

SIGNALING COMPLEXES: Biophysical Constraints on Intracellular Communication

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ABSTRACT

This review surveys the kinds of protein complex that participate in cell communication and identifies, where possible, general principles by which they form and act. It also advances the notion that biophysical constraints imposed by macromolecular crowding and diffusion have had a controlling influence on the evolution of cell signaling pathways. Complexes associated with the bacterial aspartate receptor, with eucaryotic tyrosine kinase receptors, with T-cell receptors, and with focal contacts are examined together with proteins that serve as adaptors, anchors, and scaffolds for signaling complexes. The importance of diffusion in controlling the numbers and locations of signaling complexes is discussed, as is the special role played by membranes in signaling pathways.

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Perspectives and Overview

One of the most important unifying principles to have emerged from the past decade of cell signaling research concerns the physical location of the proteins involved. It now appears that many of the protein kinases, protein phosphatases, transmembrane receptors, and so on, that carry and process messages inside a living cell are associated with compact clusters of molecules attached to cell membranes or the cytoskeleton. These clusters, variously termed signaling complexes, signal cassettes, signaling modules, signalosomes, or transduci-somes, operate as computational units. Each receives one or more inputs and generates one or more specific outputs.

Signaling complexes are important conceptually because they provide an intermediate level of organization analogous to the integrated circuits used in the construction of large electronic circuits. There is an exciting possibility that they could help us make sense of the seemingly impenetrable jungle of molecular interactions that characterizes even the simplest forms of cellular communication.

My purpose in writing this short review is to survey the kinds of protein complexes that participate in cell communication and to identify, where possible, general principles by which they form and act. I would also like to advance the notion that biophysical constraints that result from macromolecular crowding, rates of diffusion, and the energy-cost of information have an important and perhaps controlling influence on the design of cell signaling pathways. Why do cells use protein complexes? Why are these always associated with membranes or the cytoskeleton? Why do many receptor complexes form when active and dissociate when inactive? Answers to these questions may be more easily found in the biophysics of the participating molecules than in the chemical details of the transformations they undergo.

Tar Complex: Solid-State Circuitry

To set the stage, it is helpful to examine one signaling complex in detail. The cluster of proteins associated with the chemotactic receptor of coliform bacteria, here called the *Tar* complex, is particularly well understood and illustrates many features found also in larger eucaryotic complexes [reviewed in (48)]. The *Tar* complex is built around a dimeric transmembrane receptor (*Tar*) that has an extracellular binding site for aspartate (34). The receptor is multifunctional and monitors not only (a) the concentration of aspartate in the surrounding fluid, but also (b) the concentration of maltose, through a specific interaction with the maltose binding protein, (c) repellents, such as nickel ions, (d) ambient pH, and (e) ambient temperature. On its cytoplasmic domain, *Tar* is associated with a cluster of “*Che*” (for chemotaxis) proteins and, together with these proteins, generates a signal that is sent to the flagellar motors (Figure 1). The magnitude

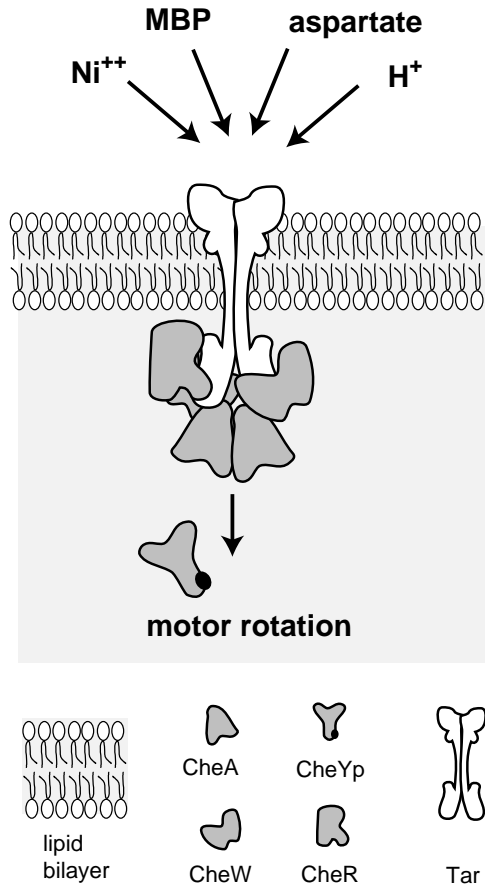


Figure 1 The core Tar complex. Two other proteins, CheY, CheB, are known to associate with the complex transiently, and there is some evidence that CheZ may also bind.

