Splice-Site Selection and Decoding: Are They Related?

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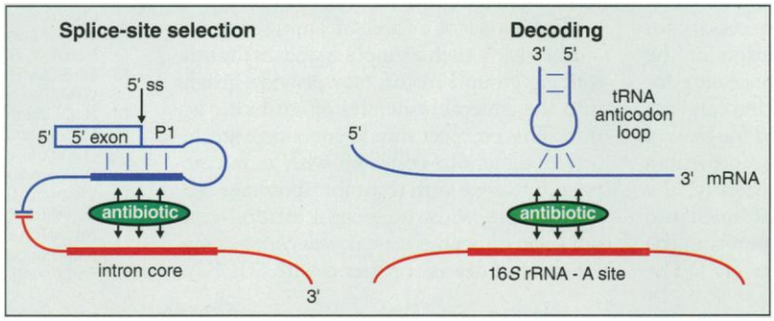
Aminoglycoside antibiotics such as neomycin, gentamicin, and streptomycin bind to the 16S RNA component of prokaryotic ribosomes and disturb their ability to decode mRNA and synthesize the specified new protein (*1*). Recently, self-splicing group I introns (sections of RNA that excise themselves from newly synthesized RNA) have been shown to be a second target of the aminoglycoside antibiotics (*2*). The fact that the self-splicing process is also inhibited by these antibiotics led to the first speculations that self-splicing group I introns and ribosomal RNA (rRNA) are related (*3*, *4*). In a report in this issue of *Science* (*5*), von Ahsen and Noller have now mapped the interaction sites of these antibiotics that inhibit splicing and find that these antibiotics interact with the catalytic core of the intron RNA. Thus, aminoglycoside antibiotics act on functional RNA elements both in 16S rRNA and in the group I intron core. Do these two RNAs share common structural and functional elements? We believe they may and here present a model showing a general mechanistic principle common to both decoding and splicing.

A growing body of evidence indicates that the RNA moieties of the ribosome harbor essential functional elements for translation of mRNA into protein. By cross-linking and footprinting studies, 16S rRNA has been shown to interact with the RNA anticodon loop and 23S rRNA to interact with the acceptor end of the tRNA (*1*). The peptidyl transferase activity of the ribosome is unusually resistant to protein extraction procedures, whereas it is highly sensitive to ribonuclease treatment, suggesting that the 23S rRNA bears the functional activity (*6*). The importance of the 16S rRNA for translation is emphasized by the fact that this molecule is among the most highly conserved molecules known. The anticodon loop of the tRNA recognizes its cognate codon on the mRNA. During decoding, this complex then interacts with the decoding site (or A site) on 16S RNA to enable the correct translation of the nucleotide sequence of the mRNA into the corresponding protein sequence, a process that requires high precision. By binding to the 16S RNA, aminoglycoside antibiotics interfere with decoding by increasing the incorporation of incorrect amino acids into the peptide chain. They act at the decoding site, which is a cluster of conserved nucleotides around positions 1400 and 1500 of the 16S rRNA, and probably cause miscoding by distorting correct codon-anticodon recognition. The fact that antibiotic resistance is often due to mutations or alterations of the rRNA further strengthens the notion that the 16S rRNA is essential for ribosomal function (*1*, *7*). Thus, the aminoglycoside antibiotics interact with conserved functional elements of rRNA.

