528g Dynamic Relationships among Pi 3-Kinase Signaling, Contact Area Spreading, and Cell Polarization Following the Attachment of Fibroblasts to Surfaces

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Cell adhesion and migration are essential steps in numerous biological processes such as wound healing. Cell spreading and migration involve both surface attachment and cytoskeletal reorganization, leading to membrane extension and ultimately cell polarization, processes which are stimulated and coordinated through intracellular signal transduction pathways mediated by adhesion and chemoattractant receptors. In fibroblasts and many other cell contexts, activation of the phosphoinositide (PI) 3-kinase pathway is strictly required for motility. PI 3-kinases generate specific 3' PI lipid products, which act as membrane second messengers, and the spatial pattern of 3' PI density in the membrane is thought to control the directionality of membrane protrusion and cell migration. As a step towards an integrated understanding of fibroblast migration, we have quantitatively followed the time course of PI 3-kinase activation, membrane spreading, and morphological polarization of mouse fibroblasts following their initial attachment to a surface. Two imaging modes, total internal reflection fluorescence and epifluorescence microscopies, were used in conjunction with fluorescent 3' PI and nonspecific membrane probes to evaluate the relationships among these processes for various plating conditions. We report the following findings: 1) spontaneous PI 3-kinase activation generally foretells the dramatic acceleration of cell spreading rate at localized regions of the contact area; 2) cells plated on fibronectin versus poly-lysine show similar qualitative behavior in this respect but spread at different rates; and 3) stimulation with platelet-derived growth factor (PDGF), a chemoattractant for fibroblasts during wound healing, enhances the overall PI 3-kinase signaling and cell spreading rate.