Ncking BCR-mediated PI3K activation

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The development of adaptive immune responses to combat infection depends on the specific recognition of foreign substances by antigen receptors expressed on T and B lymphocytes. Activation of these receptors, including surface immunoglobulin—the B-cell receptor (BCR)—on B cells and the T-cell receptor (TCR) on T cells, leads to the propagation of downstream signalling cascades, resulting in cytoskeletal reorganization, cell division, gene expression and the induction of antigen-specific immunoglobulins (antibodies) from B cells and effector cytokines from T cells. These effector molecules are crucial for activating other arms of the immune system and thereby eliminating pathogens. Antigen-receptor signalling cascades are triggered by, and thus dependent on, the rapid activation of tyrosine kinases. As such, adaptor molecules that interact with tyrosine-phosphorylated proteins are crucial components of antigen-receptor signalling cascades.

The non-catalytic region of tyrosine kinase adaptor protein (Nck) family of adaptor proteins, which includes Nck1 and Nck2, is thought to have a central role in T cells by integrating TCR-mediated activation with actin reorganization [1,2]. However, relatively little is known regarding the functional role of Nck proteins in B cells—the cells responsible for antibody production and the generation of humoral immunity. A study from Batista and colleagues, published in *Nature Immunology*, has addressed this question at both the molecular and organismal levels, revealing a major role for Nck in linking the BCR to activation of phosphoinositide 3-kinase (PI3K)—a crucial regulator of B-cell development, survival and proliferation [3]. Surprisingly, these functions of Nck seem to be independent of actin cytoskeleton regulation, raising the possibility of distinct pools of Nck in antigen-receptor signalling.

TCR and BCR signalling begins when the polymorphic antigen receptors are engaged, which triggers the rapid activation of Src family tyrosine kinases that phosphorylate immunoreceptor tyrosine-based activation motifs (ITAMs) in the cytoplasmic tails of the antigen-receptor complexes (Fig 1). Tyrosine phosphorylation of ITAMs on Igα

and Igβ of the BCR and CD3 chains of the TCR leads to the recruitment and activation of Syk family kinases, which, in turn, phosphorylate crucial downstream adaptors, including SLP65 in B cells and SLP76 and LAT in T cells. Together, these molecules serve as platforms for recruiting other molecules involved in activating phospholipase C-γ, Ca²⁺ mobilization, actin cytoskeletal rearrangement and downstream kinase cascades leading to gene transcription. The recruitment and assembly of signalling proteins and adaptor molecules into signalling microclusters is essential for the activation of multiple downstream effector pathways.

Antigen-receptor signalling also activates PI3Ks. Class I PI3Ks, in particular, are a crucial family of lipid kinases that produce 3'-phosphorylated phosphoinositides. These lipids act as second messengers that recruit PH-domain-containing proteins, including the kinases Btk—a crucial regulator of Ca²⁺ in B lymphocytes—and Akt [4], to cellular membranes, in which they are activated [4,5]. PI3K activation has been linked in B cells to engagement of the co-receptor CD19, which modulates positively and sets a threshold of BCR signalling, and the B-cell adaptor for PI3K (BCAP; [4,5]). How these molecules integrate PI3K activation with BCR engagement is unresolved.

As a scaffolding protein with a modular architecture, consisting of a single SH2 domain and three SH3 domains, Nck is recruited to the SLP65/BLNK complex in B cells and the SLP76/LAT complex in T cells. In T cells, SLP76-associated Nck has been implicated in the recruitment and activation of the Wiskott-Aldrich syndrome protein (WASP) and WASP-binding proteins, which are crucial for actin cytoskeleton reorganization and full T-cell activation [2]. Batista and colleagues used the DT40 chicken B-cell line, which has high rates of homologous recombination, to examine the effects of *Nck* gene disruption on functional responses to BCR stimulation. Although Nck-deficient DT40 cells formed filopodia-like structures and cell spreading on surface-bound antigen was impaired, the phenotypes were only mildly different from wild-type. Notably, Nck-deficient DT40 cells also showed an intrinsic impairment in BCR signalling,

as indicated by decreased BCR-induced Ca²⁺ mobilization. Surprisingly, Nck was recruited to sites of antigen stimulation and active BCR-signalling microclusters in an SLP65- and Syk-independent manner, suggesting its involvement in BCR signalling upstream from SLP65 [3].

By using analytical mass spectrometric analysis and reconstituted Nck-deficient DT40 cells, Batista and colleagues established that the SH2 domain of Nck interacts with a specific non-ITAM phosphorylated tyrosine on the BCR-associated Igα signalling chain in a Syk-independent manner [3]. In T cells, Nck can also be directly recruited to the TCR, through an interaction between its first SH3 domain and a non-canonical proline-rich sequence in the CD3ε chain, which occurs before tyrosine phosphorylation. This recruitment is associated with a conformational change of the CD3ε chain that occurs on TCR ligation, facilitating Nck binding [1]. Thus, both BCR and TCR signalling show evidence for receptor-associated Nck.

