PERSPECTIVES: MICROBIOLOGY

Signaling Antibiotic Resistance in Staphylococci

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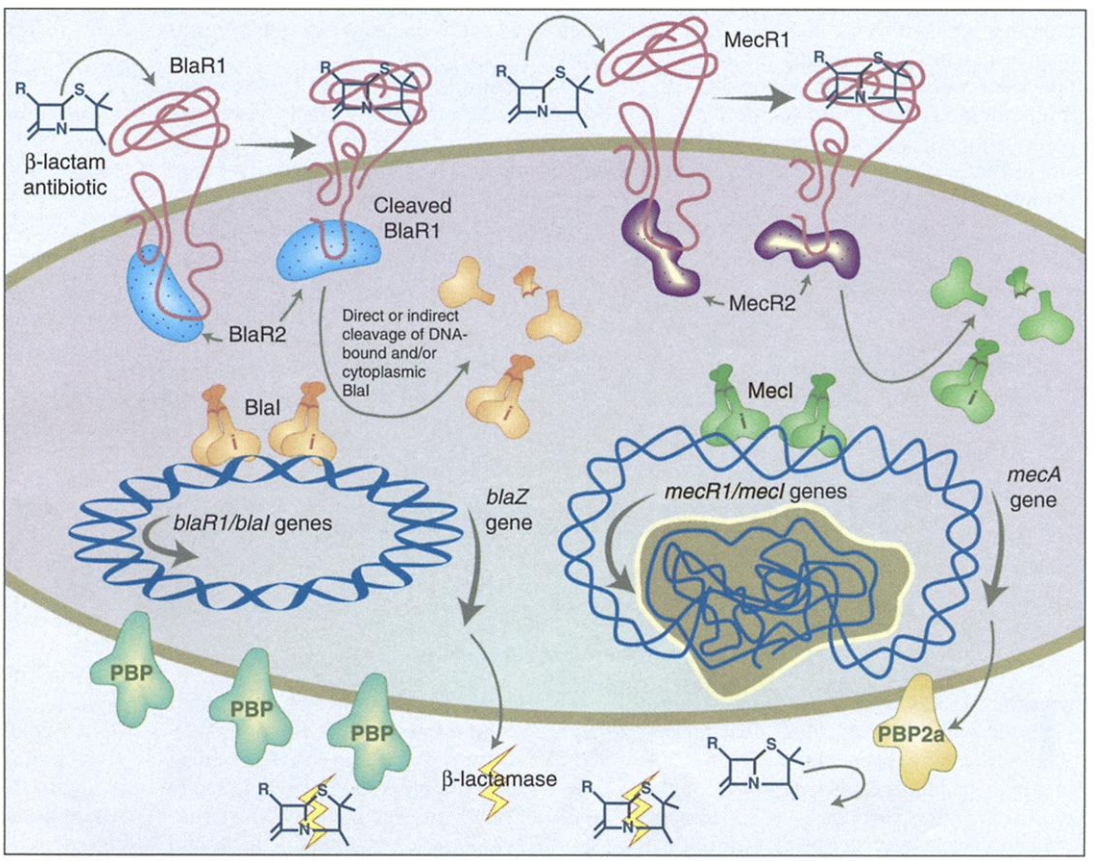
Staphylococci are the most common cause of bacterial infections in hospitalized patients (*1*). These spherical Gram-positive bacteria persist in hospitals because they acquire genes that render them resistant to most clinically useful antibiotics, including the β-lactams (penicillins and cephalosporins). The bacterial targets of β-lactam antibiotics are transpeptidase enzymes (penicillin-binding proteins) that maintain the structural integrity of the bacterial polysaccharide cell wall. Staphylococci resist attack by β-lactam antibiotics in two ways: They produce β-lactamases, enzymes that inactivate the β-lactam antibiotics, and they make new transpeptidase targets that are impervious to antibiotic activity. The signaling pathway that regulates production of β-lactamases has been studied for more than 30 years. On page 1962 of this issue, Zhang *et al.* (*2*) fill in several blanks in this pathway with their finding that production of β-lactamase depends on serial proteolytic cleavage of several key signaling components.

