How actin/myosin crosstalks guide the adhesion, locomotion and polarization of cells☆

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Cell–tissue interaction is determined by specific short range forces between cell adhesion molecules (CAMs) and ligands of the tissue, long range repulsion forces mediated by cell surface grafted macromolecules and adhesion-induced elastic stresses in the cell envelope. This interplay of forces triggers the rapid random clustering of tightly coupled linkers. By coupling of actin gel patches to the intracellular domains of the CAMs, these clusters can grow in a secondary process resulting in the formation of functional adhesion microdomains (ADs). The ADs can act as biochemical steering centers by recruiting and activating functional proteins, such as GTPases and associated regulating proteins, through electrostatic–hydrophobic forces with cationic lipid domains that act as attractive centers. First, I summarize physical concepts of cell adhesion revealed by studies of biomimetic systems. Then I describe the role of the adhesion domains as biochemical signaling platforms and force transmission centers promoting cellular protrusions, in terms of a shell string model of cells. Protrusion forces are generated by actin gelation triggered by molecular machines (focal adhesion kinase (FAK), Src-kinases and associated adaptors) which assemble around newly formed integrin clusters. They recruit and activate the GTPases Rac-1 and actin gelation promoters to charged membrane domains via electrostatic–hydrophobic forces. The cell front is pushed forward in a cyclic and stepwise manner and the step-width is determined by the dynamics antagonistic interplay between Rac-1 and RhoA. The global cell polarization in the direction of motion is mediated by the actin–microtubule (MT) crosstalk at adhesion domains. Supramolecular actin–MT assemblies at the front help to promote actin polymerization. At the rear they regulate the dismantling of the ADs through the Ca++-mediated activation of the protease calpain and trigger their disruption by RhoA mediated contraction via stress fibers. This article is part of a Special Issue entitled: Mechanobiology.

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1. Introduction

Cell adhesion and locomotion are fascinating albeit complex processes since they require the ongoing reorganization of the composite cell envelope and the associated intracellular macromolecular scaffolds by a network of cell signaling pathways. The biochemical and genetic control of the micro-organization of the membrane and the cytoskeleton plays a key role for numerous life processes, including embryonic development, immunological responses and the transmission of protrusion forces to substrates during cell migration through tissue or on surfaces. Dysfunctions of this interplay between cell signaling and material properties of the cell frequently results in the development of cancer and metastatic cells. Over the last 15 years insight into the physical basis of cell adhesion has been gained by combined studies of biomimetic systems containing the basic ingredients of cell adhesion and amoeboid cells adhering and crawling on bio-functionalized surfaces.

In the first part I show how by analyzing the adhesion induced shape changes of soft elastic shells (giant vesicles and cells) free energies of adhesion can be measured, yielding quantitative insight into the control of adhesion by interplay of interfacial, osmotic and elastic forces. The model membrane studies showed that due to the competition between short range attraction and long range repulsion forces adhesion is inevitably associated with the decay of the contact zone between cells and tissue into micro-domains of strong adhesion (with interfacial distances of h ≈ 15 nm) separated by non-adhering zones (exhibiting distances of >50 nm). The transition from the free to the bound state of soft shells can be described in terms of the Cahn theory of first order wetting [1,2].

The model membrane studies can only provide insight into the primary step of cell adhesion driving the nucleation of integrin clusters. They provide quantitative information on important regulators of cell adhesion such as (i) the control of adhesion strength by membrane elasticity, (ii) the unbinding of adhesion domains by antagonists of CAMs, and (iii) the control of force-induced unbinding of adhesion domains by the linker mobility [3]. The secondary step of cell adhesion and locomotion, such as the growth, stabilization and functionalization of the adhesion domains, cannot be realized by biomimetic systems. Thus, to
gain insight into the physical basis of cell locomotion and the role adhesion domains we have to study cells moving on biomimetic tissues. Due to the modern techniques of manipulating the expression and activity of specific proteins, this has become feasible now. The model of cell locomotion presented below is based on previous comparative studies of model membrane and cells [4] and a survey of more recent literature until 2014.

In the second part I show first how adhesion domain formation can help cells to move most economically by generation of protrusion forces through sequential actin gelation pulses emanating from adhesion domains. The ADs formed by integrin clusters serve first as biochemical reaction centers which activate specific actin polymerization promoters (such as WASP and WAVE) through GTases of the Rac family. They serve simultaneously the force transmission between cells and tissue. I then discuss the chemo-mechanical control of the coordinated motion of the progressing cell front and the retracting end in terms of a shell-string model of cells. The cell body hovering over the adhering cell envelope is moved by traction forces arising in microtubules which extend from a central force center (called microtubule organization center (MTOC) or centrosome) to actin gel patches. The traction forces can be generated either actively by microtubule based motors of the dynein family, or passively by MT–actin binding proteins (such as the MT plus end binding proteins CLIP170) which couple the MT plus ends to adhesion domains.

In the third part, I summarize experiments showing that the repeated spreading of the cell front is controlled by the antagonistic interplay of the GTases Rac-1 and RhoA. Owing to the microtubule actin crossstalk at the ADs the impact of the two activated switches, Rac-1* and RhoA*, is restricted to the opposite ends of the cell. Rac-1* drives the quasi-periodic switching of the actin polymerization at the newly formed integrin clusters, while RhoA* activates the stress fibers which help to disrupt (partially dismantled) adhesion domains at the trailing end. The disruption of the rear ADs is triggered by the protease calpain which is activated by the local release of Ca<sup>2+</sup> from the endoplasmatic reticulum. At the front the effect of Ca is suppressed by actin MT crossstalk mediated by IQGAP, a MT binding protein harboring a Ca<sup>2+</sup>-binding calmodulin domain. The mature ADs in the center of the cell tissue interface are either internalized by coated pits or act as strain sensitive force transmission centers of actin–myosin stress fibers.

Cell adhesion is an example showing a fundamental difference between biological and technical material design. Engineers have to establish and control the specific conditions under which a certain material is designed, such as a metal alloy with specific thermo-mechanical properties. Living matter is designed by genetically controlled self-organization through the logistic delivery of modules to specific sites at the right time. Material properties are constantly repaired by ongoing decomposition and renewal of components.

