Changes in protein synthesis during the development of *Xenopus laevis*

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Summary

Patterns of protein synthesis during the development of *Xenopus* were studied by two-dimensional gel electrophoresis. Up to the end of the blastula stage we find no newly synthesized proteins which are not already made in the oocyte. The first new proteins are seen during gastrulation, and they increase in number during neurulation. Some of these are restricted to the 'ectodermal' region, and some to the 'endodermal' region of embryos divided into two parts. These new, region-specific proteins include α-actin. When the oocyte matures the number of detectable newly synthesized proteins decreases, reaching a minimum in the unfertilized egg. Some, such as β- and γ-actin, re-appear at the end of cleavage. This could not be shown to be a recovery artifact. The relation of the total rRNA to these changes in protein synthesis was studied by translation in the lysed reticulocyte cell-free system. The mRNAs that code for oocyte proteins that cease synthesis in the unfertilized egg and re-appear in blastulae are nevertheless detectable in total RNA made from eggs. These proteins therefore seem to cease and resume synthesis through translational control. mRNAs for new proteins first appear after gastrulation, just when these proteins are first detected *in vivo*. This strongly suggests, though it does not prove, that new gene activity is involved. It is therefore likely that region-specific gene activity is already present by the gastrula stage of development, and has an impact on the most abundant kinds of proteins made in the embryo.

Introduction

Cell determination is believed to be under immediate genetic control, that is to be brought about by patterns of gene activity changing in space and time. The effective expression of gene activity is primarily in the synthesis of particular proteins. It is therefore reasonable to attempt to define the genetic programme for cell determination by studying the way protein synthesis changes during cell differentiation and by finding how it differs in populations of cells with different developmental fates.

In spite of the long-established popularity of amphibian embryos in the study of cell differentiation, surprisingly little is known about the way in which protein synthesis changes during development. Information is available for a number of total enzyme activities characteristic of all cells, for example RNA polymerase

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(Wasserman, Hollinger & Smith, 1972; Roeder, 1974; Hollinger & Smith, 1976) and DNA polymerase (Benbow, Pestell & Ford, 1975). These nuclear proteins are stored during oogenesis and change little in amount during early development, but nothing is known about their synthesis and turnover. Contractile proteins like tubulin behave in an analogous fashion (Smith & Ecker, 1969; Pestell, 1975). More is known about another class of nuclear proteins, the histones (Adamson & Woodland, 1974, 1977; Woodland & Adamson, 1977), where there is also a developmental store, but where the rate of synthesis is known to change dramatically at maturation by a post-transcriptional process. This whole subject of protein synthesis in early development has been reviewed in detail by Davidson (1976). Less is known about the very early appearance of histospecific proteins. Green, Goldberg, Schwartz & Brown (1968) showed that collagen synthesis becomes established at a rapid rate during the gastrulation of *Xenopus* embryos. However, it is not certain that this synthesis is restricted to a fraction of the cells, or that new gene expression is involved. The best studied amphibian histo-specific protein is probably tyrosinase. This is restricted to neural cells and melanocytes (Smith-Gill, Richards & Nace, 1972) and detectable levels first appear at the neurula stage in the development of *Rana pipiens* (Benson & Triplett, 1974a, b). Inhibitor studies suggest that new gene expression is involved in the appearance of this protein.

We may conclude that at least a few new proteins appear at the neurula stage, some being characteristic of particular cell types. To assess just how much the overall pattern of protein synthesis changes in development, it would be useful to have much more general information about the synthesis of individual proteins. An overall picture of the 'complex' class of gene products, that is those present in high diversity and at low concentrations, is best obtained by RNA/DNA reassociation methods. This approach has been applied most completely to echinoderms among developmental systems (see Davidson, 1976). Although it is an invaluable tool there is always a doubt as to whether all of the RNA molecules are functional messengers. The more prevalent class of gene products may be studied by two-dimensional gel electrophoresis of proteins (O'Farrell, 1975). It is this approach that we have used in the work described below. A similar, but less extensive, series of experiments has recently been reported by Brock & Reeves (1978). Their results were similar to ours, but because they did not examine oocytes their conclusions were rather different.

