A Genetic Screen Links the Disease-Associated Nab2 RNA-Binding Protein to the Planar Cell Polarity Pathway in Drosophila melanogaster

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ABSTRACT Mutations in the gene encoding the ubiquitously expressed RNA-binding protein ZC3H14 result in a non-syndromic form of autosomal recessive intellectual disability in humans. Studies in Drosophila have defined roles for the ZC3H14 ortholog, Nab2 (aka Drosophila Nab2 or dNab2), in axon guidance and memory due in part to interaction with a second RNA-binding protein, the fly Fragile X homolog Fmr1, and coregulation of shared Nab2-Fmr1 target mRNAs. Despite these advances, neurodevelopmental mechanisms that underlie defective axonogenesis in Nab2 mutants remain undefined. Nab2 null phenotypes in the brain mushroom bodies (MBs) resemble defects caused by alleles that disrupt the planar cell polarity (PCP) pathway, which regulates planar orientation of static and motile cells via a non-canonical arm of the Wnt/Wg pathway. A kinked bristle phenotype in surviving Nab2 mutant adults additionally suggests a defect in F-actin polymerization and bundling, a PCP-regulated process. To test for Nab2-PCP genetic interactions, a collection of PCP mutant alleles was screened for modification of a rough-eye phenotype produced by Nab2 overexpression in the eye (GMR>Nab2) and, subsequently, for modification of a viability defect among Nab2 nulls. Multiple PCP alleles dominantly modify GMR>Nab2 eye roughening and a subset rescue low survival and thoracic bristle kinking in Nab2 zygotic nulls. Collectively, these genetic interactions identify the PCP pathway as a potential target of the Nab2 RNA-binding protein in developing eye and wing tissues and suggest that altered PCP signaling could contribute to neurological defects that result from loss of Drosophila Nab2 or its vertebrate ortholog ZC3H14.

KEYWORDS Drosophila melanogaster Nab2 RNA binding protein planar cell polarity GMR eye screen

Mutations in genes encoding RNA-binding proteins often lead to tissue-specific pathology, particularly within central nervous system (reviewed in Castello et al. 2013). Inactivating mutations in the human ZC3H14 gene, which encodes a ubiquitously expressed poly(A) RNA-binding protein, are linked to a monogenic form of intellectual disability (reviewed in Fasken and Corbett 2016). Studies of murine ZC3H14 and its Drosophila melanogaster homolog, Nab2, indicate that Nab2/ZC3H14 share a conserved function in brain neurons. Nab2 loss alters structure of the brain mushroom body (MB) lobes and impairs memory (Kelly et al. 2016; Bienkowski et al. 2017), while ZC3H14 loss in mice alters hippocampal morphology and decreases working memory (Rha et al. 2017; Collins et al. 2019).

Drosophila Nab2 protein and its homologs in other species concentrate in the nucleus, with a small fraction of the protein also detected in the cytoplasm of various cell types, including neurons (Anderson et al. 1993; Leung et al. 2009; Van Den Bogaart et al. 2009; Guthrie et al. 2011; Bienkowski et al. 2017; Rha et al. 2017). Nuclear forms of Nab2/ZC3H14 proteins in various species are implicated in control of RNA poly(A) tail length, splicing, and export into the cytoplasm (Kelly et al. 2014; Soucek et al. 2016; Rha et al. 2017; Morris and Corbett 2018). In Drosophila neurons, cytoplasmic Nab2 localizes to messenger ribonucleoprotein particles (mRNPs) that contain the Fragile-X protein homolog Fmr1 and are implicated in
### Table 1 Summary of tested alleles and their effect on GMR>>Nab2 eye phenotypes

