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Invited Review

The SH2 and SH3 adapter Nck: a two-gene family and a linker between tyrosine kinases and multiple signaling networks

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Summary. SH2 and SH3 adapter proteins connect cell surface tyrosine kinases to intracellular signaling networks. For instance, the SH3-SH2-SH3 adapter Grb2 links receptor tyrosine kinases to the Ras pathway. Nck, composed of three SH3 domains and one SH2 domain, represents a two-gene (alpha and beta) family in mammals. Ncka and Nckß are expressed in the same cells and appear to have distinct signaling specificity. Studies show that Nck plays a role in cell mitogenesis and morphogenesis. The former uses Ras-dependent and Ras-independent pathways. The latter appears to coordinate with the Cdc42/Rac>PAK1/WASp>actin cytoskeleton pathway. Understanding the specificity of Ncka and Nckß signal transduction will provide answers for the previously often conflicting observations.

Key words: Src-homology domains, Adapters, Tyrosine kinases, Signal transduction

Introduction

Protein tyrosine phosphorylation/dephosphorylation is an evolutionarily-conserved chemical switch and widely used in early events of signal transduction by almost all cell surface receptors. It appears to play two major roles in regulation of a protein function. First, phosphorylation/dephosphorylation changes conformation of a protein, resulting in increase or decrease of its intrinsic enzymatic or other functional activity. For example, dephosphorylation of the phosphorylated tyrosine-527 in the carboxyl terminus of c-src disrupts its head-to-tail interaction and opens up the folded inactive molecule to an unfolded active kinase. Second, phosphorylated tyrosines serve as binding sites for the peptide modules called Src-homology 2 (SH2) and phosphotyrosine binding (PTB) domains. These "lock and key" interactions result in formation of new protein signaling complexes and often relocation of the phosphotyrosine (pY)-bound proteins (Pawson, 1995). For example, phospholipase Cy binds via its SH2 domains to pY-1009 and pY-1021 in the human PDGF receptor-beta and translocates to the plasma membrane. It then becomes tyrosine phosphorylated and activated by the PDGFR (Schlessinger and Ullrich, 1992). In addition to the pY residue, three to six amino acid residues immediately carboxyl to the pY residue, pY-$(X)^{3-6}$, play a critical role in determining the binding affinity and specificity to an SH2 domain. In contrast, the aminol terminal residues of the pY participate in binding to a PTB domain. In vitro binding studies using a pY peptide library show the κ_{ds} of submicromolar/micromolar range (Songyang et al., 1993). Src-homology domains also include SH3 domain. SH3 domains bind proline-rich segments of target molecules with the minimum consensus of P-X-X-P (Pawson, 1995). Each proline is often preceded by an aliphatic amino acid residue and the aliphatic-proline pair binds to hydrophobic pocket on the SH3 domain. In vitro, P-X-X-P peptides of optimal sequences bind SH3 domains with κ_{ds} in micromolar range. Therefore, when SH2 and SH3 domains work together, they connect pY signaling at the cell surface to a variety of signaling pathways via P-X-X-P-SH3 interactions.

A group of mammalian SH2- and SH3-containing proteins is composed exclusively of SH domains and does not have any enzymatic or known functional motifs. They include Crk, Grb2 and Nck. These proteins are therefore called SH domain-containing adapters (reviewed by Birge et al., 1996). The mechanism of action by these adapter proteins has best been demonstrated by studies on Grb2. Following EGF stimulation, Grb2 binds via its SH2 domain to a pYXNV motif in the EGFR and/or via the same motif in tyrosine phosphorylated SHC. Grb2 in turn translocates its SH3 domain-associated (constitutively) Ras guanine nucleotide exchange factor Sos to the plasma membrane.

