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Gene Expression and Macromolecular Synthesis During Preimplantation Embryonic Development

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The most extensively studied systems of early embryonic development are ones far removed from man and other mammals and include, most notably, the echinoderms and amphibia. Based on work with these and other organisms, there has been built-up an overall picture in which much, if not all, of early post-fertilization development is controlled by messenger RNAs (mRNAs) which are synthesized in the egg but are not activated until after fertilization has occurred (Gross, 1967; Davidson, 1968; Brown and Dawid, 1969; Brachet and Malpoix, 1971). The presence and activity of such masked messenger RNAs (mmRNAs) were originally defined in experiments in which early development was shown to be invulnerable to the effects of actinomycin D, an inhibitor of RNA synthesis. However, the physical existence of these mmRNAs now seems to be firmly established, and it has been possible to isolate and use them to govern protein synthesis *in vitro* (Gross, et al. 1973; Skoultchi and Gross, 1973).

With the development of techniques for the biochemical analysis of the mammalian embryo, it is natural that the question of how early *mammalian* development is controlled should be of great interest. While the presence or absence of mammalian mmRNAs has not as yet been established, the available information indicates that all stages of preimplantation mammalian development are affected by the genetic activity of the embryo itself. It will be the purpose of this paper to summarize the relevant information obtained principally from our own studies on the preimplantation mouse embryo and, when applicable, from the studies of others with mouse and rabbit embryos. Unless otherwise stated, the material will refer to the developing mouse embryo, between fertilization and implantation. Recent reviews of this subject have been published by Wolf and Engel (1972), Fowler and Edwards (1973), and Biggers and Stern (1973).

The evidence will be reviewed in order from the least to the most direct, and the major point to be considered will be the role of embryonic gene action in governing early mammalian development. However, although our emphasis is on genetic control, it is well to keep in mind that gene activity by itself is unlikely to be the whole explanation of development and differentiation. This point was well stated in a recent book by Wright (1973). "Application of the words 'cause' or 'trigger' to particular components within a differentiating system may delude the user into feeling that there exist certain 'spontaneous' or independent factors uniquely capable of causing differentiation to occur.... There is no *a priori* reason for singling out any particular biochemical event on which differentiation depends as being more essential or necessary than others.... Such an approach renders an objective analysis difficult and stresses the role of components which may or may not be limiting the rate of the differentiation process in question. It appears to be no more justified or useful to choose selective gene activation as 'the' basis of differentiation than to choose, for example, substrate availability."

MORPHOLOGY

Perhaps the least direct but still highly pertinent fact about early development and differentiation that should be considered is that it occurs. During the 4½–5 days between fertilization and implantation of the mouse

embryo, there are a series of specific changes which accompany the transformation of the single cell zygote into a blastocyst ready to implant. Considering just those detected morphologically (Fig. 1), light microscopic methods demonstrate cleavage, formation of the blastocyst cavity, segregation of the embryonic cells into trophoblast and inner cell

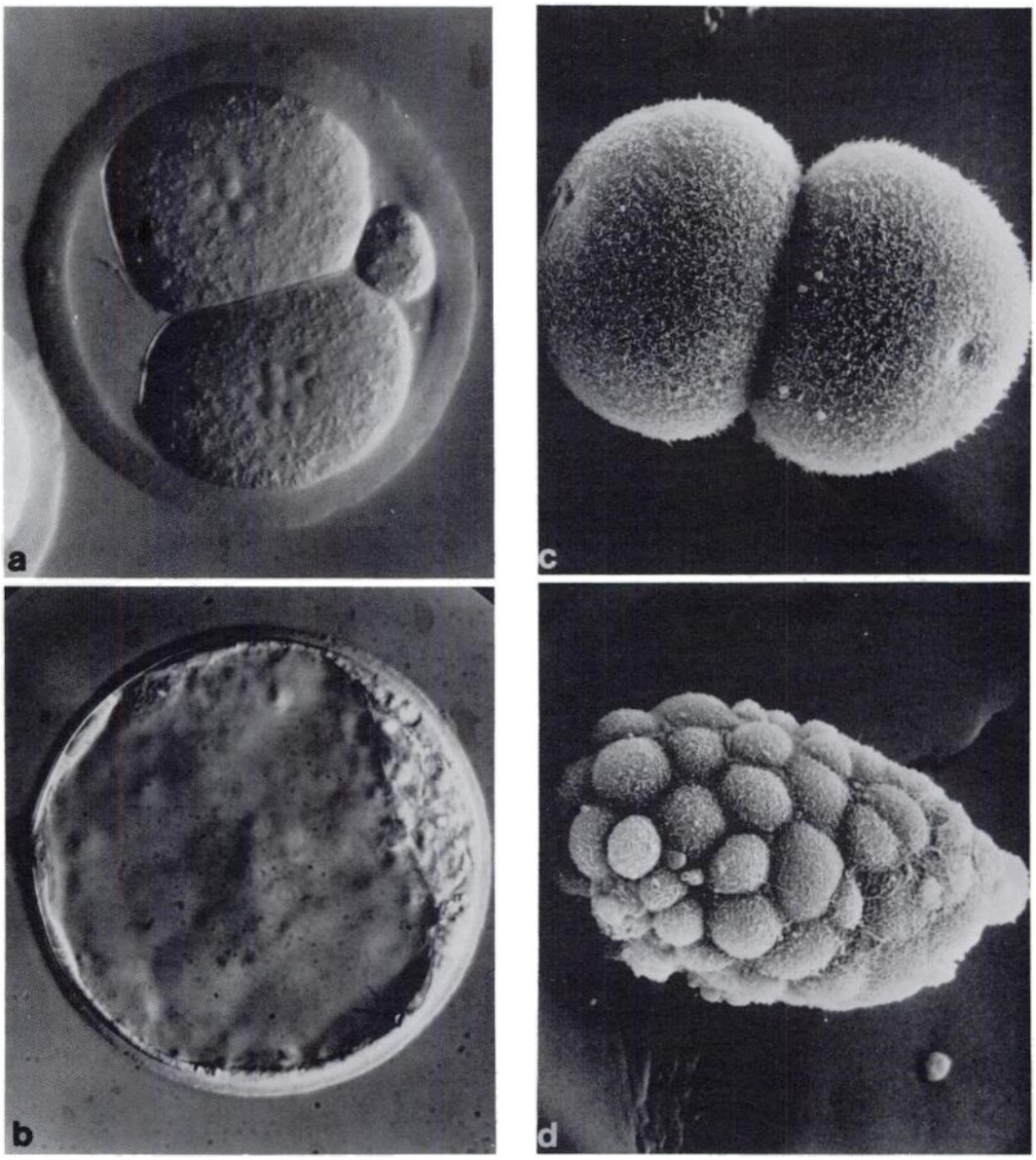
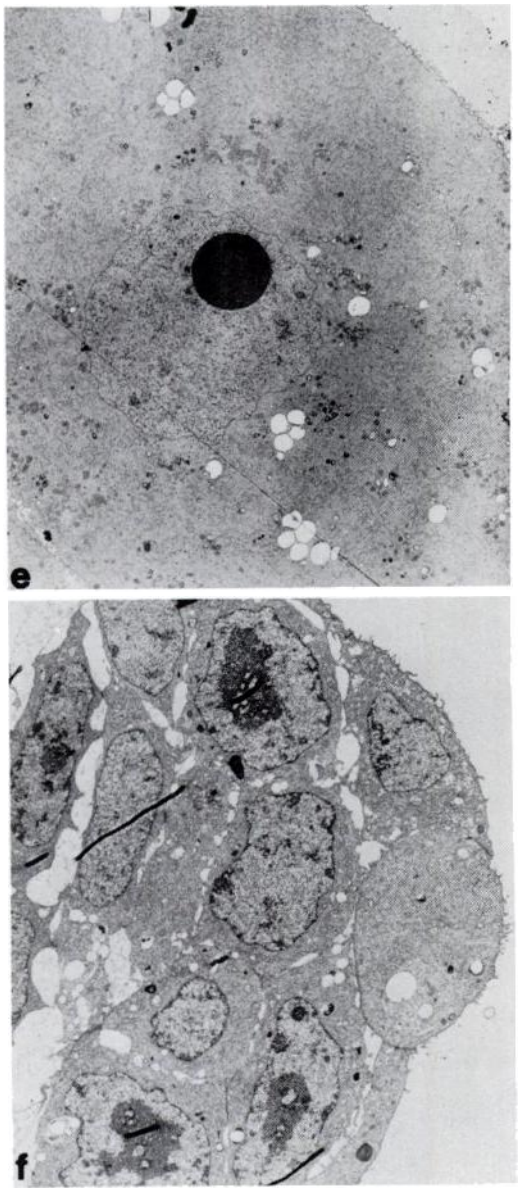


FIG. 1. a) Two cell mouse embryo photographed using Nomarski differential interference optics. The nuclei contain several round nucleoli. × 275. b) A mouse blastocyst photographed using Normarski differential interference optics. × 540. c) Scanning electron micrograph of a 2 cell mouse embryo after removal of the zona pellucida. × 1350. d) Scanning electron micrograph of a mouse blastocyst (day 4). × 2310. e) Transmission electron micrograph of a 2 cell embryo. The nucleus containing a spherical agranular nucleolus is visible in the center of one blastomere. The zona pellucida is at the upper left; a portion of the second blastomere at the lower right. × 2,590. f) Transmission electron micrograph of the inner cell mass of a 4 day mouse blastocyst. × 3150. All photomicrographs were provided by Dr. Patricia G. Calarco.



mass, changes in the nature of cell contacts, and loss of the zona pellucida. With the scanning electron microscope changes in the microvilli and in the cell junctions on the surface of the embryo became apparent, as do differences between the trophoblast cells on the outside and the inner cell mass cells facing the blastocyst cavity (Calarco and Epstein, 1973; Calarco, 1974). Transmission electron microscopy confirms these observations and reveals, in addition, changes in the structure of mitochondria and nucleoli (Calarco and Brown, 1969).

