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Local BMP signaling: A sensor for synaptic activity that balances synapse growth and function

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Abstract

Synapse development is coordinated by intercellular communication between the pre- and postsynaptic compartments, and by neuronal activity itself. In flies as in vertebrates, neuronal activity induces input-specific changes in the synaptic strength so that the entire circuit maintains stable function in the face of many challenges, including changes in synapse number and strength. But how do neurons sense synapse activity? In several studies carried out using the *Drosophila* neuromuscular junction (NMJ), we demonstrated that local BMP signaling provides an exquisite sensor for synapse activity. Here we review the main features of this exquisite sensor and discuss its functioning beyond monitoring the synapse activity but rather as a key controller that operates in coordination with other BMP signaling pathways to balance synapse growth, maturation and function.

1. BMP signaling pathways

Bone morphogenetic proteins (BMPs) are a functionally diverse group of potent secreted molecules belonging to the TGF-β superfamily of growth and differentiation signaling factors. Originally identified in bone extracts as critical inducers of bone deposition (Urist, 1965), BMPs are now recognized to regulate a variety of cellular processes from cell fate specification, renewal and maintenance of stem cell populations to axis determination, organogenesis and tissue patterning (Hogan, 1996). BMPs also play crucial roles in the development of the nervous system, including neuroectoderm induction, neural stem cells and neural crest cells specification and patterning (Bond, Bhalala, & Kessler, 2012). Dysregulation of BMP signaling is associated with many developmental abnormalities and disease states highlighting the need for tight control of this pathway.

BMPs are secreted as pro-proteins with large pro-domains that are removed by furin-type enzymes to generate biologically active ligands (Cui, Jean, Thomas, & Christian, 1998). The pro-domains are required for the proper trafficking and folding of these molecules and formation of several disulfide bonds that (i) stabilize the characteristic cysteine-knot configuration of individual secreted monomers and (ii) covalently link biologically active BMP homo- and hetero-dimers (Degnin, Jean, Thomas, & Christian, 2004; Goldman et

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al., 2006). The BMP signaling cascade is initiated by binding of active dimeric ligands to a multi-component signaling complex composed of at least two different types of transmembrane Ser/Thr kinases, type I and type II receptors (Ehrlich, Gutman, Knaus, & Henis, 2012; Massague, 1990). Within this complex, Type II receptor is a constitutive kinase that phosphorylates and activates Type I receptor. In the canonical BMP pathway, activated Type I receptor binds to and phosphorylates the intracellular R-Smad effectors (Smad 1, 5, or 8 in vertebrates, and Mad in *Drosophila*) (Feng & Derynck, 2005; Schmierer & Hill, 2007). Phosphorylated R-Smads (pSmads) have a propensity to form trimeric complexes that favor their dissociation from the receptors (Kawabata, Inoue, Hanyu, Imamura, & Miyazono, 1998). Cytosolic pSmads associate with highly related co-Smads and translocate into the nucleus where, in conjunction with additional transcription factors, they activate or repress transcription of target genes (Hill, 2016; Zhang, Feng, We, & Derynck, 1996).

Activated BMPRs can also signal independently of Smads through non-canonical pathways that include mitogen-activated protein kinase (MAPK), LIM kinase, phosphatidylinositol 3-kinase/Akt (PI3K/Akt), and Rho-like small GTPases (Derynck & Zhang, 2003; Foletta et al., 2003; Moustakas & Heldin, 2005; Zhang, 2009).

More recently, pSmad accumulation at cell membranes has been reported in at least two instances: (i) at tight junctions during neural tube closure (Eom, Amarnath, Fogel, & Agarwala, 2011), and (ii) at *Drosophila* NMJ (Dudu et al., 2006; Smith, Machamer, Kim, Hays, & Marques, 2012). During neural tube closure, pSmad1/5/8 binds to apical polarity complexes and functions as a bridge to mediate stabilization of BMP/BMPR complexes at tight junctions (Eom et al., 2011); prolonged BMP blockade disrupts tight junctions and disrupts epithelial organization (Eom, Amarnath, Fogel, & Agarwala, 2012). At the fly NMJ, pMad accumulates at the active zones, which are highly specialized membrane regions where synaptic vesicles dock to release the neurotransmitter. Each presynaptic active zone forms an asymmetric tight junction with a postsynaptic density, where neurotransmitter receptors concentrate. In both cases, the Smad-dependent junctional complexes do not participate in any transcriptional regulation activities. Instead, these local BMP signaling complexes appear engaged in interactions that control the integrity and function of the respective cellular junctions.

In epithelial cells, immunofluorescent staining of pSmads reveals very strong nuclear signals, overpowering any junctional pSmads signals. In contrast, at the fly NMJ, junctional pMad localizes at synaptic terminals whereas nuclear pMad accumulates in MN nuclei located in the ventral ganglion, the fly counterpart of the spinal cord. The spatial separation between nuclear and synaptic pMad was crucial to uncovering the function of this Smad-dependent local/junctional BMP signaling pathway in monitoring and modulating the synaptic junction (Sulkowski et al., 2016; Sulkowski, Kim, & Serpe, 2014).

The synaptic BMP signaling is genetically distinct from the other canonical and non-canonical BMP pathways that the fly motor neurons (MNs) must receive and integrate during development (Fig. 1). The NMJ, the synapse between a motor neuron (MN) terminal and a muscle fiber, is established during embryogenesis (Keshishian, Broadie, Chiba, & Bate, 1996). Once it hatches from the egg case, a first instar larva experiences rapid

growth expanding its larval muscle ~160-fold during time it takes until it reaches the third instar larval stage, the last stage of larval development. Canonical BMP signaling pathways coordinate the growing muscles with the MNs, adjusting the NMJs size and neurotransmitter release to ensure adequate depolarization of the muscle membrane and therefore proper muscle contraction and locomotion. These signaling pathways are triggered by the BMP7 homolog, Glass bottom boat (Gbb), secreted from the muscle (retrograde signaling) (McCabe et al., 2003) or from the motor neurons (autocrine signaling) (James et al., 2014) and require two type-I BMP receptors (BMPRI), Tkv and Sax, one type-II BMP receptor, Wit, and the downstream effectors Mad and Medea (Aberle et al., 2002; Marques et al., 2003; McCabe et al., 2004, 2003; Rawson, Lee, Kennedy, & Selleck, 2003). Loss of either Gbb, Sax, Tkv, Wit, Mad or Medea results in small NMJs with reduced number of synaptic boutons that are unable to depolarize the muscle fiber to normal levels. These mutant NMJs have reduced evoked excitatory junction potentials (EJPs) but fairly normal spontaneous miniature potentials (mEJPs, or minis in short). Beside canonical BMP signaling, a non-canonical BMP signaling mediated through BMP7/Gbb and BMPRII/Wit connects the presynaptic structures with the cytoskeleton to ensure structural stability (Eaton & Davis, 2005; Piccioli & Littleton, 2014). This pathway does not involve Mad or Medea. Finally, synaptic BMP signaling does not require Gbb, but relies on type-I and type-II BMPRs and Mad.

In the next sections we will review molecular mechanisms of synapse assembly and maturation then discuss the role of synaptic BMP signaling as a sensor of synapse activity and as a modulator of synapse maturation and plasticity. In the past, we and others have referred to this this pathway as non-canonical BMP signaling. However, this term is technically incorrect since non-canonical BMP signaling is defined as Smad-independent signaling. Albeit not implicated in transcriptional control, Smads are at the center of this local signaling modality, prompting us to revise our nomenclature and refer to this pathway as “local BMP signaling,” or in the case of the fly NMJ “synaptic BMP signaling.”

2. Synapse assembly and recruitment of neurotransmitter receptors at the fly neuromuscular junction

The NMJ is one of the most studied synapses in both vertebrate and invertebrate systems, primarily because of size and accessibility toward histological assays and electrophysiological recordings. In particular, the fly NMJ has been a favorite system to study synapse development and function since 1978, when Jan and Jan performed the first recordings at the larval NMJ and discovered that, like all insects and crustaceans, flies use the amino acid L-Glutamate as neurotransmitter at their NMJ (Jan & Jan, 1976a, 1976b). Subsequent studies revealed that *Drosophila* NMJ is similar in composition and physiology to mammalian central glutamatergic synapses and could serve as a powerful genetic system to analyze and model defects in the structural and physiological plasticity of glutamatergic synapses, which are associated with a variety of human pathologies from learning, memory deficits to autism.

Synapse assembly begins during embryogenesis, as soon as MN axons complete their navigation into the muscles field and arrive at their postsynaptic targets. The MN axon collapses its growth cone into a terminal with varicosities called synaptic boutons which begin to fill with neurotransmitter-packed synaptic vesicles. On the postsynaptic muscle, prior to the MN arrival, the ionotropic glutamate receptors (iGluRs) form small, nascent clusters distributed in the vicinity of future synaptic sites. The arrival of the MN triggers formation of large synaptic iGluR aggregates and promotes expression of more iGluRs which enable synapse maturation and growth. Lack of iGluRs clustering effectively halts the formation of the synapse at a specific time point, after the motor neurons have completed the navigation to their muscle target and the future synapse site has already been prefigured/pre-patterned (Burden, 1998; Kim, Bao, Bonanno, Zhang, & Serpe, 2012; Schmid et al., 2006). A mature synapse contains 40–60 individual iGluR complexes organized by a myriad of postsynaptic proteins; together they form a compact, electron-dense structure, called postsynaptic density (PSD). Each PSD juxtaposes an active zone, with a characteristic presynaptic T-bar structure, where glutamate-filled synaptic vesicles dock prior to release (Fig. 2). One bouton contains up to 20 individual synapses. During growth, the MN terminals add more synaptic boutons along one or more branches that innervate the target muscles and assume “beads on a string” morphologies. One “beads on a string” structure is commonly referred to as an NMJ, but each of these “NMJs” actually include hundreds of individual synapses. It is important to emphasize that synapse assembly and NMJ development are very different processes which are coordinated during development but are controlled by largely different signaling pathways. Several excellent reviews go into more details on NMJ development (Harris & Littleton, 2015; Menon, Carrillo, & Zinn, 2013).