### 2. Functional modules of cell envelopes and intracellular macromolecular scaffolds controlling adhesion

Cell adhesion and locomotion are membrane based processes. Therefore the basic physical concepts controlling these processes can be understood on the basis of the simplified shell–string-model shown in Fig. 1a. Cells are considered as viscoelastic shells enclosing a soft plastic body. The cell envelope is composed of the lipid protein bilayer (called plasma membrane; PM) which is mechanically stabilized by coupling to a 200–300 nm thick macromolecular network (the actin cortex) composed of semi-flexible actin filaments. The cell body is stabilized by coupling of the aster like arrangement of microtubules, emanating from the centrosome to the actin cortex, in an interactive way (called actin MT crosstalk [3–5]). Cell envelopes are stratified shells composed of three coupled layers: (i) the ~4 nm thick plasma membrane (a two dimensional fluid); (ii) the extracellular macromolecular film (called glyocalix) mediating the communication with the environment and (iii) the viscoelastic actin cortex (Fig. 1a). Structural changes within and the functions of the three subshells are intimately correlated.

**Fig. 1.** (a) Top: Cell string model of cells. The global mechanical stability is determined by coupling of the aster shaped microtubule assembly (MT) to the actin cortex. Traction forces (τ) in the soft (20 nm thick) MTs are balanced by tangential stresses in the viscoelastic actin cortex (Σ), which is generally isotropic. The microtubules are coupled to actin gel patches by passive linkers (denoted by X in the inset at the bottom right) and actively by linear motors of the dynein family forming complexes with the actin coupling protein dynactin or other proteins such as CLIP 170 (bottom left). (b) Coarse grained image of the composite cell envelope composed of (i) the central lipid–protein bilayer (called plasma membrane; PM) which harbors many functional proteins (not shown) and cell adhesion molecules CAMs; (ii) the glyocalix modulating the communication with the environment and (iii) the actin cortex, a percolated viscoelastic network of mesh size ξ ≈ 100 nm. (c) High resolution structure of the PM adhering on tissue via lock-and-key force between clusters of CAMs (such as integrin) and specific ligands exposed by tissue. As shown on the left, the affinity of the integrins is increased dramatically by binding of the FERM domain of talin (or ezrin) to the integrin J-1 chain which opens the binding pocket by breaking a salt bridge [6]. As shown on the right isolated integrins bind only weakly.
The lipid bilayer moiety of the plasma membrane is a multicomponent lipid–protein alloy which can control the lateral organization of the receptors and repellents and their in-plane mobility. About 20% of the phospholipids are negatively charged and are all assembled in the lipid monolayer of the PM and the intracellular organelles facing the cytoplasmic space (surface charge density 0.1 – 0.2 e nm⁻²). These lipids play a key role for the activation of proteins through their recruitment to the membrane by electro-hydrophobic forces. These are mediated by the electrostatic interaction of the acidic lipids with the polybasic sequences of the protein and membrane anchoring of fatty acid chains (see example discussed in Fig. 3).

The glycocalix is composed of the head groups of glycoproteins (negatively charged oligosaccharide – peptide co-polymers), some of which can extend 40 nm into the extracellular space, such as sialophorin (CD43). The head groups of many cell adhesion molecules are much shorter and are therefore hidden within the glycocalix, such as the integrins which expose 15 nm long head groups. Moreover the glycocalix contains also about 5% of acidic glycolipids which contribute to the high negative surface charge of the cell surface.

The cytoplasmic side of the PM is coupled to a quasi-two dimensional (200 – 300 nm thick) network (or heterogel) composed of the semi-flexible actin filaments (exhibiting persistence lengths of 10 nm or bending moduli B = 5 × 10⁻²⁶ J m⁻³). Actin filaments are locally and reversibly coupled to the intracellular domains of the CAMs (such as integrins). This occurs by actin–membrane linkers, such as talin and ezrin. They expose specific binding domains, called FERM-domains, that recognize intracellular tails of the CAMs (see Fig. 1c and reference [6]). These linkers are highly dynamic. Similar to many proteins involved in the manipulation of the actin cortex they reside in the cytoplasm in a self-inhibited conformation (hiding the FERM domain). They are activated by phosphorylation of specific sites which exposes the FERM domains, the polybasic sequence and the fatty acid anchor resulting in the binding to the PM (see Fig. 1c). The membrane association is often enforced by a specific protein domain, called pleckstrin homology domain, which binds strongly to the phosphoinositides (PI-4, 5-P₂ and PI-3, 4, 5-P₃). Most importantly, the binding of the FERM domain can increase the affinity of the integrin head group for ligands of the tissue by a factor of 5 (see [6]).

3. Bio-analogue model systems and measurements of adhesion energies by micro-interferometry

To generate biomimetic systems giant vesicles are used as test cells. They are doped either with cell adhesion molecules (CAMs) or constituents of tissue, specifically recognized by the CAM chosen. Lipids exposing macromolecular head groups (composed of polyelectrolyte oxide of n ≥ 2000) mimic the function of the glycocalix. Solid supported planar membranes doped with conjugate CAMs or ultrathin polymer cushions exposing specific ligands of tissue serve as target cell or tissue, respectively. To account for the softness of biomaterials and to avoid strong Van der Waals attraction by the solid, the membranes are separated from the solid surface by ultrathin polymer cushions [3]. The micro-interferometric technique “Reflection Interference Contrast Microscopy” (RICM) allows us to reconstruct the contour of adhering vesicles or cells close to the surface with nm height resolution as a function of lift forces applied via magnetic tweezers. (see Fig. 2a and [2]).

