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Maturase and endonuclease functions depend on separate conserved domains of the bifunctional protein encoded by the group I intron aI4α of yeast mitochondrial DNA

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Intron 4α (aI4α) of the yeast mitochondrial *COXI* gene is a mobile group I intron that contains a reading frame encoding both the homing endonuclease I–*Sce*II and a latent maturase capable of splicing both aI4α and the fourth intron of the cytochrome *b* (*COB*) gene (bI4). The aI4α reading frame is a member of a large gene family recognized by the presence of related dodecapeptide sequence motifs called P1 and P2. In this study, missense mutations of P1 and P2 were placed in mitochondrial DNA by biolistic transformation. The effects of the mutations on intron mobility, endonuclease I–*Sce*II activity and maturase function were tested. The mutations of P1 strongly affected mobility and endonuclease I–*Sce*II activity, but had little or no effect on maturase function; mutations of P2 affected splicing but not mobility or endonuclease I–*Sce*II activity. Surprisingly, the conditional (temperature-sensitive) mutations at P1 and P2 block one or the other function of the protein but not both. This study indicates that the two functions depend on separate domains of the intron-encoded protein.

Keywords: endonuclease/group I intron/maturase/yeast mitochondria

Introduction

Many group I introns contain reading frames that encode proteins (Lambowitz and Belfort, 1993; Mueller *et al.*, 1993). Some of these proteins are DNA endonucleases that confer mobility to the introns that encode them, while others are maturases required for efficient splicing of the intron. Many of the reading frames belong to a large gene family, all of whose members have a conserved amino acid sequence motif originally called LAGLIDADG (Hensgens *et al.*, 1983)—often referred to as a dodecapeptide. Nearly all such proteins have two non-identical dodecapeptide sequences, called P1 and P2, separated by ~100 amino acids. Those motifs have four nearly invariant amino acids, two glycines and two acidic amino acids (usually Asp), but the amino acid(s) at most of the other positions vary a lot, even between P1 and P2 of a given intron (Cummings *et al.*, 1989a,b; Lazowska *et al.*, 1989). All group I intron maturases belong to this gene family. Interestingly, there are some related endonucleases that are not encoded by group I intron reading frames; some are 'free-standing' genes (Kostriken *et al.*, 1983; Nakagawa *et al.*, 1991) and others are encoded by protein introns (Shub and Goodrich-Blair, 1992).

Where it has been possible to test a given intron reading frame for endonuclease and maturase functions, nearly all were found to have just one or the other (Lambowitz and Belfort, 1993). The fourth intron of the *COXI* gene (aI4α) of mitochondrial (mt) DNA of *Saccharomyces cerevisiae* was the first intron found to encode both functions. Relatively recently it was found that aI4α is mobile and encodes the I–*Sce*II endonuclease (Delahodde *et al.*, 1989; Wenzlau *et al.*, 1989). Earlier studies have shown that the splicing of aI4α in wild-type strains depends on the maturase encoded by intron 4 of the *COB* gene (bI4; Dhawale *et al.*, 1981; De La Salle *et al.*, 1982; Weiss-Brummer *et al.*, 1982). The splicing of both bI4 and aI4α is blocked in mutants of bI4 that lack the maturase activity, but splicing can be restored by second-site suppressor mutations. One type of suppressor is a missense mutation in the aI4α reading frame (the *MIM2-1* mutation; Dujardin *et al.*, 1982). In addition, dominant suppressor alleles of the nuclear *nam2* gene restore splicing (Dujardin *et al.*, 1982). The *NAM2-1* suppressor that restores aI4α and bI4 intron splicing, by activating the latent maturase activity of the aI4α reading frame, is a missense mutant of the gene encoding the mitochondrial leucine tRNA synthetase (Labouesse *et al.*, 1987).

