Brief Communication

Actin Filament-Stabilizing Protein Tropomyosin Regulates the Size of Dendritic Fields

Wenjun Li and Fen-Biao Gao

Gladstone Institute of Neurological Disease, Neuroscience Program, University of California, San Francisco, San Francisco, California 94141-9100

Dendritic arbors of different neuronal subtypes cover distinct spatial territories, known as dendritic fields, to receive specific inputs in a nervous system. How the size of dendritic fields is determined by cell-intrinsic factors during development remains primarily unknown. To address this issue, we used the *Drosophila* embryonic peripheral nervous system. In each hemisegment, six dorsal cluster dendritic arborization (DA) neurons elaborate stereotypic dendritic branching patterns underneath the epidermis. Here we report the identification of loss-of-function mutations in the tropomyosin II gene (*TmII*) that result in expanded dendritic fields of DA neurons. Mosaic analysis with a repressible cell marker demonstrated that *TmII* functions in a cell-autonomous manner to control the formation of dendritic fields. Furthermore, we show that *TmII* genetically interacted with *flamingo*, a gene encoding a membrane receptor-like molecule that affects dendritic growth. *TmII* encodes multiple isoforms of a protein that stabilizes actin filaments. Our findings suggest that tropomyosin helps control the size of dendritic fields by regulating actin cytoskeletal dynamics.

Key words: **dendritic field; *Drosophila*; size; tropomyosin; actin-binding; isoforms**

Introduction

In a nervous system, different neuronal subtypes exhibit distinct dendritic morphologies (Ramón y Cajal, 1911; Masland, 2001). The size of the dendritic fields of a specific subtype of neurons can be regulated by cell–cell interactions and by both extrinsic and intrinsic factors (for review, see Whitford et al., 2002; Gao and Bogert, 2003). For instance, the dendritic field sizes of α and β ganglion cells exhibit an inverse relationship with the density of these cells in the retina (Boycott and Wässle, 1974). In rats (Perry and Linden, 1982) and other mammals, competitive interactions between dendrites of neighboring ganglion cells play a role in defining their dendritic field sizes. Such interactions occur primarily between the same subtype of ganglion cells (Weber et al., 1998) and are likely mediated by direct dendro-dendritic contacts (Lohmann and Wong, 2001). Similar interactions are also found between dendrites of some dendritic arborization (DA) neurons in the peripheral nervous system (PNS) of *Drosophila* larvae (Gao et al., 2000; Grueber et al., 2002).

One of the genes that controls dendritic field formation is *flamingo* (Gao et al., 1999, 2000), which encodes a G-protein-coupled receptor-like molecule with a large extracellular domain containing cadherin repeats, epidermal growth factor (EGF) motifs, and laminin A globular domains (Chae et al., 1999; Usui et al., 1999). In *flamingo* mutant *Drosophila* embryos, the dorsal dendrites of DA neurons overextend toward the dorsal midline (Gao et al., 1999, 2000). Flamingo is expressed in both dendrites and axons of DA neurons and has a cell-autonomous function in postmitotic DA neurons to control dendritic growth as shown by mosaic analysis with a repressible cell marker (MARCM) (Sweeney et al., 2002). This technique allows the dendritic morphology of a single mutant neuron to be visualized in a mosaic animal (Lee et al., 1999). It remains primarily unknown what other molecules control dendritic field formation and how the signals mediated by Flamingo lead to the changes in the cytoskeleton during dendritic growth *in vivo*.

To further understand how the dendritic field sizes are specified during development, we performed a genetic screen to identify lethal mutations on the third chromosome. Our goal was to identify genes that regulate dendritic growth in a way similar to *flamingo*. Using this approach, we found that mutations in the gene *tropomyosin II* (*TmII*) increased the dendritic field sizes of DA neurons in *Drosophila* embryos. The gene encodes multiple isoforms of actin filament-stabilizing protein tropomyosin; some of them are expressed in neurons (Hanke and Storti, 1988; Tetzlaff et al., 1996; Cooper, 2002). Additional analysis using the MARCM technique demonstrated that *TmII* functions in a cell-autonomous manner to control the dendritic field sizes of DA neurons. Because of the similarities in dendritic phenotypes between *flamingo* and *TmII* mutants, we examined the genetic interaction between the two genes and found that the dendritic overextension phenotype caused by *TmII* mutations could be further enhanced by reducing the dosage of *flamingo*. The actin cytoskeleton reorganization plays an important role in neuronal morphogenesis (for review, see Luo, 2002; Gao and Bogert, 2003). Our studies demonstrate a direct role for the actin-filament-stabilizing protein tropomyosin in controlling dendritic field sizes.

Received April 9, 2003; revised May 21, 2003; accepted May 22, 2003.

