Molecular evolution of Multi Copper Blue Proteins

Kensuke Nakamura

Metallome

いくつかの金属元素は生命現象に必須

Fe, Zn, Cu, Mn, Co, V, Mo, W　遷移金属

Ca, Mg, Na, K　アルカリ（土類）金属

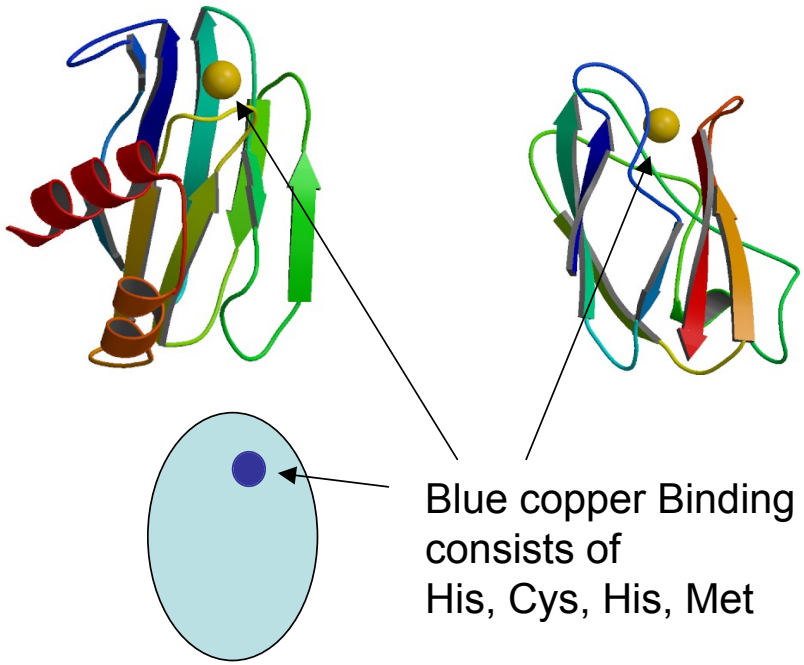
銅イオンは活性酸素などのラジカル種を作り出すので有毒.
だが、様々なタンパク質が銅イオンの酸化還元能力を利用している

シトクロム酸化酵素
活性酸素不活化酵素
チロシナーゼ
ヘモシアニン
など

Blue Copper Proteins

Double Greek-Key eight β-strands

Blue-Copper center for Redox Function



Blue copper Binding site
consists of
His, Cys, His, Met

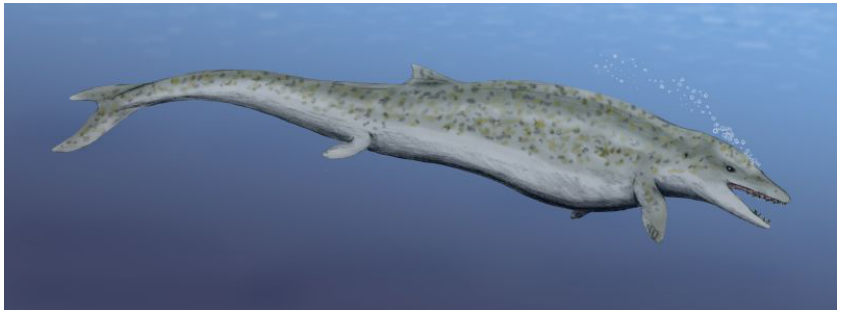
Cupredoxins mono-domain
- Azurin
- Pseudoazurin
- Amicyanin
- Plantacyanin
- (Basic Blue Protein)
- Plastocyanin
- Auracyanin
- Rusticyanin
- Stellacyanin
- UCLAcyanin !!
- Nodulin

Cu-oxidases multi-domain
- Nitritereductase
- Laccase
- Ascorbate Oxidase
- Ceruloplasmin

