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A mutation in the GTPase domain of the large subunit rRNA is involved in the suppression of a -1T frameshift mutation affecting a mitochondrial gene in *Chlamydomonas reinhardtii*[*]

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Abstract

The *dum19* mutation isolated in *Chlamydomonas reinhardtii* is due to the deletion of one T at codon 152 of the mitochondrial *cox1* gene sequence. Phenotypically, the *dum19* mutant is characterized by a lack of cytochrome *c* oxidase activity and is unable to grow under heterotrophic conditions. A spontaneous pseudo-revertant that grows slowly in the dark was isolated from the *dum19* mutant strain. A genetic and molecular analysis allowed us to demonstrate that the revertant phenotype is the consequence of two additional mutations that together act as a frameshift suppressor: an *m* mutation affecting a mitochondrial gene other than *cox1* and an *n* mutation affecting a nuclear gene. On its own the *n* mutation does not act as a suppressor, whereas the *m* mutation very slightly compensates for the effect of the -1T mutation. Sequencing analysis showed that the *m* mutation affects the GTPase-associated domain of the large subunit (LSU) of mitochondrial rRNA. Surprisingly, two substitutions, A^{1090} to G and A^{1098} to C, were found in the LSU rRNA of the revertant, the latter one being already present in the *dum19* mutant strain itself. The A^{1090} to G substitution is thus involved in the suppression of the frameshift mutation, but it is not clear whether the change at position 1098 is also required for the expression of the suppressed phenotype. To our knowledge, this is the first example of a mutation in the GTPase-associated domain acting as a suppressor of a frameshift mutation.

Keywords : Suppressor ; Frameshift mutation ; GTPase center ; Mitochondrial LSU rRNA ; *Chlamydomonas*

Introduction

The identification of suppressors that compensate more or less efficiently for frameshift mutations is of major importance for our understanding of the basic mechanism of translation. Genetic and molecular studies, mainly performed in bacteria, phages, viruses and in the yeast *Saccharomyces cerevisiae,* have shown that +1 or -1 alterations in the reading frame can be suppressed by several quite different mechanisms. In addition to the familiar frameshift suppressor tRNAs with modified anticodons, other suppressors have also been identified, including mutant forms of ribosomal RNAs, ribosomal proteins, or translational factors, as well as modifications of the mRNA sequence that introduce "slippery" codons (recently reviewed by Farabaugh 2000).

The unicellular green alga *Chlamydomonas reinhardtii* presents interesting features for the study of the process of frameshift suppression, especially for mutations that affect the mitochondrial DNA. This very compact genome is only 15.8 kb long, and its sequence has been fully determined (reviewed by Remacle and Matagne 1998). It encodes seven proteins (components of different respiratory complexes and a reverse transcriptase-like protein), only three tRNAs and the RNAs of the large and small mitochondrial ribosomal subunits. The structures of the LSU and SSU rRNA genes are very bizarre, since each gene is discontinuous, being split into several pieces or "modules" (L1-L7, S1-S4) which encode individual rRNA segments (Boer and Gray 1988).

Several frameshift mutations affecting mitochondrial genes that encode components of the respiratory-chain complexes have been characterized at the molecular level (Remacle and Matagne 1998; Duby and Matagne 1999; Remacle et al. 2001). Phenotypically, mutants that lack an active ubiquinone-cytochrome *c* oxidoreductase or cytochrome *c* oxidase are obligate photoautotrophs: they are unable to grow heterotrophically (in the dark in the presence of acetate as an exogenous carbon source) despite their capacity to respire via the alternative oxidase. Two of these mutations affect subunit 1 of cytochrome *c* oxidase: *dum18* is a +1T mutation at codon

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145 and *dum19* is a -1T mutation at codon 152 of the *cox1* gene sequence (Colin et al. 1995). We previously isolated a revertant of *dum18* that is able to grow slowly in the dark. In that revertant, the +1T mutation was still present, and a base-pair substitution creating a rare codon that promotes frameshifting was found nearby in the *cox1* sequence (Remacle et al. 1998).

We here describe a slowly growing revertant derived from the mutant *dum19*. The revertant still has the -1T deletion in the *cox1* sequence but possesses two additional mutations, one of mitochondrial origin, the other affecting a nuclear gene, that allow the cells to grow in the dark. The mitochondrial suppressor mutation alone only slightly suppresses the effect of the *dum19* frameshift mutation. This mitochondrial suppressor mutation is localised in the highly conserved GTPase-associated domain of the LSU ribosomal RNA.

