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Fasciclin II Signals New Synapse Formation through Amyloid Precursor Protein and the Scaffolding Protein dX11/Mint

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Cell adhesion molecules (CAMs) have been universally recognized for their essential roles during synapse remodeling. However, the downstream pathways activated by CAMs have remained mostly unknown. Here, we used the *Drosophila* larval neuromuscular junction to investigate the pathways activated by Fasciclin II (FasII), a transmembrane CAM of the Ig superfamily, during synapse remodeling. We show that the ability of FasII to stimulate or to prevent synapse formation depends on the symmetry of transmembrane FasII levels in the presynaptic and postsynaptic cell and requires the presence of the fly homolog of amyloid precursor protein (APPL). In turn, APPL is regulated by direct interactions with the PDZ (postsynaptic density-95/Discs large/zona occludens-1)-containing protein dX11/Mint/Lin-10, which also regulates synapse expansion downstream of FasII. These results provide a novel mechanism by which cell adhesion molecules are regulated and provide fresh insights into the normal operation of APP during synapse development.

Key words: Fasciclin II; amyloid precursor protein; X11; adhesion; signaling; *Drosophila*; neuromuscular junction

Introduction

Although homophilic cell adhesion molecules (CAMs) of the Ig superfamily (IgCAMs) play critical roles in synapse plasticity (Murase and Schuman, 1999; Huntley, 2002; Packard et al., 2003; Welzl and Stork, 2003), the molecular mechanisms by which IgCAMs regulate synapse dynamics remain poorly understood (Garcia-Alonso et al., 1995; Beggs et al., 1997; Schmid et al., 1999). Mice deficient for the mammalian IgCAM neural CAM (NCAM) show impaired spatial memory as well as reduced long-term potentiation (Luthl et al., 1994; Muller et al., 1996). In *Aplysia*, long-term facilitation of the gill- and siphon-withdrawal reflex results in the formation of new synaptic connections between the presynaptic siphon-sensory neuron and the target cells. Underlying this process is the downregulation of apCAM, an *Aplysia* IgCAM, in a manner that involves mitogen-activated protein kinase (MAPK) and a ubiquitin-dependent degradation pathway (Bailey et al., 1992, 1997).

At the *Drosophila* neuromuscular junction (NMJ), as muscle fibers increase in size, synaptic efficacy is maintained by the expansion of the synaptic arbor, which continuously sprouts new synaptic boutons throughout development (Packard et al., 2003).

This expansion is regulated by local changes in Fasciclin II (FasII) levels by a process that involves presynaptic activity, changes in FasII clustering, MAPK pathway-dependent FasII downregulation, and changes in FasII exocytosis (Budnik et al., 1990; Schuster et al., 1996a; Thomas et al., 1997; Koh et al., 1999, 2002; Mathew et al., 2003).

A widely accepted model posits that enhanced FasII-mediated cell adhesion constrains synaptic growth, whereas decreased FasII-mediated adhesion partially lifts this constraint, allowing for new synapses to form (Schuster et al., 1996a; Mayford and Kandel, 1999). Although this model is consistent with the observations from the above studies, it has not yet been tested fully. Indeed, almost nothing is known about the signaling mechanisms that may be activated by FasII-mediated cell adhesion. Moreover, as with other CAMs, the presence of an intracellular domain suggests that the ability of transmembrane FasII to modulate synapse formation may also involve the activation of intracellular signaling mechanisms (Biederer et al., 2002; Panicker et al., 2003).

Here, we demonstrate that FasII requires the fly homolog of amyloid precursor protein (APPL) to regulate synaptic growth and that its influence on synaptic bouton formation cannot be simply explained in terms of adhesion but by additional signaling through APPL. We show that FasII and APPL are in the same protein complex in *vivo* and that FasII–APPL signaling depends on interactions with the APPL-binding protein dX11/Mint/Lin-10/dX11L. Furthermore, we find that enhancement of new synapse formation depends on a balance of FasII levels at both sides of the synapse rather than a change in absolute levels, as suggested previously. Conversely, an imbalance of FasII levels at either side of the synapse interferes with new synapse formation and leads to gross abnormalities in bouton structure, including microtubule...
tangles, membranous inclusions, and abnormal APPL deposits. These studies unravel a signaling pathway activated by FasII during synapse formation and establish a link between two important modulators of synapse growth: FasII and APPL.

Materials and Methods

Fly strains. Flies were reared in standard Drosophila medium. The following mutant stocks were used: hypomorphic fasII107 (Schuster et al., 1996a), null allele Appd (Luo et al., 1992), double-mutant Appd’/ fasII107 obtained by recombination, P(1L)8895-3 (Bloomington Stock Center, Bloomington, IN), and Df(1)BK10 (Bloomington Stock Center), a deficiency of the x11 region. We used the following upstream activator sequence (UAS)–APPL strains [described by Torroja et al. (1999)]: UAS–APPLSD, containing a form of APPL that cannot be proteolytically processed, UAS–APPL952, a Gal4 activator strain in which the intracellular domain of APPL is deleted, and UAS–APPLACi, in which the cytoplasmic internalization sequence GY ENPTY has been removed. We also used the following: dX11, UAS–dX11APPTB, in which the phosphorysine-binding (PTB) domain had been deleted, and the FasII UAS strain UAS–FasII– glycosylphosphatidylinositol (GPI) (see below). Gal4 activator strains used to drive motoneuron-specific expression of APPL and FasII were C164, which has Gal4 expression in type I motoneurons during larval development (Torroja et al., 1999), and C380, which has Gal4 expression in type I motoneurons (Koh et al., 1999). For expression of transgenes in body-wall muscles, we used the Gal4 drivers BG487 and C57 (Budnik et al., 1996).

Generation of transgenic flies. To generate UAS–dX11, Drosophila dX11 cDNA was digested with Not1 and Kpnl, and the resulting insert, containing the entire dX11 coding region, was directly cloned into pUAST vector for germline transformation (Spradling, 1986). To generate dX11 lacking the APP-binding PTB domain (dX11ΔPTB), site-directed mutagenesis was performed using the Stratagene (La Jolla, CA) QuickChange kit. Briefly, oligos were used to introduce XhoI sites on either side of the PTB domain coding sequences (amino acids 766–986). The PTB domain was digested out, and sticky ends religated before subcloning into pUAST. To generate a UAS–GPI-linked FasII, cDNA LP01422 was digested with EcoRI and ligated into the pUAST vector.

