Antibodies against a fused '*lacZ*-yeast mitochondrial intron' gene product allow identification of the mRNA maturase encoded by the fourth intron of the yeast *cob-box* gene

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Communicated by A. Danchin

Several missense or nonsense mutations have been localized in the fourth intron open reading frame (ORF) of the yeast mitochondrial cytochrome b gene. These results and the phenotypes of mutants strongly suggested that a mRNA maturase, controlling the expression of both cytochrome b and cytochrome oxidase subunit I (COXI) genes, is encoded in this ORF. To investigate more directly the biosynthesis of mRNA maturase we raised antibodies against a part of the putative ORF translation product. For that purpose we inserted a fragment of the ORF sequence, in phase, into the C-terminal *EcoRI* site of *lacZ* gene. The hybrid gene was then expressed in *Escherichia coli* under the control of either the wild-type *lac* promoter or the thermoregulated lambda system $P_R/cI857$. The hybrid protein was partially purified and antibodies were raised against it. These antibodies recognized a mitochondrially coded protein, p27, in intron mutants, whereas no such protein was detected in the wild-type cell. These results demonstrate that the p27 protein, previously shown to be associated with the mRNA maturase activity, is actually translated from the intron ORF. The autoregulated mRNA maturase synthesis model is discussed in relation to these results.

Key words: *lacZ* gene/p27 protein/mRNA maturase/yeast/intron open reading frame

Introduction

The penultimate intron of the gene coding for cytochrome b in yeast mitochondria (intron bi4 in the strain 777-3A or intron bi2 in the strain D273-10B) is particularly interesting in many respects. Like several other fungal mitochondrial introns it contains a long, 1158 bp, open reading frame (ORF) in phase with its preceding exon (Nobrega and Tzagoloff, 1980; De la Salle *et al.*, 1982). Numerous splicing-deficient mutants localized in the intron bi4 have been characterized and found to belong to two classes: *trans*-recessive and *cis*-dominant. The first indicate that an impairment of biosynthesis or activity of a protein, mRNA maturase, encoded by the intron's ORF and essential for splicing is the cause of the deficiency, while the second are interpreted as modifying the productive folding of the substrate, i.e., pre-mRNA, still containing the intron (Jacq *et al.*, 1980; Lamouroux *et al.*, 1980; De la Salle *et al.*, 1982; Weiss-Brummer *et al.*, 1982, 1983; Anziano *et al.*, 1982).

In contrast, however, to other optional introns for which there is evidence, introns bi2 (Lazowska *et al.*, 1980) and ai1

(Carignani *et al.*, 1983), or only belief, introns ai5 alpha and ai5 beta (Hensgens *et al.*, 1983), that they encode mRNA maturases, the intron bi4 appears as an obligatory one in yeast mitochondria. It is responsible for the 'box' phenomenon: the control of expression of the *oxi3* gene coding for the subunit I of cytochrome oxidase (Kotylak and Slonimski, 1976; Pajot *et al.*, 1976; Church *et al.*, 1979; Dhawale *et al.*, 1981; Labouesse and Slonimski, 1983). All *Saccharomyces cerevisiae* strains analysed until now contain this intron and the cytochrome b gene devoid of all introns does not synthesize cytochrome oxidase, although it makes cytochrome b itself. It suffices to introduce the intron bi4 alone in order to restore the synthesis of oxidase (Labouesse and Slonimski, 1983). All this points to the idea that the maturase encoded by the intron bi4 is essential for splicing of two introns and by this pleiotropic activity it regulates the expression of two structurally unrelated but functionally inter-dependent respiratory enzymes (see Dujardin *et al.*, 1982 for further discussion).

