Mechanisms of Protrusion and Cell Locomotion*
(Actomyosin-based Contraction in the Cell Body and Uropod Rather than Actin-based Motors at the Leading Front Drives Locomotion of Walker Carcinosarcoma Cells)

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Earlier models explaining cell locomotion are briefly reviewed. Then, a model explaining locomotion of non-adhesive Walker carcinosarcoma cells is proposed based on the following data: 1) Walker carcinosarcoma cells, which normally form lamellipodia, can produce forces for movement by at least two distinct actin-based mechanisms, 2) Lamellipodial motility is driven by local actin polymerization, but lamellipodia and actin-based mechanisms (polymerization or contraction) at the front are redundant for locomotion, 3) actomyosin-dependent contraction at the rear (body and/or uropod) is sufficient and necessary for locomotion, 4) fluid pressure can generate protrusion (blebs), 5) an intact cortical layer at the front tends to reduce the speed of locomotion, 6) there is no biologically significant difference in the efficiency of locomotion (speed, persistence, net displacement) of migrating cells showing either lamellipodia, blebs or no morphologically recognizable protrusions, 7) polymerized actin is concentrated in the cortical actin layer. Myosin II A is preferentially associated with the actin cortex at the rear part of the cell. The data suggest that actomyosin-based contraction in the form of cortical contraction generates protrusion and locomotion in Walker carcinosarcoma cells as previously described in Amoebae. The role of actomyosin-dependent contraction and of fluid-driven mechanisms in other metazoan tissue cell lines is discussed.

Key Words: Cell Migration, Persistence, Lamellipodia, Blebs, Contraction, Actin, Myosin, Fluid Pressure

1. Force Generation for Locomotion of Tissue Cells: Where is the Motor and How are Forces Generated?

The major concepts of cell locomotion have first been developed in amoebae. The model developed by Mast in 1926 suggested that locomotion is driven by contraction of the rear of the cell, the model proposed by R.D. Allan (fountain zone contraction theory) in 1961 suggested that frontal contraction drives the cell forwards (for review see Ref. (1)). The present knowledge summarized by Grebecki(10) is that forces in *Amoeba proteus* are generated in the cell body by actomyosin-dependent cortical contraction and that the front relaxes in a coordinated manner. Later, the question whether the motor for locomotion is at the front or the rear has been once again debated with respect to metazoan tissue cells(21,22). Based on the work of Abercrombie, many investigators concluded

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that the forces required for locomotion of metazoan tissue cells are generated primarily or exclusively at the front, in particular in lamellipodia or at the basis of lamellipodia (for review see Refs. (4), (5)). Data suggesting that the cell body but not the lamellipodium generates forces for locomotion in metazoan cells have received little attention(9). More recently, the concepts implying that lamellipodia are primary locomotor organs and that the cell body acts as passive passenger have been questioned. It has been proposed that different cell types may use different mechanisms(21,20). Furthermore, a single cell type can use two or more different motors(80–109). Forces can be generated either by actin polymerization, actin-filament sliding and/or actomyosin-dependent contraction, by gel-osmotic pressure generated by actin polymerisation/depolymerisation cycles at the front of the cells or by hydrostatic pressure(12,9,25,34,84,111,12). In the last decade, the role of myosin-based mechanisms has been recognized(25,91–103). Actomyosin dependent contractions produce forces for locomotion by contraction or by transport(114). The contractile model is based on polarized cortical contraction and relaxation, the transport model postulates that myosin generates forces in an inherently polarized manner acting directly to pull the cell forward(89). The cortical contraction/fronatal expansion model is widely accepted for Amoeba proteus(110), but not for metazoan tissue cell. In the present review, I will summarize evidence to show that the actomyosin-driven contraction suffices to produce locomotion of Walker carcinosarcoma cells, a metazoan cell line, in a way reminiscent of the cortical contraction/fronatal expansion model developed in Amoeba(110).

In the past, formation of cellular fragments has been used to locate the motor(10–18). Since fragments detached from the front are capable of locomotion, it has been concluded that the motor is at the front, particularly in lamellipodia. Recent data point out the limitations of this approach. The motive force may also be generated in the cell body(73,34) and there is growing evidence that cells may have more than one motor, possibly in different locations(91–98). For instance, Walker carcinosarcoma cells can migrate in the absence of lamellipodia by means of actomyosin-dependent contraction at the rear, but migrating fragments can nevertheless be derived from the front of blebbing cells (Keller and Bebie, in preparation). Furthermore, suppression of lamellipodial expansion has been shown to stop cell displacement but not cell body motility of keratocytes and chick fibroblasts(73,80). This suggests that cell body motility is not sufficient to move the entire cell. Likewise, one has to consider that lamellipodial motility may be sufficient to move thin fragments derived from lamellipodia, but it may not be sufficient to move the entire cell which has a much greater mass.