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Sos then activates the membrane-attached Ras (Pawson and Schlessinger, 1993). A general working model for SH2- and SH3-containing adapters is schematically represented in Figure 1. A and B represent activator and effector, respectively. While Grb2 has been shown to link a wide range of surface receptors to the Ras signaling pathway leading to DNA synthesis, the cellular functions of the rest of the adapter proteins remain largely undefined. Nck is composed almost entirely of three SH3 domains and one SH2 domain. Since the first cloning of the human Nck gene in 1990 (Lehmann et al., 1990), there have been more than 150 publications in a wide variety of biological systems. Nck-like genes have been identified in Xenopus, Drosophila and *C. elegans*. Recent genetic studies in Drosophila and studies in mammalian cells have pointed out that Nck plays an important role in signal transduction from protein tyrosine kinases to the actin cytoskeleton. We hope that this review serve as an introduction for a new era of studies that will aim at the cellular function and signaling specificity of Nck family genes.

Nck is a two-gene family in mammals and is evolutionarily conserved

A Nck cDNA was first isolated in 1990 from a λgt11 cDNA expression library constructed from mRNAs of a human melanoma cell line, resulting apparently from a monoclonal antibody (against melanoma-associated antigen MUC 18) cross reaction with the Nck (Lehmann et al., 1990). The deduced amino acid sequence revealed an SH3-SH3-SH3-SH2 polypeptide with variable lengths (30 to 50 amino acids) of interval sequences, but showed no enzymatic or other known functional motifs. The mRNA of the Nck gene was detected in all tissues and cell lines studied. In 1992, a partial cDNA fragment of a mouse Nck homologue was reported as one of the in vitro EGFR-binding proteins (Grb4) (Margolis et al., 1992; Margolis, 1994). The fragment of Grb4 showed an overall 64% amino acid identity with the human Nck. Since 64% identity was thought to be too low for Grb4 to be the mouse orthologue gene of the human Nck, high stringency screening of mouse cDNA libraries using the human Nck as the probe and screening of human cDNA libraries using Grb4 as the probe were conducted. This approach led to discovery of a novel mouse and a novel human Nck genes (Chen et al., 1998). The newly identified mouse Nck and human Nck share more than 96% amino acid identity with the previous human Nck and the mouse Grb4, respectively (Chen et al., 1998). Chen and colleagues named or renamed the two pairs of human and mouse Nck genes as hNckα and hNckß and mNckα and mNckß. The mammalian Nck homologue and orthologue genes are schematically shown in Figure 2. Cloning of the mNckß (the full length Grb4) and hNckß/Nck2 has also been reported by three other groups (Park, 1997; Tu et al., 1998; Braverman and Quilliam, 1999). Nckα/Nck and Nckß/Grb4/Nck2 are encoded by two distinct genes, because the human Nckaα gene is located at 3q24, whereas the human Nckß gene resides at 2q21 (Huebner et al., 1994; Vorabieva et al., 1995; Chen et al., 1998). The amino acid variations between Nckaα and Nckß fall largely into the interval sequences between the SH domains. Comparisons within the SH3 and SH2 domains show higher amino acid identity between Nckα and Nckß (Table 1). However, noticeable changes between Nckα and Nckß are found in some of the key sequences, which are believed to play important roles in determining the binding specificity of SH2 and SH3 domains (Waksman et al., 1992, 1993; Lee et al., 1994). There are amino acid substitutions between Nckα and Nckß in "RT-loop" in all the three SH3 domains (VAQQEQ (in Nckα) to TAQQDQ (in Nckß) in SH3-I; MAERED (in Nckα) to VAERED (in Nckß) in SH3-II; and SSSNDE (in Nckα) to SSVTEE (Nckß) in SH3-III). In the SH2 domains, the GQRKFS sequence of "EF loop" and "F strand" in Nckα is changed to GQRRFH in Nckß. The APIFTSEQGEKL sequence of the "BG-loop" in Nckaα is replaced with APIFTSEH GEKL in Nckß. All these amino acid variations between Nckaα and Nckß are 100% conserved between the humans and the mice. It remains to be studied if these changes indeed confer binding specificity upon the Ncks. Two studies showed that Nckß binds significantly stronger than Nckaα to tyrosine phosphorylated EGFR, PDGFR and p62^{dok} (Chen et al., 1998; Tu et al., 1998). It suggests that Nckß-SH2 and Nckα-SH2 bind different target proteins in vivo.

