

Signaling Through Scaffold, Anchoring, and Adaptor Proteins

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The process by which extracellular signals are relayed from the plasma membrane to specific intracellular sites is an essential facet of cellular regulation. Many signaling pathways do so by altering the phosphorylation state of tyrosine, serine, or threonine residues of target proteins. Recently, it has become apparent that regulatory mechanisms exist to influence where and when protein kinases and phosphatases are activated in the cell. The role of scaffold, anchoring, and adaptor proteins that contribute to the specificity of signal transduction events by recruiting active enzymes into signaling networks or by placing enzymes close to their substrates is discussed.

The speed and precision of signal transduction are often taken for granted. Yet, understanding the mechanisms that allow intracellular signals to be relayed from the cell membrane to specific intracellular targets still remains a daunting challenge. Many protein kinases and protein phosphatases have relatively broad substrate specificities and may be used in varying combinations to achieve distinct biological responses. Thus, mechanisms must exist to organize the correct repertoires of enzymes into individual signaling pathways. One such mechanism involves restriction of certain polypeptides to localized sites of action. This function can be achieved either by recruitment of active signaling molecules into multiprotein signaling networks (Fig. 1A) or activation of dormant enzymes already positioned close to their substrates (Fig. 1B). Simply stated, either the enzymes go to the signal or the signal goes to the enzymes.

The assembly of signaling proteins into biochemical pathways or networks is typified by the association of autophosphorylated receptor tyrosine kinases with cytoplasmic proteins that contain specialized protein modules that mediate formation of signaling complexes (Fig. 2) (1). For example, src homology 2 (SH2) domains bind specific phosphotyrosyl residues on activated receptors (Fig. 2A), and src homology 3 (SH3) domains bind to polyproline motifs on a separate set of target proteins (Fig. 2D) (2). This permits simultaneous association of a single protein containing both SH2 and SH3 domains with two or more binding partners, and hence, the assembly of complexes of signaling proteins around an activated cell-surface receptor (Fig. 1A). Similarly, subcellular organization of serine-threonine kinases and phosphatases occurs

through interactions with the targeting subunits or anchoring proteins that localize these enzymes (3). In addition, kinase binding proteins such as 14-3-3 proteins serve as adaptor proteins for signaling networks, whereas proteins such as Sterile 5 (Ste 5) and AKAP79 maintain signaling scaffolds of several kinases or phosphatases (Fig. 1B) (4). Hence, the cell uses related mechanisms for controlling the subcellular organization of tyrosine and Ser-Thr phosphorylation events. In this review, we compare and contrast the protein modules, adaptor molecules, targeting subunits, and anchoring proteins that coordinate signaling networks.

Mechanisms for Recognition of Phosphotyrosine and Peptide Motifs

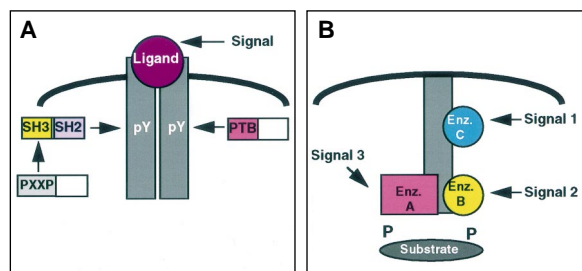
SH2 domains are protein modules that recognize short, phosphopeptide motifs composed of phosphotyrosine (pTyr) followed by three to five COOH-terminal residues, such as those generated by autophosphorylation of activated receptor tyrosine kinases (Fig. 2A) (5). According to this scheme, the sequences of the SH2 docking sites on a given receptor tyrosine kinase dictate which SH2-containing targets associate with the receptor and will therefore help determine which signaling pathways the

receptor can activate. These modules are coupled directly or indirectly to downstream signaling molecules, including enzymes that control phospholipid metabolism, Ras-like guanosine triphosphatases (GTPases), protein kinases, transcription factors, and polypeptides that regulate cytoskeletal architecture and cell adhesion.

A genetic test of this concept has been provided in mice by use of the Met receptor tyrosine kinase, which has two closely spaced autophosphorylation sites within its COOH-terminal tail that bind a number of signaling proteins with SH2 domains. Conversion of these Tyr residues to Phe results in the same phenotype as a null mutation, despite the fact that the activity of the kinase domain is unaltered. In contrast, a single substitution of an Asn two residues COOH-terminal to one of the phosphotyrosine sites (the +2 position) selectively uncouples the receptor from its ability to bind the Grb2 SH2/SH3 adaptor protein and yields a hypomorphic phenotype affecting myoblast proliferation and muscle formation (6). Thus, the creation of docking sites through autophosphorylation is absolutely required for Met function in vivo, and specific binding to a particular SH2-containing protein is required for one of the receptor's biological activities.

