Relocation and reorganization of germ plasm in *Xenopus* embryos after fertilization

R. E. RESSOM and K. E. DIXON

School of Biological Sciences, The Flinders University of South Australia, Bedford Park, SA 5042, Australia

Summary

In the unfertilized egg, germ plasm is widely distributed throughout the vegetal subcortex in small islets. Following fertilization or artificial activation, the location and organization changes, and by the 4- to 8-cell stage the germ plasm forms a small number of large patches overlying the vegetal pole. We distinguish three processes that produce these changes. The first of these is aggregation which involves the islets moving towards the vegetal pole to form large patches by coalescence. This phase requires microtubules but does not depend on cleavage or dynamic microfilaments. The second phase is ingression during which the patches of germ plasm move to the interior of the egg. The movement is due to a flow of cytoplasm from the vegetal pole internally and the cytoplasmic current does not require either microtubules or dynamic microfilaments. In the third phase, the germ plasm is trapped in the vegetal hemisphere by microtubular arrays – in normal development, the mitotic spindle.

Key words: germ plasm, cytoskeleton, primordial germ cells, *Xenopus*, fertilization.

Introduction

The initial differences between cells in developing embryos commonly arise as a result of the inclusion of specialized regions of egg cytoplasm, termed cytoplasmic localizations, in some cells and not others. Germ plasm of anuran amphibians is one such cytoplasmic localization. It is generally thought that this substance is responsible for the differentiation of the primordial germ cells (reviewed in Dixon, 1981; Smith *et al.* 1983), although conclusive evidence is not yet available. In fertilized eggs, the germ plasm is distributed over a wide area of the vegetal hemisphere as small cortical islets, which, because of their size, can only be identified with certainty in the electron microscope (Czolowska, 1972). During the second cleavage cycle, larger patches of germ plasm can be seen in the light microscope (Bounoure, 1934; Blackler, 1958; Whittington & Dixon, 1975), at this stage located close to the vegetal pole. Usually, one patch is included in each of the first four blastomeres. As cleavage continues, each patch is segregated to only one of the daughter cells and in this way, by late cleavage, the germ plasm remains in approximately four cells. This group of cells has been called the founder clone of presumptive primordial germ cells by Dixon (1981).

The aim of this investigation was to study in greater detail the reorganization of the small islets of germ plasm into large patches and their relocation from a diffuse distribution over much of the vegetal hemisphere to a concentration overlying the vegetal pole. In particular, we wished to discover the mechanisms involved in these processes.

Materials and methods

Fertilization and artificial activation

Eggs were obtained from frogs injected with hormones and inseminated using a macerated testis (Cleine & Dixon, 1985). Eggs were artificially activated by direct application of 2–3 drops of calcium ionophore A23187 (1 mM in 1:1 DMSO, ethanol) to the medium surrounding them. The jelly coats were removed with 4% sodium thioglycollate in 25% MMR (after Kirschner & Hara, 1980, but omitting EDTA), pH 7.8, and rinsed in several changes of filtered tap water. Embryos were allowed to develop at 20–22°C and staged according to the Normal Table of Nieuwkoop & Faber (1967).
Microinjection

Eggs and early embryos were injected with various drugs in 100 mM-Hepes buffer using a glass micropipette (Dziadek & Dixon, 1978) while immersed in 10% Ficoll in 25% MMR to reduce leakage (after Cleine & Dixon, 1985).

Histology

All material was fixed in Smith's fixative and embedded in paraffin. Serial sections 6 μm thick were stained in Aurantia (2% in 70% ethanol) for 10 min followed by Naphthalene Black 12B (0.5% in 1% aqueous chromium potassium sulphate) for 5 min. Germ plasm stained deep blue with nuclei and cytoplasm pale blue and yolk platelets yellow.

