Project 3 : RNA maturation enzymes

Permanent staff involved

Béatrice Golinelli-Pimpaneau (DR2 CNRS), Bruno Faivre (IE CDF), Marc Fontecave (Pr CDF).

For about ten years, the importance of chemical modifications of RNA—which allow it to contain more than just the four canonical nucleosides—has been widely recognized. Recently, RNA modifications were at the center of a major scientific breakthrough that affected not only our view of science but also society as a whole, since the 2023 Nobel Prize was awarded "for discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19." Our laboratory studies the mechanism and structure of RNA-maturation enzymes (ribonuclease and tRNA-modifying enzymes).

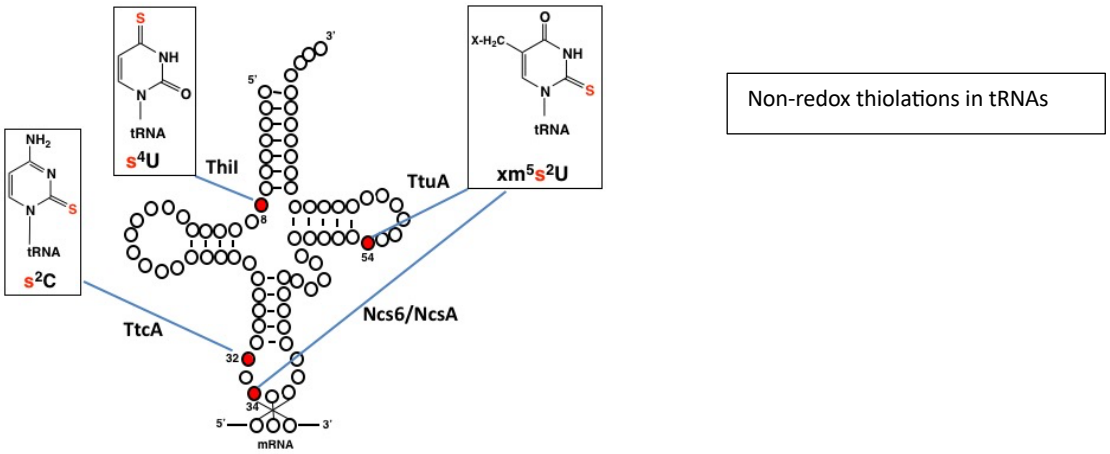
I. tRNA Modification

Numerous and diverse chemical modifications, especially in transfer RNAs, play a key role in the fidelity and efficiency of genetic translation. It was recently shown that the distribution of these modifications in tRNAs is dynamically regulated according to the cellular environment, particularly in response to oxidative stress. In humans, abnormalities in RNA modifications or in the enzymes catalyzing them are often associated with diseases involving metabolic defects, mitochondrial dysfunction, neurological disorders, or cancer.

In recent years, our work has focused on the biochemical and structural study of various non-redox tRNA sulfurization enzymes that depend on a [4Fe-4S] cluster.

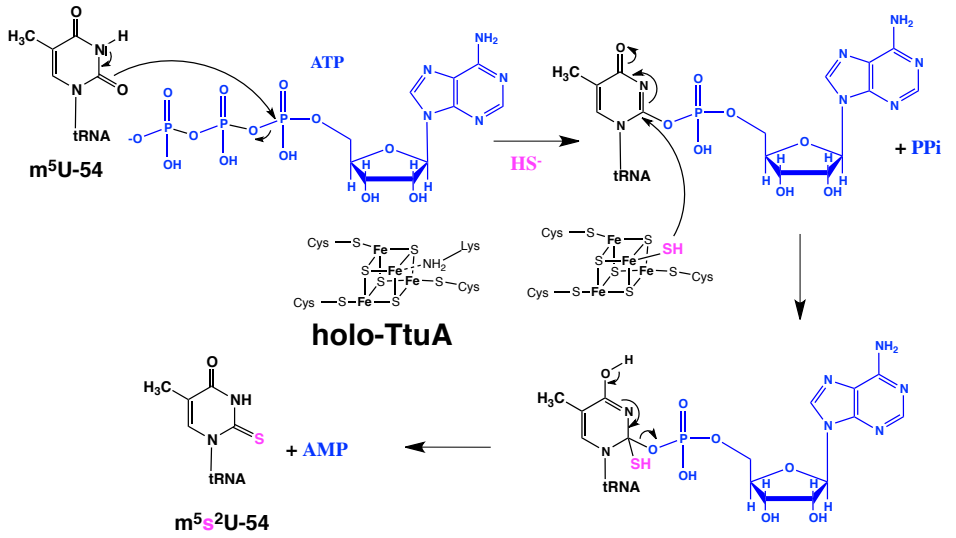
Sulfur is found on uridine at positions 8, 34, and 54, and on cytidine at position 32 in several tRNAs. Formation of the corresponding thionucleosides is catalyzed by specific enzymes: ThiI/TtuI (s^4U8) in bacteria and archaea, TtuA (s^2U54) in archaea and thermophilic bacteria, and TtcA (s^2C32) in bacteria and some archaea. The s^2U34 modification is catalyzed by MnmA-type enzymes in bacteria and mitochondria and by NcsA-type enzymes in archaea and the eukaryotic cytosol.

