DEVELOPMENTAL BIOLOGY 100, 190–196 (1983)

Transcripts of Paternal and Maternal Actin Gene Alleles Are Present in Interspecific Sea Urchin Embyro Hybrids

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Received March 22, 1983; accepted in revised form May 23, 1983

Analysis of actin-coding RNAs in interspecific hybrid sea urchin embryos of *Strongylocentrotus purpuratus* and *Lytechinus variegatus*, and *S. purpuratus* and *S. droebachienis* has revealed the presence of transcripts from both paternal and maternal *S. purpuratus* actin gene alleles. In the *L. variegatus* ♀ × *S. purpuratus* ♂ embryos transcripts from at least two different paternal actin gene alleles are present in both the blastula and prism stages. In the reciprocal *S. purpuratus* ♀ × *L. variegatus* ♂ embryos, the same two maternal (*S. purpuratus*) alleles were also expressed as RNA in blastula. The *S. droebachiensis* ♀ × *S. purpuratus* ♂ embryos appear to contain transcripts from at least one paternal actin gene allele at the blastula stage. The paternally derived actin-coding RNAs are the same size as the mature actin mRNAs expressed in normal *S. purpuratus* embyros. Since all known *S. purpuratus* actin genes contain at least two introns, the paternal alleles are not only transcribed in the hybrid embryos, but also the primary transcripts are probably processed to mature mRNA. An explanation of the diversity of observations in the literature on paternal genome expression in hybrid sea urchin embryos is discussed.

INTRODUCTION

Echinoid interspecies hybrid embryos display a complex and mystifying mixture of expression of the maternal and paternal genomes, with the maternal genome generally dominating early embryonic gene expression. While in some hybrids this maternal dominance may be due to a selective loss of portions or all of the paternal genome, Tufaro and Brandhorst (1982) have shown that this is not the case in a cross between the distantly related species, *S. purpuratus* and *Lytechinus pictus*. Since it is generally accepted that early embryonic development is highly dependent on stored maternal gene products, it is not surprising that detection of paternal gene products is somewhat difficult. Paternal genome expression is, however, documentable in both early and late embryos, demonstrating the potential for transcriptional activation of these alleles. The detection of paternal histone mRNA in hybrid 2-cell embryos by Maxson and Egrie (1980) verifies that paternal alleles can be transcribed quite early in hybrid embryos. In this case they found similar amounts of maternal and paternal histone messages in the embryo and that paternal message was on polysomes by the 16-cell stage. Furthermore, it has been demonstrated that male pronuclei of fertilized sea urchin eggs synthesize RNA (Longo and Kunkle, 1977), indicating that early paternal genome transcription is not an aberration of hybrid embryos. Several examples of paternal genome expression in later stage hybrid sea urchin embryos have also been detected using a variety of approaches. In crosses between *Arbacia lixula, Paracentrotus lividus*, and *Psammechinus microtuberculatus*, Harding *et al.*, (1954) detected paternal antigens as early as late blastula. Assay of aryl sulfatase activity in hybrids of *S. purpuratus* and *Allocentrotus fragilis* demonstrated the presence of paternally derived enzyme by mesenchyme blastula (Fedecka-Bruner *et al.*, 1971). Using a cell adhesion assay McClay and Hausman (1975) found that paternal genes affecting cell surface adhesiveness were being utilized by the mesenchyme blastula stage in hybrids of *L. variegatus* and *Tripneustes esculentus*. Most recently Tufaro and Brandhorst (1982) have shown, by two-dimensional gel analysis, that some distinctly paternal proteins are synthesized in hatching blastula and pluteus stages in hybrids of *S. purpuratus* and *S. droebachiensis*, and *S. purpuratus* and *L. pictus*. In this case only 2 to 7% of the protein spots that were distinguishable between the parental species showed paternal expression.

While the number of examples of paternal genome expression in hybrid embryos is limited, there can be little doubt that the paternal genome is active in most if not all hybrids. An understanding of the degree to which the paternal genes are correctly activated and regulated in hybrid embryos will be important to analysis of the activation of embryonic gene expression. If particular paternal alleles are appropriately regulated in hybrid embryos, then control of embryonic expression of these alleles must either be predetermined in the sperm genome or result from the interaction of con-

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0012-1606/83 $3.00
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served or nonspecific effectors. By specifically examining expressed paternal alleles in hybrid embryos, it should be possible to identify which of these categories of mechanisms are important in controlling their expression.

