Actin synthesis during the early development of *Xenopus laevis*

By E. A. STURGESS[1], J. E. M. BALLANTINE[1],
H. R. WOODLAND[1], P. R. MOHUN[2], C. D. LANE[2] AND
G. J. DIMITRIADIS[2]

*From the Department of Biological Sciences, University of
Warwick, Coventry, and the National Institute for Medical
Research, London*

SUMMARY

Cytoskeletal β and γ-actin are amongst the most rapidly made proteins of oocytes, blastulae and later embryonic stages of *Xenopus laevis* but, relative to other proteins, the rate of synthesis is low in the egg or cleaving embryo, although the messenger RNA is present in comparable amounts at the different stages. Actin synthesis therefore involves post-transcriptional regulation. α-actin, the actin class characteristic of striated muscle cells, is first detectable in late gastrulae and it is an abundant newly synthesized protein from the neurula stage onwards. mRNA template activity for this protein is not detectable before the gastrula stage. Thus α-actin synthesis probably reflects new gene action, confined to part of the embryo, for α-actin only appears in the section which includes presumptive skeletal muscle cells. It therefore constitutes the earliest cyto-specific protein so far demonstrated in Amphibia. When tadpole tail poly(A)-containing mRNA is injected into oocytes and eggs α-actin synthesis is seen in both cases. Extensive evidence for the identification of the actins is presented. This is based on location of synthesis, DNase-I binding and partial peptide mapping.

INTRODUCTION

The actins are a family of related contractile proteins. The control of the family of genes (Vandekerckhove & Weber, 1978) coding for them is of particular interest, for multiple forms of actin have been shown to exist in a particular cell. Further they differ in different cells of the same species, and in the same cell at different stages of development. The actins may be partially separated by electrofocusing which yields three fractions called, in order of decreasing negative charge, α, β and γ (Whalen, Butler-Browne & Gros 1976; Garrels & Gibson, 1976; Rubenstein & Spudich, 1977; Storti *et al.* 1978), thus supporting evidence of actin heterogeneity obtained by peptide mapping and sequencing (Gruenstein

& Rich, 1975; Gruenstein, Rich & Weihing, 1975; Elzinga & Lu, 1976). Translation of actin mRNA *in vitro* indicates that α, β and γ-actins are different gene products (Hunter & Garrels, 1977), as does amino acid sequencing data, which reveals at least six actin genes in vertebrates (Vandekerckhove & Weber, 1978). These latter authors have also shown that α-actin, which is only found in skeletal and cardiac striated muscle, is itself heterogeneous, the skeletal and cardiac forms differing in primary sequence in at least two positions. β and γ-actins are found in all the cell types examined, including smooth muscle, but again there is tissue-specificity, since chicken gizzard actin differs in primary sequence from the actins of non-muscle cells even though they have the same iso-electric point.

Two-dimensional gel electrophoresis reveals the changing patterns of protein synthesis in developing *Xenopus* embryos. Among the proteins most clearly seen on these gels are those tentatively identified as actins (De Robertis & Gurdon, 1977; Brock & Reeves, 1978; Ballantine, Woodland & Sturgess, 1979). In this paper we identify the actins and describe their synthesis during early development.

MATERIALS AND METHODS

Xenopus oocytes, eggs and embryos were prepared as described by Ballantine *et al.* (1979). Embryonic stages were according to Nieuwkoop & Faber (1956). Electrophoretic analyses were performed as described previously and were derived from the original procedures of O'Farrell (1975).

Preparation of tadpole tail RNA

The procedure is derived from that of Ullrich *et al.* (1977). 300 stage-40 tadpole tails were prepared, washed with distilled water then blotted with paper and placed immediately in 100 ml of homogenization buffer (4 M guanidinium thiocyanate, 1 M β-mercaptoethanol, 100 mM sodium acetate, pH 5·0). The tails were homogenized in a glass vessel, then centrifuged at 22000 rev/min for 1 h in an SW40 rotor at room temperature. The resulting supernatant was layered over a cushion of 5·7 M-CsCl, 0·1 M-EDTA (Gilsin, Crkvenjakon & Byus, 1974) using 2 ml of cushion for each 12 ml supernatant. The cellulose nitrate tubes used were centrifuged in the SW40 rotor at 22000 rev./min for 24 h at room temperature. Under these conditions the RNA is pelleted, the DNA bands and the protein forms a pellicle at the top of the CsCl cushion. The pellet obtained was dissolved in 0·12 M-NaCl, 2 mM-Tris-HCl, pH 7·5, 0·5 % sodium dodecyl sulphate by heating at 60 °C for 30 sec. It was precipitated by the addition of two volumes of ethanol followed by an overnight incubation at − 20 °C. Polyadenylated RNA was prepared on columns of oligo(dT) cellulose by the method of Singer & Penman (1973). The total RNA was dissolved in sterile distilled water at 1·25 μg/μl and polyadenylated RNA at a concentration of 0·1 μg/μl.

Purification of actins on DNase-I Sepharose columns

The method of Lazarides & Lindberg (1974) was adapted for extracts of *Xenopus* embryos. Thus embryos were homogenized in column buffer 3 (150 mM-NaCl, 1 mM-CaCl₂, 50 mM-Tris-HCl, pH 7·4) using a ratio of eight embryos to 0·5 ml buffer. The extract was centrifuged for 2 min in an Eppendorf microcentrifuge and the clear supernatant between the pellet and lipid skin was collected by piercing the tube.

Products from translation of oocyte and neurula RNA in the nuclease-treated rabbit reticulocyte lysate (Pelham & Jackson, 1976) were diluted fourfold with column buffer before loading onto the columns. Non-radioactive oocyte or neurula supernatant (equivalent to 3–5 oocytes or embryos/column) was added as carrier in some experiments.

Samples in column buffer were supplemented with 60 μg/ml phenyl methyl sulphonyl fluoride (PMSF) and incubated for 5 min at 27 °C. Bovine serum albumin was then added to a final concentration of 500 μg/ml and the incubation continued for a further 5 min.

Columns of DNase-I Sepharose (0·5 ml) were washed with 2·5 ml column buffer 1 (4 M guanidinium chloride, 0·35 M sodium acetate, 30 % glycerol, pH 6·5) and then with 2·5 ml of column buffer 2 (1 mM-CaCl₂, 50 mM-Tris-HCl, pH 7·5). 0·5 ml samples, prepared as described above were loaded at 4 °C and washed with column buffer 3 (see above) until all visible haemoglobin was removed from the column. Eluates were reloaded and washed again. The resulting eluate constitutes *Fraction 1*. Columns were eluted, again at 4 °C, with five further steps to yield a total of six fractions:

Fraction 2(i): 2·5 ml column buffer 3.

