indiscriminate, and the key cargo was not clearly identified.

In this issue of *Science*, Viola *et al.* (*5*) show that the cargo for the actin-based transport mechanism is the 70-nm-diameter lipid rafts also referred to as caveolae or detergent-insoluble glycolipid domains (*6*). The rafts are initially distributed evenly on the T cell surface and remain so after engagement of TCRs by beads coated with antibody to the TCR. Remarkably, engagement of CD28 together with the TCR recruits essentially all the rafts to the contact area. This correlates with an increased lifetime for tyrosine phosphate, which may occur through phosphatase exclusion, and increased consumption of the Lck kinase, indicative of greater tyrosine kinase activation. It has been suggested recently that engaged TCRs migrate into rafts (*7*). Viola *et al.* now demonstrate that it is the rafts that migrate to engaged TCRs and CD28.

These studies suggest that costimulation modulates the signaling environment around the engaged TCRs. Rafts are rich in kinases and adapter molecules that are required for T cell activation (*8*). In addition, the rafts' topological features are also compatible with their promoting sustained TCR engagement. Because glycolipids and small glycophosphatidylinositol-anchored molecules such as CD59, DAF, alkaline phosphatase, and Thy-1 are concentrated in rafts, these domains may represent regions of reduced steric hindrance where interaction of the short TCR and MHC would be favored. In addition, cholesterol in lipid rafts may increase membrane rigidity and enhance the affinity of membrane protein interactions.

The costimulation-initiated transport mechanism appears capable of transporting anything linked to actin. Because both positive and negative regulators of T cell signaling may be associated with the actin cytoskeleton (*9*), how does the process achieve selectivity? One type of selectivity is demonstrated in the extreme by the movies of Wülfing and Davis (*10*): size selectivity. Large beads are transported to the edge of the synapse, but are excluded because they are too big to enter. On a molecular scale, integrins, the group of adhesion molecules that includes LFA-1, can generate effective occlusive barriers that exclude large molecules from contact areas (*11*). We and others have proposed that molecules such as CD2 that interact with ligands to generate very small gaps (<15 nm) between apposed membrane are also involved in large-molecule exclusion (*1*, *12*). If the actin-based transport process can convey molecules to the center of the immunological synapse, then these barriers could be conceived of as molecular filters allowing only small molecules to enter the contact area, while excluding molecules with larger ectodomains (see the figure).

The conventional view of T cell signaling was that each type of receptor generates its own distinct signal or has its own "voice." This collection of independent voices from the surface was then harmonized (integrated) in the nucleus to regulate transcription. The new concept that is emerging suggests that the immune synapse functions to tune, adjust, and amplify a single voice, the signal transduced by the TCR. TCR signaling is intimately associated with contact formation because extended cell contact is required to maintain TCR engagement. This new concept is supported by recent studies of molecular organization of components in the immunological synapse and by the demonstration that specific transport mechanisms organize the contact area. Although we do not yet know whether CD28 and LFA-1 produce specific biochemical signals to initiate this transport process, a new paradigm for immunological costimulation is emerging that is built around the central role of contact formation in T cell activation.

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PERSPECTIVES: SIGNAL TRANSDUCTION

The Path to Specificity

Charles S. Zuker and Rama Ranganathan

Signal transduction systems in a typical eukaryotic cell consist of a network of proteins that transform multiple external stimuli into appropriate cellular responses. Molecules that form this network can be placed into ordered biochemical pathways in which signal propagation occurs through the sequential establishment of protein-protein and small molecule–protein interactions. A major challenge in the study of intracellular signaling has been the elucidation of the physical and biological principles by which the network of signaling molecules is assembled to execute temporally and spatially ordered signaling programs.

How does specificity arise in connecting a given input signal with the appropriate cellular response? How is "crosstalk" between pathways avoided when detrimental but promoted when necessary? In addressing these questions, recent work has begun to focus on the organization signaling components into macromolecular assemblies. These assemblies are mediated by multifunctional adapter proteins that are critical for both efficiency and specificity of signaling. By recruiting the appropriate assortment of signaling proteins together, adapters organize signaling pathways into distinct functional entities (*1*, *2*). Adapter molecules range from very simple to complex multidomain proteins that contain different numbers, varieties, and combinations of modular protein-protein interaction motifs.

