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SH2 Domains, Adapter Proteins and Tyrosine Kinases: Back to the Future

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Introduction – Domains as Common Features of Human Proteins

I wish to understand the principles underlying cellular organization, and particularly the mechanisms by which proteins are organized into signalling networks that convey information from receptors at the plasma membrane to targets within the cell. This is important not only in understanding normal cellular behaviour, but also in defining the alterations in cell signalling characteristic of disease states such as cancer. As a starting point for this line of inquiry, we have exploited the protein products of retroviral and cellular oncogenes, that can single-handedly induce marked changes in many aspects of cellular function, such as growth and proliferation, adhesion, migration and gene expression (Figure 1). These observations had suggested that cancer-causing oncoproteins could directly link to multiple signalling pathways within the cell, and that intracellular signalling proteins were likely highly interconnected.

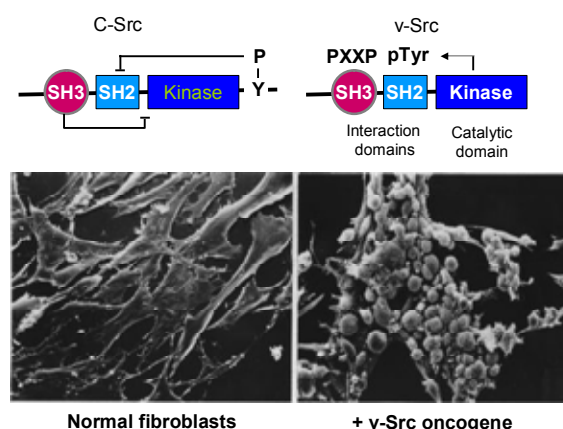


Figure 1. The Src oncoprotein has a modular domain-based structure, capable of altering many aspects of cellular behaviour. c-Src is the normal cellular protein, that is autoinhibited by intramolecular interactions involving the SH3, SH2 and kinase domains, whereas v-Src is a constitutively active oncogenic variant.

Subsequent work, as I will discuss, revealed that a majority of cellular proteins are composed of discrete domains, corresponding to modular structures that retain their structure and biochemical properties when expressed in isolation from their host proteins. Such domains can have catalytic functions, such as protein kinase activity, or can mediate interactions with proteins or other biomolecules. Interaction domains commonly have a cassette-like structure, with their N- and C-termini closely juxtaposed in space; during the course of evolution, this feature may have facilitated their facile incorporation into pre-existing proteins, which consequently acquired new biological functions. In addition, signalling proteins frequently possess short peptide motifs located in unstructured regions that serve either as sites of post-translational modifications, notably phosphorylation, or as binding sites for protein interaction domains, or both in the case of motifs that are first modified and then recognized by specific interaction domains. These findings have suggested two general concepts regarding signalling proteins and signalling pathways. Firstly, such proteins generally have a modular domain-based organization (see Figure 1), and secondly, protein-protein interactions provide a general mechanism to connect proteins into specific biochemical pathways and networks.

Function of Protein Interaction Domains

The prototypic interaction module is the Src homology 2 (SH2) domain, which binds selectively to short phosphotyrosine-containing peptide motifs. At least 100 families of interaction domains have now been described, many of which recognize short peptide ligands, including sites of post-translational modification, proline-rich sequences and C-terminal motifs, while others mediate homo- or heterotypic domain-domain interactions in a fashion that requires the folded structure of both domains. In addition, a number of domains bind non-protein ligands, including phospholipids, especially phosphoinositides, nucleic acids, second messengers such as cAMP, or metabolites (Figure 2). Protein interaction domains can thereby interpret the dynamic state of the cell, by recognizing modified protein, phospholipids and small molecules, and couple these signals to the regulation of key signalling pathways, which in turn control core cellular activities such as gene expression, protein synthesis, metabolism and cytoskeletal architecture, amongst others.

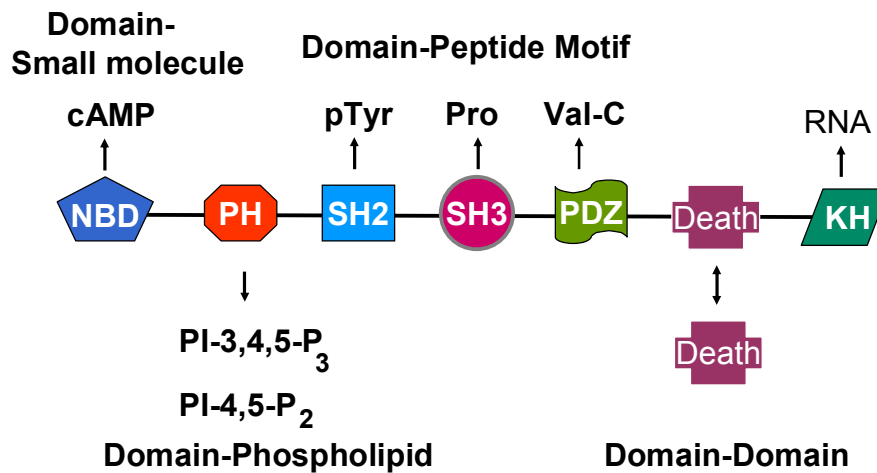


Figure 2. Representation of protein interaction domains, and their ligands.

