Protein Lipidation in Cell Signaling

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The ability of cells to communicate with and respond to their external environment is critical for their continued existence. A universal feature of this communication is that the external signal must in some way penetrate the lipid bilayer surrounding the cell. In most cases of such signal acquisition, the signaling entity itself does not directly enter the cell but rather transmits its information to specific proteins present on the surface of the cell membrane. These proteins then communicate with additional proteins associated with the intracellular face of the membrane. Membrane localization and function of many of these proteins are dependent on their covalent modification by specific lipids, and it is the processes involved that form the focus of this article.

The classic view of membrane-associated proteins is one in which the protein is inserted into the membrane bilayer so that it spans the membrane. Such proteins are synthesized with a sorting signal at their NH_2-terminus, which results in their trafficking through the endoplasmic reticulum–Golgi apparatus pathway on the way to the surface (*1*). However, studies over the past decade in particular have highlighted additional pathways for directing proteins to cell membranes that involve co- or posttranslational modification by specific lipids. This process, termed lipidation, is especially evident when one examines the protein machinery involved in transmembrane signaling. It is now clear that the lipids attached to these signaling molecules play crucial roles in their functions. This article reviews the current status of protein lipidation and its roles in cell signaling. After a brief overview of the types of lipids involved and the mechanisms for their attachment to proteins, the major signaling processes in which these proteins participate will be discussed. The emphasis is on signaling in animal cells, but the contributions to the field from studies on lower eukaryotes cannot be overstated. The reader is directed to several recent reviews that cover these aspects in more detail than is possible here because of space limitations (*2*–*4*).

Classes of Lipidated Proteins

Lipid-modified proteins are classified on the basis of the identity of the attached lipid (Table 1). Each type of lipid used in protein modification has unique properties in terms of its chemistry, which can impart distinct functional attributes to its protein host. Palmitoylated proteins contain a 16-carbon saturated fatty acyl group attached by a labile thioester bond to cysteine residues (*5*, *6*). Although a more appropriate term for this class would be *S*-acylated proteins, because other fatty acyl chains may substitute for palmitoyl (*5*–*7*), they will continue to be referred to as palmitoylated here. The acylation is posttranslational and its lability allows the process to be reversible, a unique property of this modification that gives cells the potential to control the modification state of the protein. The molecular machinery involved in protein palmitoylation has not yet been identified, but there is evidence for enzymes that catalyze both attachment and removal of the lipid (*6*, *8*). In addition, acylation of cysteine thiols can occur nonenzymatically (*5*, *9*), although the physiologic relevance of this process is not yet clear.

Myristoylated proteins contain a saturated acyl group of 14 carbons added by a quite distinct process involving cotranslational modification of the NH_2-terminal glycine residue through amide bond formation. The enzyme responsible, *N*-myristoyltransferase, has been the subject of extensive investigation in regard to substrate specificities and properties (*3*). Myristoylation is generally regarded as a constitutive process resulting in a stably modified protein, although there is at least one report of removal of the lipid from a mature protein (*10*). In addition to a role in targeting proteins to membranes, an attached myristoyl can also serve a structural role in proteins (*11*, *12*). For one of these proteins in particular, the retinal protein recoverin, the myristoyl moiety can apparently "switch" in a Ca^{2+}-dependent fashion between a form that contacts the protein and a liberated form that is available for use in membrane association (*13*).

Prenylated proteins contain one of two isoprenoid lipids, either the 15-carbon farnesyl or the 20-carbon geranylgeranyl. The lipids are attached to cysteine residues at or near their COOH-terminus through stable thioether bonds in a posttranslational process (*2*, *14*, *15*). As with myristoylated proteins, this constitutive process results in a stably modified protein. The molecular details of protein prenylation have recently been elucidated. Proteins containing a cysteine residue fourth from the COOH-terminus (the so-called Cys-A-A-X motif) can be modified by either farnesyl or geranylgeranyl, depending on the identity of the COOH-terminal residue (the X). Specific Cys-A-A-X-motifs are recognized by two closely related cytosolic enzymes, one specific for farnesylation [farnesyltransferase (FTase)] and one for geranylgeranylation [geranylgeranyltransferase I (GGTase I)] (*16*, *17*). Following prenylation, most Cys-

Table 1. Major classes of lipidated proteins. The structure of the lipid and the attachment residue are shown, except for the complex GIP moiety. The subunit to which the lipid is attached is indicated in parentheses after the trimeric G protein.

