Macrolides: The Plug Is Out

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Macrolide antibiotics are thought to clog up the ribosomal tunnel and thereby block general protein synthesis. By using a combination of elegant in vivo and in vitro approaches, Kannan et al. show that the inhibitory action of these drugs on bacterial protein synthesis is selective rather than global.

The discovery of erythromycin in the 1950s was the starting point for the development of the clinically important antibiotics of the macrolide class. Macrolides consist of 14- to 16-membered lactone rings to which various sugar moieties are attached. These drugs specifically inhibit the activity of the bacterial protein biosynthetic machinery, the ribosome. The cellular target of macrolides is a narrow tunnel of the large ribosomal subunit known as the nascent peptide exit tunnel (NPET). Nascent polypeptides synthesized by ribosomes must exit through this ribosomal "birth canal" to reach the extraribosomal cellular environment (Ban et al., 2000; Milligan and Unwin, 1986; Yonath et al., 1987). Binding of macrolides to the inner wall of the NPET is thought to occlude the tunnel lumen, leading to a complete shut-off of protein synthesis (Schlünzen et al., 2001). In contrast to this prevailing "plug-in-the-bottle" model of macrolide action, in this issue, Kannan et al. (2012) demonstrate that a distinct subset of polypeptides can slither through the drug-obstructed tunnel and that the inhibition of protein synthesis by macrolides is protein selective rather than general.

The bacterial ribosome is a primary target of antibiotics. The binding sites and the modes of action on ribosomes, however, are different among the different antibiotic classes. Many clinically relevant antibiotics, like chloramphenicol and the lincosamides, directly interact with and block the ribosomal peptidyl transferase center (PTC), where amino acids are assembled into proteins by peptide bond formation (Schlünzen et al., 2001). Antibiotics of the macrolide (and streptogramin B) class are unique among the

ribosome-targeting antibiotics, as they do not block peptide bond formation in the PTC directly. Macrolides bind within the NPET at the mouth of the exit tunnel between the PTC and a tunnel constriction formed by the extended loops of the ribosomal proteins L22 and L4 (Figure 1) (Hansen et al., 2002; Schlünzen et al., 2001; Tu et al., 2005). This portion of the tunnel is the narrowest, and the prevailing view of macrolide action is that the drugs form an impassable barrier at the tunnel constriction that blocks the path and, thus, the synthesis of all nascent polypeptide chains. Crystallographic studies of macrolide-bound ribosomes confirm that macrolide binding dramatically narrows the tunnel (Schlünzen et al., 2001). However, after modeling short nascent peptides into the erythromycin-obstructed NPET, Steitz and coworkers (Tu et al., 2005) hypothesized that there is potentially still enough room for a peptide to pass the antibiotic. In line with this model, Kannan et al. provide compelling evidence that a distinct subset of proteins can indeed efficiently bypass the macrolide-obstructed tunnel in vivo and that full-size proteins can be synthesized by drug-bound ribosomes. Moreover, Mankin and coworkers (Kannan et al., 2012) show that not all proteins that bypass the antibiotic with their N-terminus are translated to full size. Elongation, in particular of larger polypeptide chains, can be arrested by macrolides also at later stages of translation, resulting in the formation of large truncated proteins (Figure 1). The finding that macrolides allow selective synthesis of full-size and truncated proteins in bacteria could be a highly important aspect contributing to the bacterial toxicity of this antibiotic

class. The unregulated residual and fragmented translation of polypeptides very likely leads to a dramatic imbalance of the cellular proteome that potentially impairs essential survival pathways more rapidly than the global shut-down of protein synthesis. Consistent with this view, Kannan et al. demonstrate that the more potent and newest generation of macrolide antibiotics—the ketolide group—obstruct the ribosomal tunnel less tightly than macrolides, allowing more residual protein synthesis. However, further experimental data are clearly needed before drawing the conclusion that selective protein synthesis inhibitors are killing bacteria more efficiently than global inhibitors. If verified, this might open up an entirely new direction for antibiotic drug development.