In flies as in humans, synapse strength and plasticity is determined by the interplay between different postsynaptic receptor subtypes with different channel properties. For the scope of this review, we will focus on the two types of postsynaptic iGluR complexes, called type-A and type-B receptors, which are present at relevant NMJ PSDs. Both receptors form Ca^{2+} permeable ligand-gated channels that open upon binding glutamate. However, this is an oversimplification of the system as this synapse is also modulated by presynaptic iGluRs, which function as auto-receptors, and by metabotropic glutamate receptors, which are G-protein-coupled receptors with modulatory roles at larval NMJ (Bogdanik et al., 2004; Kiragasi, Wondolowski, Li, & Dickman, 2017).

Type-A and type-B receptor channels consist of four different subunits: three shared subunits GluRIIC, -IID and -IIE, and either GluRIIA (type-A receptors) or GluRIIB (type-B) (DiAntonio, Petersen, Heckmann, & Goodman, 1999; Featherstone et al., 2005; Marrus, Portman, Allen, Moffat, & DiAntonio, 2004; Petersen, Fetter, Noordermeer, Goodman, & DiAntonio, 1997; Qin et al., 2005). Phylogenetic analysis indicates that all these subunits are closely related to the vertebrate kainate receptors, though the *Drosophila* receptors have strikingly different ligand binding profiles (Han, Dharkar, Mayer, & Serpe, 2015; Li et al., 2016). Like vertebrate kainate receptors, type-A and type-B receptors are modulated by an auxiliary subunit from the Neto (Neuropilin and Tolloid-like) family of proteins (Kim et al., 2012). These highly conserved auxiliary proteins modulate the gating properties of kainate-type glutamate receptors from worms to humans (Ng et al., 2009; Tomita & Castillo, 2012; Wang et al., 2012; Zhang et al., 2009). Neto proteins are single pass transmembrane proteins

with two extracellular CUB (for complement C1r/C1s, UEGF, BMP-1) domains followed by an LDLa (low-density lipoprotein receptor domain class A) motif, a transmembrane domain and fairly divergent intracellular portion. At the fly NMJ, the postsynaptic iGluRs absolutely depend on Neto for their function and recruitment at synaptic sites indicating that Neto functions as an obligatory non-channel subunit for the postsynaptic iGluR complexes (Han et al., 2015; Kim et al., 2012, 2015).

The type-A and type-B receptors have similar single channel amplitude, but their kinetics are strikingly different, with type-B receptors desensitizing 10 times faster than the type-A (DiAntonio et al., 1999). Type-B channels can desensitize even before reaching the open state (Heckmann & Dudel, 1997), producing a much-reduced amplitude of the synaptic response comparing with the type-A channels (DiAntonio et al., 1999; Petersen et al., 1997). Because of this difference in channel properties, the dose of type-A vs type-B at individual synapses is a critical determinant of quantal size (or minis amplitude, mEJPs—the amplitude of the postsynaptic response to the spontaneous fusion of a single synaptic vesicle). Also, GluRIIA and GluRIIB compete with each other for the limiting shared iGluR subunits (GluRIIC, -IID and IIE) and the obligatory Neto. Muscle overexpression of GluRIIA triggers reduction of synaptic GluRIIB-containing receptors and a net increase in the quantal size, whereas overexpression of GluRIIB reduces the levels of synaptic GluRIIA/type-A receptors as well as the quantal size (Marrus & DiAntonio, 2004). Since different mechanisms modulate the synaptic recruitment and the activity of type-A and type-B receptors, flies can adjust synapse strength by differentially regulating these two receptor channels.

The life journey of glutamate receptors starts in ER, where all ionotropic glutamate receptors presumably assemble as tetramers followed by traffic to the cell surface, then to the synapse and stabilization at synaptic sites. Reconstitution studies in *Xenopus* oocytes indicate that only the NMJ iGluR complexes with four distinct receptor subunits are delivered to the cell surface (Han et al., 2015). This explains why in mutants lacking any of the shared subunits (GluRIIC, -IID or -IIE) or GluRIIA and GluRIIB together, none of the other iGluR subunits localizes to nascent synapses; such mutants never form NMJ synapses and die as late embryos, unable to move and hatch into the larval stages (DiAntonio et al., 1999; Featherstone et al., 2005; Marrus & DiAntonio, 2004; Qin et al., 2005). The surface delivery of either GluRIIA/C/D/E and GluRIIB/C/D/E reconstituted tetramer in heterologous systems is quite inefficient suggesting that additional chaperone(s) may facilitate the surface expression of these receptors *in vivo*. Neto has only a modest role in promoting the surface expression of both receptor channels (Han et al., 2015). Instead, Neto plays key roles during the subsequent steps in synapse assembly and function, including regulation of receptors recruitment, synaptic stabilization and function (Han et al., 2015; Kim et al., 2012, 2015; Ramos, Igiesuorobo, Wang, & Serpe, 2015). Genetics and live imaging studies show that Neto forms clusters at nascent NMJs at the time when iGluRs begin to accumulate and cluster (Kim et al., 2012). Moreover, Neto and iGluRs depend on each other for trafficking and stabilization at synapses: in the absence of Neto, the iGluR subunits fail to accumulate at the nascent NMJ, and, conversely, in the absence of iGluRs, Neto is not recruited either. Similar to mutants lacking iGluRs, *neto* mutants never form functional NMJs and die as completely paralyzed embryos. *neto* and *iGluR* mutants

have also distinguishable phenotypes, as iGluR clusters are entirely absent from the muscle membrane in the *iGluR* mutants, whereas small, nascent receptor clusters can be detected in the vicinity of the prepatterned synaptic site (Kim & Serpe, 2013). Together these phenotypes indicate that Neto engages the iGluRs on the muscle membrane and together they traffic and are stabilized at the nascent synapse. Once at the synapse, Neto further modulates the receptors properties and, as will be discussed below, provides a dynamic scaffold for recruitment of PSD proteins and stabilization of selective receptor subtypes (Han et al., 2015; Kim et al., 2012, 2015; Ramos et al., 2015).

In flies as in vertebrates, MNs arrival of at the target muscles trigger clustering of postsynaptic neurotransmitter receptors and formation of large, stable receptor aggregates (Broadie & Bate, 1993; Chen & Featherstone, 2005; McMahan, 1990). Vertebrate MNs accomplish this task partly through the secretion of Agrin, a large glycoprotein implicated in the aggregation of postsynaptic (acetylcholine) receptors (Kim et al., 2008; Reist, Werle, & McMahan, 1992; Zhang et al., 2008). To date, no Agrin-like functions have been discovered at the *Drosophila* NMJ. Dynamic studies indicate that, upon MNs arrival at nascent synapses, fly NMJ iGluRs are incorporated into stably aggregates, with very little if any turnover. In contrast, most PSD components show significant mobility (Rasse et al., 2005). How iGluRs/Neto complexes are stably incorporated into large synaptic aggregates remains an open question. One key player appears to be the obligatory auxiliary subunit Neto which must be activated before being capable to mediate iGluRs clustering (Kim et al., 2015). Neto activities are restricted by an inhibitory prodomain which must be removed by Furin-mediated proteolysis. When the prodomain cleavage is blocked, Neto is properly targeted to the muscle membrane and engages the iGluR complexes *in vivo* but fails to enable the incorporation of iGluRs in stable synaptic clusters. The extracellular N-terminal CUB1 domain of Neto appears to be crucial for iGluR clustering: Neto mutants lacking the CUB1 domain, or its Ca^{2+}-binding capabilities, traffic to the synapses but cannot support formation of iGluR stable clusters and NMJ function (R. Vicidomini et al., unpublished data). Thus, Neto-enabled extracellular interactions provide a “receptors clustering capacity” function which is essential for synaptogenesis. Although the molecular nature of the iGluR clustering mechanism remains to be determined, the results from our laboratory indicate that this process requires an exposed, Ca^{2+}-binding, CUB1 domain.

Why is clustering of the receptors a critical step at the onset of synaptogenesis? There are at least two kinds of arguments for it: From a physiology perspective, the efficacy of the synaptic transmission decreases with the square distance between the site of neurotransmitters release and the site of their reception. Building a tightly packed postsynaptic receptor field that perfectly juxtaposes the release site is therefore paramount for the efficacy of the synaptic function. From a cell biology point of view, aggregates of membrane proteins could remain at the cell membrane and evade endocytosis if they are larger than the opening of the clathrin-coated pits. By clustering and building a large receptor aggregate, the nascent synapse may delay receptors internalization until postsynaptic scaffolds could be recruited and organized to enable further stabilization of postsynaptic receptors. It’s not enough for postsynaptic neurotransmitter receptors to properly traffic to synaptic sites; aggregating these receptors at synaptic locations may hold them in place and initiate the cascade of events that stabilize the receptors at synaptic

sites. In flies as in mammals, the postsynaptic densities function as both synaptic scaffolds and signaling hubs, ensuring the proper density for the neurotransmitter receptors, as well as their composition (in this case, the ratio of type-A: type-B receptors) (Albin & Davis, 2004; Lee & Schwarz, 2016; Liebl & Featherstone, 2008; Morimoto, Nobechi, Komatsu, Miyakawa, & Nose, 2009; Parnas, Haghighi, Fetter, Kim, & Goodman, 2001; Ramos et al., 2015). Later on, these mature postsynaptic structures are thought to function as grids where removal/ turnover of receptors leave empty “slots” and new receptors could slide right in with minimal disruption for the overall synaptic structure and physiology (Nicoll, 2017).

