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Protein synthesis and messenger RNA levels along the animal–vegetal axis during early *Xenopus* development

ROSAMUND C. SMITH*

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

SUMMARY

The patterns of proteins synthesized in animal and vegetal regions of *Xenopus* oocytes, eggs and embryos were examined by 2D gel electrophoresis. In oocytes and eggs, the only proteins synthesized asymmetrically along the animal–vegetal axis were a small number of proteins synthesized predominantly in the vegetal hemisphere. At the cleavage stage there were a total of four proteins synthesized unevenly in animal and vegetal regions: three synthesized predominantly in the vegetal hemisphere and one synthesized predominantly in the animal hemisphere. By the gastrula stage, when maternal messages have largely been replaced by embryonic transcripts, the number of differences in proteins synthesized in the animal-derived ectoderm and mesoderm, and the vegetal-derived endoderm started to increase rapidly with time of development with many more animal-characteristic proteins than vegetal-characteristic proteins appearing. Comparison of protein synthesis patterns with those obtained when extracted RNA was translated *in vitro* and run on 2D gels, showed that the asymmetry in protein synthesis along the animal–vegetal axis in the oocyte and early embryo reflected directly the distribution of their mRNAs along the axis. There was no evidence for localized 'masked' abundant messages along the animal–vegetal axis of oocytes and cleavage embryos.

INTRODUCTION

The animal and vegetal hemispheres of the *Xenopus* oocyte and egg have different developmental fates. The animal region is destined to develop into ectodermal and mesodermal structures whereas the vegetal region is destined to develop into endodermal structures (Nieuwkoop, 1977). The animal–vegetal axis is established during oogenesis and a polarized state exists from the very beginning of oocyte formation (Heasman, Quarmby & Wylie, 1984). Restriction of external pigmentation to the animal hemisphere of the oocyte, egg and early embryo makes these two regions of different developmental fate easy to identify even before morphological differences between the two regions become apparent. This makes the animal and vegetal hemispheres ideal for a study of localizations at the molecular level in two regions of different developmental fate before morphological differentiation occurs; localizations which may account for the differences in gene expression that arise between these two regions. Maternal masked mRNA

* Present address: Department of Biological Sciences, Columbia University, New York, NY 10027, USA.

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and proteins have often been proposed as candidates for morphogenetic determinants, and their asymmetric distribution in oocytes, eggs and early embryos of many systems is consistent with this role (Rodgers & Gross, 1978; Carpenter & Klein, 1982; Kandler-Singer & Kalthoff, 1976; Jeffery, Tomlinson, Brodeur & Meier, 1984).

Previous investigations into localizations along the animal–vegetal axis of *Xenopus* have revealed differences in the content of soluble proteins along the animal–vegetal axis of unfertilized eggs. 25 % of protein bands resolved on one-dimensional (1D) gel electrophoresis are present along only part of the egg's animal–vegetal axis (Moen & Namenwirth, 1977; Jäckle & Eagleson, 1980). Carpenter & Klein (1982) showed that the vast majority of poly(A)$^+$RNAs is not segregated along the animal–vegetal axis of the *Xenopus* egg, although they did show that 3–5 % of the poly(A)$^+$RNAs were enriched 2- to 20-fold in the vegetal region of the egg. However, individual mRNAs have not been examined, and no single study so far has combined an investigation into the distribution of mRNAs with an examination of protein synthesis in animal and vegetal regions.

This paper describes experiments that examine in detail protein synthesis along the animal–vegetal axis in the oocyte, egg and five stages of early development in *Xenopus laevis*. Protein synthesis was studied directly by labelling embryos with [^{35}S]methionine using two-dimensional (2D) gel electrophoresis to analyse the proteins. For pregastrula stages mRNA levels were also estimated by translating RNA *in vitro* and running the translation products on 2D gels. Such an analysis has made it possible to describe localizations of individual mRNAs along the animal–vegetal axis, and to determine if any differences in protein synthesis along the axis are caused by mRNA segregation, and also if there are mRNA localizations along the axis that are not reflected in the pattern of protein synthesis. Any identifiable localization or asymmetry of proteins or mRNAs in the oocyte, egg and early embryo may possibly be involved in the determination and subsequent differentiation of the animal and vegetal regions of the *Xenopus* embryo.

MATERIALS AND METHODS

Production of oocytes, eggs and embryos

Mature oocytes and eggs were obtained as described by Gurdon (1977). Embryos were obtained by *in vitro* fertilization using standard methods (Gurdon, 1977) and were dejellied with 2 % cysteine–HCl at pH 7·8. They were staged according to Nieuwkoop & Faber (1967).

Radioactive labelling of proteins

Oocytes were labelled by incubation for 6–8 h at 18 °C in Barths' saline containing approximately 300 μCi ml^{-1} of [^{35}S]methionine (800 Ci mmol^{-1}; Amersham). Eggs and embryos were injected with label, each receiving about 0·5 μCi in a maximum of 50 nl, and were incubated at 20 °C in 1/10th De Boer's solution (Wolf & Hedrick, 1971) for 90 min. Only eggs and embryos that were in good condition and had reached the same stage as uninjected controls were analysed.

Dissection into animal and vegetal regions

Accurate dissection of oocytes, eggs and embryos is essential for this study. Dissection of unfixed gastrulae, neurulae and tailbuds into ectoderm/mesoderm and endoderm derivatives is possible (Ballantine, Woodland & Sturgess, 1979), but difficult. Moen and Namenwirth's freezing technique for dissection cannot deal with stages past blastula when dissection into animal and vegetal derivatives requires more intricate separation than transverse sectioning (Moen & Namenwirth, 1977). Dissections were facilitated by fixing all samples in 70 % ethanol for a minimum of 10 min prior to dissection (Smith & Knowland, 1984). This simple method greatly increases the accuracy of dissection, a necessity if region-specific differences are to be observed. Fig. 1 shows how the dissections were performed.

Oocytes, unfertilized eggs, and cleavage and blastula embryos were divided into animal and vegetal regions by a single transverse cut. Later stage embryos were initially bissected and the yolky endoderm (vegetal region) then separated from the remaining ectoderm and mesoderm (animal region). Due to the relative simplicity of these dissections the presumptive areas of development contained in each animal and vegetal sample at each stage does vary. For example, the vegetal region of the pregastrula embryo does contain some regions of presumptive mesoderm development. However, these dissections separate the main animal and vegetal regions of the oocyte, egg and embryos, with the animal samples including the majority of presumptive ectoderm and mesoderm areas, and the vegetal regions consisting mainly of presumptive endoderm.

Protein extraction

Ten labelled oocytes, eggs or embryos were homogenized in 200 μl of lysis buffer containing 0·1 % SDS but no NP40 (O'Farrell, 1975). After 10 min an equal volume of lysis buffer containing 5 % NP40 was added. The homogenate was centrifuged at 10 000 g for 5 min to remove the yolk, and the supernatant was analysed. It has previously been shown (Smith & Knowland, 1984) that this procedure extracts approximately 95 % of the labelled protein.

RNA extraction

RNA was extracted from fixed oocytes or cleavage embryos using hot phenol–SDS as described by Hunter, Hunt, Knowland & Zimmern (1976). Oocyte RNA was labelled by incubation with [^{3}H]uridine and the percentage recovery of RNA during phenol extraction was followed. 80 % of the radioactivity in the oocyte RNA in the initial homogenate was recovered in the final RNA precipitate.

