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Domain requirements for the Dock adapter protein in growth-cone signaling

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ABSTRACT **Tyrosine phosphorylation has been implicated in growth-cone guidance through genetic, biochemical, and pharmacological studies. Adapter proteins containing src homology 2 (SH2) domains and src homology 3 (SH3) domains provide a means of linking guidance signaling through phosphotyrosine to downstream effectors regulating growth-cone motility. The *Drosophila* adapter, Dreadlocks (Dock), the homolog of mammalian Nck containing three N-terminal SH3 domains and a single SH2 domain, is highly specialized for growth-cone guidance. In this paper, we demonstrate that Dock can couple signals in either an SH2-dependent or an SH2-independent fashion in photoreceptor (R cell) growth cones, and that Dock displays different domain requirements in different neurons.**

The establishment of precise neuronal connections during development requires the proper navigation and targeting of growing axons. It is the growth cone, a sensorimotor structure at the leading edge of the axon that plays a key role in translating guidance signals into changes in motility. These signals can be either attractive or repulsive and can influence growth-cone behavior over both short and long range. Numerous guidance signals have been identified, including cell adhesion molecules and other cell surface proteins, extracellular matrix components, and diffusible chemoattractants and chemorepellents. Receptors on the surface of the growth cone must detect signals, and the signal transduction machinery, in turn, must coordinate the activity of cytoskeletal regulators that modulate growth-cone movement.

A number of guidance receptors have been identified, including cell adhesion molecules, receptor tyrosine kinases, receptor tyrosine phosphatases, as well as receptors for extracellular matrix components and diffusible guidance cues (1–3). Although regulation of the actin and microtubule dynamics underlie the changes in growth-cone motility, little is known about the mechanisms by which these receptors regulate changes in cytoskeletal structure. Pharmacological studies have demonstrated the importance of heterotrimeric G-proteins (e.g., refs. 4 and 5), tyrosine phosphorylation (e.g., refs. 6–8), and calcium (e.g., refs. 9 and 10) in growth-cone signaling. Genetic and biochemical studies have implicated several cytoplasmic proteins in transducing extracellular signals into changes in growth-cone behavior. For instance, disruption of the gene encoding GAP43 in mice specifically affects growth-cone steering at the optic chiasm (11), whereas CRMP, the vertebrate homolog of *Caenorhabditis elegans* guidance molecule UNC-33, is required for collapsin-induced growth-cone collapse (12). In *Drosophila*, disruption of Ca^{2+}/calmodulin signaling affects both growth-cone extension and guidance (13), and ectopic expression studies suggest that Rho-family GTPases regulate growth-cone motility (14). We recently reported that a growth-cone localized adapter protein, Dreadlocks or Dock, is essential for photoreceptor (R cell) guidance and target recognition in the *Drosophila* visual system (15) and proposed that it provides a link between signaling through tyrosine phosphorylation and changes in the structure of the growth-cone cytoskeleton.

The R cells of the compound eye elaborate a precise pattern of connections in the optic ganglia of the brain. The eye has a crystal-like structure comprising ≈800 facets, called ommatidia, each containing eight identified neurons, R1–R8. R cells project into the optic ganglia during the third and final stage of larval development. The R cell axons from each ommatidium form a single fascicle, which projects in a topographic fashion into the optic ganglia. R1–6 growth cones terminate within the lamina, whereas R7 and R8 growth cones continue to extend into a deeper region of the optic ganglia, the medulla. Dock protein is highly localized to growth cones (15). In *dock* mutants, R cell axons exhibit pathfinding errors leading to defects in retinotopy, and many R1–6 axons show targeting defects, extending past their normal target sites and projecting into the medulla. In addition, R8 axons show defects in growth-cone morphology and topographic map formation in the medulla. The projections of R7 neurons have not been analyzed. Although Dock is required for growth-cone guidance and targeting, it is not required for growth-cone extension. In addition to its role in R cells, Dock is required for growth-cone guidance by motoneurons in the embryo (C. Desai, S.L.Z., and K. Zinn, unpublished data), and for the establishment of normal fiber patterns in the neuropil of inner optic ganglia of the visual system (see *Results*). Together, these data argue that Dock plays an important role in growth-cone steering.

