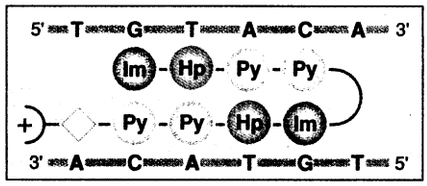
cationic porphyrins, which improve oligonucleotide cell penetration.

Another class of compounds, the PNAs (peptide nucleic acids), is quite different in that it has a peptide-like backbone instead of the normal sugar and phosphate groups of DNA. One of the inventors of PNA, P. Nielsen (Copenhagen University), showed that PNA could enter bacterial cells and kill them. This may be an important finding since increasing numbers of bacterial strains become resistant to antibiotics and alternative treatments are needed. He speculated that PNA may also be used to turn on specific genes by binding to a promoter region of a gene to initiate RNA transcription. E. Uhlmann (Hoechst) tries to combine the best of both worlds by making chimeric molecules of PNA and DNA. The DNA portion will allow enzymes attacking DNA-RNA hybrids to cut the RNA part of the complex into pieces (leading to dissociation of the drug molecule, which can then be reused), whereas the PNA portion will contribute stability and selectivity.

Genetic drugs can also be directed at the gene itself. The code for targeting the RNA copy (single stranded) of a gene was outlined almost 50 years ago by Watson and Crick. The DNA of the gene is double-stranded, and here the molecular recognition problem is not as straightforward. Since there is only one copy of many genes in a cell, this "antigene" approach is, however, a very attractive one.

The first chemical approach to target double-stranded DNA has been to use oligonucleotides to bind in the major groove of DNA and form a specific local triple helix. C. Helene (Museum National d'Historie Naturelle) demonstrated successful tests of blocking transcription of the HIV genes *nef* and *pol* in cell cultures by using oligonucleotides linked to intercalators. Some PNA sequences bind to double-stranded DNA by an invasion mechanism; two PNA molecules form a triplex structure with the complementary DNA target sequence, whereas the other strand of the DNA duplex is displaced into a single-stranded loop. Once formed, such PNA-DNA complexes are extremely stable. This type of binding is limited to pyrimidine-rich PNA sequences, and therefore B. Norden's team (Chalmers University) is applying spectroscopic techniques to try to understand the mechanism in detail so that it can be extended to direct PNA molecules to any DNA sequence.

An elegant solution to selective targeting of double-stranded DNA was presented by P. Dervan (Caltech). His group designs minor-groove binding polyamides that contain combinations of three different aromatic amino acids, which pair and uniquely recognize each of the four Watson-Crick base pairs (see the figure). Hairpin polyamides bind specifically to predetermined DNA sequences with the affinity and specificity of protein transcription factors. Remarkably, cells are permeable to these polyamides, a property that may be related to the fact that they are significantly smaller in size than oligonucleotide analogs used in antisense approaches. The most recent work shows that these synthetic DNA binding ligands can enter the nucleus and inhibit expression of specific genes by blocking promoter-specific transcription factors.



Deadly hairpins. Hairpin polyamides can be designed to bind double-stranded DNA at any desired base-sequence; upon binding to a promoter sequence the expression of protein from that gene can be blocked. Py, pyrrole; Im, imidazole; and Hp, hydroxypyrrole.

Where is this field going in the next few years? Because pathogen sequence information could lead directly to drug design, C. Cantor (Boston University) suggested that gene-targeting compounds should be an extremely important class of potential therapeutics against unknown biological weapons. P. Dervan argued, however, that the future success for gene-targeted drugs requires continued fundamental research and that more work needs to be done before these revolutionary drugs will be readily available.

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PERSPECTIVES: PROTEIN SYNTHESIS

All You Need Is RNA

Paul Schimmel and Rebecca Alexander

The genetic code is an algorithm that relates triplets of nucleotides called codons—ATG, CGG, CAA, for example—in genes to specific amino acids that, in turn, are linked with one another by peptide bonds to make proteins. Because all of life depends on this algorithm, its chemical basis and that of protein synthesis can tell us something about how life arose on Earth. A significant advance on this front is presented in the article by Nitta *et al.* on page 666 of this issue (*1*). The authors demonstrate that fragments of the ribosome (the organelle that executes the algorithm of the genetic code) containing only RNA are sufficient to catalyze peptide bond synthesis between amino acids, adding weight to the idea that early life systems could have emerged from a world in which RNA molecules coupled amino acids to make peptides (*2*).

