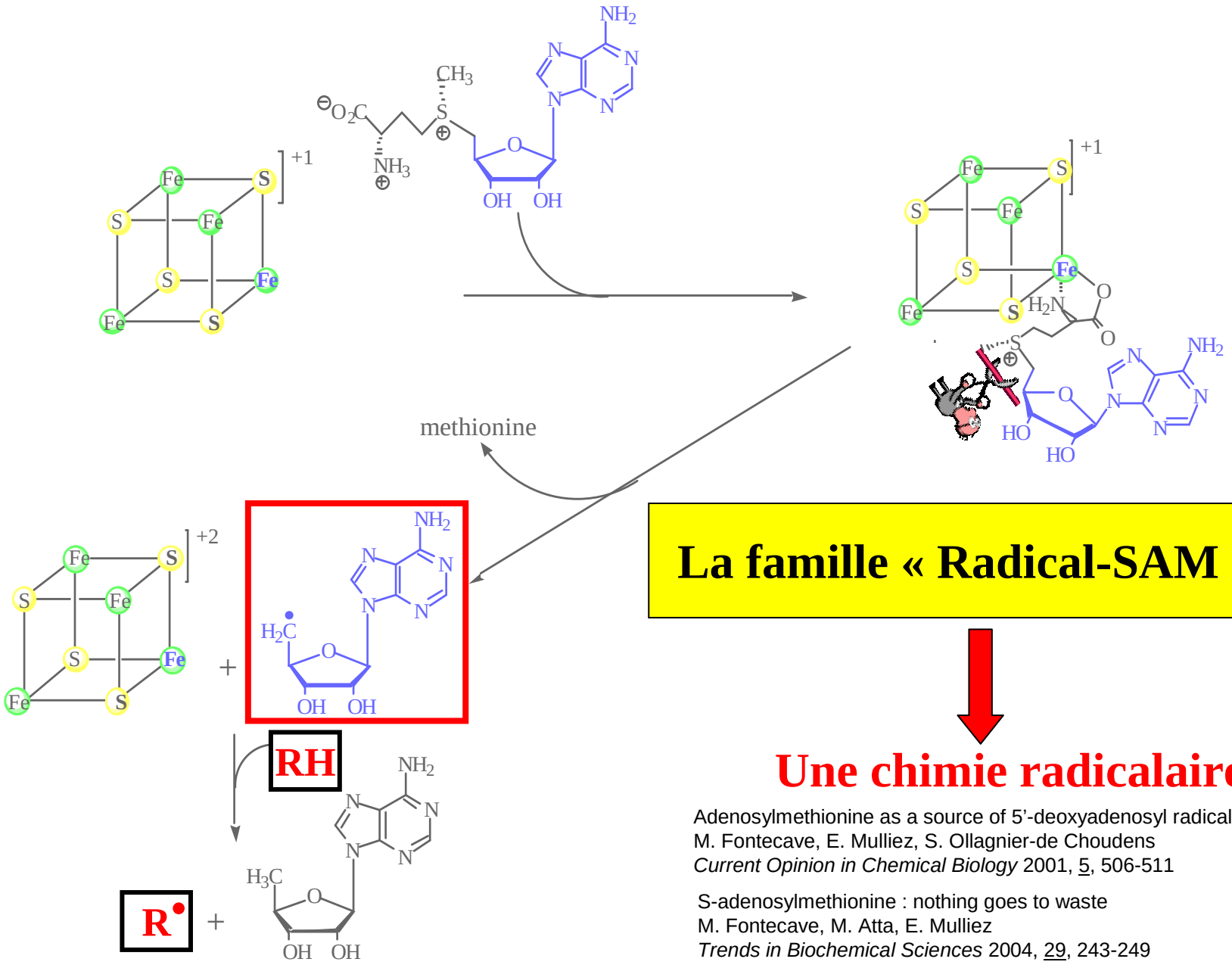
Une chimie radicalaire et des centres fer-soufre pour la biosynthèse de produits naturels soufrés

Marc Fontecave

Laboratoire de Chimie et Biologie des Métaux, Université Joseph Fourier, CNRS, CEA/DSV/iRTSV
CEA-Grenoble 17 rue des martyrs 38054 Grenoble cedex 9, France
mfontecave@cea.fr; Phone: (0033)438789103 ; Fax: (0033)438789124

Collège de France, 11 Place Marcelin Berthelot, 75231 Paris Cedex 05



La famille « Radical-SAM » :

Une chimie radicalaire

Adenosylmethionine as a source of 5'-deoxyadenosyl radicals
M. Fontecave, E. Mulliez, S. Ollagnier-de Choudens
Current Opinion in Chemical Biology 2001, 5, 506-511

S-adenosylmethionine : nothing goes to waste
M. Fontecave, M. Atta, E. Mulliez
Trends in Biochemical Sciences 2004, 29, 243-249

S-Adenosylmethionine-dependent radical-based modification of biological macromolecules
M. Atta, E. Mulliez, S. Arragain, F. Forouhar, J. F. Hunt, M. Fontecave
Curr. Op. Struct. Biol. 2010, 20, 684-692

La même chimie radicalaire pour:

Biosynthèse de:

-Cofacteurs (lipoate, PQQ, molybdopterin…)

-Antibiotiques (desosamine, mitomycine, fosfomycine,…)

-Vitamines (biotin, thiamin,…)

-Alkaloïdes

-Chlorophylle

Metabolisme de:

-Sucres

-Amino-acides

-Hydrocarbures

Modification de:

-ARNs de transfert

-Enzymes

Réparation de:

- ADN

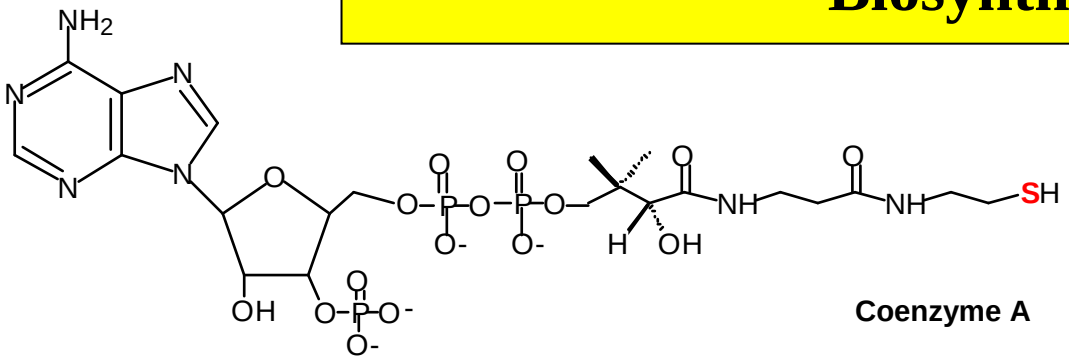
Enzymes Radical-SAM:
Biosynthèse de produits naturels soufrés
MiaB, MtaB, RimO: methylthio-transferases

Hamid Atta

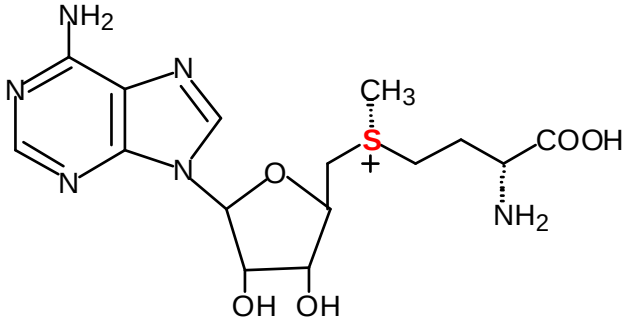
Etienne Mulliez

F. Pierrel
S. Arragain

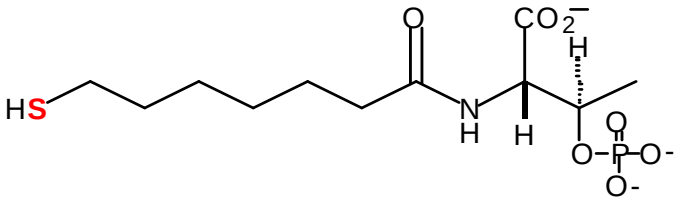
Du soufre dans les composés biologiques
Biosynthèse ?



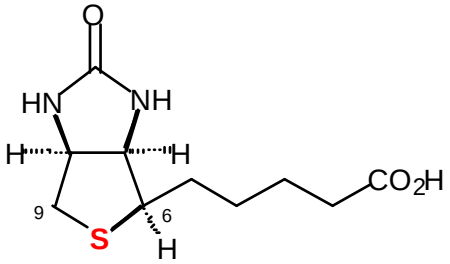
Coenzyme A



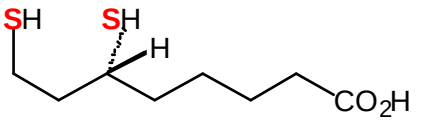
(*S*,*S*)-adenosylmethionine



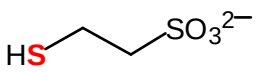
Coenzyme B
N-(7-mercaptoheptanoyl) threonine phosphate



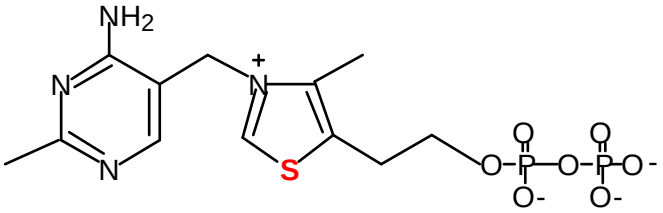
Biotin



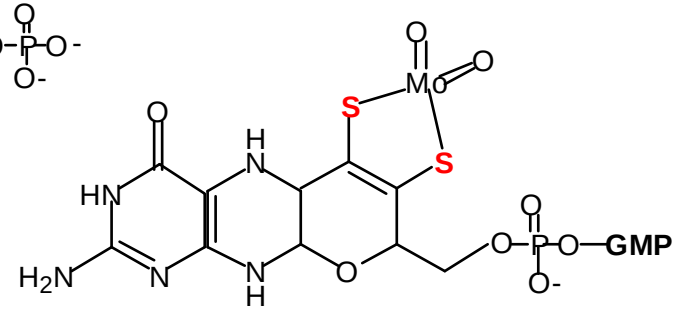
Lipoic acid



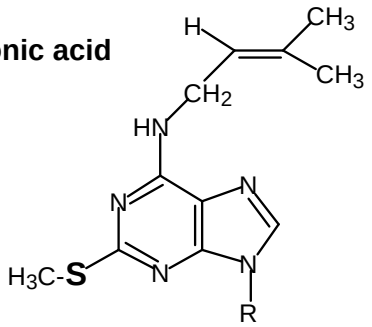
Coenzyme M
2-mercaptoethane sulfonic acid



Thiamin pyrophosphate



Molybdopterin



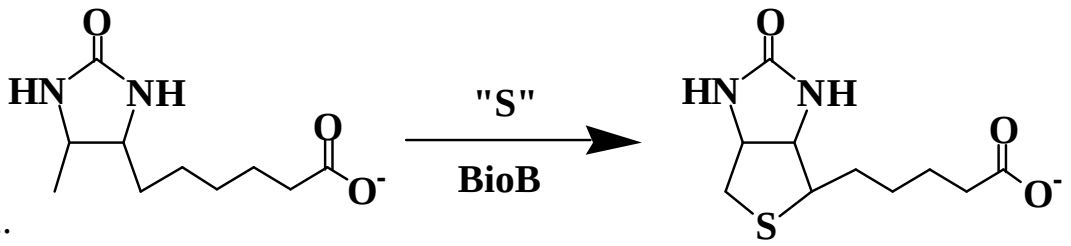
ms^{2}i^{6}A
N^6-(4-isopentenyl)-2-methylthioadenosine

Biosynthèse de composés naturels soufrés: conversion de C-H en C-S

Biological radical sulfur insertion reactions
M. Fontecave, S. Ollagnier-de Choudens, E. Mulliez
Chem.Rev. 2003, 103, 2149-2166

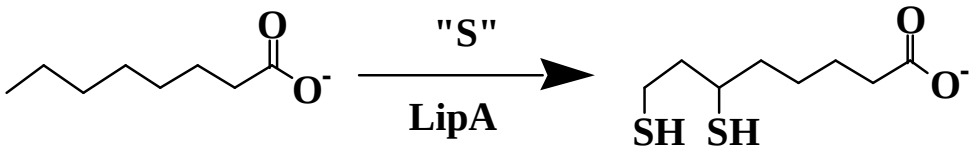
New light on methylthiolation reactions
M. Fontecave, E. Mulliez, M. Atta
***Chemistry and Biology* 2008,15, 209-210**

⇨ Biotine synthase



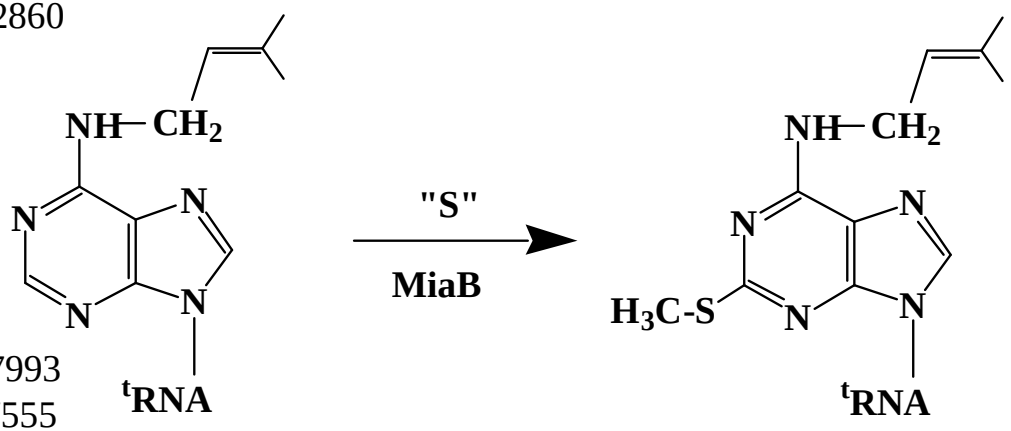
JT Jarrett: (2001) *Biochemistry* 40, 8352.
(2001) *Biochemistry* 40, 8343

⇨ Lipoate synthase



SJ Booker: (2004) *Biochemistry* 43 11770
(2005) *J. Am. Chem. Soc.* 127 2860

⇨ MiaB

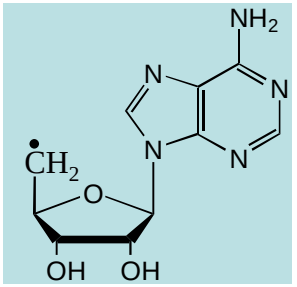


M. Atta et al: (2003) *J. Biol. Chem.* 278 17993
(2004) *J. Biol. Chem.* 279 47555
(2007) *Biochemistry* 46, 5140

Question:
C-H → C-SH (→ C-S-CH_3)

Activation radicalaire: **C-H → C°**

Abstraction d'atome H?:
Radical 5'-deoxyadenosyl
Dérivé de la SAM

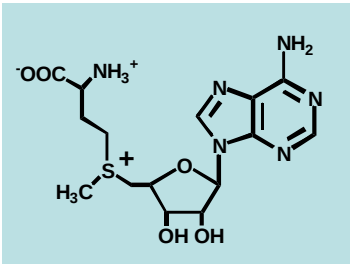


Insertion de soufre: **C° → C-SH**

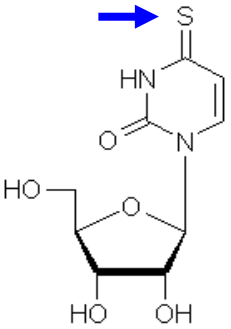
Donneur d'atomes de S?:
Origine? Nature ?
mécanisme?

(Méthylation): **C-SH → C-S-CH_3**

Donneur de méthyle?:
S-adenosylméthionine

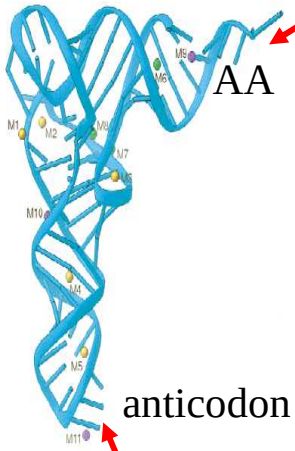


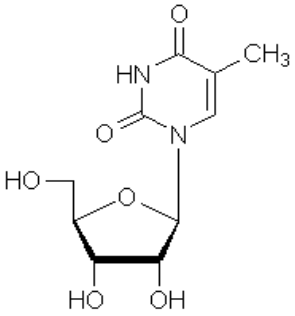
Nucléosides modifiés dans les ARN^{t}



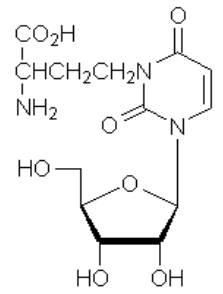
4-thiouridine : $^{4}_{S}$U

E. coli tRNA^{phe}

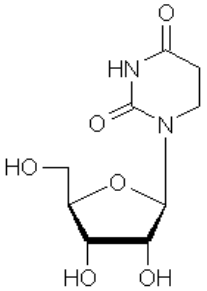




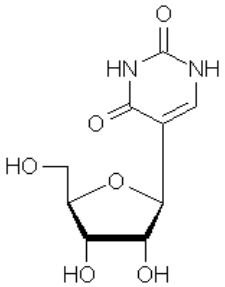
5-methyluridine : ribothymidine, T, m^{5}U