| Allele | BDSC Stock # | Allele class (chr) | Effect on GMR>>Nab2 | # of F1 adults scored* | Notes on rationale |
|--------|--------------|--------------------|---------------------|-----------------------|--------------------|
| dsh1   | 5298         | hypomorph (X)      | E* slight size      | full                  | 43m carriers w*/K417M in DEP domain disrupts PCP (Axelrod,1998) |
| dsh3   | 5299         | amorph (X)         | S+++ size,structure and pigment | full                  | 48f              |
| dsh5   | 5297         | amorph (X)         | S+++ structure, pigment slight size | full                  | 34f carries w*/J534bp causing fs after N94 (Yanagawa,1995) |
| fz1    | 1678         | hypomorph (3)      | S* size pigment     | na                    | 41f carries w*/lesion unknown (Perrimon,1987) |
| fz8    | na           | amorph(3)          | S++ size,structure and pigment | partial              | 41m,46f |
| vanGai153 | 6919    | hypomorph (2)      | E* slight size      | na                    | 23m,64f lesion unknown (Perrimon,2005) |
| vanGai6 | 6918         | amorph (2)         | E* slight size      | na                    | 42m,46f lesion unknown (Wolff,1998) |
| stanGai3 | 6967    | hypomorph (2)      | S* slight,structure and pigment | -                    | 25m,67f |
| stanGai59 | 41776   | amorph (2)         | E* pigment loss S* size | full                  | 46m,36f  |
| pkGai32 | 44229        | Strong hypomorph (2) | S* slight,structure and pigment | full                  | 52m,68f, Q1306bp removes pk and sple function (Green,2000) |
| pkGai32-sple13 | 44230 | hypomorph (2)      | S* slight,structure and pigment | -                    | 28m,40f |
| pkGai32-sple14 | na   | hypomorph (2)      | S* slight,structure and pigment | full                  | 34m,41f |
| DAAM4a | 52348        | lethal hypomorph (X) | S+++ size,structure and pigment | full                  | 42f D360v in FH3 domain (Haelterman,2014) |
| DAAM51547 | 33546   | potential hypomorph (X) | S+++ size pigment  | full                  | 52f EP insertion into 5' region (Belen,2004) |
| Appld  | 43632        | amorph (X)         | S+++ structure pigment | full                  | 34m internal deletion (Torroja,1996) |
| Wnt4Gai23 | 6650     | amorph (2)         | S+++ size,structure pigment | full                  | 29m,37f Q343 Ter (Cohen,2002) |
| Wnt4Gai1 | 6651        | hypomorph (2)      | S* slight size      | na                    | 23m,34f in-frame deletion of E299 (Cohen,2002) |
| tapGai541 | 55498   | hypomorph         | S+++ size,structure and pigment | full                  | 54m,63f MIC gene trap 5' UTR (Nagarkar-Jaiswal,2015) |
| pucGai541 | 56272   | hypomorph (3)      | S+++ size,structure and pigment | full                  | 58m,61f MIC gene trap intron 3 (Nagarkar-Jaiswal,2015) |
| RbsnGai17 | 39628   | amorph (2)         | S* slight size      | na                    | 20m,27f Q241 Ter (Morrison,2005) |
| Pabp2Gai5 | 39628    | amorph (2)         | E+++ semi-lethal    | full                  | 8m,5f Deletion of coding sequence (Benoit,2005) |

mRNA translational repression (Bienkowski et al. 2017). Murine ZC3H14 also localizes to the neuronal cytoplasm, where it co-sediments with puromycin-sensitive ribosomal fractions and localizes to axons and dendritic spines (Rha et al. 2017). These data indicate that Nab2/ZC3H14 likely has effects on nuclear pre-mRNA processing and cytoplasmic translation of mRNAs involved in neurodevelopment. The majority of Nab2-regulated neuronal mRNAs are unde

PCP link in the brain. The PCP pathway involves two apically localized transmembrane complexes, Starry Night (also Flamingo)-Van Gogh-Prickle (Stan-Vang-Pk) and Starry night-Frizzled-Dishvelled-Diego (Stan-Fz-Dsh-Dgo), that interact across cell:cell junctions but are mutually antagonistic within cells, resulting in a polarized pattern of complex accumulation that propagates across an epithelium (reviewed in Yang and Mlodzik 2015). PCP controls the planar orientation of cells via localized effects on the F-actin cytoskeleton and contributes to a number of developmentally programmed processes, including proximal-distal hair cell orientation and axon guidance in the nervous systems of multiple species (Sato et al. 2006; Srahna et al. 2006;
Inaki et al. 2007; Hollis and Zou 2012; Ng 2012; Vandewalle et al. 2013; Ackley 2014; Gombos et al. 2015; Avilés and Stoeckli 2016).

