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CELLS FROM XENOPUS LAEVIS GASTRULAE ADHERE TO FIBRONECTIN-SEPHAROSE BEADS AND OTHER LECTIN COATED BEADS

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Abstract

Cells from Xenopus laevis blastulae have a poorly developed ability to adhere to Sepharose beads covalently coupled to bovine plasma fibronectin (FN-beads). They do, however, have the ability to adhere to con A-beads and cytodex-1 and cytodex-3 beads. Beginning at the early gastrula stage, there is a progressively increasing ability of cells to adhere to FN-beads. Gastrula cells adhere to FN-beads by the formation of large ruffling lamellipodia. These cells can translocate on the surface of FN-beads; and when attached to both beads and the surrounding glass substratum of culture vessels, have the ability to move the beads extensively. Gastrula cells also have the ability to adhere to but not move upon con A-beads, wheat germ agglutinin-beads, and soy bean agglutinin-beads. They do not adhere significantly to Tetragonolobus purpureas agglutinin-beads. These results suggest that there are increasing numbers of fibronectin receptors present on the surface of embryonic amphibian cells during the period of gastrulation. They may explain the differential distribution of fibronectin-containing fibrils in vivo as observed by scanning electron microscopy.

KEY WORDS: Xenopus laevis, Gastrulation, Cell Adhesion, Fibronectin, Lectins, Cell Movement

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Introduction

Prior to the beginning of gastrulation in amphibia, the basal surface of the epithelial cell layer comprising the roof of the blastocoel is a naked cell surface. At the beginning of gastrulation, a network of 0.1 μm extracellular fibrils begins to accumulate on this basal surface. In urodeles, these fibrils form an anastomosing array now known to contain fibronectin (FN) (Boucaut and Darribere, 1983) and laminin (Nakatsuji et al., 1985). In the anuran Xenopus laevis there is also a network of fibrils present (Nakatsuji and Johnson, 1983; Johnson, 1984), although they are much less extensive than those present in urodeles. These fibrils also contain fibronectin (Lee et al., 1984; Nakatsuji et al., 1985). These fibrils are even less sparse in Rana pipiens but nevertheless are present in increasing numbers throughout gastrulation. Furthermore, in certain interspecific arrested hybrid embryos where gastrulation is defective and migration of mesodermal cells does not occur, these fibrils are either less prominent than in normal control embryos or are entirely absent (Nakatsuji and Johnson, 1984a).

In urodeles, where these networks are most abundant, they are known to have a preferential orientation parallel to the animal pole-blastopore axis (Nakatsuji et al., 1982). When deposited on plastic substrata, these fibrillar networks maintain their orientation and can direct the migration of gastrula mesodermal cells seeded onto such conditioned substrata in vitro (Nakatsuji and Johnson, 1983). It is possible to exert tension on conditioning explants, alter the orientation of the deposited fibrils, and consequently alter the orientation of the paths of migrating mesodermal cells seeded onto such distorted arrays of fibrils (Nakatsuji and Johnson, 1984b).

Keller (1975, 1976) has produced detailed fate maps of gastrulation in Xenopus laevis and has then used these fate maps to guide his dissection of embryos into fragments with known fates. By doing this analysis and by a painstaking application of explant culture, time-lapse cinemicrography, and scanning electron microscopy, he has been able to show that the convergent extension of the dorsal marginal zone and of the involuting marginal zone are important morphogenetic movements during gastrulation. The deep mesoderm

has been shown to undergo shape changes and interdigitation of cells after their involution, resulting in this region constituting the main engine which drives gastrulation (Keller, 1984; Keller et al., 1985). As the involuting mesodermal mass moves across the inner surface of the roof of the blastocoel, the push generated by interdigitation and cell shape changes may well be directed by fibrillar networks of fibronectin on the basal surface of the cells making up the roof of the blastocoel.

One of the central questions addressed by this study is how do fibronectin-containing fibrils become preferentially distributed along the inner surface of the roof of the blastocoel, given the fact that they are synthesized in many different parts of the embryo (Lee et al, 1984). Also, we wanted to see if there were alterations in cell surface characteristics during gastrulation as manifested by embryonic cell's changing ability to adhere to different kinds of beads. For these investigations, we covalently coupled fibronectin and several lectins to CNBr-Sepharose beads. We also examined the adhesion of cells from different developmental stages to cytodex beads. We used these different kinds of beads because we wanted to know if developmental changes in adhesion to fibronectin-Sepharose beads were specific for fibronectin.