3-(3-amino-3-carboxypropyl)uridine: acp3U

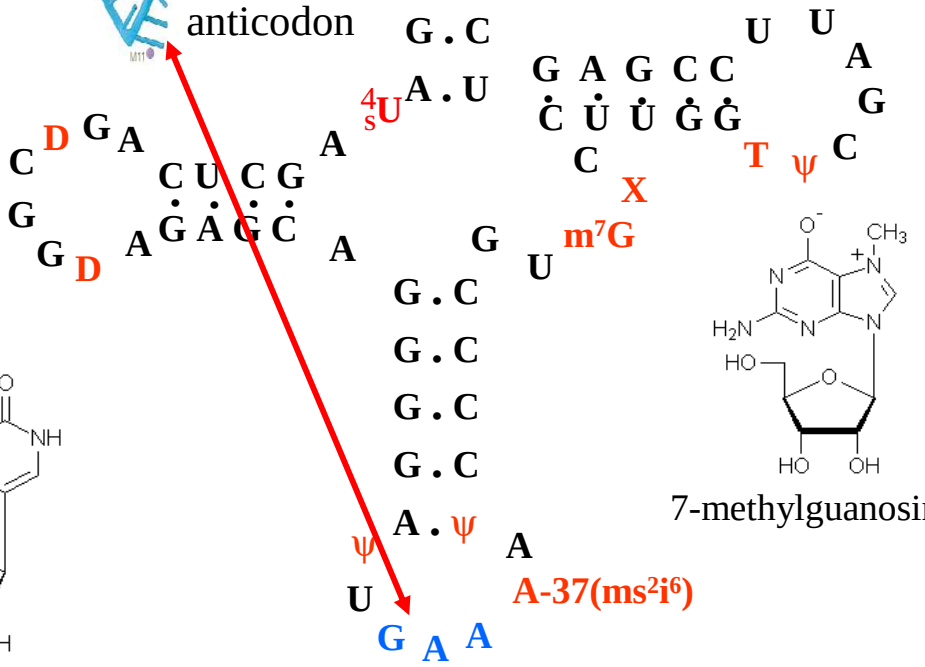


Dihydrouridine : D

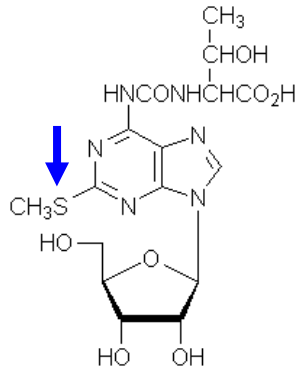


Pseudouridine : ψ

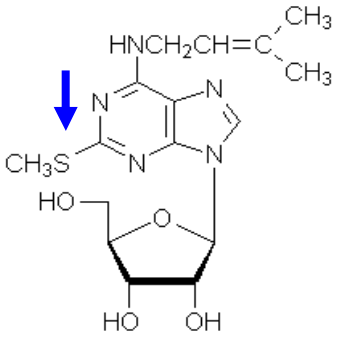
anticodon



7-methylguanosine : **m^{7}G**



2-methylthio-*N*6-Threonylcarbamoyladenosine:ms^{2}t^{6}A



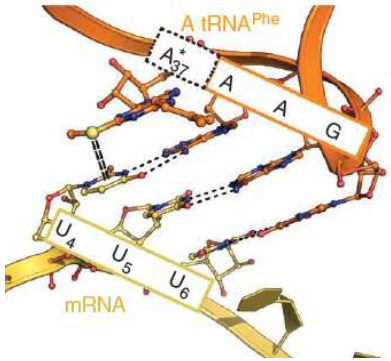
2-methylthio-N6-isopentenyladenosine : ms^{2}i^{6}A

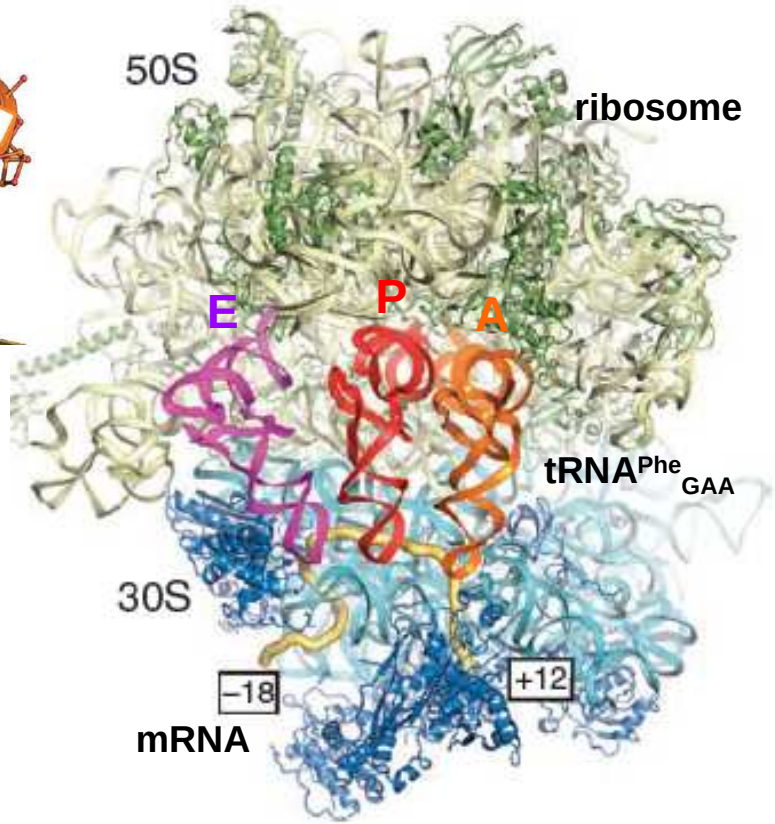
Importance de ms^{2}i^{6}A pour

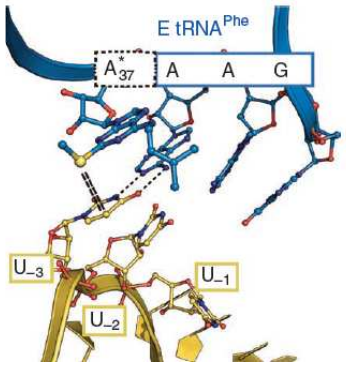
-Stabiliser les interactions tRNA/mRNA/rRNA

-maintenir the bon cadre de lecture

-Décroitre la fréquence de déplacement de cadre

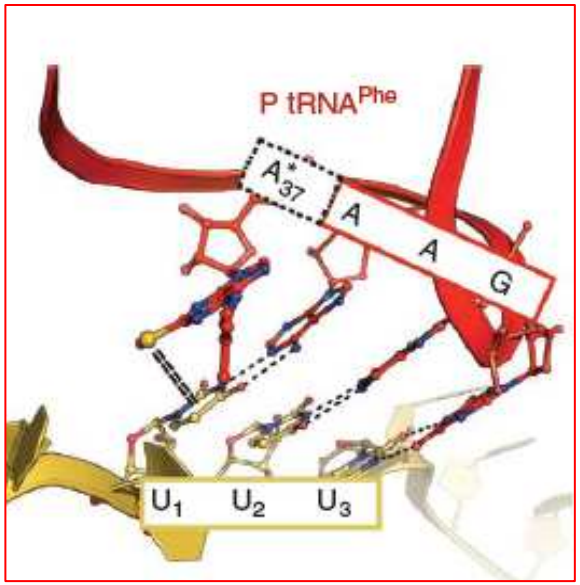




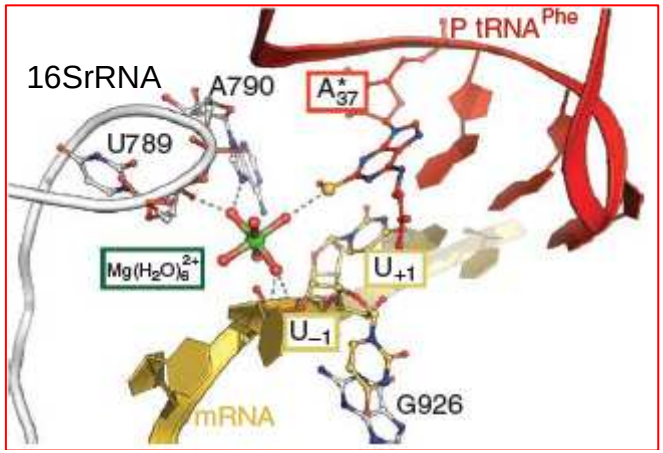


Nature Str & Mol Biol 2010, 17, 555

« Stacking » avec la base du premier nucléotide du codon

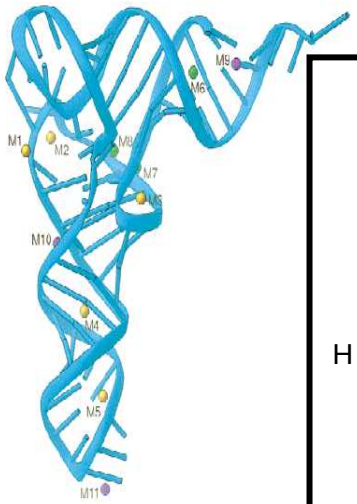


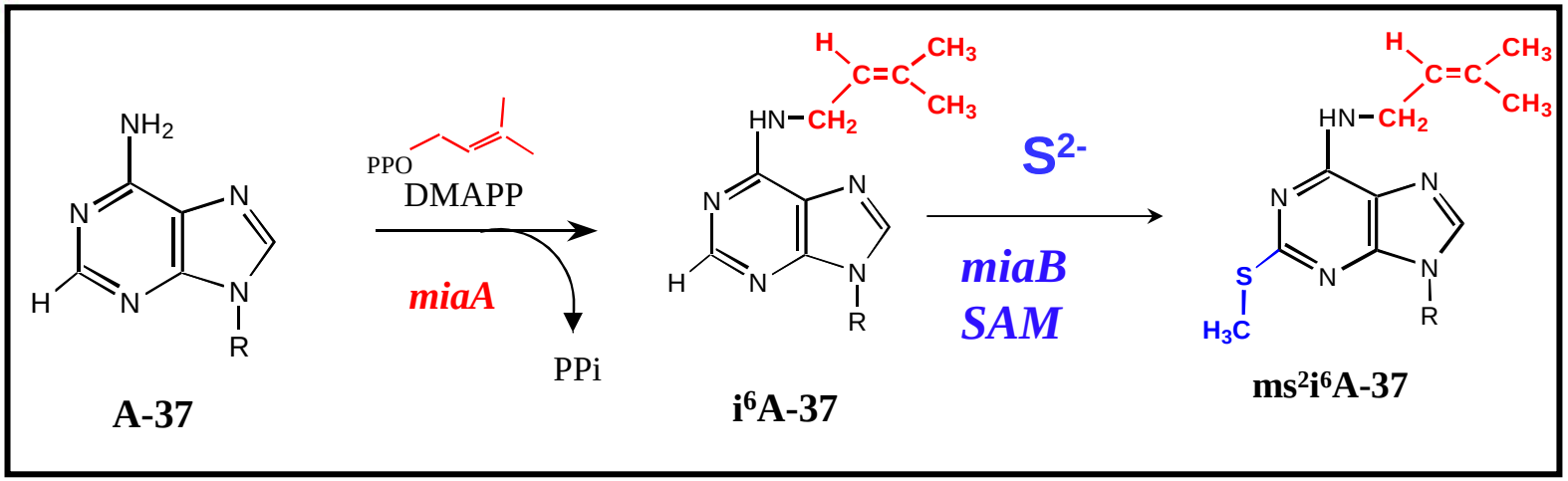
interactions autour du premier nucléotide du codon P



MiaB: une enzyme Radical-SAM

→ **Un substrat acide nucléique (ARNt)**



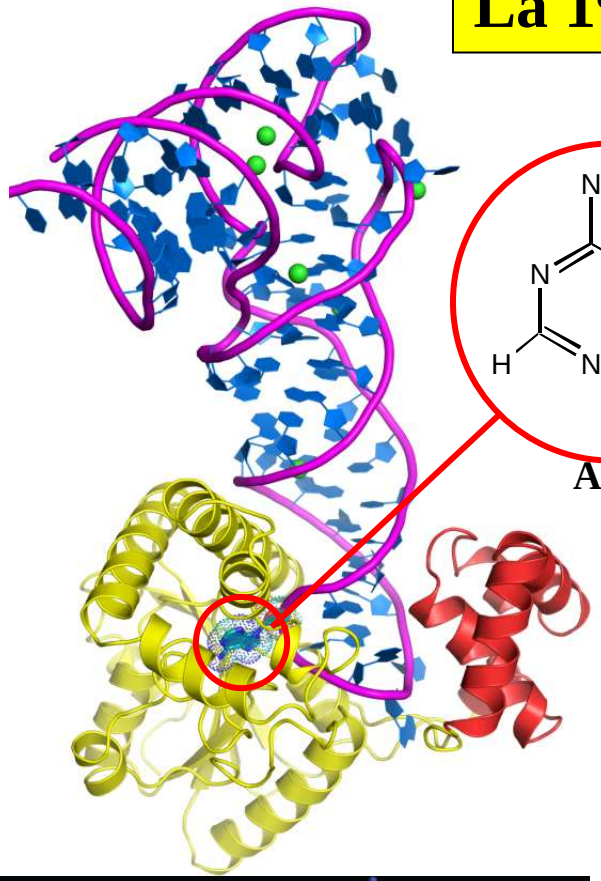


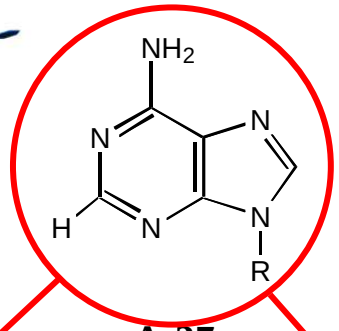
→ **Deux functions: sulfurase et methyltransferase (SAM-dépendantes)**

→ **Deux centres [4Fe-4S]**

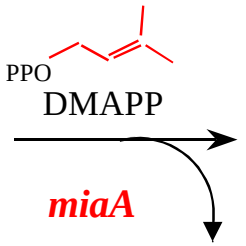
J. Biol. Chem. (2002) 277 13367
J. Biol. Chem. (2003) 278 17993
J. Biol. Chem. (2004) 279 47555
Biochemistry 2007, 46, 5140-5147

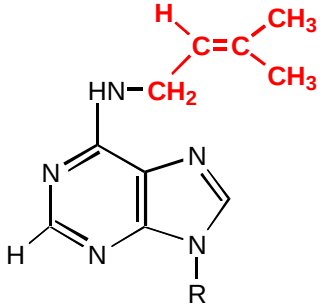
La 1ère étape de N6-alkylation : MiaA



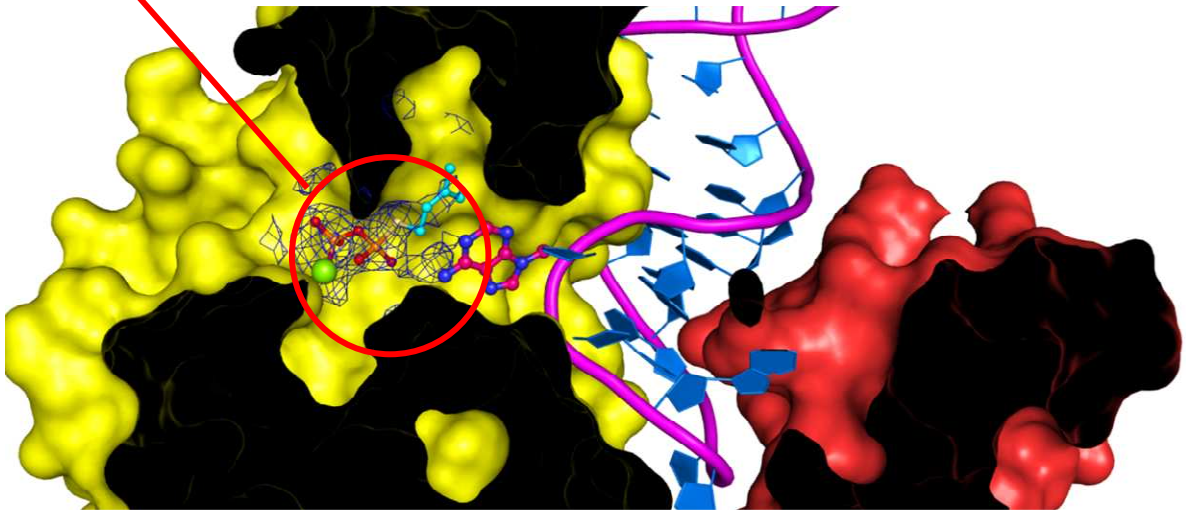


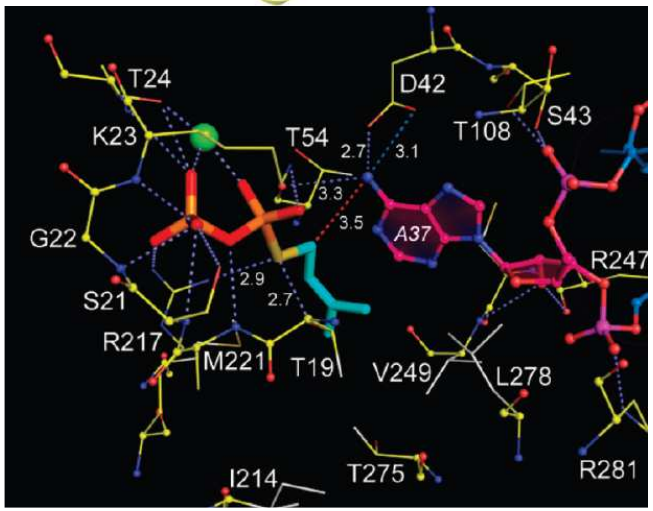
A-37



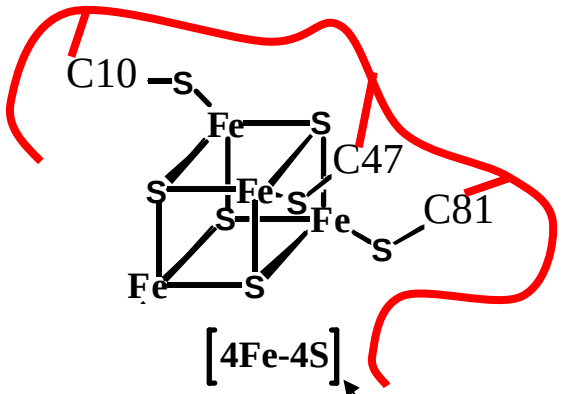


i6A-37





(M. Atta, E. Mulliez, J. Hunt,M. Fontecave et al, Biochemistry, 2009 48 5057)

