Proteins involved in the cmnm^{5}U-modification at the wobble-position of tRNA

A. Scrima and A. Wittinghofer

Max-Planck- Institut für molekulare Physiologie, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany

GidA is a FAD-binding protein of a size of 70 kDa. This protein, together with the GTP-binding protein TrmE, is believed to be directly involved in an enzymatic reaction, the modification of tRNA at the wobble U34 position of tRNA in bacteria, yeast and mammalia. Bacterial strains lacking either GidA or the 50 kDa TrmE protein are deficient in the biosynthesis of tRNA modified at position 5 [1]. The modification of U34 at the 2 and 5 position of the uridine base leads to 5-methylaminomethyl-2-thiouridine in bacteria, 5-carboxymethylaminomethyl-2-thiouridine in yeast and as 5-taurinomethyl-2-thiouridine in human. The modification at the 5 position allows interaction of U with G and A, but restricts base-pairing with C and U [2]. This is extremely important in mixed codon box families (Glu, Gln, Lys, Leu and Arg) for which base-pairing of U with C or U would lead to misincorporation of amino acids. Furthermore the modification influences frameshifting during the translation process [3].

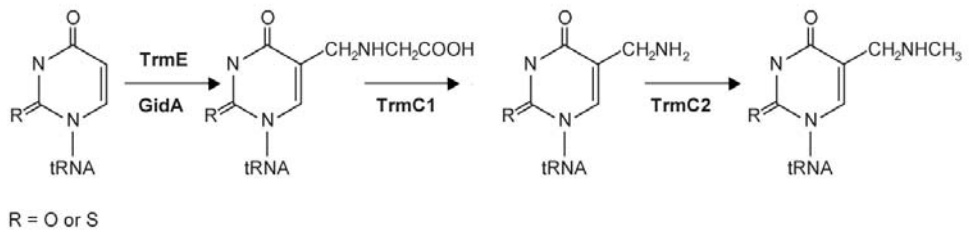

Figure 1: Proposed pathway for the biosynthesis of 5-methylaminomethyl-uridine at U34 in tRNA. R can be oxygen or sulfur.

The modification of U34 requires many different proteins: MnmA catalyzes the thiolation of U34 at the 2-position leading to s^{2}U [4]. GidA is believed to be involved in the first modification step at the 5 position, the formation of 5-carboxymethyl-aminomethyl-2-thiouridine (cmnm^{5}s^{2}U), which also requires the protein TrmE (also called MnmE) [1,5]. In a following modification step the TrmC protein is believed to catalyse the formation of 5-methylaminomethyl-2-thiouridine (mnm^{5}s^{2}U) (Fig. 1) [6].

Mutant alleles of the GidA and TrmE homologues Mto1 and MSS1 in *S. cerevisiae* reveal a respiratory-deficient phenotype. Recent studies on GTPBP3 and Mto1, the human homologues of TrmE and GidA, lead to the suggestion that those proteins may also be involved in several human diseases like the non-syndromic-deafness or different clinical forms of myofibrillar myopathy (MERRF: myoclonic epilepsy; ragged red fibres / MELAS: mitochondrial encephalomyopathy; lactic acidosis; stroke) which are based on mutations in mitochondrial tRNA genes [7-9].

After having solved the structure of the GTP-binding protein TrmE from *Thermotoga maritima* [10], we will now try to get new insights into the whole reaction mechanism by analyzing the different components of the reaction pathway.

References

[1] D. Elseviers, L.A. Petrullo and P. Gallagher, *Nucleic Acids Res*, 12, 3521–3534 (1984)
[2] S. Yokoyama and S. Nishimura, in *tRNA: Structure, Biosynthesis and Function* (1995)
[3] J. Urbonavicius, Q. Qian, J.M.B Durand, T.G. Hagervall and G.R. Björk, *EMBO J.* 20, 4863–4873 (2001)

[4] M.A. Sullivan, J.F. Cannon, F.H. Webb and R.M. Bock , *J Bacteriol*, 161, 368–376. (1985)
[5] D. Brégeon, V. Colot, M. Radman, F. Taddei, Genes Dev. 15(17):2295-306 (2001)
[6] T.G. Hagervall, C.G. Edmonds, J.A. McCloskey and G.R. Björk ,*J Biol Chem*, 262, 8488–8495 (1987)
[7] X. Li and M.X. Guan, *Mol Cell Biol* 22(21):7701-11 (2002)
[8] X. Li, R. Li, X. Lin and M.X. Guan, *J Biol Chem*. 277(30):27256-64 (2002)
[9] T. Suzuki, T. Suzuki, T. Wada, K. Saigo and K. Watanabe, *EMBO J.* 21(23):6581-9 (2002)
[10] A. Scrima, I.R. Vetter, M.E.Armengod and A. Wittinghofer, in publication