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This paper is dedicated to Professor Maciej Wiewiórowski

Yeast nuclear *PET127* gene can suppress deletions of the *SUV3* or *DSS1* genes: An indication of a functional interaction between 3′ and 5′ ends of mitochondrial mRNAs[o]

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***Saccharomyces cerevisiae* nuclear genes *SUV3* and *DSS1* encode putative RNA helicase and RNase II, respectively, which are subunits of the mitochondrial degradosome (mtEXO): a three-protein complex which has a 3′ to 5′ exoribonuclease activity and plays a major role in regulating stability of mitochondrial RNA. Lack of either of the two gene products results in a respiratory negative phenotype, while on the molecular level it causes a total block of mitochondrial translation, loss of the *in vitro* exoribonuclease activity and changes in stability and processing of many mtRNAs. We have found that the yeast nuclear gene *PET127* present on a low or high copy number vector can effectively suppress the effects of the *SUV3* or *DSS1* gene disruptions. Since the product of the *PET127* gene is involved in processing of the 5′ ends of mitochondrial mRNAs, we suggest that there is a functional coupling between the 5′ and 3′ ends of mitochondrial mRNAs.**

Turnover of RNA in yeast mitochondria is regulated by the protein complex called degradosome or mtEXO [1, 2]. It is composed of three protein subunits and *in vitro* shows a 3′-5′ exoribonuclease activity. Two of the three genes coding for the constituents of the

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Abbreviations: mtEXO, mitochondrial degradosome; mtRNA, mitochondrial RNA; ORF, open reading frame.

degradosome have been identified: the nuclear gene *SUV3* codes for a 84 kDa protein which is a NTP-dependent, putative RNA helicase from the DExH box family [3]; the nuclear gene *DSS1* codes for a 110 kDa protein showing homology to RNaseII from *E. coli* [1, 4]. The third subunit of the degradosome, a 75 kDa protein, has not been identified yet.

Deletion of either the *SUV3* gene or the *DSS1* gene results in a deregulation of the degradosome and leads to many phenotypes. The deletion strains are respiratory negative and quickly lose their mitochondrial genomes. Intronless mitochondrial genomes are more stable, but translation in such strains is blocked and no *in vitro* activity of the 3′-5′ exonuclease can be detected. In addition significant changes in mtRNA stability and processing can be observed: steady-state levels of COB mRNA and of 16S rRNA are very low and precursors of 21S rRNA and *VAR1* mRNA are accumulated [1]. In the deletion strains where intron-containing mitochondrial genomes were introduced accumulation of group I introns was observed, while COX1 and COB mRNAs were increasingly unstable depending on the amount of introns present [5].

We have proposed a model for functioning of the mitochondrial degradosome [1] assuming that it interacts with the 3′ ends of mtRNAs and participates in RNA turnover depending on interactions with RNA structures and with three protein complex of dodecamerase – the enzyme which generates 3′ ends of mitochondrial mRNAs by cleaving close to the conserved dodecamer sequence AAUAAUAUUCUU [6].

Several other nuclear-coded proteins affecting mRNA stability were found in yeast mitochondria. A specific factor interacting with the 5′ end has been described for the COB mRNA, and it is coded by the nuclear gene *CBP1* [7]. The putative roles for the Cbp1 protein include processing of precursor RNA to yield the mature 5′ end and protection of the mRNA from a nuclease activity. The nuclear-coded protein Soc1 has been also suggested to play a role in this process, but the *SOC1* gene has not yet been identified [8].

Another protein affecting mitochondrial RNA stability is the product of the yeast nuclear gene *PET127* [9]. The Pet127p is localized in the mitochondrial membrane and it is necessary for efficient 5′ processing of several precursor transcripts. It has been suggested that Pet127p functions in mRNA surveillance system, eliminating precursors with unprocessed 5′ ends.

In this study we show that the *PET127* gene can act as a low-copy number or high-copy number suppressor of the deletion of either *SUV3* or *DSS1* gene. It restores respiration, translation and mtRNA processing. Our findings suggest that there is a functional coupling between proteins interacting with 5′ and 3′ ends of mtRNAs.

MATERIALS AND METHODS

Yeast strains used in this study are listed in Table 1. YPGly medium contained 2% glycerol, 1% yeast extract and 2% bacto-peptone (Difco); molecular biology methods were according to [10]. Plasmids used as molecular

Table 1. Yeast strains used in this study

Strain	Nuclear genotype	Mitochondrial	Source
BWG	*MATa, his1, ade1, leu2, ura3*	intronless, Δi	[3]
ΔSUV3	*MATa, his1, ade2, leu2, ura3, SUV3::URA3*	intronless, Δi	[3, 4]
ΔDSS1	*MATa, his1, ade2, leu2, ura3, DSS1::URA3*	intronless, Δi	[4]

probes were described [5]. Plasmids pGW808 and pGW810 containing the wild-type *PET127* gene cloned on a low-copy number vector Ycplac111 and a high-copy number vector YCplac181, respectively [9], were kindly provided by Dr. G. Wiesenberger (University of Vienna, Austria).