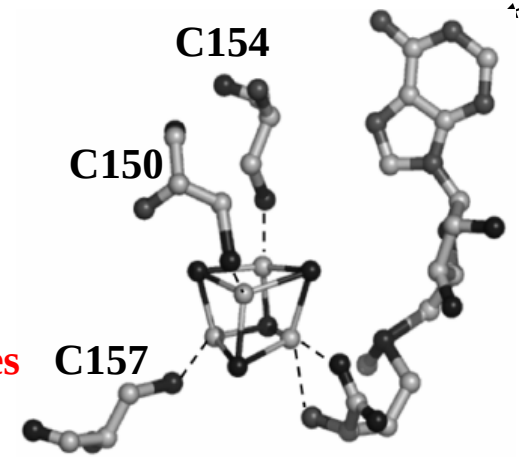


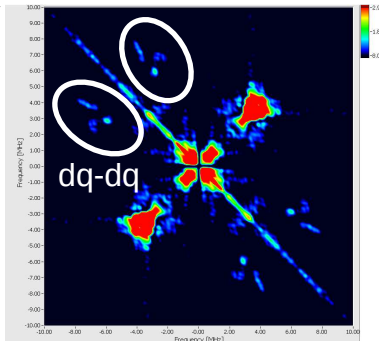

```
E.coli      MTKKLHIKTWGCQMNEYDSSKMADLLDATHGYQLTDVAEEADVLLLNTCSIREKAQEKVFHQLGRWKLLKEKNPDLIIGVGGCVASQEGE 90
S.typh      MTKKLHIKTWGCQMNEYDSSKMADLLDATHGYQLTDVAEEADVLLLNTCSIREKAQEKVFHQLGRWRLLKEKNPDLIIGVGGCVASQEGE 90
H.inf       MTQKLHIKTWGCQMNEYDSSKMADLLLSTHGLELTEAPEEADVLLLNTCSIREKAQEKVFHQLGRWKELKKNNPNLVIGVGGCVASQEGE 90
A.ae        MSKKFFIKTFGCQMNFNDSERIRGLLK-TIGYEQTDNWEEADLIILNTCTIREKPDQKVLSHLGEYKKIKEKNPKALIAVAGCLAQRTGW 89
T.mari      --MRFYIKTFGCQMNENDSEAMAGLLV-KEGFTPASSPEEADVVIINTCAVRRKSEEKAYSELGQVLKLKKKK-KIVVGVAGCVAEKERE 86
               ::.***:*****  **. : .**  . *   :   ****:::***::*.*.::*.   **.   :*::: . ::.*.**:*.:

                                                                          motif CxxxCxxC
E.coli      HIRQRAHYVDIIFGPQTLHRLPEMINSVRG--DRSPVVDISFPEIEKFDRLPEPRAEGPTAFVSIMEGCNKYCTYCVVPYTRGEEVSRPS 178
S.typh      HIRQRAHYVDIIFGPQTLHRLPEMINSVRG--DRSPVVDISFPEIEKFDRLPEPRAEGPTAFVSIMEGCNKYCTYCVVPYTRGEEVSRPS 178
H.inf       HIRHRAPYVDIIFGPQTLHRLPEMINQIRG--GKSSVVDVSFPEIEKFDRLPEPRAEGPTAFVSIMEGCNKYCTFCVVPYTRGEEVSRPV 178
A.ae        ELVKKAPVIDIMFSSFNMHQLPELINQAQAGYKAIAILDELPQDEDKIWEYPVERDNKYCAYVTIIKGCDKNCTYCVVPRTRGKERSRAL 179
T.mari      KFLEKG--ADFVLGTRAVPRVTEAVKKALEG-EKVALFEDHLDEYT--HELPRIRTSRHHAWVTIIHGCDRFCTYCIVPYTRGRERSRPM 171
            .: .:.   *.:::.  : ::.* ::         .:.:    :     . *  * .   *:*:*:.**:: **:*:** ***.* **.
```

E. Coli et *T. maritima*

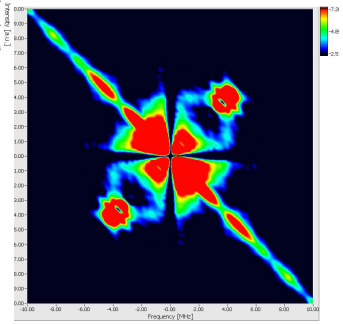
-UV-Vis
-RPE
- Raman resonance
-Mössbauer
- mutagénèse dirigée:

les 6 Cys conservées sont essentielles





MiaB +SAM + tRNA
a_N~5 MHz



MiaB +SAM

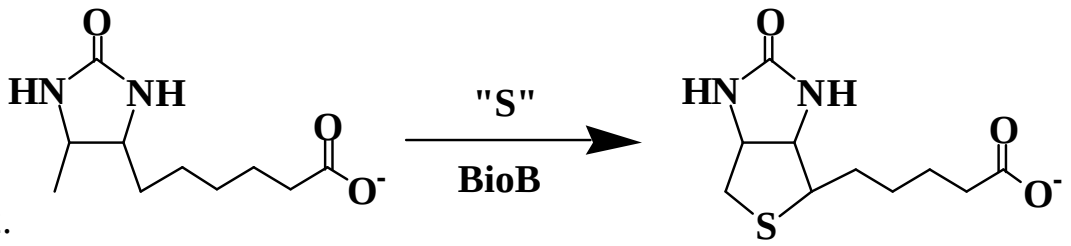
Biosynthèse de composés naturels soufrés: Enzymes avec deux centres [Fe-S]

Biological radical sulfur insertion reactions
M. Fontecave, S. Ollagnier-de Choudens, E. Mulliez
Chem.Rev. 2003, 103, 2149-2166

New light on methylthiolation reactions
M. Fontecave, E. Mulliez, M. Atta
***Chemistry and Biology* 2008,15, 209-210**

Biotine synthase

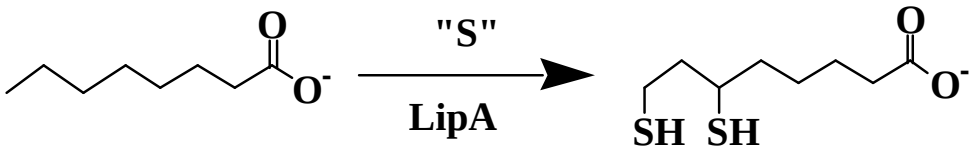
-Cluster SAM
-[2Fe-2S]



JT Jarrett: (2001) *Biochemistry* 40, 8352.
(2001) *Biochemistry* 40, 8343

Lipoate synthase

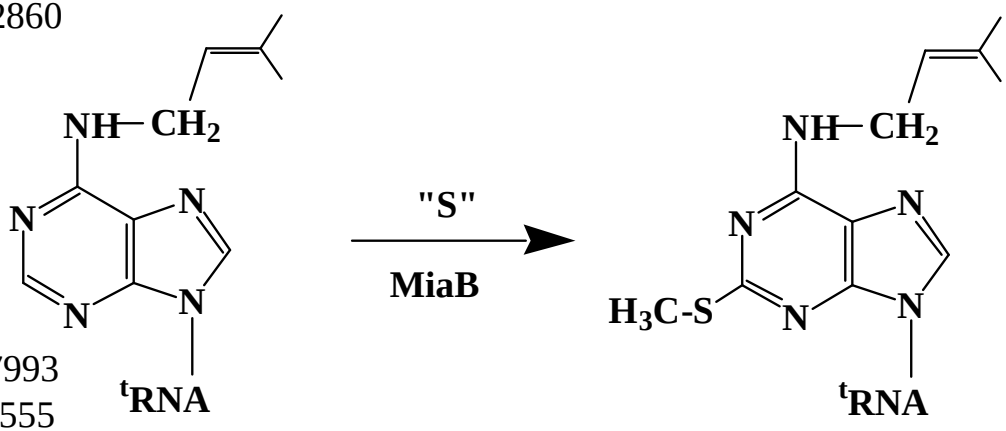
-Cluster SAM
-[4Fe-4S]



SJ Booker: (2004) *Biochemistry* 43 11770
(2005) *J. Am. Chem. Soc.* 127 2860

MiaB

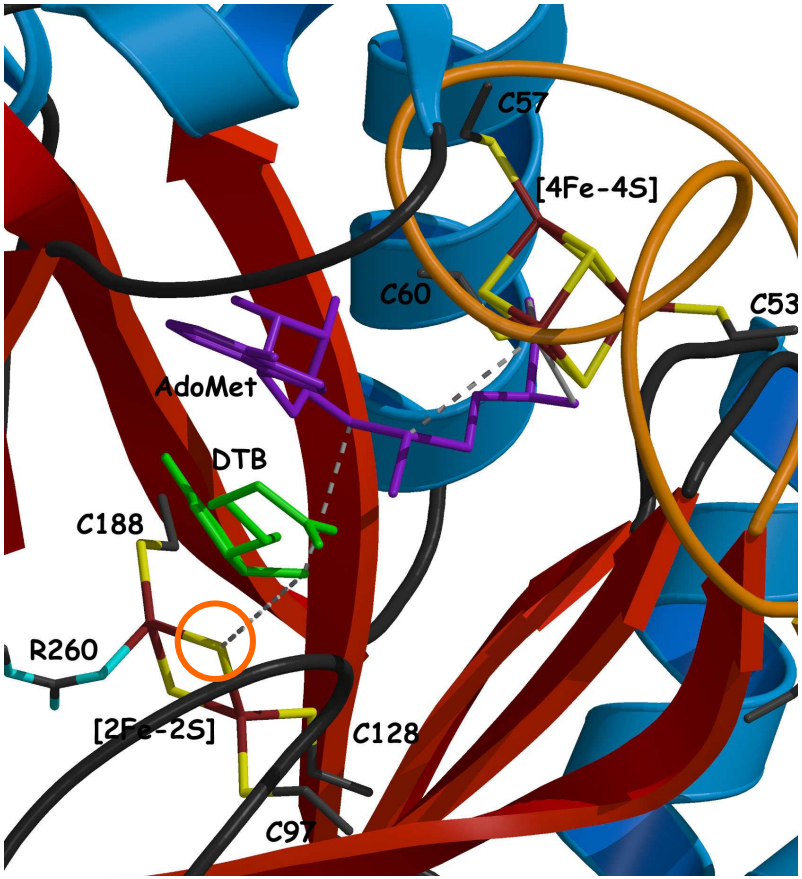
-Cluster SAM
-[4Fe-4S]



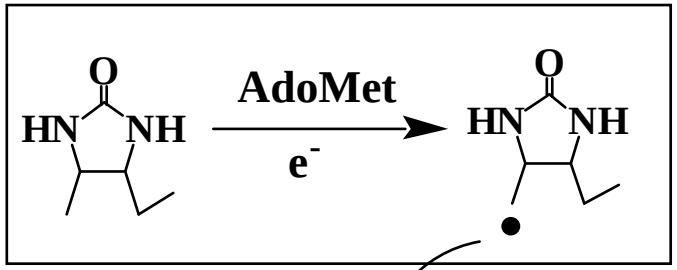
M. Atta et al: (2003) *J. Biol. Chem.* 278 17993
(2004) *J. Biol. Chem.* 279 47555
(2007) *Biochemistry* 46, 5140

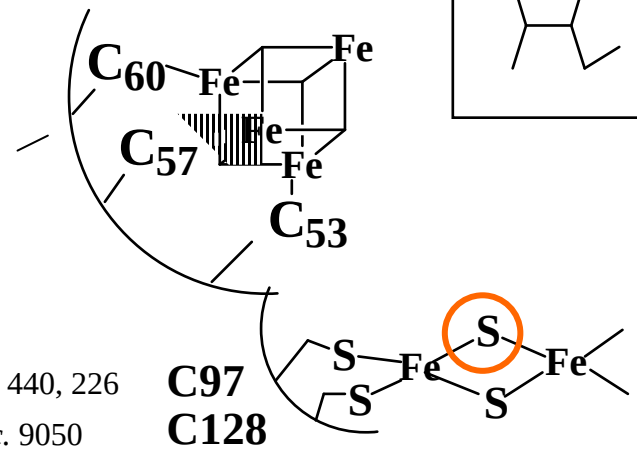
Une nouvelle fonction des centres fer-soufre? Transporteurs et donneurs d'atomes S

Le cas de la biotine synthase

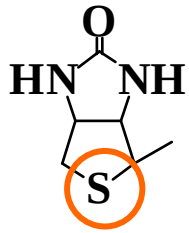


Drennan et al. (2004)
Science 303, 76-79.

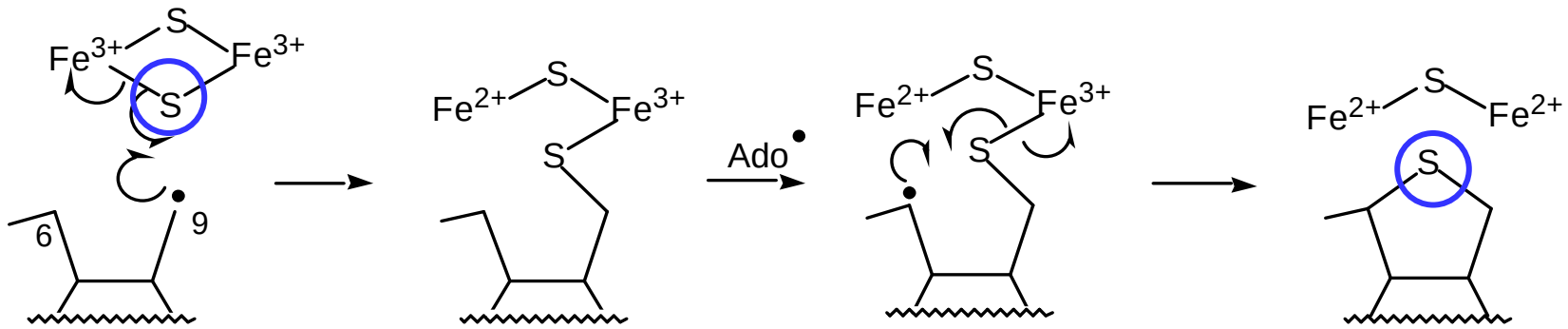




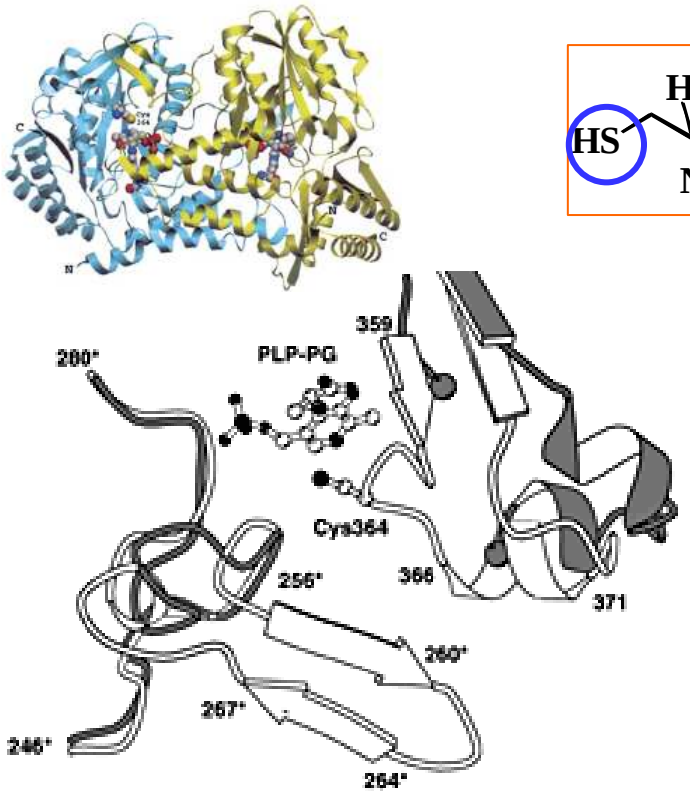
C97
C128
+ C188

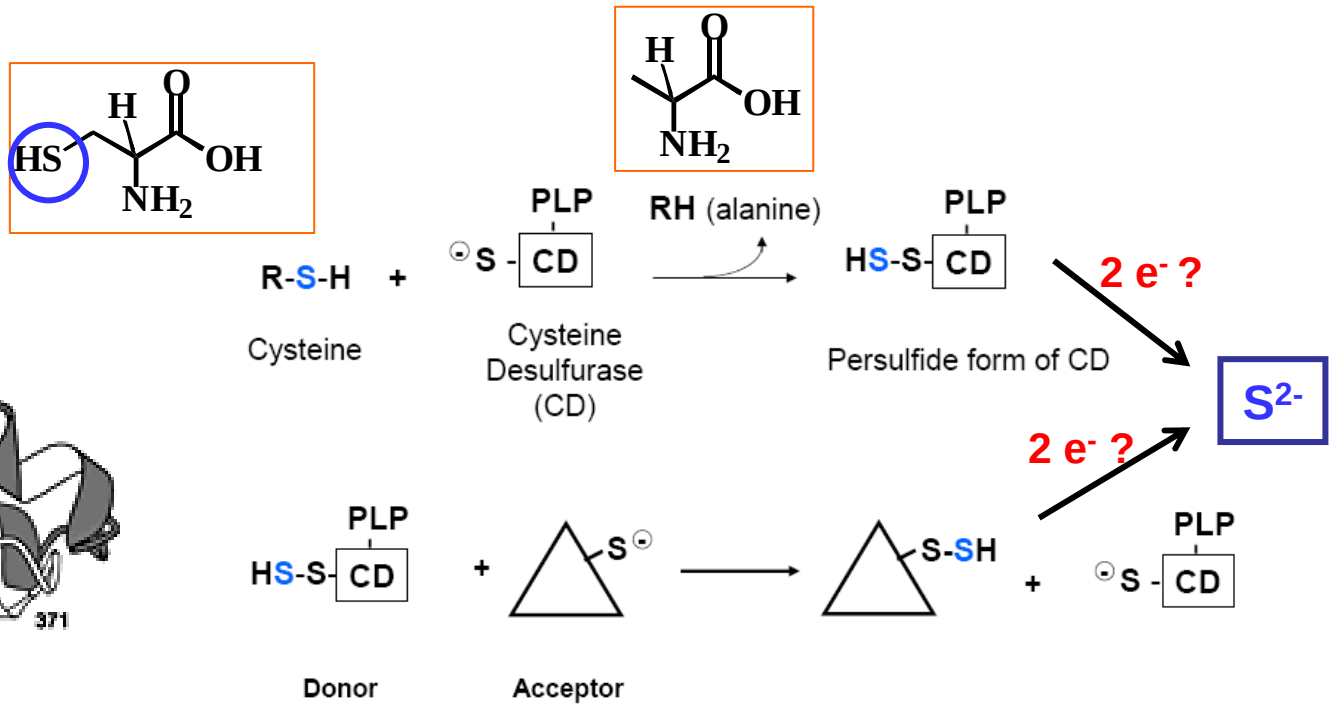


Marquet, A. et al, (1998) *FEBS Letters* 440, 226
Jarrett, J. et al (2002) *J. Am. Chem. Soc.* 9050
Johnson, M. et al (2004) *Biochemistry* 43, 2007