Missing Link

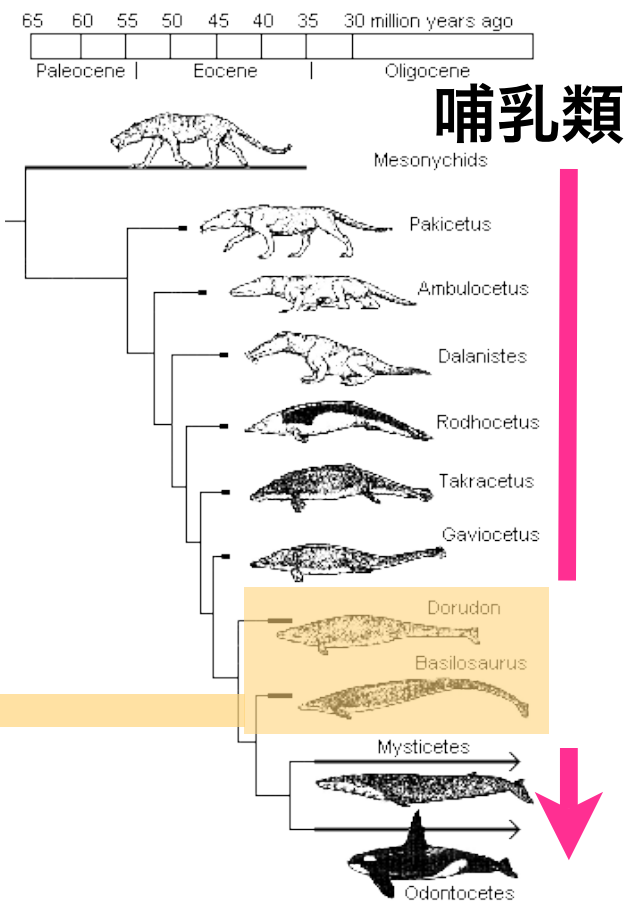
博物学としての生物学からの例

Dorudon



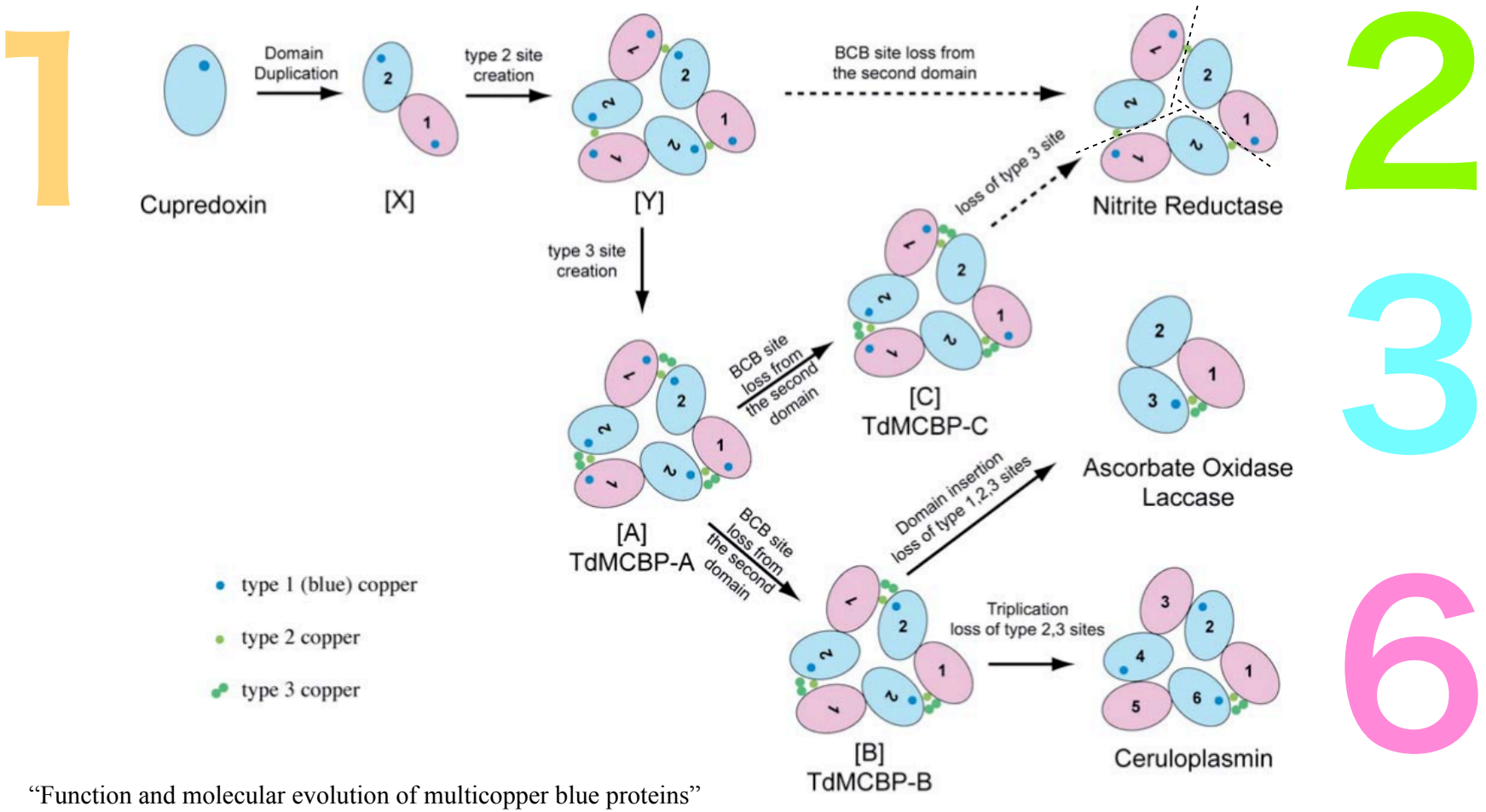
四脚哺乳類からクジラへの進化上の

ミッシングリンク



バイオインフォマティクスへ応用

分子進化
マルチ銅ブルータンパク質



"Function and molecular evolution of multicopper blue proteins"
K. Nakamura, N. Go CMLS 62, pp2050-2066 (2005)