Mutations inactivating the intron-encoded functions have been reported for most of the LAGLIDADG proteins of yeast mtDNA (Lazowska *et al.*, 1980, 1989; Anziano *et al.*, 1982; De La Salle *et al.*, 1982; Macreadie *et al.*, 1985; Wenzlau *et al.*, 1989; Moran *et al.*, 1992). In most cases, the mutations truncate the intron reading frame, so that little has been learned about the functional domains of the proteins. Interestingly, the two missense maturase-deficient mutants that have been described alter the invariant glycine residues of the P2 motif: one mutation inactivates the maturase function of the bI3-encoded protein (Lazowska *et al.*, 1989), while the other renders the bI4-encoded maturase temperature sensitive (Anziano *et al.*, 1982; Lamb *et al.*, 1983). Several mutations of the P1 motif block the endonuclease activities encoded by two inteins (Hodges *et al.*, 1992; Gimble and Stephens, 1995), but so far no P1 motif mutations have been studied in an intron reading frame. Here, we report the effects of the P1 and P2 motifs of the aI4α-encoded protein on the functions that it encodes. The results show that the P1 motif is important for endonuclease but not maturase function, while the P2 motif is important for maturase but not endonuclease function.

Results

Missense mutations in the P2 motif of aI4α block splicing but not mobility

This study was initiated by analyzing site-directed mutations of the two highly conserved glycines of the P2

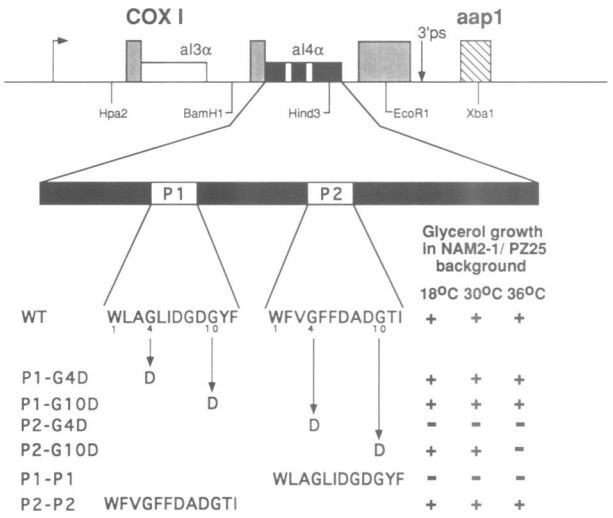

Fig. 1. The CI34 allele of *COXI* and mutations in the aI4α reading frame. The diagram at the top shows the organization of the CI34 allele of the *COXI* gene. It contains two group I introns, aI3α and aI4α. Exon sequences are shown as shaded boxes, intron reading frames as rectangular boxes, and non-coding sequences as lines. The location of the *COXI* gene promoter and the 3′ end processing site is indicated. Restriction sites shown were used to construct the plasmids described in Materials and methods. The locations and sequences of the P1 and P2 dodecapeptide motifs of the aI4α reading frame are shown; the amino acids of each motif are numbered 1–12 so that P1–G4 refers to the fourth amino acid (glycine) of the P1 motif, and so on. Below the sequences are shown the amino acid substitutions present in each mutant strain constructed for this study. To the right of the mutant designations is summarized the glycerol growth phenotypes of the set of strains carrying the *NAM2-1* nuclear mutation and the PZ25 mutation of the *COB* gene.

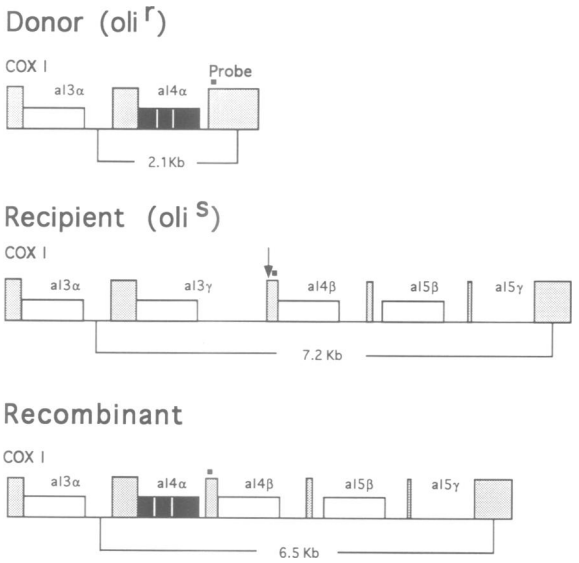

Fig. 2. Diagram of parental and recombinant *COXI* alleles for an analysis of intron mobility in crosses. The diagram shows the donor, recipient and recombinant alleles of *COXI* scored in the crosses of Figure 3. Digestion of mtDNA with *Bam*HI and *Eco*RI yields allele-specific restriction fragments detected here using an exon 5-specific probe (indicated in the diagram). The arrow denotes the aI4α insertion site in the recipient gene. None of the aI4α reading frame mutations alters the size of the donor or recombinant restriction fragments relative to the wild-type strain.

