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Cellularization in locust embryos occurs before blastoderm formation

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SUMMARY

In *Drosophila* intracellular gradients establish the pattern of segmentation by controlling gene expression during a critical syncytial stage, prior to cellularization. To investigate whether a similar mechanism may be exploited by other insects, we examined the timing of cellularization with respect to blastoderm formation in an insect with extreme short-germ development, the African desert locust, *Schistocerca gregaria*. Using light and electron microscopic techniques, we show that the islands of cytoplasm surrounding cleavage nuclei are largely isolated from their neighbours, allowing cleavage to proceed asynchronously. Within a short time of their arrival at the surface and prior to blastoderm formation, nuclei become surrounded by complete cell membranes that block the free uptake of dye (10,000 kDa) from the yolk. Our results imply that the formation of the blastoderm disc involves the aggregation of cells at the posterior pole of the egg and not the migration of nuclei within a syncytial cytoplasm. These findings suggest that the primary cleavage syncytium does not play the same role in patterning the locust embryo as it does in *Drosophila*. However, we do identify a syncytial nuclear layer that underlies the forming blastoderm and remains in continuity with the yolk.

Key words: locust, *Drosophila*, actin caps, cleavage energids, insect embryogenesis

INTRODUCTION

All pterygote insects go through a syncytial stage early in development (Anderson, 1972a,b). The role of this syncytial stage in establishing the insect body plan is best understood in *Drosophila* (reviewed by Pankratz and Jackle, 1993). Here, dividing nuclei of the early zygote share cytoplasm, allowing patterning molecules to diffuse between them. Gradients of transcription factors provide positional cues for these nuclei, which respond with target gene transcription that reflects their position along the A-P axis. During the syncytial blastoderm stage (mitotic cycles 9-13), this mechanism establishes the striped pattern of 'pair-rule' gene expression, (and hence the boundaries of all body segments) within the shell of about 6,000 nuclei that occupy the periphery of the egg. Cellularization occurs only after this, at cycle 14; the plasma membrane invaginates around each nucleus and closes off to form individual cells (reviewed by Foe et al., 1993). Thereafter, patterning proceeds via signal transduction mechanisms (reviewed by Martinez Arias, 1993).

It has been suggested (Davidson, 1991) that this syncytial stage of patterning is a highly derived mode of development, unique to those higher insects that generate most of their segments at the blastoderm stage (the so-called 'long-germ' insects: Sander, 1976). Many other insects generate only the more anterior segments before gastrulation. Commonly, segments of the head and thorax are specified in the blastoderm, but segments of the abdomen are not generated until after gastrulation, presumably in a cellular environment (the 'intermediate-germ' condition). In some extreme 'short-germ' insects, all of the trunk segments appear to be generated after gastrulation. Only head and trunk regions are distinct in the blastoderm.

These differences suggest that the molecular mechanisms of segmentation may differ in different insects. However, several beetles that show a range of apparent germ types all display patterns of segmentation gene expression similar to those in *Drosophila* (Tautz et al., 1994; Tautz and Sommer, 1995; Patel et al., 1994). The major differences are the timing of the appearance of these patterns in relation to gastrulation. In the short/intermediate-germ beetle *Tribolium*, the stripes of pair-rule gene expression appear sequentially, rather than almost simultaneously as they do in *Drosophila*, and only the first three (mouthparts and thorax) are formed in the blastoderm. The remaining stripes are generated after gastrulation. It is not yet known how these patterns are generated in the presumably cellular environment of the *Tribolium* abdomen.

Flies and beetles are both representatives of one evolutionary lineage that gave rise to most of the higher insect orders – the endopterygotes (Kristensen, 1991). Segmentation has been studied at the molecular level in only one insect that does not belong to this lineage – the extreme short-germ embryo of the grasshopper *Schistocerca*. In *Schistocerca*, the two pair rule segmentation genes that have been examined do not show the same patterned expression as in flies and beetles (Patel et al., 1992; Dawes et al., 1994), suggesting that these Orthopteran insects may not make segments in the same manner as *Drosophila*. Further studies of these, and other lower insects, are required to resolve what aspects of segment patterning are common to all insects and which are derived characteristics of

particular orders. A prerequisite for such studies is a knowledge of the cell biology of early development.

The cell biology of the *Drosophila* embryo has been described in detail, using a range of modern techniques (Foe and Alberts, 1983; Warn et al., 1984), but no recent study has examined early development in *Schistocerca* or any representative of the more basal insect orders. Older studies of numerous species described cleavage energids (i.e. nuclei with associated cytoplasm) moving through the yolk to reach the surface of the egg, where they aggregate at a characteristic position to form the blastoderm anlage of the germ band, generally near the posterior pole (Roonwal, 1936; Johannsen and Butt, 1941). These studies examined cleared whole mounts or sections of wax-embedded embryos, and could not therefore visualise cell membranes. It is generally assumed that the embryo is cellular by the onset of gastrulation, but the timing of cellularization with respect to blastoderm formation has not been determined. Thus any role of the syncytium in patterning the embryos of lower insects remains unclear.

Here we investigate the timing of cellularization in *Schistocerca*. We use rhodamine-conjugated phalloidin to stain cortical actin (Karr and Alberts, 1986; Miller et al., 1989). We use tubulin staining to visualise the distribution of energid cytoplasm, and electron microscopy to determine the presence or absence of membranes around energids during the cleavage stages of *Schistocerca* embryogenesis. Our results suggest that cellularization occurs during early cleavage. We confirm this by studying the uptake of dyes from the yolk into cleavage energids.

