chromes and how similar will either be to the light-dependent redox reaction mediated by photolyases? Darwin, surprisingly uninterested in his blue light experiment described above, would surely be excited by these latest findings.

Note added in proof: A recent report (30) confirmed and extended earlier observations concerning the role of cryptochromes in mammalian circadian rhythms.

References and Notes
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39. J. Kissinger for valuable assistance in the phylogenetic analysis. We are also indebted to N. Bonini, M. Bucan, F. Daldal, E. Fichersky, A. Sehgal, and M. Jorns for their reading of the manuscript and for helpful suggestions.

REVIEW

A Cell’s Sense of Direction

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In eukaryotic cells directional sensing is mediated by heterotrimeric guanine nucleotide–binding protein (G protein)–linked signaling pathways. In Dictostelium discoideum amoebae and mammalian leukocytes, the receptors and G-protein subunits are uniformly distributed around the cell perimeter. Chemotaxants 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.

All living cells can sense their environment. The term “directional sensing” refers to the ability of a cell to determine the direction and proximity of an extracellular stimulus. Directional sensing is needed to detect morphogens that control differentiation and attractants that direct cell migration, as in chemotaxis. This fascinating response is critical in immunity, angiogenesis, wound healing, embryogenesis, and neuronal patterning. Chemotaxis is strikingly exhibited during the life cycle of the social amoebae, D. discoideum (1). During growth, these cells track down and phagocytose bacteria. When starved, they move toward secreted adenosine 3′,5′-monophosphate (cAMP) signals, form aggregates, and differentiate into spore and stalk cells. The fundamental role of chemotaxis in this simple eukaryote provides a powerful system for its genetic analysis. Recent observations in D. discoideum, as well as in yeast and mammalian leukocytes, have clarified views of directional sensing. In this review, we focus on the signal transduction events involved in gradient detection. Other important aspects of chemotaxis, such as the regulation of adhesion, motility, and cell shape, have been reviewed and will not be discussed (2).

Both leukocytes and amoebae use G protein–linked signaling pathways to detect chemotaxants (Fig. 1). Binding of the attractants to receptors of the seven–transmembrane helix class leads to the dissociation of the G proteins into α and βγ subunits. It is likely that chemotaxis is mediated through the βγ subunits. In both leukocytes and amoebae, chemotaxants elicit rapid and transient increases in Ca2+ influx, in the intracellular messengers inositol 1,4,5-trisphosphate (IP3), cAMP, and guanosine 3′,5′-monophosphate (cGMP), and in the phosphorylation of myosins I and II. Chemotaxants also induce actin polymerization, most likely through the activation of the Rho family of small guanosine triphosphatases (GTPases). All these events rapidly subside in the presence of persistent stimulation. This rapid inhibition may allow a migrating cell to “subtract” the ambient concentration of attractant and more accurately sense the direction of a gradient.

Models of chemotaxis should take into account the following behaviors of chemotactic cells (3). First, chemotactic cells are extremely sensitive. The accuracy of chemotaxis depends on the relative steepness of the gradient rather than the mean concentration of the attractant, and concentration differences as low as 2% between the front and the back of the cell can direct movement (4). Second, cells can regulate polarity. Although they display sensitivity at all points on their perimeter, when amoebae are oriented by...
Conserved mechanisms of directional sensing in eukaryotic cells. Directional sensing is mediated by receptors that activate heterotrimeric G proteins (30). The Gβγ subunits trigger various biochemical responses (31). In amoebae and leukocytes, addition of chemoattractants gives rise to transient increases in the proportion of polymerized actin, the production of cAMP, cGMP, Ca2+ influx, and IP3, the phosphorylation of myosin I and II, as well as rapid changes in cell shape and adhesion (1, 3, 32). The inset depicts how receptor activation leads to transient responses. It does not account for the complex kinetics for many of the responses. Receptor occupancy regulates two counteracting biochemical processes: excitation and inhibition. Whereas excitation rapidly peaks and plateaus, inhibition rises slowly. Both processes eventually reach an activity specified by the fraction of occupied receptors, and the response shuts off. Further increments in receptor occupancy will trigger additional transient responses. When the stimulus is withdrawn, the level of inhibition declines and the cell regains sensitivity (33).