Immunocytochemistry and antibody production. Immunocytochemistry and laser-scanning confocal image acquisition and analysis were as performed by Budnik et al. (1996) using a Bio–Rad (Hercules, CA) MRC600 or a Zeiss (Oberkochen, Germany) LSM confocal microscope and Zeiss PASCAL 3.0 software. To quantify synapse number, third instar body-wall muscle preparations were stained with a neuronal marker (anti-HRP, which cross-reacts with several presynaptic membrane antigens, thus labeling the entire presynaptic arbor), and boutons were counted at muscles 6 and 7 of abdominal segment 3. To quantify the number of buds, the following criteria were used to identify buds: (1) buds were much smaller than the parent boutons, and (2) they appeared to sprout from a neighboring “parent” bouton of mature bouton size. To measure muscle surface, the width and length of muscles 6 and 7 were measured under epifluorescence using a calibrated ocular grid. For the expression of FasII, dX11, or APP variants using the UAS/Gal4 system (Brand and Perrimon, 1993), crosses and rearing of larvae were performed at 29°C to maximize levels of Gal4 expression. Controls at this temperature were also performed for wild-type, homozygous Gal4 strains, homozygous UAS–APPL strains, and Gal4/UAS–LaCZ heterozygotes. No significant differences in bouton number were observed in these controls. The number of buds and boutons was expressed (mean ± SEM) and compared using the Student’s t test. To quantify APPL levels inside a bouton, a single confocal slice at the bouton midline was selected, and APPL fluorescence intensity in the ring of HRP staining at the bouton membrane (APPLm) was measured. APPL fluorescence at the internal region of the bouton (APPLi), the area circumscribed by the ring of HRP staining, was then added to the intensity value by using the histogram function of the Zeiss LSM software. Then background intensity, obtained by measuring APPL fluorescence intensity in surrounding muscle, was subtracted, after normalizing the intensity measurements to surface area. Numbers were expressed as percentage of total APPL fluorescence (normalized APPLi/normalized APPLm plus APPLi). The following antibodies were used to label synaptic terminals: FITC- or Texas Red-conjugated anti-HRP (1:200), anti-FasII (1:3500) (Koh et al., 1999), anti-APPL952 (1:500) (Torroja et al., 1996), anti-dX11 (1:100; see below), anti-tubulin (1:1000; Sigma, St. Louis, MO), and monoclonal antibody 2C10 (1:100; a gift from S. Benzer, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA). Secondary antibodies (Jackson Immunoresearch, West Grove, PA) were used at 1:200.

Anti-dX11 antibodies were generated by immunizing rats and rabbits with bacterially generated and affinity-purified His-tagged protein (dX11 N-terminal amino acids 137–266), produced using the pET system (Novagen, Madison, WI). The rabbit antisera was subsequently affinity purified (Tang, 1993).

Immunoprecipitations. For immunoprecipitations, 20–40 body-wall muscle preparations per genotype were homogenized at 4°C in radiomimoprecipitation assay (RIPA) buffer containing protease inhibitors and 100 μm Na3VO4. After centrifugation (3000 × g for 5 min), the supernatant was precleared with Protein A– or Protein A/G-agarose beads for 1 h. Cleared lysate was subsequently incubated with beads bound to anti-dX11 or rabbit anti-APPL952 at 4°C for 1–2 h and then washed three times with RIPA buffer, and boild in loading buffer. Proteins were separated in an 8% SDS–PAGE gel, transferred to Immobilon-P (Millipore, Billerica, MA) nylon membrane, and sequentially blotted with rabbit anti-APPL952 (1:1000), rat anti-dX11N1 (1:1000), mouse anti-tubulin (1:5000; Sigma), and rabbit anti-FasII (1:1000).

Schneider cell transfection. dX11 and fasII cDNAs were cloned into the pAcV5/HisB vector (Invitrogen, Carlsbad, CA) for transfections. Drosophila Schneider S2 cells were cultured in HYQ SFX–Insect cell culture medium (HyClone, Logan, UT) containing 10% FBS, penicillin (100 U/μl) and streptomycin (100 μg/μl). Three wells (2 ml each) per sample of 60–80% confluent S2 cells were transfected with 1.5 μg of DNA using Cellfectin (Invitrogen). Thirty-six hours after transfection, cells were harvested, resuspended in 300 μl of RIPA buffer, and homogenized, and immunoprecipitations were performed as described above for body-wall muscles. For RNA interference (RNAi) experiments, dX11 double-stranded RNA (dsRNA) was generated using dX11 primers containing a 5’ T7 polymerase-binding site and applied to S2 cells, as described by Clemens et al. (2000).

Electron microscopy. Ultrastructural analysis was as performed by Jia et al. (1993), with the exception of the use of 2 mM Mg2+–Trump’s fixative (Torroja et al., 1999). Transverse ultrathin serial sections (70–80 nm) were cut from ventral longitudinal muscles 6 and 7 at abdominal segments A3 and A4. Three wild-type (10 boutons), three [FasII, APPL]-pre (12 boutons), and three [FasII]-pre (eight boutons) samples were used for this analysis.

Electrophysiology. All experiments were performed on wandering third instar larvae raised at 29°C. Larvae were dissected under ice-cold hemolymph-like (HL-3) saline (Stewart et al., 1994) containing 0.3 mM calcium. Body-wall muscles were visualized under a Zeiss Axioscope 200 long-working distance objective and continually superfused with HL-3 saline containing 0.5 mM calcium at 22°C. Recordings were done by impaling body-wall muscle 6 in abdominal segment 3 with a 15–20 MΩ electrode and were amplified using an Axoclamp 2A amplifier (Molecular Devices, Union City, CA). Recordings were exported to both an ITC-16 computer interface (InstruTech, Port Washington, NY) for data storage. Data were imported via Pulse software (HEKA Electronik, Lambrecht/Pfalz, Germany) and analyzed using both Mini Analysis software (Synaptosoft, Decatur, GA) and Origin software (OriginLab, Northampton, MA). Evoked excitatory junctional potentials (EJPs) were induced through application of a 10-μm-diameter lumen glass–suction electrode to a cut segmental nerve and stimulated with a 1 ms suprathreshold stimulus at 1 Hz by an S48 stimulator and an SIU-5 stimulus isolation unit (Grass–Telefactor, West Warwick, RI). Only muscle cells with a resting potential of −60 to −63 mV were used for analysis, and at least five cells were analyzed for each genotype. Statistical analysis was performed using the Student’s t test.
Results
Does FasII constrain synaptic growth? Previous studies of NMJ expansion have suggested a basic model of FasII function at the NMJ, in which enhanced FasII-mediated cell adhesion constrains synaptic growth, whereas decreased

changes in bouton number were not the result of defects in targeting and stabilization of ectopic synapses, which occur after changing FasII levels during initial embryonic synaptogenesis (Davis et al., 1997). Thus, a simultaneous decrease or a simultaneous increase in FasII levels in both the presynaptic and the

FasII-mediated cell adhesion partially lifts this constraint, allowing for new synapses to form (Schuster et al., 1996a,b). In those studies, FasII levels were decreased by using hypomorphic fasII alleles as well as mutations that decrease FasII expression at the NMJ, which resulted in greater synaptic growth. However, the consequences on synaptic bouton number of enhancing FasII expression either presynaptically or postsynaptically, or both simultaneously, was not examined in those studies. In subsequent work, Davis et al. (1997) examined the effects of changing FasII levels in the stabilization of ectopic innervation.

