The Nab2 RNA-binding protein patterns dendritic and axonal projections through a planar cell polarity-sensitive mechanism

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Abstract

RNA-binding proteins support neurodevelopment by modulating numerous steps in post-transcriptional regulation, including splicing, export, translation, and turnover of mRNAs that can traffic into axons and dendrites. One such RNA-binding protein is ZC3H14, which is lost in an inherited intellectual disability. The Drosophila melanogaster ZC3H14 ortholog, Nab2, localizes to neuronal nuclei and cytoplasmic ribonucleoprotein granules and is required for olfactory memory and proper axon projection into brain mushroom bodies. Nab2 can act as a translational repressor in conjunction with the Fragile-X mental retardation protein homolog Fmr1 and shares target RNAs with the Fmr1-bonucleoprotein granules and is required for olfactory memory and proper axon projection into brain mushroom bodies. Nab2 can act as a translational repressor in conjunction with the Fragile-X mental retardation protein homolog Fmr1 and shares target RNAs with the Fmr1-interacting RNA-binding protein Ataxin-2. However, neuronal signaling pathways regulated by Nab2 and their potential roles outside of mushroom body axons remain undefined. Here, we present an analysis of a brain proteomic dataset that indicates that multiple planar cell polarity proteins are affected by Nab2 loss, and couple this with genetic data that demonstrate that Nab2 has a previously unappreciated role in restricting the growth and branching of dendrites that elaborate from larval body-wall sensory neurons. Further analysis confirms that Nab2 loss sensitizes sensory dendrites to the genetic dose of planar cell polarity components and that Nab2-planar cell polarity genetic interactions are also observed during Nab2-dependent control of axon projection in the central nervous system mushroom bodies. Collectively, these data identify the conserved Nab2 RNA-binding protein as a likely component of post-transcriptional mechanisms that limit dendrite growth and branching in Drosophila sensory neurons and genetically link this role to the planar cell polarity pathway. Given that mammalian ZC3H14 localizes to dendritic spines and controls spine density in hippocampal neurons, these Nab2-planar cell polarity genetic data may highlight a conserved path through which Nab2/ZC3H14 loss affects morphogenesis of both axons and dendrites in diverse species.

Keywords: Nab2; RNA-binding protein; planar cell polarity; mushroom body; axon; ddaC neuron; dendrite; intellectual disability

Introduction

While many key developmental events are triggered by extracellular factors that signal through cytoplasmic cascades to alter nuclear gene transcription, other key events are triggered by shifts in post-transcriptional processing or localization of mRNAs that guide cell fates and differentiation. Importantly, the fidelity of these mRNA-based developmental mechanisms relies on RNA-binding proteins (RBPs) that associate with nascent RNAs and regulate splicing, export, stability, localization, and translation (Schliebeck et al. 2021). These key regulatory mechanisms are particularly evident in the developing nervous system, where mutations in genes encoding RBPs are often linked to human diseases. Examples of this linkage include Fragile-X mental retardation protein (Gross et al. 2012), the survival of motor neuron protein (Edens et al. 2015), and the TAR DNA-binding protein 43 (Agrawal et al. 2019; Gebauer et al. 2021). Sensitivity of the central and peripheral nervous systems to loss of RBPs has been attributed to the importance of post-transcriptional mechanisms, such as local mRNA translation and 3’-UTR extension (Mattioli et al. 2017; Thelen and Kye 2019; Engel et al. 2020), that enable fine-tuned spatiotemporal control of neuronal gene expression. This spatiotemporal control of mRNA processing and translation plays an important role in forming complex dendritic architectures and the uniquely polarized morphology of neurons (Lee et al. 2005). Accordingly, neurological diseases caused by mutations in genes encoding RBPs often include defects in axonal or dendritic morphology (Jung et al. 2012; Hornberg and Holt 2013; Holt et al. 2019), and in some cases, these axonal and dendritic defects can be traced to defective post-transcriptional control of one or a few mRNAs normally bound by the corresponding RBP.
The human ZC3H14 gene encodes a ubiquitously expressed zinc-finger, polyadenosine RBP (ZnF-Cys-Cys-Cys-His #14) that is lost in an inherited form of intellectual disability (Pak et al. 2011). Studies in multiple model organisms have begun to define functions for ZC3H14 in guiding neuronal morphogenesis. Analysis of the sole Drosophila ZC3H14 homolog, Nab2, detects cell-autonomous requirements in Kenyon cells (KCs) for olfactory memory as well as axonal branching and projection into the brain mushroom bodies (MBs; Kelly et al. 2016; Bienkowski et al. 2017), twin neuropil structures that are the center for associative olfactory learning in insects (Thum and Gerber 1999). Significantly, transgenic expression of human ZC3H14 only in fly neurons is sufficient to rescue a variety of Nab2 null phenotypes (Pak et al. 2011; Kelly et al. 2014, 2016), supporting a model in which Nab2 and ZC3H14 share critical molecular roles and mRNA targets. The Zc3h14 gene is not essential in mice but its loss results in defects in working memory (Rha et al. 2017) and dendritic spine morphology (Jones et al. 2021). An accompanying proteomic analysis of Zc3h14 knockout hippocampi identified several proteins involved in synaptic development and function that change in abundance upon ZC3H14 loss (Rha et al. 2017) and are thus candidates to contribute to Zc3h14 mutant phenotypes. Intriguingly, ZC3H14 localizes within dendritic spines in hippocampal neurons in culture and homologs of ZC3H14-regulated proteins in the mouse hippocampus are also sensitive to Nab2 loss in the developing Drosophila pupal brain (Corgiat et al. 2021), suggesting conserved links between Nab2 and ZC3H14 and neurodevelopmental pathways.

