Ribosomal RNA Gene Amplification—an Unresolved Process

DURING oogenesis in the toad *Xenopus laevis* the total number of ribosomal RNA genes is dramatically increased. Somatic cells possess only a few hundred copies of the basic ribosomal DNA unit strung along one after another in a tandem fashion. Each rDNA unit codes for an 18S and a 28S rRNA molecule and also contains a "spacer". By contrast, oocytes contain some thousand times more of these rDNA genes spread out over about a thousand nucleoli. The arrangement of the rRNA genes and spacers seems to be identical in both somatic cells and oocytes. The purpose of this selective amplification of rDNA genes is to facilitate production of ribosomes which are stored in large numbers in the developing oocyte.

What is the molecular mechanism of this amplification? Formally two possible distinct routes can be identified. The simplest of these uses the entire rDNA complement of the chromosomal nucleolar organizer as a template for DNA replication in the classic semi-conservative manner. Such a mechanism might be characterized by the incorporation of newly synthesized DNA into the chromosomal rDNA at the nucleolar locus. An alternative route would be to copy a single rDNA unit into a complementary polynucleotide—either RNA or DNA—which would then act as a template for the ensuing replication of the rDNA unit.

In 1970 Tocchini-Valentini and Crippa (*Lepetit Colloquia*, **2**, 237) plumped for the second model and put forward the attractive proposal that the first step in the amplification process is the formation of an RNA transcript of the entire rDNA unit which would then serve as a template for an RNA-dependent DNA polymerase (or reverse transcriptase). This polymerase would first generate an intermediate RNA-DNA duplex which would then be used to form a double stranded DNA copy of the repeating unit of rDNA. Crippa, Tocchini-Valentini and their associates further proposed that the final polycistronic rDNA molecules were formed by the stringing together or ligation of these monocistronic units.

This model has several predictions. First, a complete (47S) RNA transcript of a repeating rDNA unit must be present at some stage of the amplification process. Second, the replicative intermediate should contain both this 47S RNA and newly synthesized DNA. Third, a specific RNA-dependent DNA polymerase should be responsible for the initial production of the rRNA:rDNA hybrid and, finally, monocistronic rDNA units might transiently exist in a free state. On these last two points evidence is virtually non-existent. In accordance with the first two predictions, however, Crippa and Tocchini-Valentini (*Proc. US Nat. Acad. Sci.*, **68**, 2769 ; 1971) identified in amplifying ovaries a 47S RNA species containing sequences complementary to both the 28S and 18S cistrons and the spacer region. This RNA was clearly distinguished from the normal 37S rRNA precursor on the basis of sequence composition. Furthermore, the same workers isolated an RNA-DNA complex in which most of the RNA component sedimented at 45S.

Although these experiments are entirely consistent with their proposed model, they do not prove that RNA-dependent DNA synthesis is the mechanism actually responsible for rDNA amplification in the oocyte. In an attempt to nail down the actual process more precisely Bird, Rogers and Birnstiel describe in this issue of *Nature New Biology* (page 226) the results of a reinvestigation of the problem. First, they report that they cannot detect the 47S RNA species corresponding to a transcript of an rDNA unit by fractionating *in vivo* labelled RNA on polyacrylamide gels or by sensitive hybridization techniques. Second, they find that they cannot even locate the RNA-DNA complex although they have attempted to label such a putative entity with both radioactive uridine and thymidine as precursors. Indeed, they find that the uridine precursor is actually incorporated into DNA in the form of deoxycytidine. This latter observation does not seem to explain the incorporation of uridine into 47S RNA, observed by Crippa and Tocchini-Valentini, because that polynucleotide was clearly sensitive to ribonuclease. Thus, on the face of it there is a straight conflict of evidence between the two groups for which no completely consistent explanation is immediately apparent.

The data of Bird *et al.*, taken together with their final statement that amplification of rDNA can proceed by a classical semi-conservative mode of DNA replication, must imply that the bulk of rDNA amplification is achieved by a conventional DNA replication process. The possibility that the DNA which initially serves as a template for this replication is, however, itself first synthesized by an RNA-dependent DNA polymerase is certainly by no means excluded. It follows that if the actual mechanism were an initial RNA-dependent synthesis of DNA succeeded by DNA-dependent DNA replication, perhaps by a rolling circle process, then the apparent conflict of data would simply be a consequence of the two groups looking at different stages of the amplification process. Nevertheless, it is very clear that on the basis of the evidence available there is as yet no conclusive answer to the crucial question of the nature of the first intermediate in the process of rDNA amplification. Is it simply a copy of the entire rDNA region, or is it a copy of a single rDNA unit, and, if so, is it RNA or DNA? The answers to questions such as these could be of more general significance than simply explaining the amplification of rDNA in a rather specialized amphibian.

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