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# Nck adaptors at a glance

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## ABSTRACT

The non-catalytic region of tyrosine kinase (Nck) family of adaptors, consisting of Nck1 and Nck2, contributes to selectivity and specificity in the flow of cellular information by recruiting components of signaling networks. Known to play key roles in cytoskeletal remodeling, Nck adaptors modulate host cell–pathogen interactions, immune cell receptor activation, cell adhesion and motility, and intercellular junctions in kidney podocytes. Genetic inactivation of both members of the Nck family results in embryonic lethality; however, viability of mice lacking either one of these adaptors suggests partial functional redundancy. In this Cell Science at a Glance and the accompanying poster, we highlight the molecular organization and functions of the Nck family, focusing on key interactions and pathways, regulation of cellular processes, development, homeostasis and pathogenesis, as well as emerging and non-redundant functions of Nck1 compared to those of Nck2. This article thus aims to provide a timely perspective on the biology of Nck adaptors and their potential as therapeutic targets.

**KEY WORDS: Actin dynamics, Nck, Signaling, Tyrosine phosphorylation, SH2 domain, SH3 domain**

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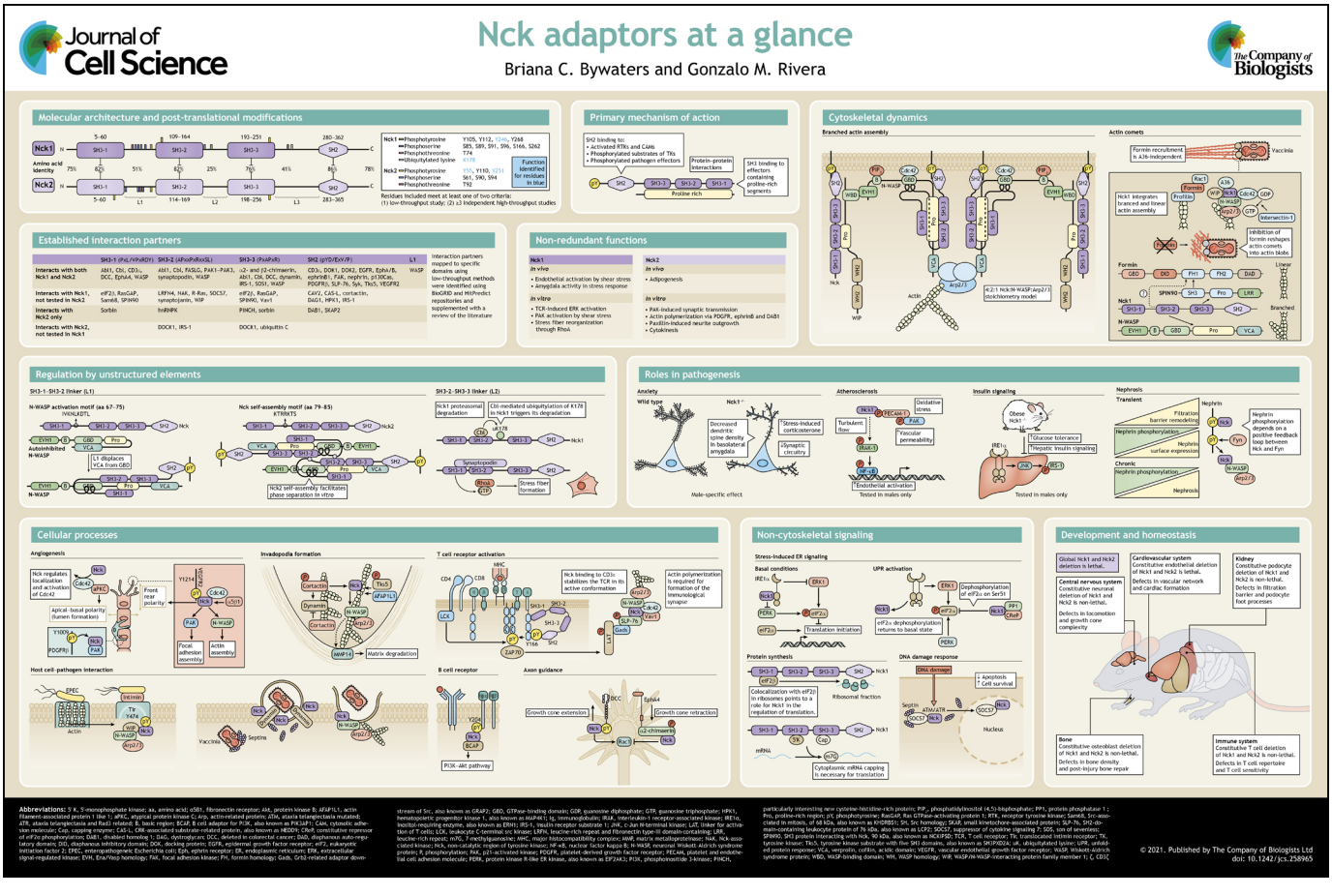
## Introduction