Lipid	Structure	Representative proteins modified	Position of modification
N-Myristoyl	O N-Gly H	Trimeric G proteins (α) NRTKs	NH_2-terminus
S-Palmitoyl (*S*-acyl)	O S-Cys	Trimeric G proteins (α) GPCRs Ras	Internal, no defined consensus
S-Prenyl	S-Cys Geranylgeranyl S-Cys Farnesyl	Trimeric G proteins (γ) Small G proteins Retinal GRK	COOH-terminus
GPI	Complex structure containing ethanolamine, sugars, and phosphatidylinositol	Many cell-surface proteins	COOH-terminus

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A-A-X–type proteins are directed to a membrane compartment for further processing involving removal of the three COOH-terminal residues and methylation of the now-exposed COOH group of the prenylcysteine (*14*). A second mechanism for prenylation exists for guanosine triphosphate (GTP)–binding proteins of the Rab family, which are involved in membrane trafficking in cells. These proteins are geranylgeranylated at two cysteine residues at or very near the COOH-terminus by a distinct enzyme, GGTase II or Rab GGTase (*18*).

The final class of lipidated proteins are those containing the complex glycosylphosphatidylinositol (GPI) moiety (*4*). As the name indicates, these proteins contain an entire phospholipid with associated sugars and ethanolamine. Proteins are initially directed into the secretory pathway by means of an NH_2-terminal signal sequence, where they acquire the GPI moiety. The GPI moiety is apparently preassembled on the cytoplasmic face of the endoplasmic reticulum and then transferred into the lumen (*19*), where it is attached to a COOH-terminal residue of the protein. The entire procedure, which involves proteolytic processing of the host protein to expose the GPI addition site, is quite complicated and the gene products involved are just now being identified (*20*, *21*). For the purpose of this discussion, the major point is that essentially all GPI-linked proteins are destined for the cell surface.

Signaling Through Trimeric GTP-Binding Proteins

In the majority of signaling events initiated by extracellular ligands such as hormones, neurotransmitters, and growth factors, the binding of these molecules to membrane-spanning receptors results in activation of specific trimeric GTP-binding proteins (G proteins) associated with the inner face of the plasma membrane (see Fig. 1). Members of the trimeric class of G proteins are activated through direct interaction with cell surface proteins termed G protein–coupled receptors (GPCRs). In the activated state, these trimeric G proteins regulate the activities of effector molecules such as cyclic nucleotide-metabolizing enzymes, phospholipases, and ion channels that produce the second messengers involved in producing the physiological response to ligand binding to the GPCR (*22*).

Protein lipidation appears at numerous steps in the process of signaling through trimeric G proteins. Many GPCRs are subject to palmitoylation near their COOH-terminus. In at least one case, that of the β-adrenergic receptor, the palmitoylation state of the receptor is influenced by agonist occupation (*23*). This finding suggests a dynamic role for the lipid modification, which has been implicated in receptor coupling to G proteins and in downregulating activity of a receptor by influencing its removal from the cell surface [(*24*) and references therein]. Another potential role is in the agonist-mediated desensitization of receptors triggered by phosphorylation. Specific receptor kinases, termed GRKs, can phosphorylate agonist-occupied receptors at sites near the COOH-terminus in a way that leads to their uncoupling from G proteins (*25*), and palmitoylation in this region could influence recognition by GRKs. Protein lipidation is also clearly involved in the actions of GRKs themselves. These kinases are typically located in the cytosol but move to the membrane upon receptor activation. Farnesylation of the retinal GRK has been implicated in this process (*25*), whereas translocation of other GRKs is facilitated by their binding the lipid-modified βγ complex (see below) released upon receptor activation of G proteins (*25*, *26*). In addition, at least one GRK is subject to palmitoylation (*27*). These findings highlight the view that distinct lipid modifications can apparently serve similar purposes [see also (*28*)]. Why nature chooses one type over another in particular pathways remains a mystery.