This work was supported by grants from the Alfred P. Sloan Foundation, the Esther A. and Joseph Klingenstein Fund, the Sandler Family Foundation, and the McKnight Endowment Fund for Neuroscience. We thank S. Ordway and G. Howard for editorial assistance, Kathleen Anderson for manuscript preparation, and Gao laboratory members for comments.

Correspondence should be addressed to Fen-Biao Gao, Gladstone Institute of Neurological Disease, Neuroscience Program, University of California, San Francisco, San Francisco, CA 94141-9100. E-mail: fgao@gladstone.ucsf.edu.

 0270-6474/03/236171-05$15.00/0

Materials and Methods

Fly stocks and reagents. All the genetic crosses were performed at 25°C with standard food medium. Mutant fly lines with *P*-element insertions on the third chromosome were obtained from the Szeged *Drosophila* Stock Center (Szeged, Hungary). Monoclonal antibody against the N terminus of Flamingo was kindly provided by Dr. T. Uemura (Kyoto University, Kyoto, Japan). The following stocks were obtained from the Bloomington Stock Center (Indiana University, Bloomington, IN) or other laboratories: (1) *GAL4 109(2)80, UAS-GFP* (Gao et al., 1999); (2) *TM3, Sb, Krüppel-Gal4, UAS-GFP* (kindly provided by T. Kornberg, University of California, San Francisco, San Francisco, CA); (3) *GAL4 109(2)80, UAS-GFP, flamingo*^{51}*/Cyo, Krüppel-Gal4, UAS-GFP* (Gao et al., 1999); (4) *TmII*^{el4}*/TM3, Sb, Ser* (Erdélyi et al., 1995); (5) *cTmII/TM3, Sb, Ser* (Tetzlaff et al., 1996); and (6) *GAL4*^{C155}, *UAS-mCD8::GFP, hs-FLP1*, and *FRT*^{82B}, *tubP-GAL80/Cyo* (Bloomington Stock Center).

Genetic screen for mutations affecting the sizes of dendritic fields. Mutant fly lines with *P*-element insertions on the right arm of the third chromosome were crossed with *GAL4 109(2)80, UAS-GFP; +/TM3, Sb, Krüppel-Gal4, UAS-GFP.* In the F1 generation, *GAL4 109(2)80, UAS-GFP/+; P{lacZ, w*$^{+}$*}/TM3, Sb, Krüppel-Gal4, UAS-GFP* flies were selected and self-crossed. Staged embryos from this cross were collected on grape agar plates and processed as described previously (Gao et al., 1999). Briefly, embryos were collected at 25°C overnight, dechorionated with 2.5% sodium hypochlorite, and mounted in 90% glycerol in PBS. The dendritic morphology of dorsal cluster DA neurons was assessed by confocal microscopy (Radiance 2000; Bio-Rad, Hercules, CA).

Plasmid rescue. Genomic DNA sequences flanking the sites of *P*-element insertions were isolated by plasmid rescue. Genomic DNA was digested with *Eco*RI or *Hin*dIII, self-ligated, and recovered by transforming *Escherichia coli* competent cells. For each plasmid rescue experiment, three independently generated clones were sequenced to determine the genomic DNA sequences adjacent the *P*-element insertions.

Semiquantitative reverse transcription-PCR. Messenger RNA from embryos of the desired genotypes was used to generate cDNA in reverse transcription (RT) reactions, which served as the template for PCR. PCR was performed with oligonucleotide primers corresponding to exons specific for different TmII isoforms. The PCR products were collected after 22 cycles and analyzed by Southern blotting with digoxigenin-labeled DNA probes.

Quantitative analysis of ddaE neuron dendritic fields. Images of green fluorescent protein (GFP)-labeled dorsal cluster DA neurons in wild-type or *TmII* mutant embryos were obtained with a Bio-Rad confocal microscope (Radiance 2000). The dendrites of ddaE neurons were traced, scanned, and converted into Photoshop (Adobe Systems, San Jose, CA) images. For statistical analyses, one ddaE neuron per larva was analyzed, and ANOVA and Student's *t* test were used. Dendritic branch length was calculated with Photoshop software (6.0).