Fig. 1. Conserved mechanisms of directional sensing in eukaryotic cells. Directional sensing is mediated by receptors that activate heterotrimeric G proteins (30). The Gβγ subunits trigger various biochemical responses (31). In amoebae and leukocytes, addition of chemoattractants gives rise to transient increases in the proportion of polymerized actin, the production of cAMP, cGMP, Ca2+ influx, and IP3, the phosphorylation of myosin I and II, as well as rapid changes in cell shape and adhesion (1, 3, 32). The inset depicts how receptor activation leads to transient responses. It does not account for the complex kinetics for many of the responses. Receptor occupancy regulates two counteracting biochemical processes: excitation and inhibition. Whereas excitation rapidly peaks and plateaus, inhibition rises slowly. Both processes eventually reach an activity specified by the fraction of occupied receptors, and the response shuts off. Further increments in receptor occupancy will trigger additional transient responses. When the stimulus is withdrawn, the level of inhibition declines and the cell regains sensitivity (33).

Fig. 2. Uniform distribution of chemoattractant receptors during chemotaxis. (A) Endogenous chemoattractant receptors in D. discoideum amoebae were replaced by a cAR1-GFP fusion construct. Green fluorescence was observed uniformly around the cell perimeter and covering the finest pseudopods and filopods. Cells were exposed to a gradient formed by a cAMP-filled micropipette. The upper two images were captured at 15-s intervals. The pipette was repositioned and the lower two images were captured at 15-s intervals. An animated version of this figure can be seen at Science Online (34). (B) Chimeric C5a-GFP receptors were ectopically expressed in differentiated PLB-985 cells. Green fluorescence was observed uniformly around the cell perimeter and covering the finest lamellipodia and filopods. Cells were exposed to a gradient formed by a ChaCha (NH2-Met-Phe-Lys-Pro-dCha-Cha-dArg)-filled micropipette (Cha, cyclo-hexylalanine). The two images were captured at a 154-s interval. The white dot indicates the position of the micropipette tip. [Reprinted from (35) with permission of the American Society for Cell Biology.] Bars, 10 μm.
membrane, covering the finest filopods and pseudopods. The receptors do not redistribute during random or chemotactic movements, even when the cells are induced to reverse direction or turn by repositioning of a micropipette filled with the chemoattractant cAMP (Fig. 2A). The complement 5a (C5a) receptor fused to GFP expressed in a monocyte cell line behaves similarly. Again redirecting the chemotactic movements of the cells by repositioning a chemoattractant micropipette does not alter the surface distribution of the receptors (Fig. 2B). Moreover, when either cell type is exposed to a high concentration of chemoattractant, a condition that leads to receptor desensitization, the receptors remaining on the surface do not cluster. In view of the active membrane rearrangements taking place in these phagocytic cells, it is remarkable that the chemoattractants taking place in these phagocytic cells in the absence of chemoattractants. As was observed with the chemoattractant receptors, a GFP-GRP fusion protein expressed in Gβ-null cells remains evenly distributed in chemotactically oriented cells (12). The uniform distribution of receptors and G proteins is consistent with the fact that both amoebae and leukocytes are chemotactically responsive at all points on their perimeter. The dispersal of the upstream components of the sensory apparatus to all points on the cell surface is ideally suited for directional sensing through a spatial mechanism.

In contrast to receptors and G proteins, actin and the actin-binding proteins filopodin (a talin homolog), coflin, coronin, and cyclase-associated protein (CAP) transiently accumulate in pseudopods at the leading edge of chemotaxing cells (13, 14). However, the same redistribution of these cytoskeletal components also occurs in randomly moving cells in the absence of chemoattractants. Even in cells lacking the Gβ subunit, coronin (and probably the other proteins) localizes to newly extended pseudopods and to the “crowns” of macropinosomes, large, actin-rich membrane ruffles involved in liquid uptake (12). In amoebae, null mutations for these and other cytoskeletal components show defects in motility rather than in directional sensing (14, 15). Taken together with the data on receptors and G-protein β subunits, these observations suggest that localization of the signal for directed movement must occur by selective activation of the pathway upstream of the modification of the actin cytoskeleton.