Volume and distribution of germ plasm

The volume of germ plasm in an embryo was estimated by measuring the area in successive sections with an eyepiece grid and multiplying the sum of the areas by the thickness of the sections. The number of patches of germ plasm was counted directly from serial sections. The position of each patch of germ plasm in the egg was plotted on a circle representing an egg, using a 100 μm square eyepiece grid superimposed on the section and then projected onto a single median plane.

Results

These results are based on more than 50 experiments using eggs from approximately 80 females. In each experiment, three replicate series with 10 embryos in each were used, except where noted otherwise. The changes that take place after fertilization in the distribution and location of germ plasm are considered under three headings: (i) aggregation, (ii) ingestion and (iii) localization.

Aggregation - the process

In fertilized eggs, germ plasm was identified with certainty at 2 h postfertilization (approximately the 2-cell stage). It consists at this time of numerous (92.7 ± 8.2) small islets of granular yolk-free cytoplasm that stains heavily with Naphthalene Black (Table 1). They lie in a subcortical band about 25–30 μm thick. By 4 h postfertilization (p.f.), at the 16-cell stage, the number of islets had decreased to an average of 4.25 ± 0.3 per embryo (Table 1) an estimate consistent with those of Whitington & Dixon (1975), Akita & Wakahara (1985) and Ikenishi & Nakazato (1986). At 6, 8 and 10 h, the number of patches had not changed (Table 1), indicating that aggregation, i.e. coalescence of the original islets into larger patches, was complete by 4 h p.f.

As the islets coalesced, the patches grew larger (Fig. 1). Between the 2- and 4-cell stages, their area parallel to the surface of the embryo increased approximately 25 times from an average of 30.7 μm² to the 2-cell stage to an average of 754.8 μm² in 4-cell embryos. In the direction perpendicular to the surface, i.e. the thickness of the aggregates, the increase was of the order of three times (4.51 ± 1.5 μm–15.7 ± 8.2 μm). This suggests that movement of the islets is restricted to a narrow subcortical region and hence end-to-end fusion of islets is the major process in coalescence. Coalescence also led to a reduction in the area (in projection) of the egg occupied by germ plasm. Treating the area as a circle, the diameter decreased from 281 ± 47 μm at 2 h p.f. to 164 ± 89 μm at 4 h p.f., i.e. a contraction to approximately 60% of the original area (cf. Fig. 2A, B). In actuality, in 4-cell embryos, patches are slightly ovoid with the ratio of short to long axes being 0.8 on average (51 patches measured).

As a consequence of aggregation, the germ plasm was arranged in four patches, lying above the vegetal pole, one in each blastomere.

Aggregation - mechanisms

(1) Cleavage

Eggs activated with calcium ionophore (A23187) do not cleave and hence provide a means to assess the role of cleavage in aggregation of germ plasm.

In activated eggs, germ plasm first became visible at the same time as in fertilized eggs and its distribution was similar, but spread over a slightly greater area (e.g. at 2 h postactivation (p.a.), 398 ± 58 μm² diameter compared to 281 ± 47 μm in fertilized eggs – 10 eggs measured in each) (cf. Figs 2A, 3A). At 4 h p.a., the number of patches and their distribution (Fig. 3B) was again similar to that in fertilized eggs (Fig. 2B) but once more they occupied in total a larger area of the egg (306 ± 43 μm diameter compared to 164 ± 89 μm – 10 eggs measured in each). These differences may reflect less-efficient aggregation in activated eggs. However, at least part of the variation is probably due to the inwards curvature of the plasma membrane at the cleavage furrow in fertilized eggs. The effect of this is to decrease the projected area occupied by germ plasm in fertilized eggs (and also to make it appear as if some germ

<table>
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<tr>
<th>Time after fertilization h</th>
<th>No. embryos</th>
<th>No. patches/embryo mean ± s.e.</th>
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<tbody>
<tr>
<td>2</td>
<td>7</td>
<td>92.7 ± 8.2</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>4.25 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>4.6 ± 0.7</td>
</tr>
</tbody>
</table>

Serial section of embryos fixed at 2 h intervals following fertilization were examined. Patches were scored as discrete entities when they were clearly separable at a magnification of 500×.