Could all of these morphological changes, and the biochemical ones to be described below, occur in the absence of activity of the embryonic genome? Considering what is known about lower species, the answer could well be "yes" if the existence of masked mRNAs or, at least, of various stable mRNAs, whether masked or not, were postulated. In theory, the answer could be "yes," even if such mRNAs were not invoked, if all of the necessary enzymes and other essential proteins were present in either active or inactive forms. However, even in the slime mold, *Dictyostelium discoideum*, which can undergo much of its differentiation under conditions in which new mRNA cannot be made, there is a point in the developmental cycle before which interference with mRNA synthesis does inhibit differentiation. This suggests that pre-existing proteins, enzyme and otherwise, and metabolites alone are not sufficient to carry through all of the complex development processes which are taking place. If this analogy is extended to the developing mammalian embryo, it seems reasonable to postulate, unless firm evidence for pre-existing masked or stable mRNAs can be obtained, and not necessarily even then, that expression of the embryonic genome will be required for control of development.

METABOLISM

During the preimplantation period of mouse development there are significant changes in intermediary metabolism, the most striking of which are in the ability of the embryo to use various substrates as sources of energy. These changes have been well described and reviewed by other investigators (see the reviews of Biggers, 1971, and of Biggers and Stern, 1973) and by Wales in this Symposium, and will be considered only briefly here.

Very early (1 cell) embryos cannot utilize glucose or lactate as their sole energy sources, but can use pyruvate and oxalacetate. Late 2

cell embryos can also use lactate, and 8 cell embryos are able to exist on glucose as well (Biggers, et al, 1967). Changes in the transport of these substrates and in the activities of some of the enzymes involved in their metabolism, while they do exist, have not been sufficient to explain the observed metabolic differences. It has recently been proposed that there is a block in glycolysis at the point of formation of fructose-1,6-diphosphate by 6-phosphofructokinase (PFK) (Barbehenn, et al, 1974), even though the measured activity of PFK does not change (Brinster, 1971a).

In the context of the present discussion, the major question is whether these changes in metabolic activity can be taken as strong evidence for activity of the embryonic genome. At the present state of knowledge, the answer must be "no." As Wright's (1973) analysis indicates, the changes observed can be explained on the basis of progressive orderly and interrelated variations in substrate and metabolite concentrations and in enzyme activities, without invoking the synthesis of new mRNAs, and Barbehenn, et al (1974) do suggest such possibilities in their report. On the other hand, changes in metabolic patterns are not inconsistent with and are still most easily explained by expression of the embryonic genome.

PRECURSOR TRANSPORT

Although the preimplantation embryo can be cultured *in vitro* in relatively simple media and seems to require few metabolic precursors from the outside (Whitten and Biggers, 1968; Cholewa and Whitten, 1970), there are, nevertheless, significant developmental changes in the ability of the embryo to transport a number of metabolites from the medium. The first studied of these were the transport processes for the various energy sources already referred to above. Although the determinations were not always carried out under conditions where saturation of the transport system was assured or assessed, the results do indicate an increase in glucose uptake between the 1 and 2 cell stages, but not between 2 and 8 cells (Wales and Brinster, 1968), and increases in citrate, malate, and 2-oxoglutarate uptake between 2 and 8 cells, with further increases in malate and oxoglutarate uptake to the morula stage (Wales and Biggers, 1968; Kramen and Biggers, 1971). Since uptake was largely inhibited by incubating the embryos at low temperatures, the existence of a facilitated transport system, perhaps active in nature, has been postulated but not further defined.

Work on the transport of nucleic acid precursors has been more extensive and was stimulated by the need to dissociate observed changes in the incorporation of radioactive precursors into RNA from changes in precursor uptake (see below). By a combination of developmental and competition studies, it has been possible to define a series of apparently independent transport systems (Fig. 2). Even under conditions of high substrate concentration, the uptake of all of the tested nucleic acid precursors is very low during the 1 and 2 cell stages, although some increase is noted for the adenine-guanine system between these

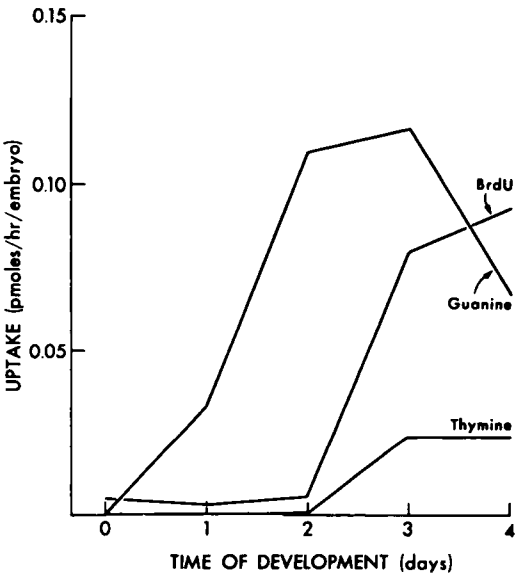


FIG. 2. Uptake of RNA precursors by preimplantation mouse embryos. The patterns shown are representative of the three transport system present in the embryos: nucleosides (BrdU), purine bases (guanine), and pyrimidine bases (thymine). Data from Epstein, et al (1971), Epstein and Daentl (1972), and Golbus and Epstein (1974).

two stages (Epstein and Daentl, 1971; Epstein, et al, 1971). Following the 2 cell stage there are major divergences in developmental patterns. The adenine-guanine (purine base), system increases greatly in capacity, with the 8–16 cell embryos having a several-fold higher uptake and the blastocysts having the same or only slightly more than the 8–16 cell embryos. By contrast, the nucleoside transport system, which appears to transport both oxy- and deoxyribonucleosides, increases only minimally between 2 and 8–16 cells, but shows a great increase in capacity coincident with morula and early blastocyst formation (Daentl and Epstein, 1971; Epstein and Daentl, 1972; Daentl and Epstein, unpublished data). The third system, for pyrimidine bases (exemplified by thymine and uracil), also increases but still remains quite low in capacity at the time of blastocyst formation (Epstein and Daentl, 1972; Daentl and Epstein, unpublished data). In writing of transport systems, rather than the behavior of individual precursors, use is being made of an extensive series of competition studies. The essence of such studies is to show that one member of a group effectively competes for uptake by another member. Thus, the uptake of uridine at a concentration of about 2 μM is 80–95 percent inhibited by 20–25 μM concentrations of cytidine, adenosine, deoxyadenosine, deoxyuridine, and thymidine (Daentl and Epstein, 1973), and similar competitive effects exist within other systems as well (Daentl and Epstein, unpublished data). Competition between different systems is virtually non-existent. While the nature of the nucleic acid precursor transport systems has not been precisely defined, largely for technical reasons, it is clear that they are facilitated systems mediated by constituents contained within the cell membrane.

Again because of its relevance to biosynthetic studies, this time of protein, the transport of amino acids has also been examined in some detail (Fig. 3). With these compounds, there is greater uniformity of behavior during early development. All amino acids tested are taken up, although in small amounts, by unfertilized eggs and zygotes, and the rate of uptake actually appears to decrease somewhat between the 1 and 2 cell stages. Then, similar to the nucleosides, there is a small increase between 2 and 8–16 cells and a very major increase between the latter stage and the early blastocyst (Brinster, 1971b; Epstein and Smith, 1973). However, unlike all of the nucleic acid precursor systems, which remain roughly constant or even decrease in capacity, the rate of amino acid transport continues to increase and is about 2–3 times greater in the late day 4 blastocyst than in the early blastocyst (Epstein and Smith, 1973). Although some competition studies have been done, the number of different amino acid transport systems operating is not known.

Kinetic and inhibitor studies have been used to define further the amino acid transport systems. While the results have not been unequivocal, the systems are temperature sensitive and appear active in nature (Epstein and Smith, 1973; Borland and Tasca, 1974). Changes in the parameters, Km and Vmax, suggest an increase in the number of carrier molecules or transport sites (higher Vmax) for leucine and methionine and perhaps the

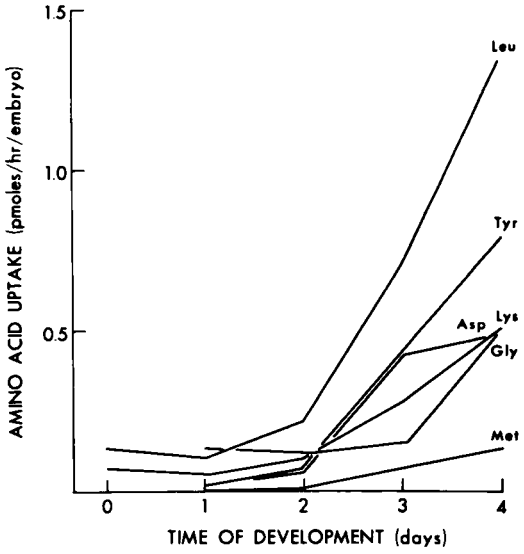


FIG. 3. Uptake of amino acids by preimplantation mouse embryos. In general, the rate of uptake increases during the third day of embryonic development and, unlike protein synthesis, continues to increase during the blastocyst period. Data from Epstein and Smith (1973).

appearance of a new type of carrier or site (different Km) for methionine (Borland and Tasca, 1974).

A frequent question is whether changes in membrane transport can be accounted for by changes in the area of the embryonic surface. This is clearly not the case. Although the early cleavages do result in marked overall increases in surface area, changes in transport are, except for the purine base system, relatively small. To the contrary, the greatest transport changes generally occur at the time of blastocyst formation. But, it is at this time that tight intercellular junctions develop, and the embryonic surface exposed to the outside is little greater, if any, than the surface of the 1 cell zygote. Moreover, the number of surface microvilli, the presence of which really preclude an accurate estimation of surface area, appears less in the blastocyst than in earlier stages (Calarco and Epstein, 1973). Still another consideration is that the changes in the capacities of the various transport systems do not occur coordinately, thereby ruling out any single alteration in the embryonic membrane as a common basis. The inference, therefore, is that each transport system is separately controlled, much the same as different enzymes appear to be (see below).