Materials and Methods

Adult Xenopus laevis were obtained from Nasco (Fort Atkinson, Wisconsin). They were induced to breed in the laboratory by the injection of human chorionic gonadotrophin(Sigma) as described by Gurdon (1967). Embryos were collected in 10% Wolf-Quimby balanced salt solution (WQBSS)(Wolf and Quimby, 1964) and raised to appropriate stages at 22-24° C. Jelly coats were removed by a 5 minute treatment with 0.7% mercaptoacetic acid in 50% WQBSS with the pH adjusted to 8.6 with 5 N NaOH. After removal of jelly, embryos were rinsed 10 times in 10% WQBSS and raised to appropriate stages for experiments. Embryos were staged according to Nieuwkoop and Faber (1967). For dissection and dissociation, embryos were transferred to Ca++, Mg++-free WQBSS (CMF) with 2 mM EDTA added, had vitelline membranes removed manually with sharpened Dumont #5 watchmaker's forceps, and were dissected into appropriate fragments, and then were incubated for 1 h at 22° C. for dissociation. Dorsal and ventral fragments were produced by dissecting embryos approximately along the equatorial plane as the embryos rested in their natural position after removal of their jelly coats. The dorsal halves of Stages 8, 9, and 10 embryos contained predominantly prospective ectodermal and mesodermal cells from the animal pole and upper marginal zone. The dorsal halves of Stages 11 and 12 embryos contained uninvoluted ectodermal and mesodermal cells as well as involuted mesodermal cells and, in Stage 12 embryos, even large yolky endodermal cells. The dorsal halves of neurula stage embryos contained the roof of the archenteron and medullary plate once neurulation had begun. The ventral halves of Stages 8, 9, and 10 embryos contained predominantly prospective endodermal cells from the vegetal pole and lower marginal zone cells. The ventral halves of Stages 11 and 12 embryos contained the bulk of the ventral endodermal mass, some involuted mesodermal cells, and ventral ectodermal cells that had spread below the equator of the embryo due to epiboly.

CNBr-Sepharose beads for cell adhesion assays were prepared according to manufacturer's instructions (Pharmacia). Bovine plasma fibronectin (Sigma)(FN) was dissolved at 1 mg/ml in coupling buffer giving a final concentration of FN of 0.62 mg/ml as determined by the O.D. at 280 nm using an absorbance coefficient of 12.8 for FN (Mosesson and Umfleet, 1970). After dissolving FN in coupling buffer, 1 ml of this solution was mixed with 1 ml packed volume of washed and swollen beads. Bovine serum albumin (Sigma)(BSA) and crude porcine stomach mucin (Sigma)(PSM) were dissolved at 10 mg/ml in coupling buffer and 1 ml of this solution was mixed with 1 ml of beads. Blank beads were prepared by incubating washed and swollen beads in 1 M ethanolamine in coupling buffer. Concanavalin A-Sepharose beads were purchased from Pharmacia and washed extensively in culture medium before using in adhesion assays. Cytodex-1 and cytodex-3 beads were purchased dry from Pharmacia and hydrated and washed extensively in culture medium. Wheat germ agglutinin (WGA) (L-1005, Sigma), soy bean agglutinin (SBA)(L-8004, Sigma) and Tetragonolobus purpureas agglutinin (TPA) (L-9245, Sigma) were dissolved at either 1 mg/ml or 2 mg/ml in coupling buffer and then coupled to CNBr-Sepharose beads as before. We used cytodex beads and lectin-beads to test the ability of all stages of cells to adhere to such beads. For instance, we were concerned that the inability of blastula stage cells to adhere to FN-beads was a reflection of blastula stage cells general inability to adhere to beaded substrata. Sephadex G-200 beads were swollen in WQBSS and washed three times in WQBSS before use. Sepharose CL-4B beads were purchased pre-swollen and were washed three times in WQBSS. All WQBSS for washing and storing beads as well as culture medium added to cell adhesion assay plates contained penicillin G (100 IU/ml) and streptomycin sulfate (1 µg/ml). After all coupling and washing steps, beads were allowed to settle out of suspension in graduated centrifuge tubes. Supernatant washes were removed carefully with a Pasteur pipette and enough WQBSS with antibiotics was added to make a 50% v/v suspension of beads.

To set up cell-bead adhesion assays, Falcon Multiwell tissue culture plates (#3047) with 24 wells and a capacity of 2 ml/well were coated with agarose by adding 200 µl of warm 1% aqueous agarose in WQBSS with antibiotics. Preliminary experiments showed that agarose coating was essential to prevent cells from sticking to the bottom of the wells. After the agarose cooled and solidified, plates were stored in a moist chamber in the refrigerator. Just before an assay was performed, each well of a test plate received 100 µl of 50% v/v bead suspensions. It is important to begin assays by addition of test beads immediately before addition of cell suspensions so that the agarose coatings don't detach from the test plates and float in the culture medium,