MATERIALS AND METHODS

Locusts

Schistocerca gregaria (Forskål) egg pods were obtained from a laboratory colony maintained by G. Ballard at the Department of Zoology, Cambridge University. The eggs pods, containing 20-60 eggs, are laid in moist sand and are incubated at 22°C or 29°C. Staged collections of eggs were made by changing egg pots every 4 to 6 hours, except those used for Fig. 1A-C, which were timed from the precise moment of egg laying.

For experiments, eggs were separated using blunt watchmaker's forceps under a dissection scope in Locust Embryo Saline (LES: 8.76 g/L NaCl, 0.22 g/L KCl, 0.29 g/L $CaCl_2 \cdot 2H_2O$, 0.25 g/L $MgSO_4 \cdot 7H_2O$, 1.15 g/L TES (Sigma, catalogue number T 6541)). They were kept on LES-moistened filter paper (Whatman, 3MM) in Petri dishes in a humid incubator. Eggs were incubated at 22°C, 29°C or 33°C but reference to the age of eggs in the text and figure legends refers to eggs kept at 29°C unless otherwise stated. 18 hours at 29°C correspond to 33 hours at 22°C, or 12 hours at 33°C.

Fixation and peeling of whole eggs

Eggs were normally fixed by pricking them 15-20 times with a 5-20 μm diameter glass needle in 4% formaldehyde in PBS. For electron microscopy, eggs were fixed in an aldehyde-acrolein fixative (0.2 M Pipes pH 6.8, 0.5% paraformaldehyde, 2.0% glutaraldehyde, 5.0% acrolein monoaldehyde: recommended by Dr Jeremy Skepper, Department of Anatomy, Cambridge) and, after pricking, the posterior 2 mm of each egg was severed in fixative to allow for maximum permeation of tissue. Eggs were fixed for at least 24 hours at 4°C with gentle shaking. (The acrolein-aldehyde fixative gave much better preservation of cellular fine structure than aldehyde-cacodylate fixative (4% paraformaldehyde, 0.5% glutaraldehyde, 0.1 M sodium cacodylate buffer, pH 7.4) as evidenced by the presence of mitochondrial and nuclear membranes, Golgi apparatus, ribosomes and other cytoplasmic components. To our knowledge, this is the first time this fixative has been used to prepare yolky eggs for electron microscopy).

To remove the chorion after fixing, an incision was made with a No.10 surgical blade (Swann-Morton) along 3/4 of the length of the egg. Sharpened watchmaker's forceps were then used to peel back small slivers of chorion from the yolk.

Staining of whole eggs

Mouse anti-tubulin antibodies (a gift from J. Raff) were used at a dilution of 1:500 in PBTx (PBS with 0.5% Triton X-100 and 0.1% bovine serum albumin). FITC-conjugated goat anti-mouse secondary antibodies (Sigma) were used at a concentration of 1:20 in PBTx. The protocol for antibody staining of whole eggs is described by Dawes et al. (1994, 1996).

To stain chromatin, fixed, peeled eggs were kept in a 2 ng/ml solution (final concentration) of Hoechst 33258 (Molecular Probes) in PT (PBS with 0.1% Tween-20) for 2 hours at 4°C. Eggs were then washed briefly in PBS and mounted in agarose wells on slides for visualisation. A similar procedure was used to stain both DNA and RNA with propidium iodide. In this case, eggs were incubated in 2 μg/ml solution of propidium iodide (Sigma P4170) in PT for 20 minutes.

To stain actin, fixed, peeled eggs were incubated overnight in rhodamine phalloidin (Molecular Probes, 40 μg/ml in PT) at 4°C with gentle shaking, washed twice in PT (30 minutes each wash) and once in PBS, mounted in agarose wells on a glass slide and visualised using epifluorescence (Rhodamine filter, 450-490; FT510; 515-565).

Transmission electron microscopy

Fixed egg specimens were stored in Pipes buffer (0.2 M Pipes, 2 mM $CaCl_2$) for up to 3 days. Then eggs were postfixed in 2% OsO_4, stained en bloc with 2% aqueous uranyl acetate, dehydrated in an ethanol series and embedded in Spurr's resin.

Semithin sections were stained with 2% methylene blue in 1% boric acid and examined using standard light microscopy. Ultrathin sections were stained with uranyl acetates and lead citrate and examined with a Phillips 300 electron microscope.

Injection of dextran dyes into whole eggs

Pods of eggs were washed thoroughly with LES, submerged in 10% chlorox (10 seconds), rinsed in LES, washed in 80% ethanol (20 seconds) and rinsed with copious amounts of LES. Washed eggs were incubated at 22°C in Petri dishes with LES-moistened filter paper until they had reached the desired stage to be injected. Eggs were lined up in 2% agarose moulds (similar shape to a gel comb) with their posterior ends all pointing in the same direction. They were injected using a PLI-100 pico-injector (Digitimer) with 10-20 nl of 5 mg/ml of 10,000 kDa dextran tetramethylrhodamine (lysine fixable, Molecular Probes D-1817) in PBS. The eggs were left to recover for 7 hours at 22°C and then fixed overnight. Their chorions were then peeled and the whole eggs were stained with Hoechst 33258 and viewed under a fluorescence microscope (Filters used: Hoechst 33258, G365 FT 395-LP420 and rhodamine, 450-490 FT510 515-565).