Returning to the central question of genetic control, the information obtained from analysis of membrane transport is compatible with but does not at this point constitute evidence of a role for the embryonic genome. Kramen and Biggers (1971) have suggested that the developmental changes in the uptake of tricarboxylic acid cycle intermediates are related to genetically determined alterations in mitochondrial structures, but there is no solid basis for this conjecture. In studies of fertilization in sea urchins, increases in transport rates have been shown to occur within minutes of sperm entry under conditions where no gene activation could have occurred (Epel, 1971). These changes are presumably mediated by shifts in the distribution and concentration of intracellular metabolites and by the activation of intracellular enzymes. A similar situation can be visualized as applying in the mouse embryo as well, although it must be noted that the observed changes occur much more slowly in mammalian embryos—over days rather than minutes. Both an increase in the amount of a membrane carrier and an appearance of a new carrier could, in principle, be explained by activation and alteration of pre-existing carrier molecules or by the synthesis of more and/or different molecules utilizing pre-existing messengers. These questions will be considered further in the dicussion of embryonic enzymes in the next section.

ENZYMES

Since the initial measurements of lactate dehydrogenase (LDH) activity by Brinster (1965), developmental changes in the activities of close to 20 enzymes have been analyzed. These are summarized in Fig. 4. Several salient points emerge from these studies. The first is that the one-cell zygote is already endowed with a large complement of enzymes so that it is possible, indeed it is likely, that it can go for some period of time without requiring the synthesis of many of the enzymes required for early development. The second is that the measured amounts of enzyme activity present in the preimplantation embryos cover several orders of magnitude. It must be realized, however, that *in vitro* assays are carried out under conditions of high substrate concentration and, consequently, of maximal enzyme activity. As a result of lower substrate concentrations and the possible presence of inhibitors, activators, and other regulators of enzyme activity, the actual enzyme activities *in vivo* may be considerably lower. The third point, one already made with regard to the transport systems, is that the changes in enzyme activity do not occur coordinately, either in time or amount. To the contrary, virtually any type of change can and does take place, but, as with the transport systems, the time of maximum change, whether up or down, seems to be at and just before the time of blastocyst formation.

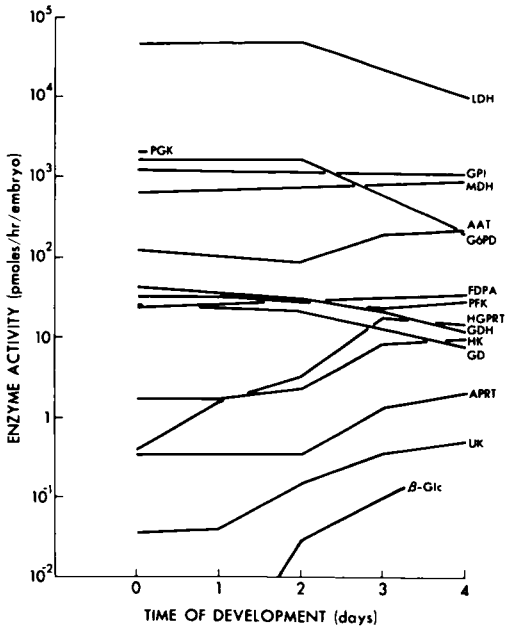


FIG. 4. Enzyme activities in preimplantation mouse embryos. The activities are plotted on a logarithmic scale to make possible the display of a wide range of activities on a single graph. PGK, phosphoglycerate kinase (Kozak, et al, 1974); LDH, lactate dehydrogenase (Epstein, et al, 1969); GPI, glucose phosphate isomerase (Brinster, 1973); MDH, malate dehydrogeanse (Epstein, et al, 1969); G6PD, glucose-6-phosphate dehydrogeanse (Epstein, et al, 1969); AAT, aspartate aminotransferase (Moore and Brinster, 1973); FDPA, fructose-1,6-diphosphate aldolase (Epstein, et al, 1969); PFK, phosphofructokinase (Brinster, 1971a); HGPRT, hypoxanthine guanine phosphoribosyltransferase (Epstein, 1970); GDH, glutamate dehydrogenase (Moore and Brinster, 1973); HK, hexokinase (Brinster, 1968); GD, guanine deaminase (Epstein, et al, 1971); APRT, adenine phosphoribosyltransferase (Epstein, 1970); UK, uridine kinase (Daentl and Epstein, 1971); β-Glc, β-glucuronidase in C57BL/6J (Chapman and Wudl, 1974).

It is common, but not necessarily correct, to equate measured enzyme activities with the amounts of enzyme protein present in the embryo and to relate changes in activity to changes in enzyme concentration. In actuality, the latter is possible only if it is shown that enzyme activators and inhibitors are not present in the assay system and that changes in activities are accompanied by equivalent changes in enzyme protein. The best studied enzymes from this point of view are LDH and glucose-6-phosphate dehydrogenase (G6PD). For these enzymes, both of which remain constant in activity between the 1 and 8–16 cell stages and then decrease exponentially, no evidence for activators or inhibitors could be found in mixing experiments (Epstein, et al, 1969). Furthermore, immunochemical titrations using an anti-mouse-LDH-1 (Spielmann, et al, 1974a) and an anti-human-G6PD have shown that the amounts of antisera necessary to inactivate the embryonic enzymes are proportional to the amounts of enzyme activity added to the assay mixture, irrespective of the stage of development from which the enzymes were obtained (Spielmann, et al, 1974b). If constant specific activity is assumed (a reasonable assumption since inhibitors and activators were shown not to be present), this means that the decreases in enzyme activity result from decreases in enzyme molecules. Since not all enzymes decrease in amount at the same time, the decreases in LDH and G6PD are clearly specific events, but the mechanisms and signals involved have yet to be understood.

Unfortunately, LDH and G6PD, while present in high concentrations, are not optimal subjects for investigation of gene activity since they are decreasing in quantity during the period of interest. No studies have yet been carried out that demonstrate that those enzymes which increase in amount do so as the result of an increased rate of synthesis or, at least, of a rate of synthesis in excess of that of degradation. However, even if it could be shown that such were the case, it would still not be established that the increase is the result of gene expression. Again, the same problems as have already been mentioned recur. Could the enzyme have existed in an inactive or "blocked" form, perhaps as a "proenzyme?" If it is assumed that such are not involved the question becomes one of the source of the mRNA responsible for synthesis of the enzyme. For those enzymes detectable in unfertilized eggs, it is clear that the appropriate mRNAs must exist during oogenesis.

It is also possible that these mRNAs might still be present in the embryo, albeit in an inactive mmRNA form or in a form from which there is only a low rate of translation. Were either of these the case, the increase in enzyme synthesis could result from a "translational" control mechanism, one in which the rate of protein synthesis is being regulated, rather than by a "transcriptional" mechanism in which changes in gene expression are involved. Such translational mechanisms have been invoked in various enzyme induction systems and are, of course, implicit in the masked messenger RNA hypothesis.

The existence of genetically determined enzyme variants has provided one method for circumventing these difficulties. Since the origin of putative masked mRNAs is maternal—i.e., they are made during oogenesis and carried over into the zygote after fertilization—the appearance of paternally determined products would necessitate the conclusion that the embryonic genome had been activated. It is assumed, of course, that there are no paternally determined mmRNAs or enzymes brought with the sperm, and analyses of sperm have not suggested that such are present. Two enzymes in which genetic variants are known have been found in sufficient amounts in preimplantation source embryos to allow for analysis, and the approach has been a simple one. Females homozygous for one allele are mated with males homozygous for another, and the time at which the allele transmitted from the male becomes evident is determined. This time constitutes the latest at which activation of the gene for that enzyme can have occurred; in all probability, the actual time is probably earlier. Using this approach, Chapman and collaborators (1971) were able to show that the locus for glucose phosphate isomerase-1 (*GPI-1*) was active by the late blastocyst stage, even though there was no increase in enzyme activity. This estimate of the time of paternal enzyme appearance was later reduced to the 8 cell stage by Brinster (1973). Further, Chapman and Wudl (1974) have shown that the paternal form of β-glucuronidase (*Gus*) is also detectable by the 8 cell stage, again with only a slight increase in enzyme activity. These findings with mouse embryos are in contrast to those in other species in which paternal alleles were not shown to be active until the hatching stage in Drosophilia (Wright and Shaw, 1970), the tailbud stage in frogs (Wright and Subtelny, 1971), and the primitive streak stage in Japanese quail (Ohno, et al, 1968).

Using a somewhat different approach, we have obtained findings analogous to those obtained with enzyme variants. The synthesis of hypoxanthine guanine phosphoribosyltransferase (HGPRT) during oogenesis is controlled by the number of X-chromosomes present in the oocyte prior to meiosis. Eggs of mice with two X-chromosomes have twice as much HGPRT activity as do eggs of mice, the so-called XO mice, with only one (Epstein, 1972). The same is also true of G6PD (Epstein, 1969) and phosphoglycerate kinase (Kozak, et al, 1974). HGPRT increases greatly in amount between the 8 cell and blastocyst stages (Epstein, 1970) (Fig. 5). If this increase results from activation of preexisting maternally derived mRNAs or of inactive enzyme precursors, it would be expected that the same dosage relationship should hold in morulae and blastocysts from XX and XO mothers, i.e., XO/XX = 0.5. On the other hand, if the mRNA were embryonic in origin, a higher ratio would be expected, the precise value depending on the state of X-chromosome expression and the relative survivals of XO and YO embryos. The observed ratio of XO/XX for HGPRT in day 3 morulae and blastocysts was 0.93 (Epstein, 1972) (Fig. 5). Therefore, the evidence is again for embryonic gene activation by the 8 cell stage.