**MATERIALS AND METHODS**

**Biological materials**

Oocytes, oocytes matured in vitro, eggs and embryos of *Xenopus laevis laevis* were obtained, handled, and radioactively labelled, as described previously (Adamson & Woodland, 1974, 1977; Gurdon, 1974; Gurdon & Woodland, 1975). Oocytes were labelled by incubation in 30–50 μl of solution containing
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freeze-dried $^{[35S]}$methionine (300–800 Ci/m-mole, The Radiochemical Centre, Amersham) dissolved at 2 mCi/ml in a saline normally containing 88 mM-NaCl, 1 mm-KCl, 2.4 mm-NaHCO$_3$, 0.33 mm-Ca(NO$_3$)$_2$, 0.41 mm-CaCl$_2$, 0.8 mm-MgSO$_4$, 15 mm-Tris-HCl, pH 7.6 and 0.01 g/l each of sodium benzylpenicillin and streptomycin sulphate. The NaCl molarity was raised to 0.11 for matured oocytes. Eggs and embryos were injected with 40 nl $^{[35S]}$methionine at 10 mCi/ml in 0.1 M-KCl, after softening the jelly with u.v. irradiation at pre-hatching stages (Elsdale, Gurdon & Fischberg, 1960; the u.v. lamp must produce very short wavelength light, the production of small amounts of ozone being a good indication that the light will be effective). After radioactive labelling the embryos were dejellied with 1% cysteine-HCl, pH 8.0. Incubations were terminated by freezing on solid CO$_2$ and the embryos were stored at −80 °C.

Protein synthesis in parts of embryos was examined after dissection. The embryos were removed from the jelly and vitelline membrane manually and separated into an animal and vegetal half, called for convenience ‘ectodermal’ and ‘endodermal’, as described by Woodland & Gurdon (1968). The aim of the dissection was to partition most of the somitic mesoderm into the ‘ectodermal’ half. About five halves were incubated in 20 μl of the saline solution indicated above, containing 12.5 mCi/ml $^{[35S]}$methionine. After 3–5 h they were washed, frozen and stored at −80 °C. In one experiment the embryos were labelled by injecting $^{[35S]}$methionine before the dissection.

Sample preparation

Our procedures were similar to those of O’Farrell (1975), except that 0.1% sodium dodecyl sulphate (SDS) was present in the homogenization medium. Twelve oocytes, eggs or embryos were homogenized in a small glass homogenizer in 0.5 ml lysis buffer containing 9.5 M urea (Schwarz–Mann, Ultrapure), 2% (w/v) Nonidet P40, 2% (v/v) ampholines (pH range 3.5–10:5–7, 1:2), 5% (v/v) 2-mercaptoethanol. The homogenate was centrifuged at 10000 g for 10 min and the clear supernatant removed by puncturing the tube. This was run on gels without further treatment.

The parts of embryos were processed identically except that five parts were homogenized in 0.3 ml lysis medium.

Aliquots were taken from the clear supernatants to determine radioactive incorporation. Total radioactivity was measured by drying duplicate 5 μl aliquots onto Whatman GF/C filters. Acid-insoluble radioactivity was measured by soaking 5 μl aliquots onto Whatman GF/C filters, then placing them in 20% trichloroacetic acid. They were washed in two changes of acid, and once each in ethanol and ether. Radioactivity was measured by scintillation counting in a toluene-based fluor.
First dimension separation by iso-electric focussing

Iso-electric focussing gels 0.25 x 11 cm were prepared according to O'Farrell (1975) using a combination of LKB ampholines, pH range 3.5–10 and 5–7, in the ratio 1:2. The pH gradient at the end of every run was monitored immediately by cutting a control gel into 1 cm pieces and shaking them with 2 ml boiled distilled water in capped tubes. After 30 min the pH was measured. The gradient was found to be from pH 4.0 to 6.7. Longer shaking times gave higher pH measurements.

