Elucidation of the reaction mechanism of tRNA thiolation involving iron-sulfur cluster

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Research background

Transfer ribonucleic acid (tRNA) is a molecule that transfers amino acids according to the genetic code for protein synthesis. Immediately after tRNA is transcribed, tRNA is in an immature state and cannot play their physiological activity. Various maturation processes such as sulfur modification (thiolation) are necessary to mature tRNA[1]. In humans, abnormalities in mitochondrial translation induced by the lack of thiolation were found from patients with incurable mitochondrial diseases[2]. Additionally, overexpression of thiolation enzymes in skin and breast cancer cells promotes cellular invasion and metastasis[3]. Therefore, tRNA thiolation plays important roles in cells.

The sulfur-modified tRNA (s^2T) in the cytoplasm was discovered in 1971, but the details of s^2T biosynthesis remained unclear[4]. In 2006, cellular biological experiments revealed that s^2T was synthesized by 2-thiouridine synthase A (TtuA) and sulfur donor protein (TtuB)[5]. However, purified TtuA and TtuB could not produce s^2T *in vitro* without cell extracts. These results suggested that TtuA requires unknown factors for its enzymatic activity (Fig. 1).

In this study, we have performed the structural-functional analysis of TtuA and found that sulfur from TtuB is transferred to tRNA using an unstable factor "iron-sulfur (Fe-S) cluster" that decays when TtuA is oxidized[6]. Furthermore, we also revealed that the exposed iron (the unique Fe) in the Fe-S cluster which is not bound to the cysteine residues of TtuA receives the sulfur from TtuB[7].

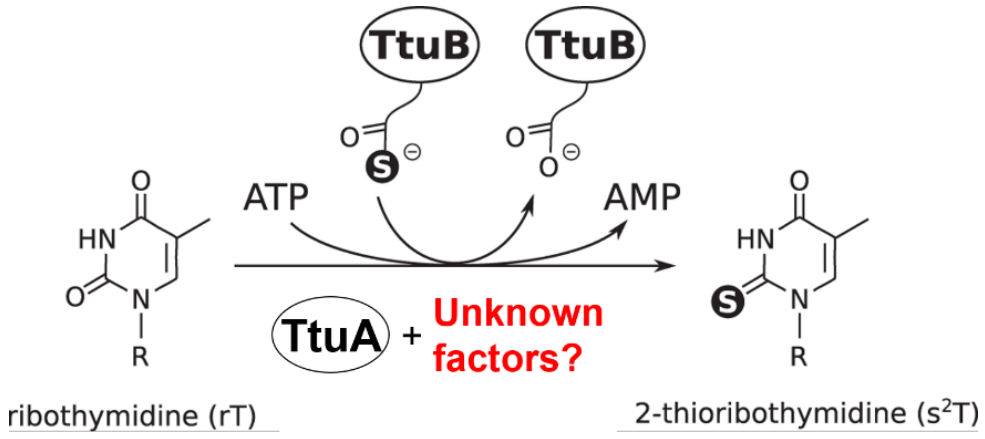

Fig. 1: Scheme of tRNA thiolation by TtuA and TtuB. TtuA transfers sulfur from sulfur donor TtuB to substrate tRNA by consuming ATP. The enzymatic activity of TtuA is dependent on a [4Fe-4S] cluster.

Research results

We could purify a large amount of TtuA expressed in *Escherichia coli* with the yellow color characteristic of the Fe-S clusters by strict controlling redox conditions in an oxygen-free chamber. Next, we determined the structures of Fe-S cluster-TtuA and Fe-S cluster-TtuA-TtuB complex at 2.8 Å and 2.2 Å resolution, respectively. The complex structures showed that TtuA had an iron-sulfur cluster consisting of four irons and four sulfurs ([4Fe-4S]), the [4Fe-4S] cluster had exposed iron (the unique Fe) unbound to the cysteine residues of TtuA, and TtuA bound to TtuB via the unique Fe (Fig. 2).