PROTEIN SYNTHESIS

Following the initial autoradiographic studies of Mintz (1964a) and Weitlauf and Greenwald (1967), which suggested an increase in protein synthesis at the time of

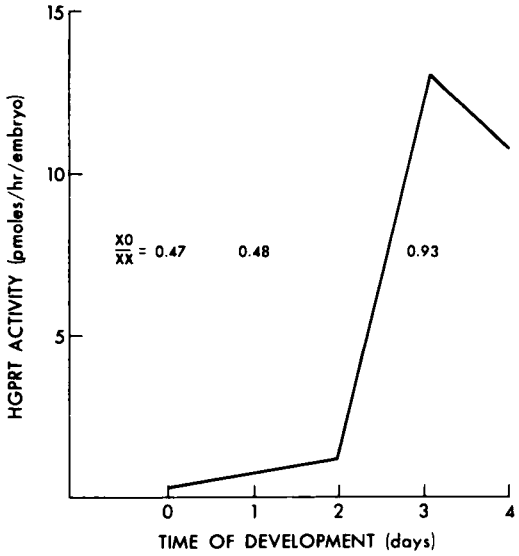


FIG. 5. Developmental changes in hypoxanthine guanine phosphoribosyltransferase (HGPRT) activity of normal embryos and in the ratio of HGPRT activities in eggs and embryos of XO and XX females. In unfertilized ova (day 0) and 2 cell embryos (day 1), the ratios are close to 0.5, indicating that enzyme synthesis is proportional to the number of X-chromosomes which are present. The dosage effect disappears in the early blastocysts (day 3), indicating that new post-fertilization messenger RNA synthesis has occurred. Data from Epstein (1970) and Epstein (1972).

blastulation, Monesi and Salfi (1967) and Tasca and Hillman (1970) published quantitative data which led them to the same conclusion. Interpretation of all of these studies is complicated by the absence of information about precursor handling and the relationship between uptake and incorporation of the radioactive precursors. As has already been discussed in the section on precursor transport, there are marked changes in the capacity of embryos to transport amino acids. The greatest increase in transport capacity occurs concurrently with the increase in incorporation into protein, and one interpretation of the data might be that the increased incorporation is a direct result of increased uptake. The work of Brinster (1971), in which different precursor concentrations were used, suggested that this is probably not the case; our own investigations have brought us to the same conclusion (Epstein and Smith, 1973). One important observation was that the rate of incorporation of precursor into protein does not increase between the early and late blastocyst stages although the rate of uptake does. Thus, for this period of time, there is a clear dissociation between uptake and incorporation. In addition, by manipulating the concentration of precursor in the medium, it was possible to dissociate uptake from incorporation during the earlier and more important time between 8 cells and blastulation, and the general strategy for this is described below and in Fig. 6. To obtain a valid estimate of the rate of macromolecular synthesis using radioactive precursors, it is necessary to know the specific activity of the precursor at the site of synthesis. One way of assessing this specific activity

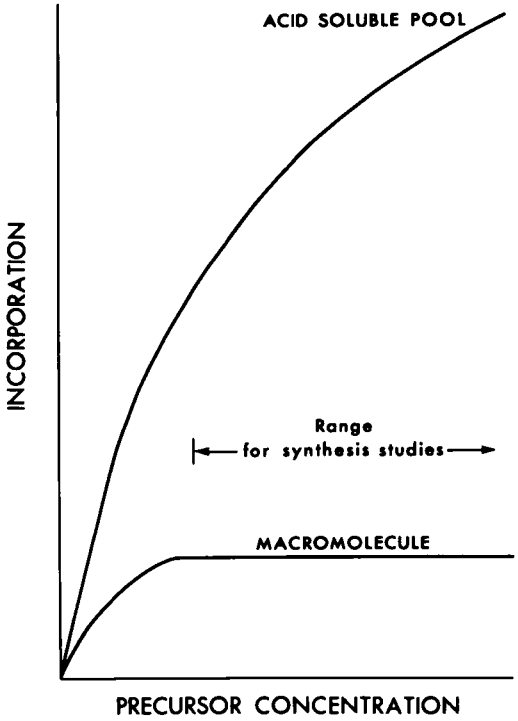


FIG. 6. Strategy for overcoming problems with endogenous precusor pools in measurement of macromolecular synthetic rates. Embryos are incubated in various concentrations of precursor, and uptake into the acid soluble pool and macromolecules determined simultaneously. Over the range in which incorporation into macromolecules remains constant while uptake into the acid soluble pool increases, the endogenous precursor pool makes a minimal contribution and the precursor specific activity is effectively that of the precursor in the medium.

is by actually measuring it, but such measurements require estimation of the intracellular concentration of the appropriate precursor, sometimes a difficult task when working with mouse embryos. The other approach, and the one we have adopted, is to work under conditions where the specific activity of the precursor within the embryo is the same as that in the medium. To achieve this state, it is necessary that the amount of precursor taken up during the course of the study rapidly produces an intracellular concentration far in excess of that of the endogenous precursor concentration, so that the latter does not significantly dilute the former. With a great enough transport maximum, Vmax, it is possible to obtain the desired rates of uptake by working at high substrate concentrations in the medium. Verification that the desired intraembryonic specific activity has been reached is obtained by measuring incorporation at a series of precursor concentrations which produce different rates of uptake. If the rate of incorporation is constant and independent of the changes in the rate of uptake thus produced (Fig. 6), it can be taken as an appropriate measure of the rate of protein synthesis.

Using this method, we have shown, as suggested by the earlier data, that there is a significant increase in the rate of protein synthesis between the 8 cell and blastocyst stages (Epstein and Smith, 1973). When expressed on a whole embryo basis, the increase is 3- to 4-fold with leucine as a precursor and 6-8-fold with lysine (Fig. 7). However, the increase is considerably less on a *per cell* basis and is more compatible with the establishment of a constant rate of synthesis per cell than with a marked increase in synthetic activity. In quantitative terms, the hourly rate of synthesis of proteins by 8-16 cell embryos and blastocysts are 0.035 and 0.125 pmoles leucine, respectively (Epstein and Smith, 1973). Assuming that leucine comprises 8.2 percent of protein amino acid residues (data from Handbook of Biochemistry, 1970), this corresponds to a synthetic rate of 1.34 and 4.79 ng protein/day.

While the main interest in the quantitative aspects of protein synthesis has been in the changes which are occurring, a finding of considerable importance is that there is detectable synthesis at all times from fertilization onward and even in the unfertilized egg. This indicates that the egg does contain mRNAs which are being translated. Although the times of synthesis during oogenesis, the stabilities, and the identities of these mRNAs are not known, it seems reasonable to postulate that at least some of the protein synthesis during very early embryogenesis is indeed maternally controlled. This does not, however, mean that these maternal messengers are necessarily masked mRNAs in the same sense as applies to sea urchin development. Rather, the fact that measurable protein synthesis is going on implies that they probably are not and that they may represent stable mRNAs.

Quantitative determinations give no information about what proteins are being made nor about whether there are developmentally related qualitative changes. To obtain such information it is necessary to use an analytical system which separates the proteins in a

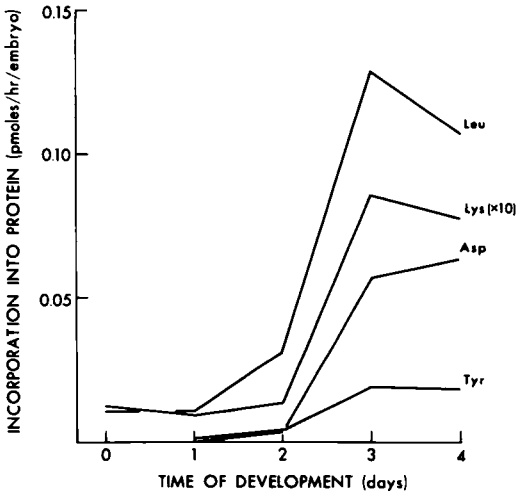


FIG. 7. Synthesis of protein by preimplantation mouse embryos. The incorporation of four different amino acids, all of which exhibit a marked increase in the rate of incorporation during the third day of development, is shown. Protein synthesis is detectable at all preimplantation stages. Data from Epstein and Smith (1973).

reproducible manner. Of these, the most convenient is polyacrylamide disc gel electrophoresis. When carried out in the presence of sodium dodecyl sulfate (SDS), proteins are separated roughly according to their molecular weights. It is thus possible to determine which molecular weight classes are represented in the synthetic product and whether there are developmentally associated differences among the classes. This approach has been applied to developing preimplantation mouse embryos, and radioactive amino acids have been used to label the newly synthesized proteins (Epstein and Smith, 1974). To facilitate comparisons between different stages of development, a double labeling method has been employed. Embryos at one stage are labeled with a ^{3}H-amino acid and those of another with a ^{14}C-amino acid, the amino acid being the same for both. The labeled embryos are then mixed and analyzed together, and the radioactivity in each protein fraction derived from the two different stages is ascertained by appropriate counting and computational techniques. By these methods, it is possible to obtain valid quantitative comparisons of the relative amounts of radioactivity in the several fractions. Since radioactivity is being measured, the results are indications only of the rates at which newly synthesized proteins are appearing and not of the protein composition of the embryo *per se*.

