PERSPECTIVES: SIGNAL TRANSDUCTION

Signals to Move Cells

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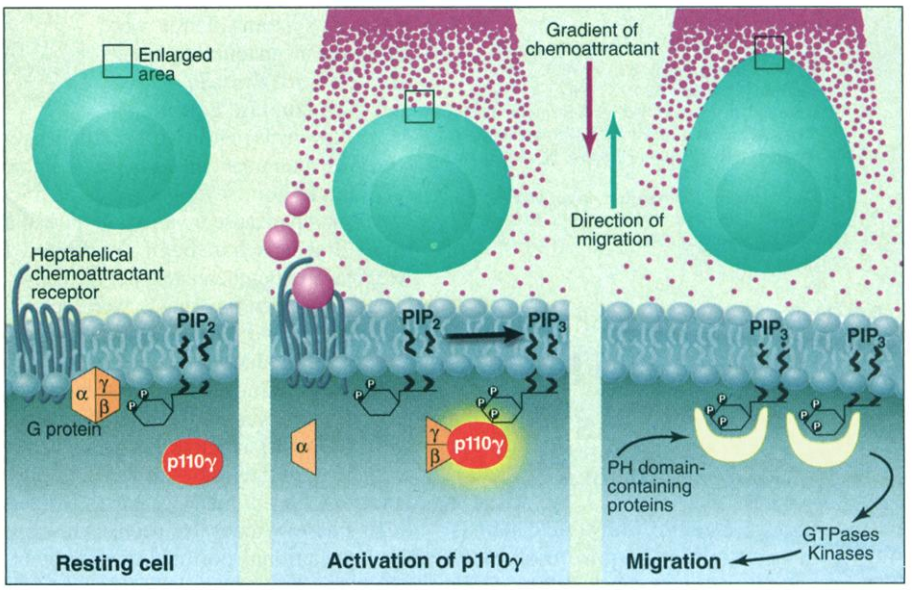
Directed movement along a concentration gradient of chemical attractants is essential for the survival of microbes and for the effectiveness of the immune system's phagocytic cells that pursue and engulf them. This directional motility (called chemotaxis) is governed by chemoattractants such as interleukin-8 (IL-8), C5a, and the formylated peptide *N*-formyl-Met-Leu-Phe (*f*MLP), which bind to receptors on the surface of cells. Chemoattractant receptors belong to the seven-transmembrane helix receptor family. After binding their chemoattractant ligands, these receptors become activated and transmit their signals to heterotrimeric G proteins (see the figure). The G protein complex dissociates into the α subunit and the βγ subunits, which in turn bind and activate target enzymes such as phospholipase C, phosphoinositide 3-kinase (PI 3-kinase) or adenylyl cyclase. These enzymes generate intracellular messengers that initiate a cascade of events culminating in the biological response to the receptor signal. Five papers in this issue of *Science* (*1*–*5*) investigate how PI 3-kinase is involved in heptahelical receptor signaling and chemotaxis. Together they give us new insights into the intracellular machinery that chemoattractant receptors harness to produce their downstream effects.

Phosphoinositide 3-kinase converts the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP_2) into phosphatidylinositol-3,4,5-trisphosphate (PIP_3) (*6*). Multiple PI 3-kinase isoforms, characterized by their catalytic subunits (p110α, β, δ, γ), catalyze this reaction (*6*). Of these, the p110γ catalytic subunit is activated by the βγ complex of heterotrimeric G proteins (*7*), which suggests that it has a function downstream of heptahelical chemoattractant receptors. The others (p110α, β, δ) are activated by tyrosine kinase receptors through an adapter molecule that acts as a bridge between the p110 subunit and phosphotyrosine residues on the activated receptor. This classification of the PI 3-kinase isoforms on the basis of their mode of activation is useful but it should not be taken as absolute because tyrosine kinase–activated PI 3-kinases are in some cases also under the control of seven transmembrane helix receptors [(*8*) and references therein].

The variation in PI 3-kinase isoforms is likely to reflect the different functions of the cells in which they are active. Currently available reagents for investigating p110 function have the drawback that they suffer from crossover effects between isoforms. Li *et al.* reporting on page 1046 (*1*), Sasaki *et al.* on page 1040 (*2*), and Hirsch *et al.* on page 1049 (*3*) overcome this problem by genetically engineering mice to lack the p110γ catalytic subunit of PI 3-kinase γ. With these knockout mice it has been possible to rigorously assign the activation of PI 3-kinase isoforms to different receptor classes and to investigate the role of PI 3-kinase γ in the downstream biological responses to receptor activation.

