179. Ribonucleic Acid (RNA) Synthesis in Dissociated Embryonic Cells of *Xenopus Laevis*. II^{*)}

Inhibitor of Ribosomal RNA Synthesis

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It has been shown that in amphibian embryos, soluble, and ribosomal RNA are synthesized at rates characteristic of their developmental stages.^{1),2)} They synthesize mostly soluble RNA up to the late blastula stage but, with the onset of gastrulation, ribosomal RNA synthesis takes place and becomes more and more active from then on. The involvement of some cytoplasmic factor in regulation of embryonic RNA synthesis has recently been pointed out.^{3),4)} The previous reports^{5),6)} of the present authors have shown that the use of isolated cells of *Xenopus laevis* embryos are suitable for investigations on such a problem. In the present communication, the results will be described which have been obtained using the dissociated embryonic cell system and it will be suggested that in cleaving embryos there exists a factor that inhibits specifically ribosomal RNA synthesis.

Experimental. The methods used for dissociating embryos, culturing and labeling isolated embryonic cells were those described previously.^{5),6)} Two groups of animals were given gonadotropic hormone on three consecutive days to get both late neurulae and mid-blastulae at the same time. Cells obtained from blastulae and neurulae are designated as before as B-cells and N-cells, respectively. In some experiments, N-cells were cultured in a medium containing an "extract" (conditioned medium) of B-cells. The extract was prepared by exposing the culture medium to B-cells for 5 hours.

Results. It has already been revealed that B-cells synthesize soluble RNA but ribosomal RNA synthesis is negligible, in contrast to active synthesis of both RNA species in N-cells.^{5),6)} Thus, in B-cells, the ratio of synthesized ribosomal RNA to soluble RNA (rRNA/sRNA) is about 0.5; this is, however, as high as 5 in N-cells.

N-cells were cultured in a medium containing H³-uridine, together with B-cells from varying numbers of embryos (Fig. 1). An addition

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Fig. 1. Incorporation of H^3 -uridine into ribosomal and soluble RNA in mixed cultures. N-cells obtained from 25 embryos were cultured for 5 hours with B-cells. Ordinate: Counts per minute per culture. Abscissa: Figures show numbers of embryos from which B-cells were obtained. (●) Ribosomal RNA; (○) Soluble RNA. Two lines represent the amounts of ribosomal (upper) and soluble (lower) RNA to be synthesized, on the basis of independent synthesis in both N-cells and B-cells.

Fig. 2. Effects of B-cell extract on incorporation of H^3 -uridine into ribosomal and soluble RNA in N-cell cultures. N-cells obtained from 25 embryos were cultured for 5 hours in the medium containing B-cell extract. Ordinate: Counts per minute per culture. Abscissa: Figures show numbers of embryos with which B-cell extract was prepared. (●) Ribosomal RNA; (○) Soluble RNA. Two lines represent the amounts of ribosomal (upper) and soluble (lower) RNA to be synthesized in the normal medium.

of B-cells to a N-cell culture reduces ribosomal RNA synthesis; on the other hand, soluble RNA synthesis is indifferent to it. Indeed, the amount of ribosomal RNA synthesized in these cultures is consistently and significantly less than that of N-cells alone. Thus, the ratio (rRNA/sRNA) is lowered from 5.4 to 2.0. A reciprocal experiment has given essentially the same result: the presence of B-cells does not interfere with soluble RNA synthesis, but with ribosomal RNA synthesis, in N-cells. These results would demonstrate that B-cells have some inhibitor for ribosomal RNA synthesis of N-cells.

Then, N-cells were incubated in the medium containing a B-cell extract. The results obtained are shown in Fig. 2. With an increase in amount of the cell extract added to a culture, an apparent decrease

Fig. 3. Effects of B-cell extract on RNA, DNA, and protein synthesis in N-cells. N-cells obtained from 5 embryos were cultured in the presence of B-cell extract. The extract was prepared with 10 blastulae. Ordinate: Counts per minute per culture. Abscissa: After an indicated period, the cells were removed to obtain acid-insoluble materials, which were then measured of radioactivity. (a) Total RNA (H^3 -uridine); (b) DNA (H^3 -thymidine); (c) protein (C^{14} -protein hydrolyzate). Labeled in the presence (●) or the absence (○) of the extract.

in ribosomal RNA synthesis of N-cells is observed. The synthesis of soluble RNA, on the other hand, does not change: the ratio shifts from 4.9 to 2.6. The results reveal that the inhibitor can be released from B-cells into the medium and remains active in inhibiting ribosomal RNA synthesis of N-cells.

If such inhibition is not attributed to some general damage of N-cells during incubation in the presence of B-cell extract, removal of the extract should recover ribosomal RNA synthesis. It was observed that, after the ratio (rRNA/sRNA) had been reduced to 3.2, the resumption of ribosomal RNA synthesis occurred and the ratio returned back to 5.4 within 6 hours. If it is a specific site of the inhibition, RNA synthesis should be impaired by the inhibitor before the inhibition of DNA and protein syntheses becomes apparent. Rates of the synthesis were expressed in terms of incorporation of H^3 -uridine, H^3 -thymidine and C^{14} -protein hydrolyzate. The results are illustrated in Fig. 3, which clearly show that RNA synthesis is significantly inhibited by the extract while DNA and protein syntheses are not. That the reduction in RNA synthesis is nothing other than a reflection of reduced ribosomal RNA synthesis has been confirmed. The sum of these two lines of observations would suggest that the inhibition of ribosomal RNA synthesis is perhaps not due to some unspecific, general injury of N-cells. Instead, the inhibitor in question seems to inhibit specifically ribosomal RNA synthesis.

Characterization of the inhibitor is now in progress in our laboratory; preliminary experiments have pointed out that the inhibitor is heat-stable and dialyzable. It occurs also in other animal tissue cells, such as those of rat liver.

These facts stated above seem to provide us with a picture of one aspect of the mechanism by which the regulation of ribosomal RNA synthesis is realized in the amphibian embryo during development. The synthesis of ribosomal RNA could be arrested in the cleaving embryo because of the presence of the inhibitor and, when the embryo arrives at the gastrula stage, the inhibitor would decrease in concentration either by inactivation or by the cessation of its synthesis, to give rise to the synthesis of ribosomal RNA.

References

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