REVIEW

Cell Biology of Embryonic Migration

Satoshi Kurosaka and Anna Kashina*

Cell migration is an evolutionarily conserved mechanism that underlies the development and functioning of uni- and multicellular organisms and takes place in normal and pathogenic processes, including various events of embryogenesis, wound healing, immune response, cancer metastases, and angiogenesis. Despite the differences in the cell types that take part in different migratory events, it is believed that all of these migrations occur by similar molecular mechanisms, whose major components have been functionally conserved in evolution and whose perturbation leads to severe developmental defects. These mechanisms involve intricate cytoskeleton-based molecular machines that can sense the environment, respond to signals, and modulate the entire cell behavior. A big question that has concerned the researchers for decades relates to the coordination of cell migration in situ and its relation to the intracellular aspects of the cell migratory mechanisms. Traditionally, this question has been addressed by researchers that considered the intra- and extracellular mechanisms driving migration in separate sets of studies. As more data accumulate researchers are now able to integrate all of the available information and consider the intracellular mechanisms of cell migration in the context of the developing organisms that contain additional levels of complexity provided by extracellular regulation. This review provides a broad summary of the existing and emerging data in the cell and developmental biology fields regarding cell migration during development. **Birth Defects Research (Part C) 84:102–122, 2008.** © 2008 Wiley-Liss, Inc.

INTRODUCTION

Cells of the developing embryo undergo a highly complex chain of events that define their correct shape and positioning during organogenesis and in the tissues and organs of the adult body. Among these events, a crucial role belongs to coordinated cell migration that involves movement of cells of different lineages over short and long distances throughout the body. Defects of migration at all stages of development lead to severe embryonic malformations and result in drastic overall consequences, ranging from early embryonic lethality to birth defects and accounting for multiple human syndromes, including neurological disorders, congenital heart diseases, and physical and mental retardation.

Migration is an evolutionarily conserved mechanism that underlies the development and functioning of uni- and multicellular organisms and takes place in normal and pathogenic processes, including various events of embryogenesis, wound healing, immune response, cancer metastases, and angiogenesis. Despite the differences in the cell types that take part in different migratory events, it is believed that all of these migrations occur by similar molecular mechanisms, whose major components have been functionally conserved in evolution for over a billion years, from protozoa to mammals.

Molecular mechanisms of migration have been extensively studied over the past decades (see Pollard and Borisy, 2003; Ridley et al., 2003; Vicente-Manzanares et al., 2005 and Cell Migration Gateway www.cellmigration.org for overviews). Although many mysteries underlying the regulatory mechanisms of migration still remain to be uncovered, significant progress has been made in the mechanistic understanding of migration and the major molecular events that occur inside the cell during directional movement. Most of these studies have been conducted in culture, where cells move on hard, two-dimensional surfaces rather than in the three-dimensional environment of the tissues composing the embryo. Special aspects of cell migration on different matrices and surfaces of different stiffness that mimic the tissue environment in situ have recently become a focus of cell biology research, but a gap still exists between the mechanistic studies of cell migration in culture and embryonic migration in situ. Vast amounts of data have been accumulated in both fields, however developmental biologists traditionally devote more attention to the external signaling molecules that control the migration on the global tissue and organismal level, while cell biologists focus more on the intracellular molecules involved in migration of individual cells. The connection between the molecular mechanisms of cell movement in

Satoshi Kurosaka and Anna Kashina are from Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

*Correspondence to: Dr. Anna Kashina, Department of Animal Biology, School of Veterinary Medicine, 143 Rosenthal, The University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104. E-mail: akashina@vet.upenn.edu

culture and in situ, as well as the general nature of the mechanisms of cell migration regardless of specific environment, is rarely emphasized in the current literature.

The goal of this review is to bridge these two fields and present a broad overview of the studies of the mechanisms of cell migration in culture and in embryogenesis, with emphasis on the similarities between the mechanisms that underlie the major embryonic migratory events. We focus on the intracellular mechanisms that control cell migration and are common for most of the migratory cell types and use mouse development as a model of the migration processes in situ.

Figure 1. Cell migration. A schematic representation of a generic cell of mesenchymal morphology migrating directionally along a two-dimensional substrate. Polarization, mediated by signaling molecules, defines the leading and the trailing edge of the cell and the direction of migration. Lamella, lamellipodia, and filopodia are responsible for the activity and directionality of the leading edge, whose protrusion is mediated by the branched actin network. A cell attaches to the substrate via dynamic focal adhesions. Actin filaments in the cytoplasm to the rear of the lamella form stress fibers that transmit the myosin-mediated tractional forces to propel the cell movement and regulate the trailing edge retraction via actomyosin contractility. See the explanation in the text for further details.
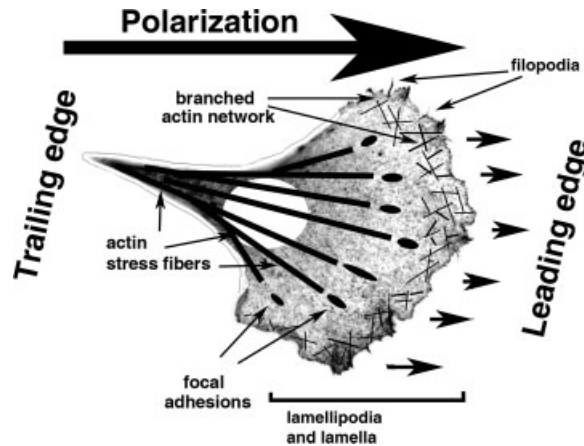
> **Although on the organismal level migration is initiated and coordinated by global extracellular signaling, for each individual cell, it is regulated from within, by forming transient structures that allow the cell to polarize, protrude, and retract in response to its environment.**

BASIC PRINCIPLES OF CELL MIGRATION
Overall Structure of the Cell-Leading Edge

To initiate migration in a developing organism, individual cells receive signals, which set in motion the complex and highly coordinated molecular machinery that drives these cells to move in the right direction with the appropriate speeds and to arrive at their destinations at exactly the right time. Although on the organismal level migration is initiated and coordinated by global extracellular signaling, for each individual cell, it is regulated from within, by forming transient structures that allow the cell to polarize, protrude, and retract in response to its environment.

Migration is a cyclical process, in which the cell polarizes to form an active leading edge that extends dynamic protrusions toward the cell front, and a trailing edge that retracts as the cell moves forward (Fig. 1) (Lauffenburger and Horwitz, 1996; Pollard and Borisy, 2003; Ridley et al., 2003). The major structures that define the leading edge activity of a migrating cell are lamella, lamellipodia, and filopodia. Lamella is a broad, highly active zone that encompasses the area inward from the leading edge. Many of the mechanistic and regulatory events responsible for cell migration and leading edge activity take place in the lamella. Its outer zone, a thin area at the extreme cell edge, is defined as lamellipodia. In a polarized migrating cell, lamellipodia is highly active in defining the direction of the cell movement. Another type of leading edge structures, thin fingerlike protrusions called filopodia, are believed to be responsible for exploring the environment during motility (Faix and Rottner, 2006; Gupton and Gertler, 2007). Although it has been suggested that the inhibition of lamellipodia (Gupton et al., 2005; Yang et al., 2007) or filopodia (Yang et al., 2007) does not inhibit cell motility, it results in defects in cell migration speed and directionality. Although apparently subtle in vitro, such defects are likely to result in a critical handicap during in situ migration through tissues. Both lamellipodia and filopodia are always seen at the edge of the locomoting cells, and their relative balance is believed to regulate specific aspects of migration of different cell types.

Four Steps of Migration: Polarization, Protrusion, Adhesion, and Retraction

Polarization. To move in a certain direction, a cell first needs to polarize in that direction, that is, to define its front (that will move forward) and its back (that will remain in the rear and retract as the leading edge protrudes). The central regulators of establishing this polarity are Rho family small GTPase Cdc42 (Etienne-Manneville and Hall, 2001; Itoh et al., 2002), Par proteins, and atypical protein

kinase (aPKC) (see (Ridley et al., 2003) for overview). Additional signals are provided by a gradient of phosphatidylinositol triphosphate (PIP3) (decreasing from front to rear), produced by the action of phosphoinositide-3 kinase (PI3K), and regulated by PTEN, a PIP3 phosphatase, which is located to the cell rear and controls the lower level of PIP3 at the trailing edge. Thus, polarization is a complex, highly coordinated process that encompasses the entire cell, from front to rear.

Signal gradients during cell polarization are closely followed by cytoplasm rearrangement and organelle repositioning. The central role in this rearrangement belongs to the actin filaments that provide the major driving force for the subsequent cell movement, but other organelles and cytoskeletal systems also participate in this process. The microtubule organizing center reorients to the front of the nucleus to serve as rails for vesicle transport toward the leading edge. This reorientation is closely coordinated with the reorientation of Golgi apparatus to the front of the nucleus. Both events occur rapidly after cell polarization (Gotlieb et al., 1981; Kupfer et al., 1982, 1983; Gundersen and Bulinski, 1988; Etienne-Manneville and Hall, 2001). In the cell rear, the increased PTEN activity is correlated with the activity of myosin II—an actin-dependent molecular motor that ensures the trailing edge contractility and retractive behavior.

Remarkably, cell fragments that lack nuclei and centrosomes are also capable of directional motility (Euteneuer and Schliwa, 1984; Verkhovsky et al., 1999), suggesting that the organelle rearrangement is secondary to other polarization events and may not be required for cell motility. Enucleated centrosome-free cell fragments have been shown to possess a functional cytoskeletal array (Rodionov and Borisy, 1997; 1998; Vorobjev et al., 2001; Malikov et al., 2005), suggesting that the critical role in motility of cells as well as cytoplasmic fragments belongs to the cytoskeleton and regulatory molecules, regardless of the presence or absence of other major organelles.

It has been traditionally believed that cell polarization, especially when it occurs in response to external stimuli (e.g., chemotaxis), originates at the cell front and is initiated by protrusion of the leading edge. However, in spontaneously polarizing cells, the contraction of the rear end actually precedes the leading edge protrusion (Chen, 1979; Dunn and Zicha, 1995; Verkhovsky et al., 1999; Yam et al., 2007). Recent quantitative analysis of spontaneous polarization in fish keratinocytes (Yam et al., 2007) suggests that cell polarization signals are initiated near the nucleus and at the cell rear rather than at the leading edge. Because, during development, the migratory cell types are often not polarized at all before the initiation of the migration at the tissue and organismal level, it appears highly likely that polarization signals in situ may originate at different cell locations to define the leading and trailing edge, and that multiple mechanisms may exist to ensure that this polarization occurs promptly, uniformly, and unambiguously in all cells of the migrating layer.

Protrusion. Protrusion of the cell leading edge is the actual start of the cell migration cycle. The mechanical force for protrusion is provided by the polymerization of actin filaments at the leading edge, either as long parallel bundles to form filopodia, or as a branched dendritic network during lamellipodia formation (Svitkina and Borisy, 1999; Pollard and Borisy, 2003; Ridley et al., 2003; Ponti et al., 2004; Vicente-Manzanares et al., 2005). In both cases, addition of actin subunits onto the fast-growing "barbed" ends of the actin filaments physically pushes the membrane forward in the direction of migration. As the cell moves, the leading edge filaments are disassembled from the rear-facing, slow-growing "pointed" ends, creating a polymerization-depolymerization-driven "machine" often referred to as the "actin treadmill." In such a "treadmill," the force of actin polymerization at the front is coupled to the disassembly of the actin network at the rear of the lamellipodia, propelling the leading edge forward along the substrate (Watanabe and Mitchison, 2002; Pollard and Borisy, 2003; Ponti et al., 2004; Machacek and Danuser, 2006). Based on actin filament stiffness, it has been calculated that the "free" length of the actin filaments pushing the leading edge has to be quite short (less than 150 nm) to create sufficient force for membrane protrusion (Mogilner and Oster, 1996), suggesting that the filament length in the treadmill must be very tightly regulated to achieve the most efficient forward movement.

Assembly of different types of actin structures at the leading edge is achieved by two different actin-nucleating mechanisms (Pollard, 2007). The first mechanism is mediated by the Arp2/3 complex (Welch et al., 1997; Mullins and Pollard, 1999; Welch and Mullins, 2002; Goley and Welch, 2006) that interacts with the surface of the existing actin filaments to create sites of branching for new actin polymerization and ensure actin assembly into the dendritic network that drives the protrusion of lamellipodia. The second mechanism is mediated by formins (Zigmond et al., 1998; Pruyne et al., 2002; Zigmond, 2004; Goode and Eck, 2007) that drive actin assembly into parallel cross-linked bundles that serve as a structural basis for filopodia.

