Sea Urchin Tube Feet: Unique Structures That Allow a Cytological and Molecular Approach to the Study of Actin and Its Gene Expression

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ABSTRACT Actin is the major extractable protein component from the tube feet of four different species of sea urchin: *Arbacia punctulata*, *Strongylocentrotus purpuratus*, *Strongylocentrotus droebachiensis*, and *Diadema setosum*. Actin made up as much as 60% of the total Coomassie Blue-staining material after SDS polyacrylamide gel electrophoresis and densitometer analysis. Two-dimensional gel electrophoresis resolved two, and possibly three, species of actin for each sea urchin of which the dominant component was analogous to the beta form in vertebrates. In a cell-free system from rabbit reticulocytes, total RNA from tube feet stimulated the synthesis of one protein that represented 80% of the total methionine incorporation, migrated with the properties characteristic of actin in a two-dimensional gel system, and on proteolysis yielded fragments identical to purified rabbit actin. The mRNAs from the tube feet of two divergent species of sea urchin, *Arbacia punctulata* and *Strongylocentrotus purpuratus*, synthesized actins differing by <0.02 pH unit for each isospecies. 90% of the DNA copied from tube foot RNA by reverse transcriptase represented a highly abundant sequence class judged by copy DNA(cDNA)-RNA excess hybridization. At least two-thirds of this class represented a low-complexity component, with a $R_0t_{1/2}$ about three times that expected for actin messenger RNA. The remarkable degree of conservation of the actin protein is reflected in concomitant conservation of the protein-coding nucleotide sequences of the messenger RNA, which has allowed the use of a cDNA probe to isolate actin sequences from a human phage library.

Sea urchin tube feet are sensory, locomotive, and anchoring appendages that can extend and contract between 2 and 150 mm in length (1) (Fig. 1). The remarkable contractility of this appendage suggests the involvement of muscle; morphological and histochemical investigations presented here have confirmed this. Histologically, the tube foot, of which there are about 2,000 per urchin, is composed of three obvious cylindrical layers around a central lumen (Fig. 2). The large innermost layer (excluding a single-celled endothelium) composed at least 50% of the cellular volume of the tube foot and appeared to be histologically analogous to vertebrate smooth muscle. A large basement lamina could be seen as well as a richly pigmented epithelium. This morphology is characteristic of echinoderm tube feet (2–4). Electron microscopy revealed the muscle cells to be full of closely packed filaments of the diameter expected of actin (7 nm), which were confirmed to be actin by immunofluorescence assay (data not shown).

This biochemical investigation of the tube feet from four different species of sea urchins confirmed them to be unique structures in that they were mainly composed of actin. Actin is a ubiquitous, evolutionarily conserved protein making up, in most cells and particularly vertebrate smooth muscle, the major abundant protein component (5, 6). In no other tissue source, however, has the abundance of actin been reported to be as high as that found in sea urchin tube feet by our observations. This led us to determine that the tube feet of the sea urchin are a source of naturally enriched messenger RNA for this protein. A readily accessible abundant source of actin messenger RNA facilitates a molecular analysis of the actin gene, because the actin-coding nucleotide sequences are well conserved throughout evolution. This allows the abundant actin messenger RNA from tube feet to be used to search for actin genes from recombinant DNA libraries containing actin sequences from other species.

THE JOURNAL OF CELL BIOLOGY · VOLUME 89 APRIL 1981 109-114

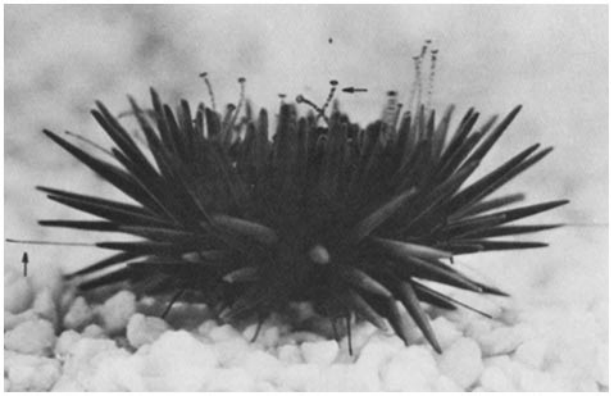

FIGURE 1 *Arbacia punctulata* inverted and submerged in seawater. Arrows indicate representative tube feet in extended state.

