DEVELOPMENTAL BIOLOGY 98, 429–436 (1983)

Conserved Pattern of Embryonic Actin Gene Expression in Several Sea Urchins and a Sand Dollar

FREDERIC D. BUSHMAN[1] AND WILLIAM R. CRAIN, JR.[2]

Cell Biology Group, Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, Massachusetts 01545

Received December 14, 1982; accepted in revised form March 10, 1983

An examination of the size and relative abundance of actin-coding RNA in embryos of four sea urchins (*Strongylocentrotus purpuratus, Strongylocentrotus droebachiensis, Arbacia punctulata, Lytechinus variegatus*) and one sand dollar (*Echinarachnius parma*) reveals a generally conserved program of expression. In each species the relative abundance of these sequences is low in early embryos and begins to rise during late cleavage or blastula stages. In the four sea urchins, actin-coding RNAs increase between approximately 9- and 35-fold by pluteus or an earlier stage, and in the sand dollar about 5.5-fold by blastula. A major actin-coding RNA class of 2.0–2.2 kilobases (kb) is found in each species. A smaller actin-coding RNA class, which accumulates during embryogenesis, is also present in *S. purpuratus* (1.8 kb), *S. droebachiensis* (1.9 kb), and *A. punctulata* (1.6 kb), but apparently absent in *L. variegatus* and *E. parma*. In *S. droebachiensis*, actin-coding RNA is relatively abundant in unfertilized eggs and drops sharply by the 16-cell stage. This is in contrast to the other sea urchins where the actin message content is relatively low in eggs and does not change substantially in the embryos throughout early cleavage. The observations in this study suggest that the pattern of embryonic expression of at least some members of this gene family is ancient and conserved.

INTRODUCTION

Examination of the evolution of gene structure and organization is important to understanding the processes and mechanisms which gave rise to modern genes. The identification of essential features of gene structure should make it possible to make inferences regarding the control of expression of, at least, particular genes. Such structural analyses by themselves will not, however, yield direct information on the evolution of the pattern of expression of genes or gene families, or on the association of their expression with developmental programs of gene expression. If the products of specific genes are involved in essential and phylogenetically conserved functions, their pattern of expression during similar developmental processes might also be conserved among organisms. Furthermore, such genes might be expected to have become associated with particular programs of gene expression early in evolution, so that major features of their expression would be generally similar in divergent species.

It is well established that actins and the genes which encode them are highly conserved among eucaryotic organisms (Pollard and Weihing, 1974; Vandekerckhove and Weber, 1978; Cooper and Crain, 1982), and that they are involved in essential cellular functions which are probably similar in most species (Pollard and Weihing, 1974; Goldman *et al.*, 1976). With these considerations in mind we have examined the accumulation of actin-coding RNA during embryonic development in several echinoderms, whose divergence from each other ranges from approximately 10 to 220 million years (my) ago. The results of RNA blot analysis at several embryonic stages indicate that the actin-coding sequences accumulate similarly in both the closely related and the distantly related species. The observations suggest that the general pattern of embryonic expression of particular members of the actin gene family is conserved between the closely related species, *S. purpuratus* and *S. droebachiensis*. According to the analyses presented here the expression of some actin genes was probably associated with the program of early embryonic development at least 220 my ago.

MATERIALS AND METHODS

Living materials. Embryos were cultured in filtered seawater with stirring, *Echinarachnius parma* (15°C) and *Strongylocentrotus purpuratus* (15°C), or in shallow trays without stirring, *Strongylocentrotus droebachiensis* (4°C), *Lytechinus variegatus* (22°C), and *Arbacia punctulata* (22°C).

RNA blotting experiments. RNAs were isolated as described by Chirgwin *et al.* (1979) and RNA blotting experiments were conducted essentially according to Thomas (1980). Ten micrograms samples were loaded into each lane except where noted. The filters were hybridized 12–18 hr at 66°C in 0.32 *M* sodium phosphate

[1] Present address: The Biological Laboratories, Harvard University, Cambridge, Mass. 02138.

[2] To whom all correspondence should be addressed.