Group I introns are autocatalytic RNA elements within rRNA, tRNA, and mRNA genes. Correct expression of these genes requires precise excision of the introns. Many of these group I introns are self-splicing and do not require protein factors for their excising activity. On page 1500, von Ahsen and Noller report the mapping of the sites of interaction between aminoglycosides, which inhibit splicing, and the group I intron RNA by footprinting experiments (*5*). Two sites were detected where antibiotics protect the intron RNA against modifying agents: one at the substrate guanosine binding site (G-binding site) (*8*) and the other at the joining region 8/7, which interacts with the 5' splice site (*9*). Binding of antibiotics to the G-binding site could explain their competition with the substrate guanosine, whereas binding of the antibiotics to joining region 8/7 may interfere with contact between the 5' splice site and the catalytic core. Both sites of antibiotic interaction are located at essential parts of the group I intron core. The 5' splice site selection in group I introns involves both formation of a double-stranded RNA helix (P1 stem) and contact of this helix with the catalytic core of the intron. The P1 helix is formed via Watson-Crick base pairing between the nucleotides surrounding the 5' splice site and the internal guide sequence of the intron. The correctly folded P1 stem contacts the catalytic core of the intron by interaction of 2'-OH groups of nucleotides upstream of the 5' splice site with joining region 8/7 of the catalytic core of the intron (*9*, *10*) (see figure, left). The P1 stem is not conserved in sequence among group I introns, whereas the sequence of the intron core is extremely conserved. Thus, the highly conserved sequence of the intron core recognizes a large number of different P1 helices, not via their bases, but rather via ribose-specific 2'-OH groups (*9*).



Splice-site selection and decoding. (**Left**) 5' splice-site selection in group I introns occurs via Watson-Crick base pairing of sequences surrounding the 5' splice site with the internal guide sequence (bold blue), forming a double-stranded RNA stem called P1 (blue). This short, double-stranded RNA helix interacts with the conserved intron core (bold red) via ribose 2'-OH groups (black arrows) (*9*). Aminoglycoside antibiotics interact with the intron core (bold red) (*5*), interfering with the splicing reaction. 5' ss, 5' splice site. (**Right**) During decoding, the anticodon of the tRNA base pairs with the cognate codon on the mRNA, forming a short double-stranded RNA helix (blue). This short helix, which differs in sequence for each codon, is recognized by a conserved region of the 16*S* rRNA, called the decoding site or A site (bold red). Aminoglycoside antibiotics interact with this site and induce miscoding.

Decoding may involve a similar recognition process. The anticodon loop of the aminoacylated tRNA base pairs with a codon of the mRNA, forming a short double-stranded RNA helix. This short helix, which differs in sequence for each codon, has to contact the conserved decoding region of the 16S rRNA (see figure, right). The codon-anticodon helix can be considered analogous to the P1 stem of group I introns, whereas the conserved decoding region of 16S RNA is analogous to the intron core. How the codon-anticodon helix actually interacts with the 16S rRNA is as yet unknown. However, as the decoding region has to recognize a different base-paired triplet at every codon, the bases of the codon-anticodon triplet cannot be responsible for interaction with the 16S rRNA. It is more likely that, when codon and anticodon are correctly paired, the backbone has a defined structure, which fits into the decoding site. Thus, the ribose and phosphate moieties of the backbone are candidates for interactions with the decoding region.

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This recognition between the bases of a functional, sequence-conserved RNA and the backbone of a sequence-variable element may be a general principle in RNA-RNA interactions (9). Both splice-site selection in group I introns and decoding during translation follow this principle and both processes are affected by the same aminoglycoside antibiotics. This similarity is underlined by an intriguing coincidence: the $tRNA^{leu}$ gene of the cyanobacterium *Anabaena azollae* contains a self-splicing group I intron inserted into the anticodon loop between the wobble base and the second base of the anticodon (*11*). Here an anticodon loop is also a 5' splice site, which base pairs with the intron's internal guide sequence and interacts with the intron core. Thus, this single region is involved both in the formation of the P1 stem necessary for self-splicing and in the formation of the codon-anticodon interaction necessary for decoding. Many different functions and activities have been demonstrated for group I introns, showing that their core represents a multipotent ribozyme (*10*). Recently, the *Tetrahymena* ribozyme was transformed into an RNA enzyme capable of promoting the hydrolysis of a carboxylate ester (*12*). The yeast U2 small nuclear RNA, an RNA component of the spliceosome, shows a structural similarity to the catalytic core of group I introns (*13*). Thus, splicing of group I introns may be similar to splicing of nuclear mRNA introns by the spliceosome (*14*). Additionally, group I introns may bear some tRNA-like features; two tRNA synthetases have been identified that, in addition to loading tRNAs, also promote the splicing reaction of group I introns (*15*). This family of potentially related RNAs can now be extended to include 16S rRNA, on the basis of the similarities between decoding and splice-site selection. These similarities could be due to a common ancestral RNA activity or could be due to evolutionary convergence of their structures as a consequence of their related functions.

