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Telling bacteria: Do not LytTR

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Abstract

Transcriptional regulators containing the LytTR-type DNA-binding domain control production of virulence factors in several bacterial pathogens. In this issue of Structure, Ann Stock and colleagues report the crystal structure of this elusive domain in complex with its DNA target.

The recent emergence of "superbugs", pathogenic bacteria that are resistant to most commonly used antibiotics, serves as a painful reminder that the struggle against bacterial infections is never over. During most of the 20th century, bacterial diseases were successfully controlled through the use of increasingly powerful antibiotics. This could not last forever, and in the past several years many bacterial pathogens have developed resistance to one or more commonly used drugs. Because drug-resistance genes give pathogenic bacteria a better chance of survival, they are under positive Darwinian selection, which ensures rapid spreading of these genes in the bacterial population, e.g. in hospital environments. To make things even worse, we are running out of effective antibiotics and there are very few new ones in the pipeline (Projan and Bradford, 2007).

One of the goals of the recent efforts in bacterial genome sequencing was to achieve a better understanding of the bacterial cell and harness this understanding towards developing new avenues for fighting bacterial infections. Bacterial genomes have provided unprecedented insights and offered a plethora of new potential drug targets. Our cells lack the ability to synthesize vitamins, nucleotides, and certain amino acids. Therefore, there are many enzymes that are essential for bacterial growth but are missing in humans and, hence, could be used as drug targets. However, genomic data have also revealed the extreme diversity of pathogenic mechanisms. Despite the recent progress, we often lack a basic understanding of what drives bacterial colonization of the host, how do bacteria interact with susceptible tissues, and, most importantly, what factors regulate bacterial virulence.

One of the most dangerous newcomers has been MRSA (methicillin-resistant *Staphylococcus aureus*), a versatile gram-positive bacterium that causes infections of skin, wounds, and soft tissues, food poisoning and, once it makes its way into the bloodstream, toxic shock syndrome (Foster, 1996). Given that *S. aureus* is part of normal skin microflora, it hardly makes sense to try killing it in the absence of infection: this only leads to further spreading of antibiotic-resistant strains, such as MRSA. If we only could render the bacteria harmless by switching off production of their virulence factors, there would be much less need for killing the bugs.

The article by Ann Stock and colleagues (Sidote et al., 2008) in this issue of Structure offers a valuable insight into the mechanisms of transcriptional regulation of toxin production by some nasty bacterial pathogens, including the infamous MRSA. Most bacterial transcriptional regulators combine a signal input (ligand-binding or phosphoryl-accepting) domain with some

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version of the DNA-binding helix-turn-helix (HTH) domain (Aravind et al., 2005). The external signal induces a conformational change of the signal input domain, which affects binding of the associated HTH domain to its recognition site(s) on the chromosomal DNA (Gao et al., 2007). However, virulence of *S. aureus* and several other pathogens is controlled by unusual transcriptional regulators that contain a non-HTH DNA-binding domain (Nikolskaya and Galperin, 2002). This domain was dubbed LytTR ("litter") domain, after transcriptional regulators LytT (*Bacillus subtilis)* and LytR (*S. aureus*), which regulate cell wall turnover in the respective bacteria. LytTR-containing proteins have several domain architectures (see http://pfam.sanger.ac.uk/family?acc=PF04397), but by far the most numerous of them are two-component response regulators whose DNA-binding properties are controlled by environmental (extracellular) signals (Gao et al., 2007).

A recent survey showed that LytTR-containing proteins account for ~2.7% of all prokaryotic response regulators (Galperin, 2006). Although LytTR domains are typically found in just one or two proteins per genome (see http://www.ncbi.nlm.nih.gov/Complete_Genomes/LytTR.html), they regulate production of many important virulence factors: toxins, bacteriocins, fimbriae, and extracellular polysaccharides (Table 1). Such genes are not essential for cell growth, which is why the protein studied by Ann Stock and colleagues is called the accessory gene regulator, AgrA. This protein is 100% conserved in all known strains of *S. aureus*, including MRSA, and is found in related pathogens: staphylococci, clostridia, and listeria. There already have been attempts to tweak this system to make the bacteria less virulent (Lyon et al., 2000). The availability of the LytTR structure will allow doing this much more efficiently.

The crystal structure of the LytTR domain of AgrA confirmed that it has a novel, non-HTH structure (Sidote et al., 2008). It also provided a role for the conserved sequence motifs FxRxHrS and SKHR, previously implicated in DNA binding (McGowan et al., 2003).