Bioinformatic analysis suggests that about 70% of human proteins have one or more interaction or catalytic domains, which can be identified through their conserved amino acid sequences. Each family of interaction domains usually has between 10 and 300 members in the human proteome, suggesting that in the course of evolution specific domain types have been selected and expanded, likely because they have desirable biochemical properties, and are also flexible scaffolds that can readily acquire new ligand-binding properties. Consistent with this view, interaction domains can show a striking expansion in their numbers as organisms become more complex. For example there are 28 SH3 domains in yeast, but at least ten times this number in human proteins. This raises a related point, that although the total number of proteins encoded by eukaryotic organisms such as yeast, worms and mammals is remarkably similar, individual proteins have often become more complex during the course of evolution through the acquisition of new protein domains, which confer new biological functions.

Interaction domains can have diverse properties, which I briefly summarize here.

1) They can regulate the localization of signalling proteins, for example through the ability of modules such as PH domains to bind phosphoinositides such as phosphatidylinositol (PI)(3,4,5)P₃. In this scheme, the activation of PI 3'-kinase by growth factor receptors increases the concentration of PIP₃ in the plasma membrane, which consequently recruits the serine/threonine protein kinase PKB/Akt, and its activating kinase PDK1, both of which have PH domains that selectively bind PIP₃. Once activated at the membrane, PKB is a key regulator of cell growth, survival and metabolism.

2) Interaction domains mediate many of the effects of post-translational modifications, by direct and selective recognition of covalently modified peptide motifs. In addition to SH2 domains, there are multiple interaction domains that selectively bind to peptide sequences that have been phosphorylated at tyrosine or serine/threonine residues. In each case, binding is dependent on phosphorylation of the appropriate hydroxyamino acid, and on the nature of the amino acids that flank the phosphorylated site, which provides a degree of specificity. In addition, other types of post-translational modifications, including lysine acetylation, lysine/arginine methylation, proline hydroxylation, ubiquitination and sumoylation, all create sites that are selectively recognized by modification-dependent interaction domains. One of the principal mechanisms by which modifications such as phosphorylation alter cellular function therefore appears to be through the creation of binding sites for interaction domains.

3) Different types of interaction domains can be covalently linked within a single polypeptide chain, to yield proteins with adaptor properties. Through its various interaction domains, an adaptor can couple an upstream protein, such as an activated growth factor receptor, to downstream targets that control intracellular signalling pathways. For example, proteins composed exclusively of SH2 and SH3 domains, such as Grb2, Nck and Crk, link phosphotyrosine-containing sites on receptor tyrosine kinases to targets with SH3-binding proline-rich motifs, such as the Ras guanine nucleotide exchange factor Sos in the case of Grb2. The use of adaptor proteins appears to be a common device through which the flow of information within the cell is directed.

4) Two domains, once they are linked together, can potentially undergo intramolecular interactions that result in allosteric regulation. For example, as I will discuss later, the SH2 domain of the Fes cytoplasmic tyrosine kinase interacts with the adjacent catalytic domain to stimulate an active kinase conformation and also to bind potential substrates, and thus to promote the phosphorylation of specific targets. Such cooperative effects can therefore endow multi-domain proteins with complex modes of regulation.

Modular Interaction Domains as an Evolutionary Device

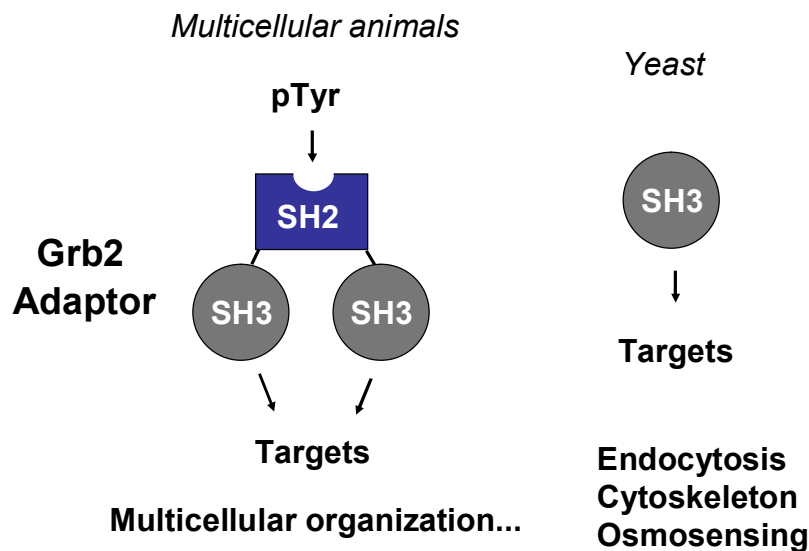


Figure 3. Interaction domains may be combined in new ways in the course of evolution to create novel signaling pathways.

