The Mitochondrial Reading Frame RF3 Is a Functional Gene in *Saccharomyces uvarum*

Bertrand Séraphin, Michel Simon, and Gérard Faye

From the Institut Curie, Section de Biologie, Centre Universitaire, Bâtiment 110, 91405 Orsay, France

The yeast mitochondrial genome contains three reading frames, RF1, RF2, and RF3, which are related to the group I maturases, though they are not intronic sequences. In the *Saccharomyces cerevisiae* strain D273-10B/A, these reading frames are interrupted by G+C-rich sequences (GC clusters) which break the frames. In the present work we described a *Saccharomyces uvarum* strain which possesses a RF3 continuous sequence devoid of GC clusters. Moreover, our results strongly suggest that in the same strain RF2 and RF1 are also continuous sequences. As all three RFs belong to transcription units which are highly expressed, it is most reasonable to assume that RF1, RF2, and RF3 are functional genes. Furthermore, we have discovered a rule which seems to explain the transposition of GC clusters, considered as mobile elements, in the mitochondrial genome.

In *Saccharomyces cerevisiae*, the mitochondrial genes encoding 21 S ribosomal RNA, cytochrome *b*, and subunit 1 of cytochrome *c* oxidase are split by intronic sequences. In the largest mitochondrial genomes 13 introns have been identified, 10 of them containing an open reading frame encoding the maturases (Jacq et al., 1982). These 13 intron sequences can be arranged into two distinct groups (Michel et al., 1982; Michel and Dujon, 1983). Members of the same group share distinctive nucleotide stretches and may be folded up into similar secondary structures (Michel and Dujon, 1983; Davies et al., 1982; Waring and Davies, 1984). Two short conserved oligopeptide sequences which are characteristic of the open reading frame of group I introns were called P1-P2 by Michel et al. (1982), decapeptides in Waring et al. (1982), and LAGLI-DADG by Hensgens et al. (1983). Genetic studies and biochemical analyses have disclosed or suggested four different functions for the intron-encoded proteins (Kotylak et al., 1985). (a) Some maturases are essential for the correct splicing of mitochondrial intervening sequences (Banoque et al., 1986). (b) The maturases of group II introns show a significant homology with reverse transcriptase (Michel and Lang, 1988) and are supposed to play a role in the deletion of intervening sequences from the genes (Gargouri et al., 1983). (c) The intron-encoded protein in the 21 S rRNA intron is necessary for the duplicative transposition of this intron (Colleaux et al., 1986). (d) A maturase was shown recently to be involved in the induction of genetic recombination between homologous exons (Kotylak et al., 1985).

Besides the intron-encoded proteins, three open reading frames related to the group I maturases exist which are not intron sequences (Coruzzi et al., 1981; Michel, 1984; Séraphin et al., 1985). They were named RF1, RF2, and RF3. They are part of three multigenic transcription units, and each of them is, respectively, located downstream of a gene which is not mosaic: ox1 (subunit 2 of the cytochrome oxidase); ox2 (subunit 3 of the cytochrome oxidase); and oli2 (subunit 6 of the mitochondrial ATPase) (Coruzzi et al., 1981; Thalenfeld et al., 1983; Michel, 1984; Simon and Faye, 1984b).

The organization of the RF2 and RF3 sequences, in the strains so far studied, is very peculiar (Fig. 1). Each of them is composed of four fairly large open reading frames which overlap within G+C-rich sequences (GC clusters) (de Zambrocy and Bernardi, 1986). Furthermore, a shift of +1 or −1 base was found between each pair of consecutive reading frames. A unique GC cluster is present in the RF1 sequence that could also introduce a −1 frameshift since some uncertainty remains in the sequence published by Coruzzi et al. (1981). The odd structure of the RF2 and RF3 sequences suggests that they could be silent genes inactivated by the insertion of GC clusters and that the loss of their functions could be compensated for by other coding sequences located elsewhere in the mitochondrial genome. However, if they are functional genes we have to think of some mechanisms (acting on transcription or translation) able to introduce changes in the reading frames at the level of the GC clusters so as to correct the frameshifts. In the present work, we describe a yeast strain which possesses a continuous RF3 sequence, i.e., it is not interrupted by GC clusters. Moreover, our results strongly suggest that in the same strain RF2 and RF1 are also continuous sequences devoid of GC clusters. As all three RFs belong to transcription units which are highly expressed (Simon and Faye, 1984b; Thalenfeld et al., 1983; Coruzzi et al., 1981) it seems most reasonable to assume that RF1, RF2, and RF3 are functional genes. Furthermore, we have discovered a rule which appears to explain the transposition of GC clusters in the mitochondrial genome.