We studied several of these enzymes and showed that they use a [4Fe-4S] cluster to catalyze tRNA thiolation. We then published a review in 2024 summarizing all biochemical and crystallographic results obtained for these enzymes (Gervason et al., *Biochim. Biophys. Acta. Mol. Cell Res*. 2024, 1871, 119807, doi:10.1016/j.bbamcr.2024.119807*).* In 2025, we wrote a perspective article explaining the theory and comparing data from different groups on tRNA sulfurization enzymes (Gervason et al., *Accounts Chem. Res.*, 2025, doi: 10.1021/acs.accounts.5c00485).



1) The crystal structure of TtuA bound to its [4Fe-4S] cluster reveals a new function for Fe-S clusters in biology

We first showed that a [4Fe-4S] cluster can be reconstituted in TtuA enzymes under anaerobic conditions and characterized it using spectroscopic methods. We then obtained several crystal structures of TtuA under anaerobic conditions, which showed that the [4Fe-4S] cluster is coordinated by only three cysteines, allowing the fourth iron atom to bind a hydrosulfide ion. This enabled us to propose an original mechanism for sulfurization in which the [4Fe-4S] cluster acts as a Lewis acid to bind and activate sulfur, implying formation of a catalytic [4Fe-5S] intermediate (Arragain et al., *PNAS,* 2017, 114, 7355, doi:10.1073/pnas.1700902114).



Proposed mechanism for the reaction catalyzed by TtuA in which the [4Fe-4S] center acts as a sulfur transporter, enabling multiple catalytic cycles (Arragain et al., *PNAS,* 2017, 114: 7355-7360, doi: 10.1073/pnas.1700902114).

2) The [4Fe-4S] cluster of MnmA, bound by two cysteines and an aspartate, is required for tRNA sulfurization

After characterizing the [4Fe-4S] cluster of TtuA, we re-examined several enzymes in this tRNA-thiolation family for which a mechanism had been previously proposed, to determine whether they could also use a [4Fe-4S] cluster for catalysis. We showed that *E. coli* MnmA, for which a persulfide-chemical mechanism had been proposed (Numata et al., *Nature* 2006, 442, 419, doi: 10.1038/nature04896), in fact also uses a [4Fe-4S] cluster to catalyze its sulfur-transfer reaction when inorganic sulfide is used as the sulfur donor (Zhou et al., *Nucleic Acids Res.* 2021, 49, 3997, doi: 10.1093/nar/gkab138; Gervason et al., *J. Inorg. Biochem*. 2025, 274, 113064, doi:10.1016/j.jinorgbio.2025.113064). This new mechanism of *E. coli* MnmA could only be uncovered by maintaining the holo-protein under strict anaerobic conditions. However, the system that matures MnmA remains unknown, since *in vivo* studies show that U34 thiolation occurs even in the absence of the ISC and SUF [Fe-S] biogenesis systems.

In collaboration with Prof. F. Barras (Institut Pasteur), we also showed that the s^2U34 thionucleoside formed by *Escherichia coli* MnmA is involved in cellular stress resistance: under acidic pH or oxidative conditions (H_2O_2), lack or slowdown of cell growth was observed in the *mnmA* mutant strain compared with the wild type.

3) ThiI and NcsA depend on a [4Fe-4S] cluster rather than a [3Fe-4S] cluster

We showed that a subclass of ThiI/TtuI proteins also uses a [4Fe-4S] cluster for catalysis *(*He et al., *Nucleic Acids Res*. 2022, 50, 12969, doi:10.1093/nar/gkac1156) rather than a [3Fe-4S] cluster as previously reported (Liu et al., *PNAS,* 2016, 113, 12703, doi: 10.1073/pnas.1615732113*)*. The [3Fe-4S] cluster therefore likely results from degradation of the [4Fe-4S] cluster by residual oxygen.