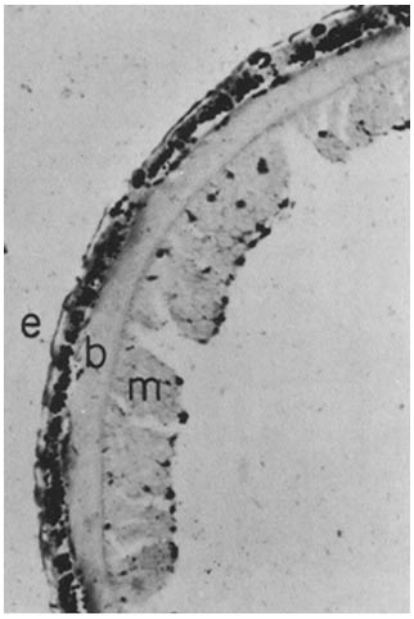

FIGURE 2 Histological section of a tube foot (paraffin, toluidine blue): *m*, muscle; *b*, basement membrane (collagen); *e*, epithelia. × 400.

MATERIALS AND METHODS

Sea Urchins

Arbacia punctulata was obtained from the Marine Biological Laboratories, Woods Hole, Mass. *Strongylocentrotus purpuratus* was obtained from Pacific Biomarine Labs, Venice, Calif. *S. droebachiensis* was obtained by collection in St. Thomas, V. I.

Histology and Electron Microscopy

Extended tube feet were removed with forceps, fixed immediately in a solution of seawater containing 5% glutaraldehyde, rapidly dehydrated through a series of alcohols, and embedded in glycol-methacrylate before being sectioned and stained in aqueous toluidine blue.

Analysis of the Tube Foot Protein

Removed tube feet were homogenized in 9.5 M urea, 2% Triton X-100, 2% SDS, and 1 mM dithiothreitol, and the clarified supernate was then diluted into the appropriate buffer for gel electrophoresis. Typically, tube feet obtained from two sea urchins and homogenized in 1 ml of the above extraction solution gave protein concentrations of ~10 mg/ml by Lowry assay. For quantitation of the actin content, the total solubilized tube foot protein was subjected to electrophoresis on an SDS polyacrylamide slab gel, with actin and myosin standards in adjacent wells, and stained with Coomassie Blue. The gel was sliced into strips and scanned in a Gilford spectrophotometer at 550 nm (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). The peaks in the densitometer tracing were measured by area triangulation, using the Wang "digitizer" program.

Tube Foot RNA Extraction

Removed tube feet were homogenized in 8 M guanidine hydrochloride, 25 mM ethanol precipitation, the RNA was resuspended in 1% SDS, 0.1 M NaCl, and 10 mM Tris (pH 7.4), phenol-extracted, and then precipitated with 3 M sodium acetate (pH 6). After centrifugation, the RNA was resuspended in 0.2 M sodium acetate, pH 5, and then precipitated with 2 vol of ethanol at −20°C. Before use, the RNA was centrifuged, washed twice with 80% ethanol to remove residual salt, dried under vacuum, and resuspended in 4°C sterile water at the desired concentration. 50 sea urchins yielded ~250 μg of tube foot RNA.

Cell-free Protein Synthesis

Rabbit reticulocyte lysate was prepared by the method of Woodward et al. (8) and treated with micrococcal nuclease according to Pelham and Jackson (9). Reaction mixtures contained, in a final volume of 25 μl:140 mM potassium acetate, 1.5 mM magnesium acetate, 500 μM spermidine (free base), 8 mM creatine phosphate, creatine kinase (155 U/mg; Sigma Chemical Co., St. Louis, Mo.) at 8 μg/ml, 20 mM HEPES at pH 7.6, 25 μM amino acids minus methionine, 2 mM dithiothreitol, 10–50 μCi of [^{35}S]methionine (400–600 Ci/mmol, Amersham-Searle, Chicago, Ill.), and 1–5 μg of total tube foot RNA isolated by 8 M guanidine extraction (7). Reactions were carried out at 37°C for 1 h, and incorporation was assayed by hot TCA precipitation as described by Roberts and Paterson (10).