**Materials and Methods**

**Yeast and Bacterial Strains**—The yeast strains used are listed in Table I. The *E. coli* used are strains RR1 (Bolivar et al., 1977), JM105 (Yanisch-Perron et al., 1985), and JM103 (Messing et al., 1981).

**Preparation of DNA**—Mitochondrial DNA of strains D273-10B/A, NCYC74, and DS802 was purified by bismethidime CsCl buoyant density centrifugation (Simon and Faye, 1984a).

The rapid yeast DNA procedure used was adapted from the method described by Davis et al. (1980).

**Cloning and Sequencing**—Preparation of plasmid DNA, restriction analysis, and cloning in *Escherichia coli* were carried out as described
Nonintronic Maturase-like Genes in Yeast Mitochondria

FIG. 1. A, organization of the multigenic transcription units containing RF5 (a), RF2 (b), and RF1 (c). Genes are represented by thick open boxes, and wavy lines symbolize primary transcripts. Arrowheads indicate the RNA cleavage sites AATAAATTCTCT, oxil, oxil2, and oxil are, respectively, the subunits 1, 3, and 2 of cytochrome c oxidase. aapl, ATPase subunit 8; OLi2, ATPase subunit 6. B, organization of the RF1, RF2, and RF3 sequences of strain D273-10B/A. RF1, RF2, or RF3 DNA segments are depicted by thick open boxes. Thinner open boxes drawn as arrows represent open reading frames. The position of GC clusters is shown in each RF. +1 (or -1) indicates a +1 (or -1) frameshift introduced in the reading frames by the GC clusters. If some mechanism is able to correct these frameshifts, then translation products (corresponding to the junction of the reading frames) are formed, either for RF1, RF2, or RF3.

Southern and Northern Transfers—Southern transfers were carried out as described by Maniatis et al. (1982). Mitochondrial RNA of yeast cells grown in 1% yeast extract, 1% Bactopeptone, 2% raffinose, 0.1% glucose was extracted as described (Faye and Simoa, 1983). RNA transfers to nitrocellulose filter and RNA-DNA hybridization were performed according to the Southern method as modified by Thomas (1980).

S1 Mapping—S1 nuclease protection experiments were carried out according to Sharp et al. (1980).

RESULTS

Southern Blots—The mitochondrial DNA of 20 laboratory yeast strains from different sources (Table I) was analyzed in order to know whether RF1, RF2, or RF3 sequences devoid of GC clusters could be found. The total DNA from each strain, extracted by a rapid procedure, was digested with appropriate restriction endonucleases. The DNA fragments obtained were then separated on an agarose gel, transferred onto a nitrocellulose filter using the method described by Thomas (1975), and then hybridized with probes labeled by nick translation. The hybridization pattern from each strain was then compared to that of strain D273-10B/A which was used as a reference pattern since the nucleotide sequence of the RF1, RF2, and RF3 regions was mainly determined in this strain (Coruzzi et al., 1981; Michel, 1984; Séraphin et al., 1985).

To study the organization of the RF1 region, the DNA preparations were digested with the enzymes PvuII and HpaII. The probe used, to reveal the Southern blots, was the mitochondrial DNA from the rho- strain DS302 (Coruzzi et al., 1981). The autoradiogram is shown in Fig. 2. An important point to note is that the RF1 sequence is present in the 20 strains studied. Pattern A is shared by strains D273-10B/A (lane 1), 777-3A (lane 2), and X004-3A (lane 10). Twelve out of the 20 strains studied exhibit the pattern B where the 616-bp mtDNA fragment is cut by the HpaII (or PvuII) restriction enzyme to generate two fragments about 220- and 400-bp long. Among the other patterns, pattern D obtained with Saccharomyces uvarum (lane 5) is the most interesting; there is no HpaII site in its RF1 open frame. This means that it is not interrupted by a GC cluster since the HpaII sites are found in the corresponding G+C-rich sequence of D273-10B/A.

