Geometry Shapes Cell Signaling Network Output

Raquell M. Holmes[1,*] and Leslie M. Loew[1,*]
[1]University of Connecticut Health Center, Center for Cell Analysis and Modeling, 263 Farmington Avenue, Farmington, CT 06032-1507, USA
*Correspondence: rholmes@uchc.edu (R.M.H.), les@volt.uchc.edu (L.M.L.)
DOI 10.1016/j.chembiol.2008.06.001

Through live cell imaging and computational modeling, Neves et al. (2008) demonstrate that molecular interactions alone are insufficient to explain the patterns of MAPK activation: a model must include the cell morphology and spatial distribution of signaling molecules.

Theoreticians have worked to develop models that mathematically test hypotheses about cellular behaviors. It has often been the case that the model is developed independently and then demonstrated to adequately reproduce previous experimental results. This pattern arises from the fact that theory and experiment generally do not coexist in the same laboratory. Exploration of biological questions through the joint approaches of computation and experiment is increasing, but rarely shows the synthesis seen in the work of Neves et al., 2008. To address what can be an otherwise intractable question of the interplay between spatial and biochemical components during cell signaling, the authors of the recent *Cell* paper combine live cell imaging in hippocampal neruons with simulations of signaling networks in realistic cellular geometry.

The intricate architecture of neuronal cells begs the question, what role do these specialized structures play in cellular function? In addition to specializations in cell shapes such as lamellipodia, microvilli, and filopodia, cells also have geometric specialized subcellular structures: endopolasmic reticulum, Golgi, and mitochondria. Each organelle has a unique geometry that is intricately connected to the biochemistry associated with it. With new live-cell imaging techniques and computational frameworks for spatial modeling, we are poised to develop in depth quantitative understandings of the role of cellular geometries on reaction-diffusion systems.

Neves et al. use the Virtual Cell which makes it possible for researchers to bring cellular geometries obtained from experimental data together with reaction networks and solve the resultant partial differential equations (Slepchenko et al., 2003). From multiple detailed spatial simulations, in concert with in vivo imaging experiments, they are able to demonstrate that microdomains (regions of high concentration defined by length and slope of gradient) can be established by a cellular architecture that includes the subcellular distribution (membrane-bound and cytoplasmic) of the reaction network within the complex cellular morphology of a neuron.

Previous reports on the significance of cell shape and size on gradients of molecular substrates have used models to demonstrate that when the opposing reactions in a reaction-diffusion system are spatially separate, a gradient of the substrate can form. For example, when inositol-3,4,5-trisphosphare (IP3) is produced at the plasma membrane and degraded in the cytosol, gradients of IP3 can be produced; these gradients are further exaggerated by the differing surface to volume ratio of neurites and soma in neuronal cells (Fink et al., 1999, 2000). Fink et al. analyzed the consequence of this spatial IP3 gradient on the downstream calcium dynamics by combining model predictions with experimental imaging of neurons containing fluorescent calcium indicators.

Similar effects were predicted in models where kinase and phosphatase are localized, respectively, to plasma membrane and cytosol (Brown and Kholodenko, 1999; Meyers et al., 2006). The gradient for the phosphoproteins is a result of the interplay of diffusion with the rates of phosphorylation and dephosphorylation. Simple analytic shapes were used to show that changes in either cell size (circles with increasing diameters) or shape alter the gradient (Meyers et al., 2006). In both cases, the size of gradients is a function of the relationship between the decay gradient, diffusion coefficients, and cell shape. From these theoretical efforts it became clear that distinct geometries within a cell can be sufficient to create distinct steady-state distributions of phosphoprotein (Brown and Kholodenko, 1999; Meyers et al., 2006).

In the recent *Cell* paper, Neves et al. use loss of FRET with the live cell cAMP sensor ECAP1 (Nikolaev et al., 2004) to study changes in cAMP in hippocampal tissues. The authors begin with an ordinary differential equation model of the biochemical reactions for β-adrenergic receptor stimulation of cyclic AMP in a well-mixed environment. However, reaction networks cannot reproduce the experimental patterns of MAPK activation because they fail to account for diffusive gradients. When the biochemical reaction model is placed within the physical constraints of a realistic cell morphology, a cell tracing or comparable analytic geometry, cAMP microdomains are predicted and can be directly compared to the experimental biosensor images.

