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Cytoskeletal mechanisms responsible for invasive migration of neoplastic cells

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ABSTRACT Cytoskeletal reorganizations, especially alterations of contractile tension generated by the actin-myosin cortex, are of central importance in the development of the phenotype of morphologically transformed neoplastic cells with invasive behavior. These reorganizations can be regarded as genetically determined aberrations of the physiological reactions of normal cells which are responsible for their ability to undergo exploratory migrations, including epithelio-mesenchymal transformations, invasion of matrix by epithelial tubules *etc*. It is suggested that these physiological and neoplastic transformations are based on Rho-dependent alterations in contractility. A decrease or an increase in contractility may result in the development of distinct types of invasive phenotypes. These contractility-dependent phenotype alterations may be modified by alterations in the expression of other genes, especially of those coding for components of adhesive structures.

KEY WORDS: *microfilament, microtubule, epitheliocyte, fibroblast, tension*

Introduction

Cell locomotion, including invasion of the foreign tissue territories, is closely associated with dynamic morphology of this cell and, especially with the dynamics of its cytoskeletal and adhesive structures. The two main morphological types of tissue cells, epitheliocytes and fibroblasts, acquire the ability for invasion mostly under special conditions, especially in the course of neoplastic evolution. In this review we will briefly discuss morphology and migration of these two types of cells in culture before and after neoplastic morphological transformations. We will also describe reversible transitions between epitheliocytes and fibroblasts; these transitions play important roles in normal morphogenetic processes and may be regarded as normal prototypes of irreversible genetic transformations of morphology and locomotion responsible for invasive behavior of neoplastic cells. These transformations will be discussed in the last part of the review.

The review discusses mainly the results of the studies of locomotion as analyzed in the experiments with 2D cultures. Results of molecular studies are reviewed in other papers of this series and will be only briefly recapitulated here. Comprehensive review of all aspects of earlier studies of invasion was presented by Mareel and Leroy (2002). Studies of invasion in 3D systems are extremely important but at present they are still scarce. Whenever possible, their results will be compared with those of 2D studies.

Abnormal invasive migrations as aberrations of developmental exploratory reactions

Normal invasive migrations may be regarded as a large group of exploratory mechanisms playing essential roles in evolution of Metazoa. Defining characteristic of these mechanisms as formulated by Gerhardt and Kirshner (1997) is generation of large amounts of variations of cells, from which other factors select a single functional outcome.

The outcome of exploratory migrations occurring in the course of normal development are multiple final choices of differentiation, cell shape and cell position. One may say that all developmental migrations are exploratory, but to a different degree (Fig. 1). For instance, when epitheliocytes migrate as a coherent sheet into a wound made in culture, the degree of "exploration" is minimal, as mutual positions of these cells locked to one another by cell-cell contacts remain almost unchanged in the course of migration. Wound healing accompanied by formation of outgrowths (Omelchenko *et al.*, 2003) involves somewhat larger changes of cell-cell positions and greater degree of environment exploration.

Degree of exploration is much higher during migration of the cells of another type, fibroblasts. These individually moving cells

Abbreviations used in this paper: EMT, epithelio-mesenchymal transformation; HGF/SF, hepatocyte growth factor/scatter factor.

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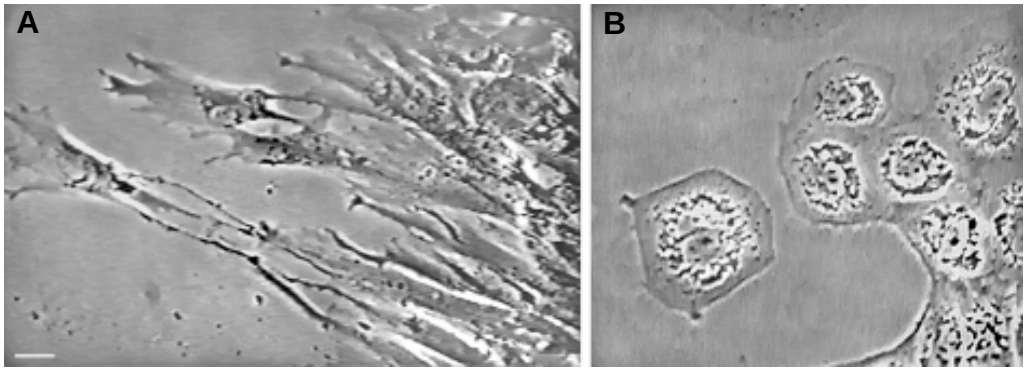

Fig. 1. Cells moving into the wound. **(A)** *Individually moving elongated fibroblasts.* **(B)** *Epitheliocytes moving as a sheet. Courtesy of O.Y. Ivanova and A.Y. Alexandrova.*

continually change their mutual positions, orientations and shapes. These changes are a result of searches performed by pseudopodial reactions, that is, by extension, attachment, and retraction of pseudopods at the leading edge of the cells. These pseudopodial reactions are based on polymerization of actin microfilaments and subsequent other reorganizations of actin cytoskeleton controlled, among other factors, by a special group of small GTPases, such as cdc42, Rac, and Rho proteins. Pseudopodial extensions are initially rather random but eventually they lead to adaptation of cell position and orientation to certain environmental factors, such as adhesiveness and rigidity of various areas of the substrate (so-called contact orientation), contacts with other cells (so-called contact inhibition of movement), and chemotactic gradients of certain proteins in fluid medium. All these environmental cues modulate distribution of pseudopodial extensions, and the choice of cell shape and direction of movement is gradually stabilized by a microtubule-dependent mechanism. The cell can also modulate its area and shape in response to alterations of substrate adhesiveness. All these choices are reversible.

In the next paragraph we will briefly review the cellular mechanisms responsible for migratory behavior of epitheliocytes and mesenchymal cells or, more exactly, of their prototype variant, cultured fibroblasts.

Dynamic morphology of fibroblasts

The characteristic phenotypic features of fibroblasts are elongated cell shape and antero-posterior polarity manifested by formation of one or several peripheral flattened lamellas and division of the cell edge into active, pseudopodia-forming zones and stable, non-active zones. Moving fibroblast usually has single wide anterior lamella with leading active edge at its pole; the narrow tail is located at the opposite pole. This posterior pole often has small lamella at its end so that when the cell meets some obstacle at the anterior end, it can easily enlarge the posterior lamella and start moving in the opposite direction without turning the cell body.

Active edge and lamella

Active edge zone is a cambial structure where all or almost all actin microfilaments are polymerized from monomeric actin. The process of polymerization involves formation of complexes of specific proteins at the edge. In particular, WASP/Scar complex activates Arp2/3 complex which initiates polymerization of new microfilaments at the sides of pre-existing microfilaments so that a branched "microfilament tree" is formed (Borisy and Svitkina, 2000; Pollard, 2003). This unique design determines co-operative action of newly formed microfilaments. These microfilaments push the membrane forward forming the flattened protrusions (lamellipodia). From the membrane, microfilaments start to move backwards, gradually replenishing all other parts of actin cortex and actin bundles in the cell body.

Dynamic observations of centripetal movements of microfilaments (Alexandrova *et al.*, 2002) indicate that their mechanisms are different in two zones of the active edge. In the distal zone (designated as lamellipodium) the velocity of actin movement is high (7-8μm/sec), and actin microfilaments are not associated with myosin II molecules. In the proximal zone (designated as lamella), the microfilaments form more regular bundles and in these bundles they are associated with myosin II and with certain other actin-binding proteins. Actin and myosin continue to move centripetally within the bundles but their movement is much slower than that in the lamellipodium. The boundary between distal and proximal zones is formed by adhesive structures (focal complexes and focal contacts, see below). The centripetal movement of actin stops at the boundary of lamella with the cell body. In the course of centripetal movement part of actin microfilaments is gradually "debranched" and depolymerized; possibly, this de-

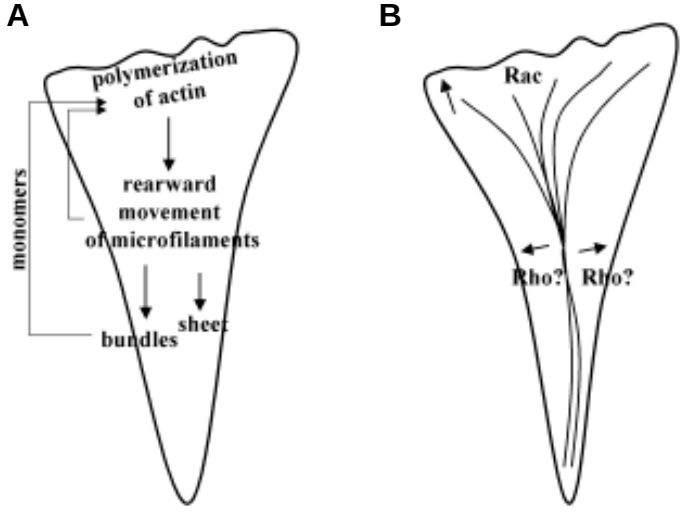

Fig. 2. Organization of actin cortex dynamics. **(A)** *Scheme of the circuit of actin in fibroblasts.* **(B)** *Hypothetical scheme of activation of Rac at the ends of microtubules and of Rho at the sides of microtubules.*

polymerization is associated with the ageing of these microfilaments manifested by the dissociation of actin bound ATP to ADP; depolymerization is also promoted by a special protein, cofilin (Pollard and Borisy, 2003).