To investigate a Nab2-PCP link in vivo, a group of alleles of core and accessory PCP components was screened for modification of phenotypes produced by eye-specific Nab2 overexpression (GMR>Nab2) (Pak et al. 2011). This candidate approach identifies multiple PCP alleles that dominantly interact with GMR>Nab2, and a subset of PCP alleles that also modify Nab2 loss-of-function phenotypes. A PCP-Nab2 link is further supported by our discovery that Nab2 mutant adults exhibit wing hair misorientation, a feature of many Drosophila PCP factors (reviewed in Yang and Mlodzik 2015). Collectively, these data identify the PCP pathway as a potential target of the Nab2 RNA binding protein in neurons.

MATERIALS AND METHODS

Drosophila genetics:

Crosses were maintained in 25º humidified Shel•Lab incubators with 12hr light-dark cycles. The Nab2 alleles ex3 (null), pex41 (control, precise excision 41), and the EP3716 line carrying UAS sites upstream of the Nab2 coding sequence, have been described previously (Pak et al. 2011). For autosomal alleles, five males of each candidate modifier stock (‘paternal transmission’ in Table 1; balancers correspond to those listed for each BDSC stock) were crossed to five virgin GMR>Nab2 females (w;GMR-Gal4/Cyo2,Nab2EP3716/TM6B,tub-Gal80). For X-linked lethal alleles and the two X-linked viable alleles, five virgin females of each candidate modifier stock (‘maternal transmission’ in Table 1; balancers correspond to those listed for each BDSC stock, except for dsh6, dsh1 and AppI chromosomes, which were rebalanced over FM7a) were crossed to five GMR>Nab2 males (w;Y,GMR-Gal4/Cyo2,Nab2EP3716/TM6B,tub-Gal80). Three independent crosses were set up per genotype. Lines obtained from Bloomington Drosophila Stock Center (BDSC): GMR-Gal4, dsh1 (Axelrod et al. 1998), dsh3 (Yanagawa et al. 1995), dsh6 (Perrimon and Mahowald 1987), fz1 (Park et al. 1994), fz1b (Povelones et al. 2005), vangemb153 and vangab13 (Wolff and Rubin 1998), stanf3 (Rawls and Wolff 2003) stanf4 (Lu et al. 1999), pk30 (Green et al. 2000), pk6-psi13 (Gubb et al. 1999), DAAM01 (Haelterman et al. 2014), DAAM1157 (Bellen et al. 2004), Appf1 (Torroja et al. 1999), wnt4emer and wnt4f1 (Cohen et al. 2002), tap9101 and puc9101 (Nagarkar-Jaiswal et al. 2015), Rbsn3-47 (Morrison et al. 2008), Pabp255 (Benoit et al. 2005), fz1b and pk6-psi14 alleles were a gift of G. Pierre-Louis (present address Valencia College, FL) and J. Axelrod.

Image and data collection

Images of adult eyes were captured with a Leica MC-170 HD digital camera mounted on a Nikon SMZ8000 microscope and processed with Adobe Photoshop to standardize brightness and sharpness. Eye phenotypes were categorized as ‘Enhancer’ (E), ‘Suppressor’ (S), or ‘no effect (-)’ based on visual assessment of pigmentation loss, disorganization of the retinal honeycomb structure, and quantitative measure of 2D eye size (see Table 1 and Figure 3). Eye size was determined for five eyes per condition (i.e., genotype and gender) using Photoshop and standardized to the average size of a wildtype eye (BDSC #5298) provided as a control for the apricot eye color and PCP-defective genetic background of the (P) w,dsh1/Y, GMR>Nab2 genotype. Images to scale.
App/+;FM7,Actin-GFP, pkl-pickle14/CyO,Df(3L)GFP, or Wnt4/+;CyO,Df(3L)GFP were crossed to Nab2e23/TM6B,Stud females, and the viability of modifier/+Nab2e23/Nab2e23 females was determined by manual counting of viable adults as described above. To calculate the penetrance of bristle kinking in Figure 5, control (Nab2e23), Nab2e23, and dsh1/+;Nab2e23 females collected from crosses described above were examined for kinking of humeral and scutal macrochaetae characteristic of Nab2e23 adults (Pak et al., 2011). Females with at least one kinked thoracic bristle were scored as ‘positive’ for the phenotype. Kinking penetrance was determined by dividing the percent of adult females with kinked bristles over the total number of adult females observed.

