Mesoderm migration in the *Xenopus* gastrula

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ABSTRACT During *Xenopus* gastrulation, the mesoderm involutes at the blastopore lip and moves on the inner surface of the BCR toward the animal pole of the embryo. Active cell migration is involved in this mesoderm translocation. *In vitro*, mesoderm cells migrate non-persistently and intermittently by extending and retracting multiple lamellipodia, which pull the cell body in their direction. Lamellipodia formation is induced by FN. FN fibrils are present on the BCR as part of the *in vivo* substrate of mesoderm migration. Mesoderm cells can attach to the BCR independently of FN, but interaction with FN is required for lamellipodia extension and cell migration on the BCR. In contrast to preinvolution mesoderm, involuted migrating mesoderm always stays on the surface of the BCR cell layer: migrating mesoderm cells do not mix with BCR cells, and a stable interface between tissues is maintained. A corresponding change in cell sorting behavior occurs during mesoderm involution. In *Xenopus*, the mesoderm moves as a multilayered coherent cell mass held together by cadherin-mediated cell adhesion. Aggregate formation changes mesoderm cell behavior, rendering it more continuous, persistent and directional, i.e. more efficient. The mesoderm possesses an intrinsic tissue polarity which biases the direction of its movement. In addition, the fibrillar FN matrix of the BCR contains guidance cues which also direct the mesoderm toward the animal pole. Haptotaxis is most likely not involved in this substrate-dependent guidance of the mesoderm, but intact FN fibrils seem to be required. A polarity of the BCR cell layer which underlies this anisotropy of the BCR matrix develops under the influence of the marginal zone in the late blastula. Although in other amphibian species, gastrulation depends critically on mesoderm cell migration, in *Xenopus*, convergent extension of the axial mesoderm seems to provide the main driving force for gastrulation.

KEY WORDS: *gastrulation, mesoderm, cell migration, Xenopus*

Introduction

Although gastrulation as a whole appears characteristically different in the various vertebrate groups, mesoderm movement during gastrulation exhibits profound similarities. Particularly, migration of the mesoderm across the inner surface of the outer embryonic layer is a well conserved feature of vertebrate gastrulation (Winklbauer, 1994). This essential morphogenetic process is perhaps best understood in the amphibian embryo.

In the amphibian blastula, a ring of prospective mesoderm surrounds the embryo below the equator. The activities of this mesoderm drive much of the gastrulation process. At the lower, vegetal margin of the mesoderm, a blastopore invaginates, first dorsally and then laterally and ventrally, to encompass eventually the whole embryo. The mesoderm above it begins to involute. It rolls over the blastopore lip, becomes apposed to the inner surface of the blastocoel roof (BCR), and moves away from the lip toward the animal pole of the embryo. This movement involves cell migration, i.e. active crawling of mesoderm cells on the inner surface of the BCR. In *Xenopus*, all mesoderm is covered by a layer of suprablastoporal endoderm which moves passively with the mesoderm (Fig. 1) (Keller, 1986).

Migration of the amphibian mesoderm is characterized by three basic features. First, the mesoderm moves as a multi-layered coherent cell mass, and not as a loose stream of individually migrating cells. Second, cells migrate on a planar substrate, the BCR cell layer, which is covered by a network of extracellular matrix fibrils. Fibronectin (FN) is a major component of these fibrils and plays an important role in mesoderm cell migration (Boucaut and Darribere, 1983a,b; Nakatsuji and Johnson, 1983a,b; Boucaut *et al.*, 1984a,b, 1985; Darribere *et al.*, 1985, 1988, 1990; Nakatsuji *et al.*, 1985). Third, mesoderm movement away from the blastopore lip and toward the animal pole region of the gastrula is goal-directed. In the present article, we review our work on mesoderm migration in the *Xenopus* gastrula and discuss it in the context of the results of others.

Motile activities of mesoderm cells

Cell translocation by crawling is very common, but it is not a single, distinct mechanism. Instead, different cell types show dif-

Abbreviations used in paper: BCR, blastocoel roof; FN, fibronectin; HM, head mesoderm; RGD, Arg-Gly-Asp.

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0214-6282/96/$03.00

ferent types of crawling locomotion. In the opaque *Xenopus* embryo, cell movement cannot be observed directly. To obtain basic data on how mesoderm cells translocate, mesoderm cell motility has to be studied *in vitro*. The first cells of the mesoderm to engage in migration are the prospective head mesoderm (HM) cells. It is mainly these cells that we examine.