Analysis of Cell-free Products

Samples from cell-free reactions were analyzed by one- and two-dimensional gel electrophoresis. For one-dimensional analysis, 10 or 12% SDS polyacrylamide slab gels were used, following the method of Laemmli (11). For two-dimensional analysis, the method of O'Farrell (12) was used as described. Isoelectric focusing was done at constant power (ISCO [Instrumentation Specialties Co.], Lincoln, Nebr.), 3 W overnight. The pH was found to be linear with length of the gel, using a contact electrode (LKB Instruments, Inc., Rockville, Md.). Purified actin from rabbit, chick, or sea urchin was mixed with the radioactive samples. For direct comparison of the first and second dimension of the gel electrophoresis, 26-cm-wide slab gels were used to accommodate the tube gels (18 cm) and to allow use of adjacent wells.

The SDS polyacrylamide slab gels were fixed and stained for 0.5 h with a 10% acetic acid, 30% methanol, and 0.2% Coomassie Blue solution and then destained in a solution containing 10% acetic acid, 50% methanol overnight. The stained marker actin spots were marked with India ink. The gels were then analyzed by fluorography after the method of Bonner and Laskey (13) and Laskey and Mills (14). The fluorograms were then scanned at 550 nm for quantitation by densitometry.

Partial Proteolytic Digestion and Electrophoresis of the Actin Translation Product

The procedure described by Cleveland et al. (15) was followed. Samples from tube foot RNA-stimulated cell-free reticulocyte lysate reactions were mixed with unlabeled rabbit marker actin (15–20 μg; Worthington Biochemical Corp., Freehold, N. J.) and subjected to electrophoresis on an SDS polyacrylamide slab gels. The gel was stained with Coomassie Blue for 10–20 min and destained for 30 min. The actin bands were cut out with a razor blade and incubated in 10 ml of 0.125 M Tris (pH 6.8), 0.1% SDS, 1 mM EDTA for 30 min. For analysis by partial proteolysis, the gel slices were placed in the wells of a stacking gel (1% acrylamide, 0.5% agarose) containing 1 mM EDTA. *Staphylococcus aureus* V8 protease (Miles Laboratories Inc., Elkhart, Ind.), trypsin, or papain (Worthington Biochemical Corp.) was then added to each well, and the mixture was subjected to low current for 30 min. The proteolytic fragments were then subjected to electrophoresis in a 15% acrylamide (1 mM EDTA) separating gel. Identical amounts of enzyme without actin samples were introduced into adjacent wells. The gel was analyzed by Coomassie staining and subsequent fluorography. The stained marker actin fragments (after subtraction of enzyme-specific bands) and the [^{35}S]methionine-labeled translation product fragments were then compared.

Synthesis of Copy DNA (cDNA)

cDNA was synthesized using purified avian myeloblastosis virus (AMV) reverse transcriptase, as described by Friedman and Rosbash (16). The reaction mixture (25 μl) consisted of: 50 mM Tris (pH 8.3), 20 mM dithiothreitol, 6 mM

$MgCl_2$, 60 mM NaCl, 1 mM each of dATP, dTTP, dGTP, 200 μM dCTP (220 Ci/mmol), 5 μg/ml oligodeoxythymidine$_{10}$ (Collaborative Research, Waltham, Mass.) 122 μg/ml actinomycin D (Sigma Chemical Co.), 2 μl of AMV reverse transcriptase (720 U/ml final concentration), and 2 μg of total tube foot RNA. The reaction mixture was incubated for 45 min at 37°C, and RNA was hydrolyzed by addition of 150 μl of 0.5 N NaOH and boiling for 3 min at 100°C, and then neutralized with 150 μl of 1 N NaH_2PO_4. Nucleotides were separated from the cDNA by an SP-50 (Pharmacia Inc., Piscataway, N. J.) column (0.7 cm × 10 cm) equilibrated with 300 mM NaCl, 10 mM sodium acetate (pH 5.5). Excluded peak fractions were pooled and precipitated with 2 vol of ethanol after addition of 20 μg tRNA. The product was centrifuged, resuspended in water, and stored in siliconized glass tubes at −20°C. The specific activity of the cDNA was 2×10^7 cpm/μg. The cDNA was found to be 500 nucleotides in length, judged by centrifugation through an isokinetic alkaline sucrose gradient (17).