The RF2 region was analyzed by digesting the DNA preparations with EcoRI, PvuII, and MboI (Fig. 3). The fragments obtained were probed with the plasmid pN-RF2 (cf. “Materials and Methods”). Only strain M41-7B (lane 20) displays the pattern A which is characteristic of strain D273-10B/A (lane 1). In the strains showing pattern B, the band corresponding to the 468-bp-long fragment of strain D273-10B/A is slightly larger indicating that a second GC cluster might be present in this fragment. S. uvarum (lane 5, pattern C) is the sole strain in which bands corresponding to the 498- and 468-bp-long fragments show a small reduction in size suggesting the absence of GC clusters in its RF2 open reading frame. The RF2 sequence is absent in half of the strains studied (pattern E). In fact, it is known that the RF2 segment is missing in some strains of S. cerevisiae (Thalenfeld et al., 1983; Fox and Reif, 1983).

Concerning the RF3 region, the DNA preparations were

1 The abbreviation used is: bp, base pair(s).
The probe used was the plasmid pN1. Eleven strains do not possess the RF3 sequence. We have previously sequenced the mitochondrial DNA of one of them, strain JM6, in the region immediately downstream of the oli2 gene and in that overlapping the deleted RF3 sequence (Séraphin et al., 1985). Seven strains share the pattern of D273-10B/A (Fig. 4, lane 1). There is no HpaII site in the RR8 EcoRI fragment of S. uvarum (lane 5), which is about 100 bp shorter than the corresponding RR8 fragment of D273-10B/A. Thus, it seems that the RF3 open reading frame of S. uvarum does not contain any GC clusters. The mitochondrial genome of strain 4870-6B (lane 8) possesses a RR8 EcoRI fragment which is about 1.8 kilobases long (data not shown), whereas the pN1 probe reveals only three EcoRI-HpaII fragments.

Table II shows that the strain polymorphism, with respect to the presence or absence of the RF2 and RF3 sequences in the mitochondrial genome and the presence or absence of GC clusters in the three regions studied is rather high, since 11 different arrangements are observed. At least three among the seven strains which share the same arrangement (arrangement VII) were obtained from the Yeast Genetic Stock Center (Berkeley). It is known that many mutant and segregant strains from this collection have been derived from the same progenitor strain (Mortimer and Johnston, 1986). This could explain why arrangement VII was observed more frequently on our Southern blots.

S. uvarum appears to be the bona fide candidate strain we were looking for; it seems to have no GC clusters in its RF1, RF2, and RF3 reading frames. However, this strain does contain numerous GC clusters elsewhere in its mitochondrial genome; whereas 200 GC clusters are scattered throughout the mitochondrial genome of S. cerevisiae (de Zamaroczy and Bernardi, 1986), their number is only slightly smaller in S. uvarum. Thus, it seems that the RR8 restriction fragment from this strain was cloned in the vector M13lg130 and was sequenced according to the strategy described under "Materials and Methods." The 1673-bp-long RR8 fragment does not contain any of the three G+C-rich clusters which punctuate the RR8 sequence of D273-10B/A. The comparison of the two RR8 sequences shows that in strain D273-10B/A the three GC clusters have been inserted at three AG sites. The RF3 open reading frame of strain NCYC74 is continuous and has the potential to code for a 476-amino acid-long protein, with a calculated molecular weight of 58,870 (Fig. 5). Except for the three GC clusters, only one nucleotide transition was observed between strains D273-10B/A and NCYC74.
namely a G for an A changing a glycine codon for a glutamic acid, respectively. This confirms that S. cerevisiae and S. uvarum are phylogenetically related and should belong to a single species. In fact, the mitochondria of S. uvarum can be transferred by cytoduction (Lancashire and Mattoon, 1979). The number marking each lane refers to the name of the strain as it is written in Table I, whereas the letters refer to the restriction maps depicted in the lower part of the figure. The sizes of the PvuII-HpaII restriction fragments from D273-10B/A (lane 1), as known from the sequence data, are indicated in the margin. Concerning the maps B-E, only the restriction fragments differing in size from those of D273-10B/A are indicated. The small black boxes and the small open boxes represent GC clusters whose existence is certain or assumed, respectively. H, HpaII restriction sites; P, PvuII restriction sites; OX11, gene of the subunit II of cytochrome oxidase; RF1, RF1 gene.

