Molecular Biology of the Sea Urchin Embryo

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Summary. Research on the early development of the sea urchin offers new insights into the process of embryogenesis. Maternal messenger RNA stored in the unfertilized egg supports most of the protein synthesis in the early embryo, but the structure of maternal transcripts suggests that additional functions are also possible. The overall developmental patterns of transcription and protein synthesis are known, and current measurements describe the expression of specific genes, including the histone genes, the ribosomal genes, and the actin genes. Possible mechanisms of developmental commitment are explored for regions of the early embryo that give rise to specified cell lineages, such as the micromere-mesenchyme cell lineage.

The early development of the sea urchin has been more intensively studied at the molecular level than has any comparable embryonic system. The reasons for this are partly historical in that a deep background of useful developmental knowledge has accumulated since sea urchin embryos became a favorite subject of experimentation about a century ago. Modern investigators capitalize on a number of practical advantages. The eggs of certain sea urchin species can be obtained in the laboratory the year round (*1*), and other conveniently accessible species are gravid at alternate seasons. In the United States the main, although not the only, sea urchins used for research are *Strongylocentrotus purpuratus, Lytechinus pictus, Arbacia punctulata*, and *Tripneustes gratilla*, referred to in this article respectively as *Sp*, *Lp*, *Ap*, and *Tg*. Eggs can be fertilized in vitro, and rapid, highly synchronous development ensues. Cultures in excess of 10^8 embryos (*Sp*) are routinely grown in our laboratories. The ready availability of such large amounts of material facilitates quantitative studies of the macromolecules of the embryo, as do the ease of demembranation, homogenization, and cell fractionation, the relative lack of yolk, the permeability of the embryos to radioisotopes, and the relatively small genome size, which is about 8×10^8 nucleotide pairs or approximately one-fourth the size of the mammalian genome. The embryo is rather transparent and (in *Sp*) has only about 600 cells at the gastrula stage, compared, for example, to more than 10^4 in amphibian gastrulas. Not only can all the cell types be observed in living embryos throughout early development, but the embryo can be disaggregated in ways that permit preparative recovery of some of these cell types and the structures they form. There are also some disadvantages, the principal one perhaps being the lack of genetic data, although sea urchin embryos can easily be carried through advanced larval stages including metamorphosis in the laboratory (*2*), and adults can be maintained routinely in suitable culture systems for years (*1*). Some inbred strains have indeed been produced, including several displaying developmental mutations, but inbred strains have so far been used only for a few specific investigations (*3*).

Embryonic and Larval Development

Most research on sea urchin embryos concerns the period between fertilization and the stage when feeding can begin. The rate of development is temperature-dependent, and the following abbreviated description refers in particular to embryos of the purple California sea urchin (*Sp*), cultured at 15°C. Immediately after fertilization a thick membrane rises off the surface of the egg (Fig. 1a), and within a short time sclerotizes, thus providing physical protection during cleavage. Cleavage begins about 1½ hours after fertilization. The first cleavage furrow divides the egg into equal halves along what is called the "animal-vegetal" axis. The top of the egg (animal pole) later becomes the site of a plate of apical cells bearing a tuft of especially long cilia, and the opposite pole (vegetal pole) later becomes the site of gastrular invagination. At fourth cleavage (5 hours after fertilization) four very small cells, the micromeres, are formed at the vegetal pole (Fig. 1b). Together these include only about 8 percent of the total volume of the embryo, and they are of particular interest because they are the specific progenitors of a lineage of cells that ultimately gives rise to the larval skeleton.

A logarithmic rate of cell division is maintained until there are about 200 cells (12 hours after fertilization). Shortly thereafter the embryo secretes a protease that dissolves the fertilization membrane and hatching occurs (about 18 hours after fertilization). The swimming blastula stage embryo contains about 400 cells, and is organized as a hollow ciliated ball bearing the prominent ciliary tuft at the animal pole. During the blastula stage approximately 30 primary mesenchyme cells, descendants of the original micromeres, invade the blastocoelic cavity (Fig. 1c). The development of a thickened, flat plate of cells at the vegetal pole marks the site of invagination and the subsequent onset of gastrulation. This process leads to the formation of the archenteron (embryonic gut), which can be seen in Fig. 1d (46 hours after fertilization). Morphogenesis of the differentiated structures of the larva ensues. The gut tube bends forward across the blastocoel, preceded by strands of secondary mesenchyme cells, and where it makes contact with the embryo wall the mouth forms inductively. The initial site of invagination becomes the anus. The internal skeleton forms as a pair of tripartite spicules, secreted initially as a calcium carbonate–protein complex within the vacuoles of primary mesenchyme cells that are situated in linear arrays along the blastocoel walls. During the pluteus stage (Fig. 1e) the embryo elongates, a stomach region differentiates, and the four skeletally supported arms grow anteriorly, bearing rows of cilia that beat in a coordinated fashion. At about 70 hours after fertilization the pluteus contains approximately 1500 cells, and is sufficiently developed to feed and exist as a

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free living, pelagic larva. Until feeding begins the mass of the protein and of the RNA in the embryo remains essentially constant. Most of the ribosomes used throughout embryogenesis were originally present in the unfertilized egg. Prior to feeding, embryogenesis is a process of cell division, reorganization of maternal components, and the appearance of new transcripts and their products, all occurring within a closed system that is not increasing in mass.

In the laboratory, *Sp* larvae require about 6 weeks of feeding to attain the stage at which metamorphosis may occur (*2*, *4*). The larva at this point contains about 5×10^4 cells (*4*). Figure 1f shows an *Sp* larva about halfway through its growth period, and the cover photo shows a nearly mature larva, in which the ventral portions of the imaginal structures that on metamorphosis will give rise to the juvenile sea urchin can be seen clearly (*5*). Metamorphosis is dramatic and rapid, occurring within a few hours after the larva settles down on an appropriate surface. Most of the external larval structures, such as the eight arms, disappear, although in addition to dorsal and ventral imaginal derivatives, it is probable that some coelomic elements and portions of the larval gut are also included in the emergent juvenile sea urchin. Further morphogenesis takes place after metamorphosis, including completion of the adult digestive tract and mouth, and gonadal differentiation (*6*).

The general form of embryogenesis in echinoderms places this group in the great branch of the animal kingdom, the deuterostomes, that also includes chordates. In deuterostomes the site of gastrular invagination becomes the anus, and the mouth forms secondarily. Other basic similarities between echinoderm and some chordate embryos include the manner in which gastrulation occurs, and the origin and disposition of the larval mesoderm. These classically observed morphological relations have been supported by a particular homology in the organization of the genes coding for actin. The sea urchin actin genes contain intervening sequences that are present at precisely the same locations as those in the coding regions of mammalian and avian actin genes, while intervening sequences appear at totally different positions in the actin genes of *Drosophila* (that is, a protostomial invertebrate), and in several lower invertebrates (*7*). It is difficult to estimate the extent to which the molecular mechanisms of sea urchin embryogenesis will prove relevant to mammals because there is as yet insufficient knowledge of mammalian development to permit general comparisons.