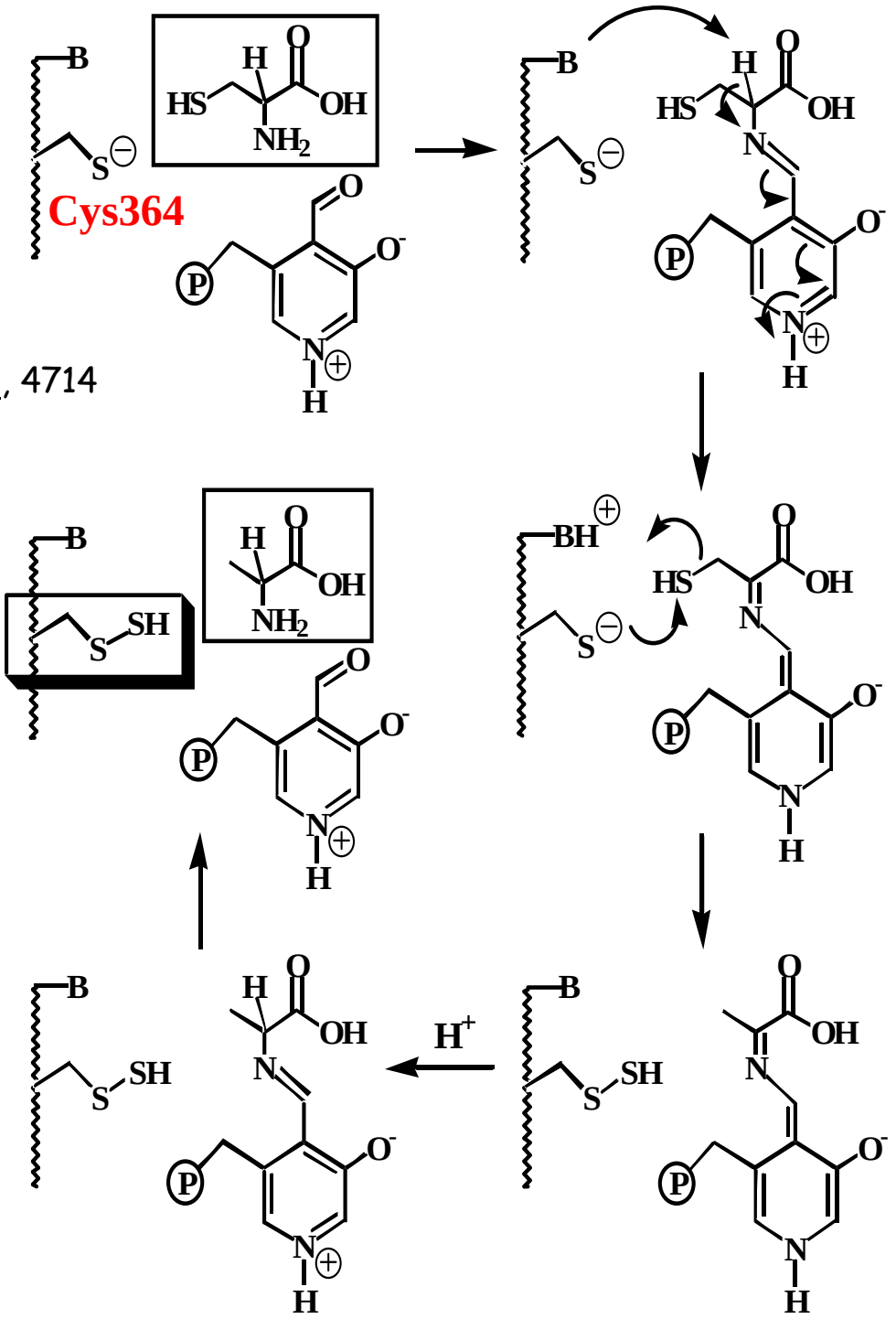
S^{2-} = Cystéine + cystéine désulfurase ??



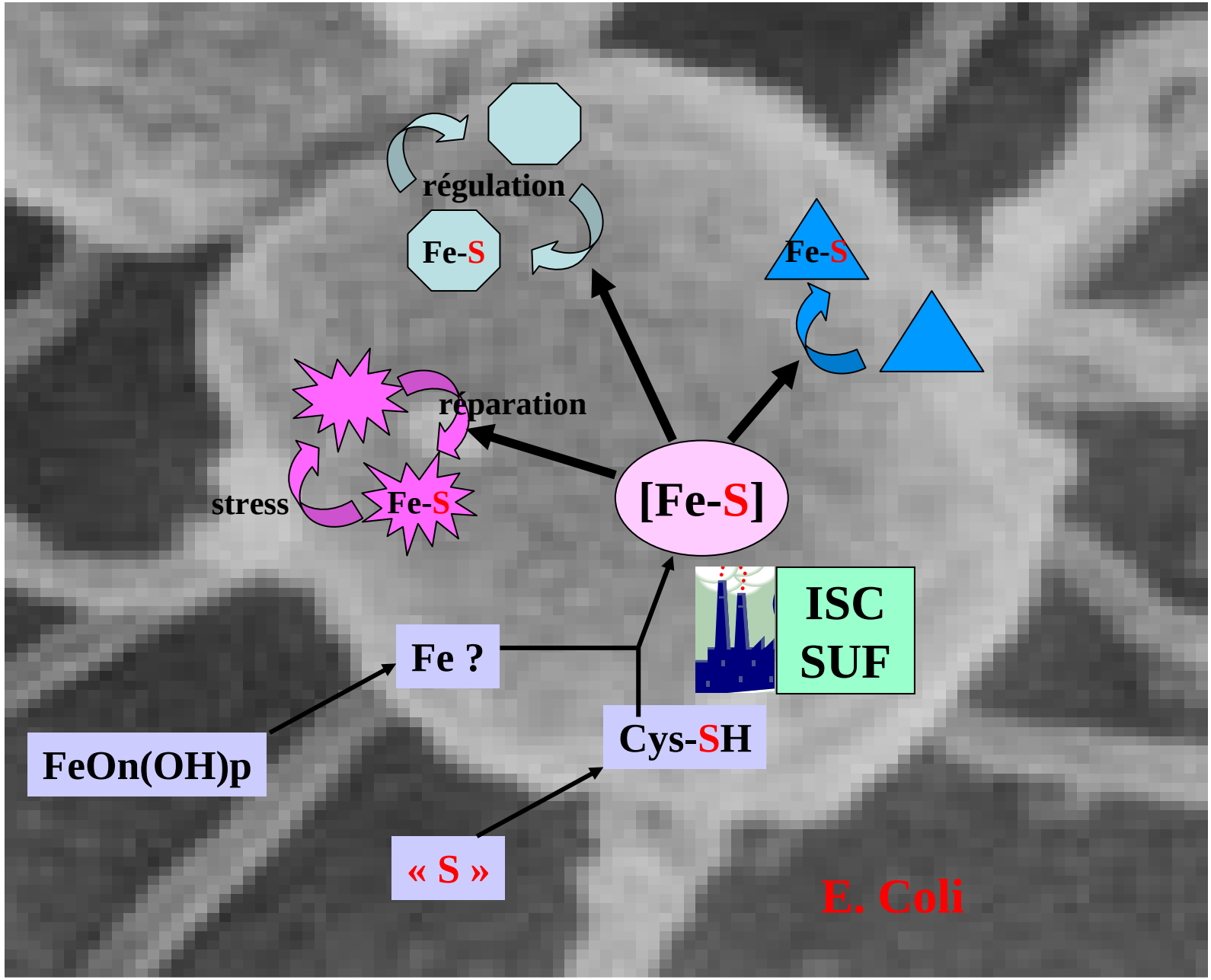


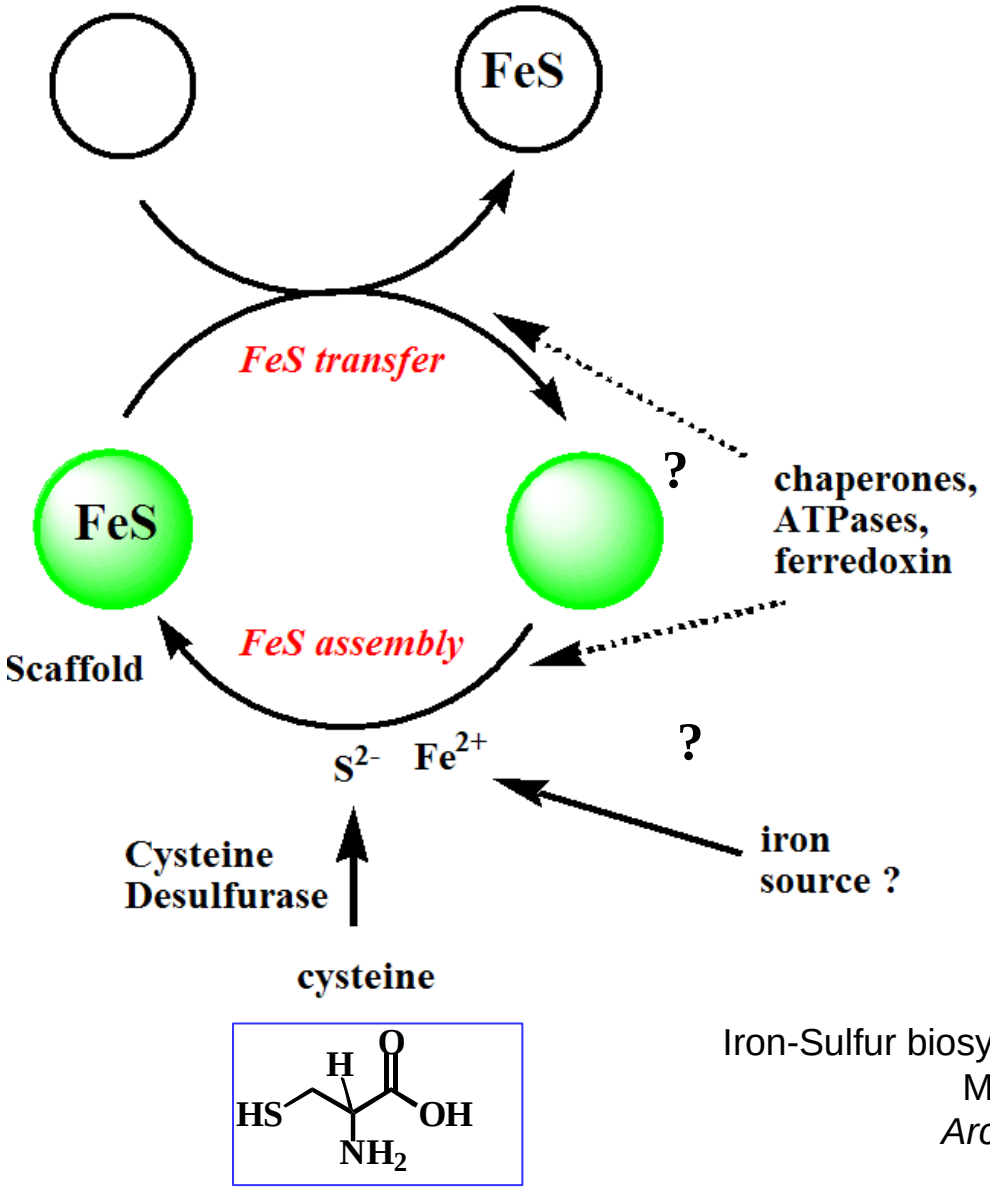
Mécanisme des cystéine desulfurases

Zheng L, Dean DR et al. *Biochemistry* (1994)33, 4714



Biosynthèse et réparation des centres fer-soufre





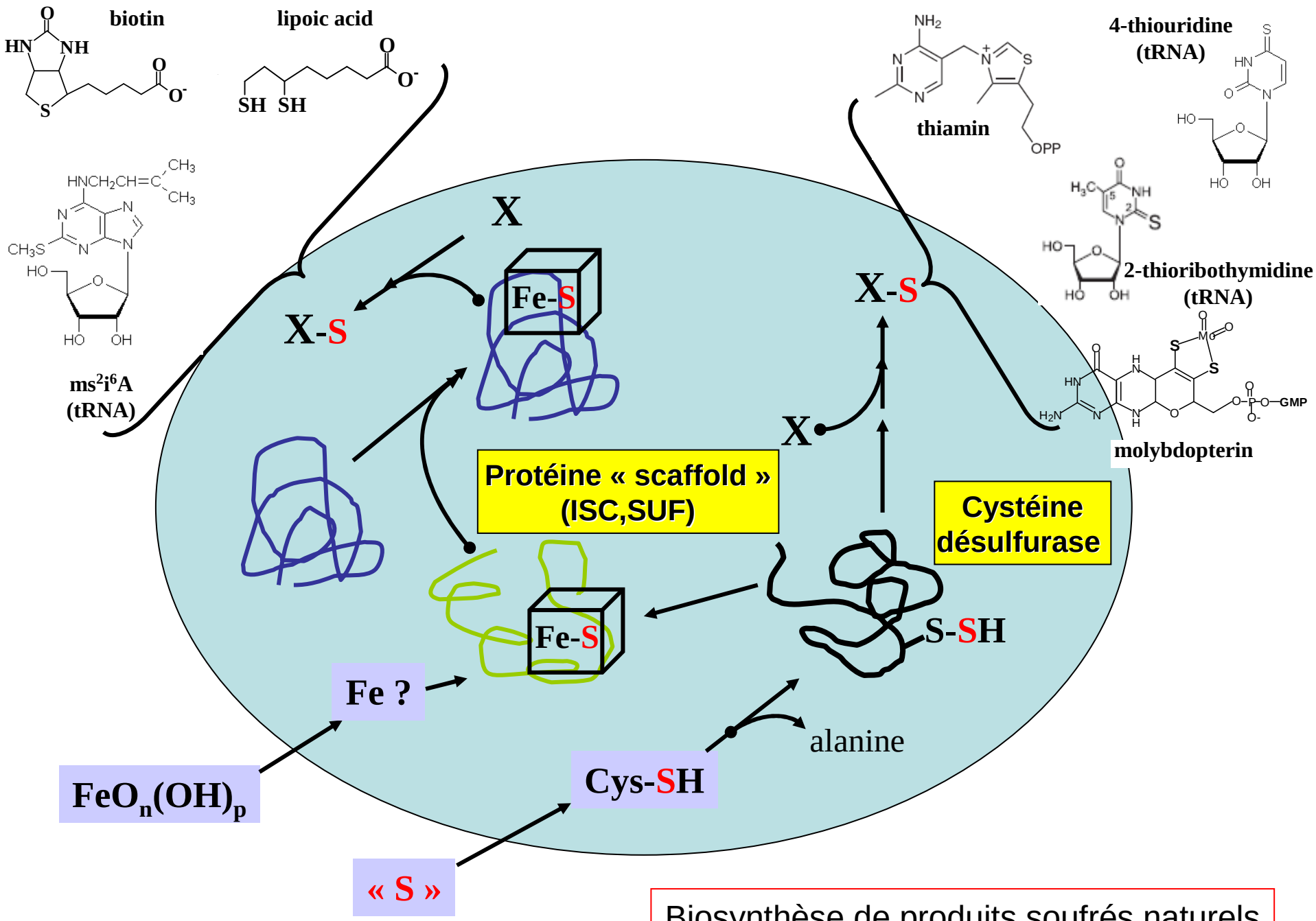
Deux acteurs majeurs

Protéine d'assemblage (Scaffold):
Construit le cluster et le transfère à une apoprotéine cible

Cystéine desulfurase:
Extrait les atomes de S de la cystéine et les donne à la « scaffold »

Iron-Sulfur biosynthesis: mechanisms of cluster assembly and transfer
M. Fontecave, S. Ollagnier-de-Choudens
Arch. Biochem. Biophys. 2008, 474, 226-37

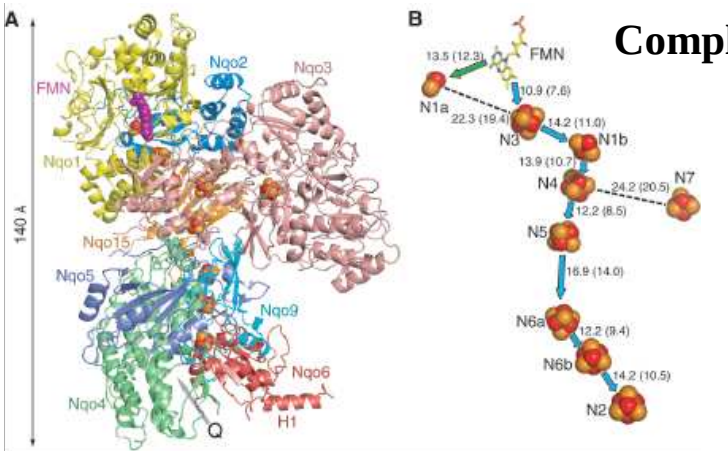
From iron and cysteine to iron-sulfur clusters: the biosynthetic protein machineries
M. Fontecave, S. Ollagnier-de-Choudens, B. Py, F. Barras
Escherichia coli and *Salmonella: cellular and molecular biology* 2008 Chapter 3.6.3.14



Biosynthèse de produits soufrés naturels

Clusters fer-soufre en biologie

Iron-sulfur clusters : ever expanding roles
M. Fontecave
Nature Chemical Biology 2006, 2, 171-174

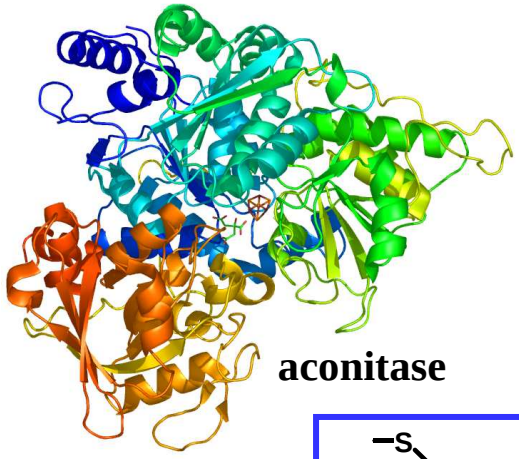


Complex I

➢**Transport et transfert d'électrons (photosynthèse, respiration,…) (1960….)**

$$e^- + [xFe\text{-}xS]^{2+} \rightleftharpoons [xFe\text{-}xS]^{+}$$

➢**Catalyse non rédox (déhydratases, ACONITASE,…) (1970….)**

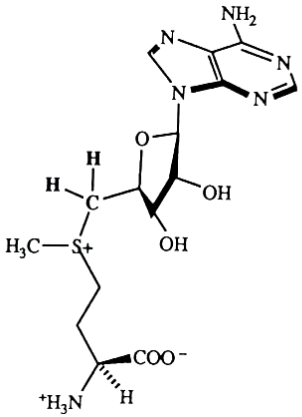


aconitase

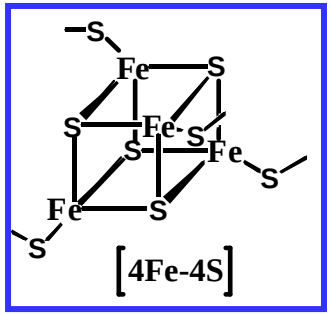
➢ **Modulation de l'expression des gèn (FNR, SoxR, IRP,…) (1980….)**