Gastrula-stage HM cells of *Xenopus* are large, measuring about 50-100 µm in diameter. The cell body is packed with yolk platelets and other formed inclusions. On a non-adhesive substrate, two types of attachment-independent, constitutively expressed motile activities can be discerned on isolated cells. First, the globular cell body shows a constant kneading motion (Winklbauer and Selchow, 1992). Since actin microfilaments are concentrated at the cell membrane, we presume that HM cells possess a typical contractile cell cortex (A. Selchow, unpublished results). The second type of motile activity of non-attached HM cells is the spontaneous formation of cytoplasmic processes. Filiform protrusions extend singly or in groups from the cell surface into the medium and retract again after a few minutes. New processes usually appear close to the site of previous ones, thus defining an active region on the cell surface (Winklbauer *et al.*, 1991; Winklbauer and Selchow, 1992). The filiform processes contain also actin filaments and appear continuous with the cell cortex (A. Selchow, unpublished results).

The protrusive activity of HM cells is altered through interaction with an adhesive substrate: processes become lamelliform, and they extend along the substrate surface instead of protruding freely into the medium (Winklbauer and Selchow, 1992). This modulation of protrusive activity is brought about most effectively by FN, which is part of the *in vivo* substrate of HM cells. Several minutes after contact with a FN substrate, two cytoplasmic lamellae appear simultaneously at opposite ends of an HM cell. Movement of lamellae in opposite directions leads to bipolar spreading. Additional lamellae may thereafter appear. Eventually, HM cells show a bipolar or multipolar morphology (e.g. Fig. 5b) (Winklbauer *et al.*, 1991; Winklbauer and Selchow, 1992).

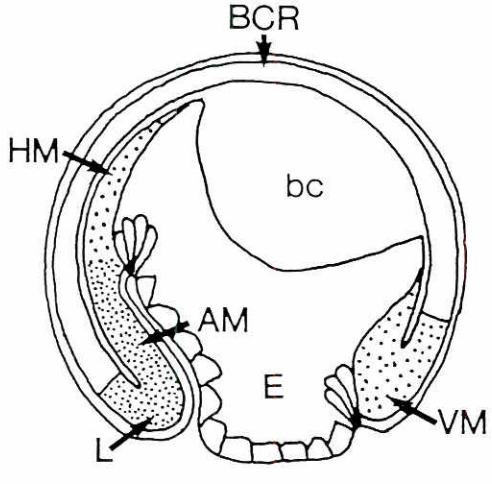

Fig. 1. Schematic drawing of a sagittal section through a *Xenopus* middle gastrula. *Future dorsal side to the left, animal pole to the top. bc, blastocoel; BCR, blastocoel roof; HM, prospective head mesoderm; AM, prospective axial mesoderm; E, prospective endoderm.*

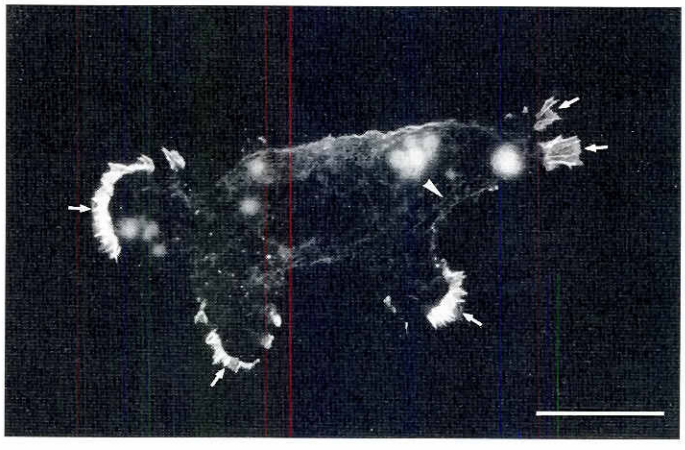

Fig. 2. Filamentous actin in HM cells. *Cells migrating on FN were fixed, and their upper parts were removed by exposing cells to the surface tension of the fixative solution. The lower, substrate-apposed cytoskeleton remained in place. Filamentous actin was visualized by staining with rhodamine-phalloidine. Lamellipodia (arrows) show prominent staining. Weakly staining actin filament bundles, but no stress fibers, are present in the lower cell cortex (arrowhead). Bar, 25 µm.*

The distal margin of cytoplasmic lamellae is densely packed with polymerized actin, including radially oriented actin filament bundles, as is typical for lamellipodia (Fig. 2). These lamellipodia are connected to the cortex of the cell body by a less dense array of actin filament bundles (A. Selchow, unpublished results). Like the protrusions of non-attached HM cells, the lamellipodia are dynamic, short-lived structures which are constantly extending, retracting, dividing, or moving laterally along the cell margin (Winklbauer and Selchow, 1992).

