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Control of rRNA Production in Non-growing Cells

COOPER has summarized the recent evidence that the asymmetric labelling of 28S and 18S rRNA in non-growing human lymphocytes reflects the rapid turnover ("wastage") of rRNA[1]. His summary has been presented in response to an alternative interpretation that such asymmetries reflect very long transcription times for the synthesis of rRNA precursor molecules[2]. I have examined in detail the experimental basis of each of these arguments and I still do not find the evidence for "wastage" in lymphocytes sufficiently conclusive to eliminate an extended transcription time interpretation. My views on each of his four points are summarized briefly.

(1) The evidence that the rates of rRNA synthesis are regulated in lymphocytes and that this regulation occurs independently of the asymmetric labelling of rRNA has been based on measurements of the incorporation of radioactive extracellular precursors into RNA. But this method for determining rates of cellular RNA synthesis is not valid. No relationship has been demonstrated between the rates of incorporation of such precursors and the actual rates of RNA synthesis[3–5]. The quantitative measurement of the rates of cellular RNA synthesis requires determination of the specific activity of the intracellular radioactive precursors (nucleotide triphosphates or S-adenosylmethionine) in addition to measurement of the rates of incorporation of radioactivity into RNA. Determination of the specific activity of the intracellular pools is particularly important in these experiments because lymphocytes in very different metabolic states are being compared.

(2) It is difficult to interpret changes in the relative radioactivity in 32S (28S) and 18S rRNA during an actinomycin D chase since this drug interferes with the normal processing of rRNA in lymphocytes, causing the abnormal and rapid degradation of newly synthesized rRNA[6]. Furthermore, actinomycin D causes the preferential degradation of 32S (28S) rRNA in another human cell (HeLa)[7] and such preferential degradation may also occur in lymphocytes.

(3) The differences in the kinetics of labelling of total and "cytoplasmic" rRNA of growing and non-growing lymphocytes are incompatible with a "wastage" interpretation. The linear labelling kinetics of rRNA in the total as well as the "cytoplasmic" fractions indicate that all labelled molecules are stable and accumulate during the labelling period. If "wastage" were occurring during this time, a non-linear decreasing rate would be observed. The reduced but linear rate of labelling of the "cytoplasmic" fraction may reflect a slower transport of rRNA or a decrease in the recovery of labelled RNA in the arbitrarily defined "cytoplasmic" fraction[8].

(4) The apparent increase in asymmetry of labelling of rRNA does not distinguish between "wastage" and extended transcription time interpretations. Such an increase would be predicted under an extended transcription time interpretation if, as is likely, the specific activity of the precursor pools were increasing at a rapid rate during these initial labelling periods.

Asymmetric labelling of 28S and 18S rRNA has been correlated with growth of chick skin cells in culture and has been interpreted to reflect variations in the transcription times for rRNA precursor[2]. The anomalous lag observed in the initial rates of labelling of rRNA precursor was also consistent with this transcription time interpretation. Cooper has suggested that this lag could have resulted from systematic errors in estimating the amounts of radioactivity associated with rRNA precursor. This criticism, however, is without basis. The amounts of radioactivity measured in rRNA precursor during the lag periods (up to 20 min of labelling) were 2 to 4 times below the expected values and represented deficiencies of 50 to 1,000 c.p.m. These deficiencies were equal to and greater than the amounts of radioactivity assigned to these peaks of rRNA precursor and were much in excess of the error (10–20%) involved in these graphical estimates. A more detailed treatment of such kinetic data is available[9]. The interpretation most consistent with the anomalies observed in the labelling of both rRNA precursor and 28S and 18S rRNA would be that the transcription time (the total time a nascent rRNA precursor chain spends on the gene) varies under different states of growth of the chick skin cells; however, this is not meant to imply that this hypothesis should not and cannot be tested further.

It is to be hoped that the disagreement about the interpretation of the asymmetric labelling of rRNA will not obscure the conclusion that the rate of synthesis of rRNA precursor is regulated during regulation of growth of chick skin cells in culture. Quantitative measurements of RNA synthesis, based on determinations of the specific activity of nucleotide precursor pools, demonstrated that the observed regulation of both the rate of rRNA precursor synthesis and of the rate of catabolism of 28S and 18S rRNA can account completely for the regulation of rRNA accumulation which occurs during contact inhibition of cell division.

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