Dock belongs to a growing family of adapters consisting exclusively of src homology 2 (SH2) and SH3 domains, including Grb2/Drk/Sem-5 (16–20), Nck (21), Crk (22, 23), and SLAP (24). Dock protein contains three SH3 domains and a single SH2 domain and is highly related to mammalian Nck. SH2 and SH3 domains are found in a wide variety of intracellular signaling proteins and mediate specific protein–protein interactions (25). SH2 domains bind to specific phosphotyrosine-containing peptide motifs on proteins, whereas SH3 domains bind to consensus PXXP sites. Grb2/Drk/Sem-5 has been shown to play an essential role in cell growth and differentiation by linking upstream tyrosine kinase signaling events via SH2 binding to specific phosphotyrosines on activated receptors and SH3 binding to distinct polyproline regions in the downstream effector Sos, leading to the activation of Ras (26, 27). Although other adapters have been shown to bind to various proteins *in vitro* their roles *in vivo* are not known. By

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Abbreviations: SH, src homology; Dock, Dreadlocks; UAS, upstream activation sequence.

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analogy with Grb2, we proposed that Dock mediates growth-cone guidance by transmitting upstream tyrosine phosphorylation signals through its SH2 domain to changes in the actin-based cytoskeleton via its SH3 domains (15). To assess this model, we have tested altered forms of Dock for rescue of *dock* mutants. These studies revealed a single essential SH3 domain and marked flexibility in adapter function, including dispensability of the SH2 domain in R cells caused by its functional redundancy with specific SH3 domains, and differential domain requirements in different neurons.

MATERIALS AND METHODS

Plasmid Construction. *dock* and human *nck* cDNAs were subcloned into the p*UAS*T plasmid, which contains the *GAL4*-responsive upstream activation sequence (*UAS*) (28). Site-directed point mutations were generated by using the Chameleon Double-stranded, Site-Directed Mutagenesis Kit from Stratagene. The ΔSH2 construct was made by deletion of amino acids from glutamic acid 283 to lysine 406 by using the *Nco*I site in the *dock* cDNA.

P-Element Transformation and Rescue Experiments. Germ-line transformation of *Drosophila* was performed by using standard methodology (29). Multiple independent lines carrying *UAS*-transgenes were obtained and crossed into a $dock^{P1}$/*In(2LR)Gla Bc Elp* background. To assess the rescue activity of *dock* mutant transgenes, lines carrying *UAS-dock* transgenes in $dock^{P1}$/*In(2LR)Gla Bc Elp* background were crossed with *yw*;$dock^{P1}$/*In(2LR)Gla Bc Elp*; *elav-GAL4*/*elav-GAL4* line. Eye-brain complexes from non-*Bc* larvae were double-stained by using mAb 24B10 and anti-Dock antibodies.

Western Blotting. Western blot analysis was performed as described (30). Anti-Dock and anti-human Nck antibodies were used at a dilution of 1:2,000.

Immunohistochemistry. R cell projection patterns in third instar larvae were visualized by using mAb 24B10 and horseradish peroxidase-coupled goat anti-mouse secondary antibody as described (31). Eye discs from third instar larvae were stained by using rabbit anti-Dock polyclonal antibody (1:2,000 dilution) and horseradish peroxidase-coupled goat anti-rabbit secondary antibody as described (31). Adult heads were sectioned and stained as described (32).

RESULTS

Expression of Mutant Transgenes in *dock* Mutant Larvae. Neuron-specific expression of *UAS* wild-type *dock* driven by *elav-GAL*4 rescued the *dock* mutant phenotype in the visual system (Fig. 1). This finding established that *dock* is required in postmitotic neurons consistent with its role in guidance and that it is not required for earlier stages of development (i.e., proliferation and cell fate determination). *dock* mutant transgenes containing point mutations in SH3 and SH2 domains were tested for their ability to restore the normal pattern of R cell axonal projections in *dock* null mutants. Each SH3 domain binding pocket was disrupted by substituting lysine for a conserved tryptophan residue [SH3–1 (W48K), SH3–2 (W151K), and SH3–3 (W225K)]. This amino acid substitution has been shown previously to inhibit binding of Nck SH3 domains to polyproline-containing polypeptides *in vitro* (33). That the binding properties of mammalian Nck and Dock are likely to be very similar is supported by the finding that neuron-specific expression of human Nck rescues the *dock* mutant phenotype (Fig. 1, Table 1). SH2 function was disrupted by substituting a glutamine for an invariant arginine at position βD5 (R336Q) deep within the phosphotyrosine binding pocket (25). This mutation, placed into the SH2 domain of PI3 kinase, more efficiently inhibited binding to phosphotyrosine-containing polypeptides than the more commonly used lysine substitution (34). All mutant transgenes were expressed