To begin an examination of the expression of the paternal genome in sea urchin embryo hybrids, we have assayed for the presence of paternally derived actin-coding RNAs in hybrid embryos of *S. purpuratus* and *S. droebachiensis*, and *S. purpuratus* and *L. variegatus*. In this report we use the term expression to mean the presence of actin-coding RNA. This is not meant to imply that these RNAs are translated into protein, since we have no evidence on this point. This analysis focuses on the expression of two actin messages, which normally accumulate from 10- to 20-fold during *S. purpuratus* embryogenesis (Crain *et al.*, 1981). Both of these developmentally regulated RNAs are expressed at blastula and prism stages in *L. variegatus* ♀ × *S. purpuratus* ♂ embryos and at least one is present at the blastula stage of *S. droebachiensis* ♀ × *S. purpuratus* ♂ embryos. The paternal genes which encode these RNAs must thus be transcribed in the hybrid embryos. Furthermore, since the RNAs detected are the size of *S. purpuratus* actin mRNA, it is probable that the primary transcripts are processed into mature mRNA in the hybrid embryos.

MATERIALS AND METHODS

Living materials. Animals were induced to shed gametes by injecting 1–5 ml 0.5 *M* KCl into the coelomic cavity. Fertilization between *L. variegatus* and *S. purpuratus* generally did not proceed spontaneously, although individual females occasionally showed a substantially higher percentage of fertilization than the average. To routinely obtain *L. variegatus* ♀ × *S. purpuratus* ♂ and *S. purpuratus* ♀ × *L. variegatus* ♂ embryos, eggs were aged overnight in sea water containing 10–25 units/ml penicillin (22°C for *L. variegatus*, 4°C for *S. purpuratus*), dejellied by four to eight passes through 102 μM Nitex mesh, washed, soaked in egg jelly from the heterologous species for about 5 min, then fertilized with one to two drops of undiluted heterologous sperm (Harvey, 1956). This procedure yielded 10–70% fertilization for *S. purpuratus* ♀ × *L. variegatus* ♂ hybrids and 15–27% fertilization for *L. variegatus* ♀ × *S. purpuratus* ♂ hybrids. Unfertilized *L. variegatus* eggs decomposed rapidly in embryo cultures and by 48 hr after fertilization were present only as debris. To obtain *S. droebachiensis* ♀ × *S. purpuratus* ♂ embryos, *S. droebachiensis* eggs were washed several times and then fertilized with two drops of undiluted *S. purpuratus* sperm, routinely yielding 90–100% fertilization. The *S. purpuratus* ♀ × *S. droebachiensis* ♂ reciprocal cross yielded very low percent fertilization spontaneously. Hybrid embryos were obtained by aging *S. purpuratus* eggs overnight at 4°C in sea water containing 10 u/ml penicillin, dejellying eggs by four to eight passes through 102 μM Nitex mesh, adding *S. droebachiensis* egg jelly to *S. purpuratus* eggs, and then adding one to two drops fresh *S. droebachiensis* sperm. This procedure yielded greater than 50% fertilization in the *S. purpuratus* ♀ × *S. droebachiensis* ♂ cross. Hybrid embryos were grown at the maternal temperature (Bushman and Crain, 1983) in Millipore-filtered sea water containing 20 (*S. droebachiensis* crosses) to 80 (*L. variegatus* crosses) u/ml penicillin. When harvesting embryos for RNA isolation, active developing embryos were decanted away from settled unfertilized material.

RNA isolation and blotting experiments. RNA was extracted using the procedure of Chirgwin *et al.* (1979). RNA blotting experiments were conducted essentially as described by Thomas (1980), using modifications described by Bushman and Crain (1983). Ten-microgram samples were loaded into each lane, unless otherwise noted.

Preparation of ^{32}P-labeled probes. Two different hybridization probes were used. In some experiments the entire recombinant plasmid pSpG17, which contains a complete actin-coding sequence, was used (Cooper and Crain, 1982; Durica *et al.*, 1980). The protein-coding sequence should recognize any actin-coding RNA from these species. In the other experiments a *Bst*EII-*Hin*dIII restriction fragment of pSpG17, which is known to contain untranslated 3′ actin mRNA sequence, was used (Cooper and Crain, 1982; Crain *et al.*, 1982). This sequence is present only once in the haploid *S. purpuratus* genome (Paz-Aliaga and Crain, in preparation). It has also been found that a related sequence is present in the *S. droebachiensis* genome (Bushman and Crain, 1983). Both probes were nick translated using three α-^{32}P-labeled nucleotides as described by Rigby *et al.* (1977).

