# Dynamics of key signaling molecules during gradient sensing in the social amoeba Dictyostelium

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**The fundamental process(es) underlying gradient sensing in eukaryotic chemotaxis are poorly understand, though many of the molecular components have been identified. In this study, we quantitatively follow the dynamic localization of these components during gradient sensing with the goal of understanding how cells process information about a chemoattractant gradient and decide in which direction to move. To facilitate fluorescence confocal imaging of live cells, we have developed microfluidic devices that flatten cells, while providing precise stable gradients that can be rapidly switched on/off and reversed.**

***Keywords*** **—eukaryotic chemotaxis, microfluidics**

Cells respond to a variety of secreted molecules by modifying their physiology, growth patterns, and behavior. Motile bacteria and eukaryotic cells can sense extracellular chemoattractants and chemorepellents and alter their movement. In this way fibroblasts and leukocytes can find their ways to sites of injury and cancer cells can home in on sites that are releasing growth factors. Upon starvation, the social amoeba Dictyostelium initiates a developmental cycle and becomes highly chemotactic to cAMP. Dictyostelium is a genetically tractable and well-studied model system for eukaryotic chemotaxis and can respond to a difference as little as 1% in the concentration of cAMP across a cell. However, the fundamental process(es) underlying gradient sensing and the exquisite sensitivity of these cells are still not understood.

Many of the components of the gradient sensing network in Dictyostelium have been identified, but what is missing is an understanding of how the components interact to process information about the gradient. In this study, we use a combination of microfluidics and confocal fluorescence microscopy to follow the dynamic localization of key signaling molecules in response to controlled spatiotemporal gradients to infer their interactions. Several genetic lines of evidence indicate that activation of the small G-protein, Ras, by ligand bound receptors is an early step in gradient sensing [1]. We study the localization of RasGTP using the Ras Binding Domain of Raf1 linked to Green Fluorescent Protein [2]. By similarly tagging other key signaling molecules with Red Fluorescent Protein, we correlate the dynamics of pairs of signaling molecules in living cells.

Following the dynamic localization of signaling molecules using fluorescence confocal imaging is limited in highly motile cells, such as Dictyostelium, by the requirement to take many confocal sections to fully capture movement in the z direction and the resulting problem of phototoxicity. To facilitate fluorescence confocal imaging of live cells over extended periods of time, we have developed microfluidic devices that flatten cells, so that they can be imaged in a single confocal plane, while providing precise stable gradients that can be rapidly switched on/off and reversed [3].

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