RESULTS

The *Schistocerca* egg is approximately 0.75 mm in diameter and 7-8 mm long. The sperm entry point is at the micropyle, marked by a ridge of sculptured chorion that runs around the posterior tip of the egg, about 1 mm from the posterior pole. After fertilisation, male and female pronuclei fuse within the yolk near the posterior pole of the egg (described for the

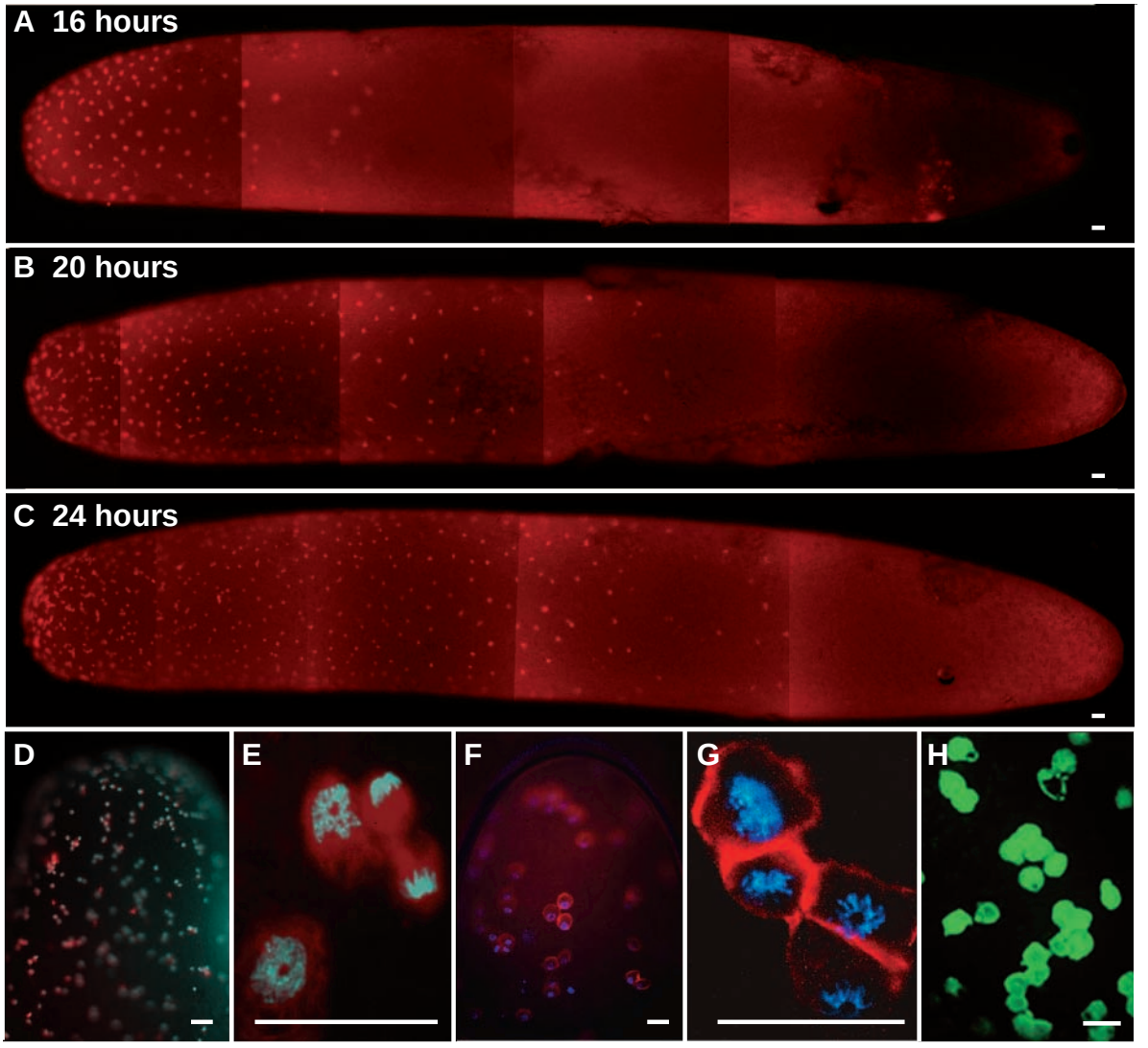

Fig. 1. Early development of *Schistocerca gregaria*. (A-C) Propidium iodide staining of whole eggs at 16, 20 and 24 hours (at 29°C) after egg laying, showing the initial emergence of superficial energids near the posterior pole of the egg and their subsequent appearance more anteriorly. (D, E) Energids from 20 hours embryos stained with propidium iodide and Hoechst 33258, showing nuclei (light blue) within islands of cytoplasm. (F,G) Energids stained with Hoechst 33258 (blue) and phalloidin (red) show that actin caps surround each superficial energid at the cleavage stage (16 hAEL at 33°C which is equivalent to 24 hAEL at 29°C). Note in E and G that mitoses are not synchronous. (H) Anti-tubulin staining during blastoderm formation (20 hAEL at 33°C which is equivalent to 35 hAEL at 29°C) shows that the cytoplasm of cells is still discrete at this stage. Bar represents 100 μm in A-C, 60 μm in D-H.

closely related Locusta by Roonwal, 1936**).** Mitoses take place within the yolk until, at about 14 hours after egg laying (hAEL), the first energids appear on the surface of the yolk near the posterior pole. Over the next 24 hours, energids populate the more anterior part of the egg (Fig. 1A-C; E shows a higher magnification of the energids). It is not clear whether superficial energids migrate anteriorly along the egg periphery or whether energids emerge from the interior of the yolk throughout the length of the egg. The first energids reach the anterior pole of the egg by 42 hours (Dawes, 1996).

