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The transition from maternal to embryonic control in the 2-cell mouse embryo

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The development of the early 2-cell mouse embryo to the late 2-cell stage is marked by the appearance between 23 and 26 h post-insemination of a complex of polypeptides of mol. wt. ~67 K. Addition of α-amanitin between 18 and 21 h post-insemination prevents or reduces the subsequent appearance of these polypeptides. Addition of α-amanitin after 21 h does not obviously affect the appearance of the ~67 K polypeptides. A major change in synthetic profile occurs between 29 and 32 h post-insemination involving many polypeptides. Addition of α-amanitin to 2-cell embryos prior to 29 h post-insemination prevents the appearance of the new polypeptides observed during this major change but does not prevent the disappearance of the old polypeptides. In contrast, addition of α-amanitin after this time does not affect the appearance of the new polypeptides. This result, together with other evidence presented, suggests that during the 2-cell stage the embryonic genome shows transcriptional activity in two phases at 18 – 21 and 26 – 29 h post-insemination, that these transcripts are utilized soon after their synthesis, and that most maternal transcripts used before the second phase of embryonic transcription become ineffective soon afterwards.
Key words: mRNA/embryo/mouse/amanitin

Introduction

The earliest developmental events in embryos of various species, including the mouse, appear to occur largely or exclusively under the control of the maternal genome (Denny and Tyler, 1964; Brachet *et al.*, 1968; Woodland *et al.*, 1979; Braude *et al.*, 1979; Ballantine *et al.*, 1979; Rosenthal *et al.*, 1980; Woodland and Ballantine, 1980; Wells *et al.*, 1981). During this period, the embryonic genome is either inactive or its activity appears to be irrelevant to proximate developmental events (Van Blerkom, 1981; Johnson, 1981), and qualitative changes observed in the proteins synthesised are explicable on the basis of post-transcriptional control mechanisms (Jenkins *et al.*, 1978; Braude *et al.*, 1979; Rosenthal *et al.*, 1980; Schultz *et al.*, 1980; Johnson, 1981; Van Blerkom, 1981). Here we examine the timing of the transition from maternal to embryonic control during the 2-cell stage of mouse development. We suggest that the activation of the embryonic genome occurs in two phases, a limited activation occurring between 18 and 21 h post-insemination and a major activation occurring between 26 and 29 h post-insemination. We further suggest that most maternal mRNA may be inactivated over the period 29 – 48 h post-insemination.

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Results

The effects of α-amanitin on the quantity of protein synthesised between the 1- and 8-cell stages

Populations of 1-, early 2-, late 2-, 4-, and 8-cell mouse embryos were recovered, and some embryos from each group were placed immediately in [^{35}S]methionine for 3 h. The remaining embryos were cultured in the presence or absence of 11 μg/ml α-amanitin, shown at this concentration to be a specific and irreversible inhibitor of RNA polymerase II in mouse embryos (Levey and Brinster, 1978). Samples were removed and radio-labelled after 9 or 21 h and the incorporation of methionine into acid precipitable material counted. The results are recorded in Table I. Three patterns are observed depending on the age of the embryos at initiation of culture. The first pattern, in which α-amanitin exerts no obvious effect on methionine incorporation, is found in embryos cultured from the early 1-cell to the early 2-cell stage (Group 1, Table I) and from the late 2-cell to the early 8-cell (Group 3, Table I). Such a pattern would be expected if, during the periods analysed, a population of stable mRNAs was present and translated, and little mRNA turnover was occurring. A second pattern, in which α-amanitin prevents an increase in methionine incorporation shown by the control embryos (and may cause a slight decrease), is found in embryos cultured from the early 4-cell to the late 8-cell and from the early 8-cell stage to the late 16-cell stage (Groups 4 and 5, Table I). This effect of α-amanitin around the time of compaction confirms previous observations (Johnson *et al.*, 1977; Braude, 1979) and would be explained if the net increases in protein biosynthesis occurring over these periods were contingent upon new RNA production. The third pattern, in which α-amanitin almost completely eliminates incorporation of methionine into acid-precipitable material, is shown uniquely by embryos cultured from the early 2-cell to the late 2-cell/early 4-cell stage (Group 2, Table I). In these embryos, in contrast to embryos cultured over the immediately preceding or succeeding periods (pattern 1), the *in situ* template activity of mRNA appears to decline to very low levels. The three distinct patterns of incorporation were shown not to be a consequence of differential effects of α-amanitin on uptake of methionine, which was unaffected at all stages examined (data not shown).