This behavior of lamellae is intimately linked to the mechanism of HM cell translocation. What may be thought typical of crawling locomotion, that a cell is following a more or less continuously advancing leading lamella, is very rarely observed with HM cells. Usually, several lamellae are present simultaneously, which exert traction and deform the cell body until equilibrium of forces is reached. Translocation occurs then only when the number or arrangement of lamellae is changed, which disturbs the balance of forces. The elastic and contractile cortex of the cell body apparently aids in rapidly attaining a new equilibrium, by moving the cell contents in the direction of resultant traction forces. The body of crawling HM cells is less strongly attached than lamellae (Winklbauer and Selchow, 1992). No focal contacts can be observed underneath the cell body (A. Selchow, unpublished results), and no stress fibers develop (Fig. 2). Its weak attachment certainly facilitates hauling of the cell body by the lamellae. The presence of several independently acting lamellae could be a consequence of the large relative size of the cell body. The dependence of movement on lamella turn-over leads to a step-wise and non-persistent mode of translocation (Winklbauer *et al.*, 1991; Winklbauer and Selchow, 1992).

Before involution, prospective mesoderm cells are part of the BCR which forms the substrate layer for mesoderm migration (Fig. 1). Interestingly, preinvolution mesoderm cells are completely stationary. They attach to FN substrates *in vitro*, spread and extend processes, but do not migrate. In contrast, after involution, the same cells translocate on FN *in vitro* (Winklbauer,

1990). Apparently, the ability to interact with FN is not sufficient to initiate mesoderm cell migration, but some presently unknown change in the motile apparatus of the cells is required.

Interaction of mesoderm cells with the blastocoel roof substrate

Crawling cells need a substrate for translocation which resists the traction exerted by their locomotory protrusions. The *in vivo* substrate for the migrating mesoderm is the FN fibril matrix on the BCR and the surface of BCR cells exposed between fibrils. As seen in the electron microscope, mesoderm cells can be in direct, close contact with the basal surface of BCR cells (Nakatsuji, 1976). Accordingly, when cell-FN interaction is prevented (e.g. by RGD-containing peptides or FN antibodies), mesodermal cells still attach to the BCR. However, they remain globular and do not spread or extend lamelliform protrusions (Winklbauer, 1990; Winklbauer *et al.*, 1991; R. Winklbauer, unpublished results). FN-independent attachment to the BCR shows that adhesion of mesoderm cells does not by itself lead to rapid cell spreading and lamella formation. Instead, these latter processes depend on cell-FN interaction. Thus, FN fibrils on the BCR seem to have a similar effect as FN substrates *in vitro*, namely to induce the extension of lamellae along the substrate surface.

The step-wise, non-persistent mode of HM cell migration observed *in vitro* is not altered when isolated cells move on their *in vivo* substrate. Cell trails show characteristic, abrupt turns, like cells moving on FN *in vitro*, and cells migrate persistently only over short distances (Fig. 3a). However, even this limited persistence is lost when cell interaction with FN is inhibited by an RGD-containing peptide, and cells move on convoluted, random pathways (Fig. 3b) (Winklbauer, 1990; Winklbauer *et al.*, 1991). As noted above, cells attach to the BCR under these conditions, but do not extend lamellae. Apparently, FN-induced lamella formation stabilizes the movement of HM cells such that some degree of persistence is attained. Thus, although FN will certainly contribute to the attachment of mesoderm cells to the BCR, due to its adhesive properties, its more specific role in mesoderm migration is the induction of lamellipodium formation. In this way, the BCR substrate not only provides adhesiveness and resistance to mesoderm cell traction, but also regulates the protrusive activities of migrating cells.

Migrating mesoderm cells remain always on the surface of the BCR layer, regardless of whether interaction with FN is inhibited or not. They do not integrate into the BCR cell layer, although they are not physically isolated from it. Obviously, this is a necessary condition for mesoderm migration to be effective. On the other hand, such behavior is by no means trivial. Thus, when preinvolution mesoderm, which is then still part of the BCR, is placed on a BCR explant, it does not stay on its surface, but it reintegrates completely into the BCR cell layer within minutes (Fig. 4) (R. Keller, R. Winklbauer, S. Wacker, unpublished results). Apparently, for gastrulation to proceed normally, a change in the sorting out behavior of mesoderm has to occur during involution, such that a stable BCR-mesoderm interface can develop. By testing small mesoderm explants from different regions and developmental stages, we found that the postulated transition in mesoderm behavior does indeed take place during involution. The transition does not occur autonomously in the mesoderm, but requires signalling from more vegetal parts of the gastrula (S. Wacker, unpublished results). An understanding of this change in cell sorting behavior and its regulation will be fundamental to an understanding of *Xenopus* mesoderm involution.