Table 1. Change in the number of patches of germ plasm in embryos after fertilization.
but no role in the movement and coalescence of the islets of germ plasm.

But cleavage plays a role in the distribution of germ plasm between the blastomeres and also in the position of the patches within the embryo. In fertilized eggs, when aggregation is completed, the patches of germ plasm are separated from one another by blastomere membranes and, moreover, they are aligned along the incurring cleavage furrow. In this way, the germ plasm is partitioned between the first four blastomeres and is positioned in readiness for the next phase of ingress.

(2) Cytoskeleton – microtubules

Colcemid was injected into fertilized eggs and activated eggs as a solution in 100 mM-Hepes buffer. When fertilized eggs were injected with buffer alone they cleaved normally and germ plasm aggregated, but the distribution of the patches differed slightly from that in uninjected controls, some patches being located more internally than normal. This result shows that injection per se has a slight effect on aggregation.

When colcemid was injected into activated eggs (20 nl of 1 mg ml\(^{-1}\)) at 0-2 of the first cleavage cycle, none of the characteristic events associated with activation (i.e. movement of animal hemisphere pigment, pseudocleavage), was observed. At 2 h p.a. visible patches of germ plasm had not formed and at 4 h p.a. a similar result was obtained. Injection of colchicine into fertilized eggs prevented cleavage and spindles were not visible in sections. Once again patches of germ plasm did not form (15 embryos from three experiments). Lumicolchicine, which has no effect on microtubules, did not prevent cleavage or aggregation of germ plasm. Thus the effects of colchicine are due to the effects on microtubules. Furthermore, another anti-microtubule drug, podophyllotoxin (20 nl of 5 mg ml\(^{-1}\) in 100 mM-Hepes buffer injected at \(t = 0.2\)) also blocked cleavage and aggregation of germ plasm.

We conclude that aggregation of germ plasm depends on the presence of polymerized microtubules. Presumably the drugs used prevented movement of the individual islets and hence they could not aggregate into patches.

(3) Cytoskeleton – microfilaments

Fertilized eggs and activated eggs were immersed continuously in 10 \(\mu\)g ml\(^{-1}\) cytochalasin B in 25% MMR or injected with approximately 50 nl of 100 \(\mu\)g ml\(^{-1}\) cytochalasin B in 25% MMR (2 groups of 10 embryos) at 0-2 of the first cleavage cycle. Preliminary experiments showed that DMSO in which the cytochalasin B was dissolved, at the concentration used, had no discernible effect on development and the distribution of germ plasm was the same as in controls.

After exposure to cytochalasin B for 2 or 4 h, the location and distribution of germ plasm was not different from controls (Fig. 5A,B) and the denser concentration of germ-plasm islets and patches over the vegetal pole at 4 h compared to 2 h indicates that aggregation had continued (c.f. Fig. 3A and B, respectively. Treated embryos did not cleave, showing that the drug penetrated into the egg and similar results were obtained after injection of the drug. We conclude, therefore, that dynamic microfilaments do not have any role in aggregation of germ plasm. Furthermore, examination in the electron microscope of patches of germ plasm did not reveal any microfilaments although they were clearly visible in the cortex of untreated eggs. These experiments also confirm our earlier conclusion that aggregation is independent of cleavage.

(4) Requirement for protein synthesis

After incubation of fertilized eggs in cycloheximide (200 \(\mu\)g ml\(^{-1}\) in 25% MMR) beginning at 0-2 of the first cleavage cycle, none of the developmental changes normally seen after fertilization was observed and the eggs did not cleave. On histological examination at 2 and 4 h p.f., aggregates of germ plasm were absent. We conclude that protein synthesis is required for aggregation.