Changes in the quality of proteins synthesised between the 1- and 8-cell stages

Changes in the nature of the proteins synthesised over the 1- to 8-cell period were examined by electrophoresis of radiolabelled polypeptides in one or two dimensions. As shown in Figure 1, the polypeptides synthesised by unfertilized eggs (1a) or early 1-cell zygotes (1b) show only a few differences from those made by early 2-cell embryos 24 h later (1c). A most marked change is the increased appearance of a set of polypeptides, of mol. wt. 35 K, which has been reported previously (Braude *et al.*, 1979; Cullen *et al.*, 1980; Van Blerkom, 1981; Cascio and Wasserman, 1982). Between the early 2-cell embryos and the late 2-cell/early 4-cell embryos 24 h later (1d), there is a major change in the pattern of poly-

Table I. Effect of α-amanitin on incorporation of methionine by cleaving mouse embryos into acid insoluble material

Age of embryos (hours post-insemination or fertilization)[a] at start of experiment (embryonic stage)		Continuous presence of α-amanitin (11 μg/ml)	Incorporation (fmol total methionine/embryo/h) at			Analysis of variance	
			Time zero + 3 h	zero + 12 h	zero + 24 h	Time	Drug
1[b]	1	−	1.2	1.8	1.0	$F=8.09(2,10)$	$F=0.06(1,10)$
	(1-cell)	+		1.6	1.0	$p<0.01$	$p=$N.S.
2	22	−	1.8	1.1	1.0	$F=130.65(2,10)$	$F=472.93(1,10)$
	(early 2-cell)	+		0.3	0.1	$p=0.001$	$p=0.001$
3[b]	38	−	2.5	3.3	3.0	$F=1.18(2,10)$	$F=0.87(1,10)$
	(late 2-cell)	+		2.7	4.2	$p=$N.S.	$p=$N.S.
4	45	−	6.3	10.1	10.6	$F=33.35(2,10)$	$F=109.04(1,10)$
	(4-cell)	+		7.8	5.1	$p<0.01$	$p=0.001$
5	59	−	11.2	22.4	44.8	$F=18.49(2,10)$	$F=79.39(1,10)$
	(8-cell)	+		11.9	6.8	$p<0.01$	$p<0.01$

[a]Embryos in groups 1 and 2 were produced by fertilizing eggs *in vitro*, and in groups 3, 4, and 5 were flushed from oviducts after *in vivo* fertilization. The labelling period was 3 h in each experiment.
[b]Analysis of variance indicates no significant effect of α-amanitin on incorporation rates in these groups (see columns 6 and 7).

