

Yeast Nfs1p is Involved in Thio-Modification of Mitochondrial and Cytoplasmic tRNAs

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Inorganic sulfur atoms of iron-sulfur clusters are provided from cysteine via the desulfuration reaction catalyzed by IscS/NifS, a pyridoxal phosphate (PLP)-containing enzyme. Analyses of the bacterial IscS/NifS proteins have revealed a unique desulfuration mechanism of this enzyme; a sulfur atom of the sulfide group of cysteine is eliminated by the PLP-catalyzed reaction and binds transiently to the active site cysteine residue of IscS/NifS to form an enzyme-bound persulfide. The sulfur atom is then transferred to certain partner proteins (e.g. IscU/NifU) as a sulfan sulfur to make a final product. Beside being involved in the iron-sulfur cluster biosynthesis, this characteristic sulfur transferring ability of IscS can also allow it to be participated in other physiologically important thiolation reactions. One of such IscS-mediated sulfur transfer reactions is that coupled to the post-transcriptional thio-modification of tRNA molecules. Thio-modification of tRNAs are found in almost all organisms and considered to be important to maintain efficiency and rate of translation.

Recently we have found that the yeast orthologue of IscS (Nfs1p), which is mainly localized to mitochondria, is required not only for the mitochondrial but also for the cytoplasmic tRNA thio-modification *in vivo*¹. Depletion of Nfs1p from yeast cells resulted in an immediate decrease in production of 2-thio-modification of 5-carboxymethylaminomethyl-2-thiouridine (cmnm⁵s²U) at the wobble positions of two mitochondrial tRNAs, mt-tRNA^{Lys}_{UUU} and mt-tRNA^{Gln}_{UUG}. In addition, we also observed a severe reduction in the 2-thio-modification of 5-methoxycarbonylmethyl-2-thiouridine (mcm⁵s²U) of two cytoplasmic tRNAs, cy-tRNA^{Lys2}_{UUU} and cy-tRNA^{Glu3}_{UUC}. Interestingly, impairment of thio-modification under Nfs1p-depleted condition was somewhat delayed compared to that seen in mitochondrial tRNAs. Mass spectrometric analysis revealed an increase in 5-methoxycarbonylmethyluridine (mcm⁵U) concomitant with the decrease in mcm⁵s²U in cytoplasmic tRNAs that were prepared from Nfs1p-depleted cells. These results suggest that Nfs1p is involved in the 2-thio-modifications of both cmnm⁵s²U in mt-tRNAs and also mcm⁵s²U in cy-tRNAs. We are now investigating in detail the Nfs1p-involved thio-modification of cytoplasmic tRNAs.

¹Nakai, Y. *et al.* (2004) *J. Biol. Chem.* 279, 12363-12368.