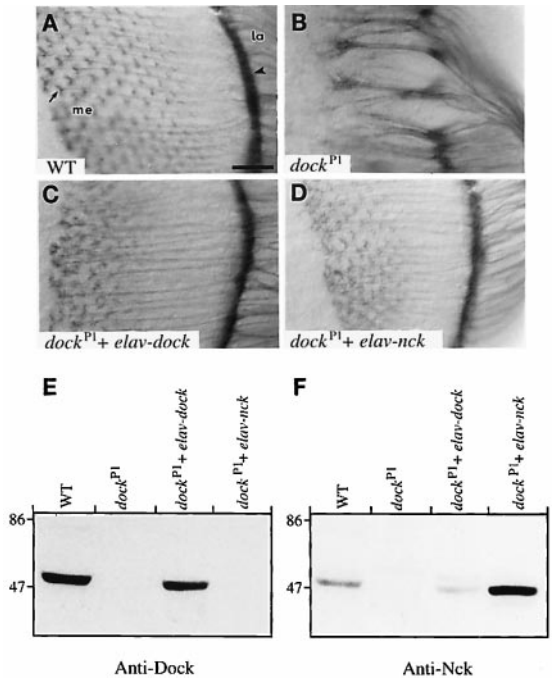

FIG. 1. Neuron-specific expression of *dock* and *nck* transgenes rescues R cell projection defects in *dock* mutants. (*A–D*) R cell projection patterns in third-instar larvae were visualized by using mAb 24B10. The eye comprises an array of cell clusters, each containing eight R cells (R1–8). In wild type (WT) (*A*), R1–6 axons terminate in the lamina (la), whereas R7 and R8 axons pass through the lamina and project into the medulla (me). The expanded growth cones of R1–6 form a plexus in the lamina (arrowhead). The R8 growth cones (arrow) form a precise array in the medulla. At this early stage, few R7 growth cones have entered the medulla. (*B*) In homozygous $dock^{P1}$ mutants, R cell projections in both the lamina and medulla are disorganized. Large axon bundles form. These are particularly visible between the lamina and medulla. Previous studies indicated that these bundles contain R1–R6 neurons that fail to terminate in the lamina (15). R cell axons in the medulla do not elaborate expanded growth cones. Neuron-specific expression of *dock* (*C*) and human *nck* (*D*) rescued the defects in homozygous $dock^{P1}$ larvae. Genotypes: (*A*) wild type; (*B*) $dock^{P1}$/$dock^{P1}$; *elav-GAL4*/+; (*C*) $dock^{P1}$/$dock^{P1}$; *elav-GAL4*/*UAS-dock*; (*D*) $dock^{P1}$/$dock^{P1}$; *elav-GAL4*/*UAS-nck*. (*E*) Western blot of larval extracts (25 μg total protein/lane) probed with anti-Dock antibody. *elav-dock* and *elav-nck* refer to *UAS-dock* and *UAS-nck* driven by *elav-GAL4*. No Dock protein was detected in homozygous $dock^{P1}$ or $dock^{P1}$ larvae carrying an *nck* transgene. (*F*) The blot in *E* was stripped and reprobed with anti-Nck antibody. Nck protein was detected in $dock^{P1}$ larvae carrying an *nck* transgene. Note anti-Nck antibody also shows weak crossreactivity with Dock protein. (Bar = 20 μm.)

in postmitotic neurons by using the *UAS-Gal4* system with *elav-GAL4* as the driver. Multiple transgenic lines for each mutant construct were established. The expression levels of the transgenes were assessed in homozygous mutant larvae by using Western blotting (Figs. 1 and 2). Mutant proteins were expressed at levels comparable to wild-type Dock. Immunohistological studies also demonstrated that the mutant proteins were made in R cells (see Figs. 2 and 4), and transported to the growth cone (not shown). R cell projections to their targets in the optic ganglia were assessed in whole-mount preparations stained with mAb 24B10.

