Project 3 : RNA maturation enzymes

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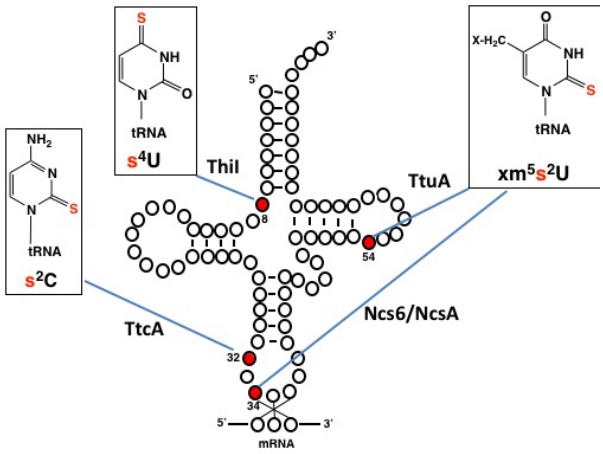
RNA is implicated in various biological processes such as coding, decoding, regulation, and expression of genes. In particular, transfer RNAs (tRNAs) are key molecules of the translational machinery that ensure decoding of the successive codons in messenger RNA (mRNA) inside the ribosome, thus allowing the synthesis of the protein corresponding to the mRNA sequence. In most organisms, non-coding genes such as those of tRNA are transcribed as RNA precursors that undergo further processing, whereas mRNA decay is crucial for the control of gene expression. Using the tools of molecular biology, protein chemistry, biophysics and structural biology, we are studying several RNases and post-transcriptional modification enzymes involved in mRNA degradation and stable RNA maturation.
It has recently been shown that the dynamic control of post-transcriptional RNA modifications (epitranscriptome) corresponds to a new level of gene expression regulation. Among all RNA species, tRNAs present the largest variety of chemical modifications and the highest degree of modification. Under stress conditions, tRNA modification reprogramming greatly contributes to cell survival by orchestrating the synthesis of specific proteins vital to stress response. Several pathologies have been shown to be linked to abnormal tRNA modifications. For example, some tRNA modifications are overexpressed in cancers tissues whereas deficiency in several others is at the origin of specific diseases, such as mitochondrial myopathies.
We aim at characterizing these various RNA maturation enzymes, biochemically and structurally. To understand the structural basis of the catalytic mechanism, we are trying to crystallize these proteins alone, and in complex with their RNA substrate. The knowledge of the tridimensional structure by X-ray diffraction, coupled to biochemical and biophysical studies and to molecular modeling, allow us to unravel the mode of substrate activation, the catalytic mechanism and the dynamics of catalysis, bringing a better understanding of the recognition mechanisms between the enzymes and their specific RNA substrate. We are also studying the interaction of these enzymes with their protein partners.

1- Biosynthesis of sulfur- and selenium-containing nucleosides

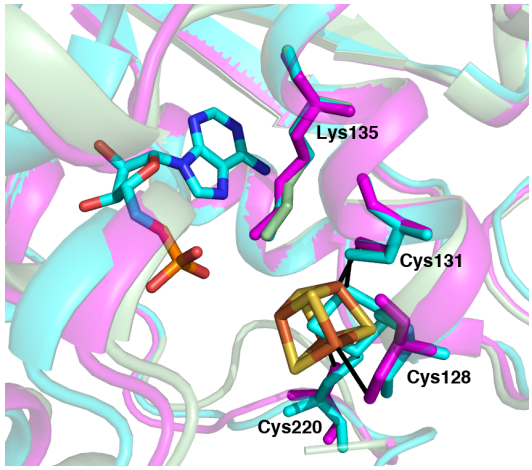
Principal Investigator: Béatrice Golinelli-Pimpaneau

