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Systematic screening of nuclear encoded proteins involved in the splicing metabolism of group II introns in yeast mitochondria

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Abstract

Studies of yeast, algae and plants have provided genetic and biochemical evidence that the splicing reaction of organellar localized group II introns either depends on proteins encoded by the introns themselves ('maturases') or encoded by other genes of the host organisms. However, only a few of those proteins have been identified to date and characterized in more detail.

In order to find new nuclear encoded proteins that assist group II splicing, we screened a complete knockout library of *Saccharomyces cerevisiae* strain BY4741 consisting of 4878 viable haploid clones. The strain contains a *rho*$^+$ mitochondrial genome with a set of 13 introns including the three group II introns (aI1, aI2, aI5γ) in the gene encoding cytochrome-*c*-oxidase subunit 1 (*COX1*) and the single group II intron (bI1) in the gene encoding cytochrome *b* (*CYTB*). In our screen and initial molecular analysis, we focus on intron aI5γ, the last intron in the *COX1* gene.

Keywords: Yeast knockout library; *Pet* mutants; Nuclear encoded splicing factors; Group II introns; Intron aI5γ

1. Introduction

Group II introns represent an independent structural class of large catalytic RNAs that were discovered in organelles of certain protists, fungi, algae and plants, and were also shown to be widespread in the bacterial world. The splicing reaction of group II introns is characterized by two consecutive transesterifications joining the flanking exons and releasing the intron in a typical lariat form. Alternatively, first-step hydrolytic cut could be observed leading to a release of the intron in a linear form (for reviews, see: Bonen and Vogel, 2001; Lehmann and Schmidt, 2003). Despite the large and still rapidly growing number of known group II introns, only a few were found to splice autocatalytically from their pre-RNAs in vitro. In general, the in vitro reaction depends on extreme unphysiological conditions (high salt, high temperature, etc.) and even when optimized turnover rates are slow, indicating that those conditions might at least partially compensate for the absence of proteins. Thus, it is generally accepted that proteins contribute to the folding and catalysis of most, if not all group II introns in vivo. The splicing factors already known are either encoded by the introns themselves ('maturases') or encoded by nuclear localized genes. Genetical and biochemical studies of yeast, algae and plants revealed some of the nuclear encoded factors, which are required for the in vivo splicing reaction of organellar group II introns (reviewed in Grivell, 1995; Lambowitz and Perlman, 1999; Barkan and Goldschmidt-Clermont, 2000; Lehmann and Schmidt, 2003). However, only a few of them have been characterized in more detail.

Abbreviations: aI5γ, last intron in the *COX1* gene; *COX1*, *2* and *3*, mitochondrial genes encoding different subunits of cytochrome-*c*-oxidase; CYGD, comprehensive yeast genome database; *CYTB*, mitochondrial gene encoding cytochrome *b*; GII-0, mitochondria carrying no group II introns in their genome; GII-5γ, mitochondria carrying intron aI5γ as the only group II intron in their genome; mtDNA, mitochondrial DNA; ORF, open reading frame; *pet*, yeast mutant carrying a mutation in a nuclear gene required for maintenance of respiratory-competent mitochondria; rho$^+$, yeast cells containing a complete mtDNA; rho°, yeast cells lacking mtDNA; SGD, *Saccharomyces* genome database.

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Séraphin et al. (1987) screened a large collection of nuclear *pet* mutations more than 17 years ago for their effects on the respiratory phenotype of yeast strains with and without the complete set of mitochondrial introns. They estimated that about 18 nuclear genes seem to be essential for the splicing reaction of the mitochondrial introns. A similar number of yeast mitochondrial splicing factors were calculated 5 years ago on the basis of more than 340 nuclear genes from the *Saccharomyces* data bases known to code for mitochondrial localized proteins (Grivell et al., 1999).

In order to find new nuclear encoded group II intron splicing factors, we adapted the above screening strategy of Séraphin et al. (1987). We extended the search by screening all *pet* mutants of a complete yeast knockout library for those mutants that suppress the *pet* phenotype in a mitochondrial background without any group II introns. We also report on the initial analysis of the effects of the same mutants on the splicing metabolism of one of the four mitochondrial group II introns, intron aI5γ.

2. Materials and methods

2.1. Media and genetic procedures

Standard media and methods were used for genetic manipulation of yeast (Sherman, 1991). Yeast cells were grown on complete medium YPD (yeast extract, peptone, dextrose), on YPG containing 3% glycerol as a non-fermentable carbon source and on YNBD (yeast nitrogen base with 2% dextrose) plus required supplements, as noted. Manipulation of the mitochondrial genome (induction of the rho° state, cytoduction) was done following the protocols of Perlman and Mahler (1983).