Materials and methods

Strains and culture conditions

The *dum19* mt^- mutant (strain 239) was isolated in 1994 from wild-type *C. reinhardtii* 137c mt^- (strain 2) after treatment with acriflavine (Colin et al. 1995).

The following strains were also used: wild-type mt^+ (strain 1); arg7-8 mt^+ (strain 115, auxotrophic for arginine); *dum19* mt^+ (*strain 238*); *dum19* NR^+ mt^- (strain 192); *arg7-7 dum19* mt^- (strain 248); and *arg4 dum19* mt^- (strain 256). The last four strains were obtained from crosses between strain 239 and other strains, at different times after the isolation of strain 239. Strains 266, 268, 280, 281, 616, 617, 618, 619, 620 and 621 were isolated in the course of this study.

Cells were grown on agar plates (15 g/l Gibco agar), under cool-white fluorescent light (80 or 5 µE per m^2/s) or in the dark at 25°C. TRIS-acetate phosphate (TAP) culture medium (Harris 1989) was used, supplemented with arginine at a concentration of 100 µg/ml, when required. A nitrogen-free minimal medium was also used for zygote maturation (VanWinkle-Swift 1977).

The number of cell divisions that had occurred during growth on solid agar medium in the dark was estimated from the mean number of cells per colony using a dissecting microscope. When the colonies contained more than 32-64 cells, the mean cell number per colony was determined as follows. A piece of agar bearing a defined number of colonies (between 80 and 120) was removed from a plate and the cells were dispersed in 5-10 ml of distilled water using a glass "hockey stick". After centrifugation, the cells were resuspended in 50 µl of 1 % formaldehyde and counted microscopically using a standard hemocytometer.

Genetic analysis of meiotic products

The transmission pattern of the mutations was determined by random analysis of meiotic products (Harris 1989) from ~ 50 germinated zygotes. After 10 days of culture, cells from individual meiotic clones were sampled and transferred to the wells of microtitre plates containing ~100 µl of distilled water. A few microliters of each cell suspension were spotted (42 at a time) onto agar plates, which were incubated under illumination or in darkness to determine the phenotypes of the meiotic products.

Sequence analyses

Total DNA was prepared according to the procedure of Newman et al. (1990). Three segments of the mitochondrial genome (GenBank Accession No. CRU03843: positions 8413-10401, 11170-11670 and 12723-15123) containing all rRNA modules and tRNA genes were amplified by PCR according to a standard protocol using a total of 10 primers and Taq DNA polymerase from Qiagen in a GeneAmp PCR System 9700 (PE Biosystems). Amplified products were sent to Genome Express (Paris, France) for sequencing on both strands. Overlapping segments were assembled with the aid of the GCG (Genetics Computer Group) software package (available at the Belgian EMBL Node, ULB/VUB) and final consensus sequences were aligned with the MAP alignment tool available online at the Human Genome Center (Baylor College of Medicine, Houston, Tex.).

For the analysis of the GTPase-associated region of the LSU rRNA, a segment of DNA (8892-9129) containing the major part of the L5 module was amplified by PCR and sequenced. A segment of the *cox1* gene (nucleotides 274-637) was amplified and sequenced following the same protocol.

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Results

As mentioned in the Introduction, the *dum19* mutant is unable to grow heterotrophically (in the dark on acetate as a carbon source) because of the inactivation of the cytochrome *c* oxidase. The enzyme defect results from the deletion of one T at codon 152 of the mitochondrial *cox1* coding sequence (Colin et al. 1995). A revertant (strain 266) that grows very slowly in the dark ($dk^{+/-}$ phenotype) appeared spontaneously five years after the isolation of the *dum19 mt* mutant. As the capacity to grow heterotrophically was not fully restored, it was assumed that the phenotype of strain 266 was not the result of a true reversion. Sequencing analysis confirmed that the -1T deletion was still present in the *cox1* sequence of strain 266 and that no other sequence alteration had occurred within ~200 bp upstream or downstream of the mutation site. Thus, the partial restoration of the capacity to grow in the dark was most probably due to the presence of an extragenic suppressor mutation (*su*).