The SH2 Domain Is Not Required for R Cell Connectivity. The requirement of each domain was assessed by testing the ability of singly mutant transgenes to rescue the *dock* phenotype. Of the three SH3 domains, only SH3–2 was essential for the restoration of normal connectivity. Not only did the SH3–1 and SH3–3 mutants fully rescue the *dock* mutant defects (Fig. 2 *A* and *D*), even doubly mutant transgenes in which both

Table 1. Rescue activity of *dock* mutant transgenes

Transgenes	R-cell projection			Inner optic lobe		
	Rescue activity[a]	N[b]	Lines[c]	Rescue activity[d]	N[b]	Lines
None	−	0/44		−	0/9	
Nck (SH3-SH3-SH3-SH2)	+++	19/26	4[e]	+++	9/9	2[g]
Dock	++++	15/15	2	++++	15/15	2
W48K	++++	24/24	4	++++	15/15	4
W151K	−	0/18	3	−	0/11	2
W225K	++++	13/13	3	++++	18/18	3
R336Q	++++	20/20	4	−	0/14[h]	2
ΔSH2	+++	20/20	3	−	0/12	2
	+++	24/24	4	+++	12/15	3
	−	0/13	3	−	0/8[i]	2
	+/−	9/26	3[f]	−	0/10	2
	+/−	19/28	4	−	0/10	3
	−	0/28	5	−	0/11	3
	−	0/32	2	ND	ND	ND[j]
□-⊠-□-□ + ⊠-□-⊠-⊠	−	0/11		ND	ND	ND

ND, not determined.

[a]−, Many gaps were present in R1–6 growth cone array in the lamina. R-cell terminal field in lamina and medulla was disorganized. Large fiber bundles were observed in both lamina and medulla. R8 axons did not elaborate expanded growth cones in the medulla. +/−, A few axons in medulla were able to form expanded growth cones. The number of large fiber bundles seen in *dock* mutants decreased. +++, Normal R1–6 growth cone array in lamina. Most R8 growth cones were expanded. Minor disruption in the R8 terminal field in the medulla was seen. Occasionally abnormal bundles were seen. ++++, Complete rescue.

[b]Number of rescued or partially rescued individuals/number of individuals examined.

[c]Number of independent lines examined.

[d]−, Severely disrupted fiber pattern in inner medulla, lobula, and lobula plate. +++, Inner medulla, lobula, and lobula plate were relatively normal, but minor defects were observed. ++++, Complete rescue.

[e]Of six independent lines examined, two lines showed no rescue activity. The expression level of Nck protein in these two lines is much lower than that in lines with rescue activity.

[f]Of three independent lines examined, only one line weakly rescued the defects. The other two did not.

[g]Among two independent lines examined, only one line rescued the defects.

[h]Two individuals showed some weak rescue activity.

[i]One individual that was completely wild type and likely represents a contaminant was not included.

[j]No *dock*^{P1} homozygous adults carrying this transgene were obtained.

SH3–1 and SH3–3 were inactivated efficiently rescued the R cell guidance defects (see Fig. 4*F*). Surprisingly, the *dock* transgene containing the R336Q mutation in the SH2 domain fully rescued the R cell innervation pattern in *dock* mutants (Fig. 2*E*). To assess whether rescue reflected residual binding of the mutated SH2 domain to its cognate ligand, a *dock* transgene in which the SH2 domain was deleted in its entirety was introduced into *dock* mutants. This construct largely, but not completely, rescued the mutant phenotype (Fig. 2*F*). These findings are in marked contrast to the essential requirement of the SH2 domain of the *C. elegans* and *Drosophila* homologs of the Grb2 adapter, Drk and Sem-5, respectively (16, 19). That the SH2 point-mutant and deletion transgenes did not rescue lethality (not shown) suggested that the SH2 domain was required in other neurons.

The SH2 Domain Is Required in Inner Optic Ganglion Neurons. To determine whether these domain requirements apply to all neurons, we assessed the ability of mutant transgenes to rescue neuronal connectivity defects in the inner optic ganglia of *dock* mutants (Fig. 3*B*). This phenotype can be rescued in its entirety by expression of wild-type *dock* specifically in postmitotic neurons (Fig. 3*C*). Consistent with a direct role in guidance of these neurons, Dock protein is highly enriched in their growth cones and axons (15). However, because of the lack of cell-type specific markers in and the cellular complexity of the inner optic ganglion, we cannot rule out a role for Dock in other aspects of postmitotic neuronal differentiation. As seen for the R cells, the SH3–2 domain is essential for the formation of fiber patterns in the inner optic ganglia (Fig. 3*D*) and transgenes singly or doubly mutant for SH3–1 and SH3–3 rescue the phenotype (Fig. 3*F*, Table 1). In contrast to R cells, however, the SH2 domain is essential for the fiber patterns in the inner optic ganglia (Fig. 3*E*). Hence, Dock couples guidance and/or differentiation signals in different ways in different neurons.