The predominant role in the signaling events that regulate cell protrusion during migration belongs to small GTPases of the Rho family (Etienne-Manneville and Hall, 2001). When bound to GTP, they are active and interact with their downstream targets, including protein kinases, lipid-modifying enzymes, and activators of the Arp2/3 complex. The major players in the cell protrusion machinery are Rac and Cdc42, whose downstream targets of the WASP/WAVE family activate the Arp2/3 complex that regulates the forma-

tion of branches on actin filaments at the leading edge. Additional factors that are important in the network formation and maintenance are regulators of actin polymerization, such as profilin that binds and targets actin monomers to the barbed ends, therefore preventing self-nucleation and ensuring actin incorporation into the proper network, and ADF/cofilin that severs the filaments at the branch points (debranching) and promotes depolymerization from the pointed ends. In filopodia, anticapping proteins, such as Ena-VASP and their partners (Bailly et al., 1999; Krause et al., 2004; Lafuente et al., 2004) and bundling proteins, such as fascin (Adams, 2004a,b), are especially important. A large number of other actin-binding proteins participate in the leading edge actin dynamics, facilitating different stages of actin assembly, disassembly, sequestering, and cross-linking. Identification of these proteins and their role in the leading edge dynamics constitutes an exciting field of research.

Adhesion. Once the protrusion of the leading edge has formed, it needs to attach to the surrounding matrix and stabilize, so that the moving cell can use it as a "push-off" point to move forward. The central role in this attachment belongs to integrins, transmembrane proteins that indirectly bind the ends of the actin filaments on the inside and attach to the extracellular matrix on the outside, providing stable points of the cell interaction with the environment.

Integrins are heterodimeric proteins, consisting of different combinations of α and β chains that are responsible for binding to different matrices (Delon and Brown, 2007; Takada et al., 2007; Lock et al., 2008). As they bind to their extracellular ligands, they change conformation, promoting activation of intracellular signaling cascades that lead to changes in all the signaling molecules that participate in the leading edge activity. Integrin activation on the cytoplasmic side is mediated by

talin binding and involves protein kinase C (PKC), small GTPases (Rap1), and PI3K signaling pathways. Such activation is followed by integrin clustering (achieved by binding to multivalent ligands and regulated by Rac) and recruitment of structural and signaling proteins to form nascent adhesions that eventually mature to focal adhesion.

Focal adhesion formation and maturation is coupled to the assembly of prominent actin stress fibers and is believed to be mediated by actin interaction with the molecular motor myosin II (Rottner et al., 1999; Schoenwaelder and Burridge, 1999; Geiger and Bershadsky, 2001; Galbraith et al., 2002; Giannone et al., 2007). Myosin and its regulatory machinery play a major role in creating and maintaining tractional forces that enable the cell to attach and propel itself over the substrate (Even-Ram et al., 2007; Iwasaki and Wang, 2008). The strongest forces in a migrating cell are transmitted through the focal adhesions in the leading lamella and the retracting regions in the cell rear (Beningo et al., 2001).

As the cell moves forward, focal adhesions disassemble to release the cell for a new phase of the migration cycle. At the leading edge of the cells, focal adhesion disassembly accompanies the formation of new protrusions and assembly of new adhesions at the front (Webb et al., 2002). Some adhesions, mature and persist to form larger, more stable structures that provide firm attachment sites. Although little is known about the mechanisms that determine the fate of each particular adhesion, it has been suggested that some focal adhesions are targeted for disintegration by the dynamic ends of the microtubules in a process sometimes referred to as the "kiss of death" (Kaverina et al., 1999; Small and Kaverina, 2003). Activation of protein kinases FAK and Src, as well as signaling via Rac and ERK, have also been shown to contribute to the adhesion turnover at the leading edge.

Trailing edge retraction. As the cell moves forward, the trailing edge must retract to enable the cell to advance. An exception to this rule is the migration of the neuronal growth cones, which occurs by the mechanisms similar to those described above, but without the movement of the cell body that remains attached to the advancing neurite. This feature prompted some researchers to refer to growth cones as "lamellae on a leash."

Persistent adhesions form at the front and stay as the cell moves forward to maintain the connection to the substratum. Their release at the cell's trailing edge is accompanied by stretching, driven by myosin II contractility, and regulated by FAK and Src signaling (Henson et al., 1999; Verkhovsky et al., 1999; Zhang et al., 2003; Vallotton et al., 2004; Medeiros et al., 2006). The stretching force created during the focal adhesion release is believed to be sufficient to open the stretch-activated Ca^{2+} channels that trigger activation of the calcium-dependent protease calpain that participates in focal adhesion disassembly by cleaving a number of focal adhesion proteins including talin, vinculin, and FAK (Franco et al., 2004; Franco and Huttenlocher, 2005). The release of adhesions at the cell rear contributes to maintaining the polarity of the leading edge and promoting the leading edge activity, providing positive feedback for the continued cycle of migration.

Posttranslational Regulation of Cell Migration

In addition to the activity of protein kinases and proteases that posttranslationally regulate proteins involved in the cell migration cycle by phosphorylation or cleavage, emerging data suggest contribution of other posttranslational modifications to various migration-dependent events. Of those, a special place belongs to posttranslational modification of cytoskeletal proteins. It has been long known that tubulin undergoes acetylation that correlates with

microtubule stability and microtubule-based organelle transport (Reed et al., 2006; Bulinski, 2007), which is important for the delivery of membranous components for cell polarization and protrusion to the cell-leading edge. Tubulin is also modified by post-translational phosphorylation and detyrosynation, as well as addition of tyrosine (Raybin and Flavin, 1977a,b), mono- and poly-glutamine (Edde et al., 1990; Audebert et al., 1993), and glycine (Plessmann and Weber, 1997) [see also (Luduena, 1998) for an overview of tubulin modifications]. Some of these tubulin modifications have been associated with microtubule stability and found to serve as markers for cell polarization (Gundersen and Bulinski, 1986; Khawaja et al., 1988; Infante et al., 2000; Erck et al., 2005). Tubulin modifications by amino acids have also been implicated in the modulation of microtubule functions in various types of motile cells (Raybin and Flavin, 1977a,b; Redeker et al., 2005).

Actin also undergoes a number of posttranslational modifications that have been implicated in normal and pathological actin-mediated processes (see Sheterline et al., 1998 for an overview). N-Terminal aminopeptidation and acetylation is involved in the early maturation of the actin monomers and shown to be essential for normal actin function (Hennessey et al., 1991; Rubenstein and Martin, 1983; Redman and Rubenstein, 1984). An emerging role in cell motility belongs to posttranslational arginylation of actin and actin-associated proteins (Wong et al., 2007). Arginylation has been shown to modulate lamella formation in motile cells and determine directionality and speed of cell migration (Karakozova et al., 2006).

CELL MIGRATION IN A THREE-DIMENSIONAL ENVIRONMENT

Although the basic principles of cell migration described earlier are generally applicable to all types of migratory events in different motile cells throughout the body, several aspects of cell migration in the developing organism are distinct from those in culture, which have served as the foundation for the model of the cell migration cycle described earlier. These differences have to do with the fact that during embryogenesis cells migrate through three-dimensional tissues and matrices that possess distinct physical and chemical properties, which in themselves could vary during migration. The dynamic properties of these environments introduce an

---

During embryogenesis cells migrate through three-dimensional tissues and matrices that possess distinct physical and chemical properties, which in themselves could vary during migration.

---

additional level of complexity into the signals and molecular interactions that influence the migration of different cell types. In the section below, we give a general overview of the special aspects that distinguish cell migration in a three-dimensional environment of an organism compared to a two-dimensional culture.

Extracellular Signaling: Coordinated Migration of Layers and Lineages

Cells are capable of chemical sensing, which enables them to migrate toward favorable directions and/or in response to extracellular chemical stimuli. Although cells in culture often exhibit spontaneous, stimuli-independent polarization, and migration, in the developing embryo cell migration is precisely coordinated by extracellular signals that define the timing, the direction, and the final destination for the migrating cells. Such signals control the complex events that occur during embryogenesis, including gastrulation, patterning, and tissue and organ formation. Perturbation of these signals and the underlying molecular mechanisms of their generation lead to drastic abnormalities of embryogenesis (Gilbert, 2000).

Extracellular signals define the initial steps of cell polarization to ensure that large populations of cells in a certain layer or lineage initiate their migration synchronously on a tight temporal cue. However, while external signals indeed induce preferred directionality and provide the overall cues for the embryonic migratory events, their role in the migration cycle is restricted to the activation of the intracellular signals that drive the subsequent migration steps. Given their strictly extracellular role, such signals were therefore excluded from the current review.

Extracellular Matrix and Force Sensing: Paving the Pathways of Migration

Arguably, the most important aspect that distinguishes between cell migration in culture and in the embryo is related to the fact that in situ cells migrate through a three-dimensional network of the extracellular matrix. Although all cells basically use similar mechanisms of migration, their pathways and the speeds of movement through the tissues of the developing embryo vary a great deal between cells of different lineages and types (Table 1). It is highly likely that this variability is achieved in large part by the properties of the surrounding matrix, which forms structures of different thickness, strength, and density, as well as biochemical composition, facilitating differen-

TABLE 1. Major Types of Embryonic Migratory Events

Cell types	Embryonic events	Stages	Duration (hr)	Origin	Destination	Maximum range	Distance[a] (μm)	Speed[a] (μm/hr)	Reference
Epiblast cells	Gastrulation	E6.5	~24	Primitive streak	Mesoderm	Neighboring cell layers	1	<0.04	Gilbert, 2000; Nagy et al., 2003
Primordial germ cells	General embryo patterning and organogenesis	E7.5–E12	96	Base of the allantois	Genital ridge	Within the tail region	1500	15	Gilbert, 2000; Molyneaux and Wylie, 2004; Molyneaux et al., 2001; Nagy et al., 2003
Cranial neural crest		E8–E9	24	Mid-diencephalon to somite 5	Forebrain, craniofacial mesenchyme	Neck to front head	1000	40	Hutson and Kirby, 2007; Nagy et al., 2003; Serbedzija et al., 1992; Trainor, 2005
Vagal neural crest		E9–E14.5	120	Somites 1–5	Neurons in the gut	Neck to end of gut	10000	85	Anderson et al., 2006a; Durbec et al., 1996; Heanue and Pachnis, 2007; Young and Newgreen, 2001
			120	Somites 6–7	Neurons in the esophagus	Neck to stomach	3000	25	
Trunk neural crest		E8.5–E10.5	48	Somite 5 to caudal tip of neural tube	Sensory and sympathetic neurons, adrenal gland, schwann cells, pigment cells	Back to front	1500	30	Hutson and Kirby, 2007; Nagy et al., 2003
Sacral neural crest		E10–E10.5	12	Caudal region to somite 24	Hindgut	Back to front	1000	85	Anderson et al., 2006a,b; Heanue and Pachnis, 2007; Kapur, 2000
		E14.5–E15.5[a]	12[a]	Hindgut	Spread within hindgut	Within an organ	1000	85	
Cardiac neural crest	Heart development	E8.5–E13.5	120	Otic placode to the end of somite 3	Cadiac outflow tract, septae, parts of ventricular myocardium via pharingeal arches	Neck to heart	4000	35	Brown and Baldwin, 2006; Hutson and Kirby, 2007; Jiang et al., 2000
Extraembryonic mesoderm, endothelial, smooth muscle	Vasculogenesis and angiogenesis	E7–postnatal		Extraembryonic tissues and primitive streak	Mature circulatory system	Circulatory system	NA	NA	Gerthoffer, 2007; Gilbert, 2006; Mu et al., 2005; Patan, 2000
Nerve cells	Brain development	E14.5–adulthood		Brain and ganglia	Nerve endings in the brain and throughout the body	Up to adult body length	NA	NA	da Silva and Dotti, 2002; Gilbert, 2006; Skaper, 2005

[a]Estimated.

tial migration of cells intended for various destinations (Trelstad, 1984). Such differentially paved "roadways" in the embryo participate in the complex embryonic mechanisms that regulate the formation of different tissues and organs (Gilbert, 2000).

It has been found that cells migrating through three-dimensional matrices assume a different morphology compared to those migrating on hard two-dimensional surfaces, have different migration speeds, and may use additional migratory mechanisms (Even-Ram and Yamada, 2005), such as proteolysis of the matrix at the leading edge, shown to be used by endothelial cells during angiogenesis (van Hinsbergh and Koolwijk, 2008). It has also been found that even on flat, matrix-coated surfaces, the amount of extracellular matrix determines the speed of migration by balancing the protrusion and adhesion steps of the cell migration cycle (Gupton and Waterman-Storer, 2006). When the concentration of the extracellular matrix is too low, cultured cells exhibit a decrease in the actin flow and form few highly dynamic adhesions that cannot sufficiently "stick" to the substrate. When the concentration of the extracellular matrix is too high, cells form too many adhesions that are less dynamic and cannot retract enough to ensure movement. An optimal, medium, concentration of extracellular matrix ensures a normal migration cycle and results in the highest motility rates. It appears likely that similar mechanisms are used in the developing organism, where varying amounts of extracellular matrix can ensure great variability in the migration speeds that are often observed for cells of the same lineage during the formation of different organs (Table 1).

