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A gastrulation center in the ascidian egg

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Summary

A gastrulation center is described in ascidian eggs. Extensive cytoplasmic rearrangements occur in ascidian eggs between fertilization and first cleavage. During ooplasmic segregation, a specific cytoskeletal domain (the myoplasm) is translocated first to the vegetal pole (VP) and then to the posterior region of the zygote. A few hours later, gastrulation is initiated by invagination of endoderm cells in the VP region of the 110-cell embryo. After the completion of gastrulation, the embryonic axis is formed, which includes induction of the nervous system, morphogenesis of the larval tail and differentiation of tail muscle cells. Microsurgical deletion or ultraviolet (UV) irradiation of the VP region during the first phase of myoplasmic segregation prevents gastrulation, nervous system induction and tail formation, without affecting muscle cell differentiation. Similar manipulations of unfertilized eggs or uncleaved zygotes after the second phase of segregation have no effect on development, suggesting that a gastrulation center is established by transient localization of myoplasm in the VP region. The function of the gastrulation center was investigated by comparing protein synthesis in normal and UV-irradiated embryos. About 5% of 433 labelled polypeptides detected in 2D gels were affected by UV irradiation. The most prominent protein is a 30 kDa cytoskeletal component (p30), whose synthesis is abolished by UV irradiation. p30 synthesis peaks during gastrulation, is affected by the same UV dose and has the same UV-sensitivity period as gastrulation. However, p30 is not a UV-sensitive target because it is absent during ooplasmic segregation, the UV-sensitivity period. Moreover, the UV target has the absorption maximum of a nucleic acid rather than a protein. Cell-free translation studies indicate that p30 is encoded by a maternal mRNA. UV irradiation inhibits the ability of this transcript to direct p30 synthesis, indicating that p30 mRNA is a UV-sensitive target. The gastrulation center may function by sequestration or activation of maternal mRNAs encoding proteins that function during embryogenesis.

Key words: ascidian egg, gastrulation center, protein synthesis, UV irradiation.

Introduction

Classic studies on gastrulation and embryonic axis formation have been carried out in amphibian, teleost fish and bird embryos, which begin gastrulation after the egg has cleaved into thousands of cells (see Trinkaus, 1984 for review). *Drosophila* (Leptin and Grunewald, 1990; Kam et al., 1991) and sea urchins (Hardin, 1987; Ettensohn, 1985), the major invertebrate systems used to study these processes, also begin gastrulation at stages containing a relatively large number of cells. In contrast, gastrulation starts after the fourth or fifth cleavage in some polychaete annelid (Anderson, 1973) and tunicate (Berrill, 1955) embryos. These simple systems provide an opportunity to study gastrulation and axis formation under conditions of low cellular complexity. In addition, the short interval between fertilization and the beginning of gastrulation is favorable for examining the relationship between morphogenesis and earlier developmental events, such as fertilization, ooplasmic segregation and precocious cell determination.

Ascidians begin gastrulation between the sixth and seventh cleavages (110-cell stage) and then undergo a series of morphogenetic movements resulting in the formation of a tadpole larva. The ascidian tadpole larva contains only a few thousand cells and six different cell or tissue types: muscle, mesenchyme, notochord, neural, endoderm and epidermis. Despite this simplicity, ascidian larvae exhibit typical chordate features, including a dorsal nervous system and a tail containing a notochord and flanking bands of striated muscle cells. Because of their determinate cleavage pattern, invariant cell lineage and the existence of localized egg cytoplasmic regions, ascidians are a model system for studying autonomous cell determination (see Venuti and Jeffery, 1989 for review). The cytoplasmic regions of ascidian eggs are thought to contain determinants that cause embryonic cells to adopt a specific fate. For example, muscle determinants may reside in the myoplasm, a cytoplasmic region distributed to the tail muscle cell progenitors during cleavage (Whittaker, 1982). It is also apparent, however, that cell fates can be established by induction in ascidian embryos. The larval brain and its sensory organs are examples of tissues specified by inductive cell interactions (Rose, 1939; Reverberi et al., 1960; Nishida and Satoh, 1989; Nishida, 1991).

In this article, the relationship between ooplasmic segregation, gastrulation and axis formation is examined in ascidian embryos. Evidence is presented that gastrulation is preceded by the establishment of a gastrulation center in fertilized eggs. The gastrulation center may function by sequestering or activating maternal mRNAs encoding proteins that function during morphogenesis.

Experimental section

Ooplasmic segregation

Ascidian eggs are radially symmetric, however, bilateral symmetry is evident shortly after fertilization, coincident with the completion of ooplasmic segregation (Conklin, 1905; Jeffery, 1984a; Sardet et al., 1989). As first described by Conklin (1905), three cytoplasmic regions are distributed in a distinct spatial pattern along the animal-vegetal axis of unfertilized ascidian eggs. In a few ascidian genera, including *Styela* and *Boltenia* (Conklin, 1905; Jeffery, 1984a), these regions contain pigment granules and can be distinguished by color. The animal hemisphere contains the ectoplasm, a clear cytoplasmic region derived from the germinal vesicle during oocyte maturation. The vegetal hemisphere contains the endoplasm, which is filled with yolk granules. Except for a small area around the animal pole (AP), the egg cortex contains the myoplasm (Figs. 1 and 2, Row A), a yellow cytoplasmic region that enters the tail muscle cells during embryogenesis. The description of ooplasmic segregation below will focus on the myoplasm because this region is involved in the establishing the gastrulation center.

The myoplasm is a domain consisting of a peripheral submembrane cytoskeleton and a deeper cortical cytoskeleton (Fig. 2, Row B). The submembrane cytoskeleton stains with actin antibodies and phalloidin, interacts with myosin and can be disrupted by treating extracted eggs with DNase I, suggesting that its integrity is dependent on F-actin (Jeffery and Meier, 1983; Sawada and Osanai, 1985). The cortical cytoskeleton is a three-dimensional network of filaments containing pigment granules, mitochondria, endoplasmic reticulum and maternal mRNA (Jeffery and Meier, 1983; Jeffery, 1984b; Gualtieri and Sardet, 1989), which is attached to the submembrane cytoskeleton and the underlying ectoplasm. NN18, a monoclonal antibody that reacts with vertebrate neurofilaments, stains the cortical cytoskeleton, suggesting that it has structural affinities to intermediate filaments (Swalla et al., 1991). Although both parts of the myoplasmic cytoskeletal domain segregate together after fertilization, the motive force for the first movement is supplied by actin filaments (also see below) in the submembrane cytoskeleton. This was demonstrated by showing that the submembrane domain could segregate without the cortical cytoskeleton after the latter is displaced by low speed centrifugation (Jeffery and Meier, 1984).

