

Watching Signaling in Action: Single Molecule Studies of a Reaction Circuit Involved in Chemotaxis

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Just as you move to the kitchen in the morning when you smell coffee brewing, single-cell animals move to their meal once they sense nutrients. In both cases, you and the amoeboids use surface sensory molecules, such as G protein-coupled receptors, to detect critical nutrients (i.e., coffee), which trigger a series of protein associations and reactions on the inner surface of the plasma membrane that ultimately allow for actin rearrangement and forward movement.

In this article, Ziemba et al. (1) quantitatively describe an early portion of the reaction circuit that propagates chemotaxis of amoeboids using single-molecule fluorescence. Specifically, they focus on four key steps of the reaction pathway (see Fig. 1) that are initiated when the cell commits to forward movement by reconstituting the purified components and quantifying the association and dissociation kinetics as the reaction proceeds. Their analysis begins after external stimuli have mobilized the signaling pathway that activates PKC (protein kinase C). PKC has many targets and one of these is MARCKS (myristoylated alanine-rich C kinase substrate) (2). The unstructured effector domain of MARCKS is very basic (+13) and can

sequester ~3 molecules of the acidic signaling lipid PIP₂ (phosphatidylinositol 4,5 bisphosphate) by nonspecific electrostatic interactions (3,4). It is therefore not surprising that phosphorylation of the PIP₂ binding region of MARCKS by PKC (step 1, Fig. 1), releases it from the plasma membrane while concurrently releasing sequestered PIP₂ in the plasma membrane (step 2, Fig. 1). The released PIP₂ is a target for the key regulatory enzyme, PI3K (phosphatidylinositol 3 kinase), which phosphorylates PIP₂ to PIP₃ (step 3, Fig. 1). PIP₃ then recruits a number of cellular proteins, causing actin rearrangement at the leading

edge of the chemotaxing cell (step 4, Fig. 1). Previously this reaction scheme was purely hypothetical because it was based on measurements of the individual steps, on cell biology studies of cell movement under different physiological conditions and treatments, and on inference (5,6). This study provides clear and quantitative support for the series of concerted events. The authors placed fluorescence tags on purified full-length PKC α and PI3K, the effector domain of MARCKS, and PIP₂ and PIP₃-sensors. They then followed the diffusion trajectories of the species, the activities of the individual enzymes in real time, and the

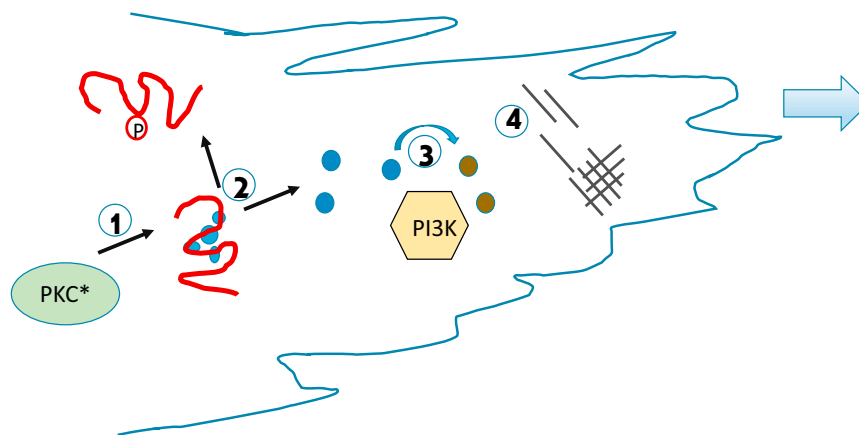


FIGURE 1 Surface view of a plasma membrane of a chemotaxing cell showing the portion of the feed-forward events studied. (1) Activated PKC α (PKC*, green ellipse) phosphorylates the effector domain of MARCKS (red squiggly line) on at least one site resulting in (2) dissociation of MARCKS from the membrane surface and the dissociation of the sequestered PIP₂ (blue circles) into other regions of the plasma membrane. (3) PIP₂ acts as an activator and substrate of PI3K (yellow hexagon) to produce PIP₃ (brown circles), causing a further series of events that results in actin rearrangement (4) and forward movement. To see this figure in color, go online.

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generation of lipid substrates and products, all under buffer conditions that closely mimic the physiological. By direct observation, they find that activated PKC α drives PI3K activity but only in the presence of membrane-bound MARCKS and PIP₂, and that membrane dissociation of MARCKS by PKC α activity short-circuits the reaction.

Considering the combined rates of the reaction pathway, their results support the putative biological pathway (Fig. 1), and pave the way for future studies in cellular systems. In a more general sense, these studies demonstrate that a working concerted biological pathway can be tested using

single-molecule studies to give quantitative support for a proposed biological pathway. Additionally, these studies readily show how the system can easily be short-circuited, like when you finally reach that coffee pot.

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