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ROLE OF THE CYTOSKELETON IN CELL LOCOMOTION.

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INTRODUCTION: Understanding the consequences of cell locomotion is central to understanding embryonic development and most of the body's healing processes as well as many disease conditions such as cancer. However, the role of cell locomotion in these processes is very complex and we don't yet even fully understand how cells move - though there has been rapid progress in understanding the molecular interactions that underlie cell movement. One problem that confronts us is the large variability between individual cells and the large variation in motile characteristics with time. Another is that the main supramolecular structures involved in cell movement and are not stable but are constantly disassembling, relocating and re-assembling: sometimes with a lifetime of only a few seconds. Our strategy is to develop new methods of microscopy and imaging to enable data from large numbers of cells to be gathered automatically and analysed by computer and to reveal the molecular activity within living cells as they move around.

METHODS: DRIMAPS (Digitally Recorded Interference Microscopy with Automatic Phase Shifting) is a technique we developed at the Randall Institute that that enables growth and motility data from large numbers of cells to be gathered automatically and analysed by computer. The images that it produces are calibrated maps of the distribution of cellular material (dry mass) within all cells in the field of view.

We have recently developed a method called FLAP (Fluorescence Localisation After Photobleaching) which uses laser light to label any specific protein molecule at any chosen location in the cell and then allows us to follow the fate of these labelled molecules as they relocate and participate in new structural configurations. Microinjection or transfection were used to introduce cDNA fusion constructs of β-actin with cyan (ECFP) and yellow (EYFP) fluorescent protein into cells. A laser-scanning confocal microscope allowed rapid localised bleaching and subsequent near simultaneous acquisition of cyan and yellow FP signals. Multi-channel detection allows the simultaneous acquisition of transmission phase contrast images to show cell morphology and interference reflection images to visualise areas of close contact between the cell and the substrate.

RESULTS: The FLAP method is beginning to tell us how cells protrude or push out a process which is the first phase in enabling them to crawl to a new location. The chief cytoskeletal protein involved in this process has long been known to be actin but the mechanism by which unpolymerised actin is transported to the leading edge has been poorly understood. FLAP was used to study actin dynamics within fibroblast-like cells and various malignant cell types. It has allowed us to follow both the fast relocation of monomeric G-actin and the much slower dynamics of filamentous F-actin simultaneously in living cells. We show several different behaviours of actin within the cell specific to different cell morphologies. By visualizing the ratio of bleached-to-total molecules, we found that actin was delivered to protruding zones of the leading edge at speeds exceeding five micrometers per second. Monte-Carlo modeling confirmed that this flow cannot be explained by diffusion and must involve active transport. Using fluorescence bleaching experiments, we have also discovered that actin polymerisation is confined to a very narrow band within 1 µm of the leading edge. We are now combining fluorescent methods with the DRIMAPS method to obtain estimates of the relative actin concentrations in different regions of the leading lamella.

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