2. Protrusion and Locomotion

Cells with different protrusions such lamellipodia, blebs, filopodia and cells without morphologically recognizable protrusions have been distinguished based on morphological criteria(96,109). The dynamic appearance of lamellipodia, blebs or filopodia at the front is so impressive that these structures have been termed pseudopods and are considered to be crucial for locomotion of tissue cells. However, formation of protrusion occurs very frequently in stationary cells, where it is associated with cell functions such as pinocytosis or phagocytosis. Protrusions of locomoting cells occurs preferentially and continuously on one side of the cell.

Different protrusions are formed by distinct forces and formation is regulated by distinct pathways(96,109,113,120). It seems widely accepted that forces protruding lamellipodia are generated by local actin polymerization, whereas blebs are fluid-driven(119). Blebs protruding at the front are either driven by hydrostatic pressure generated by cortical contraction at the rear (i.e. at a site distant from the protrusion) or by locally generated gel-osmotic pressure. I have formulated the hypothesis, that the degree and/or the rate of actin polymerization determines the morphology of the protrusion. Extensive or excessive actin polymerization may result in formation of lamellipodia, low polymerization in blebs or absence of morphologically recognizable protrusions(120).

Migrating metazoan tissue cells usually form lamellipodia. Therefore, many investigators have suspected that other types of protrusions, particularly blebs are upphusiological or a sign of apoptosis (for review see Ref. (19)). This rather dogmatic attitude may have been motivated by the view that the motor driving locomotion of metazoan tissue cells was believed to reside exclusively in lamellipodia. If one assumes that a particular cell type produces the forces for locomotion exclusively in lamellipodia, one must expect that a switch from lamellipodia to blebs at the front of polarized cells stops migration. However, our recent studies have shown that lamellipodia are redundant for locomotion, at least in Walker carcinosarcoma cells(96,109). A systematic analysis of Walker carcinosarcoma cell migrating with different types of protrusions (lamellipodia, blebs and cell without morphologically recognizable protrusions show only relatively small differences in the locomotor behavior (speed, persistence and net displacement) which are unlikely to be physiologically relevant (Keller and
Bebie manuscript submitted). Furthermore, locomotion of untreated cells with lamellipodia, of blebbing colchicine-treated cells and/or Walker carcinosarcoma cells without morphologically recognizable protrusions is equally inhibited by a large variety of agents including hypertonic treatment\(^{(23)}\), inhibitors of actin polymerization\(^{(33)}\), the myosin inhibitor BDM\(^{(29)}\), the protein kinase inhibitors staurosporin or K252a\(^{(23)}\), the diacylglycerol kinase inhibitor R-5902\(^{(24)}\) and by PMA which activates PKC\(^{(25)}\). There is no significant difference in the inhibitory effect (IC\(_{50}\)) of these agents on cells with different types of protrusions. Therefore, it is not justified to a priori disqualify data obtained with migrating cells exhibiting protrusions other than lamellipodia as non-representative. It should be documented in each case whether a particular type of protrusion (lamellipodia, blebs, filopodia and others) is necessary or redundant for locomotion.

Protrusion can be defined in different ways. One definition implies that protrusion is territory gained, retraction is territory lost\(^{(31)}\). This can be applied to any moving object including a space rocket. An alternative definition is that protrusions are morphologically identifiable structures (lamellipodia, blebs, filopodia and others) projected at the cell surface by a shift of cellular mass from the body into the newly forming structure. This shift can be quantified if the protrusions extend beyond a morphologically recognizable boundary which is stationary with respect to the substratum\(^{(27)}\). However, this definition can only be applied to the cells producing morphologically recognizable protrusions. The shift of volume from the body into blebs was found to quantitatively determine the rate and the direction of locomotion of Walker carcinosarcoma cells\(^{(27)}\).

No comparable studies have been performed with cells forming lamellipodia and in cells without morphologically recognizable protrusions because no stationary boundary at the rear of the protrusion could be identified\(^{(20,27)}\). This does not exclude that protrusion takes place. However, we are at present unable to identify and measure this process in this way.