Trans-synaptic signaling and several layers of trans-synaptic interactions mediate the coordinated assembly of presynaptic and postsynaptic structures and control the proper alignment of the active zones with the postsynaptic receptor fields (Banovic et al., 2010; Dalva, McClelland, & Kayser, 2007; Giagtzoglou, Ly, & Bellen, 2009; Li, Ashley, Budnik, & Bhat, 2007; Mosca, Hong, Dani, Favaloro, & Luo, 2012; Ramesh et al., 2021; Thomas & Sigrist, 2012). Closer to the PSDs, presynaptic Nrx and postsynaptic Nlgs are engaged in dynamic interactions that control the sequential recruitment of type-A and type-B receptors to a growing PSD (Owald et al., 2012) and limit the size of the postsynaptic receptor fields (Banovic et al., 2010; Xing et al., 2014). A second layer of trans-synaptic interactions involves integrins and teneurins and anchors the synaptic specializations to the pre- and postsynaptic skeletons ensuring synaptic stability (Koch et al., 2008; Mosca et al., 2012; Stephan et al., 2015; Wang, Han, Nguyen, Jarnik, & Serpe, 2018). Finally, successful establishment of the synapse triggers transcriptional signals in both pre- and post-synaptic compartments and initiates the synthesis and trafficking of new components to the growing synapses.

3. Distinct mechanisms recruit type-A and type-B glutamate receptors

Coordinated locomotion in fly larvae requires several types of neurons, including tonic (Ib) and phasic (Is) glutamatergic MNs, type II octopaminergic and type III peptidergic neurons. Each muscle is innervated by only a single Ib MN that is the primary driver for contraction (Newman et al., 2017). Each Is MN innervates a group of 7–8 muscle fibers and appears to provide coordinated control within each hemisegment as well as along segments. The Ib and Is MNs have different composition of iGluRs: more type-A receptors at Ib and more type-B receptors at Is synapses (Marrus & DiAntonio, 2004). This suggests that the two types of glutamatergic MNs employ different mechanisms for the recruitment of glutamate receptors at their synapses.

During development, type-A receptors are the first to arrive at a nascent synapse and usually form the core of an individual synapse. Type-B receptors arrive after type-A and tend to accumulate at the periphery of a PSD (Akbergenova, Cunningham, Zhang, Weiss, & Littleton, 2018; Rasse et al., 2005). Studies in many laboratories uncovered a rich variety of mechanisms that ultimately impact the synaptic iGluRs abundance and composition (Liebl & Featherstone, 2005; Liebl et al., 2006; Zhao et al., 2020). However, most of this regulation seems to be primarily directed toward modulation of type-A receptors, starting from the stability of the *GluRIIA* transcript and the GluRIIA protein to the synaptic trafficking and retention of the type-A glutamate receptor channels. In this section we will review some of

these mechanisms and highlight the complexity of efforts to control the level and activity of type-A receptors.

The signaling cascade that initiates synaptogenesis and mediates contact-dependent postsynaptic glutamate receptor expression remains unknown. However, this pathway signals to muscle nuclei and triggers a large yet transient burst of iGluRs transcription (Ganesan, Karr, & Featherstone, 2011). Upon neuron arrival, iGluR transcripts abundance increases rapidly in the muscle, but within a few hours decreases and continues to fall throughout larval development.

Several effectors that modulate the iGluRs mRNA abundance have been identified. For example, Lola (longitudinal lacking), a BTB-Zn finger transcription factor, has been implicated in the transcription of the iGluR subunits GluRIIA, -IIB, IIC, as well as dPak (p21 activating kinase), a PSD component (Fukui et al., 2012). Lola is negatively regulated by neural activity, which may account for the limited production of iGluR transcripts as development progresses.

TGF-β signaling promotes transcription of both *GluRIIA* and *GluRIIB* mRNAs but not *GluRIIC* or other postsynaptic components (such as dPak and Dlg) (Kim & O'Connor, 2014). This pathway, activated by either Activin, secreted from MNs, or Maverick from the glia, requires the type-I receptor Babo, the type-II receptor Punt and the dSmad2/Smox effector (Kim & O'Connor, 2014; Sulkowski et al., 2016). In the absence of Smox or Babo, the quantal size is strongly reduced due to the low abundance of both postsynaptic receptors. At the same time, Mav and an activin-like ligand, Dawdle, activate the TGF-β signaling pathway in the muscle to promote the secretion of Gbb and therefore upregulate NMJ growth (Ellis, Parker, Cho, & Arora, 2010; Fuentes-Medel et al., 2012). TGF-β signaling also controls GluRIIA synaptic abundance through post-transcriptional mechanisms; muscle overexpression of a *GluRIIB-GFP* transgene in *Smox* mutants restored the GluRIIB synaptic accumulation, whereas overexpression of *GluRIIA-GFP* did not, with the net GluRIIA-GFP muscle levels remaining undetectable (Kim & O'Connor, 2014). Thus, additional TGF-β transcriptional target(s) in the larval muscle function to stabilize either the GluRIIA *mRNA* or the GluRIIA protein.

Negative regulators of *GluRIIA* and *GluRIIB mRNA* levels include Dicer-1, the endoribonuclease necessary for microRNA synthesis, and microRNAs such as *mir-284* (Karr et al., 2009). Removal of *dicer-1* or *mir-284* de-represses the *GluRIIA* and *GluRIIB* transcripts and induces production of excess extra-synaptic receptors. However, there is no increase in synaptic receptors, consistent with additional mechanisms that limit the synaptic accumulation of these receptors. The translational repressor Pumilio (Pum) and its co-repressor partner Nanos (Nos) act in opposition to each other to regulate glutamate receptor subunit composition and synaptic physiology (Menon, Andrews, Murthy, Gavis, & Zinn, 2009; Menon et al., 2004). Pum binds to the 3′ untranslated regions of *Nos* and *GluRIIA* transcripts repressing their translation, whereas Nos represses GluRIIB. Since GluRIIA and GluRIIB compete with each other for limited, shared iGluR subunits, Pum and Nos form a regulatory network that controls postsynaptic receptor composition. This network which

also involves the translation factor eIF-4E (Menon et al., 2009), has additional presynaptic functions in the control of NMJ growth.

Recent studies revealed that the postsynaptic levels of GluRIIA protein are down-regulated by calpains, which are calcium-activated proteases (Metwally, Zhao, Li, Wang, & Zhang, 2019). Since iGluRs are Ca^{2+}-permeable channels, the idea that calcium negatively regulates postsynaptic GluRIIA abundance is very appealing. It is still unclear whether calpains directly cleave GluRIIA *in vivo*; *in vitro* studies map putative processing sites within an extracellular (ligand binding) domain of GluRIIA, whereas calpains are primarily Ca^{2+}-sensing cytoplasmic enzymes.

During development, individual synapses grow until they reach a maximum size and do not split; instead, the larvae initiate a *de novo* synapse (Rasse et al., 2005). The limited size of mature synapses presumably reflects a limited capacity for receptor clustering and may contribute to the competition between type-A and type-B receptors for synaptic stabilization. Indeed, as described above, muscle overexpression of GluRIIA leads to reduction in GluRIIB synaptic abundance and vice versa (DiAntonio et al., 1999; Petersen et al., 1997). A growing body of literature indicates that the synaptic recruitment of type-A receptors depends on multiple postsynaptic components and on the activity of type-A receptor channels themselves (Lee & Schwarz, 2016; Liebl & Featherstone, 2005, 2008; Ljaschenko, Ehmann, & Kittel, 2013; Morimoto et al., 2009; Parnas et al., 2001; Petzoldt et al., 2014; Ramos et al., 2015). In contrast, type-B receptors appear to be incorporated by default and appears limited only by individual synapse's capacity for clustering receptors in stable aggregates. In other words, it takes work to capture/stabilize type-A receptors in stable synaptic aggregates, whereas type-B receptors seem to be incorporated by default, when the synaptic recruitment of type-A receptors fails. Examples supporting this view are abundant throughout embryonic and larval stages of development, indicating a consistent strategy for the synaptic recruitment of NMJ iGluRs.

For example, during late embryogenesis stages, Coracle, the *Drosophila* homologue of the mammalian cytoskeletal protein 4.1, binds to the GluRIIA cytoplasmic domain and appears to anchor type-A receptors to the actin cytoskeleton, promoting their synaptic accumulation (Chen, Merino, Sigrist, & Featherstone, 2005). The p21-activated kinase (PAK) co-localizes with the iGluR complexes at PSDs and, in conjunction with the guanine nucleotide exchange factor Pix and the adaptor protein Dreadlocks, promotes the synaptic accumulation of GluRIIA-containing receptors (Albin & Davis, 2004; Parnas et al., 2001).

The ability to recruit and stabilize type-A synaptic receptors appears to rely heavily on Neto-β, the predominant Neto isoform in the postsynaptic muscle (Ramos et al., 2015). Neto-β associates with iGluRs but also provides a dynamic postsynaptic scaffold through its large cytoplasmic domain rich in putative protein interaction motifs and docking sites. Animals lacking parts of Neto-β intracellular domain fail to recruit dPAK (Ramos et al., 2015) and dPix (Vicidomini et al, unpublished) at synaptic sites and have significantly reduced postsynaptic structures. These mutants have normal muscle levels of *GluRIIA* and *GluRIIB* transcripts but fail to concentrate type-A receptors and instead show increased synaptic accumulation of type-B iGluRs. Complete removal of Neto-β further decreases

the ability of these synapses to recruit GluRIIB-containing receptors. These phenotypes support the view that at individual synapses Neto-mediated extracellular interactions limit the iGluRs clustering capacity, whereas Neto-dependent intracellular interactions provide a dynamic scaffold that controls PSD assembly and composition.

