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Turnover of embryonic messenger RNA in preimplantation mouse embryos

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SUMMARY

We have estimated the average half-life of embryonic messenger RNA in mouse embryos at two stages of preimplantation development. Embryos were collected at 48 and 75 h post-hCG and cultured overnight in the presence of [^{3}H]uridine. Beginning at 65–68 h (morulae) or 92–94 h (early blastocysts), the label was withdrawn and replaced with unlabelled uridine, and samples were taken at intervals thereafter for RNA isolation. Label in cytoplasmic, poly(A)-containing RNA was measured after binding to oligo(dT)-cellulose, and was normalized to label in 28S and 18S ribosomal RNA, separated on sucrose gradients. The stability of rRNA in both stages was verified directly, as was the integrity and purity of the isolated mRNA. With morulae, the mRNA decay curve was monophasic, with an average half-life of $9{\cdot}5 \pm 0{\cdot}9$ h. In three experiments with early blastocysts the decay curve appeared to be biphasic, consisting of short-lived (less than 6 h) and long-lived (30–50 h) components; in two other experiments a short-lived component was not evident. In all cases, however, the overall average half-life of mRNA in early blastocysts, determined by linear regression assuming monophasic kinetics, was greater than that in morulae. Our data indicate that the stability of embryonic mRNA increases by at least twofold during the morula-to-blastocyst transition. The results are considered in terms of the transcriptional dependency of early mouse embryos and the regulation of maternal and embryonic mRNA.

INTRODUCTION

One of the most interesting features common to animal embryos is the dual nature of genetic programming for early biochemical and morphological events: both maternal (oogenetic) and embryonic gene transcripts serve to direct developmental change (reviewed by Davidson, 1976). Mammalian embryos can be included in this generalization, although in mammals, the influence of the embryo's genome can be seen phenotypically much earlier than in other forms, even as early as the first few cleavages (reviewed by Sherman, 1979). In the mouse, the pattern of changes in polypeptide synthesis during the first cell cycle as revealed by two-dimensional electrophoresis (Levinson, Goodfellow,

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Vadeboncoeur & McDevitt, 1978; Cullen, Emigholz & Monahan, 1980) is almost certainly maternally programmed, since transcription of the zygote's genes during this 24 h period is undetectable (Moore, 1975; Young, Sweeney & Bedford, 1978). Furthermore, Braude, Pelham, Flach & Lobatto (1979) have demonstrated that a set of polypeptides whose synthesis increases between the late 1-cell and early 2-cell stages is translated from maternal mRNAs present in the oocyte. Yet by the late 2-cell stage, embryonic transcription can be detected and measured (Woodland & Graham, 1969; Knowland & Graham, 1972; Moore, 1975; Clegg & Pikó, 1977), and the effect of this transcription can be seen beginning at least by the 8-cell stage in the form of paternal allelic forms of enzymes (reviewed by Sherman, 1979). The necessity of early embryonic gene activity for normal development is demonstrated by the sensitivity of cleaving mouse embryos to transcriptional inhibitors such as actinomycin and α-amanitin (Sherman, 1979), and the fact that embryo-lethal mutations have been characterized which affect development in cleavage stages (reviewed by McLaren, 1976; Pedersen & Spindle, 1980; Bennett, 1975; Gluecksohn-Waelsch, 1979; Sherman & Wudl, 1977).

Between the 2-cell stage, when embryonic transcription is just beginning, and the yet-to-be determined time when the early mouse embryo is reliant exclusively upon its own gene products, there must be a phasing-out of maternal transcripts from the protein-synthesizing machinery. Although measurements have been reported of the amount of maternal mRNA remaining in the mouse embryo at several points in the preimplantation period (Bachvarova & DeLeon, 1980), these data do not tell us much about the relative contributions of maternal and embryonic mRNAs in programming developmental events. Can a definite transition period be discerned, during which developmental change becomes more thoroughly dependent on embryonic transcription?

One approach to answering this question involves the use of transcriptional inhibitors such as α-amanitin to block the synthesis of embryonic mRNA. This approach has already seen widespread use (reviewed by Sherman, 1979); however, interpretation of such experiments in terms of the role of embryonic transcripts in programming early development has been hindered by a lack of information on the stability of embryonic mRNA. The data reported here begin to fill that gap. Our results indicate that gene expression in the pre-implantation embryo is regulated in part at the level of mRNA turnover, with the average mRNA half-life increasing between the morula and early blastocyst stages. We can now begin to use these data as the basis for experiments to test the dependence of morphological and biochemical changes on embryonic transcription.