A volume containing approximately half an oocyte, egg or embryo was loaded onto each gel and the gel's were run at 400 V for 19 h. Other details were according to O'Farrell (1975). The gels were removed and run immediately in the second dimension or, more usually, stored at -80 °C.

Second dimension separation on SDS-acrylamide gels

First dimension electrofocussing tube gels were prepared for running in the second dimension by equilibration for 1 h in 18 ml of buffer containing 0.0625 M-Tris-HCl, pH 6.8, 2.2 % SDS, 5 % (v/v) 2-mercaptoethanol.

Polyacrylamide/SDS slab gels (approximately 15 cm wide and 20 cm high) were made and run essentially as described by Laemmli (1970). Gels containing an exponential gradient of 10–30 % acrylamide were made as described by O'Farrell (1975). The high percentage acrylamide (30 % acrylamide/0.15 % bis-acrylamide) was stirred in a 20 ml constant volume mixing chamber, into which the lower percentage acrylamide (10 % acrylamide/0.27 % bis-acrylamide) was pumped. Non-gradient gels contained a single percentage of acrylamide (12 % acrylamide/0.3 % bis-acrylamide). A 3 cm 3 % acrylamide stacking gel was poured on top of the main slab gel and the equilibrated iso-electric focussing gel was fixed on top of it with 1 % agarose in 0.0625 M-Tris-HCl, pH 6.8, 0.2 % SDS coloured with 0.1 % bromophenol blue. Molecular weight marker proteins with molecular weights in the range 14500–130000 were run in a slot at the side of the gel. The gels were run overnight at a constant current of 19 mA/gel for gradient gels until the cytochrome c was 1 cm from the bottom of the gel, and 12 mA/gel for 12 % gels until the cytochrome c had reached the bottom of the gel.

Staining, fluorography and quantification of gels

Gels were routinely stained with Coomassie brilliant blue in 45 % methanol/10 % acetic acid and destained in 45 % methanol/10 % acetic acid. The gels were prepared for fluorography by the procedure of Bonner & Laskey (1974) and Laskey & Mills (1975).

The amount of radioactivity incorporated into the gel and the percentage of acid-insoluble counts located in discernible radioactive spots was determined as follows. A photocopy of the autofluorograph was fixed to the gel by alignment with markers and stained spots then the radioactive spots were punched out of
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Table 1. Incorporation of \[^{35}S\]methionine by rabbit reticulocyte lysates supplemented with total RNA prepared from different developmental stages of Xenopus laevis

| Stage  | \(\mu g\) total RNA added per 50 \(\mu l\) incubation | cpm incorporated per 2 \(\mu l\) between 0 and 60 min | Stimulation
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<tr>
<td>—</td>
<td>0 (blank)</td>
<td>17411</td>
<td>1</td>
</tr>
<tr>
<td>Oocyte</td>
<td>10·6</td>
<td>62116</td>
<td>3·6</td>
</tr>
<tr>
<td>Egg</td>
<td>10·8</td>
<td>63524</td>
<td>3·7</td>
</tr>
<tr>
<td>Blastula</td>
<td>9·8</td>
<td>57375</td>
<td>3·3</td>
</tr>
<tr>
<td>Gastrula</td>
<td>13·0</td>
<td>53201</td>
<td>3·1</td>
</tr>
<tr>
<td>Neurula</td>
<td>10·4</td>
<td>58974</td>
<td>3·4</td>
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the gel using the photocopy as a template. The punched out pieces of gel and the remaining ‘non-radioactive’ background were swelled in standard toluene-based scintillation fluid containing 9% Soluene (Packard) and 1% water. The radioactivity was measured by scintillation counting.

Extraction of RNA and translation in the rabbit reticulocyte lysate system

RNA was phenol-extracted directly from oocytes, unfertilized eggs and embryos as described by Ruderman, Woodland & Sturgess (1979). A nuclease-treated, rabbit reticulocyte cell-free system (Pelham & Jackson, 1976) was supplemented with approximately 10 \(\mu g\) RNA, and 32 \(\mu Ci\) \[^{35}S\]methionine per 50 \(\mu l\) incubation. Five microlitre aliquots of the incubations were mixed with 35 \(\mu l\) lysis buffer. These samples contained approximately 150000 cpm in TCA-insoluble products and were loaded onto iso-electric focussing gels which were prepared and run as previously described. Slab gels with a single percentage of acrylamide (12%) were used in the second dimension.

