References

1. Theriot, J.A. (2000). The polymerization motor. Traffic *1*, 19–28.
2. Smith, G.A., Portnoy, D.A., and Theriot, J.A. (1995). Asymmetric distribution of the Listeria monocytogenes ActA protein is required and sufficient to direct actin-based motility. Mol. Microbiol. *17*, 945–951.
3. Kocks, C., Marchand, J.B., Gouin, E., d'Hauteville, H., Sansonetti, P.J., Carlier, M.F., and Cossart, P. (1995). The unrelated surface proteins ActA of Listeria monocytogenes and IcsA of Shigella flexneri are sufficient to confer actin-based motility on Listeria innocua and Escherichia coli respectively. Mol. Microbiol. *18*, 413–423.
4. Cameron, L.A., Footer, M.J., van Oudenaarden, A., and Theriot, J.A. (1999). Motility of ActA protein-coated microspheres driven by actin polymerization. Proc. Natl. Acad. Sci. USA *96*, 4908–4913.
5. Peskin, C.S., Odell, G.M., and Oster, G.F. (1993). Cellular motions and thermal fluctuations: the Brownian ratchet. Biophys. J. *65*, 316–324.
6. Mogilner, A., and Oster, G. (1996). Cell motility driven by actin polymerization. Biophys. J. *71*, 3030–3045.
7. Gerbal, F., Chaikin, P., Rabin, Y., and Prost, J. (2000). An elastic analysis of Listeria monocytogenes propulsion. Biophys. J. *79*, 2259–2275.
8. Gerbal, F., Laurent, V., Ott, A.,Carlier, M.F., Chaikin, P., and Prost, J. (2000). Measurement of the elasticity of the actin tail of Listeria monocytogenes. Eur. Biophys. J. *29*, 134–140.
9. Bernheim-Groswasser, A., Wiesner, S., Golsteyn, R.M., Carlier, M.F., and Sykes, C. (2002). The dynamics of actin-based motility depend on surface parameters. Nature *417*, 308–311.
10. Dickinson, R.B., and Purich, D.L. (2002). Clamped-filament elongation model for actin-based motors. Biophys. J. *82*, 605–617.
11. Mogilner, A., and Oster, G. (2003). Force generation by actin polymerization II: The elastic ratchet and tethered filaments. Biophys. J. *84*, 1591–1605.
12. Bernardini, M.L., Mounier, J., d'Hauteville, H., Coquis-Rondon, M., and Sansonetti, P.J. (1989). Identification of icsA, a plasmid locus of Shigella flexneri that governs bacterial intra- and intercellular spread through interaction with F-actin. Proc. Natl. Acad. Sci. USA *86*, 3867–3871.
13. Goldberg, M.B., and Theriot, J.A. (1995). Shigella flexneri surface protein IcsA is sufficient to direct actin- based motility. Proc. Natl. Acad. Sci. USA *92*, 6572–6576.
14. Cameron, L.A., Svitkina, T.M., Vignjevic, D., Theriot, J.A., and Borisy, G.G. (2001). Dendritic organization of actin comet tails. Curr. Biol. *11*, 130–135.
15. Kuo, S.C., and McGrath, J.L. (2000). Steps and fluctuations of Listeria monocytogenes during actin-based motility. Nature *407*, 1026–1029.
16. Pistor, S., Chakraborty, T., Walter, U., and Wehland, J. (1995). The bacterial actin nucleator protein ActA of Listeria monocytogenes contains multiple binding sites for host microfilament proteins. Curr. Biol. *5*, 517–525.
17. Welch, M.D., Rosenblatt, J., Skoble, J., Portnoy, D.A., and Mitchison, T.J. (1998). Interaction of human Arp2/3 complex and the Listeria monocytogenes ActA protein in actin filament nucleation. Science *281*, 105–108.
18. Laurent, V., Loisel, T.P., Harbeck, B., Wehman, A., Grobe, L., Jockusch, B.M., Wehland, J., Gertler, F.B., and Carlier, M.F. (1999). Role of proteins of the Ena/VASP family in actin-based motility of Listeria monocytogenes. J. Cell Biol. *144*, 1245–1258.
19. Smith, G.A., Theriot, J.A., and Portnoy, D.A. (1996). The tandem repeat domain in the Listeria monocytogenes ActA protein controls the rate of actin-based motility, the percentage of moving bacteria, and the localization of vasodilator-stimulated phosphoprotein and profilin. J. Cell Biol. *135*, 647–660.
20. Mullins, R.D., Heuser, J.A., and Pollard, T.D. (1998). The interaction of Arp2/3 complex with actin: nucleation, high affinity pointed end capping, and formation of branching networks of filaments. Proc. Natl. Acad. Sci. USA *95*, 6181–6186.
21. Skoble, J., Portnoy, D.A., and Welch, M.D. (2000). Three regions within ActA promote Arp2/3 complex-mediated actin nucleation and Listeria monocytogenes motility. J. Cell Biol. *150*, 527–538.
22. Suzuki, T., Miki, H., Takenawa, T., and Sasakawa, C. (1998). Neural Wiskott-Aldrich syndrome protein is implicated in the actin- based motility of Shigella flexneri. EMBO J. *17*, 2767–2776.
23. Steinhauer, J., Agha, R., Pham, T., Varga, A.W., and Goldberg, M.B. (1999). The unipolar Shigella surface protein IcsA is targeted directly to the bacterial old pole: IcsP cleavage of IcsA occurs over the entire bacterial surface. Mol. Microbiol. *32*, 367–377.
24. Robbins, J.R., Monack, D., McCallum, S.J., Vegas, A., Pham, E., Goldberg, M.B., and Theriot, J.A. (2001). The making of a gradient: IcsA (VirG) polarity in Shigella flexneri. Mol. Microbiol. *41*, 861–872.