The myoplasm segregates in two phases after fertilization (Fig. 2, Row A). During the first phase, a transient cap of yellow cytoplasm is formed in a lobe of cytoplasm near the vegetal pole (VP) of the zygote (Fig. 1). Concomitant with the first phase of segregation, the male pronucleus is

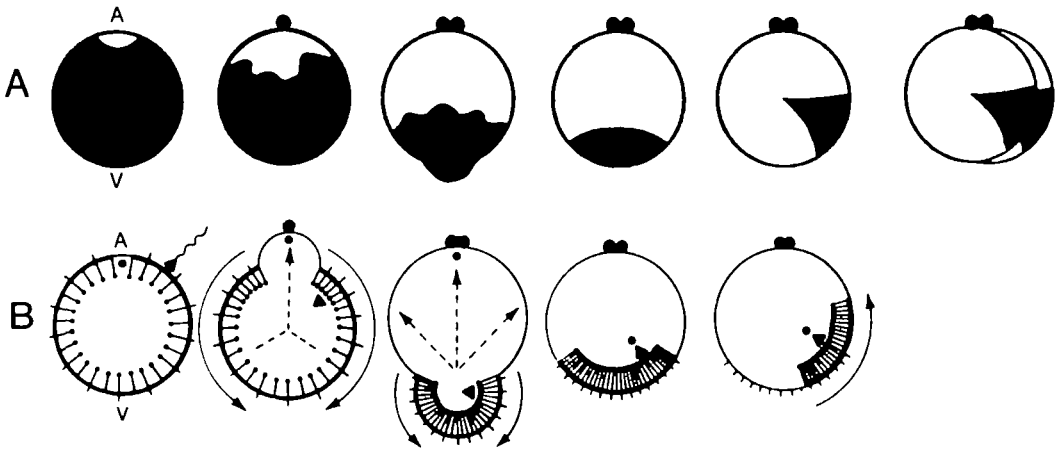

Fig. 2. Diagrams showing changes in the distribution of the myoplasm (Row A) and the myoplasmic cytoskeletal domain (Row B) during ooplasmic segregation in ascidian eggs. Row A. The myoplasm (dark areas) segregates from the egg cortex to the vegetal pole (V) during the first phase of ooplasmic segregation and then is translocated to the posterior of the zygote where it spreads out as the yellow crescent. The yellow crescent is bisected by the first cleavage plane. Row B. The translocation of the myoplasmic cytoskeletal domain in eggs sectioned through the animal-vegetal axis at stages corresponding to A. The filled spheres on the outside of the eggs represent the polar bodies. Small A: animal pole. The thick boundaries represent egg plasma membrane underlain by the sub-membrane cytoskeleton, and the thin boundaries egg plasma membrane without this cytoskeleton. The structures drawn as thin filaments on the outer surface of the plasma membrane represent egg surface components translocated with the underlying myoplasm during the first but not the second phase of segregation. The structures drawn as thin filaments topped with filled spheres on the internal surface of the plasma membrane represent the cortical cytoskeleton and associated organelles that participate in both phases of segregation. The filled spheres inside the egg represent the female pronucleus. The filled triangles represent the sperm head (outside the egg) or the male pronucleus (inside the egg). The arrows with continuous tails outside the egg represent the direction of myoplasmic movement during ooplasmic segregation. The arrows with broken tails on the inside of the egg represent the direction moved by other cytoplasmic components. From Jeffery and Swalla (1990).

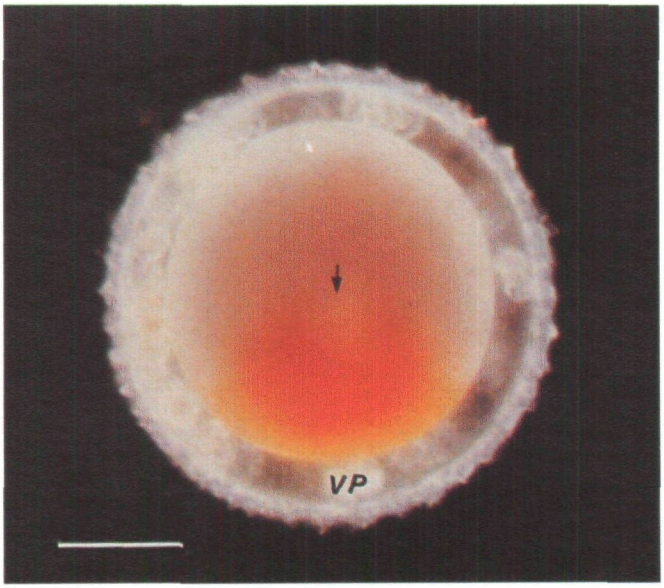

Fig. 1. An *S. clava* egg at completion of the first phase of ooplasmic segregation showing a cap of yellow myoplasm localized at the vegetal pole (VP) and an adjacent clear spot (arrow) representing the site of sperm aster formation. The egg is viewed from a lateral side of the vegetal hemisphere. Scale bar: 50 μm.

translocated into the VP region and a sperm aster appears adjacent to the cap of myoplasm (Fig. 1). The first phase can be blocked by cytochalasin, indicating that it is mediated by actin filaments (Zalokar, 1974; Sawada and Osanai, 1981). During the first phase of segregation, microtubules are rare or disorganized in the myoplasm. After the myoplasm is focussed at the VP, however, it is penetrated by microtubules growing from the sperm aster (Sawada and Schatten, 1988). During the second phase of segregation, most of the myoplasm moves into the sub-equatorial zone where it is extended into a yellow crescent (Fig. 2, Row A). The second phase is blocked by colchicine and likely to be mediated by aster microtubules (Zalokar, 1974; Sawada and Schatten, 1989). As the myoplasm moves into the equatorial zone, it shifts with respect to the cell surface (Fig. 2, Row B). This shift was demonstrated by applying chalk particles to the surface of denuded *Styela plicata* eggs before and during ooplasmic segregation (Bates and Jeffery, 1987). When chalk particles were applied to regions of the cell surface underlain by myoplasm before the first phase of segregation, they were transported into the vegetal hemishpere. In contrast, when chalk particles were applied to the cell surface over the vegetal cap of myoplasm after the first phase was completed, they did not move toward the equator during the second phase. These results suggest that cell surface components undergo the first phase of segregation in concert with the myoplasm, but afterwards remain stationary near the VP.