There are a number of intermediary points at which the response could become localized. First, the small GTPases of the Rho family can mediate actin remodeling (16). In yeast, Far1p links the Rho exchange factor Cdc24 and Cdc42 with G-protein βγ subunits, although it is not known whether this complex is localized (17). In amoebae and higher eukaryotic cells, the steps linking the heterotrimeric and small GTPases could involve proteins that contain both regulators of G protein signaling (RGS) and Rho exchange factor domains (18). Many exchange factors have pleckstrin homology (PH) domains that could promote their association with βγ subunits or membranes (16, 19). Cdc42 and Rac accumulate on phagosomes in a manner similar to coronin’s association with macropinosomes and they may also translocate to the leading edge of moving cells (20). Second, cGMP metabolism can alter the myosin cytoskeleton. In wild-type cells, cGMP promotes translocation of myosin II heavy chain (MHC) and MHC kinase (MHCK) to the cell cortex; ensuing phosphorylation of specific residues on the MHC tail causes it to return to the cytosol (21). Genetic manipulations that lower intracellular concentrations of cGMP or increase MHCK activity maintain a cyto-

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**Fig. 3.** Chemoattractant-mediated recruitment of PH domain–containing proteins. Chemoattractant receptors or GTP-γS [guanosine 5′-O-(3′-triotophosphate)] activate G proteins, generating a binding site for the PH domain–containing protein CRAC. CRAC, together with Gβγ subunits, activates adenylyl cyclase. The Gβγ subunits may be the CRAC binding site or the Gβγ subunits may lead to the synthesis of inositol lipids that are the binding site. PKB behaves like CRAC and may bind the same site. Other cytosolic proteins, such as the Rho exchange factor VAV, may also be drawn to the CRAC binding site and trigger other responses besides the activation of adenylyl cyclase.

**Fig. 4.** Translocation of PH domain–containing proteins to the plasma membrane at the leading edge of chemotaxing cells. Endogenous CRAC of *D. discoideum* amoebae was replaced with a CRAC-GFP fusion protein. Green fluorescence was evenly distributed in the cytosol. (A) Chemoattractant was applied by increasing the injector pressure on the micropipette. The cells respond with uniform translocation of CRAC to the plasma membrane. The colors were obtained by merging frame 1 (in blue) with frames 2 or 3 (in yellow). Values of time in the bottom left corner are in seconds. (B) These three images are from the same series as in (A). The gradient of cAMP was restored, and a polarized CRAC-GFP signal was observed as the cells moved up the gradient. The micropipette was located just outside the bottom of the frames. Values of time in the bottom left corner are in seconds. An animated version of (A) and (B) can be observed at Science Online (34). (C) Wild-type amoebae expressing the PH domain of PKB linked to GFP were placed in the gradient of chemoattractant. As the cells were moving up the gradient (the white dot represents the micropipette tip), the fluorescent signal was observed at the front of the cells. Cell sizes are as described in Fig. 2.
solic distribution of myosin, whereas those that increase the concentration of cGMP or decrease the kinase activity promote persistent association of myosin with the cortex (21, 22). These manipulations impair chemotaxis, suggesting that cGMP, through its effects on myosin, may restrict the activity of the actin cytoskeleton to local sites on the periphery. It is puzzling, however, that a mutant completely lacking myosin II shows more robust chemotaxis than mutants deficient in guanylyl cyclase activity (23). Third, binding sites for PH domains are sharply localized on the stimulated edge of chemotactic cells (9). Because a number of diverse signal transduction proteins contain PH domains, it is possible that the localization of binding sites for these protein modules is an initial event in directional sensing.

Localized Appearance of Binding Sites for PH Domains
In D. discoideum, receptor or G-protein activation causes the transient localization of the cytosolic regulator of adenylyl cyclase protein (CRAC) to the plasma membrane (24). This association of CRAC with membranes is an indication of the rapid appearance of binding sites for the PH domain of CRAC on the inner face of the plasma membrane (Fig. 3) (24). This process can be visualized in living cells expressing a chimeric CRAC-GFP or PH-GFP construct and does not occur in cells lacking the G-protein β subunit (Fig. 4A) (9, 24). Up to 10% of the cytosolic protein binds to the membrane, resulting in a transient depletion in the cytoplasm (9). The PH domain of D. discoideum protein kinase B (PKB or Akt) fused to GFP also transiently associates with plasma membranes after stimulation of cells with chemoattractant (25). Although the identity of the binding sites is unknown, several studies have suggested that PH domains could bind to Gβγ subunits, phosphorylated inositol lipids, or both (19, 26). CRAC-GFP associates with rims of macropinosomes in both wild-type and Gβ-Null cells (9). This suggests that, although the receptor-mediated generation of these sites requires Gβγ, they can also be generated independently from Gβγ and that Gβγ is not itself the binding site.

