ELSEVIER

Prespecification and plasticity: shifting mechanisms of cell migration

Peter Friedl

Cell migration is a universal process involving different morphologies and mechanisms in different cell types and tissue environments. Prespecified cell-type-specific patterns of cell migration can be classified into single cell migration (amoeboid, mesenchymal) and collective migration modes (cell sheets, strands, tubes, clusters). These intrinsic molecular programs are associated with a characteristic structure of the actin cytoskeleton, as well as the cell-type-specific use of integrins, matrix-degrading enzymes (matrix metalloproteinases and serine proteases), cell–cell adhesion molecules (cadherins and activated leukocyte adhesion molecule), and signaling towards the cytoskeleton (carried out by RHO GTPases). In response to the gain or loss of these key molecular determinants, significant adaptation reactions can modify the cell's shape, pattern, and migration mechanism; examples of this include the epithelial–mesenchymal transition, mesenchymal–amoeboid transition and collective–amoeboid transition.

Addresses
Molecular Cell Dynamics Laboratory, Rudolf-Virchow Center, DFG Research Center for Experimental Biomedicine, and the Department of Dermatology, University of Würzburg, Josef-Schneider-Str. 2, 97080 Würzburg, Germany
e-mail: peter.fr@mail.uni-wuerzburg.de

Current Opinion in Cell Biology 2004, **16**:14–23

This review comes from a themed issue on
Cell structure and dynamics
Edited by John A Cooper and Margaret A Titus

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2003.11.001

Abbreviations

2D	two-dimensional
3D	three-dimensional
CAT	collective–amoeboid transition
EC	endothelial cell
ECM	extracellular matrix
EMT	epithelial–mesenchymal transition
MAT	mesenchymal–amoeboid transition
MMP	matrix metalloproteinase
MT-MMP	membrane-type MMP
ROCK	Rho-associated serine/threonine kinase
uPA	urokinase-type plasminogen activator
uPAR	uPA receptor

Introduction

Most cell types in the body, at a given time and tissue space, are capable of — and perform — migration. Initially established for fibroblasts and keratinocytes moving across 2D substrata, the principle events leading to cell migration within 3D extracellular matrix (ECM) environments are regarded as five interdependent steps that form a continuous cycle [1,2]. Initial cell polarization is driven by localized actin polymerization to filaments that is followed by the extension of a leading pseudopod (step 1). Once the protruding pseudopod touches ECM ligands, adhesion receptors of the β1 and β3 integrin families cluster, attach to the substrate and engage cytoplasmic adaptor, signaling and cytoskeletal proteins towards the adhesion site (step 2). These circumscribed interaction zones, termed focal adhesions or focal contacts, recruit surface proteases, such as MT1–MMP (membrane-type 1 matrix metalloproteinase) and the complex of urokinase-type plasminogen activator (uPA) and its receptor (uPAR), to execute local proteolysis towards adjacent ECM proteins. Pericellular proteolysis is thought to widen extracellular scaffolds for the forward-expanding cell body (step 3). During or shortly after integrin–ligand binding, actin filaments engage with cross-linking and contractile proteins, such as myosin II, which stabilize and contract the actin strands (step 4). The shortening of membrane-anchored actin filaments results in local cell contraction, slow forward gliding of the posterior cell pole and cell translocation along the substrate (step 5). These five steps do not represent a stereotypic program but rather provide an adaptive platform that undergoes cell-type-specific modification dependent on the inherent molecular repertoire and the type of ECM environment.

This review summarizes cellular and molecular signatures of cell migration as well as their reprogramming and reactive compensation in migrating cells.

Prespecified migration mechanisms

In the process of cell differentiation and acquisition of specific tissue phenotypes, different patterns and mechanisms of cell migration emerge. These include the mechanisms controlling the migration of single cells (Figures 1a-c) or, alternatively, collective migration (Figures 1d-g). The central molecules that govern and specify such diverse migration processes comprise the following (Figure 1): matrix-binding adhesion receptors, most notably those belonging to the β1 and β3 integrin families; matrix-degrading proteases of the matrix metalloproteinase (MMP) and serine protease families (uPA/uPAR); molecules that enable cell–cell adhesion and communication (i.e. E-, N- and VE-cadherins and gap junctions); and signaling proteins that control the actin cytoskeleton, most notably the small GTPase RHO and

Box 1 Viewpoint: amoeboid movement in mammalian cells

The sequencing of the *Dictyostelium* genome will be finished soon (see Dictyostelium Genome Analysis Consortium website at http://genome.imb-jena.de/dictyostelium/). To date, no predicted homologues of integrins or of secreted or surface metalloproteases have been identified. *Dictyostelium* is therefore a likely model organism for studies on cell migration processes that do not depend on integrin and MMP functions. In a recent genetic screen at least one novel pattern recognition receptor was identified to contribute critically to adhesive interactions with substrate and migration [3•]. Termed SadA, this receptor contains several EGF-like repeats and a predicted talin binding site, thereby providing putative links between the cytoskeleton and the extracellular substrate. It is reasonable to assume that, in addition to integrins, pattern recognition receptors are retained in higher mammals to provide interactions with ECM ligands and to contribute to cell adhesion and migration in the absence of integrin function.

Amoeboid cells, such as stem cells and most leukocytes, are able to enter and move through many different organs, including skin, gut and brain. They hence retain a powerful capacity for dissemination in connective tissue and recirculation between organs and the blood system as well as the lymphatic system. Other examples of amoeboid movement in the human body are cells from lymphoma, small-cell lung carcinoma and certain mammary carcinoma [2,6]. Because of their striking capacity for efficient dissemination from very small tumor lesions, amoeboid cancers represent systemic diseases metastasizing to distant organs at their earliest stages. Taken together, amoeboid migration in mammals is a feature of nonresident cells that recirculate towards different tissues and migrate therein, yet possess cell–matrix interactions of low stringency and, hence, have limited tissue specificity.

its downstream effector, the RHO-associated serine/threonine kinase ROCK.

