



## Adaptor proteins

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The specific and appropriate response of cells to external stimuli requires the integration of multiple signaling pathways. Stimulation of cell surface receptors will initiate cellular signals that are governed by post-translational modifications (e.g., phosphorylations), recruitment of protein binding partners to specific subcellular domains, such as the membrane and through protein–protein interactions. One of the major goals of scientists who study signal transduction is to determine the mechanisms that control cross-talk between signaling cascades and to determine how specificity in signaling is achieved. An emerging class of proteins that are major contributors to these processes are adaptor (or adapter) proteins. Adaptor proteins contain a variety of protein-binding modules that link protein-binding partners together and facilitate the creation of larger signaling complexes. By linking specific proteins together, cellular signals can be propagated that elicit an appropriate response from the cell to the environment. Specificity in signaling would be achieved by the type of protein binding modules encoded by the adaptor protein, the sequence of these domains or motifs that would dictate specificity in binding, as well as the subcellular localization and the proximity of binding partners. Thus, adaptor proteins are positioned to regulate cell signaling in a spatial and temporal fashion.

An appreciation for the emergence and recognition of adaptor proteins can be traced back to earlier studies that defined functional domains within proteins and the subsequent identification of their function. Based on studies of glycolytic enzyme pathways, the eminent X-ray crystallographer Michael G Rossman proposed that ‘domains’ within a protein could be defined based on (1) homologous sequences in other proteins, (2) structure, (3) spatial separation within a protein, (4) function and (5) an active center (Rossman, 1981, *Phil. Trans. Roy. Soc. Lond.*, pp. 191–203). Not long afterward, Pawsons and colleagues used computer-

based searching techniques to identify the src-homology 2 (SH2) domain within the amino terminus of the Src nonreceptor tyrosine kinase and verified the presence of this domain in other signaling proteins. Biochemical analyses by this group demonstrated the function of the SH2 domain and not long afterward, a number of other protein binding modules were identified, such as the SH3, PH, WW and other domains, as well as the conservative structure of the opposing binding motifs. Deletional mutagenesis studies by Parsons and colleagues as well as others demonstrated that the integrity of these protein-binding modules was functionally required for v-Src or Src527F to transform cells. Here, mutations in the SH2 and SH3 domains of v-Src or Src527F were able to block the ability of these oncogene products to transform cells while having little effect on the high protein tyrosine-specific kinase activity of Src that is associated with transformation-competence. Ultimately, the structural analysis of these modular domains using X-ray crystallography or NMR demonstrated the active centers of these domains and the mechanisms by which they facilitate protein-protein interactions. Collectively, these data demonstrated the structure and function of protein binding domains and underscored the importance of protein-protein interactions in modulating cellular signaling.

Interestingly, it was the identification of Crk by Mayer and colleagues in 1988 that offered an initial example of a signaling protein that encoded little more than a series of protein binding modules (SH2 and SH3 domains). The ability of Crk to transform cells indicated that this protein served to link signaling proteins together that effected cell growth and cell shape. Here, we used Crk as a model to define the class of adaptor proteins that are covered in this review. A case could be made for proteins such as Src as having the qualities of an adaptor protein, based on the observations by Varmus and colleagues of the function of cSrc in cSrc<sup>-/-</sup> cell lines, where the integrity of the SH2/SH3 domains and not the kinase domain were important for facilitating cell spreading. However, for our purposes, we limit the definition of adaptor proteins to those proteins that lack an enzymatic function and contain two or more protein binding modules that serve to link signaling proteins together which effect cell growth and shape. This ability to bind to two or more proteins at once provides the cell with a mechanism to link signaling proteins to each other and propagate a cellular signal.

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We begin this special edition of *Oncogene* with a review of adaptor proteins that promote receptor-mediated signaling, as exemplified by the T cell receptor signaling adaptor proteins, LAT, GADS, and SLP-76. Wonerow and Watson initiate this series with a discussion of the transmembrane adaptor, LAT, which is recognized to play a central role in linking T cell receptor mediated activation of Lck, Fyn and Zap 70 at the cell membrane to stimulate downstream signaling. McGlade follows with a review of the structure and function of GADS, which serve to link LAT to SLP-76. Subsequently, Judd and Koretzky describe SLP-76 structure and function in T cell signaling, as well as the role of this protein in modulating outside-in signaling through integrin  $\alpha$ IIb $\beta$ 3 in collagen-induced platelet activation. Two other interesting examples of adaptor proteins that modulate signals emanating from the cell membrane are intersectins and NHERF. O'Bryan describes the intersectins and their role in facilitating endocytosis following receptor activation. Lastly, Voltz *et al.* describe the role of an interesting Na<sup>+</sup>/H<sup>+</sup> ion exchange regulatory factor called NHERF, which contains tandem PDZ domains and can bind to the ERM family of cytoskeletal adaptor proteins. Although NHERF was originally described as a regulator of Na<sup>+</sup>/H<sup>+</sup> exchangers, it is now evident that this protein plays an important role in cellular growth by regulating the localization and turnover of G-protein coupled receptors, PDGF receptor and other ion transporters.

