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Three Sea Urchin Actin Genes Show Different Patterns of Expression: Muscle Specific, Embryo Specific, and Constitutive

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The expression of three different actin genes in the sea urchin, *Strongylocentrotus purpuratus*, was monitored in embryos and adult tissues by using untranslated mRNA sequences as specific hybridization probes. Three distinct patterns of expression were found: muscle specific, embryo specific, and constitutive (i.e., present in all tissues examined). The actin genes encoding the muscle-specific and constitutively expressed genes were each found to be present once in the haploid genome. The embryo-specific probe could derive from either a single gene or a small subset of actin genes. These data demonstrate that at least three members of the sea urchin actin gene family are expressed in distinct ways and thus are probably associated with different regulatory programs of gene expression necessary for development of this metazoan.

Differential expression of actin gene family members is well documented in metazoans, with the translated proteins falling into two general categories, muscle and cytoplasmic or cytoskeletal (8, 10, 11, 13, 17, 20–23). Within these two categories, which have been defined largely by expression and not function, there are additional actin isoforms. Thus, there are at least two vertebrate cytoplasmic actins and at least four vertebrate muscle actins (22). In *Drosophila melanogaster*, an invertebrate, there are two cytoskeletal and four muscle actin genes which are expressed in characteristic patterns in developing and adult flies (11, 17). Expression of particular actin genes thus seems to be associated with specific developmental pathways. Examination of these associations in a variety of metazoans is necessary to identify particular features of these genes which are important in modulating their expression in concert with appropriate groups of other genes.

Previous examination of expression of the actin genes of sea urchins has demonstrated temporal (7, 15, 18) and spatial (2; S. G. Ernst, F. D. Bushman, and W. R. Crain, unpublished data) differences in actin mRNA accumulation during embryogenesis. In this report, we have extended these observations in two ways. First, we have used untranslated mRNA sequences from three different actin genes as hybridization probes, making it possible to specifically follow the expression of these genes. Second, we have assayed the expression of these genes in four adult tissues, coelomocytes, gut, lantern muscle, and tube feet, and in embryos. These experiments have shown that one actin gene is expressed only in muscle-containing tissue, one only in embryos, and one in each adult tissue and embryos.

MATERIALS AND METHODS

RNA preparations. Two methods were used to obtain total RNA. In the first, gut, lantern muscle, and tube foot tissue were dissected from the animal and quickly frozen in liquid nitrogen. Coelomocytes were collected as described by Galau et al. (12). The frozen tissue was ground in a mortar with dry ice and equilibrated with a mixture of 2 to 3 volumes of buffer containing 0.1 M Tris-hydrochloride (pH 7.8), 0.1 M NaCl, 1.0 mM EDTA, and 2 to 3% sodium dodecyl sulfate (SDS) and an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1). The pelleted coelomocytes were also suspended in this mix. After the organic and aqueous phases were separated by centrifugation, the aqueous phase was diluted with the same buffer, without SDS, to a final concentration of 0.5% SDS. Predigested proteinase K was added to a final concentration of 50 μg/ml and incubated at 37°C for 15 to 30 min. The solution was extracted several times with phenol-chloroform-isoamyl alcohol and ethanol precipitated. The pelleted RNA was suspended in distilled water and precipitated in 2.0 M NaCl–5 mM EDTA (pH 7.2). The RNA was pelleted again by centrifugation, resuspended, and ethanol precipitated once more before being stored in distilled water. The second method, which was used for embryos and adult tissue, was a modification of that described by Chirgwin et al. (4). The dissected tissues (or pelleted coelomocytes or embryos) were homogenized in 2 to 3 volumes of lysis buffer containing guanidine hydrochloride. The homogenate (3 to 3.5 ml) was layered on a 1-ml cushion of 5.7 M CsCl and centrifuged in a SW50.1 rotor at 30,000 rpm at 18°C for 16 to 20 h. The RNA pellet was dissolved in water and ethanol precipitated before being stored in water. In some cases this material was further digested with proteinase K (as described above). In some experiments polyadenylic acid-containing RNA from the adult tissues was obtained by passing total RNA over a single oligodeoxythymidylic acid-cellulose column, as described by Aviv and Leder (1).