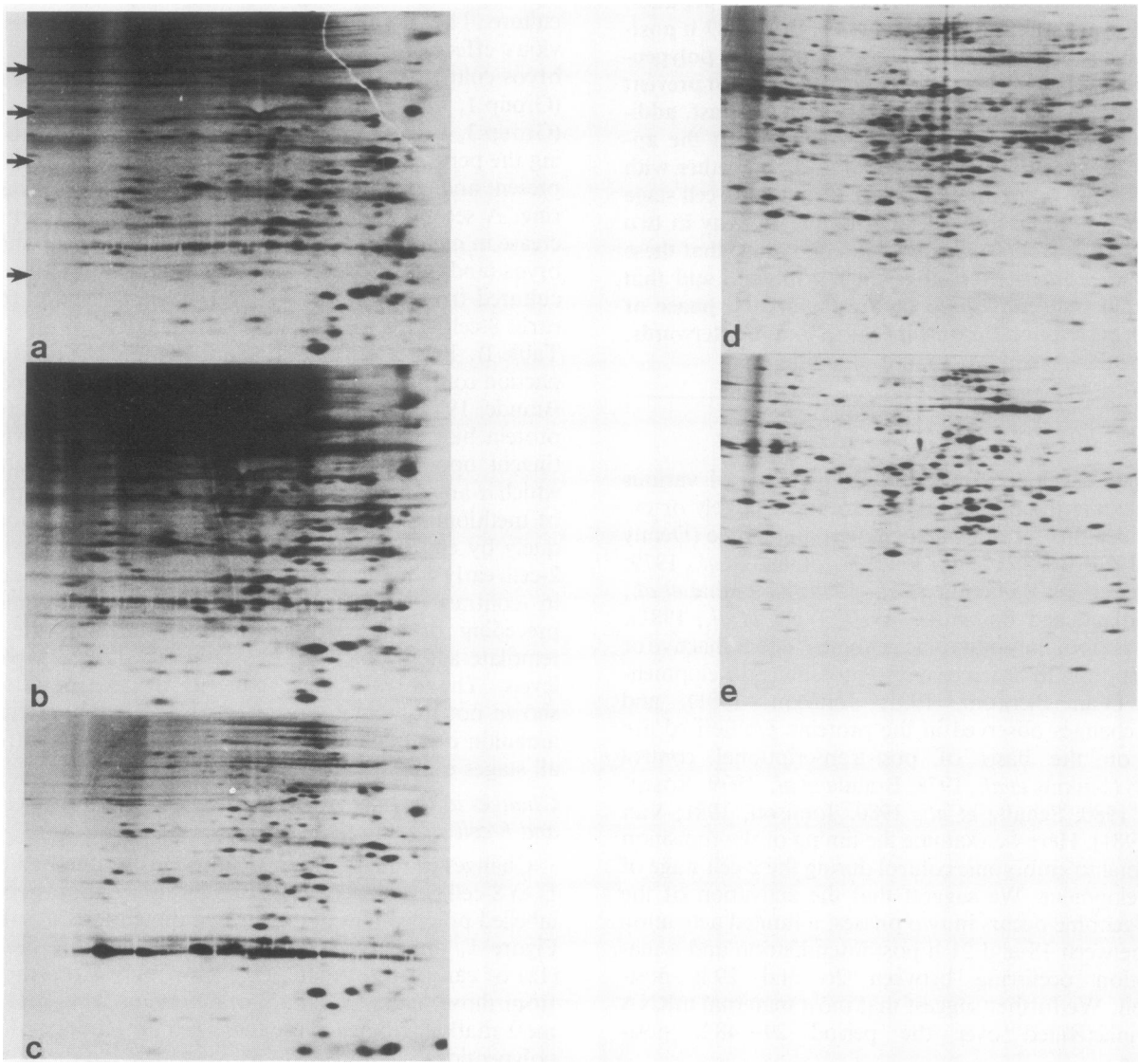

Fig. 1. Two-dimensional electrophoretic separation under denaturing conditions of radiolabelled polypeptides. Labelled samples were **(a)** unfertilized egg, 5 h post-ovulation, **(b)** newly fertilized 1-cell zygote (2 h post-fertilization), **(c)** early 2-cell embryo (22 h post-fertilization), **(d)** late 2-cell embryo (39 h post-fertilization), and **(e)** early 8-cell embryo (62 h post-fertilization). The arrows **(a)** represent mol. wt. markers (from top to bottom) of 92.5 K, 69 K, 46 K, and 30 K. Isoelectric point separation range displayed is (from left to right) pH 6.5 to 4.0.

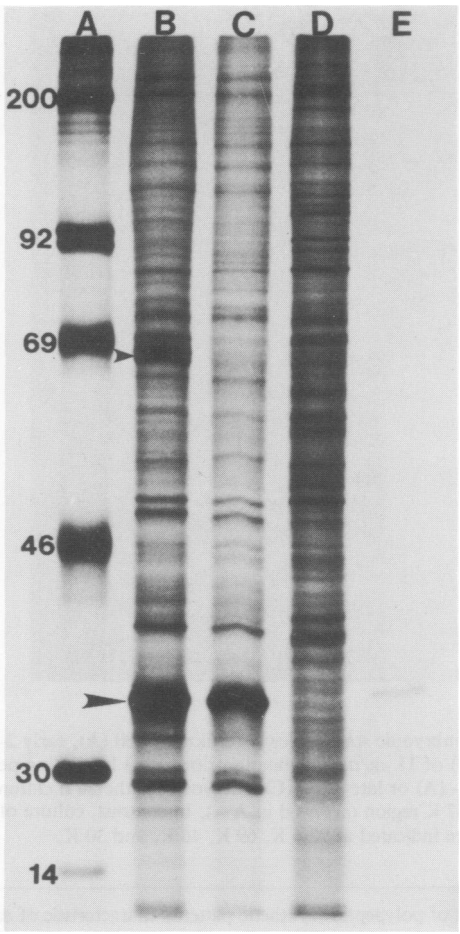

Fig. 2. One-dimensional SDS 10% polyacrylamide gel electrophoresis of radiolabelled polypeptides. **Track A**: mol. wt. markers 200 K, 92.5 K, 69 K, 46 K, 30 K, and 14.3 K. **Tracks B – E**: [^{35}S]methionine-labelled polypeptides synthesized by early mouse embryos derived by fertilization *in vitro* in the presence (**C** and **E**) or absence (**B** and **D**) of 11 μg/ml α-amanitin. The spermatozoa were removed at 4 h post-insemination and the embryos cultured for a further 19 h (**B** and **C**) or 43 h (**D** and **E**) before a 3 h labelling period. Each track represents the synthetic activity of 20 embryos and an exposure time of 14 days. Arrows indicate the positions of the 35 K and 67 K polypeptide complexes referred to in the text.

peptides synthesised, many no longer being evident, and many new ones appearing. This result also confirms previous observations (Van Blerkom and Brockway, 1975). In contrast, when the polypeptides synthesised by late 2-cell/early 4-cell embryos are compared with those made by early 8-cell embryos 24 h later (1e) few differences are observed. Thus, the 2-cell period over which embryos show greatest sensitivity to the effects of α-amanitin on net synthesis of protein is characterised by major changes in the species of polypeptides being synthesised.

The timing during the 2-cell stage of this qualitative change in polypeptide synthesis was determined more precisely by fertilizing eggs *in vitro*, culturing to the 2-cell stage, and placing groups of embryos into label for 3 h at various times post-insemination. The pattern of proteins synthesised at each time point was analysed on one dimensional SDS-polyacrylamide gels. Embryos placed in label at 21 h post-insemination showed a pattern indistinguishable from track C (Figure 2). Embryos placed in label at 23, 26, and 29 h post-insemination showed a pattern (illustrated in track 2B for embryos placed in label for 3 h at 23 h post-insemination) which differed only in the presence of a complex of polypeptides of mol. wt. ~67 K. Embryos placed in label at 44 h post-insemination showed a quite distinct pattern (track 2D) in which many new bands were present and in which many bands that were previously present, including the 67 K band, were no longer evident or prominent. Embryos placed in label at 32 or 35 h showed a mixed pattern with features of both b and d present. This result appeared to suggest that, whilst the earliest detectable change in 2-cell protein synthesis occurs at 23 – 26 h (the 67 K complex), most qualitative changes occur over the period of 29 to 32 h post-insemination.