A variety of intercellular signaling mechanisms plays required roles in sensing extracellular cues that guide the complex axonal and dendritic structures that characterize specific areas of the central and peripheral nervous system (CNS and PNS). These cascades can respond to long-range directional cues, such as Netrin signaling, or to short-range directional cues from the Slit-Robo, ahl-Ena, and Semaphorin pathways (Puram and Bonni 2013; Stoeckli 2018). One pathway with an emerging role in both axonal and dendritic development is the planar cell polarity (PCP)-non-canonical Wnt pathway (Zou 2004; Andre et al. 2012; Zou 2012; Gombos et al. 2015; Misra et al. 2016). PCP signals are based on the asymmetric distribution of 2 apically localized transmembrane complexes, which in Drosophila correspond to the Stan-Vang-Pk complex (Starry Night aka Flamingo–Van Gogh–Frickle) and the Stan-Fz-Dsh-Dgo complex (Frizzled–Disheveled–Diego); these complexes are intracellularly antagonist but intercellularly attractive, leading to apical polarization across an epithelial plane (Taylor et al. 1998; Boutil and Mlodzik 1999; Vladar et al. 2009; Goodrich and Strutt 2011; Adler 2012; Peng and Axelrod 2012; Adler and Wallingford 2017; Mlodzik 2020). Core PCP components signal to downstream effector molecules that exert localized signal to downstream effector molecules that exert localized

Materials and methods
Drosophila genetics
All crosses were maintained in humidified incubators at 25°C with 12 h light-dark cycles unless otherwise noted. The Nab2ex3 loss-of-function mutant has been described previously (Pak et al. 2011; Schmitt et al. 2006; Shafer et al. 2011; Cang and Feldheim 2013; Yoshioka et al. 2013; Hagiwara et al. 2014; Yasumura et al. 2021). For example, loss of the murine Vang homolog Vangl2 leads to defects in axon guidance of spinal cord commissural axons (Shafer et al. 2011), and dsh mutants in C. elegans cause neuronal projection and morphology defects (Zheng et al. 2015). In Drosophila, loss of the core PCP components stan, Vang, pk, fz, or dsh individually disrupt α and β axon projection into the MBs (Shimizu et al. 2011; Ng 2012). Intriguingly, loss of stan or its LIM (LIN-11, Isl-1 and MEC-3)-domain adaptor espinas (esn) also disrupts dendritic self-avoidance among the class IV dendritic arborization (da) neurons (Matsubara et al. 2011), demonstrating a requirement for PCP proteins in both axon and dendrite morphogenesis within sets of neurons in the CNS and PNS.

Integrating data from 2 of our recent studies provide evidence for pathways through which the Nab2 RBP could guide axonal and dendritic projections. These analyses, one a genetic modifier screen based on a GMR-Nab2 rough eye phenotype (Lee et al. 2020) and the other a proteomic analysis of Nab2 null pupal brains (Corgiat et al. 2021), each suggests a link between Nab2 and the PCP pathway. The GMR-Nab2 modifier screen identified alleles of PCP components, both core components and downstream effectors (e.g. Vang, dsh, fz, stan, pk, Appl, and the formin DAAM), as dominant modifiers of Nab2 overexpression phenotypes in the retinal field (Lee et al. 2020). In parallel, gene ontology (GO) analysis of proteomic changes in Nab2 null brains detected enrichment for dendrite guidance and axodendritic transport GO terms among affected proteins (Corgiat et al. 2021), which include the core PCP factor Vang and the PCP accessory factor A-kinase anchor protein 200 (Akap200). Significantly, Drosophila Vang and its murine homolog Vangl2 are one of 6 pairs of homologs whose knockouts mouse hippocampi (Rha et al. 2017; Corgiat et al. 2021), suggesting a conserved relationship between Nab2/ZC3H14 and the PCP pathway in the metazoan CNS.

Considering observations outlined above, we have investigated interactions between Nab2 and PCP genes in 2 neuronal contexts—CNS axons of the Drosophila pupal MB α- and β-lobes, and in larval dendrites of class IV dorsal dendritic arbor C (ddaC) neurons—which provide complementary settings to analyze the Nab2-PCP link in axonal and dendritic compartments. We detect enrichment for PCP proteins among brain-enriched proteins affected by Nab2 loss, and a pattern of genetic interactions between Nab2 and multiple PCP alleles in both MB axons and ddaC dendrites that are consistent with Nab2-regulating axon and dendrite outgrowth by PCP-linked mechanisms. However, differences in how individual PCP alleles modify axonal vs dendritic Nab2 mutant phenotypes suggest that the Nab2-PCP relationship may depend on cellular context (i.e. pupal KCs vs larval ddaC neurons). Cell type-specific RNAi indicates that Nab2 acts cell autonomously to guide axon and dendrite growth, implying a potentially direct link between Nab2 and one or more PCP components within KCs and ddaC neurons. Collectively, these data demonstrate that Nab2 is required to regulate axonal and dendritic growth through a PCP-sensitive mechanism that has the potential to be conserved across species.
Alleles and transgenes: Nab2^{EP3716} [referred to as "Nab2 oe"; Bloomington (BL) #17159], UAS-Nab2^{ENAI} (Vienna Drosophila Research Center, #27487), UAS-fz^{RNAi} (BL #27568), appi^{2} (BL #43632), dsh^{1} (BL #5298), Vang^{st-6} (BL #6918), pk^{kk-ple-13} (BL #41790), Vang^{GFP-C} ("Vang-GFP") (gift of D. Strutt), ppk-Gal4; UAS-mCD8::GFP (gift of D. Cox), and w^{1118} ("control").