To form the full "actin circuit", monomers formed during the centripetal flow should be brought back to active edge for repolymerization (Fig. 2A). As shown by Zicha and collaborators (2003), labeled monomeric actin injected into the cytoplasm of the cell body reaches the active edge at a very high velocity, passing 5 μm per second. As suggested by these authors, most probably, monomeric actin solved in the cytosol is moved forward by the hydrostatic pressure of the cortex.

The main gap in this emerging picture of actin dynamics is the scarcity of knowledge about the architecture of submembraneous actin cortex in general and, especially, near active edge, where monomeric actin penetrates into the zone of polymerization.

This problem of the organization of the cortex is closely related to another unsolved problem, that of the mechanisms determining the appearance of another form of protrusions, blebs, at the active edge.

Blebs at the edges are extended in different situations: at the early stages of apoptosis, after depolymerization of microtubules (Pletjushkina *et al.*, 2001), in the cell with deficiency of the actin-binding protein ABP-180 cross-linking microfilaments (Cunningham, 1995). Polymerized actin is not initially present within blebs, but appears later (Keller and Eggli, 1998). Possibly, blebs occur when the pressure of the cortex drives local outward expansion of the membrane and is then stopped by polymerization of actin. Functions of blebs are not clear. Probably, blebs at the anterior edge can replace lamellipodia in driving forward movement of the cell.

The third type of surface extensions are the filopodia. They are abundant at the earliest stage of cell spreading but later they are replaced by lamellipodia and in fully spread cells of most types they are not numerous. Filopodia are shown to be formed by the reorganization of branching network of microfilaments (Fig. 3). This reorganization involves fusion of plus ends of growing independently nucleated microfilaments and accumulation of the Vasp protein at these ends (so-called lambda model, Svitkina *et al.,* 2003).

Possibly, filopodia serve as "tentacles" by which the cell feels surrounding substrate. Attached filopodia may also act as "rails" directing the outward movement of lamellipodia.

Focal complexes and contacts

Different forms of focal contacts are the main adhesive structures connecting the cell with the extracellular matrix. They consist of two components: transmembrane component made by clusters of integrin molecules linked to the matrix and submembraneous component made by a complex of specific proteins such as vinculin, talin, special focal adhesion kinase (FAK), and many others. This complex is linked to the ends of actin microfilaments. Focal contacts have several stages of development: focal complexes (round spots less than 1-2 μm in diameter); mature focal contacts (elongated strips, 2-8 μm in length) and "supermature" focal contacts, up to 15-20 μm long; the last stage is characteristic for the development of the so-called myofibroblasts (Fig. 4; see Dugina *et al.,* 2001). Protein composition of focal contacts may be changed in the course of their maturation (Zaidel-Bar *et al.,* 2003).

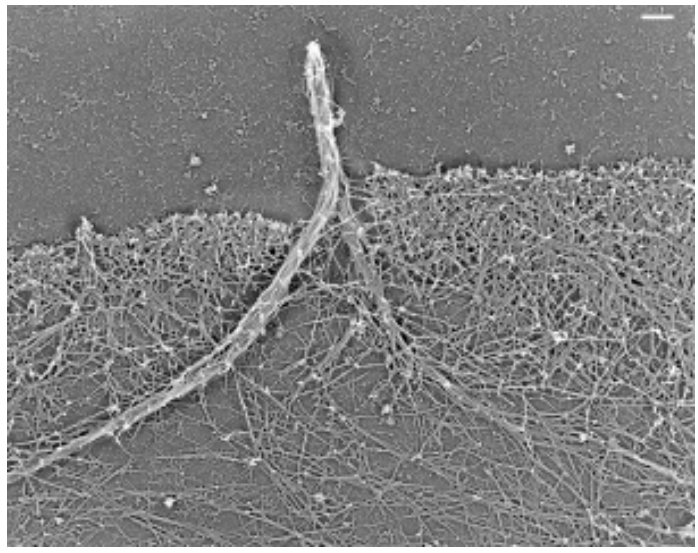

Fig. 3. Network of actin microfilaments and developing filopodium at the edge of a fibroblast. *Electron microscopic replica. Courtesy of T.M. Svitkina and E.A. Bulanova.*

Focal complexes are usually formed at the surface of lamellipodium, near the active edge (Fig. 5). Position of newly formed focal complexes determines the boundary between lamellipodium and lamella (see above); this position is changed with the appearance of each new focal complex. Formation of the new focal complexes near the active edge suggests that this part of the membrane has some special adhesive properties for the matrix (Nishizaka *et al.,* 2000). The nature of this localized adhesiveness is not clear. One possibility is that microfilaments at the active edge may be immediately linked to the integrin molecules in the nearby membrane. Contact of lamellipodium with the matrix may link these integrins to their ligands and then cluster them, initiating formation of the focal complexes.

Evolution from complexes to mature and supermature contacts is associated with the tension developed by attached actin bundles: factors increasing the tension cause maturation of contacts and vice versa (Chrzanowska-Wodnicka and Burridge, 1996; Dugina *et al.,* 2001; Geiger and Bershadsky, 2002).

At the outer side of the membrane focal contacts are also linked to matrix fibers made of fibronectin and other proteins. These fibers are stretched by the cortical tension and may be detached in the course of cell movement. Disorganization of the fibronectin matrix around the cell led to a drastic decrease of the length of focal contacts and of the size of associated actin bundles (Dugina *et al.,* 2001). Naturally, focal contacts not only react to actin-myosin tension, but also transmit this tension upon the matrix with which they are linked. Paradoxically, typical focal adhesions exert tension forces proportional to their size, while some small (less than 1 μm) adhesions produce non-proportionally high forces (Tan *et al.,* 2003).

Thus, the cytoskeletal and adhesive organization of fibroblast is determined by mechanochemical tension generated and transmitted in the triad: actin cortex, focal contacts, and cell-associated matrix. We do not know yet the molecular mechanisms of this control. Possibly, tension exerted upon focal contacts by the actin bundles and by the matrix activates integrin-associated signal

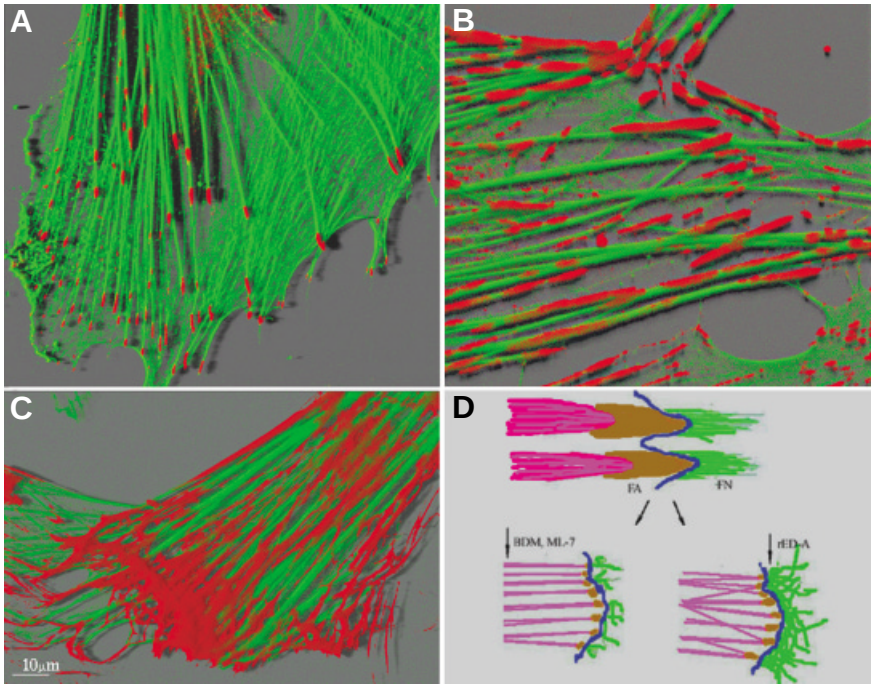

Fig. 4. Focal contacts, actin bundles, fibronectin and the active edge of a human fibroblast. (A) *Actin microfilaments (green) and mature focal complexes stained for paxillin (red) .* **(B)** *Actin microfilaments containing α-smooth muscle actin (green) and supermature focal contacts stained for paxillin (red).* **(C)** *Actin microfilaments (green) and fibronectin fibers (red) near the edge of a fibroblast.* **(D)** *Scheme showing reversible maturation of focal contacts. Top: large actin bundles (red), supermature contacts (brown), and fibronectin fibers (green). Bottom: reversal of mature structures after inhibition of contractility by BDM (left) and after disruption of fibronectin by rED-A (right). BDM is 2,3 butanedione-2-monoxime. rED-A is soluble recombinant fibronectin fragment inhibiting formation of extracellular fibronectin network. See Dugina* et al.*, 2001. Courtesy of V.B. Dugina.*

pathways eventually establishing the adequate equilibrium between these three structures.

Regulation of actin-myosin contractility

Tension and other parameters of the actin-myosin cortex are controlled by several subsystems of regulatory enzymes. The first of these systems controls directly myosin contractility by modulating the myosin light chain (MLC). Activation of myosin contractility is achieved by action of MLC kinase and the opposite action by MLC phosphatase.

These enzymes, in turn, are regulated by another subsystem, so-called Rho group of small GTPases. This group includes, among other members, Rho proteins, Rac proteins, and CDC42 proteins (Hall, 1992, 1998; Fukata *et al.,* 2001; Ridley, 2001; see reviews in Van Aelst and Symons, 2002; Caron, 2003; Riento and Ridley, 2003).