The effect of α-amanitin on the qualitative changes in protein synthesis

Having established the timing of qualitative changes in polypeptide synthesis over this period, we analysed the effects of α-amanitin on these changes. Three types of experiment were undertaken.

In the first experiment, newly fertilized eggs, early 2-cell embryos, or late 2-cell embryos were cultured for 21 h in the presence or absence of α-amanitin, placed in [^{35}S]methionine for 3 h and the labelled polypeptides analysed by two-dimensional electrophoresis. The results are shown in Figure 3. α-Amanitin has little effect on late 2-cell embryos (Figure 3C) and relatively little effect on newly fertilized eggs (Figure 3A) the only consistent changes being observed in polypeptides in the 67 K region (arrowed in 3A). In contrast, α-amanitin suppresses the synthesis, by early 2-cell embryos cultured for 24 h, of all polypeptides, only a trace of the most prominent 35 K polypeptide remaining evident (Figure 3B).

The timing of these α-aminitin sensitive events was then determined. Eggs were fertilized *in vitro* in the presence of α-aminitin and then placed in label for 3 h at 23, 26, 29, 32, or 44 h post-insemination. The profile of polypeptides synthesised at 23, 26, and 29 h differed from control profiles only in the absence of the 67 K complex (compare tracks B and C, Figure 2). However, at 32, 35, and 44 h, two features were observed: first, no new "late 2-cell" polypeptides appeared, and second there was a progressive reduction with time in the intensity of labelled polypeptides characteristic of the early 2-cell, until again by 44 h only a faint 35 K band was visible (compare tracks D and E, Figure 2). These effects of α-amanitin were not contingent upon the continuing presence of the drug as shown by its removal at various points during the culture (see Table II for summary of results).

In a third type of experiment, eggs were fertilized *in vitro* in the absence of α-amanitin, cultured *in vitro* to the late 1- or early 2-cell stage and then groups of embryos were placed in α-amanitin at 15, 16, 17, 18, 19, 21, 23, 29, 32, 35, or 47 h post-insemination. Embryos were then either cultured to 25 h or 47 h post-insemination, and then labelled for 3 h by incubation in [^{35}S]methionine. Embryos, whether 1-cell or 2-cell, placed in α-amanitin between 15 and 18 h post-insemination showed no evidence of synthesis of the 67 K complex of polypeptides at 25 h (Figure 4, track B). Two-cell embryos placed in α-amanitin at 19 or 21 h post-insemination synthesised the 67 K complex at 25 h (Figure 4, tracks C and D) but more weakly than 2-cell embryos placed in α-amanitin at 23 h or later (Figure 4, tracks E and F). Thus, the α-amanitin sensitive event which leads to the increased synthesis of the 67 K complex of polypeptides appears to occur over the period 18 – 21 h post-insemination.