Translation of RNA

A message-dependent lysate (MDL) was prepared as described by Hunt & Jackson (1974), Pelham & Jackson (1976) and T. Hunt (personal communication). It was characterized thoroughly using tobacco mosaic virus RNA in order to confirm that it synthesized full-length, high-molecular-weight polypeptides with minimal premature termination. The extent of protein synthesis was proportional to the concentration of added TMV RNA over the range 0–100 μg ml^{-1}. Total RNA from *Xenopus* oocytes and embryos was used to direct protein synthesis. Poly(A)$^+$RNA alone was not used because this class of RNA does not represent the total translational capacity of *Xenopus* mRNA, and adenylations and deadenylation of messages occur during *Xenopus* development (Ruderman & Pardue, 1977; Sagata, Shiokawa & Yamana, 1980; Ruderman, Woodland & Sturgess, 1979). This study is attempting to estimate the relative distribution of mRNAs in animal and vegetal regions, and all messages, whether poly(A)$^+$ or poly(A)$^-$, need to be translated in order for the estimates to be valid.

Translations were performed as described by Pelham & Jackson (1976).

Two-dimensional gel electrophoresis

10 μl of lysate, containing up to 100 000 cts min^{-1} in 35 μl of lysis buffer was used for each analysis of *in vitro* translation products. 40 μl of each protein sample (one oocyte or embryo

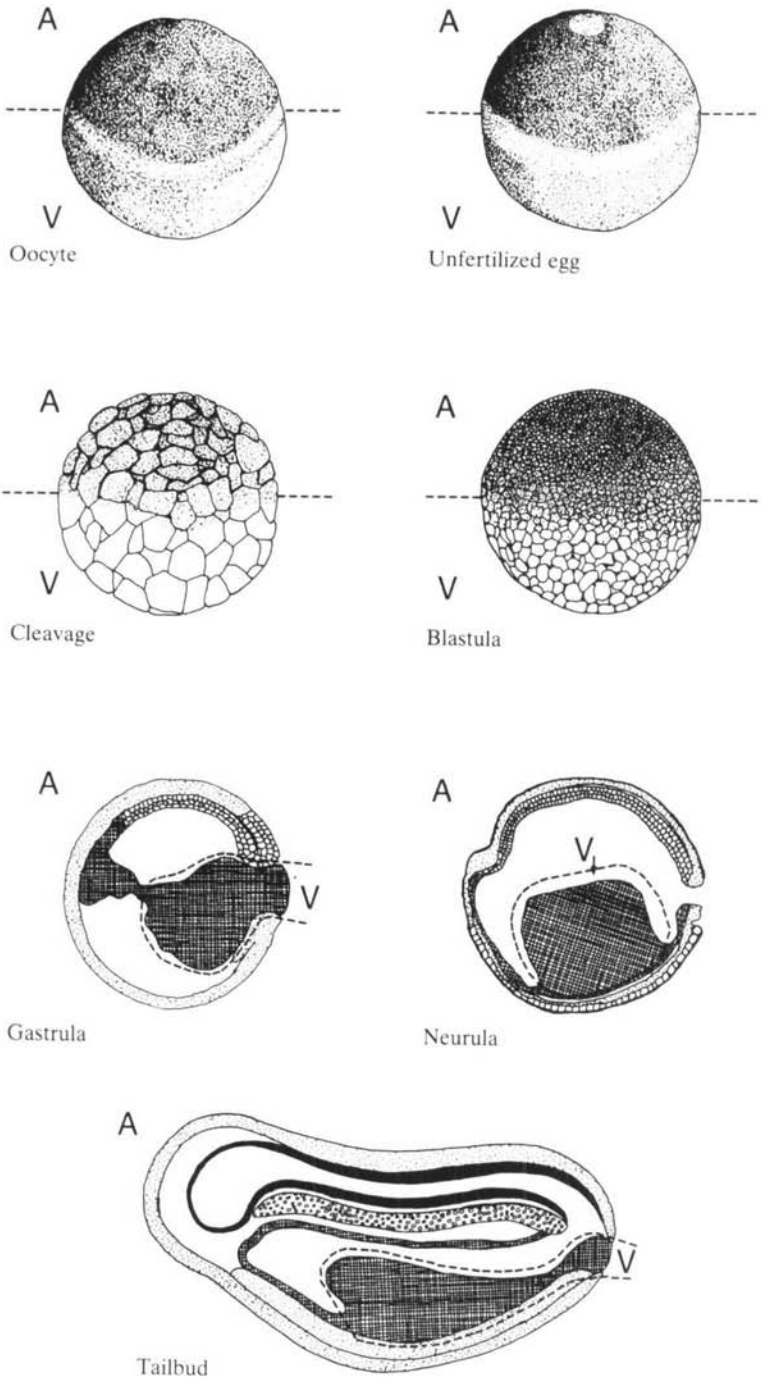


Fig. 1. Diagrammatic representation of oocyte, egg, cleavage and blastula embryos indicating by dotted lines the plane of dissection into animal and vegetal portions. For gastrula, neurula and tailbud embryos, a vertical cross section is shown indicating by dotted lines the cuts made in the dissection of the embryos into animal (A) and vegetal (V) portions. See text for details.

equivalent) containing approximately 150 000 cts min^{-1} was used for each analysis of proteins synthesized *in vivo*.

All samples were run first on isoelectric focusing (O'Farrell, 1975) or non-equilibrium pH gradient gels (O'Farrell, Goodman & O'Farrell, 1977) and then on 12% slab gels using an acrylamide:bisacrylamide ratio of 200:1 (Knowland, 1974). Gels were fixed and radioactive proteins were located by fluorography, using preflashed X-ray film (Laskey & Mills, 1975). Exposure time was often very long, in the order of weeks, sometimes months.

Comparison of spot intensities on fluorographs

Individual spots on fluorographs were scanned horizontally at vertical intervals of 0·5 mm down the whole spot using a Joyce–Loebl double-beam recording microdensitometer. On the resulting scans the peaks were cut out and the area of the peaks estimated by weighing. The sum of the weights gave a relative value of the intensity of the spot which could be compared to other values in the same manner. When comparing spots on different fluorographs representing protein synthesis in different regions of the oocyte, egg or embryo, proteins whose synthesis was considered to be constant throughout the oocyte, egg or embryo were measured for spot intensity and standardized to single values. These 'constant' proteins are indicated in Fig. 2. These constant proteins were compared to other similar proteins which by visual inspection appeared to be synthesized at equal rates in animal and vegetal regions (not marked) and the relative intensity of these other proteins A:V ranged from 1:0·8–1·2. It was possible by using the same standardization factors to estimate the relative differences in intensities between spots on different fluorographs, thus obtaining estimates for differences in the rates of accumulation of newly synthesized proteins in different regions of the organism. The same technique was applied to gels of proteins synthesized *in vitro* in order to estimate the differences in mRNA levels in different parts of the oocyte and cleavage embryo.

RESULTS

For simplicity the term 'protein synthesis' will be used throughout this report to describe the *in vivo* labelled proteins analysed by gel electrophoresis. Actually the *in vivo* labelled proteins are the steady-state levels of newly synthesized proteins and represent a balance of synthesis and degradation.