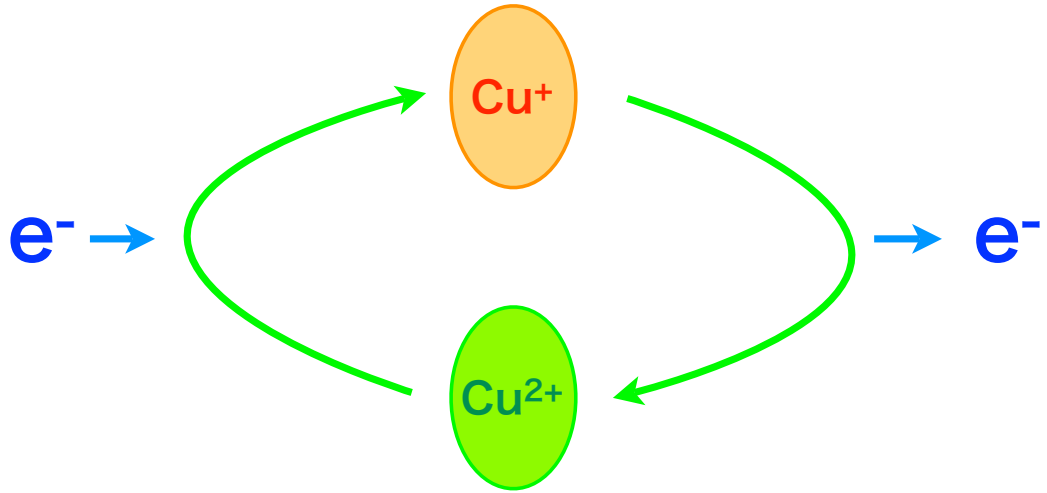
1

植物

プラストシアニン

アズリン

電子輸送



光合成系など

亜硝酸還元酵素

2

NIR

嫌気性菌

N_2

↓

NH_4^+

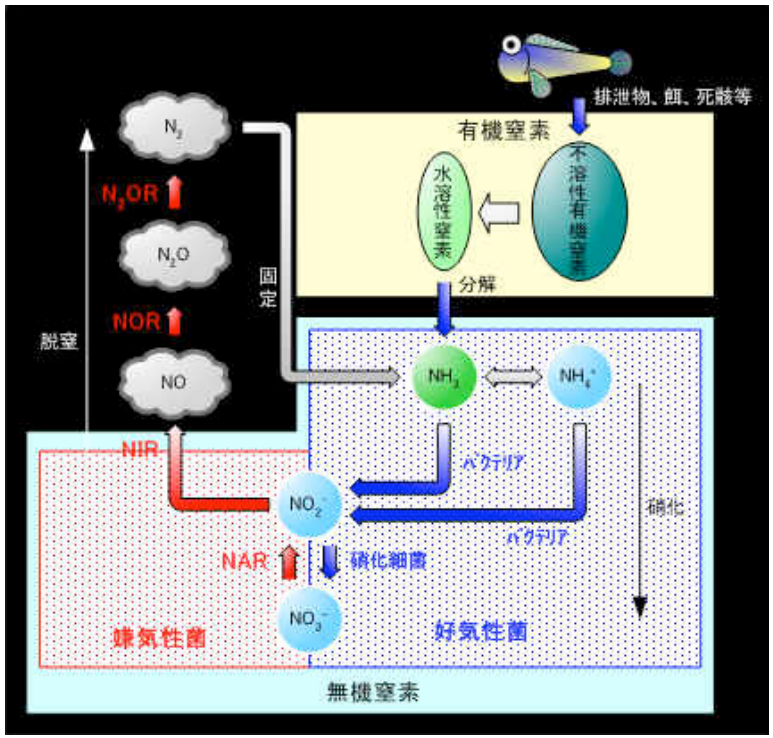
↓ 好気性菌

Nitrite

↓ 嫌気性菌

N_2 ↑

窒素循環



ラッカーゼ

リグニン　＝　植物の細胞壁

↓

分解

↓

利用

バイオ燃料、 洗剤, 浄化槽,

3

真菌
植物
バクテリア

アスコルビン酸酸化酵素

ビタミン C　　抗酸化作用

活性酸素への抵抗性・老化防止

セルロプラスミン

ヘモグロビンに鉄を供給

Fe^{2+} -> Fe^{3+}

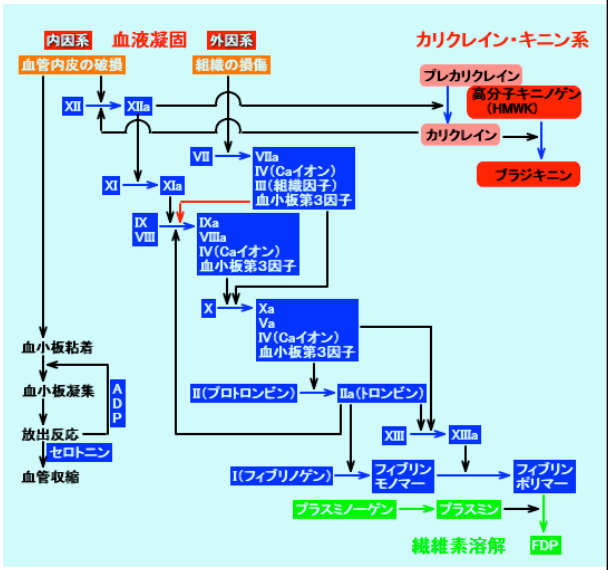
6

脊椎動物

血液凝固因子

BC5

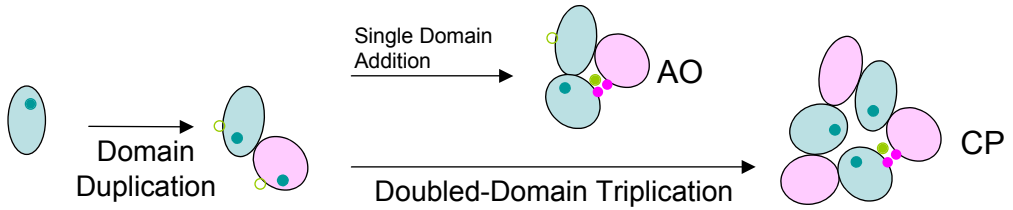
BC8



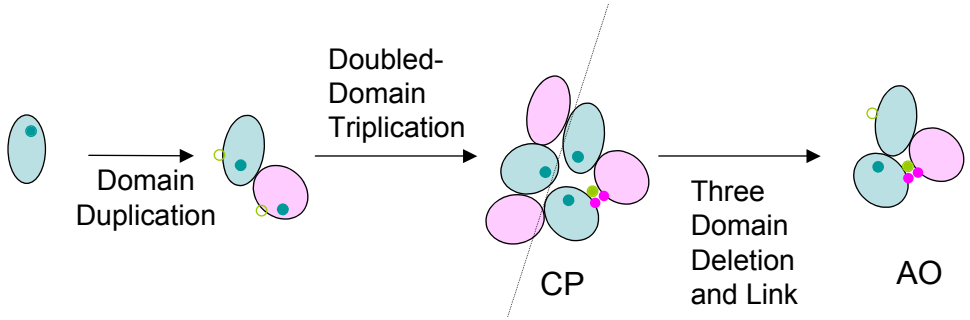
銅の必要性は不明

Previously Inferred Paths of Evolution

1. Ryden and Hunt : based on Sequence Phylogeny