Part of the RF2 sequence of strain NCYC74 located between sites HindIII and XbaI as well as the sequence of a segment overlapping the third GC cluster was determined (Fig. 5). Both sequences confirm that the three GC clusters of RF2 in strain D273-10B/A is also an AG sequence (taking into account that the last two GC clusters are oriented in a direction opposite to that of the first one (Michel, 1984)). Except for the GC clusters, the two RF2 fragments (the total length of which is 600 bp) we have sequenced in NCYC74 are identical to the corresponding sequence of D273-10B/A.
Nonintronic Maturase-like Genes in Yeast Mitochondria

clusters, the RF3 coding sequences are identical in strains D273-10B/A andNCYC74 is intriguing; if RF3 is functional in strain D273-10B/A we have to suppose that some mechanism(s) eliminates the phase discontinuities introduced by the GC clusters. One possibility is that a cut and splice process would excise the G+C-rich sequences from the RF3 transcript and would restore the continuity of the frame. To test this or 5'-ends and overlapping either the first or the third GC clusters, the RF3 coding sequences are identical in strains D273-10B/A andNCYC74 is intriguing; if RF3 is functional in strain D273-10B/A we have to suppose that some mechanism(s) eliminates the phase discontinuities introduced by the GC clusters. One possibility is that a cut and splice process would excise the G+C-rich sequences from the RF3 transcript and would restore the continuity of the frame. To test this possibility we performed S1-mapping experiments by hybridizing total mitochondrial RNA of strain D273-10B/A to selected restriction DNA fragments labeled either at their 3'- or 5'-ends and overlapping either the first or the third GC clusters. Each resulting DNA-RNA hybrid was then subjected to S1 nuclease trimming, and the length of the nuclease-resistant fragments produced was analyzed on polyacrylamide-urea gel slabs. The probes used are depicted in Fig. 8. Using as a probe the RsaI-AvaII fragment 5'-labeled at its AvaII end (Probe 1, Fig. 8), an S1 nuclease-protected fragment was detected ending just at the TAGT sequence of cluster I. The 3'-end-labeled AhaIII-AhaI11 probe overlapping cluster I (probe 3, Fig. 8) located a cleavage near the 3'-end of the AAGAG sequence (see also Fig. 9). Similar results were obtained for cluster III with the RsaI-Hinfl probe 5'-labeled at its Hinfl end (probe 2, Fig. 8) and with the 3'-end-labeled AhaIII-PstI probe (Probe 4, Fig. 8). This suggests that the DNA probes were protected by RNA species retaining the GC-rich sequences either at their 5'- or 3'-end (cf. Fig. 8, lower part). These results are unexpected. We rather anticipated that probes hybridized to spliced RNA molecules would be cleaved by S1 nuclease on both sides of the excised GC-rich RNA sequences or in the loop of their hairpin structure (de Zamaroczy and Bernardi, 1986). In any case, signals corresponding to size-protected probes were in large excess over those produced by the hybridization to "cleaved" RNA molecules with probes 1, 2, and 4.

![Fig. 4. Southern analysis of mitochondrial DNA in the RF3 region.](image)

The DNA preparations were digested with EcoRI and HpaII. The probe used was the EcoRI-EcoRI fragment from strain NCYC74 which includes the RF3 sequence. The autoradiograms of 7 out of 20 strains studied are shown. Strains 4, 6, 7, 9, 11, 12, 14, 16, 18, and 19 lack the RF3 sequence (pattern B). The dotted fragment drawn on the restriction map B corresponds to the DNA sequence localized immediately downstream of the EcoRI-EcoRI fragment of strains such as D273-10B/A that contain the RF3 sequence (Séraphin et al., 1986). The HpaII sites on restriction map D were not accurately located. E, EcoRI; H, HpaII.