of the signal depends on the size of the various inputs to the Tar molecule or, more precisely, the rate of change thereof.

Proteins associated with the Tar complex include the autophosphorylating protein kinase CheA, the transducing protein CheW, the methylating enzyme CheR, and perhaps the protein phosphatase CheZ (17, 18, 33, 44). A small, highly charged protein CheY associates transiently with the Tar complex and is phosphorylated at a rate that depends on the level of activity of that complex. The phosphorylated product CheYp then diffuses to the flagellar motor where it interacts with the switch complex, changes the direction of rotation of the

motor, and thereby modifies the swimming behavior of the cell. A second cytosolic protein, CheB, which also interacts transiently with the Tar complex, removes the methyl groups added by CheR at a rate that depends on the level of stimulation.

The mechanism of action of the Tar complex is best understood in its response to aspartate, which acts as an attractant. Aspartate molecules bind to the extracellular domain, one per Tar dimer, and are believed to favor the formation of coiled-coils between subunits by changing the relative orientation of the monomers (6, 49).

This conformational change is propagated through the membrane to the cytoplasmic domain where it is thought to cause a matching change in the CheA molecule, decreasing the rate of its autophosphorylation and hence modulating the level of CheYp. The rate of autophosphorylation is also influenced by the level of methylation of Tar at four sites on each receptor monomer (eight sites in the dimer). In this way, CheR and CheB provide an adaptation mechanism to the chemotactic response.

We see therefore that the Tar complex operates like a self-contained, solid-state processing unit. It receives environmental stimuli through its extracellular domain and information on the current state of the cell by methylation of its cytoplasmic domain. These inputs are integrated within the complex so as to produce a certain rate of phosphorylation of CheA and hence a specific rate of formation of the phosphorylated species, CheYp. From a physiological standpoint, the Tar complex greatly simplifies the aspartate pathway, reducing it to a single freely diffusing species (CheYp), a protein complex concerned with the stimulus (the Tar complex), and a second protein complex concerned with the behavioral response (the flagellar motor).

PDGF Receptor Complex: Assembly on Demand

Receptor-associated complexes also function in eucaryotic signaling pathways, as in the family of receptors activated by growth-factors and cytokines and characterized by having a tyrosine kinase in their cytoplasmic domain (13, 32). In contrast to the Tar complex just described, the protein aggregates associated with tyrosine kinase receptors are not tightly bound and form only transiently, when the receptor is bound to its cognate ligand. For example, the receptor for platelet-derived growth factor (PDGF) exists as a single transmembrane protein diffusing freely in the lipid bilayer. When it encounters a molecule of PDGF in the extracellular milieu, the receptor associates laterally with a second (presumably unoccupied) receptor to form a dimer. As the two receptor tails make contact, their kinase domains become active and phosphorylate each other at multiple sites.

Phosphorylation then triggers the assembly of an intracellular signaling complex on the receptor tail (38). Six newly phosphorylated tyrosines serve as