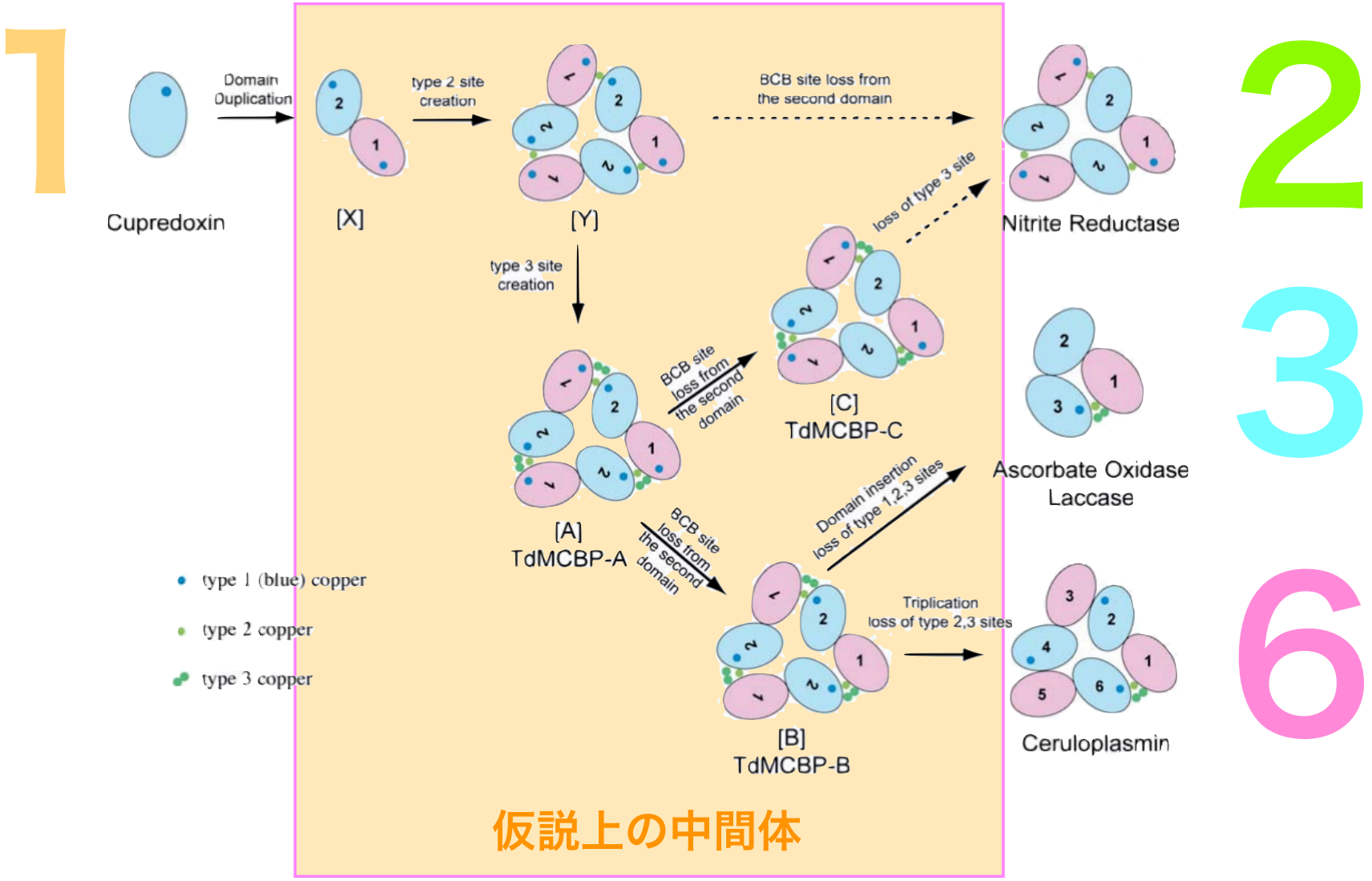


2. Murphy, Lindley Adman : based on Structural Similarity



Not consistent with the phylogenic tree

ここで提唱した進化経路



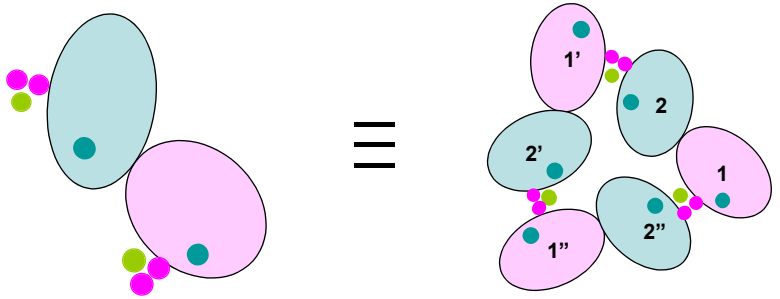
状況証拠　1：
仮説上の中間体が配列データベースに見つかった

Found [A]　*Halobacterium* NRC-1

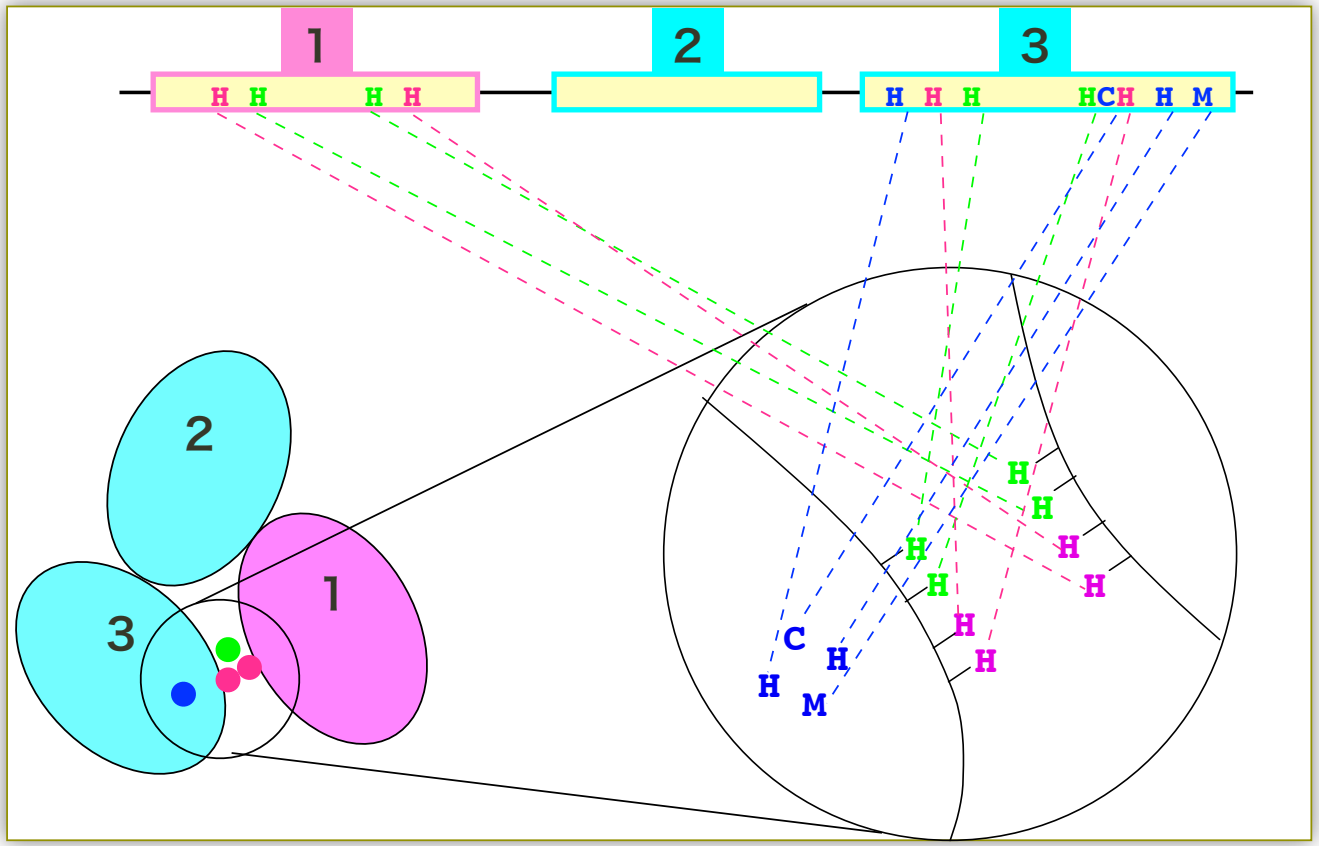
- Q9HQF4 : [A] type　2 domain MCO
  - 449 amino acids
    - Domain1 [77-203] : Blue copper, 4 interdomain Hs
    - Domain2 [206-352]: Blue copper, 4 interdomain Hs

```
Q9HQF4/ 77-203     DGKRPHTLHFHG----- ~~~ YHCHYQTQRHIDM
Q9HQF4/206-352     GGYMNHPLHIHNHRFRM ~~~ MHCHK--VNHV-M
```

Possibly:

