Integrating the Molecular Machines of Mercury Detoxification into Host Cell Biology

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(Poster Weds 18 April, Session C)
The Materials Flow of Mercury in the Economies of the United States and the World

By John L. Szeppek and Thomas G. Braden

U.S. Geological Survey Circular 1197
All forms of Hg are biologically available.
Cysteine (Cys, C)
<table>
<thead>
<tr>
<th>System</th>
<th>Protein/Process</th>
<th>Molecular Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal transduction</td>
<td>Protein tyrosine phosphatase</td>
<td>Invariant Cys215</td>
</tr>
<tr>
<td></td>
<td>Zinc Finger Proteins</td>
<td>Multiple Cysteines</td>
</tr>
<tr>
<td></td>
<td>LIM proteins</td>
<td>Multiple Cys-His domains</td>
</tr>
<tr>
<td>Metal Homeostasis</td>
<td>Metallothione</td>
<td>Multiple Cysteines</td>
</tr>
<tr>
<td></td>
<td>Menkes Disease (Cu)</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Wilson's Disease (Cu)</td>
<td>&quot;</td>
</tr>
<tr>
<td>Renal transport</td>
<td>CHIP28 Water Channel</td>
<td>Cys 189</td>
</tr>
<tr>
<td>Growth Factors</td>
<td>Trefoil, EGF-like, Cystine Knot</td>
<td>Three clustered cystine bridges</td>
</tr>
<tr>
<td>CNS</td>
<td>Membrane Cysteine String Proteins (synaptic vesicles and termini)</td>
<td>Cysteine rich proteins</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>apolipoprotein(a)</td>
<td>Cys 4057 - important for assembly</td>
</tr>
<tr>
<td>Virus(es)</td>
<td>HIV Tat protein</td>
<td>Cysteine-rich protein</td>
</tr>
<tr>
<td>Oncogenes</td>
<td>RAS</td>
<td>Thioether farnesyl linkage</td>
</tr>
</tbody>
</table>
Why study Hg resistance?

Only naturally occurring system that biotransform a toxic metal in bulk

Handles inorganic and organic Hg(II)

Widely found in eubacteria and archaea that are the major Hg transformers in highly contaminated settings.

Transposable and laterally transferrable in proteobacteria.

Highly conserved mechanically - i.e pump Hg(II) in and reduce to volatile Hg(0)

Illuminates some basic biology of enzymology, gene regulation, redox metabolism

Employed in paradigm example of engineered metallophytoremediation
Transgenic *merA* tobacco plants survive transplantation to contaminated soils and detoxify Hg(II) to less toxic Hg(0)


Poster, Weds night
The Bacterial Mercury Resistance Locus
The Bacterial Mercury Resistance Locus

MerR, a mechanistically novel regulator

MerA, curiously chimeric oxido-reductase
Hg(II) provokes MerR to underwind the MerO dyad center

Using mutants to dissect the mechanism of metal specificity


Cd(II)-Responsive MerR Mutants

Activity (Miller Units)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Water</th>
<th>Hg(II)</th>
<th>Cd(II)</th>
<th>Zn(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild Type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Derepressed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R53Q</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>E72K</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>L74S</td>
<td></td>
<td></td>
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<tr>
<td>L76F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A85V</td>
<td></td>
<td></td>
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<tr>
<td>A89V</td>
<td></td>
<td></td>
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<tr>
<td>K99Q</td>
<td></td>
<td></td>
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<tr>
<td>K99T</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>M106V</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S125P</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S131L</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
MerR and MBD bind metals other than Hg in vitro and in vivo, possibly with differing specificities.
MerR binds other thiophilic metals in vivo and in vitro so its specificity as a transcriptional activator must lie in more than just metal binding.

