Paternal gene expression in developing hybrid embryos of *Xenopus laevis* and *Xenopus borealis*

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**SUMMARY**

We have studied protein synthesis in the viable hybrid *Xenopus laevis* (♀) × *Xenopus borealis* (♂) using 2D gel electrophoresis. Fourteen *borealis*-specific proteins were studied. Two of these proteins appeared by the gastrula stage, five in the gastrula and the rest later. Where homologous *laevis* proteins were tentatively identified, androgenetic haploid hybrids were used to study whether the protein was encoded by stored maternal mRNA, and how long this mRNA persisted. The two proteins appearing in blastulae were probably initially coded by stored maternal mRNA. This was not detectable by the tailbud-tadpole stage, and presumably had been destroyed.

**INTRODUCTION**

Development is continuously directed by the expression of genetic information, but also by prior gene activity during oogenesis. In the early stages of amphibian development the number of nuclei is too small to have a major quantitative impact on gene activity during the rapid progression to the late blastula stage of development (to form a 20000 cell blastula takes only 9 h). This is not to say that genes can have no impact at all between the egg and blastula stages, but rather that the effect can only be developmentally significant where the product represents a small, but very active fraction of total protein or RNA synthesis, or is coded by highly re-iterated genes (see Woodland & Wilt, 1980). The consequence of these constraints is that proteins made in very early development, except for a hypothetical very rare class, are encoded in an oogene-tic store of RNA. These conclusions are generally true of embryos that have large eggs and/or develop rapidly (see Davidson, 1976) but even seem to apply to mammals, where development is much slower (Braude, 1979). The experiments described below provide information about the timing of new gene expression in amphibian development and about the longevity and overall impact of the stored maternal mRNA.

One approach to detecting new gene action is to look for the appearance of proteins absent from the oocyte and other early developmental stages. A disadvantage of this approach is that it does not distinguish new gene activity from

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the mobilization of previously untranslated, stored mRNA. This possibility can largely be eliminated by extracting the total cellular RNA and translating it in vitro. A second disadvantage is that it yields information only about the synthesis of species of mRNA that were not present at earlier stages of development, whereas many genes might be continuously active throughout early development. Nevertheless this approach has already provided useful information about *Xenopus* development. Brock & Reeves (1978), Ballantine, Woodland & Sturgess (1979) and Bravo & Knowland (1979) have used 2D electrophoresis (O'Farrell, 1975) to study a range of proteins synthesized as development progresses. However, the oocyte makes many more proteins than the egg, and Ballantine *et al.* (1979) were unable to identify, with any certainty, proteins consistently made by eggs and blastulae that were not already made in the oocyte. In addition, translation of the mRNA in vitro indicated that at early stages, changes in protein synthesis involved post-transcriptional control. These conclusions had previously been drawn independently with respect to a single protein class, the histones (Adamson & Woodland, 1974, 1977; Ruderman, Woodland & Sturgess, 1979). As far as new types of proteins are concerned the first examples were not detected until the late gastrula stage (Ballantine *et al.* 1979). Brock & Reeves (1978) obtained essentially the same result, but they started their study at the egg stage and therefore saw the appearance of what they called ‘new proteins’, i.e. relative to the egg, not the oocyte.

An alternative approach for detecting gene activity is to study viable interspecies hybrids. An appropriate example is the cross of *X. laevis* (♀) and *X. borealis* (♂). The hybrids develop to adults (Blackler & Gecking, 1972) and in oocytes of the two species about 30% of the newly synthesized proteins can be distinguished by 2D electrophoresis (De Robertis & Black, 1978). We have used this approach to study paternal gene expression in crosses of *X. laevis* (♀) × *X. borealis* (♂). Since the sperm cannot carry a store of mRNA, the appearance of *borealis*-specific proteins shows that the genes that encode them must be active. One advantage of this approach is that it reveals the activity of genes specifying mRNA molecules already present in the oogenetic store of mRNA.

We have also studied the activity and persistence of stored, maternal mRNA by using androgenetic haploid hybrids. In these embryos the maternal nucleus is destroyed by u.v. light (Gurdon, 1960) so the stored mRNA must all be of the *laevis* type and the newly synthesized mRNA must exclusively be of the *borealis* type. Therefore the appearance and disappearance of the *laevis*-specific proteins reveals the mobilization and replacement of stored mRNA.