The contour of adhering soft shells close to the contact line exhibits the general shape shown in Fig. 2c. Owing to the membrane bending stiffness the membrane is slightly bent at the contact line, characterized by a contact curvature R_c. It goes then smoothly over into a linear region, before it is deflected upwards. The contour is thus completely defined by the contact curvature R_c and a contact angle θ_c. The linear and the curved regimes are determined by the balance of surface

![Fig. 2](image-url)
tensions and bending moments at the contact line, respectively. For fluid membranes the balance of tensions is determined by the well-known Young’s law (Eq. (1a)), which relates the contact angle \( \theta \) to the work of adhesion \( W \). The balance of bending moments yields a relationship between \( W \) and the contact curvature \( \kappa \) (for a justification of these relationships see [2,7,8]).

\[
W = \sigma (1 - \cos \theta) \quad (1a)
\]

\[
W = \frac{1}{2} \kappa R_c^{-2} \quad (1b)
\]

The bending modulus \( \kappa \) is measured in units of joule or of \( k_BT \) (where \( k_BT = 4 \cdot 10^{-21} \) J at 25 °C). The bending modulus of vesicles with 50 mol% cholesterol is \( \kappa \approx 25k_BT \). For composite cell envelopes a typical value is \( \kappa \approx 500k_BT \) (see [9]).

By determining the geometric parameters \( R_c \) and \( \theta \), through contour analysis, the free adhesion energy \( W \) and the surface tension \( \sigma \) can be measured, provided the bending modulus \( \kappa \) and the lateral membrane tension are known [2]. If \( \kappa \) is not known \( \theta \) and \( \kappa \) can be determined by measuring the change of contact angle under hydrodynamic shear flow [9a].

4. Control of adhesion by generic interfacial forces and osmotic pressures

The adhesion strength is controlled by numerous interfacial forces (see [2]). Most of them are well known from the DLVO theory of colloid stabilization and can be accounted for by an interfacial potential \( V(h) \), where \( h \) is the interfacial distance [7,8]. An unconventional force is the entropic repulsion pressure generated by thermally excited membrane bending fluctuations (first recognized by Wolfgang Helfrich [10a]). In the case of cells they can even be enforced by active random walks. The entropic pressure preserves the stickiness of erythrocytes or macrophages on tissue [10b]. Most importantly it has been shown to drive cell adhesion by pushing the cell towards the tissue surfaces [11b]. In the absence of membrane tension it is of the order

\[
P_{\text{day}} = \frac{(k_BT)^2}{\kappa d^2}. \quad (2)
\]

It compensates the Van der Waals attraction \( P_{\text{vdW}} \approx 4\pi \frac{\rho_m}{d^6} \) at \( d \leq 20 \text{ nm} \). Most importantly, \( P_{\text{day}} \) decreases strongly with membrane tension. Therefore, adhesion can be switched on and off by changing the membrane tension [2].

Following [2] the interaction potential mediated by the repellers can be approximated by the Dolan–Edwards potential for which an analytical expression exists for two limiting situations. For distances larger than the Flory radius \( R_F \) of the macromolecule (\( h > R_F \)) it can be expressed as

\[
V_p \approx k_BT \rho_l \left( \frac{R_c}{\pi} \right)^2 \exp \left\{ -\frac{3}{2} \left( \frac{h}{R_c} \right)^2 \right\}. \quad (3)
\]

\( \rho_l \) is the lateral concentration of repeller molecules. For shorter distances (\( h \ll R_F \)) the repellers are expelled from the domains of tight adhesion one obtains: \( V_p \approx k_BT \rho_l \), where \( \rho_l \) is the linker density. This is a classical result postulated by Bell et al. [11a].

A surprising and frequently underestimated effect of the squeezing-out of repellers from adhesion domains is the weakening of the adhesion strength by the 2D osmotic pressure exerted by the repellers and ligands expelled from the zones of tight adhesion. The weakening of the binding energy can be estimated on the basis of the following simple argument. Let the energy per CAM–CAM pair be \( w \), the CAM density \( c_l \) (where the index \( l \) stands for linker) and the total repeller density \( \rho_h \) (with densities given as molecules m\(^{-2}\)). The total free energy of adhesion can be approximately expressed in terms of the chemical potentials of the repellers and CAMs as

\[
\Delta g_{\text{adh}} = wc_l + V_p \rho_h + \Pi_R \quad (4)
\]

where \( w \) is the binding energy of the CAM–CAM–pairs, \( V_p \) is the repulsion energy generated by a single repeller molecule (Eq. (3)) and \( \Pi_R \) the osmotic pressure generated by the repellers expelled from the adhesion domain. Note that the signs of the first and third terms are opposite and the osmotic pressure weakens the adhesion strength. If we assume for simplicity that all repellers are completely expelled from the tight adhesion domains and all CAMs are bound it is \( \Pi_R = k_BT \rho_h \).

The weakening may appear surprising since the molecules removed from the ADs gain translational entropy. In fact, adhesion only occurs, provided the loss of adhesion energy is larger than the gain in free energy. The effect is similar to the formation of liquid droplets by the condensation of gases below the dewpoint. It has been well established in our previous model membrane studies (see [2] and references cited there). A more rigorous model has been developed by Seifert and Smith [8b] which accounts for the finite size of the cells and the partial depletion of the reservoir of ligands and repellers in the non-adhering area. The effect of the osmotic pressure is astonishingly strong. While the expected value of \( wc_l \) is of the order of \( 10^{-4} \text{ J/m}^2 \), the measured value is \( \Delta g_{\text{adh}} \approx 10^{-3} \text{ J/m}^2 \) (for references see [2b]).

4.1. Intermediate summary on lessons learned from model membrane studies

The adhesion strength between cells mediated by a specific CAM–CAM pair depends on the density of the linkers (\( c_l \)) and repelling cell surface molecules (\( \rho_h \)). The free and adhering state of cells \( i \) was separated by a first order phase boundary in a \( c_l = \rho_h \) phase diagram (see [12]). Thus, measurements of unbinding forces between isolated CAM–CAM pairs do not necessarily tell us much about their contribution to the adhesion strength under physiological conditions. It depends (i) on the total density of repellers and CAMs; (ii) on the length difference \( (H - h) \) between linkers \( (H) \) and repellers \( (h) \) and (iii) on the bending elastic energy cost associated with the adhesion induced shape change of the elastic shells. Thus, the tightly adhering zones have to be considered as an open phase state in equilibrium with a variable reservoir of repellers and CAMs.