0012-1606/83 $3.00
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buffer, pH 6.8, 0.8 × Denhart's, 0.08% SDS, 0.8 μM of each dNTP, and 10% dextran sulfate. After hybridization, they were rinsed (3×) with 0.24 M sodium phosphate buffer, pH 6.8, 0.1% SDS at room temperature, and then with 2 liters of 0.4 M sodium phosphate buffer, pH 6.8, 0.1% SDS under suction at hybridization temperature. The sizes of the actin-coding RNAs were determined by comparison to ribosomal RNA and to the two *S. purpuratus* actin mRNAs, whose sizes were previously determined (Crain *et al.*, 1981; Scheller *et al.*, 1981).

Preparation of ^{32}P-labeled hybridization probes. Three different hybridization probes were used. The recombinant plasmid pSpG17, which contains an entire *S. purpuratus* actin gene (Cooper and Crain, 1982) and reacts only with actin mRNA (Crain *et al.*, 1981), was used in the time course experiments. Because of the conserved nature of the actin genes, this probe should recognize any actin mRNA from these animals. Two different non-protein-coding *S. purpuratus* probes were used to detect homologous actin-gene transcripts in *S. droebachiensis* embryos: a *Bst*EII–*Hin*dIII fragment from the plasmid pSpG17, which contains 3′-untranslated actin mRNA sequence (Cooper and Crain, 1982; Crain *et al.*, 1982) and is present only once in the *S. purpuratus* genome (Paz-Aliaga and Crain, in preparation); and the cDNA clone pSpec4 (a gift from W. Klein), which contains primarily 3′-untranslated actin message sequence and reacts only with a 1.8-kb actin mRNA in *S. purpuratus* (Bruskin *et al.*, 1981; Crain *et al.*, 1982). These sequences were nick translated with three α-^{32}P-labeled nucleotides as described by Rigby *et al.* (1975).

RESULTS

In this report we compare the developmental time course of accumulation of actin-coding RNA in embryos from the sea urchins *S. purpuratus*, *S. droebachiensis*, *L. variegatus*, *A. punctulata*, and the sand dollar *E. parma*. RNA was extracted from embryos of each species at selected stages of development and then analyzed for the size and relative abundance of actin-coding RNA by blot hybridization. Similar amounts of RNA from each stage were hybridized. Since RNA content per embryo is essentially constant for the stages examined, comparison of hybridization band intensities among lanes reflects the changes in relative abundance per embryo of an actin-coding RNA class. Representative RNA blots and the approximate phylogenetic relationships of these species are shown in Fig. 1. Since the fossil record for sea urchins is incomplete, the depicted phylogeny is subject to some uncertainty. Previous molecular analysis, however, confirmed some of the relationships to a first approximation (Angerer *et al.*, 1976), and it seems likely that the relationships presented are essentially correct.

Accumulation of Actin-Coding RNA in S. purpuratus Embryos

The developmental modulation of actin gene expression in *S. purpuratus* embryos has been previously characterized (Crain *et al.*, 1981, Durica and Crain, 1982). The major features of the accumulation of cytoplasmic actin-coding RNA in this species are briefly reviewed here to facilitate comparison with the other species examined. Two broad size classes of actin-coding mRNA, centered at about 2.2 and 1.8 kb, are detected in cytoplasmic RNA (Fig. 1). In egg (not shown here) and early cleavage embryos the 2.2-kb size class is more abundant, but by the hatching blastula stage (18 hr) the signals have increased 24-fold and 8.8-fold for the 1.8- and 2.2-kb messages, respectively (Table 1), and the smaller message predominates. In blastula stage embryos the hybridization bands are broad, possibly ranging over 250–300 base pairs. Actin-coding mRNAs in the 2.2- and 1.8-kb size classes are known to be the products of different genes (Crain *et al.*, 1982; Ernst, Bushman, and Crain, submitted for publication). At gastrula the actin-coding mRNA abundance is similar to that of blastula (Fig. 1) as previously reported by Merlino *et al.* (1981). This same general pattern of RNA accumulation is also observed for total polysomal, polysomal poly(A)$^+$, and cytoplasmic poly(A)$^+$ RNA fractions (Crain *et al.*, 1981). Adult tissues so far examined show only the 2.2-kb actin mRNA size class, suggesting that the 1.8-kb actin message is embryo specific (Garcia and Crain, unpublished results). Synthesis of actin has been shown to increase approximately in proportion to the increase in actin mRNA abundance during the period of actin mRNA accumulation (Crain *et al.*, 1981; Durica and Crain, 1982). It is thus likely that there is no major translational control of actin synthesis during this interval.