The axis of bilateral symmetry is visible at the end of the second phase of ooplasmic segregation. The dorsoventral axis approximates the animal-vegetal axis, with the VP region representing the future dorsal side of the embryo. The anteroposterior axis lies perpendicular to the dorsoventral axis; the yellow crescent marks the posterior of the embryo and the site of larval tail formation. The first cleavage furrow bisects the yellow crescent, dividing the embryo into left and right halves through the plane of bilateral symmetry (Fig. 2, Row A). During cleavage, the myoplasm is partitioned to specific blastomeres that generate most of the larval tail muscle cells.

Gastrulation and embryonic axis formation

The morphogenetic events resulting in formation of the tadpole larva include gastrulation, elongation of the anteroposterior axis, neurulation and tail development. Gastrulation involves three steps: invagination of the endoderm, involution of the mesoderm and epiboly of the ectoderm (Conklin, 1905; Mancuso, 1973; Satoh, 1978). The blastula is slightly flattened along the animal-vegetal axis. At the beginning of gastrulation, the vegetal cells contract at their basal margins and expand at their apical margins, which converts them from a columnar to tear-drop shape. The most extreme shape changes occur in the four largest endoderm cells (A7.1 and B7.1 cells; Conklin, 1905), which appear to lead the process of invagination (Figs. 3A, 4A). The invaginating endoderm cells evenually contact ectoderm cells in the AP region on the opposite side of the embryo (Fig. 3B). At the completion of invagination, the blastopore is bordered by presumptive notochord cells on its anterior lip, presumptive mesenchyme on its lateral lips and presumptive muscle cells on its posterior lip (Fig. 3B).

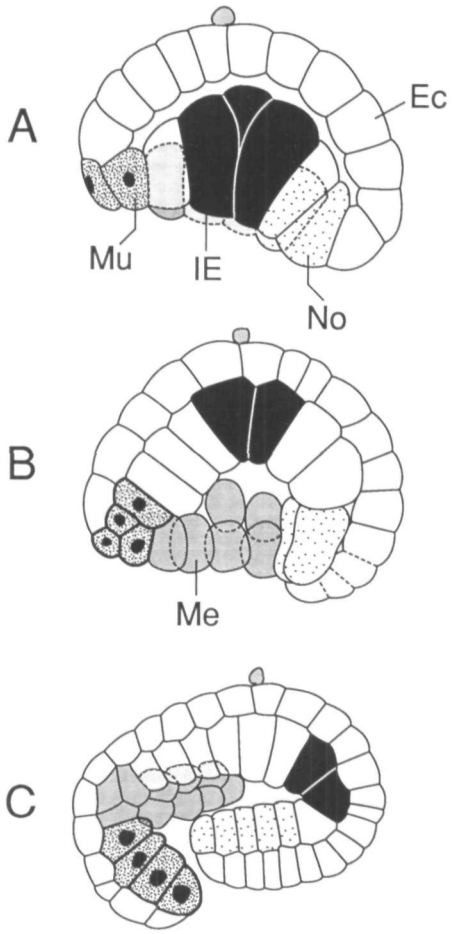

Fig. 3. Diagrammatic representation of cell movements during ascidian gastrulation. The embryos in A-C represent sections through the dorsoventral (animal-vegetal) axis. (A) Shape changes in endoderm cells initiate invagination in the early gastrula. (B) Invagination is completed in the mid-gastrula with contact being established between the endoderm and ectoderm cells in the animal hemisphere, and the mesoderm cells (prospective notochord, mesenchyme and muscle) begin to involute at the lips of the blastopore. (C) The invaginated endoderm cells move anteriorly, the mesoderm is internalized, the ectoderm cells complete epiboly and the embryo begins to elongate along the anteroposterior axis during the late gastrula stage. Gastrulae in A-C are oriented with their anterior poles facing the right and vegetal (dorsal) poles facing down. The small spheres drawn at the top of each embryo are polar bodies. IE: endoderm cells that are first to invaginate. Mu: muscle cells shown with shaded nuclei. Me: mesenchyme cells. No: notochord cells. Ec: ectoderm cells.

The mesoderm involutes over the lips of the blastopore and is replaced by epibolizing ectodermal cells (Fig. 3C).

The mechanism of ascidian gastrulation is poorly understood. However, Whittaker (1973) noted that blastomeres of *Ciona intestinalis* embryos treated with cytochalasin B after the 64-cell stage remain stationary, whereas normal cell distortions and migratory movements can occur in similar embryos treated with colcemid. These observations suggest that microfilaments, rather than microtubules, mediate cell behaviors associated with gastrulation in ascidians. In addition, it is possible that cell division, which contin-

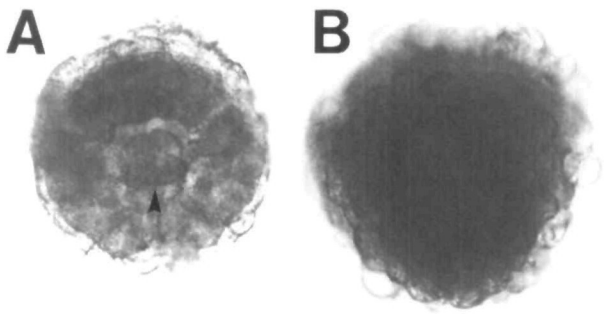

Fig. 4. Photomicrographs of normal (A) and UV-irradiated (B) *S. clava* embryos. (A) A normal embryo viewed from the vegetal side showing the bases of the invaginating A7.1 and B7.1 blastomeres (arrowhead). (B) An embryo that developed from a UV-irradiated zygote at the same stage of development as A showing no invagination. The scale bar is 20 μm; magnification is the same in A and B. From Jeffery (1990a).

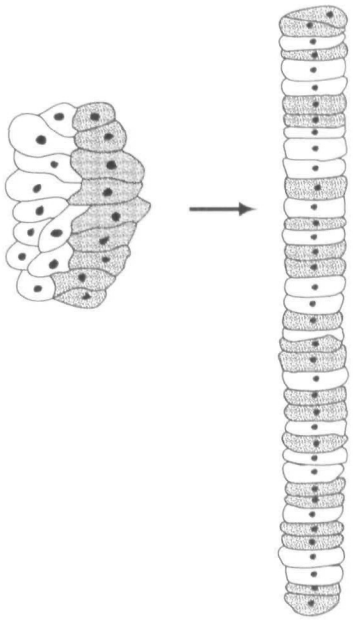

Fig. 5. Diagrammatic representation of cell rearrangements resulting in notochord formation in ascidian embryos. The notochord cells are viewed in frontal sections. Left. A mass of prospective notochord cells at the midline of a neurula. Cells that originate from the right side of the bilaterally-symmetric embryo are shaded, whereas those that originate from the left side are not shaded. Right. The notochord of a tadpole larva after interdigitation of notochord cells originating on either side of the midline and their posterior extension as a column of single cells. Note that cells orginating from either side of the midline are mixed randomly in the notochord (Nishida and Satoh, 1985). In both, orientation is with the posterior pole of the cell mass or notochord facing down.

ues in ascidian gastrulae (Nishida, 1986), may be an important factor in gastrulation.

