Review

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Ltv1 and its role in protein trafficking and ribosomal assembly in *Saccharomyces cerevisiae*

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Abstract

In *Saccharomyces cerevisiae*, *ltv1* is involved in assembling and trafficking the 40S subunit in ribosomes, as well as trafficking other proteins such as GAP1p. LTV1p helps facilitate 40S subunit assembly by binding ENP1p and recruiting other proteins. After, nuclear export of the 40S subunit occurs via the leucine-rich nuclear export signal (NES). The formation of ribosomes is essential for protein synthesis, therefore, mutating *ltv1* has many physiological consequences. Several studies have reported that the deletion mutant, *ltv1-Δ* affects growth, sensitivity to stressors and ribosomal assembly and production in *S. cerevisiae*. This review summarizes current findings on the *ltv1* gene, LTV1p protein structure and function, as well as the consequences of *ltv1* knockout mutation. Although *ltv1* is involved in several molecular pathways, the involvement of *ltv1* in plasma membrane recycling and 40S subunit assembly is not fully understood. It is also unknown how *ltv1-Δ* mutants respond to stressors such as changes in pH and increased temperature. Therefore, this review proposes a mechanism for how *ltv1* is involved in pre-40S subunit assembly and suggests what type of research can be done to broaden our understanding of the *ltv1* gene.

Introduction

In eukaryotes, ribosomal RNA (rRNA) is essential for protein translation and is made up of the small 40S subunit and the large 60S subunit, which forms the 80S ribosome. To form the 40S subunit, the pre-40S subunit needs to be assembled and shuttled from the nucleolus to the cytoplasm, where several other proteins bind to the pre-40S subunit to assemble the 40S subunit (Schafer, 2003). Several genes mediate the assembly and export of the pre-40S, as well as the trafficking of other proteins. *Ltv1* is one such gene in *Saccharomyces cerevisiae* that plays a role in 40S subunit assembly and trafficking, as well as shuttling the GAP1 protein. This review summarizes current findings on the *ltv1* gene and protein in *Saccharomyces cerevisiae*, including questions that are currently unanswered in the field and a proposed mechanism involving *ltv1*. Since translation in the cytoplasm and protein shuttling are shared features amongst eukaryotes, research done on *ltv1* may provide more insight into how ribosomes are assembled in vertebrates.

Functions of *ltv1*

In *S. cerevisiae*, *ltv1* is a conserved gene that is involved in the nuclear export and assembly of the small 40S subunit in rRNA into the cytoplasm (Merwin *et al.*, 2014; Collins *et al.*, 2018). LTV1p contains

a leucine-rich nuclear export signal (NES) at its C-terminus that is necessary and sufficient to allow the export of proteins with a nuclear localization signal (NLS) (Seiser *et al.*, 2006; Merwin *et al.*, 2014). A study conducted by Merwin *et al.* (2014) confirmed that LTV1p has a functional NES because it can replace the NES in NDM3p, which functions to export the large 60S subunit. Research done by Seiser *et al.* (2006) showed that LTV1p does not directly export the 40S subunit by itself but rather links the 40S subunit to other proteins, to export the 40S subunit. To facilitate nuclear export of the 40S subunit into the cytoplasm, LTV1p must interact with CRM1p, which usually binds to substrates containing an NES. However, CRM1 binds to LTV1p very loosely. Therefore, RanGTP proteins, specifically YRB2 in *S. cerevisiae*, increase the binding affinity of CRM1p to LTV1p. LTV1p also facilitates rRNA assembly by interacting with many different proteins. To help with rRNA assembly (Figure 1a), LTV1p binds to ENP1p, which allows the proteins RPS3p, RPS10p, ACL1p, and RACK1p to assemble into the beak of the 40S ribosomal subunit (Collins *et al.*, 2018). Therefore, LTV1p also functions as an assembly factor.