MARCM. Single-cell analysis of the *Drosophila* larval PNS was performed as described by Sweeney et al. (2002). Briefly, *FRT*^{82B}, *TmII*^{S130510}*/TM3, Ser* male flies were crossed with *GAL4*^{C155}, *UAS-mCD8::GFP*, *hs-FLP1/FM7*; *Pin/Cyo* virgin flies. Then, *GAL4*^{C155}, *UAS-mCD8-GFP*, *hs-FLP1/+*; *Pin/+*; *FRT*^{82B}, *TmII*^{S130510}*/+* male flies were crossed with *GAL4*^{C155}, *UAS-mCD8::GFP*, *hs-FLP1*; *+/+*; *FRT*^{82B}, *tubP-GAL80* virgin flies. Embryos from this cross were collected at 25°C for 3 hr. At 3–6 hr after egg laying (AEL), embryos were heat shocked in a 37°C water bath for 40 min to induce mitotic recombination. Vials were then kept at 25°C for 3–4 d. Third instar larvae were collected and examined for the presence of a single mCD8::GFP-labeled dorsal cluster PNS neuron, and images of dendritic morphology were obtained as described above.

Results

Identification of mutations that affect the size of dendritic fields

In the late stages of *Drosophila* embryogenesis, the dorsal dendrites of DA neurons cease to extend, whereas the lateral dendrites grow toward the adjacent segment boundaries. Therefore, the dendritic fields of DA neurons in contralateral dorsal clusters do not overlap and stop short of the dorsal midline (Gao et al., 1999, 2000) (Fig. 1*A*). To understand how the size of dendritic fields is specified during development, we performed a genetic screen to identify lethal mutations caused by *P*-element insertions on the third chromosome that altered the size of DA neuron dendritic fields. In line *S130510*, the dorsal dendrites failed to stop at the correct length, resulting in enlarged dendritic fields of dorsal cluster DA neurons (Fig. 1*A*). The dendritic over-

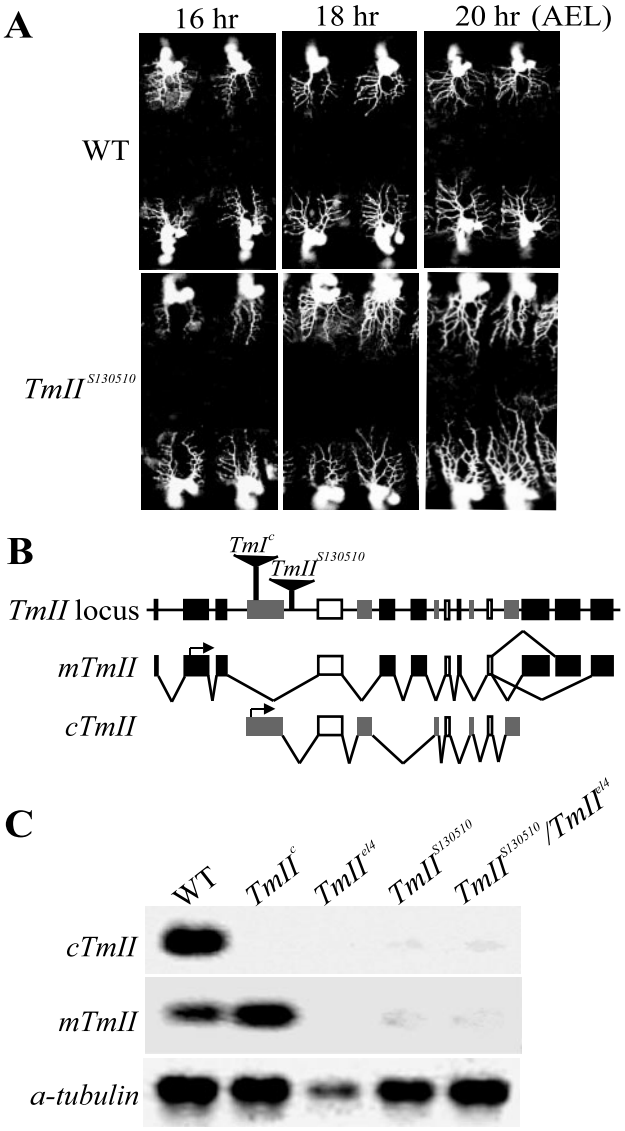

Figure 1. Identification of *TmII* mutations that alter the normal development of dorsal DA neurons in *Drosophila* embryos. *A*, The dendrites of dorsal cluster DA neurons overextend in *TmII*^{S130510} homozygous mutant embryos. These neurons were labeled with GFP whose expression was driven by *Gal4 109(2)80*. *B*, The genomic organization of *TmII*. The boxes represent exons, and the arrows indicate the start of the coding region. The black and gray boxes indicate exons unique to *mTmII* and *cTmII*, respectively; the white boxes indicate exons common to all isoforms. The *P*-element is inserted in the first exon of *cTmII* in the *TmII*^{c} line and the fourth intron in the *TmII*^{S130510} line. All three mTmII isoforms generated by alternative splicing are shown here. *C*, Semiquantitative RT-PCR analysis reveals that the expression of all *TmII* isoforms is dramatically reduced. Oligonucleotide primers corresponding to *mTmII* and *cTmII* were used for RT-PCR, and the expression level was determined by Southern analysis. α-Tubulin-specific primers served as internal controls.