The main finding in this study was that increasing FasII in specific muscles during the embryonic period led to the stabilization of inappropriate synaptic connections, but no such effect was observed when FasII levels were altered in the postembryonic period, when most synaptic boutons were formed (Davis et al., 1997).

To further explore the above hypothesis, we labeled NMJs at the last stage of larval development with a presynaptic terminal marker (anti-HRP; see Materials and Methods), and the degree of NMJ expansion was determined by counting the number of synaptic boutons (Budnik et al., 1990; Gorczyca et al., 1993). In agreement with the basic model, overexpression of FasII either presynaptically or postsynaptically resulted in a significant decrease in bouton number (Fig. 1A). Surprisingly, however, and in contrast to the above model, we found that when FasII levels were simultaneously elevated in both the presynaptic and postsynaptic cells ([FasII]-pre-post), there was a remarkable increase to >150–230% of the number of synaptic boutons compared with wild type, depending on the strength of the Gal4 drivers used to express UAS–FasII (Fig. 1A). When FasII was simultaneously decreased in both the presynaptic and postsynaptic cells in fasIIe76/+ heterozygotes, the result was a small but significant increase in bouton number, as seen in the studies of Schuster et al. (1996) (Fig. 1A). These changes in bouton number (and in all of the mutants examined in Fig. 1) were not attributable to changes in muscle size, which was indistinguishable from wild-type controls. Also, because we used Gal4 drivers that drive expression of transgenes during the postembryonic period, no ectopic synapses were observed. Therefore, changes in bouton number were not the result of defects in targeting and stabilization of ectopic synapses, which occur after changing FasII levels during initial embryonic synaptogenesis (Davis et al., 1997). Thus, a simultaneous decrease or a simultaneous increase in FasII levels in both the presynaptic and the

Figure 1. FasII signaling through APPL stimulates NMJ growth, and both proteins form a complex in vivo. A–D, Histograms showing the number of boutons at muscles 6 and 7 (abdominal segment 3) of third instar larvae with different levels of FasII and APPL. The number of samples quantified is as follows: wild type, n = 95; fasIIe76/+, n = 12; [FasII]-pre, n = 43; [FasII]-post, n = 17; [FasII]-pre-post (using the Gal4 drivers C380 and BG487), n = 13; [FasII]-pre-post# (using the Gal4 drivers C164 and C57), n = 10; Appδ, n = 49; [APPL]-pre (using Gal4 driver C380), n = 14; [APPL]-pre† (using the Gal4 driver C164), n = 85; Appδ, fasIIe76/APPδ, +, n = 24; Appδ, [FasII]-pre-post (using the Gal4 drivers C164 and C57), n = 10; [GPI–FasII]-pre-post (using the Gal4 drivers C380 and BG487), n = 6; [FasII, APPL]-pre-post† (using the Gal4 drivers C380 and BG487), n = 27; [FasII, APPL]-pre, n = 25; [FasII, APPL,ΔC]-pre, n = 10; fasIIe76/+, APPL)-pre, n = 21. Bouton numbers are mean values ± SEM. *p < 0.05; ***p < 0.0001. Low- (E–G) and high- (I–Q) magnification views of third instar larval NMJs at muscles 6 and 7 stained with anti-HRP (E, F, I, J, L–N, P, Q) and anti-FasII (G, H, O, Q) antibodies. Inset: E, F, I, J, L–N, P, Q: Wild type. F, G, H, P, Q: Larvae overexpressing only FasII in both presynaptic and postsynaptic cells. I, M: Stereoscopic images showing budding boutons in wild type (J) and [FasII]-pre-post (M). Note the dramatic increase in budding boutons in larvae overexpressing FasII either alone or in combination with APPL. Scale bars: (in Q) E–G, 50 μm; I–Q, 8 μm. H: FasII and APPL coimmunoprecipitate from body-wall muscle extracts. Extracts were immunoprecipitated with anti-APPL antibodies, and immunoblots were sequentially probed using anti-APPL, anti-FasII, anti-tubulin (Tub), and anti-spectrin (Spec). Input lanes correspond to 10% of the extract used for immunoprecipitation. Control lanes correspond to extracts in which antibody was omitted during immunoprecipitation. Note that anti-APPL immunoprecipitates FasII and that this interaction is specific if no FasII band could be detected in Appδ. Molecular weights are indicated in kilodaltons to the right of each blot. Error bars represent SEM.
postsynaptic cells results in an increase in bouton number, albeit to different extents.

An important distinction to be made, however, is that the striking increase in bouton number that we observed in [FasII]-pre-post was primarily attributable to proliferation of “satellite” boutons or buds. In the wild type, NMJs expand in part by sprouting or budding new boutons (Fig. 1 I, arrow), commonly at the distal end of a branch (Zito et al., 1999; Packard et al., 2002; Ruiz-Canada et al., 2004). These buds enlarge and then separate from the parent bouton, remaining connected only by a thin process, and eventually grow to become mature boutons. In NMJs overexpressing FasII in both presynaptic and postsynaptic cells, the dramatic increase in satellites/buds, 672% over wild type, was observed at both distal and proximal boutons (Fig. 1 M–P, arrows in M). In contrast, satellite number in fasII5/+/ decreased to 33% of the wild-type value [8.6 ± 1.1 in wild type (n = 30) vs 74.5 ± 5.22 in [FasII]-pre-post (n = 9) and 2.8 ± 0.6 in fasII6/6 (n = 12)]. To determine whether the buds observed in [FasII]-pre-post represented an enhancement of the normal process of bouton budding observed in wild type, we performed an ultrastructural analysis of buds. We found that buds in [FasII]-pre-post were similar to those observed in wild type, including the distribution of synaptic vesicles, active zones, and postsynaptic specializations (supplemental Fig. 1, available at www.jneurosci.org as supplemental material).