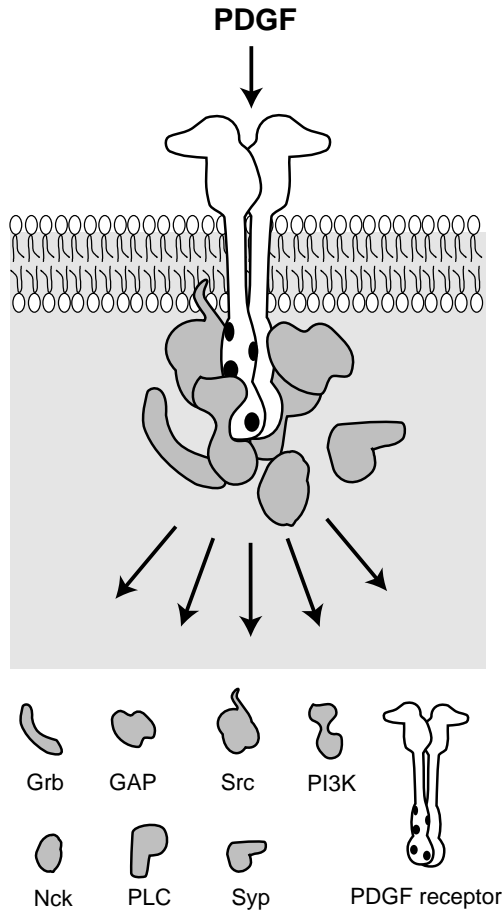


Figure 2 Signaling complex associated with the PDGF receptor. *Black patches* on the cytoplasmic domain of the receptor indicate the tyrosine phosphates to which signaling proteins bind. Note that it is not known at present how many signaling proteins are bound to any individual activated receptor at one time.

binding sites for six different intracellular signaling molecules (Figure 2). These include Src, a tyrosine kinase of broad specificity; GAP, a protein that activates small G proteins; PLC- γ , an enzyme that hydrolyzes the inositol phospholipid PIP₂; and the regulatory subunit of PI₃ kinase. These and other proteins cluster onto the active dimerized receptor, becoming themselves activated. The complex then broadcasts signals along multiple routes to many destinations inside the cell, activating and coordinating the numerous biochemical changes needed

to trigger a complex response such as cell proliferation. To terminate the activation of the receptor, the cell contains protein tyrosine phosphatases, which remove the phosphates that were added in response to the extracellular signals. Cells can also dispose of activated receptors in a more brutal way: bringing them into the interior of the cell by endocytosis and then destroying them by digestion in lysosomes.

The PDGF complex differs in a number of interesting and instructive ways from the Tar complex. To begin with, activation with PDGF requires a sequence of reactions (receptor clustering, tyrosine phosphorylation; binding of multiple signaling molecules) each of which takes time. Its response is therefore expected to be slower than the propagated conformational change of a pre-formed Tar complex. Presumably the action of a growth factor on a eucaryotic cell does not require the subsecond response of a swimming bacterium. Secondly, not every PDGF receptor carries a signaling complex on its cytoplasmic domain, only those receptors that have become activated by the binding of cognate ligand. Hence the cell can make large numbers of receptor molecules, thereby casting its net as wide as possible to catch diffusing PDGF molecules, without having to make an equally large number of copies of each of the many proteins in the signaling complex. The Tar complex, by contrast, not only must be ready to react quickly, but there is a real possibility that all or nearly all of the receptors on the cell will be occupied at the same time. The ability to respond to gradients of aspartate over a wide range of concentrations (five orders of magnitude, it is claimed) means that the bacterium must be ready to distinguish, say, 90% receptor occupancy from 95% occupancy. So all receptors must come equipped with their intracellular signaling baggage.

A third difference concerns the outputs of the two complexes. The Tar complex has only two outputs—phosphorylated CheY and phosphorylated CheB—both of which are dedicated to the same specialized response of the cell (motor switching). Moreover, there are other signaling complexes in bacteria not so far mentioned, such as the serine receptor and the ribose receptor, that also influence the same pool of phosphorylated species. The circuitry is one of multiple controlling elements converging onto a single process, like arrows to a bull's-eye. The PDGF receptor complex, by contrast, produces multiple outputs. Each PDGF molecule that arrives on its receptor fires a starburst of effects, activating protein kinases, phosphoinositide cascades, G-protein cascades, and so on. The circuitry here is highly divergent, with effects spreading throughout the cell and regulating many different processes related to the cell cycle.