Accumulation of Actin-Coding RNA in S. droebachiensis Embryos

S. purpuratus and *S. droebachiensis* are closely related sea urchin species, being within the family *Strongylocentrotidae* and having diverged from each other about 10 my ago (Durham, 1966; Angerer *et al.*, 1976). This close relationship is reflected, to some extent, by the similarities in the size and abundance of their embryonic actin-coding RNAs (Fig. 1). In *S. droebachiensis* embryos two size classes of actin-coding RNA, which are very similar in size to those of *S. purpuratus*, are detected. The larger is centered at 2.1 kb at stages prior

FIG. based o and Me coding lane; *S.* 16-cell; *variega* figure.

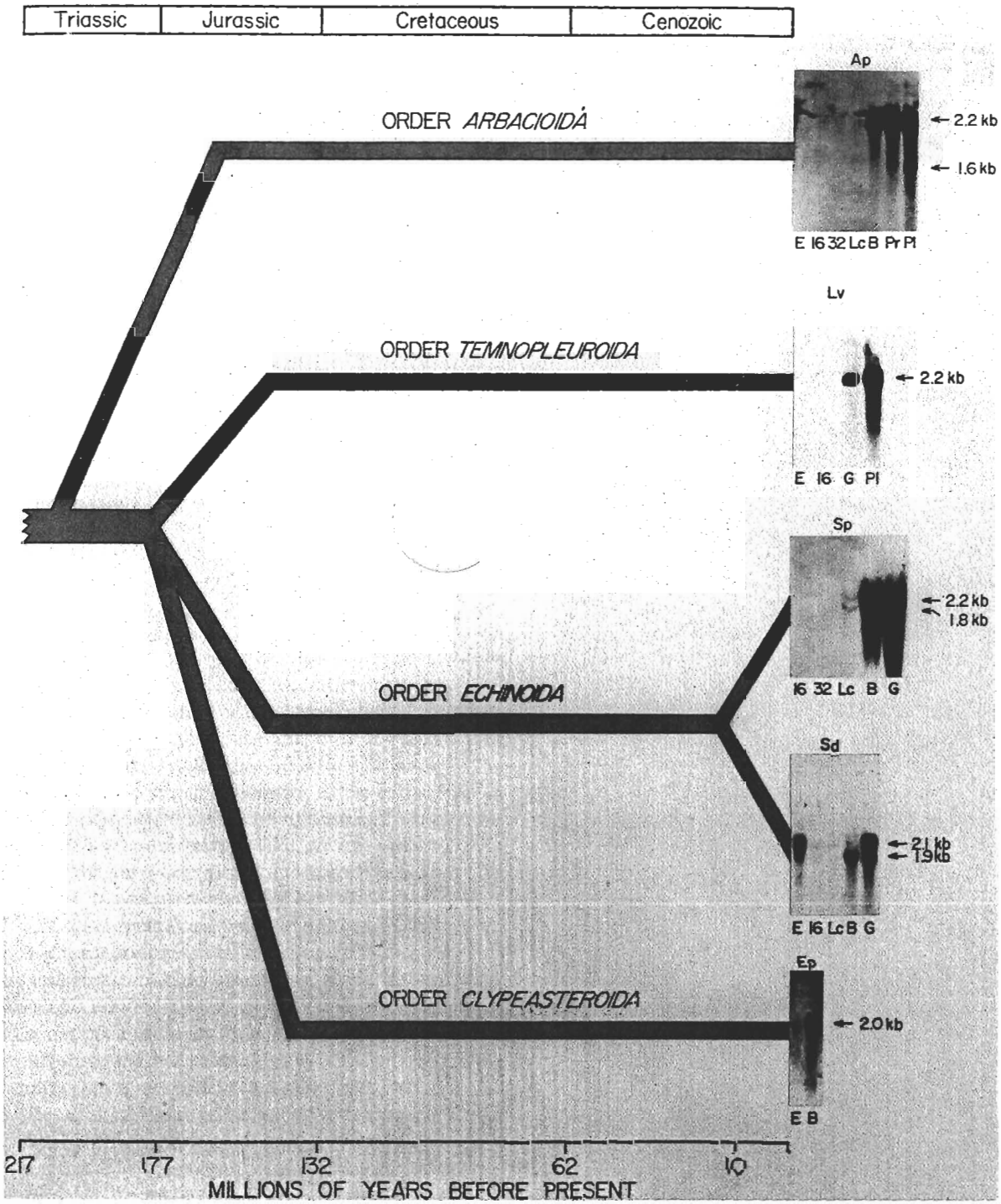

FIG. 1. Embryonic accumulation of actin-coding RNAs and phylogeny of five echinoderms. The depicted phylogenetic relationships are based on Durham (1966) and Angerer *et al.* (1976). Total egg and embryonic RNAs were subjected to blot analysis as described under Materials and Methods except for *S. purpuratus*, in which cytoplasmic RNAs were analyzed. The hybridization probe was the *S. purpuratus* actin-coding clone pSpG17 (Cooper and Crain, 1982). RNA samples were loaded as follows: *S. droebachiensis*, *A. punctulata*, and *E. parma*, 10 μg/lane; *S. purpuratus*, 30 μg/lane; *L. variegatus*, egg—6 μg, 16-cell—10 μg, gastrula—13 μg, pluteus—14 μg. Stages of development: E, egg; 16, 16-cell; 32, 32-cell; Lc, late cleavage; B, blastula; G, gastrula; Pr, prism; Pl, pluteus. Time course RNA blots were performed from four (*L. variegatus* and *E. parma*) to seven (*S. droebachiensis* and *A. punctulata*) times for each species, with a typical representative shown in the figure.

