



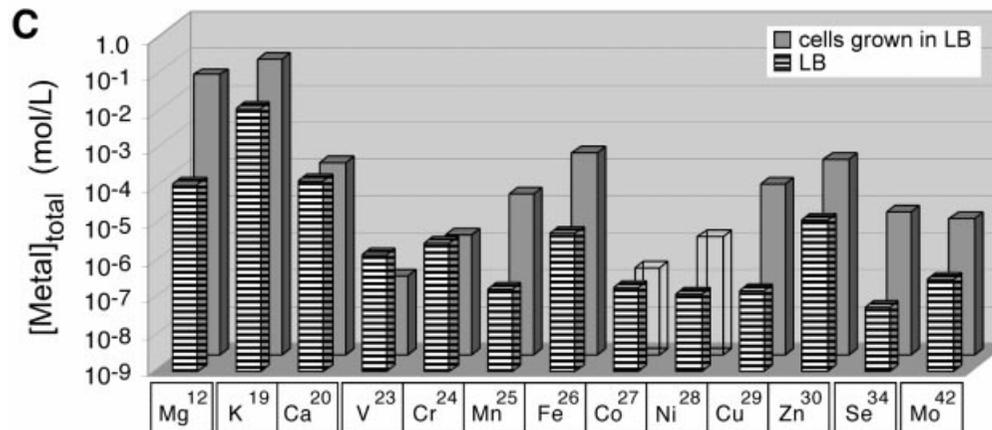
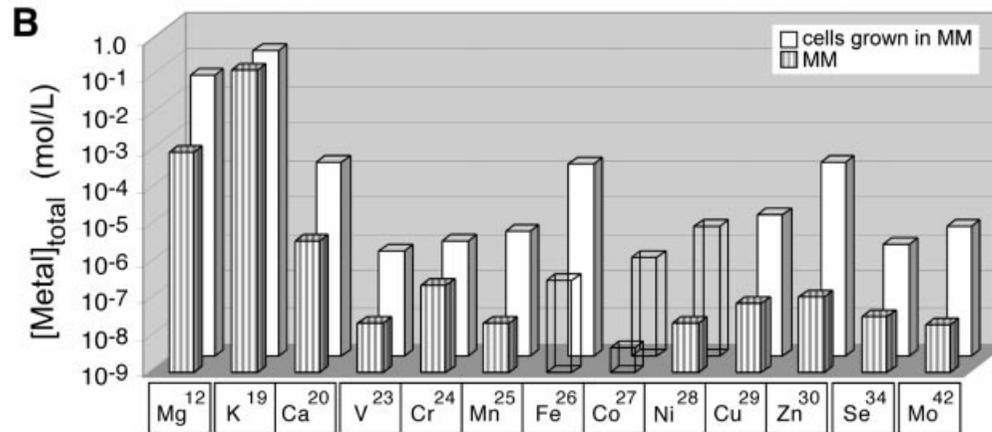
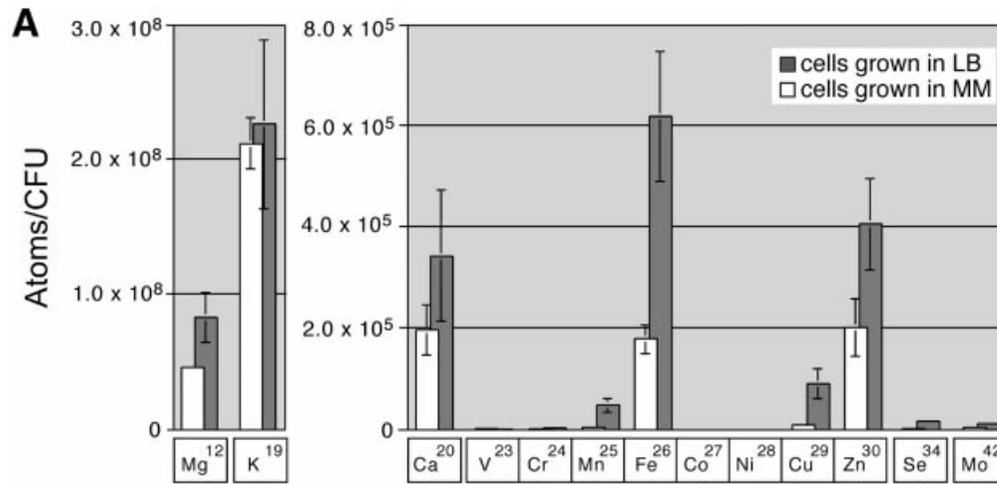
COLLÈGE
DE FRANCE
—1530—

Les ions métalliques: pèlerinages cellulaires

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Concentration cellulaires
Ions métalliques
(*E. coli*)

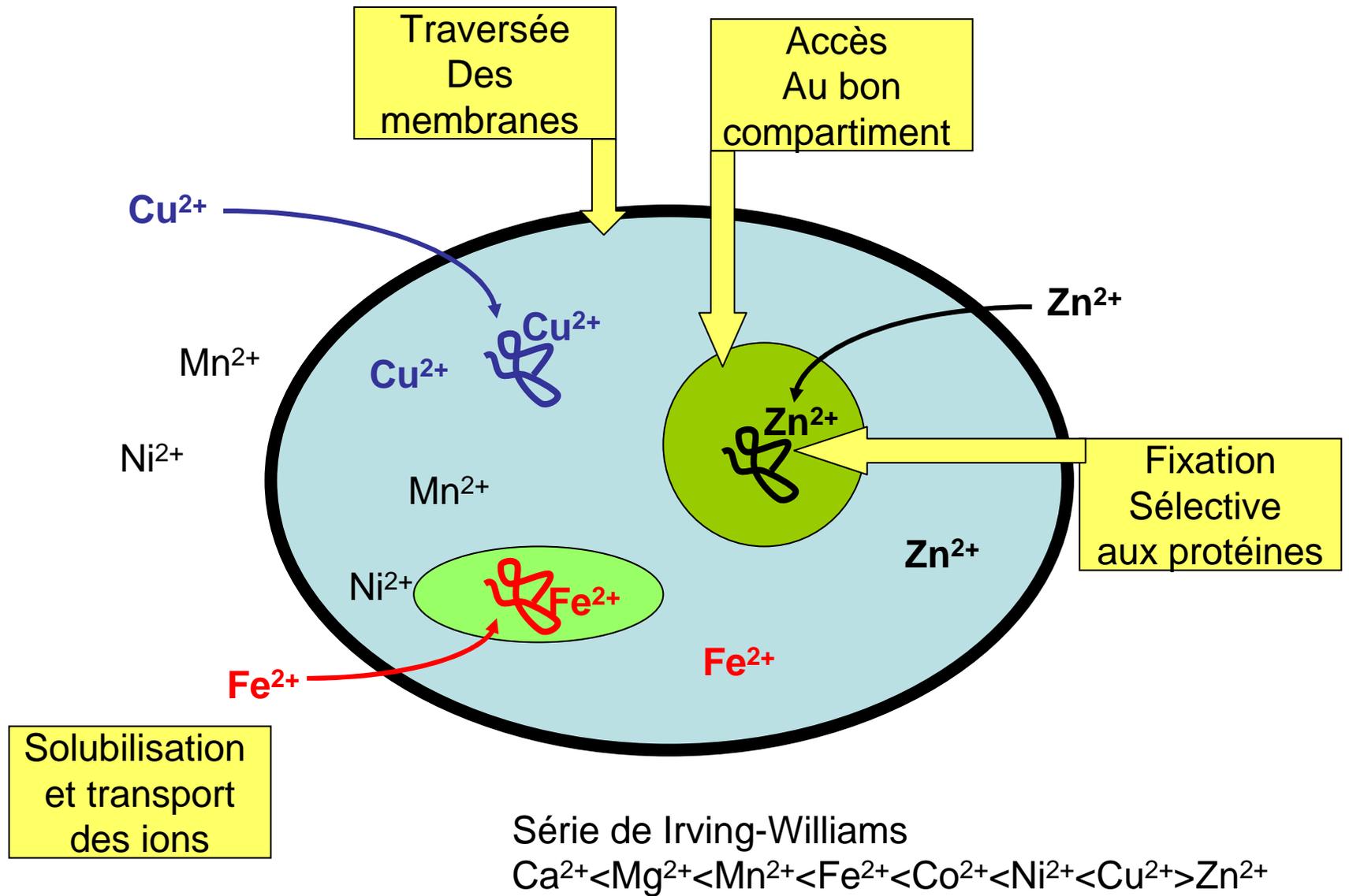
K, Mg: 10⁸ atomes/cell; > 10 mM

Zn, Fe, Ca: 10⁵ atomes/cell; 0,1 mM

Cu, Mn, Mo: 10⁴ atomes/cell; 10-100 μM

V, Co, Ni: 1-10 μM

Maturation des métalloprotéines: La question de l'adressage des ions métalliques



Traffic intracellulaire des ions métalliques: Des maladies génétiques au concept de **métallochaperones**

Maladies de **Menkes** et **Wilson**: maladies génétiques associées à des perturbations de l'homéostasie du **cuivre**

Homéostasie: tendance des organismes vivants à maintenir constants leurs paramètres biologiques face aux modifications du milieu extérieur.

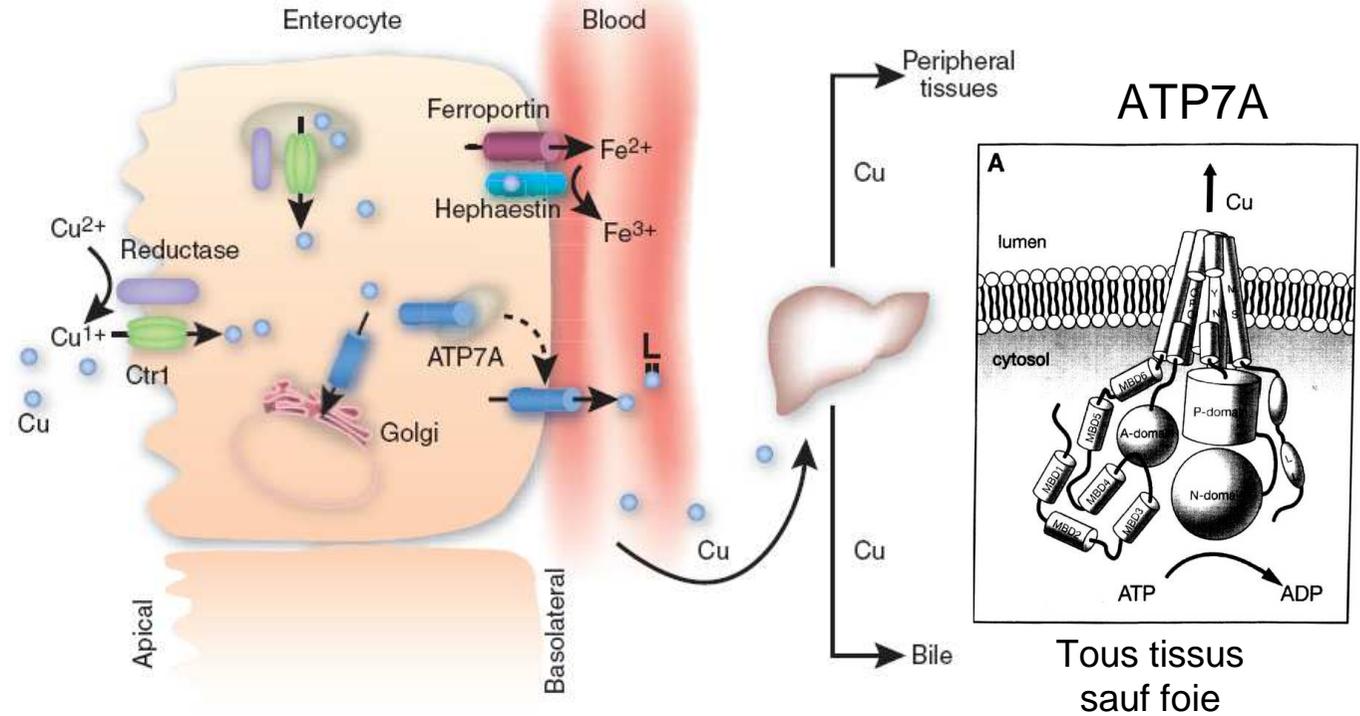
Gènes mutés: ATP7A et ATP7B

Protéines correspondantes: ATPases membranaires de transport du **cuivre**

Facilitent l'entrée du **Cu** dans l'appareil de Golgi (où sont synthétisées et maturées les protéines à Cu) et l'excrétion du **Cu** au niveau de la membrane plasmique