The above observations suggest that the influence of FasII on synaptic growth may not be purely related to its “adhesive” properties that are proposed to restrict synapse expansion, but may also involve the activation of a signaling cascade that promotes bud formation. This signaling cascade might depend on homophilic binding between FasII molecules at the presynaptic and postsynaptic cell, because overproliferation of boutons was observed only after changing FasII levels in both the presynaptic and the postsynaptic cells (Fig. 1 A and below).

Transmembrane FasII isoforms can form dimers through homophilic interactions that require their extracellular Ig domains, but FasII also contains a 136 aa intracellular domain (Grenniglohl et al., 1991), which may transduce a signal to the intracellular milieu. To determine whether the increase in bouton number in [FasII]-pre-post depended on an intact intracellular domain, we generated transgenic flies carrying the GPI-linked FasII isoform (GPI–FasII), in which the intracellular and transmembrane domains are missing, and expressed it presynaptically and postsynaptically. No increase in bouton number was observed in [GPI–FasII]-pre-post, demonstrating that the effect of FasII on bouton number depended on an intact intracellular domain (Fig. 1 A).

FasII requires APPL to modulate synaptic bouton formation

The spectacular increase in bouton buds observed at NMJs overexpressing FasII at both presynaptic and postsynaptic cells was highly reminiscent of the phenotype observed in NMJs with increased APPL (Torroja et al., 1999), a presynaptically localized homolog of APP. Overexpressing APPL in motoneurons results in an increase in bouton number to >250% of wild type, primarily because of an overproliferation of bouton buds, but also because of a proliferation of mature boutons, whereas a null mutation in Appl, Appl4, reduces bouton number (Torroja et al., 1999) (Fig. 1 B, D).

The similarity of the results between overexpression of FasII and APPL raised the possibility that FasII may activate APPL-dependent mechanisms that promote synaptic growth. We tested this possibility by looking for genetic and biochemical interac-

tions between APPL and FasII. In these experiments, we modified the levels of both proteins by generating both loss- and gain-of-function double mutants. As noted, a reduction in FasII levels in fasII3/+ resulted in a small but significant increase in bouton number (Fig. 1 B). This increase in bouton number was completely suppressed in an Appl null mutant background (Appl4, fasII3/+/Appl4, +), and furthermore, the number of synaptic boutons actually decreased compared with wild type in this double mutant (Fig. 1 B). These data lend support to the notion that Appl and FasII genes interact and that the increase in bouton number observed in fasII3/+ may result from interactions with APPL.

This hypothesis was further tested by simultaneously overexpressing FasII in both the presynaptic and postsynaptic cells in the Appl null mutants (Appl4, [FasII]-pre-post). Again, we found that in the absence of APPL, overexpressing FasII in both the presynaptic and the postsynaptic cell led to a wild-type number of boutons, in contrast to the twofold increase when APPL levels were normal (Fig. 1 C).

Another phenotype elicited by a symmetric increase or decrease in FasII was a rise in the number of NMJ branches (supplemental Fig. 2 A, available at www.jneurosci.org as supplemental material). Again, as in the case of bouton overproliferation, this phenotype was completely suppressed by the absence of APPL (supplemental Fig. 2 A, available at www.jneurosci.org as supplemental material).

To determine whether APPL was acting downstream of FasII, we performed the converse experiment, by determining whether a severe reduction in FasII function in fasII3 homozygotes could rescue the bouton overproliferation observed by increasing APPL levels. However, we found that a decrease in FasII levels in the presence of elevated APPL (fasII3, [Appl]-pre) did not rescue the increased bouton number observed in APPL gain-of-function alone (Fig. 1 D). Thus, the increase in bouton number observed when there is a simultaneous increase or decrease in presynaptic and postsynaptic FasII levels requires the presence of APPL, indicating that APPL exerts its effects downstream of FasII during synaptic bouton growth.

This hypothesis was further confirmed by experiments in which APPL expression was upregulated in larvae in which FasII was simultaneously enhanced in both the presynaptic and the postsynaptic cell ([FasII, Appl]-pre-post). In this case, the increase in bouton number was further potentiated to ~400% (Fig. 1 D, G, Q). Together, the results from the above genetic approach uncover a novel transduction pathway by which FasII regulates synapse structure and function through interactions with APPL. A potential association of FasII and APPL into an endogenous protein complex was examined by immunoprecipitation assays using body-wall muscle extracts. We found that antibodies against APPL coimmunoprecipitated FasII from body-wall muscle extracts, but this was not observed in extracts from Appl null mutants, demonstrating the specificity of the immunoprecipitation (Fig. 1 H). Furthermore, spectrin, which is particularly enriched at synaptic boutons (Ruiz-Canada et al., 2004) was not coprecipitated by APPL antibodies, demonstrating selectivity for the complex (Fig. 1 H). Thus FasII and APPL interact genetically during NMJ expansion and are present in a biochemical complex in vivo.

Functional consequences of altering FasII and APPL levels

Although APPL has been shown previously to be involved in the regulation of synaptic bouton formation (Torroja et al., 1999), the functional consequences of altering APPL levels have not been investigated. Similarly, the functional consequences of in-
creased bouton number by symmetric changes in FasII in fasII<sup>+/+</sup> and [FasII]-pre-post are not known (but see Schuster et al., 1996b). Therefore, we recorded intracellularly from body-wall muscles and stimulated segmental nerves to visualize EJPs. In fasII<sup>+/+</sup> animals, EJPs had significantly larger amplitudes than wild-type controls (p < 0.0001) (Fig. 2A,C), possibly reflecting the increased number of mature boutons in these mutants. In contrast, EJP amplitude in Appl null mutants was significantly decreased (p < 0.0001), albeit to a smaller extent (Fig. 2A,C). This phenotype may also reflect the decrease in bouton number observed in this mutant. In agreement with our hypothesis that many of the synaptic functions of FasII may occur through interactions with APPL, the increased EJP amplitude in fasII<sup>+/+</sup> was completely suppressed in the absence of APPL in Appl<sup>−/−</sup>, fasII<sup>+/+</sup>, and furthermore, it was indistinguishable from Appl<sup>−/−</sup> mutants (Fig. 2A,C).