From 30 hours onwards, nuclei begin to cluster at the posterior pole (Dawes et al., 1994) probably as a result of both aggregation and a locally increased rate of division. Around 35 hAEL, these nuclei have formed the blastoderm disc, a region about 300 μm in diameter which will give rise to the embryo proper. At this stage, the anteroposterior axis of the embryo is already defined by the expression of the homeobox protein Dax in a crescent at the future posterior side of the embryo, which generally lies on the ventral (concave side) of the egg (Dawes et al., 1994). Thereafter, the forming embryo extends along the ventral side of the egg. At approximately 48 hAEL it undergoes gastrulation. A furrow forms along the midline of the blastoderm disc, demarcating the future ventral midline of the embryo. The superficial cells that lie outside of the embryonic primordium cease to divide, become polyploid, and give rise to an extraembryonic membrane, the serosa (Falciani et al., 1996).

The structure of cleavage energids

We used phalloidin to visualise the actin cytoskeleton of

cleavage energids. Each energid is surrounded by an actin cap (Fig. 1F,G), similar to those seen in *Drosophila* (Warn et al., 1984; Miller et al., 1989). However, in other respects cleavage in this embryo is very different from the syncytial blastoderm of *Drosophila*. The energids are not part of a continuous layer of superficial cytoplasm, but are separated from one another by densely packed yolk granules that extend to the surface of the egg. Using depolymerised tubulin as a marker for cytoplasm, each energid or small cluster of energids appears as an island isolated from its neighbours (Fig. 1H). Neighbouring nuclei are not in mitotic synchrony (Fig. 1E,G), confirming that the functional interactions between them at this early stage of development are much looser than between the syncytial nuclei of *Drosophila*, which undergo co-ordinated waves of mitosis (Foe and Alberts, 1983). Similar observations have been made for a number of other Orthoptera (e.g. Roonwal, 1936; see Anderson 1972a).

Cellularization takes place during the cleavage stage

We examined embryos by transmission electron microscopy to determine whether plasma membranes surround the cytoplasm of cleavage energids. Batches of eggs at 14, 16, 20, 24 and 28 hAEL were fixed, embedded and sectioned near the posterior pole, using 6-18 eggs for each time point (Fig. 2). At 14 hAEL and 16 hAEL, there were no membranes surrounding the cytoplasm of energids in any of the eggs examined (n=12), even though the intracellular membranes of vesicles and organelles were well-preserved in the same sections (Fig. 2B). At 20 hAEL, membranes appeared around some, but not all, energids (Fig. 2D). For example, in the section from which Fig. 2D is taken four out of six energids had bounding membranes. By 24 hAEL, all energids examined were surrounded by lipid bilayers (Fig. 2F). These observations suggest that cellularization occurs during the early cleavage stage of development, well before energids have aggregated to give rise to the blastoderm disc that will form the embryo. Cellularization probably occurs asynchronously, shortly after each energid reaches the surface.

Shortly before cellularization, there is often a high concentration of vesicles in the cytoplasm of superficial energids (Fig. 3A). These vesicles are much less abundant in the cytoplasm of energids at 20, 24 and 28 hAEL. In *Drosophila*, similar vesicles line up and fuse with one another below the invaginating plasmalemma that separates each blastoderm nucleus during cleavage cycle 14 (Loncar and Singer, 1995). Loncar and Singer suggest that these vesicles provide lipid stores for the production of new cell surface during the later stages of cellularization. Aligned vesicles can be seen in *Schistocerca*, suggesting that a similar mechanism contributes to the formation of cell membranes (Fig. 3B).

Cleavage cells become isolated from the yolk

To confirm that closed cells do form during early cleavage, we injected 10,000 kDa rhodamine dextran dye into the yolk of cleaving eggs, and checked whether energids close to the site of injection became labelled within the following 7 hours. When eggs were injected at 14 or 18 hour stages (actually, 25 and 33 hours old at 22°C), the dye was incorporated into all the energids near the site of injection (Fig. 4A-E, Table 1). Eggs injected at the 26 hour stage (50 hours at 22°C) showed a very different pattern. None of the superficial energids accumulated dye, but a sparse population of slightly deeper cells still labelled (Fig. 4F-J). The dye in these remaining syncytial cells reveals a characteristic morphology, with a thin network of cytoplasmic strands spreading between adjacent nuclei.