An important property of different extracellular matrices in situ is defined by their mechanical rigidity or stiffness. The relationship of cell adhesion and motility to the substrate stiffness has become the focus of major studies in recent years (Geiger and Bershadsky,

2001; Georges and Janmey, 2005; Bershadsky et al., 2006; Vogel and Sheetz, 2006; Rehfeldt et al., 2007). It has been found that force sensing and substrate stiffness define the formation and turnover of the adhesions to the extracellular matrix and determine the speed of migration as well as cell shape and ability to differentiate (Engler et al., 2006). Loss of force sensing accompanies adhesive and migratory defects in culture and in situ, including oncogenic transformation and cancer metastases, closely correlated with the ability of cells for substrate-independent growth (Kedrin et al., 2007; Yamaguchi and Condeelis, 2007).

A special type of force sensing is related to shear stress due to the application of external mechanical force onto the migrating or stationary cells. Such force can be applied via blood flow over the endothelial cells lining the blood vessels, or via muscle contraction onto the cells within the muscle tissue. Overall, cellular responses to mechanical signals are estimated to have major influence on their behavior in culture and in tissues.

Force is transduced through adhesion sites via the actin cytoskeleton and results in major signaling changes that define cell shape, polarization, and the entire complexity of the motility events (Dobereiner et al., 2005; Georges and Janmey, 2005). Stretch applied on a cell due to external mechanical force or attachment to the stiffer substrates affects actin near the adhesion site and is followed by recruitment of adhesion complex proteins and global cytoskeletal rearrangements (Galbraith et al., 2002; Riveline et al., 2001). Multiple actin-binding proteins at the cell adhesion sites directly respond to substrate rigidity (overviewed in Dobereiner et al., 2005), resulting in changes to all steps of the migration cycle and all of the structural and signaling molecules involved (Gunzer et al., 2000; Knight et al., 2000; Beningo et al., 2004; Bhadriraju et al., 2007).

Cell–Cell Adhesion

Cell–cell adhesions have an important role in tissue morphogenesis and are critical for the formation of interconnected cell layers, such as epithelial sheets (Gilbert, 2000). Although such layers have only limited migratory capacity, they migrate jointly over short distances during gastrulation and are capable of short-distance migration with a free edge later in development and in adulthood during wound healing. Cell–cell adhesions are critical for maintaining the epithelial morphology and enabling their proper in-tissue behavior.

Cell–cell adhesion molecules are also important for the migration of cells of the mesenchymal morphology. During migration through tissues and layers of the developing embryo, mesenchymal cells do not incorporate into sheets and form stable connections with each other, but they are capable of forming transient connections to the stationary cells of the neighboring layers and with each other as they come into immediate proximity. All of these connections participate in modulating the paths and speeds of migration via cell–cell adhesion molecules, such as cadherins and catenins that, like integrins, regulate intracellular signaling cascades by sensing the environment. Information about the regulation of mesenchymal cell migration by cell–cell adhesion molecules is so far limited to the general mechanisms and signaling pathways mediated by these molecules. For this reason, we do not review these mechanisms in detail, beyond mentioning the fact that these mechanisms are likely to participate in the in situ migration cycle.

CELL MIGRATION IN THE DEVELOPING EMBRYO
Major Migratory Cell Types

Cell migration over short and long distances plays a critical role at all stages of development, from gastrulation to the formation of an adult organism (Gilbert, 2000). From the migratory point of view,
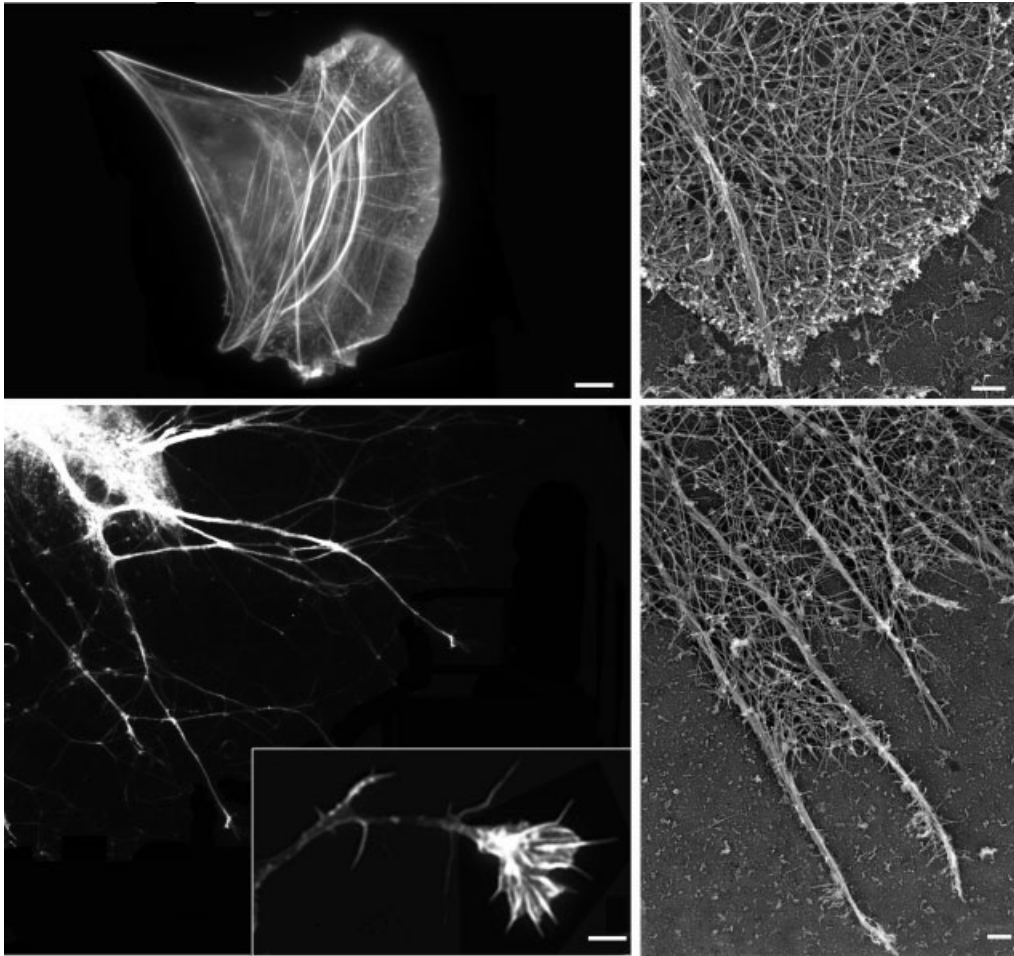
Figure 2. Migration of different cell types is driven by the same mechanisms. Top left, immunofluorescence image of a migrating fibroblast stained with rhodamine-phalloidin to visualize actin filaments. Top right, close-up electron microscopy image of a platinum replica of the leading edge cytoskeletal network of a migrating fibroblast, similar to that shown on the left. Bottom left, immunofluorescence image of neurite outgrowth and close-up image of a neuronal growth cone (boxed) illustrates the migration events during neuritogenesis and the laying down of the nervous system. Bottom right, close-up electron microscopy image of a platinum replica of the leading edge cytoskeletal network in a neuronal growth cone, similar to that shown on the left. Despite morphological differences, variations in the amount of filopodia and the relative density of the actin cytoskeleton, the major structural features of the leading edge, are similar in both cell types. Images in the top panels, bottom right panel, and the close-up of the neuronal growth cone in the bottom left panel courtesy of Dr. T. M. Svitkina (University of Pennsylvania). Bars, 2 μm for immunofluorescence images and 200 nm for the electron micrographs.

all embryonic cells are divided into cells of the epithelial morphology that form interconnected sheets and mesenchymal cells that originate from different lineages and migrate throughout the embryo as independent units to contribute to tissues and cell types throughout the body.

Interconnected epithelial sheets undergo short-range migration during gastrulation, however, part of this migration is driven not by the movement of the sheet itself, but by the repositioning of the mesenchymal cells that define the embryonic mesodermal layer.

Migration of epithelial sheets is an important part in the development of such organisms as fish and amphibia that do not dramatically increase in size during early development. Overall, the migration of epithelial sheets generally occurs at a short range at very slow speeds. Although such migration is believed to be driven by different signaling events compared to the migration of the mesenchymal cells (Locascio and Nieto, 2001), it uses similar molecular mechanisms.

Cells of the mesenchymal morphology are responsible for most migratory events that take place during development (Table 1). In general terms, mesenchymal cells share the generic features of fibroblasts, which have been the classical model for studying cell migration in culture (Figs. 1 and 2), and it is believed that different embryonic mesenchymal cells during migration generally follow the mechanisms described in the first section of the review (Molyneaux and Wylie, 2004; Gerthoffer, 2007; Pak et al., 2008) (Fig. 2).

A special type of migration takes place during development and patterning of the nervous system.

Although during predifferentiation stages neuronal precursors of the neural crest lineage follow the same migration mechanisms as any other mesenchymal cells, after differentiation the neuronal cell bodies eventually find stationary positions within the developing nervous system and only their neurites continue to migrate through tissues to reach their destinations and form connections with their effectors as part of the mature nervous system. This process can occur over considerable distances and with relatively high speeds and constitute a global and important part of development. In humans, brain development alone involves the laying down of $\sim 10^{11}$ neurons, each forming $\sim 1,000$ connections with other neurons (Williams and Herrup, 1988) and often traveling over distances of more than one meter, five orders of magnitude larger than the size of the neuron cell body. This migration, while highly specialized and morphologically distinct, is believed to occur by the same mechanisms as those described earlier for other cell types (Dent et al., 2007; Mongiu et al., 2007; Pak et al., 2008, Figs. 1 and 2). From the mechanistic perspective, the growth cone of each neurite is considered a separate leading edge and generally follows the same mechanisms as whole cells migrating through tissues (Fig. 2).

Embryonic Migratory Events

Table 1 shows an overview of the major migratory events that take place during different stages of development in a mouse. Most of these migrations take part after gastrulation and can be loosely divided into global redistribution of undifferentiated cells throughout the embryo (days 7–15 in mouse development) and local rearrangements of differentiating cells into the mature vascular and nervous system (see Figs. 3 and 4 for mouse developmental stages and migration pathways). Because the embryo continues to grow as migration takes place, both global and local migration can occur over large distances and at relatively high speeds.

Early embryonic migration is dominated by cells of the neural crest lineage (see Bronner-Fraser et al., 1991; Fraser, 1991; Le Douarin, 1983; Tucker, 2004, for overview; Saint-Jeannet, 2006) that are classified into several types based on their origin and destination in the embryo (Figs. 3 and 4 and Table 1). Cranial neural crest cells originate from the head and neck region and give rise to cartilage, bone, cranial neurons, glia, connective tissue of the face, thymus, thyroid, teeth, bones of the middle ear, and jaw (Serbedzija et al., 1992; Nagy et al., 2003; Trainor, 2005; Hutson and Kirby, 2007). Cardiac neural crest cells originate from a similar head and neck region and form cardiac outflow tract as well as heart septae and parts of the venricular myocardium (Jiang et al., 2000; Brown and Baldwin, 2006; Hutson and Kirby, 2007). Vagal and sacral neural crest cells migrate from two opposite sides to populate the gastrointestinal system and differentiate into the neurons that innervate the digestive tract (Durbec et al., 1996; Young and Newgreen, 2001; Anderson et al., 2006a). Trunk neural crest cells migrate from the back of the embryo to form sensory and sympathetic neurons, adrenal gland, Schwann cells, and pigment cells of the skin (Nagy et al., 2003; Hutson and Kirby, 2007).

In addition to the neural crest cells, primordial germ cells also migrate within the midrear area of the embryo into the genital ridges. This migration takes place over a considerable period of time (five embryonic days for a mouse embryo, which constitutes approximately quarter of the entire development), and is believed to occur in stages of rapid migration followed by relatively stationary periods (Molyneaux et al., 2001; Molyneaux and Wylie, 2004). The actual speed of this migration is difficult to estimate, because as the cells migrate, the embryo itself continues to grow and increases several times in size during the migration process (Fig. 3). Therefore, it is possible that primordial germ cells actually migrate very little, and instead "ride" the expanding tissues to reach their destination (Freeman, 2003).

Some sources refer to the positioning of the blood cell precursors in the fetal liver and bone marrow as a type of migration in embryogenesis. However, while individual blood cells (e.g., macrophages) are capable of active migration, most of the embryonic repositioning of the blood cell precursors occurs by passive circulation through the blood stream. For this reason, we are not considering this type of movement in the current review.