![Fig. 5. Nucleotide sequence of the RR8 fragment of strain NCYC74. The last 27 amino acid residues of oli2 and the sequence of the RF3 putative protein are indicated by the single-latter amino acid code, using the codon recognition rule of yeast mitochondria where TGA is used for tryptophan, CTN for threonine, and ATA for methionine. The comparison of the RR8 sequences of strains NCYC74 and D273-10B/A shows that in this latter strain the three GC clusters have been inserted at three AG sites. These signals are arranged (indicated by Roman numerals) were observed in the twenty strains studied.

| Table II |
| --- |

| Strain polymorphism in the RF1, RF2, and RF3 regions |

The numbers referring to the name of strains (Table I) are written on the first line. The letters indicate the different kinds of pattern observed in the Southern experiments described in Figs. 2, 3, and 4. Eleven different arrangements (indicated by Roman numerals) were observed in the twenty strains studied.
Nonintronic Maturase-like Genes in Yeast Mitochondria

Fig. 6. Nucleotide sequence of two segments A and B of the RF2 gene from strain NCYC74. The upper part of the figure shows a map of the RF2 region. The insertion sites of the RF2 GC clusters of strain D273-10B/A are indicated by the symbol \( \gamma \). A and B lines localize the DNA segments which have been sequenced. The comparison of the sequence of these segments from strain NCYC74 with the corresponding sequence from strains D273-10B/A (Michel, 1984) shows that in this latter strain the three GC clusters have been inserted at three AG sites (note that in strain D273-10B/A the last two GC clusters are oriented in a direction opposite to that of the first one). These sites are underlined and marked with the symbol \( \gamma \). The RF2 reading frame is continuous in the sequences A and B, H, HindIII; X, XbaI; S, SpeI; E, EcoRI.

Fig. 7. Northern blot analysis of the mitochondrial RNA of strains BS104-1 and BS104-9. Strains BS104-1 and BS104-9 are isonuclear and contain the mitochondria of D273-10B/A and NCYC74, respectively. These strains were built by cytoduction (Lancashire and Mattoon, 1979). First, K5/2 was crossed with either D273-10B/A or NCYC74; two cytoductants, K273-1 and KUVA32, harboring the nucleus of K5/2 and the mitochondria of D273-10B/A or NCYC74, respectively, were isolated. Strains K273-1 and KUVA32 were crossed with GRTF18/2, and two cytoductants BS104-1 and BS104-9 containing the nucleus of GRTF18/2 were obtained. The estimated sizes of the major transcripts (in bases) extrapolated from that of the 21 S rRNA (3270 bases) and 15 S rRNA (1680 bases) used as size markers are indicated in the margin. a, BS104-9; b, BS104-1. In the lower part of the figure the major transcripts (4100 and 3500 bases) are localized on a map of the oli2-RF3 mitochondrial region. The wavy lines schematize transcripts. Arrowheads indicate the position of the dodecamer AATAATATTCTT (cf. Simon and Faye, 1984). E, EcoRI.

Discussion

Our studies lead to two new results. First, they demonstrate that the RF3 open reading frame is continuous and transcribed in strain NCYC74 and clearly suggest that RF1 and RF2 are not interrupted by GC clusters in that strain. Consequently, these genes must be functional. Second they bring to light a rule governing the translocation of GC clusters considered as mobile elements.

RF1, RF2, and RF3 Are Maturase-like Genes—The fact that on the one hand RF1, RF2, and RF3, which are continuous open reading frames in S. uvarum, are interrupted by GC clusters that break the continuity of the phases in strain D273-10B/A and that, on the other hand, GC clusters apart, these frames may potentially code for 500 amino acid long proteins leaves us perplexed; are these genes nonfunctional in strain D273-10B/A or are there any mechanisms able to correct the frame disorder introduced by the GC clusters? We have tested the possibility that a splicing mechanism could excise the GC clusters. Our S1 nuclease-mapping experiments which were done with probes overlapping RF3I and RF3III GC clusters seem to rule out this possibility. The RNA species observed might then be intermediates in the degradation of the RF3 mRNA. As an alternative explanation the transcriptional or translational machinery could produce slippage and frameshifting when going across the G+C-rich sequences (Benne et al., 1986; Clare and Farabaugh, 1985).
The insertion sequence of GC clusters. The insertion sequences of GC clusters in the RF3, RF2, and RF1 rRNA, are underlined. GC clusters are aligned to illustrate their sequence similarity. The sources of the sequences are as follows: 21S rRNA (Dujon, 1980); ori (de Zamaroczy et al., 1984); RF2 (Michel, 1984); RF3 (Séraphin et al., 1985). The sequence of aI5p of strain KL14-4A was compared with that of strain 777-3A (Séraphin et al., unpublished results). The numbers indicating the position of GC clusters in aI5p refer to the sequence published by Hensgens et al., 1983. Note that the sequence between nucleotides 2325 and 2316 is TATTAATAAT in KL14-4A. Stars mean that the sequences were not determined.