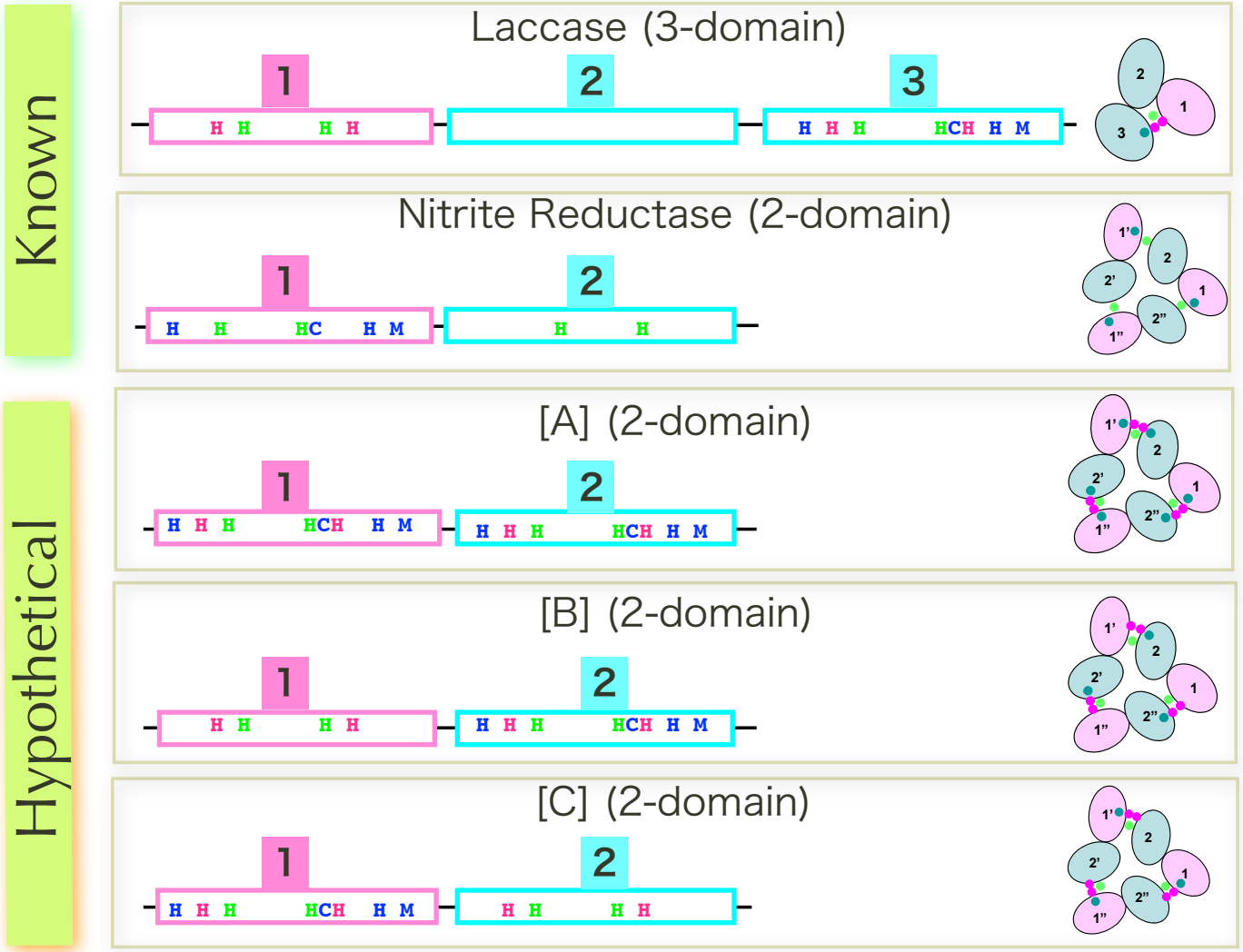


銅の配位残基の存在は配列情報だけである程度認識できる



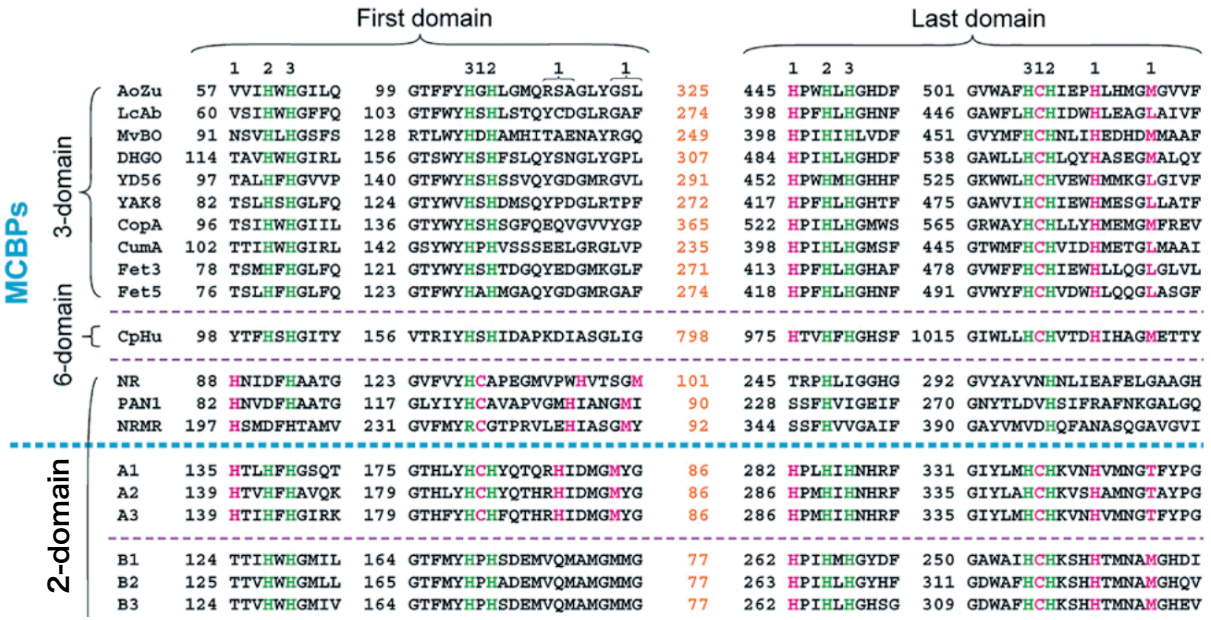
Copper-binding sites can be assigned in amino acid sequence

ドメイン構成


Known
Laccase (3-domain)
1
2
3
H H H H
H H H HCH H M
Nitrite Reductase (2-domain)
1
2
H H HC H M
H H
Hypothetical
[A] (2-domain)
1
2
H H H HCH H M
H H H HCH H M
[B] (2-domain)
1
2
H H H H
H H H HCH H M
[C] (2-domain)
1
2
H H H HCH H M
H H H H