Fraction 2(ii): 2·5 ml column buffer 3.

Fraction 3: 2·5 ml column buffer 4 (1 mM-CaCl₂, 0·5 M-CH₃COONa, 30 % glycerol, pH 6·5).

Fraction 4: 5 ml column buffer 4 plus 0·75 M-GuCl.

Fraction 5: 2·5 ml column buffer 5 (150 mM-NaCl, 50 mM-Tris-HCl, pH 7·4, 20 % glycerol, 150 μg/ml PMSF).

The columns were warmed to room temperature and eluted with two successive 2·5 ml volumes of column buffer 5 plus 0·1 % sodium dodecyl sulphate (SDS) to give *Fractions 6*(i) and *6*(ii). 9 % of each column fraction was used for the measurement of tri-chloroacetic acid (TCA) precipitable radioactivity and 1 % for total radioactivity. Then 100 μg bovine cytochrome *c* was added to each fraction as carrier and protein was precipitated for 1 h at 4 °C by adding TCA to 20 %. The resulting pellet was washed twice with acetone, dried *in vacuo* and stored at − 70 °C. Column fraction 4 was dialysed for 1 h against sterile distilled water before precipitation.

Electrophoretic analysis of the protein pellets was performed on material dissolved in either SDS sample buffer (1 D gels) or in O'Farrell (1975) lysis

buffer (2D gels), containing 0·1 % SDS for embryos, but without SDS for cell-free systems.

Peptide analysis

Actin spots from 2D gels were characterized by mapping of partial protease digests, essentially as described by Cleveland, Fischer, Kirschner & Laemmli (1977). Rabbit skeletal muscle actin, iodinated with ^{125}I according to the method of Adamson & Woodland (1974) was used as a standard. Proteins labelled with [^{35}S]methionine during synthesis *in vivo* or *in vitro*, and actins iodinated *in vitro*, were fractionated on 2D gels. The gels were usually dried down and autoradio-graphed, thereby locating the α and β plus γ actin spots: these were then cut out. In the case of the actin synthesized *in vitro* the 2D gel was fixed and stained with 0·1 % Coomassie brilliant blue in 10 % acetic acid, 45 % methanol. After destaining in 10 % acetic acid, 45 % methanol, the gel was fluorographed in a dry state to reveal the actin spots (Bonner & Laskey, 1974): again the actin spots were cut out. Part or all of the dried fragment of gel containing actin was placed in the slot of an 18 % acrylamide/SDS gel, the latter made according to Laemmli (1970), except that the bis-acrylamide concentration was 0·5 % that of the monomer. 50 μl of SDS sample buffer was added, the gel was left to swell for 20 min and then the slots were carefully overlayered with electrode buffer. Finally 20 μl of sample buffer, diluted 1:1 with 0·1 mM-EDTA and containing micrococcal V8 protease, was carefully layered over the sample buffer. The gels were then run overnight and stopped when the bromophenol blue dye had reached the bottom: the gels were dried immediately and either autoradiographed, or fixed in 20 % TCA for fluorography.

RESULTS

Changes in actin synthesis during early development

We describe putative actin synthesis during early development before pro-viding detailed evidence of the identity of the proteins. The actin regions of 2D gel (O'Farrell, 1975) separations of newly made proteins from *Xenopus* oocytes and embryos are shown in Figs. 1 and 2. Fig. 1A reveals that β and γ-actin are amongst the most abundant newly synthesized proteins in oocytes, just as they are in embryos (Ballantine *et al.*, 1979; Bravo & Knowland, 1979; Brock & Reeves, 1978; De Robertis & Gurdon, 1977). In eggs, on the other hand, very little incorporation into β, γ-actin is apparent (Fig. 1C). The failure to find radioactive actin in the egg could be an artefact produced by poor recovery of newly synthesized actin. We have virtually ruled out this possibility by injecting tadpole tail total or polyadenylated RNA into eggs and oocytes and analysing on 2D gels the products formed. Muscle is a major component of the growing tail and actin mRNA should therefore constitute one of its major mRNA species. Gel fractionation of oocytes injected with total RNA is shown in Fig. 1B.
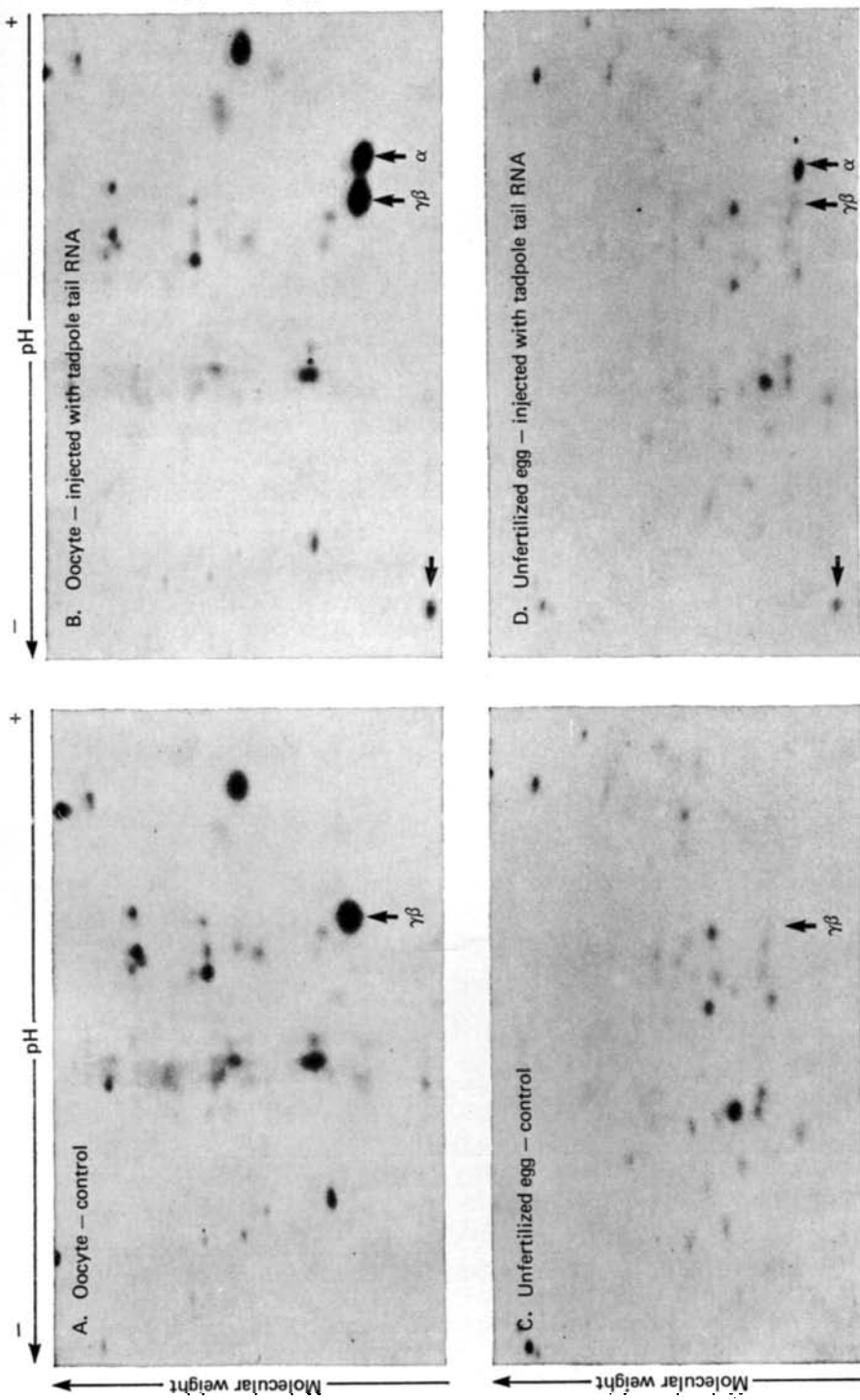
Fig. 1. Autofluorographs of 2D gel separations of proteins made in control oocytes (A) and unfertilized eggs (C) and in those injected with tadpole tail total RNA (B and D). Oocytes were injected with 50 ng of tadpole tail total RNA in sterile distilled water and incubated at 20 °C for 18 h in [^{35}S]methionine at 1 mCi/ml. Unfertilized eggs were injected with 50 ng of tadpole tail total RNA in sterile distilled water containing 15 mCi/ml [^{35}S]methionine and were incubated for 3·5 h at 20 °C. The proteins were separated in the first dimension by iso-electric focusing in gels containing ampholines pH 5–7 and pH 3·5–10 in a ratio of 4:1 and in the second dimension by SDS-gel electrophoresis on a 12 % acrylamide gel. Labelled arrows indicate the position of α, β, γ-actins and an unlabelled arrow in B and D indicates another protein found in the injected samples.