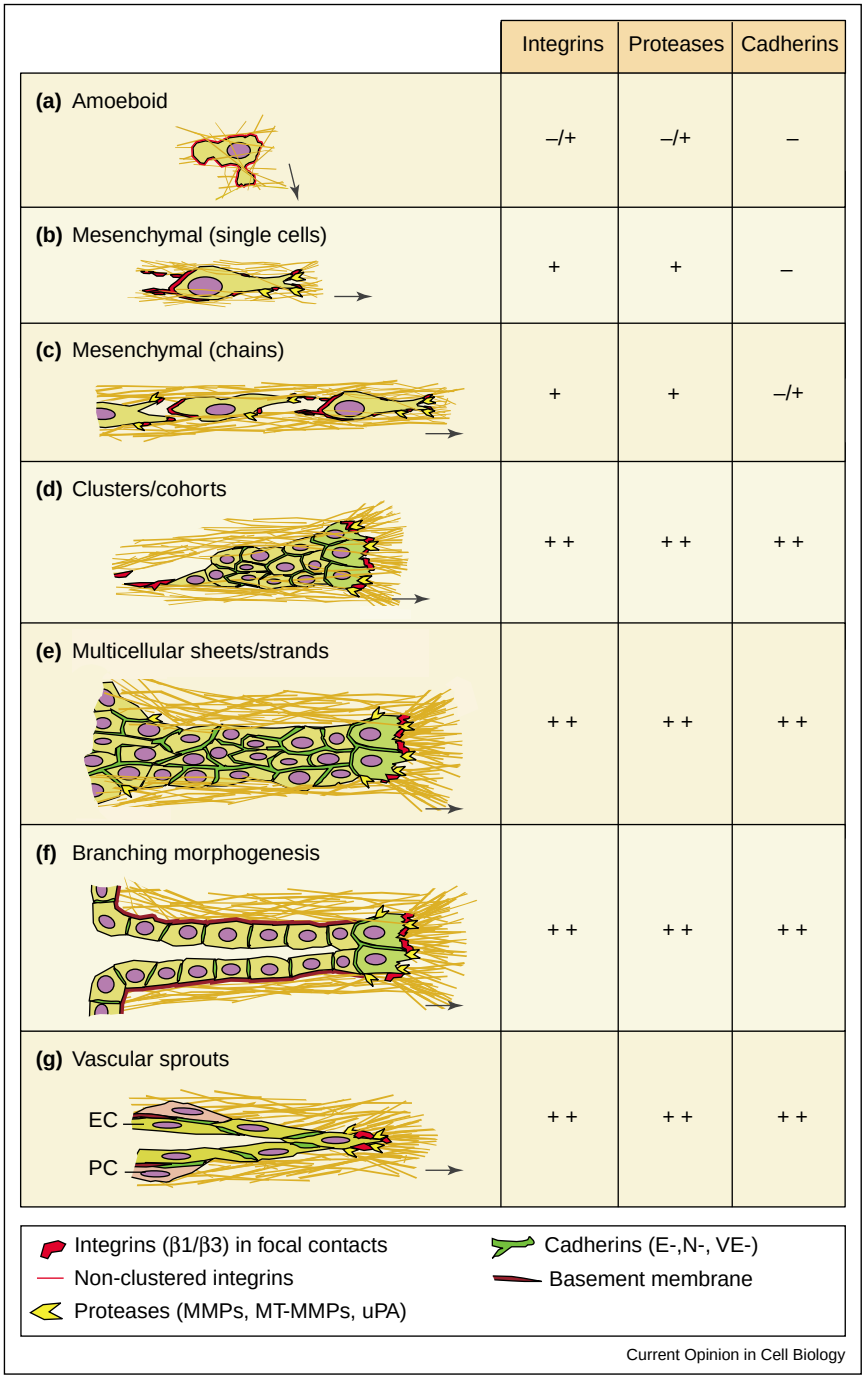
Amoeboid migration

Arguably the most primitive and in some ways the most effective form of cell migration is amoeboid movement (Figure 1a; Box 1), which mimics features of the single-cell behavior of the amoeba *Dictyostelium discoideum*. *Dictyostelium* is an ellipsoid cell that has fast deformability (within seconds) and translocates via rapidly alternating cycles of morphological expansion and contraction. Although integrins are not expressed and the binding force towards the substrate is relatively low, migrating *Dictyostelium* cells utilize one or several non-integrin pattern recognition receptors (Box 1) to bind to extracellular structures [3•]. In higher eukaryotes, amoeboid movement is carried out by hematopoietic stem cells, leukocytes and certain tumor cells [4–6] (P Friedl, unpublished). These cells use a fast 'crawling' type of movement that is driven by short-lived and relatively weak interactions with the substrate. In lymphocytes and neutrophils, β1-integrin-mediated adhesion is completely or partially dispensable for cell migration within connective tissue, both *in vitro* [7] and *in vivo* [8,9•]. Leukocytes are highly deformable and, because of their lack of stable focal contacts, move at high velocities (2–30 μm/min) [10]. Shape change is generated by cortical filamentous actin, which mediates cell dynamics as well as providing stiffness to the cell body, but mature focal contacts and stress fibers are lacking [7,11]. T lymphocytes and other leukocytes use protease-independent physical mechanisms to overcome matrix barriers, including adaptation of the cell shape to preformed matrix structures (contact guidance), extension of lateral footholds ('elbowing') [12] and squeezing through narrow spaces (constriction rings) [11]. Such shape-driven migration allows cells to glide through or circumnavigate, rather than degrade, ECM barriers [13]. The ellipsoid cell shape in amoeboid migration requires actin polymerization along the plasma membrane to stiffen and contract the cell cortex. These cortical actin dynamics are critically controlled by the small GTPase RhoA and its effector ROCK to generate cortical tension, stiffness and the maintenance of roundish cell morphology [14,15]. On the other hand, the small GTPases Rac and Cdc42 engage adaptor proteins (i.e. the WASP) that favor focalized actin assembly and generate dynamic cell protusions, such as pseudopodia, lamellipodia, and filopodia, thereby supporting cell polarization and elongation [16]. In contrast to the five-step migration paradigm, focal contacts and focalization of proteolysis are thus eliminated in amoeboid movement, whereas fast and non-focalized receptor assemblies at cell–matrix interactions are retained.

Mesenchymal migration

In contrast to cells that use amoeboid migration, mesenchymal cells accomplish the complete five-step migration sequence (Figure 1b). In 3D tissues, mesenchymal cells adopt a spindle-shaped, fibroblast-like morphology, as characteristic for fibroblasts, myoblasts, single endothelial cells or sarcoma cells [17,18••]. The elongated morphology is dependent on integrin-mediated adhesion dynamics and the presence of high traction forces on both cell poles [18••,19]. Blocking of integrins in spindle-shaped fibroblasts, endothelial cells or tumor cells by antibody or small-molecule inhibitors causes cell retraction, acquisition of spherical shape and impaired migration rates [20,21] (N Daryab and P Friedl, unpublished). Concomitant to integrin and actin focalization at substrate-binding sites, mesenchymal cells recruit surface proteases to digest and remodel ECM [22••]. β1 and β3 integrins, MT-MMPs and other proteases then colocalize at contact regions to ECM fibers, proteolytically cleave ECM molecules near to the cell surface, and generate structural matrix defects along cell migration tracks [22••,23]. Focal contact formation and turnover occur in the timescale of 10–120 minutes, resulting in relatively slow migration velocities (0.1–2 μm/min) in 3D models [10,19,24,25]. If other cells follow along the newly generated matrix defect, a moving cell chain evolves and is guided by matrix strands (contact guidance) (Figure 1c).

