Published in final edited form as:

Sci Signal. ; 5(213): pe8. doi:10.1126/scisignal.2002897.

Chemoattractant signaling in *Dictyostelium*: Adaptation and Amplification

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Abstract

The social amoebae *Dictyostelium discoideum* has long proved a powerful model organism for studying how cells sense and interpret chemoattractant gradients. Because of the rich behavior observed in its response to chemoattractants, as well as the complex nature of the signaling pathways involved, this research has attracted and benefited from the use of theoretical models. Recent quantitative experiments provide support for a popular model: the Local Excitation, Global Inhibition (LEGI) mechanism of gradient sensing. Here, we discuss these findings and suggest some important open problems.

Introduction

Chemotaxis, the ability to sense chemical gradients and use this information to direct motion, is crucial for the proper function of nearly all organisms. Chemotactic cells show a remarkable ability to interpret shallow gradients, whereby differences in receptor occupancy as small as 1% lead to polarization and subsequent motion up gradient. This precision is attributed to their ability to *adapt* – to adjust their sensitivity in response to spatially uniform changes in chemoattractant concentration – and to *amplify* the small gradient of receptor occupancy. Two recent papers in *Science Signaling* (1,2) combine quantitative experiments using the model chemotactic amoebae, *Dictyostelium discoideum*, in microfluidic chambers, and computer simulations to shed light on these two processes. In doing so, they help to discern amongst competing signaling topologies.

Adaptation

How cells adapt to persistent changes in chemoattractant has been a long standing question in biology that has attracted much interest from theoretical biologists. Two general models have been proposed, and subsequent analysis has shown that these are the only such methods (3). One class of models assumes that adaptation comes about through a negative feedback loop (4). If the feedback is based on the integral of the difference between desired and achieved response – a type of feedback known by engineers as integral control – then perfect adaptation is achieved (5). This property is robust, meaning that it does not depend on precise values of parameters, as demonstrated experimentally in bacteria (6). The second

class of models posits that adaptation results from the action of complementary excitation and inhibition processes that together regulate a response regulator. Because the scheme involves direct connections from receptor signaling to the two complementary processes, it is also known as an incoherent feedforward loop (IFFL). Though not well appreciated, the IFFL topology can be recast as an integral controller feedback loop (7,8), and has its associated robustness properties (9).

Originally proposed to explain the adaptive response to chemoattractants of *E. coli* cells (10), which sense gradients temporally, the model has been modified to explain the static spatial sensing property of *Dictyostelium* and other eukaryotic cells, by assigning differing spatial characteristics on these two processes. The resultant local excitation, global inhibition paradigm (LEGI) is now widely accepted (9,11-13). However, to date, experimental evidence for the LEGI mechanism has been mostly circumstantial. Measurements of the gradient response of *Dictyostelium* cells support one of the LEGI model predictions: the spatial distribution in the steady-state response depends on the ratio between the local and global receptor signals (14). Observations of the spatial distribution of intracellular signals after addition and removal of chemoattractant also corroborate the presence of competing processes that are locally regulated by the receptor signal, and that the excitation is slower than the slow inhibition (14,15).

By measuring the receptor-mediated activation of RasG, Takeda and colleagues have now provided further evidence for the presence of a LEGI mechanism (1). In *Dictyostelium*, one of the earliest downstream signals after chemoattractant binding to G-protein coupled receptors is the activation of Ras proteins (Fig. 1A)(16). This activity can be observed by tracking the fluorescently-tagged Ras binding domain of human Raf1, which binds to RasG, one of the *Dictyostelium* Ras proteins. Ras proteins cycle between active GTP-bound and inactive GDP-bound states (16-18). Activation is regulated by guanine-nucleotide-exchange factors (GEFs), and inactivation is regulated by GTPase-activating proteins (GAPs) that stimulate the hydrolysis of the bound GTP to GDP. Using microfluidic devices to stimulate cells, Takeda et al. demonstrated that RasG activation dynamics closely follow the LEGI-IFFL model (Fig. 1C), under the assumption that the chemoattractant receptor signal stimulates the activity of the RasG-GEF (the excitation) and RasG-GAP (the inhibitor). Importantly, they varied chemoattractant concentration and measured the corresponding temporal dose responses. A prediction of the LEGI mechanism is that the time at which the peak response occurs and the time to adapt will both be shorter as the concentration of chemoattractant is increased. This effect occurs because, in the LEGI mechanism, it is the concentration of the inhibitor that determines the time scale of adaptation. Because receptor occupancy regulates the inhibitor, higher concentrations of chemoattractant lead to higher inhibitor concentrations and faster peak and adaptation times. This prediction can be verified using an interactive applet of the LEGI mechanism, available at <http://stke.sciencemag.org/feature/legi/> (12). The observed RasG activation follows this prediction exactly.