Three new spots, two of which are shown in the diagram, may be seen quite clearly. One of these is α-actin*. β and γ-actins made on injected mRNA could not be detected, since these proteins are already made at a high rate on endogenous templates (Fig. 1A, B). The analogous experiments with eggs are shown in Fig. 1C and D. A strong α-actin spot is seen in eggs injected with mRNA and a weak β γ-spot. Thus the recovery of α-actin is comparable in eggs and oocytes. This demonstrates that at least one newly synthesized actin may be recovered from eggs, though it could be argued that β and γ-actins behave differently. Other evidence against loss of actin is that we found a good recovery of endogenous stored egg actin or embryo βγ-actin added to eggs (Ballantine *et al.* 1979). The injection of tadpole tail RNA also serves to pinpoint, accurately, the actin region of the control gel, and to reveal the weakly labelled egg actin region surrounded by neighbouring proteins (Fig. 1C). The oocyte and unfertilized egg both translate α-actin mRNA which provides further evidence that such undifferentiated cells can translate any messenger formed during cell differentiation. The injected frog cells use total and polyadenylated RNA indistinguishably and α-actin synthesis persists apparently undiminished for at least 30 h (data not shown).

Actin synthesis in dissected embryos

α-actin synthesis should be restricted to striated muscle cells: to establish that this is true of the protein identified as α-actin we dissected embryos into two halves, as described by Woodland & Gurdon (1968). The 'ectodermal' part consists of both the ectoderm and the dorsal mesoderm, including the somitic mesoderm, the latter forming the skeletal muscles. The 'endodermal' half comprises the endoderm and the rest of the mesoderm. These half embryos were incubated with [^{35}S]methionine and then analysed on 2D gels.

Figure 2 shows that β and γ-actins are made in all regions of the embryo, but α-actin is restricted, within the limits of our dissecting skill, to the region containing developing muscle cells. In the blastula/gastrula dissection the α-actin appears before it would normally be seen in a whole embryo (stage 16). The

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*It should be noted that it is possible to obtain an artefactual 'α-actin' spot (e.g. from oocyte extracts) if impure urea is used in the sample preparation.