➢**Catalyse rédox (enzymes Radical-SAM) (1990…)**

➢**Transport et transfert de soufre (Radical-SAM enzymes) (2000….)**

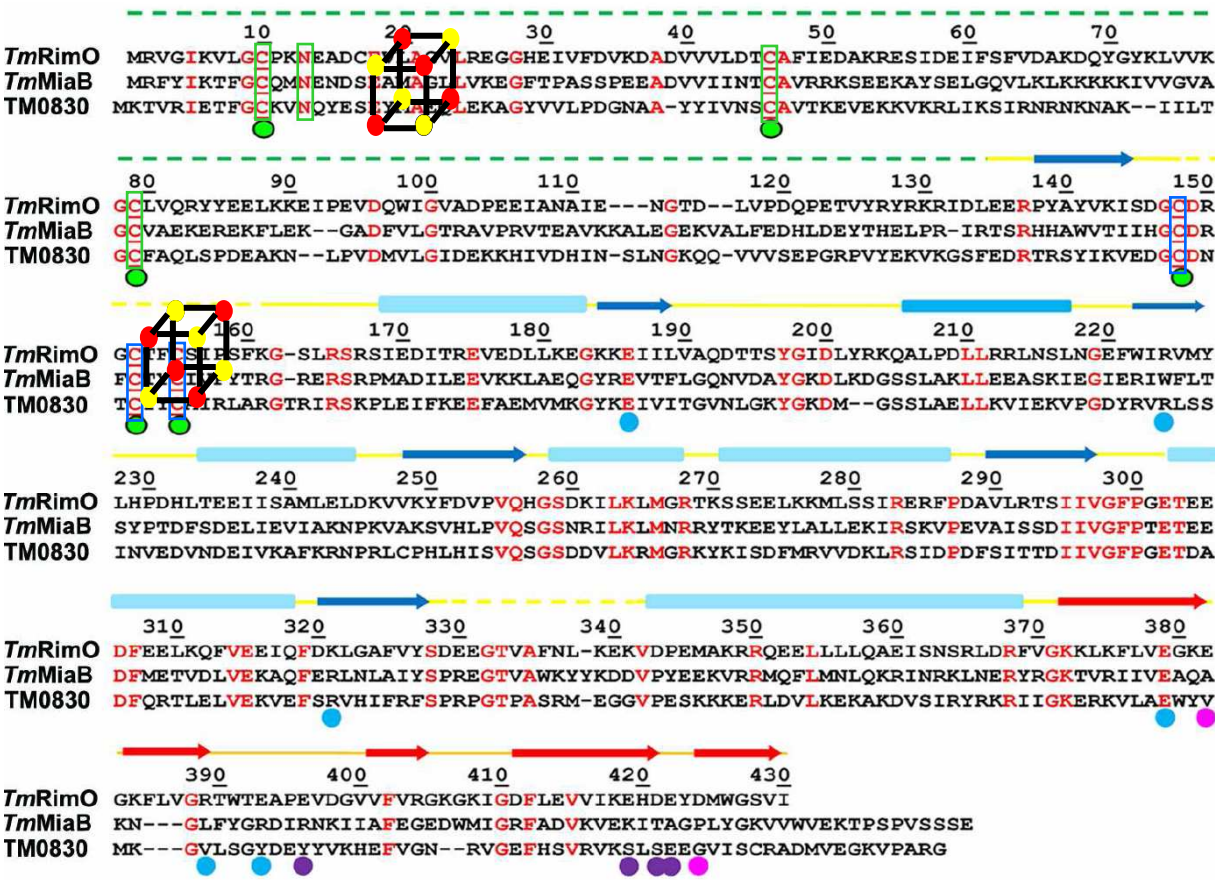


SAM: S-adenosylmethionine



[4Fe-4S]

Trois methylthio-transférases chez *T. maritima* : MiaB, TM0830, and RimO

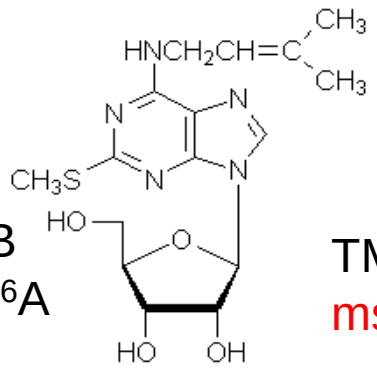


Domaine UPF0004

Domaine
Radical SAM

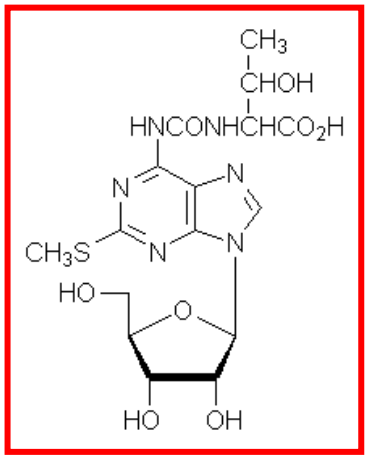
Domaine TRAM
Site de fixation du substrat

TM0830 = YqeV (*B. subtilis*) = CDKAL1 (*H sapiens*)

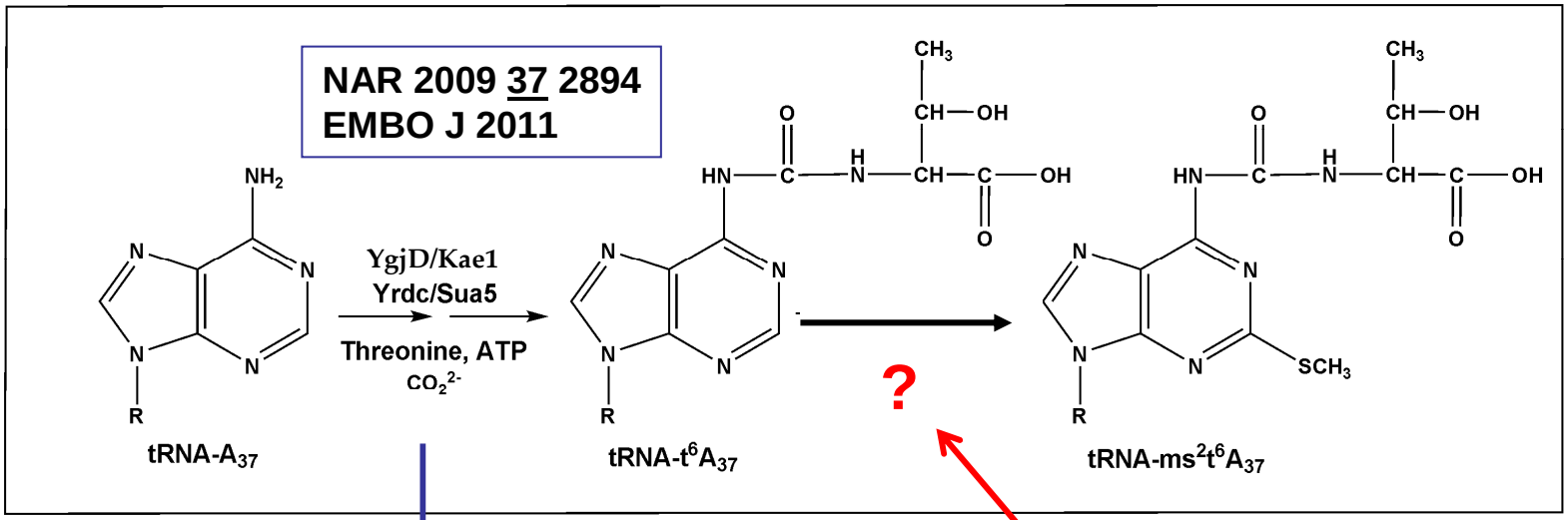


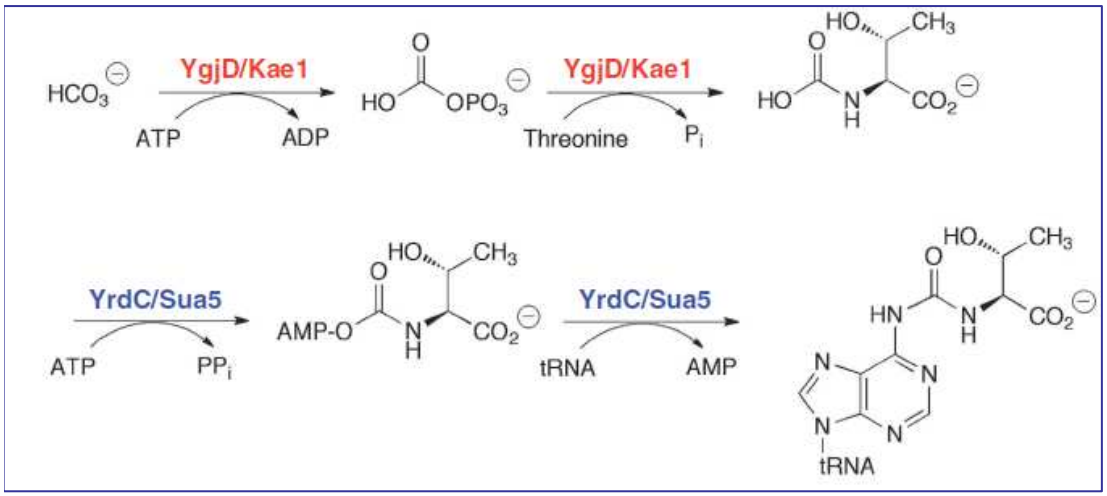
MiaB
ms^2i^6A

TM0830
ms^2t^6A **?**



Biosynthèse de ms^2t^6A





Cette methylthiolation est catalysée par TM0830/YqeV/CDKAL1
MtaB

CDKAL1(collab F-Y Wei, Kumamoto University, Japan)

Identification of eukaryotic and prokaryotic methythiotransferases for biosynthesis of ms^2t^6A in tRNA
S. Arragain, SK. Handelman, F. Forouhar, FY. Wei, K. Tomizawa, JF. Hunt, T. Douki, M. Fontecave, E. Mulliez, M. Atta
Journal of Biological Chemistry, 2010, **285**(37): 28425-28433

CDKAL1: MtaB (Radical-SAM) chez les eucaryotes !!

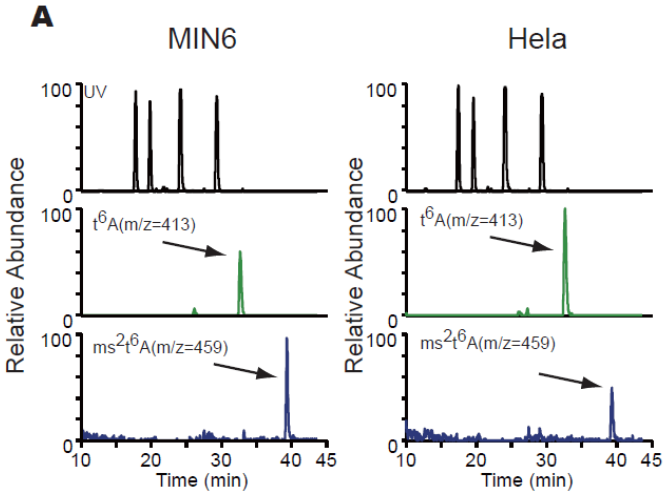
CDKAL1: un gène de susceptibilité au diabète de type 2

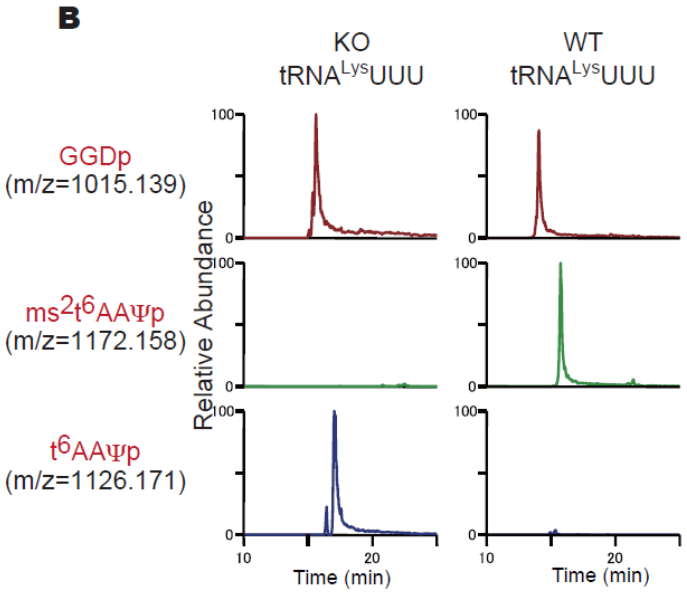
- Exprimé dans les cellules pancréatiques β et cellules immunitaires (réticulum endoplasmique)
-variants (défauts de sécrétion d'insuline) fréquemment trouvés chez les diabétiques (corrélation positive)

Nature medicine (2005) 11 1104
Nature genetics (2007) 39, 770
Science (2007) 316 1331
Genes and immunity (2009)

CDKAL1: une MTTase

- Nécessaire pour ms^2t^6A sur A^{37} de $tRNA^{Lys}(UUU)$





MIN6: lignée cellulaire dérivée de cellule β

CDKAL1: MtaB (Radical-SAM) chez les eucaryotes !!

CDKAL1: un gène de susceptibilité au diabète de type 2

- Exprimé dans les cellules pancréatiques β et cellules immunitaires (réticulum endoplasmique)
-variants (défauts de sécrétion d'insuline) fréquemment trouvés chez les diabétiques (corrélation positive)

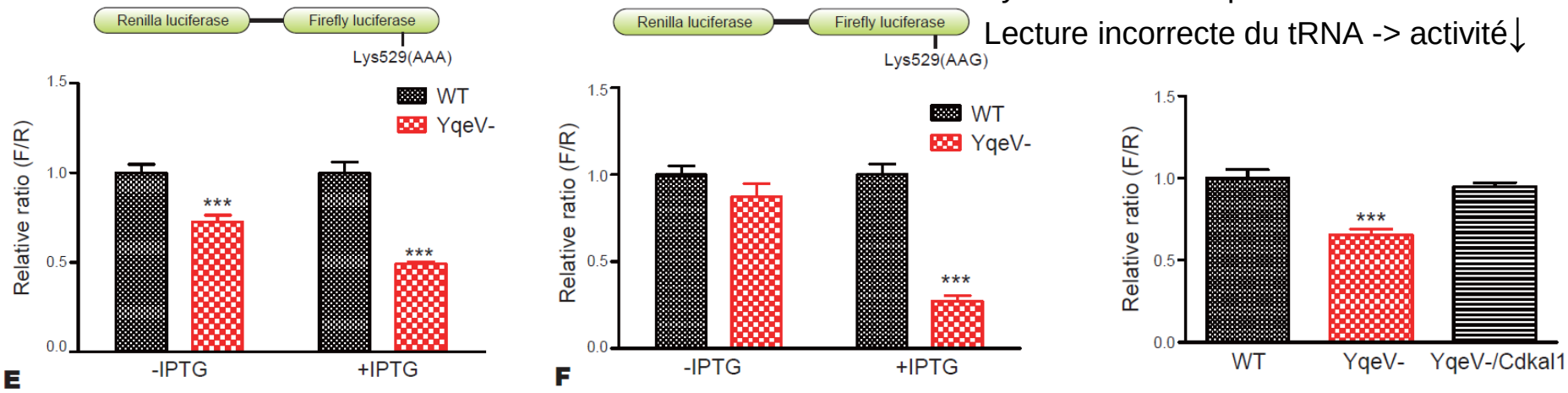
Nature medicine (2005) 11 1104
Nature genetics (2007) 39, 770
Science (2007) 316 1331
Genes and immunity (2009)

CDKAL1: une MTTase

-Nécessaire pour ms^2t^6A sur A^{37} de $tRNA^{Lys}(UUU)$

- assure la lecture correcte de tRNALys(UUU)

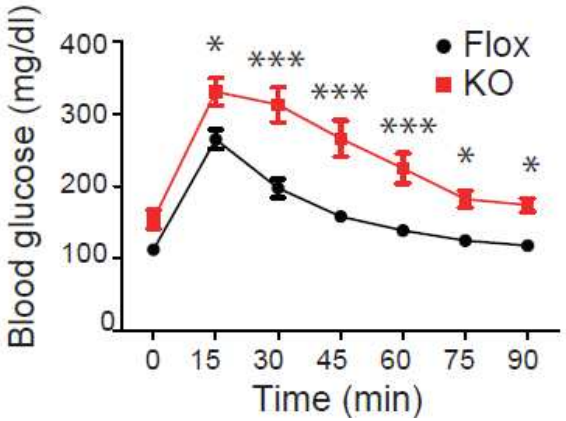
Lys259 essentiel pour l 'activité:
Lecture incorrecte du tRNA -> activité↓



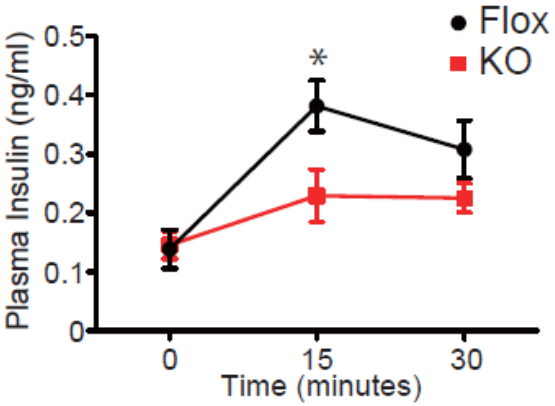
Effets sur la sécretion de l’ insuline ?