RESULTS

Proteins made by oocytes before and after maturation

Various methods of extraction and separation were tested, using \[^{35}S\]methionine-labelled proteins from oocytes as standard material. The standard homogenization procedure of O'Farrell (1975), also used by De Robertis, Partington, Longthorne & Gurdon (1977) in studies of Xenopus oocytes, was found to be satisfactory, but rather more proteins were revealed after incorporating SDS into the homogenization medium. One of our main findings is that the number of detectable newly synthesized proteins declines greatly during early stages of development. We therefore used the procedure likely to maximise the recovery of proteins. Resolution was reduced if much more than half of an oocyte, egg or embryo was run on a gel. The pH gradient (4·0-6·7) was chosen to give a maximal spread of proteins in the first dimension. In the second dimension an exponential
gradient of acrylamide was used to give a comprehensive picture of protein synthesis (Figs. 1, 2 and 3). This procedure has the disadvantage of giving great congestion in the 40000–90000 mol. wt. range, although by examination of the actual autofluorographs it is possible to resolve most of the proteins in this region. Over 750 proteins may be identified in the oocyte analyses. The proteins that differed between stages or regions were 40000–90000 daltons, so for later analyses single concentration 12 % acrylamide gels were used (Figs. 5–7).

The proteins made by oocytes, and by oocytes stimulated to mature into eggs by progesterone, are shown in Fig. 1. So many proteins (over 700) were identifiable that it is not possible to produce one figure displaying them all. Careful analysis of several experiments revealed no new types of proteins consistently appearing during maturation. On the other hand, many proteins clearly visible before maturation become greatly reduced or undetectable, as indicated in Fig. 1, even though similar amounts of radioactivity were loaded onto the gels. Amounts of radioactivity in individual proteins were measured after cutting out the spots. No consistent pattern of change emerged. Each protein varied independently, within a given experiment. Incorporation into a large number of proteins was expressed as the following ratio:

\[
\frac{\text{cpm in protein X before maturation}}{\text{cpm in actin before maturation}} \times \frac{\text{cpm in actin after maturation}}{\text{cpm in protein X after maturation}}
\]
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Clustering of the ratios obtained would have revealed any proteins behaving as regulatory classes during maturation. In fact a complete range of values was obtained. Little is served by presenting the data in more detail.

Proteins made by unfertilized eggs and blastulae

Unfertilized eggs seem to make far fewer major proteins even than oocytes matured in vitro (Fig. 2a), but some groups of proteins seen in oocytes are also seen in unfertilized eggs, e.g. M, N, Q, R, S, T, U, V. Notable amongst the missing proteins are β- and γ-actins, though very low levels of synthesis of these proteins may be detected, especially with long incubations of unfertilized eggs. Their identification is partly based on similar analyses published by De Robertis.
et al. (1977) and by Brock & Reeves (1978), but will be discussed in more detail elsewhere (Sturgess et al., in preparation). During cleavage many spots appear which are not seen in the egg, as reported by Brock & Reeves (1978), but none were distinguishable in mobility from proteins previously made in the oocyte. Proteins of this type are marked on Fig. 2b and c, e.g. A, B, C, D, E, F, and this behaviour is also clearly shown by actin. Embryos labelled from stage 2 to 7 give patterns like unfertilized eggs.

The problem to be faced is whether the reduced diversity of proteins made by unfertilized eggs and very early embryos results from artifactual loss of proteins. The fact that the patterns are reproducible, and have also been obtained by Brock & Reeves (1978), does not exclude the existence of a stage-specific extraction artifact. However, in the case of actin several observations argue against this possibility. First, staining of the gels reveals similar amounts of total actin in eggs and in oocytes, or in embryos at later developmental stages (Fig. 4). If eggs make actin, it is therefore necessary to suppose that the newly formed actin is lost selectively compared to the bulk actin, which is presumably accumulated during oogenesis. Secondly, mixing radioactively labelled unfertilized egg extract and oocyte extract does not reduce the amount of radioactively labelled oocyte actin seen after 2D electrophoresis.