As noted in the introduction model membrane studies can provide insight into the primary steps of cell adhesion, such as the nucleation and growth of CAM clusters or charged membrane domains. It provided new valuable information on the force induced control of the adhesion strength through the mobility of the CAMs and ligands, and the inhibition of adhesion by antagonistic competing with tissue ligands (see [3]). The secondary phase of cell adhesion, such as the stabilization and switching of the functionality of the adhesion domains, is determined by the adhesion induced cell signaling processes which trigger the coupling of the actin cortex and many regulatory proteins to the integrin clusters. Here the role of the lipid–protein bilayer is to mediate the recruitment of functional proteins such as GTPases and their associated proteins (as GEF) to the membrane by electrostatic–hydrophobic forces (see Supplement S1), thus enabling the formation of large biochemical reaction platforms (see Fig. 4). Many of the proteins harbor binding domains for specific lipid anchors (such as PI-3,4,5-P3 or diacylglycerols (DAG)). These are generated from the pool of lipids by enzymes (such as PI-4,5-P2 by kinases and phospholipases) which are also recruited to the membrane by electrostatic–hydrophobic forces.1

1 The electro-hydrophobic membrane coupling of proteins exposing polybasic peptide sequences and fatty acid chains acting as hydrophobic anchors has been extensively reviewed in a Lecture Note on Biological Physics (see E. Sackmann, “Physics of Functional Membrane Micro-Domains”, freely accessible via www.biophy.de).
The adhesion induced domain formation implies many advantages. Cells can change the area density of the CAMs by fusion of vesicles enriched in specific linkers in the time scale of seconds and by genetic expression within minutes. They can control the state of adhesion locally by changing either the density of repellers or CAMs by exocytosis and endocytosis. For an example see Fig. 12 of [3]. The membrane stiffness can be regulated locally via the coupling strength of the lipid–protein bi-layer to the actin cortex and by the crosstalk between the actin cortex and the star-like microtubule assembly. In the following we address the question how this manifold of control parameters is utilized by cells to control locomotion.

5. Microtubule–actin crosstalk serves the mechanical stabilization and polarization of cells

The mechanical control of many cell functions, including adhesion, can be understood in terms of the cell–string model of cells [Fig. 1a] which can be considered as an extension of the Safran–Miller model of soft shells [13]. The cytoplasmic space is considered as a soft plastic body to ensure the rapid intracellular material transport via vesicles. The MTs buckle under forces > 5 pN and cannot balance substantial bending forces. Therefore, the global mechanical stability of cells is determined by passive and active (motor-mediated) coupling of the plus ends of a fraction of the MTs emanating from the microtubule organization center (MTOC) to adhering or free actin gel patches (Fig. 1a). Most of the MT form dangling bonds and serve as trails for the transport of vesicles by MT based motors: plus–end directed kinesins and minus–end directed dynein motors.

Active coupling is mediated by the dynein–dynactin motor complexes. The minus–end directed motor tend to move towards the MTOC and therefore pull on the centrosome. The traction force $\tau$ (in N) is balanced by lateral tension $\sum$ (in N m$^{-2}$) arising in the actin cortex (see Fig. 1a).

Passive MT–actin coupling is mediated by MT plus end binding protein (also called + TIPs) forming complexes with actin binding proteins. An example is CLIP 170 which can form complexes with the actin linker CLASP (“CLIP associated proteins” [14]) and with the protein IQGAP-1 [15]. The multi-functional IQGAP protein stimulates actin gel formation by binding and activating the GTPase Rac-1 (described below in Fig. 3b and the text). Since the MTs tend to maximize the contact area with the actin cortex they can also generate pulling forces on the MTOC, as shown below and in [16a].

The active traction force $\tau_{act}$ on a bound MT tip is determined by the number ($n$) of motors involved (see Fig. 1a) and the force $f_{act}$ generated by the dynein motors: $\tau_{act} = nf_{act}$. The passive force is determined by the one dimensional analogue of Eq. (1a): $w = \tau_\cdot (1 - \cos \theta_i)$, where $w$ is the binding energy per unit length of MT and $\theta_i$ is the contact angle defined in Fig. 1a, inset bottom left. The resultant of all MT traction forces pulls on the centrosome which moves in the cytoplasmic space until the traction forces are balanced. An example of the cell body motion is shown in Fig. 5 in a recent review by Sackmann et al. [16].

The lateral tension $\sum$ induced in the cell envelope by each MT–plus end is determined by

$$\sum_i = \tau \cdot \frac{L_i}{\cos \theta_i}. \quad (5)$$

$\theta_i$ is the contact angle of the microtubule defined in Fig. 2a. $A_{ij}$ is the contact area between the MT and the actin cortex and $L_i$ is the length of the contact between the MT and the actin cortex. If we start from the equilibrium state and apply a force $\tau_i$ on one MT all MT–actin contact sites it will move tangentially until the resulting tension is zero: $\sum_i = 0$. In the resting state of cells, the plus ends of the MT move randomly in the tangential direction showing that the MTs exert random traction forces. The random force is also revealed by random motions of the centrosome [5]. Any cell shape change triggered by external forces (such as adhesion) can be balanced by changes in the traction forces on the MT. In fact, microtubules can balance forces up to 600 pN by coupling to the actin cortex.

The balance of the traction force in single MTs by the membrane tension is very rapid (~0.1 s; [5]). However, due to the ongoing formation of new ADs and actin–MT contacts and the motion of the cell body migrating cells are constitutively outside mechanical equilibrium. In particular tension gradients in the direction of motion arise at the cell front which controls the signaling processes within the adhesion domains. An example is the recruitment and activation of filamin-A to nascent ADs discussed in Chapter 9 (see Fig. 3, and Supplement S2).