Duration of each type of migration and maximum distance between origin and destination at the end of each migratory event can serve as a basis for an estimation of the overall migration speed of each cell type during different embryonic events (Table 1). The estimated speeds shown in Table 1 are based on the assumption that cells of each lineage migrate uniformly and continuously during the appropriate stages; however, for some lineages and cell types (e.g., germ cells), it may not be the case. Based on such estimation, all types of migrating cells can be classified into groups that differ from each other by their migration speeds. Sacral and most of the vagal neural crest cells that populate the digestive tract are the fastest (migration speeds >80 μm/hr), while cranial, cardiac, and trunk cells migrate at less than half the speed (30–40 μm/hr), suggesting that distinct mechanisms within the body regulate the speed of migration of these highly similar cell types.

Different mechanisms of the regulation of cell migration speeds, acting jointly, or separately could be envisioned. One mechanism involves the creation of differential extracellular matrix "pavement" for different migration pathways, making paths of different stiffness, composition, and density that could tightly control the migration speed. To regulate short "bursts"
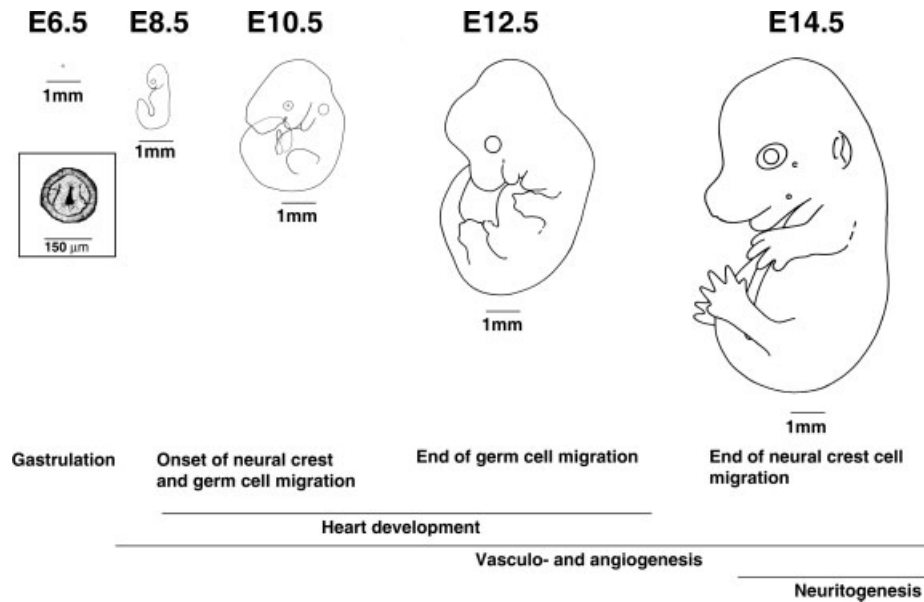
Figure 3. Timing of the major migratory events in mouse embryogenesis in relation to the embryo growth. Letters and numbers on the top denote days postcoitum. One millimeter scale bar of the same size is shown next to each embryo to enable the direct size comparison. Boxed image shows an enlargement of the gastrula shown on top. The major migratory events corresponding to those shown in Table 1 are written for the appropriate stages underneath. Images and sizes are adapted from Kaufman (1992) and have been used as the basis for the calculation of migration distances shown in Table 1.
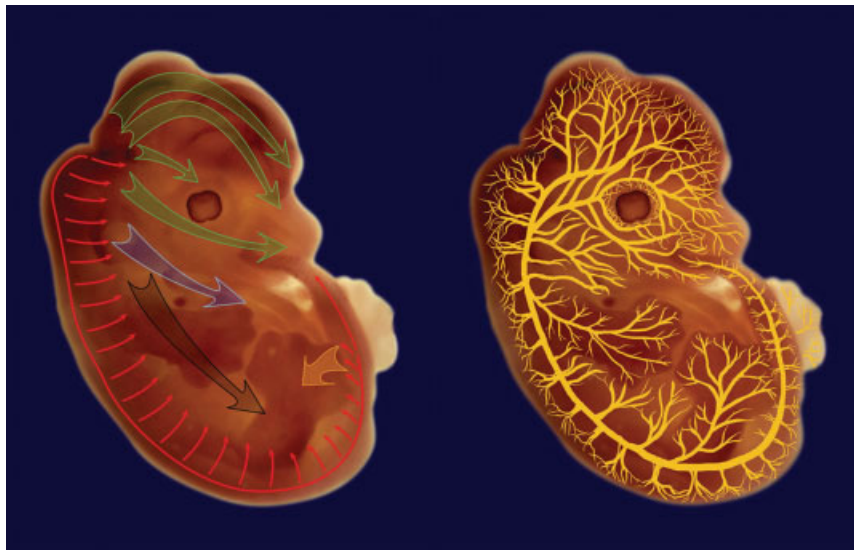

Figure 4. Pathways of neural crest migration, neuritogenesis, and angiogenesis during mouse development. Neural crest cells (left) generally migrate from the back area of the embryo, originating from different somite regions at different embryonic stages as listed in Table 1. Large green arrows in the head area denote cranial neural crest; blue arrow, cardiac neural crest; black, vagal neural crest; orange arrow, sacral neural crest; and thin red arrows; trunk neural crest. Neurites and blood vessels sprout from the structures laid out during earlier stages of development and reach to all the areas of the mature organism along the pathways illustrated on the right hand image. Illustrations were prepared by O. Karengina based on an image of a mouse embryo at E13.5.

tissue explants in chick embryos suggested that over the periods of observation, cells were able to migrate faster than the estimates shown in Table 1 for mouse cells, on the order of 170 μm/hr (Kulesa et al., 2000), arguing in favor of the "short burst" hypothesis. However, live imaging of neural crest cell migration in mouse ranged between cell populations migrating at 30–40 and 84 μm/hr, which is extremely close to our calculations presented in Table 1 (Druckenbrod and Epstein, 2005; Druckenbrod and Epstein, 2007), arguing in favor of uniform, continuous migration. Overall, it is likely that both mechanisms may contribute to the regulation of cell migration speeds in situ.

During the later stages of development, global migratory events of neuritogenesis and angiogenesis encompass the developing embryo (Fig. 4) and drive tissue rearrangements that continue after birth, and, in the case of neuritogenesis, into adulthood. Because of the extensive duration of these processes, it is difficult to estimate their distances and speeds. However, because the molecular

of migration followed by relatively stationary periods, signals may be used that would switch the migration of certain cell types on and off in coordination with other developmental events.

Live observations of in situ migration of labeled neural crest

mechanisms that drive these migratory events are similar for different cell types (Gerthoffer, 2007; Pak et al., 2008), the order of magnitude of these migration speeds is expected to be similar to those observed at the earlier embryonic stages. It seems likely that angiogenesis and neuritogenesis do not occur continuously with constant speeds, but are modulated in response to the type and availability of matrices and external signals from the neighboring cells and tissues.

MIGRATION-DEPENDENT DEVELOPMENTAL DEFECTS
Key Cell Migration Components in Mouse Embryogenesis

Because all cell types during embryonic migration use similar molecular mechanisms, perturbation of the key players in these mechanisms is expected to lead to drastic developmental defects. Over the years, numerous data on mouse knockouts of cell migration components have been accumulated (Table 2). Analysis of these data and comparison of defects that develop in response to each gene knockout provide evidence of the unique mechanisms whose perturbation results in early lethality, and those mechanisms that are either cell type specific or can be compensated by the function of different genes.

Table 2 summarizes the current data on mouse knockouts of the intra- and extracellular components that are directly relevant to the cell migration mechanisms outlined earlier. These data are grouped by functions and separated into sections based on their role at different migration stages. Actin cytoskeleton proteins play an important role in all of the mechanical steps of the migration cycle, while focal adhesion proteins, integrins, and components of the extracellular matrix predominantly participate in adhesion and retraction. In addition, knockouts of some of the regulatory molecules directly involved in the regulation of the mechanistic com-

ponents of cell migration have been described and are listed separately in the table. Knockouts of other regulatory molecules, such as PI3K and PTEN, have also been characterized (Di Cristofano et al., 1998; Stambolic et al., 2000; Katso et al., 2001; Jou et al., 2002; Suzuki et al., 2003), but are too general to be implicated directly in migration and have therefore been removed from our list.

A separate body of literature concerns tissue signaling molecules and growth factors, as well as transcription factors that regulate cell migration and coordinate global developmental migratory events (see Gilbert, 2000, for an overview; Chi and Epstein, 2002; Alva and Iruela-Arispe, 2004; Gruber and Epstein, 2004; Hofmann and Iruela-Arispe, 2007, High and Epstein, 2008; for some recent reviews). Because these knockouts do not directly affect the intracellular events of the cell migration cycle, they were not included in the tables or discussion of the current review.

As seen in Table 2, certain knockouts lead to early embryonic lethality (e.g., β actin or tropomyosin), suggesting that these proteins play a universal role in multiple embryonic events. Other knockouts result in organ-specific phenotypes (cardiac defects, placenta, neuritogenesis, or vascular patterning), suggesting that these genes either play a tissue-specific role or their function can be compensated for by similar molecules leading to normal, or seminormal development of the tissues, in which migration plays a less critical role. Analysis of these phenotypes provides insights into the underlying molecular reasons for birth defects and may allow us to predict major functional roles for proteins, whose functions have not been well established. An example of such a protein is arginyltransferase, Ate1, whose knockout in mice results in embryonic lethality with specific defects in heart development and angiogenesis that suggest that this gene and the corresponding post-

translational modification plays a critical role in cell migration in situ (Kwon et al., 2002). In agreement with that, Ate1 has been recently shown to regulate actin cytoskeleton, lamella formation, and lamellipodial protrusion (Karakozova et al., 2006); however, the exact role of Ate1 in embryogenesis and cell motility remains to be uncovered.

Cell Migration and Human Disease

The data presented earlier demonstrate the critical importance of cell migration to embryonic development. Not surprisingly, perturbations of migration can lead to severe disorders with direct relevance to human survival and health. Cancer, the second leading cause of death in the developed countries worldwide, is characterized by impaired attachment of cells to the matrix and perturbation of the cell migratory mechanisms (see Yamaguchi et al., 2005 for an overview).

On the developmental level, a number of human diseases and congenital disorders have been associated with defects in cell migration. Heart septation defects (ventricular and atrial septal defects, and persistent truncus arteriosus, abbreviated as VSD, ASD, and PTA, respectively), associated with the defects of migration of the cardiac neural crest cells (Table 1), are among the most highly occurring human congenital heart defects. DiGeorge syndrome, characterized by heart defects, craniofacial abnormalities, and severe retardation, has been linked to chromosome deletions that result in migratory defects of the neural crest cells (Epstein, 2001; Gitler et al., 2002; Epstein and Parmacek, 2005; Hutson and Kirby, 2007; Stoller and Epstein, 2005). Hirschsprung's disease, characterized by impaired bowel movement, is related to defects in migration of the vagal and sacral neural crest lineages that populate the gastrointestinal system and subsequently differentiate into the neurons responsible for gut inner-

TABLE 2. Mouse Knockouts of Cell Migration Components or Other Proteins with Direct Effects on Cell Migration

Protein	Function	Type	Phenotype	Reference
α-Skeletal muscle actin	Actin cytoskeleton	Total	Postnatal death at P1-9, marked loss of body weight; upregulation of other actin isoforms	Crawford et al., 2002
α-Smooth muscle actin	Actin cytoskeleton	Total	Viable; impaired vascular contractility and blood pressure homeostasis; upregulation of other actin isoforms	Schildmeyer et al., 2000
α-Cardiac actin	Actin cytoskeleton	Total	Perinathal lethality; cardiac hypertrophy and heart muscle abnormalities; upregulation of other actin isoforms	Kumar et al., 1997
β-Nonmuscle actin	Actin cytoskeleton	Total	Death after E9.5	Shawlot et al., 1998
γ-Nonmuscle actin	Actin cytoskeleton	Skeletal muscle-specific	Muscle weakness, necrosis and degeneration	Sonnemann et al., 2006
Tropomyosin	Actin cytoskeleton	Total	Death before morula stage	Hook et al., 2004
Mena	Actin cytoskeleton	Total	Viable, with misrouted axons and defects in the nervous system	Lanier et al., 1999
Mena, VASP, Evl triple null	Actin cytoskeleton	Total	Defects in brain development, neuritogenesis, and neural tube closure	Kwiatkowski et al., 2007
Filamin-B	Actin cytoskeleton	Total	Skeletal malformations and impaired microvascular development	Zhou et al., 2007
Gelsolin	Actin cytoskeleton	Total	Defects in fibroblast and platelet motility and lamellar responses	Witke et al., 1995
Nonmuscle myosin II-B	Actin cytoskeleton	Total	Embryonic and perinatal lethality with severe heart defects	Tullio et al., 1997
Myosin heavy chain II-A	Actin cytoskeleton	Total	Failure in embryonic patterning, embryonic lethality by E7.5	Conti et al., 2004
Cardiac α-myosin, heavy chain	Actin cytoskeleton	Total	Embryonic lethality between E11 and 12 with gross heart defects	Jones et al., 1996
Profilin I	Actin cytoskeleton	Total	Very early embryonic lethality—no viable knockout blastocysts found. Heterozygotes show reduced embryonic survival	Witke et al., 2001
Profilin II	Actin cytoskeleton	Total	Neurons have altered kinetics of synapse recycling. Mice are anxious, do not react to stress, and lack maternal behavior	Witke Group EMBL 201 Research Reports http://www.embl-heidelberg.de/emblGroup/researchReport/rr01_73.pdf
VASP	Actin cytoskeleton	Total	Mice viable, with significant reduction in cAMP- and cGMP-mediated inhibition of platelet aggregation; hyperplasia of megakaryocytes in bone marrow and spleen; abnormalities in cortical neuron positioning	Aszódi, 1999; Goh et al., 2002; Hauser et al., 1999