Statistical analysis
One-way ANOVA was used to compute significance values (p-values indicated in Legends and denoted by asterisks*) for eye size data, survival data, and bristle kink data. PrismGraphPad Software was used to generate graphs and perform statistical tests. Sample sizes (n) and p-values are indicated in figures or legends. Modifying effects of PCP alleles on Nab2e23 adult viability were quantified by as observed adult vs. expected adults (set as 100%) among three replicates of 100 sorted female larvae.

Data availability
All Drosophila transgenic and mutant lines used in this study are freely available upon request.

RESULTS
PCP alleles dominantly modify the GMR>Nab2 eye phenotype
As described in prior work (Pak et al., 2011), overexpression of Nab2 in developing eye tissue with a GMR-Gal4 transgene (chromosome 2) and an EP-type transposon located in the first non-coding exon of Nab2 (GMR-Gal4, Nab2EP3716, hereafter ‘GMR>Nab2’), leads to adult eyes that are rough, reduced in size, and lack red pigmentation in posterior domains relative to wildtype Oregon-R (OreR) or ‘GMR only’ control eyes. These phenotypes are more severe in males (Figures 1A-C) than females (Figures 2A-C) and provide a useful background to screen for alleles that dominantly interact with Nab2. This approach has proven effective in identifying factors that interact functionally or physically with Nab2, including the nuclear poly(A) binding protein Pabp2 (included as a positive control in Figures 1D and 2D) (Pak et al., 2011) and the disease-associated RNA-binding protein Fmr1 (see Bienkowski et al., 2017).

To use the GMR>Nab2 transgenic system to assess genetic interactions between Nab2 and PCP factors, a group of twenty (20) alleles corresponding to core and accessory PCP factors (Table 1) was crossed into the GMR>Nab2 background and scored for modification of eye size, roughening, and pigmentation in the F1 progeny. Fourteen autosomal alleles were screened in GMR>Nab2 males (Figure 1E-U), and four X-linked lethal alleles of the Wg/PCP factors disheveled (dsh1, dsh3) and Dishevelled Associated Activator of Morphogenesis (DAAM)G1567, DAAM*) were tested as heterozygotes in GMR>Nab2 females (Figure 2E-J). Two viable X-linked mutant alleles, dsh1 and Amyloid precursor protein-like4 (Appld), were tested both as hemizygotes in males (Figure 1N,P) and heterozygotes in females (Figure 2E,H). As summarized in Table 1, seventeen of the alleles modified, to varying degrees, one of the three GMR>Nab2 eye phenotypes tracked in this study: loss of pigmentation, disorganized ommatidial structure, and reduced eye size. Modification of the first two phenotypes was assessed by visual inspection, while the effect on male and female eye size was determined by measuring two-dimensional (2D) eye size in fixed images (Figures 3A, B). Nine of the alleles produced moderate (S++) or strong suppression (S+++) of one or more of the three scored GMR>Nab2 phenotypes: dsh3, frizzledB (fz3B), DAAM*, DAAMG1567, Appld, Wnt oncogene analog-4 homozygous (Wnt4homozygous), target of PoxnMI10541 (tapMI10541), and puckeredMI11061 (pucMI11061). One allele, vang METH, mildly enhanced (E+) GMR>Nab2 and six alleles mildly suppressed (S+) one or more of the GMR>Nab2 phenotypes: fz4, starry nightfz3 (stafz3), pk30, pk-pickel14, Wnt4h, and Rbsn5X17. One allele, starn5, mildly enhanced GMR>Nab2 pigment loss, but also mildly suppressed the reduced eye size. Finally, the dsh1 allele suppressed eye size, pigment loss and roughness in dsh1/+ heterozygous females, but had a mild enhancing effect on GMR>Nab2 in hemizygous males (i.e., dsh1/Y), although this phenotype was more difficult to score based on the apricot eye color of the w* allele (Morgan et al., 1925) carried on the dsh1 chromosome (see Figure 1C vs. O,P). Overall, of the twenty tested Wg/PCP alleles, only three, dsh6, vang METH, and pk-pickel13, had no effect on GMR>Nab2 eye phenotypes.