Possibilities:

Other metals do not provoke DNA distortion
  YES, Chuan He, U. Chicago, JACS 2004

Other metals don’t bind MerR when it is bound to DNA
  NO, Song et al., JMB 2007, in press

Does Hg(II) provoke a conformational change distinct from that of non-inducers?
**19F NMR: Watching MerR’s Tyrosines**

Y27 (Conserved)  Y40 (not conserved)

MENNLENLTIGVFAKAAGVNVETIRFYQRKGLLREPDPKPYGSIR
Y46 (conserved)

Y40 (not conserved)

RGEADVVRVKFVKSAQRLGFSLDEIAELLRLDDGT

HCEEASSL

AEHKLKDREKMADLARMETVLSELVCA

CHARKGNVSCPLIASL

QGEAGLARSAMP

SAWSHPQFEK

![Chemical structures](image)

Tyrosine  2-Fluorotyrosine (2-FY)

pKa  10.05 ± 0.04  9.04 ± 0.03
A Candidate Allosteric Signalling Pathway in MerR

**B**

\[
\begin{align*}
\alpha_1 & \text{MENNLENL TIGVFAKAA GVNVETIRFYQRKGLLRE} \\
\alpha_2 & \text{PDKPYGSIRR YGEAD VVRKFVKSAQRL GFS LDE} \\
\alpha_3 & \text{IAELLRL DDGTH CEEASLAEHKLKDVRE KMADL} \\
\alpha_4 & \text{ARMETVLSLEYCA CARKGNVS CPLIAS LQGEA} \\
\alpha_5 & \text{GLARSAMP}
\end{align*}
\]
Using substitution mutants to assign resonances

2-FY-MerR

2-FY-Y40E

2-FY-Y46F
MerR only

Hg/MerR₂ 1.0

Cd/MerR₂ 1.0

Zn/MerR₂ 1.0

ppm
MerOP DNA binding produces large chemical shift changes in Y27 and Y46 of wildtype MerR and mutant Y40E
Metal-specific changes occur at Y27 and Y46 when MerR is bound to MerOP

So DNA constrains MerR’s response to each metal…

…and C82Y in the metal-binding site ‘notices’ DNA binding.
A Candidate Allosteric Signalling Pathway in MerR

**A**

**B**

MENNLENL **TIGVFAKAA** GVNV**ETIRFYQR** KGLLRE

PDKP **Y** GSI**R** YGEAD **VVRKVFKVS**AQRL GFS **LDE**

α1

α2

α3

α4

α5

α6

ARMETVSELVCA C**HARKGNVSC**PLIAS LQGEA

GLARSAMP

**C**

Q28

Y46

Q61

Y46

Y27

Y46' Y27' Y46 Y27 K99' K99 M106' M106 C126' C126 C82 C82 C117' C117 L63 K99' M106' C126' C82 C117'
Typical Structural Components of MerA

One or two repeats of a domain that is homologous to small proteins that bind soft metals such as $\text{Cu}^{+1}$, $\text{Zn}^{+2}$, $\text{Hg}^{+2}$.

- **Catalytic Core**
  - Multidomain flavoprotein homologous with glutathione reductase, obligate dimer
  - Flexible linker

- **Tn501**
  - AA 70-95
  - AA 1-69
  - AA 96-561

- **NmerA**
  - Cysteines
C-terminal CC Remove High Affinity RS⁻ Ligands

Reduction occurs from here

NADPH
FAD
Hg\((SR)_2\)

interdomain complex

outer complex

inner complex

middle complex
NmerA Facilitates Transfer from Hg-Thioredoxin in vitro

\[ k_{\text{cat}}/K_{\text{MHg-TRX}} = 3.0 \times 10^4 \text{ M}^{-1} \text{ s}^{-1} \]

\[ K_{\text{MHg-TRX}} \sim 300 \mu\text{M} \]

\[ k_{\text{cat}}/K_{\text{MHg-TRX}} = 6.0 \times 10^3 \text{ M}^{-1} \text{ s}^{-1} \]

\[ K_{\text{MHg-TRX}} \sim 1200 \mu\text{M} \]
Potential Modes of MerB/MerA Interactions

A) Transient interaction with direct transfer to Core C-terminal cysteines

B) Transient interaction and transfer to NmerA only

C) Stable complex with Core but transfer facilitated by NmerA

Cys-S(H)  • Hg(II)
**NmerA Facilitates Transfer from Hg-MerB**

Consistent with Models B &/or C
Coming Attractions !!
Bacterial cell contents to scale.
The Mercury Shock Proteome --
With and without the *mer* Operon