Blotting experiments. RNA blotting experiments were carried out as described by Bushman and Crain (3). Hybridization was at 66°C in 0.32 M sodium phosphate buffer (pH 6.8)–0.8× Denhardt solution–0.08% SDS–0.8 μM each of deoxynucleoside triphosphate and 10% dextran sulfate. DNA blots were as described by Southern (19). Hybridization was at 65°C in 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–10× Denhardt solution–50 μg of carrier DNA per ml–0.1% SDS–10% dextran sulfate.

Hybridization probes. Several different hybridization probes were used. In experiments designed to examine all actin mRNAs, the entire actin gene-containing recombinant

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plasmid pSpG17 was used. Since the entire protein-coding sequence is present in this plasmid, it should detect mRNA transcribed from any actin gene family member. To detect sequences specifically associated with the actin gene contained in the plasmid pSpG17, two different fragments from this recombinant were used, a *Bst*EII-*Hin*dIII fragment and a *Bam*HI-*Bam*HI fragment. The *Bst*EII-*Hin*dIII fragment (about 0.75 kilobases [kb]) contains 3′ untranslated actin mRNA sequence and no protein-coding sequence (5, 6). The *Bam*HI-*Bam*HI fragment contains the first 44 nucleotides of actin-coding sequence and ca. 1 kb of 5′ adjacent sequence (5). To detect sequences specifically associated with the actin gene contained in the plasmid pSpG28, we used two *Bam*HI-*Hin*dIII fragments. One fragment includes 1.3 kb of sequence to the 3′ side of the coding sequence and does not contain protein-coding sequence (6; D. S. Durica and W. R. Crain, unpublished observation). The other fragment contains the first 44 nucleotides of actin-coding sequence and ca. 2 kb of 5′ adjacent noncoding sequence (6; A. D. Cooper and W. R. Crain, unpublished data). The cDNA clone pSpec4 (a gift from W. Klein, Indiana University), which contains sequence complementary to actin mRNA (A. M. Bruskin and W. Klein, personal communication), was also used as a hybridization probe. We have not determined whether this untranslated sequence recognizes one or a small subset of actin genes. The non-protein-coding fragments described here do not cross-react with each other under the hybridization conditions used. Those fragments which contain small amounts of protein-coding sequence show detectable cross-reaction only after long autoradiographic exposure, and this signal is far less than with the homologous sequences. The 5′ and 3′ fragments from the pSpG17 and pSpG28 plasmids were separated by agarose gel electrophoresis and recovered by electroelution.

RESULTS

Copy number of cloned actin genes pSpG17 and pSpG28. The actin genes contained in the recombinant clones pSpG17 and pSpG28 have been shown to be different, both in terms of the encoded proteins and the distribution of introns (6). To determine whether either of these genes belongs to a subset of actin genes with sequence homology in regions to the 5′ or 3′ side of the coding sequence, we determined the genome copy number of the coding-adjacent sequences by Southern blot analysis (Fig. 1 and 2). In these experiments, amounts of plasmid DNA equivalent to various numbers of actin gene copies per haploid genome were compared with genomic DNA.

The two hybridization probes used in the reconstruction copy number experiment for pSpG17 were a 5′ *Bam*HI-*Bam*HI fragment (Fig. 1A and B) and a 3′ *Bst*EII-*Hin*dIII fragment (Fig. 1C and D). The 5′ fragment contains the first 44 nucleotides of actin-coding sequence and about 1 kb of adjacent noncoding sequence (5). The 3′ fragment, which contains no protein-coding sequence, has been shown previously to contain mRNA sequences, probably including approximately the last 300 nucleotides of the 3′ untranslated mRNA sequence (5, 6). Visual and quantitative comparison of the band intensities in Fig. 1 indicates that each of these fragments is present once per haploid genome, and thus demonstrates that the actin gene contained in pSpG17 is unique in the genome. Expression of this gene can thus be specifically detected by using either of these sequences as hybridization probes. Since only one genomic sequence was detected by the 5′ *Bam*HI-*Bam*HI fragment, the 44 nucleotides of actin-coding sequence present on this fragment must not be sufficient for detectable cross-reaction with other actin-coding sequences under these experimental conditions. This is probably due to a combination of its relatively short length and to divergence of this portion of the different actin genes.