Non-catalytic region of tyrosine kinase (Nck) adaptors are ubiquitously expressed proteins that relay signals from phosphotyrosine to proline-rich effectors, thereby organizing molecular ensembles that regulate cellular functions. Nck adaptors were first isolated from a cDNA library of human melanoma cells (Lehmann et al., 1990), and the *Drosophila melanogaster* Nck homolog is required for photoreceptor axon guidance and targeting (Garrity et al., 1996), hinting at a role in development. In *Xenopus laevis,* expression of Nck mutants alters dorsoventral patterning, providing the first evidence of Nck involvement in vertebrate embryogenesis (Tanaka et al., 1997). The mammalian Nck family



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consists of two genes, Nck1 (also known as Nckα) and Nck2 (also known as Nckβ or Grb4), which we herein refer to collectively as Nck. These genes display broad and partially overlapping tissue expression pattern (Bladt et al., 2003); however, Nck2 expression appears somewhat restricted (Chen et al., 1998). Human and mouse Nck orthologs share 96% amino acid identity, whereas Nck paralogs share 68% amino acid identify (Chen et al., 1998).

Nck remodels the actin cytoskeleton (Li et al., 2001; Buday et al., 2002) and also regulates host cell–pathogen interactions (Frischknecht et al., 1999; Moreau et al., 2000), lymphocyte antigen receptors (Gil et al., 2002; Barda-Saad et al., 2010; Castello et al., 2013), intercellular junctions (Jones et al., 2006; Verma et al., 2006), cell–matrix adhesions (Chaki et al., 2013) and cell migration (Ruusala et al., 2008; Chaki et al., 2013). Recent studies suggest that Nck modulates the specific activity of biomolecular condensates that are generated by phase separation (Banani et al., 2017; Case et al., 2019a) and plays tissue-specific roles. Blending well-established and new themes in the biology of Nck, this Cell Science at a Glance article and accompanying poster outline how these adaptors contribute to signal propagation and highlight their emerging functions in development, homeostasis and disease.

## Molecular architecture

Nck adaptors mediate protein–protein interactions, thereby relocating signaling components and increasing their local concentrations. Their architecture consists of three N-terminal Src homology (SH) 3 domains and a C-terminal SH2 domain tethered by unstructured linkers (Chen et al., 1998) (see poster). Nck adaptors assemble multimolecular signaling complexes initiated by activated receptor tyrosine kinases (RTKs) and phosphorylated substrates of non-receptor tyrosine kinases (TKs) that act as docking sites for the SH2 domain (Wagner et al., 2013). Nck SH3 domains bind to proline-rich motifs constitutively to recruit additional signaling components (Mayer, 2001). Modulation of signaling by interdomain linker sequences is also increasingly appreciated (Takeuchi et al., 2010; Banjade et al., 2015; Okrut et al., 2015). Nck adaptors undergo post-translational modifications, including ubiquitylation and phosphorylation; however, their significance is only beginning to be recognized (Anselmi et al., 2012; Buvall et al., 2013; Dionne et al., 2018).

### SH2 domain

Signaling by tyrosine phosphorylation, which is critical in development and disease (Hunter, 2009), is propagated by proteins containing phosphotyrosine-binding domains (Schlessinger and Lemmon, 2003; Wagner et al., 2013). The SH2 domain (Songyang et al., 1993; Liu et al., 2006a), which consists of∼100 residues, binds to phosphotyrosine with a selectivity that is partially dependent on the identity of the surrounding amino acid sequence (Machida and Mayer, 2005; Tinti et al., 2013; Marasco and Carlomagno, 2020). Nck1 and Nck2 SH2 domains, which share ∼82% amino acid sequence identity, appear to bind *in vitro* to similar phosphopeptides with sequence pY[D/E]x[V/P] (where x indicates any residue) (Frese et al., 2006); however, comparative analysis of binding specificities *in vivo* has not been performed. Nck SH2 is recruited to activated RTKs [e.g. platelet-derived growth factor receptor β (PDGFRβ), vascular endothelial growth factor receptor 2 (VEGFR2) and EphA4] (Nishimura et al., 1993; Chen et al., 2000; Lamalice et al., 2006; Bisson et al., 2007; Fawcett et al., 2007), as well as phosphorylated substrates of TKs [e.g. p62Dok (also known as DOK1), p130Cas (also known as BCAR1), Tks5 (also known as SH3PXD2A) and cortactin (CTTN)] (Woodring et al., 2004; Rivera et al., 2006; Stylli et al., 2009; Oser et al., 2010), cell adhesion proteins (e.g. nephrin and PECAM-1) (Jones et al., 2006; Chen et al., 2015) and pathogen effectors (e.g. A36R and Tir) (Frischknecht et al., 1999; Gruenheid et al., 2001; Campellone et al., 2002) (see poster). Preferential interactions of Nck2 versus Nck1 with activated epidermal growth factor receptor (EGFR) (Chen et al., 1998), PDGFRβ (Chen et al., 1998; Chen et al., 2000) and ephrinB1 (Cowan and Henkemeyer, 2001) have been identified.