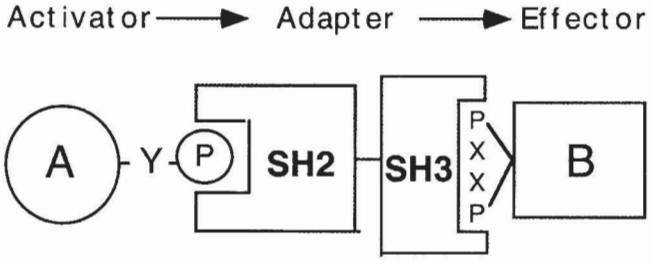

Fig. 1. A general scheme of action by SH2 and SH3 adapters. The 3-6 amino acids carboxyl to pY, whcih are involved in binding, are not shown. Additional flanking amino acids of PXXYP are invovled in SH3 binding.

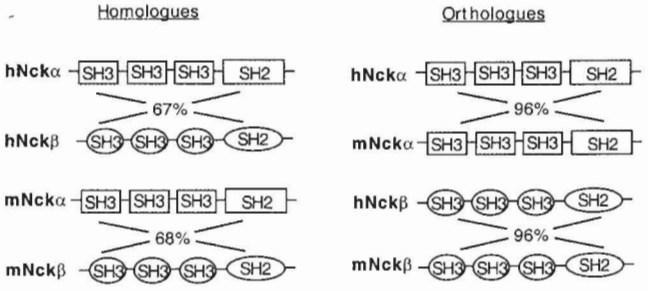

Fig. 2. Human and mouse Nck homologue and orthologue genes. The percentage given represent overall amino acid identity. "h" and "m" refer to human and mouse, respectively.

Table 1. Amino acid sequence identity (%) in Nck genes from different species.

Human	mNckα					mNckß					OTHERS Xenopus Nck					Drosophila Dock					C. elegans Nck-like				
	ov#	3-I	3.II	3-III	SH2	ov	3-I	3.II	3-III	SH2	ov	3-I	3.II	3-III	SH2	ov	3-I	3.II	3-III	SH2	ov	3-I	3.II	3-III	SH2
hNckα*	96	98	94	96	99	68	83	88	84	85	87	96	91	90	92	44	63	54	49	63	36	74	63	47	38
hNckß	67	82	95	80	85	96	96	94	96	99	68	83	85	81	82	43	69	56	52	62	33	70	61	35	40

*: hNcKα is originally called Nck; Nckß is also identified as Nck-2 and Grb4; #ov: overall.

Only a single Nck-like gene has been reported in several lower organisms, including Nck in Xenopus (Wong and Mayer, GenBank accession number U85781, 9, 10), *dreadlocks* (or *dock*) in Drosophila (Garrity et al., 1996) and a Nck-like gene in *C. elegans* (by genome sequence search, see ref. by Chen et al., 1998). Thus, the function of Nck must be evolutionarily conserved. Comparisons of overall sequences and individual domains of the Nck genes from different species are shown in Table 1. The human Nckα shows 87% and 44% amino acid identity with the Xenopus Nck and Drosophila *dock*, respectively. The human Nckß shares 68% and 43% amino acid identity with them. While the Xenopus Nck is closer related to the human Nckα, it is not obvious, from comparing the overall amino acid sequences, which human Nck is evolutionarily closer to the Drosophila *dock* and the *C. elegans* Nck gene. However, comparisons of the changes in the "F strand", the "BG-loop" and the "BC-loop" suggest that *dock* is also closer related to the human Nckα. It is possible that Xenopus and Drosophila have additional yet unidentified Nck-related genes. Nck-like gene, as well as the genes for other SH2/SH3 adapters such as Grb2 and Crk, has not been reported in yeast, possibly due to the fact that yeast does not have much protein tyrosine phosphorylation in the cytoplasm, and, therefore, there is no need to have these adapters.

