



# A Cell's Sense of Direction

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## Abstract

In eukaryotic cells directional sensing is mediated by **heterotrimeric guanine nucleotide– binding protein (G protein)**–linked signaling pathways. In *Dictyostelium discoideum* amoebae and mammalian leukocytes, the receptors and G-protein subunits are uniformly distributed around the cell perimeter. **Chemoattractants** induce the transient appearance of binding sites for several **pleckstrin homology domain**–containing proteins on the inner face of the membrane. In gradients of attractant these sites are persistently present on the side of the cell facing the higher concentration, even in the absence of a functional actin cytoskeleton or cell movement. Thus, the cell senses direction by spatially regulating the activity of the signal transduction pathway.



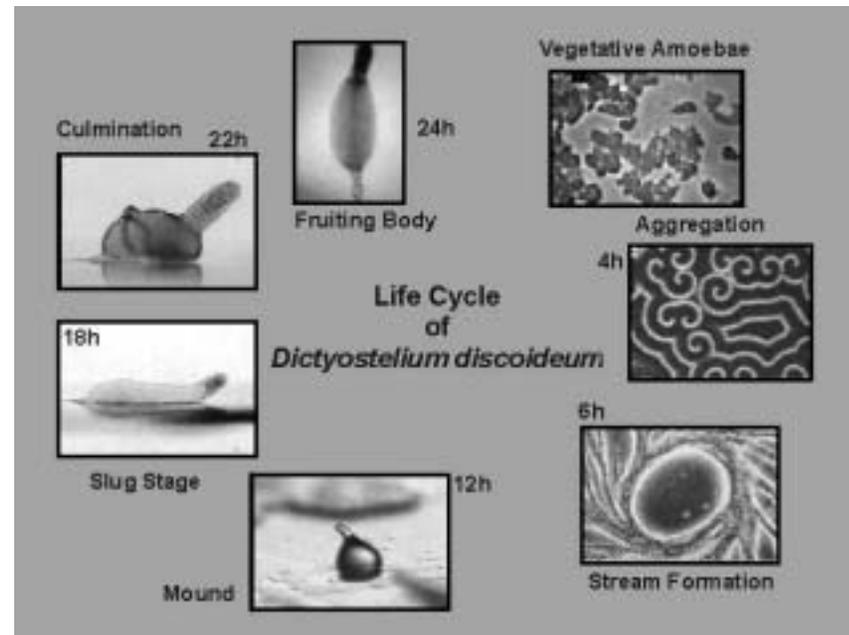
# *Dictyostelium Discoideum*

## Chemotaxis in *Dictyostelium*

- Amoebae hunt down and devour bacteria.
- **When bacteria are gone, amoebae aggregate.**
- After aggregating, cells differentiate.



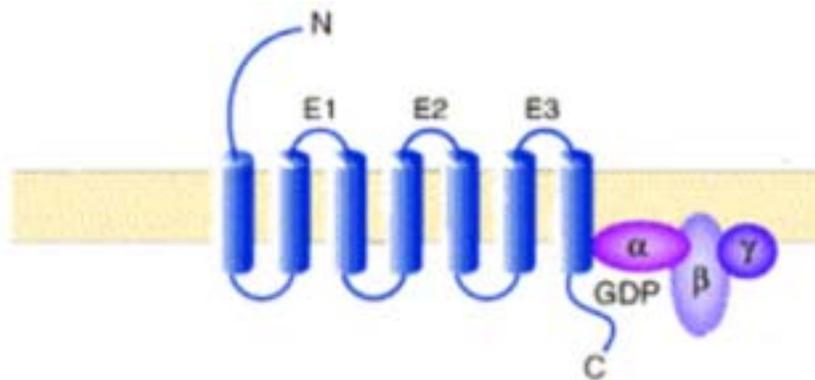
When food is scarce, *Dictyostelium* secretes cyclic adenosine monophosphate (cAMP) and will move towards increasing concentrations of cAMP, resulting in aggregation.





# G-Proteins

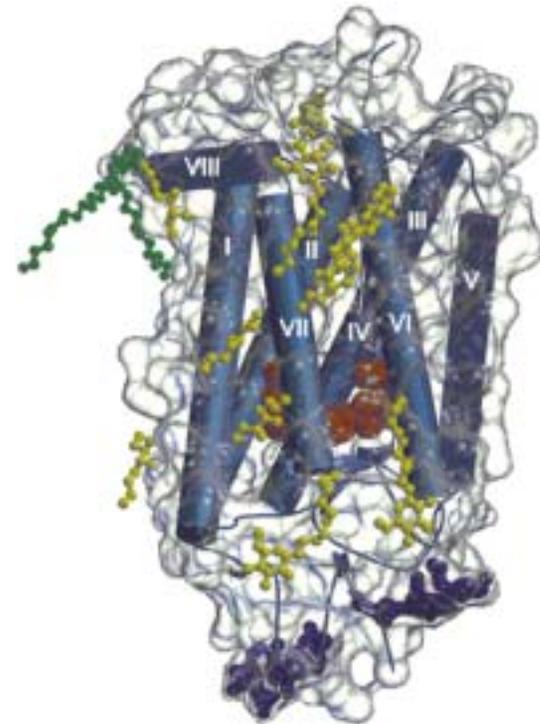
“Heterotrimeric Guanine Nucleotide-binding Protein”



G-Proteins are commonly used to receive chemical signals from outside the cell and initiate a biochemical response inside.