The genetic code is established by aminoacylation reactions in which specific amino acids are joined to their cognate transfer RNAs (tRNAs), each of which bears anticodon triplets of the code. The tRNAs are L-shaped (see the figure), with each arm of the L constituting a separate domain. One arm—the acceptor-TψC minihelix—contains the amino acid attachment site at the 3′ end, which has the universal single-stranded sequence CCA, with the amino acid esterified to the terminal A. The other arm of the L contains the anticodon triplet of the code, which is matched to the triplet nucleotide code of the mRNA. This triplet and the amino acid attachment site are separated by 75 angstroms.

The central engine for protein biosynthesis is the ribosome—a large, as yet unsolved, puzzle that makes up more than 25% of the dry mass of the bacteria. The *E. coli* ribosome consists of about 55 proteins and three RNAs—5*S* (120 nucleotides, 16*S* (1542 nucleotides), and 23*S* (2904 nucleotides) ribosomal RNA (rRNA) (*3*). These components are housed in two ribonucleoprotein subunits (large and small). Once aminoacylated, the tRNAs interact with messenger RNAs embedded in the ribosomes. Here the anticodon-containing domains of the tRNAs act as template-reading heads that decode the triplets of the mRNA by codon-anticodon binding. As a result of the lining up of the aminoacylated tRNAs along the mRNA according to the code, the charged minihelix domains are brought together so that the amino group of one amino acid attacks the carbonyl carbon of its neighbor. This coupling reaction occurs at the peptidyl transferase center of the ribosome's large subunit. This functional center is located at a junction of several helices in the fifth of six domains that make

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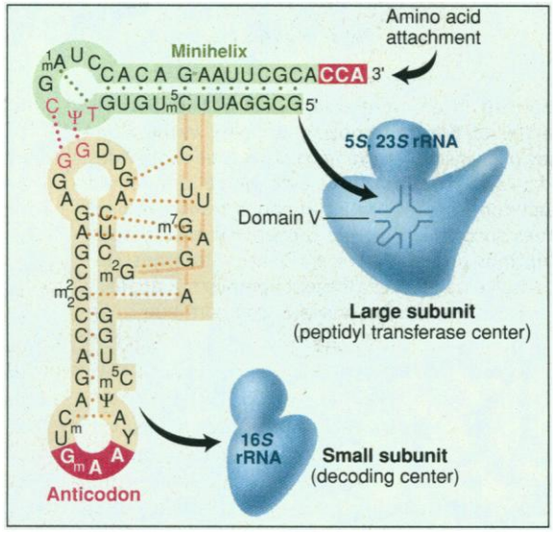
up 23*S* rRNA (*4*). A long-standing question has been whether one or more of the proteins bound to 23*S* rRNA is also required for this chemical step (*5*).

An experiment by Noller and co-workers established that protein-depleted 23*S* rRNA had transferase activity (*6*). Every attempt was made to scrub the natural RNA free of proteins, but in the end it was difficult to eliminate the last traces without also losing the peptidyl transferase activity. This result raised the possibility that the remaining traces of protein were critical for peptide bond formation. But in another study, peptide bond formation could be stimulated by a synthetic RNA (*7*); this finding further heightened the desire to demonstrate conclusively that 23*S* rRNA by itself had the transferase activity.

The most rigorous way to achieve this objective is to test a synthetic RNA derived from the gene for 23*S* rRNA. Such an RNA, never having been exposed to any of the ribosomal proteins, would be guaranteed to be free of their activities. Nitta *et al.*, taking advantage of earlier attempts by others to fold synthetic 23*S* rRNA transcripts, provided evidence that peptide bond formation could be achieved by reaction of *N*-acetyl-Phe-tRNA^{Phe} with Phe-tRNA^{Phe} to give the *N*-acetyl-Phe-Phe peptide (*8*). (If Phe-tRNA^{Phe} alone is used in this reaction, the resulting dipeptidyl group of Phe-Phe-tRNA rearranges to the diketopiperazine by attack of the amino-terminal free amino group on the carbonyl carbon of the aminoacyl ester bond; this rearrangement cannot occur with *N*-acetyl-Phe-Phe-tRNA^{Phe}.) But this study left unanswered some key questions.

