Mechanistic studies of novel thymidylate-forming enzymes

BIOLOGIE & SANTÉ 2011

Mechanistic studies of thymidylate synthase ThyX and tRNA methylase TrmFO

Biologie & santé 2011 ANR-07-PCV1-0017-02 THYMET

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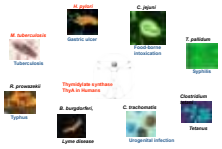
Scientific context and objectives

Enzymatic methylation of uridyl to form (ribo)thymidyl occurs during the metabolism of DNA and RNA in all organisms. We have recently discovered two novel flavoproteins, thymidylate synthase ThyX and tRNA methylase TrmFO, which catalyze the formation of thymidyl groups using methylene tetrahydrofolate as carbon donor. The discovery of these new enzymes provides an excellent example of the evolutionary convergence and versatility of the DNA/RNA modification machinery resulting from molecular tinkering. During this ANR project, we have studied mechanistic aspects of both methylation enzymes using genetic, biochemical, spectroscopic and structural approaches. Moreover, the reproduction of many human pathogens (e.g. typhus, tuberculosis...) depends on tetrameric ThyX enzymes (different from dimeric human ThyA), which makes them appealing targets for new antibiotics.



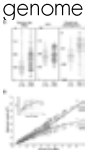
Principal results

Thymidylate synthase ThyX

- We demonstrated the influence of ThyX on DNA replication speed and prokaryotic genome composition (1, 3)

Chromosomal replacement of E. coli thyA from PBCV-1 and

filamentous phenotype



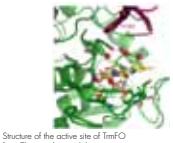
Size distribution and nucleotide counts of

thyA- and thyX-

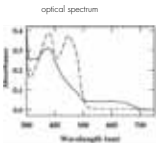
- Optimization of ThyX inhibitors towards new lead compounds and co-crystallization of ThyX with inhibitor compound (patent, 2)
- Identification of conformational changes that occur during ThyX catalysis using limited proteolysis and time-resolved spectroscopy (manuscript under revision)

tRNA methylase TrmFO

- We succeeded to express and purify high amounts of TrmFO for biophysical, biochemical and structural studies (4)
- We identified and characterized a stable catalytic intermediate present in the freshly purified enzyme (5)



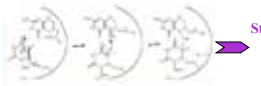
Structure of the active site of TrmFO from Thermus thermophilus (Nishimasu et al., PNAS 2009, 106:818)



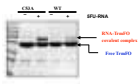
optical spectrum

mass spectroscopy

- We showed that tRNA methylation by TrmFO proceeds via a covalent complex and that Cys53 is not the nucleophile (manuscript submitted)



Strategy for trapping An RNA/TrmFO covalent complex



Conclusion and perspectives

- TrmFO and ThyX employ the same substrates, but are likely to have different catalytic mechanisms.
- In-depth structural investigation of TrmFO.
- Next generation of optimized leads for ThyX inhibition; industrial collaboration in progress.

Key publications and valorization

(1) Flavin-dependent thymidylate synthase X limits chromosomal DNA replication (2008). Escartin F, Skouloubris S, Liebl U, Myllykallio H. **Proc Natl Acad Sci U S A**. 105(29):9948-52.

(2) New anti-microbial compounds targeting ThyX (**Patent application** BET 10P0152 from 12/04/2010). International extension April 2011 (INSERM).

(3) Folate-dependent Thymidylate-Forming Enzymes : Parallels between DNA and RNA Metabolic Enzymes and Evolutionary Implications; *dans*: **DNA and RNA Modification Enzymes: Structure, Mechanism, Function and Evolution**; *ed.* H. Grosjean, Landes Bioscience, ISBN 978-1-58706-329-9, p. 275-288) (2009). Myllykallio H, Skouloubris S, Grosjean H. & Liebl U.

(4) *Expression and purification of untagged and histidine-tagged folate-dependent tRNA:m5U54 methyltransferase from Bacillus subtilis (2010).* Hamdane D, Skouloubris S, Myllykallio H, Golinelli-Pimpaneau B. **Protein Expr Purif**. 73(1):83-9.

(5) *A Catalytic Intermediate and Several Flavin Redox States Stabilized by Folate-Dependent tRNA Methyltransferase from Bacillus subtilis (2011).* Hamdane D, Guérineau V, Un S, Golinelli-Pimpaneau B. **Biochemistry** 2011 [in press].

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