Rho proteins are GTPases with many targets (Fig. 6). Targets of Rho include Rho kinase (RhoK/ROCK), mDia1, LIMK and others. Rho kinase inactivates MLC phosphatase and, probably, directly phosphorylates MLC increasing myosin activity and contractility of actin-myosin. ROCK together with mDia1 can also stimulate stress fiber formation. In addition, ROCK phosphorylates and activates LIMK and thus inhibits cofilin-induced depolymerization of actin microfilaments.

Morphologically, this Rho/ROCK induced increase of contractile tension results in formation of numerous actin bundles and associated mature focal contacts. In contrast, inhibition of Rho or RhoK activities by specific genetic constructs or by selective inhibitors leads to disassembly of actin bundles and to transformation of focal contacts into small focal complexes; this transformation is, probably, the consequence of decreased actin-myosin tension (see above). In addition to ROCK, there are several other target proteins activated by Rho. One of them, mDia1, stimulates polymerization of actin and stabilizes microtubules not linked to centrosome. mDia1, possibly decreases the rate of cytoskeleton reorganizations (Geiger and Bershadsky, 2002).

Activity of each Rho family protein is modulated by an array of special proteins: it is catalyzed by exchange factors (GEFs) and inactivated by GTPase activating factors (GAPs).

Besides Rho, other proteins of the same group, CDC42 and Rac, also play important roles in the control of actin dynamics. Injection of CDC42 induces formation of numerous filopodia, while injection of Rac induces extension of lamellipodia (Hall, 1992; Nobes and Hall, 1995; Hall, 1998). For instance, plating of suspended cells on fibronectin induces activation of Rac and CDC42, probably, via activation of integrin pathway (Del Pozo *et al.,* 2000). This activation leads to formation and attachment of extensions and, eventually, to cell spreading.

The Rho family is not the only group of proteins regulating contractility. One of the other factors involved in this regulation is caldesmon which inhibits myosin ATPase activity. Transient transfection of the DNA coding this protein into human fibroblasts decreased cell contractility and interfered with formation of stress fibers and focal adhesions (Helfman *et al.,* 1999). The role of this protein in regulation of cell motility and shape remains to be investigated.

Microtubular system

Microtubular system is another cell component essential for maintenance of cell shape and directional motility. Microtubular system controls elongated shape of fibroblast and division of its edge into active and non-active zones. Depolymerization of microtubules of fibroblast by colcemide, nocodazole or other similar specific drugs leads to the loss of elongated cell shape and to disappearance of non-active zones of the edge; pseudopodial activity is randomly distributed along all the cell perimeter (Vasiliev *et al.,* 1970). Besides loss of polarity, drug-induced depolymerization of microtubules leads to stimulation of the contractility of the cortex accompanied by the increase of the number of actin bundles in the cytoplasm (Harris, 1973; Harris *et al.,* 1980; Danowski, 1989). This stimulation of contractility is mediated by activation of Rho induced by GEP-H1 normally associated with microtubules and released into cytoplasm after their depolymerization (Krendel *et al.,* 2002). When RhoK activation and increase of contractility are prevented by a specific inhibitor, microtubule-

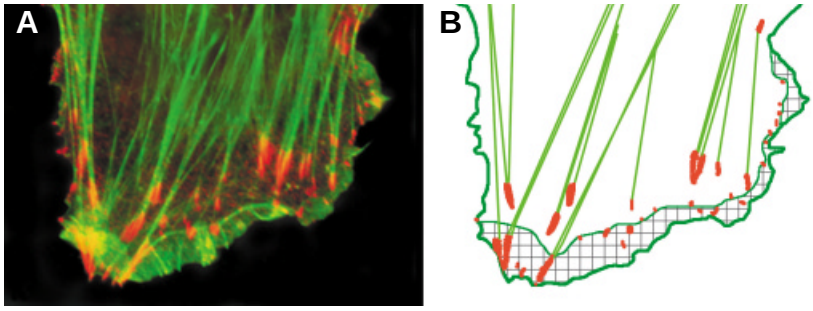

Fig. 5. Two zones of the active edge of fibroblasts. **(A)** *Photograph; green, actin; red, focal contacts.* **(B)** *Corresponding scheme showing lamellipodium zone (squares) and lamella (without squares). Courtesy of A.Y. Alexandrova.*

depolymerizing drugs still lead to the loss of cell polarity (Omelchenko *et al.,* 2002). This result shows that microtubular control of polarity is independent of Rho/ROCK regulation of contractility.

What is the mechanism of this control? One plausible possibility is that it is due to stimulation of lamellipodial activity in the zone of the cell edge near the ends of microtubules. This stimulation was observed in different situations (Bershadsky *et al.,* 1991; Waterman-Storer *et al.,* 1999) – it may be caused by activation of Rac by the ends of microtubules present in this zone. In fact, it was shown (Kawasaki *et al.,* 2003) that a Rac-activating protein, termed Asef, may bind to the APC protein which, in turn, binds to the plus ends of the growing microtubules. Thus, microtubules may control both actin polymerization at the edge and contraction of actin-myosin in the cell body (Wittman and Waterman-Storer, 2001). Repeated extension and attachment of lamellipodia in the certain zone of the edge obviously will eventually lead to elongation of a cell in corresponding direction and to directional movement.

Elongation-promoting action of microtubules counteracts the contractile action of actin-myosin cortex, and the balance of these two opposite forces seems to determine the average length of fibroblast (Levina *et al.,* 2001). Microtubules can also affect cell shape and motility by modulating focal contacts with the matrix. It was shown (Small *et al.*, 1999; Kaverina *et al.*1999; Small and Kaverina, 2003) that the ends of growing microtubules target nearby focal adhesions and then may promote their dissolution in conjunction with retraction of the cell edge. These interesting interactions may promote both protrusion at the active edge and retraction at the rear of the moving cell.

Movement of single fibroblasts

Dynamic construction of fibroblast described above is the basis of its motility. Polymerization of actin at the active edge leads to extension of lamellipodia and then to elongation of the whole cell body. Attachment of lamellipodia to the matrix, formation of focal contacts and associated actin bundles creates centripetal contractile tension. This cortical tension from time to time leads to detachment of the cell tail. Contraction of the tail part of the cell body is followed by activation of lamellipodial extensions at the opposite active edge (Chen, 1978). Most probably, this activation is due to contraction-induced stimulation of the forward flow of monomeric actin to the active edge (see above). Ingrowth of the ends of microtubules into the advancing anterior lamella activates lamellopodial extension and stabilizes polarization of their formation.

This locomotory mechanism is able to finely adapt the polarized cell shape and the direction of cell movement to very diverse changes of microenvironment. For instance, fibroblast in sparse culture on the isotropic planar substrate can extend laterally wide anterior lamella and often form several lamellar processes. In contrast, when the same fibroblast is squeezed on the narrow strip of an adhesive substrate, it acquires elongated fusiform shape. As already mentioned, the maximal length of fibroblast remains similar on the plane and on the strip due to the equilibrium of elongating action of microtubules and contractile action of cortex (Fig. 7). Similar alteration of the shape takes place when the fibroblasts are spread on poorly adhesive substrata (see below). In this case, fibroblast retains almost the same length as on standard glass, but becomes narrower and has fewer stress fibers and focal contacts. Fibroblast can exactly orient itself along the gradient of substrate adhesiveness or along the axis of cylindrical substrata. Thus, fibroblast can change considerably its transversal area (so-called transversal spreading) without changing significantly its length along the body axis (longitudinal spreading). In other words, these two components of spreading may have somewhat different mechanisms. Possibly, the ends of microtubules located in the center of the active edge near the cell axis maximally stimulate polymerization of actin and extension of lamellipodia there; this stimulation is responsible for longitudinal spreading. In contrast, transversal spreading is less dependent on microtubules but strongly dependent on the efficiency of attachment of lamellipodia to the matrix (see below about cell spreading on poorly adhesive substrata). These differences provide the basis for cell polarization and for high adaptability of cell shape and movements to the microenvironment. Due to this adaptability, mesenchymal cells *in vivo* interact with great exactitude with the various matrix structures in bones, cartilages, submucosal and dermal connective tissues *etc*.

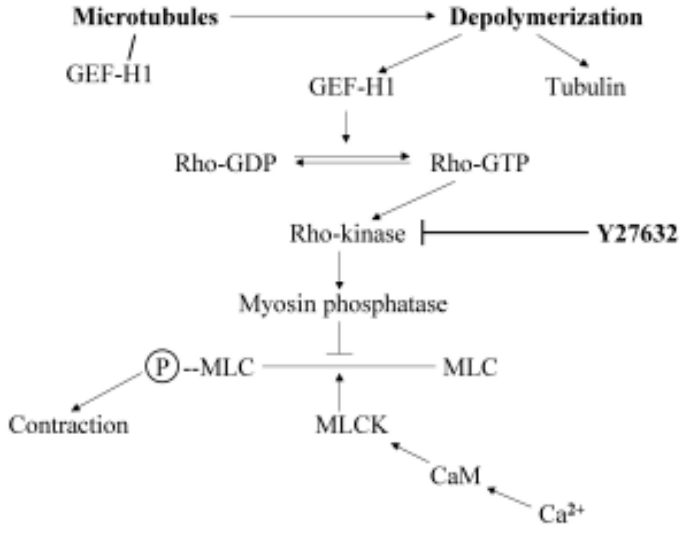

Fig. 6. Simplified scheme of the functions of Rho proteins.