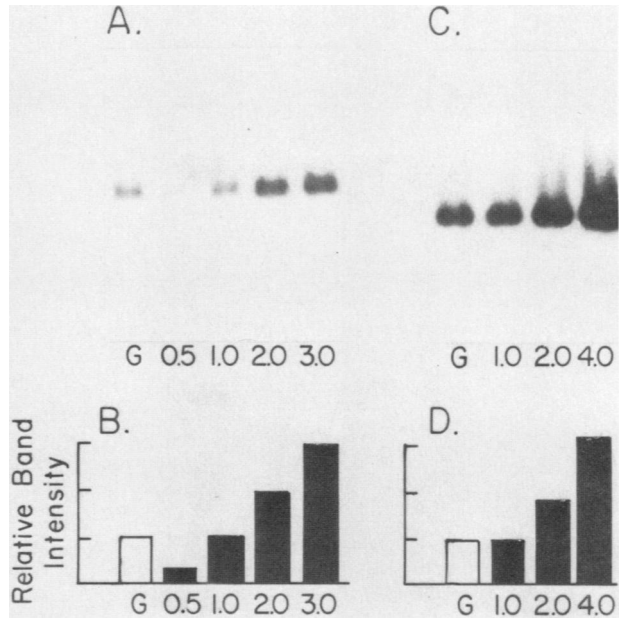

FIG. 1. Determination of the number of pSpG17 actin genes in the *S. purpuratus* genome. Southern blots were carried out on genomic DNA from blastula-stage embryos and amounts of DNA from the actin gene-containing plasmid pSpG17 which corresponded to various numbers of copies per haploid genome. (A) A 5-μg sample of genomic DNA (G) and amounts of pSpG17 DNA equivalent to 0.5, 1.0, 2.0, and 3.0 copies per haploid genome (plus 5 μg of *Escherichia coli* DNA) were digested with *Bam*HI. The hybridization probe was a *Bam*HI-*Bam*HI fragment from pSpG17, which contains the first 44 nucleotides of actin-coding sequence and about 1 kb of 5′ adjacent sequence. (B) The lanes in (A) were scanned with a densitometer, and the relative areas under the hybridization band peaks were plotted. (C) A 5-μg sample of genomic DNA (G) and amounts of pSpG17 DNA equivalent to 1.0, 2.0, and 4.0 copies per haploid genome (plus 5 μg of *E. coli* DNA) were digested with *Bst*EII and *Hin*dIII. The hybridization probe was a *Bst*EII-*Hin*dIII fragment from pSpG17 (ca. 0.75 kb) which contains 3′ untranslated mRNA sequence. (D) The lanes in (C) were scanned with a densitometer, and the relative areas under the hybridization band peaks were plotted.

The number of pSpG28-like genes was determined by using a 2-kb 5′ *Hin*dIII-*Bam*HI fragment and a 1.3-kb 3′ *Bam*HI-*Hin*dIII fragment as hybridization probes. As with pSpG17, the 5′ fragment contains the first 44 nucleotides of actin-coding sequence and showed no detectable cross-reaction with other actin genes. The 3′ fragment, which contains no actin-coding sequence (Durica and Crain, unpublished observation), contains some 3′ untranslated mRNA sequence (shown in this paper). These two fragments were mixed and hybridized together in the reconstruction Southern blot shown in Fig. 2. In this experiment, both sperm and blastula DNA were cleaved with *Bam*HI and *Hin*dIII and showed no differences in copy number or banding pattern when hybridized with these probes. In each of these genomic DNA samples, a band is present which is not in the recombinant plasmid. Since the two upper genomic bands (which correspond to the 5′ *Hin*dIII-*Bam*HI fragment) each have an intensity similar to that for 0.5 copies

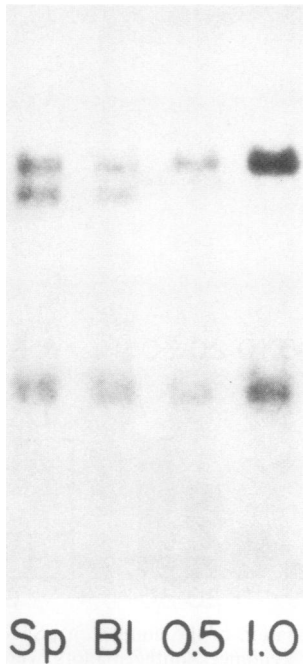

FIG. 2. Determination of the number of pSpG28 actin genes in the *S. purpuratus* genome. Genomic DNA from sperm (Sp) (5 μg), blastula (Bl) (5 μg), and amounts of pSpG28 DNA equivalent to 0.5 and 1.0 copy per haploid genome (plus 5 μg of *E. coli* DNA) were digested with *Hin*dIII and *Bam*HI. Two fragments from pSpG28, a 2-kb *Hin*dIII-*Bam*HI fragment (containing 5′ protein-coding-adjacent sequence and the first 44 nucleotides of protein-coding sequence) and a 1.3-kb *Bam*HI-*Hin*dIII fragment (which lies to the 3′ side of actin-coding sequence) were nick translated separately, and then an equal number of counts of each were mixed and used as the hybridization probe.