Unexpectedly, when FasII was expressed both presynaptically and postsynaptically, EJP amplitude was significantly decreased compared with both wild type and Appl<sup>−/−</sup> (Fig. 2A,C). This may reflect the fact that much of the synaptic bouton overproliferation in [FasII]-pre-post is attributable to the formation of immature boutons or buds. In agreement with this view, overexpressing APPL, which also causes an overproliferation of buds, also leads to a decrease in EJP amplitude (Fig. 2A,C). As in the case of fasII<sup>+/+</sup>, the [FasII]-pre-post phenotype was completely suppressed by the absence of APPL, and again, EJP amplitudes were indistinguishable from Appl<sup>−/−</sup> (Fig. 2A,C). This result demonstrates that defects in the postsynaptic signal derived from altering FasII levels depend on APPL.

To determine whether the defects in EJP amplitude were likely to be derived from changes in either presynaptic or postsynaptic function, or both, we also examined the amplitude and frequency of miniature EJPs. Although changes in mEJP amplitude are often attributable to changes in postsynaptic function, changes in mEJP frequency are associated with changes in presynaptic function. Furthermore, Drosophila larval NMJs can display compensatory mechanisms that may in certain instances adjust presynaptic and postsynaptic responses to maintain synaptic efficacy (Stewart et al., 1996; Davis and Goodman, 1998; Paradis et al., 2001). We found that, in Appl<sup>−/−</sup>, mEJP frequency and mEJP amplitude were dramatically increased (Fig. 2B,D–F), perhaps as a consequence of such a compensatory mechanism. Overall, however, junctional quantal content (QC = EJP amplitude/mEJP amplitude), a measure of synaptic efficacy, was still significantly depressed (Fig. 2F).

In fasII<sup>+/+</sup> mutants, mEJP amplitude was unchanged, and mEJP frequency was significantly decreased. However, QC was significantly enhanced (Fig. 2B,D–F). This rise in QC was suppressed by Appl<sup>−/−</sup>, again suggesting that APPL is required for at least some of the functional pathways regulated by FasII. Indeed, changes in QC in [FasII]-pre-post were not rescued by the absence of APPL (Fig. 2B,D–F). Thus, although all of the structural and many of the functional defects associated with changes in FasII levels depend on APPL, these results also suggest that changes in FasII can alter NMJ function independently of APPL.

**Asymmetric changes in FasII expression interfere with normal synapse development**

In contrast to observations in which symmetric changes in FasII at both the presynaptic and the postsynaptic cell resulted in an
increase in bouton number, we found that a grossly asymmetric change in FasII levels brought about by overexpressing FasII in either the presynaptic or the postsynaptic cell alone interfered with normal NMJ expansion. When FasII was increased only in the presynaptic or the postsynaptic cell, there was a significant decrease in bouton number (Fig. 1A). Furthermore, an asymmetric increase in FasII levels in the presynaptic cell partially suppressed the increase in bouton number observed in the APPL gain-of-function alone ([FasII, APPL]-pre) (supplemental Fig. 2B, available at www.jneurosci.org as supplemental material). These results, together with the observation that increasing FasII levels decreases bouton number in an asymmetric manner in either the presynaptic or postsynaptic cells, suggest that an imbalance in FasII expression in both cells acts in a dominant-negative manner that interferes with the ability of APPL to promote synapse formation or that inhibits APPL activation by FasII.

This view was supported by the observation that synapses were dramatically altered not only in number but also in structure when FasII was asymmetrically expressed. This was particularly evident when FasII was expressed presynaptically in the APPL gain-of-function but was also observed in the presence of normal APPL (see below). In wild type, type I boutons at muscles 6 and 7 consist of strings of boutons, which are relatively homogeneous in size, and are joined by short neuronal processes (Johansen et al., 1989). In the [FasII, APPL]-pre gain-of-function double mutants, many type I boutons were strikingly enlarged compared with controls (Fig. 3A, B, D, H, arrows in B). In addition, these NMJs were characterized by very long neuritic processes, which were devoid of boutons for long stretches (Fig. 3B, arrowheads). These “giant” boutons contained internal membranous structures that stained brightly with the neuronal membrane marker anti-HRP (Fig. 3D, H), and which contained unusual APPL (Fig. 3, compare E, I with F, J, L; arrows) and FasII (Fig. 3, compare M, P with N, Q) protein accumulation.

The above phenotype was not simply attributable to overexpressing APPL and FasII at nonphysiologically high levels, because symmetrically overexpressing FasII in both the presynaptic and the postsynaptic cell in the presence of increased levels of APPL ([FasII, APPL]-pre-post) did not result in this phenotype at the NMJ. However, to further eliminate this possibility, we expressed APPLΔC, a form of APPL lacking a domain that is required for the synapse-promoting function of APPL (Torroja et al., 1999). We found that overexpressing FasII in the presynaptic cells together with APPLΔC ([FasII, APPLΔC]-pre) did not result in the formation of abnormal synaptic boutons (Fig. 3C) or in APPL (Fig. 3G, K) and FasII (Fig. 3O, R) deposits. Thus, the phenotypes observed in [FasII, APPL]-pre are specific and depend on an intact APPL cytoplasmic domain.

The phenotypes observed in [FasII, APPL]-pre larvae were also present, although to a much lesser extent, in boutons overexpressing only FasII in the presynaptic cell alone in the presence of normal (endogenous) APPL ([FasII]-pre). These boutons had internal structures that stained with anti-HRP and that were associated with abnormal accumulations of APPL (not shown). These inclusions were virtually never observed in wild-type boutons.

**Formation of microtubule tangles and APPL deposits in giant boutons**

In Alzheimer’s disease, neurofibrillary tangles are composed of cytoskeletal components including an unusual hyperphosphorylated form of the microtubule-associated protein tau (Spillantini and Goedert, 1998; Selkoe and Podlisny, 2002). Hyperphosphorylated tau is unable to promote or maintain microtubule stability because, unlike normal tau, this form is unable to bind to microtubules (Iqbal et al., 1998). The result is the formation of collections of tangled cytoskeletal filaments throughout the cytoplasm of neurons in patients with Alzheimer’s disease and other disorders associated with senile dementia. Although alterations in APP processing have been widely accepted as playing a key role in Alzheimer’s pathology, no clear mechanistic connection has yet been made between APP and tangle formation. At the fly NMJ presynaptic microtubules are associated with the microtubule-associated protein 1B-related protein Futsch (Hummel et al., 2000; Roos et al., 2000). Futsch immunoreactivity, as determined by the monoclonal antibody 22C10, colocalizes with presynaptic microtubules at wild-type NMJs and is observed as a single filamentous bundle that traverses the center of each NMJ branch (Fig. 4A–C). At certain boutons, often located at the distal end of an NMJ branch (terminal boutons), microtubules become unbundled, and their association with Futsch is lost (Ruíz-Canada et al., 2004). We found that, in enlarged [FasII, APPL]-pre boutons, even when the giant bouton was not at the end of a branch, Futsch appeared defasciculated into multiple filaments that spread inside the giant boutons (Fig. 4E–G, arrow in F), or formed large clusters or puncta inside these giant boutons (Fig.