By taking into account the spatial distribution of components in plasma membrane and cytosol, the model accurately predicts the presence of microdomains for cAMP while upstream components (the receptor, adenylate cyclase, and Gs) are uniformly distributed. It's worth emphasizing that the cAMP microdomains are formed in the absence of additional physical barriers to uniform distribution of signaling molecules. Since cAMP is an upstream regulator of PKA and MAPK, one could imagine a story in which the initial formation of its microdomain determines the subsequent spatial pattern of downstream signaling molecules (PKA, bRaf, MEK, MAPK, and PTP). However,

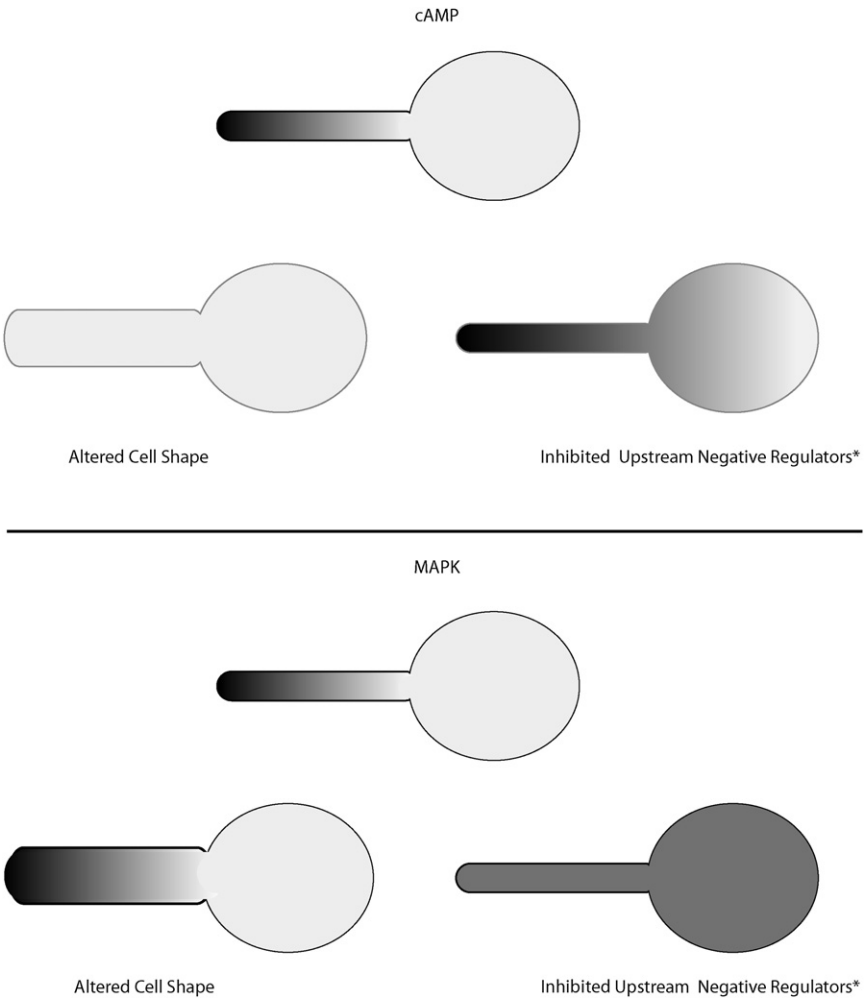

Figure 1. Idealized Cell Shapes Highlight cAMP, Upper Panel, and Phosphorylated MAPK, Lower Panel, Microdomains in Relation to Cell Shape and Upstream Negative Regulators
cAMP gradients are lost in cells with large dendritic diameters (Altered Cell Shape) and significantly modified when upstream negative regulation is lost (Inhibited Negative Regulators), in this case loss of PDE4 regulation. Phosphorylated MAPK maintains its dendritic localization despite the loss of the upstream cAMP gradient (Altered Cell Shape). The activated MAPK gradient is lost when upstream regulators (PDE4, PP2A/PP1, and PTP) are inhibited (Inhibited Negative Regulators).

simulations and experiments indicate that the propagation of ''spatial information'' (a.k.a. the replication of gradient concentrations peaks and slopes) is not transitive to downstream molecules.

Given that the molecules downstream of cAMP are cytoplasmic, diffusion may be one of the key reasons for not having a 1-to-1 relationship between microdomains of the upstream regulator and the distribution of downstream molecular concentrations. Wet lab experiments to vary diffusion rates of molecules would be extremely difficult. The authors use model simulations to alter diffusion coefficients of individual molecules in geometries of different surface-to-volume ratios and predict the behavior of the system. Surprisingly diffusion rates played little role in the establishment of microdomains for the majority of downstream molecules.

If not diffusion, then what? Neves et al. determine that the negative regulators in the network are also responsible for creating boundaries to signaling molecules. It has long been shown theoretically that the balance between reaction and diffusion can play a role in establishing gradients in biological systems (Turing, 1952). The balance of production and consumption, activation and inactivation, establishes a steady state affected by the availability of substrates due to diffusion. By disrupting these upstream negative feedback mechanisms in simulations or experiments, the microdomains of the downstream molecules, in this case MAPK, are lost (Figure 1).

Reaction-diffusion systems are given a new setting in the work of Neves et al. Simulations play a central role in predicting testable biological hypothesis on spatial dynamics of signaling molecules. Live cell microscopy both tests hypotheses and provides critical information on the spatial characteristics of signaling events in cellular geometries in vivo. The image information obtained is used to form quantitatively testable hypotheses about the significance of the physical environment on the reaction-diffusion events. The inclusion of realistic geometries puts reaction-diffusion in context and enables researchers to develop more accurate understandings of the dynamics of signaling molecules and their networks.

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