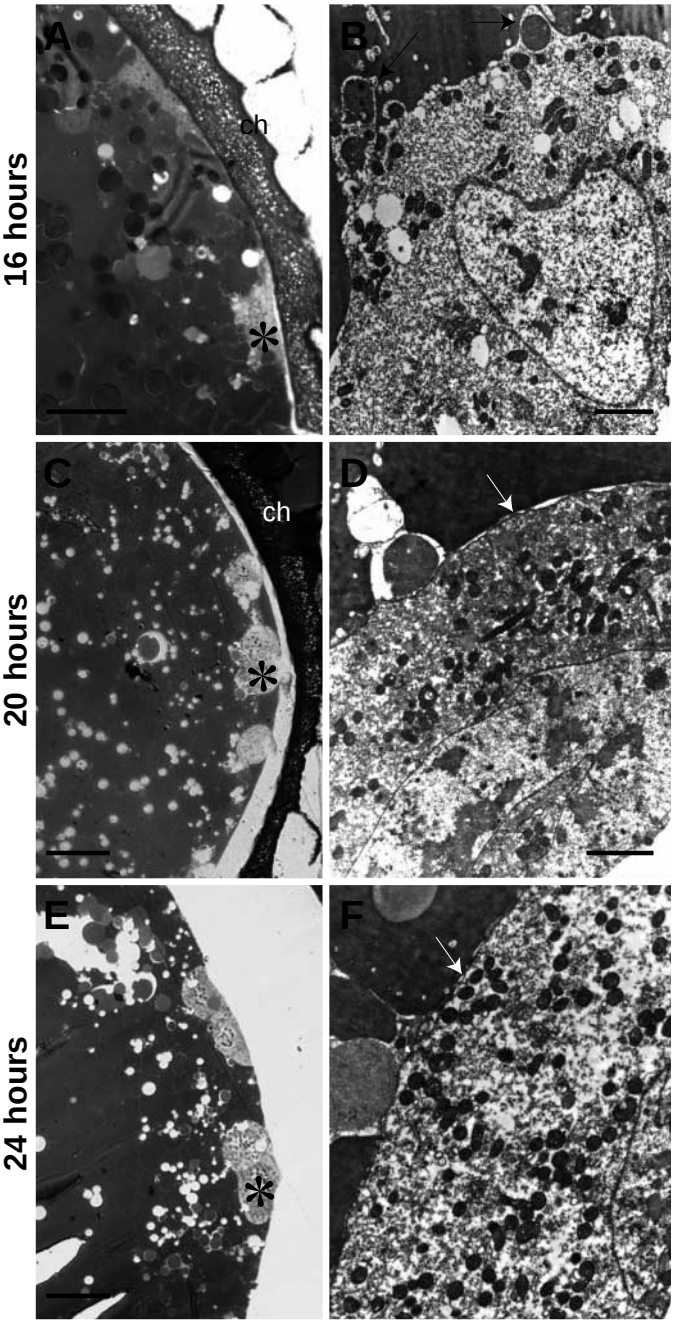

Fig. 2. Cellularization takes place around 20 hAEL. Semithin sections of superficial energids/cells near the posterior pole of *Schistocerca* eggs. The chorion can be seen in cross section to the right of plates A and C (asterisk marks energids/cells). (B,D,F) Electron microscopic images of single energids/cells from this region, showing the surface of the cytoplasm facing the yolk. Cell membranes are absent in B, present in D and F (white arrows). (A,B) 16 hAEL, (D,E) 20 hAEL, (F,G) 24 hAEL. Energid cytoplasm appears to ooze and bleb into yolk (B, black arrows). Nuclear envelopes, annuli, mitochondria and vesicles can be seen in the cytoplasm of energids and cells in B, D and F. (ch, chorion at posterior of egg). Bar (A,C,E), 50 μm; bar (B,D,F), 2 μm.

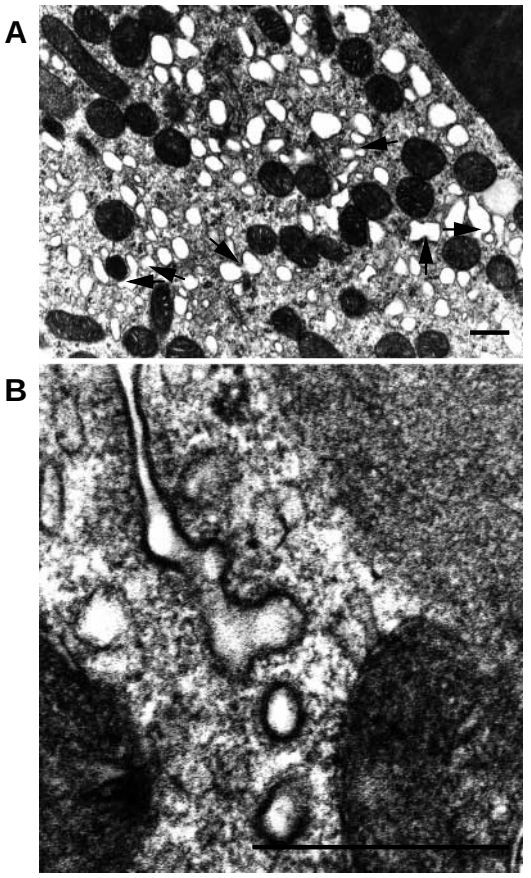

Fig. 3. Vesicles with lipid stores may contribute to the production of the cell membrane. (A) Membrane-bound vesicles (black arrows) are abundant in the cytoplasm of many energids prior to cellularization (18 hAEL). (B) Vesicles appear to be budding from, or fusing with, the cell membrane. Bar represents 400 nm.