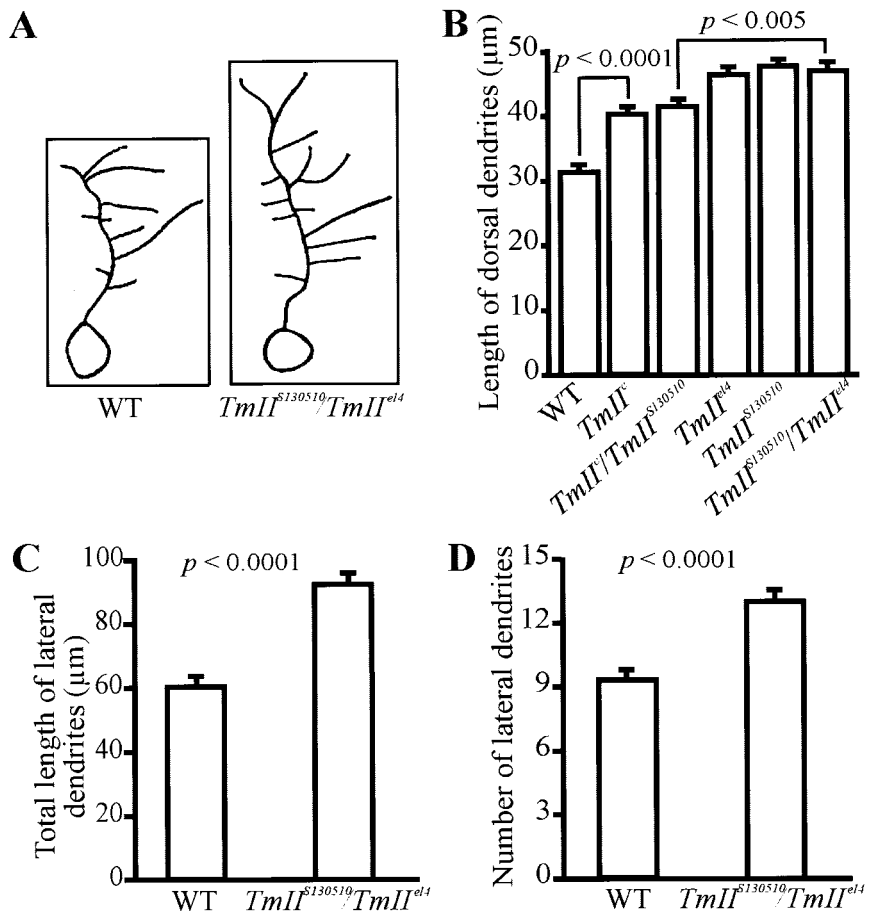

Figure 2. Mutations in *TmII* increase the size of ddaE neuron dendritic fields. All embryos were at the 20 hr AEL stage, and DA neurons were labeled by GFP under the control of *GAL4 109(2)80*. *A*, Hand tracing of ddaE neuron dendritic branching patterns in wild-type (WT) or $TmII^{S130510}/TmII^{el4}$ mutant embryos. Dendrites that extend from the cell body to the dorsal midline were defined as the dorsal dendrites; dendrites that branch out from the dorsal dendrites were defined as the lateral dendrites. *B*, The length of dorsal dendrites of ddaE neurons in A1 segment is increased in mutant embryos with combinations of different *TmII* alleles (ANOVA; $p < 0.0001$). *C*, The total length of ddaE lateral dendrites is increased in $TmII^{S130510}/TmII^{el4}$ mutant embryos (Student's *t* test; $p < 0.0001$). *D*, The number of ddaE neuron lateral dendrites is increased in $TmII^{S130510}/TmII^{el4}$ mutant embryos (Student's *t* test; $p < 0.0001$). All values are mean ± SEM.

extension phenotype was more robust at 20 hr AEL than at 16 hr AEL. In *P*-element hop-out lines, the development of dendrites from DA neurons was normal (data not shown), suggesting that the gene affected by the *P*-element insertion was responsible for the dendritic overextension phenotype.

Genomic rescue experiments indicated that two *P*-elements were present in the genome, and additional genetic analyses showed that the dendritic phenotype was associated with the *P*-element inserted between the fourth and fifth exons of *TmII* (Fig. 1*B*). There are no overlapping genes in this cytological region. Using different promoters and alternative splicing, *TmII* encodes a protein with multiple isoforms, including three muscle-specific isoforms (mTmII), one cytoplasmic isoform (cTmII) that is expressed in the nervous system and other tissues, and a few uncharacterized isoforms in *Drosophila* (Hanke and Storti, 1988; Erdélyi et al., 1995; Tetzlaff et al., 1996). The *P*-element insertion in the $TmII^{S130510}$ mutant line greatly reduced the expression of all of the known TmII isoforms (Fig. 1*C*). $TmII^{S130510}$ homozygous mutations are lethal at the late stage of embryogenesis, presumably because the absence of mTmII isoforms causes paralysis of mutant embryos.