Figure 1



Prespecified diversity in cell migration strategies. In 3D tissue environments, different cell types exhibit either individual **(a–c)** or collective **(d–g)** migration mechanisms to overcome and integrate into tissue scaffolds. **(a)** Amoeboid migrating cells develop a dynamic leading edge rich in small pseudopodia, a roundish or ellipsoid main cell body and a trailing small uropod. **(b)** Mesenchymal cells retain an adhesive, tissue-dependent phenotype and develop spindle-shaped elongation in 3D ECM (i.e. the equivalent to cell spreading on 2D substrate). For movement through 3D tissues, they form focal contacts containing focalized actin and adaptor proteins [25], whereas stress fibers are preferentially present in sessile state and contraction of anchored ECM. In the detachment zone, matrix defects and the deposition of cell surface determinants including cell fragments are seen. **(c)** Proteolytic migration tracks can be used by neighboring cells to arrange and migrate in a loose chain-like fashion. Such files are seen in neural crest cells upon morphogenesis [63] as well as in stromal tumors extending into connective tissue in a chain like pattern, such as melanoma (C Mayer, P Friedl, unpublished). **(d)** In primary cancer lesions, as detected by histopathology, detached clusters of cells are disseminated throughout the adjacent connective tissue. Leading cells provide the migratory traction and, via cell–cell junctions, pull the following group forward. **(e)** If an invading cancer collective maintains contact with the origin, for example via proliferation of cells in the inner mass, extended non-polarized collective

In migrating mesenchymal cells, such as fibroblasts, Rac and Cdc42 generate pseudopod and lamellipod dynamics at outward edges, favoring a rapid and dynamic type of β1 integrin engagement towards 2D and 3D substrata [26]. Interfering with Rac and Cdc42 activity perturbs cell extension and polarized force generation, thereby severely impairing migration [26]. Rho, on the other hand, stabilizes initial integrin–substrate linkages, increases focal contact size and strength, and further thickens actin filaments through several mechanisms [27,28]. In mesenchymal or adhesive cells interacting with 2D substrata, active Rho leads to increased adhesiveness, stress fiber formation, and retardation of migration speed [27,28]; for cell migration within 3D substrata, the functions of Rho with regard to cell shape and adhesion dynamics appear to be more complex (see below). Together, the coordination and synergy between polarized cytoskeletal dynamics at the leading edge, as mediated by Rac and Cdc42, and the somewhat opposing effect of Rho-mediated adhesion-strengthening and cell contractility is thought to play a key role in adhesion-dependent cell migration and related dynamics in cell morphology [26–28].

Collective migration modes

In collective migration, cells maintain their cell–cell junctions and move as multicellular connected strands or chords into tissues; examples include invading epithelial strands or tubes, vascular sprouts and tumor clusters (Figure 1d–g). Specialized forms of collective migration are the horizontal migration of epithelial cell sheets across 2D substrates, as observed in the gut mucosa epithelium upon self-renewal or in keratinocytes migrating across provisional wound matrix, as well as in slow border cells migrating through the cell-rich scaffold of the developing ovary in *Drosophila* [29]. Collective migration is seen experimentally in 2D *in vitro* wound scratch assays [28] and in 3D ECM cultures [30]. The leading edge of a moving cell group, both in 2D and 3D migration models, is formed by one or several cells that utilize actin-mediated ruffles and integrin-dependent traction to execute steps 1–4 of the migration cycle [31••,32]. In cell cultures, migrating cancer cell collectives develop preferential integrin and protease (MT1-MMP, MMP-2) engagement in a subset of cells at the leading edge [33]. The junctions within invading collectives are stabilized by cadherins, members of the immunoglobulin superfamily (e.g. NCAM or activated leukocyte adhesion molecule) [34–36] and gap-junctional cell–cell communication [37,38]. The rear of the leading cell(s) maintain the adhesive interaction with other cells, so the retraction of the trailing edge has an important modification: as it glides along the ECM structure, neighboring cells are dragged forward along the established migration track by means of cell–cell adhesion [30,34,39]. While the leading cells generate actin- and integrin-mediated traction, a linear cortical actin network extends along cell–cell junctions into deeper regions of the collective, suggesting that cortical actin plays a role in sustaining collective integrity [31••,32,40]. In de-differentiated tumor cell groups, rather amorphous cell strands and masses that lack an inner lumen extend within the tissue (Figures 1d,e). By contrast, non-neoplastic developing glandular ducts (Figure 1f) and blood vessels (Figure 1g) contain polarized cells that form an inner lumen and newly produce a surrounding basement membrane [41,42]. Sprouting blood vessels, unlike other groups of cells undergoing collective migration, use cadherins to recruit pericytes as a second cell type; these pericytes then participate in the *de novo* synthesis of an encircling basement membrane [43,44].

A special and more complex example of collective migration is the 'mass' movement in morphogenesis, as seen during the convergent extension of the vertebrate embryo [45] or the closure of the dorsal surface in the *Drosophila* embryo [46]. In both cases, movement is carried out by complex multicellular sheets that contain cells linked to each other by cell–cell junctions and other forms of cell–cell communication; the cells move along the underlying or surrounding tissue substrate to form epithelia or organs [45,46]. Besides the well-studied mechanisms of 'active' collective migration, morphogenic movements additionally comprise biophysically barely understood 'supracellular' forces that go beyond single cell dynamics to change the shape and position of cells and entire tissues, for example by folding cell and matrix sheets or extending and contracting entire body regions [46].

Plasticity in cell migration

While it is generally assumed that differentiated cells retain their migration mode once they have acquired it, recent data suggest that the gain or loss of generically prespecified components in the migration cycle can cause an adaptive switch in migration pattern and mechanism. This phenomenon is here termed plasticity or transition.

Physiological transitions

The process by which circulating precursor cells acquire a differentiated phenotype in the peripheral organs follows