Some of the best studied intracellular cascades are the tyrosine kinase and G protein–coupled receptor (GPCR) pathways. In the case of receptor tyrosine kinases, recruitment of specific adapter proteins (Grb2 and Shc, for example) creates a tyrosine phosphoprotein scaffold that is anchored at the plasma membrane and serves as an organizing center for components of the mitogen-activated protein (MAP) kinase pathway (*1*). Proteins assembling into this complex vary in different receptor systems, thus allowing functional diversity through modular reorganization of the signaling complex. Recently, multi–PDZ domain proteins have been shown to act as scaffolds for organizing neuronal G protein–coupled signaling proteins. In *Drosophila* photoreceptors, a five–PDZ domain protein known as InaD assembles components of the visual signaling pathway into a macromolecular complex (*3*, *4*). Flies homozygous for a null allele of InaD show mislocalization of all target proteins in photoreceptor cells and dramatic loss of signaling (*4*). Thus, in the world of intracellular real estate, location, location, and location are key determinants of in vivo function.

C. S. Zuker is at the Howard Hughes Medical Institute, Departments of Biology and Neurosciences, University of California, San Diego, CA 92093–0649, USA. E-mail: charles@flyeye.ucsd.edu. R. Ranganathan is in the Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX 75235–9050, USA. E-mail: rama@chop.swmed.edu

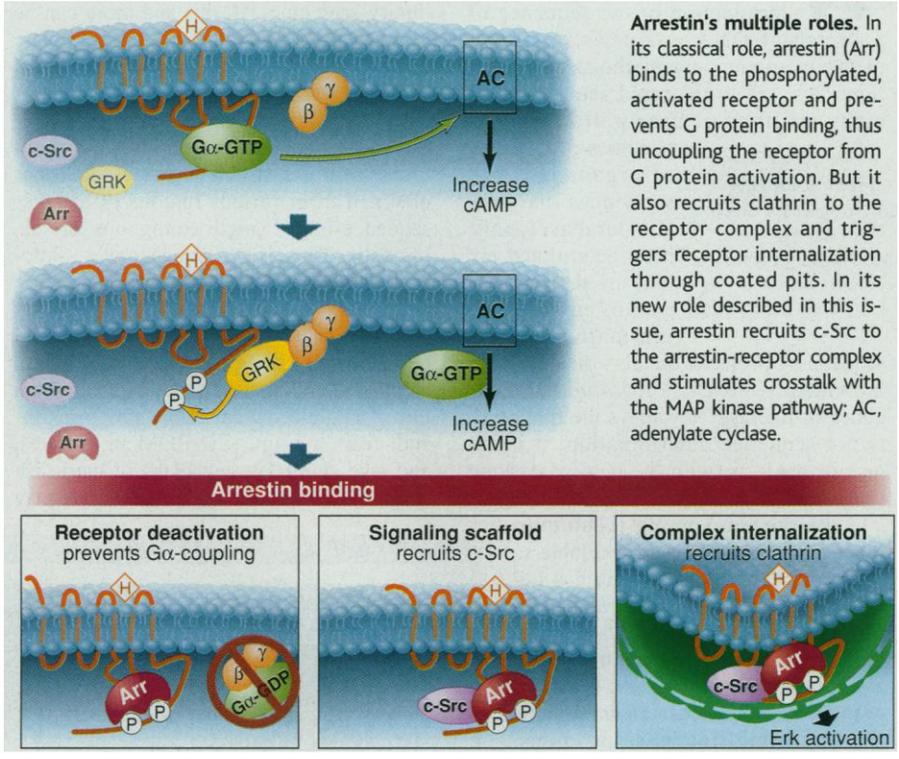
On page 655 of this issue of *Science*, Luttrell *et al.* describe the identification of a new signaling complex in which activated β-adrenergic receptors (β_2AR) couple to c-Src (*5*). These results are of significant interest because they represent a well-defined molecular example of a junction between two major intracellular signaling pathways (GPCR and tyrosine kinase). More important, they substantiate the notion that intracellular crosstalk is neither an accident nor a random consequence of "intracellular mixing." Instead, crosstalk is an active, ordered process.

