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SCO1, a yeast nuclear gene essential for accumulation of mitochondrial cytochrome *c* oxidase subunit II

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Summary. We have identified and isolated a novel yeast nuclear gene (*SCO1*) which is essential for accumulation of the mitochondrially synthesized subunit II of cytochrome *c* oxidase (CoxII). Analysis of the mitochondrial translation products in a *sco1-1* mutant reveals a strong reduction in CoxII. Examination of mitochondrial transcripts by Northern blot hybridization shows that transcription and transcript maturation of *OXI1*, the gene coding for CoxII, is not affected. Therefore the *SCO1* gene product must be involved in a post-transcriptional step in the synthesis of CoxII. We have isolated a 1.7 kb DNA fragment from a yeast gene bank which carries the functional *SCO1* gene. Two RNA species of 0.9 kb and 1.2 kb, respectively, hybridize with this DNA fragment, which is localized on chromosome II. Cells whose chromosomal 1.7 kb fragment has been replaced by the yeast *URA3* gene fail to accumulate CoxII and in addition subunit I of cytochrome *c* oxidase (CoxI). The possibility that the *SCO1* gene product is bifunctional, i.e. required for both CoxI and CoxII accumulation, is discussed.

Key words: Cytochrome *c* oxidase – Mitochondria – PET genes – Yeast

Introduction

Gene expression in prokaryotes as well as in eukaryotes seems to be primarily regulated at the level of transcription. However, there are also several examples of post-transcriptional regulation. Recently, a number of nuclear genes have been described in yeast which are specifically required for a post-transcriptional step in the expression of individual mitochondrially coded genes (for review see Fox 1986).

At least six nuclear genes are necessary for expression of the mosaic *COB* gene coding for apocytochrome *b*: the products of the genes *MRS1* and *CBP2* are necessary for removal of introns 3 and 5, respectively, from the *COB* preRNA (Kreike et al. 1986; McGraw and Tzagoloff 1983; Hill et al. 1985); the *CBP1* gene product is essential for the formation of the correct 5′end of the *COB* mRNA (Dieckmann et al. 1984a, b), and the products of the genes *CBP6*, *CBS1* and *CBS2* are involved in synthesis of apocytochrome *b* from *COB* mRNA (Dieckmann and Tzagoloff 1985; Rödel 1986; Rödel and Fox 1987). Translation of subunit 3 of cytochrome *c* oxidase (CoxIII) requires at least two nuclear genes, *PET494* and *PET54* (Costanzo and Fox 1986; Costanzo et al. 1986), and possibly a third gene, represented by complementation group 16 according to Kloeckener-Gruissem et al. (1987). Similarly, at least two nuclear genes, *PET111* and *PET112* are specifically involved in the accumulation of CoxII (Poutre and Fox 1987; Costanzo et al. 1986). CoxII is the only mitochondrially synthesized polypeptide with a transient amino-terminal presequence (Mannhaupt et al. 1983, 1985). The correct amino-terminal cleavage step depends on one or even two nuclear gene products (Mannhaupt et al. 1983; Pratje et al. 1983). For subunit 1 of cytochrome *c* oxidase (CoxI) a mutation in the nuclear gene *MSS51* has been reported to interfere with multiple steps in mRNA processing (Faye and Simon 1983; Simon and Faye 1984). Further nuclear genes are likely to be required for the correct synthesis and assembly of the mitochondrially encoded subunits of the respiratory chain (e.g. Michaelis et al. 1982; McEwen et al. 1986; Kloeckener-Gruissem et al. 1987).

The precise function of these various gene-specific factors is not yet understood. In the case of *cbs1*, *cbs2*, *pet494* and *pet54* mutants, suppressor mutants have been identified in which translation of the mitochondrial mRNA is restored by replacement of the 5′ untranslated leader in the respective RNA by the leader of another mitochondrial gene (Rödel et al. 1985; Rödel 1986; Costanzo and Fox 1986; Costanzo et al. 1986). From this observation it was concluded that the nuclear gene products themselves or factors under their control act in the 5′ untranslated leader region of the respective RNA to stimulate its translation. Similar suppressor mutations have also been identified for *pet111* mutants, but in this case the rearrangement in the mitochondrial DNA leads not only to a replacement of the 5′ untranslated leader, but in addition to a modified CoxII polypeptide with an altered amino-terminus (Poutre and Fox 1987).