motif of aI4α. mtDNA transformation was used to construct a control strain, 161/CI34-bI4$^+$, which has the nuclear genome of strain 161 and ρ^+ mtDNA with a two-intron (CI34) form of *COXI* (Figure 1). Mutations changing each of the glycines of the P2 motif of aI4α to aspartic acid (P2–G4D and P2–G10D; Figure 1) were constructed and transformed into mtDNA. Because the resulting strains have wild-type bI4 maturase, they splice efficiently and grow on glycerol medium (Gly$^+$).

The control and two mutant strains were mated to a tester strain with a *COXI* gene known to be a recipient for aI4α (Wenzlau *et al.*, 1989), and the *COXI* alleles in progeny of the cross were measured. The alleles of the *COXI* gene used in this cross are displayed diagramatically in Figure 2, and the outcome of each cross is shown in Figure 3. The input ratio of the parental mtDNAs was estimated by measuring the transmission of alleles of the *oli1* gene, located far from the *COXI* gene; the fraction of oli^S progeny (the allele from the recipient strain) is shown below each gel lane. The data show efficient aI4α mobility in the control crosses (Figure 3, lanes 3–5) and in both mutant crosses (lanes 6 and 7). Because mutant P2–G10D of bI4 is temperature sensitive for maturase function, we tested this mutant allele of aI4α for mobility in a cross carried out at 36°C; even at that temperature, there was no reduction of mobility (lane 8). Thus, these two mutations of the P2 motif of aI4α have no detectable effect on intron mobility.

The maturase activity of the aI4α-encoded protein is measurable in strains that lack the bI4 maturase and also have an activator mutation, such as the nuclear *NAM2-1* mutation. Derivative strains were constructed that carry the wild-type and the above mutant *COXI* alleles but have a mutation (PZ25) in bI4 that eliminates the splicing of aI4α and bI4. These strains were respiration deficient, showing that this wild-type aI4α allele does not encode an active maturase and that neither the P2–G4D nor the P2–G10D mutation activates the maturase. The control and mutant mtDNAs were then placed in the nuclear background of strain 161–*NAM2-1*, containing the same mutation of the *nam2* gene shown earlier to activate the wild-type aI4α maturase (Labouesse *et al.*, 1987). As summarized in Figure 1, the *NAM2-1* strain with wild-type aI4α was Gly$^+$ and the strain with the P2–G4D mutation was Gly$^-$; the strain carrying the P2–G10D mutation was temperature sensitive for glycerol growth, growing well at or below 30°C but not at 36°C.

To confirm that these mutations affect the maturase function of the aI4α-encoded protein, we analyzed Northern blots of RNA from these strains. Figure 4 shows the *COXI* transcripts detected with an exon 4-specific probe. The control strain (lane 3) splices aI4α from ~30% of the *COXI* transcripts, while >95% of the *COXI* pre-mRNA is spliced for aI4α in the related strain that has a wild-type *COB* gene (lane 1); the remainder of the *COXI* transcript detected with the exon probe is mostly a 3.0 kb RNA. The 3.0 kb RNA is also detected with an aI4α-specific probe (results not shown) so that it contains aI4α as the only unspliced intron. The 1.9 kb mRNA was not present in mutant P2–G4D, and nearly all of the *COXI* RNA was the 3.0 kb pre-mRNA (lane 4). Thus, this mutation blocks aI4α splicing, despite the presence of the activating NAM2-1 mutation. Mutant P2–G10D was conditional for aI4α splicing, accumulating some mRNA in cells grown at 30°C but none at 36°C (Figure 4, lanes