The pTyr-binding (PTB) domains of the Shc and insulin receptor substrate-1 (IRS-1) proteins (7) recognize phosphopeptide motifs in which pTyr is preceded by residues that form a β turn [usually with the consensus NPXpY (8)] (9). Specificity is conferred by hydrophobic amino acids that lie five to eight residues NH₂-terminal to the pTyr (Fig. 2B) (10) and therefore recognize their ligands in a distinct manner from SH2 domains (11). PTB domains may serve a somewhat different purpose from SH2 do-

Fig. 1. Mechanisms for recruiting or localizing signal transduction components. **(A)** Assembly of modular signaling molecules on an activated receptor tyrosine kinase. An extracellular signal input drives autophosphorylation of the receptor and leads to the recruitment of cytoplasmic proteins that contain various protein modules. pY, phosphotyrosine. **(B)** A localized signaling complex of three anchored signaling enzymes. Each enzyme is inactive when bound to the anchoring protein but is released and activated by different signals. Enz., enzyme.



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mains, because they are found primarily as components of docking proteins that recruit additional signaling proteins to the vicinity of an activated receptor. The PTB domains of proteins such as X11, FE65, and Numb can bind nonphosphorylated peptide motifs (12), indicating that PTB domains are principally peptide recognition elements, unlike SH2 domains that appear devoted to the job of pTyr recognition.

PDZ domains have a somewhat similar mechanism of ligand-binding to PTB domains, in which the peptide binds as an additional strand to an antiparallel β sheet (13). A distinguishing feature of PDZ domains is their recognition of short peptides with a COOH-terminal hydrophobic residue and a free carboxylate group, as exemplified by the E(S/T)DV motif at the COOH-terminus of certain ion channel subunits (Fig. 2C) (14). These interactions can promote clustering of transmembrane receptors at specific subcellular sites and have an especially important role in the spatial organization of voltage- and ligand-gated ion channels at synapses, because shaker-type K^+ channels and all three classes of glutamate receptors are recognized by distinct PDZ domain proteins (Fig. 3A) (15, 16). Specificity is conferred by ligand residues at the -2 to -4 positions relative to the COOH-terminus and may be regulated by phosphorylation, because the -2 residue of PDZ-binding sites is often a hydroxy-amino acid. In particular, a COOH-terminal motif (RRESAI) on the inwardly rectifying K^+ channel Kir 2.3 binds the second PDZ domain of a 95-kD postsynaptic density protein called PSD-95. PKA phosphorylation at the -2 Ser of the PDZ recognition site uncouples the channel from PSD-95 and results in inhibition of K^+ conductance (Fig. 3A) (17).

Many proteins have multiple PDZ domains (up to seven in known proteins), which may have at least two important

consequences. An individual PDZ-containing protein could bind several subunits of a particular channel, and thereby induce channel aggregation. This could be enhanced by the ability of a protein such as PSD-95 to form oligomers through NH_2 -terminal intermolecular disulfide bonds (18). Furthermore, the individual PDZ domains of a protein such as PSD-95 can have distinct binding specificities, leading to the formation of clusters that contain heterogeneous groups of proteins. Thus, the ability of the third PSD-95 PDZ domain to bind the cell-adhesion molecule neuroligin may direct the *N*-methyl-D-aspartate receptor NMDA2 and K^+ channels, which interact with the first and second PDZ domains, to specific synaptic sites (19). Two further properties of PDZ domains or proteins that contain them may expand their potential for regulating signal transduction. First, some PDZ domains may bind internal peptide sequences and, indeed, have a propensity to undergo homotypic or heterotypic interactions with other PDZ domains (20). Second, proteins with PDZ domains frequently contain other interaction modules, including SH3 and LIM domains, and catalytic elements such as tyrosine phosphatase or nitric oxide synthase domains. PDZ interactions may therefore both coordinate the localization and clustering of receptors and channels, and provide a bridge to the cytoskeleton or intracellular signaling pathways.

The sophistication of signaling networks maintained by PDZ interactions is illustrated by InaD, a polypeptide with five PDZ domains that regulates phototransduction in *Drosophila melanogaster* photoreceptors (Fig. 3B) (21). InaD associates through distinct PDZ domains with a calcium channel (TRP); phospholipase C- β , the target of rhodopsin-activated heterotrimeric guanine nucleotide-binding protein (Gq α); and protein kinase C (PKC). InaD organizes these proteins into a signaling complex that

allows efficient activation of the TRP channel by PLC- β in response to stimulation of rhodopsin and deactivation through phosphorylation of TRP by PKC (22). Thus, InaD appears to act as a scaffolding protein to organize light-activated signaling events (Fig. 3B).