Importantly, not all type-A receptors can be incorporated in stable clusters. Studies on receptor dynamics and regulations indicate that inactivated and/or channel impaired GluRIIA-containing receptors can traffic to the synapses but do not accumulate there (Petzoldt et al., 2014). Postsynaptic PKA and CaMKII activities reduced the synaptic GluRIIA levels (Davis, DiAntonio, Petersen, & Goodman, 1998; Morimoto et al., 2009; Sulkowski et al., 2014), presumably by inactivating the GluRIIA-containing receptor channels. Thus, type-A receptors must be active for proper synaptic accumulation. This emerging sequence of events and regulatory networks will likely be further explored as additional studies and genetic screens uncover new putative modulators of the synaptic recruitment of type-A and type-B receptors. It is important to note that Ib synapses contain more GluRIIA-containing receptors, whereas Is synapses contain more GluRIIB (Marrus et al., 2004). Some of the experimental settings where more GluRIIB has been observed at the NMJ may be due to increased number of Is boutons relative to Ib. The relationship between Ib and Is terminals is a hot topic today and is carefully examined in several laboratories (Aponte-Santiago, Ormerod, Akbergenova, & Littleton, 2020; Wang, Lobb-Rabe, Ashley, Anand, & Carrillo, 2021).

Why would synaptic stabilization of type-A receptors require so much control and complex regulation? The reasons behind this preferential control may reflect several very practical considerations. First, type-A receptors have the potential to allow more ion flow than type-B receptors in response to neurotransmitter release. Type-A receptors have desensitization rates an order of magnitude lower than type-B (DiAntonio et al., 1999); upon binding of glutamate, they will stay open a lot longer than the type-B receptors, evoking substantially stronger postsynaptic depolarization. Also, type-B receptors seem to be utilized only during development and are not expressed in the adult abdominal muscle (Diao et al., 2015; Li et al., 2022). During evolution, flies had little incentives to invest in building regulatory networks for recruiting the type-B receptors. Instead, a tight control of the recruitment and stabilization of type-A receptors coupled with a “default” incorporation type-B receptors may have been a more sensible strategy. Should a larva need increased synaptic strength, the immediate solution would be to incorporate more type-A synaptic receptors and increase the GluRIIA/GluRIIB ratio of synaptic receptors. This is exactly what has been observed at individual synapses upon (optogenetic) stimulation; conversely, input-specific low stimulation drove type-A receptor out of the corresponding PSDs (Akbergenova et al., 2018). Developmental studies over a longer time frame yielded similar results: the levels of synaptic type-A receptors increased in response to increased locomotor activity and decreased in less mobile animals (Sigrist, Reiff, Thiel, Steinert, & Schuster, 2003). This positive feedback appears to (i) initially promote the incorporation type-A receptors at the core of new synapses during growth (Schmid et al., 2008) then (ii) adjust the levels of type-A receptors as a function of synapse activity during maturation. The absence of type-A receptors triggers a different kind of (negative) feedback mechanism characterized by a

compensatory increase in presynaptic neurotransmitter release (reviewed in Davis & Muller, 2015; Frank, 2014).

Flies must be able to carefully monitor the type-A receptors synaptic distribution and function to efficiently adjust their postsynaptic accumulation and initiate positive or negative feedback mechanisms. How do flies accomplish this task? In the next section we will review our findings that phosphorylated Mad (pMad) accumulates at the presynaptic active zone in a pattern that mirrors the postsynaptic type-A receptors activity (Sulkowski et al., 2016, 2014), indicating that synaptic pMad functions as a sensor of synapse activity.

4. pMad as a sensor of synapse activity

Detection of phosphorylated, BMP-activated Smads is routinely accomplished with phospho-specific antibodies raised against the C-terminal sequence -IS(pS)V(pS). In flies, a variety of phospho-specific antibodies capture pMad accumulation in MN nuclei, but also in discrete puncta at synaptic terminals. While many researchers tend to examine the two populations of pMad together, the nuclear and synaptic pools represent clearly different signaling inputs and have different developmental outcomes: the nuclear pMad accumulation is a result of canonical BMP signaling and will lead to transcriptional regulation of target genes, whereas synaptic pMad appears to be a bona fide sensor for synapse activity with no function in BMP-modulated transcriptional control (Smith et al., 2012; Sulkowski et al., 2014).

Several lines of evidence support a role for synaptic pMad as sensor of synapse activity. First, pMad signals are selectively lost at NMJs with reduced levels of postsynaptic iGluR/Neto complexes, such as observed in *neto* and *GluRIIC* hypomorphs (Sulkowski et al., 2014). In contrast, nuclear pMad persists in MN nuclei, and expression of BMP target genes remains unaffected by alterations in synaptic pMad levels, indicating a specific impairment in pMad production/maintenance at synaptic terminals. Second, synaptic pMad mirrors the accumulation of postsynaptic type-A subtypes (Fig. 3). Third, synaptic pMad accumulation follows the activity and not the net levels of postsynaptic type-A receptors: a pulse of postsynaptic increase in PKA activity, which inhibits the activity of type-A receptors (Davis et al., 1998), decreases the synaptic pMad levels without changing the net levels of synaptic GluRIIA. Since pMad accumulation at synaptic terminals mirrors the activity and not the levels of type-A receptors, synaptic pMad appears to serve as an exquisite monitor for synapse activity.

Both synaptic and nuclear pMad accumulate in MNs: Expression of *Mad* transgenes in the MNs but not in the muscles restores both nuclear and synaptic pMad signals in *Mad* mutants (Sulkowski et al., 2016, 2014). However, the two pMad pools mark distinct pathways that are independently regulated, have different pathway components and different functions. Genetic manipulations of GluRIIA receptor levels in the muscle induce proportional changes in synaptic pMad and in quantal size (mEJPs amplitude) but have no effect on nuclear pMad. *GluRIIA* mutant animals have no synaptic pMad but have normal size NMJs indicating that synaptic pMad does not contribute to NMJ growth. Conversely, overexpression of Mad-GFP in MNs induces strong accumulation of nuclear pMad but has no effect on the synaptic

pMad levels. Moreover, in the absence of GluRIIA, synaptic pMad is undetectable even though excess Mad-GFP accumulates at synaptic terminals. Therefore, nuclear pMad is completely independent of the GluRIIA status, whereas synaptic pMad absolutely mirrors the levels of active, GluRIIA-containing postsynaptic receptors. Overexpression of activated Sax and Tkv receptors in motoneurons increases the EJPs (which depends on nuclear pMad) without affecting quantal size, which indicates normal GluRIIA:GluRIIB levels, therefore normal synaptic pMad (Ball et al., 2010). Nuclear and synaptic pMad share some of the BMP pathway components, such as the BMPRIs Sax and Tkv, and the BMPRII Wit. Interestingly, Gbb is only required for nuclear pMad and is dispensable for synaptic pMad (Sulkowski et al., 2016). The presence of unique and also common pathway components demonstrates that synaptic pMad marks a genetically distinguishable BMP signaling pathway that is distinct from but coordinated with the other BMP pathways via shared components.

Super-resolution fluorescence microscopy studies indicate that the pMad-positive domains distribute into thin discs of ~700 nm diameter sandwiched in between the presynaptic active zones, the sites of neurotransmitter release and the postsynaptic iGluRs fields (Fig. 4). This is a very crowded presynaptic milieu critical for docking and priming readily releasable synaptic vesicles and for clustering and positioning voltage-gated Ca^{2+} channels that control neurotransmitter release induced by action potentials (reviewed in Van Vactor & Sigrist, 2017; Zhai & Bellen, 2004). Why does a transcription factor like pMad accumulate at these synaptic specializations and what is it doing there? The size and shape of the pMad domains suggest that pMad associates with membrane-anchored complexes at the active zone. This is a very unusual distribution for Smad proteins, which are known to shuttle between cytoplasm and nucleus, depending on their phosphorylation status. The only enzyme known to phosphorylate Mad at the C terminus is Tkv, the BMPRI. Tkv can bind to and phosphorylate Mad only after it is phosphorylated by trans-activation upon the assembly of the BMP/BMPR signaling complexes. Since BMP signaling complexes are generally short lived, the synaptic pMad-positive domains likely represent pMad that, upon phosphorylation, remains associated with the activated BMP/BMPR complexes at presynaptic sites. Consistent with this model, synaptic pMad is lost upon knocking down BMPRs in the motor neurons (Sulkowski et al., 2016). This model also implies that active mechanisms must exist (i) to trap the active BMP/BMPR complexes at the active zones and (ii) to keep the product of an enzymatic reaction, pMad, associated with its own enzyme, Tkv, within the BMP/BMPR signaling complexes. Some possible mechanisms will be discussed below.