Cell-cell contacts

Homotypic cell-cell contacts are another essential factor modifying the motile behavior of fibroblasts. The main cell-cell structures contain transmembraneous component, typically N or other cadherins, linked by extracellular domains to identical molecules at the surface of contacting cell and the complex of submembraneous proteins (β-catenin, α-catenin, p120) linked to cytoplasmic domains of cadherin clusters and to the ends of actin microfilaments. These contacts are usually formed after collisions of active edges of two fibroblasts moving towards one another. When this collision occurs, the edge of one cell continues to move, overlapping the surface of another cell; however, after several minutes this forward movement stops, the pseudopodial activity of the overlapping edge is inhibited and the contact structure is formed. This inhibition of activity by cell-cell contact was described long ago and designated as "contact paralysis" (Abercrombie, 1970; Abercrombie and Dunn, 1975). The nature of signal pathways from the contacts to the sites of actin polymerization, which are responsible for this local paralysis, is still obscure.

After the paralysis, the cell usually develops pseudopodial activities at other contact-free sites of the surface and starts to move in another direction. The statistical result of many collisions of this type is that fibroblasts become oriented approximately parallel to one another forming so-called streams. Here again we know nothing about the mechanism of contact-induced change of localization of the active edge.

Cadherin-containing contacts in these cultures are typically localized at the cell poles and have the shape of numerous short sticks approximately parallel to one another and co-localized with the ends of straight actin bundles (Fig. 8). The lengths of these cell-cell "radial" contacts, like that of cell-matrix focal contacts (see above) depends on the tension of the associated actin bundles: when this tension is decreased, the contacts acquire the form of round dots (Gloushankova *et al.,* 1998; Krendel *et al.,* 1999).

Epithelial cells

A fully spread single epitheliocyte has discoid or polygonal shape; its entire perimeter has pseudopodial activity. Circular actin bundle is present near the active edge; microtubules are usually located in the central part of the cytoplasm and do not penetrate outside the circular actin bundle. Dynamic observations show that the circular bundles are continually formed from the centripetally moving actin-rich lamellipodia formed at the active edge (Krendel and Bonder, 1999). Collision of two epithelial cells immediately leads to paralysis of the contacting parts of the cell edge. Contacting lamellipodia immediately form cadherin-containing contacts which have the tendency to expand laterally and are oriented tangentially along the edges of contacting cells (Fig. 8). Certain variants of epiotheliocytes, *e.g.* skin keratinocytes, seem to form initial cell-cell contacts not by lamellipodia but by filopodia (Vasioukhin and Fuchs, 2001). Epithelial contacts contain specific E-cadherins. These contacts are very stable. Contacting cells form coherent islands and sheets and individual cells are not separated from the edge of the sheet. In these sheets, all central cells have no pseudopodial activity at the upper surface; this activity is paralyzed by cell-cell contacts. However, pseudopodia can be extended under these contacts at the lateral surfaces (Fetisova *et al.,* 1990).

Obviously, isolated discoid epithelial cells cannot move directionally, as they have pseudopodial activities along the whole perimeter. In the sheet the only cells which move actively are those at the contact-free edges. These cells expand on the cell-free substrate without breaking contacts with their lateral and posterior neighbors.

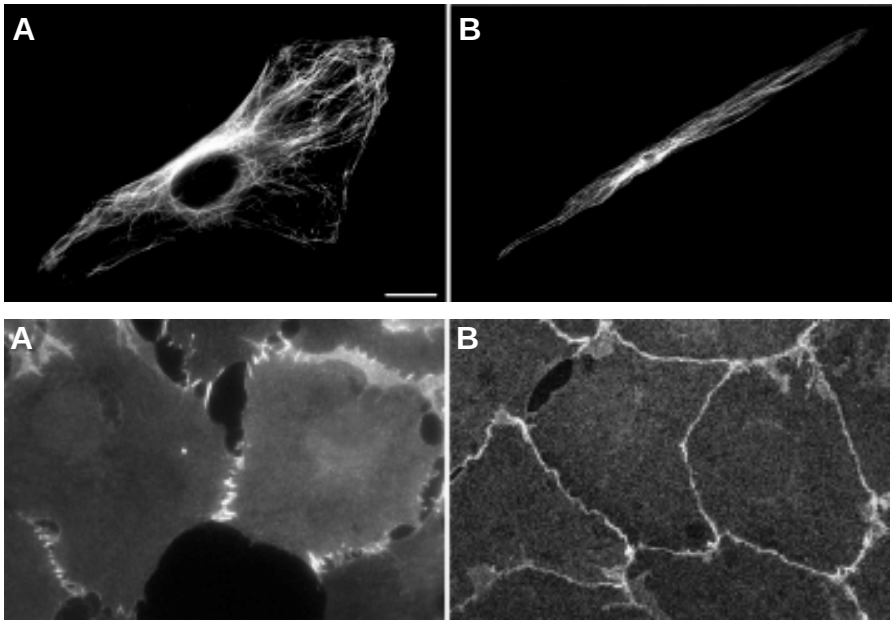

Fig. 7 (Top). Control of fibroblast cell length. *Microtubules in fibroblasts spread on the plane* **(A)** *and on the narrow strip of substrate* **(B).** *Notice that the length of both cells is similar. Courtesy of M.A. Kharitonova.*

Fig. 8 (Bottom). Different geometry of cell-cell contacts between two fibroblasts (A) and between epitheliocytes (B). (A) *Intercellular contacts stained for β-catenin in Rat-1 fibroblasts are perpendicular to the cell edges.* **(B)** *Similar contacts stained for E-cadherin in IAR2 epithelium are parallel to the cell edges. Courtesy of N.A. Gloushankova.*

Reversible transitions from the epithelial to the mesenchymal phenotype

Transformation induced by scatter factor HGF/SF

One of the most spectacular and complex exploratory processes characteristic of vertebrate ontogenesis *in vivo* is formation and evolution of neural crest (reviewed in Le Douarin and Kalcheim, 1999; La Bonne, 2002). Neural crest cells leave the boundary between neural plate and surface ectoderm and migrate individually throughout all the parts of the embryo until they finally settle in numerous sites. In response to various local signals received at these sites neural crest cells proliferate and are differentiated into variety of cell types from

chondrocytes to melanocytes and neurons. Here we have a typical example of cells linked to one another in compact tissue structure being converted into scattered migratory cells, and after exploratory migrations making many different choices.

"Epithelio-mesenchymal transformations" (EMT) are the best studied prototypes of these processes *in vitro*. In EMT epithelial cells growing as compact sheets on a standard plane substrate dissociate from the sheets and are transformed into elongated fibroblast-like cells with polarized pseudopodial activity. These cells scatter and actively move upon the substrate. EMT can be induced by various specific proteins interacting with corresponding membrane receptors. HGF/SF (hepatocyte growth factor/scatter factor) is best known among these proteins (reviewed in Trusolino and Comoglio, 2002). HSF/SF is a protein produced by many cell types, especially, many types of fibroblasts *in vivo* and *in vitro*. HGF/SF binds to a receptor protein c-met. EMT is accompanied by profound reorganization of actin cytoskeleton and, especially, by disappearance of circular actin bundles typical of epitheliocytes and by the development of straight actin bundles (Birchmeier *et al.,* 2003). Intact microtubular system is essential for transformation (Dugina *et al.,* 1995). Dissociation of epithelial cell-cell contacts is also a typical feature of EMT. However, alterations of cell shape and of motility patterns typical of HGF/SF can be induced by this protein in multinuclear single epithelial cells that, by definition, have no cell-cell contacts (Alexandrova *et al.,* 1998). Therefore cell-cell contacts are not a primary target of HGF/SF responsible for cell scattering.

HSF/SF may induce not only complete scattering of epithelia into single cells but also outgrowth of branching tubules from compact epithelial vesicles and from other tubules. This remarkable effect was observed when cultures of cells sensitive to HGF/SF were grown not as monolayered sheets on flat substrata but as epithelial cysts embedded in collagen gel (Fig. 9; see Montesano *et al.,* 1991). The picture of tubules growing in these conditions *in vitro* closely resembles many examples of tubulogenesis and angiogenesis *in vivo*. Both these groups of processes can be regarded as special variants of normal exploratory migrations.

