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Review

Analyzing the suppression of respiratory defects in the yeast model of human mitochondrial tRNA diseases

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ABSTRACT

The respiratory defects associated with mutations in human mitochondrial tRNA genes can be mimicked in yeast, which is the only organism easily amenable to mitochondrial transformation. This approach has shown that overexpression of several nuclear genes coding for factors involved in mitochondrial protein synthesis can alleviate the respiratory defects both in yeast and in human cells.

The present paper analyzes in detail the effects of overexpressed yeast and human mitochondrial translation elongation factors EF-Tu. We studied the suppressing activity versus the function in mt translation of mutated versions of this factor and we obtained indications on the mechanism of suppression.

Moreover from a more extended search for suppressor genes we isolated factors which might be active in mitochondrial biogenesis. Results indicate that the multiplicity of mitochondrial factors as well as their high variability of expression levels can account for the variable severity of mitochondrial diseases and might suggest possible therapeutic approaches.

Contents

Abbreviations: mt, mitochondrial; bp, base-pair; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; aa, amino acid(s); aa-RS, aminoacyl-tRNA synthetase; rho$^+$, mt DNA wild-type; rho, mt DNA absent; *NAM2*, gene coding for the yeast mitochondrial leucyl-tRNA synthetase; *TUF1*, *TUFM*, genes coding for the mitochondrial protein synthesis elongation factor EF-Tu in *S. cerevisiae*, in *H. sapiens* respectively; *TSFM*, gene coding for the human mitochondrial translation elongation factor EF-Ts.

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1. Introduction

Mitochondrial (mt) diseases are a heterogeneous group of hereditary disorders caused by mt dysfunction. A large proportion of these diseases is due to mutations in mt tRNA genes, resulting in the inhibition of mt protein synthesis, which can have variable severity, and for which at present no treatments exists.

Some years ago we have established a yeast model of mt diseases in order to exploit the extreme versatility of *Saccharomyces cerevisiae* classical and molecular genetics and the possibility of obtaining mt transformation in yeast by biolistic procedures (Rohou et al., 2000).

We introduced in mt DNA several base substitutions equivalent to those found in mt tRNA genes that are associated with human diseases. These mutant strains were found to exhibit respiratory defects whose severity paralleled the severity of disease observed in human (De Luca et al., 2009). Interestingly we noted that the existence of a defective phenotype and its severity were influenced by the nuclear contexts in which the mutated mitochondria were present (De Luca et al., 2009).

Moreover we have observed that several nuclearly encoded gene products having mt localization and function, could, when overexpressed, relieve the respiratory defects due to mutations in mt tRNA genes in yeast cells. Very promising results were obtained with the *TUF1* gene, encoding the mt protein synthesis elongation factor EF-Tu and with the mt aminoacyl-tRNA synthetases (aa-RS) (De Luca et al., 2006, 2009; Feuermann et al., 2003; Francisci et al., 1998, 2005; Rinaldi et al., 1997; Rohou et al., 2000). Furthermore, we found that suppression of defective respiration in yeast could be obtained by the overexpression of the orthologous human aa-RS (Montanari et al., 2010).

These effects in yeast have been validated by the results afterwards obtained in human cell lines by different laboratories. Lightowlers and colleagues partially restored the steady state level of the mutated $tRNA^{Val}$ by overexpressing the cognate mt valyl-tRNA synthetase (Val-RS) in cybrid cell lines (Rorbach et al., 2008; Park et al., 2008; Li and Guan, 2010) demonstrated that overexpression of mt Leu-RS corrects mt dysfunction of cybrids harboring the m.3243A>G mutation in $tRNA^{Leu}$(UUR), which causes the severe MELAS disease. Recently, the finding that mt Ile-RS can rescue the defective phenotype in cybrids carrying the m. 4277T>C mutation in $tRNA^{Ile}$ (Perli et al., 2012) has opened new therapeutic perspectives.

In the present paper we report a further characterization of the functionality of mt yeast and human EF-Tu factors in their catalytic and suppressing activities in *S. cerevisiae* cells. Throughout the paper we will use the term "catalytic" to indicate the activity in mt translation.

The mt EF-Tu factor is a GTPase highly conserved in evolution, having the main function of delivering the aminoacyl-tRNAs to the A site of ribosomes through the formation of a ternary complex. A chaperone function, preventing thermal aggregation of proteins and enhancing protein *refolding* in vitro, has also been reported (Suzuki et al., 2007). Several other, mainly regulatory, functions of the *TUF1* gene product have been described including aminoacyl-tRNA surveillance in mammalian mitochondria (Nagao et al., 2007).