making establishment of cultures difficult. The volume of 50% v/v bead suspension was chosen advisedly, because this is the correct volume of bead suspension to give approximately a single layer of close packed beads on the bottom of the tissue culture plate wells. Next, 1.0 ml of WQBSS + 0.5% BSA + antibiotics was added to each well. These plates were set up while tissue fragments were dissociating in EDTA. After 1 h of dissociation, cells were gently collected in Pasteur pipettes and then expelled into wells containing beads and culture medium. The dissociated fragments were aspirated up and down three times in the Pasteur pipette to facilitate dissociation. Next, cells and beads were allowed to settle out of suspension for five minutes and the 0.9 ml of medium was removed along with the contaminating EDTA and replaced with a fresh 1.0 ml of WQBSS + 0.5% BSA + antibiotics. Cultures were now transferred to a water bath maintained at 24° C. and equipped with a hood and water trap to maintain a 5% CO_2-95% air atmosphere to maintain pH at 7.4. Cultures were incubated for 2 h and then fixed by addition of 100 µl of 2.5% glutaraldehyde in 0.05 M PIPES buffer, pH 7.3 with 5 mM $CaCl_2$ added. After fixation, the contents of the well were aspirated into a Pasteur pipette and dispersed into a 60X15 mm plastic petri dish (Falcon #1007) and then examined in a dissecting microscope at 50 X magnification. The beads are large, opalescent, spherical structures that are easily distinguishable from the smaller embryonic cells (see Johnson, 1985, Figure 3 for illustration). The cells were sometimes darkly pigmented and always had a granulated appearance due to their yolk platelets. Cells attached to beads were flattened on them to a variable extent and could not be dislodged by agitation of dishes or gentle probing of beads with the tips of forceps. Each culture was scanned and the first 500 beads encountered were scored for number of cells attached. A running total of the number of beads and attached cells was kept on a differential blood counter so that after 500 beads were scored, a statistic of number of attached cells/500 beads was recorded. Five replicas were produced for each condition and the average and standard deviation for each determination was calculated.

In an attempt to perturb gastrulation, we also inserted FN-Sepharose beads into the blastocoel at the beginning of gastrulation. This experiment was performed by cutting a C-shaped flap in the roof of the blastocoel in a Stage 10 early gastrula. In one group of sham operated controls, the roof of the blastocoel was patted back in place and allowed to heal without further manipulation. In a second group, 5-10 blank beads, i.e., those treated with ethanolamine prior to any coupling reaction, were added underneath the flap and then the embryo was allowed to heal and continue development. In a third group, FN-beads were placed under the flap. Five hours at 22° C. after the operation, embryos were fixed for scanning electron microscopy (SEM).

For SEM fixation, suspensions of beads and cells or whole embryos were transferred to 2.5% glutaraldehyde in 0.05M PIPES buffer, pH 7.3 with 5 mM $CaCl_2$ added. After cells and beads settled out of suspension (5 min) most of the glutaraldehyde was removed and replaced with fresh fixative. Cells and embryos were fixed at 22° C. overnight. Next, specimens were wased in PIPES buffer and then post-fixed for 1 h at 22° C. in 1% OsO_4 in the same buffer. Following post-fixation, specimens were washed again with buffer, transferred to BEEM capsules designed to be used for embedding specimens in plastic for thin sectioning. The cover of the BEEM capsule was cut across the top so that most of the cover was cut away leaving the lower portion of the tight fitting cap as a collar around the outside of the BEEM capsule. A small piece of Nitex screening (45 µm squares) was then placed over the top of the BEEM capsule and held in place with the collar remaining from the cut top. This allowed dehydration and critical point drying of large numbers of beads without losing any of them during processing. After dehydration in a graded series of ethanol solutions and critical point drying in liquid CO_2, beads were transferred to SEM stubs coated with adhesive. Next, beads and cells were coated with 40 nm of gold-palladium and viewed in a JSM-35 SEM at 20 kV.

For time-lapse cinemicrography, standard microscope slides had a thin rectangle of petroleum jelly applied to one surface to serve as a dam for the cell-bead suspension and medium. Suspensions of living cells on beads were then transferred to the slides, excess medium was removed after cells and beads had settled out, and the preparation was sealed with a coverslip. These preparations were filmed while alive under phase contrast optics. Preparations were photographed at 8 s intervals on Kodak Plus X reversal film processed commercially and analysed on a Lafayette photoptical data analyser. Tracings of individual frames from films were made from projected films.

Results

Preliminary Control Experiments

We established the kinetics of cell adhesion to FN-Sepharose beads for normal Xenopus laevis embryos by varying the time for addition of glutaraldehyde after cultures were initiated. For cultures where glutaraldehyde was added to cultures immediately after cells were added, there was no cell adhesion to beads, indicating that cell-bead adhesions were not glutaraldehyde induced. With increasing incubation times, there is an increasing level of cell-bead adhesion reaching a plateau by 2 h and not increasing further for up to 4 h. We observed that blastula stage cells adhere poorly to FN-beads (see below) but were able to show that this minimal cell-bead adhesion was not due to limitations in numbers of cell-bead interactions because there was no substantial difference between the numbers of adherent cells in cultures receiving one, two, three, or four dissociated fragments of the roof of the blastocoel. Blastula stage cells adhere avidly to cytodex-1, cytodex-3, and con A-beads, indicating that their inability to adhere to FN-beads is not due to a generalized inability to adhere to beaded substrata. Rather, blastula stage cells appear to lack the particular

capacity to adhere to FN-beads.