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Primer

Cell migration

Rick Horwitz and Donna Webb

Directed cell migration is an integrated process that is essential for embryonic development and throughout life (Figure 1). The failure of cells to migrate or the migration of cells to inappropriate locations can result in abnormalities or have life-threatening consequences. For example, many congenital defects in brain development leading to mental disorders can be attributed to defects in neuronal migration. In the adult, cell migration is central to homeostatic processes, such as mounting an effective immune response and the repair of injured tissues. Migration can also contribute to some pathological processes, including vascular disease, chronic inflammatory diseases, and tumor formation and metastasis. Thus, there is considerable interest in understanding cell migration on a molecular level because this could lead to novel therapeutic approaches, especially in areas of biotechnology that focus on cellular transplantation and the manufacture of artificial tissues. While the emphasis in this article is on cell migration in vertebrates, migration is equally important in invertebrates, plants and some single-cell organisms.

Understanding migration presents a formidable intellectual challenge because it is the product of several complex, integrated processes that must be carefully regulated. Conceptually, the migrating cell is best viewed as a highly polarized entity with rapidly changing activities that are spatially segregated, particularly at the cell front and rear. For efficient migration to occur, these activities need to be coordinated. Therefore, the challenge is to elucidate the mechanisms of the component processes, their regulation, and the nature of their integration. Due to the difficult nature of this problem, some components of migration will require multidisciplinary approaches and concepts.

However, the benefits can be enormous since many aspects of migration research are expanding the frontiers of cell biology.

Migration and Development

Migration takes place throughout embryonic development, one example being gastrulation. This process occurs after conception and the ensuing formation of a blastocyst, a ball of rapidly dividing cells. In gastrulation, large groups of cells inside the blastocyst migrate collectively as sheets to form the three layers that comprise the resulting embryo. Cells within these layers eventually migrate to target locations throughout the developing embryo where they differentiate and form various tissues and organs. This theme of cells migrating from epithelial layers to targets where they differentiate is general and occurs throughout development. For example, in the developing cerebellum, neuronal precursor cells migrate from the epithelium that resides along a ventricle to their residences in distinct layers, where they then extend axons to their final targets and form synapses. Another example is the migration of muscle precursor cells from the somites to their targets in the limbs.

Migration is not limited to development, but occurs in the adult in both normal and pathological states. For example, skin is renewed continuously from precursors that migrate up from the basal layer. Migration also functions in the adult in immune surveillance, where leukocytes from the circulation migrate into the surrounding tissue to ingest bacteria. Tumor formation is accompanied by the invasion of blood vessels that arise from the proliferation and migration of their component endothelium. In metastatic cancer, some tumor cells acquire the ability to migrate out of the initial tumor into the circulation and move to new locations where they form a secondary tumor.

Mechanics of Migration

Migration can be thought of as a cyclical process (Figure 2). It begins with a cell's response to an external signal that leads to the polarization and the extension of a protrusion in the direction of movement. The formation of adhesions attaches the protrusion to the substratum on which the cell is migrating. These adhesions serve, in part, as traction points for migration, and they also initiate signals that regulate adhesion dynamics and protrusive activity. Contraction then moves the cell body forward and release of the attachments at the rear as the cell retracts completes the cycle.