The three papers convincingly show that leukocytes lacking p110γ are unable to produce PIP_3 in response to activation of their *f*MLP, C5a, or IL-8 receptors. Subsequent signal transduction events known to be regulated by PIP_3 are also impaired in p110γ-deficient cells. For instance, phosphorylation and activation of protein kinase B (PKB), which requires PIP_3, no longer occurs in response to chemoattractants (*2*, *3*). By contrast, in p110γ-deficient cells both PIP_3 production and PKB phosphorylation in response to activation of tyrosine kinase receptors (such as the GM-CSF receptor) were normal. This indicates that these receptors use the α, β, or δ isoforms of p110. Together these observations suggest that seven transmembrane helix receptors engage p110γ (but not the other p110s) to produce PIP_3, and that conversely p110γ is not used by tyrosine kinase receptors.

Loss of p110γ results in changes in the cells of the hematopoietic system. Sasaki *et al.* (*2*) demonstrate that there is reduced thymocyte survival and defective T lymphocyte activation in p110γ-deficient mice. In contrast, the B cell population appeared to be unaffected by the absence of p110γ (*2*). The Li (*1*), Sasaki (*2*), and Hirsch (*3*) groups show that in neutrophils, p110γ mediates activation of NADPH oxidase by the *f*MLP receptor but not by serum opsonin receptors or phorbol esters. The three groups also show that p110γ has a striking effect on the ability of neutrophils and peritoneal macrophages to migrate. Cells deficient in p110γ show a reduction in movement toward a chemoattractant (such as *f*MLP) and a reduced capacity for migration to the peritoneum in various mouse models of peritonitis. Indeed, the migration responses of cells in vivo seemed more severely compromised



Cells on the move are pipped at the post. Asymmetric signal transduction by the catalytic subunit (p110γ) of PI 3-kinase γ. A resting G protein–coupled heptahelical chemoattractant receptor (**left**) binds its ligand (red) and p110γ becomes activated through an interaction with the G protein βγ subunits. The p110γ complex then phosphorylates PIP_2, resulting in the production of PIP_3 (**middle**). PIP_3 is a target for PH domain–containing proteins, which activate kinases and small GTPases (**right**). These are important for the conversion of the initial receptor signal into the migration response.

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than those in vitro implying that p110γ may mediate signals in vivo other than those elicited in vitro.

Two of the groups (*2, 3*) observed that there were greater numbers of neutrophils in the blood of mice deficient in p110γ. This combination of a higher than normal number of neutrophils in the circulation, coupled with their marked inability to escape into the tissues, is reminiscent of a human leukocyte adhesion deficiency syndrome in which the cells lack the adhesion molecule β_2-integrin. Therefore, p110γ might be an important component of the signaling pathways of leukocytes that are activated by selectin or integrin adhesion molecules.

The outcome of an acute infection is to a large extent dependent on the result of the race between the host (to accumulate neutrophils locally) and the microbes (to proliferate). Thus, it is not surprising that the p110γ-deficient animals show a severely reduced ability to clear bacteria from the peritoneal cavity (*3*). Neutrophils and macrophages are also important instigators of the tissue damage produced at sites of inflammation—for example, in acute respiratory distress syndrome or inflammatory bowel disease. Thus, the p110γ catalytic subunit of PI 3-kinase γ might make an attractive anti-inflammatory drug target.

Although these results indicate that p110γ is an important mediator of chemotactic responses, it should be stressed that this function is not unique to this particular isoform. A recent study showed that microinjection of antibodies to p110β and p110δ into a macrophage cell line resulted in reduced cell migration in response to CSF-1, which binds and activates a receptor tyrosine kinase (*9*). Because all of these isoforms lead to the production of PIP_3, this phospholipid molecule can be seen as a general signal for migration responses (see box, below). So, the function of the various PI 3-kinase isoforms is to couple different classes of receptors to their cellular responses through synthesis of PIP_3. PIP_3 binds to pleckstrin homology (PH) domains (*10*). Kinases containing PH domains, such as phosphoinositide-dependent kinase (*11*) and PKB, are known effectors of PI 3-kinase. In the slime mold *Dictyostelium discoideum*, a homolog of PKB is rapidly activated by heptahelical receptors through a PI 3-kinase, and PKB is required for efficient chemotaxis in response to chemoattractants (*12*). This suggests that PKB may be an important regulator of chemotaxis in mammalian cells. Other PI 3-kinase effectors include exchange factors for small guanosine triphosphatases (GTPases). These GTPases are important in the regulation of the cytoskeleton (*13*) and have been implicated in migration and chemotaxis (*14*).