We mentioned above that the PDGF receptor holds its protein acolytes with a weaker grasp than the Tar receptor, and it seems probable that any single PDGF receptor will have less than a full complement of signaling proteins. Exactly how many proteins are bound at any instant, and to what extent they interact

with each other, are questions that are not resolved at present, and the answer may differ between different tyrosine kinase receptors. But in general, it seems clear that such receptor complexes have the potential for many cooperative and hierarchical interactions (21). Moreover, different tyrosine kinase receptors can also come together in a variety of heterodimers on the cell surface, each of which can stabilize a discrete receptor complex and thereby transmit a distinct biological signal to the cell (39).

Adaptors, Anchors, and Scaffolds

The formation of clusters of proteins around a tyrosine kinase receptor depends on conserved binding motifs known as Src-homology domains, SH2 and SH3. These compact regions, which are found in many different proteins, bind to salient structural features of receptor molecules (37, 38). SH2 domains bind phosphorylated tyrosines in the context of particular amino acid sequences, with different SH2 domains showing slightly different specificities. The SH3 domain (which is entirely unrelated structurally) binds sequences rich in proline residues. Other binding domains involved in signaling include pleckstrin homology (PH) domains (45); phosphotyrosine-binding (PTB) domains (51); and PDZ domains, originally found in postsynaptic density proteins (26). These and other binding domains enable proteins to be put together, like Lego bricks, in multiple combinations. In fact, there are proteins, such as Grb2 and Nck that have no enzymatic activity themselves but simply contain multiple SH2 and SH3 domains (16, 31). These proteins serve as connectors or adaptors that allow additional proteins to be recruited to a signaling complex. For example, Grb2 provides a link (via yet another intermediate protein) between the activated receptor and the important Ras proteins—small GTP-containing proteins that have a controlling influence on cell proliferation and cell differentiation. The fact that proteins such as Grb2 and Nck have evolved solely for the purpose of making protein clusters underscores the importance of the latter in the transmission of signals.

A related function is served by anchoring proteins that serve as attachment sites for protein kinases and protein phosphatases (14, 15, 24, 36). These attach their cognate enzyme to particular locations in the cell such as the nuclear membrane, the Golgi, or the actin cytoskeleton, often showing a preference for particular isoforms of the enzymes. In some cases a single anchoring protein binds multiple signaling molecules. For example, the protein AKAP79, which is enriched in postsynaptic densities, has the ability to bind protein kinase A (stimulated by cyclic AMP), protein kinase C (stimulated by Ca^{2+} -calmodulin), and PP-2B (a protein phosphatase stimulated by Ca^{2+} ions and phospholipids) (14). The implication is that AKAP79 might act as a template or scaffold that nucleates formation of a signaling complex.

One of the clearest examples of a scaffold protein emerged in a study of the visual system of *Drosophila* (50). The rhabdomeres of this organism contain a highly organized signaling complex, which includes the principal light-activated ion channels. One of the proteins of this complex, called InaD, is made primarily of five PDZ domains. Mutations in this protein lead to a dramatically reorganized subcellular distribution of signaling molecules lacking the signaling complex. This and other evidence suggests that InaD acts as a scaffold for the highly ordered visual-transduction complex.

Another scaffold protein may be present in yeasts, in a signal pathway activated by pheromones during mating (14, 30). Experiments based on the yeast two-hybrid system suggest that a protein called Ste5 (the name derives from the *sterile* phenotype of mutants lacking this protein) provides attachment sites for a series of serine/threonine kinases in a phosphorylation cascade, perhaps binding them in the same sequence as the reactions of the cascade.

The strategy of bringing various enzymes involved in the same reaction sequence together in a multienzyme complex is of course a familiar one, widely employed by cells. Since the product of one enzyme can be passed directly to the next enzyme, and so on to the final product, diffusion rates need not be limiting even when the concentration of substrate in the cell as a whole is very low (52). Multienzyme complexes are very common in cells and involved in nearly all aspects of metabolism, including the central genetic processes of DNA, RNA, and protein synthesis. It is tempting to speculate that this same principle is used in cell signaling.