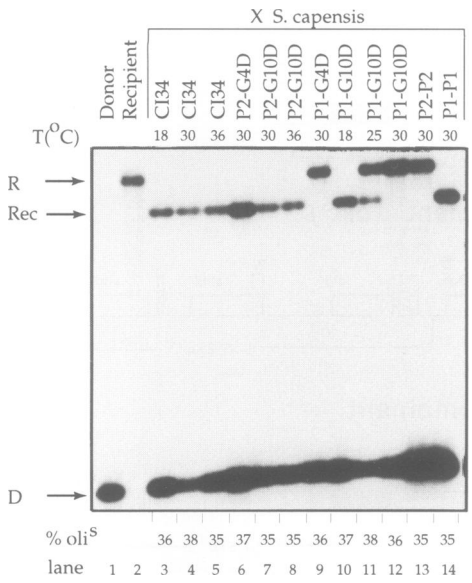

Fig. 3. Assay of intron mobility in strains with mutations in the P1 and P2 motifs of the aI4α reading frame. Southern blots of mtDNA. Cultures of the donor strains carrying the aI4α alleles shown above each lane were mated to the recipient strain carrying the *S.capensis* allele of *COXI* (see Materials and methods). The temperature at which each cross was carried out is shown above each lane. mtDNA was isolated from the resulting cultures of diploid progeny, cleaved with *Bam*HI and *Eco*RI, and analyzed on Southern blots. The three restriction fragments shown in Figure 2 are evident on the gel and are labeled (D = donor, R = recipient and Rec = recombinant). The output of the *oli1* markers in each cross is reported below the lanes.

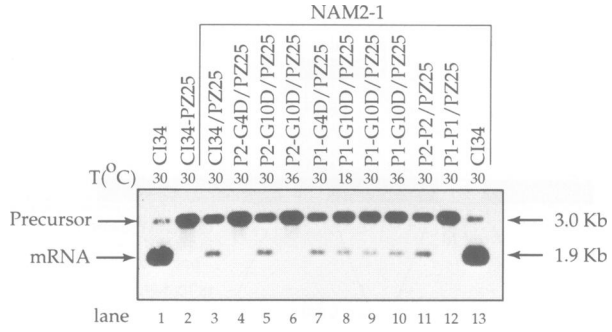

Fig. 4. Analysis of RNA splicing in strains with mutations in the P1 and P2 motifs of the aI4α reading frame. RNA was isolated from cultures of haploid strains carrying the *COXI* and *nam2* alleles shown in the figure and analyzed on Northern blots, as described in Materials and methods. The locations of the 1.9 kb *COXI* mRNA and the 3.0 kb pre-mRNA containing all exons plus aI4α are defined by the control strains in lanes 1 and 2, respectively. Glycerol growth phenotypes of the strains shown in lanes 3–12 are summarized in Figure 1. Because P1–G10D and P2–G10D are temperature sensitive for one intron-encoded function, splicing in these strains was assayed in cultures grown at several different temperatures, as indicated in the figure.

5 and 6, respectively). These data show that these mutations of the P2 motif interfere with the maturase function of the intron-encoded protein but have no detectable effect on mobility.

Missense mutations in the P1 motif of aI4α block mobility but not splicing

Next, we constructed an analogous pair of strains with mutations of the glycines in the P1 motif, P1–G4D and P1–G10D. The strains with wild-type bI4 and *nam2* were analyzed for aI4α mobility as outlined in Figure 2. The outputs of *COXI* alleles are shown in Figure 3 and the outputs of the *oli1* markers are shown below each gel lane. The data show that the P1–G4D mutation blocks aI4α mobility completely (Figure 3, lane 9). The P1–G10D mutant is conditional for mobility, showing the efficient transfer of the intron to recipient alleles at 18, less mobility at 25 and no mobility at 30°C (Figure 3, lanes 10–12, respectively).

The effects of these mutations on the maturase function of the aI4α-encoded protein were determined, as above, using strains with the PZ25 mutation of the bI4 maturase and the *NAM2-1* mutation. First, it was noted that the strains with these P1 motif mutations were Gly$^+$ (Figure 1), indicating that they can splice both introns. As shown in the RNA blot in Figure 4, the mutant P1–G4D splices aI4α about as well as the control strain (compare lane 3 with lane 7). Mutant P1–G10D grows well on glycerol medium at 18, 30 and 36°C but has somewhat less mRNA than the control sample (compare lanes 8–10 with lane 3). Clearly, the P1–G10D mutation does not block maturase function at these temperatures but does block mobility at 30°C. Together with the data from Figure 3, it appears that the P1 motif is crucial for endonuclease activity but is less important for maturase activity, while the P2 motif appears to be essential for maturase activity but unimportant for endonuclease activity.