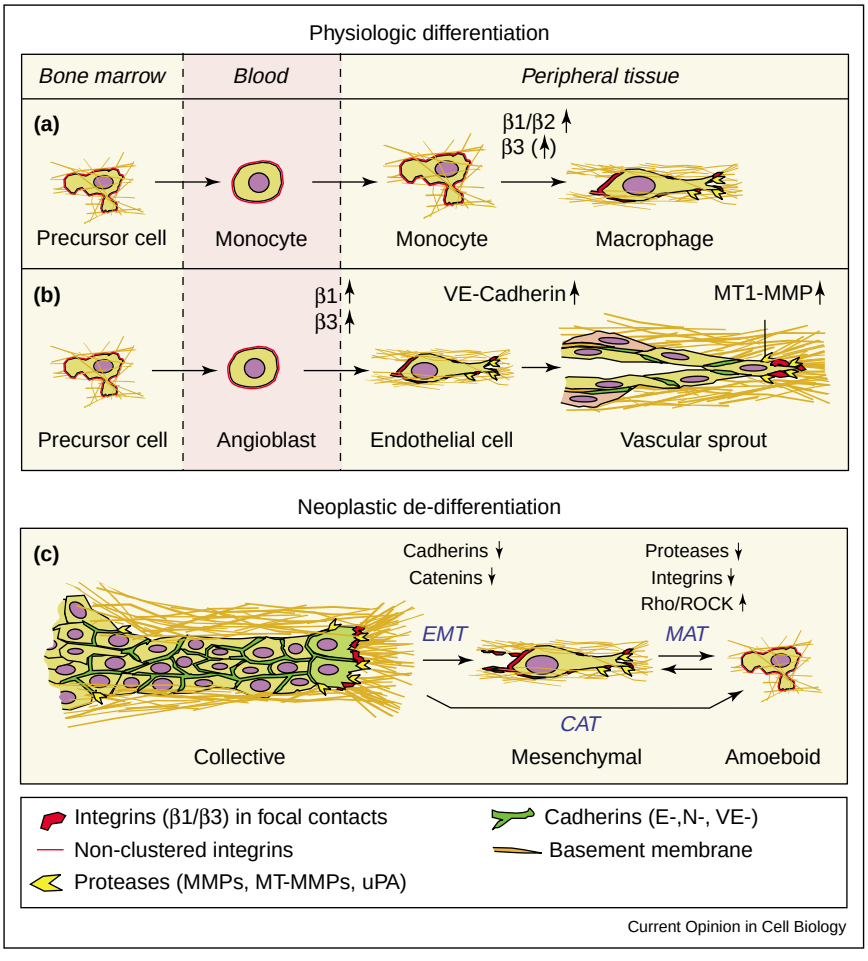
(Figure 1 Legend Continued) strands or sheets extend into the stroma. **(f)** Non-neoplastic collective migration is seen in budding epithelial ducts and glands, termed branching morphogenesis. While cells of the leading edge engage in cell–ECM interactions and proteolytic ECM remodeling, the emerging matrix defect is collectively filled by following cells. The collective then generates a basement membrane along the outside tissue interface while an inner lumen is formed. **(g)** Collective sprouts of endothelial cells form new blood vessels by moving and maintaining cell–cell junctions. Guided by one 'pathfinder' cell, the chain matures into a growing strand containing a lumen. While the strand moves forward, pericytes are recruited and engaged by cadherins. Endothelial cells and pericytes then produce a basement membrane. EC, endothelial cell; PC, pericyte. Arrows indicate the direction of migration. For the functions of integrins, proteases and cadherins, please refer to the main text.

a program of genetic prespecification that involves position change as well as alterations in the cells' molecular repertoire.

Macrophage development

An amoeboid stem cell (hemangioblast) in the bone marrow differentiates into a monocyte which, after exit from the bone marrow, blood passage and emigration into the peripheral tissue, becomes a resident macrophage (Figure 2a). Migrating monocytes express low levels of β1 or β3 integrins on their surface and use amoeboid shape change for their migration (see movies 4 and 5 at http://www.bloodjournal.org/cgi/content/full/2002-12-3791/DC1). Macrophages are stromal-type elongated cells that show strongly upregulated β1 and β3 integrins [47,48], focalized integrin–ligand interactions and an actin cytoskeleton that is rearranged from diffuse to strongly focalized [49]. The macrophage adopts a spindle-shaped yet mobile stromal phenotype and, upon granulomatous tissue reaction and cell fusion with other macrophages, can create multinucleated giant cells [49]. Although a complete dynamic mapping of these transition stages

Figure 2



Transition of cell migration in physiologic differentiation and neoplastic de-differentiation. Migratory plasticity in non-neoplastic differentiation occurs via the gain of adhesion molecules and proteases and of cell–cell interactions. **(a)** The development of a tissue macrophage requires the amoeboid stem cells in the bone marrow to differentiate sequentially into an amoeboid myeloic precursor cell and then a monocyte, which emigrates from the bone marrow. After circulation in the blood stream, the monocyte is recruited into the tissue, upregulates β1, β2 and, to some extent, β3 integrins and develops into a slowly moving macrophage of spindle-shaped morphology. **(b)** For formation of new vessel sprouts, amoeboid angioblasts are recruited from the bone marrow into the tissue to undergo differentiation into single endothelial cells (ECs) of mesenchymal phenotype. ECs interact with neighbor ECs via VE-cadherin and other adhesion receptors forming a multicellular sprout. **(c)** The reverse processes take place in neoplastic de-differentiation. After the loss of cell–cell junctions, for example through reduced expression or cleavage of cadherins, individual mesenchymal cells detach from the collective and migrate via integrin and protease usage (epithelial–mesenchymal transition, EMT). If integrin or protease functions are weakened in mesenchymal cells, as well as by an increase in ROCK activity, the transition towards amoeboid movement (mesenchymal–amoeboid transition, MAT) occurs. Collective–amoeboid transition (CAT) results from the dissociation of multicellular collectives into amoeboid single cells, as seen after inhibition of β1 integrins in melanoma explants.

is presently lacking, macrophage development represents the transition from amoeboid to mesenchymal characteristics.

Endothelial cell differentiation

Endothelial precursor cells (hemangioblasts) undergo a transition from an amoeboid phenotype through a transient mesenchymal stage to a terminal collective pattern (Figure 2b). Hemangioblasts leave the bone marrow as amoeboid cells characterized by a roundish appearance on adhesive substrates such as fibronectin, low surface levels of β1 and αv integrins, and an exclusively cortical actin cytoskeleton lacking stress fibers [50,51•] (J Varner, personal communication). At the extravasation site, the interaction of circulating angioblasts with blood vessels requires, like the transendothelial migration of leukocytes, the function of integrin α4β1 (Varner J, personal communication). Once in contact with the tissue ECM, a differentiation program towards a mesenchymal adhesive phenotype occurs, consisting of increased attachment and spreading, acquisition of stress fibers, and the strong upregulation of β1, αvβ3 and αvβ5 integrins, VCAM, and endothelial differentiation markers [51•] (J Varner, personal communication). As the final differentiation step, the gain of cell–cell junctions and the formation of multicellular sprouts and tubes results in a collective phenotype of initially primitive — later matured — vessels [52,53]. The tip of the sprout is formed by a pathfinder cell using β1 and αvβ3 integrins and MT1-MMP for collagen remodeling [54]. In all these steps, cell movement is essential for appropriate endothelial cell positioning and assembly into multicellular vessels.

Other examples of plasticity of cell shape and migration mode might include myotube formation (mesenchymal) from myogenic precursor cells (amoeboid) and fibroblast differentiation (mesenchymal) from recirculating stem cells (amoeboid) in regenerating wounds.

What happens now, if characteristics of cell differentiation are lost, as seen in neoplastic cell dedifferentiation?

Reverse transitions

Tumor cell migration models have provided useful insights into the transition stages and underlying mechanisms that allow the conversion from differentiated and collective migration towards more simple single-cell movements, here referred to as 'reverse transition'.

Epithelial–mesenchymal transition

During progressive de-differentiation in epithelial cancer, the conversion from multicellular growth and invasion to mesenchymal single cell migration is termed the epithelial–mesenchymal transition (EMT) (Figure 2c) (see [55] and references therein). The primary step is the loss of cell–cell junctions via several mechanisms. These include reduced cadherin expression, loss-of-function mutations in cadherin and catenin signaling pathways, and deregulated function of proteases leading to degradation of cadherins and other cell–cell adhesion molecules [56]. Because integrin and protease functions and promigratory signaling remain intact, detaching cells adopt a mesenchymal type of single-cell migration [55,57]. As represented by the transition of highly differentiated epithelial cancer towards a sarcomatous stromal phenotype, EMT is considered as a key event in tumor progression [55].