It is interesting to compare the p110γ-deficient phenotype described in the Li, Sasaki, and Hirsch reports in this issue, with that of the recently described mouse lacking the small GTPase Rac2. The similarities are quite remarkable (*15*)—Rac2-deficient animals have a higher leukocyte blood count, and their leukocytes are less able to infiltrate the peritoneum in experimental inflammatory models, and to migrate in vitro. Furthermore, they are severely compromised in their capacity for L-selectin–mediated endothelial rolling (something that was not tested in the p110γ knockout animals). The overlap in phenotypes suggests that Rac2 may be in the same leukocyte sig-

Asymmetric signal transduction

The complex process of directional migration involves not just relocation but also orientation of the cell along the gradient of the chemoattractant (see the figure on page 982). This in turn requires the cell to accurately detect the direction of the chemoattractant and to discriminate between small differences in chemoattractant concentration. A dramatic polarization of the cell occurs in response to a chemotactic gradient; this is reflected in morphological changes and redistribution of many intracellular components (in particular, cytoskeletal components) to the leading edge of the cell (*17*). Two further papers in this issue by Servant *et al.* on page 1037 (*4*) and Jin *et al.* on page 1034 (*5*) investigate the intracellular signals that may be important for this chemoattractant-induced morphological polarity.

Both of these studies commandeered PH domains, which in general act as binding modules for polyphosphoinositides (*10*). The investigators fused the PH domain (from PKB or the cytosolic regulator of adenylyl cyclase) to green fluorescent protein and expressed the fusion protein in HL-60 cells (*4*) or slime mold cells (*5*) so that its distribution could be studied by immunofluorescence microscopy. The localization of the PH domain depended on how the cells were stimulated. A uniform chemoattractant stimulus resulted in the uniform distribution of the fusion protein within the cell membrane. However, a shallow gradient of chemoattractant resulted in recruitment of the PH domain to the area of the cell membrane that faces the stimulus. One way to provoke this distribution would be through the spatially restricted activation of the chemoattractant receptor, resulting in the local activation of a signaling cascade involving the G protein βγ complex, PI 3-kinase (p110γ; see main text), and the generation of PIP_3. PIP_3 in turn would bind the introduced PH fusion protein. In agreement with this model, Servant *et al.* showed that an inhibitor of the heterotrimeric G proteins as well as an inhibitor of PI 3-kinase blocks PH domain recruitment in HL-60 cells (*4*).

Crucially the investigators observed that the intracellular gradient of PH domain recruitment was very steep, much steeper than would be expected on the basis of the shallow extracellular gradient of chemoattractant. That the steep gradient of PIP_3 could be a mechanism by which the receptor signal is converted into directed migration is an appealing idea. The question is now one of identifying the control mechanisms that impinge on the signaling cascade to bring about this steep gradient.

It is possible that the synthesis and breakdown of PIP_3 are regulated in different ways. For instance, PIP_3 synthesis may be governed by local receptor activity/occupancy, whereas its degradation and removal may depend on average receptor occupancy over the whole cell. The net result of such control would be the asymmetric buildup of PIP_3 at the side of the cell nearest the gradient (*17*). The Servant study (*4*) demonstrates that in HL-60 cells, the chemoattractant-induced membrane association of the PH domain is under the control of one or more of the small GTPases: Rac, Rho, and Cdc42. These small GTPases may amplify the initial receptor signal, thus creating a positive feedback loop at the leading edge of the cell. Jin *et al.* (*5*) report that in *Dictyostelium* the G protein βγ subunits themselves demonstrate some degree of polarization and are distributed along the leading edge of the membrane. Although the way in which this happens is not entirely clear, such polarization of signaling intermediates will obviously increase the sensitivity of the cells to stimulation at that end of the cell. These mechanisms may lead to the generation of the steep intracellular gradient of PIP_3 at the cell's leading edge. The asymmetric generation of intracellular messenger molecules such as PIP_3 may be important in directing the cell along the chemoattractant gradient.

naling pathway as p110γ. In vitro studies also imply that Rac is downstream of p110γ because activation of the *f*MLP receptor results in cytoskeletal rearrangements in a pathway involving Gβγ, p110γ, the Rac exchange factor vav, and Rac (*16*). However, the study by Li *et al.* (*1*) suggests that Rac activation still occurs in p110γ-deficient cells. The detailed analysis of possible downstream effector systems in p110γ-deficient mice will no doubt further elucidate the mechanisms involved in the regulation of phagocytic cell migration.

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PERSPECTIVES: EVOLUTION

Is Bigger Better in Cricket?

Pierre Capy

The size of an organism's genome—measured by the DNA content (*C* value) of egg and sperm—varies greatly among species (*1*). However, genome size does not correlate with the amount of genetic information that it contains or with the complexity of the organism (assessed by, for example, the number of different cell types) (*2*). This is called the *C*-value paradox and has puzzled biologists for decades. Now on page 1060 of this issue, Petrov *et al.* (*3*) present a possible solution to this paradox. The investigators provide evidence to show that the long-term accumulation of excess noncoding DNA (and thus total genome size) differs among species owing to the differential rates at which this nonessential DNA is eliminated.

