Localization of the G Protein βγ Complex in Living Cells During Chemotaxis

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Gradients of chemoattractants elicit signaling events at the leading edge of a cell even though chemoattractant receptors are uniformly distributed on the cell surface. In highly polarized Dictyostelium discoideum amoebas, membrane-associated βγ subunits of heterotrimeric guanine nucleotide–binding proteins (G proteins) were localized in a shallow anterior-posterior gradient. A uniformly applied chemoattractant generated binding sites for pleckstrin homology (PH) domains on the inner surface of the membrane in a pattern similar to that of the Gγ subunits. Loss of cell polarity resulted in uniform distribution of both the Gβγ subunits and the sensitivity of PH domain recruitment. These observations indicate that Gβγ subunits are not sufficiently localized to restrict signaling events to the leading edge but that their distribution can determine the relative chemotactic sensitivity of polarized cells.

Chemotactic cells can move up shallow chemoattractant gradients, indicating that they can compare and process extremely small differences in concentrations of extracellular stimuli (1). This behavior has been attributed to two characteristics of chemotactic cells. First, the cells have the ability, referred to as directional sensitivity, to spatially localize the activation of G protein–linked signaling pathways (2). This ability, in concert with a broad range of positioning cues, permits cells to choose a direction of movement. Second, chemotactic cells exhibit polarized sensitivity in which the leading edge is the most responsive region to chemoattractants, whereas the trailing end is least sensitive (3, 4). Higher concentrations of stimuli or longer times of exposure are required to elicit pseudopod formation at the rear than from the front. It has been proposed that component(s) of the receptor–G protein system may accumulate at the front of polarized cells, accounting for increased responsiveness to chemoattractants at the anterior (5–8). However, observations of living cells indicate that chemoattractant receptors remain evenly distributed on the surface of highly polarized cells (9, 10). The Gβγ complex is an essential component of the receptor–G protein system required for chemotaxis of leukocytes and Dictyostelium discoideum amoebas (11, 12). Chronic or stimulus-induced clustering of Gβγ to the front surface of a cell could possibly contribute to directional sensing and/or polarized sensitivity. To investigate the molecular basis of these properties of chemotactic cells, we determined the distribution of a G protein β subunit in living cells during chemotaxis.

Green fluorescent protein (GFP) was fused to the NH2-terminus of the unique Gβ subunit of D. discoideum (13). D. discoideum mutants that do not express the Gβ subunit (gβ− cells) are completely deficient in chemoattractant-induced responses and therefore are unable to aggregate and differentiate (12, 14). Stable expression of the GFP-tagged Gβ (GFP-Gβ) subunit rescued the chemotactic and developmental defects of the gβ− cells (Fig. 1) (15). Chemotactoant-induced expression of the early aggregation genes was similar to that of wild-type cells. Activation of adenylyl cyclase by guanosine 5′-O-(3′-thiotriphosphate) (GTPγS) in vitro was restored, and cyclic adenosine 3′,5′-monophosphate (cAMP)-filled micropipettes induced equivalent chemotactic responses in wild-type and rescued cells.

The distribution of GFP-Gβ in the rescued cells was determined during chemotaxis in the...
gradients formed by the micropipettes (Fig. 2). About 70% of the tagged Gßγ subunits were associated with the plasma membrane, and 30% remained in the cytosol (16). The fluorescent signals associated with the membrane were distributed in a shallow anterior-posterior gradient, with a higher concentration at the anterior end. When the micropipette was repositioned, the cells often turned their anterior ends toward the newly established chemoattractant gradient, indicating that these differentiated cells were highly polarized. The distribution of GFP-Gß was compared with that of the chemoattractant receptor for cAMP, cAR1, fused to GFP (cAR1-GFP). In contrast to the polarized distribution of GFP-Gß, the receptors were distributed uniformly along the cell surface (Fig. 3).

As the cells were undergoing rapid morphological changes associated with chemotaxis, the polarized distribution of the membrane-associated GFP-Gß and the uniform distribution of cAR1-GFP were maintained (17).

Activation of G protein–linked signaling systems was visualized in living cells by monitoring the recruitment of a PH domain to binding sites on the inner face of the plasma membrane (3, 4). Recruitment of PH domain-containing proteins to the membrane is an indicator of relative sensitivity to the chemoattractant. Cells expressing the cytosolic regulator of adenylyl cyclase (CRAC) or its PH domain fused to GFP (CRAC-GFP or PHcrac-GFP) were differentiated to the aggregation stage, when cells display strong morphological and behavioral polarity (6, 7). Before stimulation with cAMP, the PHcrac-GFP fluorescent signal was distributed uniformly throughout the cytosol (18). When a low concentration of stimulus was uniformly applied, PHcrac-GFP was preferentially recruited to the inner surface of the membrane at the anterior of the cell and was depleted from the cytosol in the posterior of the cell. Within seconds after stimulation, PHcrac-GFP then diffused back to the cytosol throughout the entire cell (Fig. 4).