Effects of TmII isoforms on dendritic field formation

To assess the function of different TmII isoforms in dendritic field formation, we obtained mutant lines that were allelic to $TmII^{S130510}$ (Fig. 1*B*). In the $TmII^{c}$ line (Tetzlaff et al., 1996), a *P*-element inserted in the first exon of *cTmII* abolished *cTmII* expression but did not affect the expression of muscle isoforms (Fig. 1*C*). In the $TmII^{el4}$ mutant line (Erdélyi et al., 1995), a small deletion at the *TmII* locus abolished the expression of all known TmII isoforms (Fig. 1*C*).

We measured the size of the dendritic fields of dorsal cluster DA neurons in the A1 segment of embryos at 20 hr AEL. Dendritic field size increased by 37% in the absence of cTmII isoform and by 51% in $TmII^{S130510}/TmII^{el4}$ mutant embryos. Similar phenotypes were observed in A2 and A3 abdominal segments (data not shown).

The dorsal cluster contains six DA neurons that develop distinct dendritic fields. To further characterize the dendritic phenotype caused by *TmII* mutations, we traced and quantitatively analyzed the dendrites of ddaE neurons in 20 hr AEL embryos, which have a relatively simple dendritic branching pattern and do not develop spine-like fine structures (Fig. 2*A*). In the A1 segment, the average length of ddaE dorsal dendrites was 32.2 ± 0.8 μm ($n = 34$) in wild-type embryos and 47.3 ± 1.4 μm ($n = 22$; $p < 0.0001$) in $TmII^{S130510}/TmII^{el4}$ mutant embryos, in which the expression of all known TmII isoforms was greatly reduced (Fig. 2*B*). The *cTmII*-specific mutation also caused significant increase in dorsal dendrite length, 40.3 ± 0.9 μm ($n = 34$; $p < 0.0001$) in $TmII^{c}$ homozygous mutant embryos and 41.8 ± 0.8 μm ($n = 28$; $p < 0.0001$) in $TmII^{c}/TmII^{S130510}$ mutant embryos, suggesting that cTmII contributes to the dendritic overextension phenotype. ddaE neurons in other abdominal segments (such as A2 and A3) in mutant embryos with different combinations of *TmII* alleles exhibited a similar dendritic phenotype (data not shown). The phenotypes in $TmII^{c}/TmII^{S130510}$ were less severe than those in $TmII^{S130510}/TmII^{el4}$ mutant embryos ($p < 0.005$), suggesting that other TmII isoforms also regulate the size of dendritic fields.

Next, we studied the effects of *TmII* mutations on the lateral dendrites of ddaE neurons. The total length of all lateral branches that extended from the dorsal dendrites was significantly greater in $TmII^{S130510}/TmII^{el4}$ mutant embryos than in wild-type embryos (91.6 ± 3.9 vs 59.6 ± 3.3 μm; $n = 24$; $p < 0.0001$) (Fig. 2*C*). $TmII^{S130510}/TmII^{el4}$ mutant embryos also had significantly more lateral branches than wild-type embryos (13.0 ± 0.5 versus 9.3 ± 0.3; $n = 24$; $p < 0.0001$) (Fig. 2*D*). *TmII* mutations did not affect the average length of lateral branches (data not shown), presumably attributable to the "stop signals" at the segment boundaries. These findings suggest that *TmII* mutations result in enlarged

dendritic fields by increasing both the length of dorsal dendrites and the number of lateral branches of ddaE neurons.

TmII functions cell autonomously to regulate dendritic fields

To determine whether *TmII* regulates dendritic fields in a cell-autonomous manner, we used the MARCM technique to generate single $TmII^{S130510}$ mutant DA neurons in third instar larvae (Lee et al., 1999; Sweeney et al., 2002). Because most GFP-labeled single neurons were ddaC neurons, quantitative analysis on ddaC is presented here. In most cases, only one ddaC neuron was labeled by GFP in individual third instar larvae. The dendritic field of ddaC covers the hemisegment between the adjacent segment boundaries and from the dorsal midline to the lateral cluster (Sweeney et al., 2002). Because the width of ddaC neuron dendritic fields is restricted by the width of each segment, we calculated the dorsolateral dimension of ddaC neurons relative to the anteroposterior dimension of the segment. We found that the average dendritic field of *TmII* mutant ddaC neurons was 19% larger than those of wild-type ddaC neurons ($p < 0.0001$) (Fig. 3).