RESULTS

In order to study functions of the yeast mitochondrial degradosome we were interested in finding other genes whose products may interact with the Suv3p or Dss1p. In particular we were looking for a gene *suB9*, which acts as a recessive suppressor of a deletion of the *SUV3* gene [11]. For this reason we constructed a gene bank from the *suB9* strain on a multicopy Yep351 vector and transformed the ΔSUV3 Δi strain. This approach has been described by us previously [4]. Screening of transformants was performed on YPGly medium and several respiratory competent colonies were found. In this paper we present results for one of the isolates, initially called pTW1.

The plasmid pTW1 was reisolated from the transformed yeast strain and subjected to partial sequencing. Analysis based on the known yeast gene sequences deposited in the GenBank revealed the presence of seven ORFs. Among these, the best candidate for the suppressor gene seemed *PET127*, and we subcloned it as a 3.1 kb fragment on *Hpa*I–*Hin*dIII sites. The subclone contained only the *PET127* reading frame with short flanking sequences. Transformation of this subclone into the ΔSUV3 Δi strain yielded respiratory competent colonies.

Since in our cloning procedure we used the gene bank constructed from a ΔSUV3 *suB9* mutant, we tested if the isolated fragment could bear the *suB9* mutation. For this reason we used a wild-type copy of the *PET127* gene (pGW810). It turned out that the wild-type copy of the *PET127* gene can suppress the deletion of the *SUV3* gene and that the resulting colonies grow with the same rate on YPGly medium, as those containing the pTW1 plasmid (not shown). The results indicate that we have cloned a wild-type copy of the *PET127* gene as a suppressor of the deletion of the *SUV3* gene.

Lack of the functional *SUV3* gene results in the deregulation of the mitochondrial degradosome and it can be suppressed by overexpression of the *DSS1* gene which codes for a second subunit of the degradosome [1]. Therefore we decided to check if the *PET127* gene can also suppress the deletion of the *DSS1* gene. The results are presented on Fig. 1. It can be seen that the *PET127* gene is able to suppress deletion of either *SUV3* gene or *DSS1* gene and in both cases the growth rate

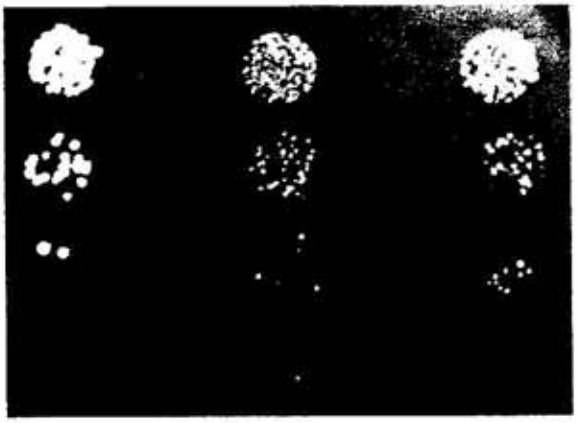

Figure 1. Growth of yeast strains on the YPGly medium at 30°C after 6 days.

1, BWG containing an empty vector YEp351; 2, ΔSUV3 containing an empty vector, ΔEp351; 3, ΔSUV3 containing plasmid YEp351-PET127; 4, ΔDSS1 containing an empty vector YEp351; 5, ΔDSS1 containing plasmid YEp351-PET127.

on YPGly medium is similar. The same results (not shown) were obtained when we used the *PET127* gene cloned on a low-copy number vector (plasmid pGW808).

Yeast strains bearing a disruption of *SUV3* or *DSS1* gene show characteristic changes in the steady state levels of mitochondrial RNAs [1]. While the levels of the COX1 mRNA re-

main stable, the levels of COB mRNA and 16S rRNA are significantly lower, and precursors of 21S rRNA appear. Therefore we tested mtRNA on Northern blots in order to check if the presence of the *PET127* gene influences RNA metabolism in *SUV3* and *DSS1* disruptransformants and in the wild-type strain. Thus for the 21S rRNA the suppressor activity restores the wild-type pattern. The same is true for 16S rRNA: low abundance of the mature transcript in the disruption strains is brought back to the wild-type level. In con-

