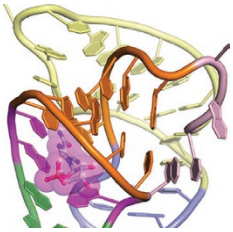
RIBOSWITCHES

Sound the alarm

Chem. Biol. **22**, 829–837 (2015)
Structure **23**, 1375–1381 (2015)
Nat. Struct. Mol. Biol. doi:10.1038/nsmb.3073

NSMB



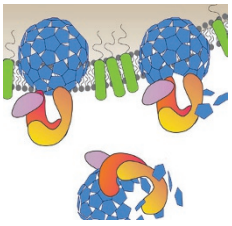
ZMP is an intermediate in purine biosynthesis that, along with its triphosphate analog ZTP, serves as an alarmone for 10-formyl-THF starvation. This 'alarm' is sensed by a riboswitch located in the upstream regions of several genes involved in purine and folate metabolism. Three research groups now report the structural basis for this interaction. All three papers found that the ZMP ligand is bound within a pseudoknot formed by two distal sequences, with nucleotide pairing primarily as predicted from prior work. The ligand forms a variety of intermolecular interactions with the RNA scaffold, including hydrogen bonds to the sugar ring and π-stacking of the ZMP nucleobase with several RNA bases. Trausch *et al.*, in their analysis of an engineered riboswitch from *Actinomyces odontolyticus*, note that this highly specific pocket explains how the riboswitch discriminates against the more prevalent and larger AMP and IMP bases. Ren *et al.*, working on the riboswitch from *Thermosinus carboxydivorans*, explore the relative contributions of these interactions by testing truncated ZMP analogs and RNA mutants, confirming the precise molecular recognition of the pair. Unlike these productive interactions, the monophosphate group is pointed out of the binding pocket, explaining why the riboswitch does not discriminate between ZMP and ZTP. All three reports also notice an unusual interaction between the carbonyl amide of the ZMP nucleobase and a coordinated Mg^{2+} ion. Finally, Jones and Ferré-D'Amaré noted that the length of the linker region between the two distal sequences varies widely; *in vitro* assays of constructs derived from the riboswitch from *Fusobacterium ulcerans* confirmed that longer linkers yielded entropic penalties in riboswitch assembly, leading to smaller modulations by ZMP in a transcription assay. These combined studies provided a detailed look at unique interactions in alarmone sensing. *CG*

VIRAL MECHANISMS

A route to the ER

PLoS Pathog. **11**, e1005086 (2015)

PLOS PATHOG.



The non-enveloped polyomavirus SV40 enters host cells via the secretory pathway, where it gains access to the ER and undergoes remodeling that makes the virus competent to integrate into the ER membrane. From there, the virus is extracted into the cytosol, where it can then travel to the nucleus to continue its path towards establishing infection. Several host proteins are known to be involved in ER-to-cytosol translocation of SV40, including an ER membrane–localized complex consisting of heat shock protein Hsc70, the ATPase-stimulating J protein called B14 and the co-chaperone SGTA. Searching to characterize the role of this complex in ER-to-cytosol translocation of SV40, Ravindran *et al.* used an immunopurification strategy to find an additional binding partner in the complex, Hsp105, whose recruitment to the complex via B14 is increased during viral infection. An RNAi approach and mutational analysis showed that Hsp105's nucleotide exchange activity is important for infection by SV40. In addition, the authors found that Hsp105 is important in promoting cytosolic arrival of SV40 in a permeabilized cytosol arrival assay. They corroborated this finding with an imaging approach that also characterized the formation of SV40-induced ER membrane foci, which could be enhanced by depleting Hsp105 or decreased by overexpressing Hsp105, suggesting that the foci represent the cytosol entry site for the virus. Hsp105 could interact with SV40 *in vitro* as well as in immunoprecipitation experiments. The SV40-Hsp105 interaction is detectable at approximately the same time as virus enters the cytosol, suggesting that the interaction takes place at the ER-cytosol interface. Indeed, membrane-associated Hsp105 binds to SV40. Sucrose gradient sedimentation assays were consistent with a mechanism whereby the Hsp105–B14–Hsc70 complex (but not the individual proteins) disassembles the virus for cytosolic entry. The authors proposed that an iterative binding-release of SV40 by Hsp105–Hsc70 initiates disassembly of membrane-embedded virus to facilitate the ER extraction process, in a mechanism that is reminiscent of ER-to-cytosol translocation of misfolded proteins called ERAD. *MB*

ANTIBIOTIC RESISTANCE

A minimal measurement

Mol. Syst. Biol. **11**, 822 (2015)

The minimum inhibitory concentration (MIC) of an antibiotic is defined as the lowest concentration that inhibits bacterial growth over a defined period of time in culture, generally starting from a standard initial cell density of 5×10^5 cells/ml (MIC*). This measurement is important in determining the resistance of bacterial cells to specific antibiotics. Artemova *et al.* argued that MIC is insufficient to quantify the fitness of a resistant bacterial strain in cases where resistance is mediated by degradation of the antibiotic, as with β-lactam antibiotics. This can be attributed to the so-called inoculum effect, whereby the cooperative inactivation of the antibiotic by the bacterial population causes the MIC to depend on the initial cell density, complicating the dynamics of the population. The authors sought a metric for quantifying antibiotic resistance that removed the effect of initial cell density. They measured the MIC for the β-lactam cefotaxime against a resistant *Escherichia coli* strain at dozens of initial cell densities. They found that the MIC varied by three orders of magnitude depending on the initial cell density: as cell density decreased, MIC asymptotically approached a limit corresponding to the level of resistance of a single cell (scMIC). The cefotaxime scMIC obtained with this liquid dilution method was within a factor of 2 of that obtained with single cells grown on agar, and both were at least an order of magnitude smaller than the MIC*. This consistency in the scMIC measurement carried over to experiments where two strains were in competition and in laboratory evolution experiments. These experiments indicated that the scMIC specifies the concentration at which selection for increased resistance (evolution) occurs. The authors built a mathematical model to describe this predictive power of the scMIC, which validated their *in vitro* and *in vivo* (in animal host) experiments. Because scMIC directly measures the fitness of an individual cell, and because selection acts on individual cells and favors cells that perform better as individuals, the scMIC is superior to MIC as a metric for quantifying cell fitness. *MB*

Written by Mirella Bucci, Catherine Goodman, Grant Miura and Terry L. Sheppard