1 Overall our conclusions are similar to those of Bravo & Knowland (1979). However, we disagree with their conclusion that new types of proteins are made in the egg, since we have found probable homologues in the oocyte and later developmental stages. It is possible that some of their differences result from polymorphisms. We also disagree with some of their results regarding actin (see Sturgess *et al.* 1980).
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The study described below represents an analogous strategy to that adopted by Woodland, Flynn & Wyllie (1979) to study histone synthesis in development.

MATERIALS AND METHODS

Methods of handling and radioactively-labelling the embryos were described by Ballantine et al. (1979). Hybrid embryos and androgenetic haploid hybrids were obtained by the methods of Gurdon (1960) and Woodland et al. (1979). Developmental stages are numbered according to Nieuwkoop & Faber (1956).

The procedures of sample preparation and the 2D gel electrophoretic analyses were described by Ballantine et al. (1979) and are based on those of O'Farrell (1975).

RESULTS

De Robertis & Black (1978) found that all of the proteins specific to the oocytes of both parents were present in oocytes of the hybrid X. laevis (♀) × X. borealis (♂). They estimated that about 30% of the proteins made in X. laevis oocytes were different from those made in X. borealis oocytes – as analysed by two-dimensional (2D) electrophoresis. We saw fewer proteins specific to each species during the early stages of development, probably because we detect fewer newly synthesized proteins in early embryos compared with oocytes. The number of proteins which can be identified as species-specific increases during the period of development studied (stage 9–42).

Table 1 details 14 representative and reproducible borealis-specific proteins whose behaviour we have investigated in detail during the development of X. borealis embryos and in X. laevis (♀) × X. borealis (♂) hybrid embryos. The positions of all of these proteins in two-dimensional gel electrophoretic analyses are shown in Fig. 1 (a hybrid neurula embryo). Where proteins are marked with a subscript they are laevis-specific and may be homologues of the borealis-specific protein with the same number. Fig. 5A and D shows the specificity of proteins 1, 2, 3, 4, 5, 6, 8, and 13 to X. borealis (protein 12 is also normally seen at this stage in X. borealis but is not visible in these Figs.) and proteins 1L, 2L, 3L, 5L, 8L, 13L to X. laevis. The probability of the proteins being homologues is highest where the two proteins only differ slightly in charge, both appear at the same stage of development and are found in the same regions of the embryo (proteins 1 and 1L, 2 and 2L, 3 and 3L, 5 and 5L). In the other cases (8 and 8L, 9 and 9L and 13 and 13L), the two proteins differ in charge and molecular weight; the laevis-specific are slightly larger (about 10 amino acids) and are more basic. These identifications are tentative without sequence data, but we do have preliminary evidence that proteins 9 and 9L have similar products of partial proteolytic cleavage (unpublished results).

The difference in borealis represents the loss of about 10 amino acids with a net acid charge. The results are not easily explained by postulating that borealis and laevis simply differ in their processing enzymes, since the hybrid is like a
Table 1. Summarized properties of the species-specific proteins studied

<table>
<thead>
<tr>
<th>Borealis protein number*</th>
<th>Stage of appearance</th>
<th>Period present†</th>
<th>Putative laevis homologue‡</th>
<th>Stage at which putative homologue appears</th>
<th>Time at which maternal homologue disappears in haploid hybrid</th>
<th>Nomenclature of homologue in Ballantine et al. (1979)</th>
<th>Localization of laevis homologue</th>
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<tbody>
<tr>
<td>1</td>
<td>9-10</td>
<td>Persists</td>
<td>1_L</td>
<td>6-7</td>
<td>16-20</td>
<td>A</td>
<td>Whole</td>
</tr>
<tr>
<td>2</td>
<td>10-12</td>
<td>Persists</td>
<td>2_L</td>
<td>10-12</td>
<td>20-25</td>
<td>C</td>
<td>Ect</td>
</tr>
<tr>
<td>3</td>
<td>10-12</td>
<td>Persists</td>
<td>3_L</td>
<td>10-12</td>
<td>Never present</td>
<td></td>
<td>Whole</td>
</tr>
<tr>
<td>4</td>
<td>10-12</td>
<td>Persists</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>10-12</td>
<td>Persists</td>
<td>5_L</td>
<td></td>
<td></td>
<td>?8-9</td>
<td>?-20</td>
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<tr>
<td>6</td>
<td>10-12</td>
<td>Persists</td>
<td>—</td>
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<tr>
<td>7</td>
<td>20-30</td>
<td>Persists</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>10-12</td>
<td>To stage 30</td>
<td>8_L</td>
<td>10-12</td>
<td>Never present</td>
<td></td>
<td>Whole</td>
</tr>
<tr>
<td>9</td>
<td>30-40</td>
<td>Persists</td>
<td>9_L</td>
<td>30-40</td>
<td>Never present</td>
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<td>Ect</td>
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<tr>
<td>10</td>
<td>30-40</td>
<td>Persists</td>
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<td></td>
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<tr>
<td>11</td>
<td>30-40</td>
<td>Persists</td>
<td>—</td>
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<td></td>
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<tr>
<td>12</td>
<td>12-20</td>
<td>Persists</td>
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<td></td>
</tr>
<tr>
<td>13</td>
<td>9-10</td>
<td>To stage 39</td>
<td>13_L</td>
<td>9-10</td>
<td>20-25</td>
<td></td>
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</tr>
<tr>
<td>14</td>
<td>30-40</td>
<td>Persists</td>
<td>—</td>
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</table>