RESULTS

Expression of Paternal and Maternal Alleles of at Least Two Actin Genes in Hybrid Embryos of S. purpuratus and L. variegatus

Reciprocal crosses of *S. purpuratus* and *L. variegatus* were carried out as described under Materials and Methods. To obtain fertilization of eggs of one species with sperm of the heterologous species it was necessary, in both cases, to age the eggs in sea water and pretreat them with egg jelly of the other species. While fertilization ranged from 10 to 70% the embryos which were finally harvested were mostly at the desired developmental stage. This was the case because many of the unfertilized eggs degraded with time, and most of those which remained were removed by allowing them to settle

away from the actively swimming embryos. When *L. variegatus* eggs were fertilized with *S. purpuratus* sperm, the embryos developed at least through the prism stage. Development of the reciprocal hybrids, *S. purpuratus* eggs fertilized with *L. variegatus* sperm, arrested during early gastrula.

L. variegatus embryos normally express only a 2.2-kb size class of actin-coding RNA (Bushman and Crain, 1983), whereas *S. purpuratus* embryos express both a 2.2- and 1.8-kb actin mRNA (Crain *et al.*, 1981). We therefore initially asked whether a 1.8-kb actin-coding RNA was present in *L. variegatus* ♀ × *S. purpuratus* ♂ hybrid embryos. The appearance of this actin-coding RNA class should be diagnostic for expression of at least one paternal *S. purpuratus* actin gene allele. Blot analysis of RNA from blastula- and prism-stage hybrid embryos clearly demonstrates expression of the 1.8-kb *S. purpuratus* actin mRNA (Fig. 1A). To ask whether there is a maternal contribution of actin-coding RNA in hybrids of these two species, RNA from the reciprocal cross (*S. purpuratus* ♀ × *L. variegatus* ♂) was analyzed. Blastula RNA from the hybrid embryos contains the 1.8-kb size class, which is characteristic of *S. purpuratus*

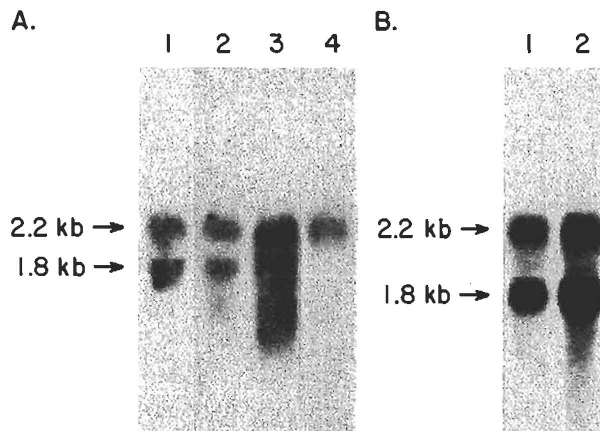

FIG. 1. Expression of 1.8-kb *S. purpuratus* actin mRNA in *L. variegatus* × *S. purpuratus* hybrid embryos. RNA blot analysis was performed using the entire *S. purpuratus* actin-gene-containing plasmid, pSpG17, as a hybridization probe. Each lane contains 10 μg of RNA. (A) Lane 1, *S. purpuratus* blastula; lane 2, *L. variegatus* ♀ × *S. purpuratus* ♂ blastula; lane 3, *L. variegatus* ♀ × *S. purpuratus* ♂ prism; lane 4, *L. variegatus* pluteus. Lanes 1–3 are from the same gel (lane 1, 17 hr exposure; lanes 2 and 3, 3 days exposure). Lane 4, from another gel, illustrates the single *L. variegatus* actin-coding RNA size class and is aligned by standards common to both gels. (B) Lane 1, *S. purpuratus* ♀ × *L. variegatus* ♂ blastula; lane 2, *S. purpuratus* blastula. For the lanes in panel B, the amount of radioactivity in each RNA class was determined by cutting the bands from the nitrocellulose and counting by liquid scintillation as described in Crain *et al.* (1981). *S. purpuratus* ♀ × *L. variegatus* ♂ blastula (lane 1) 1.8 kb—67 cpm, 2.2 kb—57 cpm; *S. purpuratus* blastula (lane 2) 1.8 kb—133 cpm, 2.2 kb—90 cpm.