Mesoderm cell-cell interaction

An aspect of amphibian mesoderm migration which has largely been neglected is that in the gastrula, the mesoderm moves as a compact, multilayered cell mass on a planar substrate. This constrains the mechanics of mesoderm translocation: only the

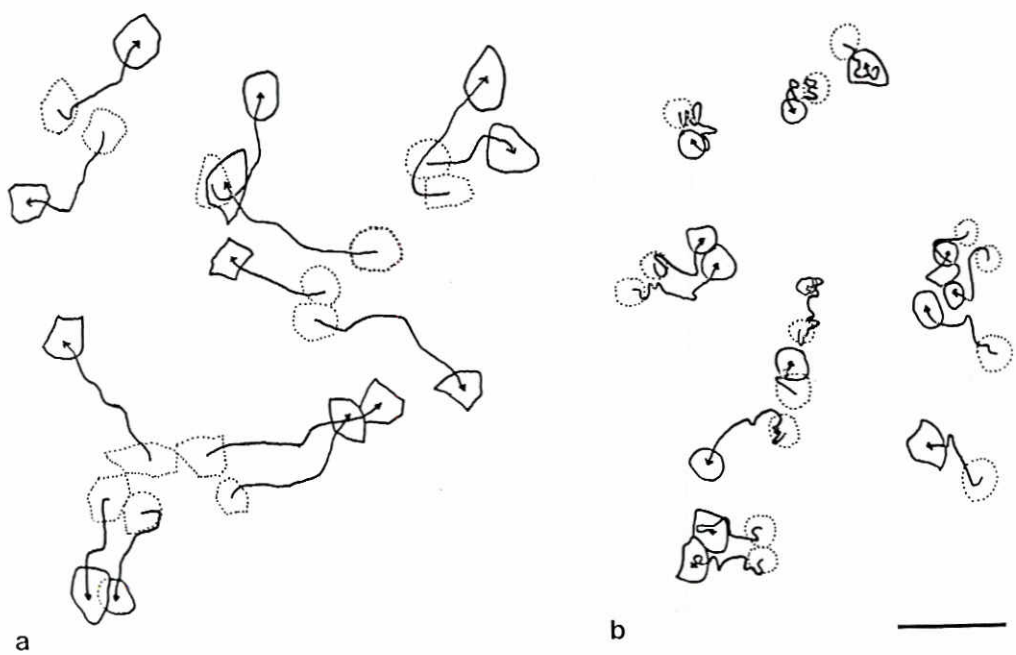

Fig. 3. Migration of isolated HM cells on the BCR. *Cells seeded onto the BCR were visualized by indirect illumination and filmed. The initial (dotted outline) and the final (solid outline) positions of cells, and the cells paths during a 1 h interval are indicated for a control explant* **(a)**, *and for cells migrating in the presence of 4 mg/ml of GRGDSP peptide* **(b)**. *Bar, 100 µm. From Winklbauer (1990) with permission from Academic Press.*

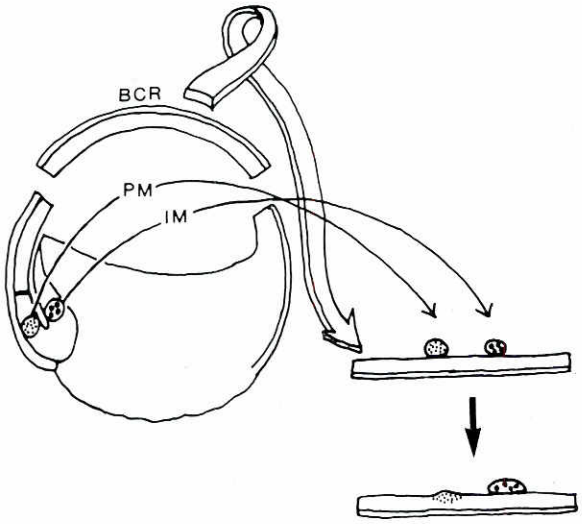

Fig. 4. Change in cell sorting behavior accompanying mesoderm involution. *Preinvolution mesoderm (PM) and involuted mesoderm (IM) is placed on a piece of BCR. After 15-30 min, preinvolution mesoderm sinks into the BCR cell layer, whereas involuted mesoderm stays on the surface.*

basal cells of the mesoderm, those in direct contact with the substrate, are in a position to migrate actively, whereas the majority of cells has to be carried along passively. This requires cohesion among mesoderm cells (Winklbauer *et al.*, 1992).

