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Proteostasis Modulators with Discriminating Taste

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Abstract

Small molecules that perturb protein homeostasis are used as cancer therapeutics and as antibiotics to treat bacterial infections. Kannan et al. (Cell 2012) describe an intriguing mechanism that enables ribosome-targeted macrolides to selectively remodel the bacterial proteome. This finding suggests the exciting possibility of targeting additional proteostasis regulators in a substrate-selective manner.

Drugs that target the ribosome are used globally for treating bacterial infections. These small molecules, most of which are microbial natural products or their derivatives, have also proven invaluable as tools for unraveling the complex biochemistry of protein synthesis (Blanchard et al., 2010). To date, all clinical classes of ribosome-targeting antibiotics bind to the decoding center on the small subunit, the peptidyl transferase center (PTC), or the nascent peptide exit tunnel (NPET). They exert their inhibitory effects through various mechanisms, including competition with substrate binding, inhibition of mRNA movement, and disruption of ribosome conformational changes. Binding of antibiotics to these sites is generally considered to result in global inhibition of protein synthesis, regardless of their mechanism of action. A recent paper (Kannan et al., 2012) describes an unexpected mechanism for the clinically important class of ribosome-targeting macrolides in which a subset of cellular proteins evade macrolide inhibition. Thus, instead of globally inhibiting protein synthesis, these drugs selectively remodel the cellular proteome. This substrate-discriminating ability depends in part on the precise structure of the macrolide and may have implications for the mechanism of bacterial cell death.

Many ribosome-targeting antibiotics bind to the peptidyl transferase center (PTC) and inhibit peptide bond formation during protein biosynthesis (Yonath, 2005). By contrast, the erythromycin family of clinically important macrolide antibiotics bind to the prokaryotic ribosome's nascent peptide exit tunnel (NPET), near the L4 and L22 protein loops. Rather than interfering with the peptide bond-forming step, erythromycin was thought to sterically block the exit tunnel and obstruct nascent chain elongation, thereby resulting in global inhibition of translation (Yonath, 2005). Contradicting this view, Kannan and co-workers report that protein synthesis persists at a low level (~5%) in the presence of saturating concentrations of erythromycin (ERY). Remarkably, an erythromycin analog with enhanced antibiotic potency, telithromycin (TEL), permits even higher levels of translation at maximum inhibition (~20%). Pulse-labeling with ^{35}S-Met, coupled with 2D gel

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electrophoresis and mass spectrometry, revealed a small subset of proteins that are resistant to ERY and TEL.

To elucidate the mechanism of this effect, the authors asked whether specific amino acid sequences near the N-terminus of the nascent polypeptide, which first encounter the bound macrolide in the ribosome exit tunnel, can promote evasion of ERY-mediated translation arrest. Experiments with H-NS, a macrolide-resistant protein identified by mass spectrometry, established that its first twelve amino acids are sufficient to confer resistance when transferred to the N-terminus of an otherwise sensitive protein. How could this be? While previous crystallographic studies had suggested that macrolide binding dramatically constricts the exit tunnel and thereby prevents nascent chain elongation (Schlünzen et al., 2001), a more recent study proposed that occlusion is incomplete and might permit wriggling of some polypeptides past the bound macrolide (Tu et al., 2005). An elegant experiment, in which an ERY-dependent translational stall sequence was fused to the C-terminus of the ERY-resistant H-NS protein, suggested that the nascent polypeptide is able to thread through the exit tunnel while the macrolide remains bound. Given the lack of sequence homology between the macrolide-resistant proteins identified in this study, an important question for the future concerns the structural or physicochemical requirements of the nascent chain for bypassing the partially occluded exit tunnel.

For nascent polypeptides that initially manage to slither past the bound macrolide, stalling can still occur at longer chain lengths due to specific internal sequences that presumably clash with the macrolide (indeed, the probability of stalling appears to increase with polypeptide length). In this case, translation arrest leads to the generation of truncated proteins. The authors suggest that partial translation inhibition by macrolide antibiotics such as TEL may result in enhanced cytotoxicity due to the accumulation of truncated proteins with altered functions. However, this provocative model remains to be tested.