While relatively slow-moving cells, e.g. fibroblasts, show these very distinct steps, the different stages are less obvious in other cell types. Rapidly migrating cells, like keratocytes and leukocytes, appear to glide over the substratum by protruding and retracting smoothly without forming noticeable attachments. Interestingly, cells migrating in sheets show features of single-cell movement; those at the front have protrusions while the cells at the rear of the sheet show features of retraction.

Recent evidence is pointing to a plasticity in migration mechanisms. For example, the migration of cells *in vivo* differs from that of cells migrating *in vitro*. Migration *in vivo* is much more directed than that *in vitro* with cells forming long, stable protrusions pointed in the direction of migration. Tumor cells also show a plasticity that depends dramatically on the environment. Under some conditions they polarize and migrate along collagen bundles, whereas under others, they become more amoeboid and use different migration mechanisms.

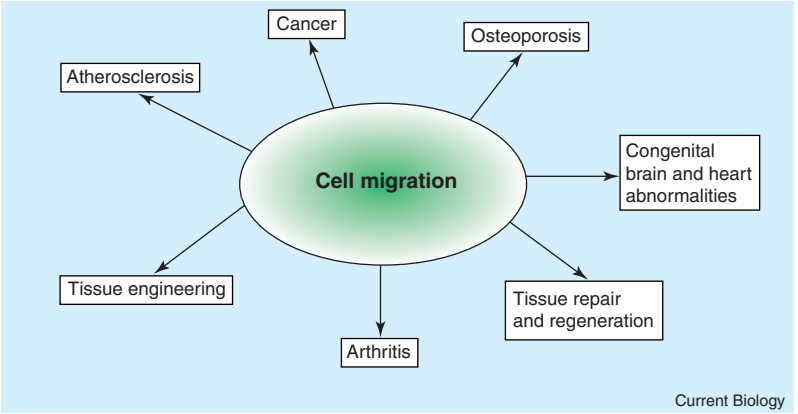

Figure 1. Migration in human health.
Migration is central to many physiological and pathological processes. A better understanding of the factors that regulate migration could lead to novel therapeutic approaches and applications.

Polarization

The centerpiece of cell migration is a polarized cell. A large variety of different molecules can serve as external agents that initiate and promote migration. Some molecules initiate a migratory phenotype (chemokinetic) while others reside in soluble (chemotactic) or substrate (haptotactic) associated gradients and lead to directed movement. These molecules and their receptors are particularly well-studied in leukocytes. Neutrophils, for example, sense the presence of even a shallow gradient in which they polarize and migrate persistently in one direction. Their persistent polarity is evident when these cells sense changing chemotactic gradients, and the whole cell turns rather than extending a new protrusion from another region. In contrast, fibroblasts seem much more plastic and can extend protrusions from any position in the cell as they change directions.

Recent studies are beginning to shed some light on how chemotactic signals are interpreted to produce directed migration. Although the chemotactic receptors themselves are not polarized in response to a gradient, they transmit the extracellular signal to the cell interior. The cell must then convert this signal to polarized internal responses. One of the first molecules to become polarized in response to a

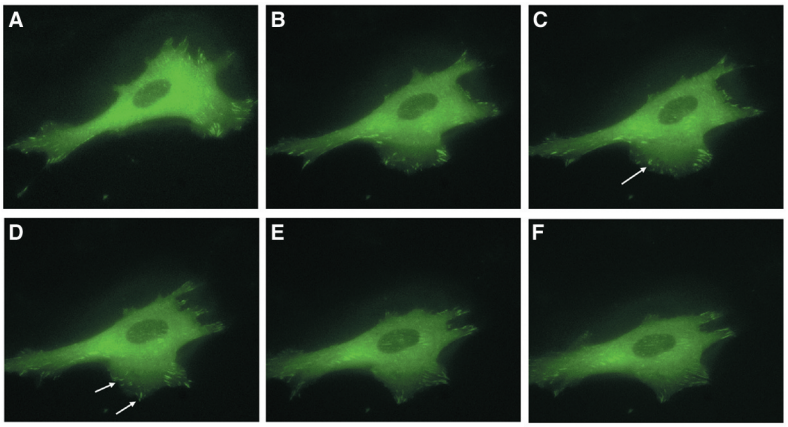

Figure 2. Steps of migration.
Images of a human fibroblast expressing paxillin–GFP, captured by time-lapse fluorescence microscopy, illustrate the mechanics of migration. (A–C) Migration begins when a cell responds to an external signal that leads to the polarization and extension of a protrusion in the direction of movement. Adhesions form to attach the protrusion to the substratum (arrows). (D–F) Contraction then moves the cell body forward and the adhesions at the rear disassemble as the rear retracts.