Hybridization of Tube Foot RNA to Tube Foot cDNA under RNA-excess Conditions

RNA-cDNA hybridization under conditions of RNA excess was performed as described by Hereford and Rosbash (18). The reactions (130 μl total volume) were incubated at 70°C in 1.5 M NaCl, 10 mM Tris (pH 7.4), 1 mM EDTA, with 2 μg total tube foot RNA and an estimated poly(A)-RNA:cDNA ratio of 100:1. Hybridization was assayed by S_1 single strand–specific nuclease digestion followed by TCA precipitation onto nitrocellulose filters. The data points were analyzed according to the least squares computer program described by Pearson et al. (19).

RESULTS

Actin Is the Major Abundant Protein of Tube Feet

Fig. 3 *a*, slot *C* shows the pattern of proteins seen on a Coomassie Blue–stained SDS polyacrylamide gel after homogenization of the tube feet and extraction as described in Materials and Methods. The most prominent band migrates with a molecular weight of 42,000, as shown by its comigration with purified rabbit skeletal muscle actin in the gel (slot *B*). Other bands are visible at molecular weights of 38,000, 180,000, and 220,000. The first has the molecular weight expected of tropomyosin. We have tentatively identified the second as collagen-chain dimers, because it comigrates with a soluble collagen preparation (not shown). Electron microscope analysis suggests that collagen is the major component of the large basement membrane of tube feet (Fig. 2). The 220,000-dalton band comigrates with purified myosin heavy chains (slot *A*). Fig. 3 *b* is a densitometer tracing of the extracted tube foot proteins assayed on an equivalent gel. A conservative measure of the areas under these three peaks using a Wang triangulation computer program, expressed as percent of the total area of the tracing, is 60% for actin and 7% each for collagen and myosin.

The gel analysis of the major band was then extended to two dimensions by use of the method of O'Farrell (12). Fig. 3 *a* (right) shows the gel pattern of the tube foot proteins after isoelectric focusing on a pH 5–7 gradient, followed by slab gel electrophoresis. Through use of this procedure, the 42,000-dalton band separated into two major components with isoelectric points between 5.6 and 5.7. A minor component is usually evident toward the acid side (5.5). This pattern is almost identical to that shown for alpha-, beta-, and gamma-actin species by Whalen et al. (20) and Hunter and Garrels (21) for verebrate muscle, and to the I, II, and III actin species from *Drosophila* of Storti et al. (22). We will identify these species by their native isoelectric values (5.50, 5.64, and 5.70). This obviates the necessity of a separate nomenclature of actin for every animal system. These putative actins are present in the tube foot in the approximate ratio of 1:90:9. Hence, the 5.50 species is not generally obvious. The actin of all four species of sea urchin examined are identical in this pattern. A spot to the right and below actin (more acidic) corresponds to the position of vertebrate "beta" tropomyosin (23).

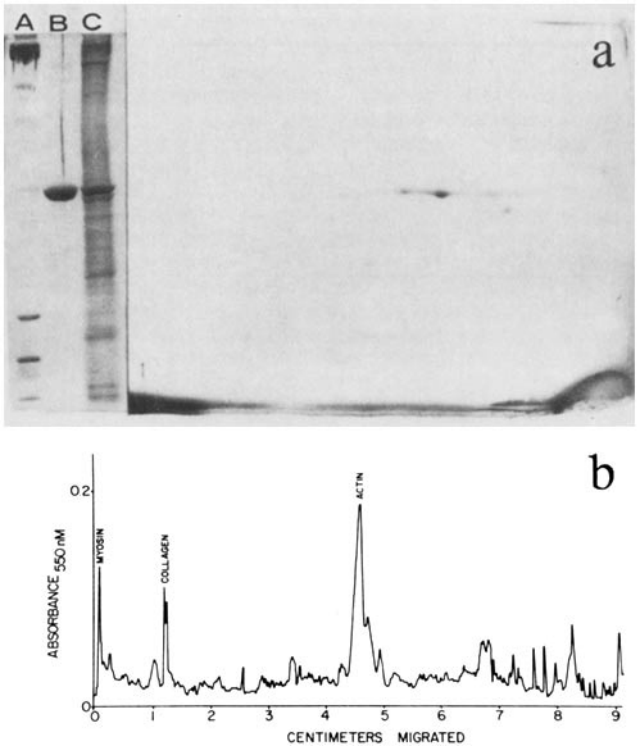

FIGURE 3 One- and two-dimensional electrophoresis of total tube foot protein (*A. punctulata*). (*A*) The top left side shows a one-dimensional analysis of marker and tube foot proteins. Slot *A*, purified vertebrate muscle myosin; slot *B*, purified rabbit muscle actin; slot *C*, total tube foot protein. Gel is a 10–15% gradient in polyacrylamide. The right side shows a two-dimensional gel electrophoresis of total tube foot protein. The isoelectric focusing gel has a pH gradient from pH 5 (right) to pH 7 (left). Gel is 12% in acrylamide. (*b*) Densitometer tracing of a Coomassie-stained, one-dimensional SDS polyacrylamide gel of tube foot proteins, scanned before drying. Gel is 8% in polyacrylamide.