Souris Cdkal1 (KO) : développement normal; morphologie normale des cellules β (ilots)

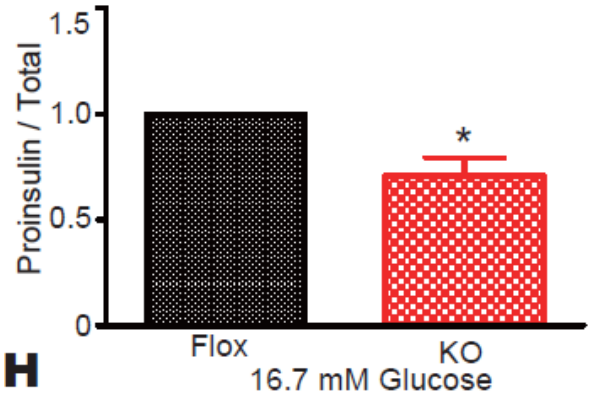
intolérance au glucose
à tous les âges



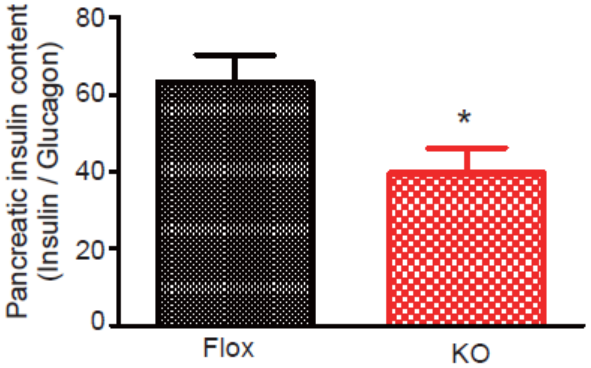
Sérum insuline ↓
(glucose challenge)



Synthèse proinsuline ↓

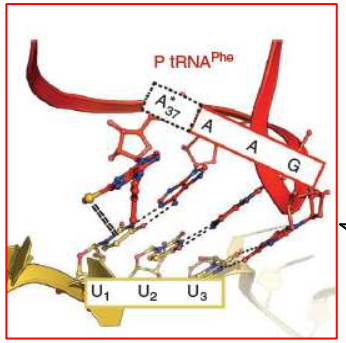


insuline pancréatique ↓



Quel lien entre modification de tRNA et sécretion d'insuline ?

ms^2t^6A important pour la stabilisation de l'interaction codon-anticodon



Empêche déplacement du cadre de lecture ou lecture incorrecte du tRNA pendant la traduction (synthès eprotéique)

CDKAL1 requis pour le décodage des codons AAA and AAG par modification de $tRNA^{Lys}(UUU)$

Perte de fonctionnelle de CDKAL1

⇩

ms^2t^6A ↓

⇩

Lecture incorrecte des codons Lys

⇩

Mauvais repliement/clivage de la proinsuline (2 codons Lys(AAG))

⇩

Synthèse incorrecte de l'insuline

⇩

Stress ER

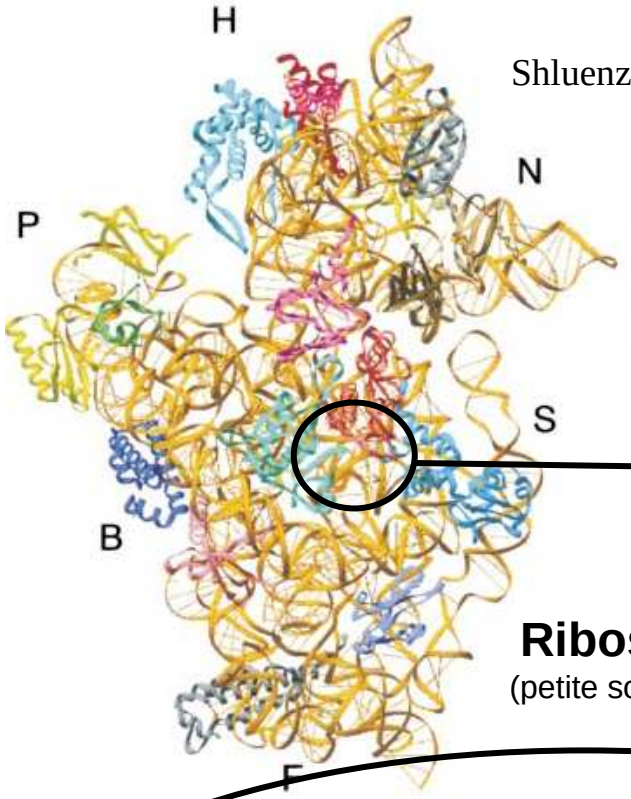
⇩

Sécretion de l'insuline ↓

⇩

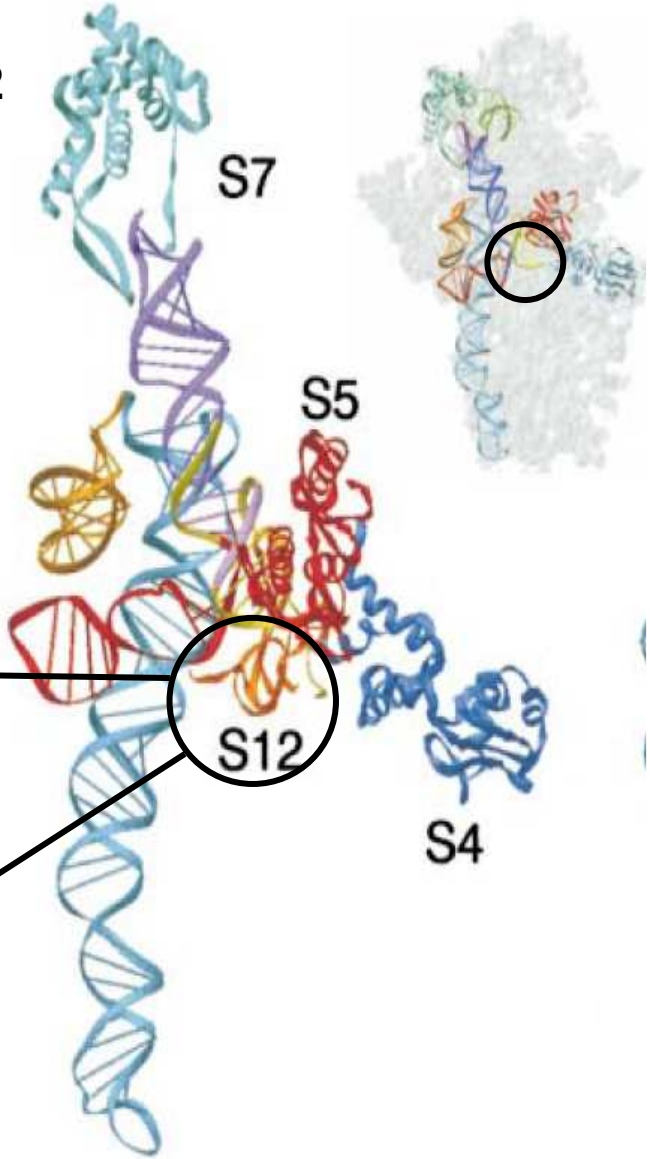
Intolérance au glucose

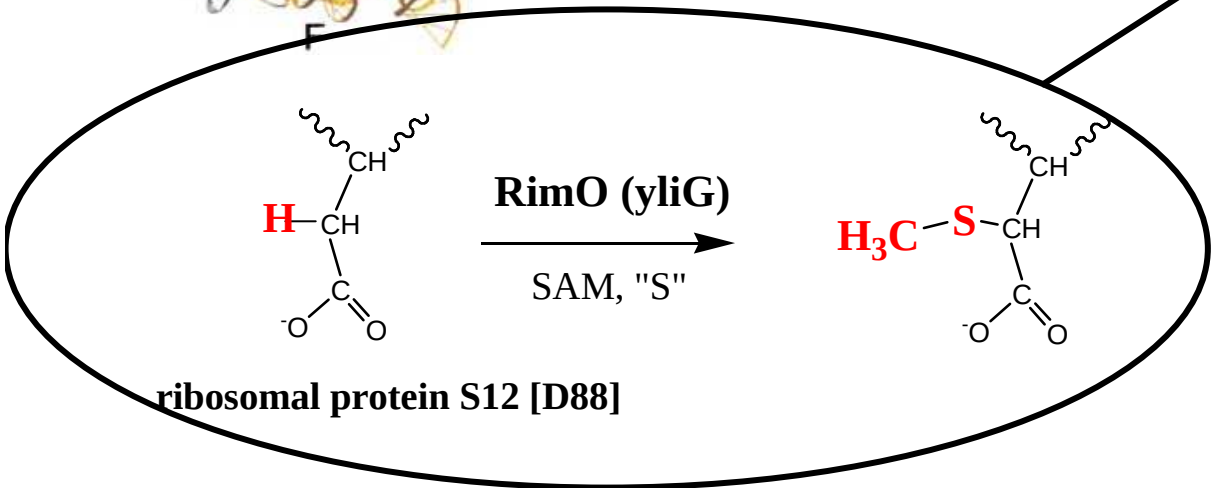
RimO: methylthiolation de la protéine ribosomale S12



Shluenzen F and coll, *Cell* 2000, 102, 615

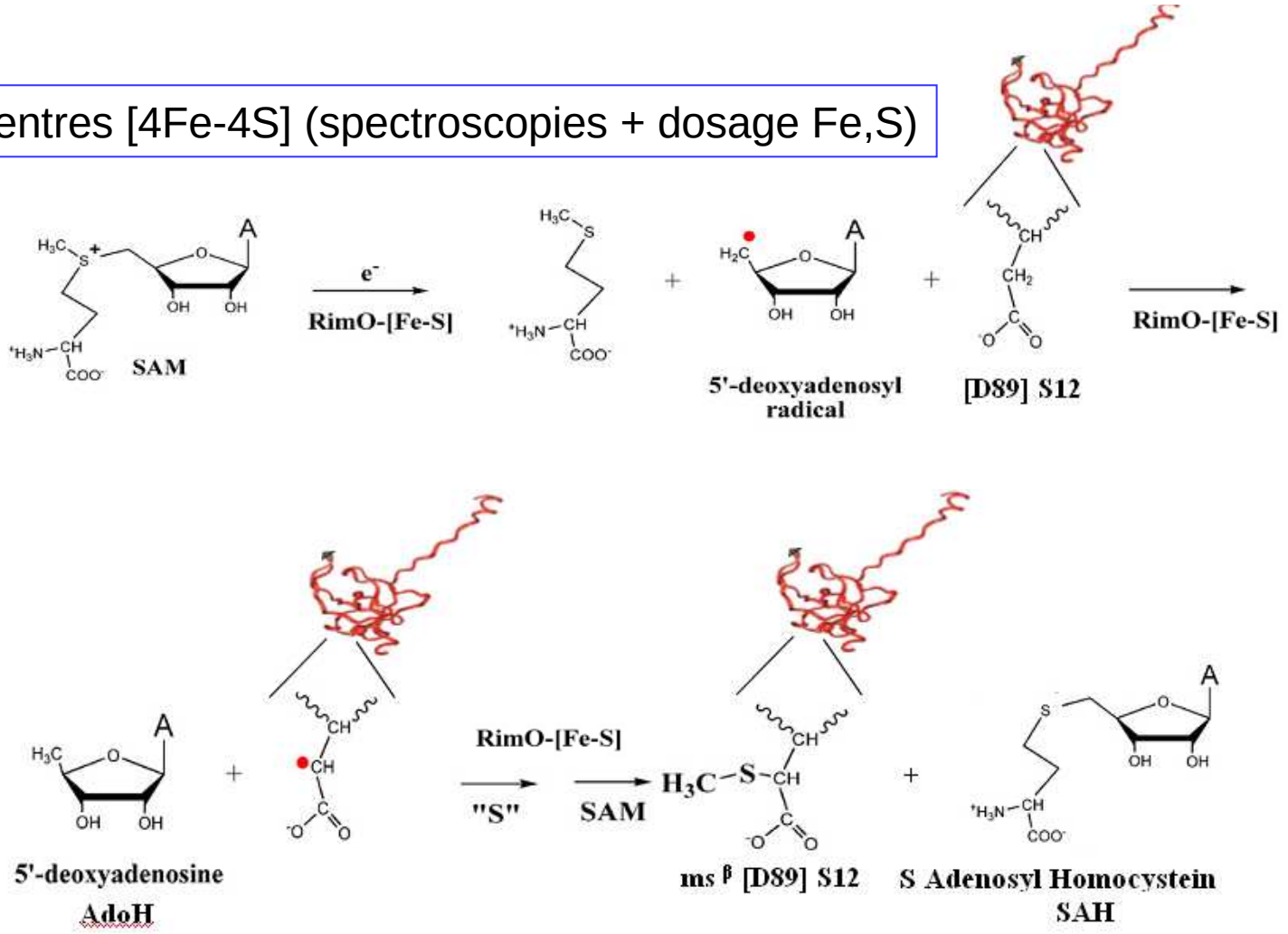
Ribosome
(petite sous unité)

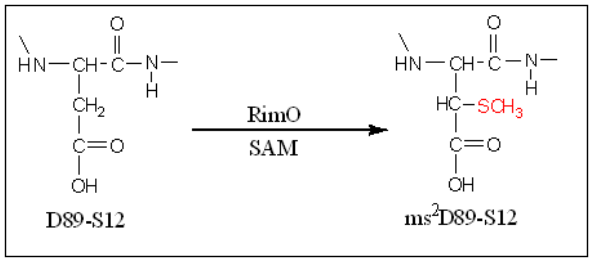




New light on methylthiolation reactions
M. Fontecave, E. Mulliez, M. Atta
Chemistry and Biology 2008,15, 209-210

RimO: 2 centres [4Fe-4S] (spectroscopies + dosage Fe,S)





Kyung-Hoon Lee et al Biochemistry 2009 48 10162

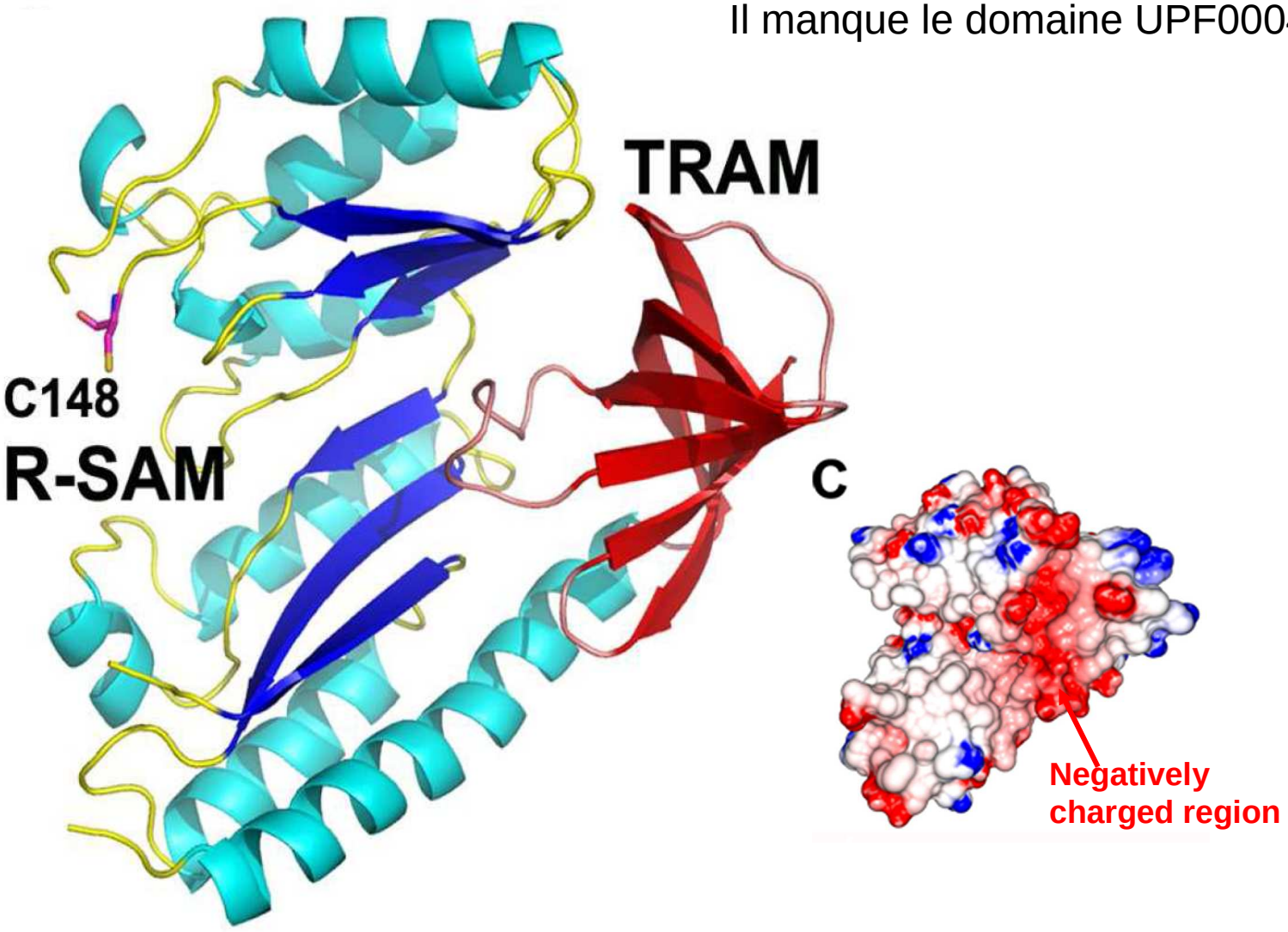
Post-translational modification of ribosomal proteins: Structural and functional characterization of RimO from *Thermotoga maritima*, a Radical-SAM methylthiotransferase.
S. Arragain, R. Garcia-Serres, G. Blondin, T. Douki, M. Clemancey, J.-M.Latour, F. Forouhar, H. Neely, G.T. Montelione, J.F. Hunt, E. Mulliez, M. Fontecave, M. Atta
J. Biol. Chem. 2010, 285, 5792-5801

LVRGGRVK**D**LPGVRYKIIRG
20 acides amlnés

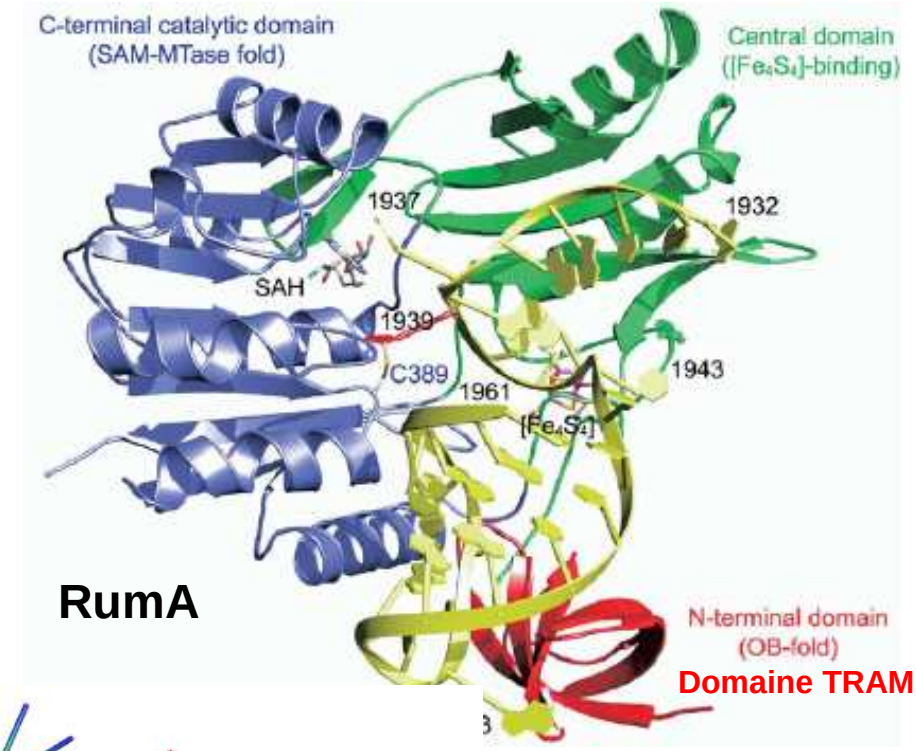
Structure de RimO (forme tronquée)
Thermotoga maritima résolution 2.0 Å (R_{free} = 25%)