Similarly, it had been reported that the NcsA enzyme from the archaeon *Methanococcus maripaludis* (MmNcsA) binds a [3Fe-4S] cluster (Liu et al., *PNAS,* 2016, 113, 12703, doi: 10.1073/pnas.1615732113*)*, but we solved the crystal structure of MmNcsA after cluster reconstitution, showing that it binds a [4Fe-4S] cluster (Bimai et al., *Sci. Rep*. 2023, 13, 5351, doi:10.1038/s41598-023-32423-9*).* The structure of the MmNcsA dimer is very close to the AlphaFold model of the human cytosolic Ctu1/Ctu2 complex, suggesting that eukaryotic U34-tRNA sulfurization enzymes use the same [4Fe-4S]-dependent mechanism as NcsA. The Ctu1/Ctu2 complex is essential for genome integrity. Moreover, U34-tRNA sulfurization activity is overexpressed in certain cancers (notably breast cancer and melanoma), promoting survival and treatment resistance, making it a promising therapeutic target.

4) LarE, a small-molecule sulfurization enzyme dependent on a [4Fe-4S] cluster

We recently showed that this sulfurization mechanism, likely involving a [4Fe-5S] intermediate, also extends to a subclass of [4Fe-4S]-dependent LarE enzymes that catalyze two successive sulfurization reactions in the precursor of the lactate racemase cofactor.

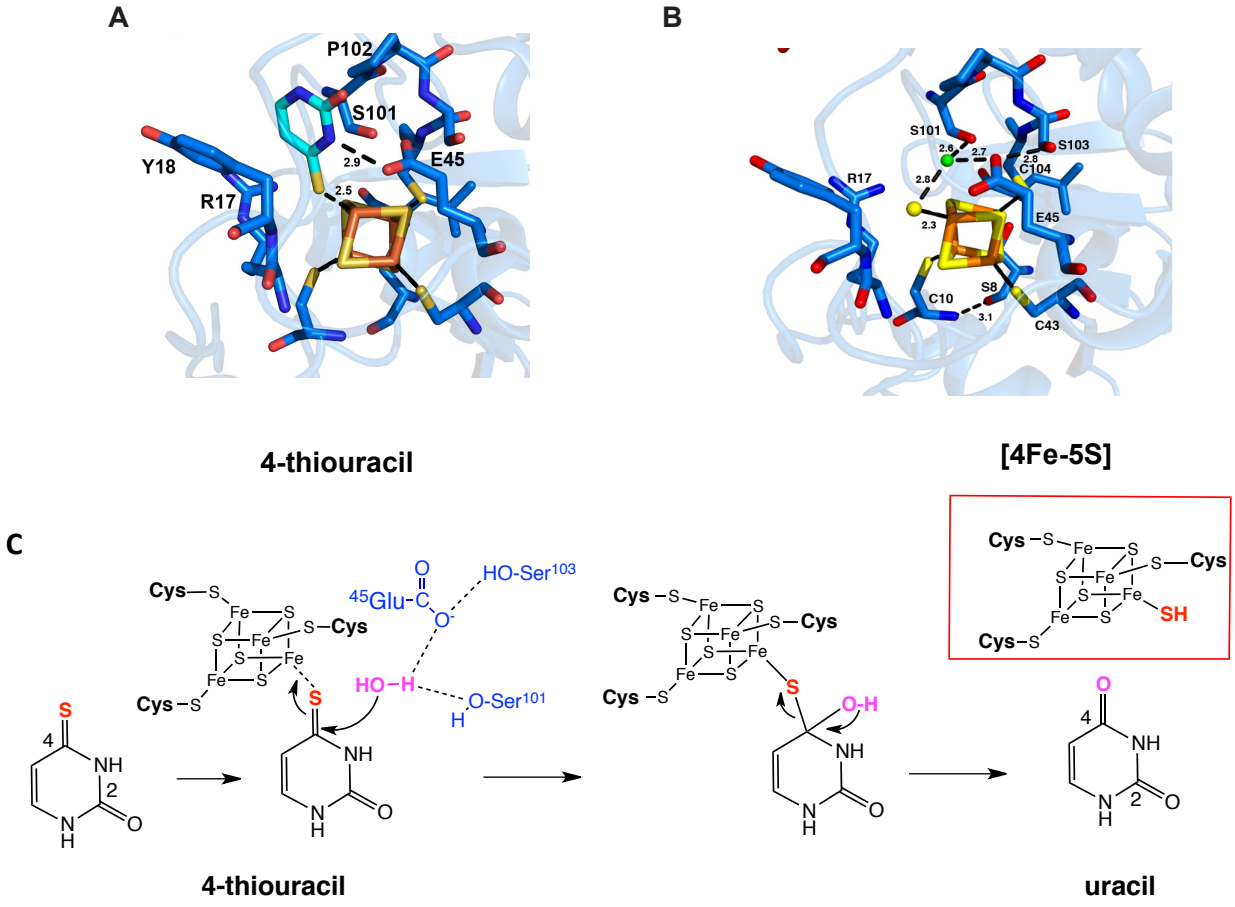
We solved the crystal structure of *M. maripaludis* LarE and observed that only three Fe atoms of the [4Fe-4S] cluster are coordinated by cysteines, while the fourth can bind an anionic ligand such as a phosphate group or chloride ion (Zecchin et al., *Protein Sci*. 2024, 33, e4874, doi:10.1002/pro.4874). The chloride-bound [4Fe-4S] cluster likely mimics the [4Fe-5S] sulfur-donor intermediate.