MATERIALS AND METHODS

Collection and labelling of embryos

Embryos were flushed from oviducts and uteri of Dub:(ICR) mice after superovulation with 5 i.u. pregnant mare's serum gonadotropin (Teikoku Zoki Pharmaceutical Co., Japan) and 5 i.u. human chorionic gonadotropin (Ayerst). For experiments involving morulae, the embryos were flushed with phosphate-buffered medium (flushing medium-I of Spindle, 1980) about 48 h post-hCG (day-2 embryos) and collected in standard egg culture medium (Biggers, Whitten & Whittingham, 1971) modified as described by Spindle, 1980. For experiments involving early blastocysts, the embryos were flushed about 75 h post-hCG (day-3 embryos) and collected in modified Eagle's basal medium (BME + AA of Spindle, 1980) containing 5% foetal calf serum (Gibco) and 5% newborn calf serum (Microbiological Associates). The embryos were later transferred to the same media containing [5,6-^{3}H]uridine (Amersham; 47 Ci/mmol) for overnight labelling (10 or 15 h) at a concentration of 10 μM uridine for morulae and 1 μM for blastocysts. Because of the inherent asynchrony of embryo batches collected after natural mating, these two embryonic 'stages' must be regarded as developmentally heterogeneous.

Following the labelling period, the embryos (300–800 morulae, 100–300 early blastocysts) were 'chased' by transferring them after extensive washing into the respective media containing 2 mM unlabelled uridine. The chase continued for 24 h after the end of the labelling period, and samples of the embryo populations we e taken for processing at regular intervals. Processing began with a brief treatment with pronase (0·5%) to remove zonae, followed by ten washes through 100 μl drops of phosphate-buffered saline containing 0·3% polyvinylpyrrolidone.

Embryo lysis and preparation of the cytoplasmic fraction

Embryo lysates were prepared and separated into nuclear and postnuclear (cytoplasmic) fractions according to the method of Howe & Solter (1979), our only modification being the inclusion of 10 mM vanadyl-nucleoside complex to inhibit ribonuclease (Berger & Birkenmeier, 1979). After spinning down the nuclei, the cytoplasmic fractions (80 μl) were stored at −70 °C. On the average, this fractionation procedure resulted in 95% of the total incorporated counts being recovered in the cytoplasmic supernatant, and in no case was the recovery less than 89%. All but about 6% of the total incorporated label could be solubilized by a 90 min treatment with 0·3 N-NaOH at 37 °C, indicating that nearly all of the incorporated label was in RNA.

Analysis of RNA

In order to minimize loss, poly(A)-containing messenger RNA was isolated directly from the cytoplasmic supernatant fractions by affinity chromatography on oligo(dT)-cellulose columns (Type 3, Collaborative Research, Inc.), without

prior purification (Pemberton, Liberti & Baglioni, 1975). The columns were washed before each use with binding buffer [10 mM Tris-HCl, pH 7·5; 500 mM-NaCl; 2 mM disodium ethylenediamine tetracetate (EDTA)] containing 100 μg yeast RNA (Sigma, Type XI), to pre-empt non-specific binding sites (Bantle, Maxwell & Hahn, 1976). The same amount of yeast RNA (in 20 μl H_2O) was added to each frozen cytoplasmic fraction, which was then thawed by adding 0·5 ml of 2 % sodium dodecyl sulphate (SDS) in binding buffer. The preparation was heated to 60 °C for 5 min, diluted to 2·0 ml with binding buffer, and allowed to cool to room temperature before loading on the column. The flow-through from the column was collected and passed through it twice again, and then the column was washed with 2·0 ml of binding buffer containing 0·5 % SDS. This was added to the first 2·0 ml of unbound material to make up the unbound fraction, which was precipitated with ethanol. After further washing of the column with at least 10 bed volumes of binding buffer with 0·5 % SDS, the bound RNA was eluted with successive 1·0 ml washes of 10 mM Tris-HCl, pH 7·5, with 0·5 % SDS. The bound/eluted samples were precipitated with 10 % trichloracetic acid (TCA) using 100 μg yeast RNA as carrier, and the precipitates were collected on nitrocellulose membrane filters for counting. Because each oligo(dT) column was used many times over, its binding efficiency was monitored from time to time using a commercial poly(A) preparation (Sigma). We did not detect any variation in binding efficiency over the course of repeated usage.