(Continued)

TABLE 2. (Continued)

Protein	Function	Type	Phenotype	Reference
N-Cofilin	Actin cytoskeleton	Total	Neural Crest cells fail to polarize and form F-actin bundles	Gurniak et al., 2005
Paxillin	Focal adhesions	Total	Embryonic lethality due to abnormal development of mesodermally derived structures; phenotype similar to fibronectin null	Hagel et al., 2002
Talin	Focal adhesions	Total	Embryonic lethality at E8.5-9.5 with disorganization of embryos at gastrulation.	Monkley, 2000
Tensin	Focal adhesions	Total	Viable and healthy at birth: weakness, renal failure and cysts in proximal kidney tubules later in life. Analysis of cysts show disruptions in cell matrix junctions and weakening of focal adhesion	Lo et al., 1997
Tensin	Focal adhesions	Total	Delayed regeneration of wounded skeletal muscle correlated with delayed expression of myosin, paxillin and dystrophin	Ishii and Lo, 2001
Fak	Focal adhesions	Total	Mesodermal defects postgastrulation. Death at E8.5 with impaired blood vessel development	Furuta, 1995; Ilic et al., 2003
Vinculin	Focal adhesions	Total	Death by E10, with embryos displaying 30–40% reduction in size, lack of midline fusion of the rostral neural tube and developmental defects in heart, somites and limbs	Xu et al., 1998
Zyxin	Focal adhesions	Total	Viable, fertile, no obvious abnormalities	Hoffman et al., 2003
α4 integrin	Integrins	Total	Embryonic lethality, defects in placenta and vascular system, cardiac hemorrhages	Yang et al., 1995
αv integrin	Integrins	Total	Partial embryonic lethality, intracerebral and intestinal hemorrhages and cleft palates	Bader et al., 1998
α5 integrin	Integrins	Total	Embryonic lethal and E10–11 with migration-type defects in posterior trunk and yolk sac mesodermal structures	Yang et al., 1993
α7 integrin	Integrins	Total	Partial embryonic lethality, cerebral vascular defects, placental defects	Flintoff-Dye et al., 2005; Mayer et al., 1997; Rooney et al., 2006; Welser et al., 2007
β1 integrin	Integrins	Endothelium	Failure of normal vascular patterning, severe fetal growth retardation, and embryonic death at E9.5–10	Lei et al., 2008; Tanjore, 2008
		Total	Death shortly after implantation	Fassler and Meyer, 1995
		In vitro studies of cells from knockout mice	Reduced neural crest cell motility	Breau et al., 2006

(Continued)

TABLE 2. (Continued)

Protein	Function	Type	Phenotype	Reference
β3 integrin	Integrins	Total	Defects in platelet aggregation and clot retraction, hemorrhages, postnatal anemia, and reduced survival	Hodivala-Dilke, 1999
β8 integrin	Integrins	Total	Embryonic or perinatal lethality with profound defects in vascular development, insufficient vascularization of the placenta and yolk sac	Zhu et al., 2002
VCAM-1	Integrins	Total	Embrynic lethality at E10–12, abnormal placental development	Gurtner et al., 1995
Syndecan-4	Extracellular matrix	Total	Blood vessel defects in the placenta; defects in angiogenesis and wound repair	Echtermeyer, 2001; Ishiguro et al., 2000; Kojima, 2002
N-Syndecan	Extracellular matrix	Total	Impaired neural migration in the developing brain	Hienola et al., 2006
Perlecan	Extracellular matrix	Total	Defects in heart integrity, invasion of brain tissue into the overlaying ectoderm, severe defect in cartilage, cleft palates and death shortly after birth from respiratory failure.	Costell et al., 1999
Tenascin C	Extracellular matrix	Total	Neural Crest cells fail to disperse laterally	Tucker, 2001
Laminin γ1	Extracellular matrix	Total	Death at E5.5, lack of basement membranes	Smyth et al., 1999
Laminin β2	Extracellular matrix	Total	Postnatal death between P15–30, neuromuscular junctions and glomerular defects	Noakes et al., 1995a,b; Patton et al., 1997
Laminin α2	Extracellular matrix	Total	Death by 5 weeks postnatal, severe muscular dystrophy and peripheral neurophathy	Miyagoe et al., 1997
Laminin α2 (dy/dy)	Extracellular matrix	Spontaneous	Adult lethality, severe muscular dystrophy and peripheral nerve dysmyelination	Patton et al., 1997, 1999
Laminin α3	Extracellular matrix	Total	Death at P2–3, epithelial adhesion defect	Ryan et al., 1999
Laminin α4	Extracellular matrix	Total	Transient microvascular defect with hemorrhages and misalignment of neuromuscular junctions	Patton et al., 2001; Thyboll et al., 2002
Laminin α5	Extracellular matrix	Total	Death at E14–E17, with placental vessel, neural tube, limb, and kidney defects	Miner et al., 1998; Miner and Li, 2000
Fibronectin	Extracellular matrix	Total	Death before E14.5, shortened anterior–posterior axes, deformed neural tubes, and defects in mesodermally derived tissues.	George et al., 1993
Collagen XVIII	Extracellular matrix	Total	Eye abnormalities modeling Knoblock syndrome	Fukai et al., 2002
	Extracellular matrix	Total	Basement membrane defects	Utriainen et al., 2004
Collagen XV	Extracellular matrix	Total	Skeletal myopathy and cardiovascular defects	Eklund et al., 2001
β-Glycan	Extracellular matrix	Total	Embryonic lethality with heart and liver defects	Stenvers et al., 2003
Connexin 43	Cell–cell adhesion	In vitro studies of cells from knockout mice	Increased cell protrusive activity, loss of polarized cell movement, rearrangement of actin stress fibers	Xu et al., 2006

(Continued)

TABLE 2. (Continued)

Protein	Function	Type	Phenotype	Reference
PECAM-1	Cell–cell adhesion	Total	Die at birth from severe pulmonary outflow tract obstruction	Reaume et al., 1995
		In vitro studies of cells from knockout mice	Reduced migration, inability to undergo capillary morphogenesis in matrigel	Kondo et al., 2007
β-Catenin	Cell–cell adhesion	Total	Mice viable, defects in retinal vasculature	Dimaio et al., 2008
		Conditional EC-specific knockout	Death between E11.5 and E13.5; impaired vascular patterning in the head, vitelline and umbilical vessels, and placenta	Cattelino et al., 2003
N-Cadherin	Cell–cell adhesion	Conditional NC specific	Defects in remodeling of cardiac outflow tract	Luo et al., 2006
		Total	Death by E10	Radice et al., 1997
		Conditional EC specific	Death at midgestation, severe vascular defects, decrease of VE-cadherin	Luo and Radice, 2005
E-Cadherin	Cell–cell adhesion	Total	Early lethality, fail to form trophectoderm.	Larue et al., 1994
VE-Cadherin	Cell–cell adhesion	Total	Death at E9.5, endothelial cells are assembled in vascular plexi, but their subsequent remodeling and maturation are impaired	Carmeliet et al., 1999
L1-CAM	Cell–cell adhesion	Total	Malformations of the nervous system, decreased nerve sensitivity, weak and uncoordinated hindlegs.	Dahme et al., 1997
Ate1	Arginyltransferase	Total	Embryonic lethality at E12–17 with defects in cardiovascular development and angiogenesis	Kwon et al., 2002
Calpain IV (Capn4)	Protease	Total	Embryonic lethality at midgestation with defects in the cardiovascular system, hemorrhaging, and accumulation of erythroid progenitors	Arthur et al., 2000
C3G	Guanine nucleotide exchange factor 1	Total	Death before E7.5; Death at E11.5 due to hemorrhages and vascular integrity defects	Ohba, 2001; Voss et al., 2003
Rap1b	Small GTPase	Total	Decreased level of Matrigel and neonatal retinal neovascularization, impaired endothelial cell motility in culture	Chrzanowska-Wodnicka et al., 2008
ROCK-II	Small GTPase	Total	90% embryonic lethal 13.5 days postcoitum due to placental abnormalities	Thumkeo et al., 2003
Csk	C-srk tyrosine kinase	Total	Neural tube defects, death between E9 and E10; developmental arrest in the 10–12 somite stage	Imamoto and Soriano, 1993; Nada et al., 1993
Ephrin B1	Transmembrane signaling	Total	Neural crest cell misguidance (cranial and cardiac, but not trunk)	Davy et al., 2004
Angiomotin	Transmembrane signaling	Total	Death between E11–E11.5, severe vascular insufficiency in intersomitic regions, dilated vessels in the brain	Aase et al., 2007

vation (Tucker, 2004; Heanue and Pachnis, 2007; Amiel et al., 2008). Waardenburg's syndrome, characterized by abnormal pigmentation, results from the impaired migration of neural crest cells that give rise to the melanocytes of the skin (Tachibana et al., 2003; Tucker, 2004). Other human syndromes, including Alagille, Carpenter, CHARGE, Ivemark, and Leopard/Noonan syndromes, result in abnormalities that suggest impairments of the neural crest migration, with known or unknown molecular reasons (Hutson and Kirby, 2003). Increasing molecular understanding of the embryonic migratory mechanisms provides means for the development of new therapies for these diseases in humans.

CONCLUSIONS

Cell migration is a highly conserved mechanism that is critical for normal embryonic development and the functioning of an adult organism. Despite the differences between cell types and line-

> **All cells migrate by similar mechanisms, whose perturbation leads to severe developmental defects.**

ages that undergo migration, all cells migrate by similar mechanisms, whose perturbation leads to severe developmental defects. These mechanisms involve intricate molecular machines that can sense the environment, respond to signals, and modulate the entire cell behavior. Molecular machines driving migration have a cytoskeleton-based "mechanical drive" and the regulatory molecules that provide direction and determine the migration speed.

A big question that has concerned the researchers for deca-

des relates to the coordination of cell migration in situ and its relation to the intracellular aspects of the cell migratory mechanisms. Indeed, all cells migrate by similar mechanisms, yet in a developing organism they migrate along highly specified paths with different speeds that can vary by orders of magnitude for different cell types (Table 1). Traditionally, this question has been addressed by researchers that considered the intra- and extracellular mechanisms driving migration in separate sets of studies. As more data

> **Molecular machines driving migration have a cytoskeleton-based "mechanical drive" and the regulatory molecules that provide direction and determine the migration speed.**

accumulate, researchers are now able to integrate all of the available information and consider the intracellular mechanisms of cell migration in the context of the developing organisms that contain additional levels of complexity provided by extracellular regulation. This synthesis of the existing and emerging data would eventually enable us to understand the complexity and the mystery of cell migration during development.

Note on References

Because the current review contains a very broad overview of several extensive fields, we have tried, where possible, to cite recent reviews on the appropriate subjects rather than original papers. We apologize to these authors whose very important articles were not included in our reference list due to space constraints.

Note on Nomenclature

In the two fields overviewed here, different conventions have been in place to define in vitro and in vivo studies. In cell biology, in vitro is usually related to the biochemical tests done in a cell-free system or with purified proteins, while in vivo defines studies of living cells in culture. In developmental biology, in vitro usually refers to cells and tissue explants in culture, and in vivo is used to describe studies in a living embryo. To side-step these conventions, we used "in culture" when referring to cultured cells and in situ when referring to cells in an embryo.

REFERENCES

Aase K, Ernkvist M, Ebarasi L, et al. 2007. Angiomotin regulates endothelial cell migration during embryonic angiogenesis. Genes Dev 21:2055–2068.

Adams JC. 2004a. Fascin protrusions in cell interactions. Trends Cardiovasc Med 14:221–226.

Adams JC. 2004b. Roles of fascin in cell adhesion and motility. Curr Opin Cell Biol 16:590–596.

Alva JA, Iruela-Arispe ML. 2004. Notch signaling in vascular morphogenesis. Curr Opin Hematol 11:278–283.

Amiel J, Sproat-Emison E, Garcia-Barcelo M, et al. 2008. Hirschsprung disease, associated syndromes and genetics: a review. J Med Genet 45: 1–14.

Anderson RB, Newgreen DF, Young HM. 2006a. Neural crest and the development of the enteric nervous system. Adv Exp Med Biol 589:181–196.