Analysis of gels

Any asymmetries in protein synthesis or mRNA levels within oocytes, eggs or embryos must take cytoplasmic volume rather than total volume into account because of the large amounts of insoluble yolk which early embryos contain. When the volumes of the animal and vegetal hemispheres of oocytes, eggs and pre-gastrula embryos are corrected for yolk the animal hemisphere is 1·5 times larger than the vegetal hemisphere (calculated from data taken from Phillips, 1982), which means that protein synthesis patterns and mRNA levels derived from 1·5 vegetal hemispheres should be compared to 1 animal hemisphere equivalent. But since a difference in intensity of 1·5 between spots on a fluorograph is not large enough to be identified consistently by visual inspection, the difference in non-yolk volume between animal and vegetal regions can in practice be disregarded. For most samples taken from animal and vegetal regions, the cts min^{-1} loaded multiplied by exposure time was a constant, and when this could not be achieved, differences in spot intensity on different fluorographs were corrected for under-exposure or overexposure by the microdensitometer procedure outlined in the

Materials and Methods section. In this way, localizations of mRNAs, or alterations in rates of protein synthesis, detected by gel analysis can confidently be taken to represent true asymmetries between animal and vegetal regions. For postblastula stages, however, due to the differences in volume between animal and vegetal regions, which were undetermined, analysis was restricted to identification of region-specific synthesis and predominant synthesis based on comparison of the pattern of synthesis from both regions. Absolute asymmetries in levels of newly synthesized proteins per unit volume of cytoplasm were not determined.

To assist description of the patterns of protein synthesis all the proteins whose synthesis is animal- or vegetal-characteristic have been numbered. The numbering system scans from the left to right and from top to bottom, starting at the top left. Proteins detected after isoelectric focusing in the first dimension (pH gradient 5–6·5) are collectively termed the acidic fraction in tables and are individually suffixed by the letter 'a', while those separated by non-equilibrium pH gradient electrophoresis in the first dimension (pH gradient 7–10) are referred to as the basic fraction and suffixed by the letter 'b'. Only those proteins that were reproducibly synthesized and behaved consistently were included in the analysis. In total over 150 gels were run and analysed. Protein extracts from several different batches of oocytes, eggs and embryos were analysed. RNA was extracted from over 200 oocytes obtained from one sacrificed female frog. Cleavage-stage RNA was extracted from over 200 embryos derived from one spawning. *In vitro* translation and gel analysis of protein products were performed at least three times for each RNA sample. Qualitative differences, and quantitative differences that were easily detected by visual inspection and estimated later by densitometry to exceed a factor of 2·5, were analysed further. Differences in protein synthesis between animal and vegetal regions were examined at seven stages: in the mature oocyte, unfertilized egg, and in the cleavage, blastula, gastrula, neurula and tailbud embryos. The tables summarize data taken from several different original fluorographs. The photographs show examples of the fluorographs used to compile the tables.

Animal- and vegetal-characteristic differences in protein synthesis in vivo

Examples of the patterns of [^{35}S]methionine-labelled proteins extracted from animal and vegetal regions of oocytes, eggs, cleavage, blastula, gastrula, neurula and tailbud embryos are shown in Fig. 2.

Certain proteins were synthesized at unequal rates in animal and vegetal regions of the embryo. Those proteins that were synthesized at higher rates in the animal hemisphere reflect animal-characteristic differences in protein synthesis. Those that were synthesized at higher rates in the vegetal hemisphere reflect vegetal-characteristic differences. The animal- and vegetal-characteristic differences in protein synthesis at the seven stages were examined and are listed in Table 1. The majority of differences were quantitative (e.g. 47a, 48a, 46a) although a number appeared to be qualitative (e.g. 63a, 64a, 7b). Because the embryos were divided only into animal and vegetal regions the large number of quantitative differences is

not altogether surprising. Due to the nature of the dissection the number of animal- or vegetal-characteristic proteins identified in this study is most likely an underestimate, but by the same token, those that are detected are likely to represent major differences. All the proteins identified are unknown in name or function, although 59a is tentatively identified as alpha-actin due to its position on the 2D gel and its appearance in the animal-derived region at the gastrula stage (Sturgess *et al.* 1980; Mohun *et al.* 1984; Gurdon, Brennan, Fairman & Mohun, 1984).

The number of differences in protein synthesis between animal and vegetal regions of oocytes, eggs and embryos is represented diagrammatically in Fig. 3.

In the oocyte and unfertilized egg all the differences were vegetal characteristic. Because these are single cells this result can be interpreted in two ways; either translocation of newly synthesized proteins to become localized in the vegetal hemisphere, or predominant synthesis in the vegetal hemisphere with little movement of synthesized protein. The fact that one of these proteins (48a) continued to be localized in later, multicellular embryonic stages suggests that for this protein the latter is most likely and that there is asymmetric synthesis of proteins along the animal–vegetal axis. The preponderance of the mRNAs for the vegetal-characteristic proteins in the vegetal hemisphere (see later) also supports the view that there is an asymmetry in protein synthesis between animal and vegetal regions, although translocation of newly synthesized proteins remains a formal possibility.

In the cleavage embryo there were three vegetal-characteristic proteins being synthesized, with the synthesis for the first time of an animal-characteristic protein. From the blastula stage onwards the number of proteins synthesized asymmetrically along the animal–vegetal axis started to increase dramatically with more animal-characteristic than vegetal-characteristic proteins synthesized.

Concerning the pregastrula vegetal-characteristic differences in protein synthesis, 15b was synthesized only in the vegetal region of the oocyte. 22b is a vegetal-characteristic protein in the oocyte and during blastula and gastrula stages of embryogenesis. 47a and 49a were synthesized in the vegetal regions of oocytes and eggs. Neither of these proteins were synthesized at later stages of development. Synthesis of 48a was vegetal-characteristic in the oocyte through to the blastula stage. 12b is synthesized predominantly in the vegetal region of the unfertilized egg, but is synthesized throughout the oocyte and pretailbud embryo. 46a, a vegetal-characteristic protein synthesized for the first time at cleavage continued synthesis through gastrulation but was no longer synthesized at the neurula stage.

Animal-characteristic differences did not appear until cleavage. Fig. 3 shows that the number of animal-characteristic differences increased dramatically at blastula and increased approximately linearly as development proceeded. The vegetal-characteristic differences, apart from those proteins in the oocyte through to cleavage, increased a little later at gastrulation and increased at about the same rate as the animal-characteristic differences.