What are the general determinants that enable nascent proteins to slither through the macrolide-obstructed ribosomal exit tunnel? We don't have a definitive answer yet, but Kannan et al. have made a great effort to define the features that confer macrolide resistance at least to some proteins. It turns out that the bypass efficiency depends on key sequences in the N termini of proteins. A peptide segment composed of the first twelve N-terminal amino acids is sufficient to mediate the bypass of the protein through the drug-obstructed tunnel. Fusion of this peptide segment to the N terminus of a short macrolide-sensitive protein results in its full translation by the drug-bound ribosomes. This finding is indeed remarkable, as the newly resistant protein still has the "problematic" macrolide-sensitive protein sequence of its original N terminus to be translated. This indicates that, once a nascent peptide slips by the
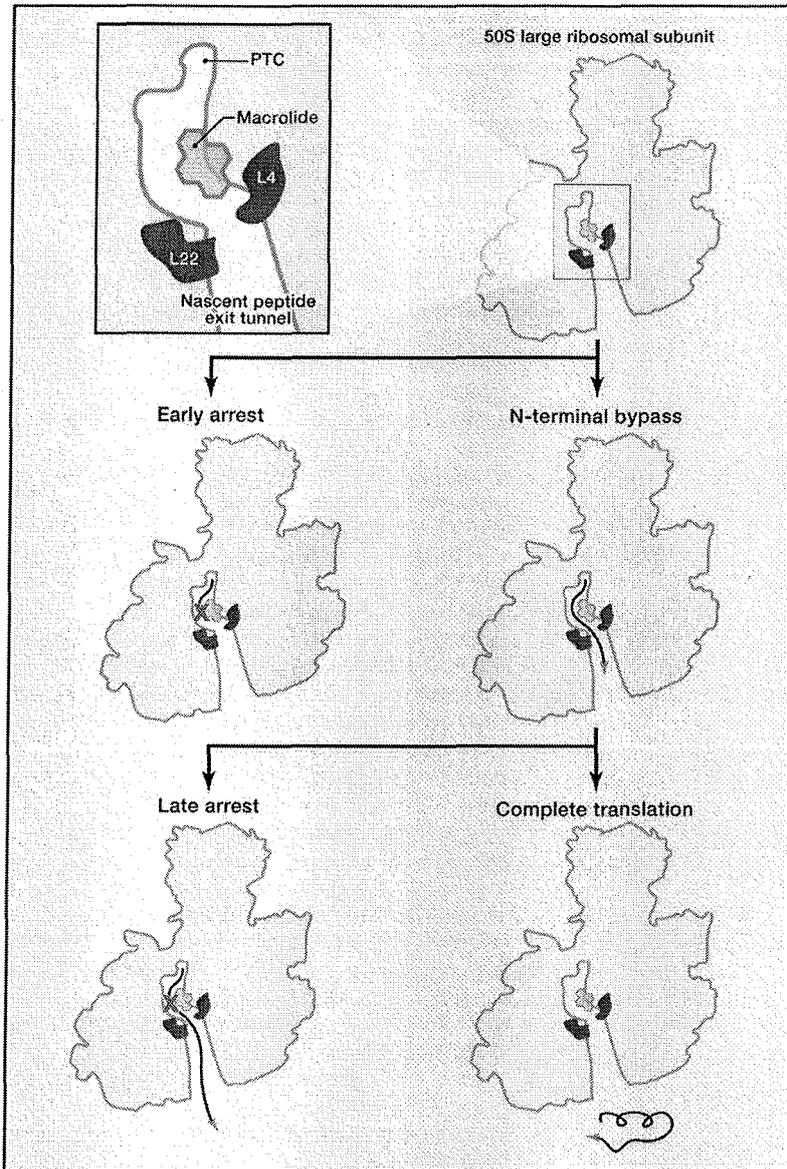
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Figure 1. Selective Blockade of Protein Synthesis by Macrolide Antibiotics
Nascent polypeptides in the macrolide-obstructed ribosomal tunnel have multiple potential fates. The side view of the 50S large ribosomal subunit (cut in half) shows the binding site of the macrolide antibiotics (yellow) within the nascent peptide exit tunnel (NPET). Macrolides specifically bind at the inner wall of the exit tunnel proximal to the peptidyl transferase center (PTC) and distal to a tunnel constriction, where loops of the ribosomal proteins L4 and L22 protrude into the tunnel lumen. Most nascent peptides have critical protein sequences at their N terminus (red cross) that cannot pass the macrolide-obstructed tunnel part resulting in early translation arrest. However, some proteins feature distinct physicochemical or structural properties at the N terminus (green arrow) that allow the growing polypeptide to pass by the antibiotic. The synthesis of polypeptides that bypass the macrolide molecule initially with their N terminus can either be arrested at later stages of translation when critical downstream sequences (red cross) enter the macrolide-bound tunnel portion or the proteins can be translated to full size.