The most extensive investigations have been carried out over the period from the 2 cell to the late blastocyst stage, and the proteins examined have been in the molecular weight range of 25,000 to 115,000 daltons. In this range, approximately 20 to 25 distinguishable proteins are synthesized, and five of these are quantitatively the most significant. However, because of the technique being used, these represent minimal numbers, and it is highly likely that even the major components represent groups of distinct and different proteins which share common molecular weights. With this reservation in mind, it is of interest to examine what happens to the different components as development proceeds. Using two different amino acid precursors, significant changes are detectable during the preimplantation period (Fig. 8). Somewhat surprising, however, is the finding that the most marked change in the pattern does not occur between 8 cells and blastocyst, as

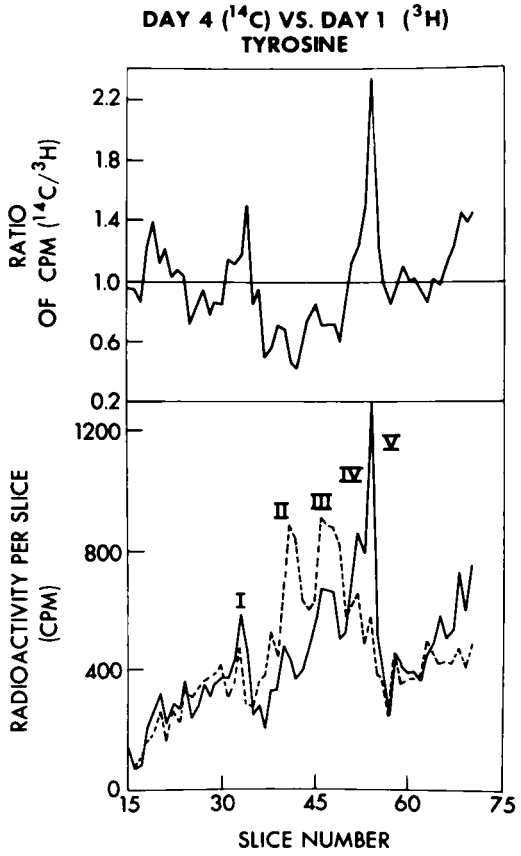


FIG. 8. Comparison of protein synthesis by day 1 (2 cell) and day 4 (late blastocyst) embryos. Day 1 embryos were incubated in ^{3}H-tyrosine and day 4 in ^{14}C-tyrosine. The embryos were then combined and processed together. Total embryo extracts were subjected to electrophoresis in SDS-polyacrylamide gels, and the radioactivity in ^{3}H and ^{14}C determined simultaneously. The various protein components are separated by molecular weight, with slice 15 corresponding to approximately 115,000 daltons and slice 70 to 25,000 daltons. The actual incorporations of radioactive amino acids are shown in the lower graph, with the solid line representing day 4 embryos and the dashed line day 1 embryos. The five major components are labeled I-V. The upper graph shows the normalized ratio of ^{14}C/^{3}H. A value greater than 1.0 represents a relative increase in the synthesis of a given component, less than 1.0 represents a relative decrease. Many differences between the day 1 and day 4 patterns are apparent. Data from Epstein and Smith (1974).

might have been expected on the basis of changes in other systems, but between 2 and 8 cells (Fig. 9). During this period of time, the relative rates of synthesis of 4 of the 5 major components change, and only one remains constant. When compared with other developing organisms, these changes are quite early. For example, major changes do not occur in the sea urchin until midcleavage (Terman, 1970), in the fish, *Fundulus heteroclitus*, until after the blastula (Schwartz and Wilde, 1973) and in the frog, *Rana pipiens*, until sometime between 2 cells and gastrula (Ecker and Smith, 1971). In the rabbit, the changes were also relatively early, but not as early as in the mouse—gross differences are found between 16 cells and the blastocyst

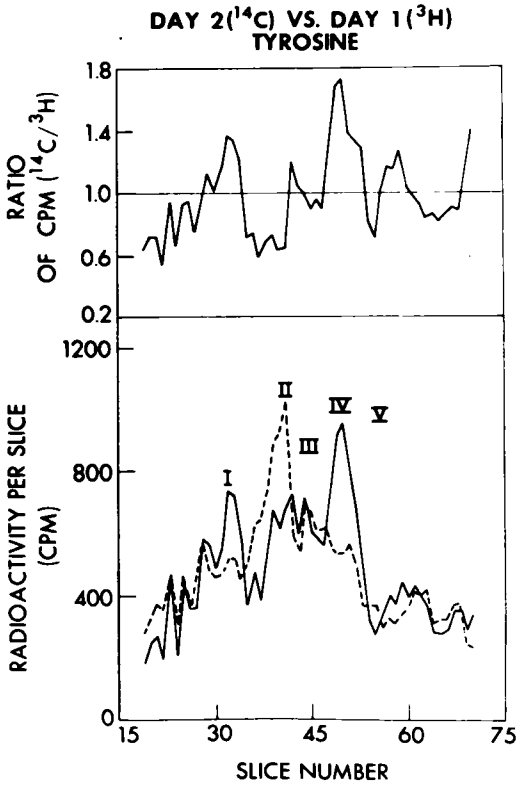


FIG. 9. Comparison of protein synthesis by day 1 (2 cell) and day 2 (8–16 cell) embryos. Experimental details and representation of the data are as in the legend to Fig. 8. Comparison of these curves with those shown in Fig. 8 indicates that the greatest changes in the patterns of protein synthesis by preimplantation mouse embryos occurs between days 1 and 2 of development. Data from Epstein and Smith (1974).

(Manes and Daniel, 1969). Unfortunately, the separation of proteins by gel electrophoresis or by any other similar technique such as isoelectric focusing or chromatography does not result in the specific identification of individual components. To obtain such identifications it is necessary to use other criteria to characterize the separated materials, but this has not yet been done. However, because of the quantities being synthesized, it is likely that the proteins separated by SDS-gel electrophoresis will turn out to be major structural components of the embryo (e.g., proteins required for membranes, mitotic apparatus, nuclear proteins) rather than enzymes or other similar products. This conjecture is also based in part on the observation that although there is little demonstrable increase in total mass—in fact, the total protein mass of the preimplantation embryo appears to *decrease* up to the morula stage (Brinster, 1967)—there is an increase in cell and nuclear number and hence in those components concerned with the formation of cell boundaries and nuclei.

The fact that significant protein synthesis is occurring even in unfertilized eggs has already been commented on, but again the question arises of what proteins are synthesized. Although ovulated eggs, even if unfertilized, cannot be considered to be in a stable state, it is likely that much of the synthesis detected at this stage and very early in development represents the replacement of proteins which are being degraded by endogenous mechanisms. The balanced synthesis and degradation of proteins occurs in all living cells, and there is no reason to believe that the egg and early embryo are an exception. In fact, the decrease in protein content (Brinster, 1967) suggests that degradation is in excess of synthesis for most of the preimplantation period, the time between morula and blastocyst being a possible exception.

For the reasons already given, the demonstration of quantitative and qualitative changes in protein synthesis does not in itself furnish conclusive evidence for activity of the embryonic genome. However, studies of the

effects of inhibitors of RNA synthesis on protein synthesis by Monesi, et al (1970) do provide pertinent information. When embryos are incubated in actinomycin D (0.1 μg/ml), the rate of incorporation of ^{3}H-leucine into protein decreases to about 50 percent of control values over a period of 12 hours, and then remains relatively constant. Taken at face value, this indicates that, while a significant fraction of protein synthesis is controlled by stable messengers (i.e., the ones continuing to code for the residual 50 percent of synthesis), a good part of it is not and relies on the continued synthesis of mRNAs which are less stable. Because of the inhibitory effects of actinomycin D on the synthesis of RNAs other than mRNA, such as transfer (tRNA) and ribosomal (rRNA), and lack of evidence that the effect is a specific one, other interpretations of the actinomycin D data are possible. What is required is evidence that, rather than a general depression in the synthesis of all proteins, there are selective decreases in the synthesis of some coupled with little change in the synthesis of others.

Before concluding this section, it must be mentioned that even though the question of the genetic control of protein synthesis is still under investigation there is no question that embryonic protein synthesis is required for development. Numerous investigators have shown that administration of inhibitors of protein synthesis such as cycloheximide and puromycin at any stage inhibits cleavage (if added early in the cell cycle) and further development (Thompson and Biggers, 1966; Epstein, et al, 1969; Monesi, et al, 1970; Molinaro, et al, 1972). Re-expansion of mouse blastocysts and the blastulation of very late morulae is not interfered with (Glass, et al, 1973), presumably because the necessary proteins have already been made.

RNA SYNTHESIS

With the consideration of RNA synthesis, we come for the first time to evidence that relates directly to the activity of the embryonic genome. Again, the earliest study of RNA synthesis was performed by radioautographic methods and intraembryonic acid insoluble radioactivity was detected at all stages, from the 1 cell zygote on (Mintz, 1964a). Incorporation into nucleolar RNA was detected at the 4 cell stage, and although there was a gradual increase in labeling from the earliest stages, the most marked increase occurred at the time of blastulation. Similar results were obtained in the quantitative studies of Monesi and Salfi (1967), with the greatest increase in total synthesis occurring between the 12–16 cell and blastocyst stages.

As with the studies on protein synthesis, the use of radioactive precursor incorporation data to estimate the rate of RNA synthesis is complicated by lack of information concerning the state of the precursor in the intracellular pool, and we therefore undertook to investigate the situation. The initial studies demonstrated that, working with concentrations of ^{3}H-uridine that produced maximal precursor uptake, there is a small increase in incorporation of label into RNA between 2- and 8–16 cells and a very large increase between the latter and the early blastocyst stages (Daentl and Epstein, 1971) (Fig. 10). The rate of incorporation then plateaus and remains essentially constant during the rest of the blastocyst period. Analysis of the state of the intracellular acid soluble radioactivity demonstrated that most of the uridine is in the form of UTP, the immediate precursor of RNA, and there was no evidence of a lag in nucleoside phosphorylation. Since the observed developmental pattern of incorporation of uridine into RNA, one quite similar to that of Monesi and Salfi (1967), is strikingly parallel to that of uridine transport itself, an attempt was made to see if the two processes could be dissociated from one another using the strategy outlined in the section on protein synthesis. While such dissociation is possible for early blastocysts, the low Vmax for uridine of the 8–16 cell stage precluded the attainment of a high enough intracellular uridine concentration. The same problem also occurred when guanine, adenine, and adenosine were used (Epstein and Daentl, 1971),

and it was not, therefore, possible to calculate the actual amount of RNA synthesis that was occurring at this stage. However, it could be inferred from the data that the actual increase in the rate of RNA synthesis was less than the apparent 15-fold increase which was indicated by the incorporation results. When the increase in cell number is taken into account, the rate of RNA synthesis per genome is found to increase at most by only a modest amount, or perhaps not at all.

The calculated rates of RNA synthesis in early blastocyts are 0.045 pmole uridine/hr/embryo and 0.030 pmole/adenine/hr/embryo. If the rates of guanine plus cytosine incorporation are assumed to be equal to adenine plus uridine, the overall rate of RNA synthesis is 50 pg/hr/embryo, or 1.4 pg/hr/cell. If it is further assumed that the cellular rates of RNA synthesis by the 8–16 cell and morula stages are of the same order and that the measurement is made 10 hours after blastulation, then approximately 750 pg of RNA would be synthesized during the transition between 8 cells and blastocyst (taken as 32 cells). These calculated values compare favorably in magnitude with the measured increase in embryonic RNA content of 912 pg between the 9 and 34 cell stages (Olds, et al, 1973).