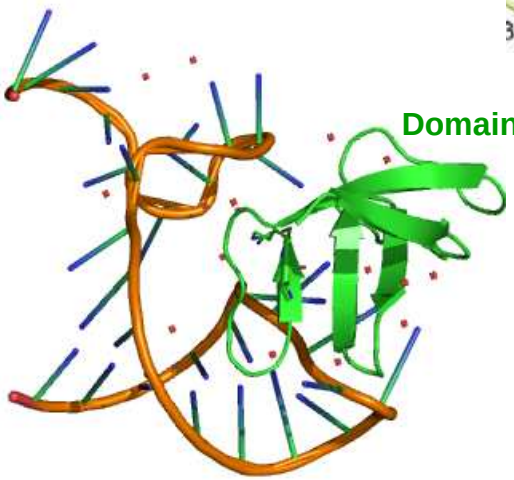
Arragain, S., *et al*. *J. B. C. 2010* 285 *5792*

Il manque le domaine UPF0004



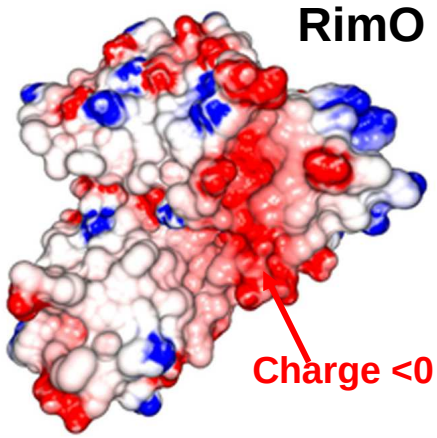
RumA: 23S ribosomal RNA methyltransferase





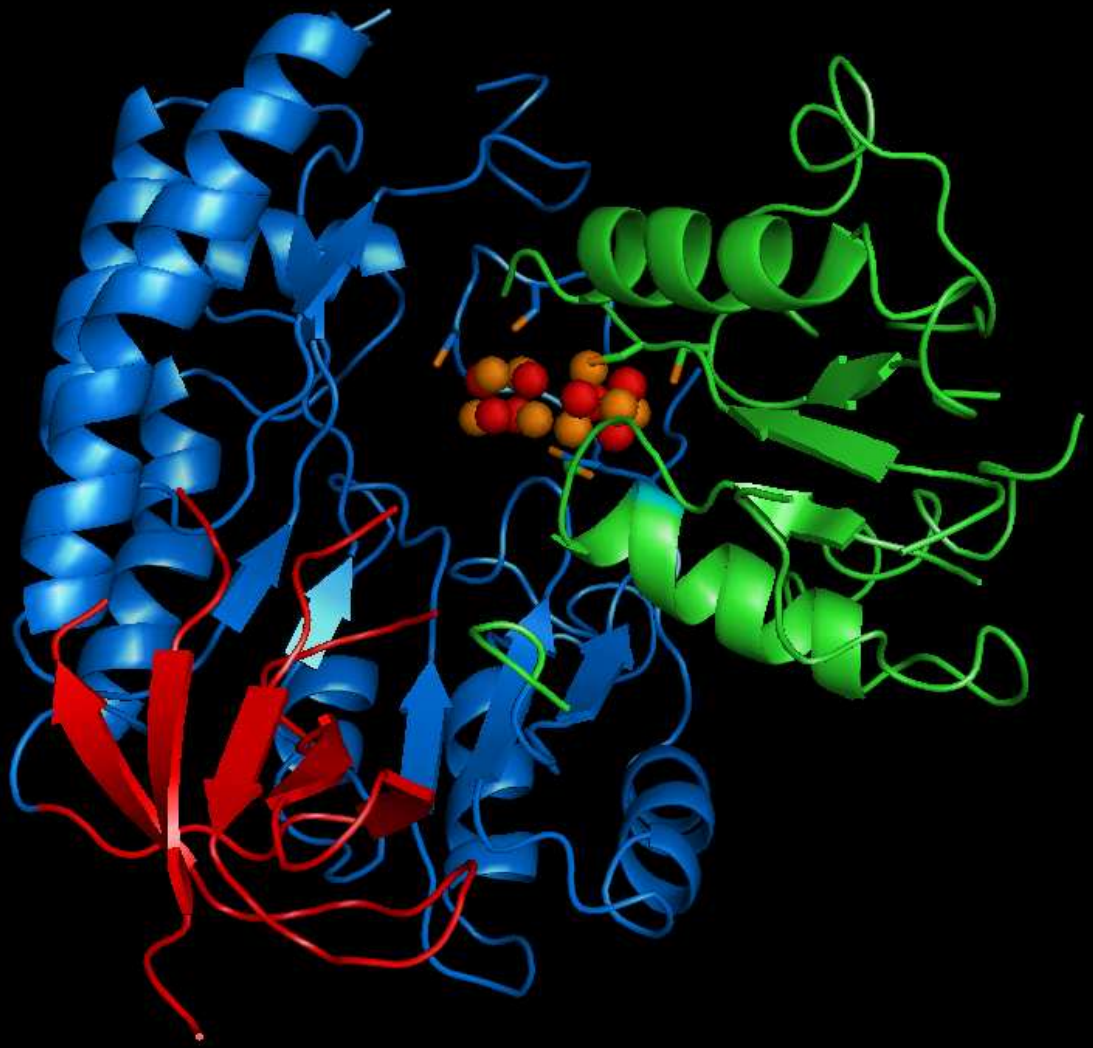
Lee T.T., *et al*, Cell, 2005,599-611

Domaine TRAM



Le domaine TRAM de RumA/MiaB (pI 11) est **basique** (chargé >0) pour fixer un tRNA **acide** (chargé <0)
Le domaine TRAM de RimO (pI 6) est **acide** pour fixer S12 qui est **basique** (pI 12)

RimO 3D structure cristallographique à 3.3 Å (non publié).



Domaine UPF0004

D ~ 8 Å

domaine
Radical SAM

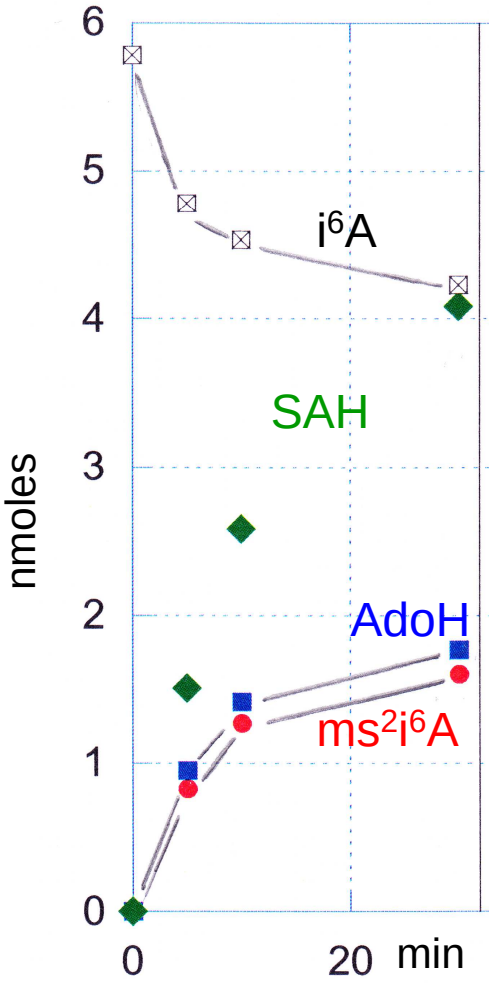
Domaine TRAM

Problème 1: le transfert de soufre ?

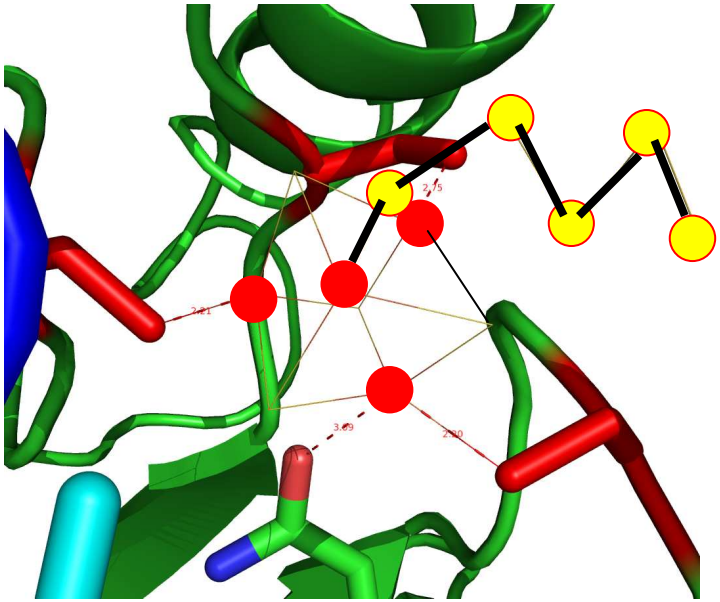
-Cystéine et cystéine désulfurase ?

-Le cluster [Fe-S] donneur de S ?

Observation : formation catalytique de ms^2i^6A !



0.5 nmol MiaB
Dithionite
$tRNA^{Phe}$(10 eq), SAM

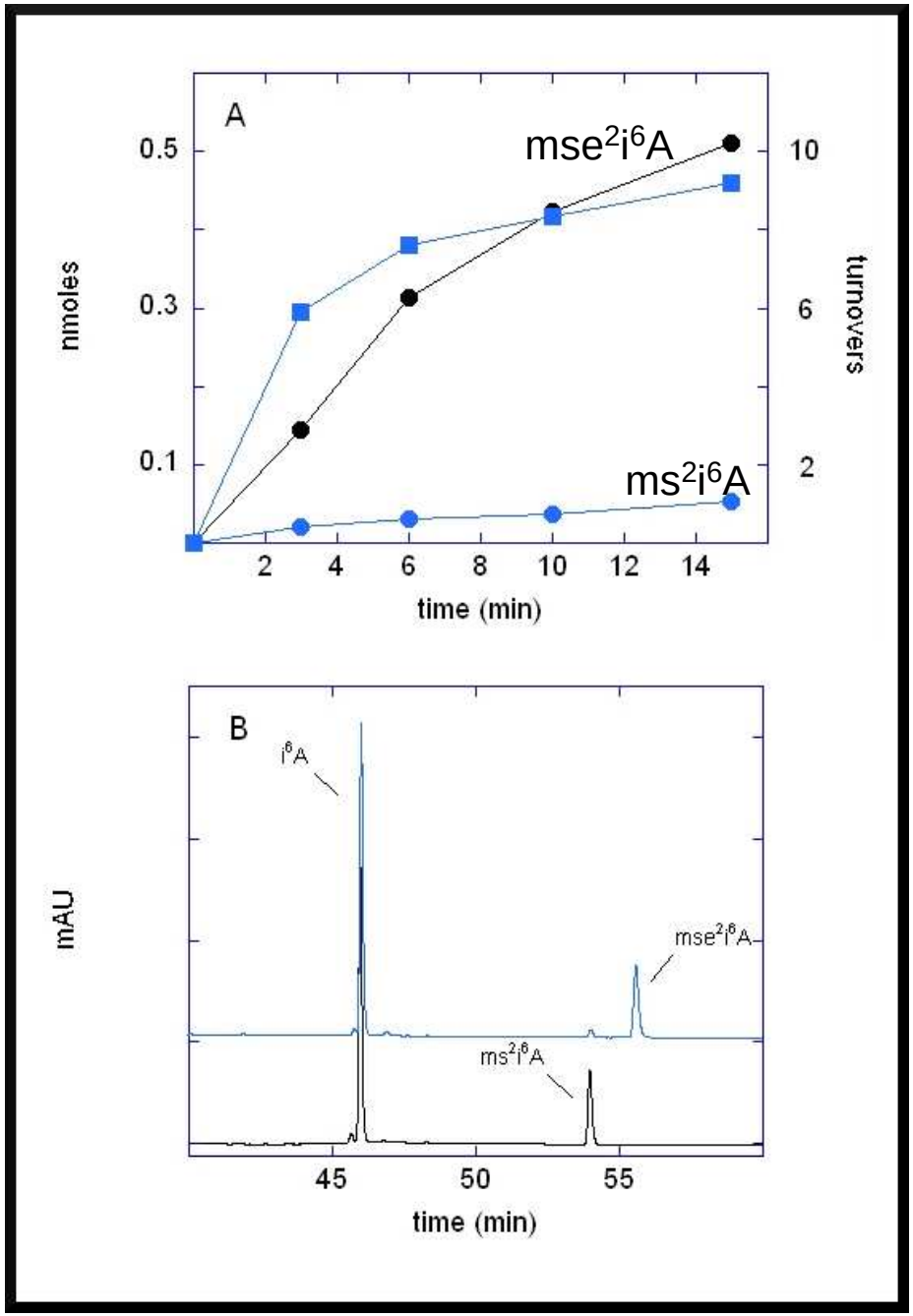


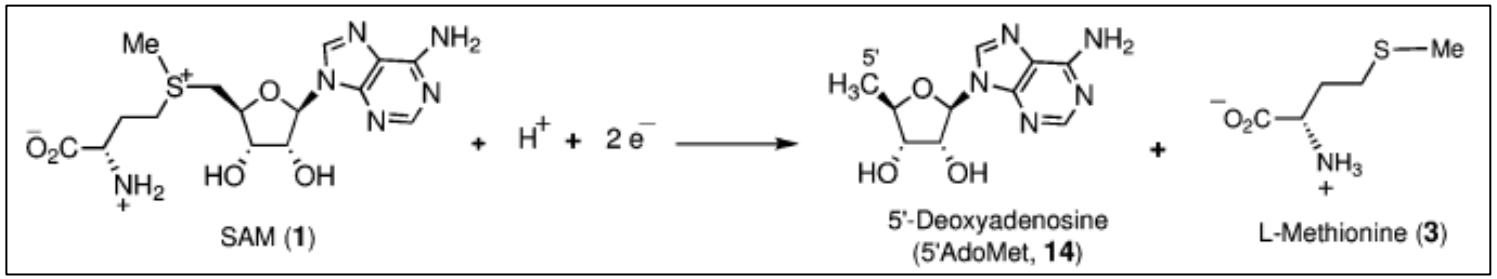
Effet du sulfure

S^{2-} (mM)	Δi^6A (nmol)	Δms^2i^6A (nmol)	TON
0	0.6	0.6	3
0.5	2.1	1.8	9

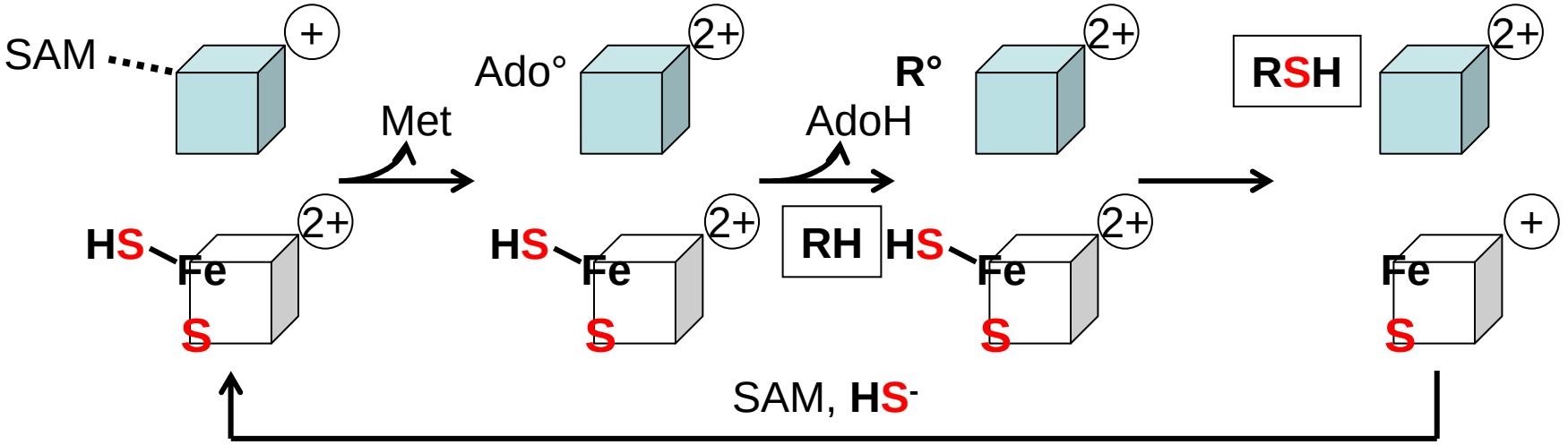
0.2 nmol MiaB
Dithionite
tRNA, SAM

Substrat
CH_3SeNa

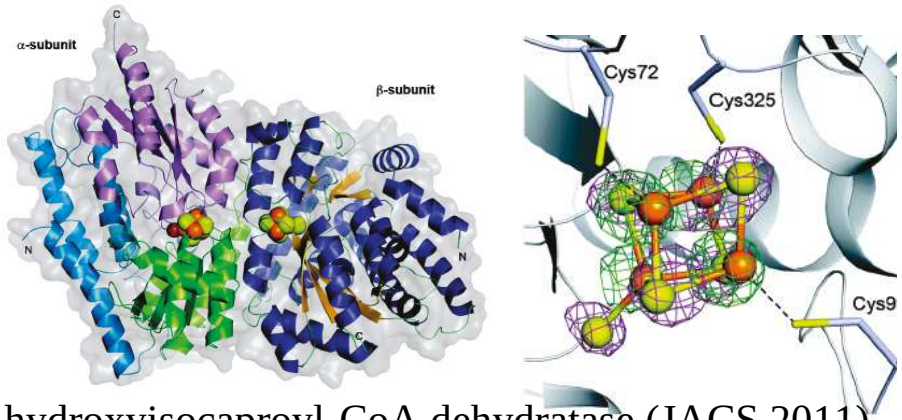




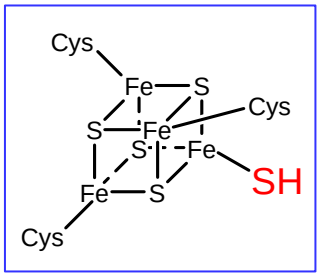
$$RH + S^{2-} \rightarrow RSH + 2\,e^-$$