Domains that bind proline-rich motifs. SH3 domains bind proline-rich peptide sequences with the consensus PXXP that form a left-handed polyproline type II helix (Fig. 2D) (23). A principal role of SH3 domains is in forming functional oligomeric complexes at defined subcellular sites, frequently in conjunction with other modular domains (2). There are certain parallels between SH3 and PDZ domains. Proteins can have multiple SH3 domains, potentially allowing clustering of several distinct ligands, and Ser or Thr phosphorylation adjacent to the proline-rich ligand may influence SH3 domain interactions (24). Serine phosphorylation of a PDZ or SH3 recognition site results in uncoupling of signaling proteins, in contrast to the autophosphorylation of receptor tyrosine kinases, which promotes the assembly of signaling complexes at the SH2 acceptor site.

WW domains are very small modules of 35 to 40 residues that also bind proline-rich motifs, commonly with the consensus PPXY or PPLP (Fig. 2E) (25). The WW domains of the E3 ubiquitin protein ligase Nedd4 bind such proline-rich motifs in an amiloride-sensitive epithelial Na^+ channel, likely leading to channel degradation (Fig. 3A) (26). Channel mutations that disrupt this interaction cause a human hypertensive disorder, Liddle's syndrome (27). WW domains may also regulate catalytic function, as suggested by a structural analysis of the peptidyl-prolyl *cis-trans* isomerase Pin1. Pin1, which interacts with cell cycle components such as the protein kinase NIMA, possesses an NH_2 -terminal WW domain that forms one part of a ligand-binding surface, which includes an α helix from the rotamase domain. The WW domain may thereby contribute to substrate

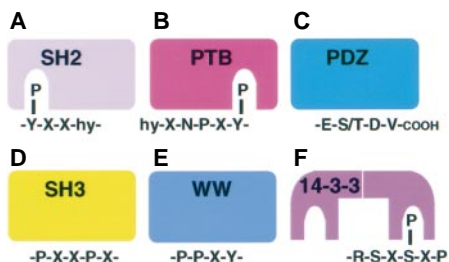
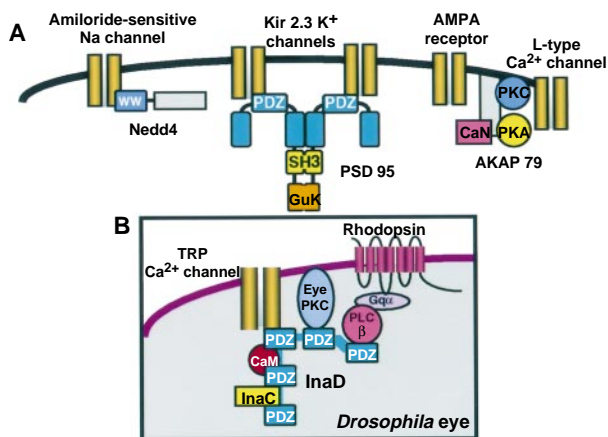


Fig. 2. Protein modules for the assembly of signaling complexes. Several modular domains have been identified that recognize specific sequences on their target acceptor proteins. These sequences, in single-letter code, are indicated for (A) SH2 domains, (B) PTB domains, (C) PDZ domains, (D) SH3 domains, (E) WW domains, and (F) 14-3-3 proteins. hy indicates hydrophobic residues.

Fig. 3. Localization of signaling molecules with ion channels. (A) Modulation of certain ion channels may be coordinated by modular docking proteins or scaffold proteins that tether signaling enzymes in proximity to ion channels. Each ion channel is identified above, and the docking or anchoring proteins are indicated below. CaM, calcineurin. (B) In *Drosophila* eye, InaD coordinates the location of PKC and phospholipase β in relation to the channel. Calmodulin (CaM) and InaC also associate with the channel.



recognition (28). An intriguing feature of Pin1 is the ability of its catalytic domain to specifically recognize phosphorylated (Ser/Thr)Pro motifs, raising the possibility that Ser-Thr phosphorylation of cell cycle regulators creates a recognition site for Pin1, which in turn could modify their conformation and functional properties (29). A conserved domain, EVH1, has recently been identified in profilin-binding proteins such as VASP and Mena, and has been shown to bind proline-rich peptide sequences such as the (E/D)FPPPPX(D/E) motif found in the ActA protein of *Listeria monocytogenes*. The EVH1 domain may couple cytoskeletal proteins such as zyxin and vinculin, and bacterial ActA, to actin remodeling (30).