Targets for the SH2 domain of Nck

In quiescent (G1-arrested) cells, both Nckα and Nckß are largely cytoplasmic proteins with a basal level of constitutive phosphorylation in serines. In response to extra cellular cues such as PDGF-bb and EGF, Nck (possibly including both Nckα and Nckß, because antibodies used in those early studies cross react with both Nck gene products) becomes hyperphosphorylated on serine, threonine and tyrosine residues. Furthermore, Nck could be detected in the same protein complexes with the activated PDGFR-ß and EGFR by antibody co-immunoprecipitations (Li et al., 1992; Meisenhelder and Hunter, 1992; Park and Rhee, 1992) The interaction between Nckα and the receptor tyrosine kinases is mediated by the SH2 domain in Nck and a phospho-tyrosine in the receptors. For example, tyrosine 751 (Y751) in the human PDGFRß has been identified as the binding site for Nckα. Since Y751 is also one of the two binding sites in the PDGFR for the p85 subunit of PI(3)K, it has been a speculation that Nckα competes with PI(3)K for binding to the PDGFR and antagonizes the function of PI(3)K (Nishimura et al., 1993). There has been so far no evidence for or against this hypothesis. Stein et al. have identified a binding site for Nck in Eph family receptor, Eph1/ELK. They showed that pY-594 in the justamembrane region of Eph1 recruits Nck to the plasma membrane. The membrane-bound Nck in turn causes activation of the JNK/SAPK pathway (Stein et al., 1998). Nckα has also been shown to bind EGFR, insulin receptor substrate-1 (IRS-1), and p130-Cas (therefore, in the same complex with focal adhesion kinase, src and Grb2) (reviewed by McCarty, 1998). In EGF and Eph ligand stimulated cells, Nck binds directly to the GTPase-activating protein (GAP)-associated protein $p62^{dok}$ (Holland et al., 1997; Tang et al., 1997). In these cases, $p62^{dok}$ appears to mediate an otherwise indirect interaction between EGFR or Eph and Nck. It should be pointed out that all the above experiments were carried out at the times when only the Nckα gene was known. Furthermore, the previously-used anti-Nck antibodies immunoprecipitated both Nckα and Nckß due to cross reactivity of these antibodies (Chen et al., 1998). Thus, the previous "in vivo" results from anti-Nck antibody co-immunoprecipitations were not able to distinguish what specific Nck was involved. It is, therefore. important to re-verify those experiments using specific antibodies against only Nckα or Nckß (see ref. by Chen et al., 1998). In conclusion, many cell surface receptor tyrosine kinases or their substrates have docking sites for the SH2 domain of Nck. A summary of the Nck-SH2 binding proteins is shown in Figure 3. It needs to be again noticed that these results came from studies in which whether or not the Nckα or Nckß or both mediated the interactions could not be distinguished at the time.

Targets for the SH3 domains of Nck

For the past few years, various experimental approaches have been used to identify the cellular targets for the three SH3 domains of Nck. At the time of this review written, some 14 either previously known or unknown proteins have been reported to interact with at least one of the three SH3 domains of Nckα in vitro. Identification of the Nckß-SH3-interacting proteins have just recently begun and no reports have yet appeared. The Abl tyrosine kinase was the first shown to bind SH3