The regulatory properties of this maturase make it particularly attractive for biochemical studies to confirm the inferences of molecular genetic studies (*loc. cit.*). To obtain more direct evidence on the synthesis of maturase in yeast mitochondria and to develop a tool that would permit its purification, we decided to raise antibodies against the mitochondrial maturase. We have therefore fused *in vitro* a fragment of the mitochondrial DNA corresponding to the bi4 ORF to the C-terminal part of *lacZ* gene, put it under the control of a lambda promoter, translated the hybrid gene in *Escherichia coli* and injected the hybrid β -galactosidase-mRNA maturase protein in rabbits. Manufacture of novel proteins by fusing together the coding sequences of different genes is now widely used, but usually the new gene is fused at the N-terminal side of the β -galactosidase gene. In our case the fusion at the 3' end of *lacZ* gene was preferable because of the presence of UGA codons in open frames of the mitochondrial genome. We show that the antibodies obtained are highly specific and recognize only one mitochondrially synthesized protein and we present evidence that this protein is the bi4 maturase. We also describe the use of these antibodies to scrutinize some general aspects of the maturase model of split-gene expression.

Results

Construction of the fused gene lacZ'-mRNA maturase and its expression in E. coli

The choice of the mitochondrial intron sequence to be expressed in *E. coli* to raise mRNA maturase antibodies is critical for two reasons. The first concerns the part of the intron that is essential for maturase activity, the second is related to the nature of mitochondrial genetic code. The intron bi4 mRNA maturase activity is well defined by the fact that *box7* mutations are *trans*-acting and block the splicing of

Fig. 1. Functional and structural features of the fragment of cytochrome b intron 4 chosen to be expressed in *E. coli*. This figure summarizes the main features of mitochondrial cytochrome b intron 4 from the yeast *S. cerevisiae* (strain 777-3A) as deduced from genetic and physical studies. This intron is composed of an ORF (hatched area) in phase with the preceding exon and of a blocked frame in its 3'-terminal part (Nobrega and Tzagoloff, 1980; De la Salle *et al.*, 1982). Two critical signal sequences *box9* and *box2*, controlling the splicing of the intron, have been identified by sequence studies of *cis*-dominant mutations (De la Salle *et al.*, 1982; Weiss-Brummer *et al.*, 1982; Anziano *et al.*, 1982); the presence of these short sequences in all group I introns from yeast and other organisms suggests their generality (Netter *et al.*, 1982; Michel *et al.*, 1982; Davies *et al.*, 1982; Burke and Rajbhandary, 1982; Helmer Citterich *et al.*, 1983; Hensgens *et al.*, 1983; Cech *et al.*, 1983). The *trans*-acting mutations (*box7* locus) have all been localized in the middle and terminal part of the intron ORF (densely hatched area) indicating that the mRNA maturase activity is encoded in this region. The generic terms LAGLI and DADG have been coined (Hensgens *et al.*, 1983) to point out the presence (dotted area) of two highly conserved short peptides found to be localized in similar positions in several intron ORFs (Michel *et al.*, 1982; Waring *et al.*, 1982). In order to be expressed in *E. coli*, the mitochondrial region (starred region) was subjected to four manipulations described at the bottom of the figure. Its subsequent insertion in *EcoRI*-*HindIII* of pBR322 gave rise to the plasmid pChI.

both introns, that of cytochrome b (*bi4*) and that of cytochrome oxidase subunit I (*ai4*). All *box7* mutations have been localized in the middle and terminal 3' part of the *bi4* open reading frame (ORF, Figure 1). DNA sequence analysis of mutants has been correlated with the electrophoretic characteristics of a p27 protein or its truncated derivatives (De la Salle *et al.*, 1982; Weiss-Brummer *et al.*, 1982; Anziano *et al.*, 1982). It is well documented that p27 is encoded by the *bi4* reading frame and its C terminus corresponds to the 3'-terminal end of the intron's ORF. Concerning its initiation codon, two possibilities exist: either p27 results from a proteolytic cleavage of a large precursor, initiated at the first AUG of cytochrome b and encompassing the translation of the fused upstream exons or it results from initiation within the intron somewhere between the *box9* and LAGLI sequences (Figure 1). Furthermore, the yeast mitochondrial genetic code differs from that of *E. coli*, in particular, UGA is a tryptophan codon in mitochondria and a termination codon in *E. coli*. For these reasons we have chosen the unique *HaeIII*-*HindIII* fragment of intron *bi4*. This fragment contains one of the longest open reading frames devoid of UGA codons; the first UGA codon is 103 codons downstream from the first *HaeIII* site. This fragment covers a substantial part of the coding capacity of p27; most of the *box7* mutations are localized in it, and it encompasses a large part of the consen-