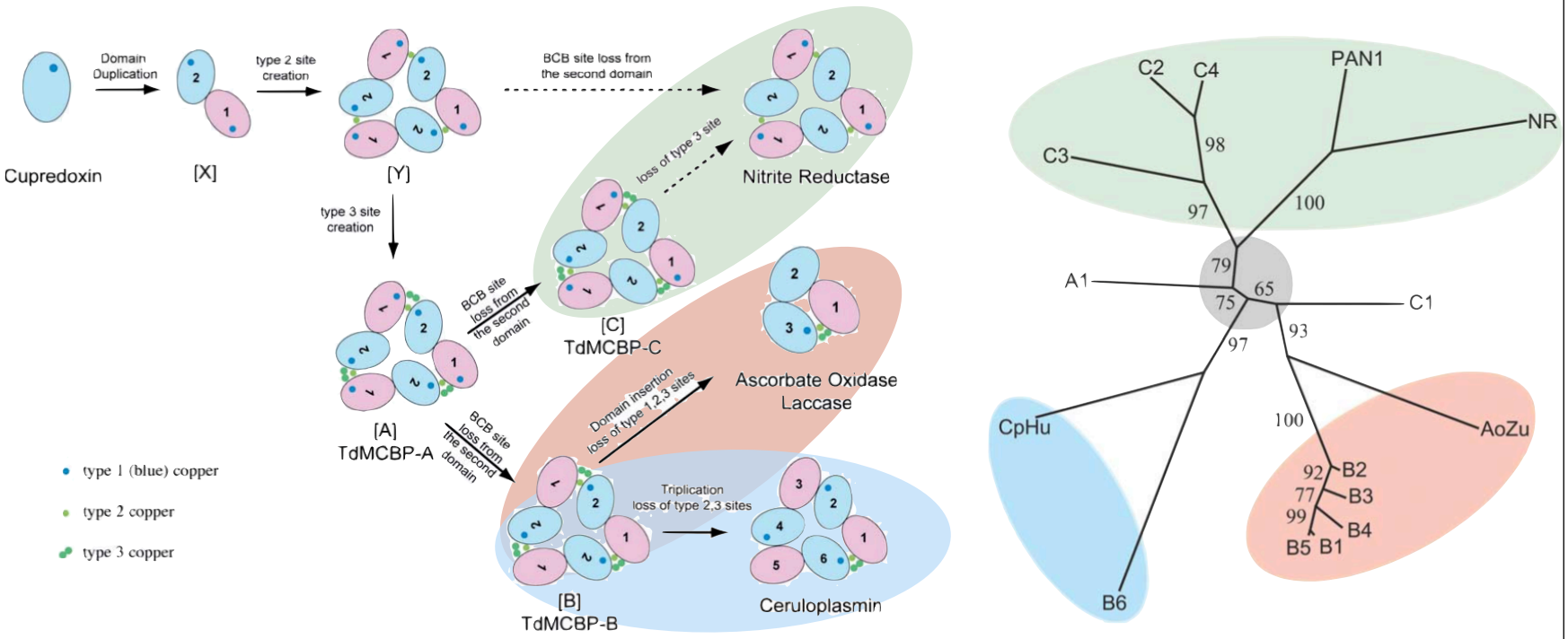
11 new types of two-domain MCO's

A	A1	: Q9HQF4	from Halobacterium strain NRC-1	(藍藻)
B	B1	: Q92S43	from Shinorhizobium meliloti	(根粒菌)
	B2	: ZP_00029340	from burkhoderia fungorum	(アニリン資化性グラム陰性菌)
	B3	: NP_768850	from bradyrhizobium japonicum USDA 110	(根粒菌)
	B4	: ZP_00052601	from Magnetospirillum magnetotacticum	(磁性細菌)
	B5	: NP_356650	from Agrobacterium tumefaciens str C58	(根頭癌腫菌 (薔薇))
	B6	: Q93HV5	from Streptomyces griseus	(ストレプトマイシン産生菌)
C	C1	: ZP_00002680	from Nitrosomonas Europaea	(硝化細菌)
	C2	: ZP_00073469	from Trichodesmium erythraeum IMS 101	(藍藻、紅色)
	C3	: NP_711736	from Leptospira interrogans serovar lai str	(家畜の病気)
	C4	: NP_487982	from Nostoc sp PCC7120	(藍藻)



4 [A]'s, 130 [B]'s, 36 [C]'s

状況証拠　２：
系統解析と進化仮説の一致



進化モデル上近い組み合わせが系統樹上でも近い位置に現れる

矛盾する実験例

SLAC = B13

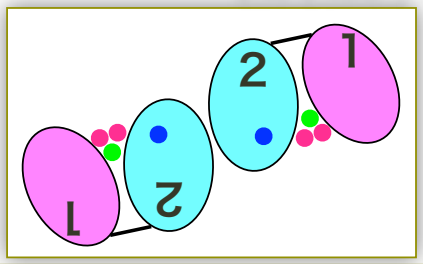
"Characterization of SLAC: A small laccase from Streptomyces coelicolor with unprecedented activity", Machczynski MC, Vijgenboom E, Samyn B and Canters GW, Protein Science, 13, Aug, pp. 2388-97, (2004).

[SLAC = B13] リコンビナントに合成し物性を調べた

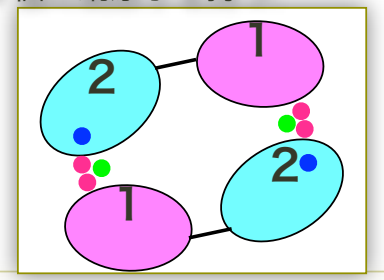
SLAC ラッカーゼ活性を示す
短いタンパク質-ShortLACcaseと命名.

SLAC はホモダイマー.

SDS-PAGE 64kDa ダイマーのバンドが観測された.
それぞれのサブユニットが 4 個の銅原子を持つ.

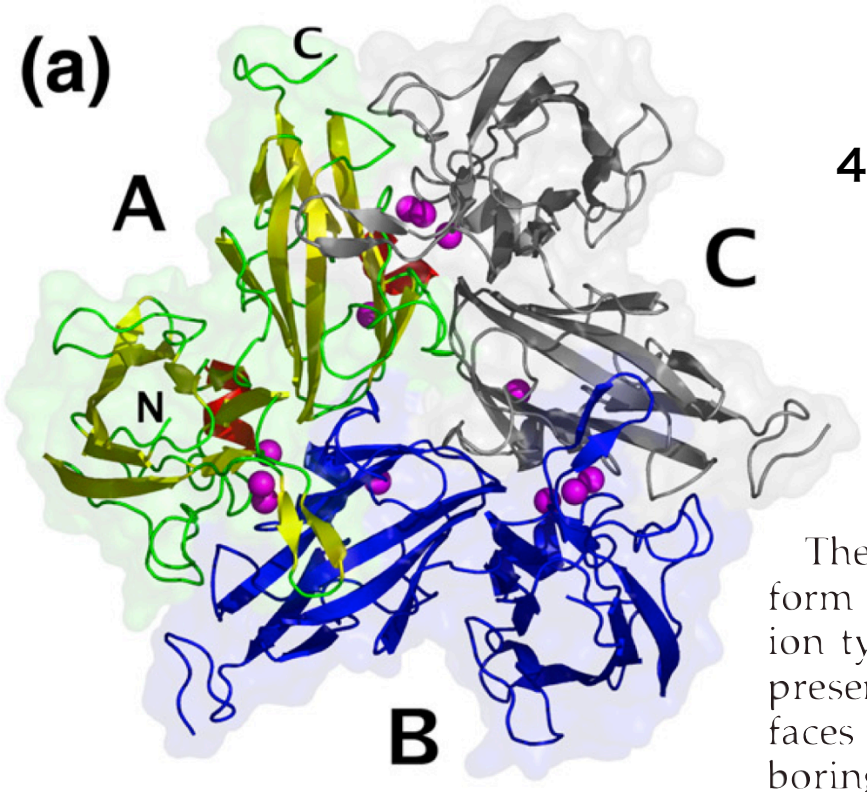


or



?

