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Drug Resistance: A Periplasmic Ménage à Trois

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The paradigm tripartite efflux transporter AcrA-AcrB-TolC confers multiple drug resistance to *Escherichia coli*. Tikhonova et al. (2011) now examine how the three components connect to unity and highlight the critical role of AcrA membrane proximal domain conformation for successful assembly.

In Gram-negative bacteria, a clever network of multidrug transporters, including tripartite efflux systems that expel drugs from the cytoplasm and periplasm to the cell exterior, secure survival under antibiotic stress and lead as a result to the occurrence of multidrug resistance. The paradigm of a tripartite drug efflux system is the *Escherichia coli* AcrA-AcrB-TolC complex. This system is composed of the membrane fusion protein (MFP) AcrA and two membrane proteins: TolC, a channel in the outer membrane, and AcrB, a proton-dependent drug antiporter of the inner membrane (Figure 1). This multimodule efflux system only confers drug resistance if all three partners are present in the periplasmic space—i.e., it is reliant on its Ménage à Trois.

X-ray structures of all three components of the AcrA-AcrB-TolC system, each elucidated individually but hitherto not as a complex, have yielded enormous insight in the atomic blueprints (Figure 1) (Koronakis et al., 2000; Mikolosko et al., 2006; Symmons et al., 2009; Murakami et al., 2006; Seeger et al., 2006). These structures and a substantial amount of functional studies (Nikaido and Takatsuka, 2009) indicated that the inner-membrane component AcrB acts as the motor for the entire assembly, being a module for both the energy transduction and substrate specificity. The current hypothesis suggests a three-stroke functional rotation of the AcrB monomers, driven by the proton-motive force, resulting in the access, binding, and extrusion of multiple drugs (Seeger et al., 2008). The structures of the MFP AcrA and its *Pseudomonas aeruginosa* homolog MexA (Koronakis et al., 2000; Mikolosko et al., 2006; Symmons et al., 2009) display a multidomain polypeptide including an α-helical hairpin, a lipoyl domain, a β-barrel domain, and the essential membrane proximal (MP) domain tethered to the inner membrane via a lipid anchor. The latter three domains interact with AcrB (Symmons et al., 2009), whereas the α-helical hairpin is postulated to form a coiled-coil interface with the TolC α-barrel, a 100 Å conduit reaching into the periplasmic space ready to interact with the TolC docking domain of AcrB (Figure 1).

However, the assembly process of the three components AcrA, AcrB, and TolC using the periplasmic space as a meeting place is not understood. The outer membrane channel TolC is used as a substrate conduit by many (tripartite) systems, sometimes in combination with ABC-transporters, sometimes with secondary H^+ (or Na^+)/substrate antiporters belonging to the Major Facilitator or Resistance Nodulation cell Division superfamilies. Despite the TolC promiscuity, complex formation appears to be highly regulated, depending not only on TolC-MFP kinetics but also on MFP oligomerization kinetics and pH (Tikhonova et al., 2009).

One recurring question is the stoichiometric composition of the tripartite system. The high resolution structure of TolC depicted an intrinsic trimer (Koronakis et al., 2000). Asymmetric as well as symmetric structures of AcrB also reveal a trimeric arrangement (Seeger et al., 2008). However, the oligomeric state of the MFP in complex with the membrane components has been highly debated. Crosslinking data combined with docking experiments lead to the postulation of a structure of a complete tripartite system (Symmons et al., 2009), suggesting a 3:3:3 stoichiometry for AcrA-AcrB-TolC. However, a recent structure of CusBA, an inner-membrane component and MFP of a tripartite heavy metal efflux system, shows a hexameric arrangement of the MFP in the bipartite complex (Su et al., 2011).

In this issue of *Chemistry and Biology*, Tikhonova et al. (2011) tackle the question of tripartite complex formation of AcrA-AcrB-TolC, using mainly surface plasmon resonance (SPR) analysis to obtain kinetic data on the interactions between the components involved.

Tikhonova et al. (2011) begin by addressing the oligomeric state of AcrA. They reveal a difference between the lipidated form of AcrA, indicating dimer formation, and the lipid anchor-less soluble form that shows monomeric features. Dimeric AcrA interacts with AcrB with an affinity in the nanomolar range, whereas the soluble monomeric version of AcrA exhibits one order of magnitude lower affinity to AcrB. This difference in affinity is explained by higher stability of the MP domain and dimerization of lipidated AcrA. Their results support a 6:3 stoichiometry of the bipartite AcrA:AcrB complex. Interestingly however, AcrA-dimer formation and AcrA-AcrB interaction are highly influenced by pH.