domains of Nckα in vitro (Ren et al., 1994). A recent study showed that the Abl kinase can be activated by membrane-bound SH3 domains of Nckα, although direct contacts between the C-terminus of Abl and SH3 domains of the Nck appeared to be unnecessary (Smith et al., 1999). Hu and his colleagues reported that overexpression of Nckα activates c-fos promoter in NIH 3T3 cells in a Ras-dependent manner (Hu et al., 1995). Moreover, they detected stable complex between Nck and Sos both in vitro and in the intact cells. These results suggested that Nck can act like GRB2 by linking receptor tyrosine kinases via its SH2 domain to guanine nucleotide exchange factor Sos by its SH3 domains, leading to Ras activation. A novel serine/threonine kinase NAK (Nck-Associated Kinase) was identified by co-immunoprecipitation followed by in vitro kinase assay (Chou and Hanafusa, 1995a,b). This 65-kDa kinase is likely related to the $p21^{cdc42/rac}$-activated kinase (Manser et al., 1994, 1995; Creasy and Chernoff, 1995; Martin et al., 1995). NAK and PAK have a similar molecular weight (65-68 kDa) and they all bind to the second SH3 domain of the human Nckα in vitro (Chou and Hanafusa, 1995a,b; Bagrodia et al., 1995b) and in living cells (Bokoch et al., 1996; Galisteo et al., 1996). PAK kinases also bind the GTP-bound Rho family GTPases, Rac and Cdc42 (Hall, 1998). Activation of PAK1 activates the Jun N-terminal kinase (JNK) and p38 MAPK (Bagrodia et al., 1995a; Manser et al., 1995). These results suggest a novel signaling pathway of Cdc42/Rac>Nck/PAK>JNK/p38-MAPK, in which Nck may "present" PAK1 to the RhoGTPases that in turn activates the PAK1>JNK/p38MAPK pathway. PRK2, a novel serine/threonine protein kinase related to the Rho effector PKN, also binds to the middle SH3 domain of Nck (Quilliam et al., 1996). PRK2 binds to Rho, but not Rac or Cdc42, in a GTP-dependent manner and cooperates with Rho to induce SRF (serum response factor) mediated transcription (Quilliam et al., 1996). Using purified GST-Nck-SH3 fusion proteins as a probe to screen cDNA expression libraries, Rivero-Lezcano et al. isolated two previously identified genes, the proto-oncogene c-Cbl (Rivero-Lezcano et al., 1994), a cellular homologue of the transforming protein of the murine Cas NS-1 retrovirus in B cell and myeloid tumors, and the human Wiskott-Aldrich Syndrome (WAS) gene (Rivero-Lezcano et al., 1995). Nck co-immunoprecipitates with c-Cbl and the Wiskott-Aldrich Syndrome protein (WASp) and Nckα binds to these proteins in vitro via its C-terminal SH3 domain (Rivero-Lezcano et al., 1995; She et al., 1997). WASp binds to Cdc42 and Rac (Aspenström et al., 1996) and participates in regulation of the actin cytoskeleton (see below). c-Cbl appears to play a negative role in cell growth (Levkowitz et al., 1998; Lee et al., 1999; Miyake et al., 1999; Waterman et al., 1999). Recent studies indicated that c-Cbl may inhibit cell proliferation by acting as an E3-like, RING finger-containing, and E2-dependent ubiquitin-protein ligase and, thereby, targeting RTKs such as PDGFR to ubiquitin system for degradation (Miyake et al., 1998; Joazeiro et al., 1999).

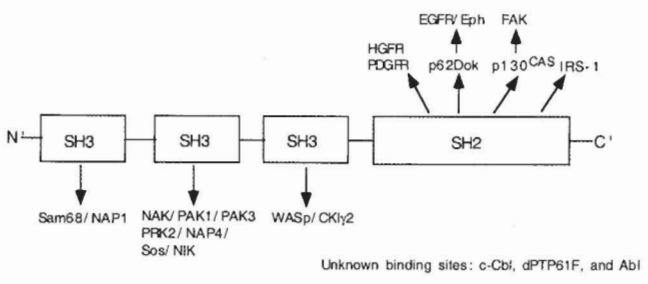

Fig. 3. Reported Nck-binding molecules. Most of the previous studies used GST fusion proteins of the human Nckα gene, since Nckß gene had not been isolated at the time. It remains to be seen whether or not Nckß are also bidns the same signaling molecules.

Other reported Nck-SH3 binding proteins include a novel serine/threonine kinase NIK (Nck-interacting kinase) (Su et al., 1997), casein kinase-1 gamma-2 (Lussier et al., 1997); Sam-68 (Lawe et al., 1997), Nap1 (Nck-associated protein 1) (Kitamura et al., 1996) and NAP4 (Nck-, Ash- and PLC-binding protein 4) (Matuoka et al., 1997). Since all the above studies were carried out prior to the recognition that Nck represents at least two independent genes in mammals (Chen et al., 1998, Tu et al., 1998), it is not clear if these SH3-binding proteins bind only to Nckα or also to Nckß. The Nck-like gene *Dock* was reported to bind a novel protein tyrosine phosphatase, dPTP61F (Clemens et al., 1996).

