Cytoskeleton and Cell Motility

**Objective:** To observe cell motility and to determine the effects of cytoskeletal disruption on motility.

**Introduction:** Many types of cells are motile. Fibroblasts, such as the 3T3 cells that we have used in previous labs, show a characteristic form of motion that consists of protrusion, adhesion and contraction. The first step, protrusion, occurs when a cell extends a lamellipodium in the direction of migration. Protrusion is driven (at least in part) by the assembly of a meshwork of actin filaments in the leading edge of the cell. Cells can extend many protrusions, some of which retract, while others adhere to the substrate. When a protrusive region adheres, then the cell can move in that direction. This motion is driven by forces generated by the contraction of actin and myosin located in the central and rear of the cell (see Figure 1). Fibroblasts migrate individually; other cells migrate not as individual cells, but in sheets. This form of motion is characteristic of epithelial cells, such as LLC-Pk1 cells. As with fibroblasts, individual epithelial cells extend protrusions, but the motion involves a group of cells, which are held together by cell-cell adherens junctions.

Cell motility can be easily studied by examining the behavior of cells in tissue culture. In phase contrast, the protrusive behavior of the cell can be observed, and time-lapse movies of these dynamic events recorded. The edges of cells often display both lamellipodia that extend along the surface and others that extend upwards into the medium. These non-adherent protrusions are referred to as ‘ruffles’ because they give the cell a characteristic ruffled appearance. Another experimental approach to examining cell motility is to make a ‘wound’ in a confluent monolayer of cells. In this type of experiment, the tip of a pipette (or similar tool) is used to remove a strip of cells from the monolayer, generating an area free of cells. With time, the cells migrate directionally, closing the wound. As the cells migrate, they extend lamellipodia into the free space. Like cells at the edge of a wound, many cells migrate directionally in response to cues in the environment. For example, amoeboid cells can migrate toward a source of food, and *Dicytostelium* migrate in response to cAMP. In embryos, cells receive diverse

![Cell locomotion diagram](image.png)
cues, from other cells and extracellular factors in their environment, to direct their motility. Other cells move in a more random manner, such as the fibroblasts that we will examine in lab.

Migration requires an intact actin and myosin cytoskeleton to generate protrusions and contractile forces. Microtubules also contribute to cell motility. When microtubules are disassembled, cells tend to round up and become less asymmetric. Cells that lack microtubules send out protrusions in all directions, as if they are attempting to migrate in multiple directions simultaneously, and fail to translocate. These experiments demonstrate that cell polarity or asymmetry is important for motility. Microtubules also serve as the tracks for the motion of various membrane bound cargoes and directed membrane delivery may also contribute to cell locomotion.

Procedure

In this lab, we will examine motility of several types of cells and use inhibitors of the cytoskeleton to examine the role of actin filaments and microtubules in motile behavior. You will have the lab period on Monday and most of Wednesday to perform these experiments, so you can plan accordingly. Cells will be available each day.

**Experiment 1:** Here, you will examine the first step in motility, the extension of a protrusion or lamellipodium. For this purpose, we will use 3T3 fibroblasts, B16 melanoma cells, and LLCPk1 cells that express GFP-actin. Because the melanoma cells are human cells you must handle them with caution. Wear gloves and place all materials that have come in contact with these cells in the orange bag to be autoclaved.

Obtain a dish of cells; the dishes have a glass bottom, so you can observe the cells directly without making a slide cover-slip preparation.

Use the 100X lens and set up your scope for phase contrast. Find a cell, and make time-lapse movies of the periphery. Try to find cells that are extending and retracing their lamella. These cells will have phase dense and phase light areas around the periphery.

Record the behavior of the cell periphery using phase contrast for each of the cell types.