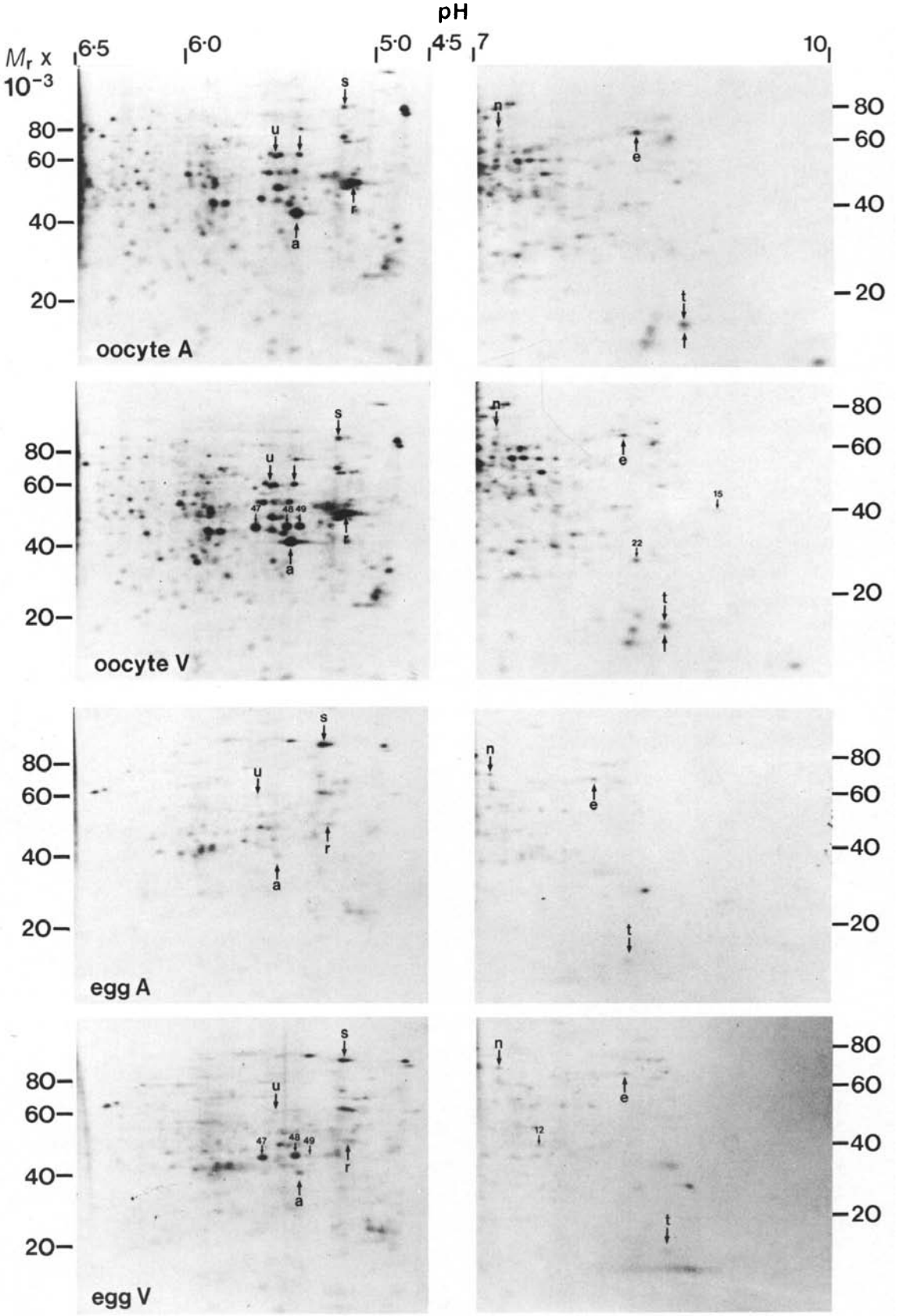


Fig. 2. For legend see p. 25

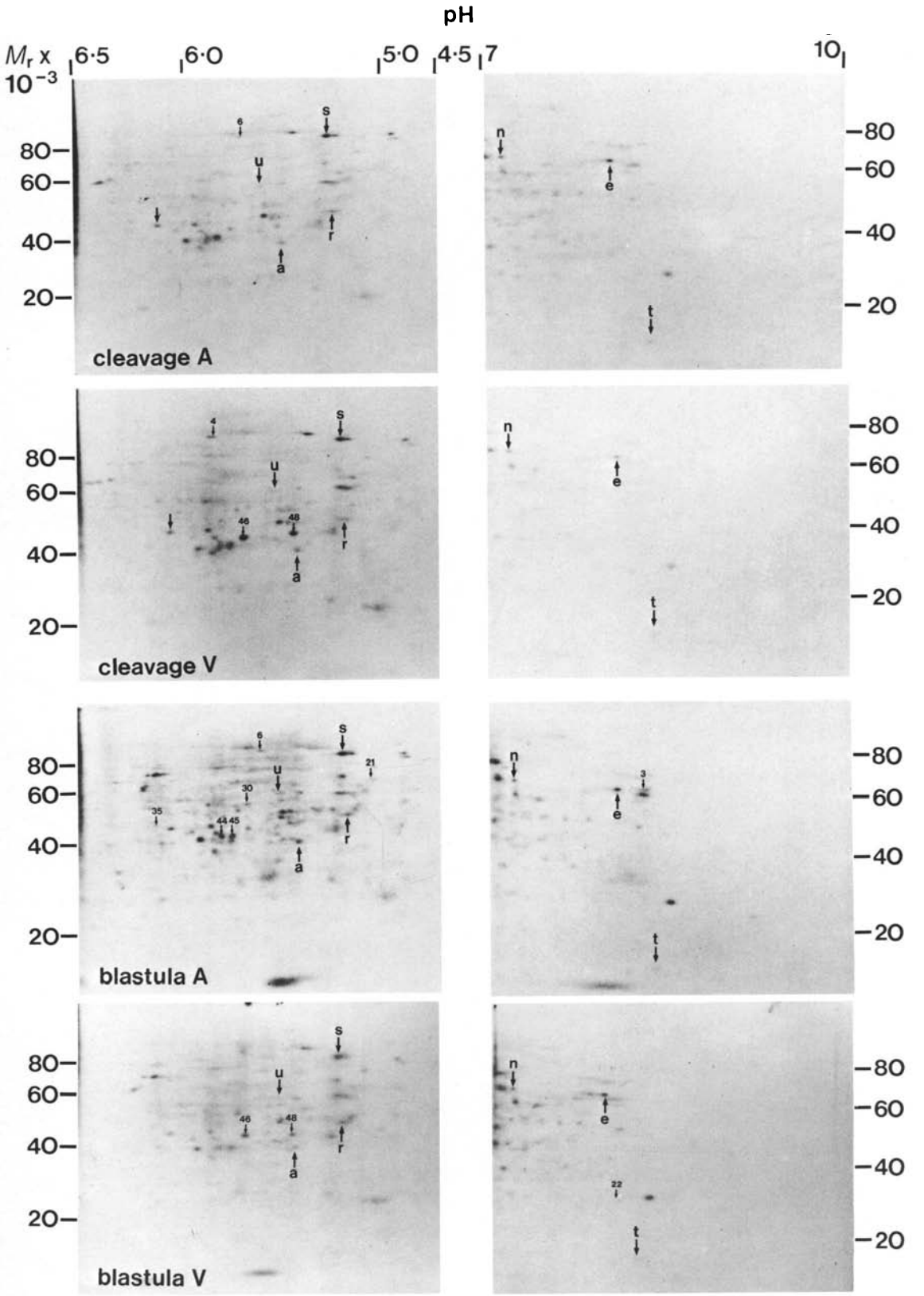
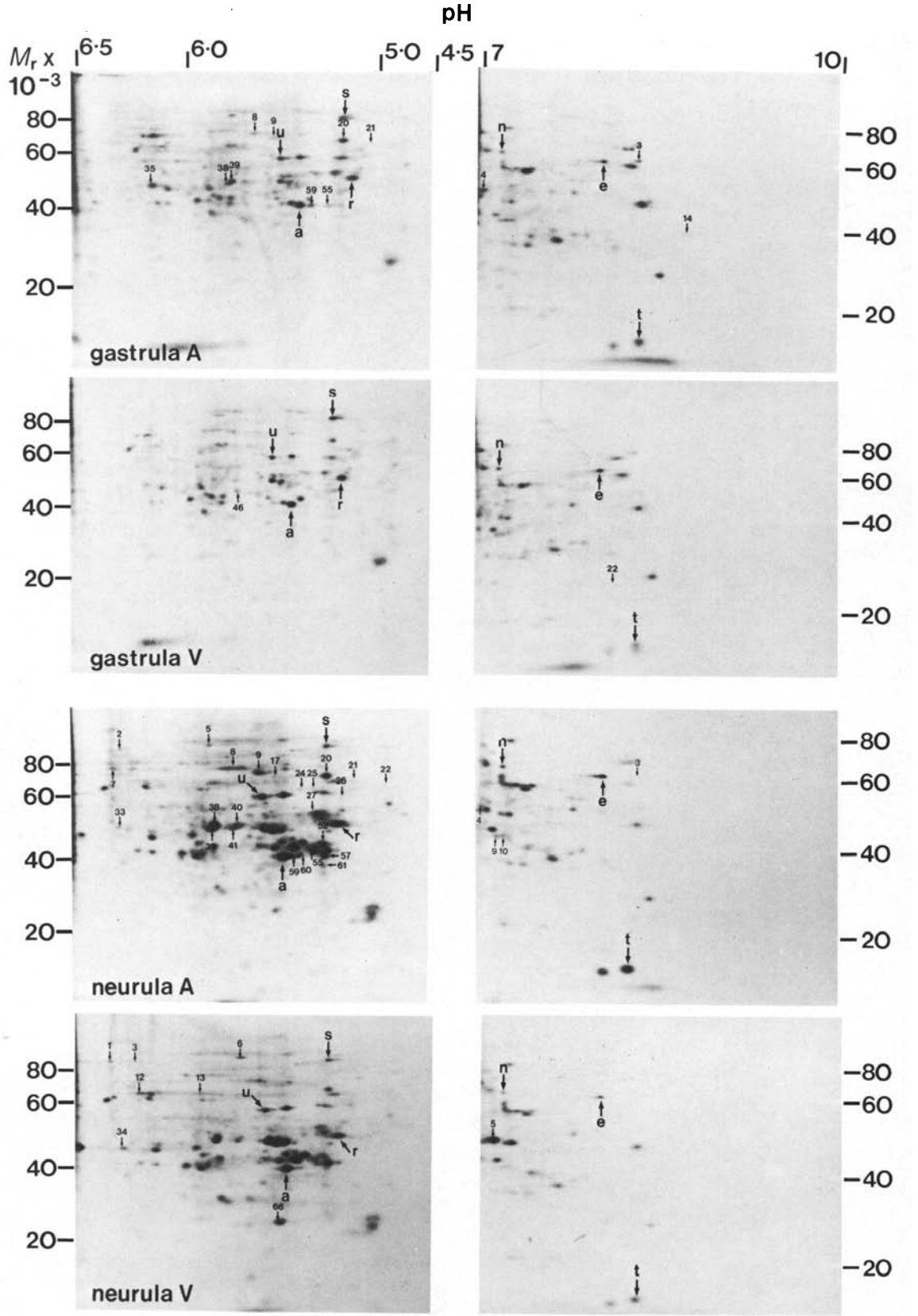