Phospholipid recognition and membrane targeting. Not all localization of signaling modules is directed by interaction with other proteins. Pleckstrin homology (PH) domains bind the charged headgroups of specific polyphosphoinositides and may thereby regulate the subcellular targeting of signaling proteins to specific regions of the plasma membrane (Fig. 4) (31). This positions such proteins for interactions with regulators or targets. In this way, PH domains can couple the actions of phosphatidylinositol (PI) kinases, inositol phosphatases, and phospholipases to the regulation of intracellular signaling (32). This is illustrated by the finding that the product of PI 3'-kinase, PI-3,4,5-P₃, binds specifically to the PH domain of a protein, Grp1, which can potentially function as an exchange factor for the small GTPase Arf (33). Because the p85 regulatory subunit of PI 3'-kinase possesses SH2 domains that control enzyme activation by tyrosine phosphorylation, it seems that the SH2 and PH domains of distinct proteins can act sequentially in a common pathway to link receptor tyrosine kinase signaling to the control of vesicle trafficking.

PH domains are also found covalently linked to other modules, such as SH2, SH3, and PTB domains, with which they may synergize in controlling the activation of specific signaling proteins. For example, the Btk cytoplasmic tyrosine kinase contains an NH₂-terminal PH domain that binds PI-3,4,5-P₃ and is covalently linked to SH3, SH2, and kinase domains (34). The production of PI-3,4,5-P₃ may therefore induce association of Btk with the membrane, facilitating the interaction of the SH2, SH3, and catalytic domains with activators and targets. Mutations in the PH domain that inhibit phospholipid recognition, or of Cys residues in an adjacent structural element that binds Zn, lead to an inherited human B cell defect, X-linked agammaglobulinemia (35).

Docking and Scaffolding Proteins in Receptor Signaling

One way receptors may amplify their signaling is to use adaptor proteins that provide additional docking sites for modular signaling proteins. Typically, docking proteins have an NH₂-terminal membrane-targeting element, either a PH domain or a myristylation site, and a PTB domain that directs association with an NPXY autophosphorylation site on a specific receptor (Fig. 4). Once it is associated with the appropriate activated receptor, the docking protein becomes phosphorylated at multiple sites that interact with specific SH2 domains of signaling proteins. For example, IRS-1 and IRS-2 (two principal substrates of the insulin receptor) have an NH₂-terminal PH domain followed by a PTB domain and 18 potential tyrosine phosphorylation sites (36). Under physiological circumstances, both the IRS-1 PH and PTB domains are required for insulin-induced tyrosine phosphorylation of IRS-1 and mitogenic signaling, suggesting that membrane targeting of IRS-1 and physical binding to its receptor tyrosine kinase facilitate insulin-mediated signaling (Fig. 4) (37). Another membrane-associated docking protein, Shc, has an NH₂-terminal PTB domain that binds both pTyr sites and phosphoinositides, as well as a COOH-terminal SH2 domain, and consequently is phosphorylated by a broad range of receptor tyrosine kinases (11). In contrast, mammalian Gab-1, which has an NH₂-terminal PH domain, may play a more specific role downstream of the Met tyrosine kinase, and p62^{dok} is a prominent substrate of the Eph receptors that control axon guidance (38). Similarly, FRS2, which is myristylated and has a potential PTB domain, is specifically phosphorylated by receptors for fibroblast growth factor (FGF) and nerve growth factor (NGF) (39).

The signaling properties of these docking proteins likely depend on the sequences of their SH2-binding motifs. Commonly, a specific Tyr-based motif is reiterated several times within an individual docking protein. Thus, Shc has two YXNX motifs, which can couple to Grb2 and the Ras pathway (40); p62^{dok} has six YXXP motifs, which may bind SH2-containing proteins that influence the cytoskeleton (38); and IRS-1 has nine YXXM motifs, which can bind and activate PI 3'-kinase. This phenomenon may allow selective amplification of specific signaling pathways. The *Drosophila* docking protein Dos, which has the potential to bind several distinct signaling proteins with SH2 domains, including Drk (the *Drosophila* ortholog of Grb-2) and corkscrew (an SH2-containing tyrosine phosphatase), was originally identified in a genetic screen for com-

ponents of RTK signaling pathways in *Drosophila* (41). This genetic analysis supports the notion that SH2-docking proteins augment and regulate tyrosine kinase signaling.