### SH3 domains

The SH3 domain, which is ∼60 residues long, recognizes proline-rich sequences in peptides and regulates intramolecular interactions, the local concentration and distribution of binding partners, and the assembly of multiprotein complexes (Mayer, 2001; Dionne et al., 2021). Canonical SH3 domain–peptide interactions involve a flat, hydrophobic patch and adjacent negatively charged binding site that interact, respectively, with a PxxP sequence and a positively charged residue (+) in ligands (+xxPxxP in class I ligands, xPxxPx+ in class II ligands) (Feng et al., 1994; Lim et al., 1994). Alternative recognition mechanisms have long been appreciated (Saksela and Permi, 2012); about half of the ∼300 SH3 domains in the human proteome exhibit non-canonical binding specificities (Teyra et al., 2017).

Nck adaptors engage various binding partners through SH3-mediated interactions (see poster). Partner selection is partially explained by the intrinsic binding specificities of individual Nck SH3 domains (Liu et al., 2006b; Carducci et al., 2012; Teyra et al., 2017). Steric and electrostatic differences contribute to selectivity; the Nck1 SH3-2 domain possesses a more negatively charged surface region than that of the SH3-1 domain (Liu et al., 2006b; Hake et al., 2008). Another specificity determinant is an intramolecular interaction between the SH3-1–SH3-2 linker (L1) and the SH3-2 domain of Nck2 that masks the proline-rich binding site (Takeuchi et al., 2010). Nck SH3-mediated interactions are modulated by phosphorylation in ligands as well as the SH3 domains; binding of the Nck SH3-2 domain to the PxxPxxRxxS peptide from the serine/threonine kinase p21-activated kinase 1 (PAK1) is inhibited by serine phosphorylation (Ser21) (Zhao et al., 2000; Zhou et al., 2003). Similarly, the interaction between the Nck SH3-1 domain and the PxxPxRDY peptide from CD3ε in the T cell receptor (TCR) complex is disrupted by tyrosine phosphorylation (Tyr166) (Kesti et al., 2007; Takeuchi et al., 2008). Phosphorylation of a conserved tyrosine residue in the C terminus of Nck SH3 domains (Tyr246 in Nck1, Tyr55 and Tyr251 in Nck2) disrupts interactions with effector molecules and abrogates downstream signaling (Dionne et al., 2018). Cooperative binding of Nck SH3 domains also regulates binding specificity (Wunderlich et al., 1999). Finally, binding partner identity and the position of the Nck SH3 domains largely determines the specificity of interactions *in vivo* (Dionne et al., 2021). Shuffling of SH3 domains in Nck2 disrupts protein–protein interactions and the ability to undergo phase separation (Dionne et al., 2021), the segregation of solution components into dense and dilute phases (Box 1).

### Unstructured elements

The notion that Nck adaptors adopt a ‘beads on a string’ architecture due to their modular organization has been challenged by the demonstration of an intramolecular interaction involving a conserved basic motif in L1 (residues ∼62–109) and the second SH3 domain (Takeuchi et al., 2010). L1 also contains an activation motif that drives actin assembly stimulated by Wiscott–Aldrich

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**Box 1. Emerging functions of Nck adaptors**

Biomolecular condensates are discrete, non-membrane-bound compartments in cells that contain densely packed macromolecules; their formation and dynamics are governed by liquid–liquid phase separation (LLPS) (Hyman et al., 2014). Importantly, biomolecular condensates regulate biological processes by diverse mechanisms, such as increased reaction specificity and kinetics, modulation of activity by sequestration or exclusion, and buffering of biomolecule concentration (Lyon et al., 2020). In imbalanced states, biomolecular condensates can contribute to pathogenesis (Wegmann et al., 2018).