Ingression – the process

Aggregation involves movement of the germ plasm...
Fig. 1. Distribution and organization of germ plasm in normal, control embryos fixed at 2 h intervals after fertilization. Patches of germ plasm (arrows). (A,B) 2 h p.f.; (C,D) 4 h p.f.; (E,F) 6 h p.f.; (G,H) 8 h p.f.; (I,J) 10 h p.f. Bars, 100 μm.
parallel to the egg surface. Ingression, as the name implies, involves movement into the interior of the egg, that is approximately perpendicular to the surface.

The distribution of germ plasm in fertilized eggs at 6 h p.f. is shown in Fig. 2C. Compared to the results at 4 h, the distribution in the horizontal dimension is somewhat narrower. The main difference, however, is that the dense concentrations immediately over the vegetal pole at 4 h are no longer present. In contrast, most of the germ plasm is located in the vegetalmost third of the egg. That is, there is a rapid change in position of the patches of germ plasm from the subcortical regions to deep within the yolky vegetal cytoplasm.

**Ingression – mechanisms**

In activated eggs at 6 h, the germ plasm was distributed more widely than in fertilized eggs at the same time (cf. Figs 2C, 3C). However, when compared...
Fig. 3. Distribution of germ plasm in eggs activated with calcium ionophore A23187. Methods as for Fig. 2. (A) 2 h; (B) 4 h; (C) 6 h; (D) 8 h; (E) 10 h.

Fig. 4. Organization of cytoplasm around germ plasm in normal 8-cell embryos. Germ plasm, large arrow; pigment granules tend to be organized in streams (small arrows) radiating from the patch of germ plasm and yolk platelets tend to be oriented with their long axis towards the germ plasm. Bar, 100 μm.

Fig. 5. Distribution of germ plasm in activated eggs incubated continuously in 10 μg ml⁻¹ cytochalasin B from 0-2 of the first cleavage cycle. Methods as for Fig. 2. (A) 2 h p.a.; (B) 4 h p.a.

with the distribution in activated eggs at 4 h, it is evident that most of the germ plasm had moved internally and to approximately the same distance as in fertilized eggs. Hence we conclude that ingestion is independent of cleavage.

When sections of fertilized or activated eggs at 6 h p.f. or p.a. were examined in the light microscope, a trail of pigment granules was noted stretching from just over the vegetal pole towards the interior. The trail was easily detected using dark-field microscopy (Fig. 6). At its inner end, patches of germ plasm were seen (Fig. 6B,C). We interpret these observations as indicating that between 4 and 6 h p.f., cytoplasm flows from the region of the vegetal pole towards the interior, carrying pigment granules and patches of germ plasm with it.

Activated eggs were injected with colcemid at 2, 4, 6 or 8 h p.a. and then examined at 10 h p.a. At this time, the cytoplasm appeared normal. In all cases, islets (2 h) and patches (4, 6 and 8 h) of germ plasm were dispersed throughout the vegetal hemisphere (Fig. 7A) showing that ingestion does not require the presence of microtubules. Similar results were obtained after immersion in, or injection with, cytochalasin B, from which we conclude that neither are dynamic microfilaments necessary for ingestion.

Activated eggs were incubated in cycloheximide (200 μg ml⁻¹ in 25% MMR), beginning at either 2, 4, 6 or 8 h p.a. and ending at 10 h p.a. when they were fixed for histological examination. In a control experiment, fertilized eggs incubated in the same way were sometimes able to complete one more cleavage but were never able to complete another, indicating that protein synthesis is inhibited under these conditions within about 30 min after treatment began. In all cases, germ plasm was observed, confirming earlier results. Moreover, with development, the islets (2 h) and patches (4, 6 and 8 h) moved inwards. Hence we conclude that new protein synthesis is not necessary for ingestion.

In summary, the aggregates of germ plasm that form over the vegetal pole during the first 4 h of development are carried into the interior of the egg by a flow of cytoplasm. This ingestion does not depend on cleavage, microtubules or microfilaments, nor require simultaneous protein synthesis.