Maladie de Menkes



- ➡ Accumulation du Cu dans l'intestin
- ➡ Déficience en Cu des enzymes à Cu
- ➡ Neurodégénération, convulsions
Cheveux anormaux
Hypothermie
Désordres du tissu conjonctif
Mort (vers 3 ans)
- ➡ Traitement: Cu-histidine

DJ Thiele Nature Chem Biol (2008) 4 176
H Kodama Metallomics (2009) 1 42

Enzymes à Cu

Table 3 Decreased enzyme activities and symptoms in Menkes disease

Cuproenzymes	Symptoms
Cytochrome C oxidase (in mitochondria)	Hypothermia Brain damage
Tyrosinase (in cytsol)	Hypopigmentation
Sulfhydryl oxidase	Hair abnormalities
Dopamine-β-hydroxidase (secretary enzyme)	Hypotension
Lysyl oxidase (secretary enzyme)	Arterial abnormalities Bladder diverticulum Loose skin and joints Osteoporosis (Wormian bones, fractures)

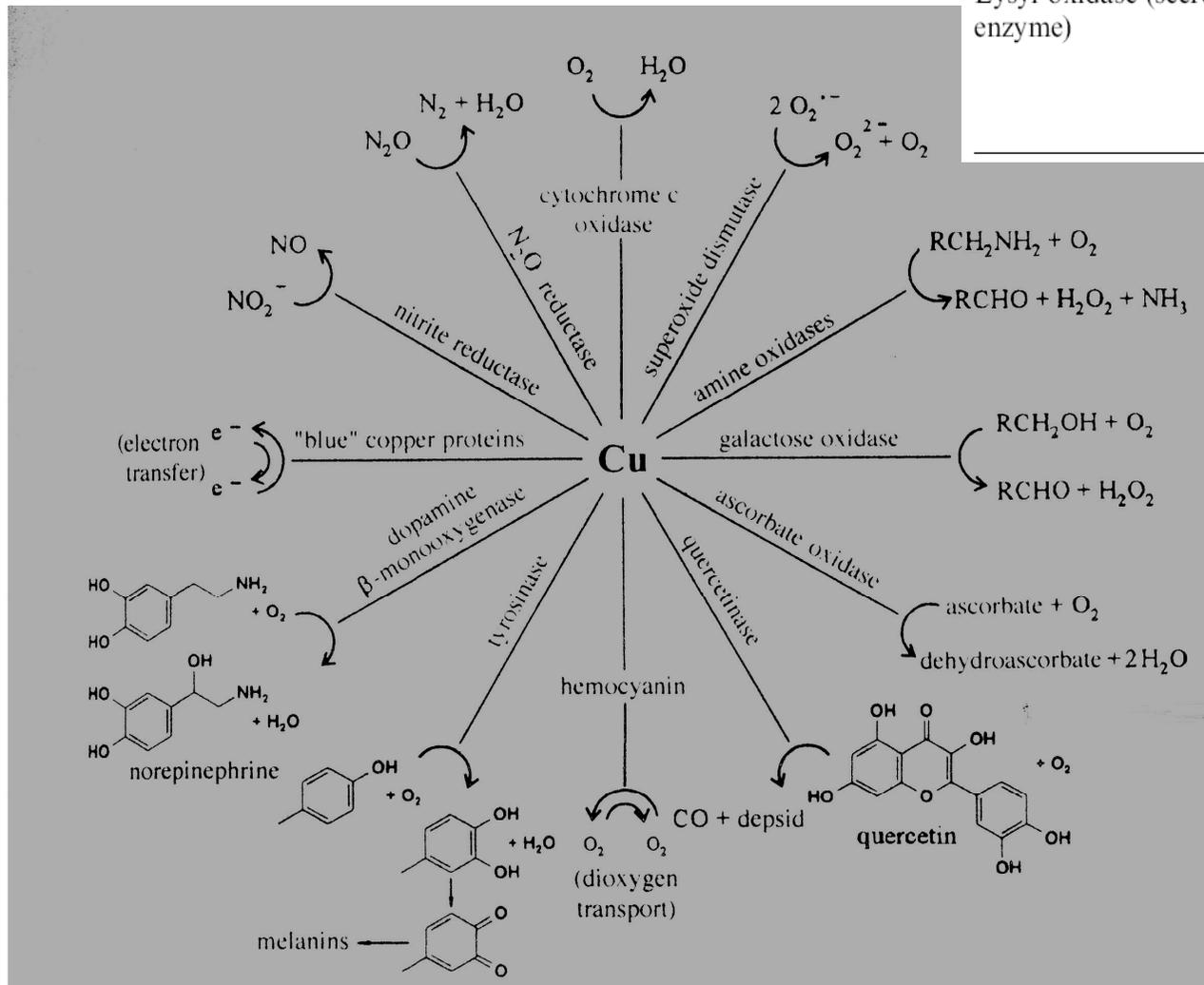
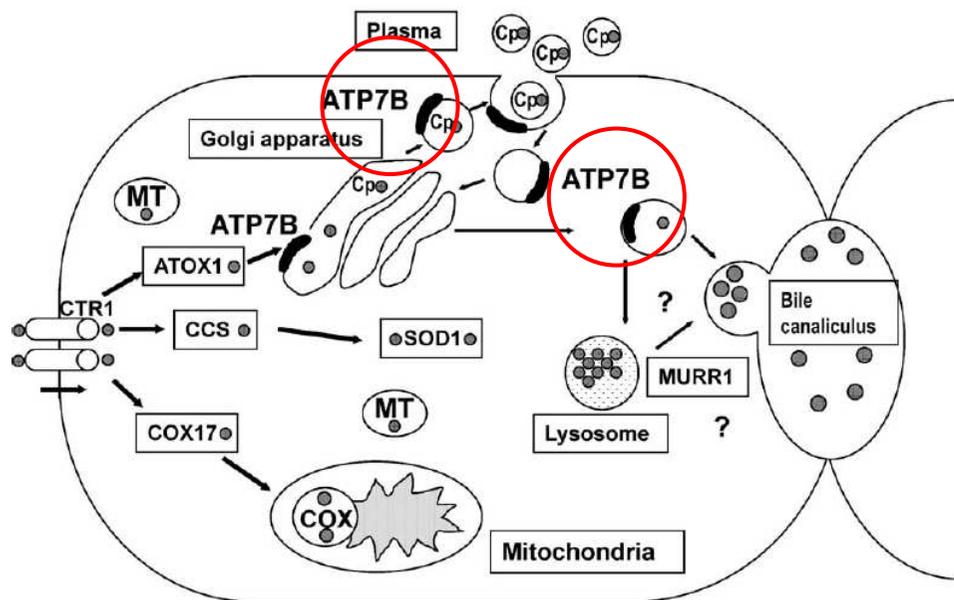


Table 1 Examples of Cu binding and Cu homeostasis proteins

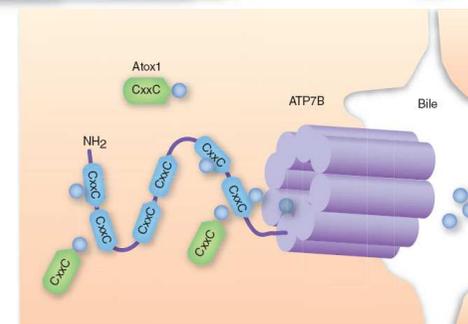
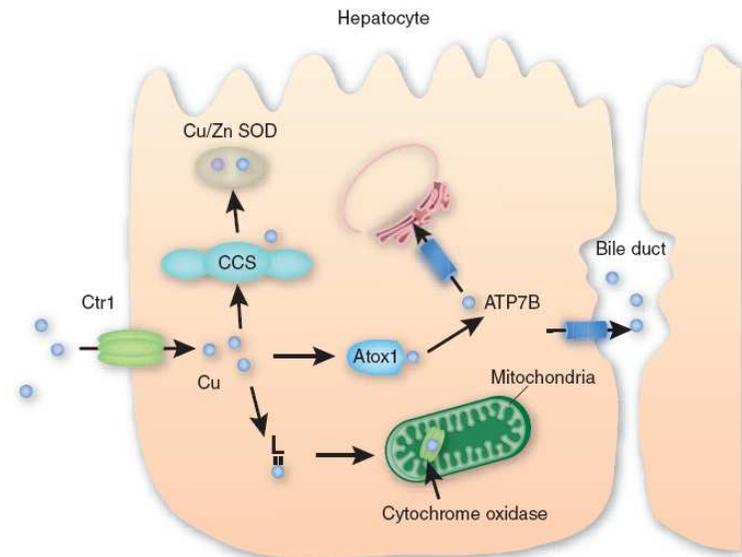
Protein	Function
Amyloid precursor protein (APP)	Protein involved in neuronal development and potentially Cu metabolism; cleavage leads to generation of A β peptide that aggregates in senile plaque associated with Alzheimer's disease
Atox1	Metallochaperone that delivers Cu to ATP7A and ATP7B Cu ¹⁺ transporters
ATP7A	Cu ¹⁺ -transporting P-type ATPase expressed in all tissues except liver
ATP7B	Cu ¹⁺ -transporting P-type ATPase expressed primarily in the liver
Carbon monoxide dehydrogenase to acetyl-CoA synthase	<i>Moorella thermoacetica</i> bifunctional enzyme; reduces CO ₂ to CO with subsequent assembly of acetyl-CoA
Ceruloplasmin	Serum ferroxidase that functions in Fe ³⁺ loading onto transferrin
Coagulation factors V and VIII	Homologous pro-coagulants present on the surface of platelets, where they nucleate the assembly of multiprotein proteolytic complexes involved in blood coagulation
CCS	Metallochaperone that delivers Cu to Cu/Zn SOD
CopZ	<i>Archaeoglobus fulgidus</i> [2Fe-2S] and Zn ²⁺ -containing Cu chaperone
Cox17	Metallochaperone that transfers Cu to Sco1 and Cox11 for cytochrome oxidase Cu loading in mitochondria
Ctr1	High-affinity Cu ¹⁺ transporter involved in cellular Cu uptake
Cu/Zn SOD (SOD1)	Antioxidant enzyme, catalyzes the disproportionation of superoxide to hydrogen peroxide and dioxygen
Cytochrome c oxidase	Terminal enzyme in the mitochondrial respiratory chain, catalyzes the reduction of dioxygen to water
Dopamine β -hydroxylase (DBH)	Oxygenase, converts dopamine to norepinephrine
Ethylene receptor (ETR1)	Member of a plant receptor family that uses a Cu cofactor for ethylene binding and signaling
Hemocyanin	Oxygen transport protein found in the hemolymph of many invertebrates such as arthropods and molluscs
Hephaestin	Transmembrane multi-Cu ferroxidase; involved in iron efflux from enterocytes and macrophages
Glucose oxidase	Pentose phosphate pathway oxidoreductase that catalyzes the oxidation of D-glucose into D-glucono-1,5-lactone and hydrogen peroxide
Laccase	Phenol oxidase involved in melanin production
Lysyl oxidase	Catalyzes formation of aldehydes from lysine in collagen and elastin precursors for connective tissue maturation
Metallothionein	Cysteine-rich small-molecular-weight metal-binding and detoxification protein
Peptidylglycine- α -amidating mono-oxygenase (PAM)	Catalyzes conversion of peptidylglycine substrates into α -amidated products; neuropeptide maturation
Prion protein (PrP)	Protein whose function is unclear but binds Cu via the N-terminal octapeptide repeats
Steap proteins/Fre1/Fre2	Family of metalloreductases involved in Fe ³⁺ and Cu ²⁺ reduction
Tyrosinase	Monophenol mono-oxygenase; melanin synthesis
XIAP	Inhibitor of apoptosis through binding and catalytic inhibition of several caspases