IV. Nck in regulation of the actin cytoskeleton

Results from a number of studies in various biological systems strongly suggest that Nck plays an important role in linking tyrosine kinases from the cell surface to the actin cytoskeleton. Rockow et al. showed that enforced overexpression of the wild type Nckα blocked NGF-induced neurite outgrowth, a Rac1/Cdc42 GTPase-dependent actin cytoskeletal reassembly, in an ERK-independent fashion in the rat adrenal pheochromocytoma cells PC12 (Rockow et al., 1996). Two of Nck-SH3-binding proteins, PAK1 (binds to the middle SH3) and WASp (binds to the third SH3), have been indicated to participate in the signal transduction by Rho family GTPases (i.e. Rac and Cdc42) to the actin cytoskeleton. Symons et al. showed that WASp binds to GTP-bound Cdc42 and clusters in polymerized actin (Symons et al., 1996), suggesting WASp acts downstream Cdc42. In this case, the role of Nck may be to translocate, by binding to receptor tyrosine kinases, WASp from the cytoplasm to the plasma membrane. WASp then could interact with Cdc42. The N-WASp (enriched in neural tissues) was involved, via Arp2/3, in Cdc42 signaling to the actin assembly (Miki et al., 1998a; Rohatgi et al., 1999; reviewed by Zigmond, 1998), although it is not clear if Nck also binds N-WASp in neuronal cells. Sells and colleagues reported that the PAK1-induced actin organization required binding to Nck, but was independent of the PAK1 kinase activity or

binding to Rac1 and Cdc42 (Sells et al., 1996). Instead, kinase-activated PAK1 appeared to inhibit the function of the non-catalytic part of PAK1 even when it still bound to Rac1 and Cdc42. Furthermore, they have shown that a kinase-dead PAK1 could mimic the effect of Rac and induced lamellipodia formation (Sells et al., 1996). Consistently, Obermeier et al. showed that the brain-specific PAK (γPAK/PAK3) induces cell spreading, membrane ruffling and increased lamellipodia formation independently of its kinase activity and binding to Rac1 and Cdc42 (Obermeier et al., 1998). They concluded that Pak3 could act both upstream and downstream of Rac, resulting in distinct outcomes. In contrast, three other groups reported that the Rac1 and Cdc42 mutants, which no longer interact with PAK1 and WASp, were still able to induce actin polymerization (Joneson et al., 1996; Lamarche et al., 1996; Westwick et al., 1997), suggesting that PAK1 and WASp are not required for the Rho GTPase signaling (Reviewed by Hall, 1998). Further studies will be needed to resolve these conflicting observations. It is possible that PAK1 and WASp regulate the actin cytoskeleton by both Rho GTPase-dependent and independent mechanisms.

So far the most significant observation that Nck links tyrosine kinases to the actin cytoskeleton came from a genetic study in the Drosophila compound eye. Each of the eight R cells (R1 to R8) of the Drosophila compound eye represents a distinct neuron and acts as a photoreceptor. Guidance and target recognition of these R cells towards axons are believed to be regulated by a tyrosine kinase receptor at surface of the growth cone, which resides at the leading edge of axon. The growth cones receive extra cellular cues and in turn control the intracellular actin cytoskeletol rearrangement. Zipursky and his colleagues found that the gene called *Dreadlocks* or *Dock* , was concentrated in the R cell growth cone and essential for R cell guidance and target recognition. Mutations in *Dock* gene disrupted the signaling from the surface of the growth cone to the intracellular actin cytoskeleton, resulting in defects in R cell fasciculation, targeting and retinotopy (Garrity et al., 1996). Moreover, loss of *Dock* function also caused a delay in synapse formation by the RP3 motoneuron in muscles (Desai et al., 1999). *Dock* is composed of three SH3 and one SH2 domains and thus is structurally related to the mammalian gene Nck (Garrity et al., 1996). Rao and Zipursky reported that during the R cell guidance and targeting the first and third SH3 domains and the SH2 domain of *Dock* were functionally redundant, whereas the middle SH3 domain was always required. Depending upon specific types of neurons, the middle SH3 domain could combine either with the SH2 or with the first and third SH3 domains (Rao and Zipursky, 1998). In mammalian cells, a recent study found that Nckß played a specific role in the PDGF-induced actin polymerization (M. Chen, H. She, A. Kim, A. and W. Li, unpublished). Enforced overexpression of the Nckß but not Nckα, blocked PDGF-stimulated, Rac-dependent membrane ruffling and lamellipodia formation in NIH3T3 fibroblasts. Mutation in the SH2 domain abolished the inhibitory effect, suggesting that binding to the activated PDGF receptor was required. Mutation in the middle SH3 domain also prevented Nckß from acting in a dominant negative fashion. More intriguingly, Nckß appeared to act in parallel with or downstream of Rac1, a mediator between PDGF receptor and the actin cytoskeleton. A membrane-bound form of Nckß (Nckß-mem) specifically blocked Rac1-induced membrane ruffling and lamellipodia formation. In the same cells, the Nckß-mem did not affect Cdc42-induced filopodia formation. These results suggest that following PDGF stimulation, the endogenous Nckß relocates critical signaling molecules from the cytoplasm to the plasma membrane, where they participate in Rac1 signaling to the actin cytoskeleton.