Localization – the process

After 6 h p.f. the distribution of germ plasm relative to the animal–vegetal axis of fertilized eggs does not change significantly (Fig. 2D,E). At 8 and 10 h p.f.
the same number of patches was present as at 6 h (Table 1) and they had the same appearance. These observations suggest that the germ plasm becomes localized to a particular region of the cytoplasm at about 6 h p.f.

Localization – mechanisms

In contrast to the observations made on fertilized eggs, the distribution of germ plasm in activated eggs changed markedly after 6 h p.a. (Fig. 3D,E). At 8 h, although most of the patches were still contained

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**Fig. 6.** Patches of germ plasm at the head of a flow of cytoplasm from the vegetal pole (arrow heads), as indicated by accumulation of pigment granules and displacement of cortical cytoplasm into the yolk mass. (A) Fertilized egg, 6 h p.f., germ plasm, arrow; (B) activated egg, 6 h p.a., germ plasm, arrow, (C) high magnification view, activated egg, pigment granules (small arrows). Bar, 100 μm.
of germ plasm being more localized than in activated eggs injected with colchicine (cf. Fig. 7B and A). We therefore conclude that as the patches of germ plasm are carried to the interior of the egg by a current of cytoplasm, they become trapped by microtubules, probably those associated with the asters in each blastomere. Whitington & Dixon (1975) observed that in cleavage-stage embryos 85% of germ plasm patches were clearly associated with one pole of a mitotic spindle. This association is primarily responsible for keeping germ plasm within the lowest third of the egg, although germ plasm has sometimes been reported just below the blastocoel (e.g. Züst & Dixon, 1975), presumably because it was not trapped by an aster. These observations also indicate that transverse cleavage furrows probably do not play a significant part in localizing germ plasm. As a consequence of localization of patches of germ plasm within the blastomeres, as cleavages continue, germ plasm is segregated to only one of the daughter cells at each cytokinesis.

**Discussion**

Maternal information present as cytoplasmic localizations must, by definition, be allocated preferentially to some cells of the early embryo. Germ plasm of anuran amphibian eggs is a classic example of a cytoplasmic localization and we have studied the processes and mechanisms whereby this material is assigned to particular cells of the early embryo. Previous studies (Czolowska, 1972) have shown that, in the unfertilized egg, germ plasm exists as a large number of widely separated, small, subcortical islets that, following fertilization, form large patches over the vegetal pole (Bounoure, 1934; Whitington & Dixon, 1975). However, the mechanism of aggregation has not been previously investigated. Our experiments have depended to a large extent on the application of inhibitors of cytoskeletal functions. The results of experiments of this type should be interpreted cautiously. If a drug has no effect, and it can be demonstrated independently that it has penetrated into the egg, then it can reasonably be concluded that the cytoskeletal function in question is not necessary. If on the other hand the drug has an effect then the question arises as to whether the action is direct or indirect.

We found that between fertilization (or activation) and the 4-cell stage approximately, the islets of germ plasm are carried by a microtubule-dependent mechanism in a narrow subcortical band towards the vegetal pole. By the 4-cell stage, they have formed four large patches, usually one in each blastomere.
Germ plasm in Xenopus

Cleavage is not necessary for aggregation and neither are dynamic microfilaments involved. Protein synthesis is however necessary.

From 4 to 6 h after fertilization, the patches of germ plasm are carried internally by a streaming of cytoplasm from the vegetal pole to the interior of the embryo. This ingression does not depend on cleavage, microtubules or microfilaments.

In fertilized eggs, the patches of germ plasm move inwards for a distance on average about one-third of the diameter of the egg (Fig. 2) and then they remain localized in this position. In activated eggs, in contrast, movement of the patches continues and many enter the animal hemisphere. When activated eggs are treated with D2O, the patches remain predominantly in the vegetal hemisphere. We interpret these results as suggesting that normally, i.e. in embryos, the patches of germ plasm are trapped by microtubular arrays such as form mitotic asters and hence remain localized in the vegetal hemisphere.