Mesenchymal–amoeboid transition

If mechanical or signaling pathways that stabilize cell–ECM interactions are weakened, mesenchymal movement can convert towards amoeboid migration (Figures 2c and 3). Known mechanisms leading to mesenchymal–amoeboid transition (MAT) are the abrogation of pericellular proteolysis using protease inhibitors, the strengthening of RHO/ROCK signal pathways, and the weakening of integrin–ECM interactions by antagonists (Figure 2c).

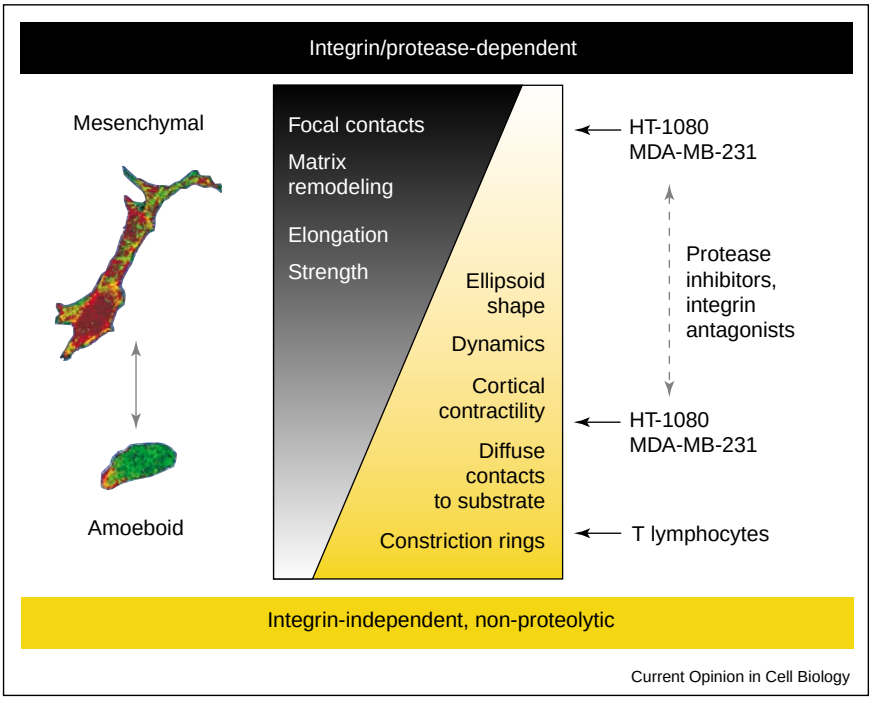
Loss of protease function

Mesenchymally migrating tumor cells such as HT1080 fibrosarcoma and MDA-MB-231 mammary carcinoma cells cease their proteolytic migration after the addition of protease inhibitors that target MMPs, ADAMs, cathepsins and serine/threonine proteases [*22*••]. Instead of widening the pericellular space and cleaving ECM fibers, the cells then switch to amoeboid behavior involving vigorous shape change and the ability to squeeze through narrow regions, thereby rescuing their migration independently of pericellular proteolysis [*22*••]. Consistent with an amoeboid phenotype, both β1 integrin distribution and filamenous actin adopt a diffuse cortical pattern, reminiscent of migrating lymphocytes [13]. MAT after inhibition of surface proteases was confirmed *in vivo* for tumor cells injected into the mouse dermis [*22*••]. A similar phenotypic change was obtained in fibroblasts populating excisional wounds in rats treated with the broad-spectrum MMP inhibitor GM6001 (Ågren MS, personal communication). Despite MMP inhibition, these fibroblasts retain the capacity to infiltrate the fibrin-rich wound matrix; however they now exhibit roundish amoeboid morphology coupled to a strongly reduced capacity to remodel the wound and generate scar contraction (Ågren MS, personal communication). How protease inhibitors interfere with the regulation of integrin and cytoskeletal dynamics and thereby reprogram mesenchymal cells towards amoeboid movement remains unknown.

Activation of ROCK

Similar transition from mesenchymal to amoeboid movement occurs in HT1080 cells that penetrate thick 3D matrigel layers after constitutive activation of the Rho-effector kinase ROCK [58••]. ROCK acts by increasing

Figure 3



Shifting adhesion and matrix degradation: the Ying and Yang of single cell migration. Migrating mesenchymal HT1080 fibrosarcoma cells and MDB-MB-231 breast cancer cells are spindle-shaped cells that use integrins and proteases for adhesive and proteolytic interactions to ECM substrate, respectively [22••]. On the other hand, T lymphocytes maintain migration by integrin-independent mechanisms and further do not generate pericellular proteolysis [10,11]. Between both extremes, cells may also fulfill the criteria for mixed phenotypes. Abrogating integrin and protease function can result in the reversible reprogramming of the migration type and the conversion from mesenchymal to amoeboid, as seen in HT-1080 and MDA-MB-231 cells [22••].

myosin-II-mediated actin filament stabilization and contraction [28,59]. Active RhoA is required for diffuse cortical actin polymerization and cell retraction in dividing cells [60] and overexpression of constitutively active ROCK causes cortical contraction and cell rounding in originally mesenchymal cell lines, such as 3T3 fibroblasts and HT1080 cells [58••,61]. Active ROCK not only causes HT1080 cells to loose their mesenchymal characteristics and convert to a roundish, contracted shape: driven by small filopodia and blebbing-type cell protrusions that contain cortical actin, the cells convert to a protease-independent migration type, reminiscent of amoeboid movement [58••]. Because ROCK activation generates cortical actin and cell rounding in some cells [15,58••,62], yet stabilizes stress fibers and causes cell spreading, flattening, and migratory cell arrest in other cell types [28], it appears that additional endogenous or environmental cofactors determine if cell–substrate adhesions are stabilized or weakened by Rho/ROCK-mediated signals.

Downregulating integrin functions

Reducing attachment forces without interfering with cell contractility prompts cell rounding and transition towards amoeboid movement. In 3D collagen substrate, such approaches include the following: selection for low endogenous β1 integrin expression by FACS sorting; titrating β1-integrin-mediated adhesion downwards by blocking antibody; interfering with the cytoplasmic β1 integrin domain by a dominant-negative peptidomimetic; and abrogating integrin expression by knockout strategies. All these procedures uniformly generate a roundish yet dynamic cell shape, an even surface distribution of (residual) β1 integrins, and a non-focalized cortical layer of filamentous actin, supporting amoeboid migration in 3D collagen lattices (N Daryab, C Brakebusch and P Friedl, unpublished).

Together, these findings indicate that cells can use a spectrum of migration modes ranging from adhesive to non-adhesive and from proteolytic to protease-independent (Figure 3) [13].