Nck in mitogenic signaling

Several studies have implicated that Nck plays a an important role in PDGF-stimulated mitogenesis. Earlier studies showed that the binding site for Nckα, Y-751, (also for PI-3 kinase) was required for the human PDGFR' s mitogenic signaling (Fantl et al., 1992; Valius and Kazlauskas, 1993). Ming et al. showed that the tyrosine-751 in the PDGFR was required for PDGF-stimulated, Ras-independent, activation of the p70/p85 S6 kinases (Ming et al., 1994). S6 kinases bind to and are activated by Rac1 and Cdc42 and have a regulatory role in control of cell growth (Chou and Blenis, 1996a,b). It remains unknown if PI-3 kinase or Nckα or both mediate the PDGF-induced S6 kinase activation. In support for a direct involvement of Nck in mitogenesis, microinjection of either purified GST-Nckα-SH2 fusion protein or anti-Nck antibodies blocked PDGF-, but not serum-stimulated DNA synthesis in NIH3T3 cells (Roche et al., 1996). Interestingly, a recent study showed that overexpression of Nckß but not Nckα inhibited PDGF-stimulated DNA synthesis in NIH3T3 cells (Chen et al., 1998). This apparent discrepancy could be due to the high concentration of the injected Nckα fusion proteins or the overexpressed Nckß, which both may cause a non-specific competition in the cells. It is now clear that most of the previously-made anti-Nck (Nckα) antibodies cross react with both Nckα and Nckß. Thus, the microinjected anti-Nck antibody likely blocked both Nckα and Nckß. An alternative way for Nck to connect PDGFR to DNA synthesis is to act as Grb2 does by translocating Sos to the plasma membrane, thereby causing activation of Ras. As previously described, Hu and colleagues showed that Nck binds to the guanine nucleotide exchange factor Sos and activate the c-fos gene promoter in a Ras-dependent manner. Dominant negative Ras blocked the overexpressed Nck-caused c-fos gene transcription (Hu et al., 1995), although different results were reported by others (Tanaka et al., 1995). A third mechanism is for Nck to link PDGFR to the JNK pathway via PAK1, which constitutively bind the second SH3 domain of Nck. PDGF stimulation

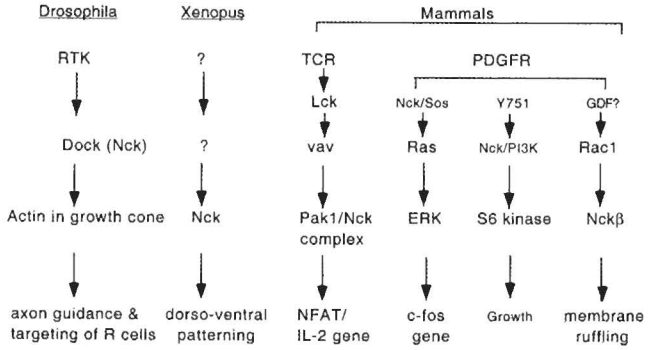

Fig. 4. Reported functions of Nck or Nck-like genes in various biological systems.

caused recruitment of PAK1 to the PDGFR signaling complex (Galisteo et al., 1996), presumably through Nck's direct binding to the PDGFR. Consistently, Lu and colleagues showed that a membrane-bound Nck could activate PAK1 in a SH3-dependent fashion (Lu et al., 1997).