chemotactic agent is phosphatidylinositol (3,4,5) trisphosphate (PIP_3), a lipid that serves to recruit other molecules, such as Akt/PKB to the membrane. The localized activation of PI 3-kinase and a low level of lipid phosphatases, such as PTEN, at the cell front produce the rapid accumulation of PIP_3 at the leading edge. The asymmetric accumulation of PIP_3 leads to activation of Rho family GTPases, including Rac and/or Cdc42, and polymerization of actin at the leading edge.

Cdc42 appears to be a key regulator of polarity because cells lose their ability to respond to a chemotactic gradient and migrate randomly when this molecule is inhibited. Par6 and its associated protein, PKCζ, are part of a signaling pathway downstream of Cdc42 that establishes cell polarity during migration. Although the downstream targets of PKCζ are not known, the pathway appears to be involved in organizing the microtubule network.

Dynamics at the Leading Edge
The local activation of Rac or Cdc42 is a key regulatory event that stimulates actin polymerization at the leading edge of the broad lamellipodia or spike-like filopodia, respectively. This polymerization serves to push the membrane forward resulting in the extension of a protrusion in the direction of migration. Other regulators of actin dynamics also localize at or near the leading edge. In the lamellipodia, these include PIP_2, WASP, Scar, and Arp2/3, which controls the formation of new actin filament branches on existing filaments. The polymerization itself is regulated by proteins that serve to cap growing filaments, sever older portions of existing filaments and control the availability of activated actin monomers.

Adhesion complexes, which are sites of attachment between the cell and the extracellular matrix (ECM), stabilize the protrusion via structural connections to actin filaments and mediate signaling to the Rho family of GTPases, ERK/MAP kinase and other regulatory molecules. Adhesion complexes are composed of a number of proteins, including adhesion receptors, kinases, adaptors, and structural molecules. They serve as traction points over which the cell moves and can transmit strong propulsive forces. The small Rac-induced adhesions at the leading edge are resposible for driving rapid cell migration. The formation of stronger adhesions inhibits migration, which is fastest at an optimum adhesion strength, i.e., strong enough to support traction but weak enough to allow rapid detachment at the rear.

Several migration-related molecules are present at the leading edge. They include activated integrins, which presumably nucleate and stabilize nascent adhesions, talin, which recruits PIP kinase type Iγ, and vinculin, which interacts with Arp2/3. Interestingly, Arp2/3 also associates transiently with nascent adhesions pointing to a role for these adhesions in nucleating actin polymerization.

Moving Forward
Actin filaments generate a myosin force at the front of the cell that serves to pull the cell body toward the protrusion. Release of adhesion connections in the rear of the cell and retraction of the tail, also a myosin-mediated process, completes the cycle. Spatial and temporal regulation of Rho GTPases controls these processes through effectors, such as ROCK, that regulate actomyosin contractility. ROCK has been implicated in the release of adhesions at the rear of the cell via regulation of myosin II. Other molecules implicated in the release of adhesions include the protease calpain, the phosphatase calcineurin, and microtubule dynamics, which serve to regulate Rac activity and adhesion disassembly.

Trafficking During Migration
The functional and molecular asymmetry that is so intrinsic to cell migration suggests a polarity in the trafficking of cellular components as old components are released and removed from the rear and new components move to the leading edge. While the relative contributions of different mechanisms are not known, there are several interesting options. These include the trafficking of endocytic vesicles, which could move material from the rear to the front of the cell or through an endosomal recycling pathway. Some signaling molecules traffic in complexes to and from functional regions of the cell via large, multiprotein complexes. Polarized actin filaments can also serve as tracks for the transport of mRNAs and in the delivery of secretory vesicles to the front of the cell.

Future Directions
While we are seeing breathtaking progress in many areas of cell

migration, some issues still provide significant hurdles that are only just beginning to be overcome. Complex cell behaviors, such as migration, result from coordinated activity of several individual component processes. While considerable progress has been made in identifying molecules related to migration and elucidating the mechanisms by which they function in a component process, understanding the integration of these various component processes presents a major challenge. Fortunately, technologies are emerging that allow us to study the spatial and temporal regulation that generates the coordination of these processes. These include biosensors that allow spatially resolved assays of signaling events in real time in living cells and photomanipulative techniques that allow local perturbations of function via photoactivation or inactivation to complement the observations made using biosensors. It is now clear that migration *in vivo* differs from that studied *in vitro*; this may reflect different signaling mechanisms or cellular mechanics. The development of three-dimensional systems that allow imaging of cellular and molecular dynamics is helping to reveal mechanisms underlying migration in physiological environments. Finally, the signals that initiate, guide, and stop migration are not well understood, especially in the context of the component processes of migration and their regulation.