Signaling Networks for Serine-Threonine Phosphorylation

The importance of protein-protein interactions is not confined to signaling steps controlled by tyrosine phosphorylation. Although some Ser-Thr kinases and phosphatases were originally thought to be constitutively attached to intracellular loci through association with targeting subunits or anchoring proteins, it is now clear that some are also recruited into signaling networks (3). Apparently, protein modules control the location and assembly of these Ser-Thr kinase signaling networks, providing an intriguing parallel with tyrosine kinase signaling events.

Protein kinase A and protein kinase C anchoring. In its inactive form, the adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase (PKA) is composed of two catalytic (C) subunits held in an inactive conformation by association with two regulatory (R) subunits. Binding of cAMP to the R subunits causes dissociation of the C subunits, which act to control a wide range of biological processes. Although cAMP is the sole activator of PKA, other regulatory proteins control where and when the kinase is turned on in response to specific stimuli. The concerted actions of adenyl cyclases and phosphodiesterases create gradients and compartmentalized pools of cAMP, whereas A-kinase anchoring proteins (AKAPs) maintain the PKA holoenzyme at precise intracellular sites. Anchoring ensures that PKA is exposed to localized changes in cAMP and is compartmentalized with sub-

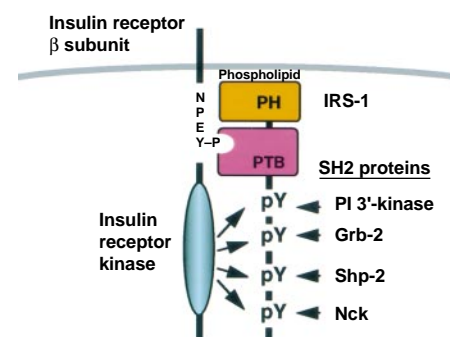


Fig. 4. The IRS-1 docking protein. The IRS-1 docking protein contains an NH₂-terminal PH domain that potentially mediates interactions with the membrane and a PTB domain that binds a specific juxtamembrane Tyr autophosphorylation site in the insulin receptor. The kinase domain of the activated insulin receptor phosphorylates Tyr residues in IRS-1 that act as docking sites for multiple SH2-domain signaling proteins.

strates. The AKAPs have two types of protein sequence that direct PKA function: a conserved amphipathic helix binds the R subunit dimer of the PKA holoenzyme and a specialized targeting region tethers individual PKA-AKAP complexes to specific subcellular structures (Fig. 5A) (42). As a consequence, PKA is held by the AKAP in an inactive state at a defined intracellular location, where it is poised to respond to cAMP by the local release of active C subunit. Thus, a pleiotropic protein kinase can rapidly phosphorylate specific targets in response to a defined signal.

Information on the AKAP motifs that direct R subunit-binding and subcellular localization has been exploited to alter the distribution of PKA inside cells. Heterologous expression of AKAP75, or its human homolog AKAP79, redirects PKA to the periphery of HEK 293 cells. This submembrane targeting of PKA enhances cAMP-stimulated phosphorylation of the $\alpha 1$ subunit of the cardiac L-type Ca^{2+} channel and increases ion flow (43). Conversely, microinjection of “anchoring inhibitor peptides,” which compete for the RII-AKAP interaction, displaces the kinase from anchoring sites and attenuates ion flow through skeletal muscle L-type Ca^{2+} channels, AMPA-kainate glutamate receptor ion-channels, and Ca^{2+} -activated K^+ channels (44). Hence, PKA anchoring seems to augment rapid cAMP responses such as ion-channel modulation. AKAPs also orchestrate the role of PKA in more complicated physiological processes such as glucagon-related peptide (GLP-1)-induced insulin secretion from pancreatic β islet cells (45).

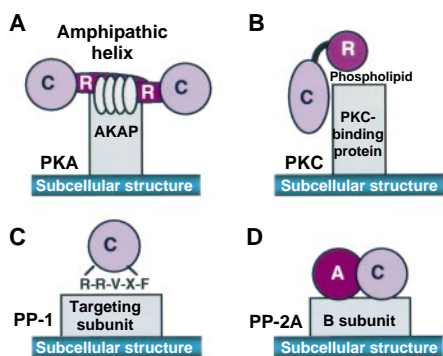


Fig. 5. Targeting proteins for Ser-Thr kinases and phosphatases. Targeting proteins are depicted for (A) the cAMP-dependent protein kinase, which is bound to its AKAPs by an amphipathic helix on the AKAP; (B) PKC, which is attached to its binding protein through protein-phospholipid interactions; (C) PP-1, which binds its targeting subunit through a consensus-binding motif (indicated in the single-letter code); and (D) the B subunit of PP-2A, which binds and targets the A and C subunit complex.