TABLE 1
ACCUMULATION OF ACTIN-CODING RNA SIZE CLASSES IN EMBRYOS OF FIVE ECHINODERMS

Animal	Message size (kb)	Relative signal intensity for each actin-coding RNA size class[a]						
		Egg	16-cell	32-cell	Late cleavage	Blastula	Gastrula	Prism	Pluteus
S. purpuratus[b]	2.2	1	1	0.9	1.2	8.8	—	—	—
	1.8	1	1	1.0	2.4	24.0	—	—	—
S. droebachiensis	2.1	9	1	—	—	2.3	22.9	—	—
	1.9	10.5	1	—	—	25.1	35.4	—	—
A. punctulata	2.2	1.1	1	0.9	0.9	4.6	—	10.5	9
	1.6	ND[c]	ND	ND	ND	1	—	4.0	10.3
L. variegatus	2.2	1.5	1	—	—	—	5.2	—	16.7
E. parma	2.0	1	—	—	—	5.5	—	—	—

[a] The relative intensities of the hybridization bands shown in Fig. 1 were determined either by cutting the radioactive bands from the filters and counting them by liquid scintillation (*S. purpuratus*, *A. punctulata*, *L. variegatus*), or by densitometric scanning (*S. droebachiensis*, *E. parma*). For *S. purpuratus* these values were taken from Crain *et al.* (1982) and derived from an RNA blot different from that in Fig. 1. Either the 16-cell value or the earliest detected value (when 16-cell was not available) was normalized to one and changes in actin-coding RNA abundance were calculated relative to this stage. *L. variegatus* values were corrected for differential RNA loading.

[b] From Crain *et al.* (1981).