In this paper we report on the identification and characterization of a third nuclear gene essential for the accumulation of CoxII. The phenotype of mutants in this gene strongly argues in favour of the respective gene product being required for a post-transcriptional step in CoxII expression.

Materials and methods

Yeast strains, media and genetic methods. Media and genetic techniques were as described (Sherman et al. 1986). The

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following yeast strains were used in this study: DL1 (*MATα, leu2-3, leu2-112, his3-11, his3-15, ura3-251, ura3-372, ura3-228*) (Van Loon et al. 1983); MK20 (*MATa, ura1, met, sco1-1*), a derivative of strain SM11-6C (*MATa, met, ura1*) (Lang and Kaudewitz 1982); NP3 (*MATα, leu2, ura3, sco1-1*) was obtained by random spore analysis after crossing MK20 with DL1. The *Escherichia coli* strain 490 (*hsd*R *hsd*M *met*$^-$ *thr*$^-$ *leu*$^-$ *recA*$^-$ *lac*$^-$ *su*$^+$, kind gift of G. Hobom) was used for *E. coli* transformations.

Analysis of mitochondrial translation products. Labelling of yeast cells in the presence of cycloheximide with [^{35}S]methionine was performed as described by Douglas and Butow (1976). Mitochondria were isolated (Needleman and Tzagoloff 1975) and electrophoresed on 10%–15% linear gradient polyacrylamide gels in the presence of sodium dodecyl sulphate (SDS) (Laemmli 1970). Gels were dried and autoradiographed on Kodak XS-5 X-ray films.

Transformation procedures. Transformation of *E. coli* (Bolivar et al. 1977) and of yeast (Beggs 1978) was done as described.

Plasmids. The yeast gene bank (which was a kind gift of F. Lacroute, Strasbourg, France) contains a partial *Sau*3A digest of chromosomal DNA from strain FL100 (ATCC28383) in the single *Bam*HI site of plasmid YEp24 (Fasiolo et al. 1981). Plasmid pU3 carries the yeast *URA3* gene (Rose et al. 1984) in the polylinker of pUC7 (Rödel et al. 1986). Plasmid pMT36 is a derivative of pBR322, with a 2.5 kb *Hae*III fragment covering the complete *OXI1* gene (Coruzzi et al. 1981) inserted into the *Pst*I site. Plasmid pMn30-1 contains a 750 bp *Mbo*I fragment of MSU494-2 (resulting from a fusion of *AAP1* and *OXI2*; Costanzo and Fox 1986), cloned into the *Bam*HI site of M13mp11. Both pMT36 and pMN30-1 are kind gifts of T. Fox, Cornell University. Plasmid pHD4, resulting from a ligation of a 1.4 kb *Bam*HI-*Hin*dIII fragment comprising exon 4 and most of intron 4 of *OXI3* into pBR322 was kindly provided by T. Pillar, Albany. The pTR262 derivative pDB262 (Roberts et al. 1980) was used for subcloning of the 1.7 kb *Eco*RI fragment of pB10 (Fig. 3).

Isolation and hybridization of DNA and RNA. Published procedures were used for mini-scale (Birnboim and Doly 1979) or maxi-scale plasmid isolation (Clewell 1972) from *E. coli*, for plasmid DNA preparation from yeast transformants (Nasmyth and Reed 1980), for isolation of yeast chromosomal DNA (Sherman et al. 1986) and preparation of mitochondrial DNA (Pillar et al. 1983). DNA fragments were separated on 1% agarose gels and blotted to nitrocellulose (Southern 1975). Preparation of cytoplasmic RNA (Sherman et al. 1986) and of mitochondrial RNA (Pillar et al. 1983) was done as described. RNA was separated on 1.5% agarose/6 M urea gels, blotted to Gene Screen Plus paper (NEN, Dreieich) and hybridized at 42° C according to the protocol of the manufacturer.

Miscellaneous. A nitrocellulose filter containing the DNA of separated yeast chromosomes of strain #140 (CBS1591) was a kind gift of D. Jäger (Biozentrum Basel, Switzerland).

The activity of complex III of the respiratory chain and the absorption spectra at low temperature of whole cells were determined as described by Lang and Kaudewitz (1982).