Sulfur is an essential element for numerous cellular components (including proteins and RNAs) in all living organisms. There are several sulfur-containing nucleosides in tRNAs: thiouridine and derivatives (s^4U8, s^2U34, m^5s^2U54), 2-thioadenosine derivatives (ms^2i^6A37, ms^2t^6A37) and 2-thiocytidine (s^2C32) (Figure below, left). It was recently shown, by different independent laboratories including ours, that **the ATP-dependent formation of s^2C32, s^2U54, s^4U8 and s^2U34 in some tRNAs depends on iron-sulfur [Fe-S]-containing enzymes named TtcA, TtuA, and archaeal ThiI and NcsA, respectively** (*Nucleic Acids Res,* 2014, 42: 7960-7970; *Proc Natl Acad Sci USA* 2017, 114: 4954-4959; *Proc Natl Acad Sci USA,* 2017, 114: 7355-7360; *Proc Natl Acad Sci USA, 2016,* 113: 12703-12708; Figure below, left). Our project aims at further characterizing *in vitro* this thiolation enzyme family that targets distinct positions within tRNA (8, 32, 34 or 54) using biochemical methods and X-ray crystallography. Indeed, crystal structures are known only for apo-TtuA (without the [Fe-S]

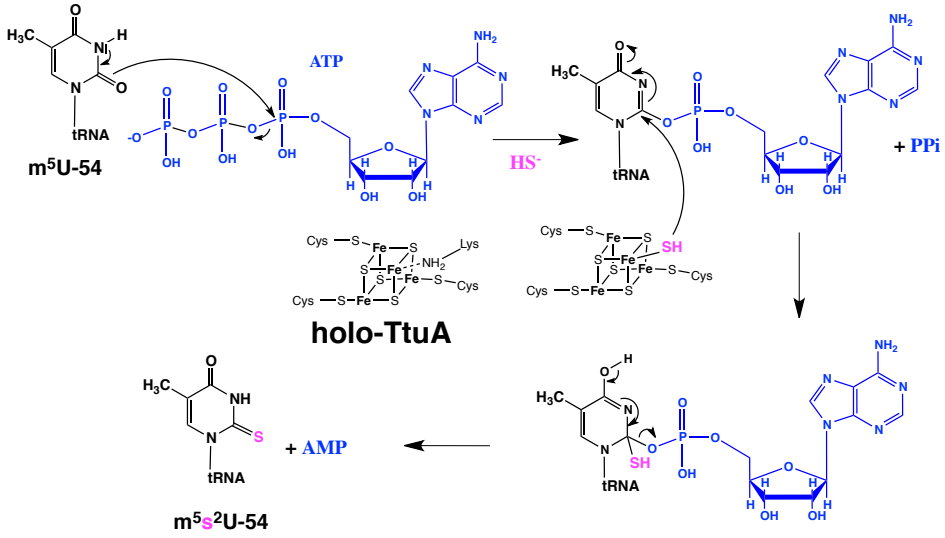
cluster), holo-TtuA (with the [Fe-S] cluster; Figure below, right) and holo-NcsA (PDB code 6SCY), and no structure in complex with RNA has been reported. Furthermore, more evidence is needed to support the mechanism for tRNA thiolation that we proposed, in which the fourth unique iron of the [4Fe-4S] catalytic cluster of TtuA serves to bind exogenous sulfide, thus acting as a sulfur carrier (*Proc Natl Acad Sci USA,* 2017, 114: 7355-7360).



[Fe-S]-dependent thiolations in tRNA.