FIGURE 2

Fig. 2. Autofluorographs of 2D gel separations of proteins made by different regions of blastulae/gastrulae (stage 9½–11), neurulae (stage 17–24), and tail-bud tadpoles (stages 32–36). The embryos were dissected into 'ectodermal' and 'endodermal' regions at the earlier stage, and incubated in [^{35}S]methionine at 12·5 mCi/ml for 4–6 h at 20 °C, when control embryos had reached the later stage indicated. The proteins were separated as described in Fig. 1, but with ampholines in the proportions 2:1, pH 5–7 to pH 3·5–10. Labelled arrows indicate the position of α, β and γ-actin.
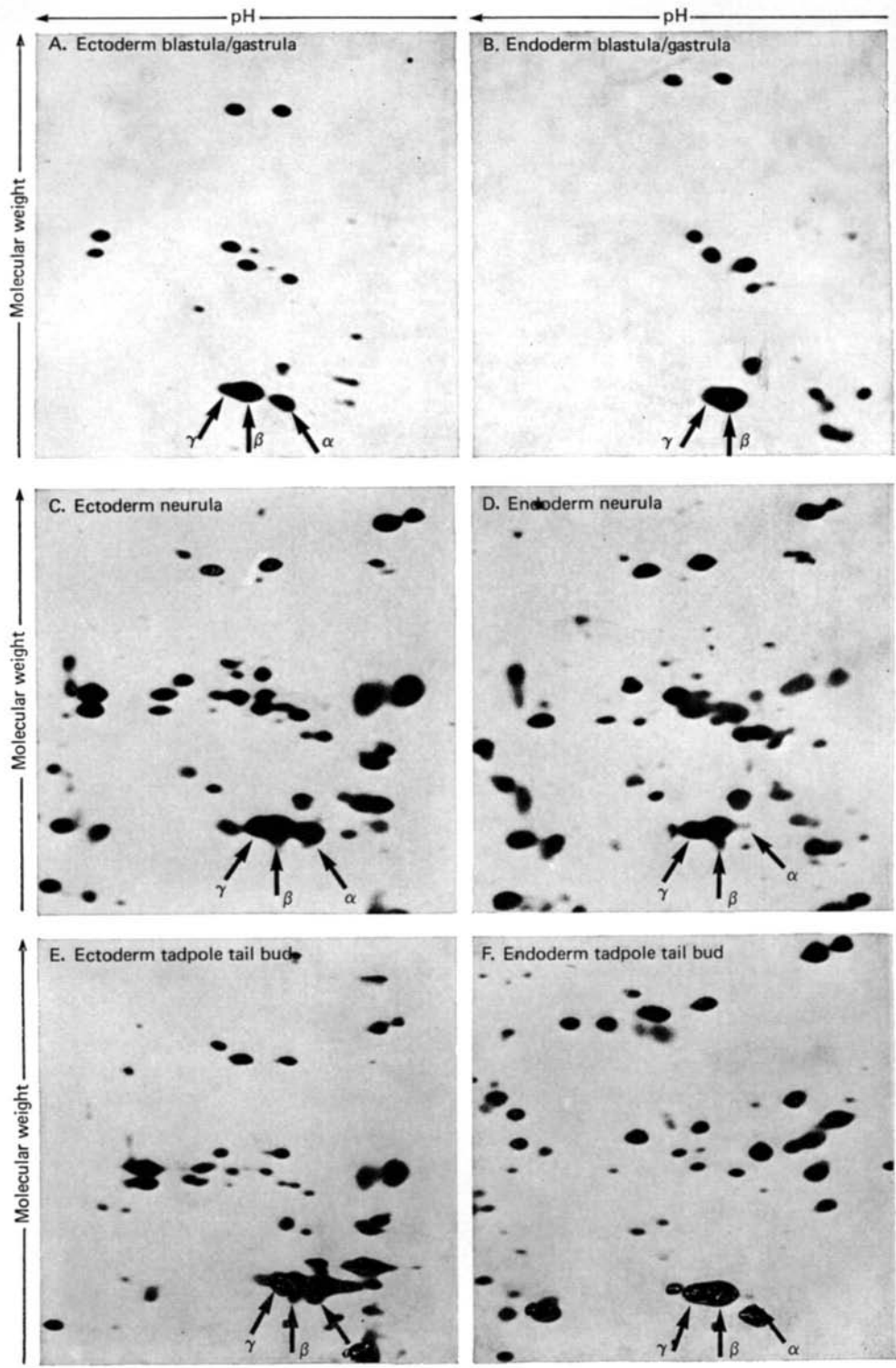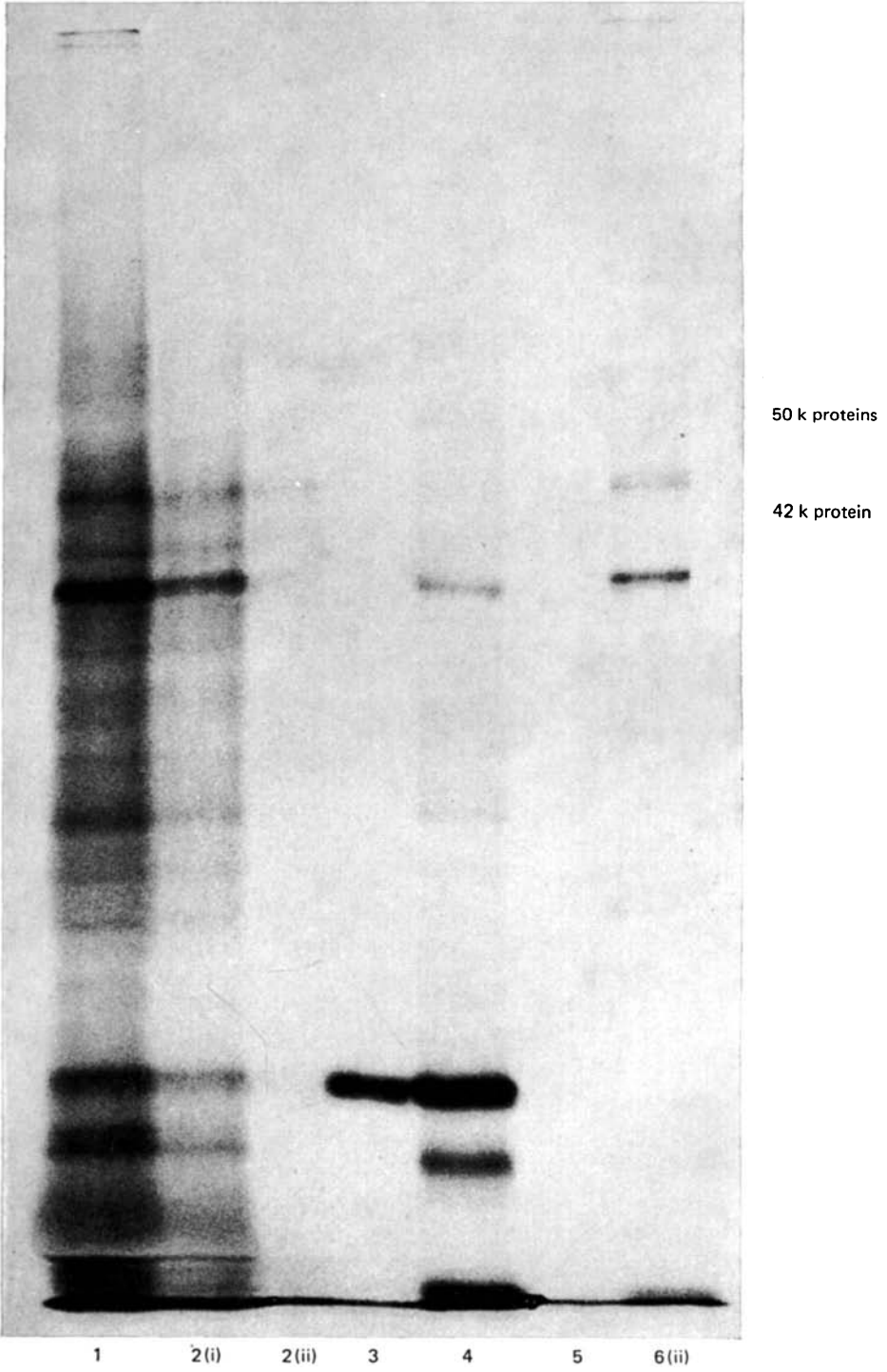
precocious appearance of α-actin is seen in embryos bisected vertically, as well as horizontally (data not shown).

DNase-I column chromatography of embryonic actins made in vivo *and in* vitro

One criterion for the identification of actins is that they bind to DNase I. If the DNase I is coupled to Sepharose an affinity column may be prepared and the bound proteins analysed by gel electrophoresis (Lazarides & Lindberg, 1974).