Empirical evidence confirms that the *Xenopus* mesoderm moves in fact as a coherent cell mass, and not as a stream of individually migrating cells. Thus, mesoderm can be excised from the gastrula and moved around as a coherent piece of tissue. *In vitro*, mesoderm explants spread on a proper substrate and move as a whole, with single cells rarely separating (Fig. 5a) (Winklbauer, 1990; Winklbauer *et al.*, 1991, 1992). In the intact gastrula, the advancing mesoderm always possesses a distinct leading edge, and single cells very rarely migrate ahead of it (Keller and Schoenwolf, 1977; Winklbauer and Nagel, 1991; Winklbauer *et al.*, 1991; Winklbauer and Selchow, 1992). To study isolated mesoderm cells, mesoderm explants must be dissociated into single cells in Ca^{++}-free medium.

Mesoderm cells do not stick to each other non-specifically. Their ability to form aggregates is founded in the expression of cadherin-type adhesion molecules on the cell surface. Closely related forms, EP/C-cadherin and XB/U-cadherin, are present in the *Xenopus* gastrula (Choi *et al.*, 1990; Herzberg *et al.*, 1990; Angres *et al.*, 1991; Ginsberg *et al.*, 1991; Muller *et al.*, 1994), and a functional antibody to XB/U-cadherin dissociates mesoderm explants into single, individually migrating cells (Fig. 5) (Winklbauer *et al.*, 1991, 1992). Thus, the molecular basis for the mechanically required cohesion of the mesoderm is to some extent understood.

It is an interesting question how aggregated mesoderm cells manage to migrate under such crowded conditions. For example, many different cell types show contact inhibition of movement. When an advancing lamellipodium contacts another cell, it immediately stops or even retracts. *Xenopus* HM cells also show contact inhibition of movement. However, inhibition occurs only when two lamellae collide. When a lamella encounters the cell body of another cell, it may continue to extend, thereby underlapping it (Winklbauer *et al.*, 1992). This can explain how mesoderm cells are able to extend protrusions when moving as coherent cell masses.

Besides being a mechanical necessity, aggregation has also pronounced effects on mesoderm migratory behavior. First, it stabilizes cell movement. Cells in aggregates migrate more persistently and more continuously than isolated cells, which amounts to cell movement being more efficient. Second, and most importantly, only when mesoderm cells form aggregates are they able to follow guidance cues in the BCR matrix which direct them to the animal pole (Winklbauer *et al.*, 1991, 1992).

Factors determining the direction of mesoderm movement

Dispersal of individual cells over a planar substrate does not require directional cues to guide cells away from the source region: random cell migration in combination with contact inhibi-