Taken together, these observations suggest a possible reason for the prevalence of protein domains in general, and of interaction domains in particular, in the proteomes of metazoan organisms. I suggest that protein domains represent fundamental units of biological function, and that their cassette-like structure may have facilitated the evolution of new and increasingly complex cellular functions. An interaction domain, for example, may gain novel properties either through a mutation that confers a new intrinsic activity, or by linkage to a new domain type as a consequence of genetic recombination. If they have a favourable impact on cellular behaviour, such mutations or new domain combinations will undergo positive selection. Analysis of SH2 and SH3 domains provides evidence for this hypothesis. As noted, the yeast *S. cerevisiae* has a number of SH3 domains that selectively bind proline-rich sequences, and are important for a variety of processes such as osmosensing, endocytosis and cytoskeletal organization. In contrast, the yeast *S. cerevisiae* has only a single domain with an SH2-like sequence, which has no detectable phosphotyrosine-binding activity, and yeast also lack dedicated tyrosine kinases and conventional phosphotyrosine-based signalling. However, during the evolution of multicellular animals, and their immediate predecessors such as the protist *Monosiga brevicollis*, SH2 domains acquired an ability to recognize phosphotyrosine, which may have helped to drive the development of tyrosine-specific protein kinases. Concomitantly, phosphotyrosine-binding SH2 domains were incorporated into proteins with novel domain combinations, such as the SH2/SH3 adaptor proteins, which may have provided a rapid mechanism to create new signalling pathways by coupling tyrosine kinases to intracellular targets (Figure 3). In support of this notion, the proximal effectors of receptor tyrosine kinases (RTK) have diverse biochemical activities, including regulation of small GTPases, control of phospholipid

metabolism, control of protein kinase pathways, cytoskeletal reorganization, ubiquitination and gene expression. Yet these very different proteins are endowed with a common ability to bind activated RTKs by virtue of the fact that they all possess an SH2 domain.

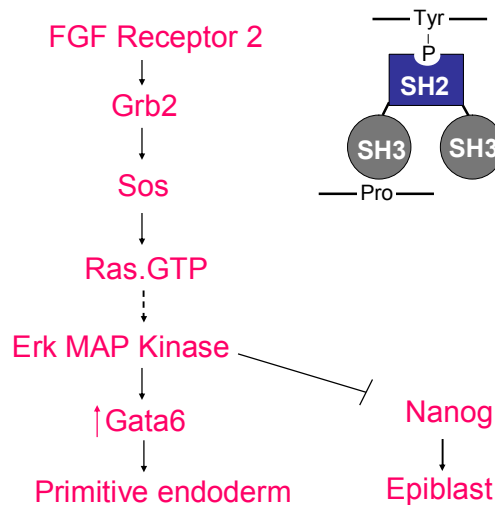


Figure 4. Grb2 adaptor is required for the formation of primitive endoderm in the E4.5 mouse embryo

If this argument is valid, one might expect that such SH2-containing proteins, especially SH2/SH3 adaptors, are important for the organization of animal cells into functioning tissues. Indeed, we have found that inactivation of the Grb2 gene in mice leads to a developmental arrest at a very early stage, at about embryonic day 4.0. This is due to a failure of the embryo to form primitive endoderm, which will go on to make extraembryonic structures. Indeed, as early as embryonic day 3.5, Grb2 can be shown to link phosphotyrosine signals, likely generated by fibroblast growth factor receptors, to the Ras-MAP kinase pathway, and thus to the upregulation of the transcription factor GATA-6 that specifies the primitive endoderm cell fate (Figure 4). Conversely, the Grb2 pathway suppresses expression of the pluripotency factor Nanog. The Grb2 adaptor, with a single SH2 domain flanked by two SH3 domains, therefore plays a key role in an early cell fate decision in the mammalian embryo. These observations are consistent with the concept that the linking of interaction domains in new combinations during the course of evolution can create new connections in the cell’s signalling network, and consequently more complex biological functions.

A model for signalling from cell surface receptors

Transmembrane RTKs are activated by a variety of ligands, including soluble growth and differentiation factors, metabolic hormones such as insulin, membrane-associated protein ligands (i.e. ephrins) and extracellular matrix components (i.e. collagen). A wealth of genetic, biochemical and structural data have suggested a model for signalling by such receptors. Binding of the extracellular ligand induces receptor oligomerization, or reorientation of the receptor chains if they are already clustered. Neighbouring receptor chains are then able to phosphorylate one another in trans, typically on tyrosine residues

in the activation loop in the large subunit of the kinase domain, which stabilizes the active conformation. In addition, tyrosine residues in cytoplasmic regions of the receptor that lie outside of the kinase domain, for example in the juxtamembrane region or C-terminal tail of the receptor, are phosphorylated at sites that serve as docking motifs for proteins with SH2 domains (Figure 5).

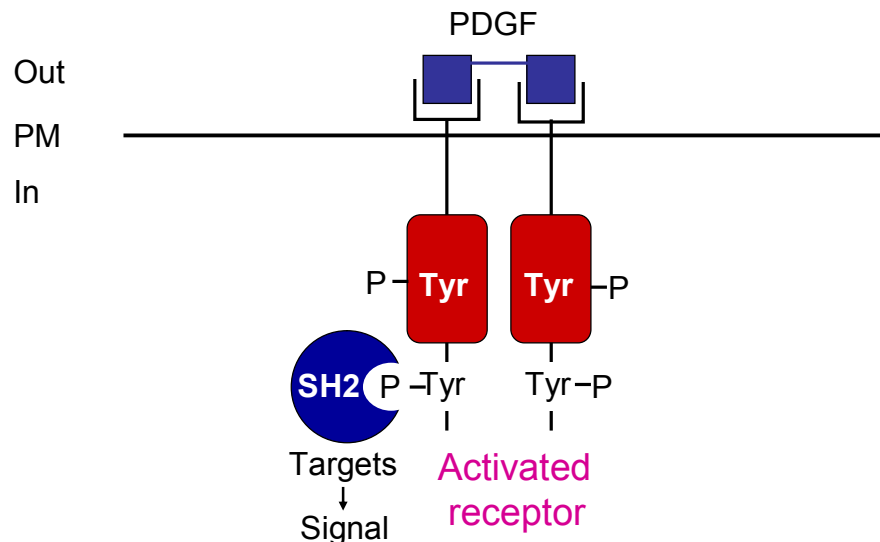


Figure 5. Receptor autophosphorylation creates docking sites for cytoplasmic targets with SH2 domains.