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**Figure 3.** Gross asymmetric changes in FasII levels interfere with APPL-mediated synaptic growth. A–C. Low-magnification views of third instar larval NMJs at muscles 6 and 7 stained with anti-HRP antibodies in wild type (A), a mutant presynaptically overexpressing both APPL and FasII (B), and a larva expressing FasII and APPLΔC presynaptically (C). B. Note the dramatically enlarged boutons in the [FasII, APPL]-pre (arrows) and the long stretches of neuritic processes lacking varicosities (arrowheads). D–R. High-magnification single confocal slices through a bouton in wild type (D, E, I, M, P), [FasII, APPL]-pre (H, L, F, J, N, O), and [FasII, APPLΔC]-pre (G, K, O, R). Note the internal inclusions and abnormal APPL accumulations in mutants expressing both FasII and APPL and the lack of inclusions and APPL accumulations in samples in which a form of APPL lacking the cytoplasmic domain (ΔC) is expressed. L. Image at higher magnification shows anti-APPL accumulations (arrows) in a [FasII, APPL]-pre bouton. M–R, High-magnification single confocal sections showing anti-FasII (M–O) and anti-APPL and anti-FasII (P–R) in wild type (M, P), [FasII, APPL]-pre (N, Q), and [FasII, APPLΔC]-pre (O, R). Note that the internal FasII-positive inclusions in boutons expressing both APPL and FasII only presynaptically is suppressed when the APPL cytoplasmic domain is absent, demonstrating the specificity of the interaction between APPL and FasII. Scale bars: (in A) A–C, 50 μm; I, 3 μm; D–R, M–R, 5 μm.
Microtubule tangles and abnormal APPL deposits are observed at NMJs of [FasII, APPL]-pre larvae. A–C, E–J, NMJs from wild type (A–C) and larvae overexpressing presynaptic FasII and APPL (E–J) showing anti-Futsch (B, F, G, I, J), anti-HRP (A, C, E, G, H, J), and merged panels (C, G, J). D, K, Anti-HRP and anti-tubulin in wild type (D) and [FasII, APPL]-pre (K). Note the disorganized appearance of Futsch and microtubules in mutant NMJs (K, arrow). Scale bar: (in K) A–C, E–J, 10 μm; D, K, 6.5 μm.

The stimulation of bouton budding by FasII and APPL depends on the cytosolic adaptor protein dX11

The above results are consistent with a FasII signaling mechanism that depends on APPL. Our previous studies have demonstrated that the ability of APPL to enhance synaptic bouton number depends on its cytoplasmic domain (Torroja et al., 1999). Overexpressing APPL results in a large increase in bouton buds. This proliferation of buds is completely suppressed when an APPL variant lacking the entire cytoplasmic region is expressed. Furthermore, expressing an APPL variant (APPLΔC), in which an amino acid sequence is deleted at the cytoplasmic region (GYENPTY), suppresses the overproliferation of buds. These observations have led to the suggestion that the GYENPTY sequence is fundamental for the regulation of bud number (Torroja et al., 1999).

In mammals, the GYENPTY sequence is conserved and is required for endocytosis of APP (Lai et al., 1998; Perez et al., 1999). Proteins that bind to the GYENPTY sequence modulate APP trafficking and/or prevent the cleavage of APP at the cell surface (Borg et al., 1996; Sastre et al., 1998; Sabo et al., 1999; Ando et al., 2001; Taru et al., 2002; King et al., 2003). One such protein is X11/Mint, which is highly expressed in neurons (King and Turner, 2004). X11 contains two postsynaptic density-95/zona occludens-1 (PDZ) domains and one phosphotyrosine

interaction/PTB domain that interacts with APP (Zhang et al., 1997). Yeast two-hybrid assays and studies in heterologous cells show that APP and X11 interact and that deleting the PTB domain prevents these interactions (Borg et al., 1996; Sastre et al., 1997; Sabo et al., 1999; Tomita et al., 1999). Similarly, in flies, it has been demonstrated that APPL and the Drosophila homolog of X11, dX11/dX11L, interact in vivo and in a yeast two-hybrid assay (Hase et al., 2002). However, the ability of the proteins to interact in an in vivo context has not been tested.

To determine whether dX11 and APPL interact in vivo and whether dX11 might be involved in the FasII–APPL signaling cascade that leads to new synaptic bouton formation, we generated polyclonal antibodies against the N-terminal amino acids 136–266 of dX11 for use in immunocytochemistry. Controls for the specificity of this antibody included Western blot analyses of body-wall muscles and S2 cell extracts showing that anti-dX11 recognized a band of the appropriate molecular weight, dsRNAi in S2 cells, which eliminated immunoreactivity in Western blots, and overexpression of transgenic dX11, which increased immunoreactivity levels in tissue and Western blots (see below).

At wild-type NMJs, we found that dX11 was localized in puncta at presynaptic boutons (Fig. 6A–E). Therefore, we performed immunoprecipitations with anti-dX11 antibodies to determine whether APPL exists in a complex with dX11 and FasII at the body-wall muscles (Fig. 7A). Consistent with this notion, in wild-type body-wall muscle extracts, immunoprecipitation with dX11 antibodies coprecipitated endogenous APPL and FasII but...
failed to coprecipitate tubulin or spectrin, demonstrating the specificity of the immunoprecipitation (Fig. 7A). When APPL levels were enhanced using UAS–APPL, the levels of APPL, but not of FasII, were also enhanced in the dX11 immunoprecipitation. This suggested that FasII may be included in the complex because of its interactions with dX11 and not with APPL. This possibility was addressed by performing the immunoprecipitations in the absence of APPL, in the Appl null mutant, as well as from larvae expressing an APPL variant lacking the GYENPTY sequence, the known site of interaction between dX11 and APPL. We found that, in both situations, the coprecipitation of FasII by dX11 was substantially increased, suggesting that dX11 can interact with FasII independent from APPL (Fig. 7A). Furthermore, because the coprecipitation between FasII and dX11 is stronger in the absence of APPL, these results suggest that APPL may negatively regulate the binding between dX11 and FasII. We also confirmed that the interaction between APPL and dX11 depended on the PTB domain in vivo, as suggested previously by using the yeast two-hybrid assay (Hase et al., 2002) (Fig. 7B).