AKAPs were thought to exclusively target the type II PKA. However, a family of dual function AKAPs has now been discovered that bind RI or RII (46). Although *in vitro* studies indicate that RI binds several AKAPs, with affinity one one-hundredth that of RII, the micromolar binding-constant interaction is within the physiological concentration range of RI and AKAPs inside cells. Thus, type I PKA anchoring may be relevant under certain conditions where RII concentrations are limiting (47). Certain AKAPs bind multiple signaling enzymes. AKAP79 functions as a signaling scaffold for PKA, PKC, and protein phosphatase 2B at the postsynaptic densities of neurons, whereas AKAP250 (gravin) targets both PKA and PKC to the membrane cytoskeleton and filopodia of cells (48). Anchored signaling scaffolds may permit the integration of signals from distinct second messengers to preferentially control selected phosphorylation events.

The Ca^{2+} phospholipid-dependent protein kinase, PKC, exerts a wide range of biological effects. Various isoforms of PKC are differentially localized inside cells. Whereas the attachment of PKC to membranes clearly requires protein-phospholipid interactions, protein-protein interactions seem to facilitate the differential localization of PKC isoforms inside cells (49). Several classes of PKC targeting proteins have been identified. Substrate-binding proteins (SBPs) bind PKC in the presence of phosphatidylserine by forming a ternary complex with the kinase (Fig. 5B). Phosphorylation of SBPs by PKC abolishes the targeting interaction, suggesting that SBPs may associate with the kinase transiently and represent a subclass of PKC substrates that release the enzyme slowly upon completion of the phosphotransfer reaction. Receptors for activated C kinase (RACKs) are not necessarily substrates for PKC and bind at one or more sites distinct from the substrate-binding pocket of the kinase. Thus, it has been proposed that PKC remains active when bound to a RACK. A third class of PKC-binding protein, termed PICKs (for proteins that interact with C kinase), have been cloned in two-hybrid screens in which the catalytic core of the kinase was used as bait. One of these proteins, called PICK-1, is a perinuclear protein that appears to only recognize determinants in the active enzyme, and also contains a PDZ domain. This is in keeping with accumulating evidence that subcellular targeting of PKC is also mediated through association with other signaling proteins (50). For example, AKAP79 colocalizes PKC with PKA and calcineurin; gravin binds PKC and PKA; and the InaD clusters PKC with phospholipase-C β , G proteins, and calmodulin

(Fig. 3). Hence, these proteins may represent an emerging class of mammalian targeting proteins that organize signal transduction events by bringing kinases together.

Phosphatase targeting. The dephosphorylation of proteins is of equal importance to protein phosphorylation for the regulation of cellular behavior, and the functions of protein phosphatases seem to be controlled by targeting interactions like those described for protein kinases. Indeed, a common feature of many intracellular tyrosine phosphatases is the presence of noncatalytic domains that direct the phosphatase to specific compartments. For example, two mammalian tyrosine phosphatases, Shp-1 and Shp-2, contain tandem NH_2 -terminal SH2 domains that both regulate phosphatase activity and allow these enzymes to be recruited into complexes of specific pTyr-containing proteins (51).

Among the Ser-Thr phosphatases, PP-1, PP-2A, and PP-2B interact with distinct targeting proteins or subunits (3, 4). PP-1 associates with glycogen particles in liver through a “glycogen-targeting subunit” (GL), whereas the skeletal muscle form of the targeting subunit (GM) targets PP-1 to the sarcoplasmic reticulum and to glycogen. Association with GM or GL has allosteric effects that modify the substrate specificity of the enzyme (3, 52). The PP-1 binding site on GL has been crystallized in a complex with the PP-1 subunit, and a consensus peptide motif, RRVXF, has been identified on the various PP-1 targeting subunits (Fig. 5C) (53). Accordingly, peptide analogs have been generated that disturb the location of the phosphatase inside cells (54). The coordination of signaling events at the glycogen particle is also achieved by a PP-1 scaffold protein called PTG, which maintains the phosphatase with some of its major substrates, phosphorylase kinase, glycogen synthase, and phosphorylase a (55). Other PP-1 targeting subunits direct the phosphatase to smooth muscle (PP-1M) or the nucleus (SDS-22 or NIPP-1), or for association with the p53 binding protein 2 [reviewed in (3, 4)].