Once recruited to the activated receptor, these proteins, or their downstream binding partners, are themselves activated, leading to the regulation of cytoplasmic signalling pathways. This can be achieved as a direct result of SH2-binding, which can relieve an autoinhibited conformation of the target protein, or by localization of the SH2 protein to its substrates at the membrane, or by phosphorylation of the SH2 protein by the receptor's tyrosine kinase domain. In addition to RTKs, a variety of different types of cell surface receptors employ a similar strategy to transmit intracellular signals. Although the details differ in each case, TGF β -receptors, TNF receptors, Fas receptors, Toll receptors, guidance receptors and others are activated by oligomerization, which drives the recruitment of modular adaptor proteins that stimulate specific downstream signalling pathways.

Allosteric Regulation by the SH2 Domains of the Fes and Abl Cytoplasmic Tyrosine Kinases

As I have already mentioned, domains that are linked within the same protein can undergo intramolecular interactions that result in the allosteric regulation of protein function. To explore this scheme, I should point out that tyrosine kinases such as Src are entirely cytoplasmic; although Src is tethered to the membrane by myristylation signal, it lacks any membrane-spanning region. Cytoplasmic tyrosine kinases include proteins

in the Src, Abl, Fps/Fes, Tec and Syk families, all of which possess a tyrosine kinase domain linked to interaction modules such as SH2 and SH3 domains. The core unit of such kinases appears to be an SH2 domain joined to a kinase domain. This is likely an ancient domain combination that emerged at the dawn of phosphotyrosine signalling, as suggested by the presence of these two domains in kinases present in the social amoeba *Dictyostelium discoideum*, that have only a very primitive phosphotyrosine signalling system. The initial function of the SH2 domain in such proteins was likely to direct the kinase domain to specific targets, potentially by recognizing proteins that already possess a phosphotyrosine site (a so-called priming site), and also to stimulate kinase activity (Figure 6).

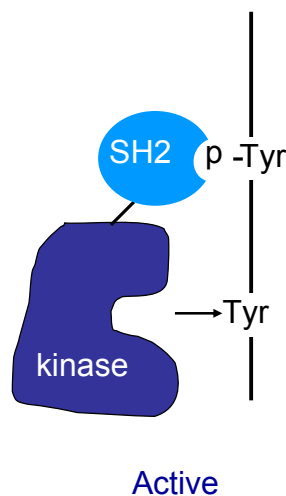


Figure 6. Hypothesis - The SH2-kinase unit is an ancient domain combination, whose ancestral function was to promote tyrosine kinase activity and substrate recognition.

In the context of proteins such as Src and Abl, the SH2 domain, in conjunction with the SH3 domain, has also acquired an ability to inhibit catalytic activity by binding to the back surface of the kinase domain, but this is apparently a more recently evolved sophistication. Indeed, we originally identified the SH2 domain through its positive effect on catalytic activity in the context of an oncogenic variant of the Fps/Fes tyrosine kinase, encoded by the Fujinami avian sarcoma virus (Fps and Fes being avian and mammalian orthologues). We have recently obtained a high resolution structure that explains how the Fps/Fes SH2 and kinase domains act cooperatively to phosphorylate substrates.

The P130^{gag-fps} protein of Fujinami sarcoma virus has an N-terminal sequence encoded by the retroviral Gag gene, joined to the cellularly-derived Fps gene. Using an insertion mutagenesis strategy, we identified three domains in the viral (v-) Fps oncoprotein that are required for its transforming activity. These are the kinase domain, a region of 100 amino acids immediately N-terminal to the kinase that is conserved in Src and Abl, which we called the Src homology 2 (SH2) domain, and an N-terminal region involved in

membrane localization, and now termed an F-BAR domain. Dipeptide insertions in the N-terminus of the SH2 domain caused a profound inhibition of kinase activity; in addition the SH2 and kinase domains of the wild type v-Fps protein formed a 45 kDa protease-resistant fragment. Taken together, these results suggested that the SH2 and kinase domains interact to form a common structure that enhances catalytic function. Mutations in the v-Fps SH2 domain also impaired the phosphorylation of specific cellular substrates, raising the possibility that the SH2 domain might be close to the kinase active site, and position SH2-binding proteins for phosphorylation. This initial identification of the SH2 domain therefore suggested two general functions, namely interaction with exogenous proteins and allosteric regulation of catalytic activity, that together regulate phosphotyrosine signalling (Figure 7).