Examples:

- Taste
- Smell
- Adrenaline response

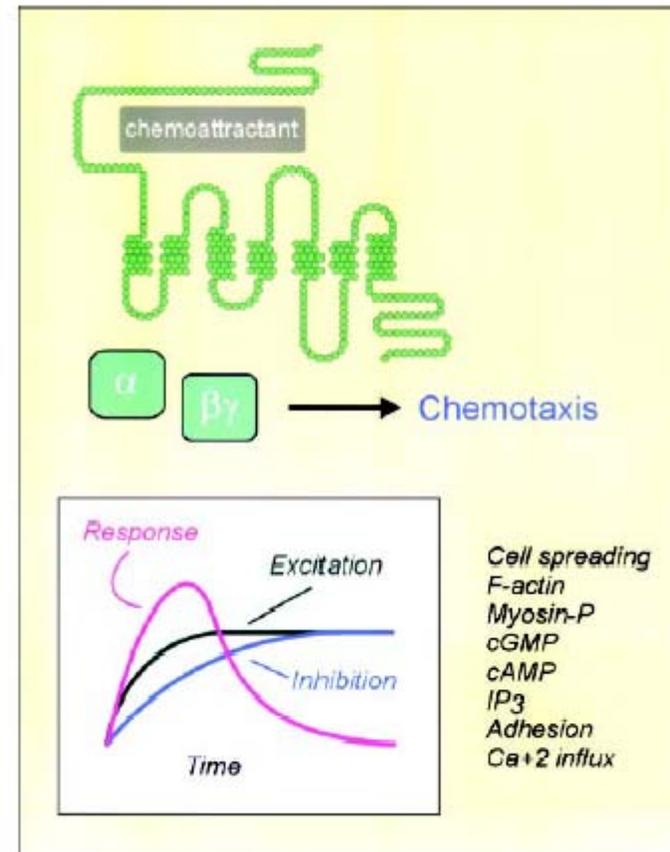


(G-Protein Coupled Receptor)



# Simple Model

- Binding of chemoattractant to the receptor releases the G $\beta\gamma$  subunit.
- G $\beta\gamma$  triggers transient increases in polymerized actin, cAMP, cGMP, Ca<sup>2+</sup> influx, IP<sub>3</sub>, and the phosphorylation of myosin I and II.
- Receptor occupancy initiates competing processes of excitation and inhibition.
- This simple model does not account for many observed responses.





# Characteristics of Chemotactic Cells

- Extreme sensitivity. Concentration differences of 2% between front and back of cell can direct movement.
- Can regulate polarity. Despite uniform concentration of receptors, areas of the cell can become more or less sensitive in response to a gradient.
- Directional sensing is not essential for movement. Take away their ability to sense direction, and they can still move, albeit randomly.
- Movement is not essential for directional sensing. Immobilized cells still try to move in the right direction.

## 2 Types of Directional Sensing Models

Temporal: The cell moves about randomly and samples the concentration at various points.

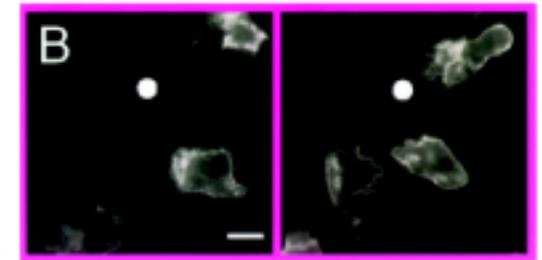
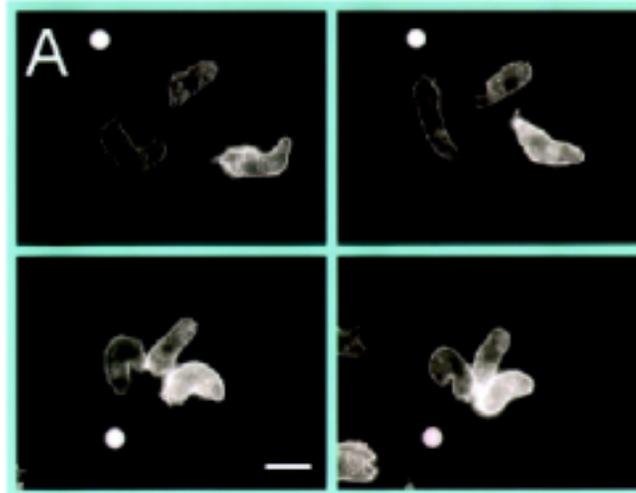
Spatial: The cell simultaneously samples the concentration at various points on its surface.