Xenopus Late Gastrula Cells Adhere to Several Kinds of Beads

We tested the ability of late gastrula (Stage 12) cells to adhere to a variety of different kinds of beads covalently coupled with different proteins. We found that Stage 12 cells adhere strongly to FN-Sepharose, SBA-Sepharose, and WGA-Sepharose beads. We were also able to show that these cells adhere even more avidly to cytodex-1 beads, cytodex-3 beads, and con A-Sepharose beads. There was slight adhesion to BSM-Sepharose and PSM-Sepharose, and none at all to gelatin-Sepharose, BSA-Sepharose, Sephadex G-200, or blank beads. These results are summarized in TABLE 1.

TABLE 1
Adhesion of Xenopus laevis Stage 12 Late Gastrula Cells to Various Beads

Beads	Number of Adherent Cells/500 Beads ± S.D. Average of 5 Determinations
FN-Sepharose	106±18
Cytodex-1	420±46
Cytodex-3	565±34
Sephadex G-200	0±0
PSM-Sepharose	23±7
BSM-Sepharose	11±3
Con A-Sepharose	175±12
SBA-Sepharose	70±17
Gelatin-Sepharose	13±5
BSA-Sepharose	7±2
Blank	1±1

Developmental Curves for Cell-Bead Adhesion to FN-Sepharose Beads

We studied the adhesion of cells from different developmental stages of Xenopus laevis embryos to FN-beads using a standard adhesion assay applied to cells from different developmental stages of embryos. We found that there was very little cell adhesion to FN-beads in Stage 8 mid-blastulae. Stage 9 late blastula and Stage 10 early gastrulae show slight increases. As development proceeds through Stages 11, 12, and 13 of gastrulation, this increasing trend continues. In Stages 14 and 15, when neurulation is progressing, there is a sharp upward trend in levels of cell adhesion. The developmental curves for cells taken from dorsal and ventral halves of embryos have a similar constant upward trend with the absolute level of adhesion always being greater in dorsal fragments than in ventral fragments (Figure 1). Detailed regional dissections of embryos were not attempted but it is clear from close observations of cultures that small, medium, and large cells in dissociated suspensions all were able to adhere to FN-beads.

Cell Adhesion to Plant Lectin-Sepharose Beads

We have studied the adhesion of gastrula cells to beads covalently coupled with several kinds of plant lectin, including concanavalin A (con A), soy bean agglutinin (SBA), wheat germ agglutinin (WGA), and Tetragonolobus purpureas agglutinin (TPA). These lectins show specificity for α-D-mannosyl and α-D-glucosyl- (con A), N-acetyl-β-D-glucosaminyl- (WGA), N-acetyl-D-galactosaminyl- (SBA), and α-L-fucosyl-residues (TPA) respectively. Xenopus laevis gastrula cells adhere avidly to beads coated with con A at 1 mg/ml in the coupling buffer. These adhere modestly to WGA- and SBA-beads at 1 mg/ml in the coupling buffer and more avidly when 2 mg/ml of WGA and SBA are used in coupling buffers. They adhere very poorly to TPA-beads at both 1 mg/ml and 2 mg/ml in coupling buffer (Tables 1 and 2).

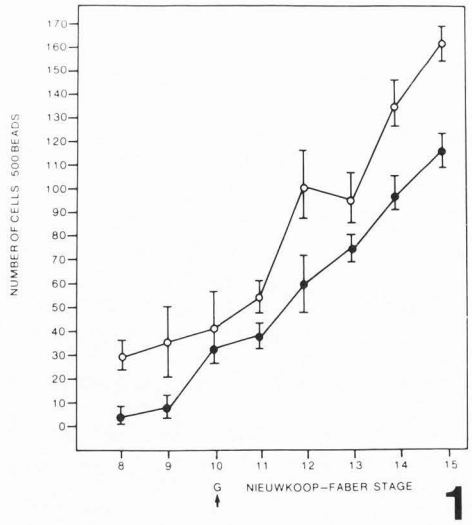


Figure 1. Xenopus laevis cell attachment to FN-Sepharose beads using 2 h incubations. For each time point, the embryos were at the indicated developmental stage at the beginning of a 1 h dissociation period. Gastrulation begins at Stage 10, as indicated by G on the horizontal axis of the graph. Dorsal halves, open circles; ventral halves, closed circles.