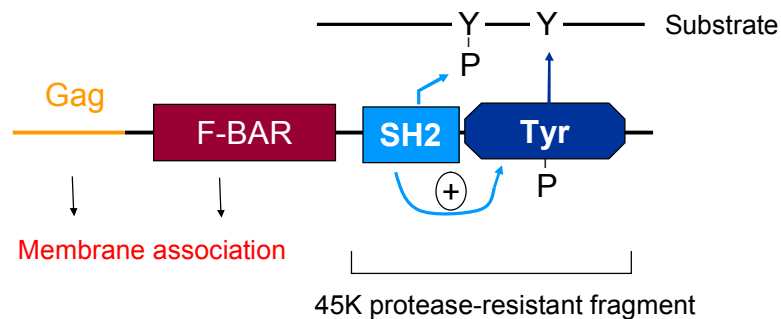
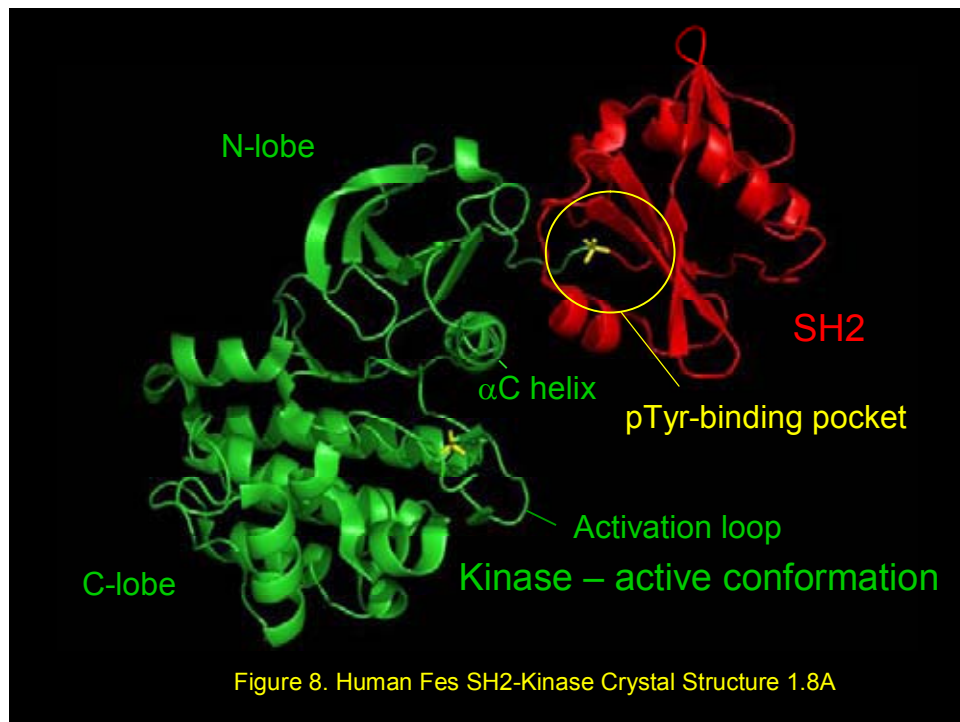


Figure 7. Model for the regulation of the Gag-v-Fps retroviral oncoprotein. The Fps/Fes SH2 and kinase domains are structurally and functionally integrated.

In a collaboration with Stefan Knapp, we have now obtained a high resolution x-ray crystal structure of the linked SH2 and tyrosine kinase domains of the normal human Fes protein. The kinase domain is in an active conformation, which is stabilized by interactions with the N-terminal region of the SH2 domain (Figure 8). In particular, two glutamic acid residues in the α A helix of the SH2 domain interact with an arginine in the α C helix, located in the small N-terminal lobe of the kinase domain. This is of particular significance, as the α C helix carries residues that are critical for catalysis, and as a rule



the correct positioning of α C is essential for kinase activity. The electrostatic interaction between the SH2 and kinase domains appears to be functionally important, since mutation of the two glutamates in the SH2 domain to lysine (EE/KK) led to a strong reduction in the tyrosine kinase activity of the full-length Fes protein in HEK293 cells. Furthermore, replacement of the α C helix arginine with glutamate restored the activity of the EE/KK mutant. The ability of this polarity switch to restore Fes tyrosine kinase activity argues that the electrostatic interface between the SH2 and kinase domains is functionally significant. A second interaction surface involves the strand at the very N-terminus of the SH2 domain, which intercalates between a loop connecting the α 1/ α 2 strands of the kinase N-lobe and the central β -sheet of the SH2 domain. This tight packing requires a glycine in the SH2 domain, and substitution of this residues with a bulkier valine leads to a complete loss of kinase activity. This G/V substitution likely forces the SH2 domain away from the kinase domain, and concomitantly disrupts the structure of the kinase N-lobe.

How might the interactions between the Fes SH2 and kinase domains be regulated? In the active SH2-kinase structure described above, two sulphate ions serve as phospho-mimetics, and help to stabilize the active conformation. One of these sulphates is located in the phosphotyrosine-binding pocket of the SH2 domain, while the second is associated with a tyrosine in the kinase activation loop, thereby mimicking autophosphorylation (see Figure 8). Of interest, in the absence of a ligand, the SH2 domain becomes much less stable, as does the kinase N-lobe, and in particular the α C-helix becomes highly mobile. These and related data suggest that binding of a phosphotyrosine-containing peptide to the SH2 domain would stabilize its conformation

in a fashion that promotes a productive interaction with the kinase domain, resulting in increased catalytic activity. As one additional point, analysis of a structure in which a substrate peptide is bound to the kinase active site shows that this peptide forms a short anti-parallel β -strand with the activation loop, which stabilizes the active conformation. Taken together, these results suggest that the fully active conformation of the Fes tyrosine kinase depends on a cooperative interaction between the SH2 domain, the catalytic domain, and a primed substrate molecule, with the SH2 domain capturing a primed phosphorylation site on a target protein, and presenting a substrate tyrosine to the active site. According to this model, the Fes kinase is only fully active in the presence of an appropriate substrate, which provides an interesting mechanism to enhance kinase-substrate specificity (Figure 9).