Initial studies linking Nck to the assembly of biomolecular condensates in membranes have shown that multivalent interactions between tyrosine-phosphorylated nephrin, Nck and N-WASP promote phase transitions that correlate with increased actin polymerization by the Arp2/3 complex (Li et al., 2012). Increased specific activity of the complex (i.e. rates of actin polymerization) is a direct function of increased dwell time of N-WASP and the Arp2/3 complex, a property shown to be independent of the density of N-WASP but dependent on the stoichiometry of molecular components in phase-separated condensates (Case et al., 2019b). Interestingly, the conserved L1 linker interacts weakly with the SH3-2 domain of Nck, thereby promoting Nck self-association and potentiating phase separation of the nephrin–Nck–N-WASP complex (Banjade et al., 2015). Intriguingly, the position of SH3 domains in Nck2 has been shown to impact the ability of the protein to phase separate; strikingly, shuffling SH3-2 to the third position increases the stability of Nck2 condensates, a finding that highlights the importance of the protein context in determining the signaling properties of modular domains (Dionne et al., 2021). Biomolecular condensates also form at the immunological synapse, the site of interaction between an antigen-presenting cell and a T cell. A peripheral branched actin network and inner concentric actin arcs of linear actin polymers can be distinguished in the synapse; centripetal movement across the synapse is powered by retrograde flow of the branched network and myosin-dependent inward contraction of the linear filaments (Hammer et al., 2019). Transport of TCR microclusters is important for signaling and maturation of the immunological synapse and effector functions of T cells (Hashimoto-Tane and Saito, 2016). Nck has been shown to contribute to multivalency-driven phase separation of LAT, a critical component of the TCR signaling complex (Su et al., 2016). In addition, LAT condensates bind to actin filaments through basic residues present in the L1 region of Nck and the polybasic motif of N-WASP, and importantly, the presence of Nck and N-WASP determines how LAT condensates are propelled through the actin networks in the synapse (Ditlev et al., 2019). It should be noted that research uncovering a role for Nck in biomolecular condensate formation and signaling has been largely based on *in vitro* reconstitution systems; therefore, the pathophysiological significance of such findings remains undetermined.

syndrome protein (WASP) and the related neuronal WASP (N-WASP, also known as WASL) (Okrut et al., 2015), actin nucleation-promoting factors that bind to G-actin and the Arp2/3 complex (Padrick et al., 2008). Activity of WASP and N-WASP is controlled by autoinhibition mediated by an intramolecular interaction between the GTPase-binding domain (GBD) and the verprolin, cofilin, acidic (VCA) domain (Rohatgi et al., 2000). The activation motif of L1 interacts directly with the GBD, thereby displacing VCA to release WASP and N-WASP autoinhibition (Okrut et al., 2015); however, full activation of N-WASP requires cooperative binding of the L1 and all three SH3 domains. L1 also promotes phase separation and Nck self-assembly through weak interactions with SH3-2 (Banjade et al., 2015). Although the functional significance of other unstructured regions remains undetermined, it is noteworthy that ubiquitylation of Lys178 in the linker between SH3-2 and SH3-3 (L2) triggers proteasomal degradation of Nck1 and concomitant modulation of RhoA activation (Buvall et al., 2013).

## Major signaling pathways and key molecular interactions

### Cytoskeletal dynamics

Nck links activated RTKs and cytosolic substrates of TKs with effectors that modulate actin polymerization (see poster). Nck1-stimulated actin nucleation by the N-WASP–Arp2/3 complex requires all three SH3 domains and is further stimulated by phosphatidylinositol (4,5)-bisphosphate ($PIP_2$) (Rohatgi et al., 2001; Rivera et al., 2009). Clustering of Nck1 SH3 domains leads to the formation of actin comets (Rivera et al., 2004), which are actin structures used for movement of endocytic vesicles and organelles (Khaitlina, 2014) as well as by intracellular pathogens for intercellular spreading (Welch and Way, 2013). This pathway also involves WASP/N-WASP-interacting protein family member 1 (WIPF1 or WIP) (Benesch et al., 2002; Donnelly et al., 2013). WIP interacts directly with Nck and N-WASP (Anton et al., 1998) and promotes the formation of actin comets (Moreau et al., 2000) and filopodia (Martinez-Quiles et al., 2001), which are thin actin-based membrane protrusions used by cells to explore their microenvironment (Mattila and Lappalainen, 2008). The exchange rate of N-WASP in the Nck–WIP-containing complex controls the rate of actin polymerization (Weisswange et al., 2009). Dimerization and/or oligomerization of Nck SH3 domains increases actin polymerization by the N-WASP–Arp2/3 complex (Padrick et al., 2008). A model including WIP and the Nck–N-WASP–Arp2/3 complex with a stoichiometry of 4:2:1 (Ditlev et al., 2012) highlights the importance of cooperative assembly for full activation of actin polymerization and suggests a mechanism that prevents activation by low-intensity signals. Initial studies implicated Nck in actin polymerization stimulated by the Scar/WAVE complex (Eden et al., 2002). Although activation of Scar/WAVE has subsequently been shown to require coincident signals of activated Rac1, acidic phospholipids and phosphorylation events (Lebensohn and Kirschner, 2009), Nck localizes Scar/WAVE during phagocytosis mediated by the granulocyte receptor carcinoembryonic antigen-related cell adhesion molecule 3 (CEACAM3; Pils et al., 2012).

Nck also cooperates with Rho GTPases (see poster). Nck1 and Cdc42, with participation of intersectin-1, stimulate actin polymerization in Fcγ receptor-mediated phagocytosis and vaccinia-induced actin comets (Dart et al., 2012; Humphries et al., 2014). A similar mechanism is coordinated by Nck during TCR ligation, wherein Vav1 is required for Cdc42 activation and stabilization of active N-WASP (Zeng et al., 2003).