The adapter protein that links β_2AR to c-Src is arrestin, the molecule long established as a deactivator of G protein–mediated signaling. In the classical role for arrestin, activation of a GPCR leads to its carboxyl-terminal phosphorylation by GPCR kinases (GRKs), which then create a high-affinity substrate for interaction with arrestin (see the figure) (*6*). Arrestin binding to GPCRs is thought to sterically prevent G protein interaction, thereby quenching (or arresting) the catalytic activity of the receptor. In this research article, Luttrell *et al.* show that agonist-mediated activation of the β_2AR leads to the formation of a protein complex consisting of receptor, arrestin, and the tyrosine kinase c-Src (see the figure). These data fit well with previous observations that β_2AR agonists stimulate activation of the MAP kinases Erk1 and Erk2 and suggest that assembly of the β_2AR–arrestin–c-Src complex is one mechanism by which this cross-pathway interaction occurs. Because arrestin shows nearly exclusive binding specificity for the active state of GPCRs, these data also indicate that c-Src activation occurs from the desensitized form of the receptor. This result nicely explains the observation that GPCR-dependent stimulation of the MAP kinase pathway does not depend on activation of GPCR-effectors (for example, phospholipase C and adenylate cyclase) (*7*). Thus, this process represents the execution of a sequential second program of signaling after GPCR activation.

Work in several laboratories had previously shown that arrestin directly binds to clathrin heavy chain and that this interaction targets arrestin-bound β_2AR for internalization through coated pits (see the figure) (*8*). Luttrell *et al.* now show that arrestin mutants that fail to interact with clathrin (that is, that prevent receptor internalization), but can still interact with receptor and c-Src, act as dominant inhibitors of β_2AR-dependent MAP kinase activation. Similarly, arrestin mutants that poorly interact with c-Src, but support receptor sequestration, inhibit β_2AR-dependent activation of MAP kinases. Thus, both c-Src recruitment and internalization of the receptor complex appear to be necessary signals for effecting MAP kinase activation.

Three mechanistic aspects of this new function for arrestin are of particular interest and require further follow-up. First, is the adapter function of arrestin a regulated process? Arrestin is a phosphoprotein. Interestingly, the free cytosolic form of arrestin is largely maintained in a phosphorylated state, but becomes dephosphorylated upon receptor interaction (*9*). Using immunoprecipitation experiments, Luttrell *et al.* showed that phosphorylated arrestin is unable to interact with c-Src. Therefore, the receptor-dependent dephosphorylation of arrestin may partially account for the recruitment and activation of c-Src. It would be valuable to determine what proteins mediate arrestin phosphorylation and dephosphorylation, and how this process is regulated. Second, what is the mechanism by which arrestin activates c-Src? Luttrell *et al.* showed that arrestin interacts with the SH3 domain of c-Src, and that binding to arrestin significantly increased c-Src's specific activity. This finding suggests that c-Src activation may result from removal of the SH3 domain–mediated inhibition of the kinase activity (*10*). Structural studies of the arrestin–c-Src complex may provide important insights into the activation mechanism. Finally, in what way is internalization of the receptor complex necessary to promote MAP kinase activation? This is particularly intriguing because c-Src binding to the arrestin-receptor complex occurs at the plasma membrane.

The emergence of adapter and scaffolding proteins as critical functional elements of cellular signaling suggests that important principles of signal transduction lie in macromolecular organization. A particularly attractive feature of signaling complex assembly through adapter proteins is simplicity through modular design. In this scenario, specificity and complexity of signaling may arise through the reorganization of signaling complexes rather than from altered activity of individual components. This work now illustrates that the same principles that govern specificity and selectivity within signaling pathways may be extended to crosstalk between signaling pathways.



Arrestin's multiple roles. In its classical role, arrestin (Arr) binds to the phosphorylated, activated receptor and prevents G protein binding, thus uncoupling the receptor from G protein activation. But it also recruits clathrin to the receptor complex and triggers receptor internalization through coated pits. In its new role described in this issue, arrestin recruits c-Src to the arrestin-receptor complex and stimulates crosstalk with the MAP kinase pathway; AC, adenylate cyclase.

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