Fig. 2. For legend see p. 25


pH
M_r x 10^{-3}
6·5
6·0
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4·5
7
10
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60
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gastrula A
gastrula V
neurula A
neurula V

Fig. 2

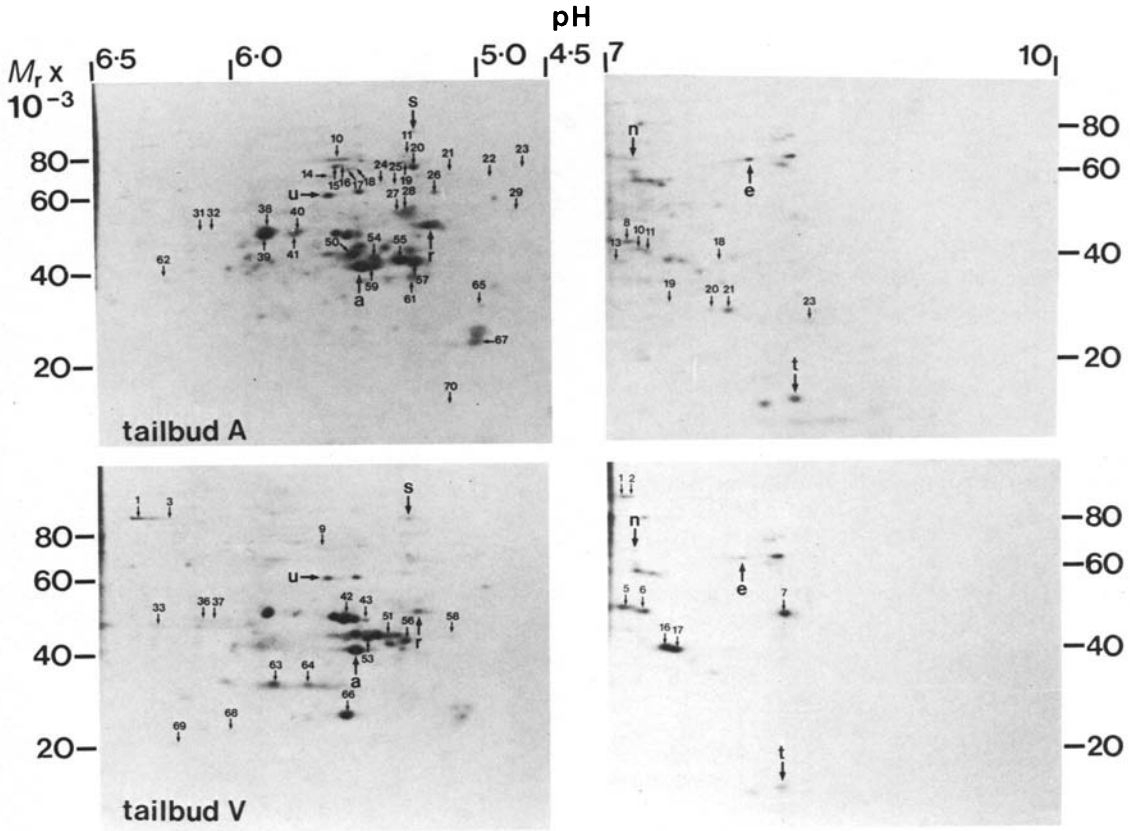

Fig. 2. Patterns of proteins synthesized in animal (A) and vegetal (V) regions of *Xenopus* oocytes, eggs, cleavage (stage 7), blastula (stage 8), gastrula (stage 11), neurula (stage 17), tailbud (stage 29) embryos. Equilibrium gels are on the left; non-equilibrium gels are on the right. Proteins synthesized asymmetrically are numbered on the gel where synthesis is predominant. Plain arrows indicate 'constant' proteins used as standards for densiometric quantitation of spot intensities. A number of 'reference' proteins common to all equilibrium or all non-equilibrium gels are marked with small letters (a, e, n, r, s, t, u) to aid in comparison of protein synthesis patterns. Proteins were labelled and extracted as described under Materials and Methods.

