particular, the early sections of this review), yeast researchers examine a posteriori the effects of mutations on petite production in terms of levels and petite types (rho$^-$ and/or rho^0). Our aim has been to find mutations which can delay and even abolish the accumulation of the deletions characteristic of premature death. To date, this has helped to identify eight genes (51, 52; D. Dequard-Chablat, personal communication), of which four have been cloned. Two encode proteins which show no similarity to known proteins and are thus absent in the yeast genome (V. Contamine, unpublished data; Dequard-Chablat, personal communication). Two others have yeast homologues (143). These are *TOM70*, encoding a component of the receptor for protein import into mitochondria (see references 191 and 236 for reviews), and *MDM10*, involved in mitochondrial morphology and distribution (283). Interestingly, mutations in the two *P. anserina* genes modify the spectrum of mtDNA rearrangements associated with cellular death and mitochondrial morphology. While effects of *tom70* mutations on petite production in yeast have not been reported, *mdm10* mutations increase petite production (Table 11) (see "Mitochondrial morphology, the cytoskeleton, and mtRNA"). This stresses the point that, although unclear (but see the previous section), the relationship between mitochondrial morphology and mtDNA maintenance has probably been conserved throughout evolution.

It may appear presumptuous and untimely to compare the well-documented situation in *S. cerevisiae* (which accumulates heterogeneous rho$^-$ deleted genomes) and the underexploited situation of premature death in *P. anserina* (which is characterized by the accumulation of mtDNA molecules lacking a specific part of the genome). However, this emphasizes that a clear comprehension of the genetic control of mtDNA maintenance remains an important challenge for the future. We are very concerned about the interest the scientific community will invest in this field in the next century.

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