Embryos placed in α-amanitin at 23 and 26 h showed no

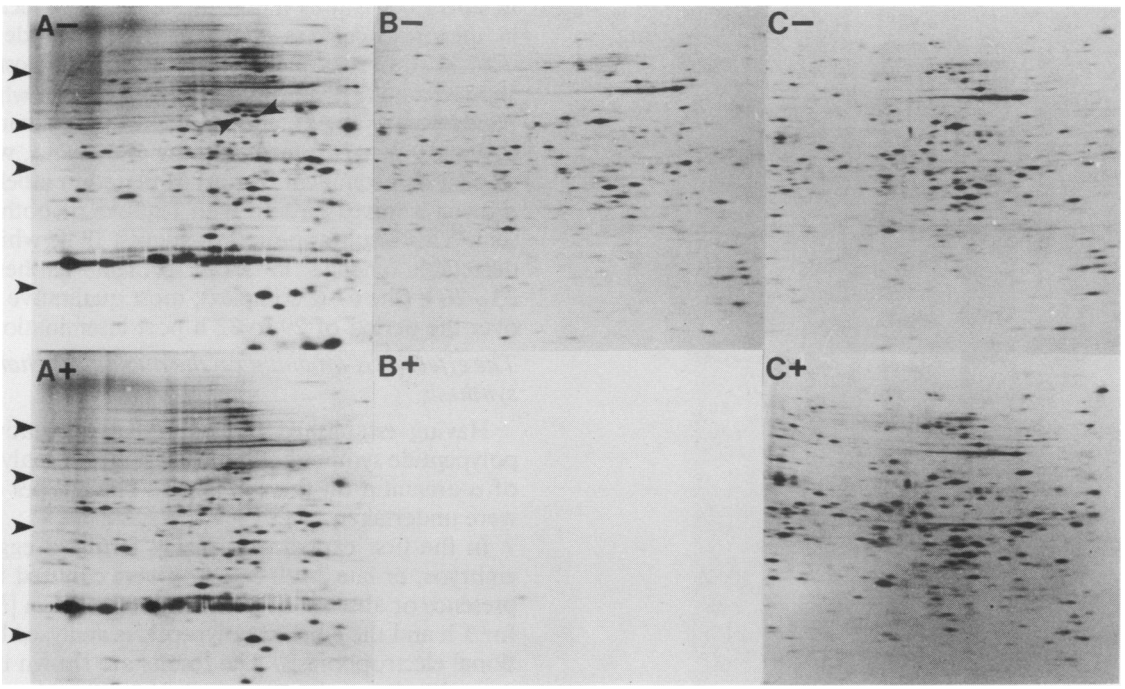

Fig. 3. The effects of α-amanitin on the two-dimensional electrophoretic patterns of different embryonic stages. Newly fertilized 1-cell **(A)**, early 2-cell **(B)**, or late 2-cell **(C)** embryos were harvested and cultured for 21 h in the absence (−) or presence (+) of 11 μg/ml α-amanitin prior to a 3 h labelling period with [^{35}S]methionine. Note that α-amanitin has little or no effect on the synthetic patterns of early 1- **(A)** or late 2-cell **(C)** embryos over the 24 h culture period, the only consistent effect on the early 1-cell pattern being the inhibition of polypeptides in the 67 K region (arrowed in **A**−). In contrast, culture of early 2-cell **(B)** embryos in α-amanitin for 24 h eliminates almost all synthetic activity. Mol. wt. markers are indicated at 92.5 K, 69 K, 46 K, and 30 K.

Table II. Effect of α-amanitin (11 μg/ml) on fertilization and cleavage rates and on development of polypeptide synthetic patterns characteristic of early and late 2-cell embryos[a]

Time (hours post-insemination) of α-amanitin		Analysis at 23 h post-insemination (early 2-cell)			Analysis at 47 h post-insemination (late 2-/early 4-cell)		
Addition	Removal	Early 2-cell polypeptides in presence of α-amanitin	Proportion of inseminated eggs that cleaved to 2-cells (%)		Late 2-cell polypeptides in presence of α-amanitin	Proportion of inseminated eggs that cleaved to >2 cells (%)	
			α-Amanitin	Controls		α-Amanitin	Controls
0	4	Yes	91/138 (66)	93/141 (66)	No	0/43 (0)	29/43 (67)
0	23	Yes	560/737 (76)	728/984 (74)	No	0/377 (0)	262/402 (65)
23	47	–	–	–	No	0/85 (0)	67/80 (84)
26	47	–	–	–	No	1/49 (2)	67/80 (84)
29	47	–	–	–	Yes	28/49 (57)	67/80 (84)
32	47	–	–	–	Yes	34/46 (74)	67/80 (84)
36	47	–	–	–	Yes	35/49 (71)	67/80 (84)

[a]All experiments used F_1 (C57B110xCBA) eggs inseminated *in vitro* with CFLP spermatozoa at 13 h post-hCG. Under the conditions used 60−90% of eggs are fertilized and of these, 90% develop to blastocysts *in vitro*.

incorporation of methionine into protein at 47 h. Embryos placed in α-amanitin at 29 h showed slight incorporation into "late 2-cell" type polypeptides only. Embryos placed in α-amanitin at later times showed full development of "late 2-cell" type patterns. Thus, the α-amanitin sensitive event, which leads to the major qualitative changes in polypeptide synthesis, appears to be localised between 26 and 29 h post-insemination. This conclusion is supported by the fact that addition of α-amanitin prior to 29 h blocks division to the 4-cell stage whereas its addition at 29 h or later is without effect (Table II).