We have shown that some strains do not possess either RF3 or RF2 and RF3. How do such strains compensate for the lack of these genes? The RF1, RF2, and RF3 reading frames are related to those of the intron maturases and that some of them may take the role of the RF2 or RF3 gene products. It has been suggested that the mitochondrial introns could result from the insertion of mobile elements both within and outside the mitochondrial genes (Hensgens et al., 1983). The gene(s) born by this putative transposon (perhaps related to the retrovirus) would have evolved to the present day maturases. This model could explain why maturases may share some properties with RF1, RF2, and RF3 gene products.

GC Clusters Are Mobile Elements—Since from one strain to another the same mitochondrial sequence is or is not interrupted by GC clusters (Dujon, 1980; Sor and Fukuhara, 1982; de Zamaroczy and Bernardi, 1986) we have to suppose that these G+C-rich sequences are either themselves mobile elements or are remnants from former mobile elements whose terminal repeats would be GC clusters. Our finding that seven GC clusters are optional in the RF1, RF2, or RF3 sequences strongly supports these hypotheses. Furthermore, we have discovered a rule which seems to explain the insertion of GC clusters. Fig. 9 clearly shows that the target site is the AG sequence. This AG sequence appears duplicated on either side of GC clusters (except for RF3II where an additional A is present, although a sequencing error is not excluded, because the palindromic sequence of GC clusters may cause “band compression” during electrophoresis (Frank et al., 1981)). However, we do not know whether one AG is brought about by the GC clusters or whether the AG sequence of the host DNA is duplicated during the insertion process as happens for the bacterial transposable elements (Calos and Miller, 1980). Most of the GC clusters of the a1 and a2 families (about 50 clusters), as described by de Zamaroczy and Bernardi (1986), are delimited by AG sequences which indicates that the insertion of a large number of GC clusters should obey the rule we have discovered.

The actual function of GC clusters has not yet been definitively established though it has been suggested that some of them are elements of replication origins (de Zamaroczy and...
References