SH3–1 and SH3–3 Domains Are Redundant with the SH2 Domain. The analysis of single-domain mutants established that SH3–1, SH3–3, and SH2 domains were not essential for Dock function in R cells. Hence, either the SH3–2 domain was sufficient for Dock function in these neurons or the loss of the SH2 domain was compensated by the SH3–1 and SH3–3 domains. To assess this possibility, a set of doubly and triply mutant forms of *dock* were tested for phenotypic rescue. Double mutants containing the SH2 point mutation in combination with mutations in either SH3–1 or SH3–3 showed only weak rescue (Fig. 4 *C* and *D*) in contrast to the near-complete rescue of the R cell connection defects observed with the singly mutant transgenes (Fig. 2, Table 1). A construct triply mutant for SH3–1, SH3–3, and SH2 did not rescue (Fig. 4*E*), and R cell projections in larvae carrying this construct were indistinguishable from *dock* mutants (Fig. 4*B*), or *dock* mutants carrying a rescue construct in which the SH3–2 domain was inactivated (Fig. 2*C*). These data demonstrate that the SH3–1 and SH3–3 domains are functionally redundant with the SH2 domain in R cells; that is, Dock function in these neurons requires either functional SH3–1 and SH3–3 domains or a functional SH2 domain.

Dock Domains Are Required in Cis. The data in the preceding sections establish that multiple, but redundant, domains are essential for Dock function. In the inner optic ganglia, neurons require both the SH3–2 and the SH2 domains. In contrast, Dock can function in R cells with the SH3–2 and SH2 domains or with all three SH3 domains in the absence of the SH2 domain. Dock may function as an adapter by binding