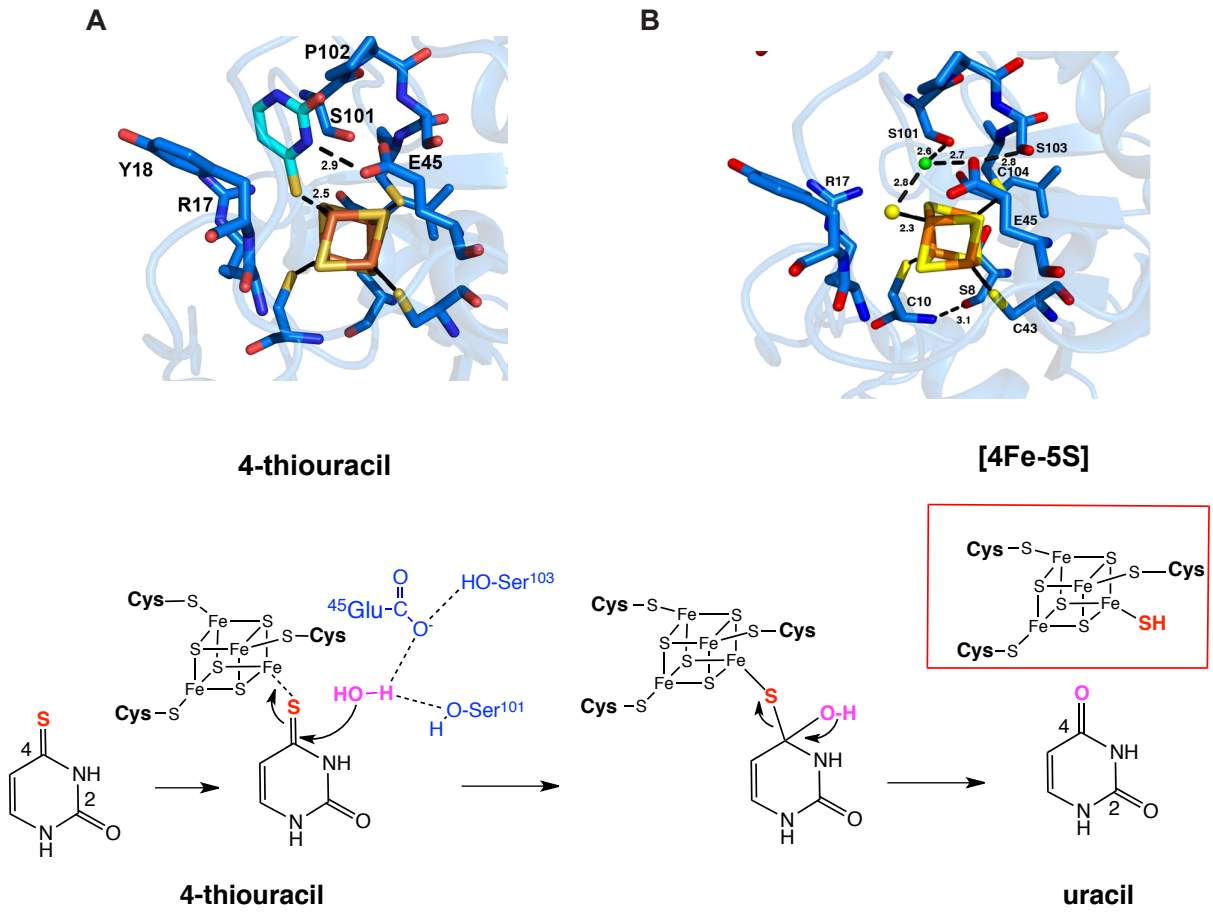
Structure of the active site of TtuA showing the proximity between AMP and the [4Fe-4S] cluster (*Proc Natl Acad Sci USA,* 2017, 114: 7355-7360).



Proposed thiolation mechanism of TtuA with the [4Fe-4S] cluster playing the role of sulfur carrier, allowing multiple catalytic cycles (*Proc Natl Acad Sci USA,* 2017, 114: 7355-7360).

In collaboration with Jaunius Urbonavičius' team, Lituania, we recently determined the crystal structure of TudS (**T**hio**U**racil **De****S**ulfidase) enzyme (Zhou et al. *Angew Chem Intl Ed,* 2020, in press), identified during the discovery of new genes encoding proteins of the Domain of Unknown function 523 (DUF523) family. This enzyme, which contains a [4Fe-4S] cluster, catalyzes sulfur transfer between its thiouracil substrate and the [4Fe-4S] cluster. We could visualize the [4Fe-5S] catalytic intermediate by X-ray cristallography at 1.5 Å de resolution and characterized it by Fe and S anomalous scattering. We have proposed a mechanism for its formation, which involves the catalytic

residues Glu45 et Ser101 to activate a water molecule that acts as a nucleophile. Our study validates a new function of iron-sulfur clusters in biology as sulfur transfer agents.

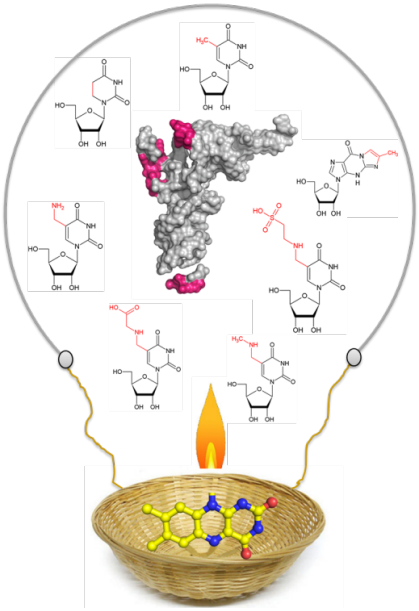


Proposed mechanism for 4-thiouracil desulfuration by TudS involving the formation of a [4Fe-5S] intermediate (Zhou et al. *Angew Chem Intl Ed, 2020*, in press). **A** Model of the 4-thiouracil/TudS complex. **B** Crustal structure of the [4Fe-5S] intermediate. **C** Catalytic Mechanism proposed for TudS. Catalysis is probably assisted by two active site bases, which were assigned to Glu45 et Ser101 based on the TudS crystal structure, docking models and site-directed mutagenesis experiments.

