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Aurora A moonlights in neurite extension

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Abstract

Aurora A, an integral mitotic kinase, is essential for microtubule dynamics of post-mitotic neurons. PKC ζ activates Aurora A, which in turn phosphorylates NDEL1 to promote neurite extension. This raises the possibility that Aurora A may also be involved in establishing cell polarity and axon/dendrite elaboration in young neurons.

During neurite extension, the cytoskeleton rapidly reorganizes and undergoes dynamic changes. Microtubule dynamics have an integral role in this process, but one less characterized than in cell division¹. On page 1057 of this issue, Mori *et al.* link Aurora A with PKC ζ and NDEL1, two proteins known for their role as regulators of microtubule dynamics during neurite extension². This finding may be the first to reveal a function for Aurora A in post-mitotic neuron differentiation. The authors previously demonstrated that Aurora A-mediated phosphorylation of the peptidase NDEL1 regulates centrosome separation and maturation³. In the present study, aPKC (atypical protein kinase C) is identified as an upstream activator of Aurora A, and phosphorylation of the peptidase NDEL1 by Aurora A is found to be necessary for neurite extension.

The PAR–aPKC signalling system was first found in *Caenorhabditis elegans*, as a necessary component for asymmetric cell division. This complex consists of aPKC, a serine/threonine kinase, and Par3 and Par6, two PDZ-domain scaffolding proteins. It has an inhibitory effect on the kinase Par1 and the putative E3 ubiquitin ligase Par2. The 14-3-3 ϵ orthologue, Par5, specifically binds to phosphorylated forms of Par3 and Par1. This balance between the Par proteins is required for the establishment of cell polarity and Par proteins are known to localize at the edge of the presumptive axon to generate neuronal polarity⁴. The PKC family is divided into three groups based on structure and regulation. Atypical kinases have a conserved kinase domain and a zinc finger, but lack the calcium-sensitive domain of classical PKCs. Broadly, the two atypical isotypes, PKC ζ and PKC λ/ι , are highly related, but mice lacking either show different phenotypes: the former is viable with lymphoid organ defects whereas the latter is embryonic lethal⁵.

The mammalian homologues of *Aspergillus nidulans* NudE (nuclear distribution gene E), NDE1 and NDEL1, are also key integrators of microtubule and centrosomal dynamics during cell division and neuronal migration. In particular, NDEL1 promotes neuronal growth and migration through interactions with cytoplasmic dynein, 14-3-3 ϵ and LIS1 (mutated in human lissencephaly)⁶. After the completion of neuronal migration, NDEL1 associates with dynein and DISC1 (linked to schizophrenia) to promote neurite outgrowth⁷.

Aurora serine/threonine kinases are an attractive target for cancer therapeutics, in part because of the apparent lack of effects other than those on cell division. However, studies have hinted that there may be more diverse non-mitotic functions of Aurora A. For example, Aurora A has been reported to interact with histone deacetylase complexes to induce cilia disassembly, and may have a role in mRNA stabilization⁸.

The study by Mori and colleagues establishes a signalling pathway in mouse dorsal root ganglia neurons, where PKC ζ phosphorylates Aurora A at Thr 287 to regulate neurite extension. This is followed by autophosphorylation of Aurora A at Thr 288, which facilitates binding between Aurora A and TPX2, another microtubule-associated protein, which binds to the catalytic domain of Aurora A and targets the activated protein to the spindle⁹. Aurora A bound to TPX2 subsequently phosphorylates NDEL1 at Ser 251. Using phospho-specific antibodies, the authors found that active forms of Aurora A, TPX2 and NDEL1 colocalize and co-immunoprecipitate *in vitro*. Importantly, phosphorylated Aurora and NDEL1 localizations overlap in an area surrounding the centrosome, which may later determine neuron polarity. Quantification of Aurora A and NDEL1 protein expression over time showed that total protein levels in cultured neurons was relatively stable, but phosphorylated forms increased, presumably as neurites were forming.

To establish whether this pathway might be relevant for neurite formation, each protein function was blocked: PKC ζ by pharmacological manipulation, Aurora A by RNAi knockdown and NDEL1 by Cre-mediated recombination in a conditional knockout mouse. PKC ζ activation was not affected by Aurora A or NDEL1 loss, and Aurora A was unaffected by NDEL1 loss, suggesting a linear pathway with no feedback (Fig. 1). Neurite elongation of dorsal root ganglion neurons was assayed using an aPKC pseudosubstrate, kinase-dead forms of PKC ζ and Aurora A, RNAi knockdown of *Aurora A* and Cre-mediated recombination of NDEL1. All these manipulations disrupted neurite extension, with the most severe shortening resulting from loss of NDEL1. Using an EB3-GFP reporter, the authors found that microtubule emanation, but not speed of microtubule growth, was severely affected, suggesting a defect in microtubule dynamics. Finally, reintroduction of an active, but not of a kinase-dead, form of Aurora A rescued neurite extension after PKC ζ inhibition or Aurora A depletion. Thus, the authors demonstrate that Aurora A and NDEL1 are crucial downstream effectors of PKC ζ in neurite extension, providing solid evidence that these kinases have diverse roles within different microenvironments of the cell.