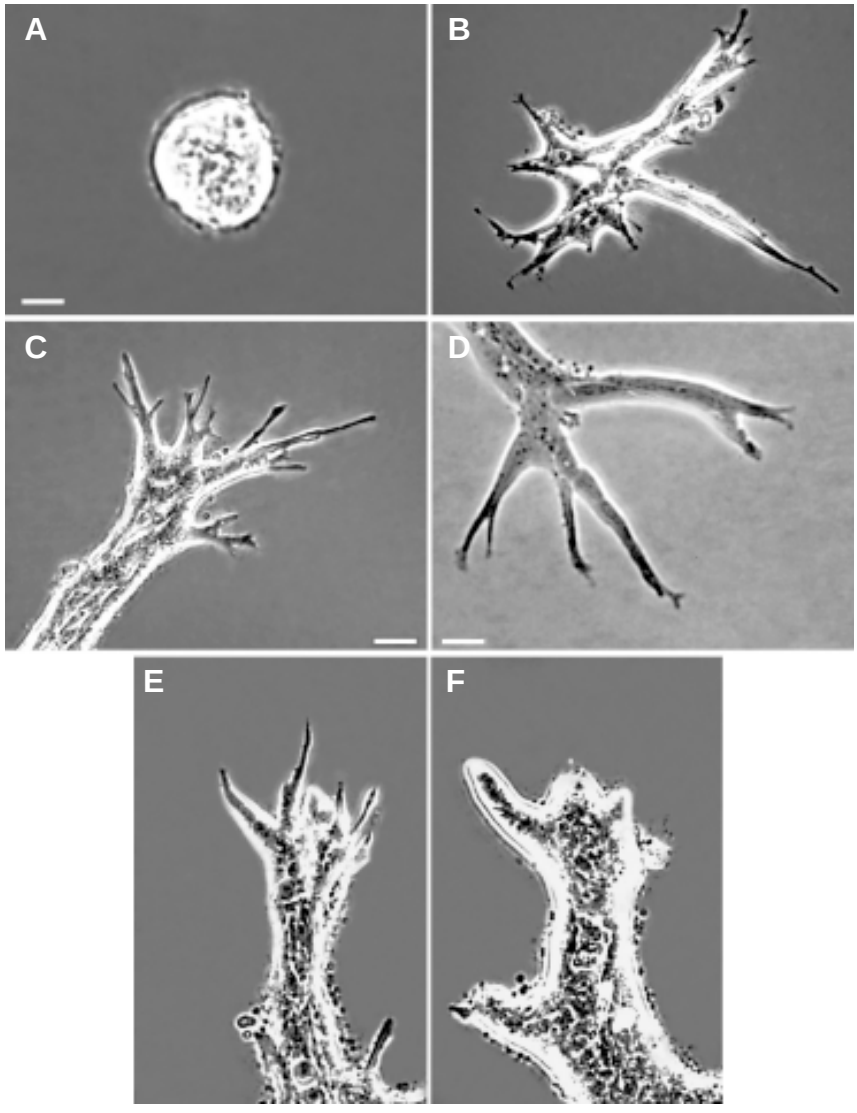

Fig. 9. Effects of scatter factor (HGF/SF) on MDCK epithelial cells growing in collagen gel. **(A)** *Control culture without HGF/SF, 24 h. Spheroid cyst without outgrowths.* **(B)** *HGF/SF, 24 h. Elongated cells radiating from the center of the aggregate.* **(C,D,E)** *Ends of epithelial tubules growing in the presence of HGF/SF for 5 days. Elongated cells at the ends of the tubules.* **(F)** *The same tubule as in E at 2 days after withdrawal of HGF/SF. Notice that elongated cells disappeared while large tubular structures were preserved. Courtesy of E.A. Bulanova.*

Thus, experiments with HGF/SF suggest that, depending on the nature of microenvironment, the same inducing factor acting upon the cells of the same type may induce different variants of exploratory migrations: either migration of single cells or outgrowth of branching tubules. (Affolter *et al.,* 2003; Zegers *et al.,* 2003). Apparently, mechanisms leading to these two variants of switches are closely related. In fact, partial transitions of epithelial cells on the tips of the outgrowths into polarized elongated cells seem to occur in the course of branching morphogenesis. For instance, branch formation in the *Drosophila* tracheal system induced by local activation of Fibroblast Growth Factor receptors of the cells on the branch tip is accompanied by formation of filopodial extensions and then of the broader extensions (Affolter *et al.,* 2003). These cells at the tips of the branch may then move forward pulling out other cells of the tube. This way of branch outgrowth closely resembles partial epithelio-mesenchymal transitions at the edge of healing epithelial sheets (see below).

Tension exerted by matrix fibers on the cells *via* contacts, in all probability, plays an important role in the mechanism of outgrowths leading to tubule formation. In experiments of Wozniak and collaborators (2003), breast cells cultivated in floating 3D collagen matrix formed tubules: in these conditions Rho was

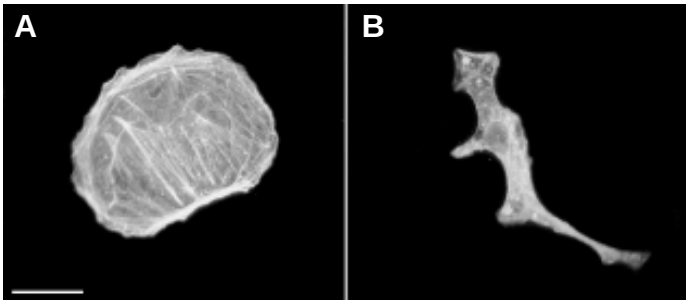

Fig. 10. Epithelio-mesenchymal transformation of cells on poorly adhesive substrate. (A) *A discoid IAR2 cell on a standard adhesive substrate.* **(B)** *An elongated IAR2 cell on a poorly adhesive PolyHEMA substrate. Staining for actin. Courtesy of T.G. Moizhess.*

down-regulated and contractility of the cells decreased. In contrast, when the matrix was made more rigid by attachment to the surface of culture dish, Rho activity remained high and tubulogenesis was disrupted. In these conditions Rho/Rac regulation of cell contractility was apparently regulated by cell-matrix attachment sites via integrin receptors. Similarity of this situation to the formation of epithelial outgrowths from epithelial sheets inhibited by high Rho activity (see below) is obvious.

Similar mechanisms may be responsible for many other morphogenetic reactions playing essential roles in development, such as gastrulation, formation of neural tube *etc*. HGF/SF and its receptor have many versatile roles in embryonic development: they are essential for the formation of many organs including liver, kidney, lung, nervous system and others. Interactions of other ligands, *e.g.* FGF, VEGF with their receptors leading to similar reactions may be responsible for other morphogeneses (Birchmeier *et al.,* 2003).

Epithelio-mesenchymal morphological transformation during normal spreading of fibroblasts

Besides "classical" SF-induced EMT described above, there are several examples of similar transformations of cell shape and cytoskeletal organization developing without application of special inducing ligands. One of them is the transformation observed in the course of normal spreading of suspended embryo fibroblasts on the standard planar culture substrata (Vasiliev and Gelfand, 1981). The surface of suspended cells is covered with blebs or, more rarely, with microvilli. When these cells touch the substrate, they extend first filopodia and then lamellipodia in all directions, their central body becomes flattened so that at 20-30 min after seeding they acquire discoid epithelioid shape. Similarity to epitheliocytes is increased by the formation of circular actin bundle in peripheral cytoplasm; microtubules are localized centrally to the bundle. After 1-2 h, the cells resume spreading, but at this stage, pseudopodia are formed only at certain sites of the cell perimeter, so that the cell acquires polygonal and then elongated shape typical for fibroblast. Simultaneously the circular actin bundle is fragmented and straight bundles crossing the cell body are formed. Microtubules grow into peripheral cytoplasm toward the cell poles.

Interestingly, discoid shape and circular actin bundles may be restored when the cell is treated with taxol disintegrating and fragmenting its microtubular system (Pletjushkina *et al.,* 1994).

Polarization of epitheliocytes on poorly adhesive substrata

When discoid epitheliocytes are spread on the substrata with decreased adhesiveness, *e.g.* on the substrata covered with PolyHEMA, a substance decreasing the adhesiveness of various surfaces, their final area on the substrate, as expected, drastically decreases. Simultaneously, the shape of these cells becomes elongated, and the circular actin bundle disappears (Fig. 10; see Moizhess and Vasiliev, 2001). PolyHEMA is usually regarded as chemically inert substance, and it seems probable that alteration of shape here is due to general decrease of the area available for spreading and not to reactivation of some special receptors.

Partial transformations of epitheliocytes at wound edges

Cultured epitheliocytes at the edge of a coherent sheet usually have small lamellas and marginal semicircular bundle of actin linked *via* lateral cell-cell contacts to similar bundles of neighbor cells. These continuous cables of filamentous actin formed along the wound margin are very similar to those formed in the wounds made in embryonic chick; they are called "purse strings". Small holes in the monolayer are often healed by coherent movement of all edge cells and constriction of a "purse string"; this process is accompanied by extension of filopodia but not by any other significant changes of organization of edge cells (Martin and Lewis, 1992; Harden, 2002).

Somewhat different cell-cell interactions were observed during closing of wounds in the upper layer of chick blastoderm (Bortier *et al.,* 1993). Here individual cells in the sheet had considerable freedom of movement. As described by these authors, they "were seen to move like sheep in the flock: individual cells in different directions, the whole flock towards the wound". This variant certainly deserves further analysis.

Yet another variant of cell movement was observed during healing of large wounds in the sheets of certain epithelial lines (Figs. 11, 12; see Omelchenko *et al.,* 2003). In this case, some edge cells undergo reorganizations that make them somewhat similar to fibroblasts: they develop large anterior lamella and lose marginal bundle of actin. Instead, they acquire several straight actin bundles. In contrast to individually moving fibroblasts, these "leader" cells remain firmly connected with neighbors and when

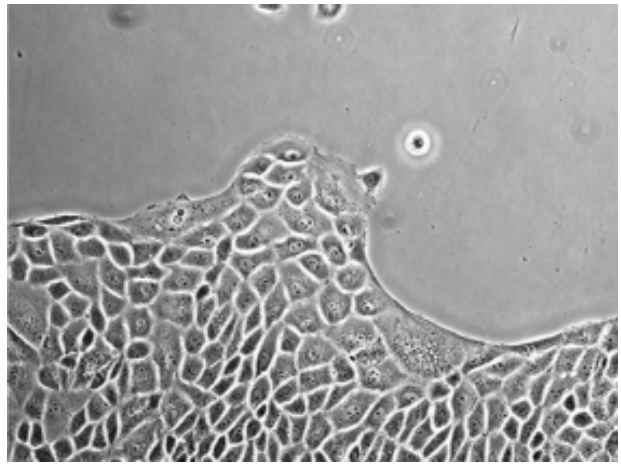

Fig. 11. Epithelial outgrowth from the edge of the wound of IAR2 monolayer. *10 hours after wounding. Notice large "leader" cell at the top of the outgrowth. Courtesy of T.A. Omelchenko.*

they move on the free substrate, they drag these neighbor cells after them, so that eventually, tongue-like multicellular outgrowths with leader cell at the top are formed.

