**Sujet du stage (sous forme de titre court)**:

**Laboratoire**
- **Nom du Responsable**: Fontecave Marc
- **Affiliation administrative (CNRS, INSERM...) et n° l'Unité**: CNRS, Collège de France UMR 8229
- **Adresse précise du Laboratoire**: Chimie des Processus Biologiques, 11 place Marcelin Berthelot, 75005 Paris

**Équipe d'accueil des Doctorants**
- **Nom de l'équipe**: Enzymologie Moléculaire et Structurale
- **Nom du Responsable**: Golinelli-Pimpaneau Béatrice
- **École Doctorale de rattachement**: Complexité du Vivant, UPMC, Paris VI

**Responsable du Stage**
- **Nom**: Golinelli-Pimpaneau Béatrice
- **Numéro de téléphone**: 06 82 36 64 59
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- **Profil de l'étudiant(e) souhaité**: biologiste moléculaire, biochimiste des protéines

**Renseignements complémentaires**
- **Perspectives de poursuite de thèse**: oui
- **Avec une bourse spécifique**: oui
- **si oui précisez**:

**Laboratoire d'accueil (Unité CNRS, INSERM, etc..) : CNRS**
- **Nombre de chercheurs**: 5 (dont 3 dans l'équipe d'accueil)
- **Nombre d'enseignants-chercheurs**: 1 (dont 0 dans l'équipe d'accueil)
- **Nombre de "HDR"**: 3 (dont 2 dans l'équipe d'accueil)
- **Nombre d'ITA**: 6 (dont 2 dans l'équipe d'accueil)
- **Nombre de "post-docs"**: 4 (dont 2 dans l'équipe d'accueil)
Sujet de stage (et principales techniques) : Mechanistic and structural study of an enzyme that substitutes sulfur for selenium in tRNAs

Besides its presence within the rare amino acid selenocysteine in enzymes, selenium is found in the form of modified nucleosides modifications in certain tRNAs (1). The most abundant selenonucleoside is 5-methylaminomethyl-2-selenouridine (mnm5se2U), present at the wobble position 34 of tRNAs belonging to the three domains of life, is thought to play a role in the fine tuning of codon-anticodon interactions and translation fidelity. During the last two decades, considerable progress has been made in identifying genes involved in tRNA modification; however the enzymology of the encoded proteins remains to be studied. The present project specifically aims at characterizing biochemically and structurally 2-seleno-uridine-tRNA synthase that catalyzes the replacement of the sulfur atom in 5-methylaminomethyl 2-thiouridine at position 34 of tRNAs with a Se atom to form 5-methylaminomethyl 2-seleno-uridine using selenophosphate as selenium-donor. The same enzyme was also shown to be responsible for the formation of the newly discovered S-geranyl-2-thiouridine modified nucleotide (ges2U) (2) that was subsequently proposed to serve as an intermediate product in the transformation of 2-thiouridine to 2-selenouridine (3).

The E. coli enzyme MmmH encoded by the ybbB gene is composed of an N-terminal catalytic rhodanese domain and a C-terminal domain containing a P-loop motif (4). Rhodaneses are sulfurtransferases, which catalyze the transfer of sulfane sulfur from thiosulfate to cyanide in vitro, via a persulfide attached to a reactive cysteine residue. The catalysis occurs via a double displacement mechanism involving the transient formation of a persulfide-containing enzyme intermediate, in which the transferring sulfur is bound to the invariant catalytic Cys residue. In archaea such as the Methanococcales, a bipartite ortholog of MmmH is present with two proteins acting in trans (5).

The goal is to investigate the molecular mechanisms by which the sulfur atom in 2-thiouridine is replaced with Se, and MmmH specifically recognizes its tRNA substrates. The different steps of the project are: (i) overexpression and production of the E. coli and Methanocaldococcus jannaschii enzymes using molecular biology and protein purification techniques (ii) Characterization of selenium-transferase and ATPase activities of the enzymes (iii) understanding the role the P-loop motif and identifying key residues, in particular cysteines involved in S/Se transfer using site-directed mutagenesis (iv) decipher the catalytic mechanisms by trapping Se-containing intermediates and using mass spectrometry analysis (v) determination of the three-dimensional structure of the enzymes alone, and in complex with substrates, in particular tRNA using X-ray crystallography

References
