Information transfer from activated heterotrimeric guanine nucleotide-binding proteins (G proteins) to downstream effectors occurs through noncovalent protein-protein interactions. Such interactions involve multiple regions of contact between the G protein and the effector. Some of these regions mediate information transfer, as defined by their ability to change the activity of their downstream binding partners, whereas other interactions appear to contribute solely to binding affinity. Such modular configurations occur in functionally diverse proteins such as myosin and a regulator of the double-stranded DNA stimulated protein kinase (PKR) called PACT. In most cases, it appears that both charge complementarity and the architecture of the interacting surfaces provide the appropriate balance between specificity of interactions and their reversibility. Information transfer regions appear to display conformational flexibility in interactions. Such flexible interactions may be essential for the local conformational changes necessary to induce change in activity by an induced fit-type mechanism. Thus, a general mechanism for information transfer by protein-protein interactions could use specific regions that induce conformational changes in the downstream partner. Other binding regions may be arranged within the protein to impart specificity of recognition and thereby maintain overall contact between the partners during the conformational dynamics that occur in the signal-transfer process.

Introduction

Cell signaling cascades rely on direct protein-protein interactions to communicate messages from the cell’s extracellular environment. These messages lead to changes in behavior. Protein-protein interactions involved in information transfer have evolved to ensure specific, transient, and successful relay of signaling information between proteins. Such communication between proteins through noncovalent interactions is used to regulate various functions in the cell, including force generation and targeting of proteins for degradation. Specificity in these protein-protein interactions is essential for organizing networks of signaling cascades within the cell and for other regulated processes. Many signaling proteins interact with multiple effector proteins, often through the same binding surface. This suggests that these binding surfaces should have structural flexibility to accommodate the varying structures of effector contact sites without compromising effector selectivity. Protein-protein interactions must be tight enough to allow for signal flow, but not so tight that reversibility and signal transmission are lost.

Exactly how protein structures have evolved to mediate protein-protein recognition and regulation is not fully understood. However, observations from a number of systems have shed new light on the basic principles that govern how proteins communicate with one another. This review analyzes data from a number of systems that contribute to our understanding of how protein-protein recognition events lead to the specific transfer of information within networks of signaling proteins in the cell.

Resolution of Functions of Regions Involved in Protein-Protein Interactions

Many proteins are composed of modular functional units, and interactions between protein binding partners often involve contacts at multiple sites. Interactions made at these distinct domains within a protein can serve different roles in protein-protein communication. During protein interactions, distinct sets of surface contacts mediate binding between partners and transfer of information as a consequence of this binding.

One of the first examples of this resolution of the functions of different protein regions involved in protein-protein interactions was described for ligand-receptor interactions. Some receptors rely on “address” surfaces to function in general binding for ligands and separate “message” surfaces to function in transmitting the signal. This is described for interactions between the chemokine C5a ligand and its cognate receptor (1), as well as the luteinizing hormone (LH) or human chorionic gonadotropin (hCG) receptors (2). The N terminus of the C5a ligand interacts with the N terminus and extracellular loops between transmembrane helices 2 and 3 of the C5a receptor, a heptahelical receptor coupled to Gt, (3). This interaction is sufficient for binding affinity and specificity between this ligand-receptor pair, but is not sufficient for receptor activation. The latter requires interaction of the C terminus of the C5a ligand with sites within the helical bundle or the receptor (4, 5). For G protein-coupled receptors (GPCRs), interactions between the C5a ligand and the receptor’s transmembrane helical bundle result in the movement of helices with respect to one another, which in turn results in movement of other receptor regions that interact with G proteins. The resultant interactions are responsible for the activation of the G protein, and thus information is transferred across the plasma membrane (6). Just as we have started to develop a detailed understanding of transmembrane information transfer, the mechanisms by which information transfer occurs within the cell are also becoming clearer.