* See Fig. 1.
† Experiments were not continued beyond stage 40.
‡ The relation between possible homologues in the two species is open to doubt. However, these proteins are laevis-specific and therefore still provide information about stored mRNAs etc., regardless of their homologues in borealis.
§ ‘Ect’ is the part of the embryo that contains ectoderm and dorsal mesoderm. ‘Whole’ implies that the protein also occurs in the remainder of the embryo.
|| Proteins 5 and 5_L vary in different series, probably because of polymorphism. These conclusions are therefore tentative.
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Fig. 1. An autofluorograph of a 2D gel separation of the [\(^{35}\)S]methionine-labelled proteins made by a diploid hybrid embryo (*Xenopus laevis* × *Xenopus borealis* 2N) at the late neurula stage (labelling between stages 16 and 23). The labelled rings allow identification of the position of all the *borealis*-specific proteins and their possible *laevis* homologues listed in Table 1. As can be seen, not all of them are present at this stage. The positions of \(\alpha\), \(\beta\) and \(\gamma\)-actins are also indicated.

mixture of the two parents. Proteins 8, 9 and 13 could be the product of the same gene, and it could be relevant that 8 and 9 are not present at the same developmental stage. Again sequence data are needed to elucidate this point.

_Paternal proteins which first appear in blastulae_

Newly synthesized protein 1 is invariably easy to detect in stage-9 hybrid embryos (Fig. 2C). Protein 13 can usually just be seen at the stage 9–10 border. Data concerning the appearance of the proteins studied are summarized in Table 1.

_Paternal proteins first appearing in gastrulae and neurulae_

The first stage-specific proteins not made in oocytes are seen in homospecific crosses at the gastrula stage (Ballantine _et al._ 1979, see Introduction). It is
therefore not surprising that we see increasing numbers of species-specific proteins in hybrids from this stage on. In the gastrula proteins 2, 3, 4, 6 and 8 become detectable, though in some cases very faintly (Fig. 3A and B). By stage 20 proteins 5 (Fig. 3C and D) and 12 are added (although protein 12 is not visible in Fig. 3D its position can be seen in Fig. 3F).
Proteins appearing at later stages

Further proteins appear as development proceeds. These include 7, 9, 10, 11 and 14 (Fig. 3E and F). Our analysis has not been extended beyond stage 40, which is the pre-feeding tadpole stage.

Behaviour of laevis-specific proteins in androgenetic haploid hybrids

In androgenetic haploids from the mating *Xenopus laevis* (♀) × *X. borealis* (♂) the cytoplasm, and hence any mRNA stored during oogenesis, will be exclusively *laevis*. On the other hand the only nuclear genes present will be from the *borealis* sperm. Thus as development proceeds, all newly synthesized mRNA molecules will be of the *borealis* type. Any newly synthesized *laevis*-specific proteins can only have been made on maternal mRNA. These androgenetic haploid hybrids thus enable us to follow the activity of stored mRNA and its replacement by new transcripts (see also Woodland et al. 1979).

Androgenetic haploids were prepared by the method of Gurdon (1960) as described by Woodland et al. (1979). The efficiency with which the maternal nuclei were destroyed was measured by counting nucleoli in control haploid *X. laevis* × *X. laevis* embryos, as well as by following the disappearance of newly synthesized maternal-type H1 histone. The experiments were performed on the same matings already described by Woodland et al. (1979), who provide the detailed data on enucleation. The success of enucleation was at least 98 %.

mRNAs coding for *laevis*-specific proteins could behave in one of three ways:

1. They could be synthesized and stored exclusively in oogenesis. This would mean that the proteins would be made in all early embryos derived from a *laevis* mother, but unless the mRNA was very stable they would cease synthesis by later stages.