embryos (Fig. 1B). It is interesting to note that the amount of radioactivity in the 1.8-kb band in the hybrid RNA is approximately one-half that in normal *S. purpuratus* blastula RNA (see legend to Figure 1). This would be expected if the maternal genome normally contributes approximately one-half of this RNA class and makes a similar contribution in the hybrids. Furthermore, the 2.2-kb RNA in the hybrids is 25–30% greater than one-half of the level found in normal blastula. This result is consistent with normal maternal contribution plus additional 2.2-kb paternal *L. variegatus* RNA. These results suggest that normal levels of maternally derived actin mRNA are present in the hybrid embryos. Since the relative abundance of these RNA classes in normal *S. purpuratus* blastula stage embryos is nearly 10- and 25-fold greater than in unfertilized eggs (Crain *et al.*, 1981), the majority of these RNAs in the hybrid must result from newly synthesized RNA rather than stable maternal actin-coding RNA.

The experiments shown in Fig. 1 demonstrate the expression of a 1.8-kb *S. purpuratus* actin-coding RNA in the hybrid embryos. We know that actin messages in the 2.2-kb, and 1.8-kb classes derive from different actin genes (Crain *et al.*, 1982; Ernst *et al.*, submitted for publication) and thus wanted to assay for expression of a second embryonically expressed actin gene, which produces a 2.2-kb actin mRNA. In this case we analyzed RNA from hybrid embryos using as a hybridization probe a 3′ untranslated message sequence from the actin gene in the recombinant plasmid pSpG17. This untranslated sequence is present once per haploid *S. purpuratus* genome, and therefore recognizes the expression of this single actin gene (Paz-Aliaga and Crain, in preparation). RNA from normal *L. variegatus* gastrula stage embryos shows little or no hybridization with this probe, whereas *L. variegatus* ♀ × *S. purpuratus* ♂ embryos express reactable RNA at both blastula and prism stages (Fig. 2A). We conclude then, that the 3′ untranslated sequence from this *S. purpuratus* actin gene does not react with an expressed RNA in *L. variegatus* embryos, and that the paternal *S. purpuratus* allele is expressed in these hybrid embryos. Since these two species may have diverged as long ago as 180 million years (Durham, 1966), the lack of cross-reaction of the non-protein-coding message sequence is not surprising. In the reciprocal cross (*S. purpuratus* ♀ × *L. variegatus* ♂) we again see expression of the maternal allele in blastula-stage embryos (Fig. 2B), further indicating that expression of the maternal genome is not repressed in the hybrids. As was seen for the 1.8-kb actin-coding RNA, the amount of this particular message in the hybrid is approximately one-half that in the normal embryo, suggesting that the normal maternal contribution is about one-half of the message.

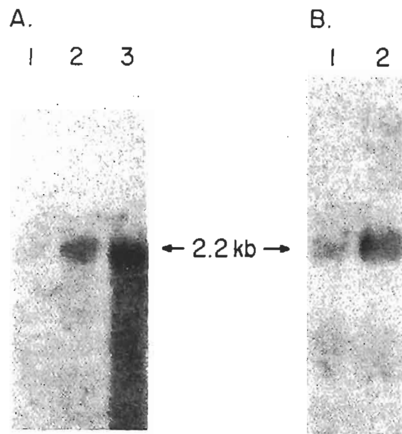

FIG. 2. Expression of an *S. purpuratus* 2.2-kb actin-coding RNA in *L. variegatus* × *S. purpuratus* hybrid embryos. The blotted RNAs were hybridized with a *Bst*EII–*Hin*dIII fragment of the *S. purpuratus* actin clone pSpG17, which contains untranslated message sequence specific to this gene. (A) Lane 1, *L. variegatus* gastrula (10 μg); lane 2, *L. variegatus* ♀ × *S. purpuratus* ♂ blastula (10 μg); lane 3, *L. variegatus* ♀ × *S. purpuratus* ♂ prism (10 μg). (B) Lane 1, *S. purpuratus* ♀ × *L. variegatus* ♂ blastula (10 μg), lane 2, *S. purpuratus* blastula (13 μg). The 2.2-kb bands in panel B were quantitated by densitometric scanning, values were corrected for differential loading, and the ratio of *S. purpuratus* blastula to *S. purpuratus* ♀ × *L. variegatus* ♂ blastula was found to be 1.7.