The full application of this procedure is illustrated by characterization of the proteins made in a reticulocyte cell-free system under the direction of neurula total RNA. Figure 3 shows the 1D gel analysis of the non-actin fractions eluted from the affinity column. In this experiment no embryo homogenate was added as carrier. Omitting carrier raises the level of actin binding to the column, and improves the resolution of subsequent 2D gel analysis. The full protocol for elution is given in the Methods section.

The run-through fraction (1) and low salt washes [2(i) and 2(ii)] show similar patterns of radioactive bands over the whole molecular weight range, including material co-migrating with unlabelled marker rabbit muscle actin. While this latter radioactive material probably includes proteins other than actin, it may also indicate that the columns were operated slightly above their maximal capacity. This was done to maximize the yield of purified actin. Washing the column with a high salt buffer (Fraction 3) removes loosely bound material, yielding mainly a single intense band with a molecular weight of about 15000–20000. Including 0·75 M guanidinium chloride in the buffer completes the removal of this protein and also elutes a variety of other proteins, which appear as bands of weaker intensity. Column buffer 5 eluted little further material, but adding 0·1 % SDS to this buffer was shown in preliminary experiments to elute authentic actin [Fraction 6(i)]. Consequently this fraction was analysed by 2D electrophoresis. Fraction 6(ii) is residual material eluted with more of the same buffer. The 1D gel shows that it contains predominantly material co-migrating with marker actin (molecular weight 42000), together with a doublet of higher molecular weight (*ca.* 50000), but weaker intensity. These three proteins were present in much larger amounts in Fraction 6(i).

The 2D gel analysis of Fraction 6(i) is shown in Fig. 4C and D. Two points emerge: firstly that a specific and limited population of proteins is selected by the column, and secondly that the putative α, β and γ-actins are the main components of this population. Non-actin proteins which bind to DNase-I Sepharose

FIGURE 3

An autofluorograph of the 1D SDS gel electrophoresis analysis of neurula proteins (made by neurula total RNA in a reticulocyte cell-free system) after passing through a DNase-I Sepharose affinity column as described in the text. The position of 42000 and 50000 molecular weight proteins is indicated.
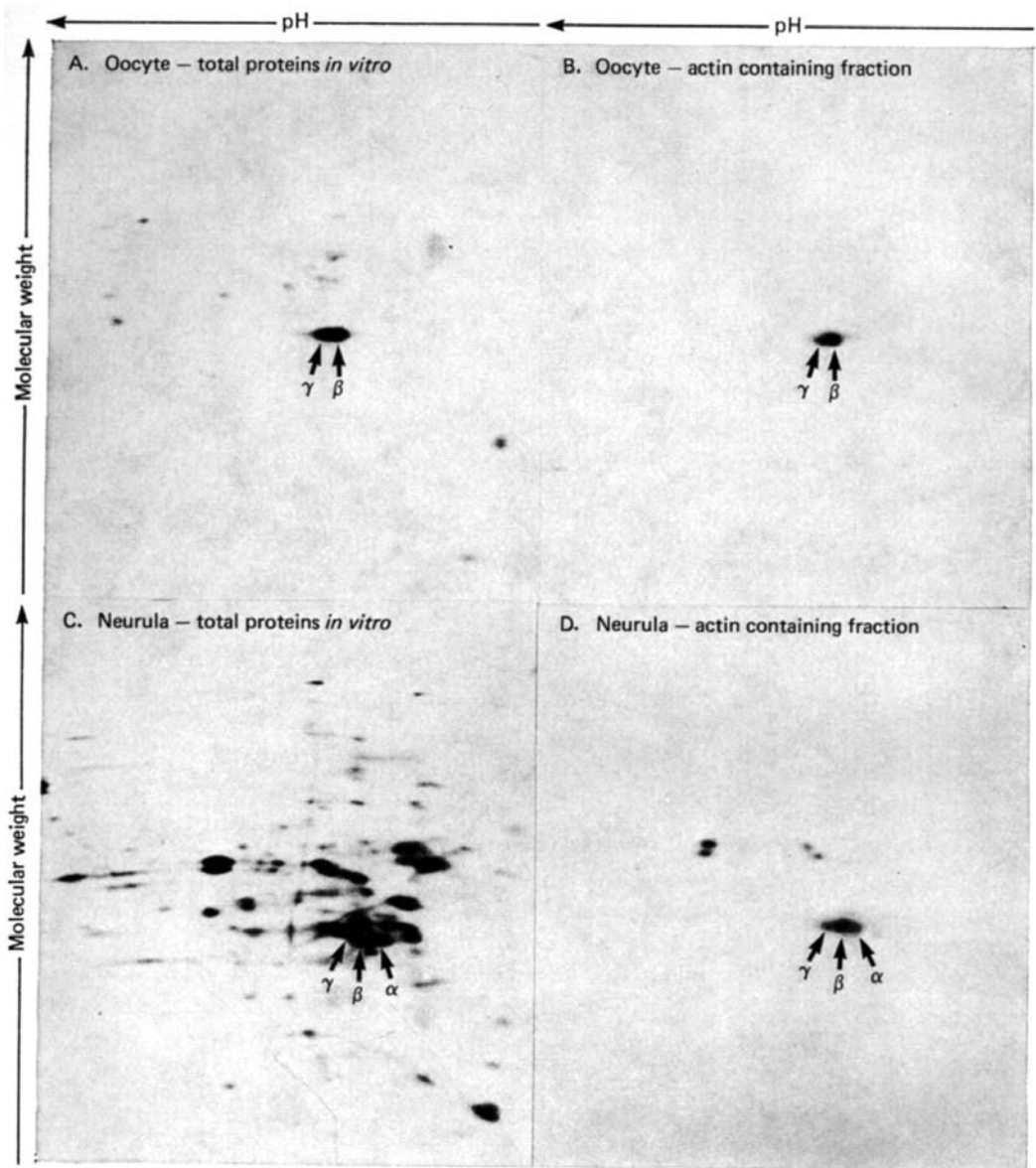
Fig. 4. Autofluorographs of the 2D analysis of the unfractionated product of a reticulocyte cell-free system programmed with oocyte total RNA (A) and neurula total RNA (C). B and D show the 2D analysis of the actin-containing Fraction 6(i) separated from the total product by binding to a DNase-I Sepharose affinity column. The proteins were prepared and separated as described in Methods. Labelled arrows indicate the position of α, β and γ-actins.