Genetic analysis

In order to determine whether the *su* mutation affects a mitochondrial, plastid or nuclear gene, the revertant was crossed to various mt^+ strains. It is well established that in *Chlamydomonas*, the mitochondrial genes and the chloroplast genes are inherited from the mating-type minus parent and the mating-type plus parent, respectively, whereas the nuclear genes are transmitted according to the Mendelian mode (Dorthu et al. 1992; Remacle and Matagne 1998).

The same transmission pattern was obtained in three different crosses (Table 1, crosses 1-3): half of the meiotic progeny were unable to grow in the dark (dk^- phenotype), whereas the other half displayed a $dk^{+/-}$(slow growth) phenotype, identical to that of the 266 parental strain. A similar segregation pattern was obtained when an arg^- $dk^{+/-}$ mating-type minus progeny clone (strain 281) obtained in cross 2 was mated to strain 238 (Table 1, cross 4). In these four crosses, the *dum19* mitochondrial mutation carried by the mating-type minus parent had to be transmitted to all the meiotic products. As 50% of the progeny were phenotypically dk^- and 50% were $dk^{+/-}$ it was concluded that the *su* mutation affects a nuclear gene.

To confirm this conclusion, two slow-growing mating-type plus isolates, one arg^+ (strain 268) and one arg^- (strain 280) recovered in crosses 3 and 4, respectively, were crossed to the *dum19* mating-type minus strain (strain 239). As both 268 and 280 cells grew slowly in the dark, these strains were assumed to carry the nuclear *su* mutation. Hence, a segregation ratio of 50% dk^- :50% $dk^{+/-}$ was expected once again. Surprisingly, all the progeny clones from both crosses displayed a dk^- phenotype (Table 1, crosses 5 and 6). In the present case, *su* was not transmitted from the mating-type plus parent and thus behaved like a mitochondrial suppressor. Moreover, we noticed that in crosses 5 and 6, about half of the progeny colonies were pale green when grown under mixotrophic conditions (light + acetate), and that this abnormal phenotype was not linked to the presence of the *arg7-8* or $arg7\text{-}8^+$ allele (data not shown).

A new hypothesis had therefore to be proposed to reconcile the two contradictory conclusions derived from the results of the six crosses. The simpler model assumes that *su* actually corresponds to two independent mutations, *m* affecting a mitochondrial gene and *n* affecting a nuclear gene, both of which are required for the expression of the revertant phenotype. According to this model, the segregation ratios obtained in crosses 1-4 must be 50% *dum19 m* (dk^- phenotype) and 50% *dum19 m n* ($dk^{+/-}$ phenotype), since the *dum19 m* mitochondrial genome will be transmitted to all progeny (Table 1). In crosses 5 and 6, where the mating-type minus parent carries the *dum19* mutation only, the progeny will segregate 50% *dum19* (dk^-) and 50% *dum19 n* (dk^-), i.e. all progeny will be phenotypically dk^- cells (Table 1). The colonies displaying the pale green phenotype in the light could correspond to the new genotype *dum19 n*.

In order to verify this model and confirm that the pale-green colonies were genotypically *dum19 n*, two pale-green, mating-type plus isolates (strains 616a and 616b isolated from cross 5 in Table 1) were crossed to strain 266. As expected, all the progeny clones grew slowly in the dark and gave rise to green cells in the light (Table 2, cross 1).

In the next step in this genetic analysis, we attempted to determine whether a strain carrying a wild-type mitochondrial genome in association with the *n* nuclear mutation displays an abnormal phenotype. Wild-type mating-type minus cells (strain 2) were mated to *dum19 m n* mating-type plus cells (strain 268) (Table 2, cross 2) and the progeny colonies (all carrying the wild-type mitochondrial genome) were tested for their capacity to grow heterotrophically. Half of the progeny grew quite well, but not as fast as the wild-type cells [$dk^{(+)}$ phenotype], suggesting that the presence of the *n* mutation slightly reduces the capacity of the cells to grow in the dark. The same conclusion was drawn for the crosses between wild-type mating-type minus (strain 2) and

two pale-green isolates (strains 616a and 616b) (Table 2, crosses 3 and 4).