Drosophila brain dissection, immunohistochemistry, visualization, and statistical analysis

Brain dissections were performed essentially as previously described (Kelly et al. 2016). Briefly, 48–72 h after puparium formation (APF) brains were dissected at 4°C in PBS (1× PBS), fixed in 4% paraformaldehyde at RT, washed 3× in PBS, and then permeabilized in 0.3% PBS-T (1× PBS, 0.3% TritonX-100). Following blocking for 1 h (0.1% PBS-T, 5% normal goat serum), brains were stained overnight in block+primary antibodies. After 5× washes in PBS-T (1× PBS, 0.3% TritonX-100), brains were incubated in block for 1 h, moved into block+secondary antibody for 3 h, then washed 5× in PBS-T and mounted in Vectashield (Vector Labs).

Antibodies used: anti-FasII 1D4 (Developmental Studies Hybridoma Bank) at 1:50 dilution, anti-GFP polyclonal (ThermoFisher Catalog# A-11122) at a 1:200 dilution, and anti-nc82 (Developmental Studies Hybridoma Bank) at 1:50 dilution. Whole-brain images were captured on a Nikon AR1 HD25 confocal microscope using NIS-Elements C Imaging software v5.20.01, and maximum intensity projections were generated in ImageJ Fiji. Mushroom body morphological defects were scored as α-lobe thinning/missing and β-lobe fusion/missing for control, Nab2^{xz}, and PCP alleles (e.g., Vang^{st-6}/+, appi^{2}/+, and dsh^{1}/+ paired with control or Nab2^{xz}). Statistical analyses for MB phenotypes and plotting performed using GraphPad Prism8. Significance is determined using Student’s t-test or ANOVA as indicated in figure legends. Error bars representing standard deviation. Significance scores indicated are *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001.

Drosophila neuron live imaging confocal microscopy, neuronal reconstruction, data analyses, and statistical analysis

Live imaging of class IV ddaC neurons was performed essentially as described in Iyer et al. (2013) and Clark et al. (2018). Briefly, wandering stage third instar ppk-Gal4, mCD8::GFP labeled larvae were mounted on a 1:5 (v/v) diethyl ether: halocarbon oil under an Olympus BX51WI upright microscope using Olympus Fluoview software v4.2. Maximum intensity projections were generated with ImageJ Fiji. Neuronal reconstruction was performed with the TREES toolbox (Theisen et al. 1994). MathWorks Matlab R2010a v7.10.0.499 (Natick, MA) was used to process 2D stacks with local brightness thresholding, skeletonization, and sparsening to leave carrier points (Cuntz et al. 2010). Dendritic roots were defined at the soma and used to create synthetic dendritic arbors. Reconstruction parameters were equivalent across neurons. Various morphological metrics were obtained using the TREES toolbox including: Sholl analysis, total cable length, maximum path length, number of branch points, mean path/Euclidean distance, maximum branch order (maxbo), mean branch order (meano), mean branch angle, mean path length, field height/width, center of mass x, and center of mass y. These metrics were extracted in batch processing using in-house custom scripts and exported into RStudio v1.1.453 (Vienna, Austria), where quantification was visualized using other in-house custom scripts. Statistical analyses for ddaC phenotypes and plotting were performed using RStudio and Matlab. Balloon plots showing phenotypic data generated using either ddaC measurements generated in Matlab or MB defect counts. Balloon plots generated using RStudio v1.1.453 ggpubr v0.2 (Alboukadel 2018; R Development Core Team 2018).

Global proteomics

MS/MS-LC data were previously described in Corgiat et al. (2021). Briefly, 10 biological replicates of 24 h aptf control (w^{1118}) or Nab2^{xz} pupal brains (60 brains per replicate) were lysed in urea buffer (8 M urea, 100 mM NaHPO4, pH 8.5) with HALT protease and phosphatase inhibitor (Pierce/Thermo Scientific) and processed at the Emory Proteomics Core. Separate samples were prepared for male and female brains. Label-free quantification analysis was adapted from a previously published procedure (Seyfried et al. 2017). Data were analyzed using MaxQuant v1.5.2.8 with Thermo Foundation 2.0 for RAW file reading capability. Spectra were searched using the search engine ANDromeda and integrated into MaxQuant against the Drosophila melanogaster Uniprot database (43,836 target sequences). Analyses presented here used RStudio v1.1.453 (R Development Core Team 2018), custom in-house scripts, and the following packages: ggrep v0.2 (Alboukadel 2018), cluster v2.1.0 (Maechler et al. 2016), and GOplot v1.0.2 (Walter et al. 2015), to examine “planar cell polarity” annotated proteins. GO analyses were performed using FlyEnrichr (FlyEnrichr: amp.pharm.mssm.edu/FlyEnrichr/; Chen et al. 2013; Kuleshov et al. 2016, 2019), a Drosophila specific GO enrichment analysis package.