3. Migration of Walker Carcinosarcoma Cells Depends on Actomyosin-dependent Contraction at the Rear (Cell Body and Uropod) but not on Actin-based Motors at the Front

Though locomoting Walker carcinosarcoma cells usually form lamellipodia at the front, suppression of lamellipodia and depletion of polymerized actin at the front as shown in Fig. 1 fail to inhibit locomotion\(^{(6,10)}\). These data present the first direct evidence that polymerized actin at the front is redundant for locomotion in a metazoan cell line. However, depletion of cortical actin at the rear (body and uropod) is associated with suppression of migration\(^{(6,10)}\). Myosin II A is concentrated in areas of marked contraction, i.e. at the uropod and the rear of the cell body. Furthermore, myosin inhibitors such as BDM and the ROCK-kinase inhibitor Y-27632 inhibit polarity and locomotion. This applies to untreated cells with lamellipodia and to cells depleted of F-actin at the front\(^{(6)}\). Therefore, actomyosin-dependent contraction driving locomotion is located at the rear rather than in lamellipodia. The data document that Walker carcinosarcoma cells have two different motors operating in different parts of the cell. Actin polymerization at the front can generate forces for protrusion of lamellipodia, but it is redundant for locomotion of Walker carcinosarcoma cells. Forces generated by actomyosin-dependent contraction at the rear (body and/or uropod) suffice to move cells at a normal rate. The following evidence suggests that the forces generated in the cell body (probably together with those formed in the uropod) are crucially important for locomotion. The formation of very elongated migrating cells requires cortical contraction of the cell body\(^{(6)}\). High concentrations of actin inhibitors compromise locomotion, the integrity of the cortical actin layer of the cell body and contraction of the cell body, whereas the uropod and polymerized actin in the uropod persist to a variable extent\(^{(6,10,23)}\).

Our data are not compatible with models assuming that actin polymerisation or actomyosin-dependent contraction at the front drive locomotion. They indicate that actomyosin-dependent contraction behind the front generates the forces driving locomotion. The analysis of shape changes including restrict-
Fig. 2 Putative mechanisms driving locomotion of Walker carcinosarcoma cells depleted of F-actin at the front.

The scheme shows a polarized Walker carcinosarcoma cell which has been depleted of F-actin at the front (top) by combined treatment with 10⁻⁷ M latrunculin A and 10⁻⁸ M colchicine. The plasma membrane (black line), the cortical actin layer (gray) and the distribution of myosin IIA (×) are shown. The first column from the left designates the different sections of the cell, the second column the corresponding contractile state. The front expands as the cortical actin layer is compromised, the midsection shows approximately isometric contraction, whereas the narrowing of the uropod is suggestive of isotonic contraction. Transition of isometric to isotonic contraction may be caused by a gradual increase in contractile forces towards the rear of the cell[39]. The mechanisms involved are summarized (right). Latrunculin A (10⁻⁷ M) and 10⁻⁸ M colchicine have synergistic effects in locally depleting F-actin at the front because latrunculin depolymerizes actin and colchicine initiates dissociation between the plasma membrane and the cortical actin layer[33,10,39,40]. Colchicine stimulates contraction of the body and the uropod by the mechanisms indicated and thereby increases intracellular pressure (↑) and traction (stippled arrows). Non-adhesive cell-substratum contacts occur mainly at the front of the cell body (not shown).

Attachment rings of locomoting cells provides information on the contractile state of the cell along the longitudinal axis of polarized cells[39]. The front expands indicating lack of contraction, the midsection shows more or less isometric contraction and the uropod shows isotonic contraction. This is likely to be caused by a contractile gradient (Fig. 2). A transport model and a contractile model of actomyosin-dependent contraction have been distinguished[33,10]. The following data suggest that the behavior of Walker carcinosarcoma cells is consistent with a contractile model. The dominant structure of the actin cytoskeleton in Walker carcinosarcoma cells is the cortical actin layer[40] and there is no evident accumulation of polymerized actin and of myosin II in the cytoplasm in front of the nucleus[6]. However, there is evidence that fluid pressure is involved in protrusion[33,10,39,40]. Furthermore, suspended cells exhibit the same type of movement and F-actin structure as cell migrating on a substratum indicating that polarized adhesion is not required for movement. Therefore, our data are best compatible with a contraction-based motility, particularly the cortical contraction/frontal expansion model which is well documented in Amoeba such as Amoeba proteus[33] and quinine-treated Dicyostelium[33]. These models imply that retrograde movement of the actin
cortex (exerting traction) as well as increased fluid pressure (driving protrusion) contribute forces for locomotion. The retrograde movement of lateral protrusions\(^\text{(29)}\) is evidence that the actin cortex flows rearward with respect to the cell and the role of pressure for protrusion has been documented in Walker carciinosarcoma cells\(^\text{(31)}\). The suction pressure required to induce blebbing is somewhat lower than the increase in intracellular pressure preceding protrusion in *Amoeba proteus*\(^\text{(32,34)}\).

