Feedback Loops at the Level of Lipid Metabolism Enhance Sensitivity and Robustness in Models of Chemotactic Gradient Sensing

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Short Abstract — **The phospholipase C (PLC)/protein kinase C (PKC) signaling pathway is required for chemotaxis of fibroblasts biased by a gradient of platelet-derived growth factor (PDGF), as during wound healing. Experiments further demonstrated that abundance of the lipid intermediate, diacylglycerol (DAG), is polarized in a shallow PDGF gradient. To identify mechanisms capable of amplifying the sensitivity of this signaling pathway, reaction-diffusion models were formulated, and simulations show that inclusion of putative feedback loops at the level of lipid availability and metabolism yields a polarization circuit that is both sensitive and robust to varying gradient conditions. Thus, we offer a framework for understanding chemotactic gradient sensing in fibroblasts and for designing experiments to reveal and characterize sources of nonlinearity.**

Keywords — **Reaction-diffusion modeling, cell signaling, polarization, chemotaxis, wound healing**

I. Background

In fibroblasts responding to gradients of platelet-derived growth factor (PDGF), an important chemoattractant in development and wound healing, signaling through the phospholipase C (PLC)/protein kinase C (PKC) pathway proved necessary for chemotaxis, whereas pathways that collaborate to activate the Arp2/3 complex were found to be dispensable [1,2]. PKC is activated through its binding to the lipid second messenger diacylglycerol (DAG), which is formed from hydrolysis of phosphatidylinositol (4,5)-bisphosphate (PIP_2) by PLC. Strikingly, in fibroblasts exposed to a shallow PDGF gradient, the density of DAG in the plasma membrane is focally enriched at the up-gradient leading edge [1], suggesting an internal amplification mechanism is at play.

In previous work, a mechanistic, reaction-diffusion model of the PLC/PKC signaling pathway was developed to identify possible mechanisms responsible for signal amplification [3]. We found that phosphorylation of myrisotylated alanine-rich C kinase substrate (MARCKS) by membrane-localized PKC constituted a positive feedback loop sufficient for local amplification of DAG and active PKC at the up-gradient end of the cell. By itself, the MARCKS feedback only weakly amplifies the signal in shallow PDGF gradients, however [3]. The system also lacks robustness to modest changes in the midpoint concentration of PDGF.

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II. Model Development and Results

The new model includes phosphatidic acid (PA), a lipid intermediate in the metabolism of DAG. PA is interconvertible with DAG by way of DAG kinases and phosphatidate phosphatases. Feedback loops incorporating PA were added to the model based on evidence that PA increases the rate of PIP_2 hydrolysis by stabilizing the recruitment of PLC [4] and that active PKC can enhance the activity of phospholipase D, another enzyme that produces PA [5]. Model simulations show that the MARCKS feedback mechanism synergizes with these new feedback loops for increased amplification even at shallow PDGF gradients and over an appreciable range of midpoint PDGF concentrations. Simulations with variations of parameter values or cell geometry further indicate that this signaling network is a highly sensitive and robust gradient sensing circuit.

III. Conclusion

Experiments will need to be performed, in concert with refinement of our modeling framework, to validate the source(s) of nonlinearity in this signaling pathway. We are currently exploring the effects of the cell's geometry on the polarization of the signaling pathway and assessing the effects of stochasticity on the performance of this system. In the future, this model will be linked to models describing the organization of the actin cytoskeleton and directionality of cell migration for a more comprehensive understanding of how fibroblast chemotaxis proceeds during physiological processes such as wound healing.

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