Results

Nab2 loss alters levels of PCP pathway proteins in the Drosophila brain

Our recent study comparing proteomic changes in Drosophila pupal brains lacking Nab2 identified PCP GO terms as one category of significantly altered factors (Corgiat et al. 2021; Fig 1a). A deeper analysis of this protein dataset detects enrichment for 5 PCP-related GO terms (establishment of planar polarity, establishment of epithelial cell planar polarity, establishment of body hair or bristle planar polarity, protein localization involved in planar polarity, and regulation of establishment of planar polarity, Fig 1b) based on 17 proteins. This set of proteins includes the core PCP component Van Gogh (Vang) and 5 putative PCP effectors: the Tumbleweed GTPase activating protein (Sotillos and Campuzano 2000; Jones et al. 2010), the neuron-specific PCP modulator Appi (Singh and Mlodzik 2012; Soldano et al. 2013; Liu et al. 2021), the anchoring protein Akap200 (Jackson and Berg 2002; Weber et al. 2012; Bala Tannan et al. 2018), the endocytic regulator X11L (Gross et al. 2013), and the muscle LIM-domain protein at 84B (Mlp84B; Weber et al. 2012). Together these factors represent 6.4% of the total differentially expressed proteins in Nab2^{xz} pupal brains relative to control (346 proteins in total; see Corgiat et al. 2021; Supplementary Table 1). The Vang protein (decreased by a factor of 5 in Nab2^{xz} versus control) and Appi protein (increased by a factor of 1.5 in Nab2^{xz} versus control) are particularly notable because alleles of these genes dominantly modify phenotypes produced by GMR-Gal4 driven Nab2 overexpression in the developing retinal field (Lee et al. 2020).

PCP components dominantly modify Nab2 axonal phenotypes

To pursue the Nab2-PCP link in the developing CNS, we tested whether axon projection defects in MBs homozygous for the
Nab2 is required to restrict dendritic branching and projection

Loss of murine Zc3h14 causes defects in dendritic spine morphology among hippocampal neurons (Jones et al. 2021) prompted us to test whether Nab2–PCP interactions in axons are also conserved in developing dendrites. For this approach, we visualized dendrites of Drosophila class IV ddaC neurons located in the larval body wall using a pickpocket (ppk)-Gal4, UAS-GFP system and quantified branching using Sholl intersection analysis (Fig. 3F, Cuntz et al. 2010). In wandering stage L3 larvae, complete loss of Nab2 leads to increased dendritic branch complexity as measured by the number of Sholl intersections relative to control (median of 200 in ppk>Gal4 vs median of 252 in Nab2ex3, Fig. 3, a, b and g), which is phenocopied by Nab2 RNAi depletion in ddaC neurons (median of 250 intersections in ppk>Nab2RNAi; Fig. 3, c and g). Nab2 overexpression in ddaC neurons using the Nab2ex3 transgene has the inverse effect of decreasing Sholl intersections (median of 179 in ppk>Nab2; Fig. 3, e and g). Significantly, RNAi depletion of the Wnt/PCP receptor frizzled 2 in ddaC neurons also increases Sholl intersections (median of 216 in ppk>frz2RNAi; Fig. 3, d and g), confirming prior work that Wnt/PCP signaling is involved in ddaC dendritic development (Misra et al. 2016). Intriguingly, the increased Sholl intersections in Nab2ex3 arbors are concentrated in distal segments (Fig. 3h), suggesting that the role of Nab2 in dendritic development becomes more significant with increasing distance from the cell soma.

The data above confirm that Nab2 and the PCP pathway are each required within ddaC neurons to control the extent of dendritic branching. To further assess whether modulation of PCP pathway activity affects this newly defined Nab2 dendritic role, we exploited the Matlab TREES toolbox and custom scripts to simultaneously quantify multiple dendritic phenotypes in Nab2ex3 homozygous larvae (Fig. 4c; Cuntz et al. 2010). This approach confirmed that Nab2 loss elevates the total number of branches compared to control (Fig. 4, a, b, and d) but also revealed an extension of overall cable length (Fig. 4, a–c) indicative of increased total projections. These data match the increase in intersections observed among Nab2ex3 ddaC cells observed using the Sholl technique (Fig. 3h and Supplementary Fig. 2b). A further breakdown of Nab2ex3 branching patterns using TREES parameters shows an increase in maximum branch order (number of branch points along a given branch from soma to distal tip; Fig. 5, i and j) and coupled decrease in mean branch length (distance between consecutive branches; Fig. 4d). Thus, Nab2ex3 ddaC arbors project and heterozygous background; the frequency of Nab2ex3 β-lobe defects drops from 88% to 33% in Vangex3/+/heterozygous background and to 35% in Appl+/heterozygous background (Fig. 2, e–f, i, j, m, and n). The PCP-specific allele dsh1 (Theisen et al. 1994; Gombos et al. 2015) lowers Nab2ex3 α-lobe defects from 85% to 63% but has no effect on the frequency or severity of Nab2ex3 β-lobe defects (Fig. 2, q and r; Supplementary Fig. 1). Intriguingly, animals with single copies of Vangex3 Appl+, and dsh1 in the Nab2ex3 homozygous background also develop an MB phenotype not observed in any single mutant: a bulbous, Fas2-positive lobe located where the peduncle splits into the 5 lobes (α, α’, β, β’, γ, arrowhead in Fig. 2, g, k, and o). The basis of this bulbous phenotype is unclear but may indicate that lowering levels of PCP proteins in KCs that also lack Nab2 leads to a novel axon guidance defect among α/β axons. In sum, these data reveal a pattern of dose-sensitive genetic interactions between Nab2 and PCP alleles that indicate that Nab2 loss sensitizes MB development to reduced PCP signaling.
branch significantly more than control across multiple parameters (Fig. 4d). Due to the increased branching, Nab2ex3 ddaC arbors exhibit reduced mean path length (−4%), smaller mean branch angles (−9%), and smaller mean branch lengths (−22%) compared to control (Fig. 4d). In view of the finding that ppp-Nab2RNAI phenocopies the effect of Nab2ex3 homozygosity on ddaC arbors (see Fig. 3, c and g), these TREES data are consistent with a model in which Nab2 is required in ddaC neurons to limit dendrite projection and branching.