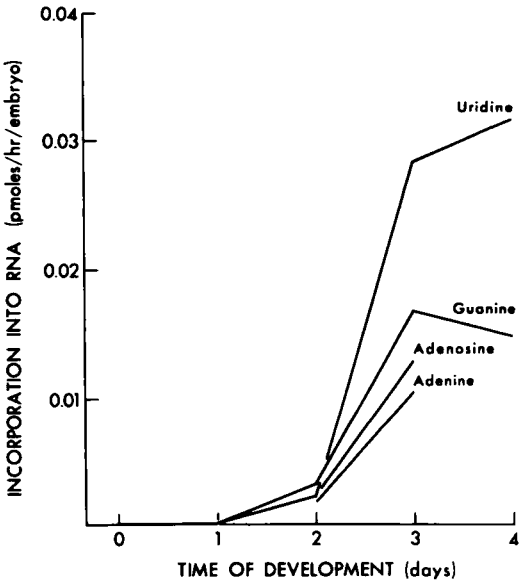


FIG. 10. Synthesis of RNA by preimplantation mouse embryos. The incorporations of four different RNA precursors, all of which exhibit a marked increase in the rate of incorporation during the third day of development, are shown. Data from Epstein and Daentl (1971) and Daentl and Epstein (1971).

Bernstein and Mukherjee (1972) have sought to elucidate the mechanisms involved in the increasing rate of RNA synthesis between the 2 and 4 cell stage. Using virus induced cell fusion, heterokaryons were formed between A9 cells and 2 or 4 cell embryos. By radioautographic means, the labeling in RNA of the A9 nuclei was shown to increase in parallel with the labeling in the RNA of the embryonic nuclei, and it has been suggested that cytoplasmic factors (to which both kinds of nuclei are exposed) govern nuclear RNA synthesis.

Several groups have analyzed RNA synthesis in qualitative terms and have identified the types of RNA actually made. Different techniques were used by each group and, as a result, the findings are not directly comparable. The most thorough investigations of the early preimplantation period were performed by Woodland and Graham (1969) and Knowland and Graham (1972). Using column chromatography, sucrose density gradient sedimentation, and polyacrylamide gel electrophoresis, they found evidence for synthesis of high molecular weight RNA (thought to be a combination of heterogeneous RNA, the fraction in which mRNA is located, and ribosomal RNA) and of a low molecular weight RNA (possibly a 4S RNA precursor) at the 2 cell stage and evidence for high molecular weight, low molecular weight 4S, and rRNA at the 4 cell and 8 cell stages. The period between 8 cells and the blastocyst were studied by Pikò (1970) using sucrose gradient sedimentation and by Ellem and Gwatkin (1968) using column chromatography. Their findings were essentially in agreement and showed that the major RNA component being made is ribosomal RNA (Fig. 11). This comprised 64 to 82 percent of the total RNA made during a 5 hour period, while DNA-like RNA (the class in which messenger RNA would be located) was 8 to 18 percent

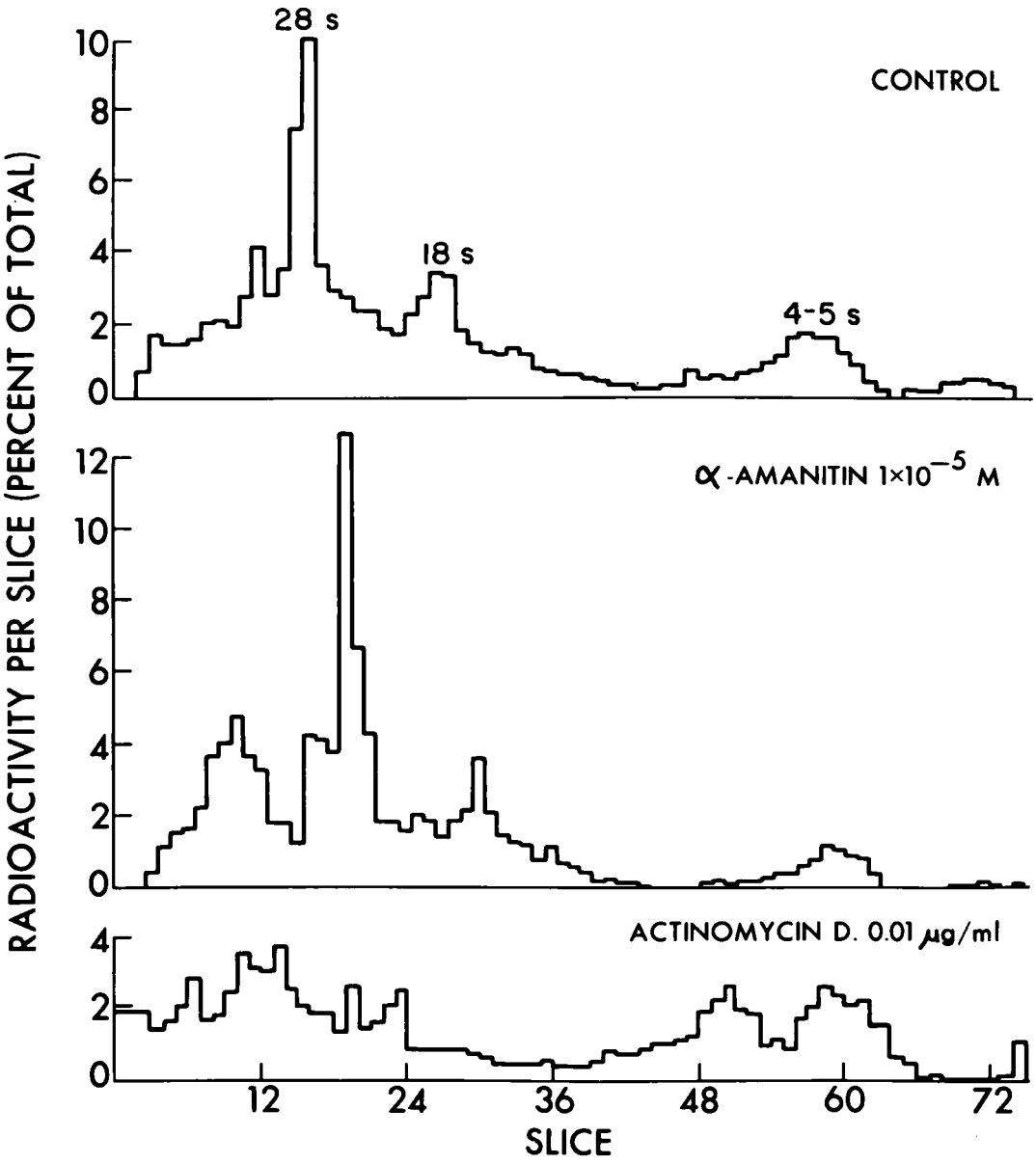


FIG. 11. Polyacrylamide gel electrophoresis of ^{3}H-uridine labeled (2 hrs) RNA from normal mouse blastocysts and blastocysts exposed to α-amanitin (10^{-5} M = 10 μM) and actinomycin D (0.01 μg/ml). Prominent 28S and 18S ribosomal subunit peaks are present in the control pattern. They are not appreciably reduced by exposure to α-amanitin (the same amount used in the experiment shown in Fig. 12), but are obliterated by a low concentration of actinomycin D. Golbus and Epstein, unpublished data.

and 4S + 5S RNA was 6 to 10 percent (Ellem and Gwatkin, 1968). Thus, the quantitative increase in RNA synthesis affects all types of RNA, although the increase in rRNA synthesis at the morula stage to 82 percent of the total is relatively greater than the increase in other categories. Since RNA sequences are directly coded for by specific chromosomal DNA sequences, the synthesis of all types of RNA can be taken as evidence of genetic activity. However, from the point of view of the genetic control of development, it is really the synthesis of messenger RNA that is of prime concern. This type of RNA is

thought to be contained in those fractions identified as high molecular weight (heterogeneous) or DNA-like RNA, and the evidence just cited indicates that such RNA species can be identified from the 2 cell stage on. Failure to identify these RNAs at an earlier stage cannot be taken as evidence for a failure of embryos to make it, since the very low uptake of RNA precursors at the 1 cell stage precludes accurate measurements from being made.

The evidence that mRNA is being made in preimplantation mouse embryos is at best inferential, but studies with preimplantation rabbit embryos have been more revealing. The rate of incorporation of ^{3}H-uridine into RNA increases with increasing cell number, with the greatest synthetic rate per cell being observed at the 28 to 1024 cell stage (morula to blastocyst transition) (Manes, 1969). By polyacrylamide gel electrophoresis, it was shown that heterogeneous and transfer RNA are synthesized from the 2 cell stage on, but, in contrast to the mouse embryo, ribosomal RNA synthesis is not detectable until the morula stage (Manes, 1971). Polyadenylic acid sequences, which are taken as a marker of mRNA, where shown to be present in RNAs synthesized from at least the 16 cell stage onward, and 20 percent of the total heterogeneous RNA (hRNA) and about 70 percent of the polysomal RNA contained such sequences (Schultz, et al, 1973a). Most of the heterogeneous RNA was derived from "non-repeated" or "unique" DNA sequences (Schultz, et al, 1973b). It therefore appears that messenger RNA is synthesized quite early in the development of the rabbit embryo, and it is quite likely that at least some of the heterogeneous RNA of the early mouse embryo is similar in type.