The focus of these reviews then shift to downstream signaling and amplification of cellular signals. Han *et al.* review the Grb7 family of proteins, an emerging family of adaptor proteins, which may play an important role in breast cancer and cell migration. Ravichandran follows this with a review of Shc, which plays a key role in modulating signals that direct Map kinase activation. Tzivion follows with a review of the 14-3-3 signaling protein, which bind to ser/thr phosphorylated residues in a specific fashion analogous to SH2 domains and plays a key role in regulating proteins involved in intracellular signaling, apoptosis and various transcription factors, by altering the targeting of these proteins. Schechtman and Mochly-Rosen review the family of PKC binding partners termed 'RACKS' and their ability to target the binding and localization of specific PKC isoforms, as well as being able to bind other signaling proteins, and their role in modulating cellular processes such as vesicle trafficking and cell-cell communication.

The Crk adaptor protein was identified as a cellular homolog to a retroviral-encoded oncogene that was capable of initiating cellular transformation. This very important family of proteins has taught us much about the structure and function of adaptor proteins. These SH2/SH3 containing proteins serve to localize and activate a number of different effector proteins, including Abl, Rap1 and Rac. Feller provides us with a comprehensive review of the Crk family and describes the important role these proteins play in

modulating cell adhesion and cell migration. Following the theme of transformation, Bustelo outlines the role of the Vav family in modulating cellular signals that effect transformation. The Vav proteins amplify receptor signaling and play a key role in modulating GDP/GTP exchange for Rho/Rac proteins. Here, it is pointed out that the Vav family may have co-evolved with tyrosine kinases as a mechanism to link changes in the cytoskeletal architecture and gene transcription that concomitantly occur in response to extracellular signaling. Tsygankov reviews the adaptor protein Cbl, which not only facilitates protein interactions, but also has a role as an E3-ubiquitin ligase that can functionally inhibit some protein tyrosine kinases.

The connection between adaptor proteins and changes in the cytoskeleton is exemplified in the review by Li *et al.* on the function of Nck/Dock adaptor proteins. Here, Nck $\alpha$  and Nck $\beta$  appear to play an important role in linking cell surface receptor signaling to the actin-based cytoskeleton as a binding partner for Pak. Continuing the theme of adaptor proteins and the cytoskeleton, four review articles follow that evolved from work with the Src oncoprotein by Parsons and colleagues. In 1989, Parsons and colleagues proposed that one or more of the tyrosine phosphorylated substrates in Src-transformed cells may play a key role in modulating the effects of Src upon changes in cell shape associated with transformation. To this end, they isolated tyrosine phosphorylated substrates from Src transformed cells, generated antibodies against these substrates and cloned and identified these tyrosine-phosphorylated substrates. From this line of experimentation, pp130cas, pp125FAK, AFAP-110, pp85 cortactin and the cadherin-associated protein pp120ctn were identified. Notably, each of these tyrosine-phosphorylated substrates are associated with the actin-based cytoskeleton, which was intriguing given that the disruption of actin filaments is a hallmark for transformation by Src. This section begins with a review of the SH3-containing protein, Cortactin, by Weed and Parsons. Cortactin appears to play an important role in regulating tyrosine phosphorylated signal transduction and cortical actin filament assembly. Interestingly, cortactin also appears to work in concert with Arp2/3 mediated actin polymerization, indicating a role for cortactin in regulating actin filament integrity at the cell membrane. This is followed by a review from Baisden *et al.* on the actin filament associated protein, AFAP-110. Originally identified as an SH2/SH3 binding partner for activated, transformation competent forms of Src, it is now apparent that AFAP-110 is capable of regulating actin filament integrity as an activator of Src. This capability may be revealed by upstream cellular signals that alter its conformation. As AFAP-110 has multiple protein binding modules, it is possible that one or more upstream signals may effect the conformation of AFAP-110 and enable it to activate cSrc resulting in changes in actin filament integrity. Another SH2/SH3 binding partner for Src is pp130cas, reviewed by Bouton *et al.* Pp130Cas plays an important role in

forging protein-protein interactions with Crk and Src, as well as a variety of other signaling proteins, enabling Cas to effect actin filament integrity and cellular proliferation. In the last part of this section Schaller reviews paxillin, an adaptor protein that facilitates interactions with focal adhesion complexes and is a binding partner for pp125FAK, where it can facilitate cellular signals that control cell spreading and motility.

In the last section, two reviews on the role of adaptor proteins in facilitating apoptosis are covered. Bradley and Pober review TRAF's, which couple tumor necrosis factor receptors to signaling pathways. The TRAF's are hypothesized to be regulators of cell death responses to stress. Nunez *et al.* review the Nod family of proteins, which play an important role in programmed cell death and regulating host immune responses against pathogens.

Unfortunately, it was not possible to cover all the interesting and important adaptor proteins within this single issue. Notably, a discussion of proteins such as Grb2, Dynamin and IRS-1 were not included in this review; however, it should be noted that very recent

and excellent reviews of these proteins are currently available in the literature. It is also noteworthy that a number of new adaptor proteins are being described in the literature each week. Emerging adaptor proteins such as Grap, Trim, Shb, Blk, Bap and others are very noteworthy and it will be of great interest to follow these and other reports of newly characterized adaptor proteins. Finally, and significantly, several research groups are engaged in studies that target rational drug design to block the ability of some adaptor proteins to form interactions with cellular signaling proteins. Although not covered in this issue, it is noteworthy that some research groups are designing small molecular inhibitors that specifically block functional interactions forged by adaptor proteins, such as inhibitors of the SH2 domain of Grb2. Given the important role of adaptor proteins in propagating cellular signals, it is quite likely that we will see much progress in this area of research in the near future and we will obtain a greater appreciation and understanding for the role of adaptor proteins in signal transduction and as potential targets for therapy.