There are two genes that render staphylococci resistant to antibiotics—*blaZ* encoding β-lactamase and *mecA* encoding the penicillin binding protein PBP2a, which binds to β-lactam antibiotics with low affinity. These two genes and their regulators are transported on mobile elements acquired from other bacteria, and an extensive evolutionary relationship exists between them. Transcription of *blaZ* and *mecA* is regulated by the sensor-transducer proteins, BlaR1 and MecR1, and their partner repressors, BlaI and MecI, which bind to DNA sequences in the two genes.

Inactivation of the BlaI repressor through proteolytic cleavage results in derepression of the *blaZ* gene and β-lactamase production (see the figure) (*3*). The BlaR1 sensor-transducer is a transmembrane protein that promotes the cleavage of BlaI (*4, 5*). Its sensor domain extends beyond the bacterial wall and contains penicillin-binding motifs (*4*). The intracellular transducer domain contains zinc metalloprotease sequences that have been presumed to cleave BlaI. Zhang *et al.* (*2*) now tie all of this disparate information together. They describe a signaling pathway that connects the binding of β-lactam antibiotics by the sensor with cleavage of the repressor by the transducer, resulting in transcription of the β-lactamase gene.

The most common way in which signals are transmitted from the environment to the inside of the bacterial cell is through phosphorylation or methylation of intracellular molecules in response to a specific stimulus applied to the exterior of the cell (*6*). These so-called two-component systems—usually a surface receptor and its cadre of associated signaling molecules—regulate the transcription of many target genes and are frequently found encoded in bacterial genomes. The



Regulation of β-lactam resistance. Two related pathways regulate resistance to β-lactam antibiotics in staphylococci. **(Left)** Production of β-lactamase is regulated by the sensor-transducer BlaR1 and the repressor BlaI, which blocks transcription of the β-lactamase gene, *blaZ*. When a β-lactam antibiotic binds to the extracellular sensor domain of BlaR1, the cytoplasmic transducer domain is proteolytically cleaved. The transducer is then free to cleave and inactivate the BlaI repressor, and transcription of *blaZ* ensues. **(Right)** The *mecA* gene encodes PBP2a, which binds β-lactam antibiotics with low affinity. Expression of *mecA* is regulated by a similar sensor-transducer and repressor system. The BlaI and MecI repressors regulate production of the β-lactamase and PBP2a genes in similar ways, but their sensor-transducers are not interchangeable. BlaR2 (blue oval) and MecR2 (purple oval) are hypothetical accessory molecules that may be required for the sensor-transducers to interact with their repressors.

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system described by Zhang *et al.* is unique among bacteria in that the transmission of an external signal and the subsequent modification of cytoplasmic effector molecules is accomplished through a series of proteolytic cleavage steps. The signal transducer, BlaR1, must first cleave itself in order for the repressor, BlaI, to become cleaved and inactivated. Although self-activation is not unusual among zinc metalloproteases (*7*), the β-lactamase pathway is the first bacterial signaling system that has been found to depend on a series of cleavage and self-activation steps.

The authors propose that, once cleaved, the cytoplasmic transducer domain of BlaR1 becomes an activated protease with direct specificity for amino acid sequences in BlaI. However, a direct association between BlaR1 and BlaI has not yet been demonstrated, and additional molecules are probably also required. It is intriguing that the BlaI cleavage site is located at one end of the repressor, far away from the region that binds to DNA, and in an area that may be essential for forming dimers. As is the case for other bacterial repressors, BlaI molecules can only bind to the DNA in pairs (dimers). It is not yet clear whether single BlaI molecules are inactivated in the cytoplasm with a consequent depletion in the repressor pool, or whether BlaI dimers are proteolytically cleaved once they have bound to the DNA.