INHIBITORS OF RNA SYNTHESIS

The demonstration that mRNA is synthesized at a certain stage of development does not in itself constitute evidence that its synthesis is essential for development. It is equally possible that the mRNA made at one stage is not translated until a later time and such has been found to be the case in the sea urchin embryo (Kedes and Gross, 1969). To obtain information about the requirements of RNA synthesis for development, use has been made of inhibitors which are thought to act specifically on RNA synthesis. Of these, actinomycin D has been the one most extensively used. At low concentrations it inhibits the synthesis of ribosomal RNA (Fig. 11), while higher concentrations inhibit the synthesis of all types of RNA. The earliest studies (Mintz, 1964a) demonstrated that cleavage of 2 cell and later stage embryos is inhibited by concentration of actinomycin D of 1 μM and higher. Inhibitory effects were also observed by later investigations (Thompson and Biggers, 1966; Ellem and Gwatkin, 1968; Skalko and Morse, 1969; Pikò, 1970; Molinaro, et al, 1972) and even the first cleavage was found to be susceptible to inhibition by actinomycin D (Golbus, et al, 1973). Detailed examination of the relationship between the time of drug administration and its effect on the cleavage immediately following revealed that cleavage was blocked only if the actinomycin was added during the early part of the cell cycle (Molinaro, et al, 1972). Taken at face value, these results indicate that species of RNA required for cleavage are synthesized early in the cell cycle and that not all of the required RNAs are present in a stable or masked form.

Because of the possibility that it is an inhibitor of respiration, the specificity of actinomycin D has been questioned (Manes, 1969). Our studies on ATP levels did not indicate that respiratory inhibition was occurring (Golbus, et al, 1973), but it was nevertheless felt that another RNA synthesis inhibitor should be used, and α-amanitin was chosen. *In vitro* α-amanitin is a specific inhibitor of type II RNA polymerase (Fig. 11), the polymerase responsible for mRNA synthesis. Incubation of preimplantation mouse embryos in α-amanitin results in a modest inhibition of the cleavage of 1 cell zygotes and in more severe inhibition of cleavage of embryos incubated from the 2 cell stage on (Fig. 12) (Golbus, et al, 1973; Warner and

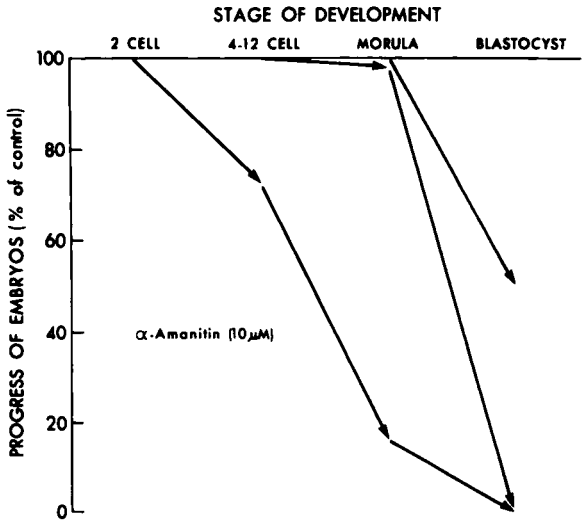


FIG. 12. Effect of α-amanitin on cleavage and blastulation of preimplantation mouse embryos. Embryos were cultured continuously in 10 μM α-amanitin from the 2 cell, 4–12 cell, and morula stages, and progress was scored as percent of control embryos. The effects of α-amanitin are first detectable at the second or third cleavage after initial exposure, except for exposure at the morula stage which leads to a decreased frequency of blastulation. Data from Golbus, et al (1973).

Versteegh, 1973). Unlike actinomycin, the inhibition of cleavage induced by α-amanitin does not become fully manifest until the second or third cleavage after exposure of the embryos to the drug, but blastulation is prevented even if the first exposure is at the morula stage. Although morulae developing in the presence of α-amanitin have the same number of cells as do those developing in its absence, neither the normal elevation in HGPRT activity nor decrease in guanine deaminase activity are found when there is continuous exposure to α-amanitin from the 2 cell stage, although the normal enzyme changes do occur when 4–8 cell embryos are similarly treated (Golbus, et al, 1973). Direct assays *in vitro* revealed that about one-third of blastocyst RNA polymerase activity (at high ionic strength) is inhibited by α-amanitin (Warner and Versteegh, 1974), a figure compatible with the amount of non-ribosomal RNA synthesized by preimplantation embryos.

Experiments with rabbit embryos (Manes, 1973) give results similar to those obtained with the mouse. α-amanitin almost completely inhibits RNA synthesis by 2 cell embryos, a finding compatible with the earlier claim the rRNA is not made at this stage. In addition, there is also an inhibition of cleavage, again with a delay until the third cleavage following exposure to the drug.

Unfortunately, the results obtained with α-amanitin, while compatible with early mRNA synthesis and utilization, are open to question. Ultrastructural examination of treated mouse embryos revealed the presence of nucleolar abnormalities (Golbus, et al, 1973), even though α-amanitin is not supposed to affect the type 1 polymerase responsible for rRNA synthesis. The embryos exposed to affect the type I polymerase respondevelop normal mature nucleoli, while the nucleoli of embryos exposed later are found to be degenerating. The reason for these nucleolar abnormalities, which have also been observed in organs of animals treated with α-amanitin, is not known, although it is possible that mRNA synthesis is in some way responsible for nucleolar integrity. Because of these problems and those already discussed above with actinomycin D, the results obtained with both actinomycin D and α-amanitin must be considered as only being suggestive of and compatible with an early role for newly synthesized mRNA in embryonic development and enzyme synthesis. The more significant finding, insofar as masked and stable mRNAs might be concerned, would have been if these drugs had failed to be inhibitory.

While not an inhibitor of RNA synthesis, 5-bromodeoxyuridine (BrdU), a thymidine analog which is incorporated into DNA, inhibits the expression of differentiated functions in many developing systems. When mouse embryos at various stages are exposed to this nucleoside, morula formation is not affected but blastulation is greatly reduced (Fig. 13) (Golbus and Epstein, 1974). With continuous exposure from the 2 cell stage, blastulation is completely inhibited. The in-

hibitory effects of BrdU can be prevented by concomitant administration of thymidine, and thymidine appears to act by competing with the BrdU at its site of action. The effect of BrdU on blastulation, even of morulae (Fig. 13) is reminiscent of the similar effect of α-amanitin, and suggests that this developmental event is one which is particularly vulnerable to interference with genetic expression. However, the fact that BrdU uptake is quite poor at stages earlier than the morula (Fig. 13) (Golbus and Epstein, 1974) makes it impossible to make any statement about the true BrdU vulnerability of earlier developmental events.

In addition to the cell nucleus, mitochondria also possess a genome which is responsible for the synthesis of ribosomal and transfer RNA. These mitochondrial RNAs are components of a mitochondrial protein synthesizing system. Mitochondrial RNA synthesis has been found to occur from the 8–16 cell stage on, but its inhibition with ethidium bromide has little effect on mitochondrial development and does not interfere with development of the embryo (Pikò and Chase, 1973). Likewise, the inhibition of mitochondrial protein synthesis by chloramphenicol has no effect on embryonic development although mitochondrial organization is affected. On the basis of these observations, Pikò and Chase (1973) have concluded that

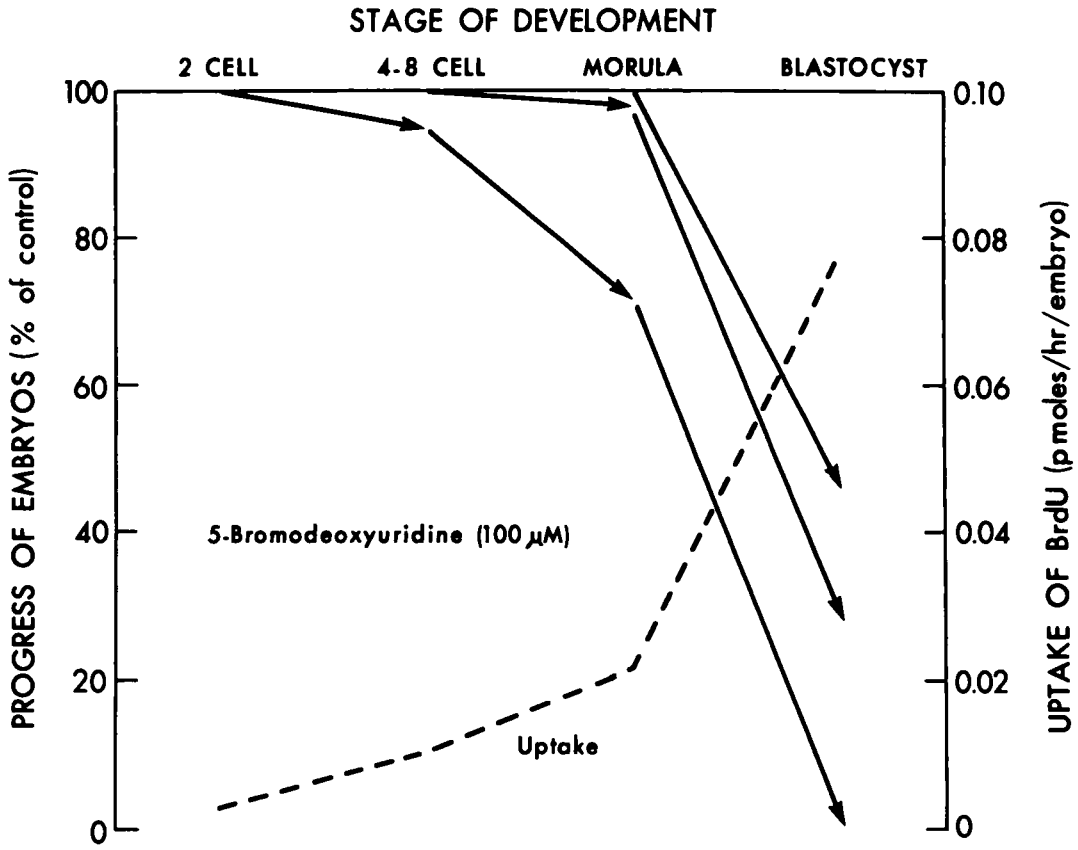


FIG. 13. Effect of 5-bromodeoxyuridine on cleavage and blastulation of preimplantation mouse embryos. Embryos were cultured continuously in 100 μM BrdU from the 2 cell, 4–8 cell, and morula stages. In each instance, the major effect was on blastulation. Comparison of the curves of sensitivity to BrdU with the developmental pattern of BrdU uptake (dashed line) indicates that the increased sensitivity at the morula stage is correlated with an increased rate of inhibitor uptake. Data from Golbus and Epstein (1974).

the mitochondrial genome does not play an important role in the development of the preimplantation embryo.