Anderson RB, Stewart AL, Young HM. 2006b. Phenotypes of neural-crest-derived cells in vagal and sacral pathways. Cell Tissue Res 323:11–25.

Arthur JS, Elce JS, Hegadorn C, Williams K, Greer PA. 2000. Disruption of the murine calpain small subunit gene, Capn4: calpain is essential for embryonic development but not for

cell growth and division. Mol Cell Biol 20:4474–4481.

Aszódi A, Pfeifer A, Ahmad M, et al. 1999. The vasodilator-stimulated phosphoprotein (VASP) is involved in cGMP- and cAMP-mediated inhibition of agonist-induced platelet aggregation, but is dispensable for smooth muscle function. EMBO J 18:37–48.

Audebert S, Desbruyeres E, Gruszczynski C, et al. 1993. Reversible polyglutamylation of α- and β-tubulin and microtubule dynamics in mouse brain neurons. Mol Biol Cell 4:615–626.

Bader BL, Rayburn H, Crowley D, Hynes RO. 1998. Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all αv integrins. Cell 95:507–519.

Bailly M, Macaluso F, Cammer M, et al. 1999. Relationship between Arp2/3 complex and the barbed ends of actin filaments at the leading edge of carcinoma cells after epidermal growth factor stimulation. J Cell Biol 145:331–345.

Beningo KA, Dembo M, Kaverina I, et al. 2001. Nascent focal adhesions are responsible for the generation of strong propulsive forces in migrating fibroblasts. J Cell Biol 153:881–888.

Beningo KA, Dembo M, Wang YL. 2004. Responses of fibroblasts to anchorage of dorsal extracellular matrix receptors. Proc Natl Acad Sci USA 101:18024–18029.

Bershadsky AD, Ballestrem C, Caramusa L, et al. 2006. Assembly and mechanosensory function of focal adhesions: experiments and models. Eur J Cell Biol 85:165–173.

Bhadriraju K, Yang M, Alom Ruiz S, et al. 2007. Activation of ROCK by RhoA is regulated by cell adhesion, shape, and cytoskeletal tension. Exp Cell Res 313:3616–3623.

Breau MA, Pietri T, Eder O, et al. 2006. Lack of $\beta 1$ integrins in enteric neural crest cells leads to a Hirschsprunglike phenotype. Development 133:1725–1734.

Bronner-Fraser M, Stern CD, Fraser S. 1991. Analysis of neural crest cell lineage and migration. J Craniofac Genet Dev Biol 11:214–222.

Brown CB, Baldwin HS. 2006. Neural crest contribution to the cardiovascular system. Adv Exp Med Biol 589:134–154.

Bulinski JC. 2007. Microtubule modification: acetylation speeds anterograde traffic flow. Curr Biol 17:R18–R20.

Carmeliet P, Lampugnani MG, Moons L, et al. 1999. Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. Cell 98:147–157.

Cattelino A, Liebner S, Gallini R, et al. 2003. The conditional inactivation of the β-catenin gene in endothelial cells causes a defective vascular pattern and increased vascular fragility. J Cell Biol 162:1111–1122.

Chen WT. 1979. Induction of spreading during fibroblast movement. J Cell Biol 81:684–691.

Chi N, Epstein JA. 2002. Getting your Pax straight: Pax proteins in development and disease. Trends Genet 18:41–47.

Chrzanowska-Wodnicka M, Kraus AE, Gale D, et al. 2008. Defective angiogenesis, endothelial migration, proliferation, and MAPK signaling in Rap1b-deficient mice. Blood 111:2647–2656.

Conti MA, Even-Ram S, Liu C, et al. 2004. Defects in cell adhesion and the visceral endoderm following ablation of nonmuscle myosin heavy chain II-A in mice. J Biol Chem 279:41263–41266.

Costell M, Gustafsson E, Aszodi A, et al. 1999. Perlecan maintains the integrity of cartilage and some basement membranes. J Cell Biol 147:1109–1122.

Crawford K, Flick R, Close L, et al. 2002. Mice lacking skeletal muscle actin show reduced muscle strength and growth deficits and die during the neonatal period. Mol Cell Biol 22:5887–5896.

da Silva JS, Dotti CG. 2002. Breaking the neuronal sphere: regulation of the actin cytoskeleton in neuritogenesis. Nat Rev Neurosci 3:694–704.

Dahme M, Bartsch U, Martini R, et al. 1997. Disruption of the mouse L1 gene leads to malformations of the nervous system. Nat Genet 17:346–349.

Davy A, Aubin J, Soriano P. 2004. Ephrin-B1 forward and reverse signaling are required during mouse development. Genes Dev 18:572–583.

Delon I, Brown NH. 2007. Integrins and the actin cytoskeleton. Curr Opin Cell Biol 19:43–50.

Dent EW, Kwiatkowski AV, Mebane LM, et al. 2007. Filopodia are required for cortical neurite initiation. Nat Cell Biol 9:1347–1359.

Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP. 1998. Pten is essential for embryonic development and tumour suppression. Nat Genet 19:348–355.

Dimaio TA, Wang S, Huang Q, et al. 2008. Attenuation of retinal vascular development and neovascularization in PECAM-1-deficient mice. Dev Biol 315:72–88.

Dobereiner HG, Dubin-Thaler BJ, Giannone G, Sheetz MP. 2005. Force sensing and generation in cell phases: analyses of complex functions. J Appl Physiol 98:1542–1546.

Druckenbrod NR, Epstein ML. 2005. The pattern of neural crest advance in the cecum and colon. Dev Biol 287:125–133.

Druckenbrod NR, Epstein ML. 2007. Behavior of enteric neural crest-derived cells varies with respect to the migratory wavefront. Dev Dyn 236:84–92.

Dunn GA, Zicha D. 1995. Dynamics of fibroblast spreading. J Cell Sci 108(Pt 3):1239–1249.

Durbec PL, Larsson-Blomberg LB, Schuchardt A, et al. 1996. Common origin and developmental dependence on c-ret of subsets of enteric and sympathetic neuroblasts. Development 122:349–358.

Echtermeyer F, Streit M, Wilcox-Adelman S, et al. 2001. Delayed wound repair and impaired angiogenesis in mice lacking syndecan-4. J Clin Invest 107:R9–R14.

Edde B, Rossier J, Le Caer JP, et al. 1990. Posttranslational glutamylation of α-tubulin. Science 247:83–85.

Eklund L, Piuhola J, Komulainen J, et al. 2001. Lack of type XV collagen causes a skeletal myopathy and cardiovascular defects in mice. Proc Natl Acad Sci USA 98:1194–1199.

Engler AJ, Sen S, Sweeney HL, Discher DE. 2006. Matrix elasticity directs stem cell lineage specification. Cell 126:677–689.

Epstein JA. 2001. Developing models of DiGeorge syndrome. Trends Genet 17:S13–S17.

Epstein JA, Parmacek MS. 2005. Recent advances in cardiac development with therapeutic implications for adult cardiovascular disease. Circulation 112:592–597.

Erck C, Peris L, Andrieux A, et al. 2005. A vital role of tubulin-tyrosine-ligase for neuronal organization. Proc Natl Acad Sci USA 102:7853–7858.

Etienne-Manneville S, Hall A. 2001. Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. Cell 106:489–498.

Euteneuer U, Schliwa M. 1984. Persistent, directional motility of cells and cytoplasmic fragments in the absence of microtubules. Nature 310:58–61.

Even-Ram S, Doyle AD, Conti MA, et al. 2007. Myosin IIA regulates cell motility and actomyosin-microtubule crosstalk. Nat Cell Biol 9:299–309.

Even-Ram S, Yamada KM. 2005. Cell migration in 3D matrix. Curr Opin Cell Biol 17:524–532.

Faix J, Rottner K. 2006. The making of filopodia. Curr Opin Cell Biol 18:18–25.

Fassler R, Meyer M. 1995. Consequences of lack of $\beta 1$ integrin gene expression in mice. Genes Dev 9:1896–1908.

Flintoff-Dye NL, Welser J, Rooney J, et al. 2005. Role for the $\alpha 7\beta 1$ integrin in vascular development and integrity. Dev Dyn 234:11–21.

Franco SJ, Huttenlocher A. 2005. Regulating cell migration: calpains make the cut. J Cell Sci 118(Pt. 17):3829–3838.

Franco SJ, Rodgers MA, Perrin BJ, et al. 2004. Calpain-mediated prote-

olysis of talin regulates adhesion dynamics. Nat Cell Biol 6:977–983.

Fraser SE. 1991. Pattern formation in the vertebrate nervous system. Curr Opin Genet Dev 1:217–220.

Freeman B. 2003. The active migration of germ cells in the embryos of mice and men is a myth. Reproduction 125:635–643.

Fukai N, Eklund L, Marneros AG, et al. 2002. Lack of collagen XVIII/endostatin results in eye abnormalities. EMBO J 21:1535–1544.

Furuta Y, Ilic D, Kanazawa S, et al. 1995. Mesodermal defect in late phase of gastrulation by a targeted mutation of focal adhesion kinase, FAK. Oncogene 11:1989–1995.

Galbraith CG, Yamada KM, Sheetz MP. 2002. The relationship between force and focal complex development. J Cell Biol 159:695–705.

Geiger B, Bershadsky A. 2001. Assembly and mechanosensory function of focal contacts. Curr Opin Cell Biol 13:584–592.

George EL, Georges-Labouesse EN, Patel-King RS, et al. 1993. Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. Development 119:1079–1091.

Georges PC, Janmey PA. 2005. Cell type-specific response to growth on soft materials. J Appl Physiol 98:1547–1553.

Gerthoffer WT. 2007. Mechanisms of vascular smooth muscle cell migration. Circ Res 100:607–621.

Giannone G, Dubin-Thaler BJ, Rossier O, et al. 2007. Lamellipodial actin mechanically links myosin activity with adhesion-site formation. Cell 128:561–575.

Gilbert SF. 2000. Developmental biology. Sunderland, MA: Sinauder Associates.

Gilbert SF. 2006. Developmental biology. 8th ed. Sunderland, MA: Sinauder Associates.

Gitler AD, Brown CB, Kochilas L, et al. 2002. Neural crest migration and mouse models of congenital heart disease. Cold Spring Harb Symp Quant Biol 67:57–62.

Goh KL, Cai L, Cepko CL, Gertler FB. 2002. Ena/VASP proteins regulate cortical neuronal positioning. Curr Biol 12:565–569.

Goley ED, Welch MD. 2006. The ARP2/3 complex: an actin nucleator comes of age. Nat Rev Mol Cell Biol 7:713–726.

Goode BL, Eck MJ. 2007. Mechanism and function of formins in the control of actin assembly. Annu Rev Biochem 76:593–627.

Gotlieb AI, May LM, Subrahmanyan L, Kalnins VI. 1981. Distribution of microtubule organizing centers in migrating sheets of endothelial cells. J Cell Biol 91(2 Pt. 1):589–594.

Gruber PJ, Epstein JA. 2004. Development gone awry: congenital heart disease. Circ Res 94:273–283.

Gundersen GG, Bulinski JC. 1986. Microtubule arrays in differentiated cells contain elevated levels of a post-translationally modified form of tubulin. Eur J Cell Biol 42:288–294.

Gundersen GG, Bulinski JC. 1988. Selective stabilization of microtubules oriented toward the direction of cell migration. Proc Natl Acad Sci USA 85:5946–5950.

Gunzer M, Schafer A, Borgmann S, et al. 2000. Antigen presentation in extracellular matrix: interactions of T cells with dendritic cells are dynamic, short lived, and sequential. Immunity 13:323–332.

Gupton SL, Anderson KL, Kole TP, et al. 2005. Cell migration without a lamellipodium: translation of actin dynamics into cell movement mediated by tropomyosin. J Cell Biol 168:619–631.

Gupton SL, Gertler FB. 2007. Filopodia: the fingers that do the walking. Sci STKE 2007:re5.

Gupton SL, Waterman-Storer CM. 2006. Spatiotemporal feedback between actomyosin and focal-adhesion systems optimizes rapid cell migration. Cell 125:1361–1374.

Gurniak CB, Perlas E, Witke W. 2005. The actin depolymerizing factor n-cofilin is essential for neural tube morphogenesis and neural crest cell migration. Dev Biol 278:231–241.

Gurtner GC, Davis V, Li H, et al. 1995. Targeted disruption of the murine VCAM1 gene: essential role of VCAM-1 in chorioallantoic fusion and placentation. Genes Dev 9:1–14.

Hagel M, George EL, Kim A, et al. 2002. The adaptor protein paxillin is essential for normal development in the mouse and is a critical transducer of fibronectin signaling. Mol Cell Biol 22:901–915.

Hauser W, Knobeloch K-P, Eigenthaler M, et al. 1999. Megakaryocyte hyperplasia and enhanced agonist-induced platelet activation in vasodilator-stimulated phosphoprotein knockout mice. Proc Natl Acad Sci 96:8120–8125.