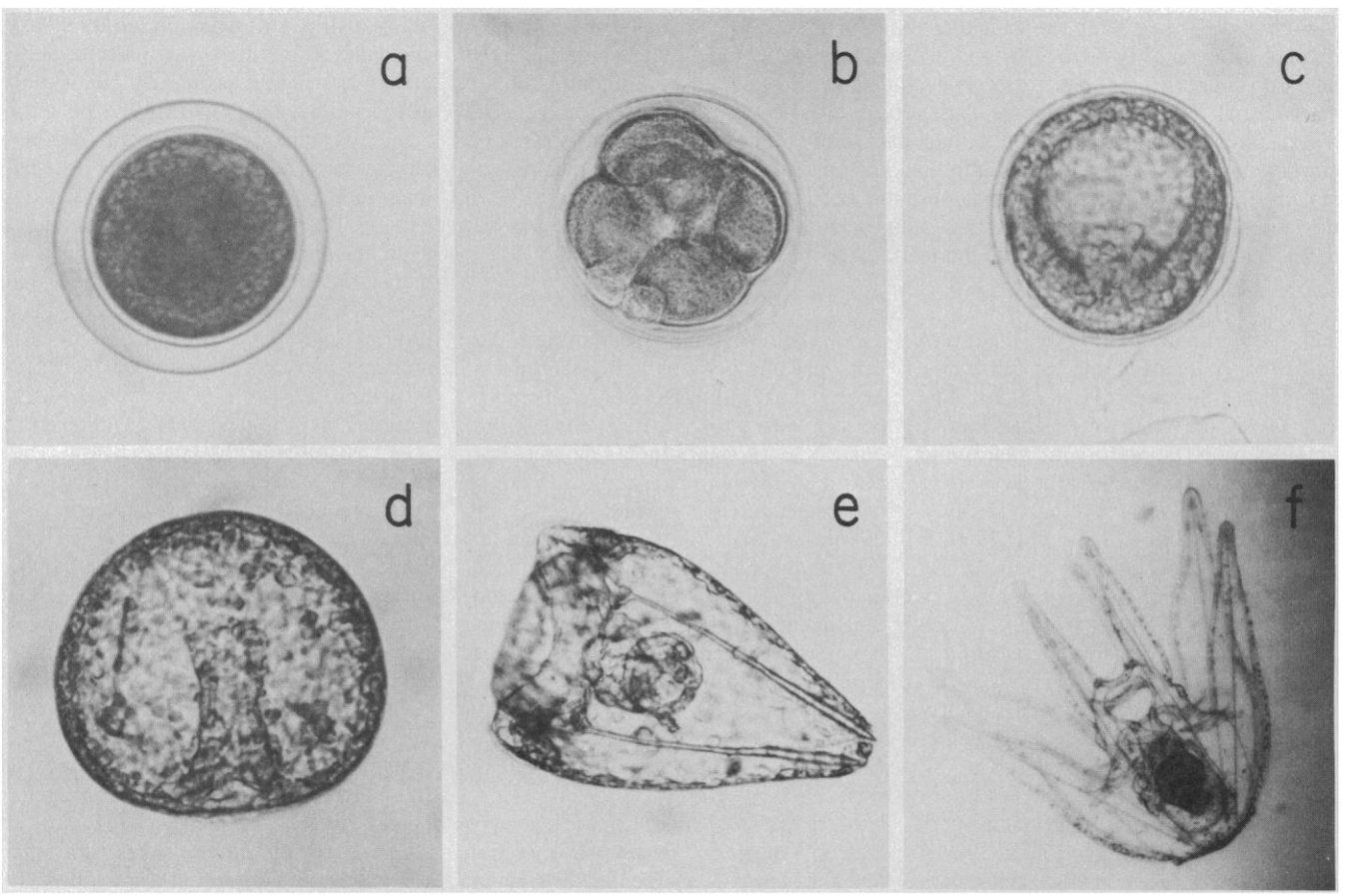

Fig. 1. Normal development of the *Strongylocentrotus purpuratus* embryo at 15°C. (a) Fertilized egg. The vitelline layer has lifted off the egg surface to form the fertilization envelope. The egg diameter is 80 μm. (a to c) All are at about the same magnification (×400). (b) The 16-cell embryo (5.5 hours after fertilization). The four small transparent cells at the lower left are the micromeres. [From (*78*); courtesy of *Developmental Biology*] (c) Mesenchyme blastula (18 hours after fertilization). Primary mesenchyme cells can be seen within the vegetal pole region of the blastocoel. Hatching occurs at about this time. (d) Late gastrula (46 hours after fertilization). The primitive gut extends most of the way across the blastocoel. On the left, one of the two early spicules is visible, with several primary mesenchyme cells associated with it. The embryos are covered with cilia and swim about vigorously. This photograph and the succeeding ones are at progressively lower magnifications. (e) Early pluteus (72 hours after fertilization), ventral view. The embryo has elongated, the gut is differentiated, and the mouth has formed between the four nascent arms toward which extend the prominent skeletal rods. The long axis of the pluteus larva is about twice the diameter of the gastrula. (f) Fed pluteus larva 3 weeks after fertilization (about 600-μm diameter). The eight arms, skeletal rods, and stomach, which is filled with ingested algae, can be observed. Dark spots along the arms are pigment granules. On the left of the stomach can be observed a small pouch that is the beginning of an imaginal structure which at metamorphosis will give rise to part of the adult.

Maternal Messenger RNA and Its Utilization after Fertilization

The sea urchin egg contains a mass of stored inactive maternal message sufficient to engage all of its ribosomes, although before fertilization < 1 percent of these ribosomes are actually assembled into polysomes (*8*). The amount of RNA that might serve as maternal message has been calculated for *Sp* as 50 to 100 picograms per egg (*9*), included in a total of about 3 nanograms of RNA, of which 85 percent is ribosomal (*10*). Maternal messenger RNA (mRNA) is identified as embryo polysomal message that derives from the unfertilized egg, or as unfertilized egg RNA that supports protein synthesis in an in vitro translation system. Much, although clearly not all, of the translatable mRNA in the unfertilized egg contains 3′-polyadenylic acid [poly(A)] tracts about 50 to 120 nucleotides long (*11, 12*). Although some RNA species are preferentially polyadenylated and others occur mainly as 3′-poly(A)–deficient egg RNA's, the same set of sequences are included in both RNA fractions (*13, 14*).

The most direct demonstration of the protein-coding capacity of unfertilized egg mRNA comes from cell-free translation experiments. Infante and Heilman visualized several hundred protein spots in two-dimensional gel analyses of the translation products of poly(A) RNA extracted from unfertilized eggs of *Sp*, and showed that these are indistinguishable from the translation products coded by the polysomal message of early embryos (*15*). There are several specific proteins for which maternal mRNA's are known to be stored. Tubulins are synthesized in the growing oocyte, and maternal tubulin message continues to be translated in the embryo even though a large quantity of tubulin protein is also inherited via the egg cytoplasm (*9, 16*). The unfertilized egg also contains maternal actin message (*17, 18*). The most detailed studies have been carried out on maternal mRNA's coding for histones. There are about 10^6 molecules of message for each of the four core histones in the egg of *Sp*, together constituting some 5 to 10 percent of the total quantity of maternal mRNA (*9, 10, 19*). Maternal mRNA for histones is synthesized and stored in growing oocytes and is also made in mature unfertilized eggs (*20, 21*). However, in the latter all the newly synthesized histone mRNA appears to be translated immediately and is then turned over (*21*). Thus the stored maternal histone message is made earlier. The maternal messages code for special early embryonic variants of the core histones and of histone H1 (*22*). Some of these histone variants are found only in the nuclei of early cleavage stage embryos while others are synthesized well into the blastula stage.

The histones and tubulins are not exceptional in that many other embryonic proteins that are coded by maternal mRNA's are also synthesized prior to fertilization and inherited by the embryo. Brandhorst (*23*) showed for both *Lp* and *Sp* that of about 400 species of proteins resolved in two-dimensional gel analyses and synthesized within an hour of fertilization (that is, on maternal messages), almost all are also being translated at a low rate in unfertilized eggs. In the sea urchin fertilization triggers a dramatic increase in protein synthesis but does not significantly alter the set of sequences being translated. It was this striking quantitative feature, which is essentially a pecularity of echinoderm biology, that led to the initial discovery of maternal mRNA (*9*).

The mature sea urchin egg has already completed its meiotic reduction divisions and is stored in the lumen of the ovary in a relatively quiescent state. RNA synthesis nonetheless continues in the pronucleus at about the same rate per haploid genome as is observed in a cleavage-stage embryo nucleus (*21*). When fertilization occurs it sets off a train of cytological, physiological, and molecular changes in the state of the egg that begins within seconds of first contact between the sperm acrosome and the egg surface. Most of these events have been reviewed by Epel and by Vacquier (*24*). Several hours after fertilization, the rate of protein synthesis in the sea urchin has increased by a factor of about 100, compared to its rate in the unfertilized eggs, as a result of two separate effects at the translational level. The major effect is on the assembly of polyribosomes from maternal components, a process that begins only minutes after contact with the sperm. By the 16-cell stage about 30 percent of the egg ribosomes are involved in polysomal structures, and at most about 10 percent of the mRNA in these polysomes is of embryonic rather than maternal origin (*8, 9, 21*). After this the polysome content continues to increase, probably due to the appearance and utilization of new embryonic mRNA, until about 60 percent of the egg ribosomes are included. Protein synthesis rate increases proportionately (*25*), and rises from about 120 pg per hour-embryo at first cleavage to ≥ 500 pg per hour-embryo at the blastula stage (*9, 25, 26*). Fertilization also increases the translational elongation rate by a factor of about 2.5, compared to this rate in the polysomes of the unfertilized egg (*27*). Neither the mechanism of this effect nor that responsible for mobilization of the stored maternal message are well understood. An immediate trigger might be the sharp increase in intracellular *p*H, from 6.84 to 7.27 (in *Lp* and similar changes take place in *Sp*) occurring about 60 seconds after fertilization (*28*). Winkler, Steinhardt, and colleagues (*29*) directly demonstrated the increase in elongation rate in vitro by measuring ribosome transit times at *p*H 6.9 and *p*H 7.4 in a cell-free system derived from unfertilized *Lp* eggs. The *p*H change and other ionic alterations that follow fertilization may also affect the messenger ribonucleoprotein (mRNP) structures that include the maternal mRNA of unfertilized eggs. These particles are fairly stable, and differ in protein content and physical properties from those containing the newly synthesized mRNA of late embryos (*30*). It has been proposed that these particles are responsible for inhibiting translation of their maternal mRNA in the unfertilized egg (*31*), but what actually happens to them in vivo remains to be discovered. There is some evidence from studies of specific actin (*15*) and histone (*10, 32*) maternal mRNA's that the rate at which mobilization into polysomes occurs after fertilization may be quite different for certain particular sequences than for the majority.

Unexplained Features: Complexity and Sequence Organization of Maternal RNA

Were the heterogeneous RNA's stored in the egg simply a collection of messages to be translated after fertilization, its sequence complexity would be that of early embryo polysomal mRNA, and its size and sequence organization would be no different from that of the mRNA's found at later stages. Observations from our laboratory on the properties of sea urchin egg RNA's suggest a more complicated—and interesting—situation, although as the foregoing discussion shows, translatable maternal mRNA's are certainly present.

