DIFFERENTIAL INHIBITION OF 28S-18S RNA AND 5S RNA SYNTHESIS IN THE CYCLOHEXIMIDE-TREATED XENOPUS LAEVIS EMBRYONIC CELLS

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SUMMARY

28S-18S RNA and 5S RNA synthesis were measured in the isolated cells from Xenopus laevis embryos whose protein synthesis was inhibited by cycloheximide. The inhibition of 5S RNA synthesis increased linearly with time, while that of 28S-18S RNA synthesis was greater in early phase but slowly increased later. Eventually, both were inhibited to a 10 per cent level of control after 5 hours. These results indicate that the sensitivity of 5S RNA synthesis toward cycloheximide is definitely different from that of 28S-18S RNA synthesis and might not be in favor of the co-ordination between them.

It has been established that the treatment with cycloheximide inhibit not only protein synthesis but also ribosomal (28S-18S) RNA synthesis(1). However, there has been no information concerning 5S RNA synthesis under such a condition. The knowledge of it might be of considerable importance in understanding the regulatory mechanism between 28S-18S RNA and 5S RNA synthesis. In fact, the co-ordination has already been suggested between them(2).

As one of the series of papers on the regulation of 5S RNA synthesis during early development of Xenopus laevis embryos, it will be reported here that 5S RNA synthesis also is inhibited by cycloheximide at the concentration which reduces over-all protein synthesis by 75-90% and is much less sensitive than 28S-18S RNA synthesis.

MATERIALS AND METHODS

Isolated cells were prepared from Xenopus laevis embryos at

two different developmental stages(stages 10, 23) and cultured as previously described(3). Labeling was done with H^3-uridine-5T(5uC/ml) or C^{14}-protein hydrolyzate(2uC/ml) for varying length in the presence of cycloheximide(150ug/ml).

Nucleic acids were extracted with phenol-SDS-bentonite at pH 5.0, and then analyzed either on sucrose density gradients as described earlier(3, 4), or by polyacrylamide gel electrophoresis(5). Gels were sectioned and the RNA was eluted with NH_4OH, then the radioactivity was measured in a ethanol-DPO-toluene mixture by a liquid scintillation spectrometer.

Ribosomal protein was extracted from the C^{14}-labeled cells with 8M urea-4M LiCl(6), and was subjected to electrophoresis on polyacrylamide gels following the modified method of Reisfeld et al.(7). The radioactivity was counted as described above. Total protein synthesis was measured by the incorporation of C^{14}-protein hydrolyzate into acid-insoluble material of the cell.

RESULTS AND DISCUSSION

The effects of cycloheximide on protein synthesis were first measured in the isolated cells derived from tailbud embryos. The maximum inhibition of total protein synthesis was realized within 3 hours and it was 90%. On the other hand, inhibition in ribosomal protein synthesis was smaller than in total protein synthesis: it ranged from 60% to 70% of control.

Among syntheses of the three RNA classes studied, 28S-18S RNA synthesis was the most sensitive to cycloheximide. After 1 hour it was reduced to a 30% level of control. Subsequently, the rate of inhibition declined slowly as the treatment was longer. The patterns of RNA synthesis after 3 hours' treatment were shown in Fig.1a,b. After 3 and 5 hours, the inhibitions were 84% and 90%, respectively (Fig. 2). That 28S-18S RNA synthesis was inhibited more severely

