LOW-SALT AND HIGH-SALT RNA POLYMERASE ACTIVITY DURING PREIMPLANTATION DEVELOPMENT IN THE MOUSE

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In a previous paper (Siracusa, 1973) we have shown that the variations in the level of total RNA polymerase activity per embryo and per blastomere in the preimplantation mouse embryo are not correlated with patterns of RNA synthesis at corresponding stages of development. We concluded that, in the mouse embryo, in contrast with the situation found in the sea urchin embryo (Roeder & Rutter, 1970), RNA polymerase apparently does not play any direct regulatory rôle in RNA synthesis.

The possibility remained, however, that a correlation could still exist for only one of the two main forms of the enzyme. A situation of this kind has been found during the development of Rana pipiens embryos (Kohl, Norman & Brooks, 1973); Polymerase I (the nucleolar form) and Polymerase II (nucleoplasmic) behave differently. The levels of Form II do not change during development despite changing patterns of DNA-like RNA synthesis, while Form I increases in the nuclei after gastrulation when ribosomal RNA synthesis begins, suggesting that the expression of the ribosomal cistrons may be controlled in this case by the availability of the corresponding polymerase.

We have attempted, therefore, to discriminate between the levels of Polymerases I and II at various developmental stages of the mouse embryo by measuring at two different ionic strengths the enzymatic activity present in embryo homogenates. The activities of RNA polymerases are markedly influenced by the ionic strength. In the presence of excess native DNA, Polymerase I shows optimal activity at 25 to 50 mM-ammonium sulphate, Polymerase II at 100 to 120 mM (Gissinger, Kedinger & Chambon, 1974).

The technical procedures used were those previously reported (Siracusa, 1973), with the following main differences.

Native DNA was used as template in the assay; tritiated uridine triphosphosphate ([³H]UTP) concentration was raised to 50 µM (NEN, sp. act. 19-8 Ci/mmol), incubation time was prolonged to 90 min. The final incubation mixture (40 µl) contained 50 mM-tris-HCl, pH 7-9; 2-5 mM-MgCl₂; 2 mM-MnCl₂; 0-2 mM-ATP; 0-2 mM-GTP; 0-2 mM-CTP; 50 µM-[³H]UTP; 0-2 mg native DNA/ml (calf thymus); 12-5% glycerol; 0-05 mM-EDTA; 0-5 mM-dithiothreitol; 2-5 mM-NaF; 0-5 mg BSA/ml; 25 or 125 mM-(NH₄)₂SO₄. A total of 4140 embryos was used.

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The results, expressed as pmol $[^3H]UTP \times 10^{-5}$ incorporated into RNA per embryo, are given in Text-fig. 1(a). In the fertilized egg, no nucleoplasmic activity could be detected and nucleolar activity was very low. Low salt activity (mainly due to Polymerase I) appeared to increase fairly rapidly until the eight-cell stage, then the rate of increase slowed down. The activity of Polymerase II was about the same as that of Polymerase I at all stages, except in eight-cell embryos when it was lower by a factor of 2.5.

The same data were plotted differently to visualize the phenomenon on a per-cell basis (Text-fig. 1b). The activity of Polymerase I per cell apparently increased between the one- and two-cell stages, remained constant between the two- and eight-cell stages, then decreased at the early and late blastocyst stages (about 45 and 260 cells, respectively). High salt activity decreased gradually after the two-cell stage.

The experiments described indicate that no apparent correlation exists between the levels of enzymatic activity present at a given stage and the amount or type of RNA synthesized at that stage. An eight-cell embryo, which synthesizes very little ribosomal RNA, contains only one-third less nucleolar enzymatic activity than an early blastocyst which is making RNA at a rapid rate. On the other hand, the increase in DNA-like RNA synthesis that occurs in the late blastocyst is not paralleled by a similar increase in Polymerase II activity.

These observations are compatible with the absence of a regulatory mechanism in which RNA polymerases are limiting within the embryo, and in which the expression of a gene is controlled by the availability of one of these
enzymes. The finding by Warner & Versteegh (1974) that the early blastocyst of the mouse probably has a higher Polymerase I: Polymerase II ratio than have liver nuclei gives little evidence, in our opinion, that a correlation between RNA polymerase levels and rate of RNA synthesis exists. Other possibilities cannot be ruled out: the enzyme might be present in the cells, but might not have access to the genes it can transcribe, being, for example, stored in the cytoplasm. Another possibility is that the enzyme is always present in excess, the regulation of specific transcription occurring by way of other limiting factors.

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