More importantly, how is the status of postsynaptic receptors relayed across the synaptic cleft? Structural studies on glutamate receptors indicate that these receptors have unusually large extracellular domains that expands ~140Å within the 200Å synaptic cleft (He et al., 2021; Sobolevsky, Rosconi, & Gouaux, 2009). These domains undergo significant conformational changes during the receptor's gating cycle (Meyerson et al., 2016, 2014). The iGluR tetramers adopt a Y-shape structure organized in layers that include (i) the amino terminal domain (ATD), which plays a role in tetramer assembly, (ii) the ligand binding domain (LBD) which forms a clam shell shaped structure that closes to trap glutamate and (iii) the transmembrane domain (TMD), which forms the ion pore. Binding

of glutamate triggers a corkscrew motion which shortens the Y tetramer and opens the ion pore (Meyerson et al., 2016). Cryo-electron microscopy studies revealed additional conformational states in which the upper arms of the Y-shaped tetramer splay apart to different extents (reviewed in Mayer, 2021). In addition, the native glutamate receptor complexes contain auxiliary subunits, such as Neto, that physically associate with the iGluRs and influence their biology (reviewed in Jackson & Nicoll, 2011; Tomita, 2010). Recent Cryo-EM studies revealed the large extent of Neto2 association with the vertebrate kainate receptor, GluK2 (He et al., 2021). Neto2 wraps around the receptor along an exposed receptor surface, with Neto's extracellular domains crosslinking different tetramer subunits within the ATD and LBD layers and the Neto's transmembrane domain associating tightly with the receptor TMD. This topology explains the role of Neto proteins in modulating channel gating (He et al., 2021). This topology also indicates that the CUB domains of Neto should be very sensitive to the channel state and should register any conformational changes within the receptor channel complex.

Expanding on this view, *Drosophila* Neto should easily distinguish between type-A and type-B postsynaptic glutamate receptors as well as their activity states. In addition, The CUB domains also bind BMPs or BMP modulators and have been implicated in a wide variety of extracellular protein interactions during development (Bork & Beckmann, 1993; Lee, Mendes, Plouhinec, & De Robertis, 2009). Neto may bind postsynaptic type-A receptors while at the same time use its BMP-binding CUB domains to reach out and engage presynaptic BMP signaling components, anchoring the presynaptic BMP/BMPR complexes at the active zones and promoting the synaptic pMad accumulation (Fig. 5). This model predicts that Neto enables trans-synaptic interactions only when bound to active postsynaptic type-A receptors. Depletion or inactivation of postsynaptic type-A receptors should trigger conformational changes that disrupts the Neto-dependent trans-synaptic interactions and restores the mobility of BMP/BMPR complexes, dampening the accumulation of synaptic pMad. By bridging both the postsynaptic iGluRs and the presynaptic BMP/BMPR complexes, Neto in a perfect position to sense the conformation of iGluR complexes and relay the status of these postsynaptic receptor channels to the presynaptic BMP signaling complexes.

The idea of Neto-centered trans-synaptic interactions raises the possibility that synaptic pMad may be a sensor of synapse activity with additional role(s) in influencing synapse composition and/or function. In the next section we will review a series of experiments that lead to the discovery of a positive feedback mechanism in which active type-A receptors induce accumulation of pMad at active zones which, in turn, promotes stabilization of type-A receptors at PSDs.

5. A positive feedback loop stabilizes glutamate receptor subtypes as a function of activity

The first hint of a positive feedback mechanism came from examining the composition of postsynaptic glutamate receptors in mutants with aberrant synaptic pMad levels. Interestingly, loss of synaptic pMad (in *impβ11*, *wit* and *mad* mutants) correlates with a

decreased GluRIIA/GluRIIB ratio and reduced quantal size, while increased synaptic pMad (such as in *nrx* mutants) correlates with increased GluRIIA/GluRIIB ratio and increased quantal size (Sulkowski et al., 2016). In contrast, the presence of synaptic pMad even in a transcriptionally impaired BMP mutant (such as g*bb*) ensures relatively normal GluRIIA/GluRIIB ratio and quantal size. This tight correlation suggests a feedback mechanism whereby active postsynaptic GluRIIA receptors induce the accumulation of pMad at active zones, which in turn promotes the stabilization of GluRIIA receptors at postsynaptic sites.

But to demonstrate this feedback mechanism, one must disrupt the local pMad accumulation, without affecting the other BMP signaling pathways canonical. This precludes the use of any BMP signaling components or known BMP modulators, as any such manipulations will affect both local and transcriptional functions of BMP pathway. One possible solution came from post-translational modifications of Mad, which have the potential to influence the association between pMad and its own kinase, Tkv, and differentially disrupt the accumulation of synaptic pMad. Indeed, among mutants with increased synaptic pMad, previous studies identified the *nemo (nmo)* locus (Merino et al., 2009; Zeng, Rahnama, Wang, Sosu-Sedzorme, & Verheyen, 2007). Nmo is a MAPK-related kinase which phosphorylates Mad at S25 and promotes its nuclear export. Lack of S25 phosphorylation (in *nmo* mutants) decreases the nuclear pMad levels, due to increased nuclear export, but increases the synaptic pMad, presumably by promoting the pMad-BMPRs association at synaptic sites. Nmo does not appear to interfere with the ability of BMP/BMPR signaling complexes to phosphorylate Mad at its C-terminal residues. Intriguingly, neuronal overexpression of Tkv, but not Mad, rescues the normal levels of nuclear pMad in *nmo* mutants. This indicates that Tkv becomes limiting in the absence of Nmo and excess amount of neuronal Mad cannot compensate for limiting enzyme.

We predicted that overexpression of a Nemo-phosphomimetic Mad variant (S25D) in the MNs should not affect the nuclear pMad levels since excess Mad^{S25D} should be efficiently exported from the MN nuclei. However, at active zones, excess Mad^{S25D} should compete (via mass action) with endogenous Mad for BMPR-mediated phosphorylation. Only endogenous pMad should remain associated with BMP/BMPR complexes, whereas $pMad^{S25D}$ should likely fall off from the presynaptic BMP/BMPR complexes. The consequence of Mad^{S25D} neuronal overexpression should be normal accumulation of nuclear pMad but diminished synaptic pMad. This is indeed what was experimentally observed: reduced synaptic pMad levels but no detectable deficits in the other BMP signaling pathways (Fig. 6) (Sulkowski et al., 2016). More importantly, selective loss of synaptic pMad was accompanied by reduced GluRIIA/GluRIIB ratio at synaptic sites and reduced quantal size. In contrast, overexpression of a phospho-impaired Mad^{S25A} variant has no influence on quantal size. These results indicate that diminished synaptic pMad accumulation in the motor neurons causes a direct reduction of postsynaptic type-A receptors. This is consistent with a positive feedback mechanism in which active type-A receptors induce accumulation of pMad at active zones which, in turn, promotes stabilization of type-A receptors at PSDs. Since GluRIIA and GluRIIB compete with each other for limiting components, reduced type-A receptors enable further synaptic stabilization of type-B receptors and induce a net change in the synaptic accumulation of iGluR subtypes toward more type-B receptors.

How do postsynaptic glutamate receptors modulate presynaptic pMad and in turn are stabilized by it? Trans-synaptic Neto-centered interactions that couple active postsynaptic type-A receptors with presynaptic BMP/BMPR complexes may provide the simplest explanation for this phenomenon (Fig. 7). Such trans-synaptic complexes could offer a versatile means for relaying iGluRs/Neto activity status to the presynaptic neuron via fast conformational modifications. At the same time, these trans-synaptic nanocolumns may function as “accumulation centers,” holding the active type-A receptors at synaptic sites and facilitating interactions that stabilize the type-A receptors at PSDs. Inactivation of type-A receptors will induce conformational changes that disrupt the trans-synaptic complexes and stop further the incorporation of type-A receptors at PSDs. Also, type-A receptors arriving at the synapse in an inactive state will be unable to engage in trans-synaptic interactions and will evade incorporation in stable clusters, retaining their mobility. Importantly, only the type-A receptors require such positive feedback for their stable incorporation at PSDs. In contrast, the recruitment of (GluRIIB-containing) type-B receptors remains fairly constant during different developmental stages or in different synapse activity states and is limited by the competing GluRIIA and by the “clustering capacity” at individual synapses.

This positive feedback mechanism provides a molecular basis for thinking about several key steps during synapse assembly, maturation and plasticity. For example, this mechanism can explain the positive feedback that promotes incorporation of type-A receptors at new synapses, then restrains the type-A receptors accumulation during synapse maturation (Akbergenova et al., 2018; Schmid et al., 2008). Type-A receptors are the first to arrive at a nascent synapse and are incorporated in stable aggregates at the center of nascent synapses for as long as they are active. Inactivation of type-A receptors will pause their further synaptic stabilization and allow for the incorporation of type-B receptor channels, which mark more mature synapses. This sequence of events has been directly observed through dynamic studies, as discussed above (Schmid et al., 2008). In addition, several indirect arguments demonstrated that channels properties and gating behavior influence the trafficking of GluRIIA-containing receptors and their stable incorporation at PSDs. First, photobleaching experiments showed that channels with reduced activity/charge transfer (such as the fast-desensitizing GluRIIA^{E783A}-containing mutant receptors) have increased mobility; these channels accumulate prematurely during early steps of synapse assembly but fail to be stabilized at synaptic locations and in time segregate away from wild-type GluRIIA-marked PSDs and from the Brp-marked active zones (Petzoldt et al., 2014). Second, the GluRIIA C-tail has been implicated in PKA- and calcium/calmodulin dependent protein kinase II (CaMKII)-dependent phosphorylation which reduces receptor activity and induces eviction from PSDs (Davis et al., 1998; Morimoto et al., 2009). Thus, postsynaptic signaling and calcium levels can immediately impact the activity of type-A receptors and therefore their ability to be incorporated in stable synaptic clusters.

This positive feedback mechanism can also explain the Hebbian mode of GluRIIA incorporation at PSDs (Ljaschenko et al., 2013). Indeed, local stimulation increases postsynaptic sensitivity by promoting synapse-specific recruitment/stabilization of type-A glutamate receptors. Conversely, GluRIIA-containing receptors are rapidly removed from synapses with reduced activity. Since the activity/ conformation status of type-A receptors can be immediately adjusted in response to (calcium) signaling, any increase or decrease in

synapse activity can be relayed to Neto-enabled trans-synaptic complexes which can adjust and fine-tune the incorporation of type-A receptors in stable aggregates.