Maladie de Wilson



CTR1 : copper transporter 1
 SOD : superoxide dismutase
 COX : cytochrome C oxidase
 ATP7A/B : copper-transporting P-type ATPase
 Cp : ceruloplasmin

(b)

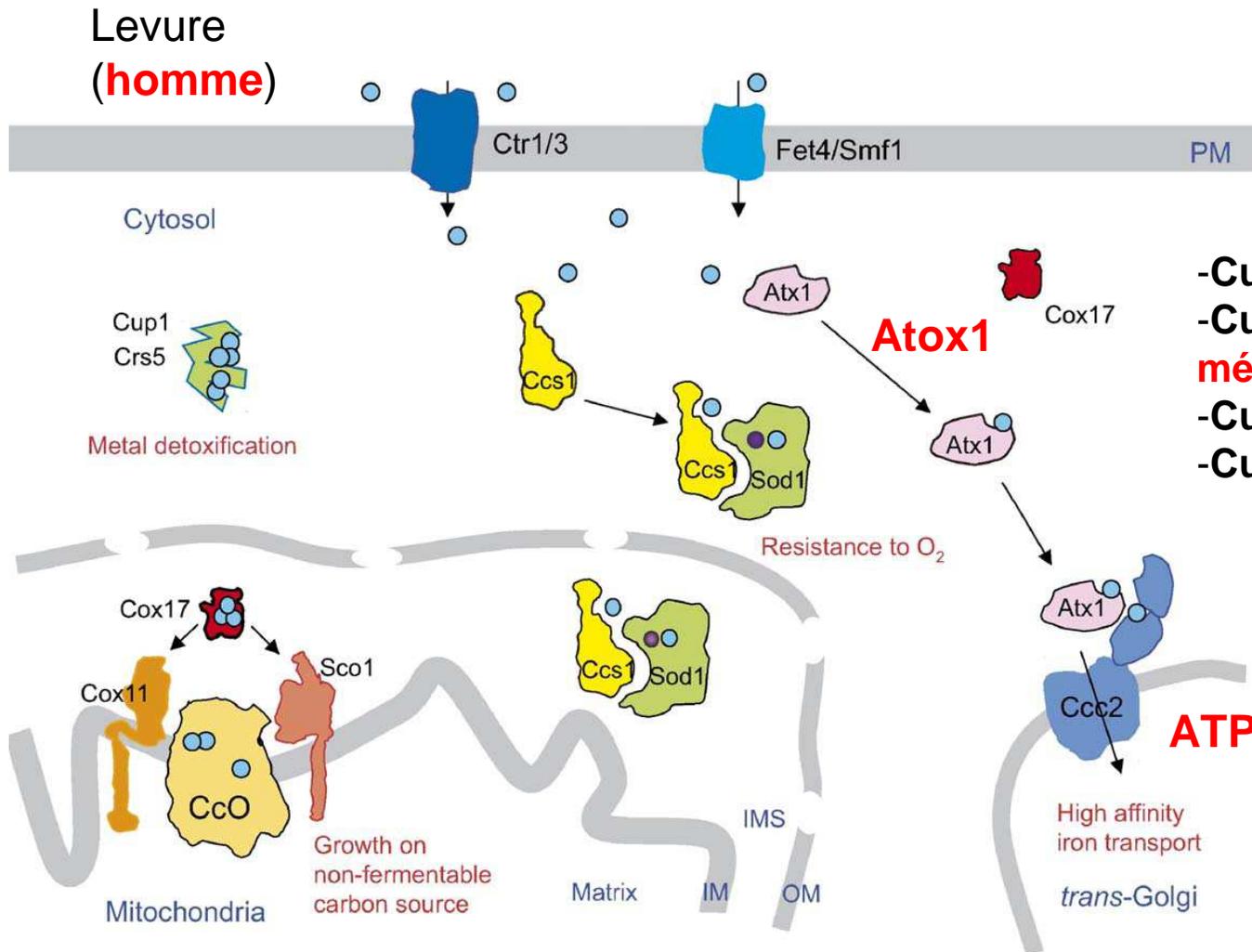
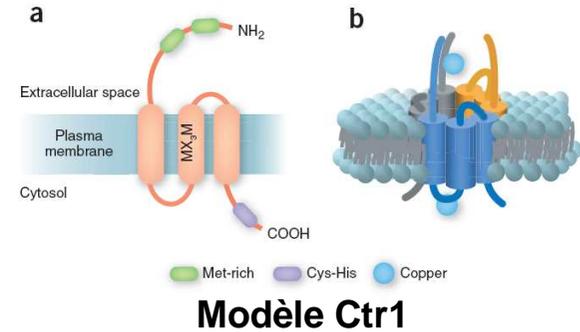


- ➡ Déficience de l'excrétion de Cu dans la bile
- ➡ Déficience dans l'incorporation de Cu dans la céruloplasmine
- ➡ Accumulation de Cu dans le foie et le cerveau
- ➡ Toxicité hépatique (cirrhose)
Dégénération neuronale
- ➡ Traitement par chélation du fer:
Pénicillamine, tétrathiomolybdate $[MoS_4]^{2-}$

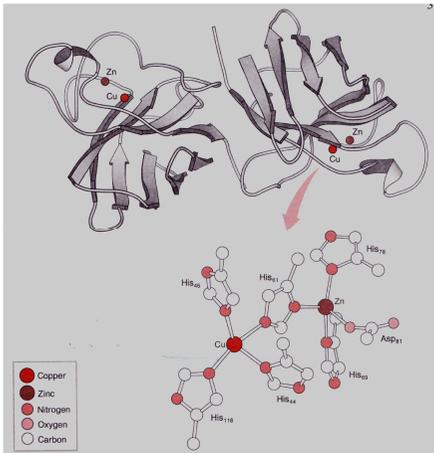
DJ Thiele Nature Chem Biol
 (2008) 4 176
 H Kodama Metallomics
 (2009) 1 42

Traffic intracellulaire des ions métalliques: Des maladies génétiques au concept de **métallochaperones**

Métallochaperones ont pour fonction de protéger et guider les ions métalliques à travers le cytoplasme jusqu'à leurs cibles protéiques auxquelles elles délivrent leurs ions



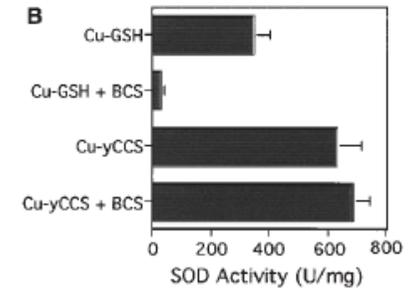
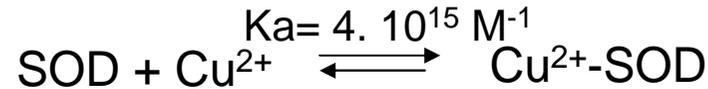
- Cu(I) pénètre (perméases)
- Cu(I) pris en charge par une **métallochaperone** (Atx1)
- Cu(I) passe de Atx1 à CCC2
- Cu(I) passe de CCC2 à Fet3



Pourquoi des **métallochaperones** ?

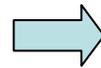
Cu_{tot} : $4 \cdot 10^5$ atomes/cellule; $100 \mu M$ ($v = 10^{-14}$ l)

SOD: 60000 molécules/cellule (50000 actives)

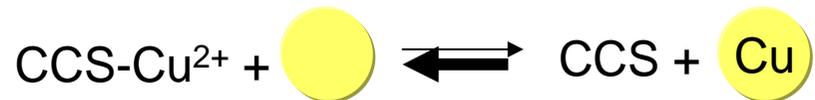
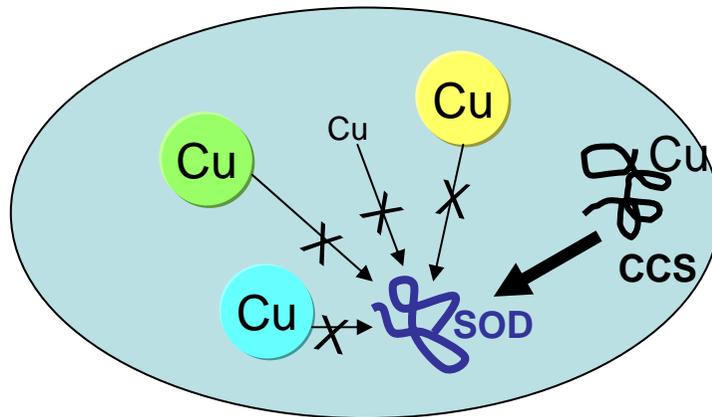


Strain	Copper (atoms per cell)	Total SOD1 (monomers per cell)	yCCS (monomers per cell)	Active SOD1 (monomers per cell)
SY1699 (WT)	$3.9 (\pm 0.2) \times 10^5$	$6.0 (\pm 2.0) \times 10^4$	$1.0 (\pm 0.2) \times 10^4$	$5.0 (\pm 3.0) \times 10^4$
VC107 (<i>sod1</i> Δ)	$3.0 (\pm 0.3) \times 10^5$	Null	$1.0 (\pm 0.3) \times 10^4$	Null
SY2950 (<i>lys7</i> Δ)	$2.3 (\pm 0.3) \times 10^5$	$5.0 (\pm 2.0) \times 10^4$	Null	$< 2 \times 10^2$
VC279 (<i>sod1</i> Δ <i>lys7</i> Δ)	ND	Null	Null	Null

$[Cu^{2+}] = 10^{-18} M$
(< 1 atome par cellule)



Cu cellulaire
Complexé (métallothionéine ?)