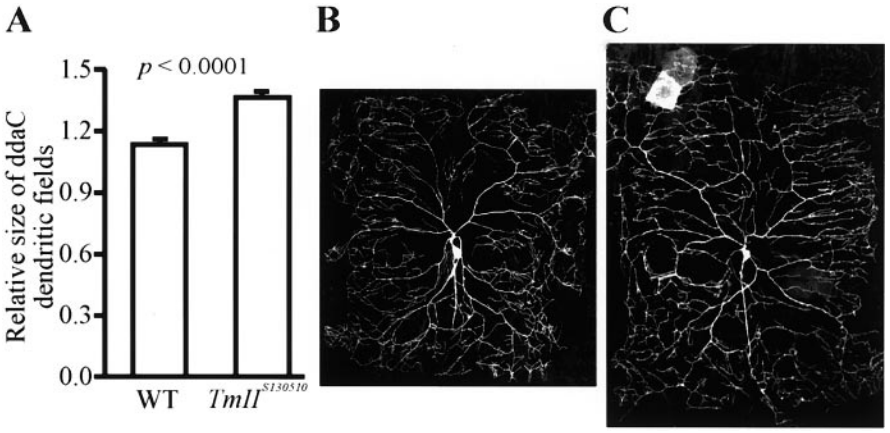

Figure 3. *TmII* has a cell-autonomous function in controlling the size of ddaC neuron dendritic fields as demonstrated by the MARCM technique. *A*, The distance between the most dorsal and the most lateral dendritic boundaries was measured as the dorsolateral dimension of ddaC dendritic fields. The ratio of the dorsolateral dimension to the width of the segment reflects the relative size of ddaC dendritic fields. The dendritic fields of ddaC mutant neurons ($n = 22$) are significantly larger than those of wild-type (WT) neurons ($n = 15$) (Student's *t* test; $p < 0.0001$). *B*, A wild-type ddaC neuron. *C*, A $TmII^{S130510}$ mutant ddaC neuron. An epithelial cell is labeled with GFP in *C*.

Dendritic overextension phenotype in *TmII* mutants was enhanced by reduced dosage of *flamingo*

Our previous studies indicate that Flamingo, a putative G-protein-coupled receptor that contains a large N-terminal domain with cadherin repeats and EGF-like domains, controls dendritic field formation in a cell-autonomous manner (Gao et al., 2000; Sweeney et al., 2002). Flamingo also affects the size of dendritic territories of mushroom body neurons (Reuter et al., 2003). To investigate potential genetic interactions between *flamingo* and *TmII*, we examined the $TmII^{S130510}/TmII^{el4}$ mutant embryos that were also heterozygous for a *flamingo* mutation. Both $TmII^{S130510}$ and $TmII^{el4}$ mutations greatly reduce the expression of all known TmII isoforms (Fig. 1). For this experiment, we identified a Flamingo protein-null allele, $flamingo^{51}$. In $flamingo^{51}$ homozygous mutant embryos, no Flamingo was detectable by immunostaining analysis with a monoclonal antibody specific for the N terminus of Flamingo (data not shown). In addition, a point mutation was identified in the coding region of *flamingo* that resulted in a stop codon at amino acid 1393, shortly after the ninth cadherin repeat. In $TmII^{S130510}/TmII^{el4}$ mutants, reducing the dosage of *flamingo* enhanced the dendritic overextension phenotype (Fig. 4), suggesting a genetic interaction between the two genes in regulating dendritic field formation. We did not see an effect in embryos that were heterozygous for both *TmII* and *flamingo* (data not shown), which is probably attributable to the function of *TmII* being less sensitive to dosage.