At the end of the gastrula stage, the embryo begins to elongate along its anteroposterior axis. Elongation involves anterior movements of the internalized endoderm cells and posterior movements of mesoderm cells. As the embryo elongates, the neural plate, which has developed in the vegetal hemisphere, invaginates to form the neural tube. The anterior portion of the neural tube later expands to form the larval brain, which ultimately differentiates two pigmented sensory organs. The posterior portion of the neural tube becomes the spinal cord, which runs along the dorsal margin of the tail. A number of investigators have shown that the larval nervous system is induced during the late gastrula stage (Rose, 1939; Reverberi et al., 1960; Nishida and Satoh, 1989). Although the identity of the neural inducer is controversial (Reverberi et al., 1960; Nishida, 1991), inductive activity has been traced to cells arising from the anterior quadrant of the vegetal hemisphere, which underlie the dorsal ectoderm after gastrulation.

The formation of the tail is driven by rearrangements of notochord cells (Conklin, 1905; Cloney, 1964; Miyamoto and Crowther, 1985; Nishida and Satoh, 1985; Swalla and Jeffery, 1990). After gastrulation, a mass of presumptive notochord, several cells wide, is positioned at the posterior midline of the neurula (Fig. 5A). During tail formation, the presumptive notochord cells interdigitate and then extend into a single row of cells that swells to form the notochord (Fig. 5B). During the extension of the larval tail, bands of striated muscle cells differentiate on either side of the notochord.

The gastrulation center

The event that links gastrulation and axis formation with ooplasmic segregation is the establishment of a gastrulation center near the VP of the fertilized zygote. The gastrulation center is defined as a region of the uncleaved egg required for the embryo to gastrulate and develop into a bilaterally symmetric larva.

The first evidence for a specialized role of the vegetal hemisphere in development was provided by microsurgical experiments with *Phallusia mammillata* and *Ascidia malaca* eggs (Ortolani, 1958; Reverberi and Ortolani, 1963). When unfertilized eggs were cut into two fragments through any plane and then fertilized, each sufficiently large fragment was capable of cleavage, gastrulation and development into a normal larva. In contrast, only the vegetal fragments were capable of normal development when the same operation was performed on fertilized eggs. The animal fragments developed into permanent blastula lacking the cell movements associated with gastrulation. These results showed that the vegetal hemisphere is the only part of the fertilized egg with the potential for gastrulation and the formation of a complete larva.

The experiments described above did not discriminate between the developmental potential of different regions of the vegetal hemisphere because *P. mammillata* and *A. malaca* eggs are not endowed with colored cytoplasmic regions that can be used as markers. Additional information about the developmental potential of the vegetal hemisphere was obtained by deleting colored cytoplasmic regions from *Styela plicata* eggs and zygotes (Bates and Jeffery, 1987). The deletion procedure involved: (1) making a hole in the chorion above the region of the egg to be

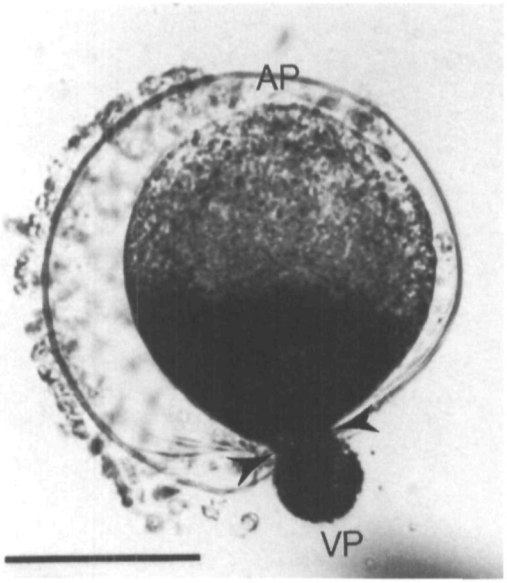

Fig. 6. Deletion of the vegetal pole region of a *S. plicata* zygote shortly after completion of the first phase of ooplasmic segregation. The position of the connection between the extruded anucleate fragment and the egg proper that is severed to produce the deletion is indicated by the arrowheads. The zygote was extracted with Triton X-100 to enhance contrast of the myoplasm (dark vegetal region) for photography. AP: animal pole. VP: vegetal pole. Scale bar: 50 μm. From Bates and Jeffery (1987).

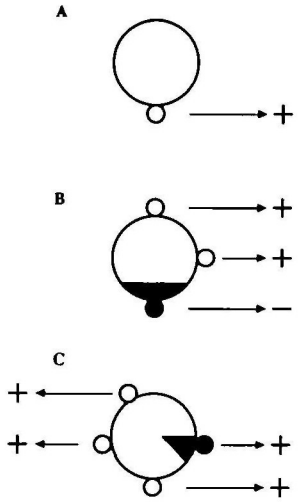

Fig. 7. A summary of deletions carried out in *S. plicata* eggs and zygotes and their effect on gastrulation and normal development. (A) Unfertilized eggs were deleted at the vegetal pole. (B) Zygotes were deleted at the animal pole, in the equatorial region and at the vegetal pole shortly after completion of the first phase of ooplasmic segregation. (C) Zygotes were deleted near the animal pole, in the anterior equatorial region, in the posterior equatorial (yellow crescent) region and at the vegetal pole shortly after completion of the second phase of ooplasmic segregation. The arrows pointing to + or – signs indicate the effect of deletions made at this position on the ability of the egg or zygote to gastrulate and develop into a normal larva: the + sign indicates that gastrulation and normal axis formation occurred, whereas the – sign indicates that gastrulation was blocked. Orientation is with the animal pole on the top and the posterior pole to the right. The shaded areas indicate the position of the myoplasm.

deleted, (2) exerting pressure on the opposite side of the egg to extrude the desired area through the hole and (3) severing the narrow connection between the extrudate and the remainder of the egg. In this way, an anucleate fragment containing plasma membrane, cortex and underlying cytoplasm could be deleted from a specific region of the egg without affecting the viability of the remaining nucleate egg fragment. Fig. 6 shows an example of a deletion in which a small portion of the egg was extruded from a point centered in the myoplasm near the VP.