Discussion

In this paper, we attempt to describe, with greater precision

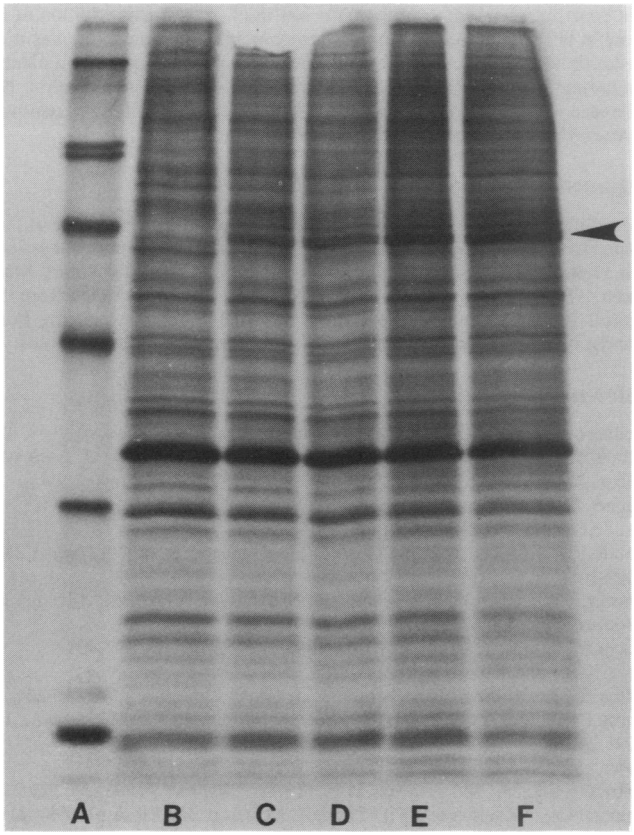

Fig. 4. One-dimensional SDS 9% polyacrylamide gel electrophoresis of radiolabelled polypeptides. **Track A**: mol. wt. markers as for Figure 2. **Tracks B – F**: [^{35}S]methionine-labelled polypeptides synthesised by early mouse embryos derived by fertilization *in vitro*, cultured to the early 2-cell stage, placed in α-amanitin prior to 18 h **(B)**, at 19 h **(C)**, at 21 h **(D)**, at 23 h **(E)**, and at 29 h **(F)** post-insemination. Each track represents the synthetic activity of ~20 embryos and an exposure time of 12 days. The α-amanitin-sensitive 67 K complex of polypeptides is indicated by the arrow. The labelling period was from 26 to 29 h post-insemination.

than has hitherto been attempted, the sequence and timing of changes in polypeptide synthetic profile during the 2-cell stage of mouse development. Heterogeneity between embryos in the timings of fertilization, zygote development, and first cleavage means that even in this study events are expressed over time ranges of ~3 h. However, despite this heterogeneity it seems clear that the changes in polypeptide synthetic profile observed over the first 18 h of development are insensitive to the action of α-amanitin applied either continuously or as a pulse over the period of fertilization. This result confirms and extends earlier experiments (Braude *et al.*, 1979; Petzoldt *et al.*, 1980; Van Blerkom, 1981) and is consistent with biochemical data. Thus, whilst very low levels of RNA synthesis may be occurring over this period (Moore, 1975; Young *et al.*, 1978; Clegg and Piko, 1982), only at some undefined point during the 2-cell stage is there evidence for synthesis of well-characterised species of RNA (Knowland and Graham, 1972; Young *et al.*, 1978; Clegg and Piko, 1982; Piko and Clegg, 1982). Fertilization, DNA replication in the 1-cell zygote, cleavage to the 2-cell stage, and the qualitative change in polypeptide synthetic profile accompanying these events seem, therefore, to be regulated largely if not exclusively at a post-transcriptional level. The first α-amanitin sensitive event to be observed is the synthesis of the 67 K mol. wt. complex of polypeptides observed on one dimensional gels between 23 and 26 h post-insemination. The appearance of this complex depends upon an α-amanitin-sensitive event 18 – 21 h post-insemination at the early 2-cell stage.

A major activation of many genes appears to occur between 26 and 29 h post-insemination. The evidence for this conclusion is as follows: (a) addition of α-amanitin at any time prior to this period completely blocks both the appearance of late 2-cell type polypeptides and the division of the embryos to the 4-cell stage; α-amanitin added any time after this period is without obvious effects on either of these parameters; (b) a major qualitative change in polypeptide synthetic pattern is first observed in embryos between 29 and 32 h post-insemination, suggesting that the production of many new embryonic transcripts is followed immediately by their translation to give detectable levels of new polypeptides. This conclusion is reinforced by genetic evidence that paternally-derived alleles are first expressed at the late 2-cell stage (Sawicki *et al.*, 1981; reviewed by McLaren, 1979; and Magnuson and Epstein, 1981), by the failure of physically enucleated zygotes to synthesise late 2-cell type polypeptides (Petzoldt *et al.*, 1980), and by the evidence that mRNA extracted from early 2-cell embryos and translated *in vitro* yields only early 2-cell polypeptides and does not code for the characteristic late 2-cell polypeptides (Schultz *et al.*, 1981).

The major activation of the embryonic genome is followed by a progressive decline in the expression of maternal mRNA between 26 and 47 h post-insemination. The evidence for this conclusion is as follows: (a) in control embryos, synthesis of polypeptides characteristic of the early 2-cell embryo declines progressively and rapidly from 26 h post-insemination onwards; (b) this decline is also observed in α-amanitin treated embryos, in which it can be shown that incorporation falls to 20% by 35 h and is almost undetectable by 47 h. This pattern contrasts with the stability of maternal mRNA observed over the period 1 – 25 h post-insemination, during which period protein synthetic activity is not reduced in the presence of α-amanitin. There is thus a distinct change in the apparent stability of maternal mRNA expression over the period at which many embryonic genes are first activated. This result is consistent with several other observations. Thus, dramatic falls in the levels of total RNA (Olds *et al.*, 1973; Piko and Clegg, 1982), polyadenylated RNA (Levey *et al.*, 1978; Piko and Clegg, 1982), and internally-labelled maternal poly(A)$^+$ RNA (Bachvarova and De Leon, 1980) have been described as occurring during the 2-cell stage. Moreover, adult mRNA injected into fertilized mouse eggs does not persist in a functional form during cleavage to the 4-cell stage (Brinster *et al.*, 1980).

