RIGging the deck of CARDs

RIG-I is a cytosolic protein that recognizes 'pathogen-associated molecule patterns' (PAMPs)—for example, a 5′-triphosphate group on a strand of RNA or a double-stranded RNA duplex—when viral RNA is present inside a cell. The detection of PAMPs by RIG-I leads to the activation of type-I interferons, which promote a robust immune response. RIG-I has several domains, including two N-terminal caspase activation and recruitment domains (CARDs) and an ATPase domain, the cellular function of which is not clear. Myong *et al.* used a single-molecule approach to probe the exact function of these domains *in vitro*, and their experiments revealed that RIG-I uses ATP hydrolysis to translocate, but not to unwind, double-stranded RNA. Deletion of part of or all of the two CARDs led to an increase in the rate of translocation, suggesting that these domains negatively regulate the activity of RIG-I. If the RNA contained a 5′-triphosphate group, the translocase activity of wild-type RIG-I increased dramatically; additional experiments revealed that this occurred only when the 5′-triphosphate group was on the RNA molecule being translocated. Although the physiological function of RIG-I's translocase activity *in vivo* is still not clear, the authors note that these findings mean that DExH-box ATPases are now known to include both single-stranded and double-stranded translocases for RNA and DNA. (*Science*, published online 1 January 2009, doi:10.1126/science.1168352) *JMF*

An alternative response to damage

Faced with an uncertain, changeable environment, bacteria use multiple promoter-specificity (sigma) factors that modulate the activity of RNA polymerase and, thereby, gene expression. A major alternative sigma factor in *Escherichia coli* is RpoS. This protein had been linked to the stress response, but its role in the response to DNA damage was unknown. In a screen for genes involved in the DNA-damage response, Lovett and colleagues identified *iraD*. Previously, IraD had been shown to inhibit RssB, a protein that targets RpoS for ClpXP-mediated degradation. In agreement with this model, they found that *rpoS* mutants were also sensitive to DNA-damaging agents, and such mutations were epistatic with *iraD* mutations. In addition, mutation of *rssB* alleviated the sensitivity of *iraD* mutant cells, consistent with the notion that the instability of RpoS in the presence of RssB is responsible for the DNA-damage sensitivity. Although IraD expression was induced by most DNA-damaging agents, it was notably not induced by mitomycin C, an agent that strongly elicits the classic SOS damage response. In the SOS pathway, DNA damage leads to formation of a RecA filament, which activates self-cleavage of the transcriptional repressor LexA, bound to several genes needed for DNA repair. Supporting the idea that IraD acts in a separate pathway, *iraD lexA* cells were hypersensitive to DNA-damaging agents. This result suggests that complementary pathways leading to upregulation of specific damage-repairing genes and a change in the RNA polymerase composition to include the RpoS sigma factor contribute to the DNA-damage response in *E. coli*. (*Proc. Natl. Acad. Sci. USA* **106**, 611–616, 2009) *AKE*

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Initiation factors

Although it is known that the eukaryotic ribosome binds the 5′ end of a message then scans to the initiation codon, the mechanism by which such a scanning ribosome would overcome secondary structure in the 5′ untranslated region (5′ UTR) has been less clear. Eukaryotic protein synthesis begins with recognition of the initiation codon by the 40S ribosomal subunit and formation of a 48S initiation complex, in which initiator tRNA is base-paired with the initiation codon. The first step in its assembly is the attachment of a 43S pre-initiation complex, composed of a 40S ribosomal subunit, four initiation factors (eIFs), 1, 1A, 2 and 3, and intitiator tRNA, to the 5′-proximal region of mRNA. Once bound, the 43S complex scans along the 5′ UTR to the initiation codon, where it forms the 48S complex. Attachment is mediated by three additional eIFs, 4A, 4B and 4F, which cooperatively unwind the mRNA to allow 43S complexes to bind and then also assist them in scanning. Together, the seven eIFs are sufficient for ribosomal scanning on an unstructured 5′ UTR, but, as Pestova and colleagues show, highly structured 5′ UTRs require an additional factor, the DExH-box protein DHX29. The authors used an *in vitro* reconstituted initiation system containing the 40S subunits and seven eIFs and found that, in the absence of DHX29, the 48S complex did not form efficiently, even on moderately stable GC-rich mRNA. Furthermore, they noticed an additional toeprint at +8–9 nucleotides from the start codon; properly assembled 48S complexes have a toeprint at +15–17 nucleotides. The authors identified DHX29 as a factor that removes the aberrant toeprint, and it is required for efficient 48S complex formation. They show that it binds to the 40S subunit and hydrolyzes ATP, GTP, UTP and CTP. They speculate that DHX29 induces a conformational change within the 48S complex that enables ribosomal accommodation of mRNA. (*Cell* **135**, 1237–1250, 2008) *MH*

Translocon quality control

The bacterial translocon core, SecYEG, is a protein-conducting channel essential for the production of most secreted and integral membrane proteins. SecYEG makes important co-translational interactions with the signal-recognition particle and ribosome, and it cooperates with the SecA ATPase during protein translocation of secretory proteins across the membrane. SecYEG can also self-associate into oligomeric complexes. The biogenesis and regulation of SecYEG remains to be fully understood. The protein Syd was originally isolated as a suppressor of a dominant-negative mutant of SecY, and there was evidence to suggest that Syd and SecY interact directly with each other. Using nanodiscs—a single membrane complex (SecYEG, in this case) placed in a small lipid bilayer supported by two membrane scaffold proteins—Duong and colleagues were able to determine that *Escherichia coli* Syd makes interactions with two cytoplasmic loops of SecY that are also known to be involved in SecY's interaction with SecA. The crystal structure of Syd reveals a charged cavity that cross-linking analysis suggests is involved in making SecY interactions. Interestingly, Syd can interact with a SecYEG monomer in nanodiscs, but it cannot compete with SecA for binding to SecYEG purified from inner membrane vesicles, which exist in an oligomeric form. Analysis of SecE mutants defective in interacting with SecY indicates that Syd preferentially recognizes misassembled SecYEG complexes, and the authors show that SecY can dissociate SecYEG dimers formed in detergent micelles. They suggest that Syd may be acting as part of a quality-control system, interacting with improperly formed complexes and thereby facilitating their degradation by the FtsH protease. (*J. Biol. Chem.* published online 12 January 2009, doi: 10.1074/jbc.m808305200) *MM*