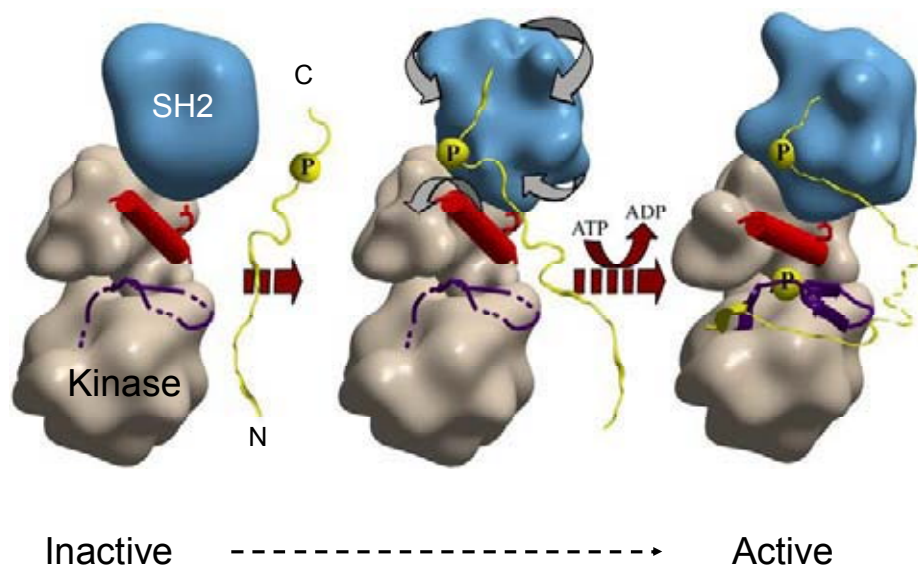


Figure 9. Model for Fes SH2-kinase regulation

We have previously provided evidence that Fes kinase activity is also regulated by the reversible association of the N-terminal F-BAR domain with membranes, which leads to clustering and autophosphorylation within the activation loop. Fes may therefore act as a coincidence detector, that is only fully active when it is localized to the right place in the cell, and juxtaposed to physiological substrates. Genetic analysis of mice deficient for Fes, and its close relative Fer, has indicated that these kinases play important roles in innate immunity, hemostasis, Fc γ R1 signaling, and axon guidance, potentially through roles in receptor trafficking, regulation of cell junctions, and control of the actin cytoskeleton. It will be of considerable interest to link the biochemical modes of Fes regulation identified in our study with the complex biological properties of this kinase.

One question raised by these observations is whether the SH2 domains of other cytoplasmic tyrosine kinases interact with the adjacent catalytic domain in the active state to promote substrate phosphorylation. Prior structural analysis of the Abl tyrosine

kinase by John Kuriyan's laboratory has identified a conformation in which the Abl SH2 domain interacts with the N-lobe of the kinase domain, principally through a conserved SH2 isoleucine. In collaboration with Giulio Superti-Furga and colleagues, we have now found that the SH2 domain stimulates Abl kinase activity in vitro, and substrate phosphorylation in cells, and that mutation of the isoleucine residue at the interface suppresses catalytic function. Thus, although the details of the interface are clearly different for Fes and Abl, the principle that SH2 domain has a positive effect on kinase function in the active configuration is apparently preserved. Ogawa et al have made a similar observation for the SH2 and SH3 domains of the Csk tyrosine kinase. These observations support the hypothesis that one reason for the selection and expansion of SH2 domains during the course of evolution is that they have sufficient versatility to undergo diverse intramolecular interactions with linked modules, such as tyrosine kinase domains, and thereby to generate varied modes of allosteric regulation. They also raise the interesting possibility that it may be possible to obtain small molecules that block the activity of such tyrosine kinases by perturbing the SH2-kinase interface.

Adaptor Proteins Direct the Flow of Molecular Information in Signal Transduction

A challenge faced by cell surface receptors is to effectively couple external signals to downstream targets. As noted previously, one way through which this problem has been solved is through the evolution of adaptor proteins and scaffolds, that possess a domain that binds directly to the receptor, and other domains or motifs that recruit one or more target proteins. In this context I have already discussed the Grb2 adaptor, and I would like to focus now on a related protein, Nck, which links phosphotyrosine signals to reorganization of the actin cytoskeleton, and thus to cell shape and movement. Mammalian genomes encode two closely related Nck proteins (Nck1 and Nck2) which possess a C-terminal SH2 domain that binds to phosphotyrosine-containing motifs such as pTyr-Asp-Glu-Val (YDEV), and three N-terminal SH3 domains that interact with proline-rich motifs in proteins such as N-WASP and PAK, which in turn control branching actin polymerization and acto-myosin contractility. We have previously made null alleles of murine Nck1 and Nck2, and a conditional floxed allele of Nck2. Using these tools we have found that Nck proteins are collectively required for early embryogenesis, likely due to a role in gastrulation, and for the formation of the actin network required for normal fibroblast movement. But perhaps of more interest, we have started to investigate the functions of Nck adaptors in specialized cell types, in particular in podocytes and neurons, and in cells infected with the enteropathogenic *E. coli* (EPEC) bacterium.