X先結晶構造により我々の推定が検証された



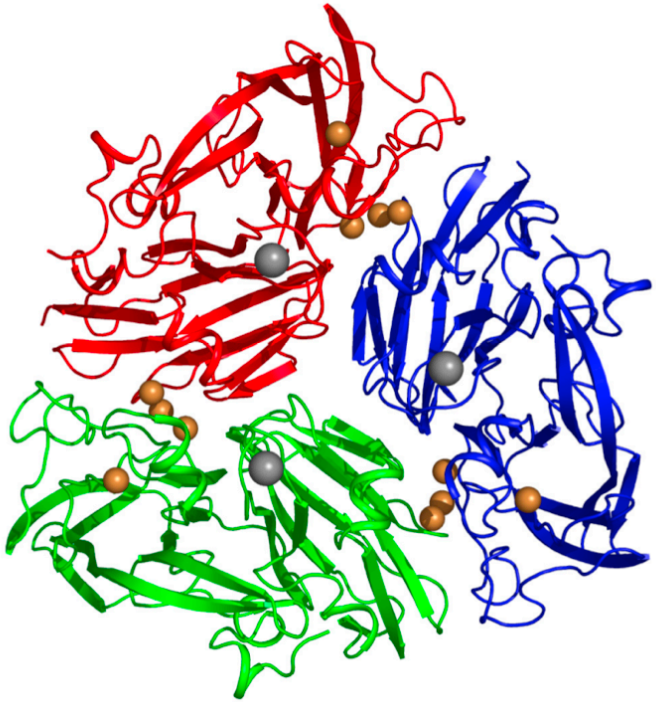
SLAC
Streptomyces coelicolor
[B]

X-ray 結晶構造により
SLAC がホモトリマーを形成し
４つの銅イオンを結合することが示された

The structure presented here confirms the trimeric form of SLAC. It also proves the presence of copper ion type 1 in each domain 2 of the trimer and the presence of the trinuclear cluster at all three interfaces between domain 1 and domain 2 of the neighboring chains, as predicted by Nakamura and Go.[8]

"The structure of the Small Laccase from Streptomyces coelicolor Reveals Link Between Laccases and Nitrite Reductase" Tereza Skalova, J. Dohnalek, L.H. Ostergaard, P.R. Ostergaard, P. Kolenko, J. Duskova, A. Stepankova, J. Hasek, JMB 385, 1165-1178 (2009)

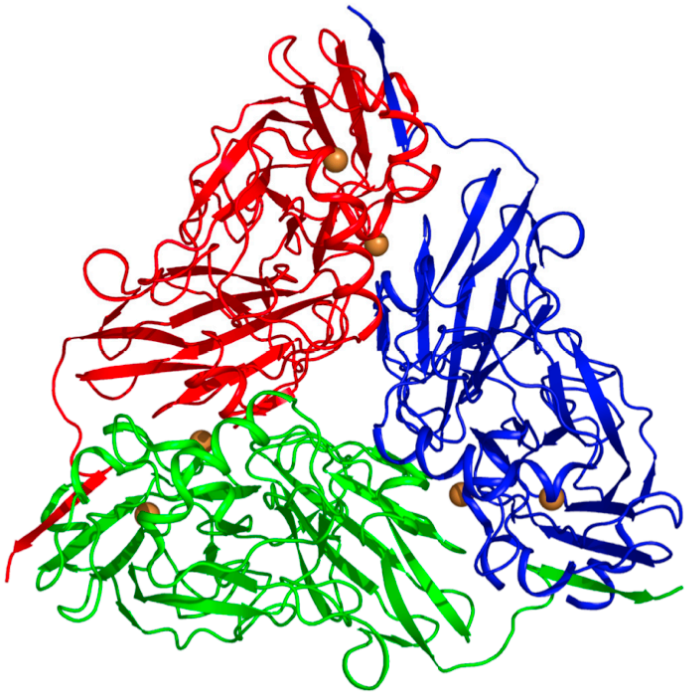
さらに、、



AMMCO

[C]

Anthrobacter sp. FB24, NC_008537



NIR

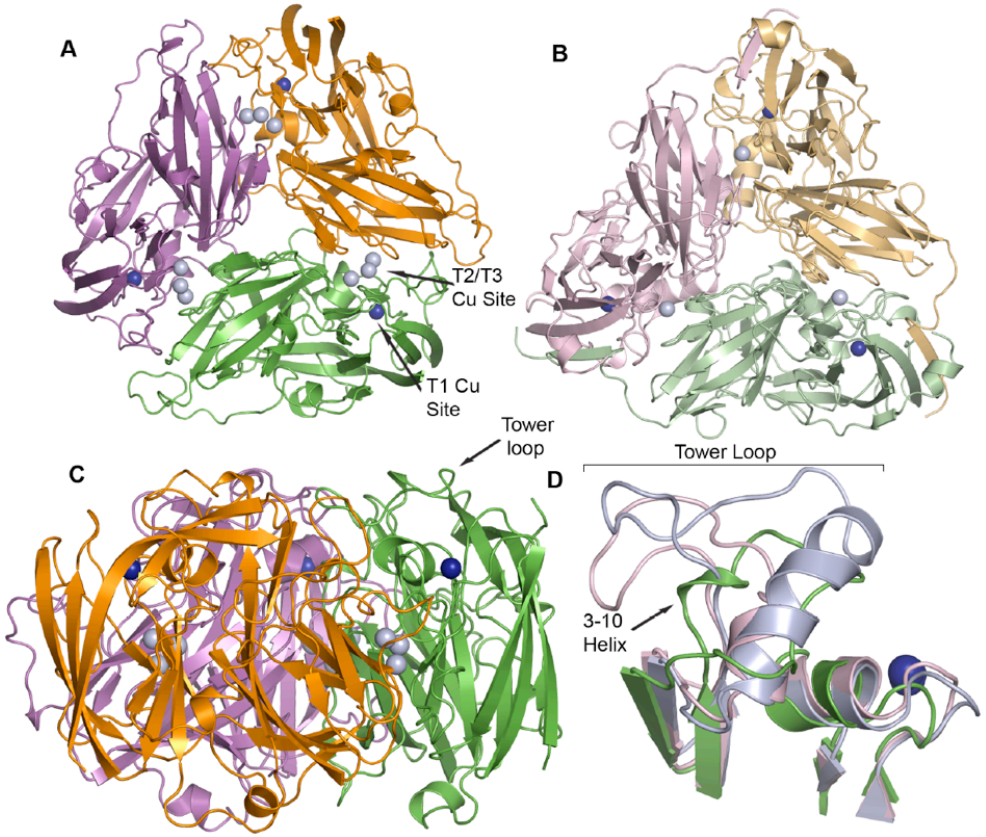
In particular, the evolutionary model of Nakamura and Go connecting NiR to three-domain MCOs is in need of experimental validation.

