Meeting report

# Where are they going? Directed cell movement in morphogenesis

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A report on the 'Cell and Tissue Morphogenesis' Spring Meeting of the British Societies for Cell and Developmental Biology, University of Sussex, Brighton, UK, 3-6 April, 2001.

Many talks at this year's joint spring meeting of the British Societies for Cell and Developmental Biology revealed the importance of regulators of cytoskeleton and signaling events, leading to rearrangement of the cytoskeletal architecture, for a number of cellular processes including morphogenesis, endocytosis and cell motility. In this report, I focus on certain presentations: in the 'cytoskeleton' session, discussions of the role cytoskeletal modulators play in wound healing; in the 'regulation of cell motility' workshop, topics ranging from the control of chemotaxis at different developmental stages of *Dictyostelium discoideum* to the regulation of the actin cytoskeleton; and in the 'signaling' session, presentations about the integrin-mediated signaling that precedes cell migration and about signaling following intercellular interactions.

## The cytoskeleton and wound healing

Paul Martin (University College London, UK) studies dorsal closure in *Drosophila* embryos as a model for understanding wound healing in epithelia. His analysis of dorsal closure in living fly embryos using actin tagged with green fluorescent protein (GFP) showed that the zippering together of the two epithelial edges relies on the activity of the Rho-family small GTPase Cdc42, a regulator of the actin cytoskeleton. His movies showing dorsal closure and wound healing indicated that the cells at the leading edge extend filopodia to find the opposing cells. The nonmuscle motor protein myosin II is essential for maintenance of the cell morphology required for dorsal closure, as revealed by Daniel Kiehart (Duke University, Durham, USA). Kiehart further demonstrated that the RhoA GTPase mediates wound healing at a time when it is apparently not required for dorsal closure.

Sandrine Etienne-Manneville (University College London, UK) demonstrated a wound-healing assay using primary rat astrocytes. In an astrocyte cell-layer model, cells at the leading edge polarize and extend protrusions towards a scratched 'wound'. Wounding induces the activation of focal-adhesion proteins, including integrins, leading to the activation of Cdc42 and members of the atypical protein kinase C subfamily (aPKCs). Etienne-Manneville was able to show that the binding of Cdc42 to aPKCs via the mammalian homolog of the *Caenorhabditis elegans* protein Par6 induces polarization of the microtubule-organizing center, independent of Cdc42-induced formation of protrusions.

## Regulators of the actin cytoskeleton

The actin cytoskeleton of motile cells is a highly dynamic meshwork of actin filaments. At the leading edge of a motile cell, this actin network is used to move the cell by prolonging and crosslinking existing filaments and thereby pushing the cell membrane forward. The main activator of actin nucleation is the Arp2/3 complex. Its activity is regulated by numerous proteins, among the most important of which are the family of the Wiskott-Aldrich syndrome proteins (WASP) and the Scar-family proteins. The expression pattern of WASP and Scar1 in different mouse tissues and during myeloid differentiation was described by Sophie Launay (University of Birmingham, UK). Furthermore, she indicated the involvement of the Arp2/3 complex in phagocytosis mediated by the complement receptor type 3 (CR3) as well as the immunoglobulin G receptor FcγRII. Myosin II seems to play an essential role in CR3-mediated phagocytosis, as blocking myosin light-chain activity inhibits particle internalization.

Recently, small Rho-family GTPases, such as Cdc42 and Rac, have been described as upstream signaling molecules involved in the activation of the Arp2/3 complex. Harry Mellor (University of Bristol, UK) presented data on a new Rho-family GTPase, Rif (Rho in filopodia), which is found

mainly in tissues containing epithelial cells. Rif induces highly dynamic, long filopodia by a Cdc42-independent pathway. The downstream targets of Rif remain to be specified: Mellor demonstrated that Rif is unlikely to bind to WASP.

Frank Gertler (Massachusetts Institute of Technology, Cambridge, USA) showed that proteins of the Enabled/vasodilator-stimulated phosphoproteins (Ena/VASP) family are not only involved in the spatial control of actin assembly, but they also influence the actin-network architecture as well. Depletion of Ena/VASP leads to a highly branched network of short filaments and increases the speed of motile cells, whereas overexpressing Ena/VASP proteins reduces cell speed and leads to a cellular actin network made of long actin filaments with little branching. Gertler showed a clear correlation between the expression level of Ena/VASP and the architecture of the actin network as well as cell speed.