Fig. 2. Construction of bacterial plasmids allowing the expression of fused *lacZ'* mRNA maturase gene. Two types of plasmids with different promoter regions controlling the expression of the fused gene have been constructed. The first type of plasmid (pDIA 9002) was obtained by inserting the *EcoRI* fragment of lambda L512 carrying the *lac* promoter and most of the *lacZ* gene into the *EcoRI* site of pChI (Leplatois and Danchin, 1983). In pDIA 9002, the mRNA maturase sequence (black area) is fused in phase with the *lacZ* sequence (hatched area) and is under the control of the wild-type *lac* promoter. The second class of plasmid was derived from the first one by inserting the *SacI*-*SalI* fragment from pDIA 9002 into plasmid pDIA 5501. In the resulting plasmid, pDIA9204, the production of the hybrid protein is under the control of the right lambda promoter operator region; this hybrid gene associated with the repressor *ci857* allows a thermosensitive control of the fused protein production.

sus LAGLI-DADG peptides (see legend of Figure 1).

For reasons which will be elaborated in the Discussion, we decided to fuse the mitochondrial fragment at the *EcoRI* C-terminal site of β -galactosidase gene. The two reading frames were fused in phase as follows. The β -galactosidase reading frame at the *EcoRI* site is G⁺AA TTC, the mRNA maturase reading frame at the *HaeIII* site is TTG G⁺CC GGT (the arrows indicate the position of the phosphodiester cut). An *EcoRI* linker, d(GGAATTCC) added at the *HaeIII* site of the mitochondrial sequence generates the sequence G GAA TTC CCC GGT which is in phase with *lacZ* gene. The *EcoRI*-*HindIII* fragment thus obtained (Figure 2) was then cloned into pBR322 and gave the intermediate plasmid pChI, the modified sequence near the artificial *EcoRI* site was checked by DNA sequence analysis (data not shown). An *EcoRI* fragment isolated from a lambda *lac* phage (Lambda L512), carrying the control region of *lac* operon and most of the *lacZ* gene was integrated at the *EcoRI* site of plasmid pChI. The resulting plasmid, pDIA 9002, in which the fused gene is under the control of the *lac* wild-type promoter region (Figure 2B) was subsequently modified to become plasmid pDIA 9204 (Figure 2D) in which the expression of the *lacZ'* mRNA maturase gene is under the control of the lambda *P_R* region. In this plasmid, the thermosensitive repressor *ci857* gene allows one to trigger the production of the hybrid protein by raising the temperature. The expression of these two

Fig. 3. Immunoprecipitation analyses of ^{35}S -labelled mitochondrial translation products in *cis*-dominant mutants. Mitochondria were labelled in the presence of cycloheximide under conditions (see Materials and methods) selective for mitochondrial protein synthesis. Mitochondrial lysates were immunoprecipitated with the antiserum raised against the hybrid *lacZ'*-*bi4* mRNA maturase (lanes A). A characteristic immunoprecipitation of p27 is apparent in mutants M4474 and W300 and not in the wild-type. The electrophoretic patterns on 15% SDS acrylamide gels of total [^{35}S]mitochondrial translation products are shown in lanes B. The wild-type, 777-3A, exhibits the typical pattern of mitochondrial products. Ribosomal protein *varI*, the three cytochrome oxidase subunits I, II, III, and the cytochrome b are visible in this strain, while cytochrome b and cytochrome oxidase *COX1* are missing in mutant W300 and cytochrome b only is absent in mutant M4474. A new protein, p27, is labelled in these two mutants.

classes of plasmids in different *E. coli* genomes has been presented (Leplatois and Danchin, 1983).