2. They could be stored during oogenesis and also made through early development. This would mean that the proteins they encode would be made at early stages in all eggs laid by *laevis* females and would continue to be made both in *laevis* embryos and in *laevis* (♀) × *borealis* (♂) diploid hybrids. They would cease synthesis in *laevis* (♀) × *borealis* (♂) androgenetic hybrids.

3. They could be made only in embryos, and never in oocytes. Therefore the proteins that they encode would be absent from early embryos of all types and would appear at specific developmental stages in *laevis* (♀) × *laevis* (♂) embryos or *laevis* (♀) × *borealis* (♂) diploid hybrids. They would never be synthesized in androgenetic haploid hybrids.

No proteins of the first type were found, but there were various proteins of categories (2) and (3). Thus in a mixture of radioactive protein preparations from stage-37 *X. laevis* and *borealis* tadpoles, 39 *laevis*-specific proteins could be seen. Ten were detectable in embryos labelled with [35S]methionine from stage 9½ to 10½ and 30 in embryos labelled between stages 12 and 20. The seven *laevis*-specific proteins indicated in Fig. 1 and Table 1, column 4, illustrate both
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of these categories are singled out because they may be homologous with *borealis* proteins (see above). There is little to be gained by describing the other 26 in detail.

Protein 1L is clearly of type 2 (Fig. 6), as are the histones (Woodland *et al.* 1979). In the haploid hybrid, 1L becomes detectable in the mid-blastula (Fig. 4A) though it can hardly be seen in the newly synthesized proteins of the egg or early morula (Ballantine *et al.* 1979, Fig. 2, protein A). This shows that 1L represents the product of a mobilized maternal mRNA under translational control. Protein 1L becomes fainter in the haploid hybrid gastrula and is indetectable after stage 20 (Fig. 4C). Since protein 1 seems to be its *borealis* homologue we interpret our observations to mean that the stored transcripts encoding protein 1L are replaced by new mRNAs synthesized from the blastula stage onwards. The status of protein 5L is uncertain (see Table 1) but proteins 13L and 2L also seem to be of type (2). They appear between stages 9 and 12 in normal *laevis* embryos, hybrids and androgenetic haploid hybrids, and then disappear between stages 20 and 25 in haploid, but not diploid hybrids. Their putative *borealis* homologues are first detectable at about the same stage, but much more maternal than paternal-type protein is seen in early diploid hybrids. The genes for 2L and 13L seem to be active in oogenesis, then through early development, from at least the late blastula stage onwards. Unlike 1L and 2L protein 13L may not be made after stage 40.

Proteins 3L, 8L and 9L are representatives of class 3. They were not detected in androgenetic haploids at any stage. They are therefore absent from the oogenetic mRNA store, at least at detectable levels, and reveal new gene activity at a range of stages from the gastrula onwards (Table 1, column 5).

**DISCUSSION**

*Onset of gene activity in development*

Once a gene becomes active, our ability to detect its protein will always depend on four variables: the sensitivity of the assay, the background, the intensity of activity of the gene and the reiteration frequency of the gene. For example, the H1 histone genes are reiterated 20–50 times (Jacob, Malacinski & Birnstiel, 1976). If all are active in development their protein should be detectable at
Fig. 4. Autofluorographs showing changes in the patterns of 2D gel separations of proteins made by haploid hybrid (\(X. \text{laevis} \times X. \text{borealis} \, 1N\)) embryos during early development. Stage 7-8 (A) shows the appearance of the \(\text{laevis}\) protein 1, followed by the appearance at stage 9-10 (B) of the \(\text{borealis}\)-specific homologues (1). By stage 10-12 (C) the relative intensity of spots 1 and 1L have changed compared to B and to the diploid hybrid (Fig. 3B). By stage 12-20 (D) spot 1L has disappeared. The absence of other \(\text{laevis}\)-specific spots at this stage (2L, 3L, 8L, 13L) is also indicated in D by labelled arrows. Radioactive labelling was as in Fig. 2.

about the 500-cell stage (Woodland & Wilt, 1979) and it can, in fact, be detected as a radioactive protein in \(X. \text{laevis} \times X. \text{borealis}\) hybrids at the 1000-10000-cell stage, only an hour or so later (Woodland et al. 1979). H1 histone represents about 2% of protein synthesis at this stage (Adamson & Woodland, 1977; Flynn & Woodland, 1980). For analyses of comparable sensitivities and against similar backgrounds other proteins made at the same efficiency should be detected at the same stage, if their genes are also reiterated 20–50 times. On the other hand there would need to be about 20000–50000 cells if the gene were single copy, i.e. the protein would first be detected at some time between stage 9 and stage 16.