For each time-point, the distribution of label among the 4S, 18S, and 28S RNAs of the unbound fraction was determined by sucrose gradient analysis. Ethanol-precipitated samples were dissolved in 10 mM sodium acetate, pH 5·1, 1 mM EDTA, 0·1 % SDS, and layered on 5–20 % sucrose gradients, which were spun at 4 °C and 40000 rev./min in a Beckman SW-40 rotor to an accumulated $\omega^2 t$ of 60700 (about 10 h). The gradient fractions were collected using an ISCO fractionator, and the samples were counted in Aquasol (New England Nuclear).

RESULTS

Design and rationale of the experiments

Our aim in these experiments was to estimate the average half-life of newly synthesized cytoplasmic mRNA. The experimental design which we chose, which involves labelling embryos with [^{3}H]uridine followed by a 'chase' with a vast excess of unlabelled uridine, is conceptually straightforward. However, in early embryos, in which the uridine transport rate is initially rather low (Daentl & Epstein, 1971; Clegg & Pikó, 1977), the chase is not likely to be immediately effective, i.e. incorporation of [^{3}H]uridine into RNA is likely to continue for some time after the start of the chase period. To correct for this, and to control for reincorporation of labelled nucleotides during the chase as well as unequal recovery of RNA from different batches of embryos, we normalized the cpm

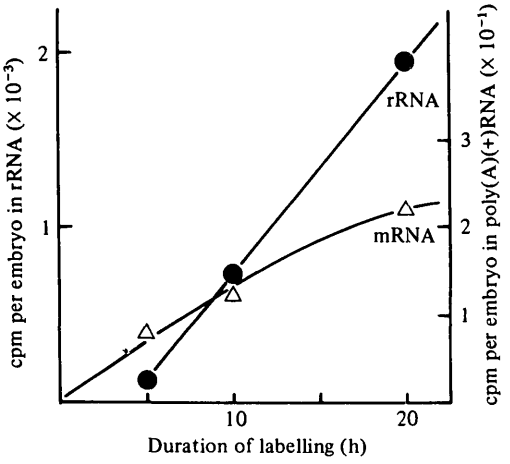

Fig. 1. Accumulation of [^{3}H]uridine in ribosomal and messenger RNA of a mixture of 150 late morulae and early blastocysts collected 92 h post-hCG.

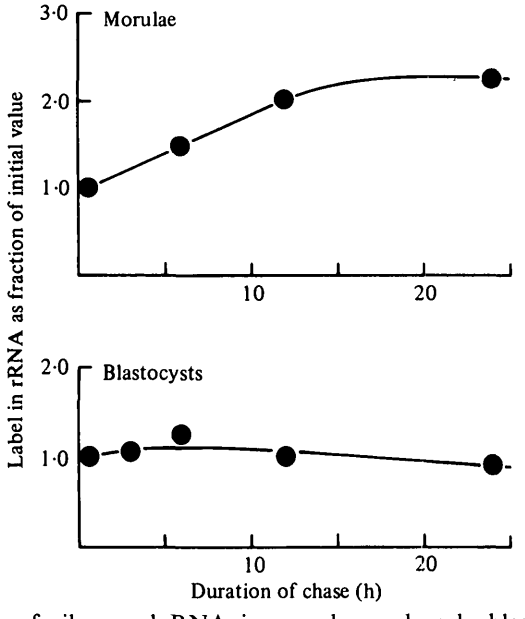

Fig. 2. Stability of ribosomal RNA in morulae and early blastocysts. For the morulae, each datum is the mean of two determinations; for the blastocysts, each is the mean of three determinations.

in mRNA for each time-point to the cpm in 28S + 18S rRNA, according to the method of Singer & Penman (1973). If the longevity of rRNA in early mouse embryos is considerably greater than that of mRNA, and the two types of RNA draw upon the same UTP precursor pool, then such a procedure permits the construction of a reliable decay plot for mRNA. Such a plot represents, of course, the sum of many decay curves for individual mRNAs, and thus is simply an