6. Phenomenology of cell crawling and directional polarization

Cell locomotion involves three coordinated, cyclically repeated, processes: The protrusion of the front by a few µm driven by actin polymerization, the retraction of the trailing end and the progression of the whole cell body hovering over the adherent zone. This requires that the actin polymerization process pushing the front forward must be switched on and off repeatedly [9,17,18,21]. Dicytostella cells move on passivated solid surfaces by spreading the front with step width of

![Fig. 3. (a) Global view of processes driving cell locomotion at leading edge and trailing end. At the cell front new adhesion domains are formed which act as activators of actin gelation. The right side shows the generation of protrusion force by the progressing actin gel front [26,27]. The left side shows the retraction of the trailing end by dismantling of the actin gel and by pulling forces generated by microtubules and actin–myosin micro-muscles. Note that the progression of the cell front can be facilitated by material flow in the PM at the top. Membrane tension can be generated by myosin I motors. The right panel shows the array of force dipoles generated at the cell front. Each dark patch is assumed to be a newly formed AD. They can be visualized by fluorescence labeling of the actin polymerization promoters VASP [4a].](image-url)
about 5 μm and speeds of 0.5 μm/s followed by the retraction of the trailing end. Due to the coordinated protrusion of the front and retraction of the end, this rhythmic process is accompanied by the quasi-periodic variation of the adhesion area (see [9]).

To push the cell front forward the protrusion force has to be balanced by shear deformation of the substrate in the opposite direction (see Fig. 3a, right side). Numerous experiments with different cell types (Dictyostelia [9,19], Fibroblasts [4] and White Blood Cells [18]) strongly suggest that the force balance is transmitted via adhesion domains (ADs) that are separated by a few μm (see [4a,20]). The tight adhesion domains are formed by assemblies of integrin in the case of mammalian cells and an integrin like CAMs in the case of Dictyostelia cells (references see [16b]). Due to this strategy cells can minimize the material changes see[16b]). Due to this strategy cells can minimize the material turnover associated with the ongoing formation of new adhesion domains at the front and the dismantling of mature adhesion sites at the rear. Interestingly the density of AD can be controlled by the internalization via coated vesicles (see Fig. 3).

To move, the cell body has to undergo global shape changes to pull the rear part forward which may help to coordinate the motion of the front and the back. These shape changes become apparent by cyclic changes of the cell–substrate contact area by a factor of 2–4 with a period of about 5 min [9b]. The whole cell body is moved forward and is mechanically stabilized by cross talk between the aster-like microtubule system and the actin cortex [4,5]. This is achieved by passive and active coupling of the MT plus ends to the actin network, such as the gel patches of the microdomains (see Fig. 1a [3]). The MT-adaptor crosstalk is also a key role for the regulation of the coordinated protrusion of the cell front and the retraction of the end as explained below.

7. Molecular machines driving cell locomotion by actin gelation

The cell front is pushed forward by sequential progression of branched actin networks (called actin gels) generated by the activation of the specific actin growth promoter WASP which activates the cross-linker Arp2/3. The progressing front of the newly formed actin gel generates a pushing force \( F_p \) [26] which is balanced by the shear stress (directed towards the rear) that is induced in the tissue through the adhesion domains. A band of force dipoles is generated at the progressing front (see Fig. 3b and [22]). Using colloidal probes coupled to artificial tissue surfaces, the force dipoles can be directly visualized by monitoring the shear deformation of the tissue on which cells move. Pushing forces can be estimated by quantitative analysis of the shear strain of the tissue at the cell front. They can also be determined by measuring the deflection of AFM cantilevers [23].

The actin gelation is triggered by acceleration of the Arp2/3 mediated growth of branched actin gels. Since the basal polymerization rate of Arp2/3 is small, specific reaction accelerators are needed to increase the actin gelation rate. Prominent examples of such promoters are WASP [24] and WAVE in mammalian cells [18] and CARMIL/myosin 1 in Dictyostelia cells (references see [25], or [16b]). These huge complexes serve simultaneously the accumulation of activated GTP-actin (bound to profilin) at the cell front. In the resting state of cell the promoters reside in the cytoplasm in a sleeping conformation. They are activated by GTPases, notably Rac-1* or Cdc42*, resulting in their recruitment to the cell envelope. The GTPase switches can be locally activated at the newly formed AD by two specific guanine exchange proteins (GEF): DOCK [28] and beta-PiX [29]. In their active states both proteins, the GEF and the GTPases, are recruited to the (negatively charged) intracellular leaflet of the plasma membrane by combined electrostatic and hydrophobic forces.

Both GEF (DOCK and beta-PiX) are switched on by the molecular switch S shown in Fig. 4b. It consists of the excited FAK*Src* complex (see Appendix A) which is associated with the integulin clusters. The excited double kinase FAK*Src* activates a signal mediator consisting of the multifunctional scaffolding (and strain sensitive) protein Cas and the adaptor protein Crk which exhibits kinase function. Cas exhibits several binding sites which are activated by tyrosin phosphorylation and act as activating docking station for activator proteins including Crk. It is activated by force induced opening of the phospho-tyrosin binding sites and their activity increases by stretching.

A unique property of activated DOCK is that it is recruited to the membrane by strong electrostatic binding of a specific basic domain DHR2 to PI-3,4,5-P3 and not by pleckstrin homology domains, which also binds to PI-4,5-P2 [28]. The PI-3,4,5-P3 generator PI-3K is also activated by the active FAK*Src* double kinase of the switch S* in Fig. 4b. The very strong and selective membrane binding of DOCK by a specific domain (instead of pleckstrin) is very important. It enables this Rac-1 activator to bind to the membrane, despite the very low density (<0.2%) of the PI-3,4,5-P3 anchor. DOCK can thus compete with many other proteins exhibiting pleckstrin domains [28]. Please note, that beta-PiX is coupled to the integrin cluster via talin and paxillin [29] and could be activated via a different pathway.