Collective–amoeboid transition

In analogy to EMT, the transition from collective invasion to amoeboid single-cell crawling is obtained if cell–cell and cell–ECM interactions are simultaneously weakened (Figure 2c). In multicellular clusters emigrating from melanoma explants, the inhibition of β1 integrins by adhesion-perturbing antibody abolishes collective movement yet induces the detachment of individual

amoeboid cells from the collective [31••]. In the continuous presence of antibody, detached cells utilized amoeboid shape change and constriction rings and developed a nonclustered, diffuse distribution of surface β1 integrins, similar to that observed in migrating lymphocytes [31••]. Although the molecular mechanism for dispersing cell collectives towards amoeboid single cells through the β1-integrin pathway requires further investigation, these observations suggest that collective migration can directly, or indirectly via a mesenchymal migration step, give raise to amoeboid single cell dissemination.

Conclusions

Taking these findings together, the conversion from multicellular to mesenchymal and/or amoeboid migration may represent stereotypic processes that can be achieved by diverse mechanisms. Although amoeboid movement is detected in cells of low differentiation state, such as stem cells and leukemia and cancer cells, it is also a feature of certain non-neoplastic differentiated cells, such as T lymphocytes, monocytes and neutrophils [11]. In higher mammals (Box 1) amoeboid migration is hence likely to embody a physically optimized migration mode that allows easy cell traffic towards and between structurally different tissue compartments. Although cell differentiation and tissue segregation lead to prespecified migration patterns that are characteristic for each cell type, a basic capacity to revert to ultimately amoeboid migration appears to be retained in some, if not many, mammalian cell types.

Molecular targeting of specific pathways may thus yield more complex adaptation reactions than previously appreciated. Pharmacological interference with adhesive, proteolytic and signaling pathways that maintain a differentiated tissue phenotype but are not essential for cell movement may favor cell reversion to a less differentiated behavior and dissemination mode (Box 2). On the basis of these concepts, the efficiency of drugs designed to interfere with, or redirect, different sorts of migration processes will depend on the target cell's capacity to compensate its migration ability by cellular and molecular adaptation. Consequently, an understanding of the mechanisms that sustain amoeboid rescue mechanisms in higher eukaryotes as well as in *Dictyostelium* will be required for a better targeting of migration processes.

Box 2 Viewpoint: essential and optional components in cell migration

For practical reasons, it will be important to distinguish between cellular and molecular mechanisms that are *essential* for cell movement and those that fulfill *optional* supportive or modifying functions in determining migratory shapes and patterns and are dispensable for migration after functional ablation.

Essential functions

For movement of virtually all cell types on 2D as well as in 3D ECM, essential functions include the acquisition of cell polarity, pseudopod protrusion, cortical stiffness, cytoskeletal contractility and dynamics, as well as physical (but not necessarily adhesive) contact with ECM environments. The capacity to change shape, the key feature of cell movement, appears to require actin polymerization and depolymerization and the function of ROCK in controlling myosin II activity. Consequently, inhibition of actin polymerization by cytocalasin D or latrunculin and/or interference with myosin-II-mediated sliding of actin filaments, for example by inhibiting ROCK [28,58••,64,65], causes profound impairment in amoeboid and other migration modes.

Optional factors

Optional factors determine a particular migration phenotype, but do not limit the capacity to migrate if cellular and/or molecular compensation mechanisms rescue motility. Some of these optional factors have previously gained major attention on the basis of their impact in 2D migration models, although in more complex 3D tissues or *in vivo* models migration may be sustained by compensation strategies. Candidate pathways include:

- Integrin-mediated attachment and traction — because loss of attachment might be compensated by less stringent cell-matrix interactions and shape change
- Proteolytic degradation of the ECM — its loss can be compensated by physical mechanisms, such as shape change, cell squeezing and seeking for trails of least resistance
- Cell–cell adhesion — because cell–cell junctions favor collective cell dynamics and migration while the loss of cell-cell contacts will prompt single cell migration
- Activating chemotactic factors and cytokines — virtually all cell types retain autochthonous cytoskeletal oscillation, polarity and random motility in the absence of migration-promoting factors

Acknowledgements

I am very grateful to Katarina Wolf and Anthea Messent for helpful suggestions on the manuscript. I further acknowledge Eva-B. Bröcker for continuous support and insightful discussions. The original work of the author's laboratory was supported by grants from the DFG (FR1155/2-3 and FR1155/5-1), Ministry for Science and Education, the Wilhelm-Sander-Foundation and the Interdisciplinary Center for Clinical Research (IZKF), Würzburg.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lauffenburger DA, Horwitz AF: **Cell migration: a physically integrated molecular process**. *Cell* 1996, **84**:359-369.

2. Friedl P, Wolf K: **Tumour cell invasion and migration: diversity and escape mechanisms**. *Nat Rev Cancer* 2003, **3**:362-374.

3. • Fey P, Stephens S, Titus MA, Chisholm RL: **SadA, a novel adhesion receptor in *Dictyostelium***. *J Cell Biol* 2002, **159**:1109-1119.
A novel non-integrin receptor provides contact with extracellular structures required for amoeba migration and other functions. This work opens new avenues for research on pattern recognition receptors in mammals.

4. Friedl P, Borgmann S, Brocker EB: **Leukocyte crawling through extracellular matrix and the *Dictyostelium* paradigm of movement — lessons from a social amoeba**. *J Leukoc Biol* 2001, **70**:491-509.

5. Francis K, Palsson B, Donahue J, Fong S, Carrier E: **Murine Sca-1$^+$/Lin$^-$ cells and human KG1a cells exhibit multiple pseudopod morphologies during migration**. *Exp Hematol* 2002, **30**:460-463.

6. Wang W, Wyckoff JB, Frohlich VC, Oleynikov Y, Huttelmaier S, Zavadil J, Cermak L, Bottinger EP, Singer RH, White JG *et al.*: **Single cell behavior in metastatic primary mammary tumors correlated with gene expression patterns revealed by molecular profiling**. *Cancer Res* 2002, **62**:6278-6288.

7. Friedl P, Entschladen F, Conrad C, Niggemann B, Zanker KS: **CD4$^+$ T lymphocytes migrating in three-dimensional collagen lattices lack focal adhesions and utilize β1-integrin-independent strategies for polarization, interaction with collagen fibers and locomotion**. *Eur J Immunol* 1998, **28**:2331-2343.

8. Werr J, Xie X, Hedqvist P, Ruoslahti E, Lindbom L: **β1 integrins are critically involved in neutrophil locomotion in extravascular tissue *in vivo***. *J Exp Med* 1998, **187**:2091-2096.

9. • Brakebusch C, Fillatreau S, Potocnik AJ, Bungartz G, Wilhelm P, Svensson M, Kearney P, Korner H, Gray D, Fassler R: **β1 integrin is not essential for hematopoiesis but is necessary for the T cell-dependent IgM antibody response**. *Immunity* 2002, **16**:465-477.

Although β1 integrins are knocked out in hematopoietic precursor cells, cell differentiation and most immune functions remain intact, including T-cell trafficking and positioning for cell–cell recognition.