Standard one-dimensional SDS gradient gel analysis of proteins made at all stages of development shows a radioactive band in the actin position. However,
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Fig. 4. Total actin extracted from unfertilized eggs and neurulae. Extracts were fractionated on 2D gels and stained with Coomassie brilliant blue. The stained spots were scanned with a densitometer.

Fig. 2 shows that a number of non-actin proteins have the same mobility as actin in the SDS dimension of a 2D gel, but are easily separated from actin by their charge in the iso-electric focussing dimension. Data from one-dimensional gels are therefore not relevant to this question.

Figure 1 shows that oocytes matured in vitro lacked some newly synthesized oocyte proteins, but had an actin spot and made some other proteins not detectable in unfertilized, or recently fertilized eggs. However, these cells were not completely equivalent to definitive unfertilized eggs; for example, they did not show the cortical activation reaction when injected.

The balance of evidence currently available favours the view that a number of proteins, for example, A, B, C, D, E, F, and actin (Figs. 2a and 3a) are reduced in synthetic rate at some time between white spot appearance during maturation and activation of the egg. Further, that many of these proteins resume synthesis at the mid-blastula stage of development. This conclusion is rather surprising and supporting evidence using other approaches is very desirable.

At the late blastula stage we cannot with certainty identify any newly synthesized proteins that were not made in the oocyte. Many proteins made in the oocyte are no longer detectable (at the late blastula stage only 300 proteins were seen, compared to 700 in the oocyte).

Newly synthesized proteins after the blastula stage

New proteins appear at the gastrula stage (Fig. 2c), but it is difficult to say that these are different from those made by oocytes. Unfortunately, so many proteins are resolved using oocyte extracts that in many regions of the gel it would, in any case, be very hard to be confident that some spots from later stages are really different from them. By the neurula stage a number of completely new proteins make their appearance, e.g. H, G. These become more numerous.
Late blastulae were dissected (see text) into 'ectodermal' and 'endodermal' parts at stages 8½ or 9½, and incubated in [35S]methionine at 12.5 mCi/ml for 4 h. They were frozen when control embryos had reached stage 10½ or 11. The analysis was similar to that in Fig. 1, except that the second dimension was a 12.5% acrylamide/SDS gel, rather than a gradient. (A, B) Embryos of a mating different from C and D. Major differences between the two series are indicated by the squares. Consistent major differences between the parts are shown by arrows, and by the α-actin labelled arrow. Nomenclature is the same as in Figs. 2 and 3.
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and more abundant through the tail-bud and early tadpole stage. The clearest of these is α-actin (Figs. 2d and 3b). The major differences and similarities between oocyte and tadpole are indicated in Fig. 3.

Proteins made in different regions of the embryo

Embryos at several stages of development were dissected into two parts as described by Woodland & Gurdon (1968), and Woodland, Gurdon & Lingrel (1974), before incubating in [35S]methionine. The aim was to separate ectodermal derivatives and most of the somitic mesoderm into one part and endoderm and other splanchnopleure components into the other. This is not easy to achieve accurately and rapidly in early embryos, so the two halves are not exactly comparable at different stages. For convenience, they are called ‘ectodermal’ and ‘endodermal’, although both contain mesoderm. At a given stage the patterns show reproducible differences.

Figure 5 shows protein synthesis by separated parts of late blastulae, incubated till they would have been early gastrulae. This figure shows firstly that the bulk of proteins yield highly reproducible patterns, although patterns from different series may show a few differences in abundant proteins. Most of these variable proteins were found at all stages of a given series and throughout the embryo.
The stage- and region-specific proteins discussed below did not vary between series.

Few differences between ‘ectoderm’ and ‘endoderm’ are detectable at the stages shown in Fig. 5 (arrowed spots). The most important is that α-actin appeared in the ‘ectodermal’ region alone. This is earlier than seen in intact embryos injected with [35S]methionine (see above). Either α-actin appears precociously in dissected embryos, or injected methionine does not reach all regions of whole embryos.