PCP alleles exhibit compartment-specific modification of Nab2 null dendritic phenotypes

Having established that Nab2 loss elicits a spectrum of ddaC branching and projection defects, we proceeded to test whether genetic reduction of PCP components could affect one or more of these parameters. Single copies of the Vangstbm6 and Appl alleles (i.e., as heterozygotes) each have no significant effects on ddaC arbors in an otherwise wild-type background, while dsh1 heterozygosity results in increased branch points,
Sholl intersections, and total cable length compared to controls (Fig. 5, a–j). When placed into the Nab2ex3 background, single copies of Vangstbm6 and Appldd alleles dominantly modify Nab2ex3 phenotypes in opposite directions: Vangstbm6 enhances the severity of Nab2ex3 ddaC branching and length phenotypes while Appldd suppresses many of the same phenotypes (e.g. total cable length and maximum branch order; Fig. 5, d, f, and I, and j). While the dsh1 allele enhances Nab2ex3 phenotypes (Fig. 5, h–j), ddaC defects in dsh1 heterozygotes suggest that this could be an additive effect. Intriguingly, Sholl analysis reveals that the Vangstbm6 allele primarily increases complexity in Nab2ex3 proximal arbors (Supplementary Fig. 2b), which are not normally affected by Nab2 loss (see Fig. 3b). By contrast, the Appldd allele has a strong suppressive effect primarily on distal Nab2ex3 arbors (Supplementary Fig. 2b). Collectively, these genetic and quantitative data argue that Nab2 acts within ddaC neurons to restrict branching and projection of their dendrite arbors, and that Nab2 loss sensitizes the proximal arbors to reduced Vang expression and the distal arbors to reduced expression of Appl.

Given that individual FCP proteins could act within signaling or receiving cells, these compartment-specific effects could reflect functional interactions between Nab2-Vang and Nab2-Appl within ddaC dendrites, or between ddaC dendrites and the cellular substrates over which they grow.
genes, Vang

link between Nab2 and one or more PCP components within

omously to guide axon and dendrite growth, implying a potential
effect. Cell type-specific RNAi indicates that Nab2 acts cell auton-
ndaC dendrites and also MB axons by a common PCP-linked
consistent with Nab2 regulating projection and branching of
affected by Nab2 loss and define a pattern of genetic interactions
for PCP factors among proteins whose steady-state levels are

showing change percent changes in

RBPs shape axon and dendrite architecture by modulating
post-transcriptional regulation of neuronal mRNAs, including
their export from the nucleus and trafficking, stability, and trans-
lation in the cytoplasm (Ravanidis et al. 2018; Schieweck et al.
2021). Of note, the analysis presented here shows that the effects
of Nab2 on dendritic morphology are exaggerated in distal
regions relative to proximal regions closer to the nucleus (Fig. 3h;

Supplementary Fig. 2, a and b). One explanation of this effect is
that Nab2 controls expression of an mRNA (or mRNAs) encoding
a factor that guides branching and projection of more distal den-
drites. While neuronal Nab2 protein is primarily nuclear (Pak
et al. 2011), the protein is also detected in cytoplasmic messenger
ribonucleoprotein granules and has a proposed role in transla-
tional repression in conjunction with the Fragile-X mental retar-
dation protein homolog Fmr1 (Bienkowski et al. 2017), suggesting
that cytoplasmic Nab2 may inhibit translation of mRNAs that
traffic to distal dendrites and encode proteins that limit branch-
ing and projection. Core PCP proteins localize to membranes at
distal tips of some Drosophila neuronal growth cones (e.g. Reynaud
et al. 2015; Misra et al. 2016) and multiple Drosophila
Wnt/PCP proteins act autonomously in ddaC cells to control den-
dritic growth [e.g. fz2 in this study and see Matsubara et al.
(2011)]. Considering these observations, Nab2 might regulate traf-
ficking, translation, or turnover of one or more mRNAs that en-
code PCP components or regulators. Molecular identification of
Nab2-bound mRNAs in the nuclei and cytoplasm of ddaC cells
[e.g. by RIP-seq] would be required to test this hypothesis and to
determine whether any candidate target RNAs encode PCP regu-
ulatory proteins.

As noted above, tissue- and compartment-specific genetic
interactions between Nab2 and PCP alleles imply that Nab2 loss
sensitizes axons and dendrites to PCP gene dosage by different
underlying mechanisms, including those that vary between cyto-
plasmic compartments of the same cell. For example, Vangstbm6
heterozygosity selectively suppresses only Nab2ex3 MB α-lobe
defects, with no effect on β-lobe morphology. MB development is
proposed to rely on a lobe-specific PCP mechanism involving the
formin DAAM (Dsh associated activator of morphogenesis) inter-
acting with Wg/Wnt receptor Frizzled (Fz) in the α-lobes and with
Vang in the β-lobes (Gombos et al. 2015). A similar type of mecha-
nism could occur for the Nab2-PCP interaction, with Nab2 either
regulating different mRNAs in α vs β lobes or regulating factors
that themselves have lobe-specific roles e.g. DAAM or the
Derailed-WntS receptor ligand pair (Reynaud et al. 2015). The α-
lobe-specific Nab2-Vang genetic interactions mirror Nab2 inter-
actions with alleles of 2 other RBPs, fmr1 and Atx2 (Bienkowski et al.
2017; Rounds et al. 2022), establishing a precedent for distinct
Nab2 genetic interactions in α vs β-lobe axons. Significantly, Nab2
associates with Fmr1 in the neuronal cytoplasm (Bienkowski et al.
2017) and limits ddaC dendrite growth, in part through an inter-
action with the mRNA encoding the PCP effector and small
GTPase Rac1 (Fanto et al. 2000; Lee et al. 2003). These data provide
one potential link between Nab2-Fmr1 and PCP activity in MB
and ddaC neurons.