Until now, the dogma implied a sequential binding of AcrA with AcrB to recruit TolC. However, from the current study, it appears that TolC forms independent interactions with AcrA as well as with AcrB. It comes as a surprise that TolC and AcrB form a high affinity complex with a K_d value similar to the AcrA-AcrB interaction. Even more astonishing is the fact that the initial rate for AcrB-TolC interaction is higher than for the AcrA-AcrB interaction. This finding strongly supports a model of AcrA-independent preassembly of TolC with AcrB, a notion that contradicts the previous perception that AcrA is essential for TolC-AcrB interaction

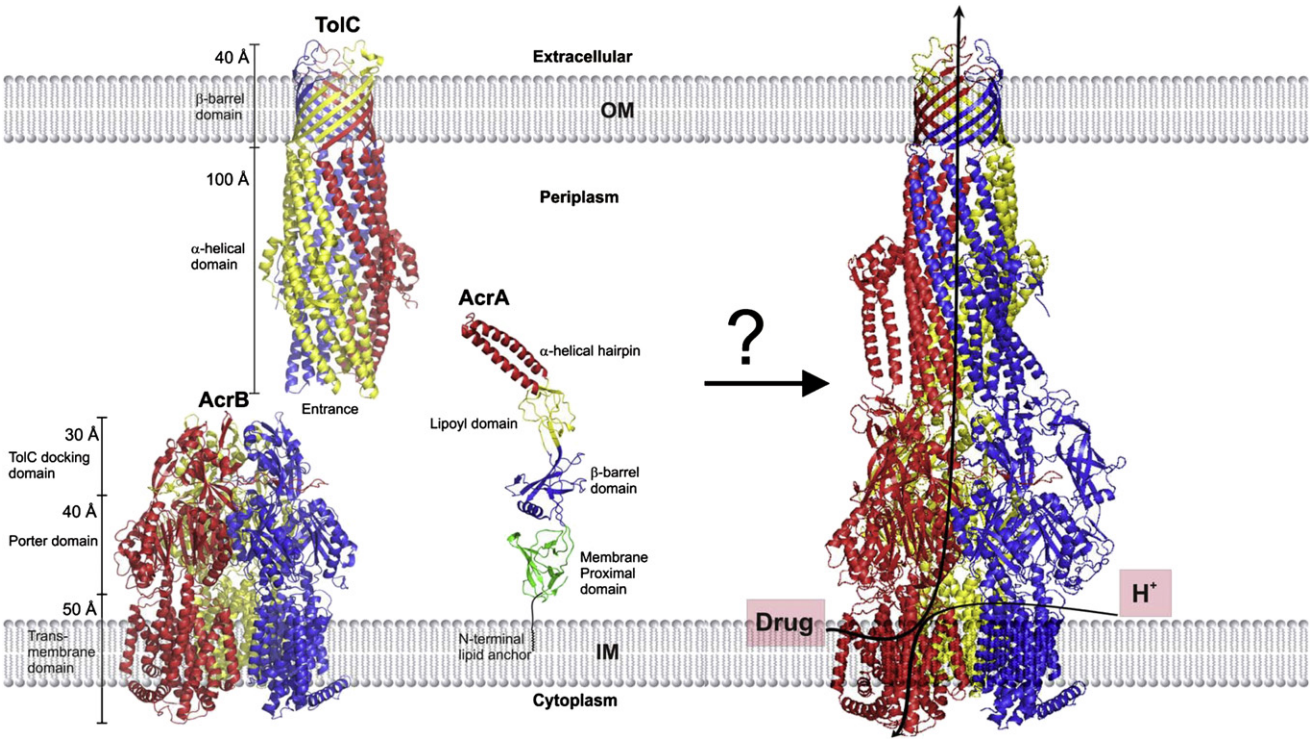

Figure 1. Ménage à Trois in the Periplasmic Space

AcrB (PDB entries: 1IWG, 2GIF) resides in the inner membrane (IM) and is composed of the transmembrane domain, the porter (pore) domain, and the TolC docking domain. TolC (PDB entry: 1EK9) is integrated into the outer membrane (OM) with its β-barrel domain and forms a long conduit in the periplasm by its α-helical domain, which narrows to a closed entrance at the proximal end. AcrA (2F1M, shown is the MexA homolog structure [2V4D] including the membrane proximal [MP] domain) is divided into the membrane proximal domain, β-barrel domain, the lipoyl domain, and the α-helical hairpin. AcrA is associated with the inner membrane via an N-terminally attached lipid anchor. The assembly of the components in the periplasmic space leads to a functional tripartite system lending Gram-negative cells the ability to resist toxic compound (e.g. antibiotics) stress. Adapted and modified from Symmons et al (2009), Seeger et al. (2008), and Eswaran et al. (2004).

(Symmons et al., 2009). The single components interact with each other upon a sequential presentation, demonstrating that the bipartite AcrA-AcrB or TolC-AcrB preassembly is favorable for final tripartite complex formation. This process occurs independently of the presence of efflux pump substrates.