Fig. 7 summarizes the results of deletions made at different stages of ooplasmic segregation (Bates and Jeffery, 1987). After each deletion, the nucleate fragments were allowed to continue development and assessed for normal cleavage, gastrulation, tail formation, and muscle and sensory cell differentiation. Consistent with the results of earlier experiments, deletion of the VP region of unfertilized eggs did not affect normal development (Fig. 7A). Deletion of as little as 5% of the total cell volume from the VP region after the completion of the first phase of segregation, however, blocked gastrulation, sensory cell induction and embryonic axis formation (Fig. 7B; also see Fig. 8A). Similar to the animal hemisphere fragments described above, the VP deficient embryos developed into permanent blastulae. The permanent blastulae did not form an axis of bilateral symmetry, but some cells expressed acetylcholinesterase (Bates and Jeffery, 1987), a muscle cell marker in ascidians (Whittaker, 1973). The site of the deletion was critical in abolishing gastrulation and axis formation. An effective deletion had to be made within the yellow cap of myoplasm (Fig. 1); deletions at other sites had no effect on development (Figs. 7B and 8). The timing of deletions was also important. Although deletions made at the VP shortly after the first phase of segregation blocked gastrulation, there was no effect on gastrulation when deletions were made at the same site at the yellow crescent stage (Fig. 7C). This result was interpreted to mean that the gastrulation center is dispersed throughout the vegetal hemisphere after sperm aster formation. It is difficult to test this possibility, however, because larger deletions remove the pronuclei, which are localized in vegetal hemisphere during ooplasmic segregation (Conklin, 1905). The results suggest that segregation of myoplasm organizes a gastrulation center in the VP region of the zygote.

The gastrulation center could mediate invagination by entering blastomeres destined to invaginate or blastomeres that ultimately induce other cells to initiate gastrulation. This question was examined by following the fate of cells that inherit the VP region in *S. plicata* embryos (Bates and Jeffery, 1987). As described above, chalk particles placed at the surface of the myoplasm when the first phase of segregation is completed do not move to the posterior pole during yellow crescent formation. By following the distribution of chalk granules placed in this position, the VP region was traced into the first endoderm cells (A7.1 and B7.1 cells) to invaginate at the beginning of gastrulation

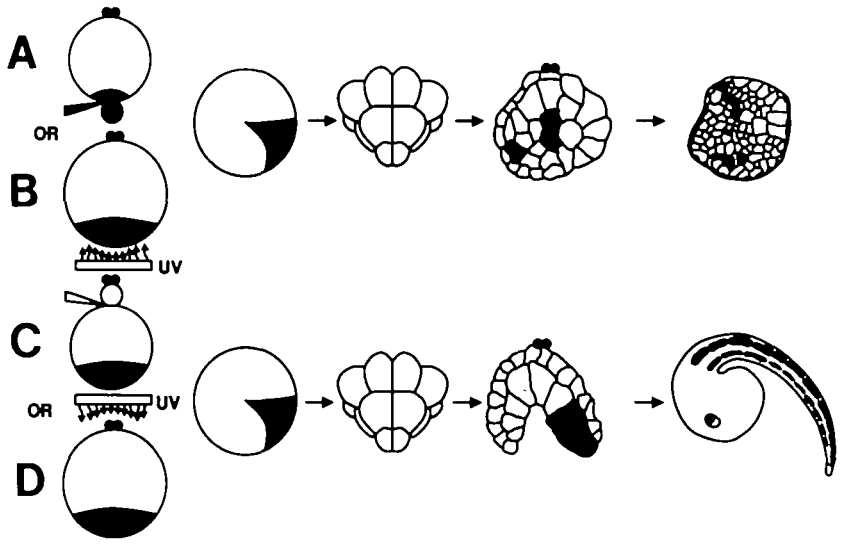

Fig. 8. A comparison of the effects of microsurgical deletion and UV irradiation of the myoplasm on embryonic development. (A) Deletion of the vegetal pole region at completion of the first phase of ooplasmic segregation. (B) UV irradiation of the vegetal pole region at completion of the first phase of ooplasmic segregation. (C) Deletion of the animal pole region at the same time as in A. (D) UV irradiation of the animal pole region at the same time as in B. Stages of normal development (lower row) from left to right: yellow cap, yellow crescent, 16-cell, mid-gastrula and mid-tailbud. Development after vegetal pole deletion or UV irradiation is shown in the upper row. The shaded areas represent myoplasm or myoplasm-containing cells. From Jeffery and Swalla (1990).

(see Fig. 3). Therefore, the gastrulation center is inherited by the cells that initiate gastrulation.

Polarization of the gastrulation center

What factors are responsible for establishing the gastrulation center near the VP? To answer this question, we must identify the cues that polarize the myoplasm during the first phase of ooplasmic segregation.

In his classic paper, Conklin (1905) proposed that the myoplasm is directed to the VP of *Styela partita* eggs because it is the site of sperm entry. In support of this proposal, it was shown that the male pronucleus is localized near the VP after fertilization. An alternate interpretation, however, is that the sperm could been carried to the VP by the advancing myoplasm after entering in another region of the egg. Conklin's observations are consistent with this possibility because his specimens were fixed for cytology relatively late after insemination.

Unfortunately, it is difficult to directly observe the site of fertilization in ascidian eggs because sperm entry is obscured by accessory cells lying between the egg surface and the chorion. Removal of the chorion provides a naked egg, but also prevents fertilization, probably because the follicle is required for sperm activation. Therefore, Bates and Jeffery (1988) tested for a restricted sperm entry site by deleting the VP region of unfertilized *S. plicata* eggs and then determining whether these eggs could be fertilized. The results showed that VP deficient eggs are fertilizable and capable of normal development. In other experiments, Bates and Jeffery (1988) examined the extent of egg surface area capable of sperm entry by inseminating multiple anucleate fragments deleted from unfertilized eggs. It was possible to fertilize three fragments obtained from different regions of the same egg, indicating the sperm entry can occur within a wide area of the egg surface. While these experiments do not exclude a preferred sperm entry site, they suggest that fertilization can occur over most of the egg surface.

The question of a preferred sperm entry site was examined directly by staining fertilized *P. mammillata* eggs with a DNA-specific fluorescent dye (Speksnijder et al., 1989). In this study, the fertilizability problem was solved by activating sperm with chorionated eggs before inseminating the naked eggs. The results showed that fertilization could occur in the animal or vegetal hemisphere, but that the animal hemisphere was the most frequent site of sperm entry (see Fig. 2, Row B). These results do not support Conklin's hypothesis that the myoplasm segregates toward a sperm entry site at the VP.