Stress fibers – bundles of unbranched actin filaments polymerized by formins (Hotulainen and Lappalainen, 2006) – are involved in adhesion and contractility of migrating cells (Livne and Geiger, 2016). Nck1 cooperates with RhoA in stress fiber formation through a mechanism dependent on inhibition of Nck1 ubiquitylation by Cbl (also known as c-Cbl) and subsequent proteasomal degradation (Buvall et al., 2013). Of note, Nck1 integrates dendritic and unbranched actin polymerization (Borinskaya et al., 2016; Pfanzelter et al., 2018). The balance between these two modes of actin assembly determines the morphology and dynamics of actin structures; inhibition of unbranched actin polymerization reshapes long, fast-moving actin comets into circular structures with low mobility (Borinskaya et al., 2016). Nck1 may interact with formins directly or through the adaptor SH3 protein interacting with Nck, 90 kDa (SPIN90, also known as WISH or NCKIPSD) (Borinskaya et al., 2016). Although most studies on actin dynamics focus on Nck1, a specific role for Nck2 in cytokinesis has been identified (Jacquet et al., 2018).

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#### Stress-induced endoplasmic reticulum signaling

Endoplasmic reticulum (ER) stress activates the unfolded protein response (UPR) (Ron and Walter, 2007), causing cells to downregulate protein synthesis and upregulate expression of genes involved in protein folding, antioxidative response, autophagy and apoptosis (Hetz et al., 2020). To prevent general translation initiation, ER stress induces phosphorylation of eukaryotic initiation factor 2α (eIF2α, also known as EIF2S1). Moderate overexpression of Nck1 facilitates translation by preventing eIF2α phosphorylation (Kebache et al., 2004; Cardin et al., 2007); in contrast, Nck deficiency decreases protein synthesis during ER stress (Kebache et al., 2004). Nck1 inhibits PKR-like endoplasmic reticulum kinase (PERK, also known as EIF2AK3), a major ER stress transducer, and so contributes to maintaining eIF2α hypophosphorylation (Yamani et al., 2014). Under ER stress, Nck1 promotes hypophosphorylation of eIF2α by forming a complex with protein phosphatase 1 (PP1) and its regulatory subunit CReP (also known as PPP1R15B; Latreille and Larose, 2006) (see poster). Under basal conditions, Nck associates with the ER membrane and forms a complex with serine/threonine-protein kinase/endoribonucleafynse 1 (IRE1α, also known as ERN1), a UPR inducer, thereby preventing downstream activation of extracellular signal-related kinase 1 (ERK1, also known as MAPK3), a critical modulator of cell survival (Nguyen et al., 2004). Of note, Nck1 is predominantly expressed in the liver and adipose tissue (Latreille et al., 2011).

#### Protein synthesis

Nck1 facilitates mRNA translation by interacting directly with the β subunit of eukaryotic initiation factor 2 (eIF2β, also known as EIF2S2), a component of the protein translation machinery, and localizing to ribosomal fractions of insulin-stimulated cells (Kebache et al., 2002). Nck1 also regulates assembly of the cytoplasmic mRNA capping complex by recruiting 5′-monophospate kinase and capping enzyme (Mukherjee et al., 2014). Capping of 5′ ends of all eukaryotic mRNAs is important for regulation of cap-dependent initiation of translation and mRNA decay (Schoenberg and Maquat, 2009). The possibility that Nck links cytoskeletal organization and translation regulation remains unexplored.

#### Genotoxic stress and the DNA damage response

Septins are GTPases involved in spatial organization of signaling components and membrane asymmetry (Spiliotis and McMurray, 2020). Septin depletion leads to nuclear translocation of Nck in association with suppressor of cytokine signaling 7 (SOCS7) (Kremer et al., 2007; Errington and Macara, 2013). Genotoxic stresses, such as UV irradiation and activation of the DNA damage response (DDR), disrupt the septin and actin cytoskeleton, thus leading to nuclear accumulation of Nck (Kremer et al., 2007). Silencing of Nck or prevention of its nuclear translocation increases phosphorylation of p53 (also known as TP53) and apoptosis in response to UV irradiation (Errington and Macara, 2013). Further studies are needed to fully elucidate the links between the distribution of Nck (cytosolic versus nuclear), cytoskeletal remodeling, cell cycle regulation and apoptosis.