GFP-tagged CRAC and PKB can be used to visualize the subcellular sites of G-protein activation in chemotaxing cells. As cells move up a gradient of chemoattractant, CRAC-GFP binds selectively to the membrane at the edge of the cell facing the higher concentration (Fig. 4B) (9). The PH domain of PKB displays a virtually identical distribution (Fig. 4C) (25). Thus, the overall localization of PH domain binding sites does not parallel receptor occupancy: The gradients used provide less than a 10% change in receptor occupancy along the length of the cell, yet the CRAC-GFP signal is observed exclusively at the front. Because CRAC is essential for activation of adenylyl cyclase, we conclude that during chemotaxis, activation of the enzyme, which incidentally is also distributed around the cell perimeter, occurs exclusively in that region (9, 27). Activation of PKB is also expected to be spatially restricted. These observations suggest that the activation of the receptor–G protein signaling pathway and all of the downstream responses triggered by chemoattractants are sharply localized at the leading edge of chemotaxing cells.

Several characteristics of the regulation of the PH-domain binding sites deserve further description. First, the intensity of the response does not depend on the distance of the cell from the micropipette tip, suggesting that it is dependent on the relative gradient rather than the absolute concentration of the chemoattractant. Second, in the absence of a gradient or in cells lacking the G-protein β subunit, the newly extended pseudopods are generally unlabeled. Third, whereas the responses to uniform increases in chemoattractant rapidly subside (Figs. 1 and 4A), the responses at the leading edge of the cell are generally persistent (Fig. 4B).

Chemotaxtulent gradients can elicit localized responses of the receptor–G protein signaling pathway in immobilized cells. In certain mutants with greatly diminished chemotactic responses, the intensity of the polarized CRAC-GFP binding is similar to or stronger than that observed in vigorously chemotaxing cells (28). Strikingly, in cells completely immobilized by inhibitors of actin polymerization such as latrunculin or cytochalasin, there is an undiminished polarized response (Fig. 5A) (9). Moreover, as long as the gradient is maintained and cell movement does not perturb the levels of receptor occupancy, the response persists. Only when the gradient is compromised, by removing the chemoattractant or by increasing its concentration uniformly across the cell, does the response subside. These observations demonstrate that a cell can detect a gradient by comparing the concentrations of chemoattractant at each of its ends and that modulation of the actin-based cytoskeleton is not required for this determination.

Sensing Spatial Gradients with “Transient” Responses
Because stimulus increments elicit biochemical responses that are transient, the ability of an immobile cell to detect a static gradient seems counterintuitive (Figs. 1 and 5A).
Imagine suddenly placing a naïve cell into a gradient. Each portion of the cell will experience an increment in chemoattractant concentration that will thereafter remain constant. The front edge will experience a slightly larger increment than the back edge and display a slightly larger response. However, within a few minutes, responses on all portions of the cell are expected to subside because no further increases in stimulus occur. Yet immobile cells not only respond to stable gradients, they respond persistently. Somehow, the presentation of the stimulus as a gradient circumvents the tendency of the responses to adapt. To explain these observations, we favor models proposing that the biochemical activity mediating excitation is local, whereas that mediating inhibition is global (Fig. 5, B and C) (3, 29). As in the excitation-inhibition scheme used to account for transient responses (Fig. 1), each process is closely controlled by receptor occupancy. The fraction of occupied receptors in the immediate vicinity determines the local level of excitation, whereas the average fraction of occupied receptors determines the amount of inhibition in all regions of the cell.