A review of the alleles with modifying effects is consistent with a link between Nab2 and the Wg/PCP pathway. Two of three dsh alleles tested (dsh1 and dsh3) suppressed the female GMR>Nab2 phenotype
PCP alleles dominantly modify Nab2 loss of function phenotypes

The genetic interactions between multiple PCP alleles and the GMR>Nab2 overexpression transgene prompted analysis of PCP allele interactions with the Nab2 loss-of-function (LOF) allele Nab2ex3, a recessive amorph that causes defects in survival, lifespan, locomotion, thoracic bristle morphology, and neurodevelopment (Pak et al. 2011; Kelly et al. 2016). Four different PCP alleles – three suppressor alleles, dsbh1, Appbh1, and pbkpk-sple14, and one weak modifier allele, Wnt4c1 - were tested for dominant effects on two readily scored Nab2ex3 null phenotypes: reduced survival to eclosion (<5% in past studies e.g., Pak et al. 2011), and thoracic bristle kinking. The Nab2ex3 survival defect was chosen because it is rescued by neuron-specific expression of either fly Nab2 or human ZC3H14 (Kelly et al. 2016), suggesting that it is an indicator of a conserved requirement for Nab2 in neurons. The Nab2ex3 thoracic bristle defect was chosen because it suggests a link to the PCP-regulated processes of F-actin assembly and bundling (reviewed in Adler and Wallingford 2017).

To analyze adult viability, the percent of pupae eclosing into viable adults was calculated among 100 non-Tubby female larvae collected from an intercross of Nab2ex3/TM6B;P[GAL4] adults. Consistent with our prior work (e.g., Pak et al. 2011), approximately 3–5% of Nab2ex3 zygotic null females eclose as viable adults, while Nab2ex3 control females (aka Nab2ex3; a precise excision of the EP3716 P-element that was used to generate Nab2ex3) display essentially full viability (~94% eclosion) (Figure 4A-B). The X-linked dsbh1 and Appbh1 alleles respectively rescue Nab2ex3 female survival to ~30% and ~25% when inherited through FM7a-balanced heterozygous mothers. Heterozygosity for either the pkpk-sple14 or Wnt4c1 alleles has no significant effect on Nab2ex3 female survival. These genetic data demonstrate that two PCP alleles that modify GMR>Nab2, dsbh1 and Appbh1, also dominantly rescue the partial lethality of Nab2ex3 females. This viability rescue in dsbh1;Nab2ex3 surviving adult females is also associated with rescue of thoracic bristle kinking compared to Nab2ex3 null females (~45% vs. ~90%; calculated as fraction of individuals showing at least one kinked humeral or scutal bristle) (Figure 5A-B).

The ability of PCP alleles to modify phenotypes associated with Nab2 gain (GMR>Nab2) and loss (Nab2ex3) prompted an assessment of whether Nab2 loss is sufficient to produce PCP-like defects in sensitive tissues e.g., altering proximal-distal wing hair orientation (Yang and Mlodzik 2015). To test this hypothesis, hairs in a fixed

**Figure 3** Effect of Wg/PCP alleles on two-dimensional size of GMR>Nab2 eyes. Quantitation of 2D eye size in adult (A) males or (B) females carrying the indicated alleles. Pixel number was determined for five eyes per test genotype using Photoshop and normalized to five GMR-Gal4 control eyes. Asterisks denote modification with P<0.0001. Other p values are noted. *n.s.* = not significant. Errors bars represent SEM.