[c] Not detected.

to gastrula and the hybridization band covers a range of about 250 nucleotides. The smaller actin-coding RNA size class is centered at 1.9 kb prior to gastrula with the band covering about 150 nucleotides. Furthermore, the relative abundance of each of these RNA classes is low in early embryos and rises considerably during embryogenesis. In contrast to *S. purpuratus* and the other sea urchins examined, both of these RNA classes appear more abundant in eggs than in 16-cell embryos (Fig. 1 and Table 1), suggesting that maternal actin-coding RNA is degraded after fertilization. This observation was made multiple times using a single egg RNA sample, prepared during a time when *S. droebachiensis* embryos were developing normally in the laboratory. Egg RNA prepared early in the breeding season, before we were able to obtain normal development, did not show a particularly high-actin-coding RNA content. We conclude that the result represented here is probably the case in mature, developmentally competent eggs. In blastula stage embryos the 2.1-kb signal has increased only slightly relative to late cleavage, but the 1.9-kb size class is approximately 25-fold more abundant (Table 1). Continued actin-coding RNA accumulation is evident at the gastrula stage. The abundance of the 2.1-kb size class has increased over 20-fold compared to the 16-cell stage and its average size has increased about 100 nucleotides. (This size shift is seen more clearly in other exposures and other unpublished experiments.) The smaller message size class rises about 40% between blastula and gastrula stages and appears to shorten by about 50–100 nucleotides. These slight shifts in the sizes of the RNA classes are not explained. They fall in a range that could result from changes in polyadenylation of these molecules, but could also result from the appearance of different actin gene transcripts.

To ask whether the embryonic actin messages in *S. droebachiensis* and *S. purpuratus* are transcribed from homologous actin genes, 3′-untranslated actin mRNA sequences from *S. purpuratus* were used as hybridization probes against *S. droebachiensis* gastrula RNA. A 3′ probe, specific for the *S. purpuratus* actin gene pSpG17, hybridizes with a 2.2-kb actin-coding RNA in both *S. purpuratus* and *S. droebachiensis* (Fig. 2A). The reduced intensity of the signal in *S. droebachiensis* RNA, as compared with the *S. purpuratus* RNA, may reflect a lower abundance of the transcript, or be due to reduced sequence homology of this untranslated sequence between these animals. A second probe, which contains primarily 3′-untranslated actin mRNA sequence from a different *S. purpuratus* gene, the cDNA clone pSpec4, hybridizes specifically to a 1.8-kb RNA in both species (Fig. 2B). The *S. droebachiensis* signal is again low compared to *S. purpuratus*, reflecting either lower abundance or sequence divergence. In at least two cases in these two species then, embryonic actin-coding RNAs which are similar in their size and accumulation are transcribed from related genes.

Accumulation of Actin-Coding RNA in A. punctulata Embryos

A. punctulata embryos show a pattern of actin-coding RNA accumulation which is generally similar to that

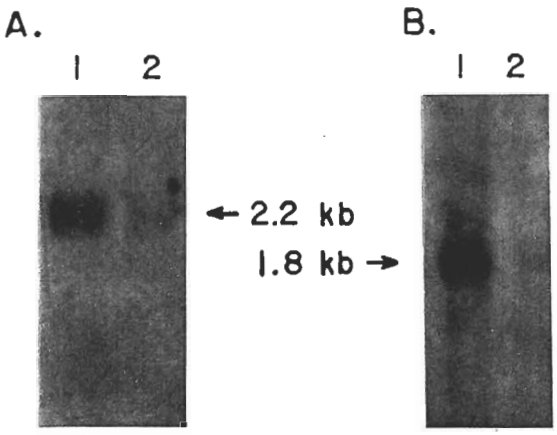

FIG. 2. Homology of 3'-untranslated sequences of *S. purpuratus* and *S. droebachiensis* actin-coding RNAs. RNA blot analysis of *S. purpuratus* blastula RNA and *S. droebachiensis* gastrula RNA was carried out using two different non-protein-coding hybridization probes. (A) A *Bst*EII-*Hin*dIII fragment, from the actin-gene-containing clone pSpG17, which contains 3'-untranslated mRNA sequence and no protein-coding sequence (Cooper and Crain, 1982; Crain *et al.*, 1982) was hybridized to *S. purpuratus* blastula RNA (Lane 1) and to *S. droebachiensis* gastrula RNA (Lane 2). (B) The cloned cDNA sequence pSpec4 (a gift from W. Klein), which contains primarily 3'-untranslated actin mRNA sequence and hybridizes to only the 1.8-kb actin mRNA in *S. purpuratus* (Bruskin *et al.*, 1981; Crain *et al.*, 1982), was hybridized to *S. purpuratus* blastula RNA (Lane 1) and to *S. droebachiensis* gastrula RNA (Lane 2).