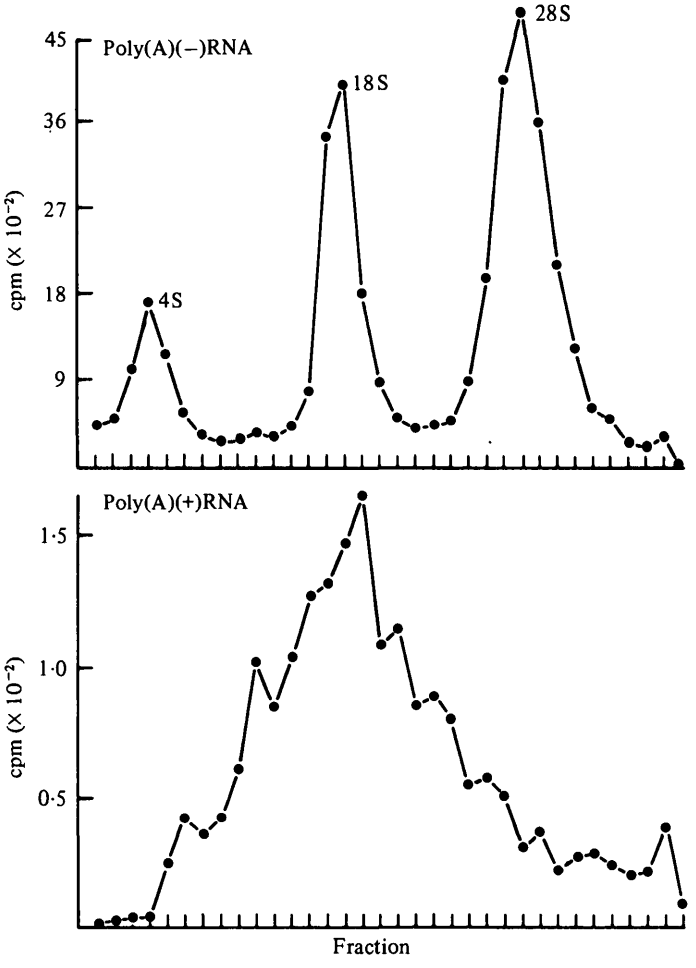

Fig. 3. Sucrose-density-gradient analysis of the unbound [poly(A)(−)RNA] and bound/eluted [poly(A)(+)RNA] fractions from oligo(dT)-cellulose chromatography. A mixture of 245 late morulae and early blastocysts had been labelled for 10 h prior to RNA isolation.

indicator of the *average* behaviour of the cytoplasmic poly(A)-containing mRNA population.

Preliminary experiments (see Fig. 1) indicated that labelled uridine accumulates in embryonic mRNA for 20 h or more. In order to facilitate detection of tiny quantities of labelled mRNA, particularly at the end of the chase period, we incubated embryos in the precursor for 15 h (day-2 embryos) or 10 h (day-3 embryos) before start of the chase. The data shown in Fig. 1 also indicate that newly synthesized rRNA accumulates in a linear fashion for greater than 20 h, indicating that it is comparatively stable over this time period. We have verified this directly for the two stages of development of interest in this report, by monitoring the label in rRNA (18S and 28S) during 24 h chase periods. After

overnight labelling, embryos (morulae, 65–68 h post-hCG; early blastocysts, 92–94 h post-hCG) were transferred into media containing 2 mM unlabelled uridine, and at intervals thereafter were sampled. The poly(A)(−)RNA fractions were analysed on sucrose gradients, with the results shown in Fig. 2. In neither case is there an indication of significant turnover of rRNA during the 24 h period. In the younger embryos, it is evident that the chase only becomes effective after 12–14 h, which presumably reflects the low rate of uridine uptake in cleaving embryos (Clegg & Pikó, 1977).

Turnover of mRNA

When quantifying mRNA based on its binding to oligo(dT)-cellulose it is essential to ensure that the mRNA is not degraded or contaminated with other forms of RNA. We thus monitored our RNA preparations with respect to both of these criteria by sucrose gradient analysis (Fig. 3). We found vanadyl-nucleoside complex to be an effective inhibitor of endogenous ribonucleases in mouse embryos, as demonstrated by the size distribution of bulk RNAs (4S, 18S, 28S; Fig. 3A) and mRNA (Fig. 3B), the latter being distributed around a modal peak of about 18S, as in other mammalian cells (Singer & Penman, 1973). Furthermore, it is clear that the poly(A)-containing mRNA fraction resulting from our chromatographic procedure is essentially free of tRNA and rRNA.