We do not now what factors determine the transformation of a fraction of edge cells into leaders. In mixed fibroblast-epitheliocyte cultures, contact of the epithelial cells at the sheet edge with fibroblast induces transformation of these cells into leader-like phenotype, but in this case, the cells do not move forward, and normal epithelial morphology is restored when the heterologous contact is broken (Omelchenko *et al.,* 2001).

Thus, in all these cases epitheliocytes temporarily acquire certain phenotypic features characteristic of fibroblastic cell but not the whole set of these features.

Mechanisms of epithelio-mesenchymal transformations

E-M transitions described above represent a wide spectrum of phenomena differing by the degree (partial and complete transitions), and by the nature of inducing factors. We do not know whether the nature of intracellular changes is similar in all these phenomena. For instance, it is not clear whether the molecular specificity of intermediate filament proteins and of cadherins, characteristic markers distinguishing epithelia from fibroblasts, is changed during all these transitions. However, the alterations of cell shape and cytoskeletal organization are certainly similar in all the variants of transitions. Obviously, these are the very changes that are most relevant to motility and invasive behavior. This is the reason to discuss possible common mechanisms of these alterations.

Analysis of the effects of changes of the activities of Rho proteins on the dynamic morphology of fibroblasts and epitheliocytes (Omelchenko *et al.,* 2002, 2003) permitted to guess the possible nature of these mechanisms. When discoid epithelial cells are incubated with low concentrations of a selective inhibitor of Rho-kinase, Y27632, they begin to extend long processes from various parts of the edge, gradually acquiring highly elongated shapes (Fig. 13). Both types of microfilament bundles, circular and straight, disappear and numerous lamellipodia remain the only actin-rich structures of these cells. Numerous microtubules fill the whole cytoplasm up to the cell borders. Presence of microtubules is essential for cell elongation, as the cells treated with microtubule depolymerizing drugs remain discoid even in the presence of Y27632.

These experiments indicate that transitions between epithelial and mesenchymal phenotypes are based on the switches of

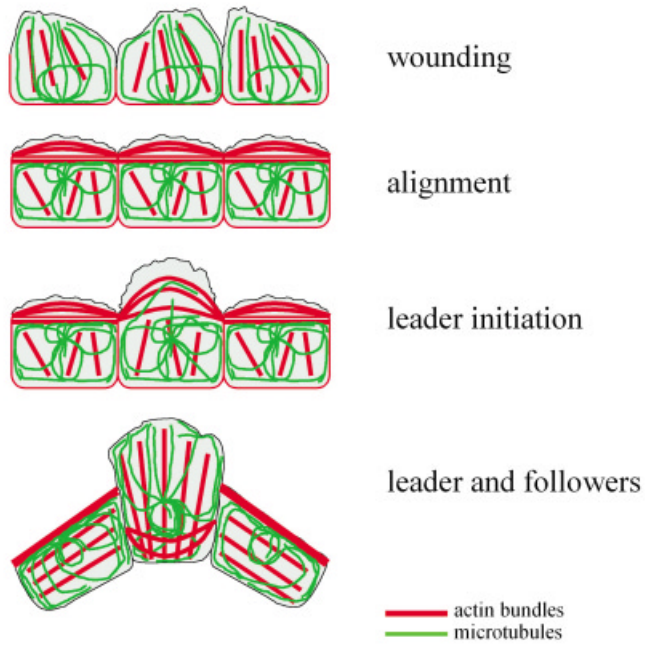

Fig. 12. Scheme showing the early stages of outgrowth from the edge of an epithelial wound. *Red, microfilament bundles; green, microtubules. Alignment of bundles along the edge of the wound is followed by the formation of leader. Courtesy of T.A. Omelchenko.*

interactions between actin-myosin cycle and microtubular system. One can formulate at least two rules of these interactions:
1) microtubules do not cross the actin bundles. This seems to be the only limitation to the ability of microtubules to grow into all the areas of peripheral cytoplasm. We do not know whether this limitation is simply mechanical or, more likely, it is due to some molecular interactions.
2) ends of microtubules, when they approach the plasma membrane, stimulate formation of lamellipodia, that is, polymerization of actin. We discussed briefly this stimulation above.

It follows from these rules that one condition for polarization of epitheliocyte is disintegration of circular actin bundle in single cell or of transversal bundle at the edge of the epithelial sheet. This dissociation can be a result of external stimuli inducing local formation of lamellipodia and of lamella distally to the bundle. This

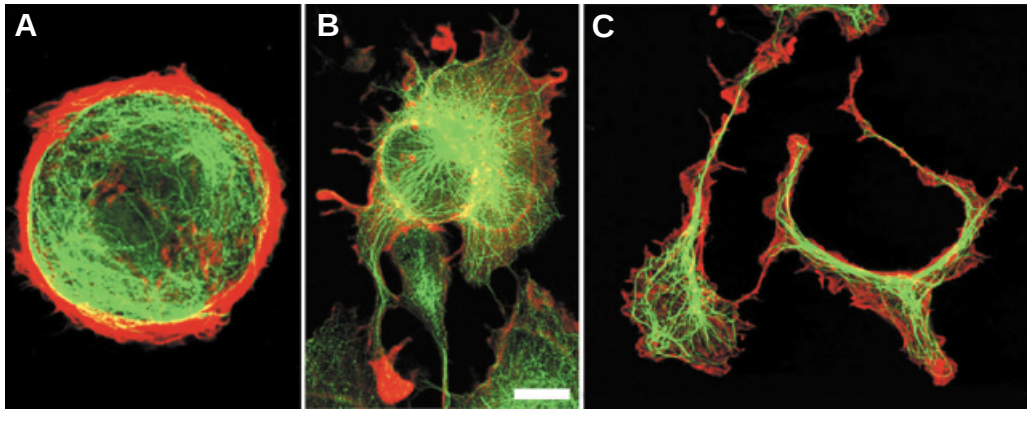

Fig. 13. Effects of inhibition of Rho kinase on IAR2 epithelial cells. (A) *A control discoid cell with circular actin bundle (red) and microtubules (green).* **(B)** *Early stage of the action of Rho kinase inhibitor Y27632. Circular bundle of microfilaments disappeared; the cells have acquired an elongated shape.* **(C)** *Advanced stage of RhoK inhibition. The cells are highly elongated. Courtesy of T.A. Omelchenko.*

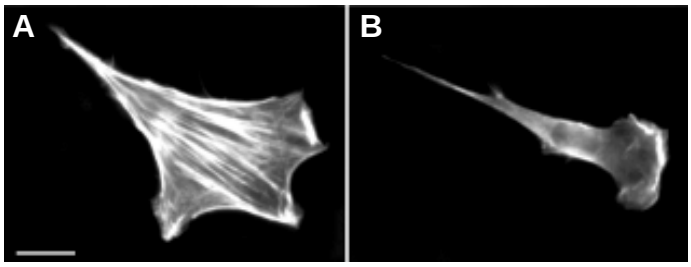

Fig. 14. Morphology of (A) non-transformed and (B) ras-tranformed 10(3) fibroblast cells. *Staining for actin. Notice that cells in (A,B) are of similar length but have different areas. Courtesy of S.A. Minina and A.Y. Alexandrova.*

new lamella locally stretches the bundle and produces a local gap in this bundle. Microtubules then grow into the gap inside the bundle and stabilize the growing extension. This stretching of the bundle by the outside lamella and production of the gap may be the main factors in polarization in several situations where distal lamellas are formed. For instance, formation of leaders at the edge of the sheet may be due to activation of lamella extension by wounding. During spreading, lamellas are extended distally to the bundle and then destroy this bundle by outward stretching. Possibly, on the poorly adhesive substrata the cell does not have enough sites for attachment of lamellipodia and therefore cannot form circular lamella, but only the local ones. Another possible mechanism of polarization is an internal one: decrease of the levels of Rho proteins or the changes of other factors regulating myosin contractility may directly induce dissociation of bundles. Both external activation of lamella formation and internal decrease of contractility possibly act during incubation with SF and other EMT-inducing ligands. Naturally, all these suggestions are still speculative and need further experimental testing.

Neoplastic morphological transformations

Dynamic morphology of transformed fibroblasts and epitheliocytes

At some stage of their multistep neoplastic evolutions, epithelial and mesenchymal cells undergo morphological transformations that most probably provide the basis for invasive migration. These transformations, like other manifestations of neoplastic evolution, are determined by genetic changes. Depending on the tissue specificity of transforming cells, one can distinguish two main groups of these transformations (Vasiliev and Gelfand, 1981):

A) Well-spread fibroblastic cells are transformed into poorly spread fibroblastic cells. The characteristic features of these transformations include:

1) decreased size of lamella and of the width of active edges on standard planar substrata. The mean area occupied by the cell on the substrate is 2-5 times less than in non-transformed parent cultures; this decrease is due mainly to much smaller size of peripheral cytoplasm, so-called lamellar cytoplasm; central part of the cell, endoplasm, is not diminished in size (Domnina *et al.,* 1972; Fox *et al.,* 1977). At the same time, the maximal length of the cells on the plane and on the narrow strips of substrate does not decrease significantly indicating that only transversal spreading but not longitudinal one is diminished (Fig. 14). Diminishment of the width of the active edge is not accompanied by diminished pseudopodial activity, that is, by diminished rate of extension and contraction of lamellipodia per unit length of the active edge. (Gloushankova *et al.,* 1997). In rare cases lamellipodia at the active edge are replaced with blebs (Cunningham, 1995).