Expression of a Paternal Actin Gene Allele in S. purpuratus × S. droebachiensis Hybrid Embryos

We constructed reciprocal crosses between the closely related *S. purpuratus* and *S. droebachiensis* species (divergence time, about 10 million years ago) and analyzed RNA from the hybrid embryos at several stages. Fertilization of *S. droebachiensis* eggs with *S. purpuratus* sperm required no special handling, and the embryos developed through at least the prism stage. The reciprocal fertilization (*S. purpuratus* eggs with *S. droebachiensis* sperm) was more difficult, requiring the eggs to be aged and pretreated with *S. droebachiensis* egg jelly. Development of these embryos arrested at blastula. *S. purpuratus* and *S. droebachiensis* each express two actin-coding RNA classes in early development, of similar but slightly different sizes (Bushman and Crain, 1983). One easily detectable difference in the embryonic expression of the actin messages in these two species is that the larger class (2.2 kb in *S. purpuratus* and 2.1 kb in *S. droebachiensis*) accumulates to a high level by the blastula stage in *S. purpuratus* (Crain *et al.*, 1981), and not until gastrula in *S. droebachiensis* (Bushman and Crain, 1983). Comparison of the actin-coding RNA in normal *S. droebachiensis* blastula and gastrula stage embryos with that of *S. droebachiensis* ♀ × *S. purpuratus* ♂ hybrids, at the same stages, reveals an abundant 2.2-kb class in the hybrid blastula, which is absent from normal *S. droebachiensis* embryos at this stage (Fig. 3). The most probable source of this message is the paternal *S. purpuratus* genome. Furthermore, by gastrula the width of the lower band in the hybrid embryo RNA appears broader than either the *S. purpuratus* or *S. droebachiensis* bands alone, and thus appears to be composed of message from both species. It is thus likely that at least two paternal actin gene alleles are being expressed in these hybrids.

To specifically examine the expression of the *S. purpuratus* actin gene pSpG17 in these hybrids, RNA was analyzed by blotting with the 3′ untranslated message sequence described in the previous section (Fig. 4). This particular hybridization probe recognizes the large actin-coding RNA in late stage *S. droebachiensis* embryos (Bushman and Crain, 1983). Comparison of blastula RNA from the hybrid *S. droebachiensis* ♀ × *S. purpuratus* ♂ embryos (Fig. 4, lane 3) with normal *S. droebachiensis* embryos (Fig. 4, lane 6) shows that the hybrid expresses a 2.1- to 2.2-kb actin-coding RNA, while this RNA is not detectable in normal *S. droebachiensis* embryos. This particular *S. purpuratus* actin gene allele, which codes for an mRNA which has accumulated in normal *S. purpuratus* blastula, therefore seems to be producing a stable RNA, of message size, in hybrid blastula. Both gastrula- and prism-stage hybrids also contain a hybridizing RNA of about 2.2 kb (Fig. 4, lanes 4, 5). This, however, is not surprising since embryos of both species contain this message by these stages. Blastula RNA from the reciprocal hybrid, *S. purpuratus* ♀ × *S. droebachiensis* ♂, also contains the 2.2-kb RNA (Fig. 4, lane 2). In this experiment the intensity of the signals was not quantitated, but visual comparison of the *S. pur-*

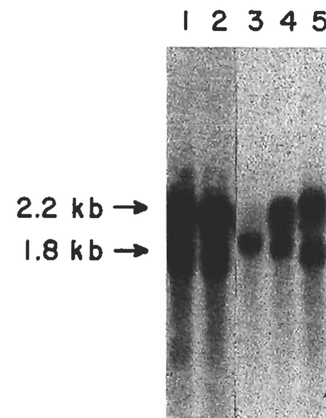

FIG. 3. Analysis of actin-coding RNA expression in *S. droebachiensis* ♀ × *S. purpuratus* ♂ hybrid embryos. The hybridization probe in this RNA blot experiment was the entire actin-gene-containing plasmid pSpG17, which should recognize all actin-coding RNA. A 10-μg sample of RNA was loaded in each lane. Lane 1, *S. droebachiensis* ♀ × *S. purpuratus* ♂ blastula; lane 2, *S. droebachiensis* ♀ × *S. purpuratus* ♂ gastrula; lane 3, *S. droebachiensis* blastula; lane 4, *S. droebachiensis* gastrula; lane 5, *S. purpuratus* blastula.