Focal Contacts: Giant Signaling Complexes?

The size of many signaling complexes cannot be defined precisely. Some proteins bind tightly, others weakly, and yet others have a casual liaison solely for the purpose of catalysis. The PDGF receptor complex mentioned above is in some ways more like a “swarm” or “penumbra” of proteins than a rigidly defined, static structure. At the other extreme are complexes that grow in size beyond that of a simple molecular assembly. Even the humble Tar complex has been found to associate, for reasons that are presently unclear, with other chemotaxis receptors to form a large cluster or patch on the surface of the bacterial cell (29). IgE receptors on mast cells aggregate when they bind antigen (23), and T lymphocytes recognize their target cells by means of an even larger protein complex comprised of multiple receptors and accessory proteins (5, 41). Each T-cell receptor in the latter complex mediates a discrete contact with the target cell and activates a distinct set of signaling events involving tyrosine phosphorylation and SH2 regulated pathways. Together, these different receptors provide an intricate fail-safe mechanism, requiring the antigen fragment

and the correct MHC molecules to be presented on the target cell, and other surface determinants to be absent, before the T cell is activated.

Other signaling complexes grow to such an extent that they reach a size visible in the light microscope, thereby becoming candidates for organelle status. Do we call kinetochores, adherens junctions, and synaptic endplates, signaling complexes? Probably not, but they certainly share some of the same properties.

Consider focal contacts: regions of attachment that form on the lower surface of a fibroblast in tissue culture. These are sites at which clusters of integrin molecules and associated proteins form links across the plasma membrane, from matrix molecules on the outside of the cell to actin filaments on the inside (25). For years, focal contacts were thought of as sites of mechanical anchorage of the cell. But abundant evidence now indicates that they also have a crucial function in cell communication. Formation of focal contacts tells the cell that it has adhered to a suitable substratum, so it can now divide, or grow, or differentiate (2). In reciprocal fashion, the physiological state of the cell, as displayed in the activity of a variety of protein kinases, has a controlling influence over the formation of focal adhesions (54).

From a structural standpoint, focal contacts are assemblies built from hundreds of thousands of protein molecules. More than 20 different types of protein have been identified in this complex, including integrins, actin-binding proteins, and several protein kinases (Figure 3). One of the latter, focal adhesion kinase (FAK), is an enzyme with a remarkably wide range of functions. FAK binds to structural components of the focal contact such as integrins as well as the tyrosine kinase Src and the adaptor protein Grb2. It also phosphorylates tyrosines in various proteins in the focal contact, including itself, thereby causing other proteins with SH2 domains to cluster in this region. The complex then generates signals that travel out from the focal contact to reach targets in the cytoplasm and nucleus. We see, therefore, that even though a focal contact is a large cytoskeletal-based structure, it also has some characteristics of a signaling complex. It receives multiple inputs from components of the extracellular matrix, mechanical tension, and phosphorylation signals, integrates them, and then produces multiple outputs that influence the growth and division of the cell as well as its state of differentiation.

Making the Complex: Macromolecular Crowding

Most of the information we have about intracellular signaling pathways comes from biochemistry. The enzymatic properties of isolated proteins, their ability to serve as substrates for kinases or methylating enzymes, and their binding affinity for ligands: These are the raw data on which the signal pathways portrayed in books and research papers are based. But chemical changes are only half the