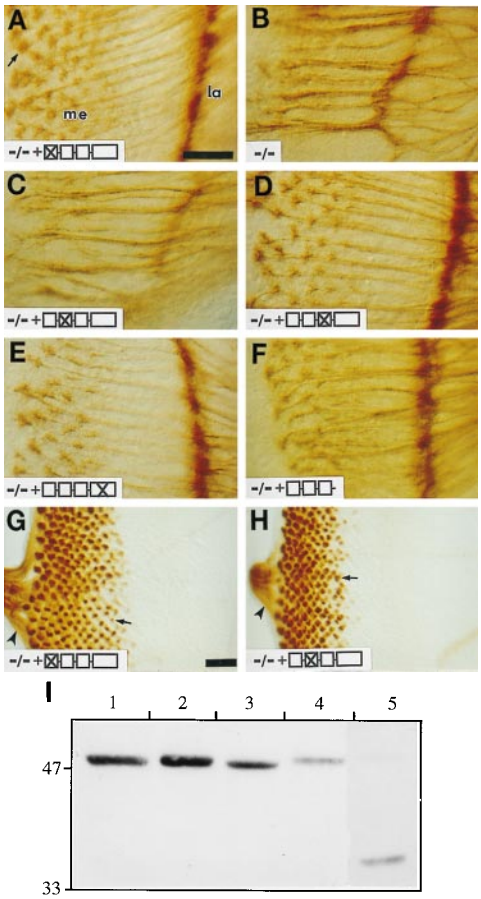

FIG. 2. Rescue activity of *dock* mutant transgenes. (*A*, *D*, and *E*) R cell projection defects in homozygous $dock^{P1}$ larvae (−/−) were rescued by the following mutant transgenes: SH3–1 (W48K) (*A*), SH3–3 (W225K) (*D*), and SH2 (R336Q) (*E*). Arrow in *A* indicates a R8 growth cone. (*C*) No rescue activity was seen with the SH3–2 (W151K) mutant transgene. (*F*) R cell projection defects were largely rescued by a transgene in which the SH2 domain was deleted. (*G* and *H*) Eye discs from third-instar larvae were stained by using anti-Dock antibody. Mutant proteins expressed from transgenes in a $dock^{P1}$ mutant background were detected in differentiated R cell bodies (arrows) and their axons (arrowheads). The expression pattern of SH3–2 (W151K) mutant protein (*H*) is indistinguishable from that of SH3–1 (W48K) mutant protein (*G*). The preparation in *G* is slightly older than that in *H*. This accounts for the increased number of R cells stained in *G*. Icons depict domain structure of Dock encoded by transgenes. (*Left to right*) Domains are SH3–1, SH3–2, SH3–3, and SH2. "X" indicates domains containing point mutation. Genotypes: Larvae were all homozygous for $dock^{P1}$ on the second chromosome and carried one copy of *elav-GAL4* on the third chromosome. In *B*, larvae carrying *elav-GAL4* but no *UAS* rescue construct were indistinguishable from $dock^{P1}/dock^{P1}$. The remaining panels contain preparations from larvae carrying the following transgenes on the X chromosome (*D–F*) and third chromosome (*A*, *C*, *G*, and *H*): (*A*) *UAS*-SH3–1 (W48K); (*C*) *UAS*-SH3–2 (W151K); (*D*) *UAS*-SH3–3 (W225K); (*E*) *UAS*-SH2 (R336Q); (*F*) *UAS*-ΔSH2; (*G*) *UAS*-SH3–1 (W48K); and (*H*) *UAS*-SH3–2 (W151K). (*I*) Western blot of third-instar larval extracts (25 μg of total protein/lane). Mutant proteins of the expected size (point-mutants: ≈47 kDa; SH2-deletion mutant: ≈37 kDa) were detected. Genotype of larvae as described above. Lanes: 1, SH3–1 (W48K); 2, SH3–2 (W151K); 3, SH3–3 (W225K); 4, SH2 (R336Q); 5, ΔSH2. Although the SH2 mutants are expressed at a lower level they still rescue the mutant phenotype. [Bars = 20 μm (*A–F*) and 30 μm (*G* and *H*).]

signaling molecules together, or alternatively, each domain in Dock may function independently. To distinguish between these possibilities, the ability of mutant transgenes to complement each other was assessed. Two mutant transgenes, one carrying the SH3–2 mutation and the other carrying mutations

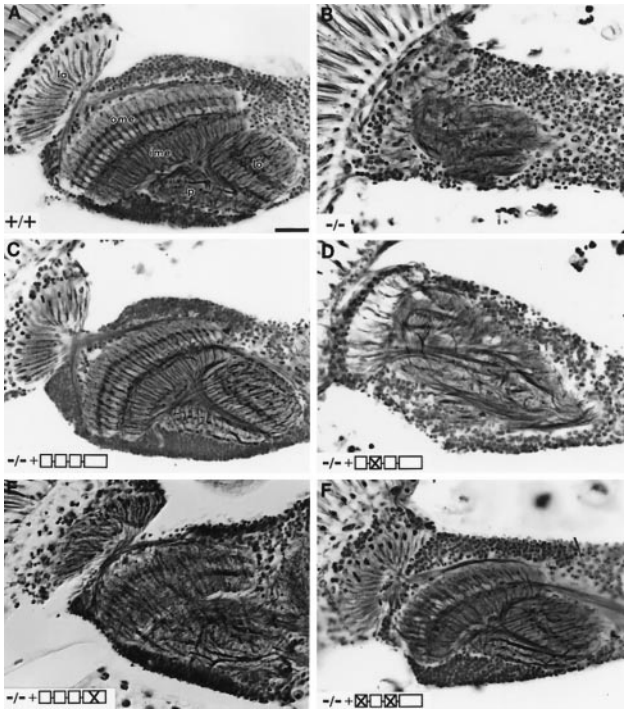

FIG. 3. Rescue of *dock* axon fiber pattern defects in the inner optic ganglia by mutant transgenes. (*A*) Horizontal section of a wild-type adult head stained with silver reveals the four highly ordered neuropil regions in the optic lobe, including the lamina (la), medulla (ome+ime), lobula (lo), and lobula plate (lp). R cell axon innervation induces the development of the outer optic lobe [i.e., lamina and outer medulla (ome)], but not the inner optic lobe [i.e., inner medulla (ime), lobula, and lobula plate] (51, 52). (*B*) In $dock^{P1}$ mutants (−/−), neuropil structures were completely disrupted. Wild-type *dock* (*C*) or SH3–1/SH3–3 doubly mutant (*F*) transgenes rescued the optic lobe defects. (*D*) SH3–2 mutant transgene exhibits no rescue activity. (*E*) The SH2 mutant largely rescued the outer optic lobe, but not the inner optic lobe, defects. Genotypes: (*A*) Wild type; (*B*) $dock^{P1}$. (*C–F*) Adult heads were from $dock^{P1}/dock^{P1}$ flies carrying one copy of *elav-GAL4* transgene and the following *UAS*-transgene rescue constructs: (*C*) wild-type *dock*; (*D*) SH3–2 (W151K); (*E*) SH2 (R336Q); and (*F*) SH3–1 (W48K)/SH3–3 (W225K). Icons depict domain structure of Dock encoded by transgenes. (*Left to right*) Domains are SH3–1, SH3–2, SH3–3, and SH2. "X" indicates domain containing mutation. (Bar = 20 μm.)

in SH3–1, SH3–3, and SH2 were introduced together into a homozygous *dock* mutant background. No rescue activity was detected (Table 1). These mutant transgenes do not function in a dominant negative fashion either alone or in combination. Hence, Dock requires multiple domains acting in cis. This is consistent with the proposal that Dock functions as an adapter to link signaling molecules together in growth cones.