The complexity or total sequence length of unfertilized egg RNA has been measured by RNA excess hybridization reactions with single-copy DNA for three species (*Sp, Ap*, and *Tg*) and by the complementary DNA (cDNA) kinetic method for a fourth (*Lp*) (*33*). The values reported all fall in the range 3.0×10^7 to 3.7×10^7 nucleotides. RNA complexities of this magnitude are characteristic

of the eggs of other groups of animals as well, such as amphibians and the house fly (*34*). The small pronucleus of the mature sea urchin egg cannot contribute sufficiently to the total mass of RNA to affect the overall observed complexity, which is in any case only about one-fifth of that measured for the nuclear RNA of immature sea urchin oocytes or of embryos (*35*). However, Hough-Evans *et al.* found that in *Sp* the complexity of the maternal mRNA, defined as that population of sequences loaded on early embryo polysomes, is only about 70 percent of that of the total egg RNA (*36*). This observation was the first indication that egg RNA might include heterogeneous sequences not directly explicable as maternal message.

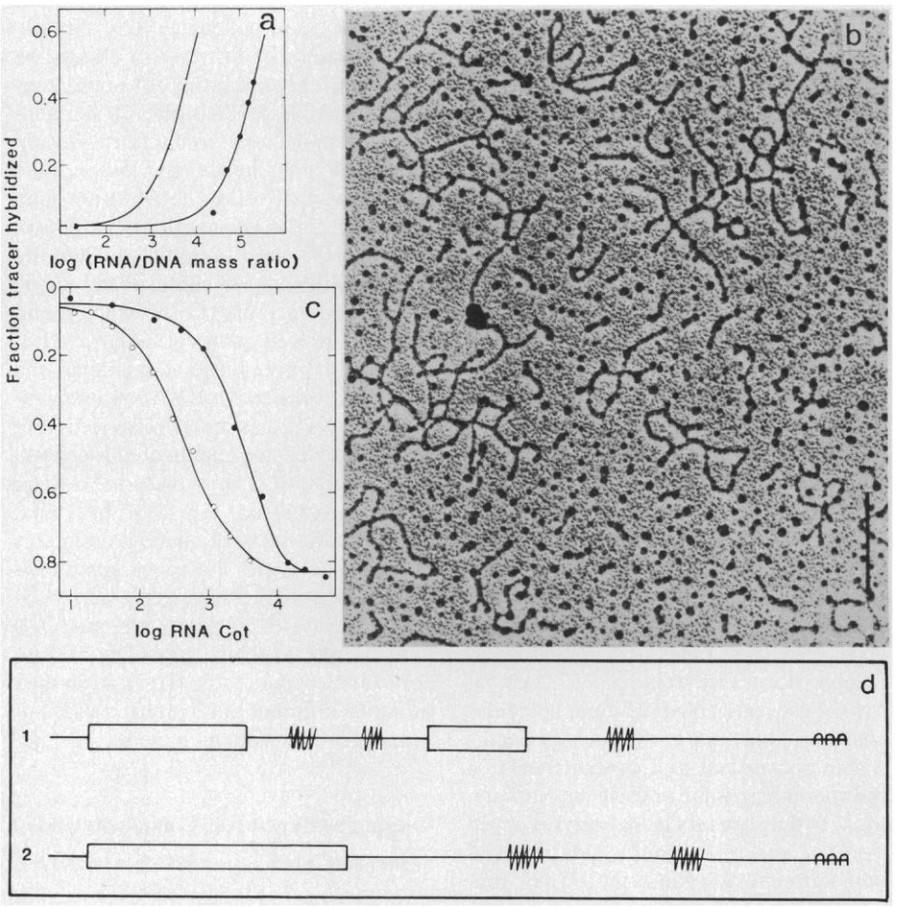

Fig. 2. Characteristics of maternal poly(A) RNA displaying an interspersed sequence organization. (a) Titration experiment demonstrating that repetitive sequence transcripts are polyadenylated. A cloned repetitive sequence called CS2109B was labeled in vitro and the strands were then separated. (Open circles) One strand was hybridized to termination with increasing amounts of egg poly(A)$^+$ RNA or (closed circles) poly(A)$^-$ egg RNA, and the reactions were assayed by hydroxyapatite chromatography. The specific fraction of poly(A)$^+$ RNA–containing transcripts of the CS2109B repeat was calculated as 1.3×10^{-4}, compared to 4.1×10^{-6} for the poly(A)$^-$ RNA. [From (*13*); courtesy of *Nature (London)*]. (b) Electron microscopy of renatured interspersed poly(A) RNA molecules of sea urchin egg RNA. The RNA was renatured to C_0t 600 (concentration × time; mole/liter-second), and duplex-containing molecules were eluted from an ethanol-cellulose column, precipitated with ethanol, and spread for electron microscopy. A representative field is shown. The circular molecules are ϕX174 DNA added as a length standard. The bar equals a single-stranded RNA length of 1000 nucleotides. [From (*42*); courtesy of *Journal of Molecular Biology*] (c) Complexity of single-copy sequence transcripts included in a selected, interspersed RNA fraction. A ^{32}P-labeled single-copy DNA tracer was prepared by hybridizing total single-copy ^{32}P-DNA with total egg RNA and purifying the hybridized fraction. The tracer was enriched about 30-fold for egg RNA sequences. It was reacted with total egg RNA (closed circle) and with RNA fractions containing interspersed repetitive sequence transcripts that had been selected by prior binding to an ethanol-cellulose column (open circle). All of the tracer that reacted with total egg RNA (about 80 percent) also reacted with the interspersed RNA. The latter reaction occurs at a faster rate because the reactive single-copy sequences are also concentrated when RNA's containing interspersed repeats are purified away from the bulk (ribosomal) RNA. [From (*13*); courtesy of *Nature (London)*] (d) Two of the possible forms of sequence organization in interspersed maternal poly(A) RNA. Diagram 1 depicts an RNA molecule in which the interspersed repetitive sequence elements are included in an intervening sequence, while diagram 2 shows an RNA molecule in which the repeat sequences are all located in a long 3′ untranslated tail. Open rectangles, coding sequence; jagged line, repetitive sequence element; *AAA*, poly (A).

Further evidence derives from a series of measurements carried out with repetitive DNA sequence probes. Costantini *et al.* (*13, 37*) showed that the RNA of unfertilized *Sp* eggs includes transcripts of at least several hundred different repetitive sequence families. Reactions with strand-separated repeat tracers demonstrated that both strands of each given repetitive sequence family are represented in the egg RNA, although in general in different molecules. Furthermore, it was found that at least 70 percent of the mass of the egg poly(A) RNA contains interspersed repeat sequence transcripts. In Fig. 2a is reproduced an experiment demonstrating that a specific repeat sequence is represented in the poly(A) RNA fraction. On the average about 10 percent of the length of a poly(A) RNA molecule consists of repetitive sequence transcript, and the remainder of single-copy sequence. If allowed to renature, such poly(A) RNA molecules will react at the complementary repeat sequences to form partially duplexed, multimolecular structures, such as are shown in Fig. 2b. Evidently more than one repeat sequence element exists in many of the egg poly(A) RNA molecules, and the repeats are frequently located in internal positions. The experiment reproduced in Fig. 2c demonstrates that the interspersed egg RNA molecules include virtually all the sequence complexity of total egg RNA. It follows that there are sets of diverse transcripts in the egg that share homologous repeat sequences, and several examples belonging to such sets have been cloned and their structure verified directly (*38*). While the number average length of *Sp* egg poly(A) RNA is about 3 kilobases (kb) (*13, 37*), RNA gel blots show that the interspersed molecules are usually much longer, often from 5 to 15 kb (*38*). The presence of interspersed repeats in such long transcripts is directly reminiscent of the structure of nuclear RNA, although as already pointed out the egg RNA complexity differentiates it sharply from total nuclear RNA. Figure 2d displays two possible kinds of sequence organization that are consistent with these observations. In one case, the interspersed repeats are located in long 3′-terminal tails, and in the other they are embedded in intervening sequences that have not yet been processed out. The repetitive sequence elements are figured outside the coding regions, because primary sequence determinations carried out on eight of nine different cloned

repeats of genomic families represented in egg RNA revealed translation stop codons in all possible reading frames (*38*).

An observation that is probably related has been reported by Duncan and Humphreys in studies of *Tg* egg RNA (*11*). The fraction of egg RNA that lacks long 3′ poly(A) tracts is approximately equal in mass to the adenylated component. An interesting feature of RNA lacking 3′ poly(A) tracts is that it contains internal A_{10} sequences (polyadenylic acid containing ten residues), just as does embryo nuclear RNA (*39*). These sequences are absent from cleavage stage polysomal RNA (*11*), that is, from the maternal message by the time it is assembled into polysomes. Whether either maternal or newly synthesized repeat-containing poly(A) RNA's are loaded directly on polysomes after fertilization remains moot, because interspersed maternal RNA species persist in the cytoplasm far into embryogenesis (*38, 40*), probably complexed with proteins, and these particles are difficult to separate completely from bona fide polysomal structures. However, embryo polysomal mRNA lacks the prevalent repetitive sequence component observed in egg poly(A) RNA (*37*), although by mass perhaps 15 percent of embryo message at gastrula stage continues to display an interspersed sequence organization, a somewhat larger figure than reported earlier (*41*).

