Hokkaido University
Leading Graduate School
Veterinary Science for One Health

The 8th Leading Special Lecture

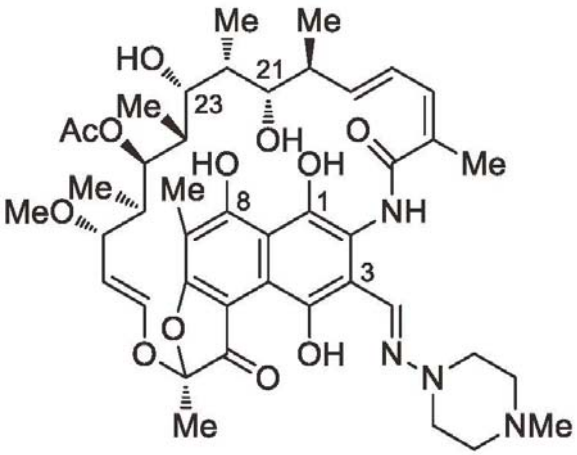
X-ray crystal structure determination of bacterial RNA polymerase toward better understandings of antibiotic mechanism of action, resistance and new antibiotic development

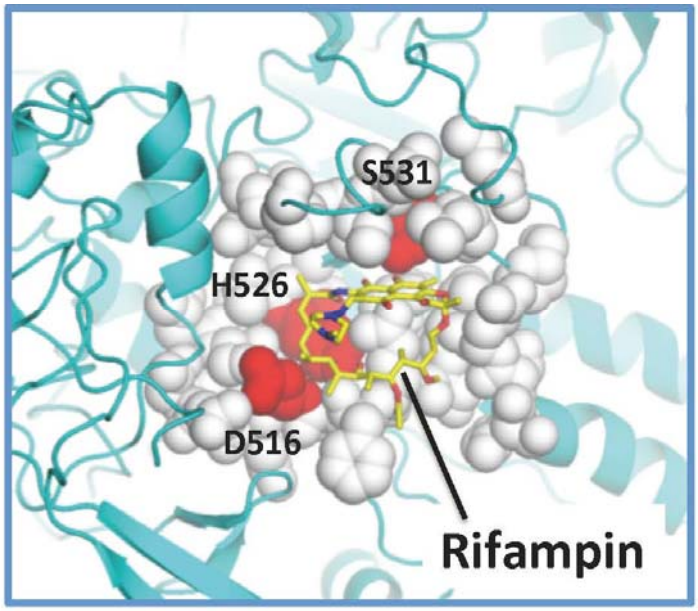
July 5(Fri), 2013, 16 : 00～17 : 30

Lecture Hall, Graduate School of Veterinary Medicine, Hokkaido University, JAPAN

Dr. Katsuhiko Murakami

Dept. of Biochemistry and Molecular Biology
The Pennsylvania State University





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July 5, 2013, 16：00～17：30

Lecture Hall, Graduate School of Veterinary Medicine,

Hokkaido University, JAPAN

Program

16:00～17:10

Dr. Katsuhiko Murakami,

(Associate Professor ; Department of Biochemistry and Molecular Biology, Pennsylvania State University)

17:10～17:30

Discussion

Katsuhiko Murakami, Ph.D.

Department of Biochemistry and Molecular Biology
6 Althouse, Pennsylvania State University
University Park, PA 16802

http://bmb.psu.edu/directory/kum14

Education

Graduate University for Advanced Studies, Japan
Ph.D., 1997, Department of Genetics, School of Life Science *Advisor: Akira Ishihama*
Yamaguchi University, Yamaguchi, Japan
Master of Chemistry, 1994, Department of Chemistry
Yamaguchi University, Yamaguchi, Japan
Bachelors of Chemistry, 1992, Department of Chemistry

Experience

2009 - present	**Pennsylvania State University**, College of Science	University Park, PA
	Department of Biochemistry and Molecular Biology	*Associate Professor*
2003 - 2009	**Pennsylvania State University**, College of Science	University Park, PA
	Department of Biochemistry and Molecular Biology	*Assistant Professor*
1998 - 2003	**The Rockefeller University**	New York, NY
		Postdoctoral Fellow - Supervisor: Seth A. Darst
1997 - 1998	**National Institute of Genetics**	Mishima, Shizuoka, Japan
		Postdoctoral Fellow - Supervisor: Yasuo Shirakihara

Award The Pew Scholars Award, HFSP postdoctoral fellowship, Norman and Rosita Winston postdoctoral fellowship

Selected Peer-reviewed Publications (from 42 publications)

