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SSD1 modifies phenotypes of Elongator mutants

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Abstract
The translational decoding properties of tRNAs are influenced by post-transcriptional modification of nucleosides in their anticodon region. The Elongator complex promotes the first step in the formation of 5-methoxycarbonylmethyl (mcm5), 5-methoxycarbonylhydroxymethyl (mchm5), and 5-carbamoylmethyl (ncm5) groups on wobble uridine residues in eukaryotic cytosolic tRNAs. Elongator mutants in yeast, worms, plants, mice, and humans not only show a tRNA modification defect, but also a diverse range of additional phenotypes. Even though the phenotypes are almost certainly caused by the reduced functionality of the hypomodified tRNAs in translation, the basis for specific phenotypes is not well understood. Here, we discuss the recent finding that the phenotypes of *Saccharomyces cerevisiae* Elongator mutants are modulated by the genetic background. This background-effect is largely due to the allelic variation at the SSD1 locus, which encodes an mRNA-binding protein involved in post-transcriptional regulation of gene expression. A nonsense ssd1 allele is found in several wild-type laboratory strains and the presence of this allele aggravates the stress-induced phenotypes of Elongator mutants. Moreover, other phenotypes, such as the histone acetylation and telomeric gene silencing defects, are dependent on the mutant ssd1 allele. Thus, SSD1 is a genetic modifier of the phenotypes of Elongator-deficient yeast cells.

Keywords Elongator complex · tRNA modification · Translation · mRNA-binding protein

Introduction
Post-transcriptionally modified nucleosides are found within all tRNA molecules. Modified nucleosides in the anticodon region usually promote proper anticodon-codon interactions and they are consequently important for the efficiency and fidelity of translation (Agris et al. 2017; Björk and Hagervall 2014). Uridine residues present at the wobble position (nucleoside 34) in eukaryotic cytosolic tRNAs are frequently modified to an xm5U-type of modified nucleoside where the xm5 moiety is either a 5-methoxycarbonylmethyl (mcm5), 5-methoxycarbonylhydroxymethyl (mchm5), or 5-carbamoylmethyl (ncm5) group (Machnicka et al. 2014). The xm5U residues sometimes also contain an additional 2′-O-methyl (xm5Um) or 2-thio (xm5s2U) group. The presence of an xm5U34, xm5Um34, or xm5s2U34 residue is generally believed to improve pairing with the cognate codon(s) (Agris et al. 2017; Björk and Hagervall 2014; Björk et al. 2007; Johansson et al. 2008; Lim 1994). In this review, we discuss the phenotypic consequences of the lack of wobble xm5 groups in *Saccharomyces cerevisiae*, focusing on the recent finding that the phenotypes are modulated by the genetic background.

The first step in formation of the xm5 groups is dependent on the Elongator complex, which is composed of two sets of the six Elp proteins (Elp1–Elp6) (Dauden et al. 2017, 2018; Huang et al. 2005; Johansson et al. 2018; Kolaj-Robin and Seraphin 2017; Setiaputra et al. 2017; Winkler et al. 2001). Elongator is thought to catalyze the formation of a cm5U34 residue, which is then further modified by additional enzymes. The xm5 moiety found in cytosolic *S. cerevisiae* tRNAs is either an mcm5 or ncm5 group. Such groups are present in 11 U34-containing tRNA species of which two carry mcm5U34, three mcm5s2U34, five ncm5U34, and one ncm5Um34 (Fig. 1) (Johansson et al. 2008 and references therein). In addition to the lack of mcm5/ncm5 groups in the 11 tRNAs (Huang et al. 2005; Johansson et al. 2008),

