mRNA RESEARCH

TOEPRINTS AND T7 HELP ELUCIDATE TRANSLATION

STONY BROOK, N.Y.—What are the features of messenger RNA-30S ribosome interaction that lead to efficient translation? New insights into this question were offered at this year's Stony Brook Symposium on Molecular Biology.

One group, headed by Larry Gold at the University of Colorado (Boulder), described work that attempts to separate elements of mRNA secondary structure from primary sequence determinants that flank initiation codons. In general secondary structures of mRNA in the -20 to +13 initiation domain have negative effects on translation. Presumably, such structures severely inhibit access of the 30S ribosome to mRNA primary determinants, such as the Shine and Dalgarno region or the initiation codon, which lie within this domain. However, Gold reported that some secondary structures are not only tolerated but appear to be necessary for efficient translation. For example, in the case of the gene 38 message of phage T4, the Shine-Dalgarno sequence is positioned 15 bases upstream of the initiator codon (compared to the

more usual eight or nine nucleotides). A hairpin in the region between the Shine-Dalgarno sequence and the initiator codon, according to Gold, is required to correctly position the message in the 30S ribosome "track."

In order to study the ribosomal components that determine this track, Gold has developed a novel method, which he calls "toeprinting." To perform a toeprint analysis, Gold synthesizes a labeled piece of single-stranded DNA which will anneal to an RNA of interest. Using reverse transcriptase, this primer is extended towards the 5' end of the message in the presence of 30S ribosomes. In theory, the polymerase should stop synthesis when it encounters a bound ribosomal subunit. In practice, the method works remarkably well, and it defines the edge of the ribosome binding region at the +15 position on the mRNA. Gold hopes that experiments aimed at locating 16S RNA nucleotides, with respect to the track within which mRNA is bound, will identify the ribosomal components that select one mRNA over another, and thus determine translational effi-

ciencies. Toeprints of the gene 32 message of phage T4 have already given the surprising result that the small ribosomal subunit is capable of selecting initiator domains without accessory factors, initiator tRNA, or the large subunit.

Also unexpected was the report from Peter Olins and colleagues at Monsanto (St. Louis, MO) that the region of mRNA immediately upstream from the Shine-Dalgarno sequence may play an important role in translation initiation. When a nine-base AU-sequence—derived from this part of the coat protein message of phage T7—is placed in a consensus initiator sequence, translation of a number of heterologous genes in *Escherichia coli* is substantially increased. This sequence allows a very energetically favorable structure—distinct from Shine-Dalgarno pairing—to be drawn between the mRNA and the 3' end of the 16S ribosomal RNA. Olins postulated that T7 has evolved mRNA binding sites that exploit the ribosome in a highly efficient manner different from normal translation.

—Harvey Bialy

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