Radioactive labelling of DNA fragments was done by nick translation with [α-^{32}P]dATP according to Rigby et al. (1977).

Results

Mutant MK20 lacks CoxII

The *Saccharomyces cerevisiae* mutant strain MK20 is unable to grow on nonfermentable substrates. This deficiency does not result from a mitochondrial mutation, as diploids resulting from a cross between MK20 and Pet$^+$ Rho^0 tester strains are respiratory competent. Instead, a mutation in a single nuclear gene is responsible for this phenotype. This is demonstrated by a 2:2 segregation of respiratory deficient and respiratory competent cells after tetrad analysis of sporulating diploids from a cross between mutant MK20 and wild-type DL1 (U. Lindner, personal communication). The low temperature absorption spectrum of whole cells of strain MK20 reveals a lack of the absorption peak at 602 nm, which is characteristic of cytochromes *a* and a_3 of cytochrome *c* oxidase (data not shown). As expected from the spectral analysis, the enzymatic activity of cytochrome *c* oxidase was reduced to 20% of wild-type activity. A closer examination of the defect was obtained by analysis of the mitochondrially synthesized polypeptides. To this end cells were labelled with [^{35}S]methionine in the presence of cycloheximide to block protein synthesis on cytoplasmic ribosomes. Isolated mitochondria were subjected to SDS-polyacrylamide gel electrophoresis and autoradiographed. As can be seen from Fig. 1, lane c, the 22 kDa band of CoxII is drastically reduced, while all the other labelled bands are present in concentrations comparable with or even higher than those of the wild-type.

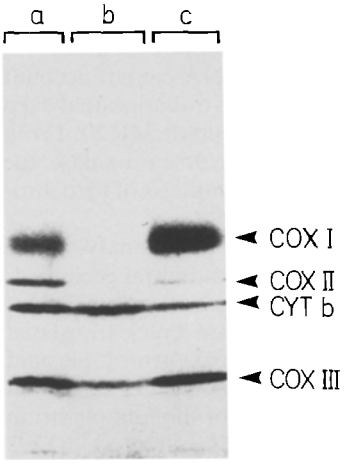

Fig. 1. Mitochondrial translation products of wild-type DL1 (**a**), GR20 (**b**) and of mutant MK20 (**c**). Cells were radioactively labelled in the presence of cycloheximide and mitochondrial proteins were isolated and separated by SDS-polyacrylamide gel electrophoresis as described (Materials and methods). The positions of apocytochrome *b* (CYT b) and of subunits 1–3 of cytochrome *c* oxidase (COXI, II, III) are indicated by *arrowheads*