Whereas this study reported no feedback mechanism between Aurora A, PKC ζ and NDEL1 in neurite extension, this does not exclude the possibility of feedback at other stages, for example during axon differentiation. Cultured neurons extend many projections during the first two stages of growth, followed by rapid growth of one of these neurites into an axon while the remaining projections become dendrites¹⁰. This process of axon selection probably involves some degree of competition and inhibition between the neurites. During this polarization process, PI3-kinase regulates the localization of Par3 and Par6 in conjunction with PKC ζ , to specify hippocampal neuron polarity⁴. This suggests that PI3-kinase signalling may be an intermediary between external cues of growth factors and calcium, and internal signalling networks. Aurora A also phosphorylates Par6 in *Drosophila melanogaster* neural precursor cells to regulate asymmetrical localization of Numb during the cell cycle¹¹.

It is possible that inhibitory cues arising from sustained Aurora A activation may also serve to regulate aPKC complex-dependent cell polarity in the neuron. Could crosstalk between these components be revealed in a broader context? Perhaps the signalling that begins in the initial steps of outgrowth might carry into the later stages of axon specification. This study and similar ones raise the exciting possibility that there are other mitotic components that may modulate neuron development beyond cell division¹².

Loss of NDEL1 had the most detrimental effect on neuritogenesis *in vitro*. This indicates that there are other pathways that may converge on NDEL1 to modulate outgrowth. During cell division in HeLa cells, activated Aurora A phosphorylates and inhibits protein phosphatase 1 (PP1) and, in turn, PP1 dephosphorylates Aurora A *in vitro* in a feedback loop during the cell cycle¹³. PP1 is found in axonal hillocks and dephosphorylates doublecortin, (a product of another gene mutated in lissencephaly) to mediate microtubule bundling. Interestingly, this is regulated by the PP1 adaptor protein spinophilin (Spn1), which is also a regulator of actin¹⁴. Thus, it would be interesting to see whether a similar interplay between PP1 and Aurora A operates during neuronal maturation. The analysis of neurons using inducible NDEL1-null neurons in this new study suggests other mouse lines, such as those lacking Spn1, LIS1 or 14-3-3ε, may be attractive models for the further study of Aurora A signalling. The potential interaction between these varied components around the centrosome may mediate crosstalk and synchronization between actin and microtubule dynamics during neuritogenesis, cell polarization and eventual neuronal maturation.

References

1. Dent EW, Gertler FB. Neuron. 2003; 40:209–227. [PubMed: 14556705]
2. Mori D, et al. Nature Cell Biol. 2009; 11:1057–1068. [PubMed: 19668197]
3. Mori D, et al. Mol Cell Biol. 2007; 27:352–367. [PubMed: 17060449]
4. Shi SH, Jan LY, Jan YN. Cell. 2003; 112:63–75. [PubMed: 12526794]
5. Moscat J, Rennert P, Diaz-Meco MT. Cell Death Differ. 2006; 13:702–711. [PubMed: 16322752]
6. Shu T, et al. Neuron. 2004; 44:263–277. [PubMed: 15473966]
7. Kamiya A, et al. Hum Mol Genet. 2006; 15:3313–3323. [PubMed: 17035248]
8. Pugacheva EN, et al. Cell. 2007; 129:1351–1363. [PubMed: 17604723]
9. Kufer TA, et al. J Cell Biol. 2002; 158:617–623. [PubMed: 12177045]
10. Goslin K, Banker G. J Cell Biol. 1989; 108:1506–1516.
11. Wirtz-Peitz F, Nishimura T, Knoblich JA. Cell. 2008; 135:161–173. [PubMed: 18854163]
12. Frank CL, Tsai L-H. Neuron. 2009; 62:312–326. [PubMed: 19447088]
13. Katayama H, et al. J Biol Chem. 2001; 276:46219–46224. [PubMed: 11551964]
14. Bielas SL, et al. Cell. 2007; 129:1227–1228.

Figure 1.

An aPKC ζ –Aurora A–NDEL1 pathway is important for neurite outgrowth. **(a)** Aurora A localizes to the base of an extending neurite. **(b)** PKC ζ phosphorylates Aurora A (AurA) on Thr 287, which facilitates Thr 288 autophosphorylation and activation. Activated Aurora A binds to TPX2. This, in turn, activates NDEL1 through Ser 251 phosphorylation, to promote neuritogenesis. Activation is depicted by a spiked outline. The lighter arrows indicate as yet unknown feedback mechanisms and the potential for this signalling pathway to affect later stages of neuronal differentiation.