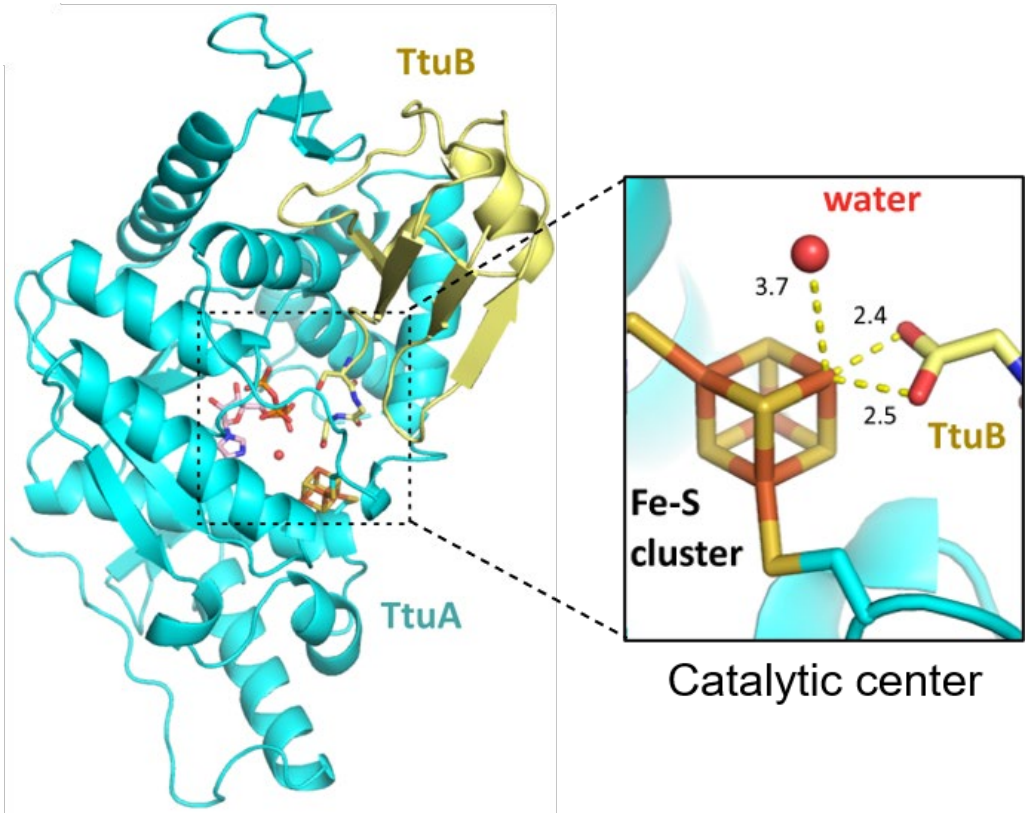

Fig. 2: Overall crystal structure of TtuA in complex with TtuB and Fe-S cluster (left), and close-up view of the catalytic center (right). The unique Fe is directly bound to TtuB and water molecule. The binding distance is shown in Å unit.

Combined with spectroscopic and biochemical analyses, we found that TtuA required oxygen-labile [4Fe-4S] clusters for s^2T synthesis. We also revealed that the activation (adenylation) of the substrate tRNA was required for the release of sulfur from TtuB. Based on the structures, we performed mutation analysis and revealed the key residues of TtuA for transferring sulfur from TtuB to the unique Fe. Taken all results together, we have proposed the molecular mechanism of the tRNA thiolation achieved by TtuA and TtuB (Fig. 3).