The trimeric G proteins themselves can have multiple lipids attached (*15*, *29*). These proteins are comprised of three subunit polypeptides, termed α, β, and γ. Upon activation, the α subunit exchanges the guanosine diphosphate bound in the basal state for GTP and then detaches from the βγ complex, which remains tightly associated. Association of α and βγ with the membrane is strongly dependent on attached lipids, which indicates a crucial role for lipidation localizing the proteins to their sites of action, predominantly the plasma membrane. All α subunits are apparently modified by saturated fatty acyl chains, either the NH_2-terminal myristoyl or a nearby palmitoyl or both. In addition to a role in membrane association, myristoylation of the α subunits so modified (that is, members of the subclass termed α_i) contributes to the binding of βγ by these forms (*30*). At least in the case of transducin, this does not seem to involve a direct protein (that is, βγ) contact for the acyl chain on α, but may involve a contact with the isoprenoid lipid attached to the γ polypeptide [(*31*) and see below]. This finding highlights the potential for multiple roles for lipids on proteins. An intriguing situation exists in the retina, where heterogenous NH_2-terminal acylation of the transducin α subunit and other retinal proteins (*32*, *33*), apparently a consequence of the acyl–coenzyme A (CoA) pool available to *N*-myristoyltransferase (*33*), may impart distinct properties to the proteins.

Palmitoylation is a near universal modification of α subunits that, at least in the case of α_s (the α subunit that mediates hormonal activation of adenylyl cyclase), can be modulated in response to activation of the G protein (*29*). This is apparently the result of a more rapid deacylation of the free α subunit as compared with when it is complexed with βγ (*34*). The functional consequence of this deacylation is not fully established, but mutations in palmitoylation sites of α subunits do impair their signaling in cells (*35*). Thus, reversible acylation may be a mechanism for modulating the signaling potential of an activated α subunit. However, the inability to manipulate the palmitoylation state of a protein in vitro has hampered

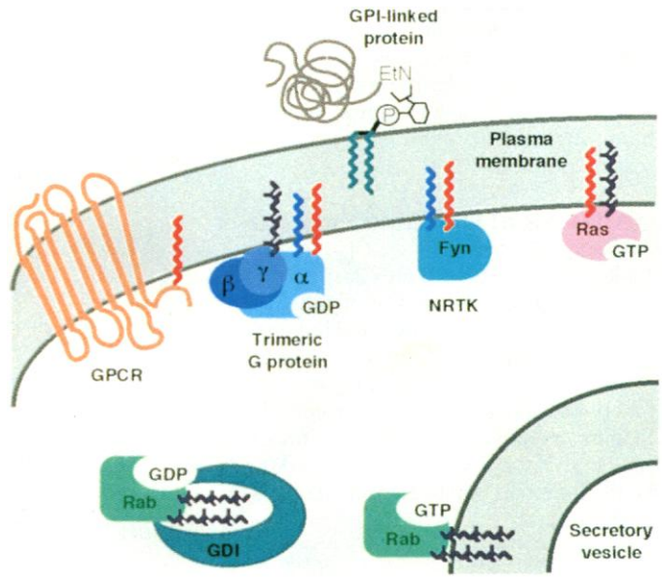

Fig. 1. Membrane localization of several lipidated proteins involved in cell signaling. Representative proteins from some of the major classes involved in signaling are shown. The attached lipids are color-coded: palmitoyl (S-acyl), red; myristoyl, blue; prenyl, purple; and unspecified acyl chains on GPI-linked proteins, green. EtN indicates ethanolamine. GPCR and NRTK refer to G protein–coupled receptors and nonreceptor tyrosine kinases, respectively. The G protein α subunit, NRTK, and Ras proteins are shown with two lipids attached, although distinct isotypes of each protein may contain only one of the lipids.

efforts to ascribe a specific functional consequence to the modification.