A second precaution concerns the viability of mouse embryos cultured in the presence of radioactive precursors (MacQueen, 1979). Although the concentration of [^{3}H]uridine used to label morulae was quite high (10 μM, or about 500 μCi/ml), they had incorporated less than 100 cpm per embryo into total RNA by the end of the labelling period. At this point, 60–90 % of the embryos were in the 4-cell stage, with the remainder in the 2-cell stage; by the mid-point of the chase 35–50 % had reached the 8-cell stage; and, like control embryos (of the same embryo population, but unlabelled), the vast majority were compacted morulae by the end of the chase. The embryos labelled for the early blastocyst measurements also developed in time with controls, and in this case the total incorporated label amounted to 1200–1800 cpm per embryo. By the end of the labelling period (92–94 h post-hCG), about half of these embryos (either labelled or controls) had cavitated, and these were selected from the population and transferred to the chase medium. (Most of the remaining morulae, which were not used for turnover measurements, eventually cavitated.) During the chase period 90–95 % of the labelled embryos underwent blastocyst expansion and hatching. Given the continued, apparently normal development of embryos of both stages during the labelling and chase period, we are confident that high concentrations of precursor and long labelling times did not adversely affect their viability.

The decay kinetics of embryonic mRNA were monitored in three batches of morulae and five of early blastocysts, and the data from these eight experiments

Table 1. *Stability of uridine-labelled messenger RNA in morulae and early blastocysts*

Experiment*	Total TCA-precipitable cpm in mRNA† remaining after					Regression analysis‡	
	0·5 h	3 h	6 h	12 h	24 h	$t_{\frac{1}{2}}$	S.E.
M-1	1615 (0·220) **137**	—	1655 (0·158) **178**	1853 (0·091) **202**	1053 (0·045) **204**	10·0 h	0·5
M-2	652 (0·194) **65**	—	742 (0·125) **62**	792 (0·078) **82**	427 (0·042) **84**	10·8 h	1·1
M-3	161 (0·513) **67**	—	160 (0·249) **70**	148 (0·117) **84**	105 (0·061) **78**	7·8 h	1·4
EB-1	124 (0·030) **14**	88 (0·025) **10**	102 (0·017) **11**	245 (0·014) **20**	171 (0·011) **34**	17·5 h	4·7
EB-2	541 (0·033) **25**	353 (0·019) **24**	271 (0·013) **25**	345 (0·011) **35**	370 (0·010) **60**	18·7 h	10·3
EB-3	1542 (0·075) **25**	1725 (0·077) **25**	2331 (0·067) **25**	1287 (0·064) **30**	805 (0·025) **40**	14·2 h	2·4
EB-4	3041 (0·077) **40**	1381 (0·031) **40**	1957 (0·041) **39**	1542 (0·034) **43**	723 (0·020) **51**	19·3 h	9·3
EB-5	4190 (0·086) **45**	5917 (0·078) **53**	5592 (0·073) **61**	3184 (0·050) **70**	3023 (0·048) **90**	26·2 h	6·5

* M, Morulae; EB, early blastocysts.

† The data are reported on a per batch basis, and the number of embryos in each batch is given in bold type. The ratio of cpm in cytoplasmic poly(A)(+)RNA to cpm in (28S+18S) rRNA is given in parenthesis.

‡ Non-linear (exponential) least-squares regression analysis was performed on each set of data [ratio poly(A)(+)RNA/(28S+18S) rRNA] to compute a single half-life ($t_{\frac{1}{2}}$) for each experiment, assuming monophasic decay kinetics; S.E., estimate of standard error.

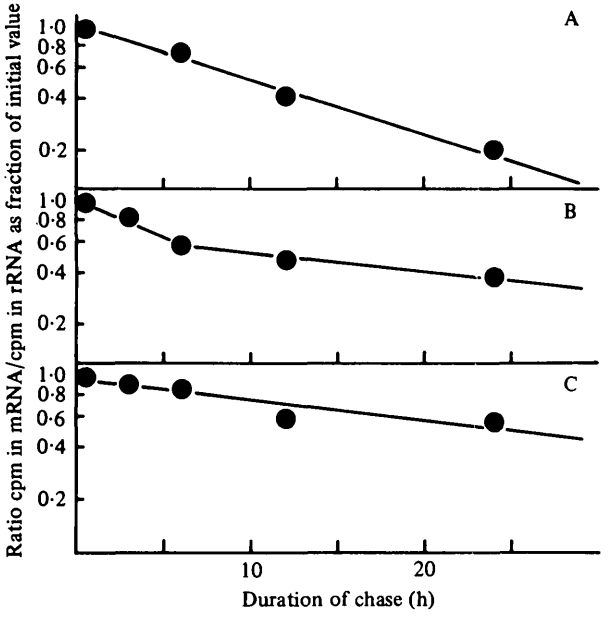

Fig. 4. Turnover of messenger RNA (cytoplasmic, poly(A)-containing RNA) in morulae and early blastocysts. The results of three representative experiments (see Table 1) are shown: (A) experiment M-1; (B) experiment EB-1; (C) experiment EB-5.