The failure to detect continuing synthesis of polypeptides on maternally-derived mRNA templates seems unlikely to result from the rapid and selective destruction of these polypeptides since, if these polypeptides are pulse-labelled at the early 2-cell stage, they are observed to persist well into the development of the morula (H.P.M.Pratt and M.Godard, personal communication; J.Van Blerkom, personal communication). Moreover, if early 2-cell embryos cultured for 24 h in α-amanitin are then subjected to RNA extraction and this RNA is translated *in vitro*, no incorporation into embryonic polypeptides is observed (data not shown). It seems probable, therefore, that the maternal mRNAs are lost rather than selectively inactivated or sequestered. It is essential to be cautious in interpreting experiments in which α-amanitin is used, since it is possible that the introduction of the drug

could disturb rates of mRNA turnover and degradation. It seems unlikely, however, that the results reported here are purely an artefact of the necessary use of the drug. First, maternal transcripts do cease to show activity *in situ* in normal late 2-/early 4-cell embryos. Second, use of α-amanitin does not reveal such a major effect either on maternal transcripts prior to 26 h post-insemination or on embryonic transcripts thereafter. The results obtained with α-amanitin do, therefore, seem to reveal underlying events characteristic of normal development over this period. The extent to which the α-amanitin might modify the kinetics of these events will be determined only by quantitative analysis of the activity of individual species of maternal mRNA both *in situ* and *in vitro*. In the meantime, the implications of these qualitative findings are considerable. Wherever evidence of a lingering maternal effect on development beyond the 2-cell stage is described (McLaren, 1979; Magnuson and Epstein, 1981), it will be important to determine whether this represents the activity of a subset of resistant or stable mRNAs not detected here or the consequences of a post-translational effect such as activation of an enzyme synthesised on maternal templates but modified post-translationally at a subsequent stage in development (Van Blerkom, 1981).

The putative transcriptional events at 18–21 and 26–29 h post-insemination observed during the 2-cell stage provide a sandwich about the second round of DNA replication which occurs in a population of 2-cell embryos over the period 18–26 h (Luthardt and Donahue, 1975; Sawicki *et al.*, 1978). We are now using synchronised populations of 2-cell embryos to determine the precise temporal relationships between DNA replication and these two phases of transcription and to determine whether these events are related causally.

Materials and methods

Embryos

One- and 2-cell embryos were obtained from HC-CFLP or (C57B110 x CBA)F_1 mice by recovering ovulated oocytes from the oviducts 13–14 h after an ovulating injection of human chorionic gonadotropin (hCG) and their fertilization *in vitro* as described by Fraser and Drury (1975). Four- and 8-cell embryos used for both quantitative and qualitative analyses were obtained by flushing *in vivo* fertilized eggs from the oviducts at ~44–48 h after an ovulating injection of hCG. All embryo recovery and culture conditions are exactly as described in detail by Pratt *et al.* (1981).

Quantitative incorporation studies

Embryos were incubated for 3 h in 5 μl [^{35}S]methionine (~800 Ci/mmol) dissolved in 50 μl of a stock of 50 or 100 μM methionine in medium 16 containing 0.4% bovine serum albumin (BSA) (Whittingham, 1971). Under these conditions, incorporation has reached a plateau whilst uptake remains linearly related to the external concentration of methionine (Holmberg and Johnson, 1979). Embryos were then washed, and placed in 50 μl of distilled water containing 50 mM methionine, to which was added 25 μl 20% trichloroacetic acid (TCA) + 20 μl BSA (1 mg/ml). After standing overnight at 4°C, samples were filtered, washed with 7% TCA containing 50 mM methionine and the filter papers counted in cocktail N (Fisons) in an ICN scintillation counter for 5 min at 35% efficiency. Each value was calculated as described previously (Holmberg and Johnson, 1979) and represents the mean of 2 or 3 replicates, each of which assayed 5–10 embryos.

Qualitative separation of polypeptides by SDS-polyacrylamide gel electrophoresis in one or two dimensions

Embryos were labelled by a 3 h incubation in 5 μl of [^{35}S]methionine (~800 Ci/mmol) dissolved in 25 μl of medium 16 containing 0.4% BSA (Whittingham, 1971) Embryos were rinsed 3x with phosphate buffered medium 1 free of BSA, and placed either in double-strength SDS buffer for one-dimensional separation or sample buffer for two-dimensional separation. Electrophoresis, autoradiography, and analysis of gels is exactly as described in detail in Johnson and Rossant (1981) as modified from O'Farrell (1975). In all cases the age in hours post-insemination specified as time of harvest includes a terminal 3 h labelling period.

In experiments in which α-amanitin was used, the time of addition of α-amanitin is always specified. In most experiments, the α-amanitin was then present up to the time of labelling. In some experiments in which α-amanitin was present during fertilization, it was removed 4 h after insemination. No difference was observed between embryos in which α-amanitin was removed or retained, confirming the irreversibility of its action.