Trans-synaptic nanocolumns that align active zone structures with postsynaptic receptor fields have been recently described at vertebrate and fly synapses. Studies that mapped vesicle fusion positions within individual vertebrate synapses indicate that action potential evoked fusion occurs within nanometer-scaled regions with higher local density of Rab3 interacting molecule (RIM), a protein that couples vesicle recruitment to release sites (Sudhof, 2012). In hippocampal neurons, these RIM nanoclusters align perfectly with high density patches of postsynaptic receptors and PSD95, a scaffold protein which stabilizes the receptors (Tang et al., 2016). Similarly, at the fly NMJ high probability release sites accumulate elevated levels of Cacophony (Cac), a voltage-gated Ca^{2+} channel, Brp, Rim and Rim-binding protein complexes (Akbergenova et al., 2018; Van Vactor & Sigrist, 2017; Zhai & Bellen, 2004). Classic electrophysiology recordings revealed that glutamate receptors preferentially cluster at sites with high release probability (Marrus & DiAntonio, 2004). More recently, dynamic studies uncovered a strong positive correlation between the levels of postsynaptic type-A receptors and the active zones with high release probability (Akbergenova et al., 2018; Fouquet et al., 2009; Rasse et al., 2005). GluRIIA concentrates in “bright” PSD fields opposing high release probability sites, marked by high accumulation of Cac and Brp. In contrast, low release probability active zones have reduced presynaptic Brp and Cac intensities and more diffuse “dim” GluRIIA levels (Akbergenova et al., 2018; Gratz et al., 2019). Within individual synapses, line profiles drawn along various PSDs captured GluRIIA intense puncta concentrated at the center of the “bright” PSD, surrounded by a ring of GluRIIB signals. At “dim” PSDs, GluRIIB was more evenly distributed across the entire receptor field (Akbergenova et al., 2018).

The striking correlation between the intensity/activity of postsynaptic type-A receptors and the corresponding levels of both (i) presynaptic pMad and (ii) presynaptic Cac and Brp at individual active zones suggests that the Cac- and Brp-marked structures surrounded by pMad and the postsynaptic type-A receptors are organized in trans-synaptic nanocolumns. Such nanocolumns appear to be at the core of a positive feedback loop that enables recruitment of type-A receptors as a function of receptors activity. This model opens a lot of exciting possibilities as it provides a molecular framework for understanding how different subtypes of postsynaptic glutamate receptors are selectively recruited and stabilized at the onset of synaptogenesis. This model also brings additional insights into how pre- and post-synaptic structures develop coordinately. Future super-resolution and protein mobility studies should bring further clarity to the subsynaptic distribution of the individual protein components. Also, is the recruitment of presynaptic pMad coordinated with the recruitment of Cac and Brp and/or with the other presynaptic molecules implicated in neurotransmitter release? Does synaptic pMad mark a more general positive feedback mechanism that monitors postsynaptic activity status and coordinates and adjusts the recruitment of presynaptic active zone components? All these possibilities remain to be explored. It is important to note that this positive feedback mechanism is completely over-ruled when genetic or other challenges alter synapse activities; such perturbations trigger negative feedback mechanisms aimed at restoring the circuit steady-state function. For example, loss of postsynaptic GluRIIA activities induces a significant enhancement of Brp

accumulation which mobilizes the readily released pool of synaptic vesicles and enables a compensatory increase of neurotransmitter release (Davis & Muller, 2015; Frank, 2014). This is in sharp contrast with the normal settings for synapse development and function, where the positive feedback mechanism coordinates the recruitment of postsynaptic type-A receptors and effectively controls key steps of synapse assembly and maturation.

At the molecular level, this positive feedback also requires that synaptic pMad remains associated with the BMP/BMPR complexes at the active zones. The active zones localize to regions of subsynaptic membranes that experience abundant exocytosis of synaptic vesicles to release neurotransmitters; however, these specialized presynaptic membranes have no endocytosis. Therefore, the BMP/BMPRs confined within this membrane microdomain should be shielded from endocytosis and from further recycling or retrograde transport to the motor neuron soma. By remaining associated with its own kinase (the BMPRs), pMad may actively anchor the BMP/BMPR complexes at active zones, protecting them from endocytosis and retrograde transport. Tissue culture-based assays revealed that pMad indeed can accumulate at cell membranes through its association with the activated intracellular domain of the BMPRI, Tkv (Nguyen, Han, Newfeld, & Serpe, 2020). Genetics and molecular modeling studies indicate that this interaction is partly mediated through the enzyme-substrate/product (Tkv-Mad/pMad) binding interface; in addition, the Tkv-pMad interaction requires a highly conserved motif within the Smad-type proteins, the H2 helix. Genetic variants within the H2 helix have been uncovered in several patients with neuronal deficits or epithelial abnormalities suggesting that this motif may be critical for local BMP signaling and the integrity of the tight junctions throughout the animal kingdom (Nguyen et al., 2020). The H2 contribution may be direct, by shaping the Mad-Tkv interface, or indirect, via recruiting other protein(s) that may stabilize Mad-Tkv complexes at specialized cell junctions. In the chick neural tube, local BMP signaling controls apicobasal polarity partly by enabling the pSmad1/5/8-dependent association of BMP/BMPR signaling complexes with the partitioning defective (PAR) proteins, the PAR3-PAR5-aPKC complex, at the tight junctions (Eom et al., 2011). Reduction of junctional pSmad1/5/8 accumulation destabilizes the PAR complexes and disrupts the tight junctions. It is interesting to point out that the opposite phenomenon, the dissolution of the tight junctions and the epithelial-to-mesenchymal transition, requires TGF-β signaling and direct phosphorylation of another PAR protein, Par6, by the type-I TGF-β receptor (Ozdamar et al., 2005). Par6 mutants that can no longer be phosphorylated block TGF-β induced tight-junction dissolution. Likewise, loss-of-function disruptions of *Drosophila bazooka* (Par-3)-Par-6-aPKC complexes produces NMJs with increased levels of synaptic type-A receptors (therefore increased synaptic pMad levels) and significantly reduced number of boutons (a hallmark of reduced levels of nuclear pMad) (Ruiz-Canada et al., 2004). Together these studies suggest a dynamic interplay between various PAR complexes and the local pSmad accumulation in regulating specialized cellular junctions.

The stability of Tkv-pMad interactions may also be influenced by Scribble (Scrib), a basolateral determinant which instructs the polarized trafficking machinery and regulates the apical-basal protein distribution of epithelial cells (Bilder & Perrimon, 2000). In pupal wing epithelia, Scrib regulates Tkv localization to the basal side, where the secreted BMP ligands —which specify the posterior crossvein structure—accumulate (Gui, Huang, & Shimmi,

2016). It was proposed that Scrib facilitates Tkv internalization and thus optimizes signal transduction after the formation of BMP/BMPR complexes. Interestingly, Scrib appears to bind directly to Tkv and together they colocalize with pMad at the basal side of epithelial cells. Scrib is expressed in MNs and has been implicated in the regulation of vesicle release at the larval NMJ (Roche, Packard, Moeckel-Cole, & Budnik, 2002). It remains to be determined whether Scrib plays any role in the MNs in regulating Tkv/pMad interactions at the active zones or within the adjacent, endocytosis-able regions. Since many BMPRs are shared among different BMP signaling modalities, mechanisms that regulate their confinement to the endocytosis-free active zones vs distribution to other membrane domains of the synaptic boutons should have profound effects on the relative contribution of various types of BMP signaling and effectively sculpt synapse development.

6. Motor neurons coordinate multiple BMP signaling to balance NMJ growth with synapse maturation/stabilization

As discussed above (Fig. 1), several BMP signaling pathways shape the *Drosophila* NMJ growth and function. Muscle secreted BMP7/Gbb binds to BMPRs (Wit, Tkv, Sax) on the motor neurons terminals and signal retrogradely to control NMJ growth (reviewed in Marques & Zhang, 2006). Gbb secreted from MNs also signals in an autocrine manner to modulate neurotransmitter release. In both cases the high-order BMP/BMPR (Gbb/Wit/Tkv/Sax) complexes are endocytosed and transported to the MN soma where they phosphorylate Mad and regulate various transcriptional programs with distinct roles in the development and function of the NMJ. Besides these canonical signaling pathways, Gbb and Wit signal non-canonically, independently of Mad, through the effector protein LIM kinase 1 (LIMK1) to regulate synapse stability and to enable addition of new boutons with increased synaptic activity (Eaton & Davis, 2005; Piccioli & Littleton, 2014). LIMK1 regulates the presynaptic actin dynamics partly by controlling the activity of the actin depolymerizing protein Cofilin. LIMK1 also binds to the C-terminal domain of Wit, to a region required for synapse stability but dispensable for Mad-mediated transcriptional control. This implies that a pool of Wit must remain at the synaptic terminals to localize the LIMK1-dependent activities. Finally, the BMPRs Wit, Tkv and Sax, but not Gbb, are required for the synaptic BMP signaling (Sulkowski et al., 2016; Zhao et al., 2015), a pathway that confines the BMP/BMPR complexes to the active zones.

Motor neurons must coordinate all these multiple BMP pathways to build appropriate NMJs. Several lines of evidence support this view. First, these genetically distinct pathways share selected components, some of them in limited and tightly controlled supplies. In both flies and mammals, posttranslational modifications such as phosphorylation and ubiquitination limit signaling activity and trigger degradation or deactivation of both Smads and receptors, keeping these signaling components in check (Alarcon et al., 2009; Dupont et al., 2009; Stinchfield et al., 2012; Zhu, Kavsak, Abdollah, Wrana, & Thomsen, 1999). Second, mutations that favor (or disfavor) one of these BMP signaling pathways appear do so at the expense of the other pathways. Finally, depending on the context, multiple modulators and regulatory feedbacks fine-tune the BMP signaling inputs/outputs and integrate this signaling network within the larger web of signaling cues that ultimately elicit specific

cellular responses. Likewise, motor neurons employ complex modulatory mechanisms to coordinate various BMP signaling modalities and integrate them with additional signaling networks to control neuronal survival and circuit function. Several mechanisms that limit the net levels, subcellular distribution and regulation of various BMP pathway components are reviewed below. The range of phenotypes induced by genetics manipulations of relevant BMP pathways modulators emphasizes the remarkable interconnectedness among different BMP signaling pathways.