To take a concrete example, consider the regulation of the sites on the inner face of the membrane that bind CRAC and PKB. Suppose the enzyme for synthesis of the sites is membrane-bound and rapidly activated to an extent specified by the local fraction of occupied receptors, whereas the enzyme for degradation of the sites is soluble and slowly activated to an extent specified by the average fraction of occupied receptors. If the cell was exposed to a uniform increase in chemoattractant, the binding sites would rapidly increase and then gradually decrease to the prestimulus value. The downstream responses would follow this time course as depicted in Fig. 1. If the same cell was placed in a gradient of chemoattractant, it would initially respond at all points. But, eventually the rate of synthesis would exceed the rate of degradation in the front of the cell while, in the back, the degradation would exceed synthesis (Fig. 5C). Thus, the binding sites for PH domains and the downstream responses could persist indefinitely at the front of the cell. As is experimentally observed, the polarized response would be relatively independent of the mean concentration of the chemoattractant and depend primarily on the relative steepness of the gradient. The directional response is expected to be extremely sensitive because it depends on the difference of two counter-acting activities, and the back of the cell will always experience an inhibition that is greater than the local activation. This model can account for sensing by immobile and mobile cells where temporal inputs from pilot pseudopods could enhance signal detection. With modifications, it may explain gradual increases in polarity that occur when cells are oriented for a longer period of time and the polarization of neutrophils in uniform concentration of attractant.

These recent observations have answered some old questions but also raised some new ones about directional sensing. Do other signaling events besides activation of adenyl cyclase and PKB, particularly those leading to actin polymerization, occur selectively at the leading edge of chemotaxing cells? Is localization again achieved by recruiting an essential PH domain–containing protein such as an exchange factor (Fig. 3)? If so, the regulation of the appearance of the binding sites would be the key to understand directional sensing. What is the chemical nature of the binding sites for these PH domains? The leading candidates are a variety of phosphoinositide lipids that could be derived from the regulation of a host of enzymes including phosphoinositide 3-kinase. Are there common binding sites or are there multiple, independently regulated distinct sites? Are binding sites for other protein modules also generated at the leading edge? Clearly, the answer to these questions and others should enhance our understanding of the mechanisms by which cells use directional sensing to perform their functions in critical physiological processes such as immunity, angiogenesis, wound healing, embryogenesis, and neuronal patterning.

References and Notes

10. S. van Es and P. N. Devreotes, unpublished observations.
12. In amoebae, there are four cAMP receptors (cAR1 through cAR4) linked to the G protein G1 (1). Double knockouts of cAR1 and cAR3 are completely insensitive to cAMP, expression of cAR2, or cAR3 restores chemotaxis with a characteristic dose curve. Cells lacking the G protein a2 subunit or the unique b2 subunit are incapable of sensing a chemotactrant gradient (6). In leukocytes, there are at least 14 chemokine receptors (CCR1 through CCR8, CXCR1 through CXCR5, and CXCR1) linked to Gz or Gq. Chemotaxis toward chemokines is inhibited by inactivation of Gq with Pertussis toxin (C. Gerard and N. P. Gerard, Curr. Opin. Immunol. 6, 140 (1994); M. Baggioli, Nature 392, 565 (1998). Knockouts of CXCR2 and CCR2 are chemotactically insensitive to specific chemokines, and expression of specific receptors in tissue culture cells confers chemotactic sensitivity to the appropriate chemokines (G. Calcagni et al., Science 265, 682 (1994); J. J. Campbell, S. Quin, K. B. Bacchi, C. R. Mackay, E. Butcher, J. Cell Biol. 134, 235 (1996); L. Boring et al., J. Clin. Invest. 106, 2532 (1997)).
14. The kinetics of these responses vary, peaking within a few seconds to 1 min after the addition of chemotactrant. In some cases, such as in actin polymerization, there is one peak after a few seconds and a second one after 90 s (J. Condeelis et al., Cell Motil. Cytoskel. 10, 17 (1987); R. Meili et al., J. Cell Biol. 95, 133 (1982); E. Albrecht and H. R. Petty, ibid., p. 343.
15. The mechanisms for inhibition of all of the responses are not known. Candidates include the GTPase activity of G proteins, in conjugation with RGS proteins,
**SIGNAL TRANSDUCTION**

Notch Signaling: Cell Fate Control and Signal Integration in Development

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Notch signaling defines an evolutionarily ancient cell interaction mechanism, which plays a fundamental role in metazoan development. Signals exchanged between neighboring cells through the Notch receptor can amplify and consolidate molecular differences, which eventually dictate cell fates. Thus, Notch signals control how cells respond to intrinsic or extrinsic developmental cues that are necessary to unfold specific developmental programs. Notch activity affects the implementation of differentiation, proliferation, and apoptotic programs, providing a general developmental tool to influence organ formation and morphogenesis.