Table 1 *Genetic analysis of the suppression of the dum19 mutation*

Crosses (phenotype, strain)[a]	Phenotypic segregation ratio
1. *dum19 su mt*$^-$ (dk$^{+/-}$, 266) × wild-type *mt*$^+$ (dk$^+$, 1)	40 dk$^-$: 44 dk$^{+/-}$
2. *dum19 su mt*$^-$ (dk$^{+/-}$, 266) × *arg7-8 mt*$^+$ (dk$^+$, 115)	44 dk$^-$: 40 dk$^{+/-}$
3. *dum19 su mt*$^-$ (dk$^{+/-}$, 266) × *dum19 mt*$^+$ (dk$^-$, 238)	43 dk$^-$: 41 dk$^{+/-}$
4. *arg7-8 dum19 su mt*$^-$ (dk$^{+/-}$, 281) × *dum19 mt*$^+$ (dk$^-$, 238)	69 dk$^-$: 57 dk$^{+/-}$
5. *dum19 mt*$^-$ (dk$^-$, 239) × *dum19 su mt*$^+$ (dk$^{+/-}$, 268)	84 dk$^-$ (half pale green)[b]
6. *dum19 mt*$^-$ (dk$^-$, 239) × *arg7-8 dum19 su mt*$^+$ (dk$^{+/-}$, 280)	84 dk$^-$ (half pale green)[b]
Putative genotypes[0]	Expected meiotic segregation ratio
Crosses 1 and 2: *dum19 m n mt*$^-$ (dk$^{+/-}$) × WT *mt*$^+$ (dk$^+$)	*dum19 m* (0.5 dk$^-$): *dum19 m n* (0.5 dk$^{+/-}$)
Crosses 3 and 4: *dum19 m n mt*$^-$ (dk$^{+/-}$) × *dum19 mt*$^+$ (dk$^-$)	*dum19 m* (0.5 dk$^-$): *dum19 m n* (0.5 dk$^{+/-}$)
Crosses 5 and 6: *dum19 mt*$^-$ (dk$^-$) × *dum19 m n mt*$^+$ (dk$^{+/-}$)	*dum19* (0.5 dk$^-$): *dum19 n* (0.5 dk$^-$ pale green)

[a]The indicated crosses involving the strain 266 and derivatives (strains 268, 280 and 281) that are able to grow slowly under heterotrophic conditions (in darkness in the presence of acetate) were carried out, and the phenotypes of the progeny were determined. Phenotypes in the dark: dk$^-$, no growth; dk$^{+/-}$, slow growth; dk$^+$, normal growth (wild-type)
[b]When grown on acetate in the light
[c]Interpretation of the segregation ratios obtained in the six crosses listed, assuming that the suppressor *su* actually corresponds to two independent mutations, *m* affecting a mitochondrial gene and *n* affecting a nuclear gene (see text)

Table 2 *Genetic analysis of dum19 suppression in crosses involving WT and dum19 strains carrying m and/or n alleles*

Crosses (phenotype, strain)[a]	Meiotic segregation ratios (phenotypes and putative genotypes)
1. *dum19 m n mt*$^-$ (dk$^{+/-}$, 266) × *dum19 n mt*$^+$ (dk$^-$, pale green, 616 a and b)	84 dk$^{+/-}$ in each case (all *dum19 m n*)
2. WT *mt*$^-$ (dk$^+$, 2) × *dum19 m n mt*$^+$ (dk$^{+/-}$, 268)	44 dk$^+$: 40 dk$^{(+)}$ (WT: WT *n*)
3. WT *mt*$^-$ (dk$^+$, 2) × *dum19 n mt*$^+$ (dk$^-$, pale green, 616a)	45 dk$^+$: 39 dk$^{(+)}$ (WT: WT *n*)
4. WT *mt*$^-$ (dk$^+$, 2) × *dum19 n mt*$^+$ (dk$^-$, pale green, 616b)	42 dk$^+$: 42 dk$^{(+)}$ (WT: WT *n*)
5. *dum19 m n mt*$^-$ (dk$^{+/-}$, 266) × WT *n mt*$^+$ (dk$^{(+)}$, 618a, b and c)	84 dk$^{+/-}$ in each case (all *dum19 m n*)
6. *dum19 m mt*$^-$ (dk$^-$, 621) × WT *n mt*$^+$ (dk$^{(+)}$, 618a)	90 dk$^{+/-}$: 78 dk (*dum19 m n: dum19 m*)
7. *dum19 m mt*$^-$ (dk$^-$, 621) × *dum19 n mt*$^+$ (dk$^-$, pale green, 616a)	38 dk$^{+/-}$: 46 dk (*dum19 m n: dum19 m*)

