Virulence and Antibiotic Resistance
Regulation in Human Pathogens

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Threats from pathogens

- **Staphylococcus aureus**
  - 12 million patients are at risk each year in US
  - Has a crude mortality rate of 25%

- **Pseudomonas aeruginosa**
  - Accounts for 10.1 percent of all hospital-acquired infections
  - Averages about 4 infections per 1000 discharges in US hospitals

- **M. tuberculosis**

Challenges:
1. Counter the rise of antibiotic-resistance
2. Control virulence

Strategies to treat infections

- Inhibitors of cell wall synthesis: beta-lactams and vancomycin
- Inhibitors of protein synthesis: aminoglycosides, tetracyclines, lincomamides, streptogramins and chloramphenicol
- Inhibitors of DNA synthesis: quinolones
- Inhibitors of RNA synthesis: rifamycins

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Antibiotic resistance

http://www.bioteach.ubc.ca/Biomedicine/CationicPeptides/antibiotresist.gif

E. coli MarR
Repressor for drug efflux pumps

Proposed mechanism: drug-binding induces dissociation of MarR from promoter DNA which leads to activation of drug efflux genes and antibiotic resistance

Martin, R. G. & Rosner, J. L., PNAS, 1995, 92, 5456-5460

Bacterial multidrug resistance regulators


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MarR family transcriptional factors in *S. aureus*

- mgrH1 (SAV2386)
- mgrH2 (SAV2265)
- mgrH3 (SAV2520)

*S. aureus* is responsible for most wound and hospital acquired infections.

Susceptibility of *S. aureus* mutant strains to norfloxacin (NOR)

Resistance to quinolone for *S. aureus* strains
Virulence test using the murine abscess model of infection

Crystal structure of MgrA

The molecular mechanism for MgrA-based regulation?

1. MgrA is a DNA-binding protein (transcription repressor?)

2. MgrA mutant shows resistance to quinolone-type antibiotics and vancomycin!!! The mutant also shows higher susceptibility towards oxacillin-type antibiotics

3. MgrA does not bind these molecules that are quite different in structure

How does MgrA regulate S. aureus response to these antibiotics and virulence? MgrA controls expression of ~350 genes in S. aureus
A unique Cys residue

Oxidation sensing?

Cysteine sulfenic acid formation in MgrA

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**Gel-shift assay to study MgrA-DNA interaction**

[Image of gel-shift assay]

CHP: cumene hydroperoxide

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**Bio-relevance: plate sensitivity assays**

[Image of plate sensitivity assays]

Oxidative stress "activates" antibiotic resistance of *S. aureus* via MgrA

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**Further in vivo results**

[Image of in vivo results]

Antibiotic resistance levels determined in the absence or presence of paraquat (PQ), a reagent known to induce oxidative stress in vivo

Induction of *norA*, a gene repressed by mgrA, by oxidative stress

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Mgra is a redox switch in *S. aureus*…

- Mgra uses an oxidative sensing mechanism to sense oxidative stress and defend *S. aureus* against antibiotics and, perhaps, our immune responses in a global manner!
- Our immune response to bacteria infection is to generate various ROS (peroxides, superoxide, NO) to kill pathogens
- The antibiotics that the Mgra mutant responds to also induce oxidative stress
- This could be a general concept true for most pathogens


SarZ, an Mgra homologue in *S. aureus*…

- Mgra regulates ~350 genes
- MgrH1 (SarZ), a homologue of Mgra, regulates ~80 genes
- Most noticeably SarZ seems to regulate switching from O\textsubscript{2}-dependent metabolisms to anaerobic energy production pathways!

SarZ is also an oxidation sensor
SarZ affects virulence, antibiotic resistance and autolysis


SarZ affects virulence, antibiotic resistance and autolysis

Crystal structure of the reduced SarZ

SarZ

MgrA

Crystal structure of the oxidized SarZ with Cys13-SOH

Oxidized

SarZ

Cys-SH

Cys-SOH

Cys-S-SR

Poor, C. B., Chen, P. R., Duguid, E. M., Rice, P. A. and He, C. J. Biol. Chem. 2009, 284, 23517-23524

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How *Staphylococcus aureus* senses host immune defense

- Molecular level signals and mechanisms that control the virulent states of *Staphylococcus aureus*
- Host signals that affect the virulence of the pathogen and regulatory pathways in bacteria in response to these signals

**Hypothesis**

- MgrA regulates ~350 genes
- SarZ, a homologue of MgrA, regulates ~80 genes

Small molecules to “trick” *S. aureus* into the low virulence form
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Strategies

PVS works like hydrogen peroxide

An epoxide as a mild and selective alkylator

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Phenyl epoxide sulphonate (PES) activates MgrA

Antibiotics plate assay:
VCM 1.4 μg/ml

Wild-type
100 μM
200 μM
300 μM
400 μM
500 μM

Northern blot assay:

Wild-type
mgrA

RNAIII: activated by MgrA

The \textit{in vivo} effect of the epoxide

Developing small molecule modulators
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FA-based HTS for non-covalent modulators

High throughput screen

Fluorescence anisotropy

\[ \frac{1}{P} = \frac{1}{P_0} \left( \frac{V}{M} \right) \]

V: molecular volume (molecular weight)

Large anisotropy P
Small anisotropy P

NSRB at Harvard

100,000 compounds were screened and over 100 hits were obtained.
NSRB: The National Screening Laboratory for the Regional Centers of Excellence in Biodefence

Other pathogens

MgA homologues can be found in S. epidermidis, B. anthracis, Vibrio alginolyticus, Brucella abortus biovar, Listeria monocytogenes, Enterococcus faecium, Clostridium perfringens, Streptococcus agalactiae, Pseudomonas aeruginosa and Mycobacterium tuberculosis

Pseudomonas aeruginosa
Mycobacterium tuberculosis
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Two MgrA homologues in *P. aeruginosa*

MgrA? PA2825

MgrH1? PA2849

OspR (oxidative stress response and pigment production regulator) in *P. aeruginosa*

Cys24 in OspR is redox active
OspR plays a role in virulence

Lan, L. and He, C. Mol. Microbiol. 2010, 75, 76.

P. aeruginosa MexR

MexR regulates antibiotic resistance in P. aeruginosa


Hypothesis

 MexR regulates antibiotic resistance in P. aeruginosa.
MexR oxidation leads to its dissociation from DNA

1 μM MexR dimer can be readily dissociated by treatment of DTT (1-2 μM)

Cys30 and Cys62 are engaged in oxidation sensing

Intermonomer disulfide bond formation in MexR

MexR oxidation leads to its dissociation from DNA

Computational analysis of the oxidized MexR structure

By Hu and Dinner

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Redox potential measurement of MexR

The redox potential was determined to be ~ -155 mV

Experiments probing in vivo response

MexR senses peroxide stress and regulates antibiotic resistance

1. Oxidation sensing is a mechanism that pathogens use to sense adverse conditions (including host immune response) and regulate responses in a global manner.

a. MgrACys12—SH → MgrACys12—SOH
   SarZ (MghH1)
   OspR in P. aeruginosa
   MgrA in Mtb

b. MexRCys30—SH → MexRCys30—S
   MexRCys30—SH
   MexRCys62’—SH, MexRCys62’—S
   E. coli MarR...

2. Fundamental signaling mechanism of the two-component system.

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