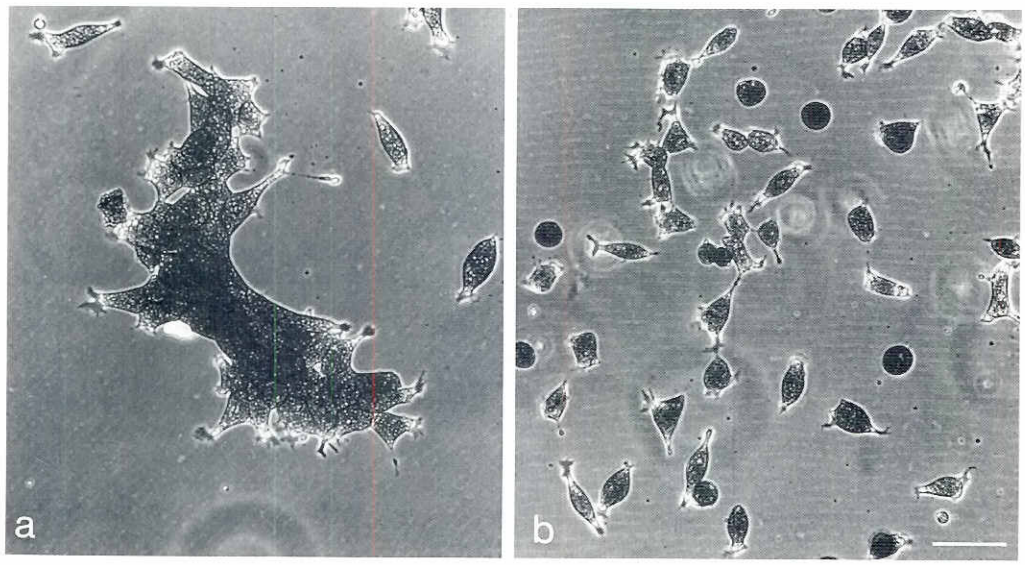

Fig. 5. Cadherin-dependent cohesion of the mesoderm. *HM explants on FN in vitro were observed. Explants were cultured in the presence of an inert P3 control antibody* **(a)**, *or of 10 µg/ml of an antibody against XB/U-cadherin, which leads to explant disintegration and single cell migration* **(b)**. *Photographs were taken after 3 hours in culture. Bar, 100 µm. From Winklbauer et al. (1991) with permission from Plenum Press.*

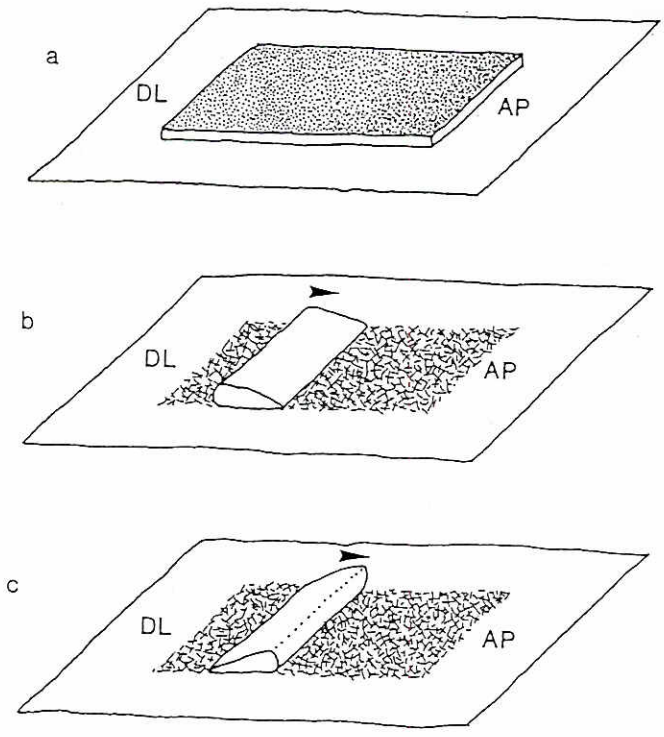

Fig. 6. Directional mesoderm migration on conditioned substrate. **(a)** *A piece of BCR (stippled), with known orientation (DL, dorsal blastopore lip; AP, animal pole), is cultured for 2 h with its inner surface down to transfer its extracellular matrix to the bottom of the culture dish.* **(b)** *After removal of the BCR explant, anterior dorsal mesoderm (HM) is placed in normal orientation (tapering anterior end toward AP) on the conditioned substrate (dashes).* **(c)** *HM on conditioned substrate in reverse orientation, with tapering anterior end toward DL. Mesoderm explants move to the AP in* **(b)** *and* **(c)** *(arrowheads). From Winklbauer et al. (1993) with permission from Plenum Press.*

tion of movement is usually sufficient. However, the situation may be different when multiple layers of cells have to be moved as a whole, as during mesoderm translocation away from the blastopore. Here, guidance mechanisms could be helpful which determine the direction of migration of each cell in contact with the substrate. These coordinately translocating basal cells could then move the attached layers of cells. Both an intrinsic tissue polarity in the mesoderm and external cues located on the BCR could contribute to this directionality of migration.

In *Xenopus*, a strip of mesoderm on FN migrates in the direction of its anterior end, as it would in the embryo (Winklbauer, 1990). This demonstrates an intrinsic mesodermal tissue polarity able to determine the direction of movement. Interestingly, a gradient of adhesiveness to FN extends along the antero-posterior axis of the mesoderm, i.e. along the axis of its movement (Winklbauer, 1990). It is not known how this gradient is related to the tissue polarity which determines the direction of migration.