Important note: All rounds of the following screening procedure were performed without reference to strain identity. The gene disruption in the selected knockout strains was not confirmed by PCR.

2.2. Screening of a yeast knockout library for respiratory-deficient strains (pet mutants)

A complete knockout library of 4878 viable haploid strains obtained from EUROSCARF, Frankfurt, Germany (http://www.uni-frankfurt.de/fb15/mikro/euroscarf/complete.html) was used for screening. These gene deletions were generated in strain BY4741 (*MATa*, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*), an isogenic derivative of strain S288C used for sequencing the yeast nuclear genome (Brachmann et al., 1998) that carries a complete set of mitochondrial introns (Fig. 1A). The knockout library was provided on 75 microtiter plates (96 wells) grown on solid YPD medium. Following transfer of the strains to 96-well plates containing liquid YPD using a 48-pin replicator and growth for 2 days at 30 °C, the cells were stamped on YPD and YPG plates. The plates were incubated for 2 days (YPD) and 4 days (YPG); respiratory-deficient strains unable to grow on YPG medium (*pet* mutants) were selected for further manipulation.

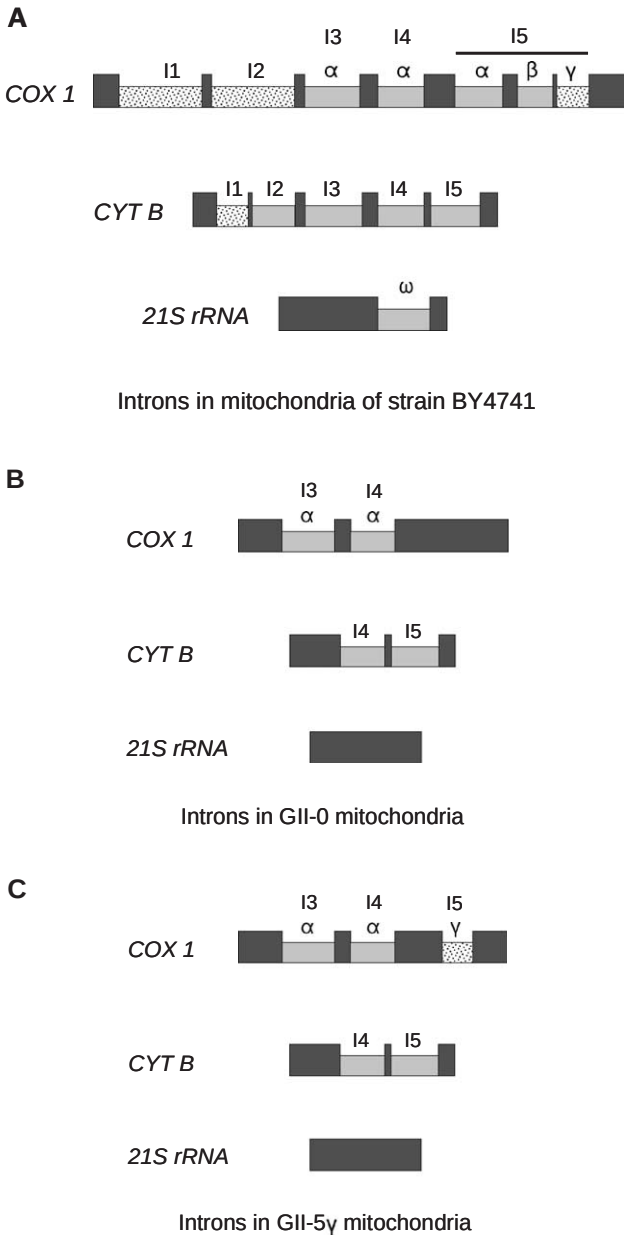

Fig. 1. Schematic drawings of the three intron-containing genes of yeast mitochondria encoding cytochrome-*c*-oxidase subunit I (*COX1*), cytochrome *b* (*CYTB*) and the *21S rRNA*. Black boxes represent exon sequences, grey boxes group I introns and dotted boxes group II introns. (A) The knockout library was constructed in strain BY4741 containing the complete set of 13 mitochondrial introns (introns 3 and 4 in the *COX1* gene were named α because two more introns between introns 3 and 4 and one more between 4 and 5 are known in other *Saccharomyces* species). (B) In the first cytoduction experiment using selected BY4741 knockout strains, the 13 intron containing mt genome was replaced by a genome with only four group I introns (GII-0 mitochondria). (C) In the second cytoduction experiment, a mt genome was introduced containing the same four group I introns and *COX1* intron aI5γ as a single group II intron (GII-5γ mitochondria).