Previous biochemical studies and the structure of *Lactobacillus plantarum* LarE had shown that the enzyme sacrifices the sulfur atom of a conserved cysteine to provide sulfur for the reaction, forming dehydroalanine (Fellner, *PNAS,* 2017, 114, 9074, doi:10.1073/pnas.1704967114). The

two LarE subfamilies therefore appear to use two very different mechanisms—catalytic or sacrificial—to sulfurize their respective substrates.

5) TudS, a tRNA desulfurization enzyme dependent on a [4Fe-4S] cluster

Interestingly, we demonstrated by crystallography and anomalous scattering the presence of a [4Fe-5S] intermediate during desulfurization of thiouracil, catalyzed by the thiouracil desulfurase TudS (Zhou et al., *Angew. Chem. Int. Ed*. 2021, 60, 424; Fuchs et al., *Commun. Biol.* 2023, 7, 1092), which catalyzes the reverse of the sulfurization reaction.



Proposed mechanism for the desulfurization of 4-thiouracil by TudS involving the formation of a [4Fe-5S] intermediate (Zhou et al. *Angew Chem Intl Ed*, 2021, 160, 424-431, doi: 10.1002/anie.202011211). **A** Model of the 4-thiouracil/TudS complex. **B** Crystal structure of the [4Fe-5S] intermediate. **C** Proposed catalytic mechanism for TudS. Catalysis is likely assisted by two bases in the active site, which can be assigned to Glu45 and Ser101 based on the crystal structure of TudS, docking, and site-directed mutagenesis.

Remarkably, it turned out that TudS is the catalytic domain of a tRNA s^4U8-desulfurization enzyme (Jamontas et al., *Nucleic Acids Res*. 2024, 52, 10543, doi: 10.1093/nar/gkae716; Munneke et al., *Cell Host & Microbe* 2025, 33, 573, doi: 10.1016/j.chom.2025.03.001). This revealed for the first time that tRNA sulfurization can be dynamically regulated in cells depending on environmental conditions, at least in bacteria.

6) CyuA, a [4Fe-4S]-dependent enzyme involved in sulfur metabolism in methanogenic archaea

The L-cysteine desulfidase CyuA catalyzes the degradation of L-cysteine into pyruvate, ammonia, and hydrogen sulfide. We studied the role of CyuA in sulfur metabolism using phylogenetic, genetic, biochemical, spectroscopic, and structural methods (Gervason et al., *Commun. Biol*. 2025, 8, 1667, doi:10.1038/s42003-025-09053-0). This study shows that Methanococcales and some other archaeal groups likely acquired CyuA through horizontal gene transfer from *Terrabacteria*.

In *M. maripaludis*, CyuA (MmCyuA) enhances growth in the presence of sulfide and enables slow growth when cysteine is the only sulfur source. MmCyuA contains a [4Fe-4S] cluster coordinated by three conserved cysteines, with the fourth iron capable of binding various small ligands. Crystallographic structures and biochemical analyses enabled us to model substrate cysteine bound to MmCyuA and propose a detailed mechanism for L-cysteine desulfurization involving a [4Fe-5S] intermediate. This intermediate could transfer sulfur from cysteine to various [4Fe-4S]-dependent tRNA-sulfurization enzymes, highlighting the central role of CyuA in sulfur transport and metabolism.