- Molodtsov, V., I.N. Nawarathne, N.T. Scharf, P.D. Kirchhoff, H.D.H. Showalter, G.A. Garcia and **K.S. Murakami** (2013). X-ray crystal structures of the *Escherichia coli* RNA polymerase in complex with Benzoxazinorifamycins. ***J. Medicinal Chem***. on-line publication. PMID: 23679862
- **Murakami, K.S**. (2013). The X-ray crystal structure of *Escherichia coli* RNA polymerase Sigma^{70} Holoenzyme. ***J Biol Chem***., **288**, 9126-9134. PMID: 23389035
- Hirata, A., B.J. Klein, and **K.S. Murakami** (2008). The X-ray crystal structure of RNA polymerase from Archaea. ***Nature*, 451**, 851-854. PMID: 18235446
- **Murakami, K.S**., S. Masuda, and S.A. Darst (2002). Structural basis of transcription initiation: *T. aquaticus* RNA polymerase holoenzyme at 4 Å resolution. ***Science* 296**, 1280–1284.
- **Murakami, K.S**., S. Masuda, E.A. Campbell, O. Muzzin, and S.A. Darst (2002). Structural basis of transcription initiation: an RNA polymerase holoenzyme-DNA complex. ***Science* 296**, 1285-1290.
- Campbell, E.A., N. Korzheva, A. Mustaev, **K. Murakami**, S. Nair, A. Goldfarb, and S.A. Darst (2000). Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. ***Cell* 104**, 901–912.

Title: X-ray crystal structure determination of bacterial RNA polymerase toward better understandings of antibiotic mechanism of action, resistance and new antibiotic development

Katsuhiko Murakami
Department of Biochemistry and Molecular Biology, The Center for RNA Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA

Tuberculosis (TB) is one of the most significant global challenges to human health. For over four decades, Rifampicin (Rif, aka Rifampin), a semi-synthetic derivative of Rifamycin, has been used as a first line antibiotic treatment of TB and is the cornerstone of current short-term TB treatment. The mode of action involves tight Rif binding to a beta subunit of bacterial RNA polymerase (RNAP) (K_d is sub nanomolar) to inhibit RNA transcription. Although many Rif resistant (Rif^R) strains with mutations in the Rif-binding pocket can be isolated in bacterial culture, only three specific Rif^R mutations account for over 80 % of *Mycobacterium tuberculosis* (MTB) Rif^R strains in clinical isolates due to Rif^R associated fitness costs.

Recently, we have shown that the *Escherichia coli* RNAP can be prepared from a convenient overexpression system and its X-ray crystal structure can be determined (1). We have also determined the crystal structures of the *E. coli* RNAP Rif^R mutants each having one of three major Rif^R mutations found in clinical isolates. Each Rif^R RNAP structure shows a unique conformation of the Rif binding pocket and their structural deviations from the wild-type Rif binding pocket are consistent with their Rif resistances suggesting that the Rif^R results from alternating the shape complementary between the Rif binding pocket and Rif in addition to disrupting hydrophilic and hydrophobic interactions. This study provides an important step toward developing superior Rif analogues for the Rif^R MTB.

Among many synthetic Rifamycin derivatives, benzoxazinorifamycins including Rifalazil have great potential for TB treatment because of their superior affinity toward wild-type and Rifamycin-resistant mutants of the MTB RNAP, and their reduced hepatic cytochrome P450 (Cyp450) induction activity for preventing drug-drug interactions. We have determined the crystal structures of the *E. coli* RNAP complexes with two benzoxazinorifamycins. The ansa-naphthalene moieties of the benzoxazinorifamycins bind in a deep pocket of the β subunit, blocking the path of elongating RNA transcript at 3 nucleotides in length. The C3'-tail of one benzoxazinorifamycin fits a cavity between the β subunit and σ factor. We propose that this novel interaction influences the template DNA conformation at the active site thereby reducing the efficiency of transcription initiation as well as blocking RNA transcripts. This study supports further expansion of structure−activity relationships of benzoxazinorifamycins against RNAP inhibition toward uncovering superior analogues with development potential.

References:

K.S. Murakami. The X-ray crystal structure of *Escherichia coli* RNA polymerase sigma70 holoenzyme. *J. Biol. Chem*. 2013, **288**, 9126-9134.

V. Molodtsov, I.N. Nawarathne, N.T. Scharf, P.D. Kirchhoff, H.D.H. Showalter, G.A. Garcia and K.S. Murakami (2013). X-ray crystal structures of the *Escherichia coli* RNA polymerase in complex with Benzoxazinorifamycins. *J. Medicinal Chem.* on-line publication. PMID: 23679862

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