2- Flavin-dependent Epitranscriptomic

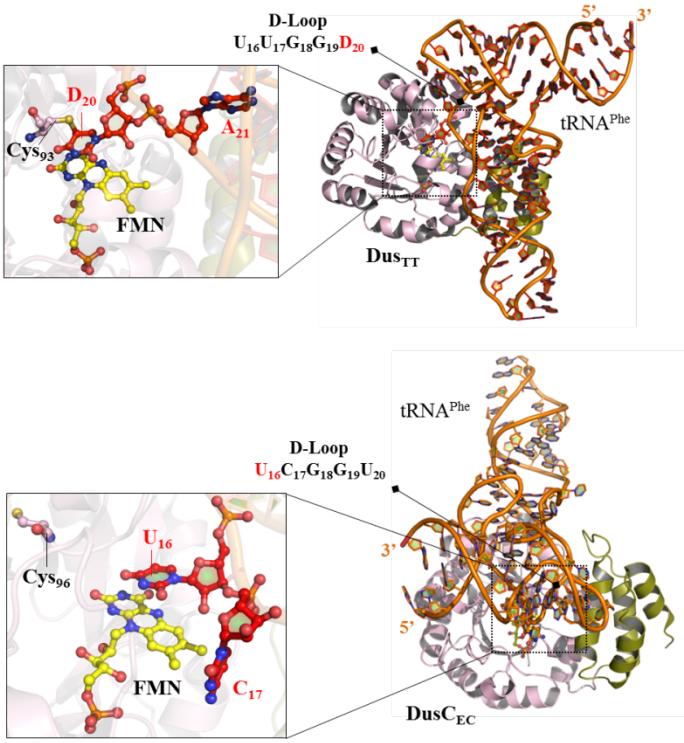
Principal investigators: Murielle Lombard and Djemel Hamdane

Presently, there are seven RNA modifications that depend on the activity of a flavoenzyme allowing the flavin cofactor to enter the new epitranscriptomic world. Our main objectives are to study exquisite biological, biochemical and structural relationship on these modifications and on their corresponding flavoenzyme catalysts.

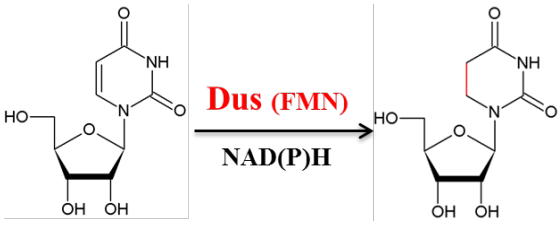


1. The dihydrouridine and the exquisite family of FMN-dependent dihydrouridine synthases

The dihydrouridine synthesis is catalyzed by a large family of FMN-dependent enzymes named dihydrouridine synthases (Dus) which employ NADPH as a flavin reducing agent. Phylogenetic analysis classified these flavoenzymes into three major groups and eight subfamilies, all of which evolved through independent duplications of an ancestral *dus* gene. The first group found in prokaryotes regroups three Dus (Dus A, B and C) while the second one is eukaryotic and contains four different enzymes (Dus 1 to 4). The last group is characterized by a single Dus observed only in archaea. Our long term goal is to characterize the structure/function relationship of the bacterial and eukaryotic enzymes.