If the LytTR domain is that important, how come its structure has not been determined before? Obviously, not for the lack of trying. According to the TargetDB database (http://targetdb.pdb.org/), AgrA-like transcriptional regulators and their LytTR domains have been subject of at least two dozen structural genomics projects. However, most of these projects have not proceeded beyond the protein purification stage and never generated well-diffracting crystals. Most likely, previous attempts to crystallize the LytTR domain failed owing to its tendency to form multimeric aggregates. The authors devised an ingenious scheme to overcome this problem: they first defined the minimal DNA target fragment for LytTR binding, a 15-bp duplex, and then co-crystallized the purified LytTR domain with a similar 15-bp DNA fragment that additionally contained 1-nucleotide overhangs at each 5′ end. This approach allowed obtaining good crystals and provided valuable data on the mechanism of DNA binding.

LytTR domain demonstrates an entirely new mode of protein-DNA interaction, whereby DNA binding is accomplished by amino acid residues located in the loops between the β-strands. These residues are poorly conserved within the transcriptional regulators of the LytR family, which probably accounts for the diversity of their DNA targets and explains our inability to deduce a single consensus binding site for all LytTR-containing response regulators (Nikolskaya and Galperin, 2002). In addition, there is an interesting observation that LytTR binding causes significant bending of its target DNA. Since DNA bending increases the chance of productive binding of the RNA polymerase, it might account for the fact that all experimentally characterized LytTR-containing proteins are transcriptional activators and not a single one appears to function as a repressor. In any case, the mechanism of DNA binding by the LytTR domain uncovered by Sidote et al. (2008) has not been seen before and might be limited to the proteins of the LytR family. If so, that would be good news for future drug design. Given that LytTR-containing proteins are not found in any known archaeal or eukaryotic

genome, and are definitely missing in humans, they make very attractive targets for fighting bacterial infections. There is no doubt that this paper by will open an entirely new chapter in studies of regulation of toxin production by *S. aureus*, *Streptococcus pneumoniae*, *Clostridium perfringens*, and other important human pathogens.

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References

Aravind L, Anantharaman V, Balaji S, Babu MM, Iyer LM. FEMS Microbiol Rev 2005;29:231–262. [PubMed: 15808743]

Foster, T. Medical Microbiology. Vol. 4th. Baron, S., editor. Galveston: University of Texas Medical Branch; 1996. p. 187-197.http://gsbs.utmb.edu/microbook/ch012.htm or http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mmed.chapter.737

Gao R, Mack TR, Stock AM. Trends Biochem Sci 2007;32:225–234. [PubMed: 17433693]

Galperin MY. J Bacteriol 2006;188:4169–4182. [PubMed: 16740923]

Lyon GJ, Mayville P, Muir TW, Novick RP. Proc Natl Acad Sci USA 2000;97:13330–13335. [PubMed: 11087872]

McGowan S, O'Connor JR, Cheung JK, Rood JI. J Bacteriol 2003;185:6205–6208. [PubMed: 14526034]

Nikolskaya AN, Galperin MY. Nucleic Acids Res 2002;30:2453–2459. [PubMed: 12034833]

Projan SJ, Bradford PA. Curr Opin Microbiol 2007;10:441–446. [PubMed: 17950658]

Sidote DJ, Barbieri CM, Wu T, Stock AM. Structure 2008;16:727–735. [PubMed: 18462677]

Table 1
LytTR-containing transcriptional regulators

Protein	Organism	Disease	Regulated process
Regulators of virulence factors			
AgrA	*Staphylococcus aureus*, other gram-positive bacteria	Wound infection, toxic shock syndrome	Production of exotoxins, hemolysins, staphylokinase, other secreted proteins
AlgR	*Pseudomonas aeruginosa*	Cystic fibrosis	Biosynthesis of extracellular polysaccharide alginate; twitching motility
VirR	*Clostridium perfringens*, *C. tetani*, *C. botulinum*	Gas gangrene	Production of exotoxins, collagenase, hemagglutinin
FasA	*Streptococcus pyogenes*	Pharyngitis, tonsillitis, necrotizing fasciitis	Expression of fibronectin-binding adhesin, streptokinase, streptolysin S
MrkE	*Klebsiella pneumoniae*, other enterobacteria	Pneumonia, urinary tract infections	Expression of type 3 fimbriae
Regulators of housekeeping functions			
ComE	*Streptococcus pneumoniae*, other streptococci	Middle ear infection, pneumonia, meningitis	Competence to DNA transformation
LytT, LytR	*Bacillus anthracis*, *Staphylococcus aureus*, other gram-positive bacteria	Antrax See above	Peptidoglycan turnover, autolysis
CbaR, PlnC	*Lactobacillus plantarum*, other gram-positive pacteria	None, many strains are probiotic (beneficial)	Production of bacteriocins (short anti-bacterial peptides)
CoxC	*Oligotropha carboxidovorans*, other soil α-proteobacteria	None	Utilization of carbon monoxide, other environmental responses