Antibiotics acting on the translational machinery

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Journal of Cell Science 116, 1391-1393
© 2003 The Company of Biologists Ltd
doi:10.1242/jcs.00365

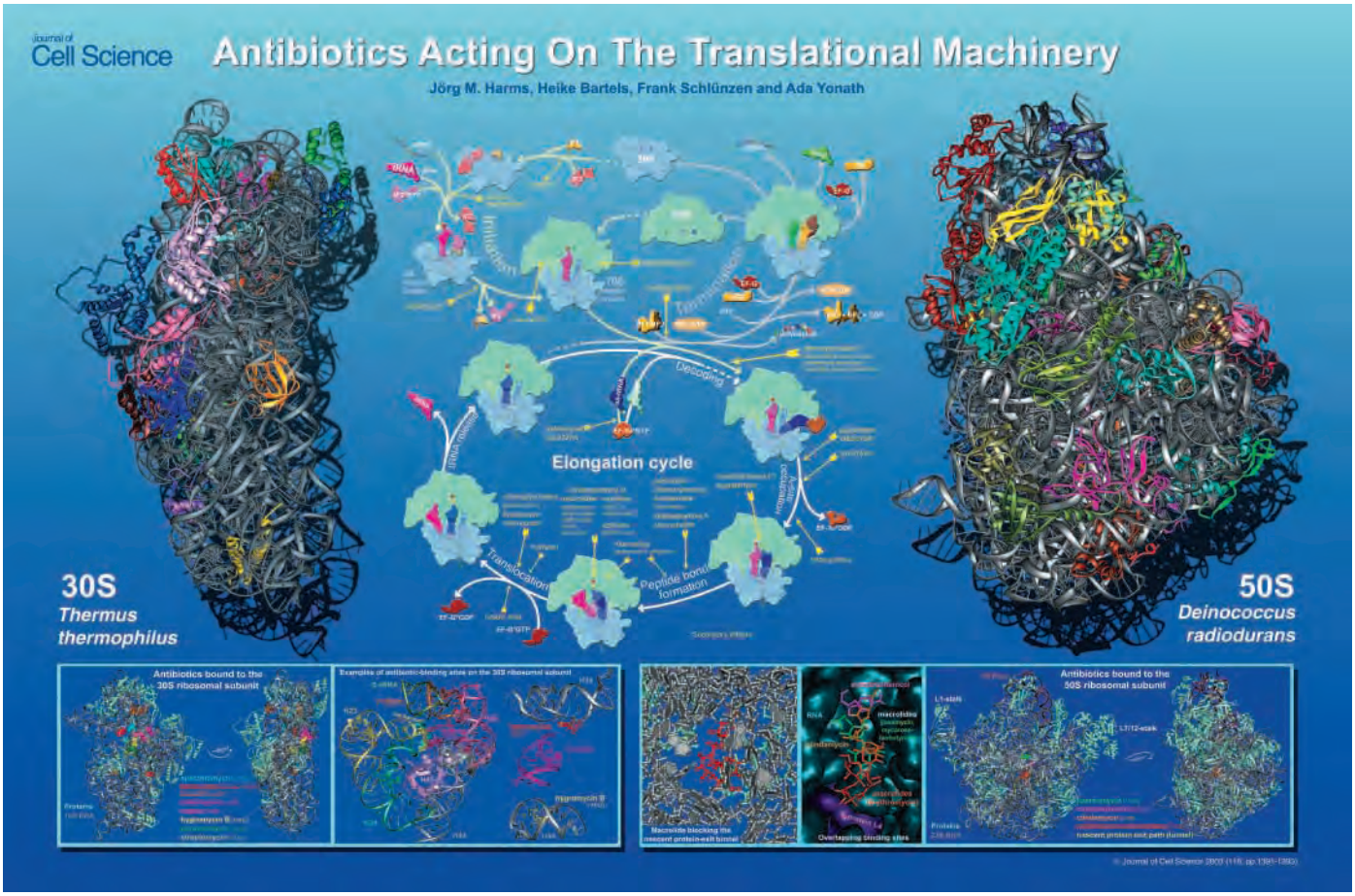
Despite the appearance of bacterial strains resistant to all clinical antibiotics, including vancomycin (the 'last resort'), development of new antimicrobial agents has slowed during recent decades. To aid design of new antibiotics, we must develop a detailed understanding of the mechanisms of antibiotic action and antibiotic resistance. Several classes of antibiotics target the ribosome and ribosomal factors, and recent structural studies of the ribosome (Ban et al., 2000; Harms et al., 2001; Nissen et al., 2000; Schlünzen et al., 2000; Wimberly et al., 2000; Yusupov et al., 2001) and complexes of ribosomes with inhibitors (Brodersen et al., 2000; Pioletti et al., 2001; Schlünzen et al., 2001; Hansen et al., 2002; Bashan et al., 2003; Schlünzen et al., 2003) are now revealing the mechanisms underlying their inhibitory activity.