Eggs injected at the 22 hour stage (40 hours at 22°C) gave variable results (Table 1). In two out of four experiments, these behaved like the older eggs but in the other two, dye was incorporated into most or all energids. In these cases, different energids incorporated dye to differing extents. We interpret these as eggs in which cellularization was incomplete at the time of injection and occurred progressively during the next few hours. The difference between pods probably reflects the range of actual ages (18-22 hours) of our nominal '22 hours' collections.

These injection experiments show that relatively small molecules (10,000 kDa) are unable to penetrate superficial energids in eggs after the 22 hour stage. This result confirms the evidence from EM sectioning, that cellularization takes place around 20-22 hAEL, prior to blastoderm formation.

DISCUSSION

Just before gastrulation, at about 40 hours, the *Schistocerca* embryo is a heart-shaped disc of close-packed cells at the posterior pole of the egg. Though it is comparable in size to a *Drosophila* embryo, it occupies only about 5% of the surface area of the egg, the remainder of the egg being covered by nuclei that will form the serosa, an extraembryonic membrane.

We anticipated, by analogy with *Drosophila*, that the blastoderm would remain syncytial until shortly before this stage, with connections to the underlying yolk, much as in *Drosophila*. Our results show that this is not the case. Cleavage energids become cells at a much earlier stage, before they have aggregated at the posterior pole. Shortly after individual nuclei reach the surface of the egg, they become surrounded by membranes that isolate them completely from the yolk syncytium. Formation of the blastoderm disc involves the aggregation of cells, and not the migration of nuclei within a syncytial cytoplasm (Fig. 5).

In some respects, the dynamics of cellularization in *Schistocerca* appear similar to those described by Takesue et al (1980) in the silkmoth, *Bombyx mori*. They reported that, in this Lepidopteran embryo, the nuclei reach the surface asynchronously and cell membranes form around each nucleus shortly thereafter. There is no stage at which the whole embryonic primordium is syncytial. However, in *Bombyx*, the nuclei emerge from the yolk into a continuous layer of cortical cytoplasm, so they are at least transiently in a common cytoplasm with their neighbours. In contrast, the first cells to form in *Schistocerca* are isolated.

Our dye injection experiments identify a distinct population of nuclei that remain below the blastoderm disc, in contact with the yolk (Fig. 6). These remain syncytial, populating a tenuous cytoplasmic layer. This layer would be analogous to the syncytial hypoblast of many fish eggs (Kimmel and Law,

Table 1. Cells are formed at 18 hAEL

		Dye incorporation into			
	Total number of eggs injected	All energids	Variable uptake in all energids	Underlying energids only	No diffusion of dye
14 hours (25 hours 22°C)	122	112 (91.8%)	0	0	10 (8.2%)
18 hours (33 hours 22°C)	85	54 (63.5%)	0	2 (2.4%)	29 (34.1%)
22 hours (44 hours 22°C)	41	4 (9.8%)	14 (34.1%)	14 (34.1%)	9 (22%)
26 hours (50 hours 22°C)	75	0	0	40 (53.3%)	35 (46.6%)

The first column shows the total number of eggs injected. The next three columns show the number of eggs in which dye incorporation into energids was observed. In some eggs at each time point the dye failed to diffuse properly. In these (column 5) the dye remained as a compact blotch at the site of injection.