Heanue TA, Pachnis V. 2007. Enteric nervous system development and Hirschsprung's disease: advances in genetic and stem cell studies. Nat Rev Neurosci 8:466–479.

Hennessey ES, Drummond DR, Sparrow JC. 1991. Post-translational processing of the amino terminus affects actin function. Eur J Biochem 197:345–352.

Henson JH, Svitkina TM, Burns AR, et al. 1999. Two components of actin-based retrograde flow in sea urchin coelomocytes. Mol Biol Cell 10:4075–4090.

Hienola A, Tumova S, Kulesskiy E, Rauvala H. 2006. N-Syndecan deficiency impairs neural migration in brain. J Cell Biol 174:569–580.

High FA, Epstein JA. 2008. The multi-faceted role of Notch in cardiac development and disease. Nat Rev Genet 9:49–61.

Hodivala-Dilke K, McHugh KP, Tsakiris DA, et al. 1999. β3-Integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. J Clin Invest 103:229–238.

Hoffman LM, Nix DA, Benson B, et al. 2003. Targeted disruption of the murine zyxin gene. Mol Cell Biol 23:70–79.

Hofmann JJ, Iruela-Arispe ML. 2007. Notch signaling in blood vessels: who is talking to whom about what? Circ Res 100:1556–1568.

Hook J, Lemckert F, Qin H, et al. 2004. γ-Tropomyosin gene products are required for embryonic development. Mol Cell Biol 24:2318–2323.

Hutson MR, Kirby ML. 2003. Neural crest and cardiovascular development: a 20-year perspective. Birth Defects Res C Embryo Today 69:2–13.

Hutson MR, Kirby ML. 2007. Model systems for the study of heart development and disease. Cardiac neural crest and conotruncal malformations. Semin Cell Dev Biol 18:101–110.

Ikegami K, Heier RL, Taruishi M, et al. 2007. Loss of α-tubulin polyglutamylation in ROSA22 mice is associated with abnormal targeting of KIF1A and modulated synaptic function. Proc Natl Acad Sci USA 104:3213–3218.

Ilic D, Kovacic B, McDonagh S, et al. 2003. Focal adhesion kinase is required for blood vessel morphogenesis. Circ Res 92:300–307.

Imamoto A, Soriano P. 1993. Disruption of the csk gene, encoding a negative regulator of Src family tyrosine kinases, leads to neural tube defects and embryonic lethality in mice. Cell 73:1117–1124.

Infante AS, Stein MS, Zhai Y, et al. 2000. Detyrosinated (Glu) microtubules are stabilized by an ATP-sensitive plus-end cap. J Cell Sci 113(Pt. 22):3907–3919.

Ishiguro K, Kadomatsu K, Kojima T, et al. 2000. Syndecan-4 deficiency impairs the fetal vessels in the placental labyrinth. Dev Dynam 219:539–544.

Ishii A, Lo SH. 2001. A role of tensin in skeletal-muscle regeneration. Biochem J 356:737–745.

Itoh RE, Kurokawa K, Ohba Y, et al. 2002. Activation of rac and cdc42 video imaged by fluorescent resonance energy transfer-based single-molecule probes in the membrane of living cells. Mol Cell Biol 22:6582–6591.

Iwasaki T, Wang YL. 2008. Cytoplasmic force gradient in migrating adhesive cells. Biophys J 94:L35–L37.

Jiang X, Rowitch DH, Soriano P, et al. 2000. Fate of the mammalian cardiac neural crest. Development 127:1607–1616.

Jones W, Grupp IL, Doetschman T, et al. 1996. Ablation of the murine α

myosin heavy chain gene leads to dosage effects and functional deficits in the heart. J Clin Invest 98:1906–1917.

Jou ST, Carpino N, Takahashi Y, et al. 2002. Essential, nonredundant role for the phosphoinositide 3-kinase p110delta in signaling by the B-cell receptor complex. Mol Cell Biol 22:8580–8591.

Kapur RP. 2000. Colonization of the murine hindgut by sacral crest-derived neural precursors: experimental support for an evolutionarily conserved model. Dev Biol 227:146–155.

Karakozova M, Kozak M, Wong CC, et al. 2006. Arginylation of β-actin regulates actin cytoskeleton and cell motility. Science 313:192–196.

Katso R, Okkenhaug K, Ahmadi K, et al. 2001. Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. Annu Rev Cell Dev Biol 17:615–675.

Kaufman MH. 1992. The atlas of mouse development. San Diego, CA: Academic Press.

Kaverina I, Krylyshkina O, Small JV. 1999. Microtubule targeting of substrate contacts promotes their relaxation and dissociation. J Cell Biol 146:1033–1044.

Kedrin D, van Rheenen J, Hernandez L, et al. 2007. Cell motility and cytoskeletal regulation in invasion and metastasis. J Mammary Gland Biol Neoplasia 12:143–152.

Khawaja S, Gundersen GG, Bulinski JC. 1988. Enhanced stability of microtubules enriched in detyrosinated tubulin is not a direct function of detyrosination level. J Cell Biol 106:141–149.

Knight B, Laukaitis C, Akhtar N, et al. 2000. Visualizing muscle cell migration in situ. Curr Biol 10:576–585.

Kojima T. 2002. Targeted gene disruption of natural anticoagulant proteins in mice. Int J Hematol 76(Suppl):36–39.

Kondo S, Scheef EA, Sheibani N, Sorenson CM. 2007. PECAM-1 isoform-specific regulation of kidney endothelial cell migration and capillary morphogenesis. Am J Physiol Cell Physiol 292:C2070–C2083.

Krause M, Leslie JD, Stewart M, et al. 2004. Lamellipodin, an Ena/VASP ligand, is implicated in the regulation of lamellipodial dynamics. Dev Cell 7:571–583.

Kulesa P, Bronner-Fraser M, Fraser S. 2000. In vivo time-lapse analysis after dorsal neural tube ablation shows rerouting of chick hindbrain neural crest. Development 127:2843–2852.

Kumar A, Crawford K, Close L, et al. 1997. Rescue of cardiac α-actin-deficient mice by enteric smooth muscle γ-actin. Proc Natl Acad Sci USA 94:4406–4411.

Kupfer A, Dennert G, Singer SJ. 1983. Polarization of the Golgi apparatus and the microtubule-organizing center within cloned natural killer cells bound to their targets. Proc Natl Acad Sci USA 80:7224–7228.

Kupfer A, Louvard D, Singer SJ. 1982. Polarization of the Golgi apparatus and the microtubule-organizing center in cultured fibroblasts at the edge of an experimental wound. Proc Natl Acad Sci USA 79:2603–2607.

Kwiatkowski AV, Rubinson DA, Dent EW, et al. 2007. Ena/VASP is required for neuritogenesis in the developing cortex. Neuron 56:441–455.

Kwon YT, Kashina AS, Davydov IV, et al. 2002. An essential role of N-terminal arginylation in cardiovascular development. Science 297:96–99.

Lafuente EM, van Puijenbroek AA, Krause M, et al. 2004. RIAM, an Ena/VASP and Profilin ligand, interacts with Rap1-GTP and mediates Rap1-induced adhesion. Dev Cell 7:585–595.

Lanier LM, Gates MA, Witke W, et al. 1999. Mena is required for neurulation and commissure formation. Neuron 22:313–325.

Larue L, Ohsugi M, Hirchenhain J, Kemler R. 1994. E-cadherin null mutant embryos fail to form a trophectoderm epithelium. Proc Natl Acad Sci USA 91:8263–8267.

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

Le Douarin NM. 1983. The neural crest (Developmental and Cell Biology Series). Cambridge: Cambridge University Press.

Lei L, Liu D, Huang Y, et al. 2008. Endothelial expression of 1 integrin is required for embryonic vascular patterning and postnatal vascular remodeling. Mol Cell Biol 28:794–802.

Lo SH, Yu Q-C, Degenstein L, et al. 1997. Progressive kidney degeneration in mice lacking tensin. J Cell Biol 136:1349–1361.

Locascio A, Nieto MA. 2001. Cell movements during vertebrate development: integrated tissue behaviour versus individual cell migration. Curr Opin Genet Dev 11:464–469.

Lock JG, Wehrle-Haller B, Stromblad S. 2008. Cell-matrix adhesion complexes: master control machinery of cell migration. Semin Cancer Biol 18:65–76.

Luduena RF. 1998. Multiple forms of tubulin: different gene products and covalent modifications. Int Rev Cytol 178:207–275.

Luo Y, High FA, Epstein JA, Radice GL. 2006. N-Cadherin is required for neural crest remodeling of the cardiac outflow tract. Dev Biol 299:517–528.

Luo Y, Radice GL. 2005. N-Cadherin acts upstream of VE-cadherin in controlling vascular morphogenesis. J Cell Biol 169:29–34.

Machacek M, Danuser G. 2006. Morphodynamic profiling of protrusion phenotypes. Biophys J 90:1439–1452.

Malikov V, Cytrynbaum EN, Kashina A, et al. 2005. Centering of a radial microtubule array by translocation along microtubules spontaneously nucleated in the cytoplasm. Nat Cell Biol 7:1213–1218.

Mayer U, Saher G, Fassler R, et al. 1997. Absence of integrin α7 causes a novel form of muscular dystrophy. Nat Genet 17:318–323.

Medeiros NA, Burnette DT, Forscher P. 2006. Myosin II functions in actin-bundle turnover in neuronal growth cones. Nat Cell Biol 8:215–226.

Miner JH, Cunningham J, Sanes JR. 1998. Roles for laminin in embryogenesis: exencephaly, syndactyly, and placentopathy in mice lacking the laminin α5 chain. J Cell Biol 143:1713–1723.

Miner JH, Li C. 2000. Defective glomerulogenesis in the absence of laminin α5 demonstrates a developmental role for the kidney glomerular basement membrane. Dev Biol 217:278–289.

Miyagoe Y, Hanaoka K, Nonaka I, et al. 1997. Laminin α2 chain-null mutant mice by targeted disruption of the Lama2 gene: a new model of merosin (laminin 2)-deficient congenital muscular dystrophy. FEBS Lett 415:33–39.

Mogilner A, Oster G. 1996. Cell motility driven by actin polymerization. Biophys J 71:3030–3045.

Molyneaux K, Wylie C. 2004. Primordial germ cell migration. Int J Dev Biol 48:537–544.

Molyneaux KA, Stallock J, Schaible K, Wylie C. 2001. Time-lapse analysis of living mouse germ cell migration. Dev Biol 240:488–498.

Mongiu AK, Weitzke EL, Chaga OY, Borisy GG. 2007. Kinetic-structural analysis of neuronal growth cone veil motility. J Cell Sci 120(Pt. 6):1113–1125.

Monkley SJ, Zhou X-H, Kinston SJ, et al. 2000. Disruption of the talin gene arrests mouse development at the gastrulation stage. Dev Dynam 219:560–574.

Mu H, Ohashi R, Lin P, et al. 2005. Cellular and molecular mechanisms of coronary vessel development. Vasc Med 10:37–44.

Mullins RD, Pollard TD. 1999. Structure and function of the Arp2/3 complex. Curr Opin Struct Biol 9:244–249.

Nada S, Yagi T, Takeda H, et al. 1993. Constitutive activation of Src family kinases in mouse embryos that lack Csk. Cell 73:1125–1135.

Nagy A, Gertsenstein M, Vintersten K, Behringer RR. 2003. Manipulating the mouse embryo: a laboratory manual, 3rd ed. Cold Sprong Harbor, NY: Cold Sprong Harbor Laboratory Press.

Noakes PG, Gautam M, Mudd J, et al. 1995a. Aberrant differentiation of neuromuscular junctions in mice lacking s-laminin/laminin β2. Nature 374:258–262.

Noakes PG, Miner JH, Gautam M, et al. 1995b. The renal glomerulus of mice lacking s-laminin/laminin β2: nephrosis despite molecular compensation by laminin β1. Nat Genet 10:400–406.

Ohba Y, Ikuta K, Ogura A, et al. 2001. Requirement for C3G-dependent Rap1 activation for cell adhesion and embryogenesis. EMBO J 20:3333–3341.

Pak CW, Flynn KC, Bamburg JR. 2008. Actin-binding proteins take the reins in growth cones. 9:136–147.

Patan S. 2000. Vasculogenesis and angiogenesis as mechanisms of vascular network formation, growth and remodeling. J Neurooncol 50:1–15.

Patton BL, Connoll AM, Martin PT, et al. 1999. Distribution of ten laminin chains in dystrophic and regenerating muscles. Neuromusc Disord 9:423–433.

Patton BL, Cunningham JM, Thyboll J, et al. 2001. Properly formed but improperly localized synaptic specializations in the absence of laminin α4. Nat Neurosci 4:597–604.

Patton BL, Miner JH, Chiu AY, Sanes JR. 1997. Distribution and function of laminins in the neuromuscular system of developing, adult, and mutant mice. J Cell Biol 139:1507–1521.