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References

Bachvarova,R., and De Leon,Y. (1980) *Dev. Biol.*, **74**, 1-8.
Ballantine,J.E.M., Woodland,H.R., and Sturgess,E.A. (1979) *J. Embryol. Exp. Morphol.*, **51**, 137-153.
Brachet,J., Ficq,A., and Tencer,R. (1968) *Exp. Cell Res.*, **32**, 168-170.
Braude,P.R. (1979) *Dev. Biol.*, **68**, 440-452.
Braude,P.R., Pelham,H.R.B., Flach,G., and Lobatto,R. (1979) *Nature*, **282**, 102-105.
Brinster,R.L., Chen,H.Y., Trumbauer,M.E., and Avarbock,M.R. (1980) *Nature*, **283**, 499-501.
Cascio,S.M., and Wassarman,P.M. (1982) *Dev. Biol.*, **89**, 397-408.
Clegg,K.B., and Piko,L. (1982) *Nature*, **295**, 342-345.
Cullen,B., Emigholz,K., and Monahan,J. (1980) *Dev. Biol.*, **76**, 215-221.
Denny,P.C., and Tyler,A. (1964) *Biochem. Biophys. Res. Commun.*, **14**, 245-249.
Fraser,L.R., and Drury,L. (1975) *Biol. Reprod.*, **13**, 513-518.
Holmberg,S.R.M., and Johnson,M.H. (1979) *J. Reprod. Fertil.*, **56**, 223-231.
Jenkins,N.A., Kaumeyer,J.F., Young,E.M., and Raff,R.A. (1978) *Dev. Biol.*, **63**, 279-298.
Johnson,M.H. (1981) *Biol. Rev.*, **56**, 463-498.
Johnson,M.H., and Rossant,J. (1981) *J. Embryol. Exp. Morphol.*, **61**, 103-116.
Johnson,M.H., Handyside,A.H., and Braude,P.R. (1977) in Johnson, M.H. (ed.), *Development in Mammals*, Vol. 2, North-Holland, pp. 67-97.
Knowland,J., and Graham,C.F. (1972) *J. Embryol. Exp. Morphol.*, **27**, 167-176.
Levey,I.L., and Brinster,R.L. (1978) *J. Exp. Zool.*, **203**, 351-360.
Levey,I.L., Stull,G.B., and Brinster,R.L. (1978) *Dev. Biol.*, **64**, 140-148.
Luthardt,F.W., and Donahue,R.P. (1975) *Dev. Biol.*, **44**, 210-219.
Magnuson,T., and Epstein,C.J. (1981) *Biol. Rev.*, **56**, 369-408.
McLaren,A. (1979) in Newth,D.R., and Balls,M. (eds.), *Maternal Effects in Development*, Cambridge University Press, pp. 287-320.
Moore,G.P.M. (1975) *J. Embryol. Exp. Morphol.*, **34**, 291-298.
O'Farrell,P. (1975) *J. Biol. Chem.*, **250**, 4007-4021.
Olds,P.J., Stern,S., and Biggers,J.D. (1973) *J. Exp. Zool.*, **186**, 39-46.
Petzoldt,U., Hoppe,P.C., and Illmensee,K. (1980) *Wilhelm Roux's Arch. Dev. Biol.*, **189**, 215-219.
Piko,L., and Clegg,K.B. (1982) *Dev. Biol.*, **89**, 362-378.
Pratt,H.P.M., Chakraborty,J., and Surani,M.A.H. (1981) *Cell*, **26**, 279-292.
Rosenthal,E.T., Hunt,R.T., and Ruderman,J.V. (1980) *Cell*, **20**, 487-494.
Sawicki,W., Abramczuk,J., and Blaton,O. (1978) *Exp. Cell Res.*, **112**, 199-205.
Sawicki,W., Magnuson,T., and Epstein,C.J. (1981) *Nature*, **294**, 450-454.
Schultz,G.A., Clough,J.R., and Johnson,M.H. (1980) *J. Embryol. Exp. Morphol.*, **56**, 139-156.
Schultz,G.A., Clough,J.R., Braude,P.R., Pelham,H.R.B., and Johnson, M.H. (1981) in Glasser,S.R., and Bullock,D.W. (eds.) *Cellular and Molecular Aspects of Implantation*, Plenum Press, pp. 137-154.
Van Blerkom,J. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 7629-7633.
Van Blerkom,J., and Brockway,G.O. (1975) *Dev. Biol.*, **44**, 148-157.
Wells,D.E., Showman,R.M., Klein,W.H., and Raff,R.A. (1981) *Nature*, **292**, 477-478.
Whittingham,D.G. (1971) *J. Reprod. Fertil. Suppl.*, **14**, 7-21.
Woodland,H.R., Flynn,J.M., and Wyllie,A.J. (1979) *Cell*, **18**, 165-171.
Woodland,H.R., and Ballantine,J.E.M. (1980) *J. Embryol. Exp. Morphol.*, **60**, 359-372.
Young,R.J., Sweeney,K., and Bedford,J.M. (1978) *J. Embryol. Exp. Morphol.*, **44**, 133-148.