Both the presence of A_{10} sequences not found in polysomes and the structure of the interspersed poly(A) RNA's suggest the rather startling conclusion that a major fraction of the stored maternal RNA's are not fully processed. Whatever their function, this phenomenon is not confined to sea urchins. We have found that a major fraction of the cytoplasmic poly(A) RNA of *Xenopus* oocytes displays the same interspersed sequence organization (*42*). Possibly the only maternal messages (in the strictest sense) are those that lack both A_{10} and in most cases repeat sequences, and are available for immediate translation (*43, 44*). On the other hand, if these sequence features reside in 3′ terminal regions of the transcripts (Fig. 2d) they might not interfere with translation. In either case the fate of these maternal RNA's, and the biological significance of the "excess" (that is, nonunderstood) sequence they contain are likely to be of basic importance in understanding the role of maternal components in development.

There are a number of interesting possibilities worth considering. For example, cytoplasmic processing in the embryo could represent a control point in the utilization of maternal information, and might be accomplished differently at various stages or in the various regions of the embryo. The maternal transcripts might include sequences (perhaps the repeat elements themselves) recognized by other macromolecules, such as proteins, small nuclear RNA's or other RNA's (*45*), and such complexes might be involved in sequestration of maternal mRNA's; or in subsequent processing; or in determining their turnover rates; or in localization of the transcripts within the embryo; or even in regulation of structure or function within the blastomere nuclei. Conceivably, some fundamental change in the regulation of transcriptional termination occurs between oogenesis, when the usual signals are not recognized, and later embryonic development, when they are (*46*). Another possibility is that these sequences are used by the embryo to monitor the quantity of maternal transcripts, and thus to set the timing of events that require the attainment of a certain nucleus-to-cytoplasm ratio as cell division proceeds within the constant mass of the maternal cytoplasm.

Patterns of Gene Expression in the Embryo

Species hybridization and actinomycin experiments initially indicated that for many hours the pattern of biosynthesis in the embryo remains essentially that determined by the maternal mRNA (*9*). This view has been substantiated in elegant detail at the molecular level. The spectrum of proteins synthesized in the embryo and resolvable on two-dimensional gels ($\leq 10^3$ species) changes very little from fertilization until the blastula stage (*15, 23*), and even thereafter the rate of synthesis of only about 15 to 20 percent of these proteins alters more than tenfold (*47*). These observations were, of course, made on extracts of whole embryos, and it is quite possible that among specific cell lineages larger differences exist in the patterns of protein synthesis. The changes that have been reported occur mainly during the transition from blastula to early gastrula, when various forms of differentiation become apparent in the embryo. Direct measurements of the population of polysomal and cytoplasmic RNA's carried out in our laboratory have shown that no very extensive switching on of new genes occurs during embryonic development. Thus Galau *et al.* (*48, 49*) discovered to their surprise that all or most gastrula mRNA sequences are already represented in the stored maternal RNA of the egg, and in later development the overall complexity of the polysomal RNA actually declines to a minor extent. About 60 percent of that set of sequences found in the maternal mRNA is still present in the newly synthesized transcripts of the late embryo (*36*). A specific rare maternal sequence that appears to be expressed only at the earliest stages was described by Lev *et al.* (*50*). This sequence is loaded on polysomes in 16-cell embryos, but thereafter it disappears from the embryo cytoplasm (Fig. 3a).

The comparisons of polysomal RNA sequence sets referred to here concern mainly the low abundance transcripts that account for most of the mRNA complexity. Such sequences are present in only a few copies per gastrula cell on the average, that is, assuming that they are equally present in all the cells at this stage, which for any given example may not be the case. Low abundance sequences amount to about 40 percent of the total mass of cytoplasmic poly(A) RNA (*48, 49, 51*). Higher prevalence RNA sequences of egg and embryo have also been compared, in measurements carried out with cDNA clone libraries (*51, 52*). Almost all of the genomic transcripts in the range 10^4 to 10^6 copies per embryo (that is, in the late embryo about 10 to 10^3 copies per cell on the average) are found to be represented to an approximately equal extent in the maternal RNA and in pluteus stage embryo cytoplasmic poly(A) RNA. Typically such transcripts decline in prevalence several-fold on a per embryo basis between egg and gastrula stages, whereupon their sequence concentration builds back up as a result of new synthesis in the late embryo (*52*). However, there are also a limited number of moderate to low prevalence RNA species that appear for the first time during blastulation, in agreement with studies of protein synthesis patterns (*47*). The appearance of poly(A) RNA sequences apparently representing newly activated genes has been verified by the RNA gel blot method with the use of cloned complementary DNA (cDNA) probes (*52, 53*), as shown for example in Fig. 3, b to d, and is also supported by a study of cDNA hybridization kinetics in blastula to gastrula stage embryos (*54*).

The high complexity of the maternal mRNA, and the persistence of much of the same complex set of polysomal RNA's throughout embryogenesis poses a very interesting developmental problem. Measurements indicate that the number average length of the polysomal poly(A) mRNA of early sea urchin em-

bryos is about 2000 nucleotides (*9*) [that is, somewhat shorter than egg or embryo cytoplasmic poly(A) RNA, the number average length of which is ≥ 3000 nucleotides (*13, 14*)]. If each polysomal poly(A) RNA molecule contains a message sequence for one protein, the codogenic capacity of the protein synthesis apparatus is probably about 12,000 diverse polypeptides at blastula stage, when the complexity is 24×10^6 nucleotides, and is more than 8000 polypeptides at gastrula stage when the complexity is 17×10^6 nucleotides (*48, 49*). One hypothesis is that morphogenesis is extremely expensive in terms of protein diversity, in that a very large number of different proteins must cooperate in the construction of three-dimensional biological structures. Relevant examples include flagella, which contain more than 120 diverse protein species; the moth egg chorion, which contains more than 180 protein species; and the T4 bacteriophage head and tail, which include more than 50 different structural gene products (*55*). It seems paradoxical that, while the structural complexity of the embryo progressively increases, the molecular complexity of the embryo mRNA is highest at the beginning, and that during development most maternal mRNA species are merely replaced by new transcripts of the same set of genes as were active in oogenesis. Perhaps a large fraction of the complex sets of proteins required for embryonic morphogenesis assembles into biological structures only long after they are synthesized. The mRNA's of the embryo might include programs for morphogenesis that begin to be transcribed and translated during oogenesis. Some of these mRNA's might continue to be expressed in the embryo genomes; perhaps different sets in different cell lineages. An example of a protein made in the oocyte for assembly in an embryonic structure might be provided by the tubulins. These maternal proteins are known to be utilized after fertilization for ciliary morphogenesis, and they continue to be synthesized during embryogenesis (*9, 16, 56*). The hypothesis that the complexity of embryonic mRNA is required by morphogenetic function might also explain why a large fraction of embryo cytoplasmic transcripts (both rare and prevalent) are not found in those adult sea urchin cell types that have so far been examined, where the total polysomal message complexity is much lower (*48, 49, 57*).