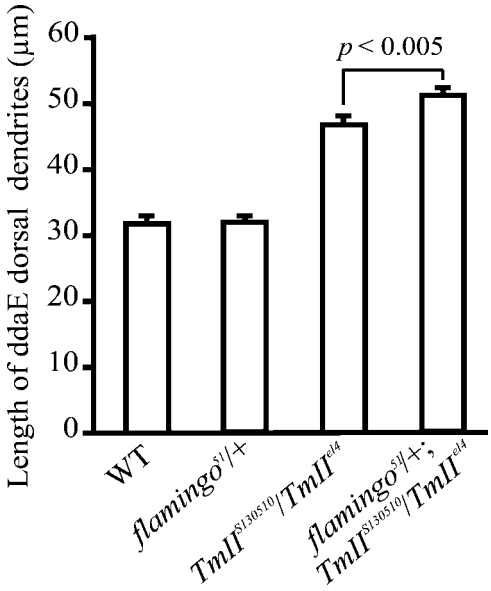

Figure 4. Genetic interaction between *TmII* and *flamingo*. All embryos were at the 20 hr AEL stage, and DA neurons were labeled by GFP under the control of *GAL4 109(2)80*. The length of dorsal dendrites of ddaE neurons in wild-type (WT) ($n = 34$), $flamingo^{51}/+$ ($n = 22$), $TmII^{S130510}/TmII^{el4}$ ($n = 22$), and $flamingo^{51}/+$; $TmII^{S130510}/TmII^{el4}$ ($n = 32$) embryos was measured as described above. There is no significant difference between wild-type and *flamingo* heterozygous embryos (Student's *t* test; $p > 0.2$). The dendritic overextension phenotype is significantly enhanced in $TmII^{S130510}/TmII^{el4}$ mutants by reducing the dosage of *flamingo* (Student's *t* test; $p < 0.005$).

Discussion

Dendritic morphogenesis can be controlled by a number of factors, such as extrinsic signaling molecules or cell-specific transcription factors (for review, see Whitford et al., 2002; Gao and Bogert, 2003). Here we provide *in vivo* evidence that tropomyosin, a protein that directly binds to and stabilizes actin filaments, functions cell autonomously in postmitotic neurons to regulate the size of dendritic fields.

Role of tropomyosin in dendrite development

Tropomyosin is a rod-shaped, coiled-coil protein that binds along the side of the actin filament (Araya et al., 2002). In non-muscle cells, tropomyosin is not associated with newly polymerized actin filaments at the leading edge of motile cells (DesMarais et al., 2002). Instead, it stabilizes actin filaments by inhibiting Arp2/3 complex-mediated nucleation in the deeper cortex (Blanchoin et al., 2001). Tropomyosin prevents the dissociation of

actin subunits from actin filaments and increases the physical strength of actin filaments (for review, see Cooper, 2002). In addition, tropomyosin regulates the association between actin filaments and other actin-binding proteins, such as Myo 1b, an unconventional myosin that localizes to the leading edge of motile cells (Tang and Ostap, 2001).

Little is known about the dynamics of actin filaments in dendritic growth and branching in developing organisms. Our findings raise the possibility that the absence of tropomyosin reduces the stability of actin filaments, resulting in increased dendritic growth. Alternatively, increased association of motor proteins with actin filaments in the absence of tropomyosin may contribute to dendritic extension. The latter possibility is consistent with the finding that a kinesin-related motor protein, CHO1/MKLP1, is required for dendrite formation in cultured neurons (Yu et al., 1997).

Both *TmII* and *flamingo* mutations caused overextension of dorsal dendrites of DA neurons (Gao et al., 2000; this study). The genetic interaction between the two genes (Fig. 4) raises the possibility that the Flamingo pathway may somehow affect the actin filaments. How the signal mediated by membrane receptor-like molecule Flamingo influences the reorganization of the actin cytoskeleton still remains primarily unknown. The identification of factors that link Flamingo and actin-binding proteins, such as tropomyosin, will be of great interest.

Different effects of tropomyosin isoforms

In *Drosophila*, multiple TmII isoforms arise as a result of alternative promoters and alternative splicing events (Hanke and Storti, 1988; Erdélyi et al., 1995). cTmII is expressed in neurons, and at least three isoforms are specifically expressed in muscles. Although cTmII affects dendritic field size, $TmII^{S130510}/TmII^{el4}$ mutants, in which all isoforms are affected by the *P*-element insertion, exhibit a more severe dendritic phenotype than $TmII^{c}$ mutants. Thus, other TmII isoforms may also contribute to the regulation of dendritic growth. It is unlikely that muscle-specific TmII isoforms have non-cell-autonomous effects on neurons. Myosin heavy chain mutations also result in the paralysis of embryos; however, the dendrites in these mutant embryos appear to develop normally (our unpublished observation). We speculate that one or more TmII isoforms that have not been characterized (Hanke and Storti, 1988; Erdélyi et al., 1995) have functions similar to those of cTmII.

Mammalian tropomyosins also have multiple isoforms, some of which are specifically expressed in neurons (Weinberger et al., 1993). Dimerization between different isoforms further increases the complexity of tropomyosin function (Araya et al., 2002). It is likely that the combinatorial action of different tropomyosin isoforms contributes to the generation of diverse dendritic morphologies in the nervous system.

References

Araya E, Berthier C, Kim E, Yeung T, Wang X, Helfman DM (2002) Regulation of coiled-coil assembly in tropomyosins. J Struct Biol 137:176–183.

Blanchoin L, Pollard TD, Hitchcock-DeGregori SE (2001) Inhibition of the Arp2/3 complex-nucleated actin polymerization and branch formation by tropomyosin. Curr Biol 11:1300–1304.