G protein γ subunits are prenylated and it is the isoprenoid that is responsible for the membrane association of the βγ complex (*36*). The 20-carbon geranylgeranyl moiety is found on all γ subunits except the retinal-specific form, for which the farnesyl group is attached. Assembly of the βγ complex is not dependent on prenylation of γ (*36*, *37*), but a requirement for the three COOH-terminal residues of prenylated γ implies that the assembly takes place before completion of the processing (*37*). Association of βγ with an α subunit to form the trimer, however, is dependent on γ prenylation (*29*, *37*). Although again the exact mechanism through which the lipid contributes to α-βγ interaction is unclear, it is possible that an interaction between the lipids attached to α and βγ is one of the determinants in trimer formation (*31*). The final step in the processing of γ, methylation of the terminal prenylcysteine, may influence the activity of βγ through modulation of its membrane-binding affinity (*38*). This finding is consistent with studies in model systems, where association of prenyl cysteines with liposomes has been shown to be markedly enhanced by their methylation (*39*).

Signaling Through Protein Tyrosine Kinases

Protein tyrosine kinases are involved in many aspects of cell growth and development (*40*). These include membrane-spanning receptors for growth factors (RTKs) as well as nonreceptor tyrosine kinases (NRTKs). Most of the latter group are found associated with the inner face of the plasma membrane in animal cells. The best studied processes here are those involving RTKs, which signal through members of the Ras family of monomeric (also known as "small") G proteins (*41*). The Ras proteins, as well as almost all of the other members of the quite diverse small G protein family, are modified by isoprenoid lipids (*14*, *42*). In addition to a role in membrane association, studies on Ras and related proteins indicate that the lipid modification is important in interaction of many small G proteins with regulatory proteins that influence the nucleotide-bound state of the G protein (*42*, *43*). Ras itself, which is farnesylated, is a proto-oncogene, and mutant forms of the protein that are oncogenic are quite prevalent in human cancers. The discovery that farnesylation is required for Ras function inspired development of inhibitors of FTase for evaluation as cancer therapeutics. Progress toward this goal has been rapid, with several compounds showing efficacy in cell and, in one case, animal models (*44*–*47*). This efficacy in the absence of general toxicity of the FTase inhibitors is somewhat surprising considering the crucial role of Ras in cell growth and the fact that several other important proteins in cells, including nuclear lamins, are farnesylated (*48*, *49*). It is likely that analysis of cells treated with these inhibitors will reveal unexpected aspects of protein prenylation to account for this. Stable association of Ras (and probably related proteins) with membranes also requires a second motif consisting of either a cysteine residue near the prenylation site that can be palmitoylated or a stretch of basic residues (*42*, *50*).

Many NRTKs also participate in signaling processes at the plasma membrane. These molecules lack the transmembrane topology of the RTKs and, not surprisingly, are targeted to membranes by lipidation. Most widely studied are members of the Src family, and these proteins are modified by myristoylation (*51*). As with the α subunits of the trimeric G proteins, nonmyristoylated forms of these proteins do not associate with membranes and cannot mediate cell signaling. Another parallel with α subunits is that most Src family members (but not Src itself) possess cysteine residues near the NH_2-terminus that compose a similar motif to that which is palmitoylated in the α subunits. Indeed, palmitoylation of two members of the family, Lck and Fyn, has been demonstrated (*52*). Recent evidence links dual acylation of these proteins to an ability to communicate with GPI-linked proteins on the cell surface (see below). The occurrence of dual lipidation in the α subunits, in NRTKs, and in certain small G proteins noted above may also reflect the relatively poor ability of single lipid modifications to stably anchor proteins or peptides to membranes that has been observed both in cells and in artificial liposomes (*9*, *42*, *51*). The second signal for stable membrane association noted for Ras may be a general requirement; in this regard, basic residues near the NH_2-terminus of Src can provide the second signal to this nonpalmitoylated NRTK as they do for some forms of Ras (*51*).

