Baz, Par-6 and aPKC are not required for axon or dendrite specification in Drosophila

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Par-3/Baz, Par-6, and aPKC are evolutionarily conserved regulators of cell polarity, and overexpression experiments implicate them as axon determinants in vertebrate hippocampal neurons. Here we examined their mutant and overexpression phenotypes in *Drosophila melanogaster*. We found that mutants neurons had normal axon and dendrite morphology and remodeled axons correctly in metamorphosis, and that overexpression did not affect axon or dendrite specification. Baz/Par-6/aPKC are therefore not essential for axon specification in *Drosophila*.

Neurons are highly polarized cells that establish two distinct compartments—axonal and somatodendritic—during their development. These differ on many levels: from their ability to synthesize proteins, to the organization of the cytoskeleton, to the proteins in the plasma membrane. Par-3 (Baz in *Drosophila*), Par-6 and aPKC have been proposed as axon determinants in vertebrate neurons. Overexpression of Par-3 or Par-6 proteins, or expression of a truncated dominant-negative Par-3 protein, results in additional long axon-like processes at this stage. These results suggest that Par-complex proteins may be axon determinants. This hypothesis has not been directly tested, however, either by examining the mutant phenotype for any of these genes, or by in vivo analysis of their neuronal function. If, as suggested by overexpression experiments, Par-3 and Par-6 are indeed crucial to axon specification, one might expect axons not to form in their absence. Here we used null mutants to eliminate *Drosophila* Baz, Par-6 and aPKC.
from developing neurons in vivo, and then assayed the neurons for axon specification and outgrowth.

We used previously characterized null alleles of baz, par-6, and aPKC for our analysis4–6, and used the MARCM (mosaic analysis with a represible cell marker) system to make GFP-marked homozygous mutant clones7,8. We assayed the mushroom body neurons, a group of several hundred interneurons that have been extensively characterized for axon and dendrite outgrowth and remodeling, and that are generated from just four precursors (neuroblasts) that produce 

\[ \alpha'\beta' \] and \[ \alpha\beta \] neurons extending into the dorsal axon lobe (Fig. 1c, upper left). In mutant clones, \[ \alpha'\beta' \] neurons showed completely normal axon projections that reached the tip of the dorsal lobe (Fig. 1c, right). We observed fewer \[ \alpha'\beta' \] and \[ \alpha\beta \] neurons, as expected, due to the failure of mutant neuroblasts to generate a full lineage. The aPKC mutant clones were about one quarter the size of wild-type clones5, consistent with the proliferation arrest that occurs at about the time that the neuroblast stops generating \[ \gamma \] neurons and starts generating \[ \alpha'\beta' \] neurons5. This small number of \[ \alpha'\beta' \] neurons allowed us to score axon morphology with single-cell resolution. We saw clearly that individual mutant-\[ \alpha'\beta' \] neurons had normal axon projections. Thus, we conclude that genetically null mutant baz, par-6 or aPKC neurons show no defects in axon specification or outgrowth.

We were concerned, however, that the stability of the Baz, Par-6 and aPKC proteins might be such that any protein present in the parental neuroblast at the time of clone induction would persist for many days and be inherited by many or all of the hundred genetically mutant neuronal progeny, where it might be present at sufficiently high levels to promote axon specification. To address this possibility, we assayed par-6 and aPKC protein levels in mutant clones at the third larval instar, 3 d after clone induction, and found no detectable protein in par-6 or aPKC mutant neuroblasts or neurons (Supplementary Fig. 1 online, and ref. 4); thus \[ \alpha'\beta' \] and \[ \alpha\beta \] neurons born subsequently must have initiated axon and dendrite formation in the absence of the proteins. In addition, we have observed that all three proteins (Baz, Par-6 and aPKC) are partitioned into the neuroblast—and out of the differentiating neurons—during each asymmetric neuroblast division4. This would make it virtually impossible for residual protein to be inherited passively by the mutant neuronal progeny. We conclude that Baz, Par-6 or aPKC are not required for axon or dendrite specification in mushroom body interneurons.