The above results suggest that FasII can interact in vivo with dX11 in the absence of APPL. However, the possibility exists that FasII and APPL can additionally interact independently of dX11. Because no dX11 null mutant is currently available (but see below), this possibility was addressed by transfecting Drosophila S2 cells. S2 cells express endogenous APPL and dX11 but virtually no FasII (Fig. 7C, input). However, we found that endogenous dX11 could be completely eliminated by using dsRNA (Fig. 7C, input). To address the possibility that APPL and FasII may interact with independence from dX11, we transfected S2 cells with dX11 and FasII. In some of these experiments, S2 cells were additionally treated with dX11 dsRNA to eliminate endogenous dX11. We found that antibodies against APPL strongly coprecipitated both FasII and dX11 in cells double transfected with FasII and dX11, and no such coprecipitation was observed in untransfected cells (Fig. 7C). However, similar levels of FasII were coprecipitated by APPL antibodies when cells were treated with dX11 dsRNA, despite the dramatic reduction in dX11 levels in treated cells (Fig. 7C). Because substantial residual dX11 was still detected in the extracts despite dX11 dsRNA treatment, we repeated these experiments in cells transfected with only FasII, with the expectation that dsRNA would completely eliminate endogenous dX11. We found that, in this case, dX11 dsRNA completely eliminated endogenous dX11, but FasII was still coprecipitated (Fig. 7C). These results suggest that APPL and FasII can interact even in the absence of dX11.

These observations show that dX11 is present at the NMJ and that it interacts with both APPL and FasII in vivo and in S2 cells. To understand the significance of dX11 during NMJ expansion, we used several approaches, including the characterization of a hypomorphic dX11 mutant, the generation of transgenic flies carrying full-length dX11 (UAS–dX11) to be used for gain-of-function studies, and the generation of flies carrying a dX11 transgene lacking the APPL-binding PTB domain (UAS–dX11ΔPTB). In our coprecipitation experiments, we found that the dX11ΔPTB transgene reduced the interactions between dX11 and APPL (Fig. 7B). For the loss-of-function studies, we used a strain carrying a P-element insertion 169 bp from the dX11 gene transcription start (dX11P). Western blot analysis of body-wall muscles from dX11P over a deficiency of the dX11 region, Df(1)BK10, demonstrated that, in this mutant, there was a substantial decrease in dX11 signal, but the levels of FasII and APPL were normal (Fig. 7D).

Notably, we found that overexpression of dX11 in motoneurons resulted in a substantial increase in bouton number [Figs. 6G–I (arrowheads in H), 7E, F]. Like NMJs overexpressing FasII presynaptically and postsynaptically, and overexpressing APPL presynaptically, the increase in bouton number was primarily attributable to an enhanced proliferation of buds (Fig. 6H, arrowheads). The opposite phenotype, a decrease in bouton number, was observed in dX11P/Df mutants (Fig. 7E, F). This phenotype is similar in extent to ApplH mutants. In both transgenic larvae and mutants, however, the size of the muscles was signifi-
The distribution of APPL was also altered in dX11ΔPTB and dX11P/Df (Fig. 8). In wild type, APPL is found at very low levels at the NMJ associated with the bouton membrane and within the bouton cytoplasm (Torroja et al., 1999) (Fig. 8A, B). Although anti-APPL immunoreactivity is low at NMJs of wild type, this signal is highly specific, because it is completely suppressed in Appl null mutants (Torroja et al., 1999). In dX11P/Df mutants, there was an increase in the levels of APPL inside the boutons, and APPL often appeared there in large accumulations (Fig. 8F, G, I, K) (supplemental Fig. 2C, available at www.jneurosci.org as supplemental material). This was even more evident in NMJs expressing the dX11ΔPTB construct, which resulted in strong APPL accumulations inside all boutons (Fig. 8R–S). In contrast, boutons had normal FasII localization in all dX11 genotypes (Fig. 8C, D, H, I, L, M, P, Q, T, U).

Discussion

Here, we demonstrate that the ability of FasII to function either as a permissive or a restrictive influence on synapse growth depends on a balance of FasII levels between the presynaptic and postsynaptic cells. Furthermore, we show that FasII and APPL form a biochemical complex in vivo and that the ability of FasII to promote new synapse formation requires an APPL-dependent transduction cascade. Finally, we show that dX11 interacts with APPL in vivo and is involved in APPL/FasII-dependent new synaptic bouton formation.

Role of APPL and FasII in promoting new synaptic bouton formation

During the development of the larval NMJ, as muscles continuously increase in size, synaptic efficacy is maintained in part by the formation of new synaptic boutons (Packard et al., 2003). In this process, FasII plays two fundamental roles: one of maintenance, as exemplified in the absence of FasII, when synaptic boutons begin to form but later retract, and a role in bouton proliferation (Schuster et al., 1996a). Although the role of FasII in synaptic maintenance might be related to its ability to mediate cell adhesion between the presynaptic and postsynaptic membranes, its ability to regulate budding mostly depends on genetic interactions with APPL: in the absence of APPL, a symmetric restriction influence on synapse growth depends on a balance of FasII levels between the presynaptic and postsynaptic cells. We found that APPL was required for both FasII-dependent synaptic growth and for many physiological abnormalities accompanying NMJ structural defects. Although the bouton number decrease in Appl null mutants was correlated with a decreased amplitude of evoked synaptic responses, the bouton number increase in fasII276/+ was correlated with increased EJP amplitude. This increase was suppressed (to ApP levels) by eliminating APPL in fasII276/+ mutants. In FasII-pre-post, however, the

Figure 6. dX11 and APPL are found in a complex at the NMJ, and changes in dX11 levels mimic changes in APPL levels. A–C, G–I, L–N. Single confocal slices of NMJs stained with anti-dX11 (A, G, L), anti-HRP (B, H, M), and merged panels (C, I, N). A–O, Specimens are wild type (A–E), larvae overexpressing dX11 presynaptically ([dX11]-pre; F–J), and larvae expressing a dX11 variant missing the PTB domain ([ΔPTB]; pre; K–O). D–F, J–K, O. High-magnification views of boutons from the above genotypes, showing the localization of dX11 in each case. Note that, in wild type and [dX11]-pre, dX11 staining is enriched at the presynaptic membrane (E, J), but only in [dX11]-pre does the protein accumulate at sites of budding (F, arrow). K. In contrast, in dX11ΔPTB, dX11 immunoreactivity is observed within the bouton cytoplasm. I, F, Arrows point to buds accumulating dX11. H, Arrowheads point to buds. Scale bar: (in G) A–C, G–I, L–N, 12 μm; D–F, J–K, O, 3 μm.
dramatic increase in buds was correlated with an EJP amplitude decrease, possibly because these boutons were buds or immature boutons. Similarly, in [APPL]-pre, there was both an increase in buds and a decrease in EJP amplitude. Remarkably, as seen in the fasIIe76/H11001 mutants, the EJP phenotype is suppressed in the Appld background (again, reaching Appld levels). Thus, as in the morphological studies, many physiological abnormalities elicited by changing FasII levels depended on APPL. Interestingly, Appld does mimic many electrophysiological phenotypes reported previously in fasIIe76 homozygotes, in that both show increased mEJP frequency, increased mEJP amplitude, and decreased bouton number (Stewart et al., 1996).