[a]Phenotypes in the dark: dk$^-$, no growth; dk$^{+/-}$, slow growth; dk$^{(+)}$, subnormal growth; dk$^+$, normal growth (wild-type); pale green refers to cells grown on acetate in the light. See text for further details

Crosses between strain 266 and three different dk$^{(+)}$ isolates from cross 3 led to the isolation of 100% dk$^{+/-}$ progeny cells in each case (cross 5, Table 2), confirming the presence of the *n* mutation in these isolates (strains 618a, b and c).

Finally, a dk$^-$ mating-type minus isolate (strain 621) from cross 1 in Table 1, assumed to be genotypically *dum19 m*, was crossed to wild-type *n* mating-type plus (strain 618a) and to *dum19 n* mating-type plus (strain 616a) cells (crosses 6 and 7 in Table 2). The segregation patterns observed in both crosses fully confirmed our model.

Table 3 gives a summary of the phenotypes observed when the strains were grown under high- or low-intensity light or in the dark. It is interesting to note that cells of the strains *dum19 m mt*$^+$ (620) and *mt*$^-$ (621) hardly divide at all when incubated for 7 days in the dark but produce 4-16 cells after 2-3 weeks. Moreover, they grow better than *dum19* cells under dim light. Hence, the *m* mitochondrial mutation alone acts as a weak suppressor of the *dum19* mutation.

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Table 3 *Growth of the various strains cultivated on TAP agar plates under the conditions indicated*

Culture conditions[a]	Genotype (strain)					
	WT (1, 2)	*dum19* (238, 239)	*dum19 m n* (266, 268)	*dum19 n* (616, 617)	*dum19 m* (620, 621)	WT *n* (618, 619)
High light	5	3	3	3 (pale green)	3	5
Low light	3	0.5	1	0.2	1	2
Darkness	3 (9-10)	0 (0)	0.5 (3-4)	0 (0)	0-0.1 (0-2)	2 (8-9)

[a]Strains were grown under high-intensity light (80 µE per m^2/s), low-intensity light (5 µE per m^2/s) or in darkness. Growth was estimated after 3 days (high light), 5 days (low light) and 7 days (darkness). The size of the colonies is indicated on a scale of 0 (no growth) to 5 (very good growth). The figures in *parentheses* indicate the numbers of divisions that the cells had undergone after 7 days in the dark. Note that *dum19 m* cells carry out 2-4 cell divisions over a period of 2-3 weeks

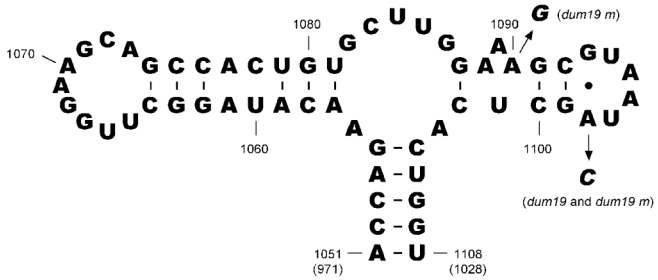
Identification of the m mitochondrial mutation

The *m* suppressor mutation affects a gene in the mitochondrial genome. To determine whether it modifies a component of the translational apparatus, i.e. one of the three tRNAs or a module of the rRNAs (see Introduction), these genes were sequenced in the two revertants (strains 266 and 268), as well as in wild-type 137c *mt*$^-$ (strain 2, see Materials and methods) as a control. The sequences were identical in all three strains, except in a segment of the L5 module corresponding to the GTPase-associated region of the LSU rRNA. In *E. coli*, this highly conserved 58-nucleotide domain, which adopts a typical secondary structure, has been shown to lie in a part of the ribosome which interacts with elongation factor G and tRNA, and is associated with GTP hydrolysis (Moazed et al. 1988).