[C]-タイプの AMMCO 結晶構造が NIRに類似

“Evolution of Copper-Containing Nitrite Reductase” I. S. MacPherson, Doctoral Dissertation U. British Columbia (2008)

もうひとつの[C]タイプ結晶構造

BCO
[C]



NIR

Nitrosomonas europaea, NC_008537

As of 2005, Nakamura and coworkers identified 21 different 2dMCOs in genome databases

"Crystal structure of a two-domain multicopper oxidase: implications for the evolution of multicopper blue proteins", Lawton TJ, Sayavedra-Soto LA, Arp DJ, Rosenzwig AC, JBC (2009)

Summary

Series of recent X-ray structures of
2-domain MCBP's supports our model

The evolutionary model is
being widely accepted

What are the function of these MCBP's in bacteria?

All 2-domain MCBP's are from Bacteria

Large number of 3-domain MCBP's are from Bacteria

Function of 3-domain MCBP's in Bacteria

CotA	*Bacillus subtilis*	Spore coat
CueO	*E. coli*	Oxidation of Cu(I)
PcoA	*E. coli*	Efflux of Cu
Fet3p	*Saccaromyces cerevisiae*	Oxidation of Fe(II)
CumA, MofA, MnxG	*Pseudomonas putida*	Oxidation of Mn(II)
Phenoxasionone synhtase	*Streptomyces lividans*	Biosynthesis of antibiotic

MCBP's in Bacillus/Lactobacillus

3-domain

Bacillus cereus; anthracis; thuringiensis; weihenstephanensis; halodurans; coagulans; pumilus; clausii;

Lactobacillus rhamnosus; casei; brevis; plantarum;

Lactobacillus lactis;

Pediococcus pentosaceus;

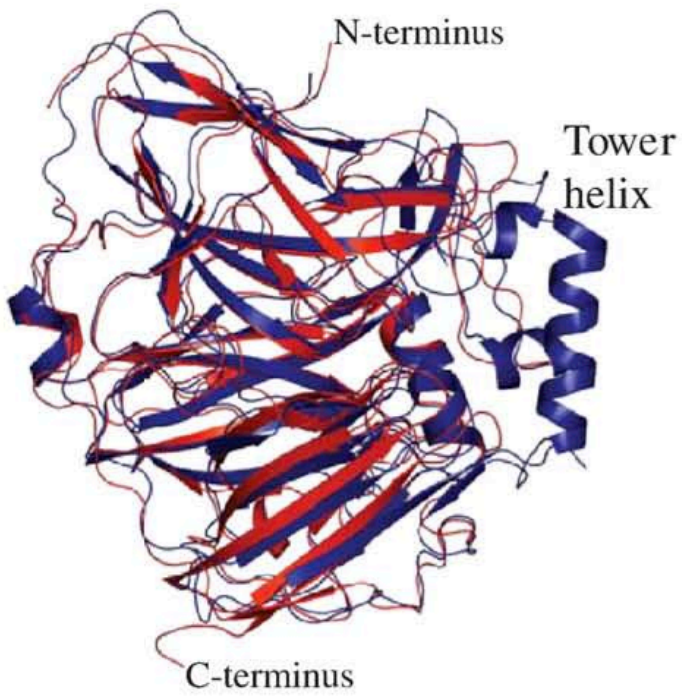
Leuconostoc citreum;

2-domain

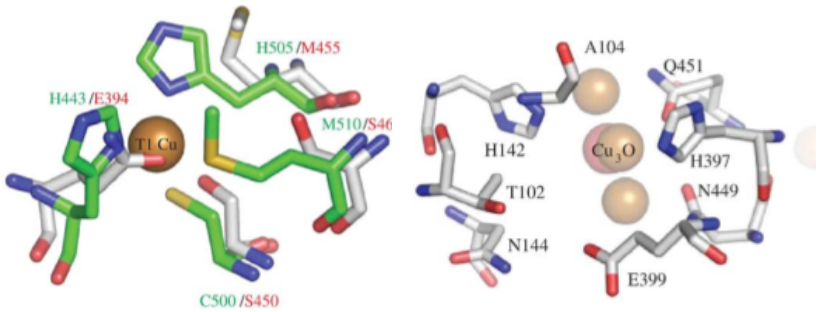
Bacillus sp. B14905;

By homology, MCBP's in Bacteria are usually assigned as
Copper resistance, Mn oxidation, Spore coat, or Cell division (SufI)

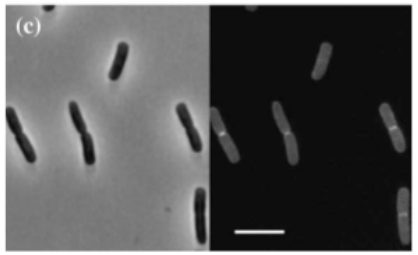
SufI(FtsP) is a 3-domain MCBP form without Cu-binding sites



SufI (red) and CueO (blue)



SufI has no Cu Binding residues



SufI is involved in Cell Division

"The Escherichia coli Cell Division Protein and Model Tat Substrate SufI(FtsP) Localizes to the Saptal Ring and Has a Multicopper Oxidase-Like Structure" Tarry M., Ryan Arends SJ, Roversi P, Piette E, Sargent F, Berks BC, Weiss DS, Lea SM, JMB 386, 504-519 (2009)

consensus is

once the whole genome is solved for one organism,
homology analysis will provide the functional annotation for all genes

GP

once the X-ray structures for all possible folds are solved
homology modeling can solve all the protein structures

SG

in reality

There are wide structural and functional variety in a group of homologous proteins

Careful inspection of each gene is important

And there is a plenty of room for Bioinformatics to contribute

Metagenome analysis should reveal
number of characteristic genes with
novel domain-organization, structure, and function