The building of an organism from a single cell to a multicellular three-dimensional structure of characteristic shape and size is the result of coordinated gene action that directs the developmental fate of individual cells. The acquisition of different cell fates orchestrates an intricate interplay of cell proliferation, migration, growth, differentiation, and death, elaborating and bringing together cellular ensembles in a precise manner. Intrinsic, cell-autonomous factors as well as nonautonomous, short-range and long-range signals guide cells through distinct developmental paths. Frequently, an organism uses the same signaling pathway within different cellular contexts to achieve unique developmental goals. How intrinsic and extrinsic factors are integrated in ontogeny to specify cell fates defines the central question of developmental biology.

Notch signaling is an evolutionarily conserved mechanism that is used by metazoans to control cell fates through local cell interactions. The realization that this signaling mechanism controls an extraordinarily broad spectrum of cell fates and developmental processes (in organisms ranging from sea urchins to humans) resulted in a veritable explosion of Notch-related studies in the past decade. Our intention here is not to review all the systems and cellular events that depend on this mechanism, because several reviews adequately cover these many issues (1–7). Instead, we present some general developmental features emerging from collective studies in vertebrate and invertebrate experimental systems as well as consider certain mechanistic aspects of Notch signaling. These studies make it apparent that signals transmitted through the Notch receptor, in combination with other cellular factors, influence differentiation, proliferation, and apoptotic events at all stages of development. Thus, Notch signaling appears to function as a general developmental tool that is used to direct cell fate and, consequently, to build an organism.

**Elements of Notch Signaling**

The gene encoding the Notch receptor was discovered in flies almost 80 years ago by virtue of the fact that partial loss of function (haploinsufficiency) results in notches at the wing margin (8). Notch received its notoriety as a result of classic embryonic analyses of lethal loss-of-function mutations, which were conducted by Poulson (9). These mutations produce a “neurogenic” phenotype, where cells destined to become epidermis switch fate and give rise to neural tissue (10). The Notch gene, first characterized in *Drosophila melanogaster*, encodes a 300-kD single-pass transmembrane receptor. The large extracellular domain contains 36 tandem epidermal growth factor (EGF)–like repeats and three cysteine-rich Notch/LIN-12 repeats. Six tandem ankyrin repeats, a glutamine-rich domain (opa), and a PEST sequence are found within the intracellular domain (11). Notch-like proteins have been identified and extensively characterized in *Caenorhabditis elegans* (LIN-12 and GLP-1) (2, 3), sea urchins, and many different vertebrates, including humans (4, 12). In all animal models tested, mutations in the Notch receptor invariably result in developmental abnormalities and thus, not surprisingly, human pathologies (13–15).

Genetic and molecular interaction studies resulted in the identification of a number of proteins that may participate in transmitting or regulating Notch signals (Fig. 1). From this increasing array of proteins, whose direct relation to Notch signaling is often unclear, a small group of elements emerges as forming the core of this signaling pathway. In *Drosophila*, the two single-pass transmembrane proteins, Delta and Serrate, have been identified as partially redundant Notch ligands (Delta and Jagged in vertebrates, LAG-2 and APX-1 in *C. elegans*) (2, 4, 16). The transcription factor Suppressor of Hairless [Su(H)] (CBF1/RJH in mammals, LAG-1 in *C. elegans*) appears to function as the major downstream effector of Notch signaling, and the genes of the *Enhancer of split* [E(spl)] locus, which encode nuclear basic helix-loop-helix (bHLH) proteins, are primary targets of Notch signaling (1, 2).

The basic picture emerging from many different studies has the extracellular domain of the ligands, expressed on the surface of one cell, interacting with the extracellular domain of the Notch receptor on an adjacent cell. As a result of receptor activation, Su(H) binds to regulatory sequences of the *E(spl)* genes and up-regulates expression of their encoded bHLH proteins (17, 18). The bHLH factors, in turn, affect the regulation of downstream target genes. One well-defined target is the *Achaete-Scute* complex, which contains proneural genes that encode proteins involved in the segregation of neuronal and epidermal lineages (19), a process affected by mutations in *Notch*. There is no doubt that this linear picture is only a skeleton, as we know that each step is embellished with additional elements and features that modulate the activity and efficacy of the signals transmitted through the Notch receptor.

At the extracellular level, the action of the ligands can be influenced by at least one molecule, Fringe (20), but it is quite possible that other extracellular factors capable of in-