TABLE 2
Adhesion of Xenopus laevis Cells to Lectin-Sepharose Beads

Stage	Lectin-Beads Concentration	Number of Cells/500 Beads Average of 5 Determinations ± S.D.
Dorsal 12	Con A 1 mg/ml	231±10
Ventral 12	Con A 1 mg/ml	119±13
Dorsal 9	SBA 1 mg/ml	58±19
Ventral 9	SBA 1 mg/ml	69±17
Dorsal 11	SBA 1 mg/ml	107±27
Dorsal 12	SBA 1 mg/ml	99±28
Dorsal 8	SBA 2 mg/ml	65±17
Dorsal 8	WGA 2 mg/ml	62±18
Dorsal 12	SBA 2 mg/ml	144±43
Dorsal 12	WGA 2 mg/ml	125±21
Dorsal 12	TPA 2 mg/ml	14±6

When observed in the light microscope, cells adhere to lectin-coated beads by flattening extensively on them without the formation of filopodia or lamellipodia at adhesion sites. Cells appear to be immobilized on lectin-beads when viewed in the light microscope in time-lapse

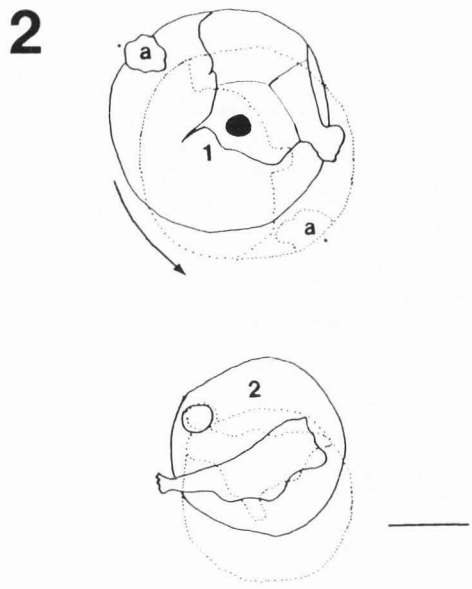
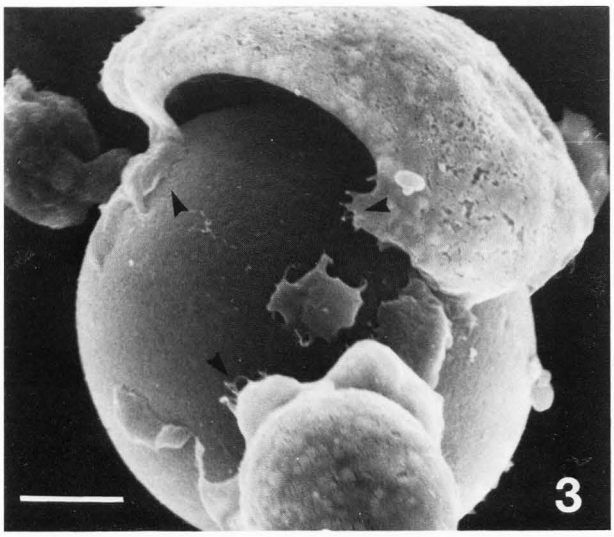


Figure 2. Tracings of individual frames from time-lapse films of Stage 12 Xenopus laevis late gastrula cells attached to and spreading upon FN-Sepharose beads. Bead 1 has a cluster of three large flattened cells attached to it. One of the nuclei of a cell in this cluster is visible in the film and is drawn in as a large dark circle. Bead 1 also has a small cell (a) attached to it. Cells underneath the bead are attached to the substratum and during the 61 minute interval shown in this drawing, the bead rotates approximately 180° counterclockwise. Eventually, after 96 minutes in the film, the entire bead had rotated more than 360° counterclockwise. Bead 2 has a cell attached to the substratum and the bead at the beginning of this sequence. During the 61 minute period of this sequence, the bead moves downward and the cell loses its grip on the substratum and crawls completely onto the bead. These two beads are together in one frame of the same film. Bar = 50 μm.



films. They do not form lamellipodia nor do they show noticeable locomotory activity when observed in time-lapse films. Instead, they form relatively smooth and featureless marginal extensions all around the cell. These marginal extensions are hyaline portions of the ectoplasm that do not show any evidence of marginal uplift or rapid changes in shape that are characteristic of filopodia and lamellipodia. These results suggest that the cell surface glycoconjugates of gastrula cells are rich in α-D-mannosyl-, N-acetyl-D-glucosaminyl-, and N-acetyl-D-galactosaminyl-residues but lack substantial amounts of α-L-fucosyl-residues.

Observations in the Light and Scanning Electron Microscope

Gastrula cells adhere to FN-Sepharose beads by the formation of active lamellipodia. They become extensively flattened against these beads and form one or more broad fan-like lamellipodial attachments to them. Once attached, cells can move about on the surface of beads. In some instances, they become extensively attached but don't show much translocation. When cells are attached to beads and the glass substratum of the culture vessel simultaneously, the contractile activities of cells are great enough to cause extensive displacement of beads and even rotation of them (Figure 2). It appears that FN-beads serve as a suitable substratum for cell attachment and locomotion. When viewed in the SEM, gastrula cells attached to FN-Sepharose beads show the formation of filopodia and lamellipodia (Figure 3). Often, cells have an irregular border consisting of smooth areas between adjacent marginal protrusions. Filopodia may occur adjacent to lamellipodia and may also be seen at the leading edge of lamellipodia. In contrast, when cells attach to lectin-beads, they flatten extensively but without the formation of filopodia and lamellipodia. Instead, cells attached to, e.g., WGA-beads, form broad attachments around the entire periphery of the cell but lacking true locomotory organelles which give an irregular outline to the edge of the cell (Figure 4).