Surprisingly, the sequence in the two revertants differed from the wild-type sequence by two substitutions: A^{1090} to G and A^{1098} to C (Fig. 1). To check whether either of these substitutions was already present in strain 239 (*dum19 mt*$^-$), from which revertants 266 and 268 derive, we sequenced the region containing the GTPase-associated domain in that strain, as well as in strains 616 and 617 (genotypically *dum19 n*) isolated during this study. In all three strains, only the change A^{1098} to C was present in the GTPase-associated region (Fig. 1). We also sequenced the same region from strains 620 and 621, containing only the *m* mutation: in those cases, both A^{1090} to G and A^{1098} to C substitutions were found. Hence, we conclude that the *m* suppressor mutation is specifically associated with the change A^{1090} to G.

We also wondered whether other *dum19* mutant strains (192, 238, 248, 256) from our stock collection contained a cytosine at position 1098 of the GTPase region. Strains 238, 248 and 256 were constructed several years ago from crosses involving the original *dum19 mt*$^-$ mutant, whereas the strain 192 was constructed more recently. We found that strains 238, 248 and 256 possessed an adenine at position 1098, as in the wild-type strain, whereas strain 192 had the C^{1098} substitution. Hence, the C^{1098} substitution was not present in the original *dum19 mt*$^-$ mutant and this change probably occurred secondarily during successive platings of strain 239.

Fig. 1 *Potential secondary structure of the GTPase-associated domain of the wild-type C. reinhardtii LSU ribosomal RNA. The sequence shown is that found in the wild-type strain (and in strains 238, 248 and 256), and is identical to the sequence published by Boer and Gray (1988). The numbering refers to the E. coli 23S ribosomal RNA. Numbers used for the C. reinhardtii sequence (Boer and Gray 1988) are given in parentheses. Changes in the nucleotide sequence found in the mutant strains studied here: strains 239, 616 and 617 (dum19 strains), A^{1098} to C; strains 266 268, 620 and 621 (dum19 strains carrying m), A^{1098} to C and A^{1090} to G (see text for further details)*



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Discussion

The *dum19* mitochondrial mutation corresponds to the deletion of a T at codon 152 of the *cox1* sequence. It causes the inactivation of the cytochrome *c* oxidase and prevents the mutant strain from growing under heterotrophic conditions. In the revertant 266 derived from *dum19* mutant, the deletion is still present and no frameshift mutation of opposite sign has been detected in the sequence of *cox1*. The genetic analysis presented here allowed us to demonstrate that the revertant phenotype actually results from two different additional mutations: an *m* mutation affecting a mitochondrial gene and an *n* mutation affecting a nuclear gene. The *n* mutation alone does not act as a suppressor, whereas, in the absence of *n*, *m* very slightly suppresses the effect of the deletion.

A sequence analysis of the mitochondrial genes encoding components (rRNAs, three tRNAs) of the translational machinery in the organelle showed that the *m* mutation affects the L5 module, which encodes a segment of the LSU rRNA. As mentioned in the Introduction, the two rRNA genes of the *C. reinhardtii* mitochondrial genome are split into pieces and it has never been demonstrated that these genes are functional. Boer and Gray (1988) have proposed that the rRNA segments probably assemble and associate noncovalently to produce the RNAs of the small and the large ribosomal subunits. The results obtained by Denovan-Wright and Lee (1995) in *C. eugametos* (a species in which the mitochondrial rRNA genes are also discontinuous) point in that direction. The present data demonstrate that the rRNA gene pieces in *C. reinhardtii* mitochondrial DNA are functional since the *m* mutation in the L5 module acts as a suppressor of the *dum19* mutation.

The identification of the *m* mutation led to a rather unexpected result. We detected in the four *dum19* strains carrying the *m* mitochondrial mutation (strains 266, 268, 620, 621) two substitutions affecting nucleotides 1090 and 1098 of the LSU rRNA sequence, respectively; one of these substitutions (A^{1098} to C) was already present in the *dum19* mutant (strain 239). Both mutations occur in the highly conserved GTPase-associated region of the LSU rRNA that interacts with elongation factor G and tRNA. The GTPase domain binds ribosomal protein L11 and is also the target site for the thiazole antibiotics thiostrepton and micrococcin, attesting to its functional importance (Rosendahl and Douthwaite 1994; Xing and Draper 1995).