Chimie de coordination du CUIVRE

$\text{Cu}^{2+} (\text{d}^9)$

Acide dur-mou

Coordinations 4, 5, 6

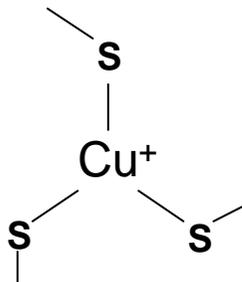
4: plan carré

5: pyramide base carrée

S: Cys, Met

N: His

O: Asp/Glu



$\text{Cu}^+ (\text{d}^{10})$

Acide mou

Coordinations 2, 3, 4

4: tétraèdre

S: Cys, Met

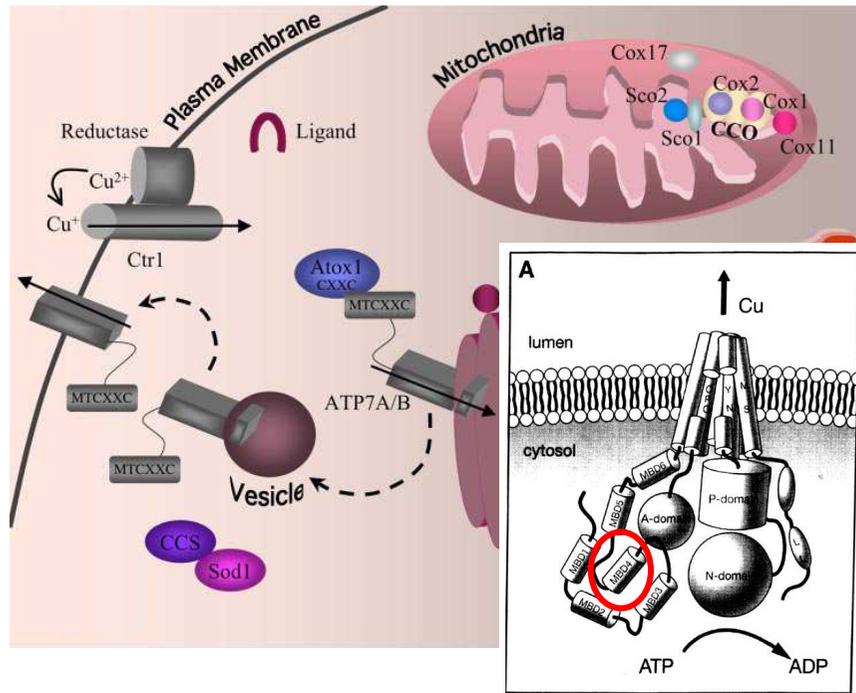
N: His

Cys

- Cysteinate/charge - (polaire)
- Liaisons fortes et courtes
- Sensible à l'oxydation

Met

- Pas de charge (hydrophobe)
- Liaisons faibles et longues
- résiste à l'oxydation



Exemple 1: Atox1 et ATP7A(B)

- La chaperone et la cible ont les mêmes repliements !! $\beta\alpha\beta\beta\alpha\beta$
- La chaperone et la cible ont les mêmes sites de complexation du Cu !!

MXCXXC

TV O'Halloran *Ann Rev Biochem* 2001 70 677
 I Bertini *J. Biol. Chem.* (2001) 276 8415
 A.C. Rosenzweig *NBC* (2000) 7 766
 Acc. Chem. Res. (2001) 34 119

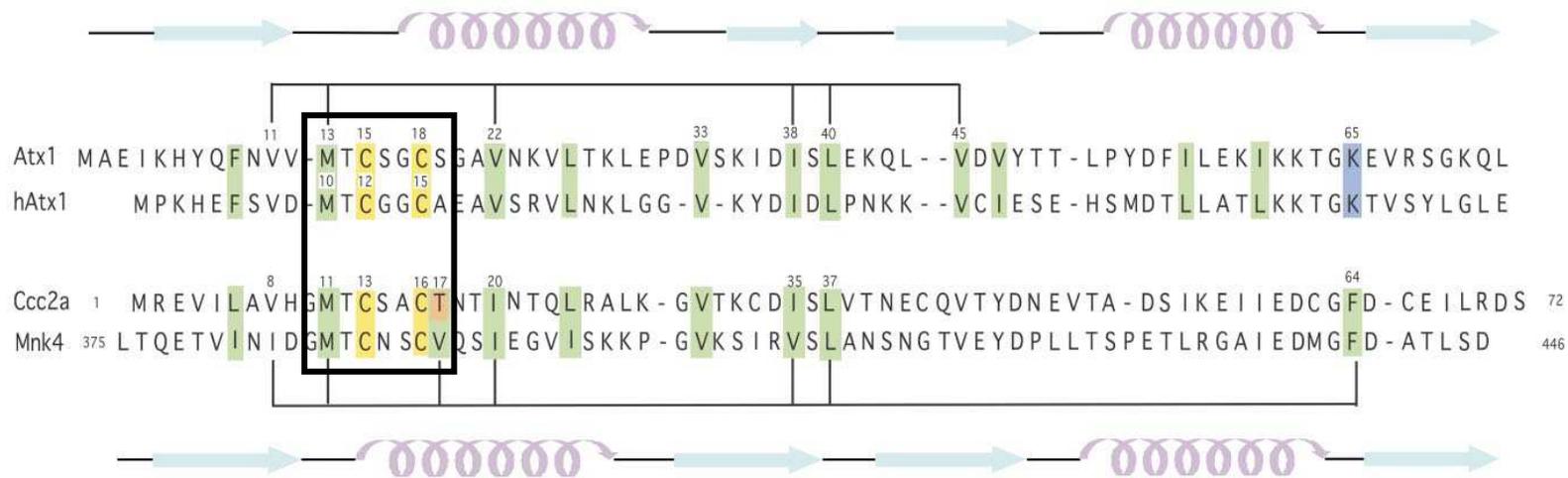


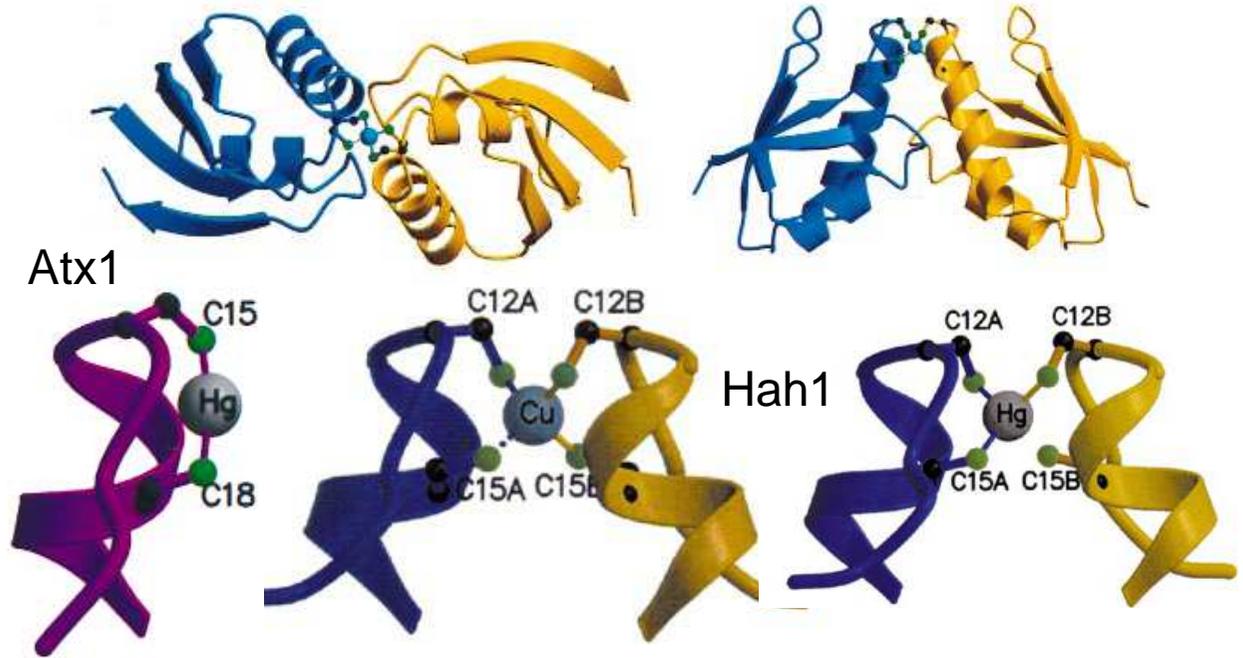
Figure 6 Structure-based alignments of Atox1, human Atox1 (Hah1), Menkes domain 4, and Ccc2a. Structural elements are represented with loop (black), helix (magenta), and β -sheet (cyan) for metallochaperones (*top*) and copper-transporting domains (*bottom*). Residues are numbered. Conserved hydrophobic residues (green), conserved lysine of the metallochaperones (purple), metal-binding cysteines (yellow), and threonine 17 (orange with green outline) of Ccc2a denote hydrophobic contact of $C\gamma$ with Met. Brackets connect conserved hydrophobic residues adjacent to Met of metal-binding motif.