Boycott BB, Wässle H (1974) The morphological types of ganglion cells of the domestic cats' retina. J Physiol (Lond) 240:397–419.

Chae J, Kim MJ, Goo JH, Collier S, Gubb D, Charlton J, Adler PN, Park WJ (1999) The *Drosophila* tissue polarity gene starry night encodes a member of the protocadherin family. Development 126:5421–5429.

Cooper JA (2002) Actin dynamics: tropomyosin provides stability. Curr Biol 12:R523–R525.

DesMarais V, Ichetovkin I, Condeelis J, Hitchcock-DeGregori SE (2002) Spatial regulation of actin dynamics: a tropomyosin-free, actin-rich compartment at the leading edge. J Cell Sci 115:4649–4660.

Erdélyi M, Michon A-M, Gulchet A, Glotzer JB, Ephrussl A (1995) Requirement for *Drosophila* cytoplasmic tropomyosin in oskar mRNA localization. Nature 377:524–527.

Gao F-B, Bogert BA (2003) Genetic control of dendritic morphogenesis in *Drosophila.* Trends Neurosci 26:262–268.

Gao F-B, Brenman JE, Jan LY, Jan YN (1999) Genes regulating dendritic outgrowth, branching, and routing in *Drosophila.* Genes Dev 13: 2549–2561.

Gao F-B, Kohwi M, Brenman JE, Jan LY, Jan YN (2000) Control of dendritic field formation in *Drosophila*: the roles of Flamingo and competition between homologous neurons. Neuron 28:91–101.

Grueber WB, Jan LY, Jan YN (2002) Tiling of the *Drosophila* epidermis by multidendritic sensory neurons. Development 129:2867–2878.

Hanke PD, Storti RV (1988) The *Drosophila melanogaster* tropomyosin II gene produces multiple proteins by use of alternative tissue-specific promoters and alternative splicing. Mol Cell Biol 8:3591–3602.

Lee T, Lee A, Luo L (1999) Development of the *Drosophila* mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. Development 126:4065–4076.

Lohmann C, Wong RO (2001) Cell-type specific dendritic contacts between retinal ganglion cells during development. J Neurobiol 48:150–162.

Luo L (2002) Actin cytoskeleton regulation in neuronal morphogenesis and structural plasticity. Annu Rev Cell Dev Biol 18:601–635.

Masland RH (2001) Neuronal diversity in the retina. Curr Opin Neurobiol 11:431–436.

Perry VH, Linden R (1982) Evidence for dendritic competition in the developing retina. Nature 297:683–685.

Ramón y Cajal S (1911) Histology of the nervous system of man and vertebrates. Oxford: Oxford UP.

Reuter JE, Nardine TM, Penton A, Billuart P, Scott EK, Usui T, Uemura T, Luo L (2003) A mosaic genetic screen for genes necessary for *Drosophila* mushroom body neuronal morphogenesis. Development 130:1203–1213.

Sweeney NT, Li W, Gao F-B (2002) Genetic manipulation of single neurons in vivo reveals specific roles of Flamingo in neuronal morphogenesis. Dev Biol 247:76–88.

Tang N, Ostap EM (2001) Motor domain-dependent localization of myo1b (myr-1). Curr Biol 11:1131–1135.

Tetzlaff MT, Jäckle H, Pankratz MJ (1996) Lack of *Drosophila* cytoskeletal tropomyosin affects head morphogenesis and the accumulation of oskar mRNA required for germ cell formation. EMBO J 15:1247–1254.

Usui T, Shima Y, Shimada Y, Hirano S, Burgess RW, Schwarz TL, Takeichi M, Uemura T (1999) Flamingo, a seven-pass transmembrane cadherin, regulates epithelial planar cell polarity under the control of Frizzled. Cell 98:585–595.

Weber AJ, Kalil RE, Stanford LR (1998) Dendritic field development of retinal ganglion cells in the cat following neonatal damage to visual cortex: evidence for cell class specific interactions. J Comp Neurol 390:470–480.

Weinberger RP, Henke RC, Tolhurst O, Jeffrey PL, Gunning P (1993) Induction of neuron-specific tropomyosin mRNAs by nerve growth factor is dependent on morphological differentiation. J Cell Biol 120:205–215.

Whitford KL, Dijkhuizen P, Polleux F, Ghosh A (2002) Molecular control of cortical dendrite development. Annu Rev Neurosci 25:127–149.

Yu W, Sharp DJ, Ryoko Kuriyama R, Prabhat Mallik P, Baas PW (1997) Inhibition of a mitotic motor compromises the formation of dendrite-like processes from neuroblastoma cells. J Cell Biol 136:659–668.