Signal-transfer regions can be resolved from general binding domains for intracellular protein-protein interactions. For Gβ7 stimulation of the effector phospholipase Cβ2 (PLCβ2), the Gβ subunit requires a different set of effector contacts to produce signal transfer, as compared to those used for general binding alone (7). Multiple domains on Gβ contribute to both binding and signal transfer.
Role of Conformational Flexibility in Effective Signal Transfer

There is emerging evidence for the role of conformational plasticity in protein-protein interactions. In many cases, proteins appear to interact with one another through an induced-fit mechanism rather than a lock-and-key mechanism. For Gβγ-PLC-β2 interactions, our studies support the hypothesis that conformational flexibility at the protein-protein interface is important for the transmission of signal information. For one signal-transfer region on Gβ, Gβ42-54, substitution of the amino acid R48 results in a decrease in apparent affinity for PLC-β2 that is accompanied by an increase in efficacy for signal transfer. One proposed explanation is that the decrease in affinity generated by the amino acid substitution R48A may increase the flexibility with which the Gβ42-54 signal-transfer region interacts with PLC and allow more fruitful communication with this effector enzyme. For the other signal-transfer region, Gβ86-105, a decrease in K_{cat} (increased affinity) results in diminished maximal stimulation. Additionally, the role of W-99 indicates that it is involved in transient contact required for efficient signal transfer.

Similar findings of low-affinity interactions for signal-transfer regions come from studies of interactions between chemokine peptide ligands and their cognate GPCRs. Much of the binding affinity between these ligands and receptors is generated by general binding domains, not by signal-transfer regions. Mutation of receptor residues in binding domains has a large impact on ligand binding, whereas mutation of receptor residues in signal-transfer regions has a large impact on signal transfer but little effect on ligand binding affinity. In these systems the specificity for ligand-receptor interactions lies mainly in the extracellular N terminus of the receptor, a region that has a major role in binding but not in signal transfer.

The overall binding affinity for signal-transfer regions may be restricted by a necessity for conformational plasticity at the inter-
Importance of Flexibility in Accommodating Diverse Binding Partners

In cell signaling pathways, a single protein surface is frequently found to bind to a number of different effector binding surfaces, with each effector having a unique structure. Such promiscuous protein-protein interactions may require a high degree of conformational flexibility for one or both of the protein interaction surfaces. For example, a single surface of the erythropoietin receptor (EPOR) binds to two sites on EPO ligand family members, as well as to a family of distinct EMPI (EPO-mimetic peptide 1) peptides (17). That a single surface can bind a multitude of target surfaces with a diverse array of structures implies a certain degree of structural plasticity.

There are similar findings for the importance of conformational flexibility for the ubiquitin conjugation enzyme (UBC) E2. UBC is involved in transferring ubiquitin to diverse substrates, including histones and cyclins. These substrates for UBC are distinct in both their structures and functions. Yet ubiquitination is a very specific event. Both nuclear magnetic resonance (NMR) and crystallography experiments for UBC9 indicate that the N and C termini are the only two flexible regions on this UBC isoenzyme, which is overall a very rigid protein. These sites correspond with surfaces of UBC9 shown to mediate substrate binding. Thus, conformational flexibility may allow UBC9 to recognize diverse substrates. This conformational flexibility may also be important for the catalytic activity of transferring ubiquitin to the substrate. Many of the residues that undergo conformational changes surround the active site of the enzyme (18).

The requirement of plasticity in protein-protein interactions may be based on the spatial organization of protein-protein interaction sites on the surface of a protein. This seems to be true in G protein signaling pathways. The Gβ subunit uses an extensive set of overlapping surfaces to recognize a multitude of effectors. The surface area of Gβγ subunits involved in interactions with effectors is one of the largest in all known protein-protein interactions. For example, the surface area of Gβ involved in direct interactions with phosducin is approximately 4550 Å² (19). This same surface is involved in regulating the activity of a multitude of structurally distinct effectors. Therefore, this binding surface of Gβ may have the flexibility to conform to different effector surfaces (20). At least part of the Gβ surface involved in interactions with phosducin undergoes a conformational change upon effector binding (19). Conformational flexibility could be a hallmark of surfaces of signaling proteins that interact with a diverse number of effector proteins, whereas those surfaces that interact with only few effectors may be more rigid in structure. This chameleonic behavior of protein-protein binding surfaces would likely allow a single protein surface to adapt to the conformations of a diverse set of target binding sites. This plastic nature of protein-protein interfaces would also be a means whereby mutations at protein-protein interfaces might be allowed for, as a mutation on one side of the interface could be accommodated by a remodeling of the protein surface on the opposite side of the interface (21).