The T cell antigen receptor (TCR) mediates antigen-binding, leading to activation of Ras and phospholipase Cγ. The latter generates the second messenger inositol 1,4,5-triphosphate and causes rapid increase in calcium concentration. Both Ras activation and calcium increase are required to activate NFAT, a transcription factor critical for transcriptional regulation of lymphokines such as interleukin-2. In addition, guanine nucleotide exchange factor (GEF) vav and Rho family GTPases Rac1 and Cdc42 have also been indicated in TCR-mediated NFAT pathway, in which vav possibly acts as GEF for Rac1 and Cdc42. Recently, Yablonski and colleagues showed that Nck-PAK1 signaling complex, a downstream target for Rac1 and Cdc42, acted downstream of vav and played an indispensable role in TCR-mediated NFAT activation. Expression of Nckα-W143K mutant (in the second SH3 domain) blocked TCR-mediated NFAT activation. In this case, the downstream target of the Nck-PAK1 appeared to be ERK2 but not the JNK (Yablonski et al., 1998). The role of Nck is likely to "present" PAK1 to Rac1 and Cdc42, which in turn activate PAK1. Production of IL-2 would cause proliferation and expansion of T lymphocyte population.

A weak transforming activity of overexpressed Nckα in NIH 3T3 and chick fibroblasts was previously reported (Chou et al., 1992; Li et al., 1992). Moreover, subcutaneous injection of the chick cells into nude mice caused formation of tumors (Chou et al., 1992). It was observed in many laboratories that no more than 3 to 5 fold increase over the endogenous Nck in stable cell lines could be possible (It is now clear that the endogenous Nck represents both Nckaα and Nckß at about 1:1 ratio, because the anti-Nck antibody's cross reactivity both Ncks. Therefore, the actual expression level of Nckα in those stable lines should be 6 to 10 fold higher than that of the endogenous Nckα). How overexpression of Nckα alone caused cell transformation still remains unknown. It is possible that a certain portion of the overexpressed Nckα becomes membrane bound via its SH2 domain by binding to a basal level of tyrosine phosphorylation, resulting in relocation and functional activation of its SH3-bound molecules. Other reported functions of Nck include determining mesodermal cell fate in Xenopus laevis development (Tanaka et al., 1997) and possibly cell attachment and migration (Schlaepfer et al., 1997). A summary of reported Nck's functions is shown in Figure 4.

Future Studies

Many questions remain. Recent gene knockout studies showed that mice without Nckaα or Nckß were apparently normal, whereas double knockout of both Nckα and Nckß genes caused embryonic lethality (T. Pawson, personal communication). These results suggest that Nckα and Nckß share redundant roles during mouse development. Why would a single cell need two functionally-similar Nck adapters ? This question can be studied by using specific cell systems. One could test the role of Nckα versus Nckß in NGF- and FGF-induced neurite outgrowth in PC12 cells. One could study if Nckα or Nckß or both mediate specific RTK-induced activation of PAK>JNK/p38MAPK pathway. One could also investigate which Nck specifically transmits cell surface RTK signals to the actin cytoskeleton. Cell lines derived from the Nck-knockout mice or embryos would provide powerful tools. Continued genetic studies in Drosophila and *C. elegans* will shed more light on the Nck signaling networks. Nck has not been reported in yeast, although homologues of Nck-interacting proteins such as STE-20 (PAK) and BEE1 (WASp) have been identified. The last but not the least, the chromosomal locations of the Nck genes coincide with mutations which are associated with a number of human diseases including cancer (Huebner et al., 1994; Vorabieva et al., 1995). It would be interesting to see if mutations in Nck genes could indeed influence the occurrence or frequency of the human diseases.

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