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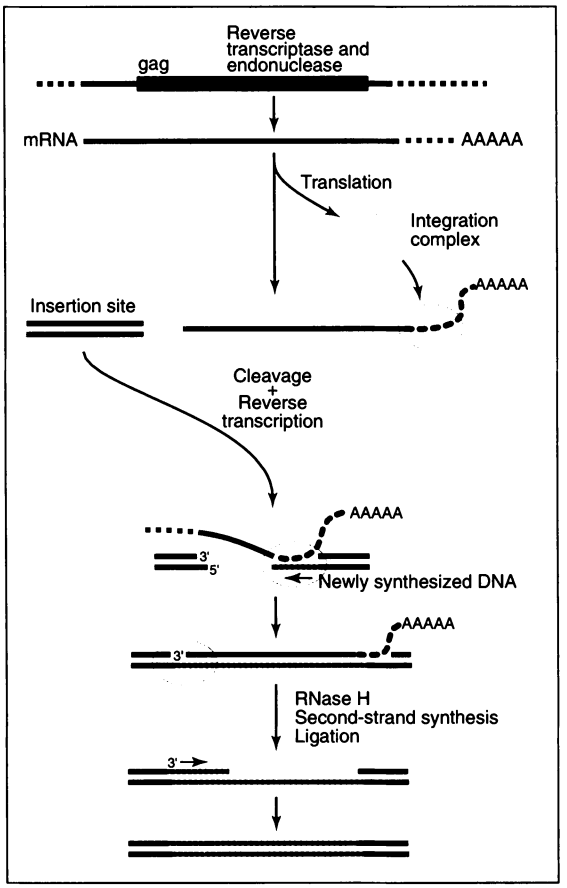
Among the smallest eukaryotic genomes are those of the yeast *Saccharomyces cerevisiae* [14 megabases (Mb)], the nematode *Caenorhabditis elegans* (100 Mb), and the fruit fly *Drosophila melanogaster* (165 to 180 Mb). Yet, the single-celled amoeba, one of the simplest of eukaryotic creatures, has an enormous genome worthy of a whale (>200,000 Mb) (*3, 4*). Plant genomes vary in size from 50 Mb for angiosperms (flowering plants) to 307,000 Mb for pteridophytes (ferns). In animals, genome sizes range from 49 Mb in sponges to 139,000 Mb in bony fishes (*3, 4*). The smallest vertebrate genome is that of the Japanese pufferfish, *Fugu rubripes* (about 400 Mb) (*4*), whereas that of *Homo sapiens* (about 3000 Mb) is fairly typical of mammals (*5*).

Several mechanisms to explain the *C*-value paradox have been proposed. These include partial or complete duplication of the genome, genetic transposition (mobility of transposable elements), retroprocessed pseudogenes, replication slippage, unequal crossing over, and DNA amplification (*4*). Although larger genomes do not contain more genes than smaller genomes, they do contain more repetitive DNA (that is, sequences present in multiple copies) (*5, 6*). Retrotransposable elements (which move within the genome as RNA intermediates transcribed into DNA by reverse transcriptase) are one of the most abundant classes of repetitive DNA. These so-called retrotransposons are estimated to account for more than 50% of the total genome of maize (*Zea mays*) and the crucifer *Arabidopsis thaliana* (*7*). Of the 280 kb between the *alcohol dehydrogenase* and *u22* genes in maize, about 60% is a jumbled mixture of retrotransposable elements (*8*).



Sneaking in the back door. Mechanism by which a non-LTR retrotransposable element (LINE) moves within the genome. The LINE is transcribed into mRNA (red). A part of this mRNA is translated into proteins involved in the integration complex, which binds to the 3′ end of the mRNA transcript. The target site (blue) is cleaved followed by reverse transcription, with the 3′ end of the target site as the primer. Newly synthesized cDNA is shown in pale green. Ligation of the cDNA occurs at the 5′ end, and the second strand is synthesized using the first cDNA strand as template and the host DNA polymerase. [Redrawn from (*12*)]

From this kind of example, it is clear that genome size may increase because of multiplication of retrotransposable elements, but what is the long-term fate of this extra "junk" DNA? By investigating the fate of non-LTR (long terminal repeat) retrotransposable elements in the cricket *Laupala* and fruit fly *Drosophila,* Petrov and colleagues provide evidence for differing rates of "junk" DNA elimination. In an evolving genome, non-LTR elements are thought to proliferate by amplification of an extremely small number of "master" genes. These genes usually give rise to inactive copies (truncated at the 5′ end) that are incapable of further transposition within the genome (*9*). The defective copies arise because of their mode of transposition through reverse transcription (see the figure), which in most cases stops replication before the 5′ end is reached. These truncated elements, called DOA ("dead on arrival"), can be used as surrogates for pseudogenes in species

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