New WAVEs in neuronal PI3K signalling

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The processes involved in the formation of the nervous system require the function of a multitude of proteins, which regulate a diverse array of cellular responses. The PI3K signalling pathway has emerged as being central to a number of steps that orchestrate the integrations of neurons into functional neuronal circuits. Consequently, the PI3K pathway is a key target in complex neurodevelopmental disorders thought to result from defects in developmental processes, such as autism, epilepsy and schizophrenia. In this issue of *The EMBO Journal*, Yamamoto and colleagues identify novel regulators of PI3K in neurons. In this work, NYAPs or 'Neuronal tyrosine-phosphorylated Adaptor for the PI3K' are demonstrated to link PI3K signalling to the WAVE1 complex.

Much progress has been made in understanding the key players and signalling systems that orchestrate the succession of neuronal events, leading to the generation of functioning neuronal circuits, several of which involve components of the PI3K signalling cascade. This truly ubiquitous signalling cascade requires protein–protein interactions to occur, which are organized in a highly context-dependent manner and are specific to cell types (Costa and Hirsch, 2010). PI3K was first characterized in 1985 (Whitman *et al*, 1985), yet it seems that our understanding of its regulation is still far from complete, especially with regard to the details required to fully comprehend its context-dependent regulation. This problem becomes particularly pertinent in neurons, which undergo defined developmental stages as part of the maturation programme required for the formation and the establishment of functioning neuronal circuits. Here, specific protein–protein interactions are likely to operate to restrict PI3K activity to confined cellular compartments, as neurons develop and maintain highly specialized structures (growth cone, dendrites, axons and synapses) that can be spatially segregated by long distances. One can only speculate that compositions of PI3K protein complexes are likely to be vital in directing requisite PI3K-dependent functions to local cellular responses.

In a nutshell, class I PI3Ks are a family of enzymes that target membranous phosphoinositide substrates at the three position of the inositol ring. PI3K activation results in the production of phosphatidylinositol (3,4,5)-trisphosphate (PIP3), which leads to the recruitment of downstream effectors to the membrane, such as AKT or RacGef (Vanhaesebroeck *et al*, 2010) (see Figure 1). Locally, these proteins may cause structural alterations in the cytoskeleton,

for example, initiating actin polymerization and causing localized zones of membrane expansion or regulating microtubule-dependent mechanisms (Ridley *et al*, 2003; Cain and Ridley, 2009; Tahirovic and Bradke, 2009). PI3Ks are activated by receptor tyrosine kinases, for example, upon stimulation with growth factors, which is achieved through binding of the regulatory PI3K subunit to phosphotyrosine residues in

Figure 1 Dual function of NYAPs in activating and recruiting PI3K to the WAVE1 complex. In neurons, the newly identified family of NYAP proteins serve a fundamental role in transmitting signals downstream of activated Fyn tyrosine kinase. Tyrosine-phosphorylated NYAPs execute a dual function in that they activate, as well as recruit PI3K into a protein complex with WAVE1. PI3K activation results in the production of PIP3, which, in turn, activates Rac and Akt pathways. Potent WAVE protein complex activation by Rac may therefore be accomplished by detaining WAVE in close proximity to activated PI3K, as well as to high local PIP3 concentrations. In this case, phosphorylated NYAPs could instruct efficient local reshaping of the actin cytoskeleton in response to external signals, for example in response to the GPI-linked cell adhesion protein Contactin5. Loss of function of all three family members of NYAPs result in aberrant neuronal morphology that may underlie the observed neurodevelopmental phenotype of brains in NYAP-deficient mice.

the receptor. Alternatively, PI3Ks can also be activated by Ras or the G $\beta\gamma$ subunits of G-protein coupled receptors or—according to an elegant and rather impressive study by Yamamoto and colleagues published in this issue of *The EMBO Journal*—by a new family of molecular adaptors, the NYAPs or ‘Neuronal tyrosine-phosphorylated Adaptor for the PI3K’ (Yokoyama *et al.*, 2011).