The A^{1098} nucleotide present in the rRNA sequence of the *C. reinhardtii* wild-type strain is extremely well conserved, being present in over 95% of all LSU rRNA sequences, including those of mitochondria (Gutell et al. 1993). It interacts with G^{1093} via non-canonical hydrogen bonds, and might stabilize the hexaloop structure $G^{1093}UAAUA^{1098}$ for the purpose of tertiary interactions (Serra et al. 1994; Huang et al. 1996). In the *dum19* mutant (strain 239) and the revertant deriving from it, A^{1098} is replaced by C, thus allowing this nucleotide to form a conventional three-hydrogen bond structure with G^{1093}. By using directed site-specific mutagenesis in *E. coli*, Xu and Murgola (1996) introduced substitutions at nucleotides 1093 and 1098 and found that several combinations, including the G^{1093}-C^{1098} base pair, cause suppression of a UGA nonsense mutation in the *trpA* gene, as well as inhibition of growth at high temperature. However, as the 1093-1098 pairs that render the loop more stable (G-C) or less stable (C•A) than G•A produced the suppressed phenotype, the authors concluded that the thermostability of the 1093-1098 hexaloop may be less important for ribosome function than the identity of the bases themselves. The presence of the A^{1098} to C substitution in the *dum19* mutant (strain 239) is puzzling, since it is difficult to assign a role to this change in the rRNA sequence. The substitution does not produce a revertant phenotype since the mutant has no cytochrome *c* oxidase activity and is unable to grow under heterotrophic conditions (Colin et al. 1995). Moreover, as other *dum19* strains (238, 248, 256) from our stock collection possess the wild-type adenine at position 1098, the C^{1098} is not required to ensure the viability of the *dum19* mutant. In short, the mitochondrial *m* suppressor mutation actually corresponds to the change of A^{1090} to G, but we do not know whether this substitution acts alone or in conjunction with the A^{1098} to C change to cause the suppression. If this last substitution is not required for the suppressed mutant phenotype, a G^{1090} A^{1098} pseudorevertant could probably be isolated from a *dum19* A^{1090} A^{1098} *n* strain.

The crystal structure of the highly conserved complex between the 58-nucleotide domain of the bacterial LSU rRNA and the RNA-binding domain of ribosomal protein L11 has been recently published (Conn et al. 1999; Wimberly et al. 1999). It reveals a precisely folded RNA structure that is stabilized by extensive tertiary contacts and contains a large interior core formed by stacked planes of bases. U^{1060}, A^{1088}, A^{1089}, A^{1090} and U^{1101}, all of which are conserved in the *C. reinhardtii* sequence, lie in a plane across the middle of the molecule, and additional stacks of three or four bases are found above and below (Conn et al. 1999). In particular, A^{1090} N6 is hydrogen-bonded to A^{1089} N3 in what was called an "A-A sidestep". The replacement of A^{1090} by G specific to the *m* mutation could modify this structural organization and be responsible for reduced translational fidelity, thus allowing frameshift suppression.

To our knowledge, frameshift suppressor mutations affecting the GTPase region of the LSU rRNA have never been described. The mutations in ribosomal RNAs so far described to act as frameshift suppressors occur in three regions of the SSU rRNA, and in the α-sarcin loop around residue 2660 of the LSU rRNA (O'Connor and Dahlberg 1996; O'Connor et al. 1997). Like the GTPase region, the α-sarcin loop is among the most highly conserved of all RNA sequences and has been identified as one of the sites in the ribosome that interacts with elongation factor G (Moazed et al. 1988; Munishkin and Wood 1997). Both domains therefore seem to play a crucial GTPase-related role, probably in EF-G-dependent translocation of mRNA and tRNAs bound to ribosomal A and P sites. We found that the efficiency of the *m* suppressor mutation was considerably increased when the *n* mutation was also present in the mutant strain. Even though the nuclear gene affected by the *n* mutation has not been identified, one could postulate that it encodes a protein that interacts with the GTPase domain, such as the L11 ribosomal protein or the elongation factor G. Interestingly, the *n* mutation alone is slightly deleterious, since it reduces the rate oft heterotrophic growth of cells carrying a wild-type mitochondrial genome and determines in the light a pale-green phenotype when associated with the *dum19* mutation. Identification of the gene carrying the *n* mutation will be difficult but is theoretically possible. It would require making a cosmid library of nuclear DNA from a suppressed strain and using it to transform a strain like 621, which contains *dum19* and *m* mutations and grows extremely slowly in the dark.

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