Vesicular Trafficking

More than two dozen different members of the small G protein superfamily have been implicated in the process of membrane traffic in cells, a process that includes secretion of neurotransmitters and other messengers in response to cell stimulation (*53*). Two distinct classes of small G proteins are involved. The largest is the Rab family; specific forms of these proteins are associated with specific membrane compartments in cells and, at least in part, mediate the targeting of membrane vesicles between distinct compartments (*53*). As noted above, Rab proteins are subject to geranylgeranylation through a process involving GGTase II; in addition, prenylation requires an accessory protein termed Rab escort protein (REP). Mutations in one REP are responsible for the retinal degeneration disease choroideremia (*54*), which suggests that defects in membrane trafficking trigger the degeneration. An interesting facet of Rab action is that they can move between membrane compartments through the cytosol, which requires that the proteins be capable of reversible association with membranes (*53*). Specific cytosolic proteins termed guanine nucleotide dissociation inhibitors, or GDIs, have been implicated in the process. Association of these proteins with Rabs is dependent on Rab prenylation (*53*, *55*), and the current view is that the prenyl groups on Rab interact directly with GDI in such a fashion as to shield them from the polar environment of the cytosol (see Fig. 1). The resulting soluble Rab-GDI complex then moves to the appropriate membrane, where dissociation of GDI exposes the prenyl groups on the Rab protein so that they can be used for membrane binding.

A second class of small G proteins involved in vesicular trafficking are the so-called Arf proteins, named for their ability to support adenosine diphosphate–ribosylation of the trimeric G protein, G_s, catalyzed by cholera toxin (*56*). Arf proteins function in vesicle budding from Golgi cisternae and probably many other membrane compartments (*56*) and can also mediate GTP-dependent stimulation of phospholipase D activity (*57*). Arf function is dependent on NH_2-terminal myristoylation, as nonmyristoylated mutants of the proteins have markedly compromised activities (*56*, *57*). Additionally, as noted previously for the retinal protein recoverin, Arf can apparently switch between a cytosolic form in which the myristoyl contacts the protein and a form in which the lipid becomes available for use in membrane association (*56*).

GPI-Anchored Proteins and Cell Surface Events

GPI-anchored proteins constitute an exceptionally diverse family of membrane molecules, with members functioning in such processes as nutrient uptake, cell adhesion, catalysis, and membrane signaling events (*4*, *58*). In terms of the signaling capacity of these molecules, the best characterized process is the activation of T cells and other lymphocytes by antibodies directed against specific GPI-anchored proteins (*59*). Antibody-mediated crosslinking of specific GPI-linked antigens on these cells stimulates tyrosine phosphorylation of numerous intracellular proteins, raising the question as

to how the signal could be transmitted across the plasma membrane by proteins restricted to the outer leaflet of the bilayer. A potential link was uncovered with the findings that two Src family NRTKs that had previously been implicated in T cell activation, Lck and Fyn, could be co-immunoprecipitated with the GPI-linked antigens after treatment of cells with nonionic detergents (59). The immunoprecipitates produced are large complexes that contain numerous GPI-linked proteins and a substantial quantity of lipid that is not solubilized by the nonionic detergent. The bulk of the lipid is apparently glycolipid and cholesterol, which accounts for its resistance to detergent extraction (60). These findings have led to speculation that both the GPI-linked proteins and the NRTKs are localized to special glycolipid microdomains in the membrane. Although this still does not provide a mechanism for signal transmission between the two types of lipidated proteins [that is, directly through lipid-lipid interactions or, more likely, indirectly through the involvement of an as-yet-unidentified membrane protein (59)] their colocalization provides support for the notion that they communicate with each other.