Cell Migration as a Discipline

Until recently, research in cell migration was split into a number of subdisciplines that did not interact as an integrated research area. Over the past few years, cell migration has emerged as its own discipline.

Recently, an NIH-sponsored Cell Migration Consortium was formed (www.cellmigration.org) with the aim of promoting migration research by addressing some major barriers to progress in the field. These include a census of the migration proteome, the structure of the supramolecular complexes that drive migration, development of novel signaling reagents that report spatially resolved signals in living cells, development of mathematical models of migration, development of novel imaging techniques, development of new biomaterials, and the production of knockout, knockin and knockdown mice and cell lines. Other Consortium activities include promoting interdisciplinary collaboration and organizing information in the field through a web-based cell migration knowledge database.

Further Reading

Etienne-Manneville, S. and Hall, A. (2002). Rho GTPases in cell biology. Nature *420*, 629–635.

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

Horwitz, A.R. and Parsons, J.T. (1999). Cell migration—movin' on. Science *286*, 1102–1103.

Iijima, M., Huang, Y.E. and Devreotes, P. (2002). Temporal and spatial regulation of chemotaxis. Dev. Cell *3*, 469–478.

Keller, R. (2002). Shaping the vertebrate body plan by polarized embryonic cell movements. Science *298*, 1950–1954.

Lauffenburger, D.A. and Horwitz, A.F. (1996). Cell migration: a physically integrated molecular process. Cell *84*, 359–369.

Pollard, T.D. and Borisy, G.G. (2003). Cellular motility driven by assembly and disassembly of actin filaments. Cell *112*, 453–465.

Rickert, P., Weiner, O.D., Wang, F., Bourne, H.R. and Servant, G. (2000). Leukocytes navigate by compass: roles of PI3Kgamma and its lipid products. Trends Cell Biol. *10*, 466–473.

Rodriguez, O.C., Schaefer, A.W., Mandato, C.A., Forscher, P., Bement, W.M. and Waterman-Storer, C.M. (2003). Conserved microtubule-actin interactions in cell movement and morphogenesis. Nat. Cell Biol. *5*, 599–609.

Ross, M.E. and Walsh, C.A. (2001). Human brain malformations and their lessons for neuronal migration. Annu. Rev. Neurosci. *24*, 1041–1070.

Roy, P., Rajfur, Z., Pomorski, P. and Jacobson, K. (2002). Microscope-based techniques to study cell adhesion and migration. Nat. Cell Biol. *4*, E91–E96.

Webb, D.J., Parsons, J.T. and Horwitz, A.F. (2002). Adhesion assembly, disassembly and turnover in migrating cells - over and over and over again. Nat. Cell Biol. *4*, E97–E100.

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Q & A

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John Gurdon is at the Wellcome Trust/Cancer Research UK Institute of Cancer and Developmental Biology in Cambridge. Educated at Eton and Christ Church, Oxford, he changed from classics to zoology. During graduate work under Michael Fischberg at Oxford, he was the first to obtain a normal adult animal by somatic cell nuclear transfer. He then spent a year on bacteriophage genetics at CalTech. From a lectureship at Oxford, he moved in mid-career first to the MRC Molecular Biology Laboratory in Cambridge and then, in 1983, to the University's Zoology Department under Sir Gabriel Horn. In 1990 he co-founded, with Ron Laskey, the Wellcome Trust/Cancer Research Campaign Institute in Cambridge. His scientific work has never strayed from the use of Xenopus*, and has concentrated on aspects of cell differentiation, including nuclear reprogramming, morphogen gradients and the community effect. He has always remained active in doing his own experiments with his own hands, trusting that members of his group are clever enough to largely guide and execute their own experiments.*

How did you come to have a career in science? Not easily. After one term of biology at school, the teacher wrote "For Gurdon to continue in biology would be a complete waste of time both for him and for those who would have to teach him". I studied classics, took the Oxford entrance exam in this subject, and was offered a place on condition that I did not read classics. Luckily for me, they were short of applicants at that time. My kind parents paid for an extra year's private teaching to enable me to start the zoology course at Oxford.

Do you have heroes? Rodney