per haploid genome, they must represent a polymorphic variation in the 5′ portion of the two alleles of this single gene. The intensity of the smaller band (corresponding to the 3′ *Bam*HI-*Hin*dIII fragment) matches with that of one copy per haploid genome, further confirming that this gene is unique in the genome. These particular fragments can thus be used to specifically detect expression of the actin gene contained in pSpG28.

Size of actin-coding RNA in adult tissues of sea urchins. It has been shown previously that two size classes of actin mRNA, 1.8 and 2.2 kb, are present in embryonic stages and tissue types of sea urchins (7, 15, 18; Ernst et al., unpublished data). To ask whether this was also true in differentiated adult tissues, we assayed for the presence of actin-coding sequences in RNA from four adult tissues: tube feet, lantern muscle, gut, and coelomocytes (Fig. 3). In this experiment, the hybridization probe was the entire actin gene-containing clone pSpG17, which will recognize actin mRNA transcribed from any actin gene family member. In contrast to embryonic RNA, each adult tissue contained only a 2.2-kb actin-coding size class. Actin gene expression, therefore, must change considerably during the differentiation of these adult tissues from their embryonic precursors.

Muscle-specific expression of actin gene pSpG28. The relative amounts of the actin mRNA size classes shift during embryogenesis (7) and are different between embryonic cell types (Ernst et al., unpublished data), and the 1.8-kb class is absent altogether in adult tissues. These observations suggest that particular members of the gene family have characteristic patterns of expression during embryonic development and differentiation. To examine the expression of the pSpG28 gene, we used sequences specific to this gene as hybridization probes in blotting experiments with RNA from four adult tissues and four embryonic stages. Figure 4A shows the results when a 3′ noncoding *Bam*HI-*Hin*dIII fragment was hybridized to RNA from the four adult tissues and blastula. A 2.2-kb actin mRNA was recognized in tube feet, gut, and lantern muscle, and none was detected in blastula or coelomocytes. Rehybridization of this same filter with an actin-coding probe (the entire pSpG17 clone) showed that the expected actin-coding RNA classes are readily detected in blastula and coelomocytes (unpublished observation). To further verify this result, a 5′ *Hin*dIII-*Bam*HI fragment from pSpG28 was used as a hybridization probe on blots of polyadenylated RNA from the adult tissues (Fig. 4B). Again, a 2.2-kb actin-coding RNA was seen in tube feet, gut, and lantern muscle and not in coelomocytes or blastula (total RNA). Longer exposure of this blot revealed a faint 2.2-kb band in coelomocyte RNA. Since this has not been seen with the 3′ probe and since the 5′ *Hin*dIII-*Bam*HI fragment contains 44 nucleotides of actin-coding sequence, we conclude that this weak signal is due to cross-reaction with the coding portion of another actin mRNA. There could, however, be a low level of expression of the pSpG28 gene in coelomocytes. Since tube feet, gut, and lantern muscle contain substantial amounts of muscle and, as far as is known, blastula and coelomocytes contain none, we conclude from these data that pSpG28 contains a muscle actin gene.