10. Friedl P, Zanker KS, Bröcker E-B: **Cell migration strategies in 3D extracellular matrix: differences in morphology, cell matrix interactions, and integrin function**. *Microsc Res Tech* 1998, **43**:369-378.

11. Wolf K, Muller R, Borgmann S, Brocker EB, Friedl P: **Amoeboid shape change and contact guidance: T cell migration mechanisms in fibrillar collagen independent of matrix remodeling by MMPs and other proteases**. *Blood* 2003, **102**:3262-3269.

12. Mandeville JT, Lawson MA, Maxfield FR: **Dynamic imaging of neutrophil migration in three dimensions: mechanical interactions between cells and matrix**. *J Leukoc Biol* 1997, **61**:188-200.

13. Friedl P, Wolf K: **Proteolytic and non-proteolytic migration in tumor cells and leukocytes**. *Biochem Soc Symp* 2003, **70**:277-285.

14. Worthylake RA, Lemoine S, Watson JM, Burridge K: **RhoA is required for monocyte tail retraction during transendothelial migration**. *J Cell Biol* 2001, **154**:147-160.

15. Smith A, Bracke M, Leitinger B, Porter JC, Hogg N: **LFA-1-induced T cell migration on ICAM-1 involves regulation of MLCK-mediated attachment and ROCK-dependent detachment**. *J Cell Sci* 2003, **116**:3123-3133.

16. Haddad E, Zugaza JL, Louache F, Debili N, Crouin C, Schwarz K, Fischer A, Vainchenker W, Bertoglio J: **The interaction between Cdc42 and WASP is required for SDF-1-induced T-lymphocyte chemotaxis**. *Blood* 2001, **97**:33-38.

17. Grinnell F: **Fibroblasts, myofibroblasts, and wound contraction**. *J Cell Biol* 1994, **124**:401-404.

18. •• Tamariz E, Grinnell F: **Modulation of fibroblast morphology and adhesion during collagen matrix remodeling**. *Mol Biol Cell* 2002, **13**:3915-3929.

This paper explores morphological and functional plasticity in fibroblasts in response to outside signals in 3D collagen matrix cultures.

19. Ballestrem C, Hinz B, Imhof BA, Wehrle-Haller B: **Marching at the front and dragging behind: differential αVβ3-integrin turnover regulates focal adhesion behavior**. *J Cell Biol* 2001, **155**:1319-1332.

20. Maaser K, Wolf K, Klein CE, Niggemann B, Zanker KS, Brocker EB, Friedl P: **Functional hierarchy of simultaneously expressed adhesion receptors: integrin α2β1 but not CD44 mediates MV3 melanoma cell migration and matrix reorganization within three-dimensional hyaluronan-containing collagen matrices**. *Mol Biol Cell* 1999, **10**:3067-3079.

21. Sims JR, Karp S, Ingber DE: **Altering the cellular mechanical force balance results in integrated changes in cell, cytoskeletal and nuclear shape**. *J Cell Sci* 1992, **103**:1215-1222.

22. •• Wolf K, Mazo I, Leung H, Engelke K, von Andrian UH, Deryugina EI, Strongin AY, Brocker EB, Friedl P: **Compensation mechanism in tumor cell migration: Mesenchymal-amoeboid transition after blocking of pericellular proteolysis**. *J Cell Biol* 2003, **160**:267-277.

The first example of MAT in tumor cells triggered by blocking matrix-degrading enzymes. Implicates amoeboid movement as the rescue mechanism of tumor cell migration during protease-inhibitor treatment.

23. Deryugina EI, Ratnikov B, Monosov E, Postnova TI, DiScipio R, Smith JW, Strongin AY: **MT1-MMP initiates activation of pro-MMP-2 and integrin αVβ3 promotes maturation of MMP-2 in breast carcinoma cells**. *Exp Cell Res* 2001, **263**:209-223.

24. Zamir E, Katz M, Posen Y, Erez N, Yamada KM, Katz BZ, Lin S, Lin DC, Bershadsky A, Kam Z *et al.*: **Dynamics and segregation of cell-matrix adhesions in cultured fibroblasts**. *Nat Cell Biol* 2000, **2**:191-196.

25. Petroll WM, Ma L: **Direct, dynamic assessment of cell-matrix interactions inside fibrillar collagen lattices**. *Cell Motil Cytoskeleton* 2003, **55**:254-264.

26. Keely PJ, Westwick JK, Whitehead IP, Der CJ, Parise LV: **Cdc42 and Rac1 induce integrin-mediated cell motility and invasiveness through PI(3)K**. *Nature* 1997, **390**:632-636.

27. Clark EA, King WG, Brugge JS, Symons M, Hynes RO: **Integrin-mediated signals regulated by members of the rho family of GTPases**. *J Cell Biol* 1998, **142**:573-586.

28. Nobes CD, Hall A: **Rho GTPases control polarity, protrusion, and adhesion during cell movement**. *J Cell Biol* 1999, **144**:1235-1244.

29. Montell DJ: **Developmental regulation of cell migration. Insight from a genetic approach in *Drosophila***. *Cell Biochem Biophys* 1999, **31**:219-229.

30. Friedl P, Noble PB, Walton PA, Laird DE, Chauvin PJ, Tabah RJ, Black M, Zänker KS: **Migration of coordinated cell clusters in mesenchymal and epithelial cancer explants *in vitro***. *Cancer Res* 1995, **55**:4557-4560.

31. •• Hegerfeldt Y, Tusch M, Brocker EB, Friedl P: **Collective cell movement in primary melanoma explants: plasticity of cell–cell interaction, β1-integrin function, and migration strategies**. *Cancer Res* 2002, **62**:2125-2130.

The first example of CAT in melanoma explants.

32. Sweeney SM, DiLullo G, Slater SJ, Martinez J, Iozzo RV, Lauer-Fields JL, Fields GB, San Antonio JD: **Angiogenesis in collagen I requires α2β1 ligation of a GFP*GER sequence and possibly p38 MAPK activation and focal adhesion disassembly**. *J Biol Chem* 2003, **278**:30516-30524.

33. Nabeshima K, Inoue T, Shimao Y, Okada Y, Itoh Y, Seiki M, Koono M: **Front-cell-specific expression of membrane-type 1 matrix metalloproteinase and gelatinase A during cohort migration of colon carcinoma cells induced by hepatocyte growth factor/scatter factor**. *Cancer Res* 2000, **60**:3364-3369.

34. Bach TL, Barsigian C, Chalupowicz DG, Busler D, Yaen CH, Grant DS, Martinez J: **VE-cadherin mediates endothelial cell capillary tube formation in fibrin and collagen gels**. *Exp Cell Res* 1998, **238**:324-334.

35. Carmeliet P, Lampugnani MG, Moons L, Breviario F, Compernolle V, Bono F, Balconi G, Spagnuolo R, Oostuyse B, Dewerchin M *et al.*: **Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis**. *Cell* 1999, **98**:147-157.

36. van Kempen LC, van den Oord JJ, van Muijen GN, Weidle UH, Bloemers HP, Swart GW: **Activated leukocyte cell adhesion molecule/CD166, a marker of tumor progression in primary malignant melanoma of the skin**. *Am J Pathol* 2000, **156**:769-774.