In summary, we suggest that the process by which widely separated islets of germ plasm are allocated to their correct position in the embryo has three phases - aggregation, ingression and localization. A diagrammatic representation of this process is shown in Fig. 9. The model integrates earlier reports that described islets of germ plasm within the vegetal subcortex of unfertilized eggs (Czolowska, 1972), the division of patches between the first four blastomeres (Whittington & Dixon, 1975), its ingression (Bounoure, 1934) and its localization at one pole of the spindle aster (Whittington & Dixon, 1975). By these mechanisms, the germ plasm is partitioned to an initial clone of four cells and then segregated at each subsequent cleavage to only one of the daughter cells, thus maintaining the clone of presumptive primordial germ cells.

But some variability can be expected and indeed has been noted in earlier studies. For instance, Whittington & Dixon (1975) reported that in some of the 16-cell embryos they examined there were 'numerous small patches of germ plasm' (up to 92) indicating that aggregation had been impaired. Similarly, as discussed earlier, Züst & Dixon (1975) observed that patches of germ plasm were sometimes located in the floor of the blastocoel, suggesting that they had avoided entrapment by the mitotic aster and hence had not been localized. It is therefore interesting that the process as a whole has selfcorrecting (i.e. 'fail-safe') aspects. For example, even if the germ plasm does not aggregate efficiently, ingestion will still take place and many of the individual patches could be trapped by the single large spindle aster in each blastomere, thus effectively correcting the error in aggregation. Similarly, if ingestion does not occur (no examples have been reported) and the patches of germ plasm remain near the vegetal surface, as the blastomeres divide and become smaller, the chance that they will become associated with a spindle aster increases. Finally, if the patches are not localized by association with a spindle aster, then late in cleavage when the germ plasm is relocated around the nucleus (Bounoure, 1934; Blackler, 1958; Whittington & Dixon, 1975), it will at that time be concentrated and

<table>
<thead>
<tr>
<th>Stage</th>
<th>Number of cells containing germ plasm (mean ± s.d.)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>3-7</td>
<td>4-5 ± 0-9</td>
<td>Whitington &amp; Dixon (1975)</td>
</tr>
<tr>
<td>8-cell</td>
<td>4-0 ± 0-0</td>
<td>Cleme &amp; Dixon (1985)</td>
</tr>
<tr>
<td>St 61</td>
<td>4-2 ± 0-2</td>
<td></td>
</tr>
<tr>
<td>8-cell</td>
<td>3-4 ± 0-2</td>
<td>Akita &amp; Wakahara (1985)</td>
</tr>
<tr>
<td>St 61</td>
<td>3-6 ± 0-2</td>
<td></td>
</tr>
<tr>
<td>32-cell</td>
<td>3-2 ± 0-8</td>
<td>Ikenishi &amp; Nakazato (1986)</td>
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<td></td>
<td>4-1 ± 1-1</td>
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Fig. 9. Model illustrating the three phases in relocation and reorganization of germ plasm in Xenopus eggs after fertilization (A-C) aggregation, (D) ingression, (E) localization.
localized in preparation for the proliferative divisions of the presumptive primordial germ cells (Dziadek & Dixon, 1975, 1977). This degree of control no doubt accounts for the small variation (standard deviation of approximately one) in the size of the founder clone of presumptive primordial germ cells (Dixon, 1981) at the late blastula stage (see Table 2).

Earlier studies have suggested that one of the mechanisms involved in aggregation of germ plasm is cleavage (e.g. Whittington & Dixon, 1975). Our observations that in activated eggs and cleavage-arrested eggs the germ plasm behaves normally invalidates this suggestion. The sole effect of cleavage is to partition the patches of germ plasm between the first four blastomeres.