Dominant suppression of Nab2ex3 mutant MB defects by the
Vangsthbm6 allele is the inverse of how this same allele affects
Nab2ex3 ddaC phenotypes. One explanation of this effect could be
that PCP signals exchanged between MB axons and surrounding
neuro-substrate differ from those exchanged between ddaC neu-
rons and their surrounding body-wall substrate, which could inver-
t Nab2 genetic interactions between MB and ddaC systems.

Another factor to consider is the nonautonomy of some PCP
alleles; e.g. while Vangsthbm6 brains show defective α and β-axon
development, projection paths of individual Vangsthbm6 axon tracts
can be rescued by adjacent Vang wild-type cells, indicating that
Wnt/PCP control of α and β-axon branching is not strictly cell-
autonomous (Shimizu et al. 2011; Ng 2012). In contrast to Vang
alleles, partial loss of Appl (ApplΔ) consistently suppresses both
Nab2ex3 dendritic and axonal phenotypes (Supplementary Fig. 3),
which parallels the increase in Appl protein detected in brain

![Fig. 4. Nab2 restricts dendritic branching and projection. Inverted intensity images of Drosophila class IV ddaC neurons from (a) control +/-, (b) Nab2ex3 larvae. Inset black boxes show high magnification views of dendritic arbors. (c) Schematic depicting measured dendritic parameters using Matlab TREES toolbox and custom scripts. (d) Balloon plot depicting 10 measurements of the Nab2ex3 dendritic arbor. Heat map shows change percent changes in Nab2ex3 vs control.](image-url)
proteomics in Nab2 mutant brains (Fig. 1b, Supplementary Table 1). Appl acts as a downstream neuronal-specific effector of the PCP pathway (Soldano et al. 2013; Liu et al. 2021) and elevated Appl protein in response to Nab2 loss could be an indirect consequence of altered core PCP pathway activity or evidence of direct regulation of the Appl transcript.

In aggregate, these data reveal a pattern of genetic interactions between Nab2 and PCP alleles and provide the first evidence that Nab2 is required for dendritic development. These interactions between Nab2 and PCP proteins in ddaC and MB cells could be cell-autonomous or reflect interactions between neurons and surrounding substrate. Changes in expression levels of core PCP proteins, such as Vang, detected in proteomic analysis suggest that Vang mRNA is a candidate target of post-transcriptional control by Nab2 both in axons and dendrites. Given that loss of the Nab2 ortholog in mice, Zc3h14, also alters levels of the Vangl2 PCP protein in the adult hippocampus, and that mutations in PCP genes including Vangl2 are linked to intellectual disabilities, severe neural tube closure defects, and microencephaly in humans (e.g. Wang et al. 2019) dysregulation of the PCP signaling in neurons is one potential mechanism to explain axonal and dendritic phenotypes in Zc3h14 mutant mice (Jones et al. 2021) and cognitive defects in human patients lacking ZC3H14 (Pak et al. 2011).

Data availability
Proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD022984. All remaining data are contained within the article.

Supplemental material is available at G3 online.
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Conflicts of interest
None declared.

Literature cited
Adler PN. The frizzled/stan pathway and planar cell polarity in the Drosophila wing. Curr Top Dev Biol. 2012;101:1–31. doi: 10.1016/B978-0-12-394592-1.00001-6.

Adler PN, Wallingford JB. From planar cell polarity to ciliogenesis and back: the curious tale of the PFE and CPLANETE proteins. Trends Cell Biol. 2017;27(5):379–390. doi: 10.1016/j.tcb.2016.12.001.

Agrawal S, Kuo P-H, Chu L-Y, Golzarroshan B, Jain M, Yuan HS. RNA recognition motifs of disease-linked RNA-binding proteins contribute to amyloid formation. Sci Rep. 2019;9(1):6171. doi: 10.1038/s41598-019-42367-8.

Alboukadel K. ggpubr: “ggplot2” Based Publication Ready Plots. R Package Ver. 0.2. 2018.

Alpatov R, Lesch BJ, Nakamoto-Kinoshita M, Blanco A, Chen S, Stützer A, Armache KJ, Simon MD, Xu C, Ali M, et al. A chromatin-dependent role of the fragile X mental retardation protein FMRP in the DNA damage response. Cell. 2014;157(4):869–881. doi: 10.1016/j.cell.2014.03.040.

Andre P, Wang Q, Wang N, Gao B, Schlitz A, Halford MM, Stacker SA, Zhang X, Yang Y. The Wnt co-receptor Ror2 regulates Wnt/planar cell polarity by modulating the degradation of the core planar cell polarity component Vang2. J Biol Chem. 2012;287(53):44518–44525. doi: 10.1074/jbc.M112.414441.

Bala Tannan N, Collu G, Humphries AC, Serysheva E, Weber U, Mlodzik M. AKAP200 promotes Notch stability by protecting it from Cbl/lysosome-mediated degradation in Drosophila melanogaster. PLoS Genet. 2018;14(1):e1007153. doi: 10.1371/journal.pgen.1007153.