2) decreased numbers of actin bundles (stress fibers);

3) decreased numbers of mature focal cell-substrate contacts. Often mature contacts disappear completely and only small focal complexes are seen near cell poles; no cell-substrate attachments are formed under the cell body.

4) decreased number of cadherin-containing cell surface contacts. Gap junctions may also disappear.

5) spread cells have less flattened upper surface than parent cells. This surface is often covered by microvilli, and more rarely with blebs.

6) matrix fibrils are formed by transformed cells much less efficiently than by their parents.

7) tension exerted by the cell upon the matrix is decreased as indicated by diminished folding of special silicone membranes used as substrates (Harris *et al.,* 1980).

B) Discoid epithelioid cells are transformed into poorly spread elongated polarized cells with active edges at the poles; often these cells have small lamellas at both poles; these are so called spindle-like or fusiform cells. Circular actin bundles disappear. Mature focal contacts and all types of cell-cell contacts are rare. In certain cases E-cadherin in cell-cell contacts is replaced by N-cadherin (Tran *et al.,* 1999).

Thus, both types of transformations have important common features. In other words, both variants of transformation lead to appearance of cells with similar, although possibly not identical, phenotype of elongated cells poorly spread on the substrate.

As already mentioned, non-transformed fibroblasts and epitheliocytes on poorly adhesive substrata significantly decrease their spread area as compared with standard adhesive substrata. They also diminish the numbers and sizes of actin cables and of focal contacts. This phenotype closely resembles that of transformed cells both on standard and on poorly adhesive substrata. One can conclude that transformation inhibits the ability of cell to undergo transition from poorly spread to well-spread phenotype in response to alteration of substrate adhesion.

One should stress that the picture outlined above is typical for advanced degrees of transformation; all variants of intermediate degrees of morphological transformations can be observed in individual lines. On the other hand, neoplastic evolution may lead to even more drastic changes, to formation of cells which lose almost completely the ability to spread on the planar standard substrata. These cells retain spherical or semi-spherical shape and are attached to the substrate by only a few lamellipodia or filopodia.

Instead of monolayer colonies with mutually oriented cells, transformed cells of epithelial and fibroblastic origin often form multilayered colonies where elongated cells criss-cross one another. It was suggested (Abercrombie, 1970) that this multilayering is due to the loss of contact inhibition of movement accompanying transformation. However, cinematographic observations of the dynamics of individual cell-cell collisions have shown (Guelstein *et al.,* 1973; Gloushankova *et al.,* 1997, 1998) that contact

inhibition is as efficient in transformed cultures as in non-transformed ones: when active lamellas of two transformed fibroblasts collide with one another extension of lamellipodia immediately stops at the site of contact and somewhat later the cells start to move away from one another. Dynamics of collisions is different when anterior lamella of one cell meets the lateral side of another cell unattached to the substrate. Then the active edge ("the head") of the first cell continues to move under the second one. These underlappings are common in the cultures of badly spread transformed cells and are probably responsible for the criss-cross shape of their colonies.

We described the morphological features characteristics of elongated fibroblast-like transformed cells. Epithelio-mesenchymal transformations are often observed in the course of tumor progression *in vivo* (Thiery, 2002). However, this seems to be not the only variant of morphological transformation. Sahai and Marshall (2003) analyzed behavior of five lines of melanoma and carcinoma cultured in 3D matrices and identified two modes of invasive migration of cells: migration of round bleb-forming cells and migration of elongated cells. Rho signaling through ROCK promoted round cell migration while elongated cell motility was associated with Rac-dependent F-actin rich protrusions and did not require Rho or ROCK function. Elongated cell but not round cell migration was associated with pericellular proteolysis and inhibited by extracellular proteases.

Invading cells in many types of non-epithelial tumors, especially, lymphomas are badly attached and have rounded ameboid shape. These cells resemble normal moving lymphocytes and *Dictiostelium* amoebae (Friedl and Wolf, 2003). Possibly, these cells are akin to spheroid cells described by Sahai and Marshall (2003) and by Omelchenko *et al.* (2003). However, this was not yet proven and the nature of mechanisms leading to formation of this phenotype still remains to be investigated.

Diversity of invading phenotypes is further increased by the fact that both elongated and rounded cells are able either to move individually or to form multicellular aggregates of different sizes and form (so-called collective migration of Friedl and Wolf). These aggregates of invading cells may move in connective tissue as non-transformed epithelial cells in regenerating sheets described above with leader cells pulling other cells of the aggregate. Still another variant of invasion may be observed in so called "organoid" highly differentiated carcinomas (Foulds, 1956) where invading structures are tubules of epithelium closely resembling morphologically normal tubules. In contrast to normal tubulogenesis, the growth of these neoplastic structures is unlimited.

Of considerable interest would be analysis of certain special cases of tumor spread such as ascites tumor variant where the cells grow *in vivo* as round cells in peritoneal ascitic fluid and sometimes reattach themselves to the peritoneal wall. Some of these ascitic lines are able to form large multicellular aggregates in the peritoneum but unable to reattach to the peritoneal wall. Ascitic tumors have been known for many decades but molecular mechanisms responsible for their formation remain unstudied.

In vivo human neoplasms arising from epithelial structures, *e.g.* breast tumors, also have many morphological variants ranging from the tumors retaining the main features of tissue organization and even of organ organization (organoid tumors, alveolar tumors) to those growing as dissociated cells (diffuse carcinomas). These pathological variants mimic different variants of physiological switches from epithelial branching tubules and alveoles to cellular strands and scattered cells.

Possible mechanisms of morphological transformations

Characteristics of transformed cells described above may provide basis to make certain suggestions about possible mechanisms of regulatory alterations responsible for morphological transformation, especially for the most studied variant, formation of elongated badly attached cells. I suggest that the critical alteration here leads to deficient formation and organization of actin-myosin cortical structures. The main symptoms of this most common type of transformation of both epitheliocytes and fibroblasts supporting this suggestion include diminished size of the active edge and disappearance of actin bundles. Decreased active edges possibly reflect diminished total rate of polymerization of actin leading to decrease of the total rate of pseudopodial extension. In contrast to actin structures, microtubules do not undergo any conspicuous changes in transformed cells. In these cells microtubules normally radiate toward the active edges and, probably, normally activate pseudopodial activity at these edges. These interactions result in formation of actively moving, highly polarized, spindle-like cells. These cells may have normal pseudopodial activity per unit length of the active edge but these edges are narrow so that total pseudopodial activity per cell is diminished (Fig. 15).

Many other symptoms of transformation such as decreased spreading, decreased formation of actin bundles and decreased tension upon the matrix, suggest that another aspect of function of actin-myosin system, its contractility, is also changed. What molecular changes can be responsible for alterations of actin-myosin organization? Decreased activities of the Rho enzymes and/or their targets seem to be the likely candidates for the changes of contractility. In fact, non-transformed fibroblasts incubated with the inhibitor of Rho-kinase (Y27632) or with other inhibitors of myosin-dependent contractility (Fig. 16) as well as cells stably transformed with the *RhoA*- plasmid acquire significant morphological similarity with the same cells transformed by activated *Ras* (Minina *et al.,* 2003): they increase degree of polarization, lose stress fibers and have diminished cell-matrix contacts.

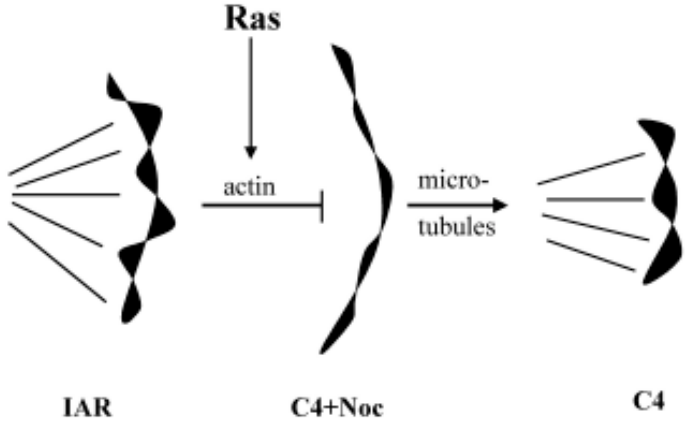

Fig. 15. Hypothetical mechanism of cytoskeletal changes after ras transformation of epithelial cells. *Inhibition of pseudopodial activity (wavy lines) and compensatory restoration of activity after concentration of microtubules in the active lamella.*