If sperm entry does not occur at the VP, how is the myoplasm oriented toward this region after fertilization? Current evidence favors the possibility that the direction of myoplasmic segregation is determined by pre-existing polarity along the animal-vegetal axis (Bates and Jeffery, 1988). This idea is supported by experiments in which the direction of myoplasmic segregation was examined in *S. plicata* egg fragments after extrusion of the AP or VP regions of unfertilized eggs. The results showed that myoplasm usually segregates toward the most vegetal region in both animal (Fig. 10) and vegetal fragments. Recent observations suggest that the sperm entry point may bias the direction of myoplasmic segregation in *P. mammillata* eggs (Speksnijder et al., 1990). According to this idea, the myoplasm can be focused up to 50° away from the VP depending on the position of sperm entry in the animal hemisphere. Even if the sperm enters in the vegetal hemisphere, however, the focal point of myoplasmic segregation is always vegetal (Speksnijder et al., 1990), indicating the primary cue for polarization must pre-exist along the animal-vegetal axis.

The identity of the axial polarization cue is currently unknown, although it has been shown that myoplasm will segregate toward an exogenously applied source of Ca^{2+} ionophore (Jeffery, 1982). Thus, a flux of free Ca^{2+}, possibly released from the cortical endoplasmic reticulum (Gaultieri and Sardet, 1989), may orient the gastrulation center toward the VP.

UV inactivation of the gastrulation center

Progress in determining the function of the gastrulation

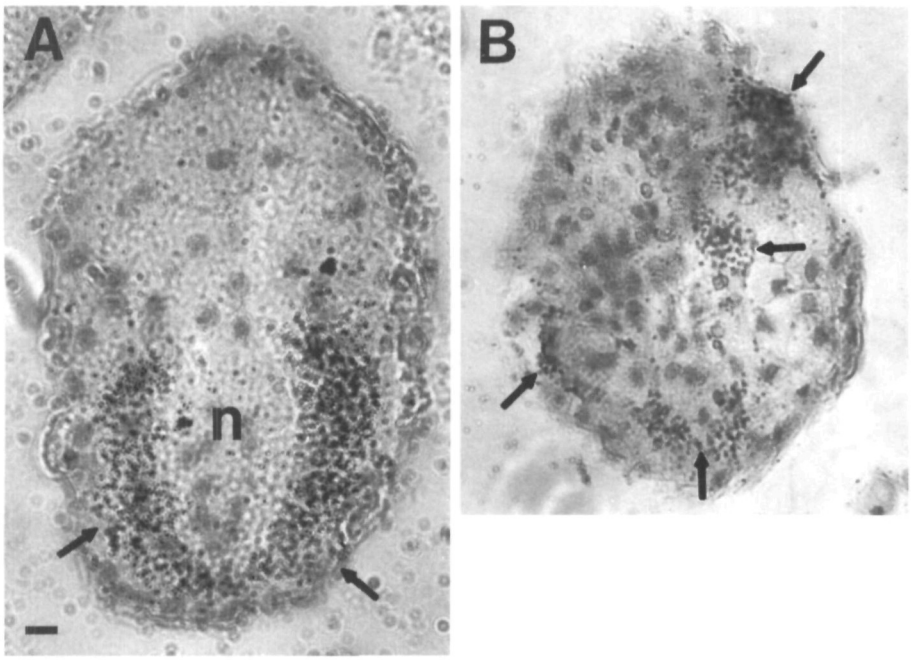

Fig. 9. Muscle cell development assessed by *in situ* hybridization with a muscle actin probe in sections of normal (A) and UV-irradiated (B) *S. clava* embryos. (A) In early tailbud stage embryos, muscle actin mRNA accumulates in muscle progenitor cells (arrows) located on either side of the presumptive notochord (n). (B) In embryos that were UV irradiated shortly after completion of the first phase of ooplasmic segregation, muscle actin mRNA accumulates in patches of muscle progenitor cells (arrows) in an embryo lacking bilateral symmetry. Scale bar: 10 μm; magnification is the same in both frames. From Jeffery (1990a).

center was aided by the development of ultraviolet (UV) irradiation methods to inactivate this region (Jeffery, 1990a). *Styela clava* was the species of choice in these experiments because it produces large numbers of synchronously developing embryos which are useful for molecular analysis. The experiments and results are summarized in Fig. 8. Similarly to cytoplasmic deletion, UV irradiation of the VP region, but not the AP region, prevented gastrulation, brain sensory cell induction and tail formation (Fig. 8B, D; also see Fig. 4B). However, neither cleavage (Fig. 8B) nor muscle cell development, as determined by in situ hybridization of the permanent blastulae with a cloned muscle actin probe (Fig. 9), were affected by UV irradiation of the VP region. These experiments suggest that UV irradiation and cytoplasmic deletion have the similar effects on the function of the gastrulation center.

The UV-sensitivity period for inactivating the gastrulation center was determined by UV irradiating zygotes at various times between fertilization and first cleavage. UV irradiation was effective in blocking the gastrulation and axis formation when administered up to the yellow crescent stage (Fig. 11). These results contrast to those obtained by cytoplasmic deletion, in which gastrulation was inhibited only if the operation was performed at the yellow cap stage or earlier. However, the results are consistent with the possibility that the dispersed gastrulation center is localized

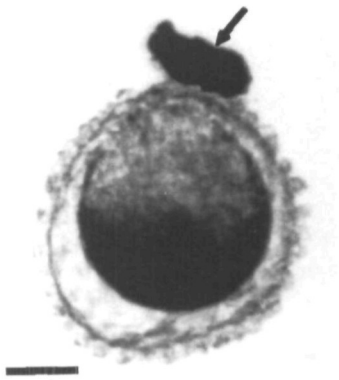

Fig. 10. The direction of myoplasmic segregation is vegetal in animal egg fragments. In this experiment, the vegetal portion of an unfertilized *S. plicata* egg was extruded before marking the animal pole with a chalk particle (arrow) and subsequent insemination. At completion of the first phase of ooplasmic segregation, the zygote was extracted with Triton X-100 to enhance contrast of the myoplasm (dark area), which has segregated to the most vegetal region of the fragment. Scale bar 50 μm. From Bates and Jeffery (1988).