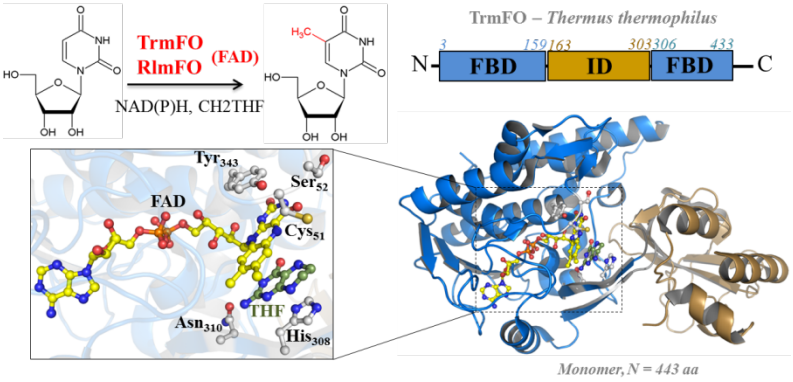


Reaction catalyzed by the Dus Enzymes



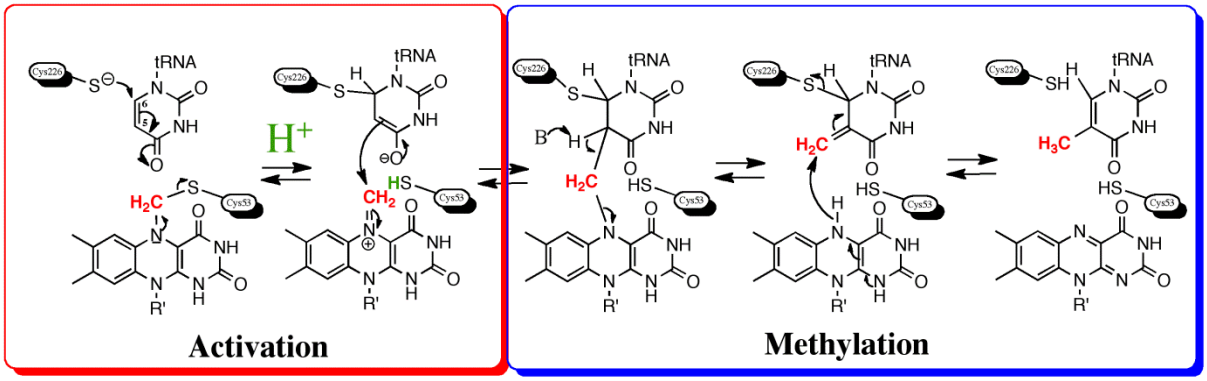
Crystal structure of bacterial DusA/tRNA and DusC/tRNA complexes

2. Reductive methylation of tRNA and rRNA by new class of flavin and folate dependent RNA methyltransferases

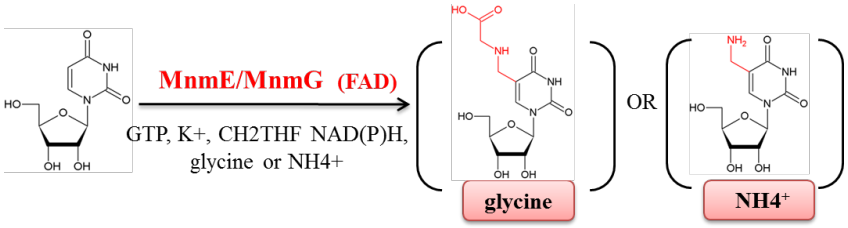


In most living organisms the m^5U is synthesized by S-adenosylmethionine (SAM)-dependent-methyltransferases, which directly transfer a methyl group from the SAM cofactor to the C5-uracil carbon via a simple SN_2 reaction. However, in Gram positive bacteria and in several mycoplasmas, this methylation proceeds by a much more complex multistep process involving the N5,N10-methylenetetrahydrofolate (CH_2THF) used as a methylene donor, and the reduced flavin adenine dinucleotide hydroquinone ($FADH^-$). This alternative type of reaction also called reductive methylation is catalyzed by characteristic flavoenzymes denoted as m^5U_{54} tRNA methyltransferase FAD/folate-dependent (TrmFO) and m^5U_{1939} rRNA methyltransferase FAD/folate-dependent (RlmFO) according to the nature of the substrate used, tRNA and rRNA, respectively. The importance of such TrmFO's catalyzed reaction as being part of organism's adaptive mechanism in response to physical changes of their environment was recently uncovered. Our extensive characterization on TrmFO has led to the recent discovery that flavin can function as an unprecedented