seen in *S. purpuratus* and *S. droebachiensis*, but varies somewhat in detail (Fig. 1). A single actin-coding RNA size class is detectable in egg and cleavage stage embryos. This size class is centered at 2.2 kb, and remains at a constant low level of abundance through late cleavage. At blastula stage the relative abundance of this size class has increased almost 5-fold, and by pluteus stage the signal has increased over 12-fold, compared to cleavage stage values (Table 1). In pluteus stage embryos the 2.2-kb hybridization band spreads over almost 500 nucleotides indicating the potential for multiple messages within this class. Oligo(dT)-cellulose chromatography of *A. punctulata* blastula RNA followed by RNA blot analysis of the poly(A)$^+$ fraction resolves the 2.2-kb size class into two bands (Fig. 3A). By blastula stage another actin-coding RNA size class is detected at about 1.6 kb. This size class increases in abundance about 10-fold by pluteus relative to its blastula value (Table 1). Hybridization of the 3'-untranslated *S. purpuratus* actin mRNA sequence from pSpG17 showed no detectable cross reaction with *A. punctulata* blastula RNA (data not shown).

The *A. punctulata* actin gene family was analyzed by Southern blot analysis of *Bam*H1 cleaved genomic DNA (Fig. 3B). When these filters were probed with the entire *S. purpuratus* actin-gene-containing plasmid pSpG17, the *A. punctulata* actin genes were found to constitute a small multigene family. Comparison of the number of hybridization bands in *A. punctulata* DNA and *S. purpuratus* DNA indicates that the *A. punctulata* and *S. purpuratus* actin multigene families are of similar size, but that the *A. punctulata* family may be slightly smaller. We have previously determined that there are 5–20 actin genes in the *S. purpuratus* genome (Durica *et al.*, 1980).

Accumulation of Actin-Coding RNA in L. variegatus Embryos

Developmental expression of *L. variegatus* actin-coding RNA shares many features with *A. punctulata* (Fig. 1). Egg and cleavage stage embryos show a single 2.2-kb message size class that is only visible on long autoradiographic exposures. By gastrula stage this signal has increased 5-fold relative to the 16-cell stage value, and almost 17-fold relative to the 16-cell value by pluteus stage. This band spans about 300 nucleotides in

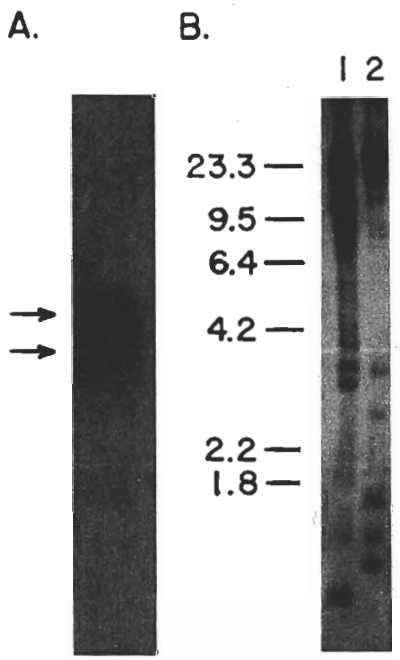

FIG. 3. Analysis of poly(A)$^+$ actin-coding RNA and actin gene number in *A. punctulata*. (A) A 50-μg sample of total *A. punctulata* blastula RNA was passed once over an oligo(dT)-cellulose column. The poly(A)$^+$-enriched RNA, which bound to oligo(dT), was eluted and the entire sample analyzed on one lane of an RNA blot. The hybridization probe was the *S. purpuratus* actin-gene clone pSpG17. The arrows indicate the position of two actin-coding RNAs, which are near 2.2 kb. (B) Ten-microgram samples of *S. purpuratus* (Lane 1) and *A. punctulata* (Lane 2) sperm DNA were cleaved with *Bam*H1 and analyzed on Southern transfers using the *S. purpuratus* actin-gene clone pSpG17 as the hybridization probe. Sizes of marker DNA fragments are indicated in kb.