Table 1. *Elution of* [^{35}S]*methionine-labelled translation products of* X. laevis *neurula total RNA from a DNase-I Sepharose affinity column*

Column fraction	Total cpm in fraction	TCA insoluble cpm in fraction	TCA insoluble cpm in fraction as % TCA insoluble cpm eluted from column
1	$35{\cdot}2 \times 10^6$	694178	59
2(i)	$62{\cdot}0 \times 10^6$	355256	30
2(ii)	166900	6833	0·6
3	24300	3722	0·3
4	40900	15111	1·3
5	5100	1644	0·14
6(i)	82600	92033	7·8
6(ii)	13300	5033	0·4

and migrate with similar molecular weights to those that we see are also clearly visible in the analyses of previous authors (Lazarides & Lindberg, 1974). From their iso-electric points and molecular weights these *ca.* 50000 molecular weight proteins may be the Z-band, actin-binding proteins called desmins (cf. Lazarides & Balzer, 1978). Their binding to DNase I is not changed by adding cold carrier neurula or oocyte homogenate to the reticulocyte lysate.

Figure 4A and B also show the analysis of Fraction 6(i) proteins made *in vitro* under the direction of oocyte total RNA. The main spot seen is that correspond- ing to β and γ-actins. Oocytes do not make α-actin (Fig. 1A), nor does their RNA programme its synthesis *in vitro* (Ballantine *et al.* 1979). They also do not make the *ca.* 50000 molecular weight proteins which figure in the neurula RNA analyses. The oocyte non-actin column fractions are qualitatively similar to those of neurulae.

The quantification of the oocyte column fractions is shown in Table 1 and reveals a high proportion (8 %) of the TCA counts in the actin fraction. In this experiment five cold neurulae were added per column as carrier: if no carrier is added to the cell-free products as much as 20 % of the radioactivity may bind to the column. We believe this to represent diverse proteins that do not show as spots on gel analyses, rather than proteins that are not solubilized by the gel lysis buffer.

We have also used DNase-I Sepharose to purify proteins made *in vivo* by oocytes and neurulae labelled with [^{35}S]methionine. Again in the neurula samples three putative actins stand out on the gel (Fig. 5). Only two of the *ca.* 50000 molecular weight proteins can be seen, but for some reason even in the original neurula extract the other DNase-I binding proteins were not well labelled. In the oocyte analysis only the β and γ-actins bound to DNase I, as expected.
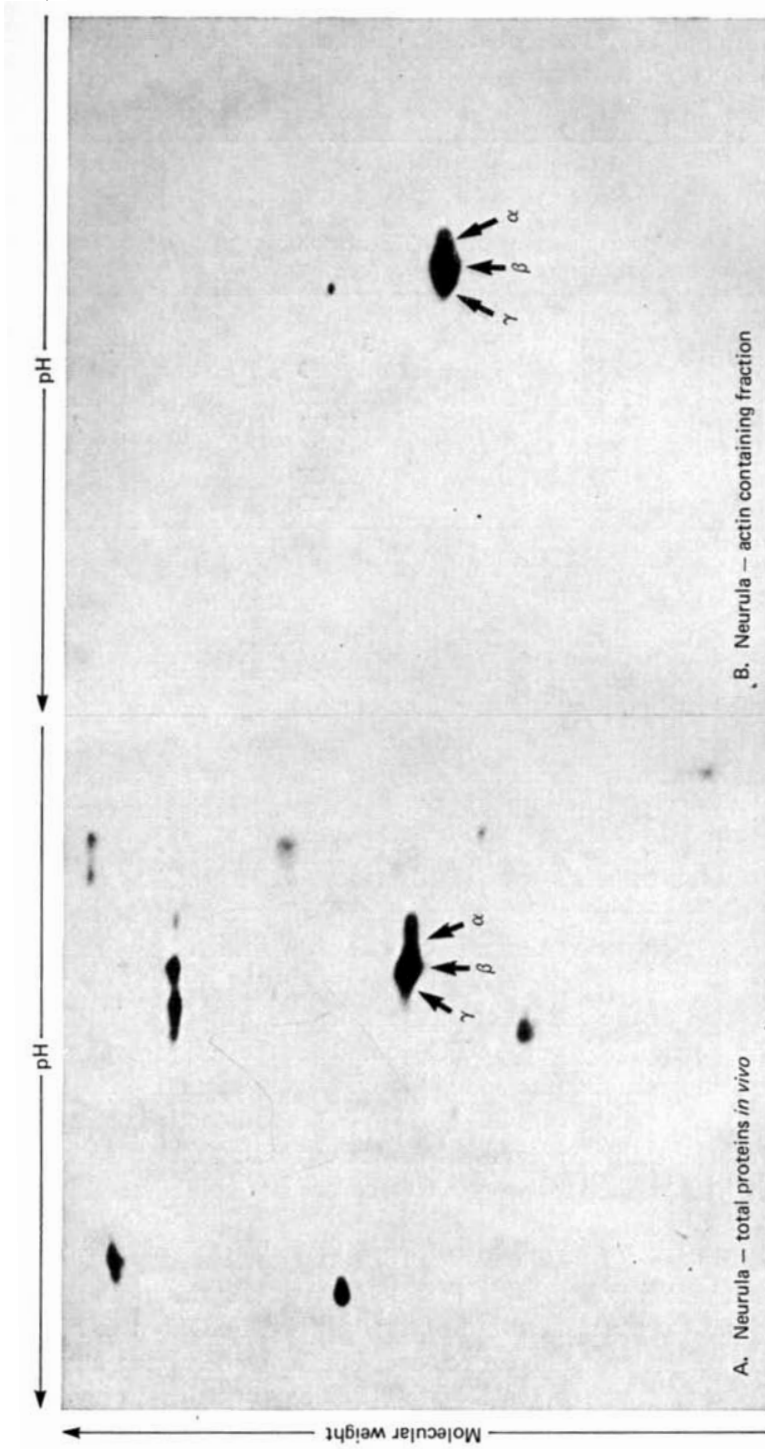
Fig. 5. Autofluorographs of the 2D analyses of: (A) the total neurula proteins made *in vivo* and (B) the proteins found in the actin-containing fraction 6(i) obtained from a DNase-I Sepharose affinity column. The proteins were prepared and separated as described in the text. Labelled arrows indicate the position of α, β and γ-actins.

In summary, the proteins tentatively identified as actins all bind to DNase-I Sepharose. This is consistent with their identification as actin, but does not prove it, since certain non-actin proteins also bind to DNase-I affinity columns.

Peptide mapping of actins made in embryos

We have checked the identity of the putative embryonic actins by analysing partial protease digests (Cleveland *et al.* 1977), an approach that was also used by Brock & Reeves (1978).

All actins are related, and sequences are highly conserved. In fact the partial cleavage products of [^{35}S]methionine-labelled α, β and γ-actins are identical. Therefore by partial peptide mapping one can only define a protein as an actin, and not as α, β or γ-actin. These sub-classes have been defined solely by their position on iso-electric focusing gels (i.e. isoelectric point). Sequencing has shown that there are several sorts of α-actin, but all of them are restricted to striated muscle cells (see Introduction). Thus by peptide mapping the protein that migrates in the α-actin position we can confirm that it is an 'α-actin'. It is a presumption that it is striated muscle-specific, but this is consistent with the results from dissected embryos presented above.