# Localized Response

At what point in the signaling pathway does the response become localized?

- Receptors and G-proteins do **not** rearrange in response to a gradient. Localization must occur after this step.



Chemoattractant receptors labeled with GFP show up uniformly around the cell perimeter during chemotaxis.

- In contrast, actin and actin-binding proteins do accumulate in the leading edge of a chemotaxing cell. However, the same thing occurs in randomly moving cells (no chemoattractant). Localization must occur before this step.



# Possibilities for Signal Localization

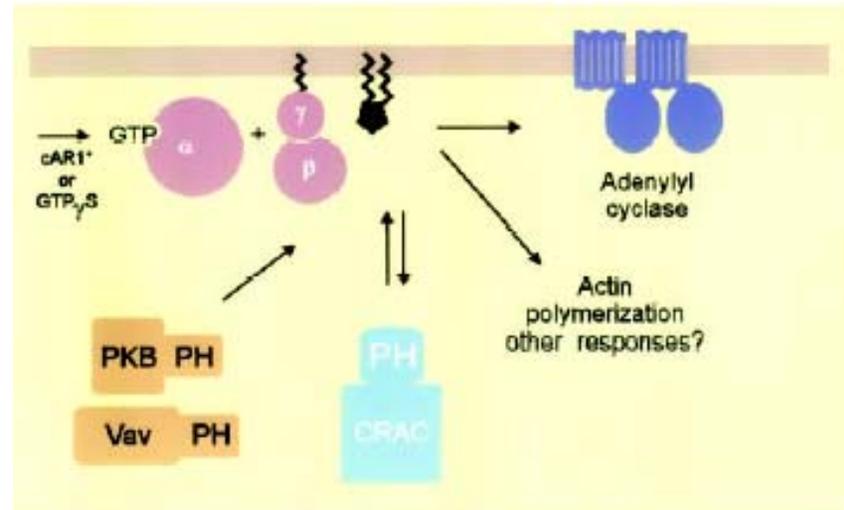
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- Actin remodeling is mediated by small GTPases, which are produced during chemotaxis. Many chemicals linking the G-protein and the small GTPases contain PH domains that could bind with  $\beta\gamma$  subunits or the cell membrane.
- cGMP, which is produced during chemotaxis, can affect the distribution of myosin in the cell. This may localize the construction of the actin cytoskeleton.
- Binding sites for PH domains are highly concentrated on the leading edge of the cell. Since many signal transduction proteins contain PH domains, this may serve to localize the response.

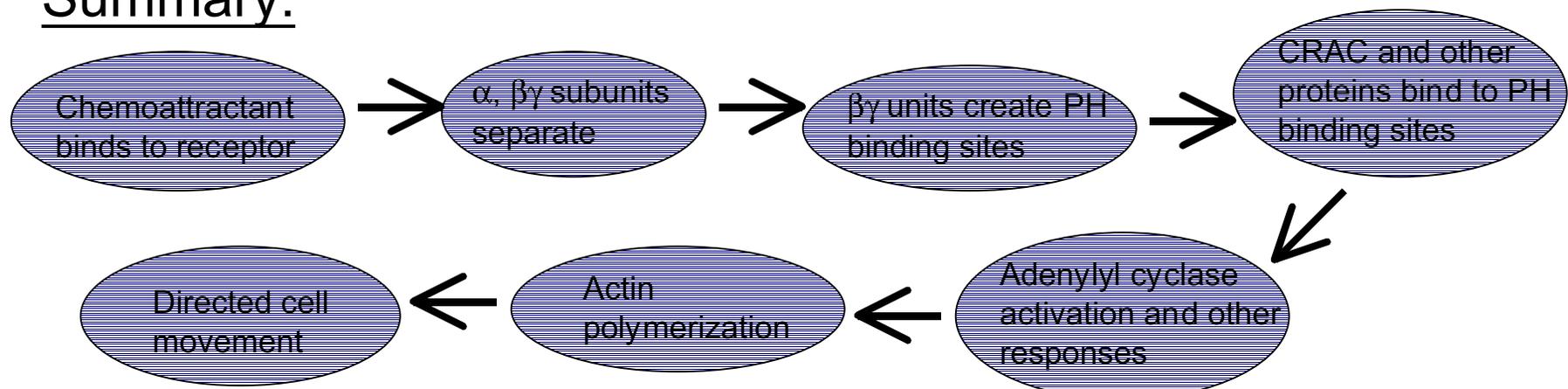


# CRAC Binding

Activation of the G-protein causes the transient localization of the protein CRAC to the cell membrane. This points to rapid generation of binding sites for the PH domain on the interior of the membrane.



