ACTINOMYCIN D-RESISTANT RNA SYNTHESIS IN ANIMAL CELLS

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In attempts to determine the fate of rapidly-labelled nuclear RNA we have encountered a paradox which we believe is best explained by postulating that in animal cell nuclei there is, in addition to Actinomycin D-sensitive RNA synthesis (1) a system which is Actinomycin D-resistant and which may be a precursor of a ribosomal component.

The experiments were conducted with LS cells (a subline of the strain L fibroblast, developed in this laboratory, which grows spontaneously in suspension). They were maintained in Eagle's medium (2) and calf serum in a tris-citrate buffer at pH 7.4. The cells were fractionated by homogenising in glycine-HCl buffer at pH3 and differential centrifugation. The method gives nearly 100% recovery of uncontaminated nuclei. Ribosomes were obtained from the supernatant by suspending in sucrose and centrifuging at 100,000 g for 1 hr. Orthodox analytical and counting methods were employed otherwise.

In preliminary experiments it was found that it required some hours for Actinomycin D to produce its full effect at the concentrations recommended by other workers. Inhibition of RNA synthesis in intact LS cells proved to be a function of time and concentration of Actinomycin D and it was necessary to expose the cells to 100 µg per ml of medium to obtain 95%

inhibition of nuclear RNA synthesis in 15 minutes. On the other hand after removing Actinomycin D and washing no recovery from its effect was noticed for at least 8 hours. A 15 minute pulse at high concentration was therefore employed.

In one set of experiments two identical suspension cultures were set up. The first was pulsed with 5 μc/ml. ^{3}H-uridine for 15 minutes and then with 100 μg. Actinomycin D for 15 minutes before transferring the cells to ordinary medium. The second was pulsed for 15 minutes with Actinomycin D and then for 15 minutes with ^{3}H-uridine and transferred to ordinary medium in otherwise identical conditions. Samples were removed immediately after the second pulse and then at intervals. The results of an experiment are shown in figure 1. In the

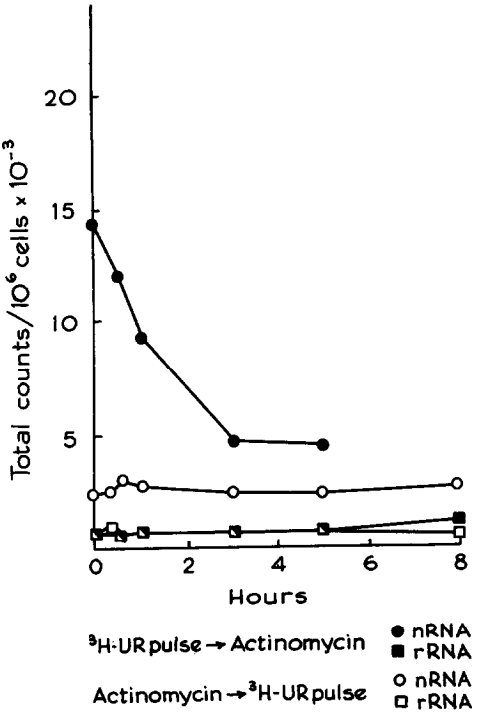

Fig. 1

culture pulsed first with ^{3}H-uridine and then Actinomycin a highly active RNA fraction was found in the nucleus and its activity rapidly declined with a half-life of just over an hour. In the culture pulsed first with Actinomycin and then ^{3}H-uridine no rapidly-labelled nuclear RNA appeared. In both very little activity was found in the ribosomes and it remained constant throughout the experiment.

Other experiments (figure 2) differed only in that ^{3}H-uridine was present in the medium throughout. One of a pair of cultures was pulsed for 15 minutes with 2 μc/ml. ^{3}H-uridine. Both were then pulsed with 100 μg/ml. Actinomycin and after washing were incubated in a medium containing 1 μc/ml. ^{3}H-uridine. Samples were removed at intervals. Both nuclear and ribosomal RNA were highly labelled in both cultures. The activity of nuclear RNA

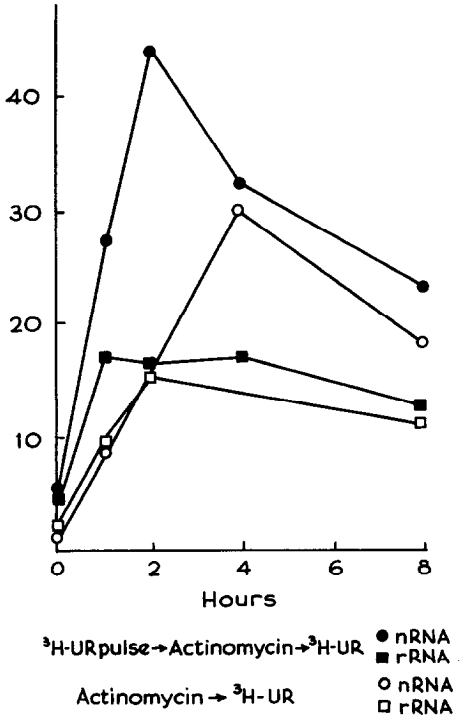

Fig. 2

reached a peak after 2 hours in the pre-labelled culture and at 4 hours in the other before declining. Activity in the ribosomes increased rapidly and remained high.

Since in both experiments the acid-soluble pool remained highly active throughout and yet the two results are very different it must be concluded that when uridine is added as a pulse it very soon becomes unavailable for RNA synthesis (either by being converted to a non-utilisable substance or by compartmentation). Consequently in the first type of experiment a true pulse-labelling of nuclear RNA is obtained. None of this finds its way to the cytoplasmic ribosomes.

In the second type of experiment, when ^{3}H-uridine is available in the medium most of the time (it is actually used at a fairly rapid rate) synthesis of nuclear RNA takes place and is not prevented by pretreatment of the cells with Actinomycin D. Active incorporation into ribosomes occurs in these conditions.

The following conclusions can be drawn in relation to these cells.

1. The nucleus contains an Actinomycin D-resistant system for RNA synthesis as well as an Actinomycin D-sensitive system.
2. Either some of the RNA synthesised by this system becomes associated with cytoplasmic ribosomes or else ribosomal RNA is formed by an independent process which is resistant to Actinomycin D.
3. Either the rapidly-labelled Actinomycin D-sensitive nuclear RNA (which is currently thought to be messenger RNA (3) never becomes associated with cytoplasmic ribosomes or else Actinomycin D interferes with such an association.

The results indicate that the syntheses of a major nuclear RNA component and a ribosomal RNA component are

resistant to Actinomycin D, and therefore probably RNA-primed. The question arises whether they are synthesised independently or whether one is a precursor of the other. Since nuclear RNA synthesised by the Actinomycin D-sensitive process does not become associated with ribosomes it is possible that, in nucleated cells, the messenger RNA which combines with ribosomes in protein synthesis is a copy of the primary messenger formed on the chromosomes.

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