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that the inactivation of the Ncs2/Ncs6 complex, which catalyzes the formation of the s² group, induces essentially the same phenotypes that are also suppressed by increased expression of the tRNA\textsubscript{\textsc{Glu}}\textsubscript{\textsc{UUC}}, tRNA\textsubscript{\textsc{Gln}}\textsubscript{\textsc{UUG}}, and tRNA\textsubscript{\textsc{Glu}}\textsubscript{\textsc{UUC}} combinations (Björk et al. 2007; Chen et al. 2011; Esberg et al. 2006; Huang et al. 2008; Leidel et al. 2009; Nakai et al. 2008; Noma et al. 2009). Moreover, ribosome profiling experiments have shown that the lack of wobble mcm\textsuperscript{5}\textsubscript{U} and/or s² groups leads to an accumulation of ribosomes with AAA, CAA, and GAA codons in their A-site (Chou et al. 2017; Nedialkova and Leidel 2015; Zinshteyn and Gilbert 2013). The mechanism by which the inefficient decoding of these codons induces the phenotypes are not well understood. One model suggests that the phenotypes may be caused by reduced expression of factors encoded from mRNAs enriched in AAA, CAA, and/or GAA codons (Bauer et al. 2012; Chen et al. 2011; Fernandez-Vazquez et al. 2013; Rezgui et al. 2013). In this model, the slower decoding of the mRNA leads to reduced protein abundance by a mechanism that may involve elevated levels of frameshifting or inhibition of translation initiation through ribosome queuing. Another model suggests that the phenotypes may be caused by the proteotoxic stress that arises from defects in co-translational protein folding and the consequent accumulation of protein aggregates (Nedialkova and Leidel 2015). As the proteins that show increased aggregation in strains lacking the mcm\textsuperscript{5}\textsubscript{U} and s² groups are not encoded by mRNAs enriched in AAA, CAA, and/or GAA codons (Nedialkova and Leidel 2015), it remains unclear if the protein aggregation is a direct or indirect consequence of the inefficient decoding of these codons.

The recent finding that the phenotypes of Elongator-deficient cells are influenced by the allelic variant at the SSD\textsubscript{1} locus provides additional information into the pleiotropic effects of Elongator (Xu et al. 2019). Several wild-type laboratory S. cerevisiae strains harbor a nonsense mutation in the SSD\textsubscript{1} gene, which encodes an mRNA-binding protein that associates with a subset of mRNAs and regulates their stability, translation, and/or localization (Hogan et al. 2008; Jansen et al. 2009; Jorgensen et al. 2002; Kurischko et al. 2011; Ohyama et al. 2010; Sutton et al. 1991; Uesono et al. 1997; Wanless et al. 2014). The notion that the SSD\textsubscript{1} locus influences the phenotypes of Elongator mutants was inferred from the observation that the temperature sensitivity (Ts) of cells deleted for ELP\textsubscript{3}, which encodes an Elongator subunit, is significantly stronger in the W303 than in the related S288C genetic background (Xu et al. 2019). Strains in the W303 genetic background contain the nonsense ssd\textsubscript{1}-d2 allele whereas those in S288C harbor an SSD\textsubscript{1} allele that encodes the full-length functional protein (Jorgensen et al. 2002; Sutton et al. 1991). Analyses of congeneric ssd\textsubscript{1}-d2 elp\textsubscript{3A} and SSD\textsubscript{1} elp\textsubscript{3A} strains, in both genetic backgrounds, showed that the ssd\textsubscript{1}-d2 allele not only aggravates the Ts...
phenotype of elp3Δ mutants but also the growth defects induced by various stress-inducing agents (Xu et al. 2019). In these assays, the effect of the ssd1-d2 mutation is comparable to an ssd1Δ allele. Further, the telomeric gene silencing and histone H3 acetylation defects of W303-derived Elongator mutants were found to be dependent on the ssd1-d2 allele, i.e., the phenotypes are suppressed by the introduction of the SSD1 gene.