### 4. Role of Protrusion at the Front and of Actomyosin-dependent Contraction at the Rear for Polarity, Persistence and Speed of Walker Carcinosarcoma Cells

The migration efficiency is of crucial importance in developmental biology and for the cellular defense against microorganisms or cancer cell metastasis. Several factors control whether and how efficiently cells migrate from one place to another. The most important factor is whether cells can be activated to migrate. Migration and polarity of Walker carcinosarcoma cells primarily depend on actomyosin-dependent contraction at the rear of the cell\(^\text{(6)}\). Spontaneous migration and polarity of Walker carcinosarcoma cells is regulated by the Rho/rho-kinase pathway\(^\text{(29)}\) which controls myosin phosphorylation. This is probably the common target for the antagonistic effects of colchicine and Y-27632 on locomotion and polarity of Walker carcinosarcoma cells\(^\text{(7)}\).

A switch from lamellipodia to cells forming blebs or no morphologically recognizable protrusions has no significant effect on the locomotion efficiency (net displacement) even though small changes in speed and persistence have been documented as outlined below (Keller and Bebie, submitted). A switch from lamellipodia to blebs or to cells without morphologically recognizable protrusions can be associated with statistically significant alterations in the turning behavior. However, these are minor changes without biological significance (Keller and Bebie, submitted). Thus, the type of protrusion has relatively little effect on persistence in the system studied. Blebbing cells and cells without morphologically recognizable protrusions tend to migrate at a higher speed than untreated cells with lamellipodia\(^\text{(6,10)}\) (Keller and Bebie, submitted). Speed depends on the strength of the contractile forces and, to a limited extent, on the resistance to deformation at the front\(^\text{(6,10)}\). An intact cortical layer increases resistance to deformation and reduces the speed of migration\(^\text{(10,36)}\). Furthermore, the mechanical properties of actin network are strongly influenced by actin binding–proteins\(^\text{(37)}\). In summary, the data show that the type of protrusion has only a small effect, or none at all, on net displacement, persistence and speed. This indicates that an intact cortical actin layer at the rear of the polarized cell rather than the type of protrusion at the front is the major factor determining the locomotor behavior of these cells.

Passive deformation of polarized cells with lamellipodia in micropipettes and/or suction pressure can produce protrusion (blebs). These blebs are formed preferentially at the cell front as compared to the uropod (tail)\(^\text{(31)}\). This documents that the front is more deformable than the rear of the cell. We have no evidence that the reduced resistance to deformation at front of cells with lamellipodia is caused by a compromised cortical actin, because polarized cells forming lamellipodia show an apparently homogeneous cortical actin layer all along the cell including lamellipodia\(^\text{(39)}\). An alternative though hypothetical explanation is that actomyosin-dependent contraction or actin-binding proteins selectively reduce deformability at the rear.