As noted above, myristoylation of Src family NRTKs is required for their association with membranes. However, myristoylation alone is not sufficient to mediate association of the molecules with the putative glycolipid microdomains, because Src itself is not detected in the immunoprecipitated complexes with the GPI-linked antigens. Experiments designed to assess why Fyn and Lck, but not Src, could be detected in the complexes revealed that cysteine residues near the NH_2-terminus of the former proteins were required (52). The demonstration that these cysteine residues could be subject to palmitoylation led to the proposal that dual acylation (that is, by both myristoyl and palmitoyl) of the NRTKs mediates targeting of these kinases with the glycolipid microdomain that contains GPI-linked proteins (61). Other investigators, however, have presented data indicating that the palmitoylation does not contribute to the association, but rather that it is a free cysteine residue near the NH_2-terminus that is important (62). It is not possible at present to reconcile the data from these reports.

In many cells, the detergent-insoluble complex that contains GPI-linked proteins is also enriched in the protein caveolin; this transmembrane protein serves as a marker for a type of membrane invagination termed caveolae (58). As caveolae themselves have been reported to be membrane sites for clustering of GPI-linked proteins, originally seen with the folate receptor, a tendency has developed among some investigators to equate the detergent-insoluble fraction from cells with caveolae. Furthermore, a number of additional signaling molecules such as trimeric G proteins, GPCRs, and small G proteins, have been detected in these detergent-insoluble fractions, leading to a proposal that caveolae could represent a specialized signaling compartment at the cell surface (63). However, recent reports cast doubt on that hypothesis. First is a demonstration that detergent-insoluble complexes containing GPI-linked proteins and glycolipids could be prepared from lymphocytes that lack caveolae (64), which indicates that detergent-insoluble complexes cannot be equated with caveolae. The second is a recent study demonstrating that a GPI-linked protein reconstituted into a glycosphingolipid-cholesterol–enriched liposome showed a similar resistance to detergent extraction as in membranes, which suggests that it is the physical-chemical property of a glycolipid subdomain and inserted acyl chains that imparts resistance to extraction (65). No data yet excludes a model whereby particular lipid-modified proteins would concentrate in the detergent-insoluble fraction in response to membrane solubilization because they favor that environment over the solubilized state; in other words, they may not normally reside in glycolipid subdomains but partition into them as a consequence of the extraction. In summary, although it is difficult at present to ascribe a functional significance to detergent-insoluble complexes from cells, the concept that specialized membrane domains exist for organizing signaling molecules is certainly fascinating and should attract considerable interest from investigators.

Conclusions and Directions

The past several years have seen a dramatic increase in studies concerned with protein lipidation. This is particularly evident in terms of our growing understanding of the mechanisms and biological consequences of lipidation. One major question that is just beginning to be addressed is the potential for multiple roles for attached lipids in protein-protein communication. Determining whether the loss in activity that accompanies elimination of lipidation for a particular protein is simply due to the reduced interaction with the membrane containing the target molecule, or reflects direct involvement of the attached lipid in the interaction with the target molecule, is quite difficult. Specific probes to detect lipid-protein interactions would be very useful in these types of studies. Additional information should come from structural studies of cytosolic lipidated proteins, because most of these probably use protein-lipid contacts for structural purposes. The recent discovery that GPI-linked proteins on the cell surface somehow communicate with intracellular proteins, together with clues that glycolipid microdomains may be involved, accent the need to obtain more detailed information on the physical-chemical properties of membranes themselves and how these properties can influence protein localization and function. Deciphering the molecular details of reversible S-acylation (also known as palmitoylation) will be required to understand what appears to be a dynamic role for this modification in many signaling processes.