We next tested whether Baz, Par-6 and aPKC are involved in axon remodeling during metamorphosis. In wild-type brains, \[ \gamma \] neuron axons are pruned back in the early pupal stage, and later grow back into the medial lobe only1 (Fig. 1a). We found that baz, par-6, and aPKC null-mutant neurons showed normal pattern of \[ \gamma \] axon remodeling, with adult-specific projections into the medial lobe only (Fig. 1c). Because the remodeling occurred many days after clone induction and after the third larval instar when no protein is detectable in clones, we could infer that no protein was present in the neurons during axon remodeling. We conclude that Baz, Par-6 and aPKC are not required for axon outgrowth during metamorphosis.

Overexpression of mammalian Par-3 (Baz) or Par-6 leads to growth of extra axon-like processes in cultured hippocampal neurons2,3. To determine whether a similar effect occurs in Drosophila neurons in vivo,
we overexpressed Baz or Par-6 in alternating segments of the embryo during axon specification, and assayed axon development in the aCC motor neuron using the aCC axon marker eve::tau–lacZ. Wild-type aCC neurons had no detectable Baz or Par-6 during axon outgrowth (data not shown), but when overexpressed, these proteins were clearly detectable in the CNS (Fig. 2a). Overexpression of Baz or Par-6 had no effect on the number, orientation or growth of aCC axons, however (arrowheads, Fig. 2a). In addition, we overexpressed Baz or Par-6 proteins at higher-than-normal levels in larval mushroom body neurons using a Gal4 driver expressed at the time of axon initiation (OK107) or a Gal4 driver expressed slightly later (201Y). In both cases we found no defect in axon or dendrite morphology (Fig. 2b). We conclude that overexpression of Baz or Par-6 in neurons does not promote axon specification or block dendrite formation.

We did not find a role for Baz, Par-6 or aPKC in axon specification, nor did we observe these proteins to be targeted to the tips of growing axons in vivo or in vitro (data not shown). We did, however, observe a polarized localization of the proteins in mature sensory or central neurons. In sensory neurons, Baz was localized at the dendrite tips whereas Par-6 and aPKC were localized around the dendrite in the support cells (Supplementary Fig. 2a). In mushroom body interneurons, Baz was axon specific, Par-6 was enriched in young axons and present at low levels throughout central interneurons, and aPKC was perisynaptic (Supplementary Fig. 2b). Rather than being co-localized as has been observed in epithelia, in Drosophila neuroblasts and in vertebrate neurons, each of the three proteins had a distinct subcellular distribution in these mature neurons. We propose that all three proteins have distinct functions in mature neurons.

We have shown that Drosophila Baz, Par-6 and aPKC are not required for axon specification in vivo, and that their overexpression has no effect on axon specification or outgrowth. In contrast, overexpression of Par-3 or Par-6 in cultured mammalian hippocampal neurons results in multiple axon-like processes, leading to the hypothesis that these proteins are axon determinants. How can we reconcile these apparently paradoxical results? One possibility is that vertebrate neurons require Par-complex proteins for axon specification, whereas Drosophila neurons do not. If this is the case, it would be interesting to learn how different molecular pathways in mammals and flies generate the same functional subcellular domain (the axon). Another possibility is that neither fly nor vertebrate neurons use Par proteins to specify axon identity in vivo; cultured hippocampal neurons are separated from normal external polarity cues and may use a different mechanism for axon specification. Polarity cues from surrounding cells may also inhibit neurons in vivo from changing polarity in response to extra Par-3 or Par-6, explaining the different effects of overexpressing these proteins in Drosophila and in hippocampal neurons. A third possibility is that the overexpression experiments, where proteins are present at higher-than-normal levels, do not reflect the in vivo functions of the proteins. Loss-of-function and overexpression experiments that examine vertebrate neurons in vivo or in slice preparations will be crucial for fully understanding the role of Par complex proteins in vertebrate axon specification.

Note: Supplementary information is available on the Nature Neuroscience website.

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