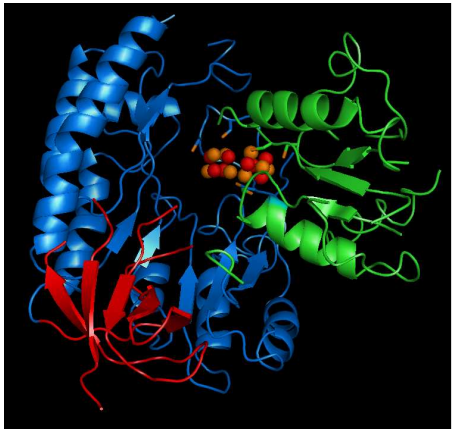


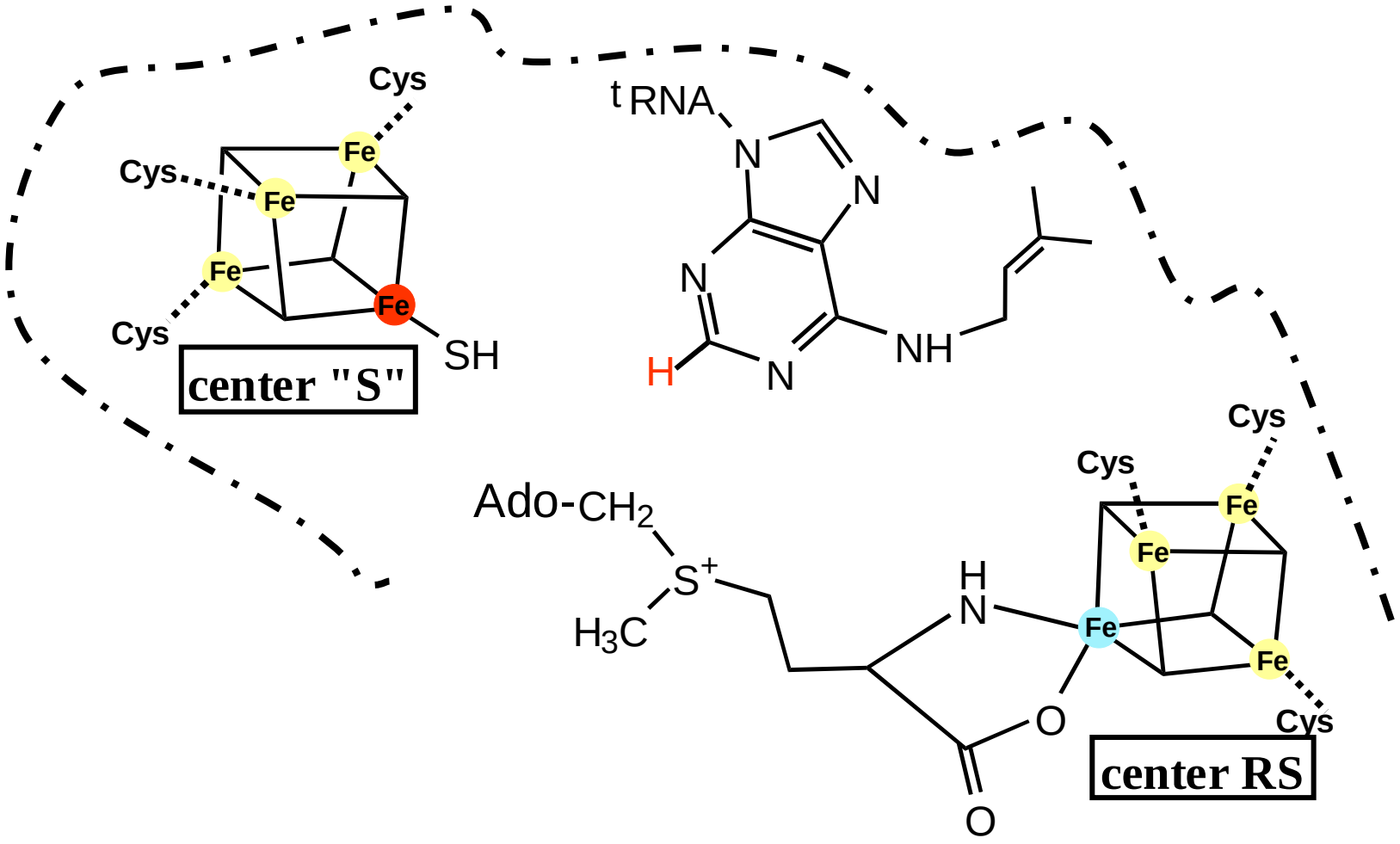
Transfert d'électron intercluster



2-hydroxyisocaproyl-CoA dehydratase (JACS 2011)

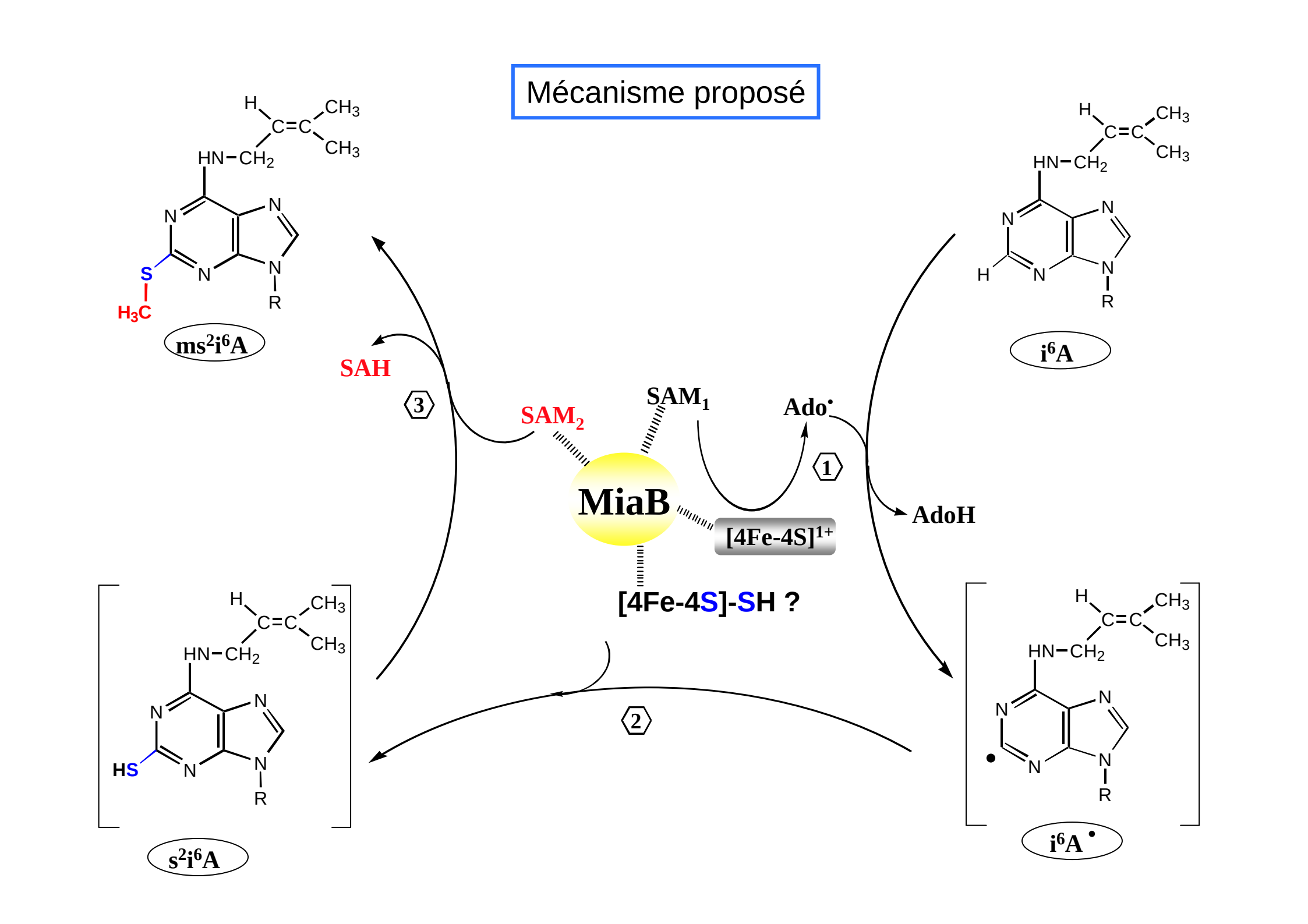


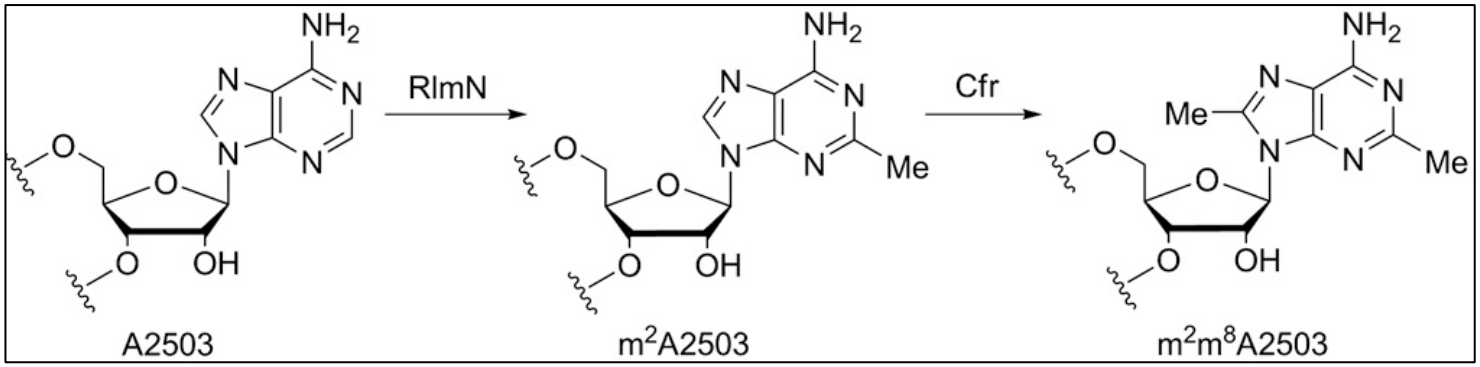



Cys
Cys
Fe
Fe
Fe
Fe
Cys
SH
center "S"
t RNA
N
N
N
H
N
NH
Ado-CH2
S+
H3C
H
N
Cys
Cys
Fe
Fe
Fe
Fe
Cys
O
O
center RS

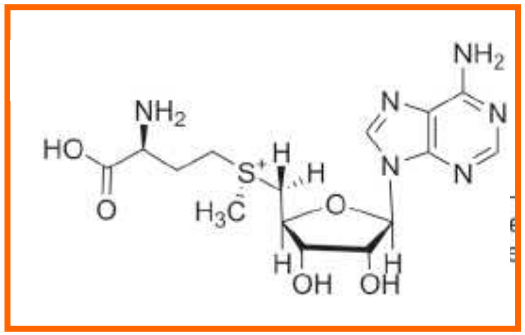
Problème 2: l’activation du substrat ?

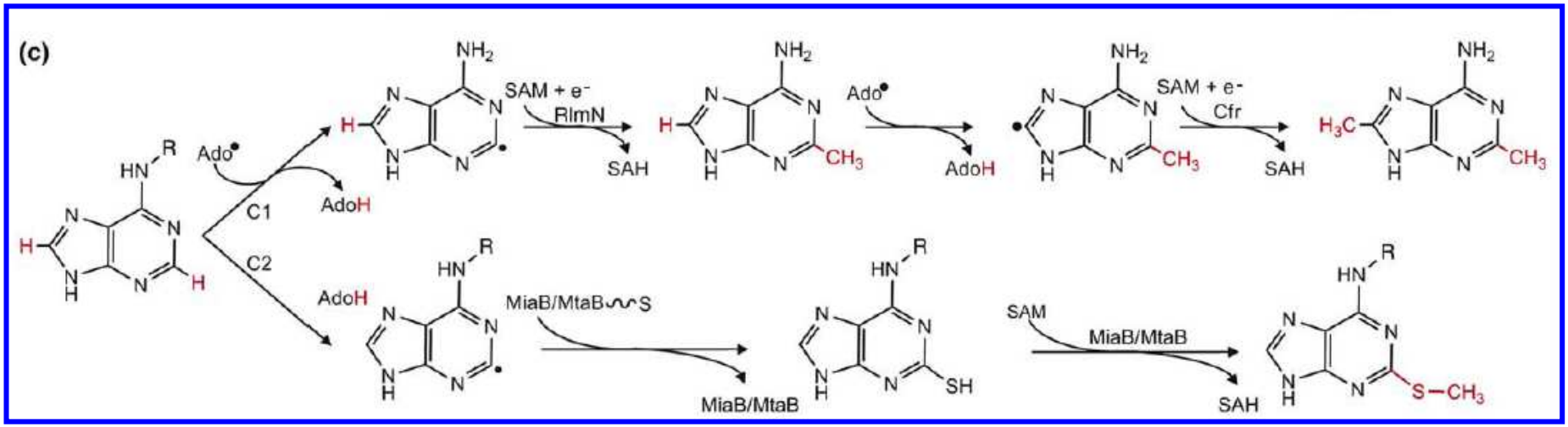
Mécanisme proposé



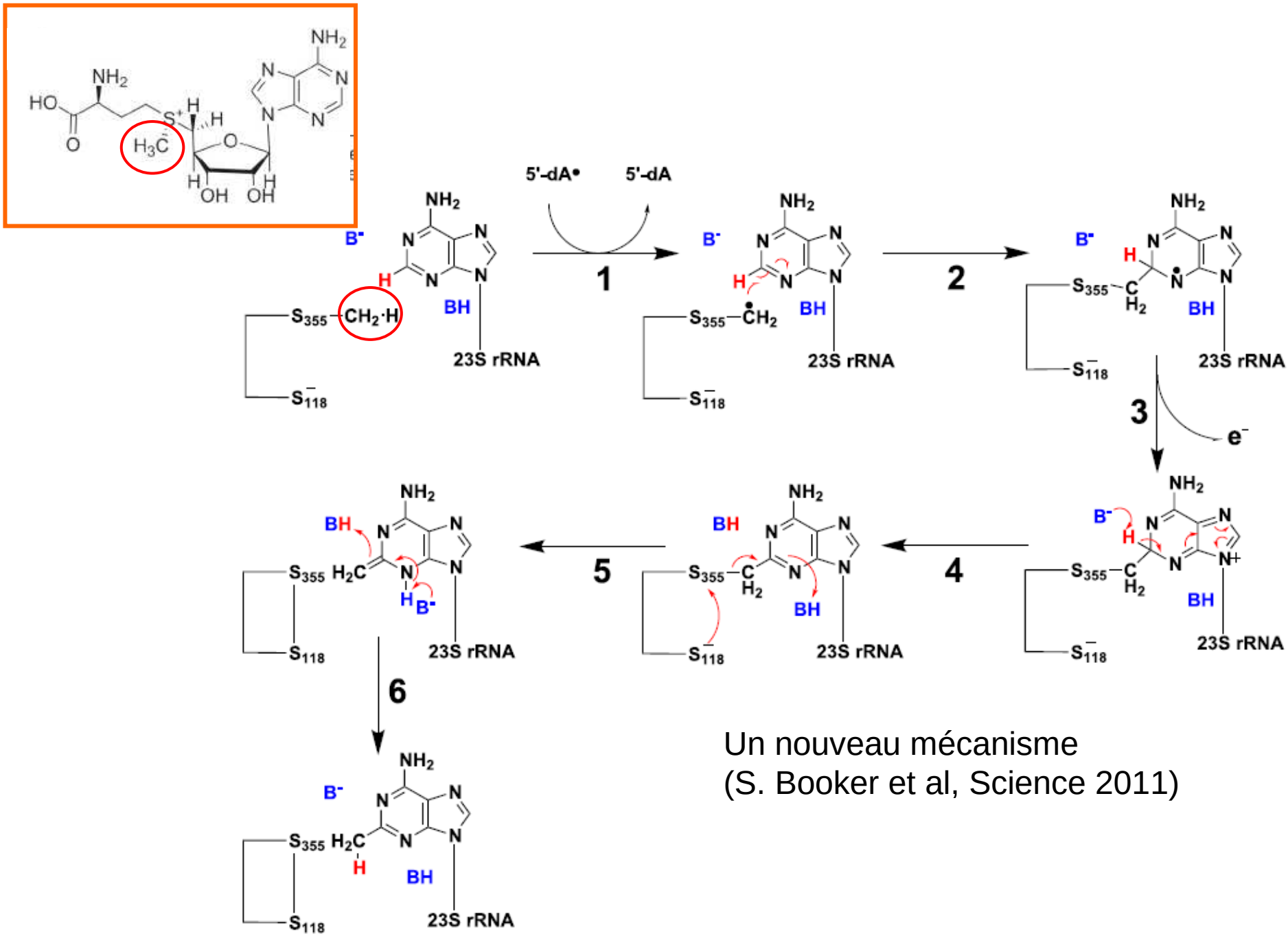


ARN ribosomal 23S
Centre peptidyl transférase
Cible d'antibiotiques



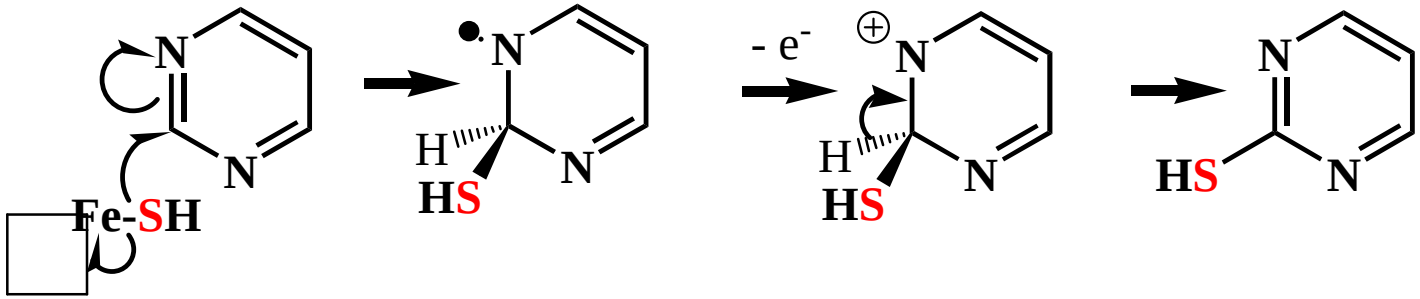


Atta, Mulliez, Fontecave et al Curr. Op. Str. Biol. 2010



Un nouveau mécanisme
(S. Booker et al, Science 2011)

Nouvelle hypothèse de travail



Une chimie radicalaire et des centres fer-soufre pour la biosynthèse de produits naturels soufrés

Marc Fontecave

Laboratoire de Chimie et Biologie des Métaux, Université Joseph Fourier, CNRS, CEA/DSV/iRTSV
CEA-Grenoble 17 rue des martyrs 38054 Grenoble cedex 9, France
mfontecave@cea.fr; Phone: (0033)438789103 ; Fax: (0033)438789124

Collège de France, 11 Place Marcelin Berthelot, 75231 Paris Cedex 05