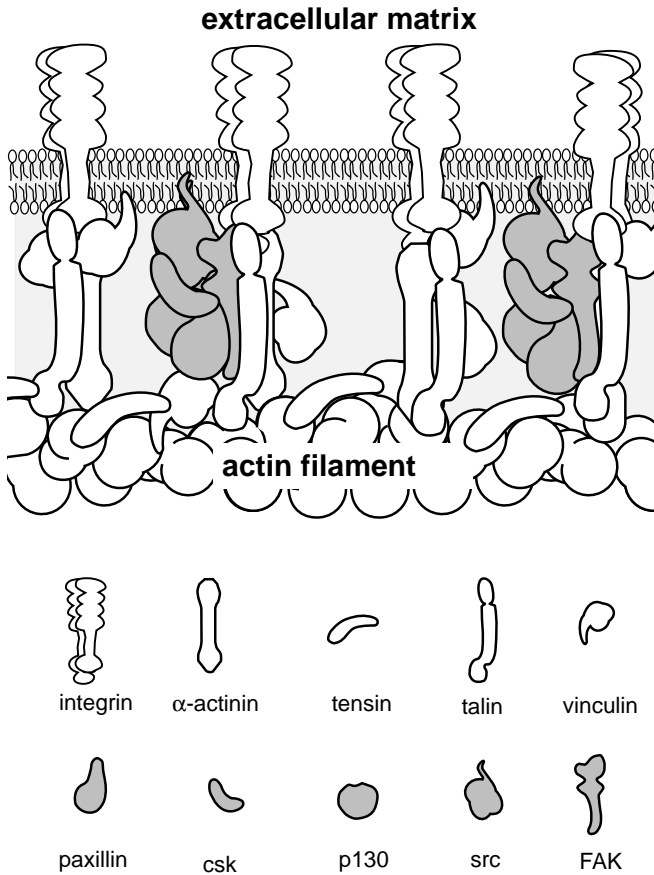


Figure 3 Schematic section of a focal adhesion (representing about 30–40 nm of a structure that might be several microns in diameter). Structural proteins such as integrin and actin are shown in *white* whereas proteins mainly concerned with signaling are shaded *gray*. Shapes and sizes are approximate, and many proteins have been omitted for clarity. Similarly, the locations of proteins in the focal contact are not known with any precision, and signaling proteins may not be clustered in the fashion shown here.

story. Unless we consider also the spatial location of molecules in the cell, their diffusion rates, and other biophysical parameters, our understanding will be incomplete.

A case in point is macromolecular crowding. Intracellular reactions do not take place in a dilute solution such as biochemists use to assay enzymes but in a concentrated slurry of molecules. The concentration of proteins in the cytosol of a living cells is comparable to that of some protein crystals (10, 19). This

high concentration of proteins and other macromolecules leads to a competition for water and has a major influence on the rate at which molecules diffuse in the cell (35, 55). It also changes the tendency of protein to associate with each other—the basis on which signaling complexes are built. Whether they are comparatively stable structures, such as a Tar complex or a focal adhesion, or short-lived, such as a PDGF receptor complex, the formation of a signaling complex requires a sequence of binding steps in which protein molecules come together in a diffusion-limited interaction governed by the law of mass action. Both experiment and theory show that the crowded conditions in the interior of cells strongly favor associations between macromolecules, often increasing the binding affinity by an order of magnitude or more (53).

If signaling complexes are made by multiple diffusion-limited binding associations, how are their numbers in the cell controlled? Recent theoretical work suggests that some proteins in a complex, such as the anchoring proteins mentioned above, might have a key role to play in this regard. Computer-based analysis of model complexes with different topologies reveals that certain proteins in a complex, typically those forming a link between two or more other proteins, inhibit complex formation if they are present in high concentrations (9). Under these conditions, the linking protein drives the formation of small, incomplete aggregates at the expense of the complete complex—an effect analogous to the familiar inhibition of antibody-antigen precipitation in the precipitin test.

An effect of this kind is thought to occur in the case of the Tar complex when CheW is expressed at high levels (8, 42). It is also the basis of suppression of dynactin, a multimeric complex involved in movement along microtubules (12). Evidently the number of bridging molecules made by a cell will control the quantity of the complete complex in the cell. Other proteins in the complex can be changed in amount over a wide range of values without affecting the quantity of the final signaling complex.

Why Signaling Complexes?

