RaPID results

By Chris Cain, Senior Writer

A Japanese team has provided new structural insights into the function of a conserved class of drug transporters and has identified cyclic peptide inhibitors of one such protein.¹ The researchers are now collaborating with **PeptiDream Inc.** to develop compounds with improved drug-like properties that hit medically relevant targets.

The multidrug and toxic compound extrusion (MATE) class of transporters exports diverse cationic chemical substrates and is conserved across all domains of life. Although the physiological roles of MATEs are still being worked out in humans and other organisms, in the lab they are capable of exporting antibiotics in multidrug-resistant pathogenic bacteria including *Neisseria gonorrhea* and *Staphylococcus aureus*, though their contribution to clinically relevant drug resistance remains unclear.

MATEs import Na⁺ or H⁺ as they export their substrate. The first crystal structure of a MATE transporter was solved in 2010, but its resolution did not allow a complete understanding of the molecular mechanism by which ion import drives drug export.²

To flesh out the mechanism, researchers at **The University of Tokyo** and the **RIKEN Advanced Science Institute** sought to capture high-resolution structures of a MATE by using a structurally stable homolog from the thermophile *Pyrococcus furiosus*. The team crystalized the transporter in multiple conformations, including in complexes with a fluoroquinolone antibiotic substrate, norfloxacin, and with newly developed macrocyclic inhibitors of the target.

The inhibitors were developed using the technology platform known as random, nonstandard peptide integrated discovery (RaPID).³ RaPID uses *in vitro* mRNA display to synthesize and screen against trillions of peptides that contain a mix of natural and unnatural amino acids. The system enabled the identification of thioether-macrocyclic peptide inhibitors that blocked *P. furiosus* MATE substrate transport at low micromolar concentrations.

RaPID was developed in the lab of Hiroaki Suga, a co-corresponding author of the study who is the cofounder and external executive officer of PeptiDream, which is commercializing the technology.⁴ Suga also is a professor in the Department of Chemistry at the University of Tokyo.

Together, the series of structures showed that the transporter adopts two distinct outward-facing conformations and suggested how a substrate is extruded by the transporter upon H⁺ import. Like most transport proteins, MATEs bind intracellular substrates in inward-facing

conformations, then transition to outward-facing conformations to release their substrates.

Co-corresponding author Osamu Nureki, professor in the Department of Biochemistry and Biophysics at the University of Tokyo, told *SciBX* that the export mechanism is the most important new piece of information provided by these structures.

Hendrick van Veen, senior lecturer in the Department of Pharmacology at the **University of Cambridge**, agreed. “One key finding of the current structure is that it provides new insights into the question of how substrate transport is coupled to the movement of protons,” he said. “Based on [the MATEs’] structures, the researchers propose that when a proton binds to the MATE transporter, it switches the conformation to allow the dissociation of the toxin molecule into the extracellular environment.”

Geoffrey Chang, whose team crystalized the first MATE transporter in 2010, said the resolution of the new transporter structures was critical

to enabling the new insights. Chang’s structure of the *Vibrio cholera* Na⁺ MATE transporter NorM was resolved at 3.65 Å. The series of structures presented in the new work range from 2.1–3 Å.

Chang is a professor of pharmacology at the **University of California, San Diego**.

Nureki now plans to conduct studies on more therapeutically relevant MATE proteins, including those from pathogenic bacteria and humans, and to solve the structures of the transporters complexed with

specific inhibitory peptides. “Our inhibitory cyclic peptide paves the way toward the development of efficient inhibitors against previously undruggable MATE transporters,” he said. “Unfortunately, because traditional antibiotics are small and readily transported out by MATEs, it was difficult to discover potent inhibitors. The thioether-macrocyclic peptides are the right molecular size to fit into the active pocket of MATE and effectively clog it and inhibit its function.”

RaPID results

Suga said the team plans to use RaPID to optimize inhibitory peptides with unnatural and D-amino acids, which are more resistant to proteases found in the blood and can increase cell permeability compared with unmodified L-amino acids.

van Veen said he was impressed that the cyclic peptides were able to block transport without entering the cell. “The researchers have identified a new type of inhibitor for MATE that acts by binding to outward-facing MATE from the exterior of the cell. This represents a very promising approach to inhibit multidrug transporters in a clinical setting because the inhibitor does not need to enter the cell to exert its action,” he said.

van Veen and Chang both said the next key advance for understanding MATE transport will come from solving the structure of an inward-facing MATE transporter.

Robert Stavenger, group leader at **GlaxoSmithKline plc** and project coordinator for TRANSLOCATION, an **Innovative Medicines**

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The University of Tokyo**

Initiative program studying drug transport in Gram-negative bacteria, said the work provides a promising model of MATE transporter action. However, he expressed concern that MATEs have not yet been linked to clinically relevant drug resistance in bacteria.

“MATE transporters have been implicated as a potential mechanism for resistance against *S. aureus* but mostly in the laboratory setting. It doesn't seem that MATE transporters are strongly involved in clinically relevant antibacterial resistance at this time. As such, I would consider them less important than, for example, NorA in *S. aureus* or the various resistance-nodulation–cell division (RND) efflux systems in Gram-negative bacteria,” he said.

NorA is a member of a distinct class of drug transporters and contributes to fluoroquinolone resistance in *S. aureus*, whereas members of the RND class of transporters have been linked to resistance in bacteria including the emerging Gram-negative pathogen *Acinetobacter baumannii*.

Suga said the concern is valid and that it is challenging to combat antibiotic resistance by targeting only one transporter because bacteria encode numerous families of drug efflux pumps. He did say the study provides proof of concept for a strategy to identify cyclic peptide inhibitors against other families of drug transporters.

Nureki said the group now plans to expand its studies beyond MATEs and conduct studies of additional efflux pumps, including ATP-binding cassette transporters associated with drug resistance.

Results were published in *Nature*. Patent applications have been filed by the University of Tokyo and are exclusively licensed to PeptiDream.

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REFERENCES

1. Tanaka, Y. *et al. Nature*; published online March 27, 2013; doi:10.1038/nature12014
Contact: Osamu Nureki, The University of Tokyo, Tokyo, Japan
e-mail: nureki@biochem.s.u-tokyo.ac.jp
Contact: Hiroaki Suga, same affiliation as above
e-mail: hsuga@chem.s.u-tokyo.ac.jp
2. He, X. *et al. Nature* **467**, 991–994 (2010)
3. Yamagishi, Y. *et al. Chem. Biol.* **18**, 1562–1570 (2011)
4. Kotz, J. *SciBX* 5(4); doi:10.1038/scibx.2012.87

COMPANIES AND INSTITUTIONS MENTIONED

GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Innovative Medicines Initiative, Brussels, Belgium
PeptiDream Inc., Tokyo, Japan
RIKEN Advanced Science Institute, Saitama, Japan
University of California, San Diego, La Jolla, Calif.
University of Cambridge, Cambridge, U.K.
The University of Tokyo, Tokyo, Japan