Microtubules are however required in two phases—aggregation and localization. Our results suggest that germ plasm may undergo a cycle of affinity for microtubules in which there is a positive attraction during aggregation followed by a loss of attraction during ingression and then affinity is regained when the patches are trapped by the spindle asters. Aggregation is the result of movement of individual patches and it seems likely that the microtubules are necessary for this movement. In other systems, a large body of evidence supports an active role for microtubules in transport of organelles e.g. in the mouse oocyte (Van Blerkom & Bell, 1986) and many other cell types (reviewed by Schliwa, 1984; Schroer & Kelly, 1985; Stebbings, 1986). In the Xenopus egg, our observations suggest the existence of a subcortical array of microtubules oriented around the vegetal pole. After fertilization, this network transports germ plasm islets and presumably associated organelles e.g. pigment granules (see below) towards the vegetal pole at a speed of approximately 200 μm h⁻¹ (assuming the most distant islets of germ plasm travel 20% of the circumference of an egg 1.2 mm in diameter). This is considerably slower than axonal transport (8 mm h⁻¹) and movement of cytoplasmic particles in the foraminifer Allogromia laticollaris (up to 46 mm h⁻¹; Allen et al. 1982), both of which depend on microtubules. One of the intriguing aspects of the action of this component of the cytoskeleton is that it brings all the patches to a localized part of the egg with some islets travelling a much greater distance than others but all aggregating together. If the microtubules of this region function in this way they must be organized more or less symmetrically around the vegetal pole. This system transports the aggregates of germ plasm to the polar region. There they are prevented from fusing to form one giant patch, presumably not only because normally the first and second cleavages partition the cytoplasm, but also probably because the system is not centred on a single MTOC but on a number of MTOCs. The concentration of pigment at the vegetal polar surface reported by Ikenishi & Nakazato (1986) and which they suggest marks the blastomeres containing germ plasm is probably also due to this system of microtubules.

In early Xenopus embryos, microtubules also play a role in axis formation (reviewed by Gerhart et al. 1986) and doubtless in many other processes. An essential step in axis formation is a rotation of the cortex relative to the subcortex during the first cleavage cycle. This rotation is mediated by microtubules. But these may be different from the microtubular array that functions in aggregation of germ plasm. The axis microtubules complete their function during the first cleavage cycle and they cause a unidirectional rotation of the subcortical cytoplasm away from the future dorsal side (Vincent et al. 1986). In contrast, the germ plasm system functions during the first and second cleavage cycles and causes convergence of vegetal subcortical cytoplasm.

The initial phase of aggregation of germ plasm is followed by its ingression which is due to a flow of cytoplasm from the surface internally. Streaming of cytoplasm is a well-known phenomenon (Allen et al. 1982), particularly in pseudopodial extension which relies on microfilaments. However, the cytoplasmic flow we have observed does not rely on any dynamic actin-based system since it persists in the presence of cytochalasin B. Neither does ingression depend on the presence of microtubules to supply motive force or even to direct the flow of cytoplasm. In this respect, cytoplasmic flow in Xenopus eggs resembles streaming in Reticulomyxa which is not affected by nocodazole, colchicine or cytochalasin D (Koonce et al. 1986). Ingression was first reported in amphibian eggs by Schechtman (1934) and later by Ballard (1955) but the nature of the ingression mechanism requires further investigation.

In eggs of some other species in which the localization of maternal information involves cytoplasmic rearrangement, microtubules have also been implicated, for example in Chaetopterus eggs (Eckberg, 1981) and in the barnacle Pollicipes (Lewis et al. 1973). In the eggs of some other species, microfilaments and not microtubules are responsible for ooplasmic segregation. For example, in Tubifex eggs, a bipolar segregation of mitochondria and plasma membrane takes place as a result of contraction of a cortical actin microfilament system (Shimizu, 1986). Similarly, in ascidian eggs, actin is involved in segregation of the myoplasmic crescent (Zalokar, 1974; Jeffery & Meier, 1983). In Caenorhabditis eggs, microfilaments participate more directly in segregation by causing the movement of germ-line P granules (Strome & Wood, 1983). The system we have described seems so far to be unique in its reliance, in part, on cytoplasmic flow.
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References


WYLIE, C. C., HEASMAN, J., PARKE, J. M., ANDERTON, B., &


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