Podocytes are specialized epithelial cells that are critical for the formation of the filtration barrier in the glomeruli of the kidney. The capillaries in glomeruli are lined by a fenestrated endothelium; this is surrounded by a basement membrane, which in turn is

overlaid by podocytes. These cells extend primary microtubule-based processes, from which project actin-rich secondary processes, also termed foot processes. The foot processes from adjacent podocytes interdigitate, and thereby form a specialized tight junction, the slit diaphragm, which together with the endothelium and basement membrane filters the plasma, and ensures that molecules such as proteins are retained in the circulation. The adhesion protein nephrin is located at the podocyte slit diaphragm, and has an extracellular region composed of eight Ig repeats that undergoes homophilic interactions, and thereby forms an essential part of the filtration apparatus. Nephrin also has a cytoplasmic tail that becomes tyrosine phosphorylated at multiple sites by Src family kinases when nephrin is clustered. Three of these phosphorylation sites are located in YDEV or YDQV motifs, that bind the Nck SH2 domain and thereby recruit actin regulatory proteins such as N-WASP. Indeed, nephrin tyrosine phosphorylation and consequent binding to Nck is associated with actin polymerization in cultured cells. In mice that are homozygous for the null allele of Nck1 and the floxed allele of Nck2, expression of the Cre recombinase from the podocin promoter leads to the inactivation of Nck2 in podocytes, which therefore lack both Nck1 and Nck2 proteins. In the absence of Nck, the actin-based podocyte foot processes fail to extend properly during development, and thus do not support the formation of functional slit diaphragms. As a consequence, such Nck-deficient mice develop a severe and lethal proteinuria. These data suggest that the rather simple Nck adaptor plays a key role in coupling adhesion proteins such as nephrin to the cytoskeletal organization of podocytes, and thus to the formation of a complex cellular architecture important for function of the kidney (Figure 10).

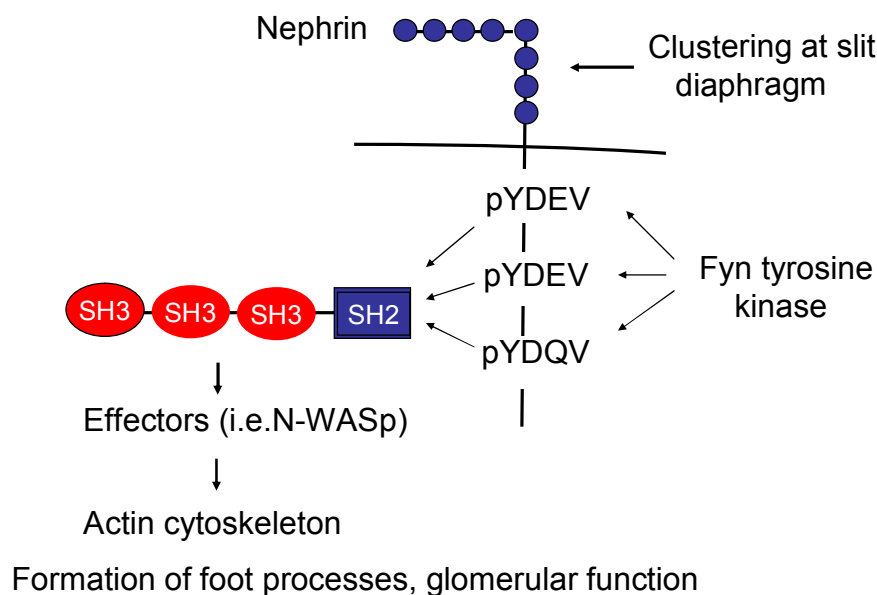


Figure 10. Nck couples Nephrin to the architecture of podocyte foot processes

The wiring of the nervous system depends on the trajectories of axons, which are determined by guidance cues and their receptors, and their effects on the cytoskeleton of

axonal growth cones. A number of such guidance receptors can interact with Nck adaptors, raising the possibility that Nck proteins might be important in the proper formation and function of the nervous system. Indeed, we have found that $Nck1^{-/-};Nck2^{flx/flx}$ mice that express the Cre recombinase in the nervous system, under the control of the nestin promoter, show aberrant projections of axons in the spinal cord, that phenocopy defects in mice that lack the EphA4 RTK. In Nck-deficient mice, cortico-spinal tract (CST) axons project aberrantly across the midline (Figure 11), as do neurons involved in the central pattern generator (CPG). The murine CPG controls the asynchronous firing of motor neurons on either side of the spinal cord that control limb movement. In the Nck-deficient spinal cord these motor neurons fire in a synchronous pattern, and as a consequence, Nck-deficient mice have a hopping gait. We propose that Nck adaptors couple signals from guidance receptors such as EphA4 to regulation of the actin cytoskeleton, and thus to the proper organization of neuronal circuits in the spinal cord. These results lend weight to the argument that Nck adaptors control signalling pathways with profound effects on cellular morphology and function.