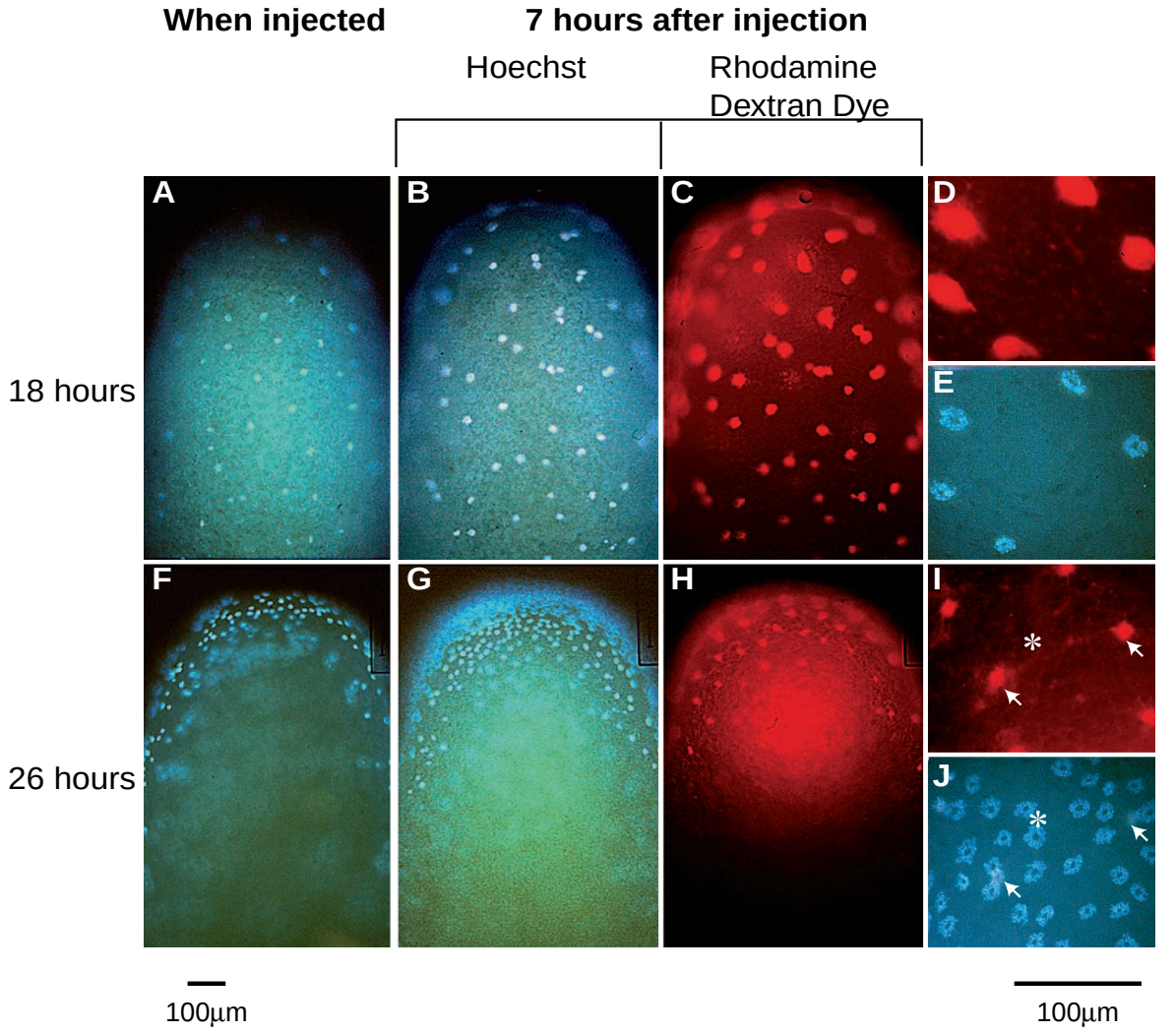

Fig. 4. Timing of cellularization as monitored by dye injection. (A,F) Control eggs stained with Hoechst 33258 showing the stage at which the sibs were injected (18 and 26 hour stages at 29°C). (B-E) In eggs injected with rhodamine dextran dye before 18 hour stage all energids near the site of injection fill with the dye. (G-J) In eggs injected at the 26 hour stage, most superficial energids are unable to take up the dextran dye, showing that cellularization has taken place (white star). A small population of energids that lie below the superficial cells can still take up dye (white arrows, marking nuclei out of focus in J).

1985). It is not clear whether the nuclei of this layer are identical to those of the previously described 'vitellophages', energids that remain within the yolk and later organise the formation of large 'yolk cells' (Johannsen and Butt, 1941).

Cells forming a distinct 'yolk cell membrane' have previously been described below the blastodisc in other Orthoptera (e.g. Locusta, Roonwal, 1936). These cells occupy a similar position to the syncytial cells that we identify in *Schistocerca*, but Roonwal suggested that they arose by invagination from the blastodisc, deriving from a transient ventral groove that appears shortly before the definitive gastrulation groove. This would be too late to give rise to the cells that we see. At present, we do not know the relationship between these two cell populations, if indeed both exist in *Schistocerca*.

Implications for pattern formation in the Orthopteran embryo

At gastrulation the future posterior pole of the embryo elongates along the ventral surface of the egg (Dawes, 1996). In many other Orthoptera, the blastodisc itself forms asymmetrically with respect to the D/V axis of the egg. Thus both embryonic axes develop in fixed relation to the axes of the egg. Therefore, the cues that define the position and axes of the embryonic primordium must be generated during oogenesis and transmitted to the forming embryo well before gastrulation. In *Drosophila*, these cues are transmitted by two routes. Maternal RNAs and proteins localised within the oocyte make the anterior and posterior poles of the egg different from one another; signals generated by the follicle cells activate surface receptors on the egg to distinguish dorsal from ventral, and termini from middle along the A/P axis (St. Johnston and Nusslein-Volhard, 1992).

The number and nature of maternal cues in *Schistocerca* are not known. Their first obvious effect is to define where in the egg the embryonic primordium will form – by guiding the aggregation of the blastoderm cells and/or by controlling their