Différentes Structures de
 - Atx1 (Atox1/Hah1)
 - Menkes 4

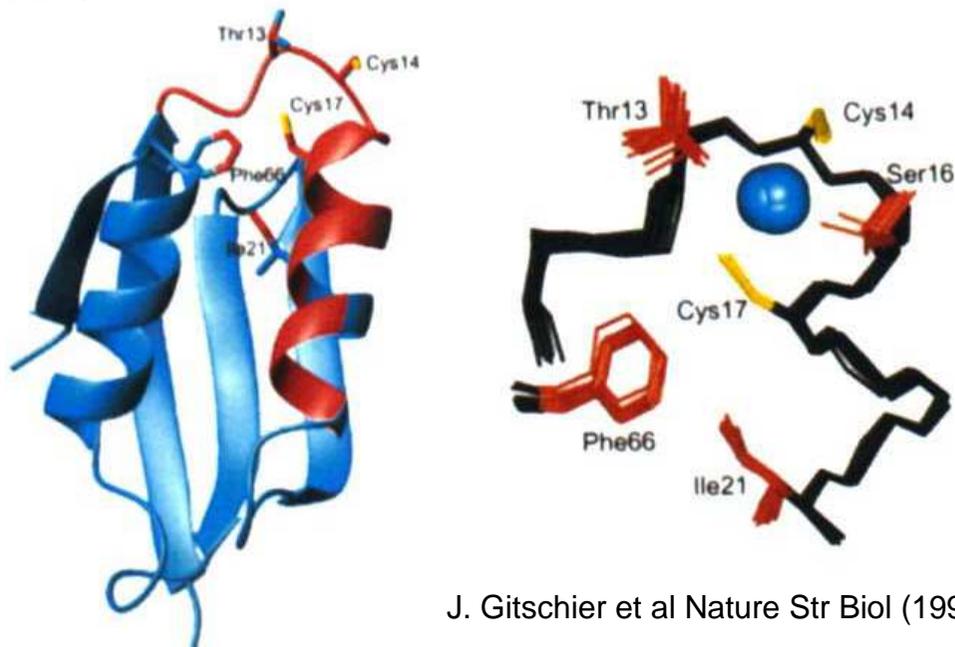
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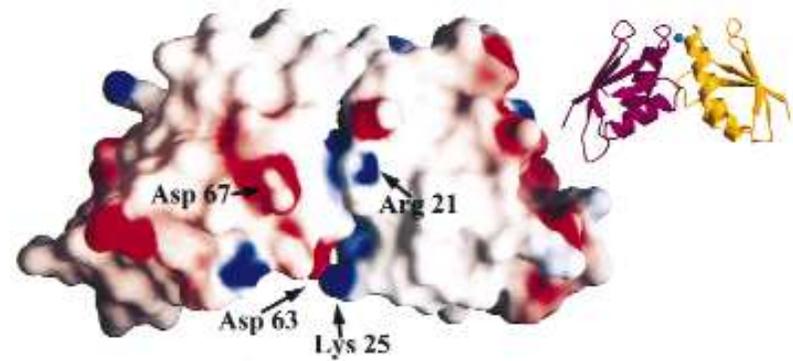
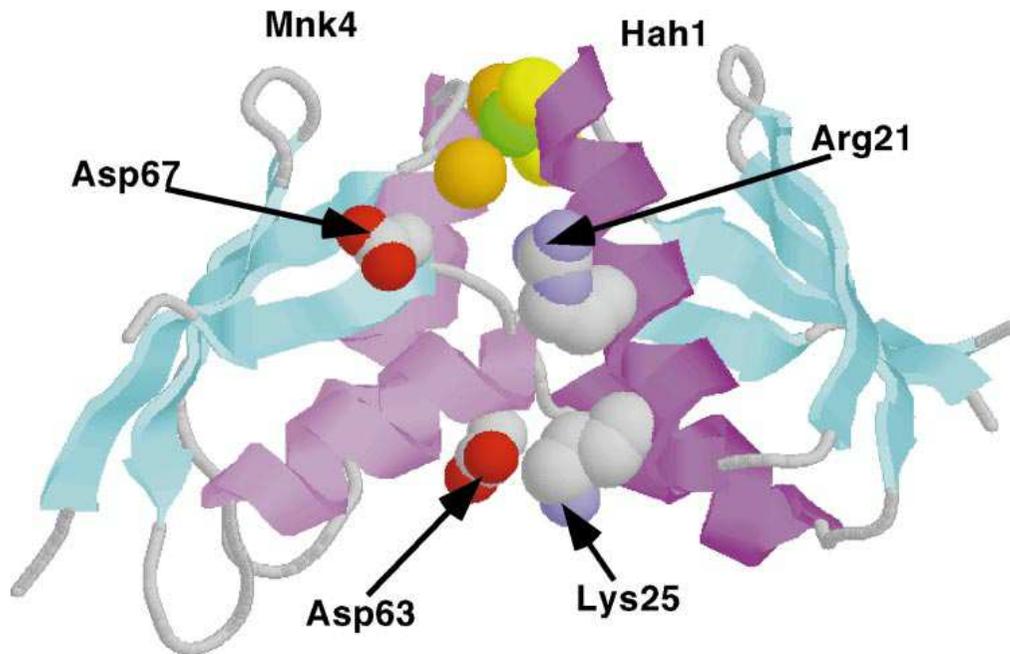
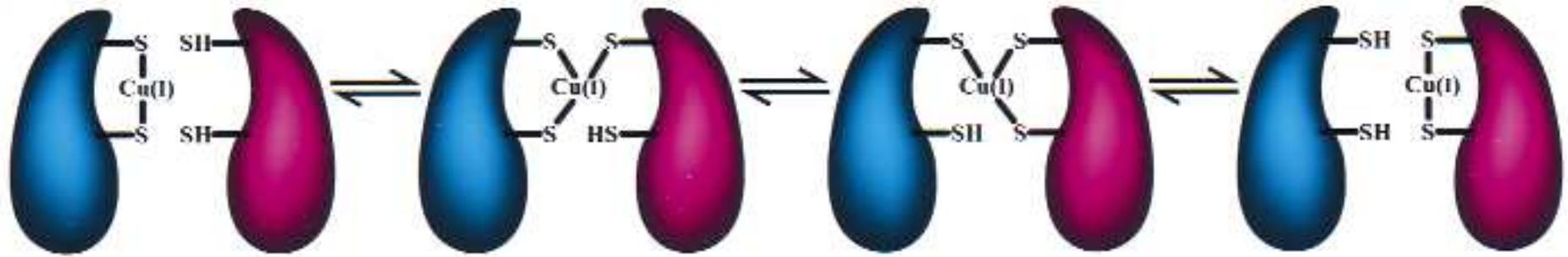


Menkes 4

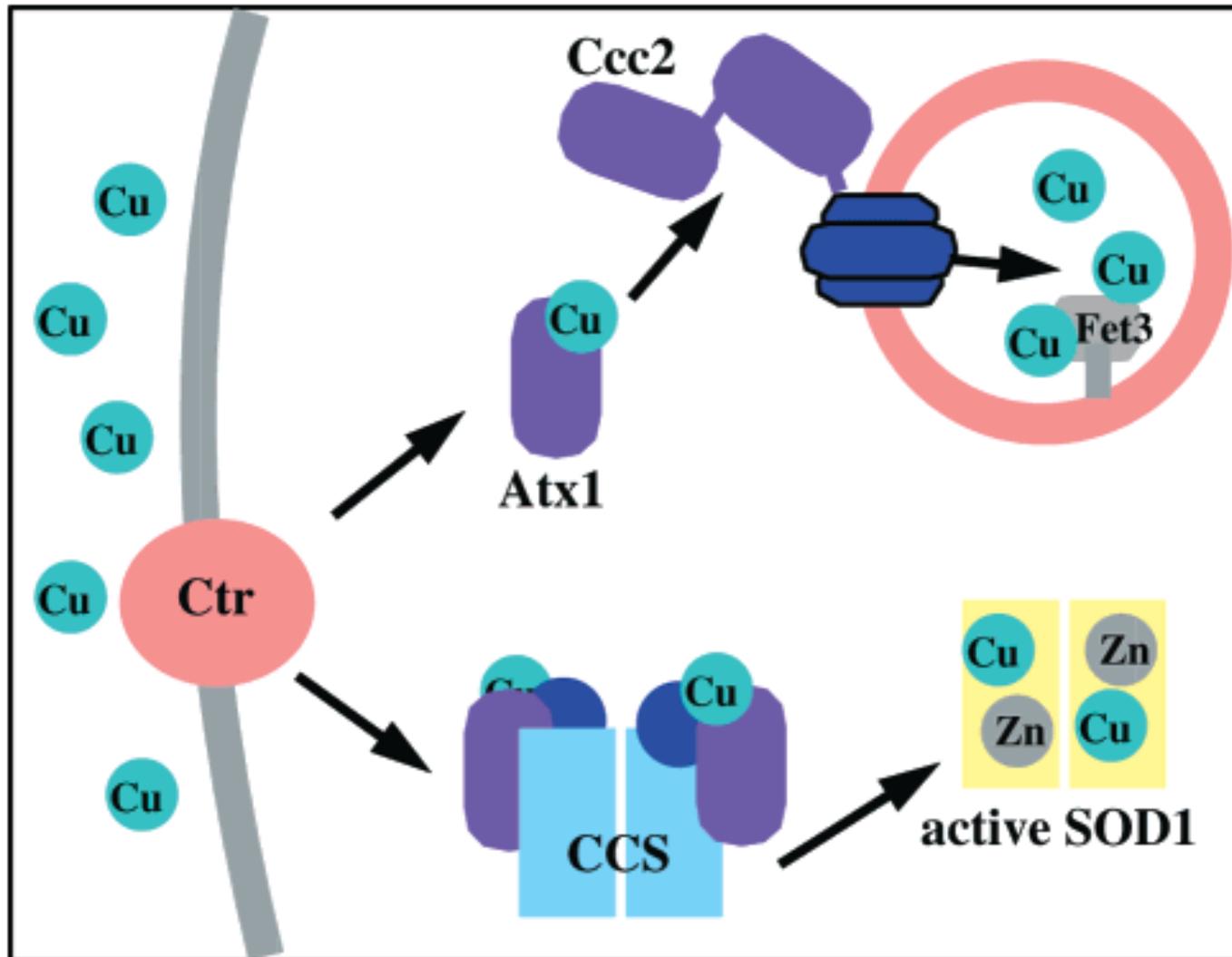


J. Gitschier et al Nature Str Biol (1998) 5 47

Mécanisme de transfert du Cuivre



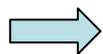
Exemple 2: CCS et SOD



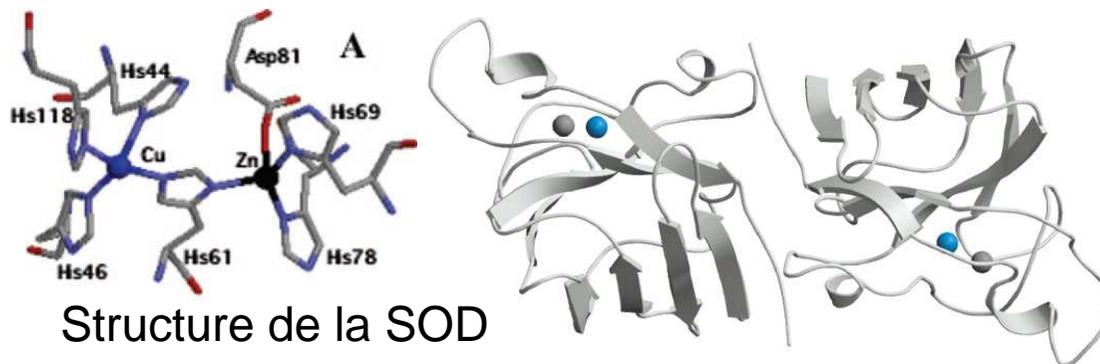
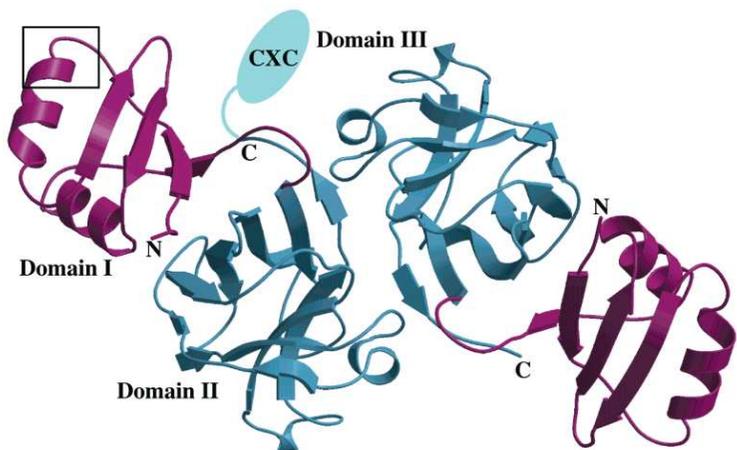
Exemple 2: CCS et SOD

Structure de CCS

A.C. Rosenzweig
 Acc. Chem. Res. (2001) 34 119
 Nature Struct Biol (1999) 6 724

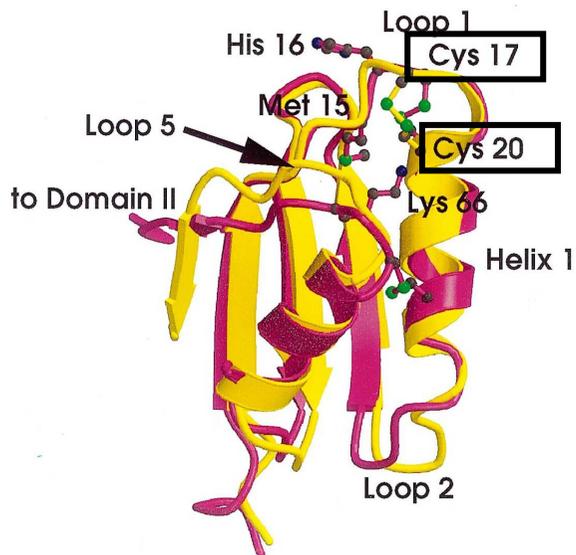


La chaperone CCS possède un domaine I **Atx1-like** (Nter), un domaine II **SOD-like** (C-ter) et un domaine III flexible (et absolument essentiel)!!!