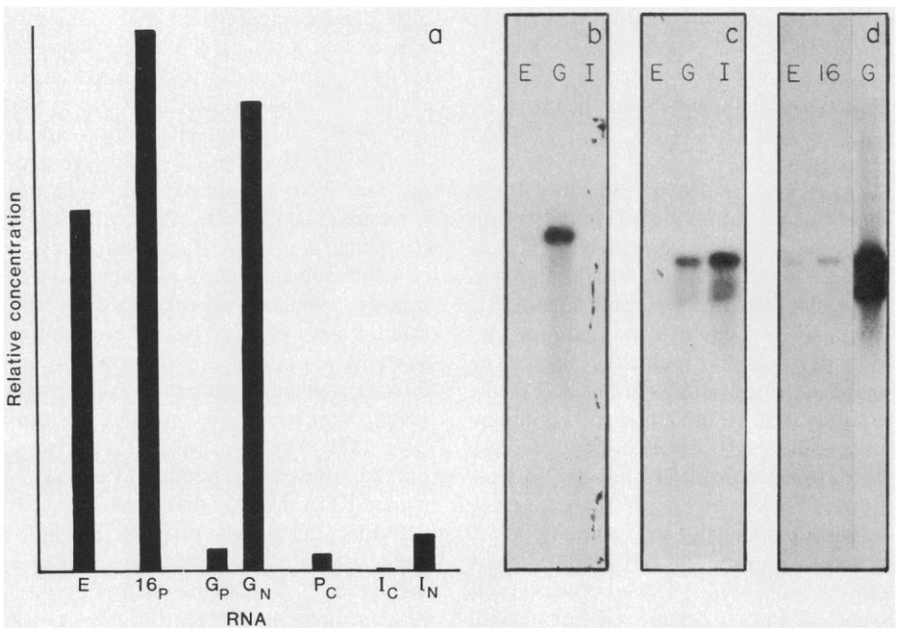

Fig. 3. Examples of patterns of gene activity observed with cloned sequence probes. (a) Representation of a rare maternal transcript in various RNA's. A cloned sequence called Sp88 that is complementary to about 1400 transcripts per egg was strand-separated and labeled in vitro. The expressed strand tracers were used to determine the concentration of complementary transcripts by titration. Data are expressed as relative concentration per unit mass RNA of Sp88 transcripts in *E*, total egg RNA; 16_P, 16-cell polysomal RNA; G_P, gastrula polysomal RNA; G_N, gastrula nuclear RNA; P_C, pluteus cytoplasmic RNA; I_C; intestine cytoplasmic RNA; I_N, intestine nuclear RNA. No Sp88 transcripts could be detected in cytoplasmic RNA of intestine cells. [From (*50*); courtesy of *Developmental Biology*] (b to d) RNA gel blot hybridizations with cloned probes. (b) cDNA clone SpG4-B9 reacted with (from left to right) egg poly(A) RNA, gastrula polysomal poly(A) RNA, and intestine poly(A) RNA. This sequence is barely represented in the maternal RNA but is expressed strongly in gastrula. It is absent in intestine. The length of the transcript is 1.45 kb. (c) cDNA clone SpG2-D12, same RNA's. This sequence is not detectably represented in egg RNA but is prevalent in the gastrula and intestine RNA's. The length of the transcript is 1.35 kb. [Parts (b) and (c) are from (*52*); courtesy of *Developmental Biology*] (d) Cloned actin gene probe, reacted with egg poly(A) RNA, 16-cell embryo poly(A) RNA, and gastrula poly(A) RNA (*85*). The two main actin transcripts are 1.8 and 2.2 kb in length (*17*).

Transcript Synthesis and Turnover Rates

It is interesting to consider more closely the role of structural gene transcription in the embryo nuclei. Measurements of the synthesis and turnover rates of embryo polysomal mRNA shows that most of the mass of this message is newly synthesized by the blastula stage (*58, 59*). However, Cabrera *et al.* (*40*) found that (in *Sp*) the actual point in development at which the maternal transcripts are replaced by new ones varies, depending on the particular sequence examined. In this work, using cloned cDNA probes we measured the cytoplasmic entry and turnover rates of a set of poly(A) RNA's of different prevalences. A general conclusion is that cytoplasmic transcript stability is an important determinant of the level of gene expression in sea urchin embryos in that most newly synthesized rare poly(A) RNA's turn over and are replaced with a half-life of several hours (*9, 40*), while prevalent ones accumulate because they are stable. Therefore, low abundance sequences can be regulated either up or down by the embryo cells, while once transcribed, most abundant sequences are there to stay, and in general their prevalence can only rise, that is, as a result of new synthesis. Since the prevalence of most sequences in the embryo is similar to that in the maternal RNA of the egg, for each sequence much the same relation between turnover rate and cytoplasmic entry rate must have existed in oogenesis as in embryogenesis. The entry rates for specific sequences are sharply different, and are so low for some prevalent transcripts that it is clear that the majority of the cytoplasmic poly(A) RNA's of such species are still of maternal origin even in pluteus stage embryos (*40*).

By far the majority of genes that are active produce low abundance sequences, and in *Sp* entry rates for such transcripts are typically about one molecule per cell every one to several hours (*40*). To maintain the steady-state concentrations of such cytoplasmic RNA's would require only occasional transcriptional initiations. Electron microscopic

examinations of the transcription complexes of *Sp* embryo nuclei were carried out by Busby and Bakken (*60*). They observed that more than 80 percent of the visualized complexes contain only single nascent transcripts. The average polymerase spacing even in the more intensely expressed regions displaying multiple transcripts was about 3.7 kb, suggesting an initiation rate of only about one molecule every 7 to 10 minutes (*61*). The cytoplasmic entry rates measured by Cabrera *et al.* (*40*) even for prevalent sequences were of the same magnitude or lower. Thus the very high rates of initiation typical of genes transcribing superprevalent mRNA's in terminally differentiated mammalian or avian cells are not generally required for the maintenance or operation of a system that is undergoing embryonic morphogenesis.

The relation between the nuclear RNA synthesized in the embryo genomes and the population of mRNA's entering the cytoplasm is not well understood. There is evidence for conservation of a major fraction of 5′ terminal RNA "cap" structures, and their transfer to the cytoplasmic mRNA compartment (*62*). However, both the complexity of the nuclear RNA and its rate of synthesis are at least tenfold higher than the corresponding parameters for the cytoplasmic mRNA of the same embryo cells (*58, 59, 63*). In addition, Wold *et al.* (*64*) found that in *Sp* many low abundance, blastula polysomal RNA sequences which are absent from the cytoplasm of adult cells are nonetheless represented in the nuclear RNA of these cells. This was also observed in *Sp* for a set of blastula-specific sequences by Shepherd and Nemer (*54*). In Fig. 3a is illustrated an example of this phenomenon, in which a specific transcript found only in the cytoplasm of early embryos remains detectable in both gastrula and adult cell nuclear RNA's. The initiation and termination sites of this gastrula nuclear transcript are approximately the same as for the predominant homologous maternal transcript of unfertilized egg poly(A) RNA (*65*). In general the nuclear RNA's of sea urchin embryo and adult cells display a very high degree of sequence overlap, although they are certainly not identical (*64, 66*). Since transcription rates (and steady-state levels) for regulated, cytoplasmically expressed polysomal sequences are so low, differential patterns of transcription that are physiologically meaningful could easily be obscured by random transcriptional "leakage" if such a phenomenon were to occur in some or all of these nuclei. However, it cannot now be excluded that regulation in sea urchin embryo nuclei occurs at the posttranscriptional as well as at the transcriptional level.

Developmental Expression of Ribosomal, Actin, and Histone Genes

There are several interesting proteins known that are likely to be involved directly in formation of biological structures during development. Examples include the proteins associated with skeleton formation, proteins that might be involved in gastrulation and morphogenesis, and the vitelline layer proteins (*67, 68*). While these are the focus of ongoing research in several laboratories, little is as yet known of the respective genes. At present the best analyzed gene systems are the ribosomal RNA (rRNA) genes, the actin genes, and the histone genes. The quantitative details of expression in each of these systems provide an instructive paradigm for gene activity in the embryo.

The ribosomal genes are present in 50 to 200 copies per genome, depending on the sea urchin species. During oogenesis the ribosomal genes function at what is probably their maximum transcriptional rate. Griffith and Humphreys showed that in *Tg* the average rate of synthesis is about 1800 rRNA molecules of each subunit per minute-oocyte, or about 450 molecules per minute in each haploid genome set (*69*). Ribosomal gene amplification apparently does not occur in sea urchin oogenesis, and the high rate of synthesis suffices to produce the maternal complement of ribosomes over the several months of oogenesis (in the egg of *Tg* there are about 4×10^8 ribosomes). In embryos the rate of rRNA synthesis per nucleus is reduced to only a few percent of that in the growing oocyte. In *Tg* this rate is about 60 molecules per minute in each (diploid) nucleus (*69*); in *Lp* about 20 molecules per minute-nucleus; in *Sp* 18 to 40 molecules per minute-nucleus (*70*). These rates are maintained all through embryological development without significant change. Electron microscope observations (*60*) suggest that in embryos a few of the ribosomal genes are highly active, while the vast majority remain repressed. Humphreys showed (in *Lp*) that on feeding as net growth resumes in the larva the rate of rRNA synthesis increases severalfold (*70*).

Actin is among the many maternal proteins inherited by the embryo (*71*), along with its maternal message. The egg of *Sp* contains relatively low quantities of a 2.2-kb transcript and an even smaller amount of a 1.8-kb species of maternal actin mRNA (*17*). Cell free translation shows that messages for several actin isoforms are stored in the egg (*15*). Crain *et al.* reported that between 8 and 18 hours after fertilization the polysomal concentration of the 1.8-kb mRNA form increases about 40-fold and that of the 2.2-kb form increases about 20-fold (*18*). This sharp developmental increase can be seen in Fig. 3d. Adult tissues so far examined in our laboratory display only a 2.2-kb actin mRNA, and the 1.8-kb form could represent an actin gene (or genes) activated specifically in the embryo. Clustered sets of actin genes have been isolated from the genome of *Sp*, and some of these clusters include actin genes of diverse types (*17*). The actin genes are distinguished from each other by nonhomologous transcribed sequences extending for several hundred nucleotides beyond the translation termination signal. Since they are expressed differentially in embryonic development and in adult tissues, these genes offer an excellent opportunity to determine the sequence features required for their developmental regulation.