The first new gene activity that we detect appears at stage 9 (10000–30000 cells, Dawid, 1965) which is as soon as one could reasonably expect, given the
methods used. This means that if such genes were equally active at earlier stages their products would not have been detected. The same is true of the more extensive gene expression detectable in the gastrula (Table 1).

Thus we may now say that H1 histone genes are not alone in being active in the amphibian blastula. Unfortunately we do not yet know the function of these other proteins. In the gastrula more genes are active. Some are region-specific and none were detectable at earlier stages. These supplement α-actin (Ballantine et al. 1979), collagen (Green, Goldberg, Schwarz & Brown, 1968),

Fig. 5. Autofluorographs of 2D gel separations of protein from late neurulae (stage 16–23) of *X. borealis* and *X. laevis* non-hybrids compared with their diploid and haploid hybrids. A and D show the presence of the spots specific to the parental types. C shows that the *borealis*-specific spots are present in the 2N hybrid together with a possible *laevis* homologue in some cases. B shows that the *laevis*-specific spots are absent from the haploid hybrid and it resembles the *borealis* parent (spot 12 is usually seen in auto-fluorographs of A, B and C at this stage). Other details are as in Fig. 2.
tyrosine (Benson & Triplett 1974) and various isoenzymes (Johnson, 1971; Johnson & Chapman, 1972; Wright & Subtelny, 1971, 1973; Wright, 1975) on the list of proteins first detectably made at the gastrula or neurula stage of amphibian development.

**Stored maternal mRNA**

Certain of the proteins studied here are *laevis*-specific. If they were synthesized by the androgenetic haploid hybrid they must have been coded by RNA synthesized by the maternal genome during oogenesis, since the embryo will contain only paternal *X. borealis* genes. If they never appear then it is most likely that they represent proteins made on transcripts synthesized only after fertilization. The majority come into the second category (Table 1), but two are certainly represented in the maternal store (proteins 1\(_L\) and 13\(_L\)). These proteins have putative *borealis* homologues. If this identification is correct, these proteins may be placed in a regulatory class with H1 histones. Each becomes detectable at the early blastula stage, as the result of mobilization of stored maternal mRNA. Each manifests paternal gene expression by the late blastula stage, although H1 is detectable slightly earlier than the proteins studied here (Woodland et al. 1979). In each case the activity of the maternal transcripts disappears after gastrulation. In the absence of maternal genes, the stored H1 transcripts

<table>
<thead>
<tr>
<th>Stage</th>
<th><em>X. laevis</em> X <em>X. laevis</em> diploid</th>
<th><em>X. laevis</em> X <em>X. borealis</em> diploid</th>
<th><em>X. laevis</em> X <em>X. borealis</em> haploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 7–9</td>
<td><img src="image1" alt="Diagram" /></td>
<td><img src="image2" alt="Diagram" /></td>
<td><img src="image3" alt="Diagram" /></td>
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<tr>
<td>Stage 9–10</td>
<td><img src="image4" alt="Diagram" /></td>
<td><img src="image5" alt="Diagram" /></td>
<td><img src="image6" alt="Diagram" /></td>
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<td>Stage 10–12</td>
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<td>Stage 12–20</td>
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<td><img src="image11" alt="Diagram" /></td>
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</tr>
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Fig. 6. Diagram illustrating the behaviour of proteins 1 (*borealis*-specific) and 1\(_L\) (*laevis*-specific) during the early development of hybrid and non-hybrid embryos. The unlabelled spot in the diagram is common to both species.
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vanish by the early gastrula stage, whereas protein 1_L disappears in the neurula and 13_L a little later. The functional instability of histone mRNA may be ascribed to its lack of a 3′poly (A) tract (Huez et al., 1978; Woodland & Wilt, 1979). The polyadenylation state of the mRNA encoding proteins 1_L and 13_L is not yet known. In any case both contrast with polyadenylated globin mRNA injected into 1- to 2-cell embryos the activity of which remains detectable to the tadpole stage (Gurdon, 1974).

REFERENCES


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