Finally, just how lipidated proteins are directed to specific membrane compartments is still an open question for most of these molecules. This process almost assuredly involves additional proteins; possibilities include cytosolic proteins that could chaperone newly modified proteins to compartments and membrane proteins already in place at the target site that might function as receptors for specific lipidated proteins. In regard to the latter mechanism, all that may be required is the presence of membrane proteins with which a particular lipidated protein interacts. A useful analogy here may be that of an individual arriving at a large party where many friends are present but also a large number of strangers. Like this hypothetical person, a lipidated protein would most likely migrate to membrane locations where it encountered the most friends and rarely stray from those locations unless some biological function required it.

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Notch Signaling

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The Notch/Lin-12/Glp-1 receptor family mediates the specification of numerous cell fates during development in *Drosophila* and *Caenorhabditis elegans*. Studies on the expression, mutant phenotypes, and developmental consequences of unregulated receptor activation have implicated these proteins in a general mechanism of local cell signaling, which includes interactions between equivalent cells and between different cell types. Genetic approaches in flies and worms have identified putative components of the signaling cascade, including a conserved family of extracellular ligands and two cellular factors that may associate with the Notch Intracellular domain. One factor, the *Drosophila* Suppressor of Hairless protein, is a DNA-binding protein, which suggests that Notch signaling may involve relatively direct signal transmission from the cell surface to the nucleus. Several vertebrate Notch receptors have also been discovered recently and play important roles in normal development and tumorigenesis.

Multicellular development is governed by the combinatorial and sequential activity of genes that gradually restrict the developmental potential of cell lineages during differentiation. Large-scale mutational analyses performed in *Drosophila* have led to the identification of genetic pathways that control the assembly of a complex multicellular organism from a unicellular oocyte (*1*). The *Drosophila* embryonic axes are established by four distinct groups of maternal-effect genes: the anterior group, the posterior group, the terminal group, and the dorsoventral group. These maternal groups regulate sets of zygotic genes, termed gap, pair rule, and segment polarity genes, which progressively subdivide the embryo into ordered segments. Finally, each segment is assigned a specific identity by the homeotic genes. Whereas these genes provide the blueprint for the overall pattern of the *Drosophila* body plan, the specification of individual cell fates within a tissue is thought to be determined by invariant patterns of cell lineage as well as by regulative events that are dependent on local cell interactions. As discussed below, regulative interactions may occur between cells that are initially equivalent (lateral specification) or nonequivalent (inductive signaling) and result in changes in intracellular physiology in response to extracellular signals.

In this review, we focus on one signaling pathway that plays a central role in the specification of cell fates that occur through local cell interactions in a wide variety of different tissues and organisms. This evolutionarily conserved pathway is mediated by the transmembrane receptor protein encoded by the *Notch* gene of *Drosophila* and its vertebrate homologs, as well as by related proteins that are encoded by the *lin-12* and *glp-1* genes of C. *elegans* (*2*, *3*). Recent studies have demonstrated the pleiotropic nature of Notch activity and its functional requirement throughout development in several species. Notch proteins have been found to function in both types of local cell interactions, namely lateral and inductive signaling. We discuss how these observations, together with data on the effects of constitutive Notch activation in several different developmental contexts, argue for a general function for Notch in the regulation of the competence of a cell to respond to more specific developmental cues. We also summarize data on the interaction of the Notch, Lin-12, and Glp-1 receptor proteins with their putative ligands in both *Drosophila* and C. *elegans*, and with putative intracellular components of the signaling pathway in *Drosophila*. Finally, we present a molecular model for some of the intracellular events in the pathway and their possible connection to nuclear events involved in Notch signaling.

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Lateral Versus Inductive Signaling

During the development of complex multicellular organisms, numerous local cell signaling events are required for proper cell-fate determination. Studies in relatively simple model organisms have distinguished signaling events that involve equivalent cells from those that involve different cell types (Fig. 1). Among a group of initially equivalent cells, a mechanism originally termed lateral inhibition (*4*) could allow an individual cell or a group of cells to be singled out from the surrounding cells. Because signals may be transmitted back and forth between the two emerging cell types, this type of signaling should more properly be termed lateral specification (*5*). The molecular details of lateral specification are still largely hypothetical,