For a standard we have used adult striated muscle actin. This was made by conventional actin polymerization methods. In fact rabbit actin was used in the experiments shown, but this gives identical results to adult *Xenopus* muscle actin. The actin was made radioactive with ^{125}I, thus labelling tyrosine residues. Actin is rich in both tyrosine and methionine, so it is not surprising that the large peptides seen in partial digests all label with both ^{125}I and [^{35}S]methionine. As one would expect the two isotopes label the various peptides to different extents, but since all are labelled this does not affect the conclusions drawn.

Embryonic actin was prepared from 'ectodermal' halves of stage-32 embryos incubated with [^{35}S]methionine. The 2D gel pattern obtained was like that shown in Fig. 2E, and the β plus γ-actin, and α-actin spots were cut out and digested with micrococcal V8 protease during analysis on 18 % acrylamide/SDS gels (see Methods). The results are shown in Fig. 6. The various slots differ slightly in their kinetics of digestion (which is hard to make exactly reproducible with differing gel slices, but is allowed for by using several protease concentrations for each sample) and the peptides vary somewhat in their intensity of labelling as expected. In addition the γ rays emitted by ^{125}I make bigger bands than the β particles from ^{35}S. However, the [^{35}S] putative actins produce all of the bands seen in the authentic [^{125}I] actins, and there are no extra bands. This analysis supports the view that the embryonic proteins are all correctly identified as actins.

A similar analysis was performed on actin made in a reticulocyte lysate programmed by neurula total RNA and bound to a DNase-I column (that shown in Fig. 4). It is clear from Fig. 7 that the putative actin made *in vitro* has the same partial digest pattern as actin made *in vivo* and as [^{125}I] rabbit skeletal muscle actin. Again this confirms our identification of the actins.
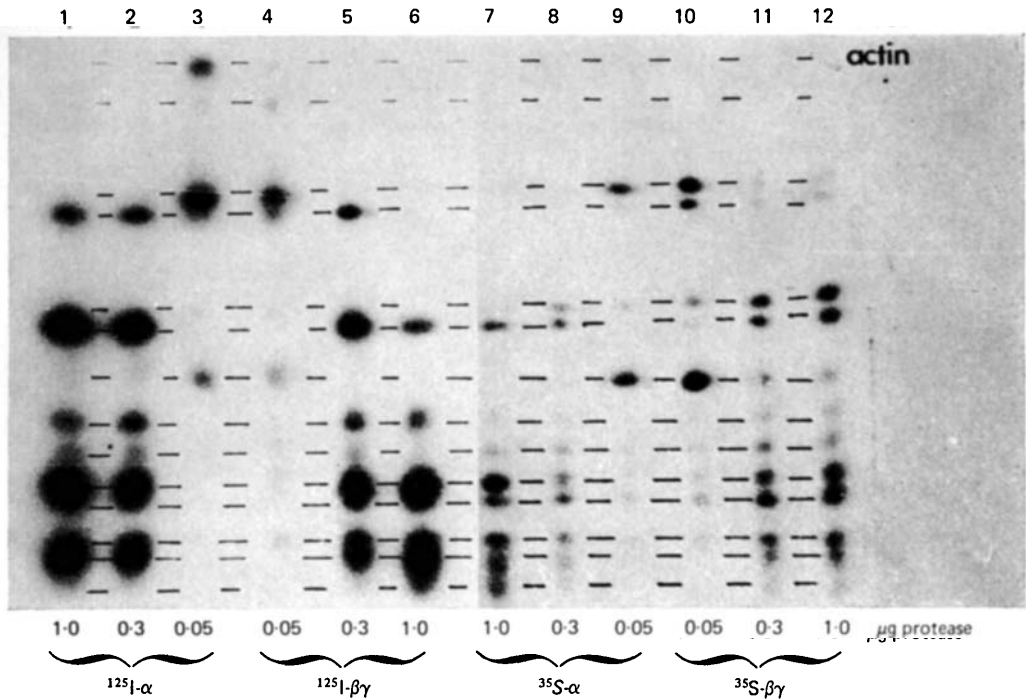
Fig. 6. An autoradiograph comparing the products of partial peptide digestion of actins from purified rabbit skeletal muscle labelled with ^{125}I *in vitro* and from tadpole total protein labelled *in vivo* with [^{35}S]methionine. The α-actin and β, γ-actins were fractionated on 2D gels and located by autoradiography. 1–3 rabbit skeletal muscle α-actin labelled *in vitro* with ^{125}I. 4–6 rabbit skeletal muscle β, γ-actin labelled *in vitro* with ^{125}I. 7–9 α-actin labelled with [^{35}S]methionine in 'ectodermal' halves of stage-32 tadpoles (cf. Fig. 2E). 10–12 β, γ-actins labelled in [^{35}S]methionine as for tracks 7–9.