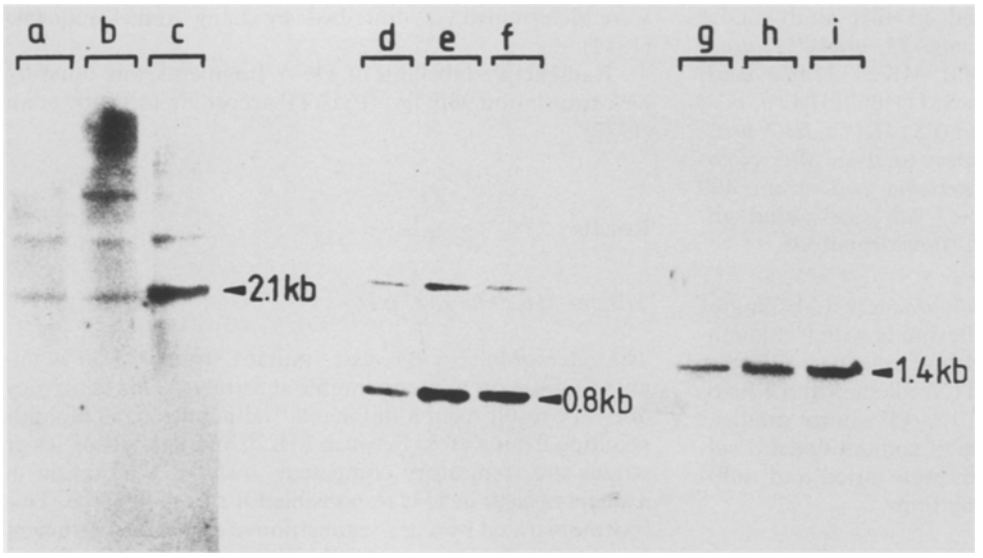

Fig. 2. Mitochondrial transcripts of mutant GR20 (lanes a, d, g), mutant MK20 (lanes b, e, h) and wild-type DL1 (lanes c, f, i). Mitochondrial RNA was prepared, separated by gel electrophoresis, blotted and hybridized as described in Materials and methods. Lanes a–c, CoxI transcripts; lanes d–f, CoxII transcripts; lanes g–i CoxIII transcripts. The probes used for hybridization are described in Results. The positions and lengths of the mature mRNAs are indicated by *arrowheads*

The lack of CoxII in MK20 does not result from a failure to transcribe or process the oxi1 *mRNA*

OXI1, the gene coding for CoxII, is part of a larger transcription unit which contains in its upstream part several tRNAs and downstream of *OXI1* an unidentified reading frame (*urf1*) (Christianson and Rabinowitz 1983); the resulting precursor RNA is then processed to yield a 0.85 kb mature *oxi1* mRNA (Coruzzi et al. 1981). To test whether transcription or maturation of the *OXI1* mRNA may be affected in mutant MK20, mitochondrial RNA was prepared from the mutant and analysed in a Northern blot experiment as described in Materials and methods. As a probe the nick-translated 0.27 kb *Pvu*II fragment of the *OXI1* gene, isolated from plasmid pMT36 (Materials and methods) was used. Figure 2 shows the results of this hybridization: in the wild-type (Fig. 2, lane f) one hybridizing band of about 0.8 kb can be detected as expected for *OXI1* mRNA (Coruzzi et al. 1981). An RNA species of exactly the same length and of comparable concentration is found in mutant MK20 (Fig. 2, lane e). Therefore a failure to transcribe and/or to process *OXI1* mRNA cannot account for the lack of CoxII; instead a post-trancriptional step must be affected by the mutation in mutant MK20. From its function in the synthesis of cytochrome *c* oxidase the respective gene was named *SCO1* (for *s*ynthesis of *c*ytochrome *c* o*x*idase).

In addition to the *COX1* transcript, we analysed the transcript pattern of the other two mitochondrial genes coding for subunits of cytochrome *c* oxidase. As a probe for *OXI2*, the gene coding for CoxIII, we used a nick-translated 170 bp *Hph*I-*Mbo*I fragment, isolated from plasmid pMn30-1 (see Materials and methods). Figure 2, lanes h and i, shows that in the wild-type and in the mutant strain a 1.4 kb mRNA, which represents the mature *OXI2* mRNA, is present. For *OXI3*, the gene encoding CoxI, a nick-translated 350 bp *Rsa*I-*Hae*III fragment of plasmid pHD4 (see Materials and methods) comprising only exon 4 sequences, was used. As can be seen from Fig. 2, lane b, in mutant MK20 the mature *OXI3* mRNA of 2.1 kb is present, although at a slightly reduced concentration as copared with the wild-type (lane c). Additional bands of higher molecular weight most probably represent splicing intermediates, which were not analysed in more detail.

Molecular cloning of the SCO1 gene

To isolate the *SCO1* gene, mutant MK20 was transformed with a yeast gene bank (see Materials and methods). About 10,000 Ura^+ transformants were selected on minimal medium lacking uracil, pooled in aliquots of 1,000 and further selected for growth in glycerol medium. From the pooled respiratory competent cells plasmids were isolated and transformed into *E. coli*. DNA prepared from individual *E. coli* clones was used to re-transform mutant strain MK20. Only one plasmid, pB20, was able to confer respiratory competence to 100% of tranformed cells of the mutant strain MK20. This result was confirmed by the fact that after growth of the respiratory competent cells in complete glucose medium for 24 h co-loss of the Ura^+ and the Pet^+ phenotypes was observed.