Embryos with FN-Beads in the Blastocoel Show Normal Gastrulation

We performed experiments to see if FN-beads in the blastocoel of an early gastrula would disrupt gastrulation. We hoped that the beads in the blastocoel might have some effect on gastrulation. We expected that we might see a stimulation of mesodermal cell migration; or, alternatively, that migrating mesodermal cells would adhere preferentially to FN-beads rather than to the inner aspect of the roof of the blastocoel, thereby disrupting mesodermal cell migration. Gastrulation appears to proceed normally in embryos that receive no beads but are operated upon (Sham Operated Controls), receive blank beads, or receive FN-beads. In the embryos that receive beads, in the blastocoel, it was not

Figure 3. SEM of two dorsal Stage 12 Xenopus laevis late gastrula cells attached to an FN-Sepharose bead. These cells form large lamellipodia on the surface of the bead (arrows). Bar = 10 μm.

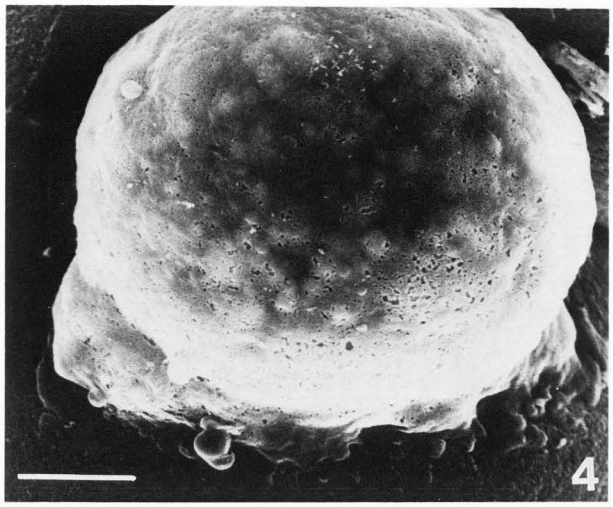
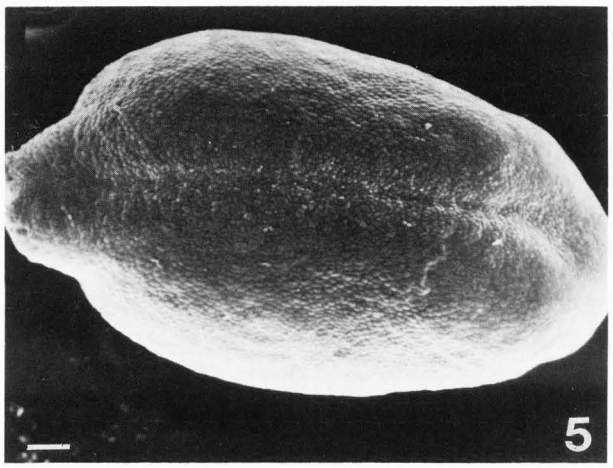
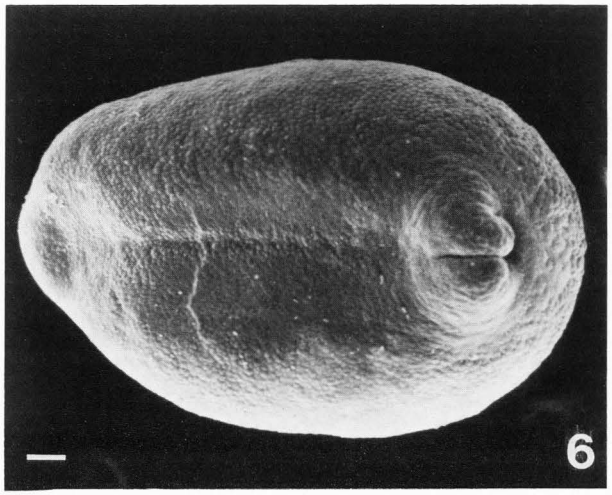


Figure 4. SEM of a dorsal Stage 12 Xenopus laevis late gastrula cell attached to a WGA-Sepharose bead. The cell spreads out on the bead but does not form locomotory lamellipodia. Bar = 5 μm.