The SSD1 gene has been genetically implicated in a diverse range of cellular pathways and processes, including cell morphogenesis, cell wall integrity, cellular aging, virulence, several signal transduction pathways, protein homeostasis, and transcription by RNA polymerase I, II, and III (Jorgensen et al. 2002; Kaeberlein et al. 2004; Kaeberlein and Guarente 2002; Stettler et al. 1993; Wheeler et al. 2003; Wilson et al. 1991). A likely explanation to the large number of genetic interactions is the function of Ssd1 in post-transcriptional gene regulation. For the Ssd1-associated mRNAs that encode factors involved in cell wall biosynthesis, Ssd1 is thought, depending on its phosphorylation status, to promote either translational repression or polarized localization (Jansen et al. 2009; Kurischko et al. 2011; Wanless et al. 2014). Moreover, the inactivation of SSD1 alters the abundance and stability of many mRNAs and this effect is not restricted to Ssd1-associated transcripts (Jansen et al. 2009; Li et al. 2009a). The precise mechanisms by which the allele at the SSD1 locus influences the phenotypes of Elongator mutants are not known, but they may involve both direct and indirect effects of Ssd1’s function in messenger ribonucleoprotein complexes. The ssd1-d2 allele does not influence the formation of the mcm3/ncm5 groups and analyses of the ssd1-d2 elp3Δ and SSD1 elp3Δ strains revealed no apparent difference in tRNA levels or the abundance of other modified nucleosides (Xu et al. 2019). Further, +1 frameshifting assays indicated that the A-site selection rate at the AAA codon is comparable in ssd1-d2 elp3Δ and SSD1 elp3Δ strains. While these observations suggest that the ssd1-d2 allele does not influence the abundance or functionality of the hypomodified tRNAs, it remains possible that the lack of Ssd1 may affect tRNA function under stress conditions. However, the two phenotypes that are dependent on the ssd1-d2 allele, the histone H3 acetylation and telomeric gene silencing defect, are detected under standard growth conditions, indicating that at least these phenotypes are not caused by a synergistic effect on tRNA function. The telomeric gene silencing defect in Elongator mutants is thought to be caused by reduced levels of the Sir4 protein, which is involved in the assembly of silent chromatin (Chen et al. 2011). The SIR4 open reading frame is enriched in AAA codons and the telomeric gene silencing defect is suppressed by increased expression of tRNAlys1UUU (Chen et al. 2011). Further, the overexpression of tRNAlys1UUU restores Sir4 levels without significantly influencing the mRNA levels (Chen et al. 2011). Even though these observations imply that the decreased silencing at telomeres is caused by reduced Sir4 levels, it is not known if the reduction is large enough to cause the phenotype and if it is a direct consequence of inefficient decoding of the SIR4 mRNA. Nevertheless, the finding that the telomeric gene silencing defect is dependent on the ssd1-d2 allele shows that the lack of the mcm3/ncm5 groups is not sufficient to induce the phenotype (Xu et al. 2019). Additional experiments are needed to investigate if the levels of Sir4 are modulated by the allele at the SSD1 locus.

The effect of the SSD1 locus also partially explained why an elp3Δ ncs2Δ double mutant, which lacks both the mcm3/ncm5 and s2 groups, is not viable in the W303 but not in the S288C genetic background (Björk et al. 2007; Klassén et al. 2015; Nedialkova and Leidel 2015; Xu et al. 2019). An ssd1-d2 elp3Δ ncs2Δ strain is, however, viable but very slow-growing in the S288C background, indicating the growth phenotype is influenced by additional genetic factors (Xu et al. 2019). Consistent with the finding that Ssd1 promotes Hsp104-mediated protein disaggregation (Mir et al. 2009), the levels of aggregated proteins were found to be higher in ssd1-d2 elp3Δ ncs2Δ than in SSD1 elp3Δ ncs2Δ cells (Xu et al. 2019). Although these observations indicate that the slow growth of the ssd1-d2 elp3Δ ncs2Δ strain may be caused by the accumulation of protein aggregates, it is not known if the aggregation is the cause or the consequence of the growth defect.

The presence of the nonsense ssd1-d2 allele sensitizes yeast cells to the translational defects induced by the lack of Elongator-dependent tRNA modifications. Future work is needed to define the mechanisms by which SSD1 modulates the phenotypes of Elongator-deficient cells. It also remains to be determined if the phenotypes of Elongator mutants in other organisms are modulated by polymorphisms in genes for mRNA-binding proteins.
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