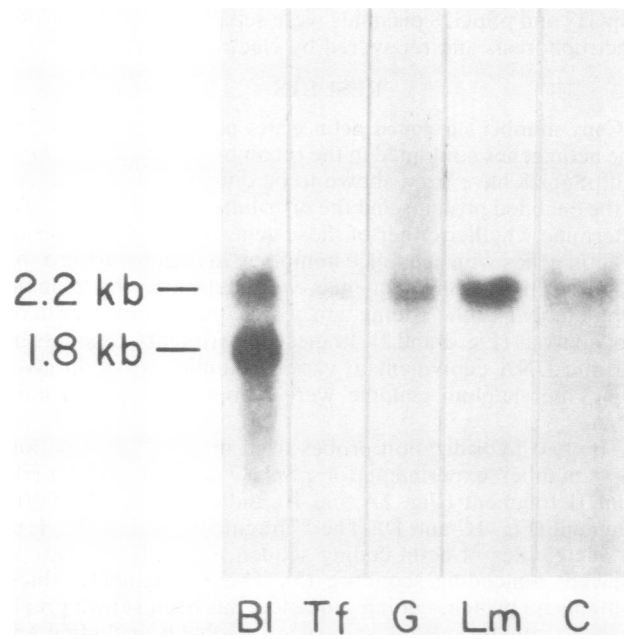

FIG. 3. Detection of actin-coding RNA in four adult tissues and blastula-stage embryos. A 10-μg sample of total RNA from blastula (Bl), tube feet (Tf), gut (G), lantern muscle (Lm), and coelomocytes (C) was electrophoresed under denaturing conditions, blotted to nitrocellulose filters, and hybridized. The hybridization probe was ^{32}P-labeled DNA from the entire actin gene-containing plasmid pSpG17, which should detect RNA transcribed from any actin gene family member. The different lanes in this figure were taken from several different experiments. Since exposure times and probe-specific activities vary, comparison of the relative intensities between lanes is not meaningful.

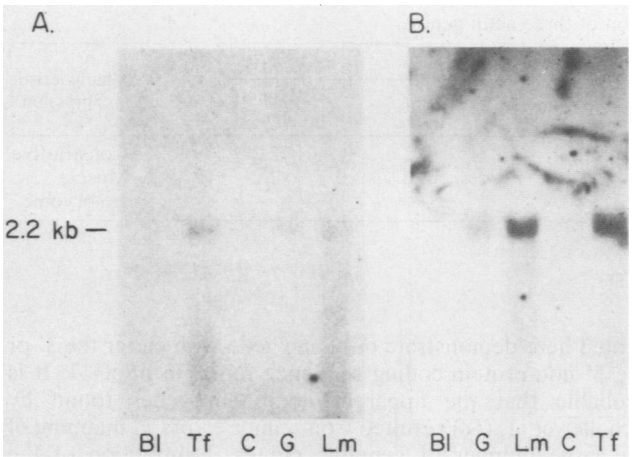

FIG. 4. Muscle-specific expression of transcripts from actin gene pSpG28. (A) Total RNA from four adult tissues and blastula (10 μg in each lane) was blotted and hybridized with a *Bam*HI-*Hin*dIII fragment from the actin gene-containing recombinant plasmid pSpG28. This fragment, which is present only once per haploid genome, lies to the 3′ side of actin-coding sequence and does not contain coding sequence. (B) Polyadenylated RNA from 50 μg of total RNA from each adult tissue and 10 μg of total blastula RNA were electrophoresed and blotted onto nitrocellulose paper. The hybridization probe was a *Hin*dIII-*Bam*HI fragment from the clone pSpG28. This fragment is single copy in the genome and contains the first 44 nucleotides of actin-coding sequence and about 2 kb of 5′ adjacent sequence. The RNAs are from blastula (Bl), tube feet (Tf), coelomocytes (C), gut (G), and lantern muscle (Lm).

Sea urchin embryos contain muscular structures by the pluteus stage (14). To determine if message from this muscle actin gene appears during embryogenesis, we used a 3′ *Bam*HI-*Hin*dIII fragment, specific to the pSpG28 gene, to probe RNA from hatching blastula-, mesenchyme blastula-, gastrula-, and pluteus-stage embryos. Actin mRNA from this gene was not readily detectable at any stage in this blotting experiment. Very long autoradiographic exposure of this blot, however, did show a faint 2.2-kb signal in the pluteus RNA (data not shown). Such a result would be expected if only a small fraction of the total cells in the embryo (the muscle cells) are expressing this gene.

Expression of actin gene pSpG17 in embryos and adult tissues. To assay the differential expression of the pSpG17 gene, RNA from blastula and adult tissues was hybridized in a blot analysis with a 3′ untranslated mRNA sequence specific to this gene (Fig. 5). A 2.2-kb transcript from this gene was present in blastula and each adult tissue. Although the size of the message transcribed from this gene was similar to that from the muscle gene, its pattern of expression was strikingly different. The presence of transcripts from this gene in each adult tissue and blastula (also in each of two blastula cell fractions [Ernst et al., unpublished data]) suggests that it is generally or constitutively expressed in sea urchin cells and encodes a cytoplasmic or cytoskeletal actin.