Table 1 shows a total of 91 animal-characteristic and 49 vegetal-characteristic differences in protein synthesis during the seven stages examined. However, because the same protein can be animal-characteristic or vegetal-characteristic at more than one stage, the actual total of region-characteristic proteins is lower. There are 58 proteins whose synthesis is predominantly in the animal region of the embryo at some time, 33 which are synthesized predominantly in the vegetal region at some stage, while two proteins are animal-characteristic at one stage and vegetal-characteristic at another. This emerges more clearly from Table 2, in which the individual proteins whose synthesis varies along the animal–vegetal axis

Table 1. *Individual proteins whose synthesis varies in rate between animal and vegetal regions during* Xenopus *development*

Stage	Animal-characteristic						Vegetal-characteristic					
	Individual					Total	Individual					Total
oocyte	—					0	48a	47a	49a	15b	22b	5
egg	—					0	48a	47a	49a	12b		4
cleavage	6a					1	48a	46a	4a			3
blastula	6a	21a	30a	35a	44a	7	48a	46a	22b			3
	45a	3b										
gastrula	8a	9a	20a	21a	35a	12	46a	22b				2
	38a	39a	55a	59a	3b							
	4b	14b										
neurula	2a	5a	7a	8a	9a	28	1a	3a	6a	12a	13a	8
	17a	20a	21a	22a	24a		34a	66a	5b			
	25a	26a	27a	33a	38a							
	39a	40a	41a	52a	55a							
	57a	59a	60a	61a	3b							
	4b	9b	10b									
tailbud	10a	11a	14a	15a	16a	43	1a	3a	9a	33a	36a	24
	17a	18a	19a	20a	21a		37a	42a	43a	51a	53a	
	22a	23a	24a	25a	26a		56a	58a	63a	64a	66a	
	27a	28a	29a	31a	32a		68a	69a	1b	2b	5b	
	38a	39a	40a	41a	50a		6b	7b	16b	17b		
	54a	55a	57a	59a	61a							
	62a	65a	67a	70a	8b							
	10b	11b	13b	18b	19b							
	20b	21b	23b									

are listed detailing the synthesis patterns of these proteins at all stages from oocyte to tailbud. They are divided into groups according to (1) when they are first synthesized, (2) whether their synthesis is continuous or intermittent, and (3) in which region of the embryo they are produced at the greatest rate. This classification reveals the existence of a large number of different groups and suggests that the control of protein synthesis along the animal–vegetal axis during early development is complex. The largest subgroup of animal-characteristic proteins is characterized by synthesis beginning at the tailbud stage and being predominantly in the animal region. The largest group of vegetal-characteristic proteins is characterized by synthesis beginning throughout the embryo at gastrulation and predominating in the vegetal region at the tailbud stage.

From gastrulation onwards embryonic transcripts have virtually replaced maternal messages. Table 2 shows clearly that there are two classes of *embryonic* genes expressed asymmetrically along the animal–vegetal axis: new genes that have not been expressed before gastrulation (50 proteins), and genes that were also expressed throughout preblastula embryos, presumably as a result of translation of maternal mRNA (43 proteins).

In vitro *translation of* Xenopus *RNA*

For RNA preparations from oocytes and cleavage stages the linear response of the MDL held for RNA concentrations over the range 1–100 $\mu g\,ml^{-1}$ as shown in Fig. 4. RNA concentrations in the MDL translation mix were within this range for all translation experiments. The stimulation of translation covered a range from ×4–13.

On comparison of *in vitro* with *in vivo* protein synthesis patterns it is apparent that many of the proteins are common to both patterns. However in certain cases, proteins synthesized *in vivo* do not have a corresponding spot on the *in vitro* gel. This is a common characteristic of cell-free translation systems (Brandhorst, Verman & Fromson, 1979; Mermod, Shatz & Crippa, 1980). Particularly there is a lack of high molecular weight proteins synthesized *in vitro*. This is most likely due to inefficient extraction of long messages. Lack of post-translational modifications in the MDL may also be an explanation for some discrepancies between *in vivo* and *in vitro* patterns. But the discrete spots of proteins that are visible on the *in vitro* gels, many of which have corresponding spots *in vivo*, show that RNA degradation is not a problem and that full-length messages have been translated to yield full-length polypeptides.

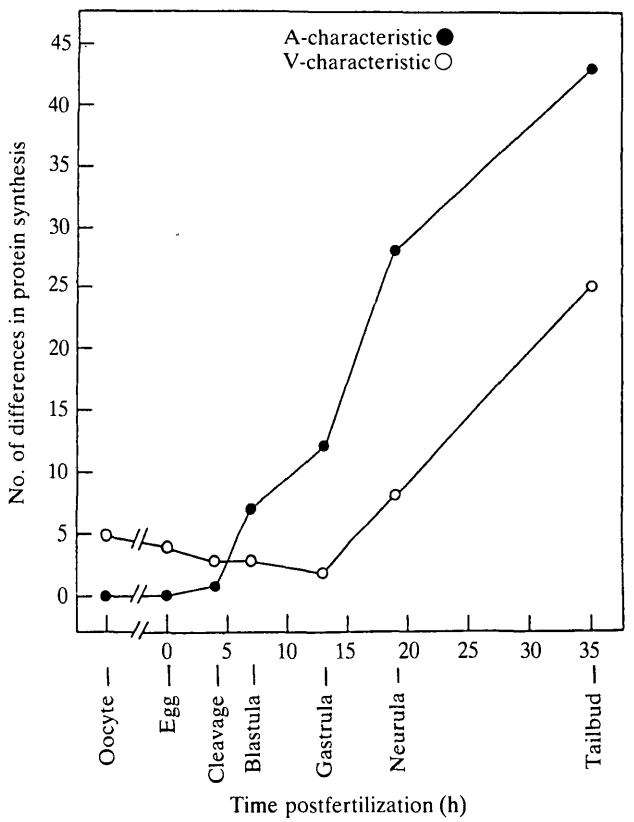

Fig. 3. The number of animal- and vegetal-characteristic proteins synthesized at various stages during the early development of *Xenopus laevis* (at 22–24 °C). See text for explanation.

Table 2. *Patterns of synthesis of animal- and vegetal-characteristic proteins during early* Xenopus *development*

Individual proteins	Total no.	Pattern of synthesis: oocyte	egg	cleavage	blastula	gastrula	neurula	tailbud
15b	1	V						
47a 49a	2	V						
44a	1	A and V			A	A and V		
22b	1	V	A and V		V			
9b	1	A and V					A	
12b	1	A and V	V	A and V				
18b	1	A and V						A
45a	1	A and V			A	A and V		
10b	1	A and V					A	
11a 7b	2	A and V						A or V
35a	1	A and V			A			
50a	1	A and V						A
4b	1	A and V				A		
27a	1	A and V					A	
20a	1	A and V				A		
28a 42a 43a 63a 67a	5	A and V						A or V
33a	1	A and V					A	
1a 3a 22a 5b	4	A and V					A or V	
29a 56a	2	A and V						A or V
52a	1	A and V					A	A and V
8b 69a	2	A and V						A or V
8a 3b	2	A and V			A			A and V
48a	1	V					A and V	
30a	1		A and V		A	A and V		
21a	1		A and V		A			
11b	1		A and V					A
4a	1			V				
46a	1			V				
54a	1			A and V				A
6a	1			A			V	
7a	1			A and V			A	
14b	1					A		
53a 6b	2					A and V		V
9a	1					A		V
5a	1					A and V	A	A and V
38a 39a 55a 59a	4					A or V		
12a 13a 60a	3						A or V	
2a	1						A	A and V
14a 16a 31a 32a 36a 64a 1b 16b 17b	9						A and V	A or V
17a 24a 25a 26a 34a 40a 41a 57a 61a 66a	10						A or V	
10a 15a 18a 19a 23a 37a 51a 58a 62a 65a 68a 70a 2b 13b 19b 20b 21b 23b	18							A or V

A = synthesized predominantly in animal region. V = synthesized predominantly in vegetal region. A and V = synthesized throughout oocyte/egg/embryo. A or V = synthesized predominantly in animal, or in vegetal region. Some proteins in the group are animal-characteristic, others are vegetal-characteristic. See Table 1 for details.