are given in Table 1. In Fig. 4 are plotted the data from three of these experiments (M-1, EB-1 and EB-5), which are representative of the three types of decay curve obtained. With the morulae, there was excellent agreement between experiments, the data fitting a monophasic decay curve with a half-life of 8–11 h (mean = 9·5 h ± 0·9 S.E.) (Fig. 4A). The data from three of the early blastocyst experiments, on the other hand, suggest the presence of two decay components (Fig. 4B; experiment EB-1, EB-2, and EB-4), one with a half-life of less than 6 h, the other considerably more stable. Since the less stable of these components was not evident in the other two experiments (EB-3, EB-5; Fig. 4C), the existence of two distinct decay components in early blastocyst mRNA cannot be considered fully substantiated. For this reason, the half-life estimates reported in Table 1 were made by regression analysis assuming monophasic decay kinetics for all experiments. In spite of the fact that this approach would lead to an underestimate of the average half-life in decay plots with biphasic kinetics, these data show that the average half-life of poly(A)-containing mRNA in early blastocysts is considerably longer than in morulae, probably by more than twofold (the mean of the early blastocyst measurements is 19·2 ± 6·9 h). Using the Mann–Whitney non-parametric *U*-test, the medians of the two sets of half-life values (morulae and early blastocysts) were judged to be significantly different ($P = 0{\cdot}05$).

DISCUSSION

Our measurements indicate that the average half-life of cytoplasmic mRNA increases considerably between the morula and early blastocyst stages of mouse development. Prior to this transition, the label accumulated in cytoplasmic mRNA decays with monophasic kinetics which offer no indication of more than one kinetic component. The average mRNA half-life of 8–11 h measured in morulae contrasts with the 14–26 h half-life in early blastocysts, indicating that embryonic mRNA must undergo stabilization during or shortly after the morula-to-blastocyst transition.

The validity of this conclusion is subject to the proviso that normalizing the data to incorporation into rRNA accurately accounts for continued accumulation of label in mRNA during the early part of the chase period. In particular, if the post-pulse accumulation of label evident in morula rRNA (Fig. 2) did not occur in the mRNA, then normalizing the data as we have done would produce an artifactually short half-life estimate for the morulae. This is a possibility, since rRNA synthesis in morulae is carried on at a rapid rate while the precursor uptake capacity is limited. Thus, precursor availability might be sufficiently near limiting for competition between different RNA synthetic systems to occur. On the contrary, our raw data suggest that incorporation into morula mRNA on a per embryo basis does continue for the first 6–12 h of the chase, the net effect of this (coupled with mRNA turnover) being that the actual amount of label in mRNA changes little during this period. It is likely, therefore, that rRNA and mRNA in morulae follow similar kinetics of continued incorporation during the chase, and our normalization procedure should provide an accurate picture of mRNA decay.

The variability observed between batches of early blastocysts is difficult to explain. As a test of the reproducibility of our procedure, we divided a population of labelled blastocysts into two groups, and sampled them at intervals during the chase period for processing in parallel. The time-points for mRNA decay (normalized to rRNA) were nearly identical between these two groups. It seems possible, therefore, that the variability between batches of early blastocysts is due to inherent biological differences between the embryos collected from different groups of mice on different days.

The method that we have used to measure mRNA decay has also been applied to HeLa cells (Singer & Penman, 1973) and rabbit blastocysts (Schultz, 1974), and in both cases biphasic decay kinetics were observed, with the short-lived component exhibiting a half-life of about 7 h. It would not be surprising, then, to find that mouse blastocyst mRNA contains both short-lived and long-lived components. Our failure to detect two components in all cases could be due to the asynchrony of embryo batches collected on different days: the different proportions of the more stable embryonic mRNA component in different batches of early blastocysts might represent different stages in the transition to

an overall slower rate of mRNA turnover. Unfortunately, when we attempted to examine this suggestion by measuring the mRNA half-life in late blastocysts, we found that ribosomal RNA in these embryos is not sufficiently stable to provide a baseline for normalizing mRNA incorporation data. Despite this uncertainty about the decay complexity of early blastocyst mRNA, however, it is clear that the average half-life of the poly(A)-containing mRNA in this stage is significantly longer than in morulae. When regression analysis was used to establish the best fit to each set of data assuming monophasic kinetics, a procedure which underestimates the average half-life of the more stable mRNA component, the difference in mean half-life between the two stages was found to be twofold.