Responsibility for the various steps of polypeptide synthesis is divided among the two ribosomal subunits (30S and 50S). The 30S subunit ensures fidelity of decoding by establishing accurate codon-anticodon interactions. The 50S subunit catalyses peptide bond formation and elongation of the nascent protein, further protecting the nascent chain by channelling it through the ribosomal exit tunnel, which links the peptidyl transferase centre to the bottom of the 50S subunit.

Initiation

Prokaryotic protein synthesis starts with the formation of an initiation complex comprising the mRNA, initiator tRNA (fMet-tRNA^{fMet}), three initiation factors (IFs) and the 30S subunit. IF3 binding prevents association of the two ribosomal subunits, verifies codon-anticodon complementarity and appears to regulate positioning of the mRNA. IF1 blocks the acceptor site (A-site) to prevent premature binding of the A-site tRNA. After binding of fMet-tRNA^{fMet}, IF3 is released; this triggers hydrolysis of IF2-bound GTP, and IF2 and IF1 are released (the exact sequence of events is not known). The 50S subunit then joins the 30S initiation complex, forming the 70S ribosome.

Edeine and pactamycin are universal antibiotics that affect protein biosynthesis in all organisms. Both hamper the formation of the initiation complex by displacing the mRNA (Brodersen et al., 2000; Pioletti et al., 2001). Edeine also affects the



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positioning of peptidyl site (P-site) tRNA and IF3 function. Evernimicin interacts with the tips of the 23S rRNA helices 89 and 91, which bind IF2, and may prevent formation of the 70S initiation complex (Belova et al., 2001).

Decoding and A-site occupation

The 30S subunit possesses a mechanism for discriminating cognate from non-cognate aminoacyl tRNAs (aa-tRNAs) that ensures the correct amino acid has been delivered by elongation factor Tu (EF-Tu). The anticodon of the aa-tRNA in the ternary complex interacts with the mRNA codon, but the acceptor stem of the tRNA is associated with EF-Tu–GTP and thus cannot enter the catalytic center in the 50S subunit. EF-Tu is released only after hydrolysis of GTP; the acceptor stem can then be placed into the A-site.

Many antibiotics target this step of the elongation cycle. Tetracycline, the first broad-spectrum antibiotic discovered, binds to multiple sites in the ribosome (Brodersen et al., 2000; Pioletti et al., 2001). Binding to the A-site in the 30S subunit sterically hinders the movement of the aa-tRNA so that it cannot simultaneously interact with the decoding site in the 30S subunit and the peptidyl transferase centre in the 50S subunit. The remaining sites may affect the assembly of the ribosomal particle but are probably not responsible for any inhibitory activity, since a single mutation in the primary binding site confers tetracycline resistance.

Aminoglycosides bind directly adjacent to the decoding site in the 30S subunit, rendering translation highly inaccurate. Paromomycin, for example, induces a conformational change that enhances the affinity of the A-site for near-cognate tRNAs. Hygromycin B and neomycin act by freezing the tRNA in the A-site (Brodersen et al., 2000). Streptomycin stabilizes the so-called ram state of the 30S subunit, in which its affinity for non-cognate tRNAs is increased (Carter et al., 2000).

Three antibiotics hamper positioning of the A-site tRNA by acting on EF-Tu: pulvomycin seems to reduce the GTPase activity of EF-Tu and inhibits its binding to tRNA; GE2270A blocks the tRNA-binding site on EF-Tu; and kirromycin prevents the release of EF-Tu, thereby stalling the ribosome (Hogg et al., 2002).

Several antimicrobial agents inhibit decoding or A-site occupation by interacting with the 50S subunit. Sparsomycin, for example, binds in the core of the peptidyl transferase centre (PTC) by stacking to the most flexible nucleotide, which induces substantial conformational alterations (Bashan et al., 2003) and affects the correct positioning of both the A-site and P-site tRNAs (Hansen et al., 2002). Linezolid, an oxazolidinone, increases frameshifting and stop codon readthrough with nonsense suppression. It presumably binds in the vicinity of the PTC at the interface between the ribosomal subunits, thereby affecting initiation and elongation, but might also affect accuracy of decoding.

Peptide bond formation

During the elongation cycle, each tRNA passes through the three ribosomal binding sites (A-site→P-site→E-site), creating two conformational states. In the pre-translocational state, two tRNAs are bound to the A-site and P-site. In the post-translocational state, the P-site and E-site are occupied. Correct positioning of the aa-tRNA stimulates its CCA end to flip towards the 3′ end of the P-site tRNA in concert with peptide bond formation. This reaction is thought to induce translocation of the deacylated P-site tRNA into the E-site (Bashan et al., 2003).