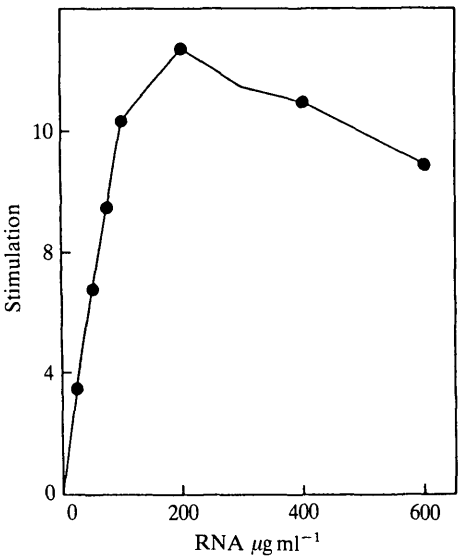

Fig. 4. Dose-response curve of MDL with oocyte RNA. Standard incubations of MDL were set up with [^{35}S]methionine and various concentrations of oocyte RNA. After 90 min incubation, samples were taken for assay of acid-insoluble counts. Stimulation of incorporation of radioactivity into acid-precipitable counts over control (no RNA) is plotted against the oocyte RNA concentration.

Animal- and vegetal-characteristic differences in in vitro *protein synthesis*

In the oocyte

The patterns of proteins synthesized *in vitro* on RNA extracted from animal and vegetal regions of oocytes are shown in Fig. 5. Relative levels of mRNAs were derived by densitometry of protein spots on the fluorographs (see Materials and Methods). This method of measurement is valid because the translation system responds in a linear fashion to changes of added mRNA (see Fig. 4), and also the preflashed film used for fluorography responds to radioactivity in a linear manner (Laskey & Mills, 1975). The results of such an examination are summarized in Table 3, which shows that the differences in *in vivo* protein synthesis of proteins 22b, 47a and 48a along the animal–vegetal axis of the oocyte appear to be a result of differences in mRNA localization in the oocyte, because the relative intensities found *in vivo* reflect those found *in vitro*. No other major differences in mRNA distribution along the animal–vegetal axis of the oocyte could be detected, and there was no sign of any *localized*, 'masked message' present in the oocyte but not translated at all *in vivo*. However, there is possibly some translational control of active mRNA, because in the case of 49a, the *in vitro* vegetal:animal difference is over three times the *in vivo* difference. The protein in the *in vitro* gel corresponding to 15b could not be identified with certainty and was not included in the analysis. Masked messages distributed throughout the oocyte were not the subject of this study. However experiments comparing *in vivo* and *in vitro* patterns of protein

synthesis at various stages of development have shown the presence, as expected, of masked messages in the oocyte and egg (R.C.S., unpublished observations).

In the cleavage embryo

The patterns of *in vitro* protein synthesis in animal and vegetal regions of the cleavage embryo are shown in Fig. 6. The synthesis of protein 48a was easily recognized by superimposition with the *in vivo* pattern for that stage. Proteins 46a, 4a and 6a however could not be confidently identified and were not included in the densitometric calculations. After correcting for differences in spot intensities (Materials and Methods), the measurements (Table 3) show that one of the major differences in protein synthesis in the living cleavage embryo (48a) is caused by mRNA localization along the animal–vegetal axis of the embryo. The distribution of mRNA for protein 48a is the same in both oocytes and cleavage embryos,

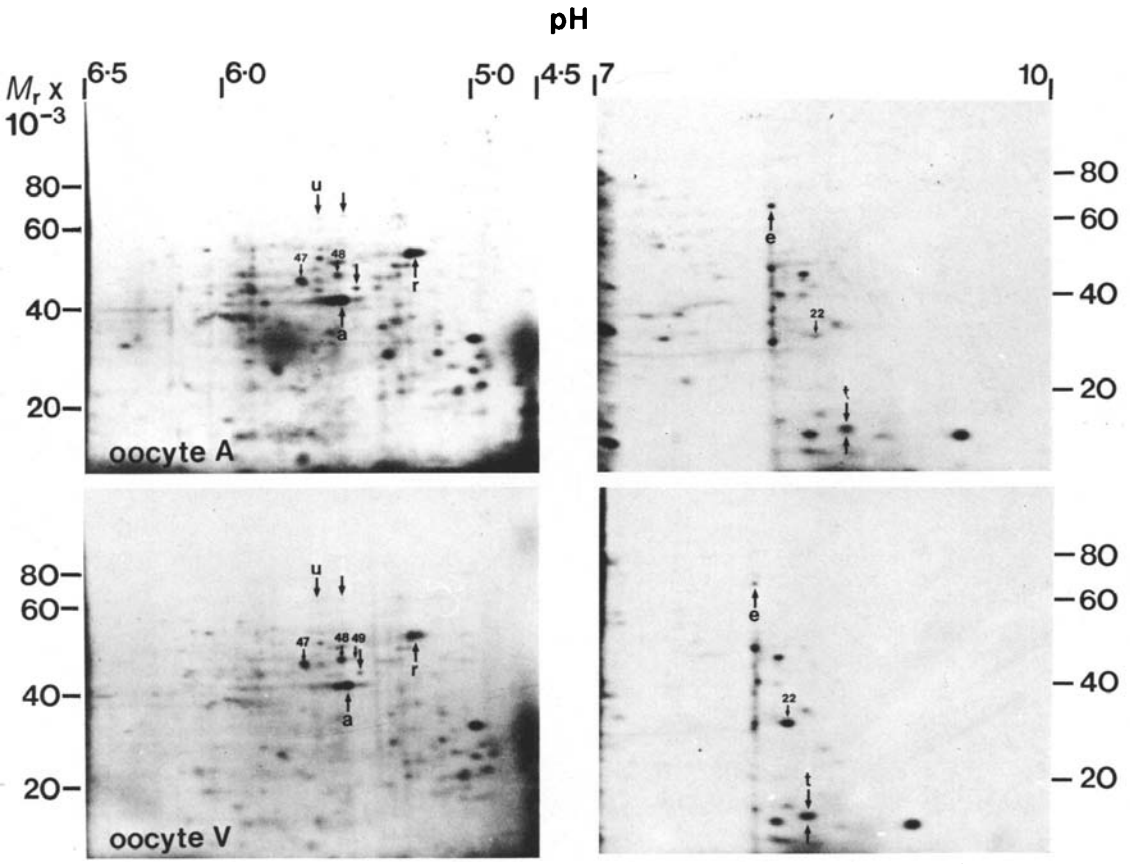

Fig. 5. Patterns of proteins synthesized *in vitro* from RNA extracted from animal (A) and vegetal (V) regions of *Xenopus* oocytes. Plain arrows indicate 'constant' proteins used as standards for densiometric quantitation of spot intensities. 'Reference' proteins (a, e, r, t, u) are marked to aid comparison of protein synthesis patterns.

presumably because of partitioning of the message after fertilization during subsequent cleavage divisions. No other major differences in mRNA distribution along the animal–vegetal axis were detectable. As in the oocyte there was no sign

Table 3. *Rate of* in vivo versus in vitro *synthesis of selected proteins as estimated by densitometric measurement of fluorographic spot intensity*

Site	Individual protein	Relative intensity vegetal: animal	
		in vivo	*in vitro*
Oocyte	22b	10·6	8·0
	47a	2·5	1·7
	48a	5·6	4·7
	49a	14·2	51·0
Cleavage embryo	48a	6·5	4·0