The biochemical steps by which living cells integrate, codify, and transduce extracellular stimuli all require molecules to come into physical contact. It is easy to see, therefore, why clusters of molecules in permanent association with each other would be well suited to perform such reactions. Solid-state computations should be more rapid, more efficient, and more noise-free than systems of diffusing molecules in which each encounter is subject to the chaotic fluctuations of thermal energy. Of course, as just mentioned, each complex will have to be constructed by diffusion-limited encounters of its constituent molecules. But, once made, the complex could work repeatedly, processing signals rapidly and accurately without further need for diffusion. Later modifications to the

assembly reflecting the current state of the cell (as in the methylation of Tar receptors described above) could be made without requiring the complex to be disassembled.

A recent estimate of the cost of transmitting information in an insect eye underscores this conclusion. Taking an ion channel as a benchmark, an individual protein can switch conformational states 10^4 to 10^6 times per second (22). If operated as a digital switch, driven by ATP hydrolysis, a single protein might therefore transmit at least 10^4 bits per second at a cost of 1 ATP per bit. In the real world of insect vision, however, experiments show that a blowfly photoreceptor cell transmits information at one tenth the rate and a million times the unit cost of a single ion channel (11). This huge difference emphasizes that the major expense is not in registering information as conformational change. The costs are incurred when information is distributed, integrated, and processed within and between systems. Limiting factors will be Brownian motion, the uncertainty of molecular collisions, and the inefficiency of diffusion as a means to carry signals.

Why Diffusion?

If molecular encounters between freely diffusing single protein molecules are so unreliable and costly, why use diffusion at all? The answer seems to lie in the extensive interconnections that characterize communication within living cells. Most extracellular stimuli do not pass in a linear chain of cause and effect from one receptor in the plasma membrane to one target molecule in the nucleus or cytoplasm. If they did, then they could be propagated most effectively as a chain of conformational changes along a protein filament. Indeed, some of the signals that travel from one end to another of nerve axons—carried as action potentials or, perhaps, as motor proteins moving along microtubules—have this one-dimensional advantage. However, the vast majority of signals in cells spread as a rapidly diverging influence from membrane-associated receptors to multiple chemically distinct targets in the cell, often at widely separate spatial locations. Sometimes the divergence amplifies a signal, whereas in others it controls different processes in the cell, a form of molecular cross-talk. But the machinery of the divergent influence in every case involves at least one molecule that diffuses as a free element.

The essential function of diffusion in signal transduction is displayed in the wide variety of signal-transduction transducers—from hormonal and synaptic systems to the receptor cells of vision and olfaction—that utilize G proteins (20, 28). The capture of a photon of light, or a molecule of odorant, hormone, or neurotransmitter, is conveyed to effector molecules (phosphodiesterase, adenylyl cyclase, potassium channel, or phospholipase C) through the intermediate activation of a GTP-binding protein freely diffusing in association with the

plasma membrane. We could easily imagine a situation in which each receptor was stably associated with one effector molecule and did not need a G protein to connect the two. In this hypothetical receptor complex, the activation step would indeed be faster and more efficient. But it would also be much smaller, since one photon or stimulant molecule would change the activity of at most one effector molecule rather than, as in the actual situation, many thousands. The cell therefore pays a price in time and consumption of ATP molecules for a huge reward in sensitivity.

Smart Membranes

Diffusion is essential to allow messages to spread widely through the cell but, at the same time, interactions based on diffusion are inefficient, error-prone, and costly in energetic terms. These conflicting constraints will determine the physical form of the signaling species and their location in the cell. I have argued that they provide partial justification, at least, for the existence of signaling complexes. Do they also tell us why these complexes, and the reactions that lead from them, are so often located on the membrane?