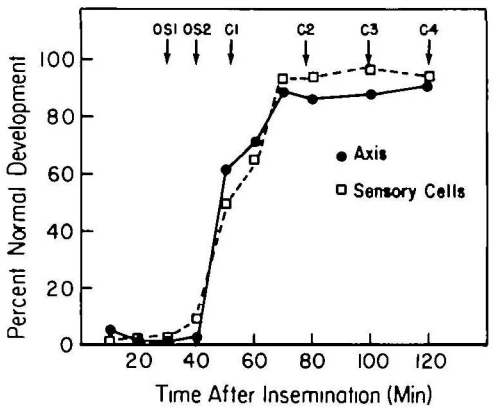

Fig. 11. The UV-sensitivity periods for axis and sensory cell development in *S. clava* embryos. Zygotes were irradiated at various times between insemination and fourth cleavage. Gastrulation was also abolished in these embryos. The timing of various developmental stages is indicated by the arrows. OS 1: completion of the first phase of ooplasmic segregation. OS 2: completion of the second phase of ooplasmic segregation. C1-C4: cleavages 1-4. From Jeffery (1990a).

near enough to the egg surface to be inactivated by UV light.

Molecular analysis of the gastrulation center

Inactivation by UV light permits a molecular analysis of the gastrulation center (Jeffery, 1990b). The first question was whether inactivation of the gastrulation center affects the pattern of embryonic protein synthesis. In these experiments, zygotes were irradiated shortly after completion of the first phase of ooplasmic segregation, allowed to cleave and then incubated with [^{35}S]methionine. Exposure to radioactive amino acids was continued until controls reached the early tailbud stage. Subsequently, proteins were extracted from the UV-irradiated and normal embryos, separated by 2D gel electrophoresis and compared by autoradiography (Fig. 12). Most of the 433 polypeptides detected in the autoradiographs were not affected by UV irradiation. For example, the three major actin isoforms synthesized by *S. clava* eggs were labelled to the same extent in UV-irradiated embryos and controls (Fig. 12). Only about 5% of the polypeptides decreased in labelling or disappeared after UV irradiation. One of the proteins whose synthesis was undetectable after UV irradiation is a 30 kDa molecule (p30). The extreme sensitivity of p30 synthesis to UV light makes it a prime candidate for a protein involved in gastrulation and axis determination.

A series of experiments was performed to determine whether there is a relationship between p30 synthesis and gastrulation (Jeffery, 1990b). The first experiment examined the effect of different UV doses. The results showed that p30 synthesis and gastrulation are abolished by the same UV dose. The next experiment compared the UV-sensitivity periods for p30 synthesis and gastrulation. The results showed that p30 synthesis and gastrulation are sensitive to UV irradiation up to the yellow crescent stage. UV irradiation after the yellow crescent stage showed labelling of p30 was equivalent to that observed in controls. Finally, an experiment was conducted to determine the timing of p30 synthesis during early development. The results showed that p30 synthesis begins at the 16-32 cell stage, peaks

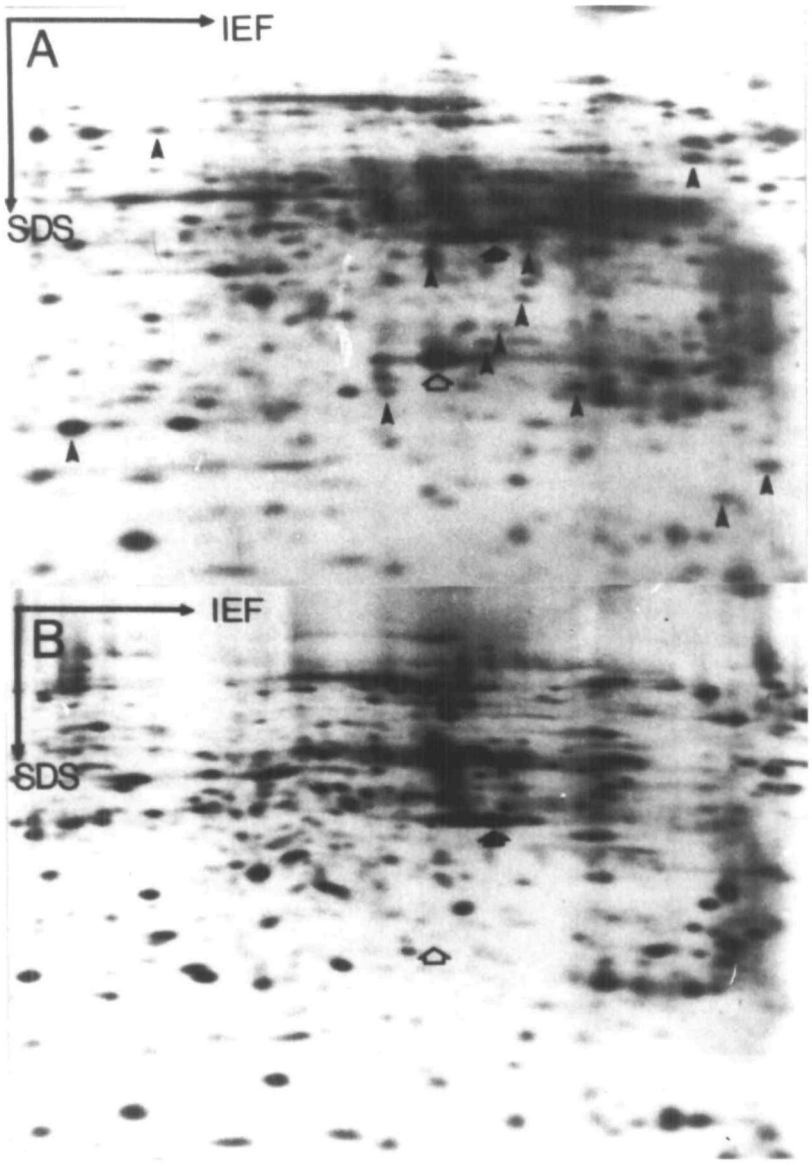

Fig. 12. Autoradiographs of gels containing proteins from normal and UV-irradiated *S. clava* embryos. (A) Proteins from normal embryos labelled with [^{35}S]methionine between the 2-cell and mid-tailbud stages. (B) Proteins extracted from embryos that developed from zygotes UV irradiated shortly after completion of the first phase of ooplasmic segregation and labelled with [^{35}S]methionine from the 2-cell stage until controls reached the mid-tailbud stage. Equal counts were applied to the gels. IEF: direction of isoelectric focussing. SDS: direction of electrophoresis through SDS gel. Solid large arrowhead: position of muscle actin marker. Open large arrowhead: position of p30. From Jeffery (1990b).