Bienkowski RS, Banerjee A, Rounds JC, Rha J, Omotade OF, Gross C, Morris KJ, Leung SW, Pak CHui, Jones SK, et al. The conserved, disease-associated RNA binding protein dNab2 interacts with the fragile X protein ortholog in Drosophila neurons. Cell Rep. 2017;20(6):1372–1384. doi: 10.1016/j.celrep.2017.07.038.

Boutros M, Mlodzik M. Dishevelled: at the crossroads of divergent intracellular signaling pathways. Mech Dev. 1999;83(1–2):27–37. doi: 10.1016/s0925-4773(99)00046-5.

Cang J, Feldheim DA. Developmental mechanisms of topographic map formation and alignment. Annu Rev Neurosci. 2013;36:51–77. doi: 10.1146/annurev-neuro-062012-170341.

Chacon-Heszele MF, Chen P. Mouse models for dissecting vertebrate planar cell polarity signaling in the inner ear. Brain Res. 2009;1277:130–140. doi: 10.1016/j.brainres.2009.02.004.

Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, Clark NR, Ma’ayan A. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics. 2013;14:128. doi: 10.1186/1471-2105-14-128.

Clark SG, Graybeal LL, Bhattacharjee S, Thomas C, Bhattacharya S, Cox DN. Basal autophagy is required for promoting dendritic terminal branching in Drosophila sensory neurons. PLoS One. 2018;13(11):e0206743. doi: 10.1371/journal.pone.0206743.

Cortigia E, List SM, Rounds JC, Corbett AH, Moberg KH. The RNA binding protein Nab2 regulates the proteome of the developing Drosophila brain. J Biol Chem. 2021;297(1):100877. doi: 10.1074/jbc.2021.100877.

Courbard JR, Djiame A, Wu J, Mlodzik M. The apical/basal-polarity determinant Scribble cooperates with the PCP core factor Stbm/Vang and functions as one of its effectors. Dev Biol. 2009;333(1):67–77. doi: 10.1016/j.ydbio.2009.06.024.

Cuntz H, Forstner F, Borst A, Hausser M. One rule to grow them all: a general theory of neuronal branching and its practical application. PLoS Comput Biol. 2010;6(8):e1000877. doi: 10.1371/journal.pcbi.1000877.

Edens BM, Ajroud-Driss S, Ma L, Ma YC. Molecular mechanisms and animal models of spinal muscular atrophy. Biochim Biophys Acta. 2015;1852(4):685–692. doi: 10.1016/j.bbadis.2014.03.040.

Engel KL, Arora A, Goering R, Lo HG, Taliaferro JM. Mechanisms and consequences of subcellular RNA localization across diverse cell types. Traffic. 2020;21(6):404–418. doi: 10.1111/tra.12730.

Fagan JK, Dollar G, Lu Q, Barnett A, Fuchsenburg J, Schlosser A, Pfieger C, Adler P, Jenny A. A novel PCP effector for Drosophila wing hair formation. PLoS One. 2014;9(9):e107311. doi: 10.1371/journal.pone.0107311.

Fanto M, Weber U, Strutt DJ, Mlodzik M. Nuclear signaling by Rac and Rho GTPases is required in the establishment of epithelial planar polarity in the Drosophila eye. Curr Biol. 2000;10(16):979–988. doi: 10.1016/s0960-9822(00)00645-x.

Gebauer F, Schwarzl T, Valcarcel J, Hentze MW. RNA-binding proteins in human genetic disease. Nat Rev Genet. 2021;22(3):185–198. doi: 10.1038/s41576-020-00302-y.

Gombos R, Migh E, Antal O, Mukherjee A, Jenny A, Mihály J. The formin DAFM functions as molecular effector of the planar cell polarity pathway during axonal development in Drosophila. J Neurosci. 2015;35(28):10154–10167. doi: 10.1523/JNEUROSCI.185–14.2015.

Goodrich LV, Strutt D. Principles of planar polarity in animal development. Development. 2011;138(10):1877–1892. doi: 10.1242/dev.054080.

Gross C, Berry-Kravis EM, Bassell GJ. Therapeutic strategies in fragile X syndrome: dysregulated mGluR signaling and beyond. Neuropsychopharmacology. 2012;37(1):178–195. doi: 10.1038/npp.2011.137.

Gross GG, Lone GM, Leung IK, Hartenstein V, Guo M. X11/Mint genes control polarized localization of axonal membrane proteins in vivo. J Neurosci. 2013;33(19):8575–8586. doi: 10.1523/JNEUROSCI.5749–12.2013.

Hagiwara A, Yasumura M, Hida Y, Inoue E, Ohtsuka T. The planar cell polarity protein Vangl2 bidirectionally regulates dendritic branching in cultured hippocampal neurons. Mol Brain. 2014;7:195. doi: 10.1186/1756-6606-7-195.

Hindges R, McLaughlin T, Genoud N, Henkemeyer M, O’Leary D. EphB forward signaling controls directional branch extension and arborization required for dorsal-ventral retinotopic mapping. Neuron. 2002;35(3):475–487. doi: 10.1016/s0896-6273(02)00799-7.

Holt CE, Martin KC, Schuman EM. Local translation in neurons: visualization and function. Nat Struct Mol Biol. 2019;26(7):557–566. doi: 10.1038/s41594-019-0263-5.
Hornberg H, Holt C. RNA-binding proteins and translational regulation in axons and growth cones. Front Neurosci. 2013;7:81. doi: 10.3389/fnins.2013.00081.

