

Order and spacing of ribosomal RNA genes

from Benjamin Lewin

THE first system to be defined in which a large precursor RNA is processed to much smaller mature molecules was that of rRNA synthesis in eukaryotic cells; as early as 1966, Greenberg and Penman (*J. molec. Biol.*, **21**, 527-535) reported that a label in nucleolar RNA first enters a 45S species in HeLa cells and only later is found in 28S and 18S rRNAs. Since then it has become apparent that the 45S RNA contains the sequences of both the 28S and 18S RNA molecules, which together occupy about 7,500 nucleotides of its total length of 14,000 bases.

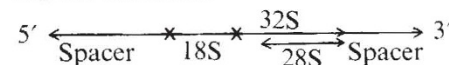
In bacteria the situation at first appeared to be different, with the 16S and 23S rRNAs each apparently transcribed independently, although their genes were located adjacent on the chromosome. But the isolation of an *E. coli* strain lacking a processing enzyme allowed Nikolaev, Silengo and Schlessinger (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3361-3365; 1973) and Dunn and Studier (*ibid.*, 3296-3300) to isolate a 30S precursor molecule that contains both sequences.

How are the mature rRNA sequences organised within the precursor? In bacteria the sequences 5' 16S-23S-3' is generally agreed on the basis of the relative sensitivity of 16S and 24S RNA synthesis to inhibition by rifampicin

(for example Doolittle and Pace, *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1786-1790; 1971). In eukaryotes the evidence has been conflicting although up to now it was generally held to favour the reverse order. In particular the identification of distinct secondary structure of the various rRNAs and their precursors in the electron microscope and the determination of polarity by digestion with a 3' exonuclease, suggested that the 5' end of the 28S molecule coincides with the 5' end of the 45S precursor (Wellauer and Dawid, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2327-2831; 1973). This seemed to be the definitive answer and a similar organisation for *Xenopus* 40S precursor was suggested by the same authors (*J. molec. Biol.*, **89**, 379-396; 1974).

But two recent experiments using different techniques suggest the opposite conclusion. Hackett and Sauerbier (*J. molec. Biol.*, **91**, 235-256; 1975) inhibited RNA synthesis in L cells by ultraviolet irradiation. The irradiation causes transcription to terminate at damaged sites in DNA; the farther a gene lies from its promoter, the more likely it becomes that a terminating event will occur before its transcription is completed. The pseudo-first order loss of 45S RNA as a function of ultraviolet dose confirmed that each

gene for the precursor represents an individual transcription unit with its own promoter. The target size of the 18S cistron suggested by these experiments was about 4,000 nucleotides, just twice the length of the 18S RNA molecule. This suggests that a spacer of 2,000 nucleotides lies at the 5' end of the 45S precursor, followed by the 2,000 nucleotides of the 18S sequence. The target size of the 28S RNA was 11,000 nucleotides; since the molecule is 5,500 nucleotides long, it must begin at a point 15,000 nucleotides after the end of the 18S RNA. This leaves a distance of 3,000 nucleotides at the 3' end of the 45S RNA, which must be part of the discarded sequences. The target size of the 32S precursor, the immediate precursor of 28S RNA, is the same as that of the 28S molecule; this suggests that the 32S precursor is adjacent to the 18S sequence, so that the 45S RNA has the structure:



Another approach to analysing sequence organisation has been taken by Liau and Hurlbert (*J. molec. Biol.*, **98**, 321-332; 1975). The mature 28S and 18S RNA molecules are methylated on the 45S precursor, when only these sequences gain the methyl groups. The