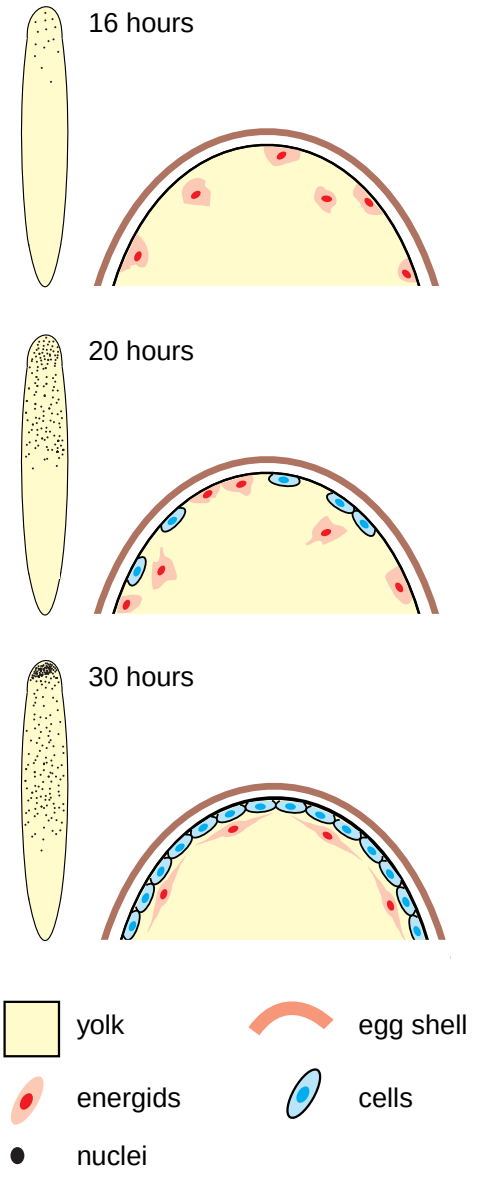

Fig. 5. Diagram summarising the early development of *Schistocerca gregaria*. At 16 hours, energids but no cells are found in the posterior pole of the egg. 4 hours later both energids and cells are present at the posterior pole. By 30 hours of development, when the embryonic primordium begins to form, two distinct populations of nuclei can be identified at the posterior tip of the egg – superficial cells and underlying syncytial energids.

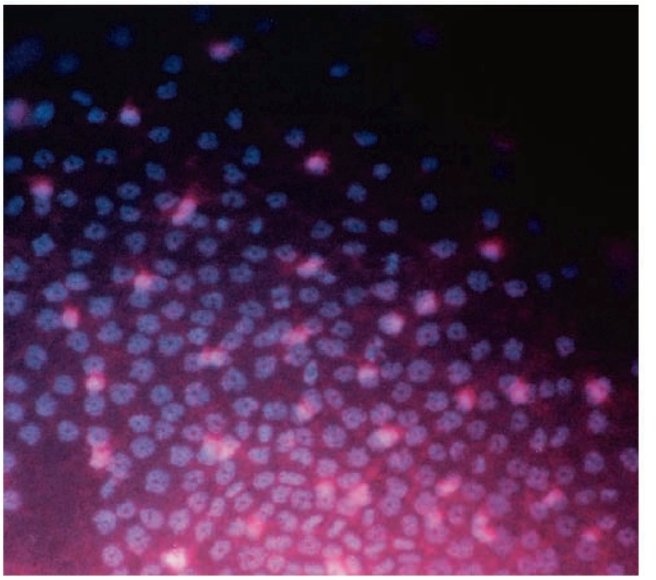

Fig. 6. In eggs injected with dextran dye at the 26 hour stage, two populations of nuclei can be seen at the posterior pole of the egg, superficial cells and an underlying population of syncytial energids.

localised proliferation. The first visible differentiation is between cells of the embryonic primordium (which remain diploid and continue dividing) and cells of the extraembryonic region, which rapidly become polyploid, stop dividing and express molecular markers for extraembryonic membrane (Falciani et al., 1996). The same or other maternal signals must also define which point on the blastoderm disc will form the head, and which the terminal structures of the abdomen. The remaining segments of the body are generated by intercalation from a subterminal patterning region (Patel et al., 1989; Kispert and Reuter, 1994).

We can consider three sources for these maternal signals: (1) cytoplasmic determinants that become localised to individual cells during cleavage (analogous to the pole plasm of *Drosophila*), (2) localised signals embedded in the vitelline membrane by the follicle cells during oogenesis that may provide signals to the egg cell after fertilisation (analogous to the D/V and terminal systems of *Drosophila* (Sprenger and Nusslein-Volhard, 1993) and (3) inductive signals from the underlying yolk syncytium (Sander, 1976).

Experiments in other Orthoptera appear to rule out a requirement for localised determinants in the cells of the embryonic primordium itself, for this primordium can regenerate from cells that would normally form extraembryonic membrane. A cricket embryo that has begun to gastrulate can be destroyed by localised X-ray irradiation. Cells of the blastodisc die, but unirradiated nuclei migrate in from the surrounding region of the egg. These nuclei resume division, populating the original site of the embryonic primordium and can form a normal embryo (Schwalm, 1965; Sander, 1976).

This suggests that cells of the future embryo acquire only limited positional information before or during cellularization, and that the pattern of the embryo is induced by signalling from the underlying yolk and/or the overlying eggshell. Our results show that a syncytial cell layer covers the yolk in *Schistocerca*, at the time when cells of the embryonic primordium aggregate at the posterior pole. This could provide a substrate for patterning interactions analogous to the syncytial blastoderm of *Drosophila*. However, specialised follicle cell populations clearly also exist, as is evident from the sculpturing of the eggshell at the posterior pole of the egg. At least four distinct types of chorion can be distinguished, arrayed in concentric circles around the posterior pole (Dawes, 1996). The role of patterning information from these two sources remains to be determined.

By the time of gastrulation, the embryo displays clear anterior/posterior and dorsal/ventral polarity, with a ventral midline in the middle of the heart-shaped embryonic disc.

Expression of the segmentation gene homologues *ftz* and *eve* reveals a subdivision into two domains ('head' and 'trunk') but no trace of segment formation. Patterning mechanisms acting on the few nuclei present at the time of cellularization could generate these broad subdivisions, but there are simply too few nuclei present at the time of cellularization for the details of the segment pattern to be specified. The first molecular signs of segmentation appear at 72 hours, with the onset of engrailed expression in the thoracic region (Patel et al., 1989). Elaboration of this pattern presumably requires interactions between cells of the gastrulating embryo.

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