For example, many BMP pathway components, including Tkv, Wit and Sax, as well as Mad and Medea, are direct targets of *mir-124*, which limits BMP signaling (Sun et al., 2012). The phenotype of *mir-124* mutants resembles that of neuronal overexpression of Mad or activated Sax and Tkv receptors together, with increased basal neurotransmission (increased nuclear pMad) but normal quantal size (normal synaptic pMad). Mad protein levels are also downregulated by *brain tumor* (*brat*), a translational repressor that limits Mad translation (Shi et al., 2013). Interestingly, *brat* loss-of-function does not mirror the *Mad* gain-of-function phenotypes; instead, depletion of Brat moderately enhances nuclear pMad levels and induces (i) increased PSD size (with more iGluRs per PSD) and (ii) a ~10-fold increase of synaptic pMad. Consistently, *brat* mutant NMJs are overgrown and show normal basal neurotransmission and increased mini amplitudes/ quantal size. These phenotypical differences suggest that, besides Mad, Brat may repress additional NMJ modulators. Alternatively, since Brat expression appears limited to a subset of MNs, Brat may elicit different signaling outcomes via neuron-specific differential regulation of Mad. Moreover, Brat NMJ activities could be further modulated by the Pum-Nos translational repressor complex, which provides additional layers of coordinated regulation between the NMJ growth and the recruitment of postsynaptic glutamate receptors (Harris, Pargett, Sutcliffe, Umulis, & Ashe, 2011; Menon et al., 2009, 2004).

In MNs, canonical BMP signaling depends on endocytosis and endosomal trafficking (reviewed in Deshpande & Rodal, 2016). BMP/BMPR complexes, but not BMPRs alone, are endocytosed at synaptic terminals and routed to signaling endosomes that are transported retrogradely along the axon to the neuron soma (Smith et al., 2012). The retrograde transport requires Dynein, a motor complex which accomplishes most of the retrograde axonal transport, and a set of cargo-specific regulators that includes Vezatin-like (Vezl) (Spinner, Pinter, Drerup, & Herman, 2020). Studies on several endocytic regulators, including endophilin, dynamin, Dap160/intersectin indicate that endocytosis negatively regulates BMP signaling (Koh, Verstreken, & Bellen, 2004; Marie et al., 2004; O'Connor-Giles, Ho, & Ganetzky, 2008). Mutations in endocytic modulators show characteristic morphologies, with overgrown NMJs with supernumerary or satellite boutons, a mark of excessive BMP signaling. These mutants have elevated levels of both nuclear and synaptic pMad as endocytosis limits BMPRs overall availability. Following endocytosis, BMPRs are sorted and routed to three destinations: signaling endosomes, recycling endosomes and lysosomes. Proteins like Liquid facets (Lqf), the *Drosophila* epsin homolog, and the Sorting nexin 16 (Snx16), promote the routing of BMP/BMPRs to signaling endosomes (Rodal et al., 2011; Vanlandingham et al., 2013). Loss of these sorting components results in excessive BMP/BMPRs directed toward lysosomal degradation and/or recycling; this reduces the levels of BMP/BMPRs in signaling endosomes and attenuates canonical

BMP signaling. Interestingly, *lqf* mutants have reduced nuclear pMad levels, but maintain significant levels of synaptic pMad, presumably because in these mutant backgrounds BMPR availability is specifically reduced within endosomal compartments but not at active zones (Vanlandingham et al., 2013). Conversely, mutations in endosome-localized proteins that interact with BMPRs and promote their lysosomal routing and degradation have excessive levels of BMPRs and consequently drastically enhanced BMP signaling in both MN nuclei and synaptic terminals (O'Connor-Giles et al., 2008; Sweeney & Davis, 2002; Tsang et al., 2009).

An additional endocytic mechanism, called micropinocytosis, contributes to Gbb-dependent BMPR internalization and degradation (Kim et al., 2019). The macropinosomes are actin-driven structures that require Abelson (Abl) tyrosine kinase, the Abl substrate and interactor Abi, and the Rac1 GTPase signaling. Mutations in any of these components (Abi, Abl or Rac1) produce satellite boutons morphologies characteristic of excess BMP signaling, accompanied by elevated nuclear and synaptic pMad levels. Tissue culture assays indicate that Gbb induces internalization of both Tvk and Wit via macropinocytosis. In addition, Tkv (but not Wit or Sax) is a substrate for Ube3A, an E3 ubiquitin ligase implicated in Angelman syndrome and autism (Li et al., 2016). Ube3A limits BMP signaling by binding and ubiquitinating Tkv for proteasomal degradation. This Ube3A function is conserved in mammalian cells, suggesting that the Ube3A-mediated downregulation of BMP signaling may explain the pathogenesis of Ube3A-associated Angelman syndrome and autism.

Mechanisms that regulate the levels and subcellular distribution of the BMP pathway components impact multiple BMP signaling pathways and may offer limited insights into their coordinated regulation. Additional BMP modulators must exist to allow the motor neuron to distinguish among different BMP signals and transduce specific pathway(s). For example, Gbb drives two different canonical BMP pathways in the MNs: Muscle-secreted Gbb signals retrogradely to control the NMJ growth, whereas neuronal Gbb signals in an autocrine manner to promote neurotransmitter release. The MNs seem to differentiate between the two different pools of Gbb via Crimpy (Cmpy), a neuronal Cysteine-rich transmembrane protein that binds to Gbb and delivers it to dense core vesicles for activity-dependent release (James & Broihier, 2011; James et al., 2014). In the absence of Cmpy, neuronal Gbb cannot sustain proper neurotransmitter release and instead induces excessive NMJ growth, augmenting the retrograde signaling. Thus, Cmpy effectively marks the pool of Gbb for autocrine signaling.

The non-canonical BMP signaling pathway appears to be selectively modulated by LIMK1 (as discussed above) and by the Fragile X mental retardation 1 (FMRP1), an RNA binding protein which causes fragile X syndrome (FXS), a common inherited form of intellectual disability and autism (Kashima et al., 2016; Zhang et al., 2001). FMRP1 binds to and downregulates the translation of two key molecules: (i) Futsch, the *Drosophila* homolog of the mammalian microtubule-associated protein MAP1B, a regulator of the microtubule cytoskeleton, and (ii) the Wit long isoform, which contains the LIMK1 binding domain. Loss of FMRP1 in larval MNs results in overgrown NMJs with elevated basal neurotransmission, presumably because of excessive (canonical and non-canonical) BMP signaling. However, *FMRP1* mutant NMJs have longer branches with increased number

of enlarged synaptic boutons—a morphology distinctly different than the satellite boutons discussed above. The mini amplitude/quantal size is also normal at *FMRP1* mutant NMJs indicating that synaptic BMP signaling is largely unaffected. Importantly, the neuronal deficits observed in fly or mouse models of FXS can be rescued by lowering the Wit/BMPRII copy number or by pharmacological inhibition of LIMK1, suggesting that FXS pathologies are linked to elevated non-canonical BMP signaling.

Finally, synaptic BMP signaling is linked via positive feedback with active postsynaptic type-A glutamate receptors. Gbb is not part of this positive feedback, even though Gbb drives all other BMP signaling pathways and promotes BMPRs internalization, limiting their availability. With BMPRs in limited supply, the MNs cannot deploy all BMP signaling pathways at once. Instead, MNs must choose among several options: (i) endocytose and route BMP/BMPRs to signaling endosomes to grow the NMJs and ensure proper neurotransmission, (ii) connect Gbb-Wit-LIMK1 perisynaptic complexes with the cytoskeleton to provide structural stability for the NMJ, or (iii) trap the BMPRs at the active zones to stabilize active type-A receptors and thus initiate formation of new synapse and modulate their maturation and plasticity. Since local BMP signaling serves as a sensor for synapse activity, the neurons may use the local signaling complexes to monitor synapse activity then deploy the other BMP signaling pathways to coordinate NMJ growth with synapse maturation and stabilization. Such a balancing act contains all the features of a finely tuned machinery capable of constantly sampling the synapse activity status and implementing swift adjustments to optimize synapse strength and function.

7. Future challenges

The powerful fly NMJ model system was instrumental in identifying and characterizing different BMP pathways, uncovering a rich modulation of levels and availability for signaling for various pathway components, as well as the subcellular distribution, sorting and trafficking of various signaling complexes. However, our understanding of the composition and regulation of local BMP signaling complexes remains sketchy. Aside from the detection of pMad at the active zones, all the other signaling pathway components have been derived from genetics arguments. With new technologies constantly improving our analyses of multimolecular complexes and detection capabilities, the next immediate challenge will be to describe the composition of local BMP signaling complexes and localize these components at the active zones. Gbb is clearly not part of these complexes, but is there another ligand involved? Ligand binding decreases the mobility of BMPRs within the cell membrane and increases the stability of hetero-tetrameric BMPRs assembles (Marom, Heining, Knaus, & Henis, 2011), promoting BMPRs trans-phosphorylation and activation. Alternatively, the density and structural constraints within the active zone may limit the BMPRs mobility and effectively confine these complexes even in the absence of a ligand.