P1 and P2 motif substitution mutations affect the functions differentially

As shown in Figure 1, the P1 and P2 motifs of the aI4α reading frame have the same amino acids at only five of the 12 positions. The above experiments show that mutations of two of the conserved amino acids (G4 and G10) in P1 and P2 have different effects on the splicing and mobility functions encoded by the intron reading frame. Next, we tested whether the other sequence differences between the two motifs were associated with either or both functions. To this end, we constructed and transformed two new alleles: in one, P1 was changed to P2 (mutant P2–P2); in the other, P2 was changed to P1 (mutant P1–P1). These alleles were then tested, as above, for mobility and splicing. The P1–P1 mutant was unaltered in mobility, as shown in Figure 3, lane 14 (compared with the control cross in lane 4); the P2–P2 mutant was blocked for aI4α mobility (Figure 3, lane 13). Conversely, the P1–P1 mutant lacked maturase function (Figure 4, lane 12), while the P2–P2 mutant had maturase function (Figure 4, lane 11). These data show that P1 and P2 are not functionally interchangeable, and extend our initial findings made with the point mutants of the conserved glycines, showing that the two motifs appear to be specific for one function or the other.

Correlation of mobility phenotypes with I-SceII endonuclease activity

To relate aI4α mobility data directly with I-*Sce*II endonuclease activity encoded by the intron, *in vitro* endonuclease assays were carried out on partially purified mitochondrial extracts, as described in Materials and methods. Because mobility was tested using strains that had bI4 maturase, aI4α splices efficiently and thus only low levels of the intron-encoded protein were present in

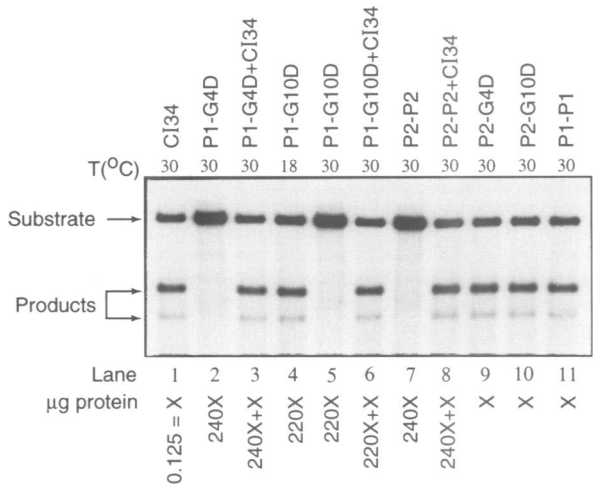

Fig. 5. I–*Sce*II endonuclease activity in mitochondrial extracts from control and mutant strains. Each yeast strain listed in the figure was grown in YEP–2% galactose medium at 30°C. Mitochondria were isolated form each cell pellet, lysed and processed to yield fraction 1a, according to Wernette *et al.* (1990). Each sample was assayed for I–*Sce*II activity, as described in Materials and methods, using the amounts of fraction 1a protein shown below each lane (30 min incubations). Reactions in lanes 3, 6 and 8 contained an amount of I–*Sce*II activity from the control strain to cleave 35–40% of the substrate during this incubation plus ~220–240 times more protein from one of the three mutants that appeared to lack any detectable activity.

these strains. The sensitivity of these I–*Sce*II assays was increased by using strains that were blocked for aI4α splicing (the ones noted above with the PZ25 bI4 mutation and *nam2* gene). Based on our experience with other strains blocked for aI4α splicing (Wenzlau *et al.*, 1989), these strains should overproduce the intron-encoded protein so that even relatively low levels of I–*Sce*II activity could be detected.

Each strain was grown on YEP–2% galactose medium. Mitochondria were purified and fractionated to yield Fraction 1a preparations from each strain (Wernette *et al.*, 1990). Each sample was assayed for I–*Sce*II activity and the results of representative assays are shown in Figure 5. The control strain and mutants P2–G4D, P2–G10D and P1–P1 had similar high levels of I–*Sce*II endonuclease activity (compare lane 1 with lanes 9–11). Mutants P1–G4D and P2–P2 had no detectable activity (lanes 2 and 7, respectively), even using 240 times the amount of protein used to assay the activity in the first four strains. From representative quantitative measures of these activities, we found that these mutations reduced the I–*Sce*II endonuclease activity by at least 10 000-fold. To determine if the P1 domain mutant extracts lacked I–*Sce*II endonuclease activity or contained an inhibitor, we assayed samples of the control extract containing 220–240 times more of the mutant extracts. As shown in Figure 5 (lanes 3 and 8), the addition of either mutant extract did not alter the activity of the wild-type enzyme. We conclude that those mutations blocked the I–*Sce*II endonuclease activity. This finding also suggests that the mutant protein binds poorly, if at all, to the DNA substrate, but this inference will require confirmation using more direct methods.