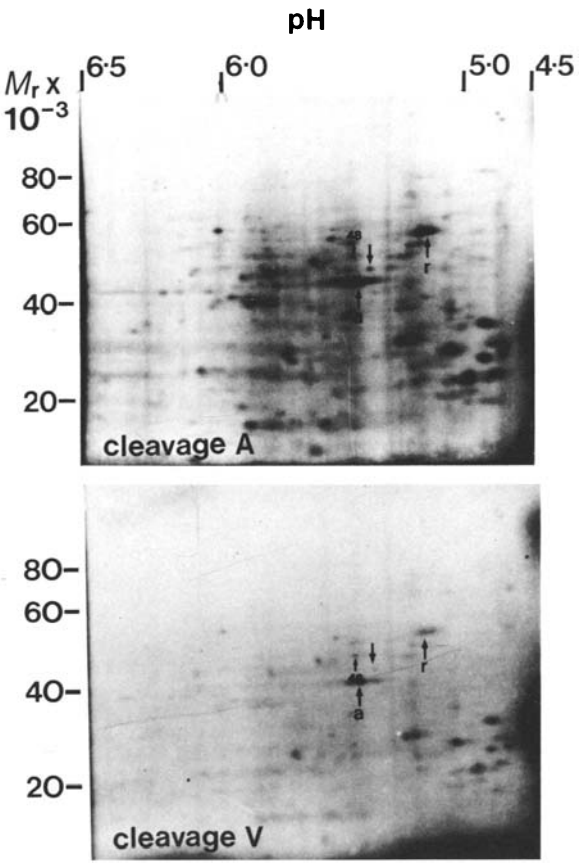

Fig. 6. Acidic proteins synthesized *in vitro* from RNA extracted from animal (A) and vegetal (V) regions of the cleavage embryo of *Xenopus laevis*. Plain arrows indicate 'constant' proteins used as standards for densiometric quantitation of spot intensities. 'Reference' proteins (a, r) are marked to aid in comparison of protein synthesis patterns.

of any localized masked message being stored, and not translated, in the cleavage embryo.

DISCUSSION

By the mid-blastula stage of *Xenopus* development the embryonic genome has become active (Newport & Kirschner, 1982*a*,*b*) and it is probable that any region-specific differences in protein synthesis along the animal–vegetal axis from this stage onwards are due to differential embryonic gene activity. The genes for alpha-actin, whose translation product is tentatively identified as spot 59a in this study, have already been shown to be activated specifically in the mesoderm at late gastrula (Mohun *et al.* 1984; Gurdon *et al.* 1984). The results presented here suggest that there is regional asymmetric expression of a large number of genes, besides alpha-actin, from blastula through tailbud, since the differences in protein synthesis between animal and vegetal regions increases dramatically during this period. This differential expression includes genes coding for proteins that have not been synthesized before gastrulation and are 'new', e.g. 59a, 14b, 38a, 55a, and also genes coding for proteins that were synthesized at some time throughout the preblastula embryo, egg or oocyte, e.g. 22a, 5b, 4b, 69a. Proteins (except 48a and 12b) synthesized asymmetrically in the preblastula embryo are no longer synthesized past gastrula and are presumably a result of translation of maternal mRNAs that are not replaced by embryonic transcripts. The majority of differences in protein synthesis in animal and vegetal regions identified were quantitative differences although some appeared to be qualitative and this may be related to the relative simplicity of the dissection into only two regions. Finer, more complex dissection would be required to distinguish true localized synthesis from gradients of synthesis. From blastula onwards the animal-characteristic differences in protein synthesis start to become prominent, followed a few hours later at gastrulation by an increase in vegetal-characteristic differences. The reason for the delay in the increase of the vegetal-characteristic differences is unclear. By late blastula there are more cells in the animal hemisphere than the vegetal hemisphere because of the faster division in the animal hemisphere that occurs after cleavage division 12, and the larger 'gene set' in the animal hemisphere may lead to a more rapid impact on the protein synthesis pattern (Ballantine *et al.* 1979). It is also possible that the larger number of cells in the animal hemisphere somehow offers a greater potential for animal-characteristic protein synthesis. In later development the endoderm gives rise mainly to gut, whereas the ectoderm and mesoderm give rise to a much more complex array of differentiated tissues, e.g. brain, somites, heart, and this may be reflected as early as the blastula stage in the size of the array of protein synthesis characteristic for each region.

The divergence at the level of protein synthesis between animal and vegetal regions of the embryo from gastrulation onwards, which accompanies morphological differentiation, is thought to be programmed or initiated by asymmetries

or localizations of maternal 'determinants' of differentiation along the animal–vegetal axis of the pregastrula embryo, egg or oocyte. An examination of protein synthesis and mRNA levels in the oocyte, egg and pregastrula embryo resulted in the identification of a small number of proteins that are possible candidates for such determinants. The rate of accumulation of these proteins in the vegetal hemisphere of the mature oocyte and egg corresponds to the distribution of abundant soluble proteins in the oocyte, egg and early embryo (data not shown). No major newly synthesized proteins were found localized in the animal hemisphere of the oocyte or egg. In the oocyte the germinal vesicle is located in the animal hemisphere and is known to contain a number of nuclear-specific proteins (De Robertis, Longthorne & Gurdon, 1978). However these proteins presumably were not synthesized and accumulated at a sufficient rate to be identified in the gel analysis of the animal protein extracts. 2D gel analysis of newly synthesized *Xenopus* oocyte proteins has previously shown nuclear-specific proteins to be barely detectable in whole oocyte extracts (De Robertis *et al.* 1978). The differences in protein synthesis that were detected within the oocyte appear to be due to corresponding segregation of mRNA within the oocyte. In all cases examined, except one, (49a), the rate of protein synthesis reflected the relative mRNA distribution along the axis. For 49a, however, there seems to be a greater difference in RNA levels along the axis than is reflected in the *in vivo* pattern, suggesting some form of translational control within the oocyte.

There is very little information on how protein and mRNA localizations within the oocyte are established or maintained. The partitioning of RNAs in embryos once cleavage has divided the cytoplasm into individual cells is easily understood. However, how mRNA asymmetries are established and maintained within cells is still obscure. Capco & Jeffery (1982) have shown that poly(A)$^+$RNAs derived from animal or vegetal regions of the *Xenopus* oocyte injected into *Xenopus* fertilized eggs migrate to their regions of origin, but the mechanism behind this movement is unclear. Capco and Jeffery have suggested the involvement of cytoskeletal elements, membrane systems, specific types of yolk particles, or other cytoplasmic organelles in the maintenance of mRNA asymmetries. Moon *et al.* (1983) have likewise proposed that an association of mRNA with the cytoskeleton may be involved in the generation of a non-random spatial distribution of maternal mRNA during early development. More recent experiments by Jeffery studying the segregation of mRNAs in the yellow crescent of ascidian eggs have shown that the pattern of mRNA distribution is maintained by the association of mRNA with a non-actin cytoskeletal matrix (Jeffery, 1984).

In summary, there were no detectable differences in mRNA levels within the oocyte or cleavage embryo apart from those that cause the differences in *in vivo* protein synthesis patterns along the axis. There was no evidence for *localized*, 'masked', stored maternal messages along the animal–vegetal axis. This suggests that the majority of abundant messages, masked and unmasked, are evenly distributed throughout the oocyte, egg and early embryo. This lack of any major segregation of stored mRNA suggests that if mRNA localizations or gradients are

important in determining developmental fate along the animal–vegetal axis in early development, and are involved in the appearance of the differences in protein synthesis described here for postblastula embryos, the important messages are not abundant or the localizations small, as they are not detected by the methods employed in these experiments. It must be remembered, however, that only a range of proteins with pI's 4–6·5 and 7–10, and molecular weights 10 000–100 000 were analysed. While the asymmetric synthesis and accumulation of abundant proteins may be involved in the developmental determination of animal and vegetal hemispheres it remains to be determined if the proteins asymmetrically synthesized along the animal–vegetal axis of the *Xenopus* oocyte, egg and early embryo, identified in this study, are of developmental significance.

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