Iyer SC, Ramachandran Iyer EP, Meduri R, Rubaharan M, Kuntimaddi A, Karamsetty M, Cox DN. Cut, via CrebA, transcriptionally regulates the COPII secretory pathway to direct dendrite development in Drosophila. J Cell Sci. 2013;126(Pt 20):4732–4744. doi: 10.1242/jcs.131144.

Jackson SM, Berg CA. An A-kinase anchoring protein is required for protein kinase A regulatory subunit localization and morphology of actin structures during oogenesis in Drosophila. Development. 2002;129(19):4423–4433.

Jones C, Chen P. Planar cell polarity signaling in vertebrates. Bioessays. 2007;29(2):120–132. doi: 10.1002/bies.20526.

Jones SK, Rida PC, Chen P. Line up and listen: planar cell polarity regulation in Drosophila. Development. 2013;140(23):4657–4671. doi:10.1242/dev.087676.

Lee A, Li W, Xu K, Bogert BA, Su K, Gao F-B. Control of dendritic development by the Drosophila fragile X-related gene involves the small GTPase Rac1. Development. 2003;130(22):5543–5552. doi:10.1242/dev.00792.

Lee SM, Corbett AH, Moberg KH. The disease-associated protein ZC3H14/dNab2, impairs neuronal function in Drosophila and humans. J Cell Sci. 2013;126(Pt 20):4732–4744. doi:10.1242/jcs.131144.

Liu T, Zhang T, Nicolas M, Bousissault I, Rice H, Soldano A, Caeys A, Petrova I, Fradkin L, De Strooper B, et al. The amyloid precursor protein is a conserved Wnt receptor. Elife 2021;10:e69199. doi: 10.7554/eLife.69199.

Mattioli F, Schaefer E, Magee A, Mark P, Mancini GM, Dieterich K, Von Allmen G, Alders M, Coutnon C, van Slegtenhorst M, et al. Mutations in histone acetylase modifier BRPF1 cause an autosomal-dominant form of intellectual disability with associated ptosis. Am J Hum Genet. 2017;100(1):105–116. doi: 10.1016/j.ajhg.2016.11.010.

Mclaughlin T, O’Leary DD. Molecular gradients and development of retinotopic maps. Annu Rev Neurosci. 2005;28:327–355. doi: 10.1146/annurev.neuro.28.061604.135714.

Misa M, Edmund H, Ennis D, Schlueter MA, Marot JE, Tambasco J, Barlow I, Sigurbjornsdottir S, Mathew R, Vallás AM, et al. A genome-wide screen for dendritically localized RNAs identifies genes required for dendrite morphogenesis. G3 (Bethesda). 2016;6(8):2397–2405. doi:10.1534/g3.116.030353.

Mlodzik M. Planar cell polarity: moving from single cells to tissue-scale biology. Development. 2020;147(24):1–4. doi: 10.1242/dev.186346.

Ng J. Wnt/FCP proteins regulate stereotyped axon branch extension in Drosophila. Development. 2012;139(1):165–177. doi: 10.1242/dev.068686.

Pak C, Garshabshi M, Kahrizi K, Gross C, Apponi LH, Noto JJ, Kelly SM, Leung SW, Tzschach A, Behjati F, et al. Mutation of the conserved polyadenosine RNA binding protein, ZC3H14/dNab2, impairs neuronal homeostasis and disease. Proc Natl Acad Sci U S A. 2011;108(30):12390–12395. doi: 10.1073/pnas.1107103108 [pii].

Peng Y, Axelrod JD. Asymmetric protein localization in planar cell polarity: mechanisms, puzzles, and challenges. Curr Top Dev Biol. 2012;101:33–53. doi:10.1016/B978-0-12-394592-1.00002-8.

Puram SV, Bonni A. Cell-intrinsic drivers of dendrite morphogenesis. Development. 2013;140(23):4657–4671. doi:10.1242/dev.087676.

Qian D, Jones C, Rzadzinska A, Mark S, Zhang X, Steel KP, Dai X, Chen P. Wnt5a functions in planar cell polarity regulation in mice. Dev Biol. 2007;306(1):121–133. doi:10.1016/j.ydbio.2007.03.011.

R Development Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; 2018.

Ravindis S, Kattan FG, Doxakis E. Unraveling the pathways to neuronal homeostasis and disease: mechanistic insights into the role of RNA-binding proteins and associated factors. Int J Mol Sci. 2018;19(9):2280. doi:10.3390/ijms19082280.

Reynaud E, Lahaye LL, Boulander A, Petrova IM, Marquilly C, Flandre E, Reynaud DRL. Wnt receptor cleavage in the brain dorsomedial lineage precursors. Cell Rep. 2015;11(8):1293–1304. doi:10.1016/j.celrep.2015.04.035.

Rida PC, Chen P. Line up and listen: planar cell polarity regulation in the mammalian inner ear. Semin Cell Dev Biol. 2009;20(8):978–985. doi:10.1016/j.semcdb.2009.02.007.

Rounds JC, Corbett EB, Ye C, Behnek JA, Kelly SM, Corbett AH, Moberg KH. The disease-associated proteins Drosophila Nab2 and Ataxin-2 interact with shared RNAs and coregulate neuronal morphology. Genetics. 2022;220(1):iyab175. doi: 10.1093/genetics/syab175.

Schieweck R, Ninkovic J, Kiebler MA. RNA-binding proteins balance brain function in health and disease. Physiol Rev. 2021;101(3):1309–1370. doi:10.1152/physrev.00047.2019.