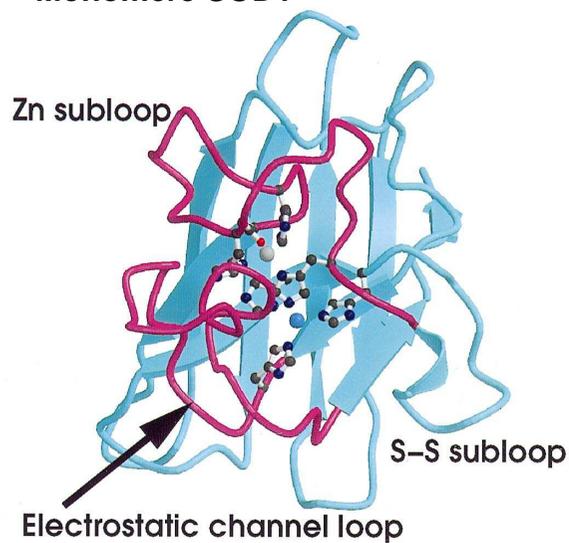


Structure de la SOD

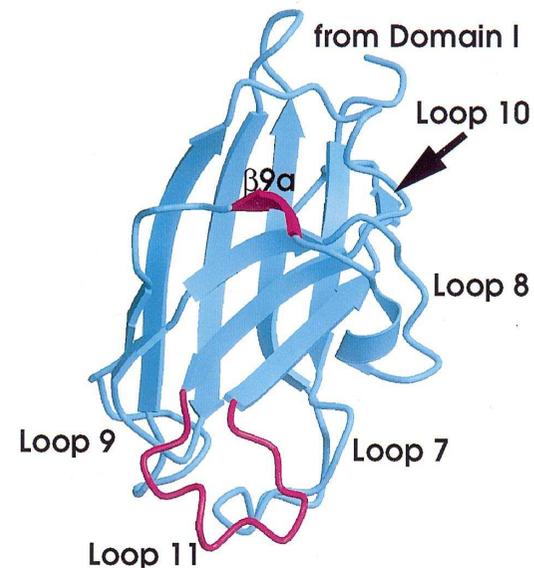
Superposition Atx1-domaine I



Monomère SOD1



Domaine II CCS Absence des ligands de Cu et Zn





yCcs	MTTNDTYEAT	YAIPMHCENC	VNDIKACLKN	VP-GINSLNF	DIEQQIMSVE	49
hCcs	MASDS GNQGTLC	FAVQMTCSQC	VDAVRKSLQG	VA-GVQDVEV	HLEDQMVLVH	54
Atx1	MAEIKHYQ	FNVVMTCSGC	SGAVNKVLT	LEPDVSKIDI	SLEKQLVDVY	48
Atox1	MPKHE	FSVDMTCGGC	AEAVSRVLNK	LG-GV-KYDI	DLPNKKVCIE	43

* *



yCcs	SSVAPSTIIN	TLRNCCKDAI	IRGAGKPN...	77
hCcs	TTLPSQEVQA	LLEGTGRQAV	LKGMGSGQLQ NL...	86
Atx1	TTLPYDFILE	KIKKTGKEVR	-SGKQL	73
Atox1	SEHSMDTLA	TLKKTGKTVS	YLGLE	68



yCcs	...SSAVATLE	TFQKYTIDQK	KDTAVRGLAR	IVQVG-ENKT	LFDITVNGVP	EAGNYHASIH	134
hCcs	...GAAVAILG	GPG-----	---TVQGVVR	FLQLTPERCL	IEGTID-GLE	PGLH-GLHVV	132
ySOD1	MVQAVAVLK	GDA-----	---GVSQVVK	FEQASESEPT	TVSYEIAGNS	PNAERGFHII	49
hSOD1	MATKAVCVLK	GDG-----	---PVQGIIN	FEQKESNGPV	KVWGSIKGLT	EGLH-GFHVH	49

* *



yCcs	EKGDVSKGVE	STGKVV----	-----	---HKFDEPI	E-CFNESDLG	KNLYSGKTFL	176
hCcs	QYGDLTNNCN	SCGNHFNPDG	ASHGGPQSD	RHRGDLG-NV	RADAD-----	---GRAIFRM	183
ySOD1	EFGDATNGCV	SAGEHFNPFK	KTHGAPTDEV	RHVGDMG-NV	KTDEN-----	---GVAKGSF	100
hSOD1	EFGDNTAGCT	SAGEHFNPLS	RKHGGPKDEE	RHVGDIGNVT	ADKD-----	---GVADVSI	100

* * * *



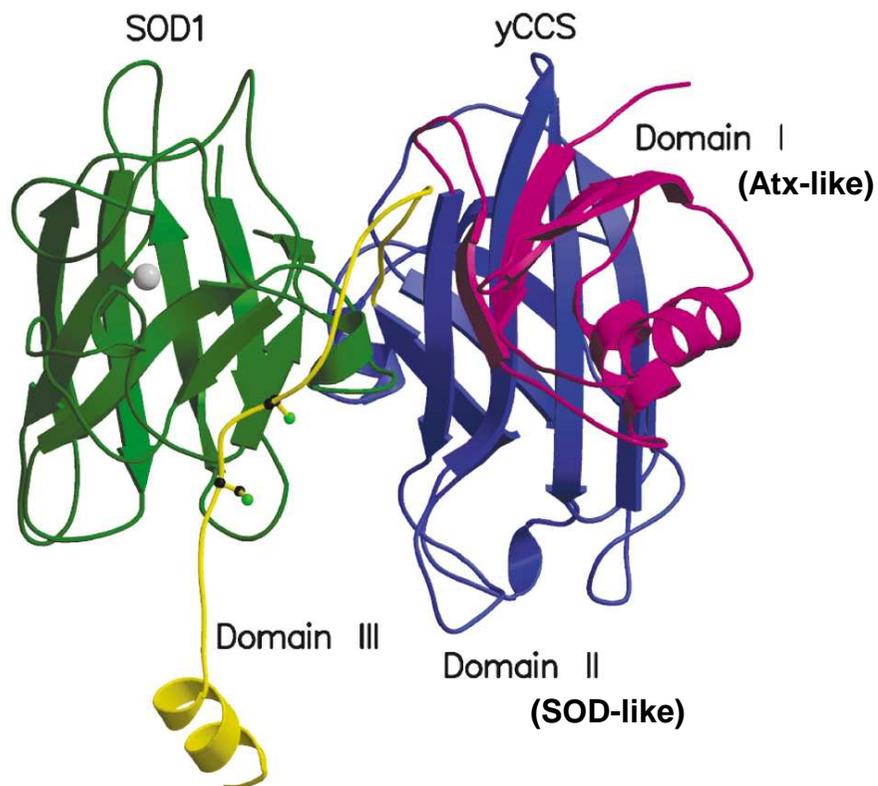
yCcs	SAP-LPTW--	-QLIGRSFVI	SKS---LNHP	ENEPSSV---	-KDYSFLGVI	ARSA ² GVWENN	225
hCcs	EDEQLKVWD-	--VIGRSLII	DEGEDDLGRG	GHPLSKITGN	SGERLACGII	ARSA ² GLFQNP	240
ySOD1	KDSLIIKLIGP	TSVVGRSVVI	HAGQDDLGGK	DTEESLKTGN	AGPRPACGVI	GLTN	154
hSOD1	EDSVISLSGD	HCIIGRTLIVV	HEKADDLGGK	GNEESTKTGN	AGSRLACGVI	GIAQ	154

*

yCcs	KQVCACTEKT	VWEERKDALA	NNIK	249
hCcs	KQICSCDEL	IWEERGRPIA	GKGRKESAQP PAHL	274

Complexes protéiques et Transfert du Cuivre intermoléculaires

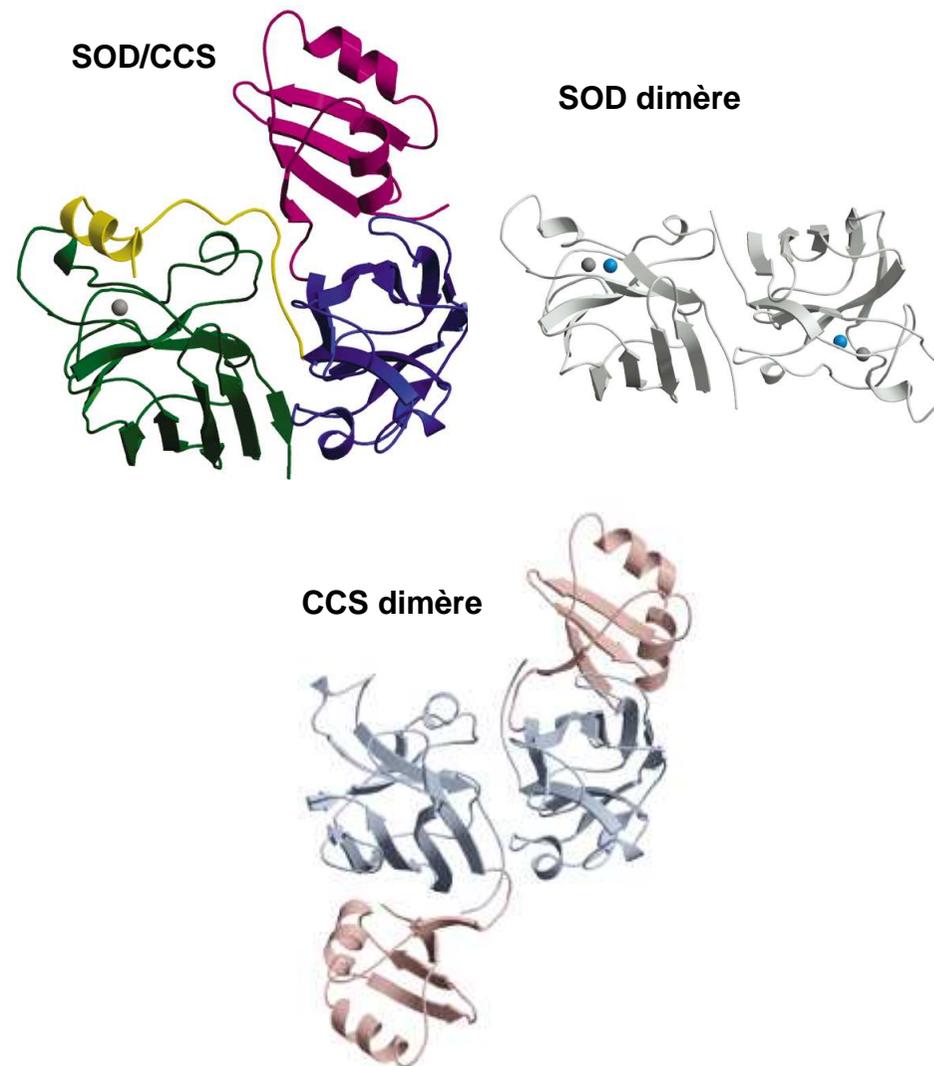
Complexe SOD/CCS



Interaction domaine II-SOD

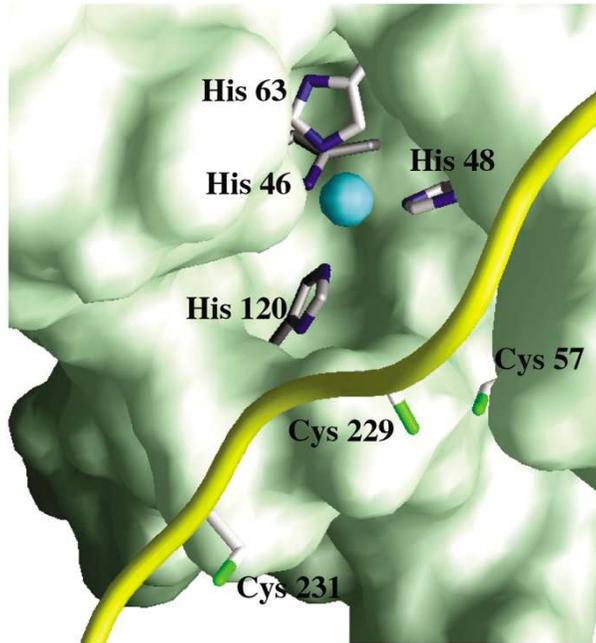
Similaire à l'interface domaine II-domaine II dans CCS