Conclusion

We have therefore revealed a new function of iron–sulfur clusters in biology and proposed that several types of [4Fe-4S]-dependent sulfurization/desulfurization enzymes use a mechanism in which the [4Fe-4S] cluster acts as a transporter and activator of exogenous sulfur by forming a likely [4Fe-5S] intermediate.

II. RNase Y

The maturation and degradation of mRNAs is crucial for the control of gene expression. Endoribonuclease Y (RNase Y), identified in 2009 in *Bacillus subtilis*, is an important factor regulating RNA metabolism in many Gram-positive bacteria. RNase Y initiates an endonucleolytic cleavage that makes RNA fragments more sensitive to exonucleases. Although functionally equivalent to *E. coli* RNase E, RNase Y shares neither sequence nor structural homology with RNase E. RNase Y is absent in eukaryotes and has been shown to regulate the expression of virulence genes in several human pathogens (Staphylococcus, Streptococcus), making it a potential target for new antibiotics.

To date, no experimental 3D structure of RNase Y exists.

RNase Y is anchored to the membrane by its intrinsically disordered N-terminal domain, which is thought to facilitate binding of multiple partners within a degradosome-like complex. Our goal is to investigate the structure of RNase Y to gain insight into its function and mechanism of action, and to understand its interactions with protein partners.

We have shown that RNase Y exists in equilibrium between a dimer (~112 kDa) and a soluble oligomer (~700 kDa) (Hardouin et al., *Biophys. J.* 2018, doi:10.1016/j.bpj.2018.10.016), but the biological function of this oligomer remains unknown.

Using multidimensional heteronuclear NMR (in collaboration with the ICSN NMR team within the Infranalytics framework) and AlphaFold structural predictions, we showed that the N-terminal BsRNaseY dimer adopts a coiled-coil-type structure (Morellet et al., *Biomolecules* 2022, 12, 1798, doi:10.3390/biom12121798). Each chain in the dimer is composed of two long helices connected by a bend. This structural organization of the N-terminal BsRNaseY domain is preserved in the AlphaFold model of the full-length RNase Y enzyme. In this model, the globular catalytic domain is composed of two helices connecting the KH module (RNA-binding domain) and the HD module (characteristic of the metal-dependent phosphohydrolase superfamily), as well as the C-terminal region. This latter region, whose function was previously unknown, is most likely involved in RNase Y dimerization. This work illustrates how very high-field NMR can answer questions inaccessible to other biostructural techniques.

We are continuing the structural study of RNase Y by cryo-electron microscopy.

Methods and expertise

• Cloning, overexpression and purification of recombinant proteins
• In vitro transcription and RNA purification
• Site-directed mutagenesis
• Enzymology
• Spectroscopy (UV, visible, IR, circular dichroism, fluorescence, light scattering)
• Mass spectrometry of nucleic acids and proteins
• Crystallization, X-ray crystallography of proteins, cryo-EM and structural analysis

Collaborations

- **Catherine Venien-Bryan**, Institut de Minéralogie, de Physique des Matériaux et de Cosmochimie, Sorbonne Université, Paris
- **Marie-Pierre, Golinelli**, Chémobiologie, Institut de Chimie des Substances Naturelles, Gif-sur-Yvette
- **Frédéric Barras**, Stress Adaptation and Metabolism in Enterobacteria Unit, Institut Pasteur, Paris
- **Jean-Luc Ravanat**, Université de Grenoble Alpes, CEA, iNAC, SyMMES, Grenoble
- **Christophe Velours**, Université de Bordeaux
- **William B Whitman**, Department of Microbiology, University of Georgia, Etats Unis
- **Volker Schünemann**, Technische Universität Kaiserslautern, Allemagne
- **Nadia Touati et Laurent Binet**, Institut de Recherche de Recherche Renard, Chimie-ParisTech, Paris
- **Pierre Legrand**, Synchrotron SOLEIL, L'Orme des Merisiers, Saint Aubin, Gif-sur-Yvette
- **Jaunius Urbonavičius**, Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lituanie
- **Carine van Heijenoort**, Structural Chemistry and Biology Team, Institut de Chimie des Substances Naturelles, Gif-sur-Yvette

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2026

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