The synthesis of RNA containing polyadenylic acid sequences in preimplantation mouse embryos

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In mammalian embryonic development, RNA synthesis commences quite early, e.g. in the mouse, RNA synthesis is detectable by the 2-cell stage (reviewed by Epstein, 1975). The types of RNA synthesized by preimplantation mouse embryos at various stages of development have been studied by Ellem & Gwatkin (1968), who used methylated albumin Kieselguhr (MAK) chromatography, by Pikó (1970), who used sucrose gradient centrifugation, and by Warner & Hearn (1977), who worked with polyacrylamide gel electrophoresis. All of these studies are in excellent qualitative agreement, showing that, from the 8-cell stage and continuing through the blastocyst stage, all classes of RNA are synthesized. The predominant class synthesized is rRNA, and the distribution of newly synthesized RNA among the different nucleic acid classes is similar for 8-cell and blastocyst embryos. However, none of these studies has unequivocally identified HnRNA or mRNA, both of which are presumably being synthesized by the embryos.

It is generally believed, but not fully proven, that in eukaryotic cells HnRNA in the nucleus gives rise to mRNA in the cytoplasm; the synthesis of mRNA is crucial for the production of new proteins. The main experimental evidence supporting this conclusion is that both HnRNA and mRNA contain poly-A at the 3' end and a cap of methylated residues at the 5' end (Darnell, Philipson, Wall & Adesnik, 1971; Rottman, Shatkin & Perry, 1974). In this paper we report the use of poly-A as a marker for the detection of newly synthesized HnRNA and mRNA in preimplantation mouse embryos.

Embryos were obtained from CF1 mice (Charles River) which had been induced to superovulate with 5 i.u. PMSG (Organon: West Orange, New Jersey), followed 48 h later by 5 i.u. HCG (ICN: Cleveland, Ohio). Embryos were collected at 64 h (8-cell stage) and 89 h (blastocysts) after the HCG injection. The embryos were pooled to give a random mixture and washed twice in the culture medium before use. Groups of 25–100 embryos were cultured under mineral oil at 37°C in a humid atmosphere of 5% CO₂ in air in 50 µl droplets of the medium described by Whitten & Biggers (1968). All incubations were for 5 h in the presence of 500 µCi [³H]5-uridine (sp. act. >25 Ci/mmol: New England Nuclear, Boston)/ml.

At the end of the incubation period, yeast RNA (Type VI from *Torula* yeast: Sigma, St. Louis, Missouri) was added as a carrier, and the RNA extracted from the embryos by the method of Lee, Mendecki & Brawerman (1971) and Perry, LaTorre, Kelley & Greenberg (1972). The method uses an extraction into chloroform:phenol (1:1 v/v) at pH 9.0, which is essential for maintenance of the integrity of poly-A sequences. These sequences are lost when extractions are performed with phenol and SDS at neutral pH, conditions used by Ellem & Gwatkin (1968) and by Warner & Hearn (1977).

In the first set of experiments, the total amount of poly-A-containing RNA was determined by binding to poly-U filters prepared as described by Sheldon, Jurale & Kates (1972). In several experiments, actinomycin D was added to block rRNA synthesis preferentially and thus increase the relative amount of poly-A-containing RNA binding to the filters. As shown in Table 1, the amount of poly-A-containing RNA was quite low compared with the total amount of RNA, which was similar for embryos of both ages over the 5-h labelling period.

In the second set of experiments, the small amount of poly-A-containing RNA from 75 mouse blastocysts was further characterized by poly-dT-cellulose chromatography and polyacrylamide gel electrophoresis. The extracted RNA was passed over a column which contained 0.15 g dry poly-dT-cellulose (Collaborative Research, Waltham, Massachusetts), suspended in 5 ml binding buffer (0.4 M-NaCl, 1 mM-EDTA, 0.01 M-tris, 0.3% SDS, pH 7.4). The column was equilibrated with binding

Table 1 The amount (mean $\pm$ S.D.) of poly-A-containing RNA in mouse embryos at the 8-cell and blastocyst stages

8-cell embryos		Blastocysts	
Actinomycin D (μ g/ml)	% binding to poly U filter	Actinomycin D (μ g/ml)	% binding to poly U filter
0	2.2 $\pm$ 0.5	0	2.3 $\pm$ 0.4
0.01	3.7 $\pm$ 0.4	0.01	5.2 $\pm$ 1.4
0.1	4.7 $\pm$ 1.4	0.1	8.8 $\pm$ 4.0

All values are the average of at least two independent determinations of the % of total RNA synthesized during the 5-h labelling period.

buffer and the labelled RNA sample was passed three times through the column. Unbound RNA was removed by washing with binding buffer at a rate of 0.5 ml/min. Thirteen 1-ml fractions were collected, and then bound RNA was removed with elution buffer (binding buffer minus NaCl), and 10 more 1-ml fractions were collected. The profile of the poly-dT-cellulose column showed that the relative amount of bound, poly-A-containing RNA was similar to that indicated in Table 1. Next, the pooled poly-A-containing fractions were subjected to polyacrylamide gel electrophoresis as described by Loening (1967), with the results shown in Text-fig. 1. In each experiment, two separate gels were run. The first contained [3 H]RNA (Schwarz-Mann: Orangeburg, New York) to serve as an external marker. The second (shown in Text-fig. 1) contained the poly-A-containing RNA and 14 C-labelled tRNA to serve as an internal marker. Essentially all of the poly-A-containing RNA was found in the mRNA region, with virtually none at the top of the gel. The apparent peak in the 4S region was due to spillover from the [14 C]tRNA internal marker.

Text-fig. 1. Polyacrylamide gel electrophoresis of mouse blastocyst RNA which had been bound to a poly-dT-cellulose column. Fractions 15, 16 and 17 from the column were pooled and analysed, as described in the text. The arrows indicate the positions of the 28S, 18S, and 4S external markers, and the 4S (14 C)tRNA internal marker.

The results described in this paper show that 8-cell and blastocyst mouse embryos do synthesize a small, but detectable amount of poly-A-containing RNA, and that the relative amount synthesized at each stage is similar. In blastocysts, virtually all of the poly-A-containing RNA is of fairly low molecular weight, in the mRNA region, with none in the high molecular weight (HnRNA) region. These results can be compared to a similar study by Schultz, Manes & Hahn (1973) on preimplantation rabbit embryos. Using very similar conditions to those described in this paper (200 μ Ci [3 H]uridine/ml

for a 4-h labelling period), they found that poly-A-containing RNAs are synthesized at least as early as the 16-cell stage, and that the portion of the heterogeneous RNA containing poly-A sequences does not appear to change markedly between the cleavage and blastocyst stages of development. They also found, by sucrose gradient sedimentation analysis, that about 20% of the poly-A-containing RNA in rabbit embryos is in the high molecular weight region. Because the incubation and extraction conditions were virtually identical in the rabbit and mouse studies, it must be concluded that rabbit and mouse blastocysts differ in the relative proportion of high molecular weight poly-A-containing RNA synthesized at this stage of development. No generalizations about poly-A-containing RNAs from preimplantation mammalian embryos can therefore be made at this time. HnRNA containing poly-A may be degraded more rapidly in the nuclei of cells of the mouse blastocyst than in the rabbit blastocyst, but the much larger size and complexity of the rabbit blastocyst may account for the smaller proportion of newly synthesized poly-A-containing mRNA in the mouse blastocyst.

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