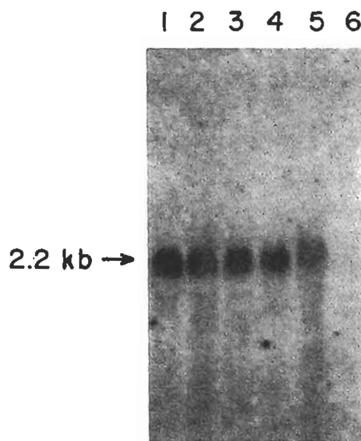

FIG. 4. Expression of a 2.2-kb actin-coding RNA in *S. purpuratus* × *S. droebachiensis* hybrid embryos. The hybridization probe for this RNA blot was a 3′ untranslated actin mRNA sequence from the *S. purpuratus* actin-gene clone pSpG17. Ten micrograms of RNA was loaded into each lane. Lane 1, *S. purpuratus* blastula; lane 2, *S. purpuratus* ♀ × *S. droebachiensis* ♂ blastula; lane 3, *S. droebachiensis* ♀ × *S. purpuratus* ♂ blastula; lane 4, *S. droebachiensis* ♀ × *S. purpuratus* ♂ gastrula; lane 5, *S. droebachiensis* ♀ × *S. purpuratus* ♂ prism; lane 6, *S. droebachiensis* blastula.

puratus ♀ × *S. droebachiensis* ♂ blastula band with the normal *S. purpuratus* blastula band (Fig. 4, lane 1) indicates a roughly similar intensity. This suggests either that the maternal contribution of this RNA in the hybrid is almost twice that in the normal (apparently in contrast to the hybrids between *S. purpuratus* and *L. variegatus*) or that the paternal *S. droebachiensis* allele is activated early in the hybrid. These alternatives cannot yet be distinguished, but are of interest for further study.

DISCUSSION

Four interspecific sea urchin hybrid crosses were constructed. Two developed through at least the late prism stage (*L. variegatus* ♀ × *S. purpuratus* ♂; *S. droebachiensis* ♀ × *S. purpuratus* ♂), one arrested in the gastrula stage (*S. purpuratus* ♀ × *L. variegatus* ♂), and one arrested at the blastula stage (*S. purpuratus* ♀ × *S. droebachiensis* ♂). The *S. purpuratus* ♀ × *S. droebachiensis* ♂ hybrid has been reported by others to develop to pluteus (Griffiths, 1965; Tufaro and Brandhorst, 1982). While we cannot explain this difference, we as others (Griffiths, 1965) had considerable difficulty obtaining efficient fertilization of *S. purpuratus* eggs with *S. droebachiensis* sperm. Furthermore, we noted considerable animal-to-animal variation in the efficiency of fertilization in each of these crosses. This type of variation in the formation of sea urchin embryo hybrids has been noted previously (Chen and Baltzer, 1975). It is possible that the particular sets of animals which were available were less compatible than usual. In any regard, each of these crosses yielded hybrid embryos which displayed substantial differentiation and development, as assessed by morphological criteria.

In the reciprocal crosses described here, between *S. purpuratus* and *L. variegatus*, and *S. purpuratus* and *S. droebachiensis*, there is evidence of accumulation of actin-coding RNA from both parental genomes. This is particularly clear in the *S. purpuratus* × *L. variegatus* hybrids, where the *S. purpuratus* actin messages can be distinguished by size and by hybridization with a 3′ untranslated mRNA sequence. In the *S. purpuratus* × *S. droebachiensis* embryos this also seems to be true, but the relative contribution of the two genomes is more difficult to assess. *L. variegatus* ♀ × *S. purpuratus* ♂ embryos contain actin-coding RNAs from at least two paternal alleles at the blastula and prism stages. Each of the RNAs which were detected, the 2.2-kb RNA (from actin gene, pSpG17) and the 1.8-kb class, are normally expressed in blastula and later stages in *S. purpuratus* at relatively high levels compared to eggs and early embryos (Crain *et al.*, 1981; Merlino *et al.*, 1981; Scheller *et al.*, 1981). A major 2.2-kb actin-coding RNA size class, which could contain multiple actin mRNAs, also accumulates after cleavage in *L. variegatus* (Bushman and Crain, 1983). Transcription and accumulation of these paternally derived actin-coding RNAs in the late embryo would thus be expected if expression of these genes is being regulated in an approximately normal manner in the hybrid embryos. Regardless of whether the timing of the expression of these genes in the hybrids is precisely correct, they are transcriptionally active and produce stable RNA products of mature mRNA size.