Plasmid pB10 contains an insert of 6.3 kb as determined by restriction analysis (Fig. 3a). When chromosomal DNA of the wild-type DL1, cut with *Eco*RI (Fig. 4a) and with *Cla*I and *Bgl*II (Fig. 4b) is hybridized with the labelled 1.7 kb *Eco*RI fragment of pB10, two bands of 1.7 kb and 3.9 kb, respectively, are detected. Fragments of this length

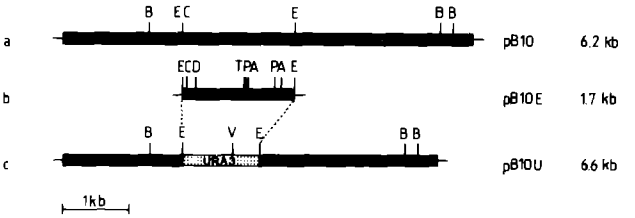

Fig. 3a–c. Restriction maps of plasmid pB10 (**a**), pB10E (**b**) and pB10U (**c**). Only the inserts are shown with the insert lengths indicated on the right. pB10 is a derivative of YEp24. pB10E was constructed by insertion of a pUC13 derivative, carrying the 1.7 kb *Eco*RI fragment of pB10, into the *Hin*dIII site of pDB262. For construction of pB10U, in which the 1.7 kb *Eco*RI fragment is replaced by the *URA3* gene, see text. Restriction sites: A, *Acc*I; B, *Bgl*II; C, *Cla*I; D, *Dra*I; E, *Eco*RI; P, *Pst*I; T, *Taq*I; V, *Eco*RV

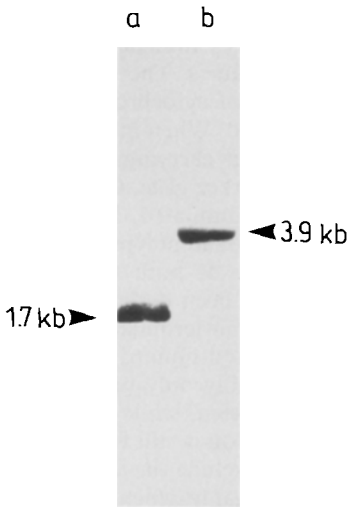

Fig. 4. Genomic Southern blot. Total DNA of wild-type DL1 was cut with *Eco*RI (**a**) or with *Cla*I + *Bgl*II (**b**), separated by agarose gel electrophoresis, blotted onto nitrocellulose paper and hybridized with the nick-translated 1.7 kb *Eco*RI fragment of pB10. The positions and lengths of the hybridizing bands are indicated by *arrowheads*

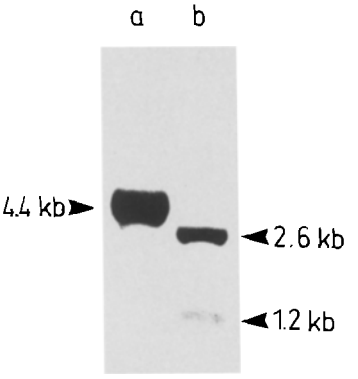

Fig. 5. Replacement of the chromosomal 1.7 kb *Eco*RI fragment by the *URA3* gene. Wild-type strain DL1 was transformed with the 3.8 kb *Bgl*II fragment of plasmid pB10U. Total DNA was prepared from the Ura$^+$ transformant GR20 (**b**) and from untransformed DL1 (**a**) cut with *Bgl*II and *Eco*RV, electrophoretically separated, blotted and hybridized with the labelled 4.4 kb *Bgl*II fragment of plasmid pB10. The positions and lengths of the hybridizing DNA fragments are indicated by *arrowheads*

are expected if the insert of pB10 is colinear with chromosomal DNA. This result demonstrates that no rearrangement has occurred in pB10 and that the DNA sequence is present in a single copy in the yeast genome (Fig. 4). In order to localize the *SCO1* gene more precisely within the insert, several subcloning experiments were performed. The smallest fragment which still retained the ability to complement the *sco1* mutation was a 1.7 kb *Eco*RI fragment (Fig. 3b).