**Experiment 2: dynamics of GFP-actin and GFP α-actinin in motile cells.** after you have examined cell protrusive activity in phase contrast, switch to fluorescence observation and examine the LLCPk1 cells expressing GFP-actin and the 3T3 fibroblasts expressing GFP-α-actinin. Record the dynamics of actin in the cell periphery; compare what you see with your observations in phase contrast.
Experiment 3: inhibition of actin polymerization. Using one of the dishes of cells that you have examined for experiment 2, remove the growth media and replace it with media that contains cytochalasin D, a fungal toxin that binds to the growing ends of actin filaments, preventing their assembly and inducing disassembly. ~1 ml of media should be sufficient for this purpose. Observe the periphery of several cells; make time-lapse movies. Examine the behavior of the cells immediately after adding the drug. What do you observe? Examine the cells at later times after drug addition. What do you observe? You can experiment with different concentrations of cytochalasin D, by diluting the stock solution with drug-free media. You can do this experiment on cells that do not express GFP-actin by using phase contrast optics; alternatively, you can use cells expressing GFP-actin, or GFP-α-actinin and make your observations in fluorescence. When making time-lapse movies with fluorescence be sure to use the shutter to minimize photobleaching.

Experiment 4: inhibition of microtubule dynamics. Take the other dish of cells that you have observed in phase, and treat them with drugs that alter the dynamics of microtubules. Here you can use either taxol, a drug purified from the pacific yew, or nocodazole, a synthetic analog of the drug colchicine, which is obtained from the autumn crocus. Taxol binds to intact microtubules, not to free tubulin dimers. The taxol binding site is within the lumen of the microtubule. Taxol stabilizes microtubules and prevents their normal dynamic instability behavior. Nocodazole binds to the tubulin dimer, not to intact microtubules. By binding to dimers, nocodazole prevents the elongation of microtubules, and shifts the equilibrium to disassembly.

Taxol and nocodazole in culture medium are available for your use. Remove the normal growth medium and replace with medium containing one of these compounds. Make observations in phase contrast, in fluorescence, or both. Compare what you observe with control cells.

Experiment 5: the B16 cells contain pigment granules, which move along the cytoskeleton. The granules are phase dense, due to the pigment, so they are easy to observe. Make observations of the edges of these cells and the interior region. Try to find a region that is sufficiently flat for you to clearly observe individual granules. Now, using the inhibitors of the cytoskeleton, test the role that microtubules play in granule motility. You can also test the contribution of actin filaments. From your observations, which cytoskeletal filament system is needed for the intracellular motion of pigment granules?

Additional experiments:
1. you can try various concentrations of inhibitors, various times of incubation, and you can try inhibition of both actin and microtubules simultaneously, or in series. You can also inhibit actin or microtubules and then observe the cells upon washout of the inhibitor (remove the drug containing media, rinse once with
some warm PBS, and then replace the PBS with warm growth medium). Do lamella extend when cytochalasin D is removed from the culture?

**Note on cell health:** these cultured cells grow best at $37^0\text{C}$. After you have examined a dish of cells for a while (~15 min), you might place the dish on the warming tray, or in the incubator, for a bit (the media will warm back up in ~5-10 min). Alternatively, you can remove the media and replace it with fresh media that we will have in the $37^0\text{C}$ incubator. When you add fresh medium containing inhibitors, use pre-warmed medium.

Data analysis: at the end of the day Monday, you will have sequences of cells extending and retracting lamellapodia for control cells and cells treated with various inhibitors. You will have data for three types of cells, and images of the actin cytoskeleton (GFP-actin and GFP-$\alpha$-actinin, a protein that binds to actin, and localizes to focal contacts). You should decide how you might want to display your findings. You can measure the rate that lamella extend, using the tools in ImageJ. You can measure the change in the area of the lamella from one frame to the next (for example for images taken a few minutes apart). You can probably think of other parameters that you can quantify. In fluorescence images, you can quantify the fluorescence of focal contacts and how this changes over time.

On Wednesday, complete any observations that you could not finish on Monday. Prepare some movie sequences, or image sequences that your group can share with the class.