possible to determine the exact number of beads present, but soon after the flaps were put back in place, it was possible to see that beads had indeed become lodged under the roof of the blastocoel. The beads gave an irregular contour to the overlying epithelium, much like one would see with several baseballs underneath a carpet. We determined that gastrulation progressed normally because there was induction of a normal looking medullary plate during neurulation in all embryos followed by the formation of a neural plate with a conspicuous neural groove along the entire cranial-caudal axis of the embryo. Wound healing was complete in all sham operated controls, so that by the end of the incubation period after the operation, there was a continuous layer of ectodermal cells over the ventral endodermal mass. Embryos that received beads in the blastocoel usually had ventral ectodermal defects after 5 h in culture. These defects were seen in embryos receiving blank beads and FN-beads, suggesting that the beads may present some physical impediment to normal wound healing. Embryos receiving

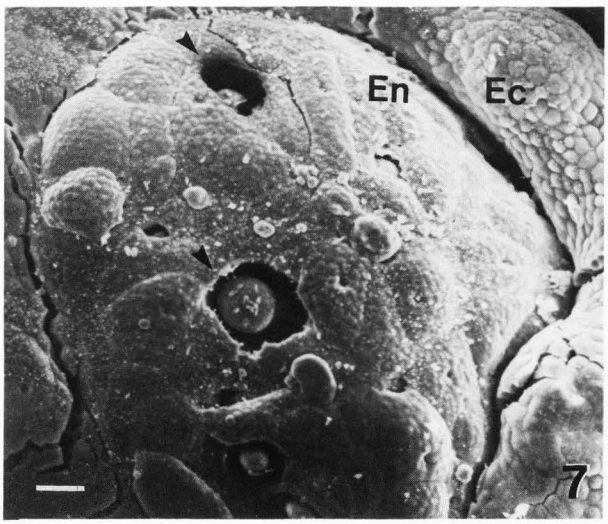


Figure 5. SEM of a sham operated control embryo. This embryo had a flap cut in the roof of the blastocoel at Stage 10 and then was allowed to develop undisturbed for 5 h. Gastrulation has been completed and a medullary plate with a neural groove has formed. The ventral surface of sham operated embryos is completely covered by fully healed surface ectoderm. Bar = 100 μm.

Figure 6. SEM of an embryo receiving FN-Sepharose beads in the blastocoel through a flap cut in the roof of the blastocoel at Stage 10 and then allowed to develop undisturbed for 5 h. Gastrulation has been completed and a medullary plate with neural groove has formed. Bar = 100 μm.

Figure 7. SEM of the ventral surface of an embryo receiving FN-Sepharose beads. The ventral endodermal mass (En) is not completely covered by surface ectoderm (Ec). Instead, there is a patch of endoderm exposed on the ventral surface of the embryo. Notice the FN-Sepharose beads embedded among the endodermal cells (arrows). Bar = 50 μm.

blank beads had no beads visible on the surface of the ventral ectodermal defects. In contrast, in embryos that received FN-beads, added beads could be seen among the surface cells in the ventral ectodermal defects. The results of these experiments suggest that beads in the blastocoel prevent normal wound healing but have no special effect on invagination of the cells responsible for primary embryonic induction. The results of these experiments are shown in Figures 5-7.

Discussion

The present results show that cell adhesion to FN-Sepharose beads is slight prior to the beginning of gastrulation but increases throughout gastrulation in Xenopus laevis. This low adhesion to FN-beads is not the result of a generalized inability to adhere to appropriate substrata, since blastula stage cells from these embryos adhere to con A-beads, cytodex-1 beads, and cytodex-3 beads. In addition, the results reported here show that gastrula stage cells have surface glycoconjugates that react with some but not all lectins. Similar results have been reported recently for Rana pipiens, R. sylvatica, and Ambystoma maculatum (Johnson, 1985). In addition, it was found that the interspecific arrested hybrid formed by fertilizing the eggs of R. pipiens with the sperm of R. catesbeiana is defective in its ability to adhere to FN-beads (Johnson, 1985). The development of the ability to adhere to FN-beads during gastrulation may be a reflection of the appearance of cell surface molecules that recognize FN in some specific fashion. If these FN-receptors were under some developmental control, their appearance, especially in cells from the dorsal portion of embryos, may account for the appearance of a network of FN-containing extracellular fibrils along the inner aspect of the roof of the blastocoel in early gastrulae in Ambystoma (Nakatsuji et al., 1982), Rana (Nakatsuji and Johnson, 1984a), Pleurodeles (Boucaut and Darribere, 1983), and Xenopus (Lee et al., 1984; Johnson, 1984).

Recently, there has been a detailed study of the synthesis of FN during gastrulation in Xenopus laevis by Lee and co-workers (1984). They examined FN synthesis and distribution during early development. They showed that FN synthesis increased following the middle of the blastula stage and that it was secreted by all parts of the embryo. Beginning at the early gastrula stage, however, they were able to detect FN localization by the use of fluorescent antibodies and were able to show that it is concentrated along the inner aspect of the roof of the blastocoel, a site known to contain fibrils that react positively with colloidal gold labelled anti-fibronectin antibodies (Nakatsuji et al., 1985). Lee et al. (1984) suggested that the differential distribution of FN as an accumulation along the inner surface of the roof of the blastocoel is achieved by "...spatially localized receptors that bind secreted fibronectin." Their immunofluorescent studies on sections of embryos also suggest that at all stages, the dorsal portions of embryos contain more FN and therefore perhaps more FN-receptors. This observation fits nicely with our observation that dorsal cells invariably bind more avidly to FN-beads than ventral cells.