The extract from an 18°C culture of mutant P1–G10D has ~0.2% of the control level of I–*Sce*II endonuclease activity when assayed at 18°C (lane 4) but has at least 10 times less when assayed at 30°C (lane 5). We also analyzed a culture of cells carrying the P1–G10D mutation grown at 30°C, the temperature that blocks mobility completely. When assayed at 18°C, fraction 1a from that sample had ~0.04% of the control level of I–*Sce*II activity; when assayed at 30°C, it was inactive (results not shown). When mixed with the wild-type sample, fraction 1a from P1–G10D did not interfere with the wild-type I–*Sce*II activity assayed at 30°C (lane 6). These data show that the P1–G10D mutation renders I–*Sce*II activity temperature sensitive both *in vitro* and *in vivo*.

As described above, different strains were used to measure mobility and I–*Sce*II activity. Mobility was measured in strains expressing a wild-type level of intron-encoded protein, while I–*Sce*II endonuclease activity was measured in strains overexpressing that protein because of a tight splicing block. We have also measured mobility in crosses using the overproducing strains as the intron donors and found that strains lacking detectable I–*Sce*II activity (P1–G4D and P2–P2) have no detectable mobility in these crosses. In agreement with the data from Figure 3, the temperature-sensitive mutant P1–G10D was mobile in this experiment at 18°C but not at 30°C. Without overexpression, this mutant was somewhat debilitated for mobility in crosses at 25°C; when overexpressed, the level of mobility at that temperature was higher.

Discussion

The dodecapeptide motifs studied here are quite highly conserved and are used to define a large and interesting gene family. While nearly all of these reading frames have two such motifs, the motifs themselves do not predict whether endonuclease or maturase function is encoded by a given intron reading frame. It has been known for some time that missense mutations of the P2 motif can block maturase function. However, because the maturase mutations were studied in introns that lack endonuclease function, it was not known whether P2 motif mutations would alter endonuclease activity if tested in another intron. No missense mutants have been reported in a P1 motif of an intron reading frame that encodes either function. Nevertheless, a missense mutation in P1 of several inteins blocks their endonuclease function (Hodges *et al.*, 1992; Gimble and Stephens, 1995).

Here we have studied a set of mutant forms of P1 and P2 motifs in an intron reading frame that encodes an endonuclease activity (I–*Sce*II) and a latent maturase activity. In agreement with earlier studies of P2 mutations in bI3 and bI4, both P2 mutations of aI4α affected maturase function. It is quite interesting that the P2–G10D mutation, first discovered as a temperature-sensitive maturase defect in bI4, also causes a temperature-sensitive maturase defect in aI4α. Mutating the homologous glycine codons to aspartate in the P1 motif did not block maturase function, indicating that only one of the motifs is involved in the splicing function of the protein. The mutations of the P2 motif had no detectable effect on mobility, while the P1 motif mutations affected both mobility and I–*Sce*II activity. These are the first missense mutants affecting an intron-encoded endonuclease of the LAGLIDADG type,

and the P1–G10D mutant is the first conditional endonuclease mutant reported.

A surprising observation is that at non-permissive temperatures, the temperature-sensitive mutants of P1 and P2 are blocked for endonuclease or maturase activity, respectively, but not both. While we do not know whether the temperature-sensitive mutations affect folding during synthesis or subsequent unfolding of the completed protein, these findings, together with the other mutations that separate the functions of the protein, suggest that P1 and P2 behave as independent domains of the protein despite being separated by only 94 amino acids. We have shown previously that active I–*Sce*II is a dimer (Wernette *et al.*, 1990), so it is possible that some of these mutations affect the quaternary structure of the protein. It is also possible that these motifs function in the interaction of the protein with its specific substrate, i.e. RNA for maturase function and DNA for endonuclease activity. Indeed, an excess of extract protein from the P1–G4D or P2–P2 mutants did not have an effect on the endonuclease activity of the wild-type protein, suggesting that the mutant proteins may be deficient in their binding function.