pluteus RNA. A close inspection of the *L. variegatus* panel in Fig. 1 reveals the presence of several faint bands. These light bands might represent minor message classes, but since they have not been observed consistently we suspect that they result from some degradation in the sample. As with *A. punctulata*, the *S. purpuratus* 3′-untranslated actin mRNA sequence from pSpG17 showed little or no detectable cross reaction with *L. variegatus* gastrula RNA (Crain and Bushman, submitted for publication).

Accumulation of Actin-Coding RNA in E. parma Embryos

To extend this investigation to more distant taxons, embryonic actin gene expression was examined in *E. parma*, a sand dollar from a different superorder from the four sea urchins analyzed. The abundance of total actin-coding RNA in *E. parma* eggs is relatively low, and increases almost sixfold by the blastula stage (Fig. 1 and Table 1). Both egg and blastula stage embryos show a single actin-coding RNA size class centered at 2.0 kb, which ranges over about 350 nucleotides. Considerable structure is seen in both the egg and blastula actin-coding RNA bands. The majority of the signal in the egg lane is concentrated at the lower border of the hybridization band, and the signal in the blastula lane is concentrated at the upper and lower margins of the band with an area of relatively weak signal in between. The significance of this internal structure is unclear, but the similarity of *E. parma* blastula actin-coding RNA to *A. punctulata* blastula poly(A)$^+$ RNA is evident.

DISCUSSION

A striking result of these comparative analyses is the similarity of the patterns of actin-coding RNA accumulation during embryogenesis among these species. In every case actin-coding RNA abundance is relatively low in early embryos and increases during development (Fig. 1). These increases are probably related to functional requirements within the embryo. In the sea urchin embryo, muscle is present in the gut of the pluteus (Gustafson, 1969), but there is no evidence of muscle-like structures prior to the gastrula stage. Since the expression of muscle actin genes in other animals is closely linked to the observable morphological changes of muscle differentiation, it is probable that the early rise in actin mRNA is due to the expression of non-muscle actin genes.

A second feature which the species share is a major actin-coding RNA size class of 2.0–2.2 kb. In each case there appears to be size heterogeneity within this class, and in *A. punctulata* this class resolves into two recognizable bands when poly(A)$^+$ RNA is examined (Fig. 3A). It is possible that actin messages from more than one gene are present in this class. In fact, we have found in *S. purpuratus* that at least two different actin genes produce 2.2-kb mRNAs, although one of these appears to be expressed only in adult tissues (Garcia and Crain, unpublished observation). Conservation of actin message size in this range is somewhat surprising since only 1125 nucleotides are required to code for actin. The *S. purpuratus* actin gene which is cloned in the plasmid pSpG17, encodes a 2.2-kb embryonic message which probably contains 374 and 515 nucleotides of 5′- and 3′-untranslated sequence respectively (Crain *et al.*, 1982; Cooper and Crain, 1982). In this case, at least, the untranslated sequence is not concentrated at either end of the message. The reason for nearly 1 kb of untranslated message sequence is unknown. Its presence on actin message in each of these species suggests that it may be required for a function which is subject to strong selective pressure, such as message stability or translatability. It should further be pointed out that actin mRNAs which fall into this size range are also seen in *Drosophila* (Fyrberg *et al.*, 1980; Zulauf *et al.*, 1981; Sodja *et al.*, 1982), rat (Katcoff *et al.*, 1980), chicken (Ordahl *et al.*, 1980; Schwartz and Rothblum, 1981), *Xenopus* (Schafer *et al.*, 1983), mouse (Minty *et al.*, 1982; Giebelhaus *et al.*, 1983), and HeLa cells (Crain, unpublished observation).