Actin Messenger RNA Is the Major Abundant Species of Message Extracted from Tube Feet

Total RNA was extracted from the tube feet of *S. purpuratus* and *A. punctulata* and purified as described in Materials and Methods, using 8 M guanidine (17). This RNA was then tested for its ability to stimulate protein synthesis in both the wheat germ (10) and the rabbit reticulocyte (9) cell-free systems. When the products from the reticulocyte cell-free synthesis were analyzed by single dimension electrophoresis and fluorography (14), the results in Fig. 4 *a* and *c* (left side) were obtained. Both *S. purpuratus* and *A. punctulata* tube foot RNA (Fig. 4 *a* and *c*, respectively) stimulated the uptake of methionine into one major band at 42,000 daltons (at the position of marker actin) that is absent in controls lacking added message (Fig. 4 *c*, second lane from left). Other proteins, synthesized in much lower amounts, were revealed in Fig. 4 *a* (left side) by increased exposure time (20 h). The radioactivity in the *S. purpuratus* putative actin band saturates the x-ray negative well before this amount of time. A densitometer tracing of the same reticulocyte fluorogram exposed so that the radioactivity does

not saturate the x-ray film (3.5 h) shows the 42,000-dalton protein to be 80% of total synthesis (Fig. 4*b*).

Fig. 4*a* and *c* (right side) also shows the two-dimensional analysis of the putative actin band on the same gel as the single-dimension analysis of the equivalent samples. The two-demensional analysis emphasizes that the 42,000-dalton protein has isoelectric points and molecular weight mobility characteristic of actin (20–22). When the actins translated in the cell-free system are analyzed on the same gel with native tube foot protein, the pattern of spots is identical, but the actins synthesized in the cell-free system are shifted +0.06 pH unit. This may be construed as a result of an in vivo modification of each isospecies of actin. The major native sea urchin tube foot protein and the beta isospecies of purified chick actin from 16-d embryonic muscle almost coincide exactly on two-dimensional analysis, to within 0.02 pH units. Both isospecies are in the middle position relative to the other actin species. Furthermore, *S. purpuratus* and *A. punctulata*, diverged evolutionarily for 200 million years (24), show all isospecies of actin to migrate coincidentally (to within 0.02 pH unit). Evident in these gels upon two-dimensional analysis is the outstanding abundance of the protein. In contrast to the complex pattern of proteins resolved by these gels after translation of total rat muscle mRNA (23), the translation of total tube foot RNA yields essentially a single protein. The reticulocyte system with no mRNA added showed no radioactive proteins in two-dimensional analysis even after long exposures. On a gel with a broader gradient from pH 3 to pH 10, no additional proteins of significant abundance were seen.