### Nck in cellular processes

#### Cell polarity, adhesion and migration

Nck regulates cell migration induced by activated RTKs, such as PDGFRβ (Rivera et al., 2006; Ruusala et al., 2008; Guan et al., 2009) and VEGFR2 (Stoletov et al., 2001; Lamalice et al., 2006) (see poster). Directional cell migration requires front–rear polarity, which is established by differential distribution and activation of Rho GTPases, phosphoinositides and polarity complexes (Nelson, 2009). Sprouting angiogenesis (Adams and Alitalo, 2007), modulated by Nck (Clouthier et al., 2015), involves recruitment of Nck to activated VEGFR2 to promote focal adhesion assembly by activated PAK (Stoletov et al., 2001). Nck-dependent directional migration of endothelial cells involves coordination of membrane protrusion and focal adhesion dynamics through active Cdc42 (Chaki et al., 2013). Nck also contributes to endothelial morphogenesis by regulating apical–basal polarization and lumenization through the Cdc42–atypical protein kinase C (aPKC) polarity complex (Chaki et al., 2015). Genetic inactivation of Nck compromises retinal angiogenesis in mice by preventing Cdc42 and PAK2 activation downstream of activated VEGFR2 and roundabout guidance receptor 1 (ROBO1) (Dubrac et al., 2016). Genetic inactivation of Nck also inhibits pericyte migration in mice by preventing ligand-induced phosphorylation of PDGFRβ at Tyr1009 and downstream PAK activation (Dubrac et al., 2018).

#### Formation of invadopodia and metastatic invasion

Invadopodia are actin-based protruding structures that facilitate tissue invasion and metastasis by cancer cells (Eddy et al., 2017). Cortactin is a protein required for formation of invadopodia (Artym et al., 2006). Whereas unphosphorylated cortactin binds and inhibits the actin filament-severing protein cofilin, phosphorylated cortactin unleashes cofilin activity to generate free barbed ends of actin filaments (Oser et al., 2009). Nck1 is involved in formation of invadopodia (Yamaguchi et al., 2005) and is recruited to phosphorylated cortactin through its SH2 domain (Oser et al., 2010) (see poster). Nck forms a complex with the adaptor Tks5 to facilitate invadopodia formation and matrix degradation (Stylli et al., 2009). In sarcoma cells, Nck2 binds to phosphorylated AFAP1L1 to enhance formation of invadopodia (Tie et al., 2016). Nck promotes invasion of breast carcinoma cells and metastasis by regulating invadopodia actin dynamics and enhancing matrix metalloproteinase 14 (MMP14) cell-surface accumulation and extracellular matrix proteolysis (Morris et al., 2017) Nck likely regulates surface levels of MMP14 through N-WASP localization and actin polymerization; N-WASP regulates trafficking of MMP14 from late endosomes to invadopodia, where it is stabilized by F-actin (Yu et al., 2012).

#### B cell receptor activation

Antigen binding to immunoglobulins at the surface of B cells activates the B cell receptor (BCR) through phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) on the cytoplasmic tails of the immunoglobulin-α (CD79A) and immunoglobulin-β (CD79B) subunits (Tanaka and Baba, 2020) (poster). Through its SH2 domain, Nck binds directly to phosphorylated Tyr204 (a non-ITAM residue) in the tail of immunoglobulin-α. Through its SH3 domains, Nck subsequently recruits the adaptor BCAP (also known as PIK3AP1) to sites of BCR signaling, thereby modulating the phosphoinositide 3-kinase (PI3K)–Akt pathway (Castello et al., 2013) required for B cell survival and maturation (Calamito et al., 2010) (see poster). Genetic inactivation of Nck reduces survival, proliferation and antibody responses of B cells due to decreased BCAP recruitment and defective PI3K–Akt signaling (Castello et al., 2013).

#### TCR activation

Nck adaptors shape the T cell repertoire to optimize antigenic coverage and T cell responses (Lettau et al., 2009). TCR consists of

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clonotypic receptor chains (α and β, encoded by *TRA* and *TRB*, respectively) and CD3 subunits (γ, δ, ε and ζ, which is also known as CD247) that are activated by binding to peptide-major histocompatibility complexes expressed on antigen-presenting cells (see poster). Recruitment of the tyrosine kinase LCK by co-receptors CD4 or CD8 leads to phosphorylation of ITAMs in CD3 subunits that serve as docking sites for signaling molecules, including the tyrosine kinase ZAP70 (Love and Hayes, 2010). Once recruited, ZAP70 phosphorylates the transmembrane adaptor protein linker for activation of T cells (LAT), leading to formation of the LAT signalosome, which includes the scaffold SLP-76 (also known as LCP2) (Gaud et al., 2018). Upon SLP-76 phosphorylation, a complex of Nck, Vav1 and Cdc42 activates N-WASP-dependent actin polymerization (Zeng et al., 2003), thereby facilitating the formation of the immunological synapse between an antigen-presenting cell and the T cell (Kumari et al., 2014). Upon TCR activation, the CD3ε subunit exposes a proline-rich segment that recruits Nck via its SH3-1 domain (Gil et al., 2002). Sequential binding of both the SH3-1 and SH2 domains of Nck1 to CD3ε (Paensuwan et al., 2016) promotes phosphorylation of CD3ζ and recruitment of ZAP70, which are required for development and activation of mature T cells (Borroto et al., 2014). A receptor kinase motif in the cytoplasmic tail of CD3ε that is exposed upon TCR activation recruits LCK through its SH3 domain (Hartl et al., 2020). The cooperative binding of Nck SH3-1 domain and the LCK SH3 domain to separate motifs on CD3ε contributes to optimal TCR signaling (Hartl et al., 2021). Genetic deletion of Nck results in T cell lymphopenia and diminished TCR response (Roy et al., 2010).