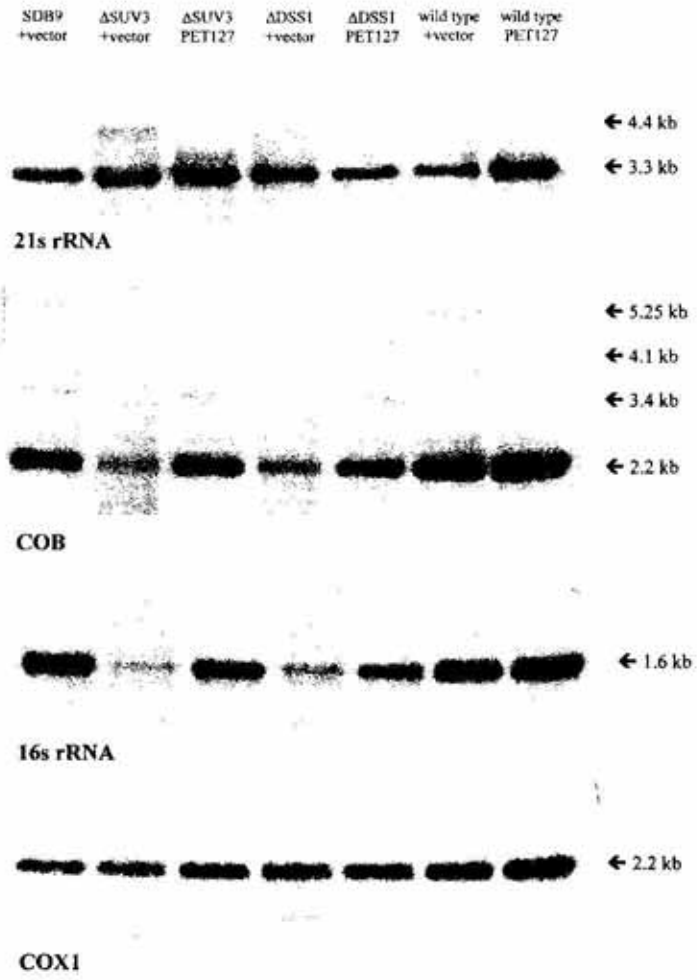

Figure 2. Northern hybridization of RNA isolated from mitochondria of strains containing the intronless mitochondrial genomes to four different probes.

RNA input was standardized for equal amounts of COX1 mRNA. Arrows indicate molecular size.

tion strains. The results are shown in Fig. 2. It can be seen that in both cases the overexpressed *PET127* gene restores the normal levels of COB mRNA and of mature 16S rRNA. The presence of the *PET127* gene in the disruptant strains also affects RNA processing, but in a manner specific for a given transcript. In the case of 21S rRNA the 4.4 kb precursor is present in the *SUV3* and *DSS1* disruption strains but is missing in the *PET127* trast to this, in the case of the COB transcripts, the low level of mature COB mRNA in disruption strains is restored to normal by the action of the *PET127* gene, but in addition a novel 3.4 kb precursor RNA appears, which is characteristic for the suppression exhibited by the *suB9* gene. Mitochondrial translation products were also brought back to wild-type levels (not shown).

DISCUSSION

The results presented here show that the nuclear *PET127* gene, coding for a mitochondrial membrane protein involved in 5′ end processing of mtRNAs, can suppress the lack of *SUV3* or *DSS1* genes. Suv3p and Dss1p are components of the mitochondrial degradosome (mtEXO) – a three protein complex exhibiting a 3′-5′ exonucleolytic activity, but our previous data suggested the involvement of this complex in RNA processing [1]. The observed suppression is surprising as for the first time molecular events at the 5′ ends of mitochondrial RNAs appear to be coupled to the ones associated with the 3′ ends. Involvement of *PET127* could also suggest that this process is associated with mitochondrial membrane.

Functional interactions between 3′ and 5′ ends were recently demonstrated for cytosolic mRNAs. The polyA binding protein Pab1p and the cap binding protein eIF-4E were found to bind to eIF-4G initiation factor [12], which in turn interacts with the eIF-3 of the 40S ribosome subunit [13]. Circular structures indicating physical contact of 3′ and 5′ ends of mRNA with a protein complex were shown by atomic force microscopy [14].

We do not yet have data if Pet127p interacts directly with the components of the degradosome (two-hybrid studies are in progress). It seems, however, that the observed effect is not a fortuitous phenotype but that it reflects *bona fide* interactions between RNA-protein complexes which participate in RNA metabolism. First, the *PET127* gene can bypass the effects of deletions of two distinct genes, second, the suppression also takes place when the gene is present on a low-copy number vector. Interestingly, deletions of the *SUV3* gene or *DSS1* gene result in a total translation block, even for COX1 mRNA, whose abundance and size are not affected in the disruption strains [1]. Thus it is possible that interactions of Suv3p, Dss1p and Pet127p play a role in ensuring mRNA translatability in a way similar to the cytosolic factors.

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