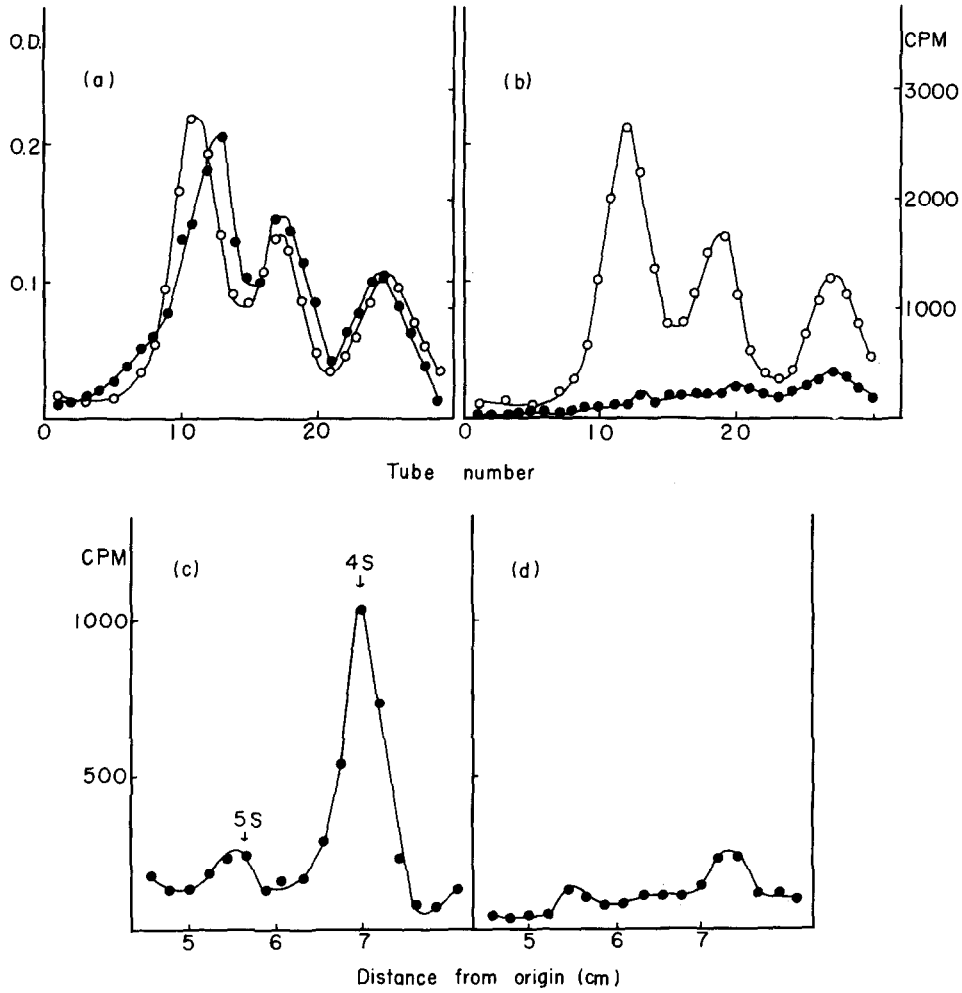

Fig. 1. Effects of cycloheximide on the three classes of RNA.
(a, b) : Isolated cells from 20 tailbud embryos were labeled with H^3-uridine (5uC/ml) for 3 hours in the presence (b) or absence (a) of cycloheximide (150ug/ml) and collected after additional 2 hours' chase. The RNA was centrifuged for 17 hours at 24000 rpm through 27 ml of 5-20 sucrose density gradient on the RSP 25 rotor of the Hitachi 55P-2 Ultracentrifuge.
(o) U.V. absorption of rat liver RNA used as carrier.
(●) radioactivity.
(c, d) : Isolated cells from 20 tailbud embryos were labeled with H^3-uridine (5uC/ml)for 5 hours in the presence (d) or absence (c) of cycloheximide (150ug/ml) and collected after additional 2 hours' chase. The RNA was submitted to electrophoresis as described in MATERIALS AND METHODS.

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than ribosomal protein synthesis might suggest some involvement in 28S-18S RNA synthesis of a mechanism which is sensitive to the agent.

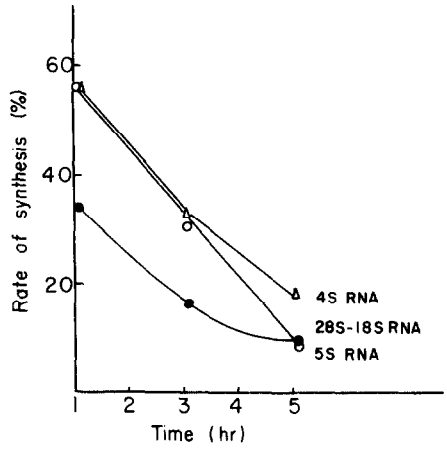

Fig. 2. Time courses of effects of cycloheximide on the three classes of RNA.
Isolated cells from 20 tailbud embryos were labeled with H^3-uridine for various length, in the presence or absence of cycloheximide. RNAs were analyzed as described in Fig. 1. The ratios of RNA synthesis in the treated-cells to that in the control cells were plotted against experimental periods.

The trend was very different in 5S RNA synthesis (Fig.2). After 1 hour, the inhibition was less than 50% ; it was followed by a sharp increase in inhibition. The synthesis was reduced to a 8% level of control after 5 hours (Fig. 1c, d). This extent is close to that in 28S-18S RNA synthesis.

As is apparent in Fig. 2, the inhibition of 4S RNA synthesis increased at the same rate as in 5S RNA synthesis during first 3 hours, but at the 5th hour it was inhibited to a less extent. In order to measure 4S RNA synthesis, the RNA samples were analyzed by the two methods, and the obtained two sets of the results are in a good agreement.

The same experiment was done with isolated cells derived from earlier embryos, gastrulae, in which 28S-18S RNA synthesis had been shown to begin at first after fertilization. The cells were cultured for 5 hours under the same condition as above. The inhibitions in total and ribosomal protein syntheses were 86% and 76%, respectively. The

extents are close to those obtained with the tailbud embryo cells, and higher resistance of ribosomal protein synthesis is again observed.

On the other hand, the inhibition in RNA syntheses was much smaller than in tailbud embryo cells. Both total RNA and 4S RNA were reduced to around 50%. This is reasonable since 4S RNA is major part of the RNA synthesized in gastrulae. 28S-18S RNA synthesis was inhibited to 23%, while the depression of 5S RNA synthesis was 60%. Here again 5S RNA synthesis is much less sensitive to cycloheximide than 28S-18S RNA synthesis.

Between the cells from gastrulae and those from tailbud embryos, remarkable differences are found in the rates of inhibition of RNA synthesis, irrespective of the rough coincidence in the extent of inhibition in protein synthesis. The difference in activity of RNA synthesis probably contributes to the observed differences in the inhibition of RNA synthesis.

The conclusions of the present experiments are as follows: (1) 5S RNA synthesis is inhibited by the cycloheximide treatment, (2) it is remarkably resistant, as compared with 28S-18S RNA synthesis, to the agent at least during shorter periods of treatment and in the gastrula cells with lower activity of RNA synthesis, and (3) the syntheses of the three classes of RNA were much less inhibited by the agent in the gastrula cells than in the tailbud embryo cells.

On the basis of the observed differential inhibition of 28S-18S RNA and 5S RNA synthesis, it seems reasonable to conclude that the strict co-ordinate synthesis does not occur between them as has been suggested. In addition to it, differential inhibition by actinomycin D and the different rates by which 28S-18S RNA and 5S RNA are being synthesized during early development of Xenopus laevis (8) are not in favor of the suggested co-ordination.

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