Achieving Flexibility Without Sacrificing Specificity

Conformational flexibility of signaling proteins must be achieved without sacrificing specificity. This may be possible because of the molecular nature of the protein-protein interface. Very “greasy” hydrophobic protein surfaces would likely yield high-affinity binding that might not be specific. This could preclude the specificity that is important for effective signal communication. However, if electrostatic charge complementarity is a primary determinant in proteinprotein binding affinity, this might allow for lower affinity interactions without a loss of specificity. Such charged amino acids do take part in protein-protein interactions between G proteins and effectors. Both signal-transfer regions on Gβ are characterized by a number of basic amino acids that are important for binding affinity. For
one of these signal-transfer regions, Gβ86-105, screening a partially random combinatorial peptide library based on the sequence of the Gβ86-105 region for binding to the effector PLC-β2 yielded no variant of the Gβ86-105 region that bound with higher affinity than the wild-type Gβ region. The positively charged residues of this signal-transfer region are very important for binding affinity. Such electrostatic interactions could provide for specific binding interactions along with functional signal transfer. This has also been found to be true for the signal-transfer region of PACT, which, like that of the PACT effector kinase PKR, is made up of a number of charged amino acids. Here, the charge complementarity would serve to balance specificity and affinity such that Gβγ subunits could specifically regulate PLC-β2 activity, even though Gβγs overall binding affinity for this effector is low.

Further evidence for the role of electrostatics in general binding comes from studies of the UBC enzyme. UBC9 conjugates ubiquitin to a wide range of effectors, yet interactions between UBC9 and effectors are very selective. Regions of UBC9 implicated in effector binding include the N and C termini, the only two flexible regions of this UBC9 isozyme. Both these termini are characterized by a positive electrostatic potential. Sequence analysis of a number of UBC9 interaction partners shows them to have a large region of negative charge consisting of about 100 amino acids. This stretch of negative charge might be attracted to the positively charged C terminus of UBC9. Thus, charge complementarity may be the driving force for determining the affinity and specificity of binding for this general binding domain within the UBC9 enzyme (18).

The spatial organization of general binding domains can also contribute to protein-protein interaction specificity. We have observed an example of this for Gβs interactions with the effector PLC-β2. Here, general binding domains on Gβ with relatively low affinity for the PLC-β2 effector are separated onto geometrically distinct surfaces of the Gβ subunit (18). Multiplicity of binding domains has also been shown for Gβ interactions with G protein-coupled receptor kinase 2 (GRK2). The crystal structure of the complex of Gβγ with GRK2 shows that Gβγ interacts with the PH domain of GRK2 at geometrically distinct sites (22). In Fig. 2, three regions of GRK2 and their corresponding Gβ contact sites are indicated; these contacts are situated at distinct sites over the protein surface. Such multiplicity of general binding domains might provide an architecture that would allow for geometry to contribute to specificity, even though the binding affinity of each of the individual general binding domains is low. Electrostatics combined with the geometry of multiple binding sites would generate an even greater degree of selectivity while still allowing for conformational flexibility at each individual binding site. Thus, the signal transfer regions would be able to propagate information flow by inducing the required local conformational change in the downstream effector.

Future Experiments

Experimental approaches that establish conformational flexibility during signaling are likely to involve NMR spectroscopy of the interacting surfaces. Studies with the E2-ubiquitin ligase UBC9 provide a good example of how NMR experiments provide useful information on dynamic regions of proteins across time scales (18). A finer level of analysis using NMR has been done for the catalytic action of the enzyme cyclophilin A (23). Here, it was possible to observe conformational fluctuations in the 0.1- to 1.0-ms range, and the conformational dynamics largely agreed with the substrate turnover rates. Application of such approaches to understanding interactions between signaling proteins should shed light on how conformational dynamics play a crucial role in signal transfer.

References and Notes

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