The organization and expression during embryogenesis of the histone gene family have been intensively investigated, and this subject has been reviewed in detail (*22, 72*). After the 16-cell stage, the maternal mRNA's for the early histone variants are replaced by new transcripts deriving from the early histone genes active in the embryo nuclei, while the genes for histones found only in early cleavage nuclei are apparently functional exclusively in oogenesis and for a short time after fertilization, and are then shut off (*22*). Recent results from the laboratories of Wilt and Kedes have added interesting quantitative details. Translation of histones rises sharply after fourth cleavage, and at its peak histone synthesis accounts for more than 30 percent of total protein synthesis (*9, 10*). At the 128-cell stage mRNA's for each of the core histone species are emerging from the average embryo nucleus at a rate of at least 800 molecules per minute (*73*) (there are about 400 genes for each of the early histone variants per haploid genome in *Sp*). Thus the number of histone mRNA molecules of each core species rises from about 10^6 per embryo at fourth cleavage, most of which are maternal in origin, to about 10^7 only 4 hours later, when 90 percent are the result of new synthesis (*19, 73, 74*). The rate of histone synthesis is keyed to the rate of cell division (*9*), and when this falls, after about the 200-cell stage, the level of

histone mRNA and of histone translation also decline, due to message turnover and a sharp decrease in the flow of new messages to about 15 percent of the peak rate (*73, 74*). At 300 cells there are again $\leq 10^6$ molecules of each core histone mRNA species present. Transcription of the early histone genes is repressed, and during blastulation there appear new "late" variants of each core histone and also histone H1 (*22*). The late histones are transcribed from a different set of genes that are present in the genome in only 1 to 2 percent of the multiplicity of the early histone genes, and at least in some cases these genes are located distantly from the major early histone gene clusters (*75*). Among the many fascinating problems of gene regulation posed by these data are the mechanisms by which first the cleavage stage histone genes and later the early histone genes are repressed, the relation between the patterns of expression and the genomic locations of these genes, the means by which early histone gene expression is coordinated with cell division rate, and the functional significance of the assembly into chromatin of the late as opposed to early histone variants. Early and late gene expression are also regulated differently during oogenesis, since the maternal message apparently does not include late histone gene transcripts. In contrast to the histones, many of the nonhistone nuclear proteins found in embryo chromatin as late as the gastrula stage are maternal in origin, and are not synthesized at all after fertilization (*76*).

The embryo applies diverse solutions to the logistic and informational demands of development. As additional genes and gene systems are investigated it becomes apparent that the basic components of these solutions, maternal mRNA's and proteins, and embryonic mRNA's and their translation products, are utilized to various extents and according to different developmental schedules.

Localization of Morphogenetic Fate in the Sea Urchin Embryo

Improvement of methods for obtaining mass preparations of micromeres and for recovering and culturing mesenchyme cells have led to a series of new experiments on the molecular basis of determination in the micromere cell lineage. An obvious proposal is that micromeres inherit a particular subset of maternal mRNA's, and thereby are endowed with special developmental potentialities. Rodgers and Gross (*77*) (in *Lp*) and Ernst *et al.* (*78*) (in *Sp*) found that only 70 to 80 percent of the maternal sequence complexity is represented in total micromere RNA, while the remainder of the embryo retains all the maternal sequences, at about the same concentrations as in the unfertilized egg. On the other hand, the sequence content of micromere polysomal RNA is just the same as that of the whole 16-cell embryo (*78*). The similarity between the translated mRNA in micromeres and in the remainder of the embryo is observed as well at the protein synthesis level. Almost all the newly synthesized proteins resolved on two-dimensional gels are synthesized alike by micromeres, macromeres, and mesomeres (*79*). While a small but potentially important subset of low abundance mRNA's would have been missed in any of these experiments, at least the simplest forms of the maternal mRNA segregation model for micromere commitment seem excluded. However, there are other differences between micromeres and the remainder of the fourth cleavage embryo, and these point in a different direction. At this stage micromere nuclei contain no high complexity nuclear RNA while nuclei of the other blastomeres synthesize such RNA actively (*78*). In addition, Senger and Gross showed that the ratio of histone to nonhistone protein synthesis is higher in micromeres, and if transcription is blocked by actinomycin this difference is abolished (*80*).

One interpretation suggested by these observations is that the basic distinction between the micromere and other cell lineages derives from precocious differences in nuclear gene expression, due initially to a cytoplasmic environment in the micromeres very different from that to which the other blastomere nuclei are exposed. The micromeres are essentially budded off from the adjacent macromeres with a very small amount of cytoplasm. The absence of a class of maternal RNA sequences found in the rest of the embryo suggests that a special cytoplasmic domain has been created in the micromeres. Furthermore, nuclear gene expression in the micromeres might be expected to dominate the pattern of protein synthesis more rapidly than in cells that contain severalfold more cytoplasmic volume and maternal mRNA (*80*). The micromere lineage is clearly committed at an early stage, and when cultured in isolation micromeres differentiate in vitro into skeleton-producing primary mesenchyme cells on the same temporal schedule as in situ (*81*). By late in embryogenesis these and other differentiated cell types no doubt contain distinct populations of cytoplasmic poly(A) RNA's and synthesize distinct proteins. As one example, Bruskin *et al.* (*53*) have described a family of ten protein species translated specifically in late embryo ectoderm cells. Transcripts coding for these proteins increase in prevalence more than 100-fold after early blastula stage, and are already concentrated in ectoderm cells by the early gastrula stage.

The molecular basis of commitment on the part of early blastomere lineages is extremely subtle. When very early sea urchin embryos are treated with lithium chloride they undergo an abnormal form of development in which gastrular invagination and skeletal formation are suppressed. Yet no differences in the patterns of protein synthesis can be detected between lithium chloride-treated and normal embryos until after the catastrophic effects of this treatment have already occurred (*82*). An approach that is promising in regard to commitment and morphogenesis is isolation of surface components from embryo cells that specify correct intercellular interactions. Giudice and his colleagues (*83*) showed that disaggregated sea urchin embryos will reassort to form blastula-like structures that subsequently complete embryological development. Recently, Noll *et al.* found that surface proteins can be extracted and then added back to promote such reaggregation (*68*), and the role and origins of these proteins can now be investigated.

The embryonic architecture of the sea urchin begins with the initial polarity of the egg. This subject was classically much investigated, and has been reexamined experimentally and reviewed by Schroeder (*84*). The egg is endowed with an animal-vegetal polarity before fertilization, and this probably originates far back in oogenesis. At fertilization (or parthenogenic stimulation) a "clock" is activated, and on a timetable that is independent of the cleavage mitoses per se, cortical cytoplasmic elements of the vegetal pole are moved upward toward the equator of the egg. The residual polar cortex is the site where the micromeres will form. A fascinating challenge that can now be considered is definition of the molecular nature of this "clock," and of the primitive axial localization resident in the unfertilized egg.

Interpretation and Future Directions

We have focused on the major concepts and puzzles that have arisen with the growth of molecular level knowledge of sea urchin embryogenesis. Clearly we

do not yet know how embryogenesis really "works" in this organism. Some major features are becoming evident, and others can perhaps be inferred. One simplified and hypothetical interpretation is as follows. During oogenesis a complex set of macromolecules that will be needed for morphogenesis and cell division begins to be transcribed and translated. They are accumulated in the growing oocyte in the quantities required for the mass of cytoplasm contained in the whole embryo, and following fertilization they are utilized until the embryo has acquired sufficient nuclei to replace them with its own transcription and translation products. The oocyte has only one (tetraploid) nucleus, and this process of accumulation requires a relatively long period of synthesis for those products needed in great abundance. Fertilization acts as a metabolic trigger that activates essentially the same pattern of biosynthesis as preceded it in the oocyte, but it also sets off a crucial series of spatial reorganizations of the cytoplasm. The result during cleavage is establishment of diverse domains in which, by some unknown process, the nuclei of different embryonic cell lineages are induced to function differentially. Most of the productive transcription (and processing) in the embryo nuclei is required for replacement of maternal RNA's and proteins, a task that is accomplished at various rates and by various means, depending on the sequence and the rate at which it is demanded. However, in these genomes there also occurs the activation of sets of cell lineage specific genes. The products of such genes might be needed for the cellular differentiations that result, by intercellular interaction, in the assembly of the three-dimensional multicellular structures of the embryo, though the actual construction of these may require extensive use of preformed components. Embryogenesis is terminated (pluteus stage) when a constant ratio of nucleus to cytoplasm is attained, when the transcripts on the polysomes are all of embryonic rather than maternal origin, when specific patterns of gene expression characteristic of the major cell lineages are established, and when the morphogenetic program that began to be read out in the oocyte nucleus has been carried to completion.

Although no other early embryonic system is nearly as well understood at the molecular level, it seems likely that additional approaches will be required if we are to be able to assay directly the functional meaning in development of the particular proteins, transcripts, and active genes of the embryo. It can be anticipated that there will be developed subembryonic and cell free systems in which the assembly of morphological structures can be studied, and other systems in which the effect of embryonic constituents on transcription and processing can be investigated in vitro. In addition, serious efforts are now being made to develop DNA transformation systems for sea urchin eggs that might permit direct investigation of the role of nucleic acid sequence features in the context of the developing embryo. It should be clear from the foregoing that early embryogenesis is a special, enormously complex process, the actual mechanism of which can scarcely be established by studying anything but embryos themselves. As it has for the past century, the sea urchin egg can be expected to continue to provide an accessible and revealing model for this endeavor.