At stages 17–24 (neurula to early tail-bud) the ‘ectoderm’ shows further specific proteins, and at least one ‘endoderm’ specific protein has appeared (arrowed spots in Fig. 6). This protein remains restricted to the ‘endoderm’ at later stages of development. The proteins marked as region-specific in Fig. 5 are the only major proteins, which continue to be made and remain region-specific at later stages. A trace of α-actin may be seen in the ‘endodermal’ region. This probably represents inadequate dissection, since it becomes less at later stages, when dissection is easier. The region-specific proteins also appear in embryos labelled from stage 14–20 (neurulae).
Fig. 8. Autofluorographs of 2D gel analysis of proteins made in rabbit reticulocyte lysates programmed with total RNA extracted from: (A) oocytes, (B) unfertilized eggs, (C) stage 8–9 blastulae, (D) stage 11–12 gastrulae, and (E) stage 17–20 neurulae as described in the Methods. The actin forms are indicated by labelled arrows and major differences between neurulae and earlier stages are indicated by labelled spots. Nomenclature is the same as for the preceding figures.
At later stages the two regions become even more divergent (Fig. 7). Comparison of Figs. 5, 6 and 7 also show the appearance of many stage-specific proteins, already mentioned in connection with Fig. 2 and 3.

In order to show that differences between regions were not an artefact of incubation after dissection, we injected embryos in parallel with those shown in Fig. 6, and dissected them at the end of the incubation. The result obtained was the same.

Changes in the mRNA complement during development

Total RNA was prepared from embryos at various stages of development by standard phenol extraction methods and the unfractionated RNA was assayed for mRNA activity in the nuclease-treated reticulocyte cell-free system (see Table 1). The products were analyzed on 2D gels in the same way as proteins made \textit{in vivo} (Fig. 8). It is clear that certain of the mRNA products remain the same during early development, but some change. Labelled spots on Fig. 8\textit{d} and \textit{e} indicate some of the new proteins which appear at the neurula stage in both mRNA \textit{in vivo} and \textit{in vitro}. These include $\alpha$-actin. Most of these proteins first become detectable at the late gastrula stage of development.

It will be noticed that both $\beta$- and $\gamma$-actin messengers were detected in the unfertilized egg.

DISCUSSION

The sort of analysis that we have used is capable only of distinguishing abundant types of proteins. We can detect the 500 or so most common proteins of the whole embryo, but proteins specific to a limited part of the embryo may be detected only if they are a very abundant species in their characteristic cells. We have quantified the proteins observed on the 2D separations of oocytes, and find that about 40\% of the radioactivity on the gel was present in discrete spots. The figure was 31\% for oocytes matured \textit{in vitro}. In a comparable analysis of sea urchin mesenchyme blastulae, Woodland & Wiggs (unpublished) obtained a value of 20\%. In the oocyte sample 27\% of the radioactivity was present in the streak leading from the origin of the first dimension. Even ignoring this, we find that only 40–50\% of the protein entering the gel is present as discrete spots. Presumably this represents products of the prevalent or abundant class of mRNA. We were therefore studying less than half of the active mRNA in the oocyte. Histones would not have entered the gels, and they have been studied by independent methods (Adamson & Woodland, 1974, 1977).

We found no consistent appearance of new proteins during maturation of the oocyte. Otero \textit{et al.} (1978) reported that several new proteins did appear at this stage, but we could not identify these changes on our gel patterns.

It is clear from our results that relatively few new sorts of abundant proteins begin synthesis during early development. The bulk of proteins made in the tailbud tadpole were already made in the oocyte. This conclusion is comparable
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to that of Brandhorst (1976), who studied sea urchin embryos, but differs from that of Brock & Reeves (1978) using the same material as our own. Brock and Reeves concluded that there were great changes in the types of protein made during early development, but the earliest stage that they studied was the unfertilized egg, which gave similar patterns of incorporation to those that we report here. Compared to the egg a blastula makes many new types of protein, but when we examined oocytes we found that proteins with mobilities like those of the 'new' blastula proteins were already made at this earlier stage. Since we have not applied further criteria to establish that all of these oocyte and blastula proteins are identical we cannot be sure that no new proteins are made at the blastula stage. However, the evidence available does not support the conclusion that new proteins are made in blastulae.

