

Cell Migration and Chemotaxis

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Crawling movement is fundamental to the behaviour of most eukaryotic cells. During tumour invasion the regulation of cell movement goes wrong - cancer cells invade other tissues, and spread through the blood and lymph systems to form metastases. This spreading behaviour is one of the most feared features of cancer and a principal driver of death in patients. Despite this, the mechanisms that control cell movement are not well understood; our group aims to improve this understanding. We use a wide range of techniques, from genetics through biochemistry and microscopy to computational modelling.

We are particularly interested in two stories. The first is chemotaxis, in which external signals orient and attract cells, and which is increasingly seen as a fundamental cause of metastasis. The second is the regulation of the Arp2/3 complex, an assembly of proteins that promotes movement by driving the formation of actin microfilaments. We study these two processes in a range of different cells. In particular, we use *Dictyostelium* amoebas for the genetic analysis of movement but we also use cultured tumour cells and computational models.

Cell movement is a central part of biology, from conception to death. Embryos form their complexity and shape from the movement of layers of cells as well as the migration of individual cells through tissues. While, adult wound healing and responses to infection require skin and immune cells to migrate to where they are needed. Metastasis, one of the most feared features of cancer, is caused when cells migrate out from a tumour into the blood, lymph or other tissues. Chemotaxis, the connection between chemical signals outside the cell and its movement, is important in these processes but remains very poorly understood. We are trying to understand cell movement - what drives it and most importantly how it is steered.

Most mammalian cells use pseudopods made of polymerised actin to power migration. Our

current research focuses on the proteins and pathways that control these pseudopods. We use three approaches. For genetic studies we use *Dictyostelium*, taking advantage of its ease of manipulation, and prominent cell movement and chemotaxis. We then extend our observations to mouse models, cultured tumour cells, and are gearing up to examine cells extracted directly from biopsies and resected melanomas. We also develop computational models in collaboration with the Mathematics Department at the University of Strathclyde. In the long-term, we will work on anything that will help us to understand the conserved and fundamental mechanisms that drive cell movement.

Regulators of the Arp2/3 complex

Actin drives nearly all cell movement, and the principal driver of actin is an assembly called the Arp2/3 complex. When turned on, the Arp2/3 complex causes new actin filaments to form and push against the membranes inside and at the leading edge of cells. We are particularly interested in the family of proteins that turns on the Arp2/3 complex.

One such regulator is SCAR. SCAR proteins - also called WAVES in mammals - are fundamentally important regulators of cell movement. Mutants in a variety of species show that SCAR is required whenever cells need to make large structures such as lamellipods; without SCAR such structures are either small

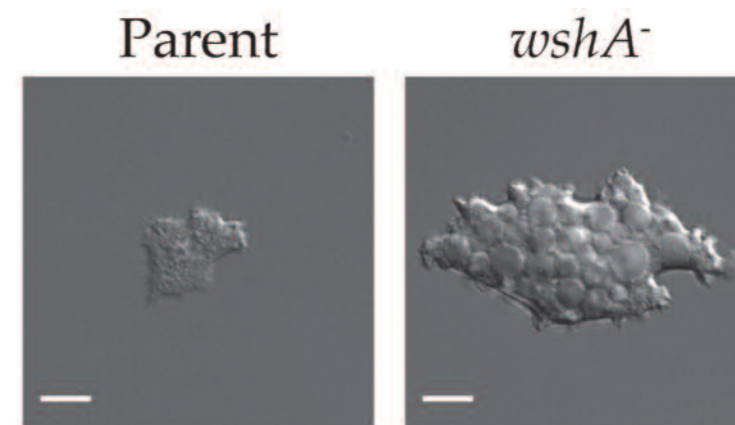


Figure 1
WASH mutants fail to exocytose indigestible material. The images show cells fed with medium containing 10% dextran. Mutants with a knockout of the gene for the actin regulator WASH take up medium at a normal rate, but the dextran is retained in large vesicles that are exocytosed from wild type cells.

and malformed, or completely absent. SCAR is found as part of a five-membered complex with the Rac-binding protein PIR121, Nap1, Abi and HSPC300. Without the other members of the complex, SCAR is rapidly removed from the cell. The prevailing view in the field is that all these proteins act simultaneously as a huge, homogenous complex that couples Rac signalling to actin polymerisation. However, our genetic studies in *Dictyostelium* show that each complex member may have a different function within the whole - with Nap1 controlling adhesion, and Abi inactivation of movement during processes like cytokinesis. These studies point to the SCAR complex being a nexus integrating multiple inputs - signalling, adhesion and a cell's internal state - and coupling the integrated output to cell movement.

Our experiments are currently focused on identifying the activators and other proteins that regulate each component of the complex. SCAR and the other complex members are phosphorylated at multiple sites but the biological significance of these phosphorylations is not understood. We have found that phosphorylation at the C-terminal end of SCAR is a particularly important regulator - dephosphorylated SCAR is activated to a far greater extent than the phosphorylated form, which predominates in cells.

We have also been making good progress in determining the role of another, poorly understood regulator of the Arp2/3 complex, WASH. Gene knockouts in *Dictyostelium* have defects in the intracellular transport of vesicles, specifically in the retrograde sorting of proteins such as the vacuolar ATPase from lysosomes. We are using genetic screens to search for WASH interactors, and unpicking the functions of the WASH regulatory complex by comparing knockout strains.

Mechanisms underlying chemotaxis

Chemotaxis, migration towards chemical signals, is emerging as a major driver of tumour

metastasis. We have recently shown that chemotaxis in *Dictyostelium* cells works by a different mechanism than that which is usually described. Pseudopods are constantly generated in random directions, then the ones that point in the best directions are selected and maintained.

We are now performing similar studies into the migration of cancer cells. We will use high-resolution, three-dimensional microscopy to ask questions including whether human tumour cells use the same mechanism as *Dictyostelium* for chemotaxis and which properties are most important to the metastatic phenotype. Parameters such as cell speed are widely used but are broadly irrelevant in our new model. Instead, we will measure the rate at which pseudopods are made and change shape, the instantaneous velocity at which pseudopods move, and the regulation of pseudopod retraction. We also want to identify which proteins are used to regulate tumour cell chemotaxis and whether they are the same for all metastatic cells, and if not, how broad the range is. It is now clear that there are at least two dissimilar mechanisms that drive tumour cell movement. We want to know whether there really are exactly two mechanisms or something more variable.

The work involves the examination of a large range of tumour cell lines and primary cultures, using a number of techniques including quantitative DIC (differential interference contrast), immunofluorescence and live-cell confocal microscopy. We will then measure a wide range of movement parameters using software we have developed and will continue to develop.

We are also collaborating with the University of Strathclyde Mathematics Department to make computational models representing moving cells. Our models already faithfully mimic the movement of *Dictyostelium* cells. We are now adding components - chiefly cell-cell adhesion and mechanical responses - to the model, with the aim of making it mimic less motile cells like tumour cells. If we can make these models behave like real cells, we can determine what the key components are in transforming a non-motile to a motile cell, and thus assess the causes of metastasis. We can also determine which components can safely be ignored, which is increasingly important - hundreds of genes are newly associated with motility and invasion every year, so we urgently need a mechanism to determine which are the most important.

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