Actin mRNA species at different developmental stages

As well as investigating proteins made *in vivo* and those made *in vitro* on oocyte and neurula templates, we have studied the proteins made *in vitro* under the direction of added egg, blastula, gastrula and tailbud RNAs. The results confirm those of Ballantine *et al.* (1979) and are not presented: moreover the identification of the actin spots by these latter authors has now been established by our DNase-I binding and peptide mapping studies of *in vitro* synthesized actins. Thus we can now conclude that β- and γ-actin messenger levels are comparable in large oocytes, unfertilized eggs, blastulae and gastrulae. At the late gastrula stage the template activity for α-actin mRNA becomes detectable. Thus changes in β and γ-actin synthesis at early stages must operate through post-transcriptional mechanisms. The tissue-specific synthesis of α-actin must either involve the activation of an RNA species that is inactive even *in vitro*, or as seems more likely, the *de novo* synthesis of functional messenger.
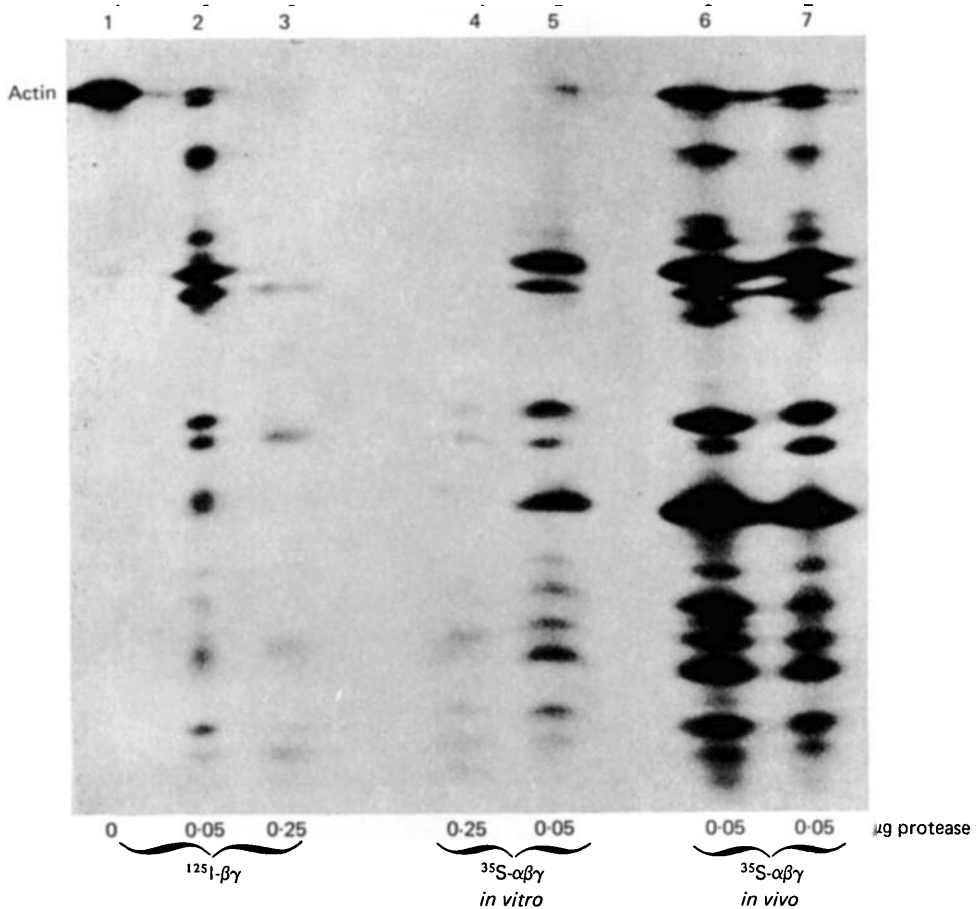
Fig. 7. An autoradiograph comparing the products of partial peptide digestion of purified [^{125}I] rabbit skeletal muscle actin, identical to that in Fig. 6, with the products of digestion of neurula actins synthesized and labelled *in vivo*, or made *in vitro* and separated by binding to a DNase-I Sepharose affinity column. 1–3 β and γ-actin from rabbit skeletal muscle labelled *in vitro* with ^{125}I. 4–5 α, β and γ-actin labelled with [^{35}S]methionine in a reticulocyte cell-free system programmed with neurula total RNA. 6–7 α, β and γ-actin synthesized *in vivo* in 'ectodermal' halves of stage-32 tadpoles.

DISCUSSION

It is possible to make a tentative identification of actin on 2D electrophoretograms of proteins solely on the basis of the abundance of the protein and its position on the gel, because actins are both abundant and highly conserved. This latter point has been established by sequencing avian and mammalian skeletal muscle α-actins, amongst others. So far no amino acid differences have been revealed (Vandekerckhove & Weber, 1978). The gel electrophoretic patterns of both the muscle and the non-muscle actins are identical in birds and mammals, and they even seem to be similar to the respective proteins of *Drosophila* (Storti

et al. 1978; Fyrberg & Donady, 1979). This led us to make a tentative identification of the actins of *Xenopus* embryos (Ballantine *et al.* 1979). We have now confirmed this identification with evidence of three kinds. Firstly, the actin-like proteins bind to DNase-I Sepharose, as predicted from their known properties, Secondly, they show a pattern of partial protease digestion products which is identical to that of authentic actin. These two criteria are met both by the actins made *in vivo* and by those made in a rabbit reticulocyte cell-free system supplemented with embryonic mRNA. Peptide mapping of the oocyte actin has previously been mentioned by De Robertis & Gurdon (1977). Lastly, the putative α-actin should be restricted to that part of the embryo where striated muscle develops, and this is indeed the case.

We are therefore in a position to outline the developmental programme of actin synthesis, though detailed quantitative information of the type available for histones (Adamson & Woodland, 1974; Woodland & Adamson, 1977) does not yet exist for actin. β and γ-actins are major constituents of oocytes and are amongst the most rapidly synthesized oocyte proteins. They make up at least 0·1–0·3 % of [^{35}S]methionine incorporated into acid-insoluble material by oocytes and about double this in terms of the radioactivity present in spots on a 2D gel. These data were obtained by cutting actin spots out of 2D gels and determining the radioactivity present. Our data indicate that actin synthesis proceeds at a low rate in eggs and at early cleavage stages. This agrees with the results of Brock & Reeves (1978) and De Robertis (personal communication). Bravo & Knowland (1979) have reported higher rates of actin synthesis, but we have consistently failed to reproduce this result. However, the rate of synthesis in eggs does seem to vary a little from batch to batch of eggs, but it is always low (Bravo & Knowland, 1979, also co-identify the major actin of heart with the β-actin of developing embryos. This seems unlikely to be correct, since all cardiac actins so far reported are of the α-type). When mRNA from tadpole tail is injected into the egg abundant newly synthesized α-actin is recovered. This and their observations, detailed in the first section of the Results, argue against recovery artifacts.

The actin mRNA content, as revealed by translation in the reticulocyte lysate cell-free system, remains constant through these early stages of development. The changes in actin synthesis outlined above must therefore be achieved by post-transcriptional control. The fact that the egg seems to synthesize little actin presents an apparent paradox. The oocyte contains large amounts of actin accumulated through oogenesis, and although accurate measurements do not exist, it is clear from stained 2D gels that actin is one of its most abundant soluble proteins. Thus making only a small amount of actin for 5 or 6 h of early development will have little effect on the actin content of the embryo. Perhaps actin mRNA (like that encoding H1 histone, Flynn & Woodland, 1980) shares regulatory sequences with other proteins which must not be made at high rates until later stages of development.

This translational quiescence during egg and cleavage stages is not unique to *Xenopus* actin (Ballantine *et al.* 1979). It is interesting to note that actin synthesis in the developing mouse is also reduced just after fertilization as reported by Abreu & Brinster (1978) and Martin, Smith & Epstein *et al.* (1978). The former authors measured radioactive incorporation, rather than absolute rates of protein synthesis. Comparison of their results on tubulin synthesis with those of Schultz, Letourneau & Wassarman. (1979), who measured absolute rates of tubulin synthesis, suggests that the experiments of Abreu & Brinster (1978) do not reflect changing amino acid pools, but reveal a true activation of actin synthesis at the 2- to 8-cell stage of development in the mouse.

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