Guidance cues directing mesoderm movement also reside in the extracellular matrix of the BCR. This has been shown first for urodele embryos. The BCR extracellular matrix can be transferred to an inert *in vitro* substrate by culturing a BCR explant with the matrix-bearing side down. In *Ambystoma*, mesoderm cells show a preference for migrating toward the animal pole position on such a conditioned substrate (Nakatsuji and Johnson, 1983a). In Pleurodeles, explanted mesoderm moves as a coherent aggregate toward the animal pole on conditioned substrate (Shi *et al.*, 1989). This is also the case for the anterior (HM) mesoderm of *Xenopus* (Fig. 6), where tissue polarity is less strongly expressed, as compared to the more posterior mesoderm (Winklbauer *et al.*, 1991, 1992; Winklbauer and Nagel, 1991). Thus, BCR extracellular matrix is somehow oriented, and this directionality is sufficient to guide mesoderm toward the animal pole. In the embryo, both the intrinsic polarity of the mesoderm and the external cues located in the substrate guide the mesoderm in the same direction.

The nature of the substrate-dependent guidance cues and their mechanism of action are unknown. One hypothetical possibility would be haptotaxis, where cells move up a gradient of adhesiveness of the substrate. In *Xenopus*, FN seems to be the only matrix component mediating mesodermal cell adhesion to conditioned substrate (Winklbauer and Nagel, 1991). However, a gradient in FN density along the blastopore-animal pole axis of the BCR could not be detected (Nakatsuji *et al.*, 1985a; M. Nagel, unpublished results). Moreover, no difference in the adhesiveness of the substrate along the pathway of migration could be demonstrated by directly measuring HM cell adhesion to different regions of conditioned substrate (Winklbauer and Nagel, 1991). This makes haptotaxis very unlikely to be involved in mesoderm guidance.

On the other hand, directional migration requires the presence of intact FN fibrils. Fibril assembly, but not FN deposition is inhibited when substrate is conditioned in the presence of RGD peptide or cytochalasin B. On such a substrate of diffusely adsorbed FN, mesoderm explants are still able to migrate. However, guidance is lost, and explants move randomly in all directions (Winklbauer *et al.*, 1991; Winklbauer and Nagel, 1991). This points to the possibility, first expressed by Nakatsuji and Johnson (1983a), that the matrix fibrils themselves are polarized. For example, FN molecules could arrange themselves in a polar manner within fibrils, and with the resultant polarity of the fibril network pointing to the animal pole, the substrate would possess proper directionality. We are currently investigating the structure of FN fibrils on the BCR with the aid of monoclonal antibodies to *Xenopus* FN.

Whatever the exact nature of the substrate-dependent cues may be, their local effects on the direction of mesoderm movement have to be coordinated over the whole BCR to ensure migration toward the animal pole from all positions on the BCR. The most likely possibility is that the BCR cell sheet possesses a globally coordinated anisotropy which can be translated into a corresponding orientation of the extracellular matrix. This underlying tissue polarity of the BCR apparently develops in the late blastula under the influence of the marginal zone. When BCR is isolated without marginal zone at the mid-blastula stage, FN fibrils are formed as normally at the time of gastrulation, but the matrix is not able to direct mesoderm migration. In contrast, when marginal zone is added back to the BCR explant, the tissue polarity required for orienting the BCR matrix is induced in the BCR (M. Nagel, unpublished results). The cellular or molecular basis of this tissue polarity is obscure.

On conditioned substrate or on the BCR, isolated mesoderm cells migrate in all directions equally well. Only aggregates of

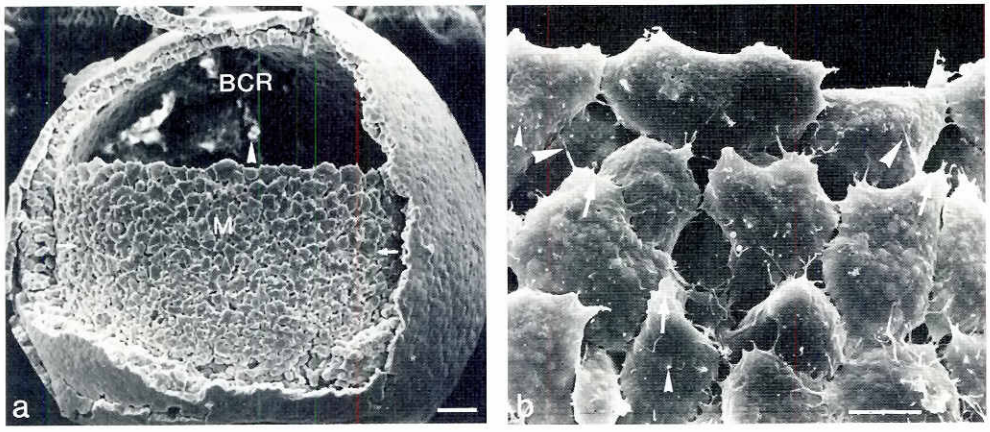

Fig. 7. Migrating dorsal mesoderm in the SEM. (a) *Middle gastrula with BCR removed from dorsal side. Animal pole to the top. The side of the migrating mesoderm (M) normally facing the BCR substrate is exposed. Anterior zone with shingle arrangement of cells above arrows. Arrowhead indicates direction of mesoderm movement. Bar, 100 µm.* **(b)** *Shingle arrangement of anterior cells, as viewed from the substrate side. Cells with filiform (large arrowheads) and lamelliform (arrows) protrusions, and small, short projections (small arrowheads). Bar, 20 µm. From Winklbauer and Nagel (1991) with permission from Academic Press.*