arrest when critical downstream sequences enter the ribosomal tunnel. Kannan et al. elegantly show that the phenomenon of macrolide-dependent late translation arrest depends on the structure of the nascent peptide proximal to the PTC and that it very likely involves interactions of the nascent peptide with the ribosomal exit tunnel as well as with the macrolide molecule.

The study of Kannan et al. addresses a very important and interesting topic: the communication of nascent peptides with the inner ribosomal landscape and the contribution thereof to cellular physiology. The inner wall of the NPET is essentially built of 23S ribosomal RNA and is thus lined with hydrated polar groups and very few hydrophobic surfaces (Ban et al., 2000). This allows the accommodation and the successful passage of most peptide sequences without regard to their chemical properties. However, the NPET is not a neutral conduit. Previous studies have shown that this tunnel is an important functional compartment that monitors the structure of the nascent polypeptide. Some nascent peptide segments interact with the ribosomal interior components proximal to the PTC and dramatically affect the rate of elongation or even terminate translation. The phenomenon of programmed translation arrest by nascent peptides has been identified not only in bacteria, but also in eukaryotes (Ito et al., 2010). A growing body of evidence indicates that nascent peptide-mediated translation arrest plays an important role in cellular physiology. Such regulatory systems that use ribosome stalling sequences are in many cases activated by small metabolites (for instance, tryptophan or arginine) operating in negative feedback loops (Fang et al., 2004; Gong and Yanofsky, 2002). Although the binding site of these metabolites on ribosomes is yet to be determined, these examples clearly show us that small molecules can modulate the discriminatory properties of the ribosomal exit tunnel. The study by Kannan et al. extends our knowledge of this phenomenon and suggests that small drug-like molecules that bind in the NPET cause structural changes and selective synthesis of a distinct subset of proteins. Targeted modification of the ribosomal tunnel resulting in selective protein

drug with its N terminus, a decision is made that allows the translation of the protein in the macrolide-obstructed tunnel. However, there is no absolute rule for this N-terminal bypass, as macrolides can also induce late translation

synthesis opens new avenues in biotechnology and medicine, far beyond the development of new antibiotics.

REFERENCES

Ban, N., Nissen, P., Hansen, J., Moore, P.B., and Steitz, T.A. (2000). Science 289, 905–920.

Fang, P., Spevak, C.C., Wu, C., and Sachs, M.S. (2004). Proc. Natl. Acad. Sci. USA 101, 4059–4064.

Gong, F., and Yanofsky, C. (2002). Science 297, 1864–1867.

Hansen, J.L., Ippolito, J.A., Ban, N., Nissen, P., Moore, P.B., and Steitz, T.A. (2002). Mol. Cell 10, 117–128.

Ito, K., Chiba, S., and Pogliano, K. (2010). Biochem. Biophys. Res. Commun. 393, 1–5.

Kannan, K., Vázquez-Laslop, N., and Mankin, A.S. (2012). Cell 151, this issue, 508–520.

Milligan, R.A., and Unwin, P.N. (1986). Nature 319, 693–695.

Schlünzen, F., Zarivach, R., Harms, J., Bashan, A., Tocilj, A., Albrecht, R., Yonath, A., and Franceschi, F. (2001). Nature 413, 814–821.

Tu, D., Blaha, G., Moore, P.B., and Steitz, T.A. (2005). Cell 121, 257–270.

Yonath, A., Leonard, K.R., and Wittmann, H.G. (1987). Science 236, 813–816.