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Review

The cytoplasmic and nuclear populations of the eukaryote tRNA-isopentenyl transferase have distinct functions with implications in human cancer

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ABSTRACT

Mod5 is the yeast tRNA isopentenyl transferase, an enzyme that is conserved from bacteria to humans. Mod5 is primarily cytoplasmic where it modifies the A37 position of a few tRNAs, and the yeast enzyme has been shown capable of forming heritable, amyloid-like aggregates that confer a selective advantage in the presence of specific antifungal agents. A subpopulation of Mod5 is also found associated with nuclear tRNA genes, where it contributes tRNA-gene mediated (tgm) silencing of local transcription by RNA polymerase II. The tgm-silencing function of Mod5 has been observed in yeast and a Mod5-deletion in yeast can be complemented by the plant and human tRNA isopentenyl transferases, but not the bacterial enzymes, possibly due to the lack of an extended C-terminal domain found in eukaryotes. In light of this additional nuclear role for Mod5 we discuss the proposed role of the human homologue of Mod5, TRIT1, as a tumor suppressor protein.

1. Mod5 has distinct functions in the nucleus and cytoplasm

Transfer RNA (tRNA) molecules undergo numerous post-transcriptional modifications, with greater than 15% of all the nucleotides of nuclear-encoded tRNAs in *Saccharomyces cerevisiae* having covalent modifications (Phizicky and Hopper, 2010). The effects of these modifications include changes in tRNA stability, translation efficiency, and translation fidelity (Phizicky and Hopper, 2010; Urbonavicius et al., 2001). Transfer RNA modifications are prevalent around the anticodon loop, particularly at nucleotide positions A34 and A37. In all domains of life, the A37 nucleotide modification of some tRNAs is conserved and suggests an important evolutionary purpose for these modifications (El Yacoubi et al., 2012; Phizicky and Hopper, 2010). One such modification is the conversion of A37 to N6-(isopentenyl) adenosine (i6A37) by a tRNA isopentenyl transferase. The enzyme responsible for this activity in *S. cerevisiae* is Mod5, which transfers an isopentenyl group from dimethylallyl pyrophosphate (DMAPP) to N6 of A37 on a small subset of tRNAs (Dihanich et al., 1987).

The *MOD5* gene is non-essential, but highly conserved and the tRNA-modifying function of its protein product is conserved in bacteria (Soderberg and Poulter, 2000), yeast (Dihanich et al., 1987; Lamichhane et al., 2011), worm (Lemieux et al., 2001), plant (Golovko et al., 2002), and human (Golovko et al., 2000). There are two highly conserved domains of Mod5 which are responsible for substrate binding (ATP/GTP and DMAPP) (Fig. 1) (Soderberg and Poulter, 2000; Zhou and Huang, 2008). Structurally, the bacterial and eukaryotic tRNA isopentenyl transferases are similar in that they both contain a large core domain and insertion domain which interact to form a channel where the anticodon stem loop of the bound tRNA resides (Soderberg and Poulter, 2000; Zhou and Huang, 2008).

The bacterial and eukaryotic homologues of this enzyme have been conserved in their tRNA modification activity (Dihanich et al., 1987; Golovko et al., 2000, 2002; Soderberg and Poulter, 2000), but the eukaryotic versions also have additional capabilities. A major difference between the bacterial and eukaryotic tRNA isopentenyl transferase sequences is that the eukaryotic proteins contain an ~100 residue zinc finger-containing, C-terminus that is absent from the bacterial enzymes (Golovko et al., 2000, 2002; Lemieux et al.,

Abbreviations: tRNA, transfer RNA; TRIT1, tRNA isopentenyl transferase 1; i6A37, N6-(isopentenyl) adenosine; DMAPP, dimethylallyl pyrophosphate; NLS, nuclear localization signal; ORF, open reading frames; tgm, tRNA gene-mediated; pol II, RNA polymerase II; pol III, RNA polymerase III; RSC, chromatin structure remolding complexes; SINES, Short Interspersed Elements; SNP, single nucleotide polymorphism.