Not only can *ltv1* help with exporting the 40S subunit, but it is also involved in trafficking other proteins, such as GAP1p (Gao & Kaiser, 2006). LTV1p forms a complex with GTPases (GSE complex) and other proteins in the endosomal membrane when shuttling GAP1p from the late endosome to the plasma

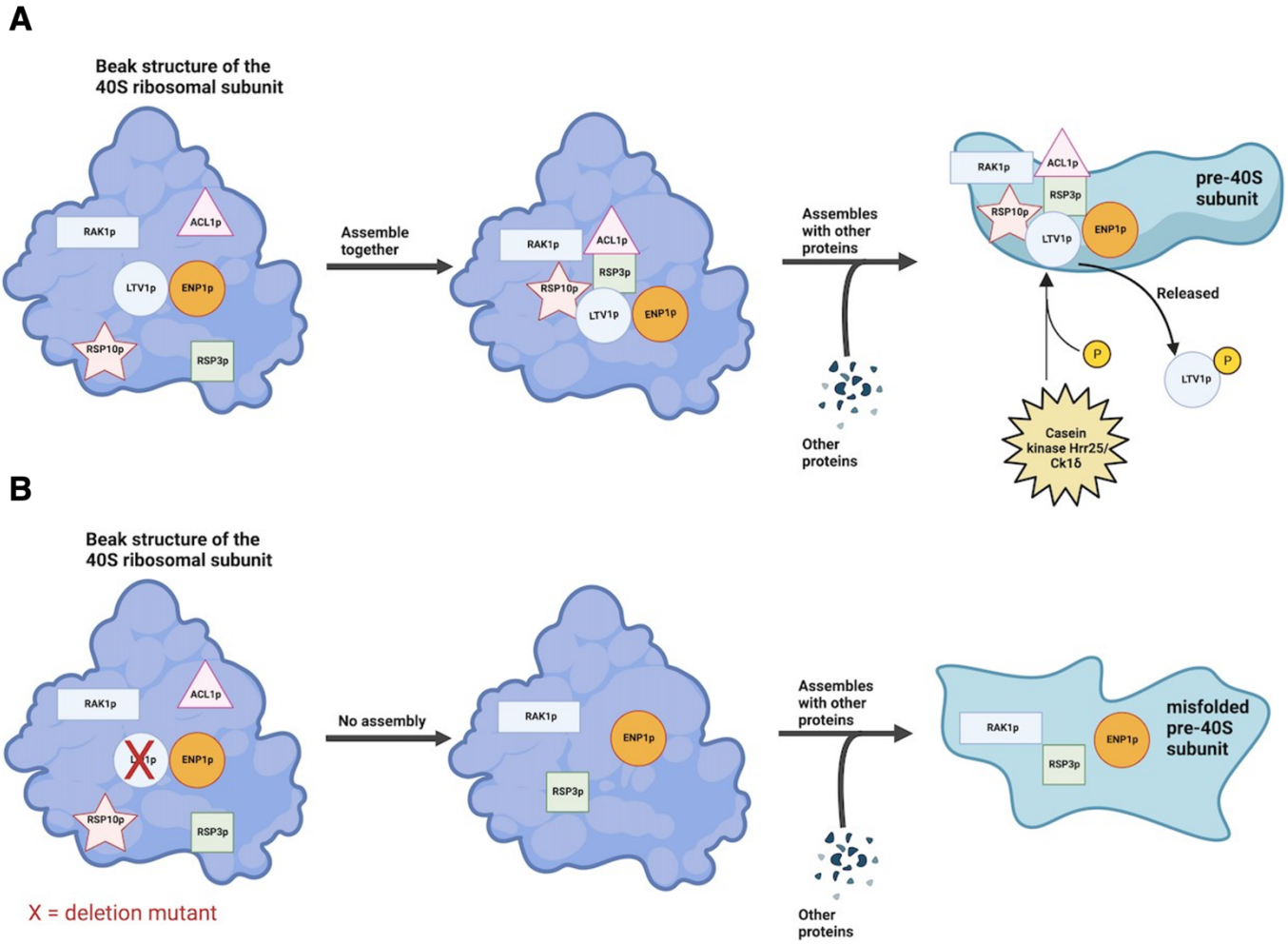

Figure 1. Modelling the assembly of the 40S rRNA subunit in the cytoplasm. (A) In the nascent beak structure of the ribosome (shown in dark blue), LTV1p binds to ENP1p which helps assemble RSP10p, ACL1p, RAK1p and RSP3p. In combination with other proteins, this complex makes up the head of the pre-40S subunit (shown in teal). Hrr25/Ck11δ is a casein kinase that phosphorylates LTV1p to release it from the pre-40S subunit. **(B)** When *ltv1* is deleted as shown by the red X, ENP1p, RSP10p, ACL1p, RAK1p and RSP3p do not assemble, resulting in a misfolded pre-40S subunit. This figure was made using BioRender.

