Mutations in \textit{rsmG}, Encoding a 16S rRNA Methyltransferase, Result in Low-Level Streptomycin Resistance and Antibiotic Overproduction in \textit{Streptomyces coelicolor} A3(2)

Nishimura, K., T. Hosaka, S. Tokuyama, S. Okamoto, and K. Ochi

National Food Research Institute, Ibaraki, Japan

Microbiology Journal Club

Youwen Pan

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GUEST COMMENTARY

Novel Links between Antibiotic Resistance and Antibiotic Production

Justin R. Nodwell

Department of Biochemistry and Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada
Introduction

• Streptomycin was discovered to be a potent drug against *Mycobacterium tuberculosis* in 1944.

• The first mutants resistant to streptomycin were reported two years after.

• The mutants could be classified into two distinct types.

Selman A. Waksman 1888 – 1973
Two Types of Streptomycin-Resistant Mutants

• Type I
  – resistant to high concentration (>100 µg/ml)

• Type II
  – resistant to much lower concentrations (MIC, 5-10 µg/ml)
Type I Resistance in *M. tuberculosis*

- Mutations are often in *rpsL* (S12), *rrs* (16S rRNA),
- Mutations lead to a hyperaccurate phenotype.
- Mutations within 16S rRNA are often in 530 loop, but are found in a limited proportion.
Interaction of streptomycin with the 30S ribosomal subunit in *E. coli*

*Nature* 407, 340-348
Why is *S. coelicolor* A3(2) used?

- *S. coelicolor* A3(2) is a special bacteria.
- “Ribosomal engineering” project.
Streptomyces

• Unique bacteria.
  – Soil organisms.
  – G⁺
  – Form branching filaments and external spores.
  – Can adapt to extreme conditions.
  – Production and secretion of numerous second metabolites, including antibiotics and enzymes.
S. coelicolor A3(2)

- Isolated by Professor Sir David Hopwood F.R.S. in 1947 at John Innes Centre, Norwich, U.K.
- Used in genetic study since 1950’s.
- Mostly used to study the regulatory mechanisms in antibiotic production.
- Best characterized strain in the genus.
- Produces at least four different antibiotics, including tripyrrole undecylprodigiosin (Red) and polyketide actinorhodin (Act).
## General features of *S. coelicolor* A3(2) chromosome

<table>
<thead>
<tr>
<th>Component</th>
<th>Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total size</td>
<td>8.7M bp</td>
</tr>
<tr>
<td>G+C content</td>
<td>72.12%</td>
</tr>
<tr>
<td>ORFs</td>
<td>7,825</td>
</tr>
<tr>
<td>Ribosome RNAs</td>
<td>6</td>
</tr>
<tr>
<td>Sigma factors</td>
<td>65</td>
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</tbody>
</table>

*Nature 417:141-7 (2002)*
Two Types of Streptomycin (Sm)-Resistant Mutants

• Type I
  – Resistant to high concentration (>100 µg/ml)
  – Due to mutations within *rpsL* (S12).

• Type II
  – resistant to much lower concentrations (5-10 µg/ml)
  – **Molecular mechanisms are unknown!**
Odd effects

• Both types of mutations on S. coelicolor confer overproduction of the secondary metabolite actinorhodin (Act).

• The strR mutations can overcome the effects of mutations in genes such as relA, relC, and brgA that impair Act production.

• Compared with the WT, type I (rspL) mutant ribosomes
  – Capability of protein synthesis is enhanced at the late growth phase.
  – 70S ribosomes are more stable. --- *Microbiology*. 149, 3299-309. (2003)
Type II Resistance

• **Molecular mechanisms are unknown!**

• **Facts**
  
  – S-adenosylmethionine (SAM) synthetase is highly expressed.
  