Embryo-specific expression of a third actin gene. The cDNA clone pSpec4, which contains primarily 3′ untranslated actin mRNA sequence, is known to hybridize with only a 1.8-kb transcript in blastula- and pluteus-stage embryos (2, 6). To determine whether transcripts complementary to this sequence are in adult tissue, we used it as a hybridization probe in blot experiments with RNA from adult tissues (Fig. 6). The data again demonstrate that pSpec4 is complementary to a 1.8-kb transcript which is expressed in blastula-stage embryos and further show that there is no detectable expression of this RNA in the adult tissues. Longer exposure of these blots revealed weak hybridization signals with 2.2-kb transcripts. Genomic Southern blot experiments have previously indicated that this clone probably contains a small portion of the 3′ end of the actin-coding sequence (2; Paz-Aliaga and Crain, unpublished observation). Since we also know that the 2.2-kb actin mRNA is abundant in RNA from these tissues, we interpret these weak signals as a cross-reaction with actin-coding sequence present on transcripts from other genes. We have not yet demonstrated whether pSpec4 derives from a single actin gene or a small subset of actin genes with related 3′ untranslated sequences. In any case, transcripts from this gene (or set of genes) have been found only in embryos.

DISCUSSION

We have presented evidence that at least three sea urchin actin gene family members exhibit distinct patterns of expression during sea urchin ontogeny (summarized in Table 1). One gene is muscle specific in its expression (pSpG28), one is embryo specific (pSpec4), and a third is expressed in embryos and all adult tissues examined (pSpG17). Furthermore, previous analysis has shown that pSpG17 is expressed in two different embryonic cell fractions (Ernst et al., unpublished data). Since both pSpG17 and pSpec4 are expressed in nonmuscle tissue, we conclude that they encode cytoplasmic or cytoskeletal actins. The pattern of expression of pSpG28 suggests that it encodes a muscle actin. The complete nucleotide sequence of pSpG17 and the partial sequence of pSpG28 demonstrate that each gene encodes a protein which is more like vertebrate cytoplasmic actin than vertebrate muscle actin. That is, 73% (16 of 22) of the most diagnostic sites encoded in pSpG17 contain cytoplasmic-like amino acids (5), and 77% (10 of 13) of those sites thus far determined in pSpG28 also contain cytoplasmic-like substitutions (unpublished observation). In *D. melanogaster*, another invertebrate, it has also been found that the muscle actin genes encode proteins which are similar to vertebrate cytoplasmic actins (10, 11, 17).

The observation of an actin gene with embryo-specific expression is thus far unique to sea urchins. Since we have

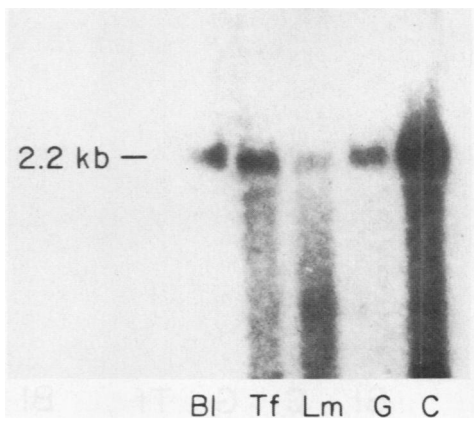

FIG. 5. Expression of transcripts from the actin gene pSpG17. The hybridization probe in this RNA blot experiment was a *Bst*EII-*Hin*dIII fragment from the actin gene-containing clone pSpG17. This fragment contains 3′ untranslated mRNA sequence and is present once in the haploid genome. A 10-μg sample of each of the following RNAs was loaded: blastula (Bl), tube feet (Tf), lantern muscle (Lm), gut (G), coelomocytes (C).