This intriguing twist in the mechanism of macrolide antibiotics is reminiscent of the cotransins, a family of cyclic peptides that includes the fungal natural product CAM-741 and its synthetic variants. Like ribosome-targeting macrolides, cotransins target a universally conserved protein biogenesis machine: in this case, the Sec61 translocation channel required for the functional expression of most secretory and integral membrane proteins (Besemer et al., 2005; Garrison et al., 2005). Cotransins potently inhibit Sec61-mediated cotranslational translocation of nascent secretory and membrane proteins into the endoplasmic reticulum (ER) of mammalian cells. Moreover, they do so in a substrate-discriminatory manner. They bind directly to the alpha-subunit of the Sec61 complex (MacKinnon et al., 2007), which recognizes the N-terminal signal sequences (or transmembrane domains) of substrate proteins. The productive interaction between Sec61 and a hydrophobic signal is required for channel gating and translocation of nascent polypeptides into the ER lumen, in addition to mediating integration of transmembrane segments into the lipid bilayer (Shao and Hegde, 2011). By analogy to the macrolide antibiotics, cotransin sensitivity of any given secretory protein is determined by specific sequences near the N-terminus, in this case the signal sequence (Besemer et al., 2005; Garrison et al., 2005). However, the precise sequence requirements for cotransin sensitivity remain unknown. Finally, cotransin structural variants with distinct substrate selectivities have been described (Harant et al., 2007; Maifeld et al., 2011). Similar to the proposed explanation for the differential effects of macrolide variants on protein translation (Kannan et al., 2012), it is likely that cotransin variants exert distinct effects on the kinetic discrimination of translocating polypeptides by the Sec61 channel.

An exciting concept emerging from these studies is the possibility of identifying small druglike molecules that modulate core regulators of protein homeostasis, not by completely shutting them down, but by enhancing their innate ability to kinetically discriminate between

different protein substrates. To fully harness the substrate-discriminatory potential of such compounds, it will be necessary to obtain a detailed understanding of (1) their binding modes (structure and kinetics), (2) the binding modes of sensitive and resistant substrates, and (3) the structure/sequence/physicochemical requirements of the polypeptide substrate for compound sensitivity. In addition to their potential therapeutic utility, these compounds can help us understand the physical principles that enable complex cellular machines to recognize and discriminate among diverse protein (and possibly, nucleic acid) substrates. Finally, the cotransin and erythromycin examples inspire the search for substrate-selective modulators of cellular machines that control other aspects of protein and nucleic acid homeostasis, including chaperone/co-chaperone complexes, the proteasome, the spliceosome, and nuclear export factors, all of which have been targeted by small molecules.

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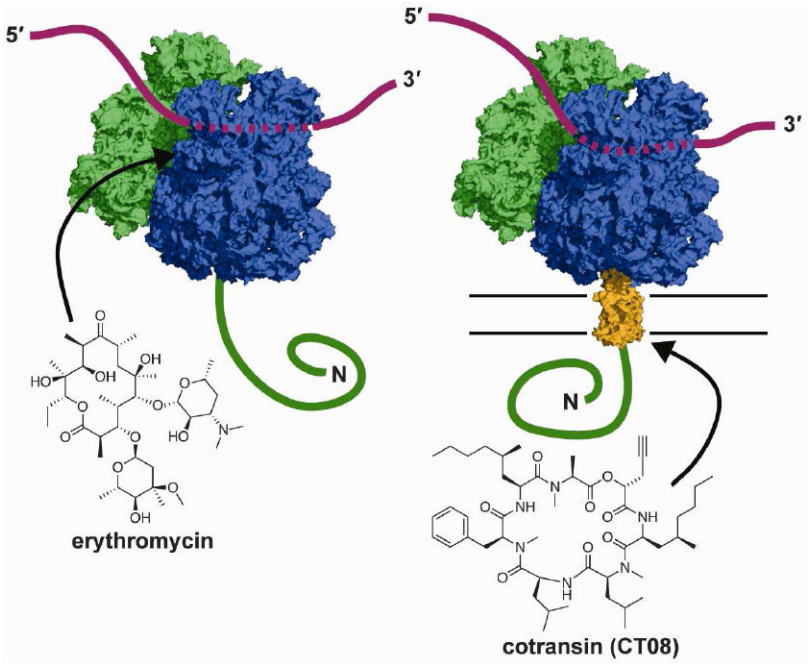

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(Left) Erythromycin-class macrolides bind to the nascent peptide exit tunnel (NPET) between the 50S (blue) and 30S (green) subunits of the bacterial ribosome. Depending on the sequence of the nascent polypeptide, this can result in (1) drop-off of peptidyl-tRNA during early rounds of translation, (2) N-terminal translation arrest, and (3) N-terminal bypass, followed by late translation arrest or synthesis of the full-length polypeptide. **(Right)** Cotransins (e.g., CT08; Maifeld et al., 2011) bind the Sec61α subunit of the mammalian translocation channel. Depending on the N-terminal signal sequence of the nascent secretory or membrane protein, cotransins can inhibit cotranslational translocation or membrane integration. The encoding mRNA is drawn in purple and the emerging polypeptide chain in green. The ER membrane is indicated as black lines.