This Week's Citation Classic®

Steitz J A & Jakes K. How ribosomes select initiator regions in mRNA: base pair formation between the 3' terminus of 16S rRNA and the mRNA during initiation of protein synthesis in *Escherichia coli*. *Proc. Nat. Acad. Sci. USA* 72:4734-8, 1975. [Dept. Vtolec. Biophys. Biochem.. Yale Univ.. New Haven. CT; and Rockefeller Univ., New York, NY]

The first experimental evidence was presented to supportthe hypothesis that bacterial ribosomes use base pairing to identify start sites for translation. A noncovalent complex including the last 50 nucleotides of 16S rRNA and an initiator region from an RNA bacteriophage message was generated and characterized. [The *SCI*® indicates that this paper has been cited in more than 530 publications.]

mRNA-rRNA Base Pairing During Translation Initiation

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Early in 19741 received from Lynn Dalgarno a preprint which represented an important turning point in our understanding of how bacterial ribosomes accurately initiate protein synthesis. It also marked the beginning of our appreciation of the central role of rRNA in ribosome function.

Starting in 1967, I had done postdoctoral work at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England. There, several years earlier, two-time Nobel Prize winner Fred Sanger had developed methods for obtaining complete sequences of short RNA molecules, such as tRNAs or SS rRNA. The challenge of sequencing much larger messenger RNAs required that suitably sized pieces be obtained. Encouraged by Mark Bretscher, I used ribosome protection to select fragments of the R17 bacteriophage mRNA that might direct the initiation of protein synthesis. The three approximately 30-nucleotide long segments I analyzed turned out to encode the beginnings of the three phage proteins.[1] But the sequences provided little insight into what besides the initiating AUGs might be critical.

After joining the Yale faculty in 1970, I had continued to try to decipher why initiating ribosomes select these particular sequences. Over the next few years the catalog of initiator regions recognized by *Escherichia coli* ribosomes grew to approximately 10 entries. But the only common feature—a purine-rich region upstream of the AUG codon—was conserved in neither position nor sequence.

Dalgarno and his student John Shine had undertaken the relatively prosaic task of sequencing the 3 terminal 12 nucleotides of *E. coli* 16S rRNA. Their results conflicted with previous reports, but their corrected sequence exhibited limited complementarity to the purine-rich regions upstream of known initiator AUGs. They therefore proposed that the 3' terminus of the 16S rRNA participates in the initiation of protein synthesis by forming several Watson-Crick base pairs with messenger RNA.[2] Dalgarno's preprint was very exciting, since I knew of many as-yet-unpublished results that fit perfectly with the Shine-Dalgarno hypothesis. But how could the idea be proven correct?

The crucial insight came during a post-Gordon Conference hike in the White Mountains that summer. While explaining the Shine-Dalgarno hypothesis to friends Bretscher and Wally Gilbert, I realized that a hydrogen-bonded mRNA-rRNA complex might be isolatable on a nondenaturing polyacrylamide gel if the 16S RNA were first cleaved with colicin E3, a toxin known to cut 16S rRNA about 50 nucleotides from its 3' end. Luckily, Karen Jakes, a Yale student who had moved to Rockefeller, had prepared large stocks of colicin E3. She immediately agreed to supply as much as might be needed.

After I finished teaching medical students in the fall, I devoted full time to the experiment. The scheme was to form initiation complexes using one of the phage R17 initiator regions that I had previously shown could rebind to ribosomes. Colicin E3 was added to cleave the 16S rRNA, and ribosomal proteins were removed by incubation with sodium dodecyl sulfate. Ittook some time to work out the gel conditions, but eventually I was able to visualize a complex which contained both the mRNA and 16S rRNA fragments. It melted at high temperature, strong evidence that the RNAs were held together by base pairs.

Many subsequent experiments have confirmed that base pairing between the Shine-Dalgarno sequence in the mRNA and the 3' end of 16S rRNA is of prime importance for initiation of translation by bacterial ribosomes.[3] We now know that rRNAs engage in a multitude of other interactions critical for ribosome function, even including catalysis of peptide bond formation.[4] Based in part on this work, I received the 1976 Eli Lilly Award in Biological Chemistry.

1. **Steitz J** A. Polypeptide chain initiation: nucleotide sequences of the three ribosomal binding sites in bacleriophagc R17 RNA. *Nature* 242:957-64. 1969. (Cited 380 times.)
2. **Shine** J & **Dalgarno** L. The 3-tenninal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. *Proc. Nat. Acad. Sci. USA* 71:1342-6. 1974. (Cilcd 2.375 times.)
3. **Dahlbcrg** A E. The functional role of ribosomal RNA in protein synthesis. *Cell* 57:525-9, 1989.
4. Noller H F, Hoffarth V & Zimniak L. Unusual resistance of peptidyl transferase to protein extraction procedures. *Science* 256:1416-9. 1992.

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