References and Notes

1. P. S. Leahy, B. R. Hough-Evans, R. J. Britten, E. H. Davidson, *J. Exp. Zool.* **215**, 7 (1981); P. S. Leahy, T. C. Tutschulte, R. J. Britten, E. H. Davidson, *ibid.* **204**, 369 (1978).
2. R. T. Hinegardner and M. M. Rocha Tuzzi, in *Marine Invertebrates* (National Academy Press, Washington, D.C., 1981), p. 291; R. A. Cameron and R. T. Hinegardner, *Biol. Bull. (Woods Hole, Mass.)* **146**, 355 (1974).
3. R. T. Hinegardner, *Am. Zool.* **15**, 679 (1975); K. A. Coffaro and R. T. Hinegardner, *Science* **197**, 1389 (1977).
4. E. Davidson, unpublished observations.
5. A good description of the developmental origin of the imaginal structures is given by G. Czihak, in *Experimental Embryology of Marine and Fresh-water Invertebrates*, G. Reverberi, Ed. (North-Holland, Amsterdam, 1971), p. 363.
6. M. S. Houk and R. T. Hinegardner, *Biol. Bull. (Woods Hole, Mass.)* **159**, 280 (1980).
7. E. H. Davidson, T. L. Thomas, R. H. Scheller, R. J. Britten, in *Genome Evolution*, G. Dover and R. B. Flavell, Eds. (Academic Press, New York, 1982), p. 177.
8. T. Humphreys, *Dev. Biol.* **26**, 201 (1971).
9. E. H. Davidson, *Gene Activity in Early Development* (Academic Press, New York, 1976), chap. 4. This chapter includes a general review of data regarding protein synthesis, maternal mRNA, and its utilization in sea urchin embryos. Species hybrid and actinomycin experiments that foreshadowed the demonstration of maternal mRNA are also reviewed in *ibid.*, chap. 2.
10. A. S. Goustin, *Dev. Biol.* **87**, 163 (1981).
11. R. Duncan and T. Humphreys, *ibid.* **88**, 211 (1981).
12. F. H. Wilt, *Cell* **11**, 673 (1977).
13. F. D. Costantini, R. J. Britten, E. H. Davidson, *Nature (London)* **287**, 111 (1980).
14. R. Duncan and T. Humphreys, *Dev. Biol.* **88**, 201 (1981); B. P. Brandhorst, D. P. S. Verma, D. Fromson, *ibid.* **71**, 128 (1979).
15. A. A. Infante and L. J. Heilmann, *Biochemistry* **20**, 1 (1981).
16. G. Cognetti, I. DiLiegro, F. Cavarretta, *Cell Differ.* **6**, 159 (1977); R. A. Raff, G. Greenhouse, K. W. Gross, P. R. Gross, *J. Cell Biol.* **50**, 516 (1971).
17. R. H. Scheller *et al.*, *Mol. Cell. Biol.* **1**, 609 (1981).
18. W. R. Crain, D. S. Durica, K. Van Doren, *ibid.*, p. 711.
19. A. Mauron, L. Kedes, B. R. Hough-Evans, E. H. Davidson, *Dev. Biol.*, in press.
20. J. V. Ruderman and D. R. Schmidt, *ibid.* **81**, 220 (1981).
21. B. P. Brandhorst, *ibid.* **79**, 139 (1980).
22. G. Childs, R. E. Maxson, L. H. Kedes, *ibid.* **73**, 153 (1979); K. M. Newrock, L. H. Cohen, M. B. Hendricks, R. J. Donnelly, E. S. Weinberg, *Cell* **14**, 327 (1978); K. M. Newrock, C. R. Alfageme, R. V. Nardi, L. H. Cohen, *Cold Spring Harbor Symp. Quant. Biol.* **42**, 421 (1978); M. Grunstein, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4135 (1978); D. Poccia, J. Salik, G. Krystal, *Dev. Biol.* **82**, 287 (1981). The specific histones for which maternal mRNA's exist are cleavage stage forms of H1, H1A, and H2B; H1α, H2Aα_1, H2Bα_1, and early forms of H3 and H4.
23. B. P. Brandhorst, *Dev. Biol.* **52**, 310 (1976).
24. D. Epel, in *The Cell Surface: Mediator of Developmental Processes*, S. Subtelny and N. K. Wessells, Eds. (Academic Press, New York, 1980), p. 169; *Curr. Top. Dev. Biol.* **12**, 185 (1978); V. D. Vacquier, *Dev. Biol.* **84**, 1 (1981).
25. A. S. Goustin and F. H. Wilt, *Dev. Biol.* **82**, 32 (1981).
26. J. C. Regier and F. C. Kafatos, *ibid.* **57**, 270 (1977).
27. M. B. Hille and A. A. Albers, *Nature (London)* **278**, 469 (1979); J. W. Brandis and R. A. Raff, *ibid.* p. 467; *Dev. Biol.* **67**, 99 (1978).
28. S. S. Shen and R. A. Steinhardt, *Nature (London)* **272**, 253 (1978); D. Epel, R. A. Steinhardt, T. Humphreys, D. Mazia, *Dev. Biol.* **40**, 245 (1974).
29. M. M. Winkler and R. A. Steinhardt, *Dev. Biol.* **84**, 432 (1981); see also J. L. Grainger, M. M. Winkler, S. S. Shen, R. A. Steinhardt, *ibid.* **68**, 396 (1979).
30. R. T. Moon, K. D. Moe, M. B. Hille, *Biochemistry* **19**, 2723 (1980); E. M. Young and R. A. Raff, *Dev. Biol.* **72**, 24 (1979); J. F. Kaumeyer, N. A. Jenkins, R. A. Raff, *ibid.* **63**, 266 (1978).
31. N. A. Jenkins, J. F. Kaumeyer, E. M. Young, R. A. Raff, *Dev. Biol.* **63**, 279 (1978). The "masked message" hypothesis that stimulated these experiments was initially suggested by A. S. Spirin [*Curr. Top. Dev. Biol.* **1**, 1 (1966)].
32. D. E. Wells, R. M. Showman, W. H. Klein, R. A. Raff, *Nature (London)* **292**, 477 (1981).
33. For *Sp*, G. A. Galau *et al.* (*48*) and B. R. Hough-Evans *et al.* (*36*); for *Ap*, D. M. Anderson, G. A. Galau, R. J. Britten, E. H. Davidson, *Dev. Biol.* **51**, 138 (1976); for *Lp*, F. H. Wilt (*12*).
34. Reviewed by T. L. Thomas, J. W. Posakony, D. M. Anderson, R. J. Britten, E. H. Davidson, *Chromosoma* **84**, 319 (1981).
35. B. R. Hough-Evans, S. G. Ernst, R. J. Britten, E. H. Davidson, *Dev. Biol.* **69**, 225 (1979).
36. B. R. Hough-Evans, B. J. Wold, S. G. Ernst, R. J. Britten, E. H. Davidson, *ibid.* **60**, 258 (1977).
37. F. D. Costantini, R. H. Scheller, R. J. Britten, E. H. Davidson, *Cell* **15**, 173 (1978).
38. J. W. Posakony, C. N. Flytzanis, R. J. Britten, E. H. Davidson, unpublished experiments; for primary sequences, J. W. Posakony, R. H. Scheller, D. M. Anderson, R. J. Britten, E. H. Davidson, *J. Mol. Biol.* **149**, 41 (1981).
39. L. M. Dubroff and M. Nemer, *ibid.* **95**, 455 (1975).
40. C. V. Cabrera, J. W. Ellison, J. G. Moore, R. J. Britten, E. H. Davidson, in preparation.
41. R. B. Goldberg, G. A. Galau, R. J. Britten, E. H. Davidson, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3516 (1973); E. H. Davidson, B. R. Hough, W. H. Klein, R. J. Britten, *Cell* **4**, 217 (1975).
42. D. M. Anderson, J. D. Richter, M. E. Chamberlin, D. H. Price, R. J. Britten, L. D. Smith, E. H. Davidson, *J. Mol. Biol.* **155**, 281 (1982).
43. An argument adduced in this connection is that all maternal poly(A) RNA is transferred to polysomes after fertilization. However, all that is in fact known is that in embryos most poly(A) is polysomal (*11*, *44*), and the matter is obscured by the rapid removal and replacement of poly(A) tracts occurring after fertilization [G. J. Dolecki, R. F. Duncan, T. Humphreys, *Cell* **11**, 339 (1977); F. H. Wilt, (*12*)]. No measurements exclude the possibility that nontranslatable poly(A) RNA from egg could be deadenylated, while other translatable molecules are readenylated in the embryo cytoplasm, since the necessary enzymes are present there [J. C. Egrie and F. H. Wilt, *Biochemistry* **18**, 269 (1979); I. Slater *et al.* (*44*); D. W. Slater, I. Slater, F. J. Bollum, *Dev. Biol.* **63**, 94 (1978)].
44. I. Slater, D. Gillespie, D. W. Slater, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 406 (1973).
45. E. H. Davidson and R. J. Britten, *Science* **204**, 1052 (1979); M. R. Lerner, J. A. Boyle, S. M. Mount, S. L. Wolin, J. A. Steitz, *Nature (London)* **283**, 220 (1980).
46. An example has been provided by M. O. Diaz, G. Barsacchi-Pilone, K. A. Mahon, and J. G. Gall [*Cell* **24**, 649 (1981)], who found that in amphibian oocyte nuclei the normal termination sites of histone genes are ignored, resulting in transcription of RNA's with lengthy 3′ terminal tails including flanking satellite sequences.
47. P.-A. Bedard and B. P. Brandhorst, in preparation.
48. G. A. Galau, W. H. Klein, M. M. Davis, B. J. Wold, R. J. Britten, E. H. Davidson, *Cell* **7**, 487 (1976).
49. G. A. Galau, R. J. Britten, E. H. Davidson, *ibid.* **2**, 9 (1974).