Our data may be compared with other average half-life measurements in mammalian embryos. In addition to the study by Schultz (1974), cited above, in which the more stable component of rabbit blastocyst mRNA was found to have a half-life of about 18 h, there is a report by Schultz & Tucker (1977), based on analysis of mRNA synthesis kinetics. These investigators estimated the average half-life of mRNA in rabbit embryos to be 16 h in the 2-day (16-cell), 4-day (morula) and 6-day (blastocysts) stages. The mouse and rabbit blastocysts would therefore seem to be quite similar in terms of average mRNA half-lives, but embryonic mRNA of the morula is less stable in the mouse than it is in the rabbit. The only direct assessment of the stability of maternal mRNA in embryos was that of Bachvarova & DeLeon (1980), who followed the disappearance of poly(A)-containing maternal mRNA labelled during oocyte growth. Although about 40% of the maternal mRNA decayed during the first 24 h of development, during the second day (from the 2-cell through the 8-cell stage) no further loss occurred; sometime during the third day (late morula to early blastocyst), a further loss of 30% was observed. It is interesting, then, that *embryonic* mRNA decays with an average half-life of 8–11 h during a period when *maternal* mRNA is reportedly stable; this implies that there is a mechanism in morula cells for the differential regulation of maternal and embryonic mRNA turnover.

A major goal in this investigation was to obtain information that would be useful in designing and interpreting experiments employing transcriptional inhibitors. The fungal toxin, α-amanitin, has recently been used to test the transcriptional dependency of cellular and molecular events in early mouse development. As a means of determining which of the polypeptides synthesized by the embryo are dependent on embryonic transcription, Johnson, Handyside & Braude (1977) (see also Braude, 1979*a*) treated embryos with this inhibitor for 24 h during the morula-to-blastocyst transition. Almost all of the polypeptides whose synthesis was apparently activated during the treatment period, including all of the inner cell mass (ICM)- or trophectoderm-specific polypeptides synthesized for the first time during this period, were sensitive to amanitin treatment. On the other hand, most polypeptides whose synthesis was already

occurring at the start of the treatment period, including a minority of ICM- and trophectoderm-specific polypeptides, were not abolished by the amanitin blockade. Since, according to our data, the average half-life of embryonic mRNA in the morula is only 8–11 h, some of those polypeptides resistant to 24 h amanitin treatment are likely to be encoded by the more stable, maternal mRNA component revealed by the experiments of Bachvarova & DeLeon (1980). Nonetheless, it remains a possibility that mRNA synthesized in the late 2-cell stage, which would have constituted a minority of the labelled mRNA molecules in our experiments with morulae, could persist into the late morula and make a significant contribution to protein synthesis.

Despite the apparent dependence of a proportion of protein synthesis on maternal mRNA during the morula-to-blastocyst transition, the morphogenetic process of cavitation is critically dependent on embryonic gene expression (Johnson *et al.* 1977; Braude, 1979*a*). The stabilization of mRNA between the morula and early blastocyst stages could be one explanation for the observation that cavitation becomes less sensitive to transcriptional blockage beginning at about 80 h post-hCG (Braude, 1979*b*). The data reported here should be of value in interpreting further experiments of this nature.

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REFERENCES

BACHVAROVA, R. & DE LEON, V. (1980). Polyadenylated RNA of mouse ova and loss of maternal RNA in early development. *Devl Biol.* **74**, 1–8.

BANTLE, J. A., MAXWELL, I. H. & HAHN, W. E. (1976). Specificity of oligo(dT)-cellulose chromatography in the isolation of polyadenylated RNA. *Analyt. Biochem.* **72**, 413–427.

BENNETT, D. (1975). The T-locus of the mouse. *Cell* **6**, 441–454.

BERGER, S. L. & BIRKENMEIER, C. S. (1979). Inhibition of intractable nucleases with ribonucleoside-vanadyl complexes: isolation of messenger ribonucleic acid from resting lymphocytes. *Biochem.* **18**, 5143–5149.

BIGGERS, J. D., WHITTEN, W. K. & WHITTINGHAM, D. G. (1971). The culture of mouse embryos *in vitro*. In *Methods in Mammalian Embryology* (ed. J. C. Daniel Jr), pp. 86–116. San Francisco: Freeman.

BRAUDE, P. R. (1979*a*). Control of protein synthesis during blastocyst formation in the mouse. *Devl Biol.* **68**, 440–452.

BRAUDE, P. R. (1979*b*). Time-dependent effects of α-amanitin on blastocyst formation in the mouse. *J. Embryol. exp. Morph.* **52**, 193–202.