as heterozygotes. The dsh1 amorph carries an internal deletion that causes a frameshift after codon 94 (Yanagawa et al. 1995), and the dsh1 hypomorph selectively blocks PCP signaling due to an amino acid substitution in the DEP domain (Dishevelled, E-gl-10, Pleckstrin) (Axelrod et al. 1998; Boutros et al. 1998; Penton et al. 2002). GMR>Nab2 male eyes are mildly enhanced by dsbh1/Y hemizygosity, perhaps due to a requirement for residual wildtype Dsh function to modify Nab2 phenotypes or to a sex-specific interaction between dsh1 and GMR>Nab2. Both fj alleles tested acted as dominant suppressors, and the fj33 amorph is a stronger suppressor than the fj hypomorph. stan2j is also a suppressor, although a second allele, stan59, has mild enhancing and suppressing effects on GMR>Nab2 phenotype. Among pk alleles tested, pk30 (also Df(2R)pk) and pkpk-sple13 mildly suppress, while a third allele, pkpk-sple14, has no obvious effect on GMR>Nab2 eyes.

Alleses of PCP accessory factors also strongly modify the GMR>Nab2 phenotype. Two alleles of the X-linked Dsh-interactor DAAM act as strong dominant suppressors of GMR>Nab2 eye phenotypes; one of these suppressors, DAAM1, contains a D360V substitution in the formin-homology 3 (FH3) domain (Haelterman et al. 2014) that is predicted to disrupt interactions with Rac GTPases during axonal outgrowth (Gombos et al. 2015). The suppressor allele Appbh1 (B-Amyloid p rotein ß-recursor-like) is an amorph that deletes the central coding region of a transmembrane protein that acts as a PCP-accessory factor in neurons and has established roles in retinal axon pathfinding and synapse formation (Luo et al. 1992; Ashley et al. 2005; Mora et al. 2013; Soldano et al. 2013). The amorphic allele Wnt4c1 (also Df(2R)Wnt4c1), which encodes a Fz ligand with roles in canonical and non-canonical Wg/Wnt signaling (Cohen et al. 2002), is a strong suppressor, while the weak hypomorph Wnt4j1 has a much weaker effect. Viable P-element insertions in the puc (pucM1060j) and tap (tapM1054j) loci, which respectively encode a component of the PCP-regulated INK pathway (Boutros et al. 1998; Martin-Blanco et al. 1998) and a regulator of Dsh levels in brain mushroom body (MB) neurons (Yuan et al. 2016), also act as GMR>Nab2 suppressors. Finally, a mutant allele of the endocytic factor Rabenosyn-5 (Rbsn5), which regulates polarized distribution of PCP proteins in wing cells (Mottola et al. 2010) has a mild suppressive effect on the size of GMR>Nab2 eyes. In sum, these data reveal a series of genetic interactions which are consistent with a model in which reducing PCP activity partially mitigates the effect of Nab2 overexpression in the developing eye.
cell polarity (PCP) pathway. The Nab2-PCP interactions detected by this approach are consistent with a role for Nab2 in restraining PCP signaling in Drosophila tissues, perhaps by inhibiting expression of a PCP component. Given that PCP signaling and Nab2/ZC3H14 are each linked to axon guidance (Sato et al. 2006; Srahna et al. 2006; Inaki et al. 2007; Pak et al. 2011; Hollis and Zou 2012; Ng 2012; Vandewalle et al. 2013; Ackley 2014; Kelly et al. 2014; Gombos et al. 2015; Aviès and Stoeckli 2016; Kelly et al. 2016; Biemkowski et al. 2017; Rha et al. 2017; Collins et al. 2019), these data argue that neurodevelopmental defects in flies, mice, and humans lacking Nab2/ZC3H14 may arise in part due to altered PCP signaling.