A variety of organisms produce antimicrobial compounds that target peptide bond formation. These are effective against many competing organisms because of the high phylogenetic conservation of the PTC; however, the originator is also attacked by its drug and must therefore develop resistance mechanisms, which are sometimes costly. Drugs inhibiting peptide bond formation act in three distinct ways: (1) by mimicking the substrate; (2) by blocking the path of the growing polypeptide chain; or (3) by alteration of tRNA positioning on the ribosome.

Puromycin is a universal antibiotic and a tool for investigating the mechanism of peptide bond formation. Mimicking the aminoacylated end of the aa-tRNA, it participates in peptide bond formation, but its non-hydrolysable amide bond cannot be cleaved and peptidyl-puromycin falls off the ribosome (Nissen et al., 2000; Bashan et al., 2003). Chloramphenicol, rarely used nowadays owing to its significant side effects, acts at the same site in the PTC. It occupies the position of the amino acid attached to the A-site tRNA, preventing peptide bond formation (Schlünzen et al., 2001).

Lincosamides (clindamycin and lincomycin) interact with both the A-site and P-site on the 50S subunit, hampering positioning of both tRNA molecules and directly inhibiting peptide bond formation. Macrolides (e.g. erythromycin) instead bind at the entrance of the ribosomal exit tunnel (Schlünzen et al., 2001), blocking the path of the nascent chain through the ribosome. This site is some distance from the PTC; thus a polypeptide chain of 3-5 residues can be produced before protein biosynthesis stalls. Semi-synthetic derivatives of erythromycin such as carbomycin A or josamycin possess a long, mycarose-isobutyrate extension of the desosamine sugar (the crucial functional group of the macrolides) that protrudes into the PTC. These compounds thus also interfere with the correct positioning of the P-site substrate and directly affect peptide bond formation (Hansen et al., 2002). Azithromycin, a rather new erythromycin derivative, binds to two sites in the *D. radiodurans* 50S subunit (Schlünzen et al., 2003). The two azithromycin molecules are in direct contact; so in this case a cooperative effect might enhance its antimicrobial activity. Streptogramins also have a dual inhibitory mechanism, but this derives from two unrelated compounds: streptogramins A and streptogramins B. These enhance each other's activity probably through a direct interaction. Streptogramins A destabilize the binding of tRNAs to the A-site and P-site; streptogramins B presumably occupy the tunnel for the nascent peptide chain – similarly to the macrolides. Macrolides, lincosamides and streptogramins B all fail to inhibit MLS_B-resistant strains possessing a single modification in the

rRNA of the PTC, which reflects the overlapping binding site of these antibiotics.

The mechanism behind the antimicrobial activity of another group of compounds, the pleuromutilins (e.g. tiamulin or valnemulin), is unclear. Since they compete with carbomycin but not erythromycin, they might prevent correct positioning of the CCA ends of tRNAs for peptide transfer.

Translocation and tRNA-release

GTP-bound elongation factor EF-G binds to the A-site of the 50S subunit. Facilitated by GTP hydrolysis, it forces the anticodon stem loop of the A-site and P-site tRNAs to move into the P-site and E-site on the 30S subunit in concert with mRNA translocation. EF-G-GDP is then released from the ribosome, and the elongation process continues with the release of the deacylated E-site tRNA and the decoding of the next aa-tRNA. The antibiotic fusidic acid stalls the EF-G-GDP complex on the ribosome. Thiostrepton and micrococcin also affect EF-G but by binding in the GTPase-associated regions of the 50S subunit. Thiostrepton appears to induce a particular conformation of the ribosomal protein L11 that greatly reduces the GTPase activity of associated EF-G or EF-Tu. It also affects IF2 but in this case enhances GTPase activity. The exact mechanism of action of these antibiotics is not yet known.

Viomycin acts at a slightly earlier stage: it seems to hamper translocation so that the A-site remains blocked. Viomycin has some additional effects, such as induction of misreading, inhibition of the peptidyl transferase reaction and inhibition of subunit dissociation during termination. Like many other drugs, it appears to bind at the interface between the subunits, thus altering the positioning of the A-site and P-site tRNAs, possibly affecting the affinity of the 50S subunit for them. Spectinomycin, another aminoglycoside antibiotic, is a rather rigid molecule. It binds to the head region of the 30S subunit, which undergoes a conformational change during elongation. Spectinomycin prevents this transition, inhibiting EF-G-catalysed translocation of the peptidyl-tRNA from the A-site to the P-site (Carter et al., 2000).

Termination

The elongation cycle normally continues until a stop codon is detected by class 1 release factor (RF1 and RF2). The class 2 factor, RF3-GTP, facilitates the cognate binding of the other release factors. This triggers the hydrolysis of the ester bond connecting nascent peptide to tRNA and release of the peptide chain. Ribosome recycling factor (RRF) in conjunction with EF-G-promoted translocation then drives dissociation of the subunits in readiness for formation of a new 30S initiation complex. To date, no antibiotic is known specifically to affect termination of protein synthesis.

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