Gene replacement of the SCO1 gene by the yeast URA3 gene

To exclude the possibility that a suppressor gene had been isolated and to test if the observed effect of the *sco1-1* mutation of mutant MK20 reflects the whole spectrum of activity of the *SCO1* gene we performed a gene deletion experiment in the wild-type strain DL1. The insert of plasmid pB10 was isolated as a *Hin*dIII-*Sal*I fragment and cloned into pBR322 from which the single *Eco*RI site had been deleted. The resulting plasmid was cut with *Eco*RI and the excised 1.7 kb DNA fragment was removed by agarose gel electrophoresis. The remaining plasmid was ligated with the 1.1 kb *Eco*RI fragment of plasmid pU3 (see Materials and methods) which carries the complete yeast *URA3* gene. From the resulting ligation product, plasmid pB10U (Fig. 3c), a 3.8 kb *Bgl*II fragment was isolated. This DNA fragment contains the *URA3* gene flanked by yeast chromosomal sequences from upstream and downstream of the *SCO1* gene. As shown by Rothstein (1983), such a DNA fragment will integrate into the yeast chromosome at the site of homology to the linearized ends with a low frequency. After transformation of wild-type strain DL1 with 5 µg of this DNA fragment, a stable Ura$^+$ transformant, GR20, was selected. To verify the correct integration leading to a replacement of the *SCO1* gene by the *URA3* gene, chromosomal DNA of the wild-type and of transformant GR20 was isolated, cut with *Bgl*II + *Eco*RV and probed after Southern blotting with the nick-translated 4.4 kb *Bgl*II fragment of pB10 (see Fig. 3a). As can be seen from Fig. 5, the 4.4 kb band of the wild-type (lane a) is replaced by two novel bands of 1.2 kb and 2.6 kb (lane b), as expected for the gene replacement. No hybridization signal was obtained with GR20 when probed with the 1.7 kb *Eco*RI fragment of pB10 (data not shown). GR20, the strain with the deleted *SCO1* gene, exhibited a respiratory deficient phenotype. When crossed with the *sco1* mutant MK20, the resulting diploid cells remained respiratory defective. The failure to complement the *sco1-1* mutant is a strong argument that the cloned gene used for the gene replacement experiment is indeed the *SCO1* gene and not a suppressor gene.

Examination of the cytochrome spectra revealed a complete absence of the cytochrome aa_3 peak at 602 nm (not shown). Analysis of mitochondrial translation products (Fig. 1, lane b) showed that in addition to CoxII the band representing CoxI is missing. Again a failure to transcribe and/or to process the respective RNAs cannot account for this observation. Figure 2, lanes a and d, shows that the mature full-sized mRNAs of both genes are present. As for mutant MK20 (lane b) the concentration of *oxi3* mRNA is slightly reduced as compared with wild-type (lane c). However, as will be outlined in the Discussion, this amount of RNA is clearly sufficient for normal synthesis of CoxI.

SCO1 is on chromosome II

A nitrocellulose filter containing DNA of separated *S. cerevisiae* chromosomes (kind gift of D. Jäger, Basel) was hybridized with the nick-translated 1.7 kb *Eco*RI fragment of pB10 (Fig. 3a). The only signal resulted from hybridization of chromosome II (according to Carle and Olson 1985) (Fig. 6). The hybridizing band was confirmed as chromosome II by rehybridizing the blot with a 1.5 kb *Cla*I-*Xho*I fragment of plasmid pLGSD5 (Guarente et al. 1982) covering the *gal10* promoter region wich has been localized on chromosome II (St. John and Davis 1981). The *SCO1* gene is therefore located on chromosome II.

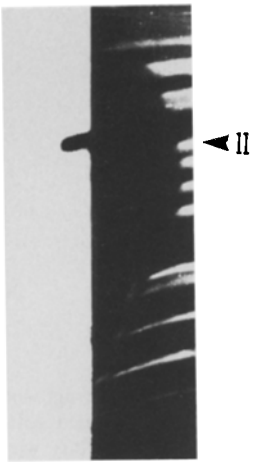

Fig. 6. Localization of the *SCO1* gene on chromosome II. The ethidium bromide-stained pattern of yeast chromosomes is shown on the right. A nitrocellulose filter containing the blotted chromosomal DNA was hybridized with the labelled 1.7 kb *Eco*RI fragment of plasmid pB10. The autoradiogram is shown on the left. The position of the hybridizing band of chromosome II is indicated