Now, Nitta *et al.* have made a new contribution by taking advantage of the organized domain structure of 23*S* rRNA. They made transcripts corresponding to each domain of 23*S* rRNA and found, remarkably, that a mixture of all six domains was active, but only when domain V was present—the very domain that contains the transferase center. In fact, domain V by itself proved active, whereas none of the other domains had significant activity alone. Moreover, the antibiotic sparsomycin (which acts at the transferase center) inhibited the observed transferase activity, whereas neomycin (which acts elsewhere) did not.

These experiments are not without pitfalls. In fact, the aminoacyl moieties of aminoacyl-RNAs can be made to react with buffer species and other ligands (*9*). Such reactions are promoted by agents like polyethylene glycol, because they create an excluded volume that crowds together the reactive species. Aware of these pitfalls, Nitta *et al.* performed several controls. For example, they determined the background from crowding-dependent reactions with high concentrations of RNAs other than domain V. They also used two chromatographic methods to show that the product seems to be *N*-acetyl-Phe-Phe, and not the other products expected from crowding reactions. In addition, total acid hydrolysis of the product yielded Phe, as expected. They also showed that with *N*-acetyl-Phe-tRNA^{Phe} as the only tRNA species in the reaction, there are no products, and that with Phe-tRNA^{Phe} alone, only small amounts of Phe-Phe are produced.



An algorithm for life. The minihelix domain (of the two-domain tRNA structure) contains the amino acid attachment site and contacts the large ribosomal subunit while the anticodon domain interacts with the small subunit.

Several questions and directions for future experiments emerge from these studies. A surprise was the finding that the activity of domain V could be stimulated by addition of domain VI. No previous studies have shown a direct interaction between domains VI and V. In contrast, some RNA cross-linking data had implicated proximity between domains II and V and between domains IV and V (*10, 11*), yet no effect of either II or IV on the activity of V could be seen. If the II-V or IV-V proximity exists within the ribosome, it may require ribosomal proteins for its stabilization or it may not be required for peptidyl transferase activity. In another vein, Nitta *et al.*'s mutational substitution at G2252, which forms a hydrogen bond with C^{74} of the universal CCA^{76} trinucleotide at the 3′ end of the tRNA (*12*), reduced the transferase activity of domain V by about 60%. Compensatory mutations (at C^{74}) that should restore activity now need to be tested.

The two domains of the tRNA structure interact with distinct rRNAs in the full ribosome (*13*). Thus, the anticodon-containing domain interacts with 16*S* rRNA in the small subunit of the ribosome, while the minihelix domain interacts with 23*S* rRNA in the large subunit. In cellular protein biosynthesis, the tRNA anticodon binds to the triplet codons of mRNA, so that protein synthesis is template dependent. In the experiments of Nitta *et al.*, peptide synthesis is template independent and presumably involves only the minihelix domain of the tRNA. Indeed, isolated minihelix domains (just the "top half" of the L-shaped tRNA structure) are substrates for aminoacylation by many of the aminoacyl tRNA synthetases (*14–16*). Because these substrates lack the anticodon triplets of the genetic code, the relationship between the amino acid and the minihelix constitutes an "operational RNA code" for amino acids that is distinct from the triplets of the genetic code that it may have preceded (*17*).

Could charged minihelices support peptide bond formation in the Nitta *et al.* system? If so, would a system consisting of domain V of 23*S* rRNA and charged minihelices reflect the early origins of peptide synthesis, before the complete tRNA and rRNA structures were assembled? The minihelix is thought to be the more ancient, historical domain of the tRNA, the one that first arose in an RNA world (*18*). Domain V of 23*S* rRNA may also be the more ancient part of rRNA and perhaps coevolved with the minihelix domain of tRNA.

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