DISCUSSION

Studies in vertebrate and invertebrate systems have led to the view that axon guidance proceeds in a stepwise fashion in which a sequence of spatial cues directs the growth cone. At specific choice points, growth cones must integrate different signals and convert them into changes in the structure of the actin- and microtubule-based cytoskeleton, which then ultimately leads to changes in growth-cone motility. Analysis of growth-cone behavior at these decision points reveals that interaction of single filopodium can reorient the growth cone and subsequent axonal elongation (35), underscoring the importance of converting localized signals into unique spatial restructuring of the growth cone. The mechanisms by which

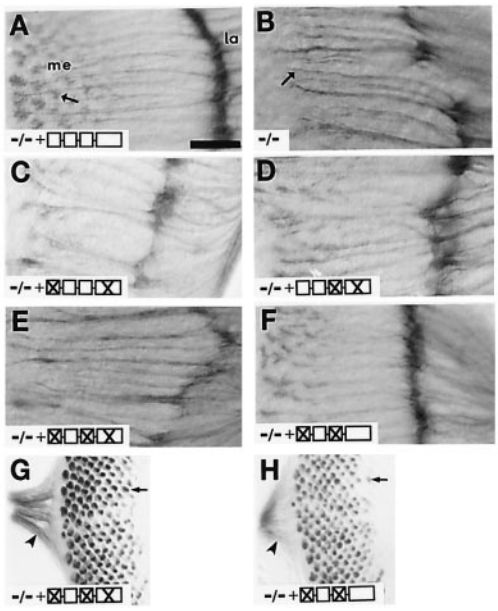

FIG. 4. Rescue activity of transgenes carrying double and triple mutations in *dock*. (*A*) Rescue of R cell projection defects by wild-type *dock* transgene. (*B*) R cell projection pattern in homozygous $dock^{P1}$ larvae (−/−). Arrows indicate R cell growth cones. Note the blunt-ended growth cones in *dock* mutants (*B*) compared with the expanded growth cones in fully rescued preparations (*A*). SH3–1/SH2 double mutant (*C*) or SH3–3/SH2 double mutant (*D*) transgenes only weakly rescued R cell projection defects. Although a few axons in the medulla formed enlarged growth cones, the R cell terminal field in the lamina and medulla remained highly disorganized. (*E*) The R cell projection pattern in homozygous $dock^{P1}$ larvae (−/−) carrying a *dock* triply mutant transgene SH3–1/SH3–2/SH3–3 was indistinguishable from that in homozygous $dock^{P1}$ larvae (*B*). (*F*) R cell projection defects were largely rescued by the doubly mutant transgene SH3–1/SH3–3. Expression of triply mutant protein SH3–1/SH3–3/SH2 (*G*) and the doubly mutant protein SH3–1/SH3–3 (*H*) in differentiated R cell cell bodies (arrows) and their axons (arrowheads). Icons depict domain structure of Dock encoded by transgenes. (*Left to right*) Domains are SH3–1, SH3–2, SH3–3, and SH2. "X" indicates domain containing mutation. Genotypes (without transgenes) were as described in the legend to Fig. 1. Transgenes were: (*A*) wild-type *dock*; (*B*) no transgene; (*C*) W48K/R336Q; (*D*) W225K/R336Q; (*E*) W48K/W225K/R336Q; (*F*) W48K/W225K; (*G*) W48K/W225K/R336Q; and (*H*) W48K/W225K. As with singly mutant transgenes shown in Fig. 2, double and triple mutant transgenes were expressed at comparable levels to the wild-type transgene (data not shown). [Bars = 20 μm (*A–F*) and 30 μm (*G* and *H*).]

receptors link signals to the cytoskeletal changes lies at the core of growth-cone guidance.