MUTATIONS AFFECTING DEVELOPMENT AND THE CELL SURFACE

If activity of embryonic genes is necessary for preimplantation embryonic development, it would be expected that mutations should exist which would be manifested during the period. Conversely, the identification of such mutations can be taken as evidence for a developmental role for the embryonic genome. Because of the extensive genetic studies which have been carried out during the past several years, the mouse has proven to be the most useful species in which to search for developmental mutations, and a few mutant genes affecting preimplantation development have been identified. Of these, the best studied has been *tailless-12* (t^{12}). In the homozygous state (t^{12}/t^{12}) this mutation leads to embryonic death at the morula or early blastocyst stage (Mintz, 1964b). An early suggestion, based on failure of RNA-related nucleolar and cytoplasmic basophilia to increase in the morula, was that there is a defect in the synthesis of ribosomal RNA. However, the finding of cytologically normal nucleoli and of an increase in the number of ribosomes during cleavage argued against a nucleolar-organizer type of mutation (Calarco and Brown, 1968; Hillman, et al, 1970), as did autoradiographic studies of nuclear and nucleolar RNA synthesis (Hillman, 1972). Also, direct biochemical studies of RNA synthesis by groups of embryos containing a significant proportion of t^{12}/t^{12} morulae did not show any significant differences from normal RNA synthesis (Hillman and Tasco, 1973; Erickson, et al, 1974). Other biochemical processes examined include uridine uptake, the uptake and incorporation into protein of leucine, and protein synthesis as analyzed by polyacrylamide gel electrophoresis, and again no significant differences have been found (Erickson, et al, 1974).

As the possibility of an intracellular biochemical defect was pursued, consideration of the range of abnormalities produced by the many alleles at the *T*-locus (Gluecksohn-Waelsch and Erickson, 1970), of the proximity of the *T*-locus to the H-2 locus which controls cell surface antigens, and of the effects of *t*-alleles on sperm segregation led to the prediction that the *T*-locus might control some aspect of the surface structure of the embryo. Recent work has shown that this prediction is likely to be correct. Antisera prepared against teratocarcinoma cells were found to react, in addition to the primitive teratocarcinoma cells, only with cleavage-stage mouse embryos and male germ cells (sperm) (Artzt, et al, 1973). The reactivity of the mouse embryos was greatest at the 8 cell to morula stages. When the antisera were absorbed with sperm, the antibodies against the teratocarcinoma cells could be removed and normal sperm were found to be twice as effective as were sperm from $+/t^{12}$ heterozygotes (Artzt, et al, 1974). These findings indicate that the reactive antigen is specifed by the *T*-locus and missing in the t^{12} mutation; in other words, it is a product of the *T-locus*. The function of this antigen and the relationship between its absence and embryonic lethality remains to be elucidated. Nevertheless, it is clear that abnormality of a specific genetic locus is accompanied by developmental arrest, indicating that this locus does have a function in early embryogenesis.

Having introduced the concept of the genetic control of cell surface structure during early development, a brief digression on biochemical, as opposed to immunological, approaches to cell surface analysis is in order. Many of the proteins of the cell surface exist as glycoproteins in which chains of carbohydrate are linked to the protein molecules in the membrane. By incubating embryos in radioactive carbohydrates, such as ^{3}H- or ^{14}C-glucosamine, and then using appropriate isolation techniques, it is possible to study the synthesis of surface glycoproteins. Using such methods, Pinsker and Mintz (1973) have shown that the surface of the blastocyst, in comparison with earlier stages, contains a

greater proportion of high molecular weight glycopeptides and have suggested that this change in surface structure might be related to the capacity for attachment to the uterine wall and implantation.

CONCLUSIONS

Despite the relative simplicity of the morphological changes which take place, the preimplantation period of mammalian devel-

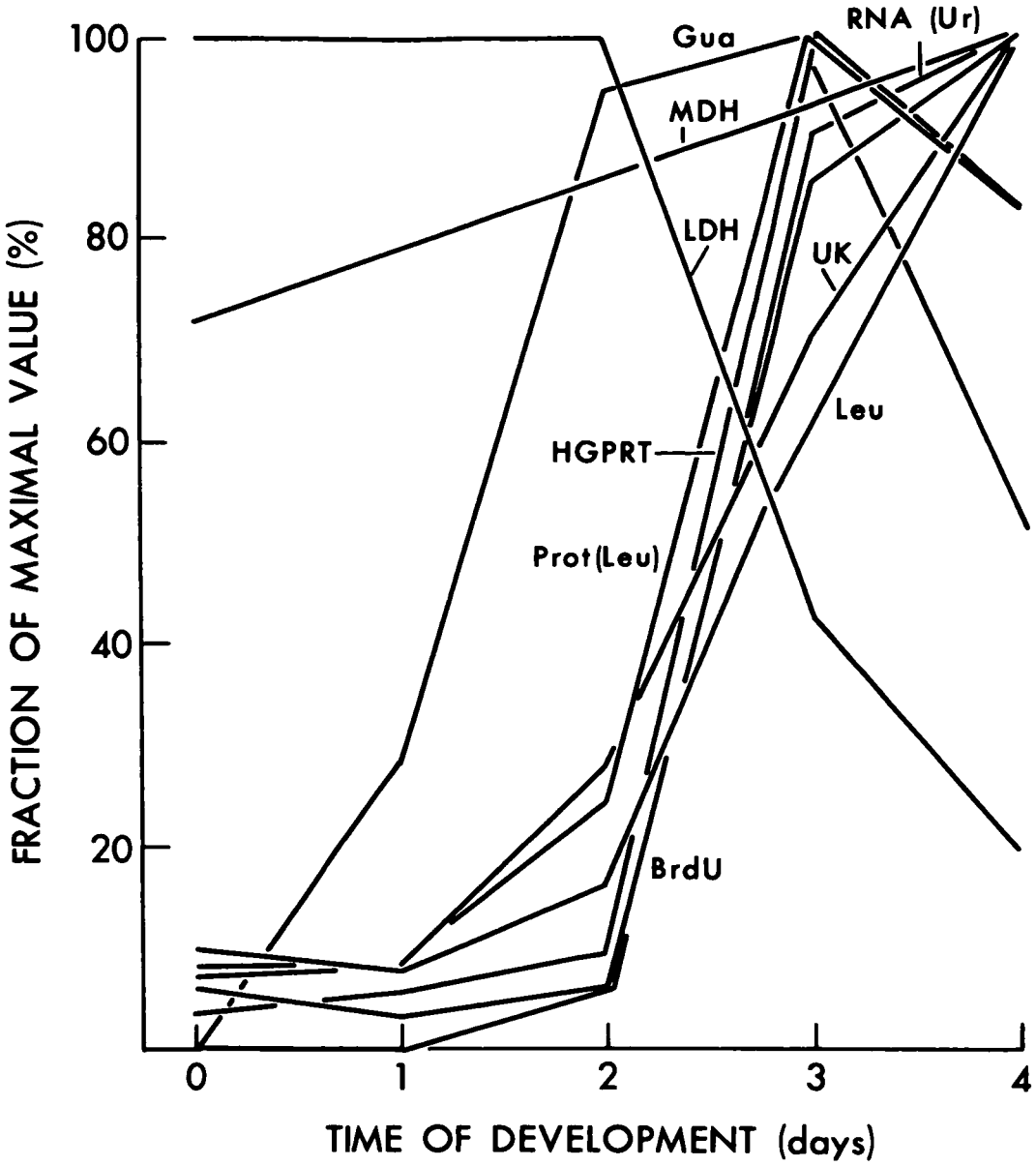


FIG. 14. Summary of biochemical changes taking place in preimplantation mouse embryos. Values are expressed as percent of maximal value attained during the preimplantation period. While the most pronounced alterations appear to be occurring between days 2 (8–16 cells) and 3 (early blastocyst), important changes also occur both prior to and following this time. Enzyme activities: HGPRT, hypoxanthine guanine phosphoribosyltransferase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; UK, uridine kinase. Transport capacities: Gua, guanine; BrdU, 5-bromodeoxyuridine; Leu, leucine. Macromolecular synthetic rates: Prot(Leu), incorporation of leucine into protein; RNA(Ur), incorporation of uridine into RNA. All values are from work of the author as cited in the text.

opment is one of great biochemical activity. The many changes which are occurring have already been discussed in detail, and some of them (from my own work only) are summarized in Fig. 14. Clearly, the time of maximum change is at or just prior to blastulation, a finding which is not surprising in view of the morphological transition which occurs. However, important changes are taking place at all developmental stages, and the overall picture is one of complex but integrated metabolic activity.

The evidence concerning the role of the embryonic genome during the preimplantation period can be summarized under three headings:

A. Developmental changes *compatible* with a role for the embryonic genome.
   1. Morphogenesis—differentiation into two cell types, organelle maturation, blastulation.
   2. Enzyme activity—particularly increases (HGPRT, APRT, uridine kinase, hexokinase, β-glucuronidase).
   3. Transport system capacity—increases for energy sources, nucleic acid precursors, amino acids.
   4. Protein synthesis—increases in rate and alterations in relative synthetic rates of specific components.

B. Developmental changes and responses *indicative* of a role for the embryonic genome.
   1. RNA synthesis—increases in rate; polyadenylation; synthesis of heterogeneous RNA.
   2. 5-bromodeoxyuridine—inhibition of blastulation.
   3. Inhibitors of RNA synthesis—inhibition of cleavage and blastulation by actinomycin D (as early as zygote) and α-amanitin (8–16 cell stage).

C. "*Conclusive*" evidence for a role for the embryonic genome.
   1. Mutations—t^{12}/t^{12} (morula stage).
   2. Expression of paternal alleles—GPI, β-glucuronidase (<8 cell stage).
   3. Change in dosage for HGPRT in embryos of XO mother (8–16 cell stage).

Taken in the aggregate, the observations to date indicate that, unlike the situation in other organisms, the mammalian embryonic genome plays an essential role in very early development, certainly by the 8 cell stage and probably at all times following fertilization.

ACKNOWLEDGMENTS

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