PP-2A is a heterotrimer consisting of a 36-kD catalytic subunit (C), a 65-kD structural subunit (A), and a regulatory subunit (B), and is compartmentalized through association with its own set of targeting subunits. Several families of B subunits have been identified that participate in directing the subcellular location of the holoenzyme to centrosomes, the endoplasmic reticulum, golgi, and the nucleus (Fig. 5D). Targeting of PP-2A to the microtubule fraction is also mediated by a specialized B subunit that associates with microtubule-associated proteins such as Tau (56). Targeting proteins also localize the protein phosphatase 2B

(PP-2B, also known as calcineurin). In the brain, the particulate form of the PP-2B is inactive, possibly because it is targeted to submembrane sites and inhibited through association with AKAP79 (48). Other studies suggest that cytosolic PP-2B associates with one of its physiological substrates, the transcription factor NFATp. T cell activation and increased transcription of genes encoding cytokines require the dephosphorylation-dependent translocation of the NFAT complex into the nucleus, possibly with the phosphatase still attached (57).

Coordination of MAP kinase cascades. Ras signal transduction pathways link activation of receptor tyrosine kinases to changes in gene expression (58). This pathway proceeds from the membrane-bound guanine nucleotide-binding protein Ras, through the sequential activation of the cytoplasmic Ser-Thr kinases Raf [a mitogen-activated protein kinase (MAPK) kinase kinase or MAPKKK], Mek (a MAPK kinase or MAPKK), and Erk (a MAPK), and leads to specific gene expression in the nucleus. Distinct MAPK cassettes, each composed of three successive kinases, are activated in mammalian cells by mitogenic or stress signals.

In *Saccharomyces cerevisiae*, the pheromone mating response is initiated through G protein-linked receptors that activate a kinase, Ste 20. This leads to the stimulation of the MAPKKK Ste 11, which phosphorylates and activates Ste 7 (a MAPKK), which in turn phosphorylates and activates the MAPK homologs Fus3 or Kss1 (59). This signaling pathway can be tightly controlled, because each enzyme associates with a docking site on a scaffold protein called Ste 5 (60). There may be additional components of the complex, because upstream activators of the pathways such as the G protein β subunit, Ste 4, and possibly Ste 20 interact with Ste 5 (61). Ste 5 may serve two functions. Dimerization of Ste 5, which requires an NH₂-terminal RING-H2 domain, can facilitate intersubunit autophosphorylation and activation of individual Ste 5-associated kinases (62). The clustering of successive members in the MAPK cascade favors tight regulation of the pathway by ensuring that signals pass quickly from one kinase to the next, thus preventing "cross-talk" between functionally unrelated MAPK units in the same cell. In addition to the indirect association of Ste 7 and Fus3 or Kss1 through the Ste 5 scaffolding protein, these two kinases may interact directly in the absence of Ste 5 through an NH₂-terminal peptide motif in Ste 7 (63). Such interactions involving a MAPKK and its cognate MAPK could increase the fidelity of the pathway and might allow the MAPKK to serve as a

cytoplasmic anchor for the MAPK.

Recently, a second yeast scaffold protein called Pbs2p was identified that coordinates components of the *S. cerevisiae* osmoregulatory pathway. One activator of this pathway is the transmembrane osmosensor Sho1, which has a cytoplasmic SH3 domain and activates a MAPK cascade containing the MAPKKK Ste 11, the MAPKK Pbs2, and the MAPK Hog1. Pbs2, in addition to acting as a MAPKK, also appears to serve a scaffolding function by interacting with Sho1, Ste11, and Hog1 (64). Indeed, the NH₂-terminal region of Pbs2 has a proline-rich motif that binds the Sho1 SH3 domain; genetic evidence indicates that this interaction is necessary for activation of the pathway in response to osmotic stress. The importance of the Ste 5 and Pbs2 scaffolds in maintaining signaling specificity is emphasized by the observation that although the mating and osmosensory MAPK pathways share a common component, Ste 11, they show no cross-talk.

So far, there is little evidence to suggest that Ste 5 orthologs exist in mammalian cells, but it seems likely that mammalian scaffolding proteins for MAPK pathways exist. Subcellular targeting of the stress-activated (Jun) protein kinase (termed JNK or SAPK) is achieved, in part, through association with JIP-1, an SH3-containing protein that prevents nuclear translocation of JNK and inhibits the bound kinase (65). This latter property is reminiscent of the AKAP signaling scaffolds, where each enzyme is maintained in the inactive state by the anchoring protein.