We studied the binding of Xenopus gastrula cells to lectin- and mucin-beads to try to learn more about the surface glycoconjugates of these cells. We know that the surfaces and extracellular spaces around gastrula cells bind lectins and contain high molecular weight glycoconjugates. The results of the binding of cells to lectin-beads also corresponds to earlier results. Johnson and Smith (1977) showed that gastrula stage cells bind FITC-con A and FITC-soy bean agglutinin when they are dissociated and then incubated in labelled lectins. Furthermore, Johnson (1984) has shown that Xenopus gastrulae show active incorporation of galactose, mannose, and glucosamine; but, are inactive in fucose incorporation into glycoconjugates of the cell surface and extracellular matrix. These glycoconjugates have been shown to be acidic mucin-like materials that cover the surface of cells and also serve as an extracellular matrix to which nearby cells can adhere. This notion is reinforced by the fact that gastrula stage cells adhere to con A- SBA-, and WGA-Sepharose beads and to mucin-Sepharose beads, but not to TPA-Sepharose beads.

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Discussion with Reviewers

G.C. Schoenwolf: In all cases, the experiments deal with mixed populations of cells. What effect did this heterogeneity have on the adhesion assay?
Authors: We are not certain what effects cell heterogeneity has on these assays. We have not performed regional dissections of embryos in an attempt to answer this question. However, we have observed that small, medium, and large cells all have the ability to adhere to FN-beads. It would be interesting to separate populations of cells on ficoll gradients to examine the effects of size heterogeneity on this assay.
G.C. Schoenwolf: Were any embryos sectioned after implantation of FN-coated beads and gastrulation had occurred to determine if the microanatomy was normal?
Authors: We have not sectioned embryos. Our sole criterion for normal morphogenesis was the establishment of a normal looking neurula. Admittedly, it would be interesting to have more information on the detailed microanatomy of treated embryos and also to have time-lapse films of embryos with implanted FN-beads.
G. C. Schoenwolf: Were statistics used to determine whether significant differences were found? I would guess that a statistically significant difference might exist in the ventral cells between stages 8 and 10, but not in the dorsal cells until stages 8-12. Could the change dorsally be more related to neurulation than gastrulation?
Authors: We are not concerned with the significance of differences from one stage to the next. Rather, we are most interested in the strong upward trend in cell adhesion during development. Linear regression analysis showed us that there was a significant upward trend in our data. We also don't know specifically how these developmental changes relate to gastrulation or neurulation in any detailed sense. Fibronectin receptors almost certainly increase in number throughout gastrulation and neurulation as the total amount of fibronectin increases in the embryo. At the present time, Prof. J-C Boucaut (Paris) and I (KEJ) are actively investigating these possibilities.
R.O. Kelley: What cells are responsible for the stage-specific secretion of fibronectin in Xenopus embryos and what mechanisms are in play which assist the cell surface in recognizing that extracellular molecule?
Authors: Lee et al. (1984) have shown that FN synthesis occurs with approximately equal intensity in all parts of the embryo, although their dissections did not divide the embryo into a large number of specific regions. This question needs to be investigated in more detail. Presumably, the cell surface recognizes extracellular FN by way of cell surface FN receptors. We are currently collaborating with J-C Boucaut to examine the distribution of FN-receptors in amphibian gastrulae, using antibodies directed against the chick cell surface FN-receptor.
R.O. Kelley: Is any organization of fibronectin apparent in the blastula? the gastrula?
Authors: In urodeles, where FN is more abundant, blastulae have a sparse network of 0.1 μm fibrils. During gastrulation, this network becomes much denser, looking much like a spider's web on the surface of cells. In anurans, where FN is less abundant, blastulae have no fibrils and gastrulae have scattered 0.1 μm strands that branch and fork on the surface of cells.
R.O. Kelley: Is the cell surface of blastomeres (archenteron, presumptive neural ectoderm, etc.) interacting with fibronectin in a manner similar to other cell systems where the link proteins have been established?
Authors: At the present time, we have no information bearing on that interesting point. In future, we plan to see if cell anchorage sites are related to the cellular distribution of vinculin and α-actinin.
R.O. Kelley: Are cell surface determinants, detected by coated bead probes, differentiating (i.e. appearing as new moieties in development) or are other surface events simply permitting their presentation at the cell surface?
Authors: Our tentative interpretation of our results is that FN-receptors increase in number during gastrulation, thus allowing increasing cell adhesion to FN-beads. Other interpretations are equally valid. As mentioned above, we plan to examine the distribution of FN-receptors during gastrulation.