### 5. The Role of Cortical Contraction/Frontal Expansion and of Fluid Driven Movement in Other Metazoan Cells

The data with Walker carcinosarcoma cells suggest that forces generated by actomyosin-based mechanisms in the body and/or the tail, probably by cortical contraction, are sufficient to move the entire cell. Contraction of the cell body and the uropod appears to cause increased pressure generating protrusions at the front and traction along the cell body (Fig. 2). The question arises how the findings in Walker carcinosarcoma cells are related to the mechanisms moving other tissue cells. Though actin polymerization can generate forces for protrusion of lamellipodia, more recent studies emphasize the role of myosin-based motility in several cell types. The type (contraction or transport mechanism) and the site of contraction (lamellipodia, transition zone, cell body, tail), the respective organization of the actin cytoskeleton and the role of myosin-based motility compared to other forms of force generation may vary with the cells type\(^\text{(5,17,38,41,42,43)}\). In fibroblasts, a transport mechanism is favored, in keratocytes a contraction model is more likely\(^\text{(46)}\). The findings in Walker carcinosarcoma cells are also in favor of a contraction mechanism but contraction appears to take place at the rear of the cell rather than in the protrusion\(^\text{(9)}\), whereas keratocytes show contractile structures in the lamellipodia/cell body transition zone in front of the nucleus and in lamellipodia\(^\text{(18)}\). The cell body of keratocytes and fibroblasts continues to move up to the position of the lamella (but not further) once lamellipodial motility is arrested. This indicates that
both, cell body motility and lamellipodial motility operate in these cells, but cell body motility is not sufficient to move the entire cell.\textsuperscript{7,10}. Walker carcinomascarcoma cells are different. They continue locomotion after suppression of lamellipodia and of polymerized actin in front of the nucleus suggesting that contraction in the body and/or uropod is sufficient to move cells at normal speed.\textsuperscript{6,10} The data suggest that the force-generating mechanisms of keratocytes and fibroblasts have different locations within the cell and that the contributions of cell body and lamellipodial motility are different from Walker carcinomascarcoma cells. The Walker carcinomascarcoma cells studied are non-adhesive, keratocytes and fibroblasts are adhesive cells. Adhesion plays an important role in regulating the migratory behavior.\textsuperscript{11} The phenotype of Walker carcinomascarcoma can be modulated to obtain non-adhesive and adhesive sublines which differ dramatically in cell behavior.\textsuperscript{19} The dominant type of contraction may also be different.\textsuperscript{18} Possibly adhesive and non-adhesive cells use different motors. An alternative possibility is that adhesiveness has different effects on the motility of the cell body and the protrusions, respectively. Formation of a thin lamella is part of cell spreading independent of locomotion.\textsuperscript{19}

Our data suggest that fluid-driven mechanisms operate in Walker carcinomascarcoma cells. So far fluid-driven mechanisms have received relatively little attention in metazoan cells. However, there is evidence that they play a role for locomotion and/or extension of lamellipodia.\textsuperscript{12,13,19} A relationship between cell speed and internal pressure has been documented in Xenopus epidermal cells.\textsuperscript{12} Furthermore, fluid-driven extension of lamellipodia can increase the speed of poorly spread myoblasts which are normally driven by actin polymerization in lamellipodia.\textsuperscript{19} One of these models proposes that lamellipodial extension is driven by fluid pressure generated by contraction,\textsuperscript{19} the other that fluid pressure can outpace actin polymerization in lamellipodia as previously described for blebs.

At present it is often difficult or impossible to determine the relative role of different force-generating mechanisms (actin polymerization, actomyosin-dependent contraction and fluid-driven movement) and/or of cell body motility vs lamellipodial motility. For instance, we have not been able to assess the relative role of actin polymerization, actomyosin-dependent contraction and fluid pressure in latrunculin A-treated cells showing gaps between actin streaks oriented perpendicular to the membrane because the putative mechanisms were not spatially separated.\textsuperscript{19} Another problem is that the site of force generation is not necessarily identical with the site where the forces are effective. In this case, motility as defined by the moving part (e.g. blebs) is not identical with motility as defined by the site of force generation (e.g. body and/or uropod contraction). Another pitfall is that locomotion may be stopped by changes in the position of cell-substratum adhesions even though the cells remain fully motile or adhesion may reversibly inhibit locomotion and polarity.\textsuperscript{18,19} Adhesion-induced spreading and arrest of locomotion can be associated with formation of a lamella.\textsuperscript{18} Useful hints can be obtained by assessing whether lamellipodia are redundant for locomotion. Migrating cells forming blebs instead of lamellipodia at the front can also be found in vivo and in vitro in other cell lines such as embryonic cells of amphibians, mouse lymphocytes or human polymorphonuclear leucocytes.\textsuperscript{18} The finding that a switch from lamellipodia to blebs does not compromise locomotion suggests that lamellipodia, and most likely local actin polymerization, are redundant and that fluid-driven mechanisms can be involved in protrusion and locomotion. We may have to change our attitude towards blebbing locomoting cells. Instead of dismissing blebs as unusual or unphysiological we can use the switch from lamellipodia to blebs as an investigative tool. The methods for estimating the forces derived from each of the different mechanisms involved have to be further developed. Assessing the localization of different motors, their mechanisms of force generation and the actual contribution of these distinct elements for locomotion of different cell types may turn out to become a major field of research in the near future.

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References


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