mesoderm cells move consistently toward the animal pole on conditioned substrate (Winklbauer *et al.,* 1992). Thus, aggregation is essential for the ability of mesoderm cells to follow the guidance cues provided by the substrate. One of the primary effects of aggregate formation seems to be on cell morphology. In contrast to isolated cells, aggregated mesoderm cells moving directionally appear unipolar when viewed from the substrate side. Cytoplasmic lamellae extend from the cell body preferentially in the direction of mesoderm movement, toward the animal pole.

Moreover, the posterior part of a cell is typically underlapped by the anterior part of the cell behind it, so that only the anterior edge of any cell is in contact with the substrate (Fig. 7). This shingle arrangement of unipolar cells is consistent with the directional migration and the high persistence of movement under these conditions (Winklbauer and Nagel, 1991; Winklbauer *et al.,* 1991, 1992). It will be important to find out how mutual cell contact allows mesoderm cells to assume a unipolar morphology.

The role of mesoderm cell migration in *Xenopus* gastrulation

Coordinated traction of mesoderm cells on the BCR should generate forces that tend to move the mesoderm toward the animal pole. Inhibition of mesoderm-FN interaction arrests gastrulation in the amphibians *Pleurodeles* (Boucaut *et al.,* 1984a,b; Darribere *et al.,* 1988, 1990) and *Rana* (Johnson *et al.,* 1993), and the mesoderm does not come to occupy its normal position. This suggests that interaction with FN is necessary for mesoderm translocation, and that gastrulation depends indeed on active mesoderm migration in these species.

The situation is different in *Xenopus*. Here, an important component of mesoderm movement is the substrate-independent convergence and extension of the more posterior dorsal mesoderm that will form axial structures like notochord and somites. Convergence of the axial mesoderm is driven by active cell intercalation within the tissue, and leads to the autonomous extension of this part of the mesoderm toward the animal pole, providing for most of its normal gastrulation movements (Keller, 1986; Keller and Winklbauer, 1992). Moreover, due to the predominant role of convergent extension in *Xenopus* gastrulation, nearly normal gastrulation is observed even when the substrate of mesoderm migration, the BCR, is removed before gastrulation (Keller and Jansa, 1993).

In view of these facts, it is no surprise that gastrulation in *Xenopus* cannot be substantially arrested by RGD peptides (Winklbauer, 1989; Smith *et al.,* 1990; R. Winklbauer, unpublished results). As discussed above, presence of RGD peptide inhibits mesoderm cell migration on explanted BCR (Winklbauer, 1990; Winklbauer *et al.,* 1991; R. Winklbauer, unpublished results). Moreover, in embryos injected with RGD peptide or antibody to FN, mesoderm cells in contact with the BCR substrate no longer extend cytoplasmic lamellae, which indicates that under these conditions mesoderm cells do not migrate (R. Winklbauer, unpublished results). Nevertheless, the mesoderm advances toward the animal pole, apparently in a substrate-independent manner, as in gastrulae from which the BCR has been removed. Injecting Fab fragments of antibodies to *Xenopus* FN has in our hands exactly the same effects as RGD peptide (R. Winklbauer, unpublished results). Antibody against FN or ß1 integrin has been reported to arrest gastrulation in *Xenopus* (Howard *et al.,* 1992), but these experiments are difficult to interpret since whole antisera were injected.

It should be mentioned that despite its overall resistance to RGD peptide inhibition, gastrulation in the absence of FN interaction is not completely normal. Although the dorsal mesoderm attains its normal position, the ventral mesoderm may not move correctly, and the vegetal yolk plug is not completely internalized (R. Winklbauer, unpublished results). This may help to explain why the migratory ability of the mesoderm did not decay during *Xenopus* evolution, despite its reduced importance for the mechanics of gastrulation in this species.

Acknowledgments

This article is dedicated to Prof. Peter Hausen on the occasion of his 60th birthday. Research of the authors is supported by the DFG.

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