Purification of the hybrid protein

To purify the β -galactosidase mRNA maturase protein we took advantage of the fact that it is one of the largest proteins in *E. coli* extracts. Assuming that the translation stops at the first UGA codon (Figure 1), the hybrid protein should be 86 amino acids longer than the wild-type β -galactosidase. As expected, upon thermoinduction of strain CAG 1139 carrying pDIA 9204, a band migrating slightly but significantly slower than authentic β -galactosidase was observed (data not shown). The main procedure to purify this protein was Biogel filtration. We did not attempt to obtain the hybrid protein in a very pure form. Thus, our preparations contained antigens

Fig. 4. Immunoprecipitation analysis of ^{35}S -labelled mitochondrial translation products in a *trans*-recessive mutant M1431 which carries an ochre mutation. Conditions are as in Figure 3. A characteristic p13 polypeptide, corresponding to the truncated maturase, is immunoprecipitated as shown in lane A.

other than the hybrid protein especially β and β' subunits of *E. coli* RNA polymerase.

Identification of maturase: immunoprecipitation of ^{35}S -labelled mitochondrially synthesized proteins

Partially purified preparations of the hybrid protein from *E. coli* were used to raise antibodies in rabbits. Antibodies from the first bleeding recognized only β -galactosidase. It took a longer time to obtain antibodies able to react with yeast mitochondrial proteins. Figures 3 and 4 show the results of immunoprecipitation experiments carried out with extracts of ^{35}S -labelled mitochondrial proteins from the wild-type strain (777-3A Figure 3) and three mutants (M4474 and W300 Figure 3, M1431 Figure 4). The antibodies did not precipitate any protein from wild-type extracts whereas a single protein of similar size was precipitated from M4474 and W300 extracts. This protein has the same electrophoretic mobility as the p27 protein visible in the total extracts of M4474 and W300 (Figure 3 lanes B). The p27 protein has been shown to be absent in a strain carrying the mutation M1431 which is localized in *box7* locus (De la Salle *et al.*, 1982). We have previously established that this mutation corresponds to a G to A transition localized in the first UGA codon (tryptophan in mitochondria) downstream from the *HaeIII* site (Figure 1). We propose that the generation of this UAA stop codon is responsible for the shortening of p27 to p13 in this mutant. The immunoprecipitation experiment carried out on M1431 extracts revealed that p13 is clearly recognized by the antibodies (Figure 4).

Identification of maturase: immunoblot analysis of total mitochondrial extracts

As far as mitochondrially synthesized proteins were concerned, the antibodies appeared to react specifically with a single protein; it was interesting to investigate whether this

Fig. 5. Western blot analysis of mitochondrial proteins in *cis*-dominant mutants. Mitochondrial extracts of two mutants (W300 and M4474) were electrophoresed, transferred to nitrocellulose, incubated with antiserum raised against the hybrid protein and revealed with [125 I]protein A as described in Materials and methods (**panel A**). Two controls were run in parallel: from strain 777-3A, which carries wild-type mitochondrial genome, and from a *rho*⁰ mutant (AB1-4A/8/55) totally devoid of a mitochondrial genome. A unique protein is immunodetected in mutants W300 and M4474, the migration of which corresponds to the [35 S]methionine-labelled p27 protein as shown in **panel B** (conditions as in Figure 3). Two other high mol. wt. bands are immunodetected by the antiserum, but they do not result from mitochondrial translation as demonstrated by their presence in the *rho*⁰ mutant.