50. Z. Lev, T. L. Thomas, A. S. Lee, R. C. Angerer, R. J. Britten, E. H. Davidson, *Dev. Biol.* **76**, 322 (1980).
51. L. A. Lasky, Z. Lev, J.-H. Xin, R. J. Britten, E. H. Davidson, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5317 (1980). In this report it was pointed out that two-dimensional gel analysis of newly synthesized embryo proteins detects the products of synthesis of those embryo transcripts present at $\geq 10^4$ transcripts per embryo (ten per late embryo cell on the average), since there are about 10^3 polypeptides resolvable and about this same number of diverse moderate and high prevalence RNA species.
52. C. N. Flytzanis, B. P. Brandhorst, R. J. Britten, and E. H. Davidson (*Dev. Biol.*, in press) noted that most prevalent poly(A) RNA species in either egg or embryo are mitochondrial in origin (*20*). These sequences were not considered in their discussion, which refers only to RNA's coded in the nuclear genome.
53. A. M. Bruskin, A. L. Tyner, D. E. Wells, R. M. Showman, W. H. Klein, *Dev. Biol.* **87**, 308 (1981); A. M. Bruskin, P.-A. Bedard, A. L. Tyner, R. M. Showman, B. P. Brandhorst, W. H. Klein, *ibid.*, in preparation.
54. G. W. Shepherd and M. Nemer, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4653 (1980); in addition, unpublished RNA excess hybridization experiments of B. Wold (author's laboratory) with selected single-copy tracer showed a small component of new sequences at the blastula stage.
55. G. Piperno, B. Huang, D. J. L. Luck, *ibid.* **74**, 1600 (1977); J. C. Regier, G. D. Mazur, F. C. Kafatos, *Dev. Biol.* **76**, 286 (1980); W. B. Wood and H. R. Revel, *Bacteriol. Rev.* **40**, 847 (1976).
56. T. Bibring and J. Baxandall, *Dev. Biol.* **83**, 122 (1981).
57. J.-H. Xin, B. P. Brandhorst, R. J. Britten, E. H. Davidson, *ibid.* **89**, 527 (1982).
58. G. A. Galau, E. D. Lipson, R. J. Britten, E. H. Davidson, *Cell* **10**, 415 (1977).
59. B. P. Brandhorst and T. Humphreys, *Biochemistry* **10**, 877 (1971).
60. S. Busby and A. Bakken, *Chromosoma* **71**, 249 (1979); *ibid.* **79**, 85 (1980).
61. A. I. Aronson and K. Chen [*Dev. Biol.* **59**, 39 (1977)] showed that in sea urchin nuclei the polymerase translocation rate is six to nine nucleotides per second. For comparison, the cloned poly(A) RNA sequence studied by Cabrera *et al.* (*40*) that enters the cytoplasm most rapidly crosses the nuclear boundary of a typical cell about every 6 minutes. Since the cell is diploid, this would require an initiation rate about half the average value calculated for the multiple transcription units (*60*).
62. M. Nemer *et al.*, *Dev. Genet.* **1**, 151 (1979).
63. B. R. Hough, M. J. Smith, R. J. Britten, E. H. Davidson, *Cell* **5**, 291 (1975); E. H. Davidson (*9*), p. 234.
64. B. J. Wold, W. H. Klein, B. R. Hough-Evans, R. J. Britten, E. H. Davidson, *Cell* **14**, 941 (1978).
65. T. L. Thomas, R. J. Britten, E. H. Davidson, in preparation.
66. K. C. Kleene and T. Humphreys, *Cell* **12**, 143 (1977); S. G. Ernst, R. J. Britten, E. H. Davidson, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2209 (1979).
67. T. T. Kawabe, P. B. Armstrong, E. G. Pollock, *Dev. Biol.* **85**, 509 (1981); G. Chow and S. C. Benson, *Exp. Cell Res.* **124**, 451 (1979); A. C. Rapraeger and D. Epel, *Dev. Biol.* **88**, 269 (1981); C. G. Glabe and V. D. Vacquier, *J. Cell Biol.* **75**, 410 (1977).
68. H. Noll, V. Matranga, D. Cascino, L. Vittorelli, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 288 (1979).
69. J. K. Griffith, B. B. Griffith, T. Humphreys, *Dev. Biol.* **87**, 220 (1981); J. K. Griffith and T. Humphreys, *Biochemistry* **18**, 2178 (1979).
70. For *Sp*, see C. P. Emerson and T. Humphreys, *Dev. Biol.* **23**, 86 (1970) and G. A. Galau *et al.* (*58*); for *Lp*, see T. Humphreys, in *Developmental Regulation, Aspects of Cell Differentiation*, S. J. Coward, Ed. (Academic Press, New York, 1973), p. 1, and S. Surrey, I. Ginzburg, M. Nemer, *Dev. Biol.* **71**, 83 (1979).
71. R. E. Kane, *J. Cell Biol.* **66**, 305 (1975).
72. L. H. Kedes, *Annu. Rev. Biochem.* **48**, 837 (1979); C. C. Hentschel and M. L. Birnstiel, *Cell* **25**, 301 (1981).
73. R. E. Maxson and F. H. Wilt, *Dev. Biol.* **83**, 380 (1981).
74. _____, *ibid.*, in press.
75. R. E. Maxson, T. J. Mohun, G. Gormezano, L. H. Kedes, in preparation.
76. O. Kuhn and F. H. Wilt, *Dev. Biol.* **85**, 416 (1981).
77. W. H. Rogers and P. R. Gross, *Cell* **14**, 279 (1978).
78. S. G. Ernst, B. R. Hough-Evans, R. J. Britten, E. H. Davidson, *Dev. Biol.* **79**, 119 (1980).
79. F. Tufaro and B. P. Brandhorst, *ibid.* **72**, 390 (1979).
80. D. R. Senger and P. R. Gross, *ibid.* **65**, 404 (1978).
81. T. Kitajima and K. Okazaki, *Dev. Growth Differ.* **22**, 265 (1980); K. Okazaki, *Am. Zool.* **15**, 567 (1975); M. A. Harkey and A. H. Whiteley, *Wilhelm Roux Arch. Entwicklungsmech. Org.* **189**, 111 (1980).
82. R. Hutchins and B. P. Brandhorst, *Wilhelm Roux Arch. Entwicklungsmech. Org.* **186**, 95 (1979).
83. G. Giudice, *Dev. Biol.* **5**, 402 (1962); _____ and G. Millonig, *ibid.* **15**, 91 (1967).
84. T. E. Schroeder, *ibid.* **79**, 428 (1980); *Exp. Cell Res.* **128**, 490 (1980).
85. J. Posakony, unpublished experiment in E. H. Davidson's laboratory.
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Fluorides and the Changing Prevalence of Dental Caries

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Dental caries is a disease associated with increasing acculturation. Along with crowding and pollution, it is one of the prices we have paid for our social and industrial development. Dental caries was not prevalent in primitive societies apparently because their diets lacked easily fermentable carbohydrates. Although caries is clearly a disease with multiple causes (*1*), the principal mode of caries initiation is acid dissolution of tooth enamel. This acid is produced by several different microorganisms, most notably *Streptococcus mutans*, with fermentable carbohydrates, especially sucrose, as the nutrient source. Although this is a simplification of a complex process, availability of sucrose in the diet is clearly the key factor in dental caries initiation.

Theoretically, the dental caries process can be interrupted or terminated in one of three ways: (i) reduction in the numbers of cariogenic bacteria or disruption of their ability to metabolize fermentable carbohydrates, (ii) dietary control of carbohydrate intake, or (iii) enhancement of the ability of tooth structure to withstand acid dissolution.

To reduce or disrupt the action of cariogenic bacteria, various antimicrobial agents have been incorporated in dentifrices and mouth rinses, and improved methods of personal oral hygiene have been recommended. A great deal of effort is also being placed on the development of an effective vaccine. Since the role of carbohydrates is well understood, it would seem relatively straightforward to restrict sucrose in the diet and substitute noncariogenic sweeteners. Both of these approaches to controlling the dental caries process, however, have serious limitations, which have already been described (*2*). It is the third approach, enhancing the ability of the tooth structure to withstand acid dissolution particularly through the use of fluorides, which has been most successful and which I will discuss in this article.

Fluorides for Dental Caries Prevention

Fluoride therapy as a means of preventing dental caries has been used since 1945, when the fluoride concentrations of community water supplies in two U.S. cities and one Canadian city were adjusted upward to 1 part of fluoride ion per 1 million parts of water (*3*, *4*). Naturally fluoridated water supplies had been protecting those who drank it for generations, but it was only in the late 1930's, with improved microanalytical techniques and large-scale epidemiological

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