At least two embryonic actin-coding RNAs in *S. droebachiensis* are transcribed from genes with 3′-untranslated message sequences which are related to those associated with *S. purpuratus* actin genes. Each of these *S. droebachiensis* RNAs is close to the same size as the related RNA in *S. purpuratus* and accumulates similarly during development. Furthermore, in the case of the 2.2-kb RNA, the gene from which it derives (pSpG17) is known to be present only once in the *S. purpuratus* genome. It seems likely then that, in these two species, homologous actin genes are producing transcripts with similar patterns of expression. The expression of these actin gene family members may thus be associated with particular developmental pathways, which are conserved between these closely related species.

We could detect no homology between a particular 3′-untranslated *S. purpuratus* sequence (from pSpG17) and *A. punctulata* and *L. variegatus* embryonic RNA. This could indicate that homologous actin genes do not have similar patterns of expression among these more distantly related species. However, if the 3′-untranslated sequence itself is not subject to selective pressure, it would have diverged freely over the 180–200 million years since the evolution of these species branched. Thus homologous genes (in their patterns of expression) might not be recognized by untranslated message sequence probes.

While a general pattern of embryonic actin-coding RNA accumulation among these echinoderm species emerges from this analysis, several interesting variations on the common scheme are also evident. The major variations are in the size and number of actin-coding RNA classes, and the kinetics of accumulation of actin-coding RNA. The two most closely related animals examined are *S. purpuratus* and *S. droebachiensis*. Among the species examined their actin message classes are most alike. However, the larger message class in *S. droebachiensis* is slightly smaller than that of *S. purpuratus* prior to the gastrula stage (about 2.1 kb as compared to 2.2 kb) and the smaller message class is somewhat larger in *S. droebachiensis* (about 1.9 kb through blastula, then shifting to about 1.85 kb in gastrula) than in *S. purpuratus* (about 1.8 kb). The accumulation of the larger message class differs somewhat between the two species in that the sharp rise in its prevalence begins during cleavage in *S. purpuratus* and between hatching blastula and gastrula in *S. droebachiensis*. The accumulation of the smaller message class is similar in the two species, 24- and 25-fold by blastula in *S. purpuratus* and *S. droebachiensis* respectively (Table 1).

Another variation seen in actin message abundance among these species is seen in the unfertilized egg. In *S. droebachiensis* both actin-coding RNA classes are present in the egg at about 9- to 10-fold higher concentration than at the 16-cell stage, and must thus be degraded early in development (Table 1). In the other sea urchin species examined there is at most 50% more actin message in the egg than the early embryo (Table 1). In sand dollar only egg and blastula were examined, so it is not possible to say whether the actin-coding RNA content drops after fertilization.

The other three species in this study, *A. punctulata*, *L. variegatus*, and *E. parma* are more distantly related to each other and to the two *Strongylocentrotus* species (Fig. 1). The major difference which we observe among these species is the lack of, or significant difference in size of, the smaller actin-coding RNA size class. In contrast to *S. purpuratus* and *S. droebachiensis*, no smaller message is seen in *L. variegatus* or *E. parma* and in *A. punctulata* a 1.6-kb actin-coding RNA becomes detectable at the blastula stage.

An understanding of the evolution of the genome and differential gene expression must include not only information on the structure and arrangement of genes in different species, but also information which relates their expression to each other and to fundamental requirements for growth and development. Association of specific genes into particular regulatory units may be essential for the creation of complex developmental pathways (Davidson *et al.*, 1982). Because of this, genes which are ancient and functionally essential to all cells, such as those which code for actin, may also have ancient and conserved patterns of expression. A sharp rise in actin-coding RNA abundance during embryonic development from a relatively low level appears to be widespread among the echinoderms. When we consider that embryos from these species contain significant amounts of maternal mRNA, it is clear that an alternate mechanism for supplying the early embryo with actin mRNA is available. That is, the production of large amounts of stable maternal actin mRNA, which could persist long into embryogenesis. In *S. purpuratus*, such a mechanism is utilized for a major fraction of early embryonic mRNAs (Flytzanis *et al.*, 1982). It may be then, that the particular pattern of embryonic actin gene expression is seen in many species because it is part of an important developmental pathway which was established early in echinoderm evolution.

We thank Susan G. Ernst and Benjamin Paz-Aliaga for helpful comments on this 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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