  – SAM synthetase in the mutant showed 5-10 fold higher than in WT.

\[ \text{metK} \]

S-adenosylmethionine (SAM) synthetase

\[ \text{actII orf4} \]

Actinorhodin (Act) gene cluster

Identification of mutation in Type II Sm resistance

• Comparative genome sequencing (CGS)
  – Microarray based DNA sequencing
  – To find point mutations in bacterial genomes
    • single nucleotide polymorphisms (SNPs)
CGS Process


(Metronidazole resistance in Helicobacter pylori)
CGS protocol (NimbleGene)

DNA extraction

Labeling

Hybridization

Imaging and analyzing

Resequencing array for the identified regions of genomic difference

http://www.nimblegen.com/
CGS analysis of the mutant strain with SignalMap (NimbleGene)

Probe intensity ratio

Wild type

mutant

SNP 488A→Δ

$rsmG$
The $\Delta rsmG$ mutant showed increased resistance to Streptomycin

$\neg Sm$  $\neg Sm$  $\neg Sm$  $\neg Sm$

$\neg Sm$  $\neg Sm$  $\neg Sm$  $\neg Sm$

$\Delta rsmG$  $\Delta rsmG$  $\Delta rsmG$  $\Delta rsmG$

$\neg Sm$  $\neg Sm$  $\neg Sm$  $\neg Sm$

$\neg Sm$  $\neg Sm$  $\neg Sm$  $\neg Sm$

$\Delta rsmG$  $\Delta rsmG$  $\Delta rsmG$  $\Delta rsmG$

$\neg Sm$  $\neg Sm$  $\neg Sm$  $\neg Sm$

$\neg Sm$  $\neg Sm$  $\neg Sm$  $\neg Sm$
RsmG Catalyzes an $m^7G$ modification within the *S. coelocolor* 16S rRNA
RsmG is a $m^7$G methyltransferase, targeting G527 of 16S rRNA in *E. coli*

- $m^7$G modification is restricted to a single position in *E. coli* 16S rRNA.
- RsmG has equivalent functions in *E. coli* and *S. coelicolor*.
- G518 in *S. coelicolor* is corresponding to G527 in *E. coli*.

*Mol. Microbiol.* 63, 1096-1106
The $\Delta rsmG$ mutant showed increased Act production and actII-ORF4 expression.
Type II Resistance

• Facts
  – S-adenosylmethionine (SAM) synthetase is highly expressed.
  – SAM synthetase in the mutant showed 5-10 fold higher than in WT.

*metK*
S-adenosylmethionine (SAM) synthetase

*actII orf4*
Actinorhodin (Act) gene cluster

RsmG has a putative SAM binding motif within its primary structure.

<table>
<thead>
<tr>
<th>Species</th>
<th>RsmG Peptide Sequence</th>
<th>晶体结构显示E. coli RsmG包含在三元结构中的SAM依赖性甲基转移酶折叠。</th>
<th>RsmG催化一个SAM依赖性的7-甲基鸟苷(m^7G)修饰G527在16S rRNA。</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.avermitilis RsmG</td>
<td>RHLLNCAVLSEVVPEGVTCDVGSGAGLPGPVLALVREDLKITLEPLRRRTNFLTEVE</td>
<td></td>
<td></td>
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<td>M.tuberculosis RsmG</td>
<td>RHLLNCAVIGELLERDRVV</td>
<td></td>
<td></td>
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<td>N.farcinica RsmG</td>
<td>RHLNCVAELMPESATVVDVGSGAGLPGPVLAIARPDLLQVELLLEPLLRTESLREMVT</td>
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<tr>
<td>E.coli RsmG</td>
<td>LDISAVP-PYLQ-RI-DVGTGPGLPGIPLSIVRPEAHFTLLDSLGKRVRFLRQVQH</td>
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<td></td>
</tr>
<tr>
<td>B.subtilis RsmG</td>
<td>YDSITAAP-FYDFNQVNTICDVGAGAGFPSPILKICFPHLHVTIVDLSNKRFLEKLSE</td>
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<td></td>
</tr>
</tbody>
</table>
ΔrsmG mutant is positive on metK expression

Measuring pyrophosphate production
Translational activity of ΔrsmG mutant ribosomes increased
Unlike rpsL mutant, ∆rsmG mutant did not show effects on RRF levels.

Unlike \textit{rpsL} mutant, $\Delta rsmG$ mutant 70S ribosomes did not show more stable than that of WT cells.
Summary

**actII orf4**
Actinorhodin ➖

**metK**
SAM ➖

**rsmG mutant**
Streptomycin resistant
Translation during stationary phase

**actII orf4**
Actinorhodin ➖

**metK**
SAM ➖

Wild type
RsmG + SAM

Streptomycin sensitive
Translation during stationary phase

*J. Bacteriol.* 2007 189: 3683-3685