The mode of action of aminoglycoside antibiotics in such a simple system as the self-splicing group I intron may provide insight into the general principles of antibiotic action. This prospect should encourage antibiotic designers to come up with new compounds to cope with resistant ribosomes and, for example, with the group I intron—containing pathogen, *Pneumocystis carinii*, a major cause of death of patients with AIDS.

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The Parallel β Helix of Pectate Lyase C: Something to Sneeze At

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Protein crystallography and nuclear magnetic resonance spectroscopy have revealed the three-dimensional organization of several hundred polypeptide chains. Typically these structures are dominated by secondary structures: α helices and β sheets, and their tertiary structure can be understood from an analysis of the packing of these secondary structure elements. Protein taxonomists have gathered four-helix bundles (*1*) (for example, human growth hormone, interleukin-4, and hemerythrin), α/β barrels (*2*) (triose phosphate isomerase, muconate lactonizing enzyme, and mandelate racemase), β sandwiches (*3*) (transthyretin, superoxide dismutase, and immunoglobulins), and jelly-rolls (*4*) (tomato bushy stunt virus coat protein) to name but a few. Most newly determined protein structures are members of an existing family of structures or a minor variation on a known structural theme. The crystal structure of pectate lyase C (PelC) by Yoder *et al.* (*5*) in this issue contains a heretofore unknown fold, the parallel β helix. This reminds us that much remains to be learned about protein folding.

Pauling (*6*) introduced the α helix and β sheet as sterically sensible geometries for dipeptides that can be propagated to form substructures with a regular network of hydrogen bonds. Ramachandran (*7*) demonstrated that the lowest energy conformations of a dipeptide correspond to the backbone dihedral angles required to form α helices and β sheets. From a detailed study of proteins of known structure, aperiodic secondary structures including β bulges (*8*), β turns (*9*), and β breakers (*10*) have been observed. However, these are minor features by comparison to α helices and β sheets that typically account for more than 50% of the protein's structure.

What is a parallel β helix? Helices are characterized by their pitch (rise per residue), period (number of residues per turn), handedness (right or left), and diameter. The familiar α helix (drawn schematically in blue) is right-handed with a pitch of 1.5 Å and a period of 3.6 residues per turn. With the appropriate backbone dihedral angles, the peptide planes of all residues in the helix align forming a macro dipole and a network of hydrogen bonds that associates the carbonyl oxygen of a residue to the amide hydrogen of its neighbor four residues downstream. The 5.4 Å spacing between rungs of the spiral is compatible with the geometry of a hydrogen bond. The diameter of the α helix is sufficiently small that the structure is more like a filled cylinder than an open spring. By contrast, the parallel β helix (shown in red) is right-handed with a pitch of 0.22 Å and a period of 22 residues per turn (on average). Consecutive peptide planes are oriented in opposite directions as is common in β sheets, and so the network of hydrogen bonds alternates between the carbonyl oxygen of residue i to the amide hydrogen of i + 22 and the amide hydrogen of i + 1 to the carbonyl oxygen of i + 22. Because of the lack of alignment of consecutive peptide planes, no macro dipole should accumulate. The 4.8 Å spacing between rungs of the parallel β helix spiral is compatible with the less linear hydrogen bonding arrangement for β sheets formed from parallel β strands.

Should the parallel β helix have a large hole along its helical axis? A little trigonometry reveals that if the parallel β helix were

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