was also true for total proteins contained in crude mitochondrial extracts. Figure 5 shows the results of the 'Western' blot experiments. In these conditions we can still detect the p27 protein in the mutants M4474 and W300 whereas it is not detectable in the wild-type strain 777-3A. These observations are in full agreement with the results obtained by immunoprecipitation analysis. Moreover two other proteins, in the 40–70 kd mol. wt. range, are recognized by the antibodies in all the strains examined. Taking into account the fact that these two proteins are also present in the strain AB1-4A/8/55 which is completely devoid of a mitochondrial genome (*rho*⁰), we can argue that these proteins are not mitochondrially coded. The reaction with these proteins might not even be relevant to the antibodies raised against the β -galactosidase RNA maturase protein since the preparations injected contained several *E. coli* proteins (cf. above).

Discussion

The fused lacZ'-mRNA maturase protein allowed us to raise antibodies able to recognize a mitochondrially synthesized protein

In this work we have shown that, starting from a mitochondrial intron sequence, we could raise antibodies against the putative translation product of this sequence by fusing it at the 3' end of *lacZ* gene. This methodology is an alternative to the widely used anti-peptide approach and may offer some advantages. Synthesis of peptides is still an arduous task whereas cloning in the *lacZ* gene is now available in most laboratories. The anti-peptide approach gives rise to antibodies elicited against a single antigenic site whereas the antibodies raised against the fused protein will recognize several antigenic sites; this may be an important advantage if one attempts to use antibodies to purify the protein. The ' β -galactosidase' approach, however, presents some difficulties which should be borne in mind. The fused proteins may not be very stable in *E. coli* though the use of *E. coli* strains carrying protease mutations, like CAG1139 (Grossman *et al.*, 1983) partly overcomes this drawback. Actually, the main drawback of the β -galactosidase method is related to the mitochondrial genome itself. The yeast mitochondrial genetic code differs from the universal code in several points (Bonitz *et al.*, 1980a; Hudspeth *et al.*, 1982). We had to choose a long mitochondrial sequence without a UGA codon – signifying Trp in mitochondria – to be able to translate it in *E. coli*. Moreover two other differences make the protein translated in *E. coli* distinct from the mitochondrial protein: CTT is read as Thr in mitochondria and as Leu in *E. coli* and ATA specifies Met in mitochondria and Ile in *E. coli*. However, in spite of these 'missense' modifications the antibodies raised against the *E. coli* made protein were able to immunoprecipitate the authentic yeast mitochondrial protein (Figures 3 and 4).

Antibodies react specifically with the intron encoded p27 protein

These results, beside being interesting from the methodological point of view, have relevance to the question of the biochemical identity of mRNA maturase. As far as cytochrome b intron 4 is concerned previous studies (De la Salle *et al.*, 1982; Weiss-Brummer *et al.*, 1982; Anziano *et al.*, 1982) have shown that *box7* mutations are localized in the intron ORF. It was further deduced that the intron encodes a *trans*-acting mRNA maturase. Sequence studies of mutations have allowed us and others to suggest that this maturase activity is carried by the p27 protein which accumulates in mutant strains. In chain termination mutants, such as M1431, this protein is shortened in relation to the position of the stop codon. The fact that the antiserum against the hybrid protein immunoprecipitates specifically the p27 protein in yeast strains carrying the mutations W300 and M4474 and the p13 protein in yeast strain carrying the mutation M1431, identifies these proteins as translation product of the intron bi4 ORF. Comparison of M4474 and W300 strengthens this point. M4474 modifies the *cis*-acting *box9* critical sequence and the strain carrying this mutation accumulates a p27 protein in its wild-type form; W300 harbours three base changes, two of which are responsible for its splicing deficiency. One base change affects the *cis*-acting *box2* critical sequence, the other one modifies the sequence coding for p27. This last change

creates a missense mutation (Gly → Asp) which is likely to alter the electrophoretic mobility of p27 in SDS-acrylamide gels (De la Salle *et al.*, 1982). Indeed, the antiserum selectively immunoprecipitates a protein in the mutant W300 which migrates slightly slower than p27 (Figure 3).