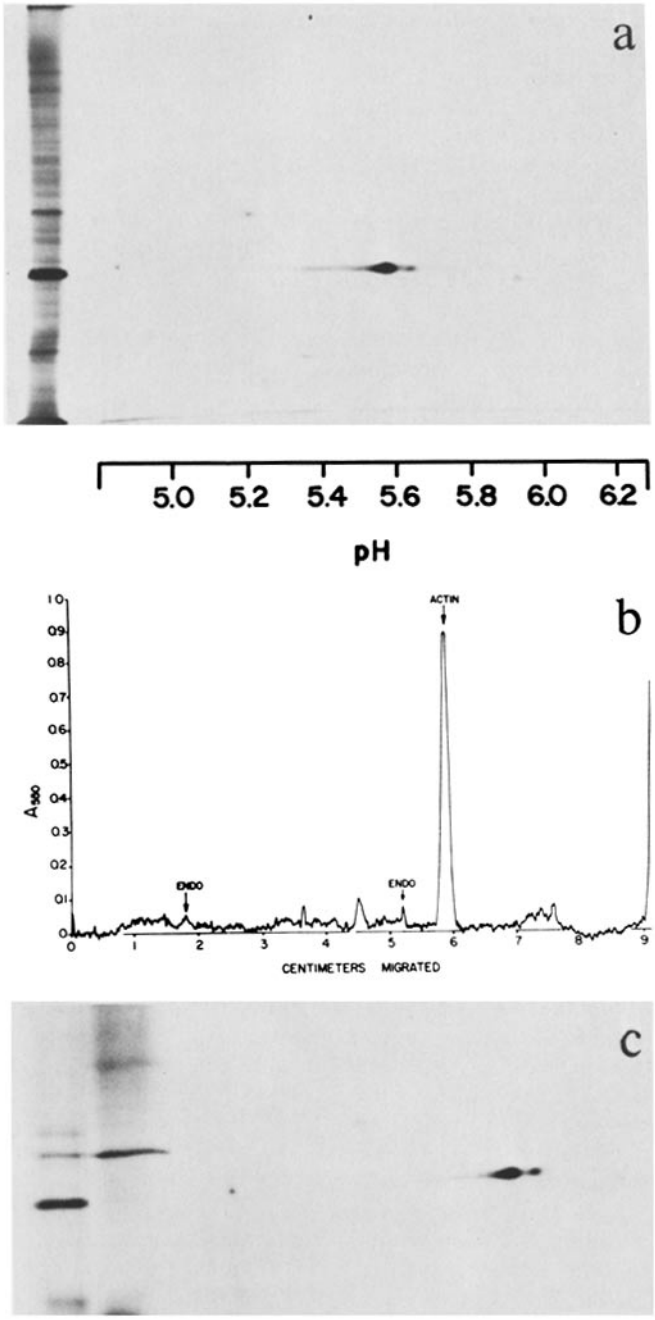

FIGURE 4 One- and two-dimensional fluorograms of the reticulocyte translation products stimulated by total tube foot RNA. (*a*) Synthesis stimulated by RNA from *S. purpuratus*. The one-dimensional gel is on the left; the two-dimensional gel is on the right, polyacrylamide is at 10%. The pH gradient was measured on an identical parallel focusing gel. Exposure time was 20 h at −80°C, using Kodak XR-5 film. (*b*) Densitometer scan of the one-dimensional gel fluorogram of *a*, exposed so that the radioactivity in the actin band did not saturate the film. Exposure time was 3.5 h at −80°C, using Kodak XR-5 film. (*c*) Synthesis stimulated by RNA from *A. punctulata*. The one-dimensional gel is on the left: first well on left, tube foot RNA-stimulated translation products; second well on left, no added RNA (endogenous control). The two-dimensional gel is on the right; the pH gradient is the same as above. Note that the polarity of *a* and *c* is the reverse of the two-dimensional gel in Fig. 3. Exposure time was 2 d at −80°C, using Kodak XR-5 film.

Identification of the Translation Product as Actin by Partial Proteolytic Digestion

Products of the cell-free synthesis in reticulocyte lysate were subjected to electrophoresis and proteolysis with purified marker actin from rabbit in an SDS polyacrylamide slab gel. The rabbit actin fragments resulting from proteolysis were detected by Coomassie stain, and the radioactive reticulocyte-synthesized protein fragments were detected by fluorography. Fig. 5*a* shows the stained gel and *b*, the fluorograph. The pattern of the nine proteolytic fragments produced by digestion with two concentrations of *S. aureus* V8 protease and with papain (slots *B*, *C*, and *D*, respectively) was identical in the gel of stained rabbit actin fragments and in the fluorograph of the fragments of the protein products from sea urchin mRNA. We conclude that the protein translated from tube foot RNA that coincides with actin upon two-dimensional electrophoresis and that has the same proteolytic fragments as purified actin is, therefore, actin. No proteolysis occurred in the presence of trypsin (slot *A*), underscoring the known relative insensitivity of actin to trypsin digestion under these conditions (25).