Similaire à l'interface monomère-monomère dans SOD

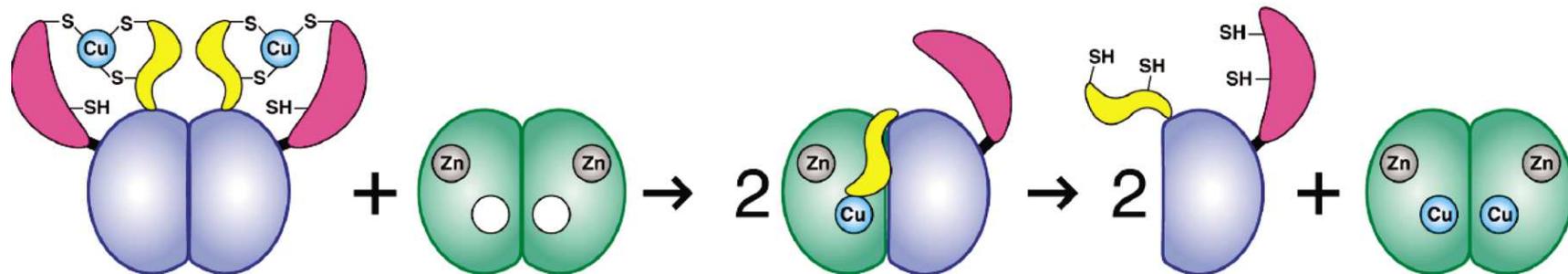
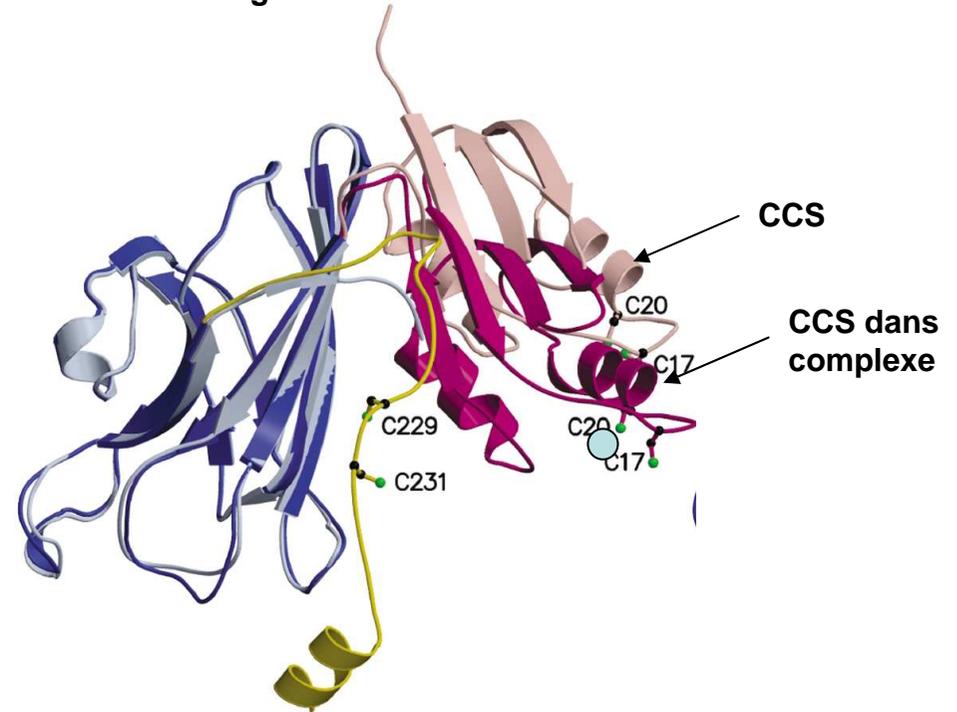


A.C. Rosenzweig *Nature Str Biol* (2001) **8** 751
Acc. Chem. Res. (2001) **34** 119

Complexes protéiques et Transfert du Cuivre intermoléculaires

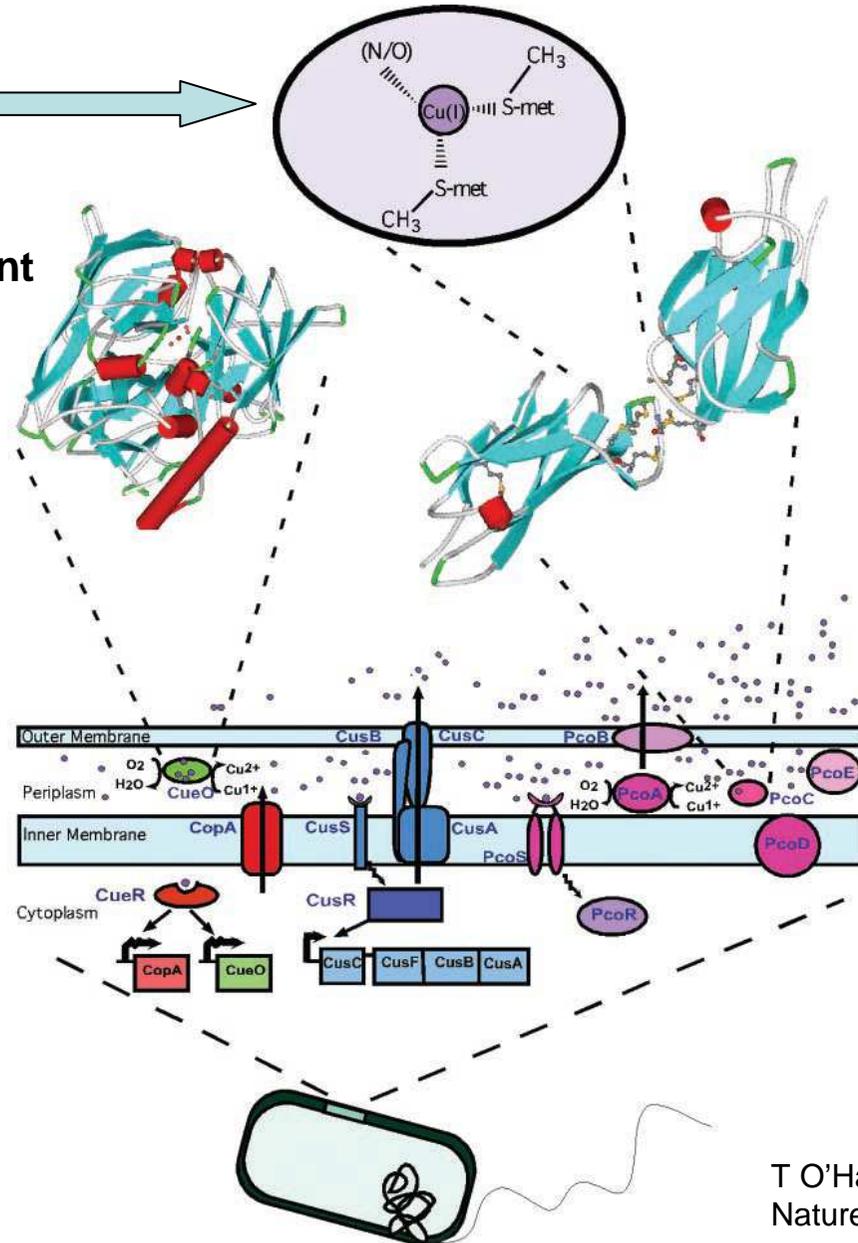


Changement conformationnel du domaine I



Transport du Cuivre chez les bactéries (E. coli)

Une coordination nouvelle: **Met**
Pour le trafic de Cu dans un milieu oxydant

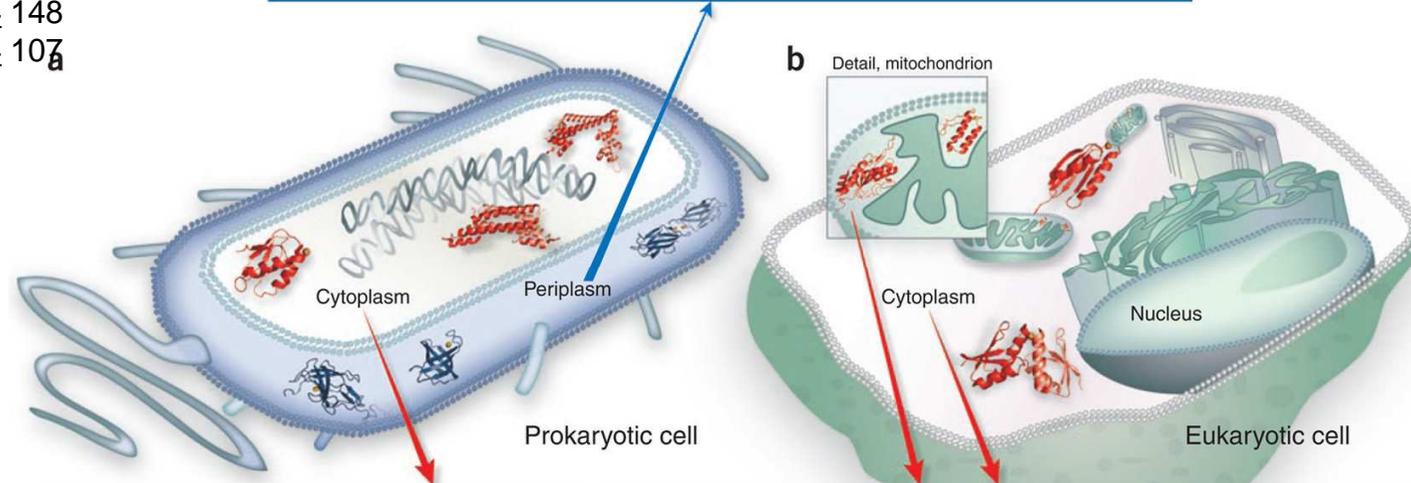
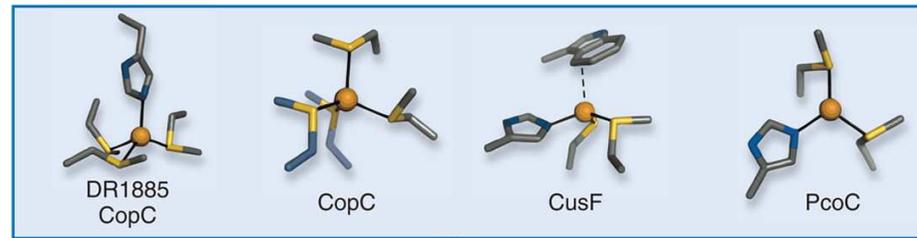