Although Nck1 and Nck2 are approximately 68% homologous at the amino acid sequence level, there is evidence that the two proteins have both distinct and redundant functions [6]. To understand further the role of Nck in B cells, the authors bred Nck1-deficient mice with mice that conditionally delete Nck2 in the B-cell lineage. Although mice that were deficient in either Nck1, or Nck1 and Nck2, had no gross alterations in B-cell development, there was a paucity of B1a cells in the peritoneal cavity, and the mice had altered basal antibody levels. Both Nck1- and Nck1 and Nck2-deficient mice also had impaired T-cell-independent responses with reduced serum IgM and IgG3 titres, despite relatively normal T-cell-dependent antibody responses [3]. B cells from these mice responded poorly to BCR engagement, including a reduction in proliferation and Ca²⁺ mobilization. Surprisingly, B-cell spreading in response to antigen and antigen processing, two actin-dependent processes, was relatively normal [3].

Taking advantage of their mass spectroscopy data, Batista and colleagues showed BCAP to be another potential Nck-binding partner. Biochemical analyses further

Fig 1 | Nck links the BCR to PI3K activation. Engagement of the BCR triggers the phosphorylation of the Igα and Igβ chains by SFK, leading to the recruitment and activation of Syk and the subsequent phosphorylation of SLP65, which recruits molecules important for downstream signalling. Among these, the Nck adaptor molecule links this complex to actin regulatory proteins, such as WASp. Batista and colleagues show that Nck also directly binds to the Igα chain to link it to BCAP, a critical regulator of PI3K pathways. BCAP, B-cell adaptor for PI3K; BCR, B-cell receptor; Nck, non-catalytic region of tyrosine kinase adaptor protein; PI3K, phosphoinositide 3-kinase; SFK, Src family kinase; SLP65, SH2-domain-containing leucocyte protein 65 kDa; WASp, Wiskott–Aldrich syndrome protein.

demonstrated that the Nck SH3 domains could bind to the proline-rich area of BCAP—a crucial regulator of PI3K in B cells. Supporting these observations, Nck-deficient DT40 cells had reduced BCAP phosphorylation and impaired recruitment of BCAP to antigen microclusters. Strikingly, Nck1-deficient B cells also had reduced phosphorylation of Akt, a downstream target from PI3K pathways. This defect was more profound in B cells that were deficient in both Nck1 and Nck2, showing the functional relevance of these interactions [3]. Together, these data suggest that Nck can function as the elusive scaffold bridging BCR to BCAP for PI3K activation.

Class I PI3Ks comprise multiple isoforms, which are important for the generation of phosphoinositide (3,4,5) trisphosphate. Of these, PI3Kδ seems to be the most important for BCR activation [4,5]. Consistent with the findings of Batista and colleagues, B cells from PI3Kδ-deficient mice have many phenotypes resembling those from Nck-deficient mice [3,5]. Moreover, mice deficient in either Btk or both Akt1 and Akt2, which require PI3Kδ for activation, also lack B1 cells and have impaired T-cell-independent responses, further supporting the connection between Nck and PI3Kδ. However, it is noteworthy that these other mutants also have a reduced number of follicular B cells and lack marginal zone B cells—phenotypes distinct from Nck1/2-deficient mice [4,5]. It is not yet obvious if other pathways compensate for loss of Nck.

It will also be of interest to discern if Nck affects additional B-cell signalling pathways. Tonic B-cell signalling, which is required for B-cell survival, has been closely linked to P110α and data suggest that CD19 is important in this process [5]. Activation of PI3K in murine B cells involves both BCAP and CD19—DT40 cells only express BCAP—and full disruption of PI3K pathways requires mutation of both proteins [7]. Whether Nck affects tonic B-cell signalling therefore awaits further evaluation.

Another intriguing open question is whether Igα-associated Nck has a different signalling potential to the pool of SLP65-associated Nck. In T cells, SLP76-associated Nck has been implicated in regulating the actin cytoskeleton through recruitment of WASp [2]. However, Nck also interacts with TCR CD3ε chains and, although the role of CD3-associated Nck remains controversial [1,8], data suggest that this interaction is important for the efficient phosphorylation of CD3ζ ITAMs [9]. Interestingly, although the role of Nck in recruiting WASp has been extensively studied in T-cell lines, analyses of Nck-deficient primary T cells demonstrate that TCR aggregation and internalization, two actin-dependent processes, are normal [10]. Indeed, defects observed in Nck-deficient T cells parallel defects identified by Batista and colleagues in B cells, including decreased TCR-induced proliferation associated with impaired Ca²⁺ mobilization [3,10]. Thus, whether Nck regulates PI3K activation in T cells is an intriguing possibility. Given

the importance of PI3K signalling in cellular activation and homeostasis, the uncovering of a crucial link in B cells provides new insight into this essential regulatory pathway, as well as keys to new functions of Nck adaptors in cellular regulation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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EMBO reports (2013) **14**, 852–853; published online 23 August 2013; doi:10.1038/embor.2013.133