The antibodies do not immunoprecipitate any mitochondrial protein other than p27. This means that, if p27 is a cleavage product of a large precursor translated from the entire sequence of the four upstream exons fused to the intron ORF, the concentration of this putative precursor would be so low that it cannot be detected by these antibodies, at least in the strains analysed (Figures 3,4).

The fourth intron, ai4, of the *COXI* gene contains an ORF which displays a considerable sequence homology to the bi4 ORF (Bonitz *et al.*, 1980b). In the wild-type, the splicing of ai4 is regulated by the presence of an active bi4 maturase (the 'box' phenomenon, cf. Kotylak and Slonimski, 1976) while in a compensatory mutant the translation product of ai4 becomes active (Dujardin *et al.*, 1982). The observation that no protein other than p27 is detected by these antibodies suggests that either the ai4 ORF is not translated in the mutant W300 (although *COXI* is not synthesised due to the non-excision of intron ai4 and accumulation of unspliced pre-mRNA, Labouesse *et al.*, in preparation) or it is translated but not recognized by the antibodies. The latter conclusion, if true, is unexpected because the sequence homology between bi4 and ai4 in the region responsible for raising antibodies is particularly high, 78% at the amino acid level. It will be of interest to investigate, with the help of these antibodies, the putative translation products of ai4 ORF in the compensatory mutant MIM2-1 (Dujardin *et al.*, 1982), or in strains devoid of all introns in the cytochrome b gene (Labouesse and Slonimski, 1983).

The mRNA maturase model of autoregulation

Antibodies raised against the protein containing the wild-type sequence of the bi4 intron ORF do not immunoprecipitate any protein from the wild-type strain (Figure 3). This paradoxical result is worth noting since it is in full agreement with the autoregulation model of mRNA maturase synthesis (Jacq *et al.*, 1980; Lazowska *et al.*, 1980). In this model it was proposed that, in the wild-type cell, the synthesis of the mRNA maturase is controlled by its own activity, since its function is to destroy its template mRNA. The consequence of this 'matricidal' activity should be an extreme scarcity of the maturase in the wild-type cell. Our results show that the maturase concentration is actually below the level of detection by this antibody preparation.

The main interest of the present report is, in our opinion, three-fold: (i) it describes, for the first time, the construction of a hybrid gene containing a mitochondrial intron and its translation in *E. coli*; (ii) it brings substance to the notion of mRNA maturases by identifying one with the help of specific antibodies and (iii) the availability of anti-maturase antibodies should greatly facilitate direct biochemical experiments on this elusive intron-encoded protein.

Materials and methods

Strains and growth conditions

The *E. coli* strain used was CAG 1139 (F-leu or *thr thi sup E44 lacY galK lon-100*) (Grossman *et al.*, 1983).

The yeast *cob-box*⁻ mutants used in this study were isolated from the strain 777-3A (α *adel opl rho*⁺) and have been described in De la Salle *et al.* (1982).

The strain ABI-4A/8/55 (a, *his4*, *OPI*, *rho*^o) was obtained from 777-3A by Kruszewska and Szczesniak (1980).

Plasmid constructions

Plasmid pChI was constructed as follows: *Hae*III fragment (Figure 1) was purified from plasmids already described (De la Salle *et al.*, 1982) in which the appropriate *Eco*RI-*Bgl*II mitochondrial fragment had been inserted in the *Eco*RI-*Bam*HI sites of pBR322. *Eco*RI linkers (GGAATTCC) from Biolabs (Beverly, MA) were then ligated at the ends of the *Hae*III fragment which was re-purified on acrylamide gel after *Eco*RI digestion. The eluted fragment was cut by *Hind*III and cloned in pBR322 to generate the plasmid pChI (Figure 1). The construction of other plasmids is described in Leplatot and Danchin (1983).