37. Graeber SH, Hulser DF: **Connexin transfection induces invasive properties in HeLa cells**. *Exp Cell Res* 1998, **243**:142-149.

38. Hsu M, Andl T, Li G, Meinkoth JL, Herlyn M: **Cadherin repertoire determines partner-specific gap junctional communication during melanoma progression**. *J Cell Sci* 2000, **113**:1535-1542.

39. O'Brien LE, Zegers MM, Mostov KE: **Opinion: building epithelial architecture: insights from three-dimensional culture models**. *Nat Rev Mol Cell Biol* 2002, **3**:531-537.

40. Davis GE, Camarillo CW: **Regulation of endothelial cell morphogenesis by integrins, mechanical forces, and matrix guidance pathways**. *Exp Cell Res* 1995, **216**:113-123.

41. O'Brien LE, Jou TS, Pollack AL, Zhang Q, Hansen SH, Yurchenco P, Mostov KE: **Rac1 orientates epithelial apical polarity through effects on basolateral laminin assembly**. *Nat Cell Biol* 2001, **3**:831-838.

42. Yang S, Graham J, Kahn JW, Schwartz EA, Gerritsen ME: **Functional roles for PECAM-1 (CD31) and VE-cadherin (CD144) in tube assembly and lumen formation in three-dimensional collagen gels**. *Am J Pathol* 1999, **155**:887-895.

43. Gerhardt H, Wolburg H, Redies C: **N-cadherin mediates pericytic-endothelial interaction during brain angiogenesis in the chicken**. *Dev Dyn* 2000, **218**:472-479.

44. Hellstrom M, Gerhardt H, Kalen M, Li X, Eriksson U, Wolburg H, Betsholtz C: **Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis**. *J Cell Biol* 2001, **153**:543-553.

45. Keller R: **Shaping the vertebrate body plan by polarized embryonic cell movements**. *Science* 2002, **298**:1950-1954.

46. Hutson MS, Tokutake Y, Chang MS, Bloor JW, Venakides S, Kiehart DP, Edwards GS: **Forces for morphogenesis investigated with laser microsurgery and quantitative modeling**. *Science* 2003, **300**:145-149.

47. Akimov SS, Belkin AM: **Cell surface tissue transglutaminase is involved in adhesion and migration of monocytic cells on fibronectin**. *Blood* 2001, **98**:1567-1576.

48. Ammon C, Meyer SP, Schwarzfischer L, Krause SW, Andreesen R, Kreutz M: **Comparative analysis of integrin expression on monocyte-derived macrophages and monocyte-derived dendritic cells**. *Immunology* 2000, **100**:364-369.

49. McNally AK, Anderson JM: **β1 and β2 integrins mediate adhesion during macrophage fusion and multinucleated foreign body giant cell formation**. *Am J Pathol* 2002, **160**:621-630.

50. Pelosi E, Valtieri M, Coppola S, Botta R, Gabbianelli M, Lulli V, Marziali G, Masella B, Muller R, Sgadari C *et al.*: **Identification of the hemangioblast in postnatal life**. *Blood* 2002, **100**:3203-3208.

51. • Guo H, Fang B, Liao L, Zhao Z, Liu J, Chen H, Hsu SH, Cui Q, Zhao RC: **Hemangioblastic characteristics of fetal bone marrow-derived $Flk1^{+}CD31^{-}CD34^{-}$ cells**. *Exp Hematol* 2003, **31**:650-658.

Fine *in vitro* characterization of all morphological and functional transition stages from endothelial precursor cells to multicellular sprouts.

52. Davis GE, Bayless KJ, Mavila A: **Molecular basis of endothelial cell morphogenesis in three-dimensional extracellular matrices**. *Anat Rec* 2002, **268**:252-275.

53. Hutchings H, Ortega N, Plouet J: **Extracellular matrix-bound vascular endothelial growth factor promotes endothelial cell adhesion, migration, and survival through integrin ligation**. *FASEB J* 2003, **17**:1520-1522.

54. Hotary KB, Yana I, Sabeh F, Li XY, Holmbeck K, Birkedal-Hansen H, Allen ED, Hiraoka N, Weiss SJ: **Matrix metalloproteinases (MMPs) regulate fibrin-invasive activity via MT1-MMP-dependent and -independent processes**. *J Exp Med* 2002, **195**:295-308.

55. Thiery JP: **Epithelial-mesenchymal transitions in tumour progression**. *Nat Rev Cancer* 2002, **2**:442-454.

56. Sternlicht MD, Lochter A, Sympson CJ, Huey B, Rougier JP, Gray JW, Pinkel D, Bissell MJ, Werb Z: **The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis**. *Cell* 1999, **98**:137-146.

57. Lochter A, Navre M, Werb Z, Bissell MJ: **α1 and α2 integrins mediate invasive activity of mouse mammary carcinoma cells through regulation of stromelysin-1 expression**. *Mol Biol Cell* 1999, **10**:271-282.

58. •• Sahai E, Marshall CJ: **Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis**. *Nat Cell Biol* 2003, **5**:711-719.

Together with [59–61], this paper shows how ROCK causes cortical actin polymerization and rounding in previously mesenchymal cells. This implicates ROCK in transition of migration modes and induction of amoeboid shape. Together with [65], this paper further shows how the amoeboid escape can be blocked by combining ROCK and MMP inhibitors.

59. Fukata Y, Amano M, Kaibuchi K: **Rho–Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells**. *Trends Pharmacol Sci* 2001, **22**:32-39.

60. Maddox AS, Burridge K: **RhoA is required for cortical retraction and rigidity during mitotic cell rounding**. *J Cell Biol* 2003, **160**:255-265.

61. Tran Quang C, Gautreau A, Arpin M, Treisman R: **Ezrin function is required for ROCK-mediated fibroblast transformation by the Net and Dbl oncogenes**. *EMBO J* 2000, **19**:4565-4576.

62. Worthylake RA, Burridge K: **RhoA and ROCK promote migration by limiting membrane protrusions**. *J Biol Chem* 2003, **278**:13578-13584.

63. Jacques TS, Relvas JB, Nishimura S, Pytela R, Edwards GM, Streuli CH, ffrench-Constant C: **Neural precursor cell chain migration and division are regulated through different β1 integrins**. *Development* 1998, **125**:3167-3177.

64. Henson JH, Svitkina TM, Burns AR, Hughes HE, MacPartland KJ, Nazarian R, Borisy GG: **Two components of actin-based retrograde flow in sea urchin coelomocytes**. *Mol Biol Cell* 1999, **10**:4075-4090.

65. Somlyo AV, Phelps C, Dipierro C, Eto M, Read P, Barrett M, Gibson JJ, Burnitz MC, Myers C, Somlyo AP: **Rho kinase and matrix metalloproteinase inhibitors cooperate to inhibit angiogenesis and growth of human prostate cancer xenotransplants**. *FASEB J* 2003, **17**:223-234.