Higher concentrations of chemoattractant resulted in a less pronounced asymmetric distribution of PHcrac-GFP and the uniform distribution of GRAC-GFP were maintained (17). The latrunculin A experiments suggest that the anterior-posterior distribution of Gßγ subunits depends on the intrinsic polarization of the cell and does not require prior exposure.
to chemotaxis to gradient, gradients. However, chemotaxis gradients were able to modulate the cellular axis. Although polarized cells often reoriented their anterior ends when micropipettes were repositioned to set up a gradient behind them, they sometimes collapsed their existing anterior ends and gradually reestablished polarity in the new direction. In these cases, the asymmetric distribution of Gβγ also dissolved and reformed on the reversed axis of the cell.

These findings prompt us to amend local excitation/global inhibition models, which have been presented to explain how gradients elicit directed responses as in chemokinesis (22). It also can explain how shallow gradients of chemotactic gradient elicit a response in the wrong direction if the combination of receptor occupancy and G protein concentration dominates on the side of the cell facing the lower concentration of chemotactic gradient. We note that when polarized cells are induced to turn by exposure to a new gradient, PH domains are recruited to the edge that leads cell movement rather than to the edge that faces the new gradient.

References and Notes

13. The coding region of GFP-Gβγ fusion protein was cloned into pMC34, an extrachromosomal expression vector of D. discoideum (12). Gβγ-null cells (gβγ- ) cells were transformed, and individual G418-resistant clones were selected.
15. The following observations suggest that the GFP-Gβγ fusion protein is expressed at a level similar to that of the endogenous Gβγ in wild-type cells. First, comparison with known numbers of cAR1-GFP molecules indicated that there are about 2 × 10^5 GFP-Gβγ molecules per cell. This is comparable to the number of endogenous Gβγ molecules estimated from purification of Gβγ (between 8 × 10^4 and 8 × 10^5 per cell) (23). Second, previous studies have indicated that massive overexpression of Gβγ mRNA does not alter the level of the protein (24). The protein immunoblot analysis with the antibody to Gβ may underestimate the level of GFP-Gβγ because the antibody is directed against the NH2-terminal fusion junction with GFP (14). The GFP-Gβγ appears to be fully functional during the aggregation stage because activation of adenylyl cyclase and chemotactic responses are normal. The functions of the Gβγ in the later developmental stages are likely affected by the GFP fusion because multicellular morphogenesis is somewhat aberrant (25).
16. Subcellular fractionation and protein immunoblot analysis of lysates of wild-type cells showed that about 70% of Gβγ associates with the membrane fraction and the remainder presents in the cytosol (23). Thus, the endogenous Gβγ and GFP-Gβγ distribute similarly. Uniform stimulation of GFP-Gβγ-Gβ γ-cells with cAMP did not alter this ratio or change the distribution of the fluorescent signal.
17. Cells were prepared and observed with conventional and confocal microscopy as previously described (3, 9).
18. A fraction of PHcrac-GFP localized in the nucleus, whereas CRAC-GFP localized exclusively in the cytosol. Nuclear PHcrac-GFP did not redistribute. Other PH domains fused to GFP also localized in nuclei of mammalian cells (V. Kenkateswarlu et al., Curr. Biol. 8, 463 (1998)).
20. An animated version of the redistribution of PHcrac-GFP in response to a uniform increase of chemotactant can be viewed in the supplemental materials (26).
22. This effect may explain the results of Varnum et al. (27), which showed that serial increments in uniformly applied chemotactant increased the rate of random locomotion of D. discoideum amoebas. PH domains are selectively recruited to the leading edge of polarized neutrophils in response to uniform concentrations of chemotactant (H. Bourne and G. Servant, personal communication).
26. Supplemental material is available at www.sciencemag.org/cgi/content/full/04/13355.13.
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Fig. 4. Translocation of PH domains in cells. (A) Translocation of GFP-tagged PH domains to the anterior surface of polarized cells. cAMP (final concentration = 10^{-6} M) was uniformly applied to polarized cells expressing PHcrac-GFP, and the cells were observed by fluorescence microscopy. Numbers in the upper left corner are seconds after the addition of cAMP. In these frames, the fronts of the cells point to the lower right corner of the images. (B) A polarized cell was exposed to uniform concentration of cAMP (final concentration 10^{-6} M), and confocal fluorescence images of PHcrac-GFP were captured at 3-s intervals. False color images were created as in Fig. 3. The front of the cell points to the top left corner of the images. (C) A micropipette containing 1 μM cAMP was placed next to a latrunculin A-rich GFP-GβγGβ γ-cell. As indicated by an arrow, the micropipette was placed at point a for 1 min and then moved to points b and c. (D) A micropipette containing 1 μM cAMP was placed next to a latrunculin A-rich cell expressing CRAC-GFP. As indicated by an arrow, the micropipette was positioned at point a and then moved around the cell to points b, c, and d. For (C) and (D), fluorescent images were captured every 5 s. Numbers in the upper right corners are seconds of selected frames. Calibration bars are 10 μm. Animated versions of (A), (B), (C), and (D) can be viewed on Science Online (26).