Plessmann U, Weber K. 1997. Mammalian sperm tubulin: an exceptionally large number of variants based on several posttranslational modifications. J Protein Chem 16:385–390.

Pollard TD. 2007. Regulation of actin filament assembly by Arp2/3 complex and formins. Annu Rev Biophys Biomol Struct 36:451–477.

Pollard TD, Borisy GG. 2003. Cellular motility driven by assembly and disassembly of actin filaments. Cell 112:453–465.

Ponti A, Machacek M, Gupton SL, et al. 2004. Two distinct actin networks drive the protrusion of migrating cells. Science 305:1782–1786.

Pruyne D, Evangelista M, Yang C, et al. 2002. Role of formins in actin assembly: nucleation and barbed-end association. Science 297:612–615.

Radice GL, Rayburn H, Matsunami H, et al. 1997. Developmental defects in mouse embryos lacking N-cadherin. Dev Biol 181:64–78.

Raybin D, Flavin M. 1977a. Enzyme which specifically adds tyrosine to the α chain of tubulin. Biochemistry 16:2189–2194.

Raybin D, Flavin M. 1977b. Modification of tubulin by tyrosylation in cells and extracts and its effect on assembly in vitro. J Cell Biol 73:492–504.

Reaume AG, de Sousa PA, Kulkarni S, et al. 1995. Cardiac malformation in neonatal mice lacking connexin43. Science 267:1831–1834.

Redeker V, Levilliers N, Vinolo E, et al. 2005. Mutations of tubulin glycylation sites reveal cross-talk between the C termini of α- and β-tubulin and affect the ciliary matrix in tetrahymena. J Biol Chem 280:596–606.

Redman KL, Rubenstein PA. 1984. Actin amino-terminal acetylation and processing in a rabbit reticulocyte lysate. Methods Enzymol 106:179–192.

Reed NA, Cai D, Blasius TL, et al. 2006. Microtubule acetylation promotes kinesin-1 binding and transport. Curr Biol 16:2166–2172.

Rehfeldt F, Engler AJ, Eckhardt A, et al. 2007. Cell responses to the mechanochemical microenvironment—implications for regenerative medicine and drug delivery. Adv Drug Deliv Rev 59:1329–1339.

Ridley AJ, Schwartz MA, Burridge K, et al. 2003. Cell migration: integrating signals from front to back. Science 302:1704–1709.

Riveline D, Zamir E, Balaban NQ, et al. 2001. Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. J Cell Biol 153:1175–1186.

Rodionov VI, Borisy GG. 1997. Self-centring activity of cytoplasm. Nature 386:170–173.

Rodionov VI, Borisy GG. 1998. Self-centering in cytoplasmic fragments of melanophores. Mol Biol Cell 9:1613–1615.

Rooney JE, Welser JV, Dechert MA, et al. 2006. Severe muscular dystrophy in mice that lack dystrophin and α7 integrin. J Cell Sci 119(Pt. 11):2185–2195.

Rottner K, Hall A, Small JV. 1999. Interplay between Rac and Rho in the control of substrate contact dynamics. Curr Biol 9:640–648.

Rubenstein PA, Martin DJ. 1983. NH$_2$-terminal processing of actin in mouse L-cells in vivo. J Biol Chem 258:3961–3966.

Ryan MC, Lee K, Miyashita Y, Carter WG. 1999. Targeted disruption of the LAMA3 gene in mice reveals abnormalities in survival and late stage differentiation of epithelial cells. J Cell Biol 145:1309–1323.

Saint-Jeannet J-P, editor. 2006. Neural crest induction and differentiation. New York, NY: Springer Science + Business Media, LLC.

Schildmeyer LA, Braun R, Taffet G, et al. 2000. Impaired vascular contractility and blood pressure homeostasis in the smooth muscle α-actin null mouse. FASEB J 14:2213–2220.

Schoenwaelder SM, Burridge K. 1999. Bidirectional signaling between the cytoskeleton and integrins. Curr Opin Cell Biol 11:274–286.

Serbedzija GN, Bronner-Fraser M, Fraser SE. 1992. Vital dye analysis of cranial neural crest cell migration in the mouse embryo. Development 116:297–307.

Shawlot W, Deng JM, Fohn LE, Behringer RR. 1998. Restricted β-galactosidase expression of a hygromycin-lacZ gene targeted to the β-actin locus and embryonic lethality of β-actin mutant mice. Transgenic Res 7:95–103.

Sheterline P, Clayton JC. SJ. 1998. Actin.4th ed. New York, NY: Oxford University Press.

Skaper SD. 2005. Neuronal growth-promoting and inhibitory cues in neuro-protection and neuroregeneration. Ann NY Acad Sci 1053:376–385.

Small JV, Kaverina I. 2003. Microtubules meet substrate adhesions to arrange cell polarity. Curr Opin Cell Biol 15:40–47.

Smyth N, Vatansever HS, Murray P, et al. 1999. Absence of basement membranes after targeting the LAMC1 gene results in embryonic lethality due to failure of endoderm differentiation. J Cell Biol 144:151–160.

Sonnemann KJ, Fitzsimons DP, Patel JR, et al. 2006. Cytoplasmic γ-actin is not required for skeletal muscle development but its absence leads to a progressive myopathy. Dev Cell 11:387–397.

Stambolic V, Tsao MS, Macpherson D, et al. 2000. High incidence of breast and endometrial neoplasia resembling human Cowden syndrome in pten+/− mice. Cancer Res 60:3605–3611.

Stenvers KL, Tursky ML, Harder KW, et al. 2003. Heart and liver defects and reduced transforming growth factor β2 sensitivity in transforming growth factor β type III receptor-deficient embryos. Mol Cell Biol 23:4371–4385.

Stoller JZ, Epstein JA. 2005. Cardiac neural crest. Semin Cell Dev Biol 16:704–715.

Suzuki A, Itami S, Ohishi M, et al. 2003. Keratinocyte-specific Pten deficiency results in epidermal hyperplasia, accelerated hair follicle morphogenesis and tumor formation. Cancer Res 63:674–681.

Svitkina TM, Borisy GG. 1999. Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia. J Cell Biol 145:1009–1026.

Tachibana M, Kobayashi Y, Matsushima Y. 2003. Mouse models for four types of Waardenburg syndrome. Pigment Cell Res 16:448–454.

Takada Y, Ye X, Simon S. 2007. The integrins. Genome Biol 8:215.

Tanjore H, Zeisberg EM, Gerami-Naini B, Kalluri R. 2008. β1-Integrin expression on endothelial cells is required for angiogenesis but not for vasculogenesis. Dev Dynam 237:75–82.

Thumkeo D, Keel J, Ishizaki T, et al. 2003. Targeted disruption of the mouse rho-associated kinase 2 gene results in intrauterine growth retardation and fetal death. Mol Cell Biol 23:5043–5055.

Thyboll J, Kortesmaa J, Cao R, et al. 2002. Deletion of the laminin α4 chain leads to impaired microvessel maturation. Mol Cell Biol 22:1194–1202.

Trainor PA. 2005. Specification of neural crest cell formation and migration in mouse embryos. Semin Cell Dev Biol 16:683–693.

Trelstad RL, editor. 1984. Role of extracellular matrix in development (society for developmental biology symposium (proceedings). New York: Wiley.

Tucker RP. 2001. Abnormal neural crest cell migration after the in vivo knockdown of tenascin-C expression with morpholino antisense oligonucleotides. Dev Dyn 222:115–119.

Tucker RP. 2004. Neural crest cells: a model for invasive behavior. Int J Biochem Cell Biol 36:173–177.

Tullio AN, Accili D, Ferrans VJ, et al. 1997. Nonmuscle myosin II-B is required for normal development of the mouse heart. Proc Natl Acad Sci USA 94:12407–12412.

Utriainen A, Sormunen R, Kettunen M, et al. 2004. Structurally altered basement membranes and hydrocephalus in a type XVIII collagen deficient mouse line. Hum Mol Genet 13:2089–2099.

Vallotton P, Gupton SL, Waterman-Storer CM, Danuser G. 2004. Simultaneous mapping of filamentous actin flow and turnover in migrating cells by quantitative fluorescent speckle microscopy. Proc Natl Acad Sci USA 9:9660–9665.

van Hinsbergh VW, Koolwijk P. 2008. Endothelial sprouting and angiogenesis: matrix metalloproteinases in the lead. Cardiovasc Res 78:203–212.

Verkhovsky AB, Svitkina TM, Borisy GG. 1999. Self-polarization and directional motility of cytoplasm. Curr Biol 9:11–20.

Vicente-Manzanares M, Webb DJ, Horwitz AR. 2005. Cell migration at a glance. J Cell Sci 118(Pt. 21):4917–4919.

Vogel V, Sheetz M. 2006. Local force and geometry sensing regulate cell functions. Nat Rev Mol Cell Biol 7:265–275.

Vorobjev I, Malikov V, Rodionov V. 2001. Self-organization of a radial microtubule array by dynein-dependent nucleation of microtubules. Proc Natl Acad Sci USA 98:10160–10165.

Voss AK, Gruss P, Thomas T. 2003. The guanine nucleotide exchange factor C3G is necessary for the formation of focal adhesions and vascular maturation. Development 130: 355–367.

Watanabe N, Mitchison TJ. 2002. Single-molecule speckle analysis of actin filament turnover in lamellipodia. Science 295:1083–1086.

Webb DJ, Parsons JT, Horwitz AF. 2002. Adhesion assembly, disassembly and turnover in migrating cells— over and over and over again. Nat Cell Biol 4:E97–E100.

Welch MD, DePace AH, Verma S, et al. 1997. The human Arp2/3 complex is composed of evolutionarily conserved subunits and is localized to cellular regions of dynamic actin filament assembly. J Cell Biol 138:375–384.

Welch MD, Mullins RD. 2002. Cellular control of actin nucleation. Annu Rev Cell Dev Biol 18:247–288.

Welser JV, Lange ND, Flintoff-Dye N, et al. 2007. Placental defects in α7 integrin null mice. Placenta 28:1219–1228.

Williams RW, Herrup K. 1988. The control of neuron number. Annu Rev Neurosci 11:423–453.

Witke W, Sharpe AH, Hartwig JH, et al. 1995. Hemostatic, inflammatory, and fibroblast responses are blunted in mice lacking gelsolin. Cell 81:41–51.

Witke W, Sutherland JD, Sharpe A, et al. 2001. Profilin I is essential for cell survival and cell division in early mouse development. Proc Natl Acad Sci USA 98:3832–3836.

Wong CC, Xu T, Rai R, et al. 2007. Global analysis of posttranslational protein arginylation. PLoS Biol 5: e258.

Xu W, Baribault H, Adamson ED. 1998. Vinculin knockout results in heart and brain defects during embryonic development. Development 125:327–337.

Xu X, Francis R, Wei CJ, Linask KL, Lo CW. 2006. Connexin 43-mediated modulation of polarized cell movement and the directional migration of cardiac neural crest cells. Development 133:3629–3639.

Yam PT, Wilson CA, Ji L, et al. 2007. Actin myosin network reorganization breaks symmetry at the cell rear to spontaneously initiate polarized cell motility. J Cell Biol 178:1207–1221.

Yamaguchi H, Condeelis J. 2007. Regulation of the actin cytoskeleton in cancer cell migration and invasion. Biochim Biophys Acta 1773: 642–652.

Yamaguchi H, Wyckoff J, Condeelis J. 2005. Cell migration in tumors. Curr Opin Cell Biol 17:559–564.

Yang C, Czech L, Gerboth S, et al. 2007. Novel roles of formin mDia2 in lamellipodia and filopodia formation in motile cells. PLoS Biol 5:e317.

Yang J, Rayburn H, Hynes R. 1993. Embryonic mesodermal defects in α5 integrin-deficient mice. Development 119:1093–1105.

Yang J, Rayburn H, Hynes R. 1995. Cell adhesion events mediated by α4 integrins are essential in placental and cardiac development. Development 121:549–560.

Young HM, Newgreen D. 2001. Enteric neural crest-derived cells: origin, identification, migration, and differentiation. Anat Rec 262:1–15.

Zhang XF, Schaefer AW, Burnette DT, et al. 2003. Rho-dependent contractile responses in the neuronal growth cone are independent of classical peripheral retrograde actin flow. Neuron 40:931–944.

Zhou X, Tian F, Sandzen J, et al. 2007. Filamin B deficiency in mice results in skeletal malformations and impaired microvascular development. Proc Natl Acad Sci USA 104:3919–3924.

Zhu J, Motejlek K, Wang D, et al. 2002. {beta}8 integrins are required for vascular morphogenesis in mouse embryos. Development 129:2891–2903.

Zigmond SH. 2004. Formin-induced nucleation of actin filaments. Curr Opin Cell Biol 16:99–105.

Zigmond SH, Joyce M, Yang C, et al. 1998. Mechanism of Cdc42-induced actin polymerization in neutrophil extracts. J Cell Biol 142:1001–1012.