#### Modulation of host cell–pathogen interactions

Nck is necessary for enteropathogenic *Escherichia coli* (EPEC) colonization and the formation of attaching and effacing lesions in intestinal cells (Vallance and Finlay, 2000). EPEC inserts translocated intimin receptor (Tir) into the plasma membrane of host cells (see poster), which is subsequently phosphorylated in the C terminal region (Tyr474), thus providing a binding site for Nck SH2 (Gruenheid et al., 2001; Campellone et al., 2004). Inhibition of actin polymerization by Crk adaptors, which competitively bind phosphorylated Tir, reduces actin pedestal formation (Nieto-Pelegrin et al., 2014). Furthermore, a peptide that binds to Cys48 in Nck SH3-2, which is involved in WIP–N-WASP recruitment and actin pedestal formation, prevents EPEC infection and protects mice against diarrhea (Qiu et al., 2020).

Poxviruses spread between host cells by stimulating actin polymerization following phosphorylation of viral proteins by host kinases and the recruitment of the Nck–N-WASP–Arp2/3 complex (Dodding and Way, 2009). Vaccinia virus release from infected cells is dependent on Nck-mediated actin polymerization, dynamin recruitment and displacement of a septin cage surrounding the virus (Pfanzelter et al., 2018) (see poster). In concert with tyrosine kinase non-receptor 2 (TNK2) and N-WASP, Nck also mediates the endocytic trafficking of picornaviruses (Jiang et al., 2019).

### Nck in development, homeostasis and pathogenesis

Although genetic inactivation of Nck in mice is embryonically lethal, development proceeds without overt abnormalities in animals lacking only a single paralog (Bladt et al., 2003). This finding suggests that Nck1 and Nck2 have partly overlapping and/or compensatory functions (see poster). In the sections below, we examine the role of Nck in organ systems, highlighting instances of known paralog-specific functions.

#### Cardiovascular system

Endothelial-specific inactivation of Nck results in embryonic lethality due abnormal vascular and heart development (Clouthier et al., 2015). Nck facilitates endocardial cushion formation by enabling endothelial–mesenchymal transition, a processes whereby endothelial cells acquire a migratory, mesenchymal phenotype. In neonatal mice, endothelial-specific inactivation of Nck decreases angiogenic sprouting, impairs retinal vascularization and prevents pathological neovascularization (Dubrac et al., 2016).

Recent studies link Nck to proinflammatory signaling in the vasculature. Nck-mediated NF-κB activation by oxidative stress and expression of proinflammatory molecules in cultured endothelial cells, as well as treatment with a Nck-inhibitory peptide in a model of ischemia–reperfusion injury, block leukocyte infiltration and vascular leakage (Chen et al., 2015). Enhanced vascular permeability in response to atheroprone hemodynamics is prevented by endothelial-specific inactivation of Nck1 (Alfaidi et al., 2020b). Moreover, endothelial Nck1, but not Nck2, has been shown to mediate expression of proinflammatory molecules and atherosclerosis development through activation of NF-κB and the serine/threonine kinase interleukin-1 receptor-associated kinase 1 (IRAK-1) (Alfaidi et al., 2020b). Of note, previous studies linking Nck1 to vascular pathology (Chen et al., 2015; Alfaidi et al., 2020a; Alfaidi et al., 2020b) have been performed in male mice exclusively.

#### Central nervous system

Initial studies *in vitro* have identified specific roles for Nck2 in neuritogenesis (Pramatarova et al., 2003; Guan et al., 2007; Thevenot et al., 2011). Consistent with phenotypes in mice deficient for the guidance receptor EphA4 (Dottori et al., 1998) and the downstream effector α2-chimaerin (encoded by *CHN1*) (Wegmeyer et al., 2007), genetic neuronal inactivation of Nck in mice causes a hopping gait, resulting from defective corticospinal tract development (Fawcett et al., 2007). Nck also relays signals from deleted in colorectal cancer (DCC), the guidance receptor for netrin-1 that promotes neurite outgrowth in commissural neurons (Li et al., 2002). Genetic deletion of Nck in the nervous system downregulates mRNA levels of DCC and reduces commissural tract thickness and growth cone complexity (Lane et al., 2015). Collectively, these findings suggest that Nck links guidance receptors and effectors controlling neuronal circuit development.