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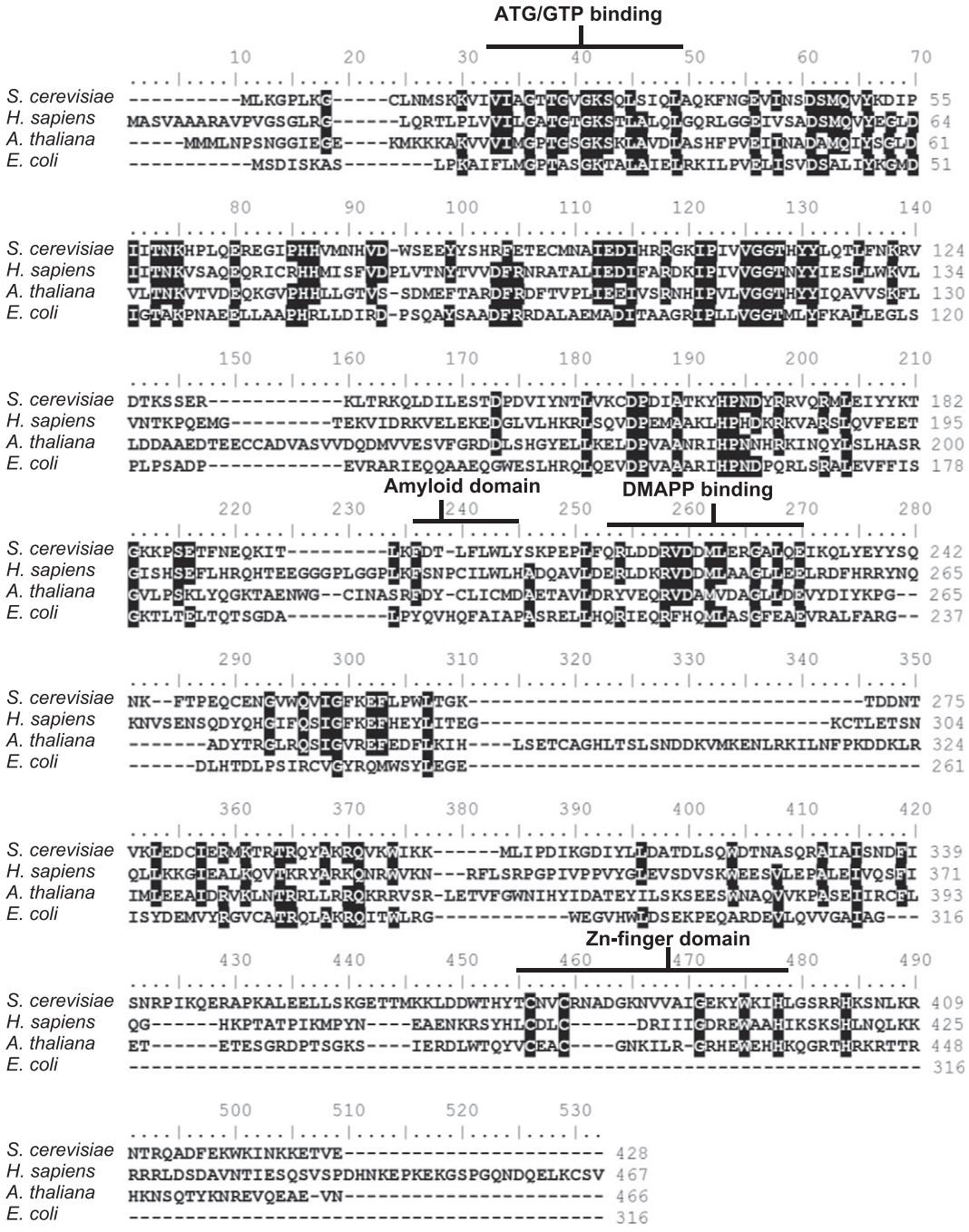

Fig. 1. The tRNA isopentenyl transferase is mostly conserved from bacteria to humans, however eukaryotes have evolved an additional C-terminal tail not present in *E. coli*. The ATP/GTP-domain and DMAPP-binding domain are conserved in all four species. The amyloid, prion-like domain required for Mod5-aggregation (Suzuki et al., 2012) is partially conserved between yeast and humans and suggests potential for human TRIT1 aggregation capabilities. A zinc-finger binding domain in the c-terminal tail is conserved among the eukaryotes and not present in bacteria (Soderberg and Poulter, 2000; Zhou and Huang, 2008).

2001; Soderberg and Poulter, 2000; Zhou and Huang, 2008) (Fig. 1). The exact function of this domain is not currently clear, but it has been proposed by Zhou and Huang (2008) to function in protecting the insertion domain from degradation and/or increasing the number of contact points that the protein makes with the tRNA as means to increase the specificity for tRNA-binding (Zhou and Huang, 2008). An increase in discrimination in binding for a tRNA modifying enzyme is in accordance with increased complexity of eukaryotic proteomes, which likely require a greater stringency during translation. Alternatively, the observation that bacterial enzymes do not contain this C-terminus but are still able to effectively modify tRNAs may suggest that the C-terminus has evolved a function for the eukaryotic enzymes in addition to the tRNA-modifying function. Interestingly, the C-terminus contains a bipartite nuclear localization signal (NLS), which directs a pool of Mod5 to the nucleus, specifically the nucleolus (Tolerico et al., 1999). The reason for this nucleolar localization was not originally clear, since it is thought this tRNA modification only occurs in the cytoplasm.

The *MOD5* gene encodes two in-frame open reading frames (ORFs), each translated from a separate AUG sites at amino acids positions 1 and 12, respectively. Mod5 protein translated from the first AUG site localizes to the mitochondria and cytoplasm (Mod5p-I), whereas Mod5 translated from the second AUG site localizes to the nucleus and cytoplasm (Mod5p-II) (Boguta et al., 1994; Tolerico et al., 1999). The