RNA Excess Hybridization of Tube Foot cDNA

Using AMV reverse transcriptase, we employed total tube foot RNA to direct the synthesis of a single-stranded cDNA. This cDNA "probe" was then hybridized to total tube foot RNA under conditions of RNA excess and single-stranded molecules digested with S_1 nuclease. Fig. 6 is a plot of the percent single-stranded cDNA as a function of the log of RNA concentration times the time ($R_o t$). The $R_o t$ analysis clearly shows that 85% of the copied sequences are highly abundant. Least-squares analysis by use of a computer program specifically developed for hybridization reactions (19) gave a fit of root mean square 0.0403 when the hybridization was assumed to reflect the kinetics of one component (85%), and 0.0209 when the hybridization was assumed to describe the kinetics of two components. In the latter case, one component makes up

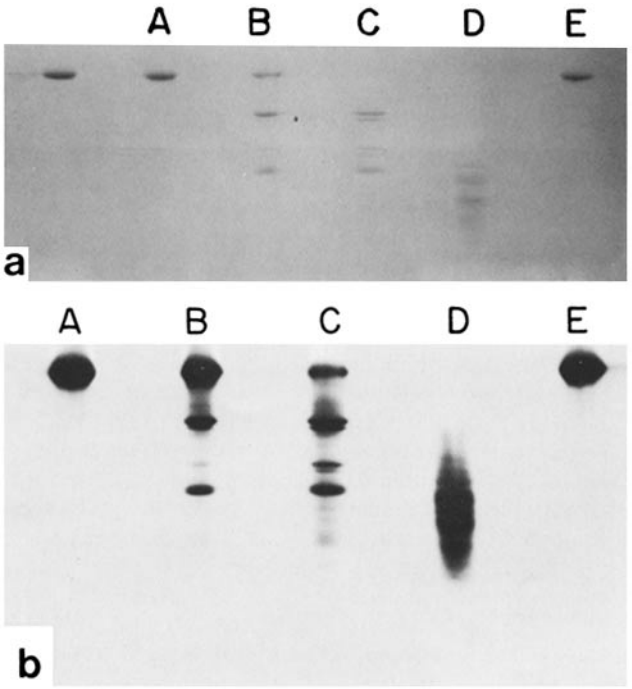

FIGURE 5 Partial proteolytic analysis of 42,000-dalton band. (*a*) Wells *A*–*D* contain rabbit marker actin bands cut from another gel and subjected to coelectrophoresis with the translated putative actins in the presence of: well *A*, 1 μg of trypsin; well *B*, 0.025 μg of *Staphylococcus* V8 protease; well *C*, 0.5 μg of *Staphylococcus* V8 protease; well *D*, 1 μg of papain. Well *E* contains translated actin with no enzyme. The immediate right of each well contained an identical amount of enzyme without actin. The unlabeled well contains rabbit marker actin alone. (*b*) The fluorograph of the same gel. The exposure time was 1 d at −80°C, using Kodak XR-5 film.

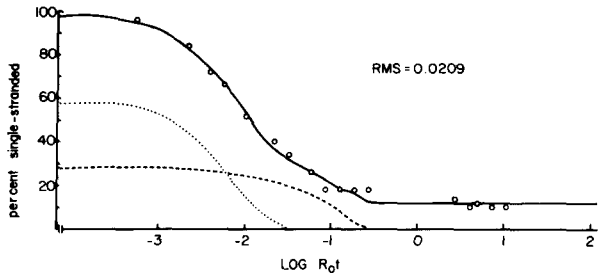

FIGURE 6 Computer plot of the hybridization of tube foot cDNA probe (*A. punctulata*) to total tube foot RNA (*A. punctulata*). The circles are the experimental points; the solid line is the best-fit two-component curve; dotted and dashed lines are the single components of the curve calculated by the computer. *RMS*, root mean square.

58% of the total copied cDNA (dotted line) and the other, 27% (dashed line). The more abundant component has a $R_0t_{1/2}$ somewhat higher than that expected of actin (6×10^{-3}). Because the cDNA is 500 nucleotides long and must be complementary to the 3′ untranslated message region, the complexity may result from a mixture of several actin messages with diverse 3′ ends (26). Therefore, it is possible that ~60% of the polyadenylated RNA in sea urchin tube feet could be considered a single protein coding sequence.

DISCUSSION

These results show that the tube feet of sea urchins contain actin in very large quantities (possibly as much as 60% of the total protein). This is more than the richest known source of actin—even when compared with vertebrate smooth muscle, which contains ~30% actin (6). Moreover, the tube foot is an organ, specialized for motility and yet composed of tissues with an unusual simplicity of protein composition. Hence, this organ is an interesting model for the role of actin as a contractile protein. The tube foot actin comigrates in a two-dimensional gel with the purified chicken beta actin isospecies from embryonic muscle, indicating the probability of a high degree of evolutionary conservation.