Takeda et al. fit their data to competing models based on LEGI-IFFL and feedback control topologies and showed a better match between simulation and experiments using the LEGI-IFFL model. Though they did not consider the cellular response to chemoattractant gradients, their model fits the spatial requirements of the LEGI model because the

Dictyostelium RasG-GAP, NF1, is globally distributed and the RasG-specific GEF, RasGEFR, is believed to be locally activated.

Amplification

In *Dictyostelium*, Ras protein activation subsequently leads to the production of PIP₃, mediated by transient increases in PI3K activity and a temporally similar loss of PTEN from the membrane (18). Wang and colleagues used the PIP₃-specific biosensor PH_{crac}-GFP to investigate the dynamic response of this downstream system when subjected to changes in chemoattractant concentration in a microfluidic device (2). As previously observed, stimulation with chemoattractant caused transient increases in PIP₃. However, by tracking the response of individual cells, Wang et al. revealed that this increase is seen only in a fraction of cells, and that this fraction increased as the concentration of chemoattractant also increased. Thus, at any given concentration of chemoattractant, two distinct populations were found. These observations point to the presence of a threshold of activation, together with a stochastic perturbation that alters the relationship between the threshold level and the basal/adapted level of the response regulator and thus causes only some cells to respond (Fig. 1D).

Previously, models have been proposed in which the presence of a switch-like circuit downstream of the LEGI module acts to discriminate between sub- and super-threshold levels in the response regulator (9,19,20). This threshold serves as an amplification step that accentuates the spatial differences sensed by the LEGI mechanism and fixes one of its deficiencies: alone, the LEGI mechanism provides no amplification. Wang et al. use such a model to fit their data and find agreement between simulations and experimental results.

Wang et al. also provide further evidence that adaptation is achieved through a LEGI-IFFL scheme, rather than integral feedback. Ramp-like increases in chemoattractant provide a means to discriminate between the IFFL topology of the LEGI mechanism and the integral feedback network. As taught to all control engineering undergraduates, a system that uses an integral control feedback loop that is subjected to a ramp increase in stimulus level will not adapt perfectly, but instead settle to a constant level at steady-state. Experiments in *E. coli* have demonstrated this behavior giving further evidence that bacteria employ integral control (21). In contrast, when Wang et al. stimulated *Dictyostelium* cells with a ramp increase in chemoattractant, cells adapted to this increasing concentration, as predicted by the LEGI model (22).

Conclusion

Together, these two papers provide convincing support that *Dictyostelium* cells sense and interpret gradients using the combination of a LEGI and an amplification step. Because the amplification step was observed downstream (PIP₃) of the RasG signal, it is tempting to suggest that RasG acts as the response regulator in the LEGI mechanism, and that the PI3K signaling circuit as the amplifier (Fig. 1A). However, there are a number of open questions that need to be addressed before we can state this model unequivocally. First, is RasGAP up regulated by changes in receptor occupancy, as required by the LEGI model? At present,

there is no direct evidence for this. As acknowledged by Takeda et al., it is possible that adaptation could occur upstream of RasG, for example, if RasGEF activity were itself adaptive. FRET studies of the G-protein have shown that the receptor-mediated dissociation between G-protein subunits is persistent and does not adapt (23), but there is no current method of testing whether these subunits continue signaling after dissociation (Fig. 1B). Second, does RasG activation possess the same degree of diversity at the single cell level as was observed in PIP₃? If true, it would argue that threshold-dependent amplification occurs upstream of PI3K signaling. One hint that this is not so is the response of cells treated by the actin inhibitor, latrunculin. Previous measurements of the spatial response have shown that amplification is seen even in latrunculin-treated cells, but that the degree of amplification increases greatly with an intact cytoskeleton (14). Takeda et al. found no difference in the response of cells with or without actin demonstrating that at least some of the amplification occurs downstream of RasG. Third, what is the source of the amplification? Recently, we and others proposed models in which a LEGI mechanism senses and interprets the gradient and uses this information to bias the triggering of a downstream excitable network (24-26). In addition to providing great amplification, this scheme accounts for the spontaneous activity of unstimulated cells. Moreover, it can explain some other aspects of the PIP₃ response seen, such as the presence of secondary patches of high activity that occur after chemoattractant stimulus (27). Determining whether such a system is indeed active in cells will also require the combination of careful quantitative experiments coupled to mathematical models reported in both these papers.

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Figure 1. Adaptation and amplification of the chemoattractant response in *Dictyostelium*.

A. chemoattractant cAMP binds to G-protein coupled receptors, causing the G-protein α and $\beta\gamma$ subunits to dissociate in a persistent manner (B). One of the earliest downstream signals is RasGTP activation, mediated by RasGEF and RasGAP. RasGTP activity adapts perfectly (blue line in C), in agreement with a LEGI model in which RasGEF acts as the excitation (green line in C), and RasGAP as the inhibitor (red line in C). Ras activates the PI3K signaling pathway which can be monitored using PIP₃-specific biosensors. The observed behavior suggests the presence of a threshold (dotted line in D). Signals above the threshold elicit responses (dotted line in D); sub-threshold signals do not (dashed line in D).