8. Mechanism of coordinated motion of cells by Rac–Rho antagonism

In this section I address the question how the local antagonistic interplay of Rac and Rho can result in global polarization and coordinate the motion at the two cell ends. Above, in Figs. 3 and 4, the protrusion of the cell front by Rac-1* was described. Below the processes at the cell front are considered in more detail. The retraction of the trailing end is propelled by two modules: the actin myosin stress fibers (acting as micro-muscles) and microtubule cables fixed to actin gel patches of mature ADs. The stress fibers are activated, as usual, by the active switch RhoA*, through the phosphorylation of the myosin light chain kinase ROCK. The microtubules can contribute to the retraction of the rear cell body in two ways. First, they can pull the centrosome and cell body forward through the activation of dynein motors at the front MT-adaptor contact sites. Second and most importantly, the giant MT-adaptor complexes at the end regulate the activation of RhoA* via the MT-regulated GEF-H1 (as shown in Fig. 5 and discussed below).

Recent experiments provided evidence that the forward-directed motion of the cell body is facilitated both by dismantling of mature adhesion domains at the cell end and the simultaneous activation of actin–myosin micro–muscle disrupting the weakly adhering adhesion domains. Both effects are mediated by the phosphatase calpain as indicated in Fig. 3. First, it cleaves the β3-integrin which results in the activation of RhoA*. Secondly, calpain cleaves the actin–integrin couplers ezrin and talin, resulting in the unbinding of the integrin β3-chains from actin gel patches and the dissolution of the integrin clusters [30, 31].

Calpain activation requires the increase of the Ca**+** level. This occurs by release of this second messenger from the ER through the binding of another GTPase switch, namely: R-Ras. R-Ras* binds to and activates a calcium channel FAM38 (see [30]). Here again the functional advantage of adhesion domains becomes very evident.

Now the question arises how the antagonistic crosstalk between the GTPases Rac-1, which triggers the actin gelation at the front, and Rho-A, which activates the actin–myosin stress fibers at the end, can be translated in a globally polarized state. First, it is very important to realize that the two molecular switches can control their state of activity via all three GTPase control proteins, namely the activators (GEF) and inhibitors (GPI) of the GDP=GTP exchange and the GTP hydrolyzing protein (GAP) that rapidly deactivates the GTP-loaded switches (see the summary of their function in Supplement S1).

The activated molecular switches Rac-1 and Rho-A* inhibit each other as shown in Fig. 5a: Rho-A* activates the Rac specific GAP protein which constantly deactivates Rac-1*. Conversely, Rac-1* can deactivate Rho A* via the protein WAVE (see [18]). The range of this biochemical mechanism of polarization is expected to be short ranged, which may be the reason why several protruding and retracting areas separated by ~10 μm often appears simultaneously (references see [17,18]).
The antagonistic interplay of GTPases Rac-1 and RhoA can be turned into a global cell polarizing through MT–actin crosstalk. Two established mechanisms are shown in Fig. 5b.

• The right side shows a mechanism mediating the Rac-1 accumulation at the front. The MT–actin coupling is mediated by the plus-end stabilizer CLIP170 which can form a complex with the Rac-1 activator IQGAP. Rac-1* activated in this way exerts two important effects. First, it stabilizes the MT plus ends by suppressing the MT destabilizing factor stathmin (reference see [14] and Figs. 13 and 14 in [3]). Secondly, the activated Rac-switch can bind and stimulate one of the actin growth promoters, such as WASP as shown in the right panel of Fig. 5b. Most interestingly, the MT coupled to the actin gel patches by the CLIP-170/IQGAP-complex can activate the actin polymerization via Rac-1*/WASP* (see Fig. 3b and [14]). By moving with the newly generated actin gel the MT plus-ends can generate a pulling force on the cell body directed towards the front.

• The left side shows a mechanism which can restrict the function of activated RhoA* to the cell end. It is based on recent studies showing that the localization of activated RhoA switches at the end can be mediated by a specific GEF, called GEF-H1, exhibiting several unique properties [32]. It harbors a specific pleckstrin homology (PH) domain which can bind to microtubules. In this bound state, the guanine exchange capacity is abolished and RhoA* is silenced. However, the GEF is liberated and RhoA* is activated if the MT becomes destabilized by switching on the calpain protease as described below (see Fig. 5b left side). The GEF-H1 cannot become active at the cell front for two reasons: The MTs are constantly stabilized by the Rac-1* mediated suppression of stathmin and Rac-1* inhibits RhoA* reaching the cell front by diffusion.

The next question concerns the signals triggering the dismantling of the adhesion domains and the simultaneous unbinding of the MT–plus ends. The first step is mediated by the protease calpain, which is activated by a local increase of a Ca++-level through the opening of specific Ca-channels (FRAM 38) in the endoplasmatic reticulum membrane [30]. This occurs by the activated GTPase Ras R. This GTPase switch is activated by recruitment of the Ras-specific GEF to the FAK*/Cas*/Crk* complex of the integrin clusters.

Finally we have to consider two questions: first, why are the MT/IQGAP/actin complexes not active at the cell end? Second, how is the effect of the R-Ras mediated Ca++-burst suppressed at the front?

1. An important consequence of the calcium bursts triggered by R-Ras* is the uncoupling of the actin–MT links mediated by the calmodulin complex coupled to IQGAP above a threshold of the Ca++-level, resulting in the destabilization of the MT plus end and the liberation of the RhoA specific GEF-H1. Moreover, the IQGAP-mediated binding

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Fig. 4. (a) Model of actin gel formation at newly formed adhesion domains. The gelation is mediated by activation of actin growth promoters (such as WASP) which enhances the actin polymerization activity of the actin cross-linker Arp2/3. WASP is activated by the GTPase Rac-1 which is switched on by the activator S* coupled to the adaptor FAK*/Src*. S* switches on Rac-1 by recruitment of Rac specific guanine exchange factors (GEF, such as DOCK). (b) The switch S is composed of the integrin coupled dual kinase FAK*/Src* (see Appendix A) which activates the adaptor pair Cas and Crk. Please note first that the adaptor pair serves as an elastic strain sensitive docking station which can assemble many functional proteins, such as DOCK. Note second, that the excited switch S* can activate both the Rac specific GEF DOCK and the PI-3,4,5-P3 generator PI-3K [37].
of MT to actin domains is restricted to the front. This results in an effective forward directed pulling force on the centrosome and the cell body.

2. Some ions of the calcium bursts generated locally by FAM38 can diffuse to the front where they are sequestered by the calmodulin associated with IQGAP.