At first sight it seems obvious that diffusion in two dimensions would be a more effective way to send a message than diffusion in three dimensions. It is far easier for a molecule to find a distant target by random walking in a plane—in fact, it is guaranteed to find such a target given enough time. A molecule diffusing in an unbounded free solution, by contrast, might never arrive: its probability of eventual capture being the ratio of the target size divided by its distance (4). Unfortunately for this argument, particle diffusion is much slower in a membrane: A small protein might have a diffusion coefficient of about $5 \times 10^{-7} \text{ cm}^2 \text{ sec}^{-1}$ in free solution, whereas in a lipid bilayer, a protein of similar size would have a diffusion coefficient of perhaps $5 \times 10^{-9} \text{ cm}^2 \text{ sec}^{-1}$ (3, 40). Because of this factor of two orders of magnitude, most calculations of the time taken for signals to travel a micron in a living cell indicate that the best route is through the cytosol.

What membranes do provide is a discrete compartment in which molecules that relay and process messages can be sequestered. Receptors and ion channels have to be in the membrane, since they interact with the outside world. It makes sense, therefore, to put the molecules that interact with these receptors in the same place . . . and the molecules that interact with these molecules, and so on. As intracellular signaling pathways evolved into systems of increasing complexity, it would have been advantageous to put newly appearing molecular species, wherever possible, in (or close) to the membrane. In present-day cells, many if not most signals are carried by proteins that are embedded in the membrane or loosely associated with it through hydrophobic or other interactions. There are even proteins that normally reside in the cytosol but move to the

membrane in order to participate in signaling response—the protein recoverin in the vertebrate rod outer segment being an excellent example (1).

It is likely, in fact, that cells exploit the principle of compartmentalization to an even greater degree. There is increasing evidence that individual proteins and protein complexes do not roam freely over the entire surface of the cell but are constrained to move within specific subcompartments. How is this regionalization achieved? One basis is the formation of domains of different lipid composition, especially rafts enriched in sphingolipids and cholesterol [it has been observed that many proteins involved in cell signaling partition into these glycolipid-enriched domains (47)]. Another way is to use cytoskeletal proteins underlying the plasma membrane to delineate small compartments (46). Single-particle tracking and manipulations with laser-based optical traps reveal that most proteins are confined to domains of $0.1\text{--}1\ \mu\text{m}^2$ (27). Given this evidence of regionalization, we can suppose that many proteins concerned with the transmission and processing of signals are sequestered into the membrane. There they will be held in close proximity to their reaction partners so that, when diffusion occurs, it will be constrained and channeled so as to obtain maximum speed and efficiency (43). According to this viewpoint, the plasma membrane acts as the “sensory cortex” of the cell: the place that receives environmental signals and processes them using protein-based computational machinery (7).

Analogy to Signaling in the Nervous System

The most effective design for a signaling pathway appears to be a combination of signaling complexes and freely diffusing molecules, associated where possible with membranes. But in this case, how do we select which molecules are to be part of the complexes and which freely diffusing? An analogy to the nervous system might suggest an answer here.

Many regions of the nervous system of vertebrates and invertebrates show a modular organization—vertebrate brain regions, invertebrate ganglia, cortical columns, canonical microcircuits (e.g. cortex, hippocampus, cerebellum, retina), all display this feature. Communication within any one of these modules is made possible by multiple short axons and dendrites, whereas messages that pass between one module and another are carried by long axons running in tracts that extend across the brain or down the spinal cord. In the first case, the computational elements are in close proximity and signals pass from one to the other with minimal delay. In the second case, the signaling elements are far apart and the messages (even though they travel in one dimension, as action potentials) take an appreciable time to pass from one to the other.

In some respects, therefore, the microanatomy of signaling pathways in a living cell is like the architecture of the brain. There are modular regions in which precise, rapid, stereotyped computations are performed by local circuitry

and carried by conformation changes and protein modifications. And there are communication channels that allow the results of local computations to be sent to often multiple distant targets, usually by diffusion. Evidently, during evolution, it has become advantageous to group together molecules that perform a specific task and that have to be active at the same time. Signaling complexes might therefore represent abstractions of the cell's environment, such as the current state of chemotactic stimulating activity, or the presence of a suitable extracellular matrix. They could, as suggested at the beginning of this review, provide elements at an intermediate level of complexity that will eventually help us decipher the signaling code used by living cells.

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