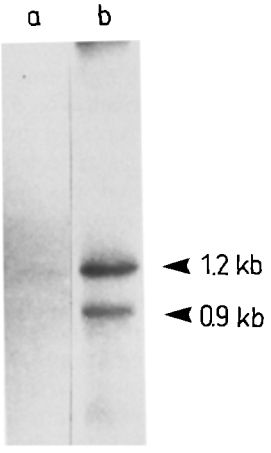

Fig. 7. Transcript pattern of the *SCO1* gene region. Total RNA of wild-type DL1 (**a**) and of a transformant of DL1 carrying plasmid pB10 (**b**) was prepared, separated by gel electrophoresis, blotted and hybridized with the labelled 1.7 kb *Eco*RI fragment of plasmid pB10. The two hybridizing RNA species of 1.2 kb and 0.9 kb are indicated by *arrowheads*

Two RNA species of 0.9 kb and 1.2 kb hybridize with the 1.7 kb DNA fragment

Total RNA of wild-type DL1 and of a transformant of DL1 carrying plasmid pB10 was prepared and used for a Northern blot experiment as described in Materials and methods. The same labelled 1.7 kb *Eco*RI fragment as used in the Southern hybridization for the chromosomal localization was chosen as a probe. In wild-type RNA (Fig. 7, lane a) two faint bands of 0.9 kb and 1.2 kb can be seen. The same bands are found at a much higher concentration in the RNA of wild-type DL1, transformed with the high copy number plasmid pB10 (Fig. 7, lane b).

Discussion

SCO1 is a yeast nuclear gene which is essential for formation of a functional cytochrome *c* oxidase. The original *sco1-1* mutation leads to a lack of subunit II of this mitochondrial enzyme complex as revealed by radioactive labelling of mitochondrial translation products. The other two mitochondrially synthesized subunits of cytochrome *c* oxidase, CoxI and CoxIII, are not affected. When in wild-type cells the chromosomal DNA fragment carrying the functional *SCO1* gene is replaced by a marker gene, CoxII and simultaneously CoxI are no longer accumulated. This could mean that *SCO1* encodes a bifunctional polypeptide required for synthesis and/or assembly of both CoxI and CoxII. A bifunctional yeast gene has been described only recently, which is involved in the amino-terminal cleavage of CoxII and cytochrome b_2 (Pratje and Guiard 1986). In the case of the *sco1-1* mutation possibly only one of two domains of the gene product are affected, while the other domain necessary for CoxI accumulation is still functional. However, at the moment we cannot exclude the alternative possibility that the deleted chromosomal fragment contains (part of) a second gene which then would function in CoxI accumulation. This would be to our knowledge the first case of a clustering of *PET* genes with related functions.

Irrespective of whether the 1.7 kb fragment carries part of another gene or not in addition to *SCO1*, the failure to accumulate CoxII (and CoxI) cannot result from reduced or missing *OXI1* and *OXI3* transcription, from defective RNA maturation or from preferential degradation of the *OXI1* and *OXI3* mRNA. This was clearly demonstrated by the analysis of mitochondrial transcripts (Fig. 2). The fact that CoxI is present in the *sco1-1* mutant MK20 in a concentration exceeding that of the wild-type demonstrates that the slight reduction in *OXI3* mRNA in MK20 is more than sufficient for synthesis and assembly of CoxI. Therefore the somewhat reduced concentration of *OXI3* mRNA observed in GR20 clearly cannot account for the lack of CoxI in this strain. From the inspection of mitochondrial transcripts we must conclude that the product of the *SCO1* gene must be involved in a post-transcriptional step of CoxII (and possibly CoxI) expression.

Both from the restriction map and from the chromosomal localization it is obvious that *SCO1* is not identical to *PET111*, a previously described yeast nuclear gene with a related function in the accumulation of CoxII (Poutre and Fox 1987). This result has been verified by the demonstration that *sco1* and *pet111* mutants complement each other after crossing (T. Fox, personal communication). The same holds true for strains carrying a mutation in the *PET112* gene, a further nuclear gene required for CoxII accumulation (T. Fox, personal communication).