membrane. Within this complex, LTV1p interacts downstream of GTR1p (MacDonald & Piper, 2017). This complex is necessary to sort GAP1p from the late endosome to eventually go to the membrane (Gao & Kaiser, 2006).

Consequences of the *ltv1-Δ* Mutation

Previous studies have also shown the consequences of deleting the *ltv1* gene (*ltv1-Δ*) in *S. cerevisiae* (Gao & Kaiser, 2006; Seiser *et al.,* 2006; Collins *et al.,* 2018). General physiological consequences of the deletion include slower cell growth, increased sensitivity to protein synthesis inhibitors (Seiser *et al.*, 2006), cold-sensitivity and resistance to certain stressors such as caffeine and ethanol (Collins *et al.*, 2018). During ribosomal assembly, *ltv1-Δ* mutants have fewer functional 40S subunits compared to wild-type, less *Gap1* activity and are deficient in RSP10p and ASCL1p (Figure 1b), resulting in problems during translation, ribosomal-mediated quality control and problems with codon recognition (Collins *et al.*, 2018). Within the GSE complex, the deletion mutation increases *Gse2* expression but does not affect the expression of any other components (Gao & Kaiser, 2006). Although the consequences of the *ltv1-Δ* mutation are known, its involvement in ribosome assembly and export could be further investigated.

Knowledge Gaps and Future Directions

Several researchers have posed gaps of knowledge regarding the function of *ltv1*. For example, Ma & Burd (2020) indicated that *ltv1*'s role in regulating plasma membrane recycling is not known. Additionally, Seiser *et al.* (2006) indicated that it is unknown whether *ltv1* is necessary for the interaction between CRM1p, YRB2p and the small 40S subunit and whether the interaction between the 40S ribosomal protein, RPS2p and LTV1p is necessary for the nuclear export of the 40S subunit. Gaps of knowledge can also be inferred based on previous findings on the *ltv1-Δ* mutation. As previously stated, *ltv1-Δ* was shown to be resistant to particular stressors. However, there are many stressors that have not been tested in *ltv1-Δ S. cerevisiae*, such as tetrahydrocannabinol (THC) exposure and different pH levels. Therefore, further research is required to determine whether stressors other than ethanol and caffeine affect resistance and growth in *ltv1-Δ S. cerevisiae.* Previous research has also indicated that *ltv1-Δ* is cold sensitive (Collins *et al.*, 2018), though not much research has been done to see whether *ltv1-Δ S. cerevisiae* is heat sensitive. Loar *et al.* (2004) have previously shown that *ltv1-Δ* mutants grow slightly slower at 30˚C compared to wild-type, however, it is not known how *ltv1-Δ S. cerevisiae* grows when heat-shocked at 37˚C. Further exploring how *ltv1-Δ S. cerevisiae* responds to higher temperatures would likely provide more insight as to how ribosomal assembly is affected in higher temperatures when *ltv1* is mutated.

Summary and Conclusion

Ltv1 in *S. cerevisiae* is a conserved gene that plays a significant role in ribosome assembly and nuclear export, as well as GAP1p trafficking. The deletion mutant *ltv1-Δ* results in physiological changes such as cold sensitivity, defects in ribosome assembly and decreased *Gap1* expression. There are currently gaps in understanding the role of *ltv1* in ribosomal assembly, suggesting potential directions for further research. Future studies can determine how *ltv1-Δ S. cerevisiae* responds to certain environmental stressors, such as exposure to high temperatures and different pH levels.

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