In a genetic screen for guidance mutants, we identified an SH2/SH3 adapter protein, Dock, the fly homolog of the mammalian proto-oncogene product Nck (15). In contrast to the well-characterized adapter, Grb2, which functions downstream from receptors regulating differentiation, patterning, and proliferation (16–20), Dock is highly specific for growth-cone function. Not only are R cell growth cones defective in guidance and targeting, growth-cone morphology differs markedly from wild type, suggesting a role for Dock in regulating growth-cone cytoskeletal structure. The outgrowth of *dock* mutant axons is not defective; indeed, in many cases, *dock* mutant axons project past their targets, supporting a model in which Dock is required to translate guidance information into changes in growth-cone motility.

To gain insights into the mechanisms by which Dock functions in the growth cone, and to obtain starting points for biochemical studies, we assessed the function of each domain of Dock through mutational analysis. These studies place specific constraints on biochemical models for Dock function and revealed surprising redundancy and functional diversity not observed in Grb2, the only other SH2/SH3 adapter protein for which a biological function has been established (16, 19, 20). We demonstrated that in R cells, different domains of Dock can be divided into two classes that function in cis: (*i*) the SH3–2 domain; and (*ii*) the remaining three domains, the SH3–1 and SH3–3 domains, and the SH2 domain. Hence, it is likely that Dock links proteins together through these two classes of domains, thereby controlling growth-cone motility. Furthermore, these studies establish that proteins comprised solely of SH3 domains can function as adapters.

A particularly surprising finding in these studies was the difference in the SH2 domain requirement between R cells and neurons in the optic lobes. Whereas the SH2 domain is largely dispensable in the R cell growth cone, it is essential for the formation of the highly ordered structure of the inner optic ganglion neuropil; its function in these neurons cannot be compensated by SH3–1 and SH3–3. These data argue that Dock participates in the formation of different signaling complexes in different neurons. Although these signaling complexes are specific for connectivity in R cells, Dock-dependent complexes in other less well-studied neurons also may play roles in other aspects of neuronal differentiation.

Redundancy between the SH2 and SH3–1/SH3–3 may reflect the existence of a single interacting protein containing three binding sites or assembly of Dock into a multicomponent complex within which Dock contacts different proteins. In this context, it is interesting to note that several proteins have been reported to contain multiple binding sites for both SH2- and SH3-containing proteins. One of these, $p62^{dok}$ (36, 37), recently has been discovered in a complex with mammalian Nck in NG108 cells (38), which forms in response to activation of the Eph guidance receptor, Nuk. In principle, Dock could link signals from guidance cue receptors through one class of sites and to downstream effectors regulating the cytoskeleton through the other. Alternatively, signals may lead to the activation of a cytoskeletal regulatory pathway component, which then recruits Dock into a complex coordinating restructuring of the growth cone. In the former case, Dock recruits specific cytoskeletal regulators to signaling complexes, whereas in the latter case, Dock functions directly to mediate interactions between cytoskeletal regulatory components.

Studies of mammalian Nck have suggested possible biochemical functions for Dock. That Nck can couple directly to receptor tyrosine kinases has been demonstrated in several mammalian tissue culture systems (39–41). Most interestingly, activation of the c-met receptor, a multifunctional receptor recently shown to regulate motoneuron guidance decisions in vertebrates (42), leads to recruitment of Nck into a receptor-containing complex (41). Several proteins implicated in the control of the actin-based cytoskeleton, including WASp, the product of the Wiskott-Aldrich syndrome gene, and PAK and PRK2, both members of the p21-activated protein kinase family, also bind to Nck (43–47). All three proteins also bind to Rho-family GTPases, which have been shown to regulate the structure of the actin-based cytoskeleton (48). Whereas WASp binds to SH3–3, both PRK2 and PAK bind to SH3–2. Sells *et al.* (49) have demonstrated that induction of cytoskeletal changes in cultured cells with activated PAK requires the presence of an Nck binding site and is correlated with increased Nck binding. Interestingly, Dock binds strongly through its second SH3 domain to DPAK (J. Xiao and S.L.Z., unpublished data), a *Drosophila* homolog of PAK, which is expressed in the nervous system and colocalizes to both tyrosine-phosphorylated proteins and F-actin. Like mammalian PAK, DPAK also binds to activated Rho-family GTPases Rac and Cdc42 (50). These studies raise the intriguing possibility that the physical interactions between Dock, DPAK, and Cdc42 may regulate growth-cone motility in *Drosophila*. Ge-

netic and biochemical studies are in progress to critically address this issue.

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