There is one remarkable difference between *pet111* mutants and the *sco1-1* mutant: fusion of the amino-terminal portion of either CoxI or ATPase9 to CoxII has been shown to suppress *pet111* mutations (Poutre and Fox 1987). The same mitochondrial suppressor genomes, *MSU111-1* and *MSU111-6*, when introduced into the nuclear background of a *sco1-1* mutant, are unable to restore respiratory competence (T. Fox, personal communication). As synthesis of CoxI and of ATPase9 is not affected by the *sco1-1* mutation, it is likely that these novel CoxII polypeptides can be synthesized in *sco1-1* mutants. Therefore the failure to accumulate mature CoxII probably results from a post-translational step. CoxII is the only mitochondrially coded polypeptide which is synthesized with an amino-terminal extension (Pratje et al. 1983; Mannhaupt et al. 1985). This precursor polypeptide is cleaved to yield the mature CoxII

polypeptide. Recently two nuclear mutations, *ts1402* and *ts2858*, have been described which interfere with this proteolytic step (Michaelis et al. 1982; Mannhaupt et al. 1983). As the respective mutants accumulate the CoxII precursor polypeptide, it is unlikely that the absence of CoxII in *sco1* mutants is due to blocked CoxII processing. The possibility that a false processing event in *sco1* mutants leads to a highly unstable cleavage product cannot be excluded. However, the different chromosomal location of *ts1402* (chromosome V), *ts2858* (chromosome XIII) (Mannhaupt et al. 1983) and of *sco1* (chromosome II), as well as the different phenotype of the respective mutants excludes *SCO1* being identical with one of these two genes.

In a recent paper, a very interesting possibility for CoxII formation has been discussed by Poutre and Fox (1987). In this model translation of CoxII is stopped after initiation by an signal recognition particle (SRP)-like particle until the complex interacts with a receptor releasing the elongation block. However, any defective component of this SRP complex or of the receptor should be bypassed by the mitochondrial suppressor mutations *MSU111-1* or *MSU111-6* as is the case for *pet111* mutants. Therefore also in this model, the block exerted by *sco1* mutations is more likely in a step after completion of CoxII synthesis. For example, the *SCO1* gene product might be essential for the proper assembly of CoxII into the mitochondrial membrane, either by constituting a factor in the still unknown pathway of cytochrome *c* oxidase assembly or by modifying the mature CoxII polypeptide post-translationally. From inspection of the published restriction maps it can be excluded that *SCO1* codes for one of the known structural components of cytochrome *c* oxidase (Wright et al. 1984; Maarse et al. 1984; Koerner et al. 1985; Seraphin et al. 1985; Wright et al. 1986; Patterson and Poyton 1986).

Beside *PET111*, *Pet112* and *SCO1* there may be further nuclear genes involved in the synthesis and assembly of CoxII (and CoxI). Of 23 complementation groups which all affect expression of one of the mitochondrially synthesized polypeptides of the respiratory chain, at least 5 somehow affect CoxII accumulation (Kloeckener-Gruissem et al. 1987). Interestingly, in some of these mutants a simultaneous reduction of CoxI is observed without a significant alteration of the mitochondrial transcript pattern. Only a detailed complementation analysis will reveal whether one of these complementation groups represents the *SCO1* gene.

As already outlined in the introduction, not only expression of the *OXI1* gene but also of the *OXI2* and the *COB* genes requires an astonishing number of gene-specific factors encoded by nuclear genes. Most, if not all of these factors act on a post-transcriptional step. Therefore, it is likely that the various steps after transcription of mitochondrial genes leading to the proper assembly of the resulting polypeptides are the primary targets for regulation of mitochondrial gene expression. So far, little is known about the regulation of the nuclear genes which code for those factors required for expression of mitochondrial genes. Only in the case of the *PET494* gene is there an indication of transcriptional repression by glucose (Costanzo and Fox 1986). We have shown that two RNA species of 0.9 kb and 1.2 kb hybridize with the 1.7 kb DNA fragment carrying the functional *SCO1* gene. This result could mean that this DNA fragment carries (part of) another gene in addition to *SCO1*, which is possibly involved in CoxI accumulation. This type of organization would readily account for the loss of CoxI in strains from which the 1.7 kb fragment has been deleted. Alternatively the smaller RNA species could represent the mature *SCO1* transcript, while the longer transcript could represent a precursor form still containing 3′ or 5′ extensions or an intron. No significant difference in the steady state level of both transcripts is observed in glucose-grown or glycerol-grown cells (M. Schulze, unpublished results). Therefore, irrespective of the nature of both transcripts, we have no indication that *SCO1* may be regulated at the transcriptional level.

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