TABLE 1. Differential expression of three actin genes

Gene	Size of transcripts (kb) in stage or tissue:						Characteristic expression
	Blastula	Pluteus	Tube feet	Coelomocyte	Gut	Lantern muscle	
pSpG17	2.2	ND[a]	2.2	2.2	2.2	2.2	Constitutive
pSpG28	–[b]	2.2	2.2	–	2.2	2.2	Muscle
pSpec4	1.8	1.8[c]	–	–	–	–	Embryonic

[a] ND, Not determined.
[b] –, No transcripts were readily detectable.
[c] Unpublished observation and Bruskin et al. (2).

examined only representative and not all adult tissues, we cannot completely exclude the possibility of expression of this gene elsewhere in the adult animal. In any regard, no embryo-specific function for an actin has yet been suggested, and this expression probably reflects a particular cytoskeletal requirement. Because of the specificity of expression of these and other actin genes, it is sometimes suggested that particular actin isoforms differ functionally. Although this may be the case, it is also possible that specific actin genes became associated with particular developmental pathways of gene expression early in metazoan evolution and now exhibit patterns of expression that are restricted by these associations and not by functional differences. In such a situation, slight variations in the protein sequence may or may not relate to functional specificity.

The number of actin genes in the *S. purpuratus* genome has not been precisely determined. We previously concluded that there are 5 to 20 family members (9). From analysis of a set of λ genomic clones, Scheller et al. (18) concluded that there are a minimum of 11 actin genes. In that analysis, they identified a subset of five actin genes (A through E [18]) which included pSpG17 and were related by homology of their 3′ protein-coding-adjacent sequences. The data presented here demonstrate only one gene with either the 3′ or the 5′ non-protein-coding sequence found in pSpG17. It is probable that the apparent additional genes found by Scheller et al. (18) resulted from minor errors in mapping of the large number of genomic clones. Subtraction of the additional four genes leaves seven different cloned actin gene. Since only one additional actin gene has been discovered in other *S. purpuratus* genome libraries (CyIIIa [14a]) or by independent screening of the λ libraries described in Scheller et al. (18; Durica and Crain, unpublished data), there are probably eight or fewer members in this gene family.

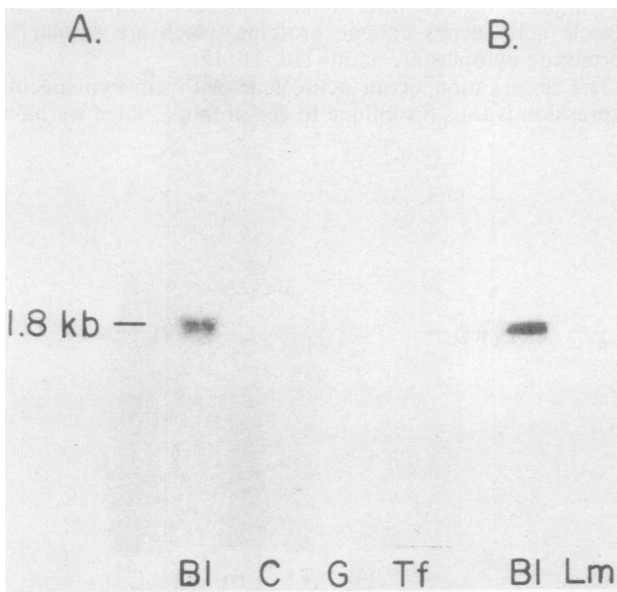

FIG. 6. Embryo-specific expression of 1.8-kb actin gene transcripts complementary to pSpec4. The RNA blot in each panel was hybridized with ^{32}P-labeled DNA from the recombinant plasmid pSpec4. (A) A 10-μg sample of total RNA from blastula (Bl), coelomocytes (C), gut (G), and tube feet (Tf) was loaded in each lane. (B) In a separate experiment, 10-μg samples of blastula and lantern muscle (Lm) RNA were loaded in each lane.

We have described here a unique pattern of expression of two different cytoskeletal actin genes (pSpG17 and pSpec4) and have discovered a single muscle actin gene (pSpG28). In an analysis of these and additional actin gene family members, it has been demonstrated that there are no additional muscle actin genes (14a, 18a). It is interesting to note that in other animals it has been found that multiple muscle actin genes are present, which are utilized in different muscle types (16, 17, 22). In fact, in *D. melanogaster*, another invertebrate, four of six actin genes exhibit muscle-specific expression (11, 17). Furthermore, there appear to be at least two muscle-type-specific patterns of expression of these four muscle genes (11). Little is known about the anatomy of sea urchin muscle, making it difficult to say whether there are different types of muscle. If there is a single muscle type, this would correlate with the single muscle gene. On the other hand, it is possible that the expression of this single gene is associated with several different muscle differentiation pathways which produce distinct structures. Such a situation would apparently be different from that found in anatomically more complex animals and would suggest different schemes for the evolution of muscle differentiation pathways.

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