Purification of the hybrid protein lacZ'-mRNA maturase

The strain CAG1139/pDIA9204 was grown at 30°C to an OD₆₀₀ of ≈ 0.8 then shaken at 42°C for 4 h. The collected cells were washed and sonicated in Tris 10 mM (pH 7.5) EDTA 1 mM, NaCl 0.15 M, pepstatin 20 μ g/ml and phenylmethylsulphonylfluoride 5 mM. The supernatant was made 33% with ammonium sulphate while the pH was maintained at 7.5, the precipitate was centrifuged, re-dissolved and boiled (5') in the same buffer containing 1% SDS. Gel filtration on Biogel A 5M 200–400 mesh (Bio Rad) was carried out at room temperature and the fractions were collected and analysed on a polyacrylamide gel.

Antibodies

200 μ g of partly purified hybrid protein were intradermally injected into rabbits every 10 days. Two injections were usually sufficient to obtain antibodies against β -galactosidase while antibodies against mitochondrial mRNA maturase were detected only after five injections.

Immunoprecipitation analyses

Immunoprecipitation of ³⁵S-labelled mitochondrial extracts obtained according to Douglas and Butow (1976) were carried out as indicated by Côté *et al.* (1979) except that the antiserum was diluted 1/20 in the hybridization buffer, protein A Sepharose (Pharmacia) was used instead of *Staphylococcus aureus* cells and the immune complex was washed with 2% Triton solution before elution.

Immunoblot analyses

Mitochondria were prepared as described by Locker *et al.* (1980) and after lysis and denaturation were electrophoresed in a 15% SDS-polyacrylamide gel. After electrophoresis, the proteins were transferred to nitrocellulose sheets using the method of Towbin *et al.* (1979). Immunostaining was performed according to Burnette (1981), ¹²⁵I-labelled protein A (Amersham) was diluted to 5 $\times 10^6$ c.p.m./20 ml for a nitrocellulose sheet of 100 cm².

Acknowledgements

We greatly appreciated the excellent secretarial help of B. Poirier and M. Even. This work was supported by grants from the Centre National de la Recherche Scientifique (ATP 'Organisation et Expression du génome'), from the Institut National de la Santé et de la Recherche Médicale and from the Ligue Nationale Française contre le Cancer.

References

- Anziano, P.Q., Hanson, D.J., Malher, H.R. and Perlman, P.S. (1982) *Cell*, **30**, 925-932.
- Bonitz, S.G., Berlani, R., Coruzzi, B., Li, M., Nobrega, F., Nobrega, M., Thalenfeld, B. and Tzagoloff, A. (1980a) *Proc. Natl. Acad. Sci. USA*, **77**, 3167-3170.
- Bonitz, S.G., Coruzzi, G., Thalenfeld, B.E., Tzagoloff, A. and Macino, G. (1980b) *J. Biol. Chem.*, **255**, 11927-11971.
- Burke, J.M. and Rajbhandary, U.L. (1982) *Cell*, **31**, 509-520.
- Burnette, W.S. (1981) *Anal. Biochem.*, **112**, 195-203.
- Carignani, G., Groudinsky, O., Frezza, D., Schiavon, E., Bergantino, E. and Slonimski, P.P. (1983) *Cell*, **35**, 733-742.
- Cech, T.R., Tanner, N.K., Tinoco, I., Weir, B.R., Zuker, M. and Perlman, P.S. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 3903-3907.
- Church, G.M., Slonimski, P.P. and Gilbert, W. (1979) *Cell*, **18**, 1209-1215.
- Côté, C., Solioz, M. and Schatz, G. (1979) *J. Biol. Chem.*, **254**, 1437-1439.
- Davies, R.W., Waring, R.B., Ray, J.A., Brown, T.A. and Schazzochio, C. (1982) *Nature*, **300**, 719-724.
- De la Salle, H., Jacq, C. and Slonimski, P.P. (1982) *Cell*, **28**, 721-732.
- Dhawale, S., Hanson, D.K., Alexander, N.J., Perlman, P.S. and Mahler, H.R. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 1778-1782.
- Douglas, M.G. and Butow, R.A. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 1083-1086.