A recent study has shown that Nck1-deficient male mice, but not females, display increased anxiety-like behaviors and elevated stress-induced circulating corticosterone compared to control mice (Diab et al., 2020). This sex-specific effect has been linked to decreased GABAergic neuronal activation in the prefrontal cortex, decreased activation of inhibitory interneurons and loss of spine density (Diab et al., 2020).

#### Kidney

Podocytes are glomerular kidney epithelial cells that form actin-based interdigitating foot processes crucial for glomerular filtration. Genetic inactivation of Nck in podocytes leads to defective foot processes and dysfunctional glomerular filtration (Jones et al., 2006). Actin organization in foot processes requires Nck recruitment to tyrosine-phosphorylated nephrin, a glomerular cell adhesion receptor (Jones et al., 2006; Verma et al., 2006). Nephrin phosphorylation, which is inversely correlated with nephrosis (Uchida et al., 2008), depends on a positive-feedback loop between Nck and the tyrosine kinase Fyn (New et al., 2013). The extent of nephrin phosphorylation and Nck complex formation regulate glomerular filtration by modulating nephrin internalization (Martin et al., 2020).

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**Box 2. Nck adaptors as potential therapeutic targets**

Several approaches have been developed in attempts to block the function of Nck adaptors *in vivo* in order to test their therapeutic potential. For example, a Nck SH3-2 domain-interacting peptide derived from the N terminus of PAK1, initially shown to block angiogenesis *in vitro* and *in vivo* (Kiosses et al., 2002), has subsequently been shown to ameliorate vascular hyperpermeability in mice (Orr et al., 2007; Chen et al., 2015). Another peptide (Borroto et al., 2014) and a small molecule compound (AX-024) (Borroto et al., 2016) that blocks the interaction between the SH3-1 domain of Nck and a proline-rich sequence in the CD3ε subunit of TCR have been shown to inhibit T cell activation. Significantly, the Nck–CD3ε-interaction inhibitor prevents psoriasis and asthma, and has a lasting therapeutic effect in a mouse model of autoimmune encephalomyelitis (Borroto et al., 2016). However, the notion that AX-024 inhibits the interaction between Nck SH3-1 and CD3ε has recently been challenged; although the drug inhibits T cell proliferation, such effects appear to result from the polypharmacological properties of the drug rather than inhibition of the interaction between Nck SH3-1 and CD3ε (Richter et al., 2020). Of interest, crystallographic studies have shown that the Nck1 SH3 domain can swap half of its residues to form a dimer that does not bind to CD3ε (Richter et al., 2020). More recently, a synthetic peptide that interacts with a unique cysteine residue in the SH3-2 domain of Nck has been shown to prevent infection by EPEC and diarrhea in human cells in culture and in mice (Qiu et al., 2020). Finally, a peptide targeting the Nck SH2 domain has been shown to protect pancreatic β cells against diabetogenic stress (Kefalas et al., 2018). Thus, a more refined knowledge of paralog-specific, as well as domain- and unstructured element-specific interactions, is necessary for the design of selective therapeutics targeting Nck.

### Skeletal system

Nck1 deficiency accelerates bone loss in response to mechanical unloading (Aryal et al., 2013). In addition, mice with osteoblast-specific deletion of Nck display osteopenia and reduced bone repair (Aryal et al., 2015).

### Metabolic regulation

Genetic inactivation of Nck1 in mice decreases ER stress in the liver and improves glucose tolerance and insulin signaling (Latreille et al., 2011). In addition, Nck1 deletion upregulates insulin synthesis and increases pancreatic β cell survival in response to diabetogenic stress (Yamani et al., 2014; Yamani et al., 2015). Genetic deletion of Nck2 has been linked to adipogenesis, dyslipidemia and loss of glucose homeostasis (Dusseault et al., 2016). Of note, the roles of Nck1 in glucose homeostasis (Latreille et al., 2011) and of Nck2 in adiposity (Dusseault et al., 2016) have only been studied in male mice.

## Conclusions and perspectives

Since the initial discoveries linking Nck adaptors to signaling via tyrosine phosphorylation and cytoskeletal remodeling, the role of these adaptors in the integration and modulation of cellular processes, tissue homeostasis and dysfunction has been increasingly appreciated. However, several important questions remain and need to be addressed, such as what are the nonredundant functions of Nck1 and Nck2 in physiopathology? What are the precise roles of the modular domains, unstructured elements and post-translational modifications in signaling specificity? In addition, the molecular basis for sex-specific differences in Nck signaling remain unclear, as does the significance of Nck-dependent modulation of molecular condensates in physiology and disease. Further insights into these themes will define their potential as therapeutic targets in specific contexts (Box 2).

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We have strived to present a balanced view on the biology of the Nck family of adaptors that reflects the significance of scientific contributions made over the last few years by multiple research groups. Regrettably, space limitations precluded a comprehensive analysis of related published work.

### Competing interests

The authors declare no competing or financial interests.

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### Cell science at a glance

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