Alleles that impair Wg/PCP signaling, and in some cases specifically perturb PCP (e.g., dsh1, Appl4, DAAM1), are able to suppress phenotypes caused by either Nab2 overexpression (GMR>Nab2) or loss (Nab2ex3). This pattern differs from other Nab2 modifier alleles that have inverse effects in Nab2 gain vs. loss backgrounds e.g., dfmr1<sup>500</sup> (as in Biemkowski et al. 2017), and could be explained if Nab2 modulates levels of a PCP factor(s). Since overexpression and loss-of-function of some PCP factors, like Frizzled, produce similar disruptions to polarity (reviewed in Yang and Mlodzik 2015), gain and loss of Nab2 might also be expected to have similar effects on PCP that are suppressed by Wg/PCP alleles. Imbalanced PCP signaling resulting from Wg/PCP overexpression or loss would then be restored by reducing the genetic dose of Wg/PCP factors. The suppressive effects of Wg/PCP amorphs like dsh3 and fz<sup>10</sup> do not distinguish whether Nab2 could affect one or both arms of the Wg/PCP pathway. The suppressive effect of the dsh1 allele, which specifically impairs PCP signaling (Axelrod et al. 1998), provides evidence of a Nab2 link to the PCP pathway. If this link is strong enough, then Nab2 null flies might thus be expected to display defects in hallmark PCP-regulated processes, e.g., wing hair polarization and ommatidial rotation. Consistent with this hypothesis, we document moderate hair misorientation defects in Nab2 null adult wings, suggesting that Nab2 may regulate PCP activity in this tissue. Nab2 is expressed ubiquitously but required in neurons for viability (Kelly et al. 2014); thus, the rescue of Nab2 null viability by dsh1 also implies that a Nab2-PCP link may also occur in neurons. This idea is further supported by the genetic interaction between Nab2<sup>ex3</sup> and the Appl4 allele, which inactivates a neuron-specific PCP component (Soldano et al. 2013) and by GMR>Nab2 modification by an allele of tap, a regulator of Dsh expression in neurons (Yuan et al. 2016). Interestingly, Nab2 and PCP alleles individually alter the trajectories of mushroom body axons that project from Kenyon cells (Ng 2012; Kelly et al. 2016), which provides a cellular context for future study of a Nab2-PCP interaction.

Nab2 may modify PCP-regulated developmental processes indirectly through post-transcriptional control of proteins (or a protein) that is not core PCP components but regulates a common process (e.g., via F-actin bundling). Alternatively, Nab2 may directly regulate post-transcriptional expression of a core PCP component. In this regard, it is notable that levels of the Vang family member Vangl2 are elevated in the hippocampal proteome of Zc3H14 knockout mice (Rha et al. 2017), and that depletion of ZC3H14 from cultured N2A cells leads to intron retention in the PSD95 mRNA (Morris and Corbett 2018), which encodes a postsynaptic guanylate kinase required for activity-dependent synaptic plasticity (reviewed in Xu 2011). Intriguingly, the fly PSD95 homolog Discs Large-1 (Dlg1) controls canonical Wg signaling in wing tissue by stabilizing Dsh protein (Liu et al. 2016). In light of these observations in mammalian systems, the corresponding Drosophila mRNAs vang and dlig1

**DISCUSSION**

Here we have used three phenotypes caused by altered dosage of the *Drosophila* poly(A) RNA-binding protein Nab2, (1) eye roughness caused by overexpression of Nab2 (GMR>Nab2), (2) a neuronal requirement for Nab2 in adult survival and (3) thoracic bristle kinking in Nab2 null animals, to screen for genetic interactions between Nab2 and genes encoding components of the Wg/planar
represent candidate Nab2 targets that may contribute to Nab2-PCP genetic interactions documented in this study.

In summary, we have carried out a candidate-based genetic screen that has identified a series of dominant genetic interactions between Wg/PCP alleles and both a Nab2 overexpression transgene and null allele. Based on data cited above, these genetic data support a molecular model in which Nab2 may regulate expression of a PCP protein or proteins in different cell types, including neurons and wing hair cells. This hypothesis is significant given that very few Nab2-target mRNAs are known and the Nab2 human ortholog ZC3H14 is lost in an inherited form of recessive intellectual disability (Pak et al. 2011). Thus, the work presented here is a key first step in exploring conserved functional and molecular links between Nab2/ZC3H14 and PCP activity that may contribute to a conserved requirement for Nab2/ZC3H14 in neurodevelopment.

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