Despite the high similarity of *TUF1* and *TUFM* both in sequence (Fig. 1) and in structure (Woriax et al., 1995) an important difference exists among the EF-Tu factors; contrary to what happens in *S. cerevisiae*, an additional exchange elongation factor (EF-Ts) is required in human, in *Escherichia coli* and in the fission yeast *Schizosaccharomyces pombe*. This difference is difficult to explain in so highly conserved EF-Tu factors, and has been the object of a very detailed genetic analysis by Chiron et al. (2005). Proposed explanations underline the higher expression level of the *TUF1* gene in *S. cerevisiae* compared to *S. pombe* and suggest that

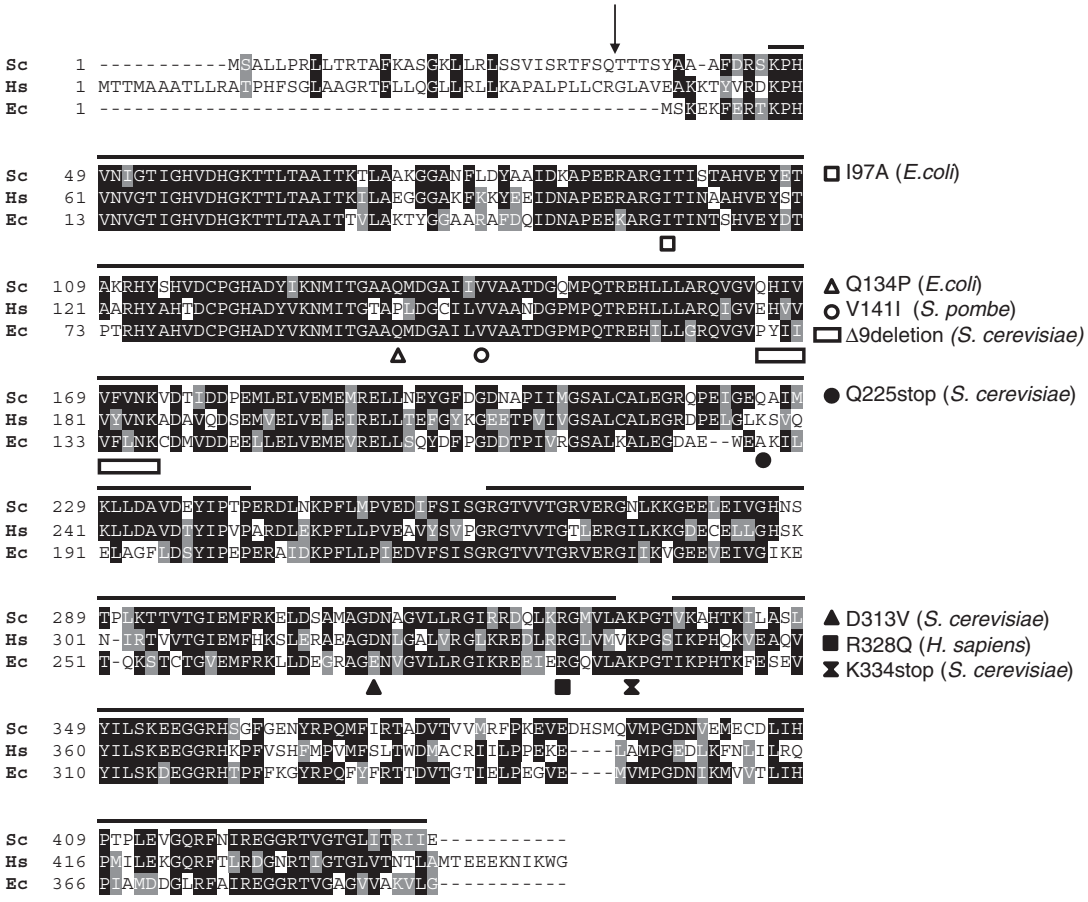

Fig. 1. EF-Tu protein alignment of *S. cerevisiae* (Sc), *H. sapiens* (Hs), and *E. coli* (Ec). Analyzed mutations are marked and numbered following the *S. cerevisiae TUF1* sequence. In brackets the species in which the equivalent mutation has been analyzed. The arrow indicates the mt pre-sequence end. The three domains are shown by horizontal lines.