We are left with an important question concerning the structure and function of the mature ADs in the central parts of the adhesion zones. Since the lifetime of excited GTPases is very long (2 × 10^3 s) Rac* is constantly active. How is Rac* prevented from activation WASP throughout the cell? At the high Ca++-level of the cell end, the Rac-1* activity of the MT stabilizer stathmin and the Ca++-level is too low to dissociate IQGAP. The isolated Rac-1* switches (coupled to the actin polymerization (see [14]). Most importantly, Rac-1* can stabilize the MT plus end by phosphorylation of the MT destabilizer CLIP 170. Left: Activation of Rho-A by the guanine exchange factor GEF-H1, which is inactive while bound to the MT but is liberated and switched-on after the onset of its dynamic instability. Note that this process can be activated by Ca++-bursts.

9. Control of on-off switching of actin polymerization by mechanical forces and dynamic Rac-1–RhoA interplay

The following experiments provide insights into the force mediated control of the cyclic on-off-switching of the actin polymerization by the dynamic antagonistic Rac-1–RhoA crosstalk. The spreading of protruding cell lobes of Dictyostelia at the front generates a spreading pressure of the order of \( \sigma_p \approx 10^{-4} \text{ N m}^{-1} \) [9] which is by over an order of magnitude larger than the cortical tension of adhering resting cells (\( \sigma_p \approx 5 \times 10^{-5} \text{ N m}^{-1} \)). This results in the transient generation of a tension gradient between the cell front and the adhesion domains which pulls at the newly formed actin patches and can exert two effects.

- **Cas is a force sensitive adaptor.** Its binding affinity (for the adaptor Crk) is enhanced by pN force-induced stretching of the internal segments harboring the active tyrosine binding sites (see Fig. 4b and references [33,35]). Therefore, the tensional stress gradient (generated by the formation of new adhesion domains at the front) enhances the Crk and the DOCK-180 (see Fig. 4b) activity resulting in the amplification of the activity of Rac-1.

- **Conversely, the activity of Rac-1 can be down-regulated in a force-dependent manner by binding of the actin linker filamin to the β1-tail of integrins.** In the force free state, the binding of filamin A to integrin is self-inhibited by internal complex formation, thus hiding the binding sites. They are exposed by forces in the 2–5 pN range, resulting in a dramatic increase of the filamin–integrin binding affinity [34]. The bound filamin-A recruits the Rac-1 deactivating guanine hydrolyzing protein (GAP) called FlnGAP which stops the actin gelation until a new AD is formed. The activated filamin A proteins mediate the coupling of the adhesion domains to the scaffolding proteins paxillin resulting in the maturing of the ADs to focal complexes. They act as force or strain sensors and become force transmission centers of stress fibers.

- **The Rac-1 activity can also be suppressed by the guanine hydrolyzing protein RacGAP which is activated by binding to IQGAP–filamin-integrin complexes (see [36] and Supplement S2).** In this case filamin-A could be maintained in the active state by stretching forces which are generated by microtubules binding to IQGAP via CLIP 170. We assume that this mechanism inhibits Rac-1* and actin gelation in the mature adhesion domains and impedes the formation of new protrusions, at least adjacent to the initial protrusion.
Elegant microfluorescence studies by Machacek et al. [37] provided new insights into the dynamics of pseudopod spreading of fibroblasts. The initial step of each cycle of spreading is initiated by the actin polymerization mediated by Rho A activation. About 100 s after this priming step, Rho A* is down-regulated while the GTPase Cdc43 activation is switched on (see Fig. 5a). With some delay (≈50 s) the membrane tension induced by the RhoA activated priming step triggers the activation of the Cas/Crk-switch resulting in the activation of Rac-1. Rac-1* turns down the RhoA* level and mediates the progression of the actin gel front by switching-on the switch S (see Fig. 4). This alternating switching on-and-off of Rac-1 (or Cdc42) for short times is assumed to be responsible for the cyclic protrusion of the cell in steps of fixed length. This conclusion is consistent with recent studies of the dynamic response of Dictyostella cells to directional changes of chemotactic gradients, showing that the induced changes of direction of motion are abolished at times shorter than 10 s [38].

Taken together, the above experiments suggest the following scenario of the cyclic generation of protrusion forces advancing the cell front by a few μm. The first step is the formation of nascent adhesion domains by integrin clustering which is accompanied by the binding of F-actin via talin. The adhesion induced membrane tension (following Eqs. (1a) and (1b)) activates RhoA (in a still unknown way) which switches on formin mediated actin polymerization. With some delay (≈50 s) the membrane tension induced by this priming step triggers the force induced activation of the Cas/Crk-switch, resulting in the activation of Rac (or Cdc42): Rac-1* turns down the RhoA* level and mediates the progression of the actin gel front which pushes the cell front forward. Now, the shear strain in the matured adhesion domains disrupts talin from the β1-tails (which occurs at about 2 pN, see [10b]). This step simultaneously activates filamin-A which binds β1-tails by unbinding talin (see Supplement S3) resulting in the down-regulation of Rac-1* (via the guanine hydrolyzing protein FlnGAP).

In the mature adhesion domains (often called focal complexes) FAK*/Src* and the adaptors (see Fig. 4) are still active. They are stabilized by vinculin mediated coupling to the actin gel patches of the ADs and act as force centers of the stress fibers [40]. The Rac-1* which remains bound to the junctions is silenced by RacGAP as noted above [36]. Smaller ADs which are not coupled to actin stress fibers are most likely internalized by clathrin coated pits. The fission of the vesicle is triggered by the GTPase dynamin which is also activated by integrin associated FAK* [39].

10. Concluding discussion

The competition between short range attraction and long range repulsion forces (mediated by the glycoproteins of the glyocalix) drives the formation of adhesion domains by clustering of the integrins. The integrin affinity is increased by talin-mediated coupling of the cytoplasmic tails to F-actin. The integrin clusters functionalized in this way act as reaction platforms which assemble the protrusion force generating machinery (Fig. 4). Moreover, the adhesion domains mediate the force transmission between the cell and tissue and control the dismantling and unbinding of the rear AD (see Fig. 5). All these functions of the adhesion domains as well as the global polarization of the cells in the forward direction and the movement of the cell body are regulated by the actin–microtubule cross-talk, whereby the MT–actin coupling complex IQGAP/Clp170 plays a key functional role.