Normally in *S. droebachiensis* embryos, the larger actin-coding RNA class (2.1 kb) does not begin to accumulate detectably until approximately early gastrula. At least one actin-coding RNA in this class is transcribed from a gene closely related to the single *S. purpuratus* actin gene pSpG17 (Bushman and Crain, 1983). In blastula stage *S. droebachiensis* ♀ × *S. purpuratus* ♂ hybrid embryos a 2.1- to 2.2-kb RNA is present at a level similar to that seen in normal *S. purpuratus* embryos. Since the *S. droebachiensis* message is not normally detectable in this stage, this RNA probably derives from the paternal *S. purpuratus* allele. This suggests the fascinating possibility that the expression of the paternal *S. purpuratus* gene in the hybrid embryos is regulated according to the *S. purpuratus* program. An alternate possibility, which is not ruled out by these experiments, is that in the hybrid embryo this gene may be transcriptionally activated prematurely, in a rather nonspecific manner which is not characteristic of the *S. purpuratus* program. We hope to clarify this issue using hybrids between more distantly related species, where the maternal and paternal alleles are more easily distinguished.

Tufaro and Brandhorst (1982) analyzed the synthesis of about 500 proteins in three sea urchin embryo hybrids. In this study they could distinguish the synthesis of 60 to 130 paternal-species proteins in the different hybrids, but found paternal expression of only 2–7% of these proteins. They could not assay synthesis of the remaining paternal proteins because of comigration of both parental gene products. Actins fall into the category of proteins which they were unable to distinguish. That is, since the actin proteins are highly conserved it is impossible to distinguish those which derive from the parental species by gel electrophoresis of the proteins. In our case, the increased sensitivity, resulting from analysis of actin-coding RNAs, demonstrates paternal allele expression which is undetectable at the protein level. The fraction of the paternal genome expression which is masked among the conserved proteins cannot be determined from the available data. An interesting possibility is that expression of the most highly conserved proteins is most likely to be properly regulated in hybrid embryos. This is not improbable if we consider that the most conserved proteins may have highly conserved and universal functions, and therefore the pattern and mechanisms of their regulation might be most conserved.

Based on these observations with actin genes and other data in the literature, we propose that there are two levels of transcriptional regulation of gene expression in early embryonic development in sea urchins. According to this hypothesis, these two categories (levels) of regulation of embryonic gene transcription differ in the timing of their determination, and possibly in their mechanism. The first category, which we refer to as "primary level regulation," would be essentially locked into both the maternal and paternal genomes at the time of fertilization. Genes falling into this category would be determined to be expressed in early embryogenesis without interaction with highly specific factors in the embryo, although certain general factors such as pH or ionic strength might be important to their activation. Possible mechanisms for the prefertilization determination of their expression could be DNA methylation patterns in the gamete genomes, or properties of the DNA sequence near the genes which cause them to be assembled into transcriptionally active chromatin in virtually all nongamete cells. Genes which would be predicted to fall into this category would be those whose products are important for universal or housekeeping-type functions such as cytoplasmic actins, histones, tubulins, etc. Since their expression would be determined before fertilization and dependent on, at most, nonspecific embryonic factors, their expression should proceed fairly normally in most hybrid embryos. The two sets of genes examined in hybrid sea urchin embryos which would be expected to fall into this category, actins and histones, clearly show paternal expression. The second category, which we call "secondary level regulation," would include genes whose expression is regulated as a result of interaction with specific factors in the embryo. Such factors would probably be newly synthesized in the embryos, deriving either from stable maternal mRNA or from primary-level-regulation products, or both. Because expression of this category of genes would be regulated in a more complicated and specific manner, the probability of their paternal alleles being properly expressed in hybrid embryos would be far less than that of the primary-level genes. We suggest that the majority of different proteins synthesized in an embryo fall into the second category, and thus have a low probability of correct expression. This could account for the observed low percentage of detection of paternal allele expression in hybrid embryos, and for the apparent accurate expression of some genes such as actin and histone genes. According to this hypothesis, other genes which encode products whose function is essential to all cells (e.g., tubulin), should show paternal allele expression in viable hybrid embryos.

We thank Susan G. Ernst for comments on the manuscript. This research was supported by NIH grants to W.R.C., by a Cancer Center Grant, and by funds from the Mimi Aaron Greenberg Memorial Institute.

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