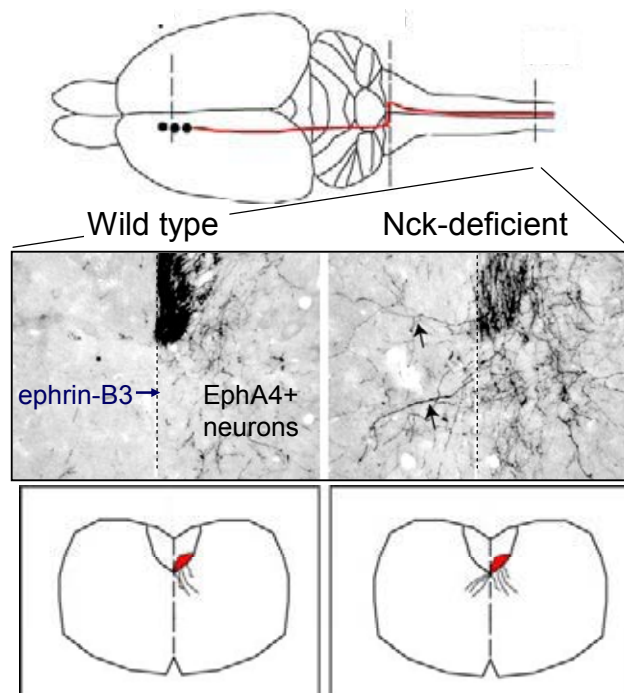
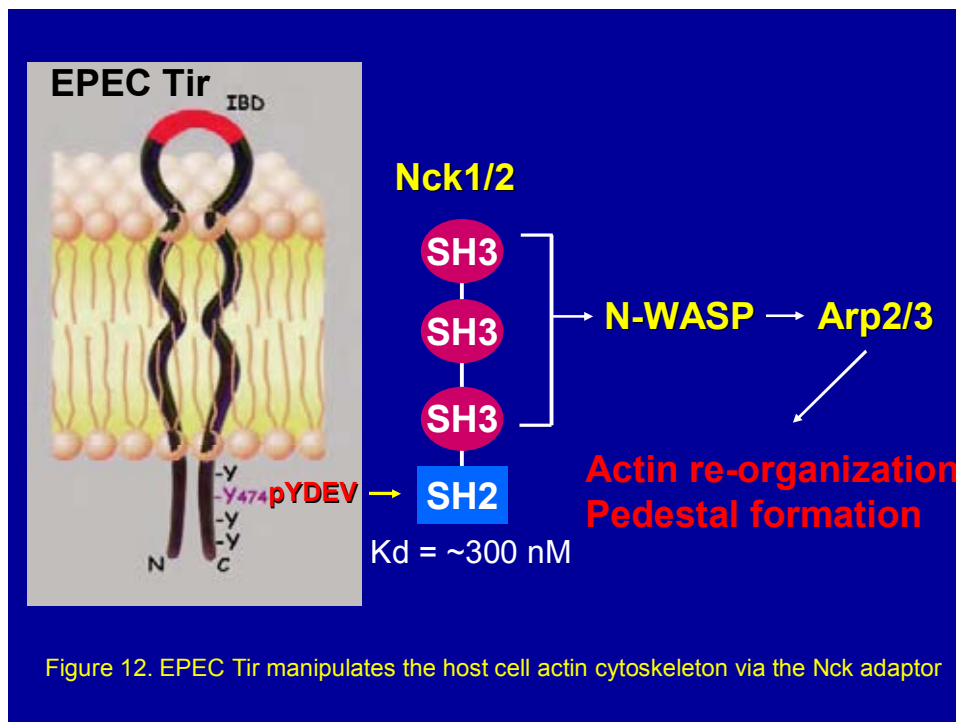


Figure 11. Nck-deficient mice show increased CST fibers re-crossing the midline

Pathogenic Microorganisms and Oncoproteins Subvert Cellular Behaviour through Adaptor Proteins

Pathogenic bacteria can respecify the properties of infected cells. EPEC, for example, inserts a bacterially encoded protein, Tir, into the plasma membrane of a host cell, and then attaches to the Tir protein through a protein displayed on the bacterial surface, intimin. Tir is organized with two membrane-spanning regions, so that both its N- and C-terminal tails are localized in the cytoplasm and interact with cellular effectors to

induce actin polymerization at the site of bacterial attachment. This results in the formation of novel actin-based cellular structures termed pedestals. Clustering induced by intimin induces phosphorylation of a tyrosine residue in the C-terminal tail of Tir, which is located in a YDEV motif that is similar to those found in nephrin and binds with high affinity to the Nck SH2 domain. Consistent with this observation, tyrosine phosphorylated Tir associates with Nck, and with its target N-WASP. EPEC bacteria that encode a Tir mutant in which this tyrosine is mutated to phenylalanine fail to recruit Nck and are impaired in their ability to induce actin polymerization; in addition, wild type EPEC does not induce efficient actin reorganization in cells that lack Nck adaptors. Taken together, these data argue that EPEC modifies the actin polymerization of infected cells through a motif that recruits the endogenous Nck SH2/SH3 adaptor, and mimics Nck-binding sites found in normal proteins such as nephrin (Figure 12). Consistent with this view, the ability of an EPEC Tir mutant lacking its intrinsic tyrosine phosphorylation site to induce actin polymerization in an infected cell can be rescued by grafting an Nck-binding motif from nephrin onto the mutant Tir protein.



Proteins that are causally involved in human cancer can also perturb cellular function through the ectopic activation of adaptor proteins. For example, the human Bcr-Abl gene that induces chronic myelogenous leukemia is a chimeric sequence that results from the a 9;22 chromosome translocation. The Bcr-Abl protein therefore has an N-terminal Bcr sequence that is fused to the Abl cytoplasmic tyrosine kinase, and stimulates Abl tyrosine kinase activity due to its propensity to oligomerize, which leads to Abl autophosphorylation. Once activated, the Abl kinase domain phosphorylates a tyrosine

observations, small molecules that stabilize, re-wire or inhibit protein-protein interactions are potentially of therapeutic value, and a number of such compounds are used clinically, or are showing significant promise in clinical trials.