BRAUDE, P., PELHAM, H., FLACH, G. & LOBATTO, R. (1979). Post-transcriptional control in the early mouse embryo. *Nature, Lond.* **282**, 102–105.

CLEGG, K. B. & PIKÓ, L. (1977). Size and specific activity of the UTP pool and overall rates of RNA synthesis in early mouse embryos. *Devl Biol.* **58**, 76–95.

CULLEN, B., EMIGHOLZ, K. & MONAHAN, J. (1980). The transient appearance of specific proteins in one-cell mouse embryos. *Devl Biol.* **76**, 215–221.

DAENTL, D. L. & EPSTEIN, C. J. (1971). Developmental interrelationships of uridine uptake, nucleotide formation and incorporation into RNA by early mammalian embryos. *Devl Biol.* **24**, 428–442.

DAVIDSON, E. H. (1976). *Gene Activity in Early Development.* New York: Academic Press.

GLUECKSOHN-WAELSCH, S. (1979). Genetic control of morphogenetic and biochemical differentiation: lethal albino deletions in the mouse. *Cell* **16**, 225–237.

HOWE, C. C. & SOLTER, D. (1979). Cytoplasmic and nuclear protein synthesis in preimplantation mouse embryos. *J. Embryol. exp. Morph.* **52**, 209–225.

JOHNSON, M. H., HANDYSIDE, A. H. & BRAUDE, P. R. (1977). Control mechanisms in early mammalian development. In *Development in Mammals*, vol. 2 (ed. M. H. Johnson), pp. 67–97. New York: North-Holland.

KNOWLAND, J. & GRAHAM, C. (1972). RNA synthesis at the two-cell stage of mouse development. *J. Embryol. exp. Morph.* **27**, 167–176.

LEVINSON, J., GOODFELLOW, P., VADEBONCŒUR, M. & MCDEVITT, H. (1978). Identification of stage-specific polypeptides synthesized during murine preimplantation development. *Proc. natn. Acad. Sci., U.S.A.* **75**, 3332–3336.

MACQUEEN, H. A. (1979). Lethality of radioisotopes in early mouse embryos. *J. Embryol. exp. Morph.* **52**, 203–208.

MCLAREN, A. (1976). Genetics of the early mouse embryo. *A. Rev. Genet.* **10**, 361–388.

MOORE, G. P. M. (1975). The RNA polymerase activity of the preimplantation mouse embryo. *J. Embryol. exp. Morph.* **34**, 291–298.

PEDERSEN, R. A. & SPINDLE, A. I. (1981). Cellular and genetic analysis of mouse blastocyst development. In *Cellular and Molecular Aspects of Implantation* (ed. S. Glasser and D. Bullock), pp. 91–108. New York: Plenum Press.

PEMBERTON, R. E., LIBERTI, P. & BAGLIONI, C. (1975). Isolation of messenger RNA from polysomes by chromatography on oligo(dT)-cellulose. *Analyt. Biochem.* **66**, 18–28.

SCHULTZ, G. A. (1974). The stability of messenger RNA containing polyadenylic acid sequences in rabbit blastocysts. *Expl Cell. Res.* **86**, 190–193.

SCHULTZ, G. A. & TUCKER, E. B. (1977). Protein synthesis and gene expression in preimplantation rabbit embryos. In *Development in Mammals*, vol. 1 (ed. M. H. Johnson), pp. 69–97. New York: North-Holland.

SHERMAN, M. I. (1979). Developmental biochemistry of preimplantation mammalian embryos. *A. Rev. Biochem.* **48**, 443–470.

SHERMAN, M. I. & WUDL, L. R. (1977). T-complex mutations and their effects. In *Concepts in Mammalian Embryogenesis* (ed. M. I. Sherman), pp. 136–234. Cambridge, Massachusetts: MIT Press.

SINGER, R. H. & PENMAN, S. (1973). Messenger RNA in HeLa cells: kinetics of formation and decay. *J. mol. Biol.* **78**, 231–334.

SPINDLE, A. I. (1980). An improved culture medium for mouse blastocysts. *In Vitro* **16**, 669–674.

WOODLAND, H. R. & GRAHAM, C. (1969). RNA synthesis during early development of the mouse. *Nature, Lond.* **221**, 327–332.

YOUNG, R. J., SWEENEY, K. & BEDFORD, J. M. (1978). Uridine and quanosine incorporation by the mouse one-cell embryo. *J. Embryol. exp. Morph.* **44**, 113–148.

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