Activation of the synapse-promoting activity of APPL by FasII depended on simultaneous changes in the presynaptic and postsynaptic cell, whereas a unilateral change in FasII in either cell alone interfered with synapse formation. This may relate to the ability of FasII to establish cis- and trans-homophilic interactions and to the exclusive presynaptic expression of APPL (Luo et al., 1990).

FasII signaling through APPL

A genetic interaction between Appl and fasII was clearly demonstrated in our studies. We also demonstrated that both proteins form an endogenous complex at the NMJ and that this complex includes the APPL-binding protein dX11. In these interactions, we found that FasII could independently interact with both APPL and dX11. In the absence of APPL, an interaction between dX11 and FasII was maintained, whereas in the absence of dX11, interactions between APPL and FasII were preserved. Precisely how APPL and FasII proteins interact physically remains unclear. However, we found that, as was the case for APPL, the FasII intracellular domain was essential for the budding phenotype, suggesting that they may interact through their intracellular domains (Torroja et al., 1999). In the case of FasII and dX11, FasII contains a PDZ-binding motif, which interacts with PDZ1–PDZ2 domains of DLG (Thomas et al., 1997). It is possible that PDZ domains of dX11 are alternative FasII-interacting domains.
Role of dX11 in FasII–APPL-modulated synaptic growth

Our studies have identified a third member of the transduction cascade that promotes synaptic growth, dX11. First, dX11 was found in the same complex with APPL at the body-wall muscles, and the lack of the GYENPTY sequence of APP or the PTB domain of dX11 suppressed or dramatically reduced this interaction, respectively. Second, a presynaptic increase of dX11 expression mimicked the effects of upregulating APPL. This effect was found at very low levels at the NMJ of wild type. In dX11 mutants and in boutons from larvae overexpressing dX11ΔPTB, there are large internal accumulations of APPL. FasII staining changes little, if any, among the various dX11 specimens. Scale bar, 3.5 μm. E, Diagram depicting likely interactions between FasII, APPL, and dX11 during bouton budding (see Discussion). Eii, Homophilic binding between FasII molecules at opposing sides of the synapse triggers the activation of APPL, an event that may involve its phosphorylation by the tyrosine kinase Abl. Eiii, APPL phosphorylation allows binding to the PTB domain of dX11, which brings or facilitates the insertion of APPL into the presynaptic membrane. dX11 also binds members of the exocytic machinery, thus bringing these components to the budding bouton. In addition, binding of APPL to FasII activates Gα protein, which modulates the microtubule cytoskeleton required for bud extension.

Figure 8. The localization of APPL at synaptic boutons is disrupted in dX11 mutants and a model of likely interactions between FasII, dX11, and APPL during bouton budding. A–D, F–U, Single confocal slices of representative boutons stained with anti-APPL (A, F, I, N, R), anti-APPL and anti-βIII-tubulin (B, G, K, O, S), anti-FasII (C, H, L, P, T), and anti-FasII and anti-βIII-tubulin (D, I, M, Q, U), in wild type (A–D), dX11/+, FasII/+, dX11/ΔPTB, dX11/ΔPTB (F–K), FasII/ΔPTB (L, M), [dX11][pre–N–Q]; and [dX11ΔPTB][pre–R–U]. Note that APPL is found at very low levels at the NMJ of wild type. In dX11 mutants and in boutons from larvae overexpressing dX11ΔPTB, there are large internal accumulations of APPL. FasII staining changes little, if any, among the various dX11 specimens. Scale bar, 3.5 μm. E, Diagram depicting likely interactions between FasII, APPL, and dX11 during bouton budding (see Discussion). Eii, Homophilic binding between FasII molecules at opposing sides of the synapse triggers the activation of APPL, an event that may involve its phosphorylation by the tyrosine kinase Abl. Eiii, APPL phosphorylation allows binding to the PTB domain of dX11, which brings or facilitates the insertion of APPL into the presynaptic membrane. dX11 also binds members of the exocytic machinery, thus bringing these components to the budding bouton. In addition, binding of APPL to FasII activates Gα protein, which modulates the microtubule cytoskeleton required for bud extension.

The notion that FasII might function not only as a cell adhesion molecule but also as a signaling molecule is not without precedence (for review, see Packard et al., 2003). Indeed, the mammalian FasII homolog NCAM has been shown to initiate a signal transduction cascade after activation of both nonreceptor tyrosine kinases (nRTKs) and RTKs that may influence neurite outgrowth (Beggs et al., 1997). Interestingly, a genetic interaction between FasII and the nRTK Abelson tyrosine kinase gene (Abl) has been reported previously in flies (Garcia-Alonso et al., 1995), and, in mammals, activated Abl interacts directly with and phosphorylates the APP intracellular GYENPTY sequence (Zambrano et al., 2001; Perkinton et al., 2004). Several studies suggest that APPL be-
seem reminiscent of intraneuronal amyloid-β accumulation, which may precede extracellular amyloid plaque formation in Alzheimer’s disease (D’Andrea et al., 2001; Glabe, 2001; Takahashi et al., 2002; Oddo et al., 2003). This phenomenon may provide additional clues toward a mechanism by which interference with normal APP function could lead to pathological events and subsequent symptoms of Alzheimer’s disease.

In conclusion, we demonstrated via genetic analysis that APPL, FasII, and dX11 are involved in the same pathway that regulates synaptic expansion at the Drosophila NMJ. Altogether, these results suggest that beyond a role in cell adhesion, FasII-mediated signaling depends on a precise balance of its levels of expression at the presynaptic and postsynaptic cell and is likely to activate intracellular transduction pathways that control synapse structure.

References