Having found this apparent specialization of function between the two motifs, we also investigated a pair of mutants in which the intron reading frame has either two P1 motifs or two P2 motifs. Each of these complex mutants was defective for one function but not the other. Again, the strain with two P1 motifs (which effectively has seven mutations of the natural P2) was efficient for intron mobility and had a level of I–*Sce*II similar to that of the control strain, but lacked maturase function. The strain with two P2 motifs had the opposite phenotype.

The intron of the 23S rRNA gene of *Chlamydomonas reinhardii* chloroplast DNA is a mobile group I intron that encodes a 19 kDa endonuclease, I–*Cre*I (Durrenberger and Rochaix, 1993). It is atypical of the dodecapeptide family of intron-encoded endonucleases because it is a small protein with just one dodecapeptide motif, located near its N-terminus. In terms of position, it is likely to be a P1 motif. This intron self-splices and is also one of the few group I introns, besides those of yeast mtDNA, that has been tested for maturase activity: decisive *in vivo* data show that the reading frame is not essential for intron splicing so that it is not a maturase (Durrenberger and Rochaix, 1993). While it has not been shown that the P1 motif of I–*Cre*I is necessary for its endonuclease activity, that activity certainly does not depend on P2.

In contrast, in a recent study of the yeast intein-encoded endonuclease PI–*Sce*I, it was found that mutations of the conserved aspartate residues of both P1 and P2 block the activity (Gimble and Stephens, 1995). Data were presented indicating that the mutant proteins still bind the DNA homing site, and it was concluded that P1 and P2 are essential for catalysis. There appears to be some fundamental difference between I–*Sce*II and I–*Cre*I on the one hand, and PI–*Sce*I on the other, because the first two do not require a functional P2 motif for endonuclease activity while the latter does.

Materials and methods

Plasmids and site-directed mutagenesis

Plasmid pMTV is a derivative of pBLSK$^+$ containing 6.3 kb of mtDNA spanning the *COXI* gene from strain GII-0 (Wenzlau *et al.*, 1989; Figure 1). The *Bam*HI–*Eco*RI insert fragment from pMTV (Figure 1) was subcloned into pGEM3zf(–) to yield plasmid pMSV. Mutations of the aI4α reading frame shown in Figure 1 were constructed by the oligonucleotide-directed mutagenesis of plasmid pMSV using a Muta-Gene Kit (Bio-Rad) and confirmed by DNA sequencing (Sequenase, USB). Mutant alleles of aI4α were transferred to pMTV by subcloning the *Bam*HI–*Hin*dIII fragment (Figure 1) from the pMSV derivatives to the larger *Bam*HI + *Hin*dIII fragment of pMTV. A plasmid containing the wild-type *nam2* gene of yeast was obtained from Dr Alex Tzagoloff (Tzagoloff *et al.*, 1988). The *NAM2-1* allele that activates the latent aI4α maturase was made by site-directed mutagenesis. The *NAM2-1* allele was transplaced into the nuclear DNA of strain 161/PZ25 (Anziano *et al.*, 1982). Gly$^+$ transformants were selected and confirmed by PCR amplification of the gene and DNA sequencing.

Yeast strains and strain constructions

Strain ID41-6/161 (called 161; *MAT***a** *ade1 lys1*) has the mtDNA genotype, ρ$^+$ ω$^-$ *cap*^{r} *oli1*^{r}, with the seven-intron form of the *COXI* gene (introns 1–5γ) and the five-intron form of the *COB* gene (introns 1–5). 161/C2107 is a mit$^-$ mutant of strain 161, with a *COXI* deletion spanning from exon 2 of the *COXI* gene through the middle of intron 5β. 161/PZ25 is a mit$^-$ mutant of strain 161 that has a nonsense mutation that blocks the maturase function of bI4 (Anziano *et al.*, 1982).