In parallel with the abundance of actin in this organ, the messenger RNA for actin is similarly abundant. Total messenger RNA extracted from *S. purpuratus* tube feet, when translated in a cell-free system from reticulocytes, directed 80% of the total incorporated methionine into actin. This actin was identified by its coelectrophoresis with purified actin in one- and two-dimensional gel systems. As with the protein extracted from the tube feet, it yielded two major (pI 5.58 and pI 5.64) and one minor (pI 5.50) isospecies of actin with isoelectric points that differed slightly from those of the native actins. 90% of the actin is present as one component, the analogue to chicken beta actin. Additionally, partial proteolytic digests of translated actin by use of three different proteolytic enzymes indicated that all fragments were identical to the fragments obtained from the digestion of purified rabbit skeletal muscle actin. Hybridization of cDNA with total tube foot RNA yields results consistent with the interpretation that probably as much as 60% of the mRNA is composed of one abundant coding sequence. Presumbly, the nonconserved diversity of the 3′ end of actin message (26) increases the complexity somewhat.

Tube foot messenger RNA contains sufficient complexity to code for 2,000 sequences (27). This was established using RNA excess with single-copy DNA tracer hybridization and did not provide information on the abundance of these species. Our approach, designed to investigate the abundant species of messenger RNA, yielded the information that actin is detectable in high abundance. Species present in less abundance (15–30% of the total mRNA) are found with the expected molecular weights for myosin, collagen, tropomyosin, light chains of myosin, and many other proteins, as detected on the gels (Fig. 3*a*). The population of tube foot messenger RNA with the highest complexity but lowest abundance most likely makes up the 5–10% of the total message complement that is not detected by cell-free translation or RNA excess hybridization.

Isolated actin messenger RNA from any organism would be useful for examining questions concerning the genetic control of actin synthesis in that organism. Actin, though a contractile protein, is also a common component in all cells (5). Actin is a very highly conserved protein (5), varying <6% in amino acid residues between evolutionarily diverged organisms (28). Because the actin genetic sequences appear to be similarly conserved (26), the actin gene family is a natural candidate for an evolutionary and histotypic study of genes for morphologically ubiquitous and abundant proteins. In an analogous system, the mRNAs for the very well conserved histone H-4 protein of two distantly related sea urchin species have diverged by 11.5% in nucleotide sequence, consistent with expected codon redundancy (29). This still allows for interspecies hybridization of these sequences. We have made use of the availability of this highly enriched messenger RNA to effect the synthesis of cDNA, to probe the genomic human library for actin sequences, and have isolated several of these sequences. This was possible because of the evolutionarily well-conserved actin

nucleic-acid sequence confirming the observations of protein conservation and the presumed lack of conservation of any contaminating species. Initial work indicates that there are <10 actin genes in the human genome. This contrasts with *Dictyostelium*, which has ~17 (30), but is similar to *Drosophila*, which has ~5 (31).

The actin genes will also serve as a general model to study gene expression. The genetic regulation of families of actin sequences include some that are constitutively synthesized (beta) and some that result from specific gene activation (alpha). The structure of the respective messenger RNA species appears to differ at the 3′ end (26). The appropriate investigations into gene structure (such as flanking and intervening sequences) may yield useful insights into the regulation of these two gene classes.

We thank Dr. J. Beard for the gift of AMV reverse transcriptase. Bruce Paterson, Robert Storti, Alan Jacobsen, Bryan Roberts, Michael Rosbash, Jeff Pudney, and Bill Crain generously lent their time, expertise and advice. Scrantz and Linda Golder in the Marine Biological Laboratory photo lab supplied Fig. 1. Lynn Golden, Ken Swartz, Bill Crain, Dave Durika, Jeff Pudney, David Pearl, and Helena Huang risked their lives to pick tube feet in Woods Hole during the great blizzard of February 1978. Barbara Schneider injured her finger while skindiving for the *Diadema* at St. Thomas, V. I.

This work was supported by the University of Massachusetts Medical School Scientific Affairs Committee for summer research in Woods Hole, Massachusetts, by National Institutes of Health Grant NS 11329 to R. H. Singer, and by a Muscular Dystrophy fellowship to J. Kabat-Zinn.

This work was first presented as a platform session at the American Society for Cell Biology Meetings, November 1979, in Toronto.

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Received for publication 27 August 1980, and in revised form 8 December 1980.

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