In searching for brain-specific substrates of the Src family of tyrosine kinase Fyn, the authors took advantage of a solid-phased phosphorylation screening, which yielded in the previously uncharacterized protein NYAP1 (Yokoyama *et al.*, 2011). Subsequent protein database screening identified two further proteins NYAP2 and NYAP3 (MYO16) with high similarities in sequences surrounding two tyrosine residues, as well as in two short stretches of amino acids enriched in prolines. Fyn is a major tyrosine kinase in the brain with a number of different roles in neuronal development (Umemori *et al.*, 1992). Similarly, the identified novel Fyn substrates NYAP1–3 are highly expressed in the embryonic brain, suggesting that NYAPs could be a major downstream signalling component of Src family of tyrosine kinases in brain development. This appears, indeed, to be the case as all NYAPs are highly tyrosine phosphorylated in brain lysate, while Fyn KO brains exhibit reduced levels in phosphorylation. What is surprising, however, is the fact that NYAPs account for a large percentage of overall tyrosine phosphorylation in the embryonic and postnatal brain, as well as in the adult brain.

Subsequent investigations establish a critical link to PI3K signalling and demonstrate that the conserved NYAP tyrosine residues exhibit the consensus sequence motif for interaction with PI3K p85 subunits. When phosphorylated, NYAPs not only associate with p85, they account for more than three-fourths of the total amount of tyrosine-phosphorylated proteins that associate with the regulatory p85 subunit of PI3Ks in the brain. Furthermore, NYAPs appear to be both required and sufficient to activate PI3Ks and downstream signalling such as Akt and Rac (see Figure 1). On the other hand, the search for possible upstream regulators of Fyn-mediated NYAP phosphorylation led the authors to the identification of a member of the Contactin family of GPI-anchored cell adhesion proteins. Contactin5, but not growth factors or other extracellular signals, enhanced tyrosine phosphorylation of NYAPs.

Importantly, the authors further identify WAVE1 complex components, which are known to mediate Arp2/3-dependent actin polymerization, as part of the brain-specific NYAP protein–protein interactions. Because Rac1-mediated signalling through the WAVE proteins is critical for the activation of Arp2/3 in lamellipodia (Takenawa and Suetsugu, 2007), the fact that NYAPs interact with both PI3K as well as the WAVE complex may highlight the importance of structural proximity in the control of the WAVE1 complex and its function in controlling actin dynamics (see Figure 1). Notably, based on

the presented expression data, this novel mode of WAVE1 regulation by ternary complex formation is specific to neurons and operates only when an intact NYAP–PI3K association is preserved.

The identification of a ternary association between PI3K, NYAPs and the WAVE1 complex provides another example of how signalling components are spatially and temporally recruited to subcellular compartments, according to cell type specific rules. In this instance, the neuron-specific NYAPs organize the conversion of PI3K signals to the activation of the WAVE effector complex. By securing close proximity of PI3K to the WAVE complex, the NYAPs may enhance efficacy of Rac-dependent WAVE activation and thus accomplish both localized as well as persistent remodelling of the actin cytoskeleton. But what, one might want to ask, is the functional significance of the ternary NYAP complex *in vivo*? Here, the authors took advantage of triple NYAP knockout mice, which, at first sight, seem healthy and fertile. However, compared with control mice, these mice displayed highly significant reduced brain size presumably caused by neuronal hypotrophy, indicating that NYAP–PI3K–WAVE1 complex formation is essential for normal brain development. NYAP deficiencies also affected neurite elongation in cultured neurons and antagonized Contactin5-induced neurite outgrowth responses. Thus, the absence of a functional NYAP–PI3K–WAVE1 complex appears to lead to specific neurodevelopmental disturbances despite (supposed) intact regulations of PI3K and WAVE1 through other mechanisms.

When aberrant PI3K signalling coincide with alterations in developmental processes of the nervous system, neurodevelopmental disorders can arise, which cover a range of diseases characterized by cognitive and behavioural deficits including intellectual disability, autism and epilepsy (Waite and Eickholt, 2010). The identification of the NYAPs proteins as dominant contributor of class I PI3K signalling in the brain adds a further layer of complexity and richness to an already complicated system. It is of note that triple NYAP KO mice show striking phenotypic similarities with mice lacking other components of the identified Contactin–Fyn–NYAP–PI3K–WAVE1 signalling module (i.e. Fyn, Rac1, WAVE1, PTP α), or, indeed, with mice harbouring deficiencies in other components of the PI3K signalling cascade (i.e. PTEN, TSC, Akt). Finally, the human NYAP1 locus (7q22.1) and MYO16/NYAP3 locus (13q33.3) show significant associations to autism. Thus, this beautiful work by Yamamoto and colleagues not only adds two more genes linked to PI3K cascade to the growing list of genes associated with high risk for ASD, it also provides a functional connection of the Contactin cell adhesion proteins, as identified ASD susceptibility genes, to PI3K signalling.

Conflict of interest

The authors declare that they have no conflict of interest.

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