Mitochondrial transformation (Belcher *et al.*, 1994) was carried out using plasmid pMTV and its mutated derivatives. Lawns of a ρ^o derivative of yeast strain MCC109 (*MAT*α *ade2-101 ura3-52 kar1-1*) were shot with a 5:1 mixture of pMTV and YEp352 (a 2μ plasmid carrying the *URA3* gene). Ura$^+$ nuclear transformants were selected (see Belcher *et al.*, 1994, for details). Plates with many Ura$^+$ colonies were then replica-mated to lawns of strain 161/C2107. Respiring recombinants were selected on glycerol-containing medium. Recombinants were subcloned and tested for nuclear markers; the desired mtDNA was usually obtained in the MCC109 nuclear background. Next, cytoduction was used to transfer the mtDNA into the nuclear background of strain 161. In these recombinants, the C2107 deletion of the *COXI* gene was replaced by the two-intron (CI34) *COXI* allele. Each transformed allele of the aI4α reading frame was confirmed by PCR amplification of the intron, followed by DNA sequencing. These manipulations resulted in a set of strains all having the 161 nuclear background and the same mtDNA except for the site-directed mutations of the aI4α reading frame.

Each of the above strains (MCC109 nucleus) was mated to a ρ$^-$ mutant carrying the *COB* gene from mutant PZ25 (161 nucleus). mit$^-$ recombinants with the MCC109 nuclear background were isolated. In these strains, the wild-type bI4 was replaced by bI4 containing the PZ25 mutation that blocks maturase function. The resulting recombinants (CI34–PZ25, CI34–P1G4D–PZ25, etc.) are respiration deficient and unable to splice both bI4 and aI4α. Next, these mtDNAs were transferred by cytoduction into the nuclear background of a derivative of strain 161 carrying the *NAM2-1* allele.

Assay for mobility of aI4α

The mobility of the wild-type and mutant alleles of aI4α was measured in crosses carried out and analyzed as described by Wenzlau *et al.* (1989). Here, the aI4α donor strains have the nuclear genome of strain 161 and the two-intron (CI34) form of the *COXI* gene (Figure 1). The recipient strain has the nuclear genome of strain GRF18 (*MAT***a** *leu2 his3*) and the mtDNA of *Saccharomyces capensis* [ρ$^+$ ω$^-$ *cap*^{s} *oli1*^{s} with a five-intron form of the *COXI* gene (introns 3α, 3γ, 4β, 5β and 5γ); Wenzlau *et al.*, 1989]. Yeast cultures were grown at 30°C, except where noted. The donor and recipient strains were grown and mated on YEPD medium. Diploids were selected in three sequential minimal medium (YNB) cultures containing 10% glucose. Cells from the third minimal medium culture were diluted into 200 ml of YEPD medium, and cells from the resulting culture were used in each experiment. Cells were plated on YEPD solid medium and the resulting colonies were replica-plated to medium containing 3% glycerol (YEPG) and YEPG medium containing 2 μg/ml oligomycin (Sigma). After incubation at 30°C for 3 days, each colony was then scored for oligomycin-resistant or -sensitive Gly$^+$ growth. The 200 ml culture was harvested. mtDNA was purified from the cells using a scaled-up version of the mini-prep procedure (Sherman *et al.*, 1986) and banded in CsCl containing bis-benzimide (Hudspeth *et al.*, 1980). mtDNA cleaved with *Bam*HI and *Eco*RI was fractionated on 0.8% agarose gels and transferred to Hybond-N paper (Amersham) using a Posi-Blotter (Stratagene). The blot was hybridized with a 5′ end-labeled oligonucleotide probe (nucleotides 8313–8329 of *COXI* exon 5; Bonitz *et al.*, 1980) to detect alleles of the *COXI*

gene (Figure 2). Proportions of the *COXI* alleles were determined by PhosphorImager scanning of the labeled blot.

Biochemical methods

Cellular RNA was purified and analyzed on RNA blots as described previously (Moran *et al.*, 1992, 1994).

For studies of I-*Sce*II levels, cells were grown on YEP medium with 2% galactose. Mitochondrial fractions were isolated as described in Wernette *et al.* (1990). Mitochondria were fractionated according to the protocol of Wernette *et al.* (1990) through the ammonium sulfate precipitation step to yield fraction 1a from each strain. Protein was measured by the method of Bradford (Bio-Rad). I-*Sce*II endonuclease activity was assayed using plasmid pRSX as a substrate, as described previously (Wernette *et al.*, 1990); 1 μg of double-stranded poly(dI–dC) (Pharmacia) was included in each 50 μl reaction. Reactions were phenol-extracted and fractionated on 1% agarose gels. The amount of product formed in each reaction was quantified using a PhosphorImager.

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