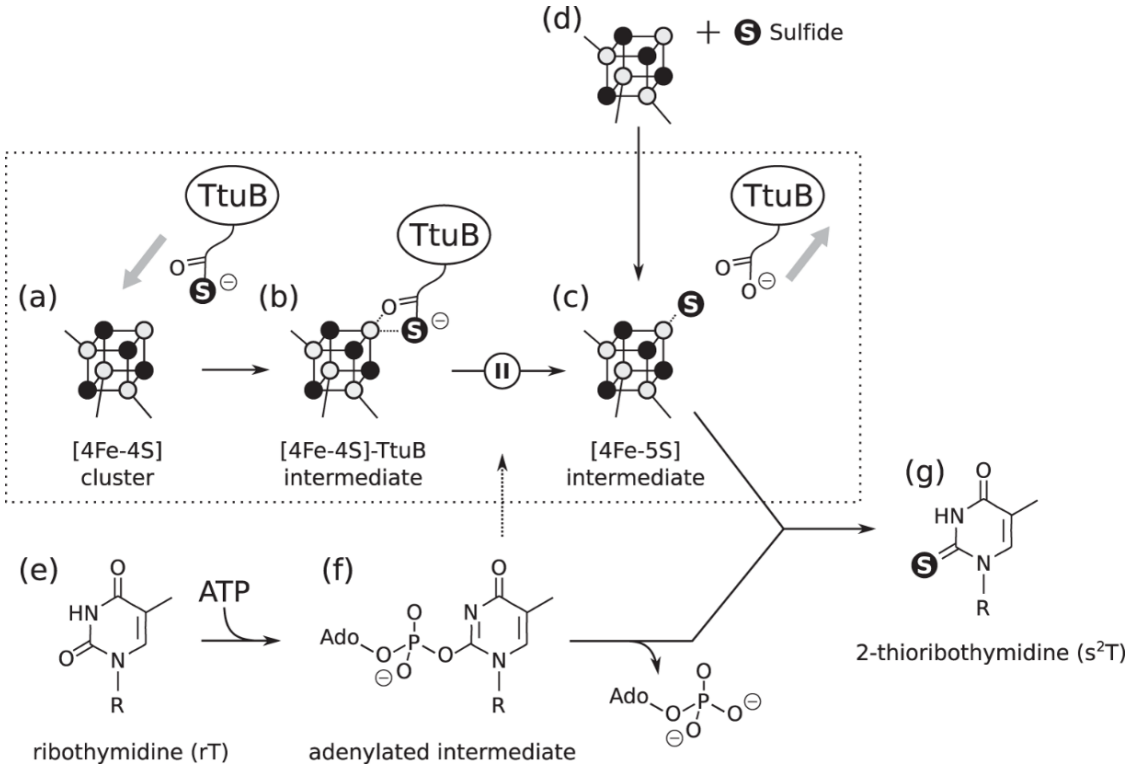

Fig. 3: Proposed tRNA thiolation mechanism. (a) TtuB accesses the catalytic center of TtuA. (b) TtuB attaches to the Fe-S cluster and forms a [4Fe-4S]-TtuB intermediate. (c) When adenylation is complete (dotted arrow), the sulfur atom is cleaved from TtuB and forms a [4Fe-5S] intermediate. (d) The [4Fe-5S] intermediate also forms in the presence of sulfide as an alternative pathway, which may occur if the organism does not possess TtuB. (e) The substrate tRNA base is activated by ATP. (f) Forming an adenylated intermediate. (g) Ultimately, the sulfur captured by the Fe-S cluster attacks the adenyl group on the rT, resulting in the formation of the s^2T product. The reaction delineated in the present study is indicated by a dotted frame.

Expectations for the future

The details of the binding mode of TtuA to tRNA and the mechanism of tRNA activation are still unclear (Fig. 3e, f). Thus, we will analyze the structure of [4Fe-4S]-TtuA-TtuB-tRNA complex to clarify them and reveal the complete reaction mechanism of the tRNA thiolation involved in Fe-S clusters. Elucidation of the mechanism of s^2T biosynthesis in the cytoplasm will enhance our understanding of the effects of tRNA thiolation on mitochondrial function.

References

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