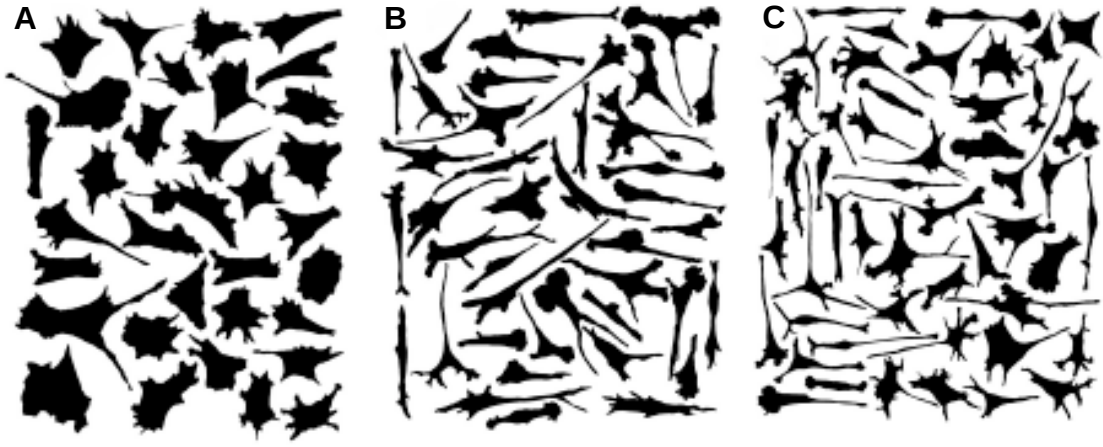

Fig. 16. Effect of contractility inhibition on cell shape. **(A)** *Contours of non-transformed 10(3) cells;* **(B)** *ras-transformed 10(3) cells and* **(C)** *non-transformed 10(3) cells incubated with the contractility inhibitor HA1077. Notice the similarity of cell shapes in B and C. Courtesy of S.A. Minina and A.Y. Alexandrova.*

As mentioned above, this phenotype is also very similar to that acquired by non-transformed cells on poorly adhesive substrata. It is possible that transition to this phenotype is an adaptive reaction to diminished signaling from matrix-attached integrins. This adaptive change in non-transformed cells may diminish actin-myosin tension upon the matrix and thus facilitate cell association with poor substrate. Here the signal for phenotypic change comes from the outside. In the case of Rho-inhibited cells, the essential part of the pathway inside the cell is suppressed. Genetic changes of neoplastic cells permanently switch off this pathway and keep the cells badly spread on all substrata. The exact targets of Rho-dependent signaling altered in the course of all these transformations remain to be found. We have also to find out what the relationship is between the mechanism diminishing lamellipodia extension and attachment and decrease of actin-myosin contractility.

In contrast to actin-myosin changes, microtubular system may be not primarily altered in the course of transformation. However, it may have essential compensatory role: microtubules growing towards diminished active edge may stimulate lamellipodial extensions and in this way secure the ability of efficient locomotion of spindle-like transformed cells.

As described in the previous paragraph, the course of neoplastic evolution, besides formation of spindle cells, may probably lead to other variants of morphological transformations associated with invasion. Of special interest is formation of spherical cells with blebbing surface described by Sahai and Marshall (2003) and by other authors (Friedl and Wolf, 2003). In the experiments of Sahai and Marshall (2003), formation of these cells was associated with increased activity of Rho.

Our experiments (Omelchenko *et al.,* 2003) show that a non-transformed epithelial cell line stably transfected by a plasmid carrying the construct increasing RhoA activity, were rounded and appeared highly contracted with abundant marginal and central actin bundles. Their migration into the wound was greatly suppressed. Although most cells in these cultures had normal looking tangential cadherin cell-cell contacts, some cells from the internal parts of the sheet detached themselves from the substrate and were released into the fluid medium (Fig. 17).

Some of these cells floating in the medium later re-attached themselves to the cell-free substratum at some distance from the wound edge and formed small colonies on this substratum. This detachment from the inner parts of the sheet was, most probably, due to increased contractility of these cells. In contrast to the cells of the same line with inhibited RhoK activity the cells with activated Rho had no increased ability to move on the substrate.

Thus, one may suggest that alternative changes of contractility may lead to different variants of invasive behavior. The elongated cells with inhibited Rho were similar to morphologically transformed fibroblast-like epithelial cells with organization suitable for directional movement (see above). At the same time, the cells with activated Rho did not show any signs of “standard” morphological transformation. In contrast, they retained the epithelial morphology. However, their behavior in contracted sheet, namely, their ability to detach, to float in the medium and to resettle on the substratum forming new colonies, closely resembled some stages of formation of metastases by malignant tumors *in vivo*, such as dissociation from the original tissue structure, floating in the fluid media, *e.g.* in lymph or blood, and formation of colonies on the new territories.

In other words, two opposite changes of contractility regulation cause two different types of the cell spread upon the new territories. Of course, not only changes of *Rho* expression but also mutations of other genes regulating cell contractility directly or indirectly, for instance, caldesmon expression changes, may theoretically induce invasive behavior.

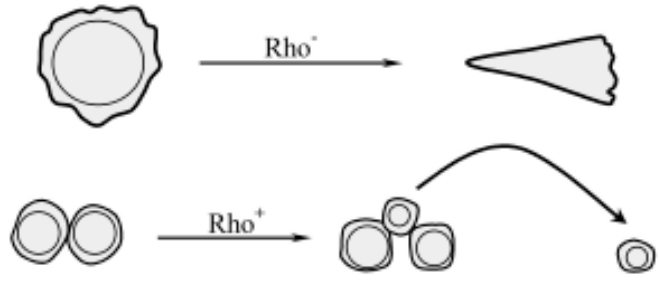

Fig. 17. Hypothetical scheme showing two ways of formation of invasive phenotypes *(see text for explanation).*

Additional variations of the morphology of invading structures may be caused by alterations of other genes. To give but one example, inactivation of cadherin expression may inhibit formation of multicellular aggregates characteristic of some tumors (see above). Mutations of cadherins and other genes coding for components of cell-cell and cell-matrix junctions were observed in many neoplasms (Braga, 2002; Hood and Cheresh, 2002; Christofori, 2003).

These examples show that transformations described above can be induced by various genetic alterations. Often a sequence of several changes of different genes is necessary for the appearance of one of invasive phenotypes. These genetic alterations involve activation of various mutated oncogenes (*ras, myc,* and many others) or inactivation of suppressor genes such as *p53* (Sablina *et al.*, 2003). Mechanisms of these changes are described in innumerable reviews (Kopnin, 2000; Blume-Jensen and Hunter, 2001; Ponder, 2000; Hanahan and Weisberg, 2000). These alterations affect many different immediate molecular targets and, in most cases, it is not clear how changes of different pathways lead eventually to similar morphological alterations. Only in few cases we can guess the nature of these pathways. One of these cases is transformation induced by mutated *met* oncogene. Normal *met* codes for receptor of HGF/SF inducing classical EMT and it is almost certain that the *met* oncogene in this case permanently activates the same pathway that is transiently activated by its normal analogue. In other cases the sequence of molecular events is less clear.

Conclusions. Old and new problems

Neoplastic invasion *in vitro* may be regarded as one of the consequences of genetically induced abnormalities of tissue structure that normally keeps each tissue well-organized and isolated from neighbor tissues. Neoplastic invasion is very similar to migration of scattered cells or invasion of collagen gels by tubules of non-transformed epithelial cell treated with HGF/SF. Morphological diversity of invading structures is accompanied by and, probably, caused by many types and combinations of genetic mutations. One may suggest that most of these mutations lead directly or indirectly to deficient regulation of the basic physiological exploratory mechanisms. Neoplastic cells are continuously exploring their environment but unable to make functionally adequate choices.

Analysis of our and literary data led to suggest that alterations of actin-myosin contractility associated with changes of Rho regulatory systems play critical roles in formation of invasive phenotypes. We also suggested that alternative alterations of Rho-dependent contractility may lead to different invasive phenotypes. Needless to say that these suggestions are personal and need many experimental tests. First of all, one needs to analyze the nature of aberrations, if any, of contractility-regulating systems in various types of neoplasms and their possible associations with different modes of invasion. Previous tests of Rho systems in tumors gave apparently contradictory data: some neoplasms showed increase of Rho while other had decreased Rho activitiy (Braga, 2002; Mareel and Leroy, 2002; Sahai and Marshall, 2002; Christofori, 2003; Horiuchi *et al.,* 2003; Lozano *et al.,* 2003; Moon and Zong, 2003). It would be important to find out whether various sorts of alterations may be associated with different types of tumor spread. One promising and important result was obtained by Clark *et al.* (2000) who showed that increased expression of RhoC increased the ability of A375 melanoma to metastasize.

We still know very little about the interrelationships between alterations of various members of Rho group of GTPases such as Rac, CDC42, variants of Rho *etc*. These interrelationships can be very important in formation of invasive phenotypes.

Alterations of cytoskeleton and related structures in neoplastic cells are, most probably, closely associated with aberrations of pathways controlling cell cycle, apoptosis *etc*. The nature of these correlations obviously needs detailed investigation.

We discussed in this paper several phenomena of cell morphogenesis closely related to neoplastic transformation but their mechanisms are still obscure and need analysis. Among these phenomena are:

A. the mechanisms of tension-induced changes in the structure of actin cytoskeleton and of cell-cell and cell-matrix contacts.

B. the nature of differences in cytoskeletal organization determining epithelial and fibroblastic morphology.

C. the mechanisms responsible for longitudinal and transverse cell spreading.

D. the mechanism determining different morphology of cells on highly and poorly adhesive substrates.

Another group of problems is that of the mechanisms of interplay between the cell microenvironment factors (matrix metalloproteases (see Chang and Werb, 2001; Seiki, 2002) contacting cells *etc*.) and regulatory systems involved in invasive behavior. At present we can only formulate these questions.

Acknowledgements

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