The proteins regulating expression of *mecA* are similar to those regulating *blaZ* transcription. Furthermore, the DNA sequences in these genes that are bound by the MecI and BlaI repressors are almost identical. These two repressor proteins are virtually interchangeable both in DNA binding experiments and in tests for repression of target gene transcription. Moreover, proteolytic cleavage takes place at the same two amino acids in the same relative position in these two repressors (*3, 8, 9*). There are considerable differences, however, when it comes to the sensor-transducer molecules, BlaR1 and MecR1. Beyond the amino acid sequences required for zinc metalloprotease activity and β-lactam binding, the proteins share only 34% amino acid identity (compared with 60% for the repressors). In addition, the kinetics of signal transduction are different: BlaR1 leads to induction of *blaZ* expression within minutes, whereas MecR1 induction of *mecA* expression takes hours. Furthermore, there are chromosomal mutations that affect induction of target gene expression by MecR1 but not by BlaR1. Although there is functional overlap between the repressors, each sensor-transducer works only with its own repressor (*9, 10*).

The Zhang *et al.* study raises many questions about the signaling pathways that switch on expression of the *blaZ* and *mecA* genes. The evolutionary origins of the components of the two pathways are obscure, but should be revealed with the increasing number of bacterial genome sequences now available for comparison. Likewise, the computerized comparison of genomes will reveal the extent to which the proteolytic cascade described by Zhang *et al.* is replicated in other bacterial signaling systems. Finally, mutations in the sensor-transducer and repressor proteins that regulate *blaZ* and *mecA* transcription would produce changes in clinical staphylococcal isolates that could alter their response to β-lactam antibiotics. Mutations in the repressors would result in the unregulated production of β-lactamase and PBP2a, with variable consequences for resistance to β-lactams (*11*). But mutations in the BlaR1 and MecR1 sensor-transducers would lead to repression of the β-lactamase and PBP2a genes. Addition of β-lactam antibiotics would not be able to relieve this repression, in effect making a resistant cell susceptible. The extent to which such sensor-transducer mutations exist among seemingly β-lactam–susceptible staphylococci has not yet been explored. Ultimately, components of the β-lactamase and PBP2a regulatory pathways may be attractive targets for developing new drugs. In this way, the machinery that staphylococci have painstakingly acquired to enable them to resist antibiotics will be turned against them.

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PERSPECTIVES: MOLECULAR BIOLOGY

RNP Remodeling With DExH/D Boxes

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The DExH/D-box proteins are nucleotide triphosphatases that are involved in most aspects of RNA metabolism and are also required for the replication of many viruses (*1*). Although their in vivo targets and mechanism of action are largely unknown, it is thought that most DExH/D proteins are RNA helicase enzymes that unwind double-stranded RNA in an ATP-dependent reaction. As the RNA unwinds, its conformation changes such that it is now able to interact with new binding partners. In contrast to DNA helicases, which unwind long stretches of double-stranded DNA, many DExH/D proteins seem likely to disrupt short RNA duplexes that may be unwound in a single step. Thus, DExH/D proteins are also called RNA unwindases to distinguish them from traditional helicases. Several DExH/D proteins are associated with large ribonucleoprotein (RNP) assemblies such as the spliceosome, which is responsible for removing the introns in pre–messenger RNA (pre-mRNA). Recently, it has been proposed that DExH/D proteins alter interactions between RNAs and proteins (*2, 3*).

But are DExH/D proteins capable of disrupting RNA-protein interactions in RNPs? Jankowsky *et al.* (*4*) have addressed this question by investigating whether the vaccinia virus DExH/D protein NPH-II can disrupt the interaction between U1A (a spliceosome protein) and RNA in vitro. NPH-II is required for viral replication and has been shown to unwind RNA duplexes in a unidirectional, processive manner (that is, NPH-II remains bound to the RNA through a series of unwinding steps rather than undergoing cycles of RNA association and disassociation) (*5*). For the RNA model substrate, Jankowsky *et al.* selected an RNA duplex with two asymmetrical internal loops, each bound to a molecule of U1A (see the figure). NPH-II unwound the RNA duplex both in the presence and absence of U1A, demonstrating its ability to disrupt highly stable RNA-protein interactions. Notably, NPH-II increased the rate of U1A dissociation by more than three orders of magnitude in an energy-dependent reaction. The

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