Britta Qualmann (Leibniz Institute for Neurobiology, Magdeburg, Germany) demonstrated that syndapins (synaptic, dynamin-associated proteins) provide a functional link between the actin cytoskeleton and endocytosis. The syndapin SH3 domain interacts with the polyproline region of N-WASP and the guanine nucleotide exchange factor (RasGEF) mSos, indicating a role for syndapins in signal transduction. Recruiting endogenous syndapins to the N-WASP polyproline region inhibits endocytosis, a phenotype that can be rescued by overexpressing syndapin. This clearly implicates a direct syndapin-N-WASP interaction.

## Cell motility and development in *Dictyostelium discoideum*

Morphogenesis of the amoeba *D. discoideum* into a multicellular slug and ultimately to form spore and stalk cells results from the coordinated movement of differentiating cells towards the chemoattractant cAMP. Cornelis Weijer (University of Dundee, UK) showed movies of aggregating cells guided by waves of cAMP, which can be visualized as waves of differing optical density. Using cells expressing GFP-tagged CRAC (cytosolic activator of adenylyl cyclase), a protein that contains a pleckstrin-homology (protein-protein interaction) domain, in an environment of untransfected cells, he showed that individual cells are highly motile in the aggregation stream and in the slug stage. In these multicellular stages, cAMP signaling occurs from cell to cell; this can be monitored with GFP-CRAC that is translocated from the cytoplasm to the cell membrane, reflecting the activation of a G-protein signaling pathway and subsequent activation of adenylyl cyclase.

The mechanical stability of the cytoskeleton, provided by myosin II, is crucial in multicellular development of *D. discoideum*, as demonstrated by David Knecht (University of Connecticut, Storrs, USA). An elegant under-agarose chemotaxis assay mimics the mechanical load of the aggregation stream. Cells lacking myosin II function cannot move under highly concentrated agarose, whereas wild-type cells can, suggesting that myosin II is an important crosslinker of the cytoskeleton in the cell cortex. Surprisingly, cells lacking the myosin essential light chain, which is required for the generation of three-dimensional cell shape, behave like wild-type cells in the under-agarose assay, showing that myosin motor function is not necessary for the chemotactic movement of *D. discoideum* under mechanical forces; instead, the crosslinking function is likely to be key.

## Integrin-mediated signaling

Integrins are transmembrane proteins that link the extracellular environment to the actin cytoskeleton via connections with adaptor proteins such as talin and filamin. Mark Ginsberg (The Scripps Research Institute, La Jolla, USA) showed an elegant method for expressing only the cytoplasmic tail of integrins. The cytoplasmic domain interacts with signaling molecules, as well as with the cytoskeleton and other intercellular proteins, and transduces signals from both outside and inside the cell. It regulates pivotal integrin-mediated functions such as cell adhesion, migration, signal transduction and gene expression. Expression of the $\beta_7$ cytoplasmic tail, which is known to interact with F-actin via talin, inhibits cell migration and reduces focal adhesions by diminishing the formation of membrane protrusions, and it also reduces cell polarity. By expressing the cytoplasmic domain of the $\alpha_4$ integrin in Jurkat T cells, cell migration is increased. The $\alpha_4$ integrin is known to interact with the signaling adaptor molecule paxillin, and loss of paxillin binding inhibits Jurkat cell migration. Ginsberg gave a short demonstration of the diverse signaling potential of different integrins, indicating the importance of the range of integrin expression patterns in different cell types.

The integrin-linked kinase (ILK), an effector protein downstream of integrins, is a regulator of both the cell cycle and apoptosis, as presented by Shoukat Dedhar (University of British Columbia, Vancouver, Canada). Overexpression of ILK in epithelial cells leads to nuclear translocation of β-catenin, a linker between cadherins (on the cell surface) and the actin cytoskeleton. After translocation of β-catenin to the nucleus, E-cadherin is transcriptionally downregulated, triggering cells to become highly invasive.

## Signaling through cell adhesions and junctions

The ability of *D. discoideum* to become multicellular makes it a useful and easy-to-handle tool for studying intercellular interactions during development. Adrian Harwood (University College London, UK) presented exciting data showing that during evolution, the ability of cells to form adherens junctions and the presence of a GSK-3/β-catenin-mediated signaling pathway arose prior to the origin of mammals. GskA, the *D. discoideum* homolog of GSK-3, stimulates

Aardvark, a homolog of mammalian β-catenins. Following the loss of Aardvark, inductive signals disappear, leading to an uncoordinated development of stalk and spore cells, as can be seen by the presence of damaged stalk tubes. Imaging of live cells that carry a loss-of-function mutation of *gskA* revealed a defect in retracting their tails, which can be rescued by an *Aardvark* null mutation. Taken together, Harwood's data provided clear evidence for a signaling pathway in *D. discoideum* similar to that seen in animals.

In the future, the use of different model organisms and comparisons of pathways in different species will give us a more complete picture of the sets of molecules involved in signaling events and regulation of cytoskeleton-dependent cellular functions.