The 14-3-3 proteins. Growing evidence suggests that Ser phosphorylation induces specific protein-protein interactions, mediated by the 14-3-3 family of adaptor proteins (66). These proteins associate to form homo- and heterodimers with a saddle-shaped structure, with each monomer possessing an extended groove that provides a likely site for peptide binding (Fig. 2F) (67). Mammalian 14-3-3 proteins can associate with a number of signaling molecules, including the c-Raf and Ksr Ser-Thr protein kinases, Bcr, PI 3'-kinase, and polyomavirus middle T antigen. Through dimerization, the proteins also may function to bridge the interaction of two binding partners, as suggested for c-Raf (66) and Bcr (68). Furthermore, association with targets such as c-Raf and Ksr requires recognition of a phosphoserine residue contained within the consensus motif RSX-pSer-XP, in a fashion reminiscent of SH2 domain interactions with pTyr-containing motifs (69).

An important function for 14-3-3 proteins in cell cycle control is suggested by their ability to recognize a pSer motif in human Cdc25C, a phosphatase that regu-

lates the activity of the Cdc2 protein kinase and thereby controls entry into mitosis. Phosphorylation of Cdc25C at Ser²¹⁶ during interphase creates a 14-3-3 binding site and inhibits Cdc25C biological activity (70). A distinct function of 14-3-3 proteins may be to potentiate the survival of mammalian cells through inducible recognition of phosphorylated Bad, a death inducer, thereby disrupting a heteromeric complex between Bad and Bcl-XL, an antagonist of cell death (71). By contrast, in plants, 14-3-3 binds and inhibits the activity of phosphorylated nitrate reductase to control nitrogen metabolism in spinach (72). These results suggest that 14-3-3 proteins exert biological effects through regulating oligomeric protein-protein interactions and protein localization, as well as through control of enzymatic activity.

Conclusions and Perspectives

Cellular responses to external and intrinsic signals are organized and coordinated through specific protein-protein and protein-phospholipid interactions, commonly mediated by conserved protein domains. Such protein modules have apparently developed to recognize determinants that are likely to be exposed within their molecular partners. By the repeated use of these rather simple lock-and-key recognition events, a complex and diverse regulatory network of molecular interactions can be assembled. The covalent association of these recognition modules—as found in adaptors, anchoring proteins, and docking proteins—allows a single polypeptide to bind multiple protein ligands. This can be used to couple an activated receptor to several downstream targets and biochemical pathways or to increase the affinity and specificity with which a single partner is engaged. As an added complexity, a single module can bind either to a motif within the same molecule or in an intermolecular fashion to other proteins. Thus, the intramolecular association of the SH2 and SH3 domains of Src family kinases with internal binding sites both represses Src kinase activity and blocks SH2/SH3 domain association with heterologous polypeptides. During Src activation, these domains are liberated for association with substrates and cytoskeletal elements. Consequently, modular domains that participate in tyrosine kinase signaling act to localize proteins to specific subcellular sites, to control enzyme activity, to direct the formation of multiprotein complexes, and to directly transduce signals.

The Ser-Thr kinases and phosphatases use a variation on this theme whereby the enzymes appear to be constitutively targeted and colocalized with their substrate at

their sites of action. Often, these anchored enzymes only become activated when their stimulating second messengers and signals become available. Recent data suggest that serine phosphorylation, like tyrosine phosphorylation, may directly regulate modular protein-protein interactions. Now that the intricacy of these interactions is understood, the challenge ahead is to understand both the physiological functions and regulation of such signaling networks.

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RESEARCH ARTICLES

Large-Cage Zeolite Structures with Multidimensional 12-Ring Channels

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Zeolite type structures with large cages interconnected by multidimensional 12-ring (rings of 12 tetrahedrally coordinated atoms) channels have been synthesized; more than a dozen large-pore materials were created in three different topologies with aluminum (or gallium), cobalt (or manganese, magnesium, or zinc), and phosphorus at the tetrahedral coordination sites. Tetragonal UCSB-8 has an unusually large cage built from 64 tetrahedral atoms and connected by an orthogonal channel system with 12-ring apertures in two dimensions and 8-ring apertures in the third. Rhombohedral UCSB-10 and hexagonal UCSB-6 are structurally related to faujasite and its hexagonal polymorph, respectively, and have large cages connected by 12-ring channels in all three dimensions.

Extensive research has led to the synthesis of zeolitic materials with previously unseen compositions and framework topologies (1), including ultralarge-pore structures VPI-5, AlPO₄-8, and UTD-1, which have pores formed of 18-, 14-, and 14-rings, respective-

ly (2). A number of structures with 12-ring channels (for example, AlPO₄-5, MAPSO-46, and CoAPO-50) have also been reported (1). Other open framework structures that have large cages or pore sizes include JDF-20, cloverite (3), and unusually low-density vanadium phosphate frameworks (4).

Unlike faujasite or its hexagonal polymorph, known materials with a zeolite