Just as in sea urchins (Brandhorst, 1976), the first new proteins were detected in gastrulae, and became easily seen in neurulae. Some of these new proteins are region-specific. The two regions of the embryos that we separated contained a number of characteristic proteins by the gastrula stage, the first being seen in the 'ectodermal' half. Some care must be observed in interpreting this observation in terms of gene activity, because the 'endodermal' part contains far fewer cells than the 'ectodermal' half, though the parts are of comparable size (Woodland & Gurdon, 1968). This means that the concentration of genes is lower in the 'endoderm' and it would therefore take longer for these genes to have the same impact on protein synthesis as genes in the 'ectoderm'. However, some early differences between the two regions seem to be real. This applies firstly to a protein seen in the 'endoderm' region and not the 'ectoderm' at the neurula stage. Here the difference is that the protein is absent from the part with the largest number of genes. Secondly, a number of proteins (such as \( \alpha \)-actin) appear exclusively in the 'ectodermal' part at very early stages, but never appear in the 'endodermal' region at levels larger than might be expected from inadequate dissection. It seems unlikely that these proteins are made transiently in gastrula or neurula 'endodermal' regions.

In incubated, dissected embryos region-specific proteins (e.g. \( \alpha \)-actin) appear earlier than in intact embryos. This may be a labelling artifact in whole embryos, or accelerated differentiation after dissection. The latter is more likely, because in vitro translation of RNA from whole embryos gives the same protein patterns as undissected embryos, whose protein has been labelled in vivo. Detectable tissue-specific differences therefore normally appear at the late gastrula stage. \( \alpha \)-Actin is made by striated muscle, which is a mesodermal derivative, and it may be significant that later differentiation of mesoderm is autonomous. In whole embryos myofibrillae have first been observed at stage 21 (late neurula, Nieuwkoop & Faber, 1956). Biochemical techniques might be expected to reveal myofibrillar proteins at an earlier stage.

Do the proteins that first appear at the gastula and neurula stages represent new gene activity? The strongest evidence for this view is that their mRNA
first becomes detectable at the same stage as the protein. Although it is conceivable that mRNA is stored in a form that cannot be translated in the reticulocyte lysate, this seems unlikely. This point can only be proved definitively by direct titration of the mRNA sequences, for example by using a cloned probe.

We pointed out above that many proteins made in the oocyte become undetectable in the radioactive protein of the unfertilized egg and cleaving embryo, and it is particularly surprising that some of them become detectable again at the blastula stage, an example being β-actin. We have attempted, without success, to show that the result is a recovery artifact. A parallel situation has been found for H1 histone, and is worth describing in detail for comparison. Oocytes and late embryos make H1 roughly in proportion to the other histones, whereas little newly synthesized H1 can be detected in eggs and cleaving embryos (Adamson & Woodland, 1974; Flynn & Woodland, 1979). This could be further evidence that proteins are easily lost from embryos at these stages, but mixing experiments have failed to reveal such a phenomenon. Additional evidence comes from the observation that sea urchin H1 histone, programmed by injected mRNA, may be recovered from eggs and early embryos of *Xenopus*, whereas the *Xenopus* H1 histones are not recovered from the same embryos (Woodland & Wilt, 1979). The *Xenopus* H1 proteins become easily detected at the blastula stage, in a fashion directly comparable with β- and γ-actin.

The mRNA for proteins like β- and γ-actin does not change greatly in amount during early development, and is certainly present in the unfertilized egg (the same is also true of H1 histone mRNA; Ruderman *et al.*, 1979). If it is correct that unfertilized eggs and early cleavage stages do not make this class of proteins, a translational control mechanism must exist which switches translation of the mRNA off in the egg and turns it back on during later cleavage.

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