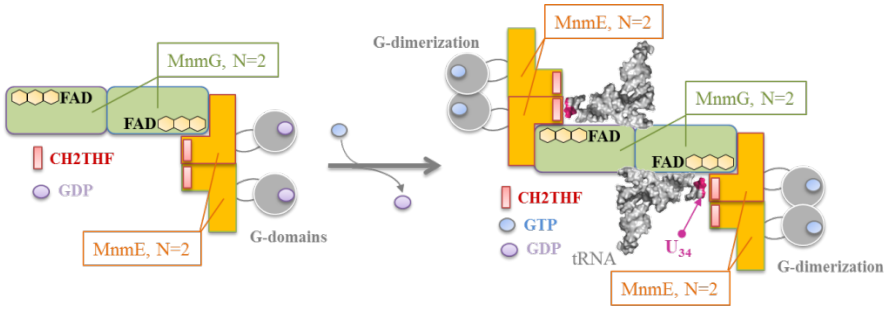
RNA methylating agent. This novel agent is in the form of a unique methylene-iminium derivative of FAD. The same CH_2=FAD species is used by the bacterial flavin-dependent thymidylate synthase ThyX, a flavoenzyme found in several human pathogens and which catalyzes the vital conversion of dUMP into dTMP. Our long term goal is to characterize in details this mechanism by using synthetic chemistry, biochemistry structural biology as well as biophysic tools.



3. A flavoenzyme complex controls translation via hypermodification of wobble uridine of tRNA



In bacteria, the 5-carboxymethylaminomethyl ($cmnm^5$) and 5-aminomethyl (nm^5) belonging to the xm^5U modifications family are synthetized by a conserved enzymatic heterocomplex involving a flavoenzyme component. For certain tRNA species, these C5 substituents can serve as intermediates in the metabolic pathways of mnm^5U modification. The heterotetrameric complex formed by two proteins, MnmE and MnmG, uses an impressively complicated mixture consisting of GTP, K^+, CH_2THF and glycine or $NH4^+$ to catalyze these xm5U modifications. Each of these proteins forms a stable homodimer both inside and outside the complex. GTPBP3 and MTO1 are the human homologs of MnmE and MnmG, respectively. These proteins are synthesized in the nucleus and transported to the mitochondria in order to modify organelle tRNAs. Currently, there is no biochemical and structural characterization of these enzymes, but it is known that the GTPBP3/MTO1 complex catalyzes the formation of 5-taurinomethyluridine 34 (τm^5U_{34}) using taurine instead of glycine. MTO1 and GTPBP3 are important since several genetic mutations of these proteins are associated to severe diseases such as mitochondrial myopathy, hypertrophic cardiomyopathy, encephalopathy, lactic acidosis. Our long term goal is to understand how the bacterial and human complexes work synergistically to catalyze these complex modifications. The structure/function relationship of these complexes would help us to understand the molecular basis of the human pathologies.



Schematic representation of MnmE/MnmG complex functioning

3- mRNA ribonucleases.

Principal Investigator: Béatrice Golinelli-Pimpaneau

The instability of mRNA is crucial for the control of gene expression. The recently discovered endoribonuclease Y (**RNase Y**) is an important player defining RNA metabolism in many Gram positive bacteria. Moreover, RNase Y is not present in eukaryotes and it has been shown to be implicated in the regulation of virulence gene expression in several human pathogens (*Staphylococcus, Streptococcus*), which makes it a potential target for the search of new antibiotics.
RNase Y is anchored through the membrane via its intrinsically disordered N-terminal domain, which is thought to facilitate binding to multiple partners within a degradosome-like complex. We aim at obtaining the crystal structure of RNase Y to get insights into its function and mode of action and to understand its interaction with its protein partners.

Methods and expertises :

- Cloning, overexpression and purification of recombinant proteins
- in vitro transcription and RNA purification
- site-directed mutagenesis
- enzymology
- thermodynamic methods (ITC) and kinetics (fast kinetics: stopped-flow)
- spectroscopy (UV, visible, IR, circular dichroism, fluorescence, light scattering)
  mass spectrometry of nucleic acids and proteins
- crystallization, X-ray crystallography of proteins and structural analysis
- molecular modeling

Collaborations

- **Damien Brégeon**, Biology of Aging and Adaptation Sorbonne Université, Paris
- **Vincent Guérineau**, Laboratoire de Spectrométrie de Masse, ICSN, Gif-sur-Yvette
- **Carine Tisné**, UMR8261 Expression Génétique Microbienne CNRS, Université Paris Diderot
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- **Frédéric Barras**,

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