- Dujardin,G., Jacq,C. and Slonimski,P.P. (1982) *Nature*, **298**, 628-632.
- Grossman,A.D., Burgess,R.G., Walter,W. and Gros,C.A. (1983) *Cell*, **32**, 151-159.
- Guiso,N., Dreyfus,M., Siffert,O., Danchin,A., Spyridakis,A., Gargouri,A., Claisse,M. and Slonimski,P.P. (1984) *EMBO J.*, **3**, in press.
- Helmer Citterich,M., Morelli,G. and Macino,G. (1983) *EMBO J.*, **2**, 1235-1242.
- Hensgens,L.A.M., Bonen,L., De Haan,M., Horst,G. and Grivell,L.A. (1983) *Cell*, **32**, 379-389.
- Hudspeth,M.E.S., Ainley,W.M., Schumard,D.S., Butow,R.A. and Grossman,L.I. (1982) *Cell*, **30**, 617-626.
- Jacq,C., Lazowska,J. and Slonimski,P.P. (1980) *C.R. Hebd. Seances Acad. Sci., Paris, Serie D*, **290**, 89-92.
- Kotylak,Z. and Slonimski,P.P. (1976) in Saccone,C. and Kroon,A.M. (eds.), *The Genetic Function of Mitochondrial DNA*, Elsevier, North Holland Biomedical Presss, Amsterdam, pp. 143-154.
- Kruszewska,A. and Szczesniak,B. (1980) *Genet. Res. Camb.*, **35**, 225-229.
- Labouesse,M. and Slonimski,P.P. (1983) *EMBO J.*, **2**, 269-276.
- Lamouroux,A., Pajot,P., Kochko,A., Halbreich,A. and Slonimski,P.P. (1980) in Kroon,A.M. and Saccone,C. (eds.), *The Organisation and Expression of Mitochondrial Genome*, Elsevier, North Holland Biomedical Press, Amsterdam, pp. 153-156.
- Lazowska,J., Jacq,C. and Slonimski,P.P. (1980) *Cell*, **22**, 333-348.
- Leplatols,P. and Danchin,A. (1983) *Biochimie*, **65**, 317-324.
- Locker,J., Morimoto,R., Synenki,R.M. and Rabinowitz,M. (1980) *Curr. Genet.*, **1**, 163-172.
- Michel,F., Jacquier,A. and Dujon,B. (1982) *Biochimie*, **64**, 867-881.
- Netter,P., Jacq,C., Carignani,G. and Slonimski,P.P. (1982) *Cell*, **28**, 733-738.
- Nobrega,F.G. and Tzagoloff,A. (1980) *J. Biol. Chem.*, **255**, 9828-9837.
- Pajot,P., Wambier-Kluppel,M.L., Kotylak,Z. and Slonimski,P.P. (1976) in Bucher,Th., Neupert,W., Sebald,W. and Werner,S. (eds.), *Genetics and Biogenesis of Chloroplasts and Mitochondria*, Elsevier/North Holland, Amsterdam, pp. 443-451.
- Towbin,H., Stachelin,T. and Gordon,J. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4340-4354.
- Waring,R.B., Davies,R.W., Scazzochio,C. and Brown,T.A. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 6332-6336.
- Weiss-Brummer,B., Rodel,G., Schweyen,R.J. and Kaudewitz,F. (1982) *Cell*, **29**, 527-536.
- Weiss-Brummer,B., Holl,J., Schweyen,R.J., Rodel,G. and Kaudewitz,F. (1983) *Cell*, **33**, 195-201.

Received on 9 April 1984