## Summary:

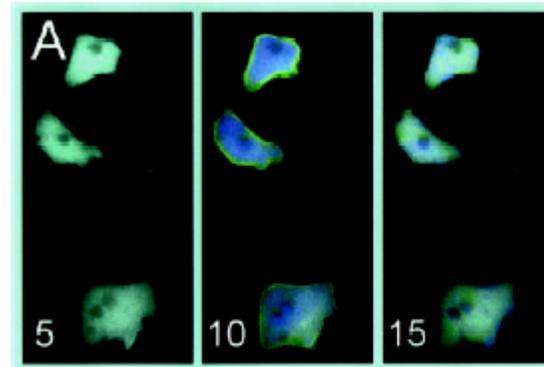




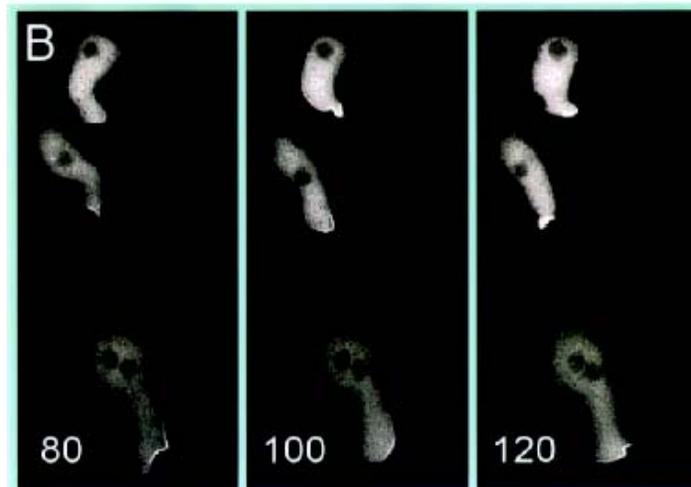
# Visualization of PH Binding

By tagging CRAC or other PH domain-containing proteins with GFP, the spatial localization of G-protein activation can be seen.

- Initially, CRAC-GFP is uniformly distributed throughout the cell.
- The concentration of chemoattractant is uniformly increased, and CRAC-GFP is uniformly bound to the cell perimeter.
- After a few seconds, the CRAC-GFP is once again uniformly distributed.



A gradient of cAMP is applied at the bottom of the frame. Increased concentration of CRAC-GFP is seen at the bottom edge of the cells.





# Immobile Direction Sensing

The localized response of the receptor-G protein pathway still occurs even if the cell is immobile.

Amoebae with CRAC-GFP are treated with a chemical that inhibits actin polymerization. A chemoattractant gradient is established from the bottom center.

GFP is localized as before, but the cells are not moving.



**But wait, wasn't this response supposed to be transient?**



# Persistent Response Model

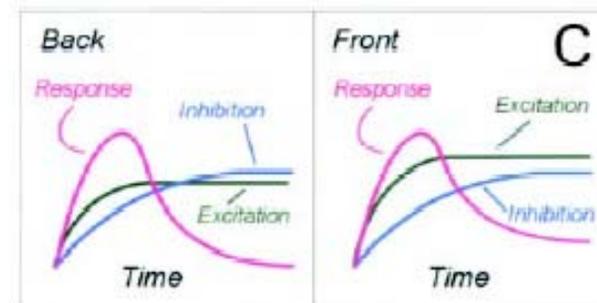
The response to chemoattractant concentration is transient, but the response is persistent in a chemoattractant gradient. This seems to be a contradiction.

## Proposed solution:

- Excitation depends on the **local** fraction of occupied receptors,
- Inhibition depends on the **global** fraction of occupied receptors.

## Example (somewhat hypothetical):

Suppose the enzyme for the generation of binding sites for PH domains is membrane-bound and rapid, thus local, whereas the enzyme for binding site degradation is slow and soluble, thus non-local. After the introduction of a cAMP gradient, there would be an initial transient response everywhere, but once the inhibition kicks in, the back side would be completely inhibited, while the front would remain active indefinitely.





# Conclusions

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- *Dictyostelium* is a useful creature in which to study chemotaxis.
- The signaling pathway for chemotaxis in *Dictyostelium* begins with a receptor-G protein complex and ends with actin polymerization. Somewhere in between, the signal becomes strongly localized.
- The observed behavior can be explained by a model in which excitation is fast and local, and inhibition is slow and global.

Unanswered Questions:

What are the specific chemicals in the signaling pathway?

Are there predictions that can be made to test this global/local model?

How does the “temporal” model of directional sensing improve on this model?