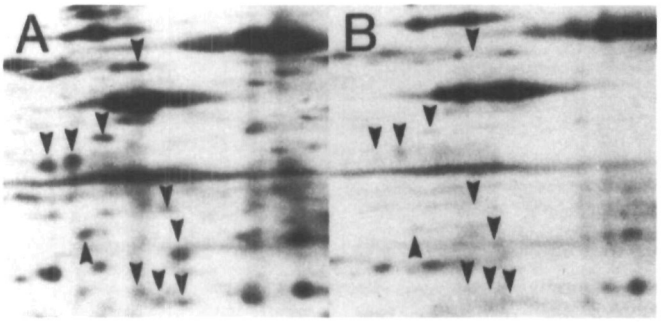

Fig. 13. The effect of UV irradiation on the ability of RNA from uncleaved *S. clava* zygotes to direct protein synthesis in a rabbit reticulocyte lysate. (A) Protein synthesis directed by RNA from normal zygotes. (B) Protein synthesis directed by RNA from UV-irradiated zygotes. p30 and a number of other polypeptides are missing or decreased in B. Upward-facing arrowheads: position of p30. Downward-facing arrowheads: position of other polypeptides whose translation was affected by UV irradiation. Only the p30 region of the gels is shown. Zygotes were UV-irradiated shortly after completion of the first phase of ooplasmic segregation. Equal counts were applied to each gel. From Jeffery (1990b).

during gastrulation and subsides by the early tailbud stage. The timing and UV sensitivity of p30 is consistent with a role in gastrulation.

Additional studies indicate that p30 is a cytoskeletal component (Jeffery, 1991). In these experiments, the distribution of labelled proteins was examined following extraction of embryos with Triton X-100. The results showed that p30 is present in the detergent insoluble fraction, suggesting it is a cytoskeletal component (Jeffery and Meier, 1983). However, p30 could be released from the insoluble to the soluble fraction when detergent extraction was carried out in the presence of DNase I, which specifically depolymerizes actin filaments. Therefore, p30 may be an F-actin binding protein.

Despite its potential role in gastrulation, p30 is not the UV-sensitive target in the gastrulation center: it is does not appear until the end of the UV-sensitive period and the chromophore has an absorbance maximum resembling a nucleic acid rather than a protein (Jeffery, 1991). Maternal RNA is a UV-sensitive target in insect eggs (Kalthoff, 1979). Thus, it was possible that p30 mRNA, rather than p30, is the UV-sensitive target. To determine whether p30 synthesis is directed by maternal mRNA, RNA extracted from unfertilized *S. clava* eggs was translated in a rabbit reticulocyte lysate and the translation products were examined by 2D gel electrophoresis and autoradiography (Jeffery, 1990b). The results showed that egg RNA directed the translation of a protein with the same molecular weight and isoelectric point as p30.

If maternal p30 mRNA is a UV-sensitive target, it should be possible to inactivate it by UV irradiation. To test this possibility, zygotes were irradiated shortly after completion of the first phase of ooplasmic segregation, RNA was extracted from the irradiated eggs and the translation products were compared with those of control eggs. The results showed that p30 and several other proteins were absent from the translation products directed by RNA from UV-irradiated eggs (Fig. 13). Thus, maternal p30 mRNA is a UV target. UV light cannot penetrate deeply into the egg cytoplasm (Youn and Malacinski, 1980), therefore, the suppression of the translation of p30 mRNA and other mRNAs (see Fig. 13) by UV irradiation strongly suggests that they are localized near the surface of the egg, possibly in the gastrulation center.

Concluding remarks

In this article, an invertebrate embryo with a low cell number and a relatively short interval between fertilization and gastrulation is used to investigate the relationship between an egg cytoplasmic region and morphogenesis. The major conclusion is that a gastrulation center is established in ascidian eggs by segregating myoplasm to the VP after fertilization. Later, as the myoplasm moves to the posterior pole, the potential for gastrulation spreads out in the vegetal hemisphere and is inherited by endoderm cells that initiate invagination. In amphibian eggs, a region is established in the vegetal hemisphere after fertilization and is then inherited by endoderm cells that subsequently induce marginal zone cells to begin gastrulation (Gimlich and Gerhart, 1984). In contrast, gastrulation is initiated by invagination of the same cells that inherit the gastrulation center in ascidians.

The experiments described here suggest that endoderm invagination in ascidian embryos is an autonomous process specified by axial determinants residing in the gastrulation center. Autonomous specification of cell fate is known to occur for some of the tail muscle cells in ascidians (see Venuti and Jeffery, 1989 for review). Further experiments are needed to prove that endoderm invagination is also an autonomous process, however. First, it would be necessary to show that endoderm cells undergo the shape changes related to invagination when they are isolated from the embryo. Second, conclusive evidence for axial determinants would require showing that the site of gastrulation could function autonomously after transplantation to another egg. At this time, however, it is possible to conclude that removal or inactivation of the gastrulation center abolishes gastrulation and axis formation, including induction of the nervous system and tail morphogenesis.

Morphogenesis of the ascidian embryo is initiated after VP blastomeres invaginate during gastrulation. The descendants of these and adjacent cells migrate through the embryo and eventually reach a position where they induce animal hemisphere blastomeres to elaborate the brain and sensory organs (Nishida and Satoh, 1989) or undergo morphogentic movements to form the notochord (Conklin, 1905). The experiments described in this article suggest that p30 is associated with the cytoskeleton and that this association is dependent on the integrity of microfilaments. Therefore, p30 may be an actin-binding protein that organizes microfilaments involved in changing the shape and/or motility of the invaginating endoderm cells.

The results support the hypothesis that gastrulation and axis formation may be mediated by maternal mRNA encoding the p30 cytoskeletal protein. During the first phase of ooplasmic segregation, p30 mRNA may be translocated to

the VP region with the myoplasm. Then, during the second phase, p30 mRNA probably remains in the VP region after the myoplasm is shifted to the posterior region of the zygote. The localization of p30 mRNA near the VP would place it in the proper location to be inherited and translated in endodermal cells that are first to invaginate and initiate axis formation during gastrulation.

The reason that p30 mRNA and other messages whose translation was abolished are particularly sensitive to UV irradiation may be explained by poor penetration of UV light into the interior of eggs (Youn and Malacinski, 1980). It follows that UV-sensitive mRNAs must be localized near the egg surface. Localized maternal mRNAs have been previously described in the myoplasm of *S. plicata* eggs (Jeffery et al., 1983). Future studies with specific probes for p30 mRNA and protein will be required to determine the spatial distribution of these molecules during development. Experiments are also in progress to identify some of the other UV-sensitive mRNAs that may be localized in the gastrulation center.

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