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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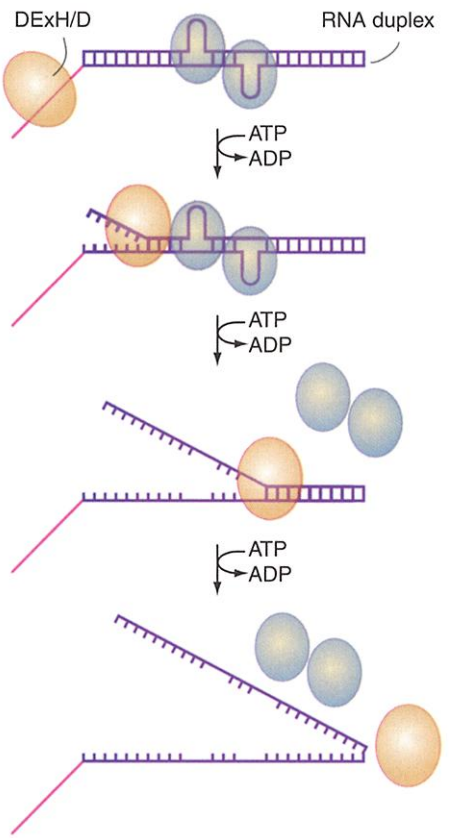
kinetic data are consistent with the notion that NPH-II directly promotes U1A dissociation by bumping this spliceosome protein from the RNA. Conceivably, NPH-II may also work indirectly by altering the conformation of the RNA binding site for U1A. The Jankowsky *et al.* study clearly demonstrates that NPH-II harnesses energy from the hydrolysis of ATP to displace U1A proteins bound to RNA and so acts as an RNPase.

Given that an RNP containing the U1A protein is an unlikely in vivo molecular target for NPH-II, the question remains whether DExH/D proteins have RNPase activity in vivo. A possible answer to this question comes from DExH/D proteins involved in pre-mRNA splicing. The spliceosome—the RNP assembly that catalyzes pre-mRNA splicing—is composed of the small nuclear RNPs (snRNPs) U1, U2, U4, U5, and U6, and numerous other proteins. These spliceosomal components interact with each other and with short, conserved regions of the pre-mRNA (the 5′ and 3′ splice sites and the so-called branch site), forming an intricate web of RNA-RNA and RNA-protein interactions. This dynamic web undergoes multiple, ATP-dependent rearrangements at specific stages of the splicing process. These remodeling events are essential for the assembly, catalytic activity, and subsequent disassembly of the spliceosome. DExH/D proteins have been implicated in many of the remodeling steps (*3*).

Recent work in the budding yeast *Saccharomyces cerevisiae* suggests that the yeast DExH/D spliceosomal proteins Prp28p and Sub2p disrupt interactions between RNAs and proteins (*6*). Prp28p promotes alterations in RNA base pairing at the conserved 5′ splice site of the pre-mRNA. During the early assembly stages of the yeast spliceosome, a short RNA duplex is formed between the 5′ splice site and U1 snRNA (the RNA component of the U1 snRNP). This base pairing is stabilized, in part, by the U1 snRNP C protein (U1C), which interacts directly with the 5′ splice site (*7*). At a later ATP-dependent stage of spliceosome assembly, this RNA duplex must be disrupted to allow the U6 snRNA to form base pairs with the 5′ splice site.

Chen *et al.* (*8*) recently shed light on how Prp28p might facilitate such remodeling. They show that the lethality accompanying deletion of the *PRP28* gene can be circumvented by introducing a mutation in U1C that reduces its affinity for pre-mRNA. Thus, Prp28p may counteract the stabilizing effect of U1C on the 5′ splice site–U1 snRNA duplex by disrupting the interaction between U1C and pre-mRNA. This destabilization may be sufficient to allow U6 snRNA to effectively compete for the 5′ splice site, which in turn would lead to displacement of the U1 snRNA.

The yeast DExH/D protein Sub2p is involved in both an ATP-independent and a subsequent ATP-dependent step of early spliceosome assembly (*9, 10*). During the ATP-dependent step, Sub2p (together with the DExH/D protein Prp5p) promotes an exchange of binding partners at the pre-mRNA branch site. This branch site is initially bound by the branchpoint binding protein, BBP (*11*). The interaction between BBP and the branch site may be stabilized by Mud2p, which binds to a region downstream of the branch site (*12*). Once BBP is displaced, a short RNA duplex forms between the branch site and U2 snRNA (*11, 12*). This remodeling event leads to a stable interaction between the pre-mRNA and U2 snRNP. Work by Kistler and Guthrie now suggests that Mud2p may be one of Sub2p's target substrates (*9*). If the gene encoding Mud2p is deleted, Sub2p is no longer essential for yeast survival. Analogous to the situation with U1C and Prp28p, Sub2p may displace Mud2p from the pre-mRNA branch site, thus destabilizing BBP and enabling the U2 snRNP to bind to the branch site.



Splitting open an RNP. The DExH/D protein of vaccinia virus, NPH-II (beige), first binds to the single-stranded region (pink) of an RNA duplex (purple). The RNA duplex contains two internal asymmetric loops that each bind the spliceosomal protein U1A (blue) with high affinity. Harnessing energy from the hydrolysis of ATP, NPH-II moves along the RNA duplex in a 3′ to 5′ direction. As it moves, the RNA duplex begins to unwind and U1A is displaced from its binding site.

Taken together, these findings raise the exciting possibility that the repertoire of DExH/D protein activities in vivo is not limited to unwinding RNA duplexes. Instead, some members of this protein family appear to disrupt RNA-protein interactions. It will be important to confirm that these DExH/D proteins are indeed RNPases and to identify their binding partners. Other questions still to be answered include whether these DExH/D proteins first interact with RNA and then protein or whether they interact with RNA and protein together, and which of these interactions triggers their ATPase/RNPase activity.

The challenge for future research is to uncover the molecular mechanisms through which DExH/D proteins couple ATP hydrolysis to the disruption of RNA-protein or RNA-RNA interactions. At first glance, members of the DExH/D protein family appear to have different ATP-dependent activities: Some are RNA unwindases, others are RNPases, and still others are both. But, on closer inspection, it becomes clear that regardless of whether the substrate is an RNA-protein complex or an RNA duplex, the end result of DExH/D protein activity is the same: The binding partner of an RNA is displaced. The ATP-powered translocation of DExH/D proteins along the RNA may bump off any proteins or other RNAs that are in the way (see the figure). Alternatively, DExH/D proteins may bind directly to their RNA duplex or RNP targets, transmitting ATP-dependent conformational changes in their own structure to nearby proteins or RNAs. More research is needed to answer the many questions that still surround the DExH/D proteins and their many targets.

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