Banroques, J., Delahodde, A., and Jacq, C. (1986) Cell 46, 837-844
Benne, R., Van Den Burg, J., Braekenhoff, J. P. J., Sloof, P., Van Boom, J. H., and Tromp, M. C. (1986) Cell 46, 819-826
Bolivar, F., Rodriguez, P., Greene, P., Betlach, M., Heykens, H., Boyer, H., Erosa, J., and Falkow, S. (1977) Gene (Amst.) 2, 95-113
Bolotin-Fukuhara, M., and Fukuhara, H. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 4608-4612
Calos, M., and Miller, J. (1980) Cell 20, 579-595
Clare, J., and Farabaugh, P. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 2829-2833
Colleaux, L., d'Auriol, L., Betermier, M., Cottarel, G., Jacquier, A., Galibert, F., and Dujon, B. (1986) Cell 44, 521-533
Coruzzi, G., Bonitz, S., Thelenfeld, B. E., and Tsagoloff, A. (1981) J. Biol. Chem. 256, 12780-12787
Davies, R., Waring, R., Ray, J., Brown, T., and Scacciozchio, C. (1982) Nature 300, 719-724
Davies, R., Thomas, M., Cameron, J., St. John, T., Scherer, S., and Padgett, R. (1980) Methods Enzymol. 65, 404-411
de Zamaroczy, M., and Bernardi, B. (1986) Gene (Amst.) 41, 1-22
de Zamaroczy, M., Faugeron-Fonty, G., Baidacchi, G., Goursot, R., and Bernardi, G. (1984) Gene (Amst.) 32, 439-457
Donahue, T. F., Davies, R. S., Luchini, G., and Fink, G. R. (1983) Cell 32, 89-98
Dujon, B. (1980) Cell 20, 185-197
Faye, G., and Simon, M. (1983) Cell 32, 77-87
Fourey, F., and Tsagoloff, A. (1976) Mol. Gen. Genet. 149, 43-50
Fox, T., and Beil, M. (1983) in Mitochondria 1983 (Schweyen, R., Wolf, K., and Kaudewitz, F., eds) pp. 441-448, Walter de Gruyter, Berlin
Frank, R., Muller, D., and Wolff, C. (1981) Nucleic Acids Res. 9, 4967-4979
Gargouri, A., Lazowska, J., and Slonimski, P. (1983) in Mitochondria 1983 (Schweyen, R., Wolf, K., and Kaudewitz, F., eds) pp. 259-268, Walter de Gruyter, Berlin
Hensgens, L., Bonen, L., de Haan, M., van der Horst, G., and Grivell, L. (1983) Cell 32, 379-389
Hinnen, A., Hicks, J., and Fink, G. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 1929-1933
Jacq, C., Pajot, P., Lazowska, J., Dujardin, G., Claisse, M., Groudinsky, O., de la Salle, H., Grandchamp, C., Labouesse, M., Gargouri, A., Guiard, B., Spyridakis, A., Dreyfus, M., and Slonimski, P. (1982) in Mitochondrial Genes (Slonimski, P., Borst, P., and Attardi, G., eds) pp. 155-183, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Kotylak, Z., and Slonimski, P. P. (1977) in Genetics and Biogenesis of Mitochondria (Bandlow, N., Schweyen, R. J., Wolf, K., and Kaudewitz, F., eds) pp. 83-89, Walter de Gruyter, Berlin
Kotylak, Z., Lazowska, J., Hawthorne, D., and Slonimski, P. (1985) in Achievements and Perspectives of Mitochondrial Research (Quagliarelli, E., Slater, E. C., Palmieri, P., Saccone, C., and Kroon, A. M., eds) Vol. II, pp. 1-20, Elsevier, Amsterdam
Kutzbach, R., Schweyen, R., and Kaudewitz, F. (1973) Mol. Gen. Genet. 125, 91-98
Lancashire, W., and Mattoon, J. (1979) Mol. Gen. Genet. 170, 333-344
Maniatis, T., Fritsch, E., and Sambrook, J. (1982) in Molecular Cloning, a Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Maxam, A., and Gilbert, W. (1980) Methods Enzymol. 65, 499-560
Messing, J. (1983) Methods Enzymol. 101, 20-78
Messing, J., Crea, E., and Seeburg, P. (1981) Nucleic Acids Res. 9, 309-321
Michel, F. (1984) Curr. Genet. 8, 307-317
Michel, F., and Dujon, B. (1983) EMBO J. 2, 33-38
Michel, F., and Lang, B. F. (1985) Nature 316, 641-643
Michel, F., Jacquier, A., and Dujon, B. (1982) Biochimie 64, 867-881
Mortimer, R., and Johnston, J. (1986) Genetics 113, 35-43
Poncz, M., Solowiejczyk, D., Ballantine, M., Schwartz, E., and Surrey, S. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 4298-4302
Prunell, A., and Bernardi, G. (1977) J. Mol. Biol. 110, 53-74
Prunell, A., Kopecka, H., Strauss, F., and Bernardi, G. (1977) J. Mol. Biol. 110, 17-52
Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463-5467
Séraphin, B., Simon, M., and Faye, G. (1985) Nucleic Acids Res. 13, 3905-3914
Sharp, P., Berk, Z., and Berget, S. (1980) Methods Enzymol. 65, 750-768
Simon, M., and Faye, G. (1984a) Proc. Natl. Acad. Sci. U. S. A. 81, 8-12
Simon, M., and Faye, G. (1984b) Mol. Gen. Genet. 196, 266-274
Sor, F., and Fukuhara, H. (1982) Nucleic Acids Res. 10, 1625-1633
Southern, E. (1975) J. Mol. Biol. 98, 503-517
Thalenfeld, B., and Tsagoloff, A. (1980) J. Biol. Chem. 255, 6173-6180
Thalenfeld, B., Hill, J., and Tsagoloff, A. (1983) J. Biol. Chem. 258, 610-615
Thomas, P. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5201-5205
Vodkin, M. (1977) J. Bacteriol. 132, 346-348
Waring, R., and Davies, R. (1984) Gene (Amst.) 28, 277-291
Waring, R., Davies, R., Scacciozchio, C., and Brown, T. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 6332-6336
Wolf, K., Dujon, B., and Slonimski, P. (1973) Mol. Gen. Genet. 125, 53-90
Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Gene (Amst.) 33, 103-119