Another interesting finding is the influence of the pH at various stages of in vitro complex formation. Not only do rate constants and affinities change upon a pH downshift, but also the underlying reaction models show pH-dependent variations by shifting from simple to more complex kinetics that account for conformational changes upon initial association events. In this process, there may be a key role for the MP domain, as Tikhonova et al. (2011) show that conformational changes are most likely to occur in this AcrA domain during complex formation. This foreshadows the notion that subsequent processes following the initial complex formation will play an important role to obtain the final drug transporting assembly (Figure 1).

Perhaps most interesting is the question of how these findings can be extrapolated to in vivo complex formation. Since the proton concentration in the periplasm is thought to be in equilibrium with the outside pH, how are AcrA-AcrB interactions influenced? Lipid modification of AcrA is facilitating complex formation, a process involving the MP domain while it is in close proximity to the inner-membrane surface. How is the charge of the inner membrane lipids and maybe even the local proton concentration so close to the membrane surface influencing AcrA stability and its interaction potential with the other components of the tripartite system?

Tikhonova et al. (2011) present unprecedented extensive biophysical analysis on the individual components of the paradigm tripartite AcrA-AcrB-TolC complex of *E. coli*. The results are indeed utmost valuable for understanding tripartite complex formation and open up new intriguing questions.

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Activity-Based Profiling for Drug Discovery

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Activity-based protein profiling (ABPP) is emerging as a game-changing tool for drug discovery, target validation, and basic biology. In this issue, Chang et al. (2011) report the ABPP-facilitated discovery of JW480, a highly selective potent and orally bioavailable inhibitor of monoalkylglycerol ether hydrolase KIAA1363 that dramatically impairs in vivo growth of human prostate cancer cell lines.

Identification and characterization of the functionally diverse enzyme complexes that coordinate and control all cellular processes is among the most important challenges of the postgenomic era. Quantitative understanding of dynamic enzyme activity, integrated from the cell up to the whole organism, is an essential step toward a unified model of life, and a powerful tool in the increasingly complex search for viable drug targets across all diseases. To understand enzyme function, we must decipher the emergent chemistry of proteins, and the application of chemical technologies to this challenge has proven particularly powerful, giving rise to the vibrant multidiscipline of chemical proteomics. An ultimate objective of this emerging field is to profile all types of enzymatic activity in whole organisms, a process commonly termed Activity-Based Protein (or Proteome) Profiling (ABPP), presenting some fascinating challenges in chemical biology (Heal et al., 2011). ABPP has origins in work from the 1980s, labeling the active site of proteases, but it is only recently that it has matured into a versatile and powerful platform technology. The Cravatt laboratory is a recognized ABPP pioneer, having demonstrated profiling across a remarkably broad range of enzyme classes. In a notable recent study, a search for selective inhibitors of members of the serine hydrolase (SH) superfamily (Bachovchin et al., 2010) was implemented using competitive ABPP, in which synthetic molecules compete with the probe for binding to the target enzyme. In this case, an SH-directed fluorophosphonate-rhodamine (FP-Rh) probe was used to profile over 70 hydrolases against more than 150 carbamate inhibitors, ultimately resulting in compounds selective toward single or small groups of SHs. Competitive ABPP is a perfect fit to the aspirations of modern drug discovery, allowing fine tuning of inhibitor selectivity and potency against numerous enzymes in parallel, directly in the native complexity of the proteome. Furthermore, even inhibitors that are selective for or against uncharacterized enzymes for which substrates have not yet been reported can be developed utilizing this methodology. The implications for understanding inhibitor on- and off-targets in vivo during drug development are evident, and ABPP holds great promise for avoiding drug attrition due to toxicity or efficacy failures in late-stage clinical trials.

In the current issue, Ben Cravatt and coworkers (Chang et al., 2011) report the development of JW480, a potent and selective carbamate-based inhibitor of KIAA1363 (also known as AADACL1), which is a membrane-bound 2-acetyl monoalkylglycerol ether hydrolase. KIAA 1363 is a member of the aforementioned abundant and diverse SH superfamily, which includes esterases, thioesterases, lipases, amidases, and proteases. Several SHs are implicated in the development and progression of tumors (Nomura et al., 2010a), but unfortunately the biological and physiological functions for many of these potential pharmacological targets remain poorly understood (Simon and Cravatt, 2010). Increased activity of KIAA1363 results in the overproduction of monoalkylglycerol ethers (MAGEs), which in turn are converted into lysophospholipids that stimulate survival, mobility, and aggressiveness of cancer cells (Chiang et al., 2006). KIAA1363 is the second SH enzyme reported recently by the same group to lead to overproduction of protumorigenic lipids. In the former study, a combination of ABPP, proteomic, and lipidomic analyses revealed a key role for monoacylglycerol lipase (MAGL) in leveling these fats (Nomura et al., 2010b).

The current study evolves from this work, focusing on the KIAA1363-MAGE pathway in prostate cancer cells with recently discovered lead compounds (Bachovchin et al., 2010), providing a starting point for rational design of more potent and selective analogs. In initial experiments, increased activity of KIAA1363,