The newly formed integrin clusters assemble and activate the double kinase FAK*/Src* which switches on an adaptor cascade (FAK*/Cas*/ Crk*) that activates the Rac-1 GTPases via the guanine exchange protein (GEF) DOCK and, through this switch, activate actin polymerization promoters (such as WASP). The activity of Crk* is enforced by the mechanical stabilization of the actin Cas by the array of force dipoles at the protruding front Fig. 3b. Cas could act as strain sensor which senses the tissue stiffness and is involved in the function residual adhesion domains [41–42].

The cyclic switching on and off of Rac-1 determines the cell protrusion length. The switching frequency is controlled by the temporal antagonistic interplay of the GTPase switches Rac-1 and RhoA. Rac-1* can be down-regulated by specific families of GAPs, which are activated by binding to filamin-A, either in a force-dependent way (via RhoA*/ ROCK* activated Fln-GAP) and a force-independent way (via RacGAP activated by IQGAP).

The cell is globally polarized by coordinated formation of new protrusions at the front and dismantling of ADs at the end, guided by the supramolecular MT–actin complexes. The dismantling of the rear ADs is triggered by the release of Ca++ burst via the opening of the calcium channels (FRAM38) through the GTPase R-Ras*. The local increase of the Ca++ level exerts two effects:

1. It activates the protease calpain, which dismantles the rear ADs by decomposing actin integrin linkers (talin and ezrin). The MT plus ends are destabilized releasing the RhoA specific guanine exchange factor GEF H1 [32] which activates RhoA. Rho A* triggers the activation of the stress fibers by ROCK whose contraction can disrupt partially dismantled ADs.

2. At the high Ca++ level at the cell end the calmodulin bound to IQGAP induces the dissolution of the Actin/IQGAP/MT complex and the liberated Rac1* is deactivated by RhoA*. Ca++ diffusing to the front is sequestered by the calmodulin associated with IQGAP and the MT plus ends are in addition stabilized by the MT-stabilizing factor stathmin which is activated by Rac-1*.

The shape of the adhering zone of the moving cell is controlled by the assembly of stress fibers polarized in the direction of motion. They generate a net force directed towards the cell center which counteracts spreading. In a pioneering work Zemel et al. [42] showed that the stationary shape of stroma cells embedded in tissue can be considered as an elastic inclusion in an isotropic matrix which is mechanically stabilized by a nematic array of force dipoles that can actively respond to external forces [41,42]. The force dipole model could explain the mechanical stabilization of the cell tissue adhesion zone by a 2D-assembly of the stress fibers extending between two adhesion domains.

The lateral tension generated by the 2D-assembly of force dipoles in the contact zone can be balanced by the tension generated in the non-adhering part by the active and passive MT–actin cross-talk. The mature adhesion domains can act both as strain sensors (due to the stretching sensitivity of the adaptor Cas) and actuators which adapt the number of F-actin fibers and myosin bundles to forces exerted by the tissue (see [4b] and references cited there).

In soft tissue stroma cells form complex shapes with slender outgrowth whose tips adhere to the matrix [43]. They protrude by parallel growth of MTs and actin gel which can also be mediated by supramolecular actin/IQGAP/Clip180/MT-complexes (according to Fig. 5b). The stabilization of the protrusions can be mediated by the balance of the retraction force generated by active stress fibers and the protrusion force developed by dynamin bound to the actin gel according to Fig. 13 of [3].

Conflict of interest

There is no conflict of interest.

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A complex between activated FAK* and Src-kinase (Src*) which forms a more versatile double head kinase than either component alone. The focal adhesion kinase (FAK) is activated through association with integrin clusters followed by the recruitment and activation of the Src-kinase as follows:

- First, FAK is weakly activated by self-phosphorylation of Tyr 397 after binding to integrin β chains. It generates an activating binding pocket for the attachment of Src, which subsequently phosphorylates other binding sites (Tyr-925 and Tyr-576/577) of FAK, thus potentiating the activity of this adaptor with kinase function.
- The Src tyrosine–kinase exposes a catalytic, a SH3 and a SH2 domain. In the resting state the enzyme is self-inhibited by mutual binding of the SH2 and SH3 domains. Activated Src* exposes a hydrophobic fatty acid chain and a polybasic segment resulting in the membrane coupling by electrostatic–hydrophobic forces.

There is a further pathway of Src activation mediated by their binding to the integrin cluster. By forming stable dimers two Src can constantly activate each other by mutual phosphorylation. In this way a high local level of activated Src* can be maintained at the adhesion domains. The Src-activity is determined by two tyrosine-groups: Tyr 416 and Tyr 527. De-phosphorylation of the Tyr 527 opens up the closed state, while the enzymatic activity is switched on by phosphorylation of Tyr 418 (through the adaptor Crk). The first activation step is triggered by the phosphatase PTP-PEST.

Appendix B. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamcr.2015.06.012.

References


Appendix A.1. Abbreviations

(*) Stars indicate activated states of molecular switches (such as GTPases) and activators, (such as Cas and Crk).

AD adhesion domains
FAK focal adhesion kinase
GAP GTPase-activating protein which de-activates GTPases
GEF guanine exchange factor; activates GTPases by GDP→GTP exchange
MT microtubule
PM plasma membrane
Src Tyrosine kinase which can associate with FAK
Crk adaptor protein recruiting cytoplasmic proteins close to Src kinases
Cas Crk-associated substrate. Itforms a forced dependent adaptor cascade with Crk
Pl-4,5-P2 phosphatidyl-inositol 4, 5 phosphate
Pl-3,4,5-P3 phosphatidyl-inositol 3, 4, 5 phosphate
Pl-3K phosphatidylinositol-3-kinase acts as PI3P → PI3P transformer

A.2. The FAK/Src tandem kinase

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