Transport et régulation du Cu: Chimie de coordination

T O'Halloran

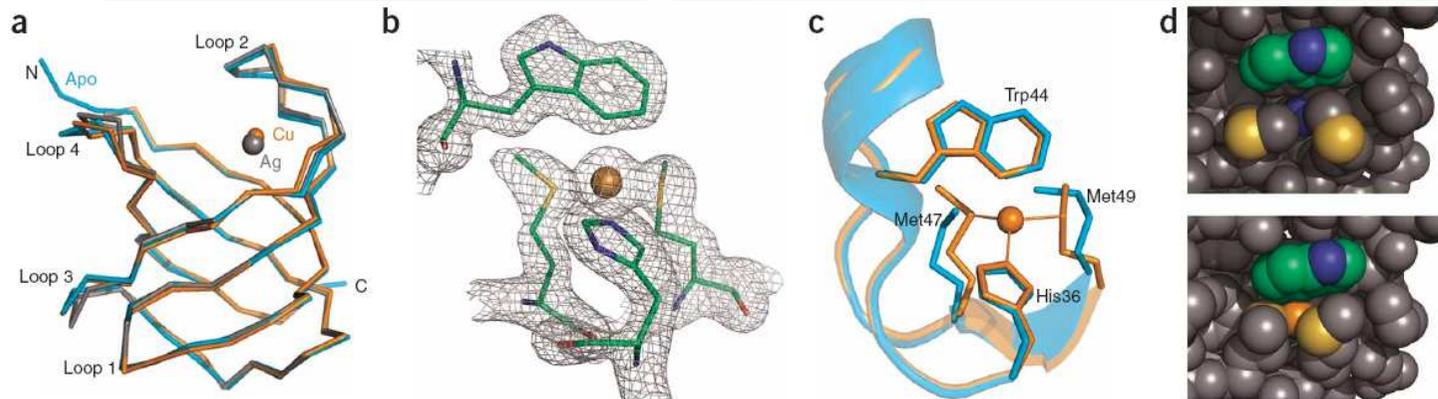
Nature Chem Biol 2008 4 148

Nature Chem Biol 2008 4 107



Rebecca Henretta

CusF (périplasm)



Pourquoi un tel contrôle intracellulaire du Cuivre ? (chaperones + $[Cu^{n+}] \sim 0$)

→ Eviter les réactions rédox du « Cu libre »



→ Maturer sélectivement les protéines à Cu

→ Eviter l'incorporation de Cu dans les autres sites métalliques

Nature (2008) 455 1138

Irving-Williams serie: $Mn^{2+} < Fe^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$

« Comment une cellule peut-elle contenir des protéines à Fe, Mn, Ni, Zn en présence de Cu ? »

Cyanobactéries



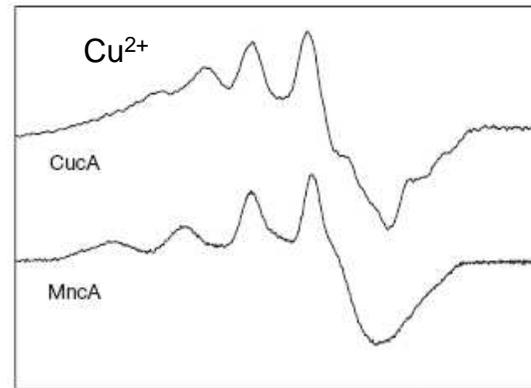
Protéines périplasmiques analysées par
 -Chromatographie à 2 dimensions
 -ICP-MS (analyse de métaux)
 -SDS-PAGE



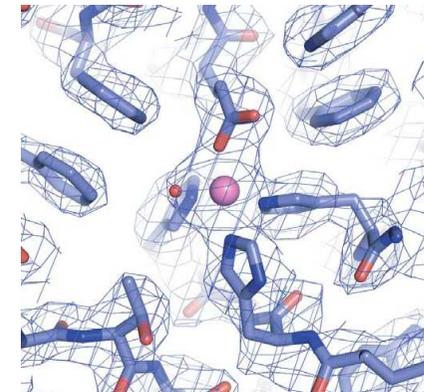
Protéine à Cu
 prédominante: CucA

Protéine à Mn
 prédominante: MncA

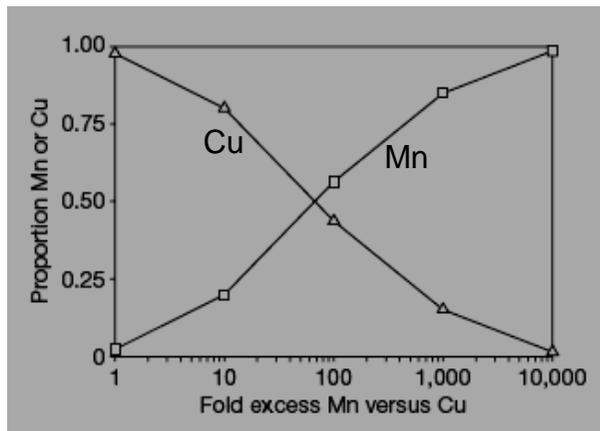
Moyennant que Cu n'interfère pas pendant la maturation de la protéine à Mn celle-ci peut être exposée à Cu car le site à Mn est protégé dans la protéine une fois maturée



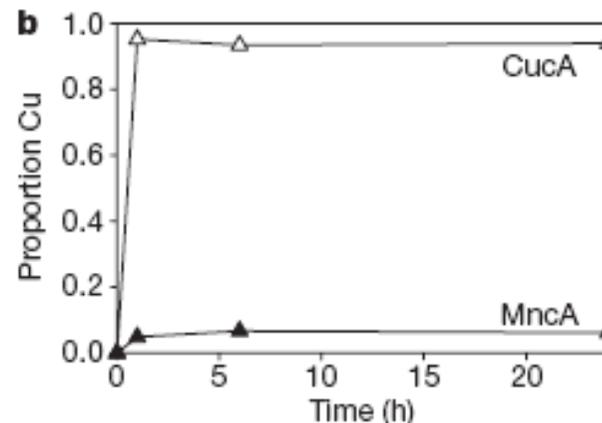
Les sites métalliques sont similaires



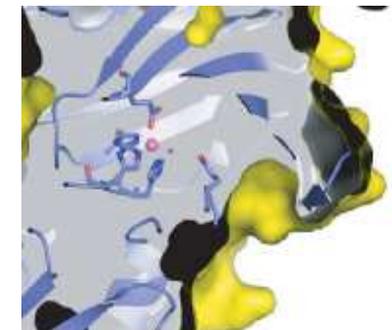
Mn²⁺-MncA



Repliement de Mn²⁺-MncA en présence d'excès croissant de Mn²⁺



Cu²⁺ remplace facilement Mn²⁺ dans Mn²⁺-CucA mais pas dans Mn²⁺-MncA

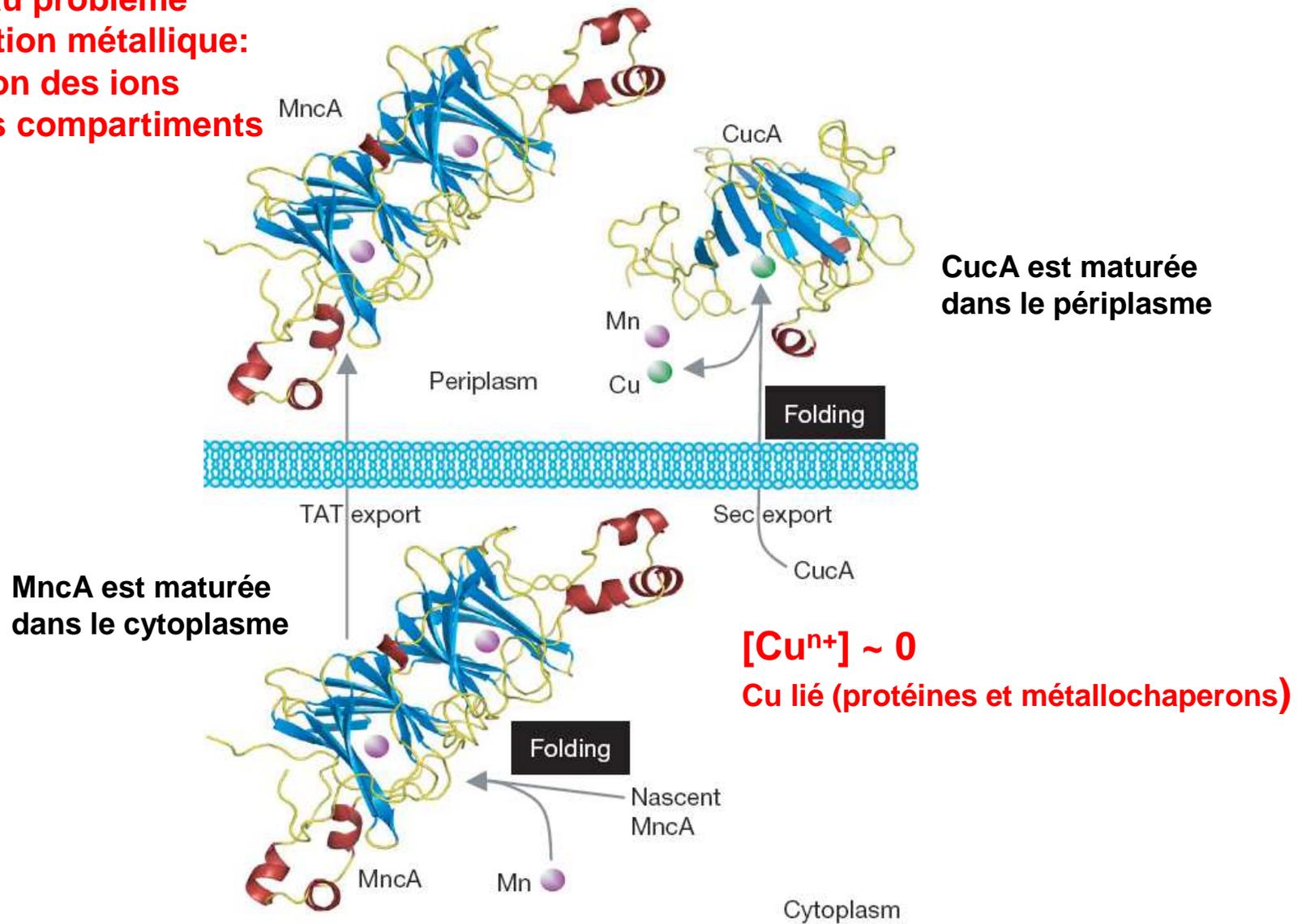


Mn²⁺ dans Mn²⁺-MncA est enfoui

N-terminal paptide signal

MncA MVNSVIGWLRRLRRFLLVGSVLLITFLGI FTPTIAQSE → Tat
CucA MLLKVKLWGIGLVLTTLTGTI LFLQNF SVA AETEIH → Sec

**Solution au problème
de la compétition métallique:
Acquisition des ions
dans différents compartiments**



Irving-Williams serie: $Mn^{2+} < Fe^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$

Et le Zinc ??

Zn cytosolique lié aux protéines [Zn] ~ 0

Métallochaperones à Zn ???

Et le Fer ??