More intriguingly, how does pMad remain associated with its own kinase after the enzymatic reaction has been completed? The Tkv/Mad binding interface seems to be crucial for the stabilization of these complexes, but additional component(s) that alter complex dissociation rate should exist. Such components may function by binding and stabilizing

the complexes directly, or they may introduce post-translational modifications in either the enzyme (Tkv) or the product (pMad) and indirectly influence their dissociation rate.

Our model also predicts that presynaptic BMP signaling complexes physically connect with postsynaptic iGluRs complexes via trans-synaptic nanocolumns. The iGluRs expand ~140Å within the 200Å synaptic cleft; the BMP-binding CUB domains of Neto expand even less, tucked just below the N-terminal domain of the iGluR complexes (as in the case of CUB1) or associated with the ligand binding domain (CUB2) (He et al., 2021). On the presynaptic site, the ectodomain of the BMPRs are ~55Å tall (Greenwald et al., 2003; Mace, Cutfield, & Cutfield, 2006). Even when BMP ligands bind to the BMPRs, these presynaptic complexes may not extend far enough to interact with the postsynaptic CUB-containing iGluRs/Neto complexes. Additional secreted molecule(s) may “bridge” these complexes and organize the proposed presynaptic BMPRs-postsynaptic iGluRs nanocolumns. In the future, screens with BioID methodologies, which label proximal endogenous proteins, may uncover both extracellular and intracellular scaffolds that configure and stabilize these nanocolumns.

As stressed above, the local BMP signaling pathway must be coordinated with the other BMP signaling modalities. But our current knowledge of transcriptional targets downstream of retrograde and autocrine BMP signaling is fairly limited. How do these BMP transcriptional targets function in adjusting the hard-wired transcriptional programs specific to each motor neuron lineage, so that each motor neuron output matches the status of the target muscle? The fast advances in RNA sequencing approaches should make such transcriptomics analyses accessible within the near future. Moreover, each muscle receives inputs from two different glutamatergic motor neurons, Ib and Is, with distinct synaptic structure, postsynaptic composition, neurotransmitter release properties and plasticity mechanisms. Recent studies reveal that the two neurons influence each other’s structural and functional output: Ablation of one neuron causes selective NMJ expansion and increased neurotransmitter release in the remaining neuron (Aponte-Santiago et al., 2020; Wang et al., 2021). Gbb appears to have both shared and distinct functions in these two types of neurons, raising the possibility that BMP signaling may further enable the coordinated response of co-innervating neurons, balancing their synaptic plasticity. Elucidating neuron-specific BMP transcriptional targets and their relationship to local BMP signaling may uncover overarching principles and developmental strategies for the assembly and maturation of plastic synapses.

The complexity of BMP signaling reviewed here is reminiscent of neurotrophin-regulated signaling in vertebrate systems (reviewed in Reichardt, 2006). First identified as neuronal survival factors, neurotrophins are secreted as pro-proteins that must be processed to form mature ligands. Like BMPs, these active dimers form cysteine-knot structures and signal by binding to transmembrane kinase receptors and inducing their activation through trans-phosphorylation. Neurotrophin/receptor complexes are internalized and transported along axons to the cell soma (Thoenen & Barde, 1980). This canonical signaling pathway controls gene expression and promotes neuronal differentiation and growth. In addition, local neurotrophin signaling mediates activity-dependent synapse formation and maturation and promotes neurotransmitter release (reviewed in Park & Poo, 2013). While the molecular details of neurotrophin-induced local signaling remain largely unknown, the remarkable

similarities between these signaling pathways suggest they may share central roles in the assembly and maturation of plastic synapses.

The local BMP signaling-based feedback mechanism reviewed here provides a molecular description of a key positive feedback loop that sculpts synapse assembly and maturation. At the same time, this positive feedback mechanism may serve as a template to elucidate other signaling networks utilized for building chemical synapses. Furthermore, a local role for BMP signaling in regulating cellular junctions has extremely broad implications, from stabilizing short lived intercellular interactions during patterning (Huang, Liu, & Kornberg, 2019) to supporting epithelia integrity and remodeling during development and homeostasis (Eom et al., 2012; Marmion, Jevtic, Springhorn, Pyrowolakis, & Yakoby, 2013). We envision this local modality of BMP signaling arose early during evolution to ensure the integrity of cellular junctions and evolved to fulfill additional functions at more specialized cellular junctions, the chemical synapses.

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References

Aberle H, Haghighi AP, Fetter RD, McCabe BD, Magalhaes TR, & Goodman CS (2002). wishful thinking encodes a BMP type II receptor that regulates synaptic growth in Drosophila. Neuron, 33, 545–558. [PubMed: 11856529]

Akbergenova Y, Cunningham KL, Zhang YV, Weiss S, & Littleton JT (2018). Characterization of developmental and molecular factors underlying release heterogeneity at Drosophila synapses. eLife, 7, e38268.

Alarcon C, Zaromytidou AI, Xi Q, Gao S, Yu J, Fujisawa S, et al. (2009). Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways. Cell, 139, 757–769. [PubMed: 19914168]

Albin SD, & Davis GW (2004). Coordinating structural and functional synapse development: Postsynaptic p21-activated kinase independently specifies glutamate receptor abundance and postsynaptic morphology. The Journal of Neuroscience, 24, 6871–6879. [PubMed: 15295021]

Aponte-Santiago NA, Ormerod KG, Akbergenova Y, & Littleton JT (2020). Synaptic plasticity induced by differential manipulation of tonic and phasic motoneurons in Drosophila. The Journal of Neuroscience, 40, 6270–6288. [PubMed: 32631939]

Ball RW, Warren-Paquin M, Tsurudome K, Liao EH, Elazzouzi F, Cavanagh C, et al. (2010). Retrograde BMP signaling controls synaptic growth at the NMJ by regulating trio expression in motor neurons. Neuron, 66, 536–549. [PubMed: 20510858]

Banovic D, Khorramshahi O, Owald D, Wichmann C, Riedt T, Fouquet W, et al. (2010). Drosophila neuroligin 1 promotes growth and postsynaptic differentiation at glutamatergic neuromuscular junctions. Neuron, 66, 724–738. [PubMed: 20547130]

Bilder D, & Perrimon N. (2000). Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. Nature, 403, 676–680. [PubMed: 10688207]

Bogdanik L, Mohrmann R, Ramaekers A, Bockaert J, Grau Y, Broadie K, et al. (2004). The Drosophila metabotropic glutamate receptor DmGluRA regulates activity-dependent synaptic facilitation and fine synaptic morphology. The Journal of Neuroscience, 24, 9105–9116. [PubMed: 15483129]

Bond AM, Bhalala OG, & Kessler JA (2012). The dynamic role of bone morphogenetic proteins in neural stem cell fate and maturation. Developmental Neurobiology, 72, 1068–1084. [PubMed: 22489086]

Bork P, & Beckmann G. (1993). The CUB domain. A widespread module in developmentally regulated proteins. Journal of Molecular Biology, 231, 539–545. [PubMed: 8510165]

Broadie K, & Bate M. (1993). Innervation directs receptor synthesis and localization in Drosophila embryo synaptogenesis. Nature, 361, 350–353. [PubMed: 8426654]

Burden SJ (1998). The formation of neuromuscular synapses. Genes & Development, 12, 133–148.

Chen K, & Featherstone DE (2005). Discs-large (DLG) is clustered by presynaptic innervation and regulates postsynaptic glutamate receptor subunit composition in Drosophila. BMC Biology, 3, 1. [PubMed: 15638945]

Chen K, Merino C, Sigrist SJ, & Featherstone DE (2005). The 4.1 protein coracle mediates subunit-selective anchoring of Drosophila glutamate receptors to the postsynaptic actin cytoskeleton. The Journal of Neuroscience, 25, 6667–6675. [PubMed: 16014728]

Cui Y, Jean F, Thomas G, & Christian JL (1998). BMP-4 is proteolytically activated by furin and/or PC6 during vertebrate embryonic development. The EMBO Journal, 17, 4735–4743. [PubMed: 9707432]

Dalva MB, McClelland AC, & Kayser MS (2007). Cell adhesion molecules: Signalling functions at the synapse. Nature Reviews. Neuroscience, 8, 206–220. [PubMed: 17299456]

Davis GW, DiAntonio A, Petersen SA, & Goodman CS (1998). Postsynaptic PKA controls quantal size and reveals a retrograde signal that regulates presynaptic transmitter release in Drosophila. Neuron, 20, 305–315. [PubMed: 9491991]

Davis GW, & Muller M. (2015). Homeostatic control of presynaptic neurotransmitter release. Annual Review of Physiology, 77, 251–270.

Degnin C, Jean F, Thomas G, & Christian JL (2004). Cleavages within the prodomain direct intracellular trafficking and degradation of mature bone morphogenetic protein-4. Molecular Biology of the Cell, 15, 5012–5020. [PubMed: 15356272]

Derynck R, & Zhang YE (2003). Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature, 425, 577–584. [PubMed: 14534577]

Deshpande M, & Rodal AA (2016). The crossroads of synaptic growth signaling, membrane traffic and neurological disease: Insights from Drosophila. Traffic, 17, 87–101. [PubMed: 26538429]

DiAntonio A, Petersen SA, Heckmann M, & Goodman CS (1999). Glutamate receptor expression regulates quantal size and quantal content at the Drosophila neuromuscular junction. The Journal of Neuroscience, 19, 3023–3032. [PubMed: 10191319]

Diao F, Ironfield H, Luan H, Diao F, Shropshire WC, Ewer J, et al. (2015). Plug- and-play genetic access to drosophila cell types using exchangeable exon cassettes. Cell Reports, 10, 1410–1421. [PubMed: 25732830]

Dudu V, Bittig T, Entchev E, Kicheva A, Julicher F, & Gonzalez-Gaitan M. (2006). Postsynaptic mad signaling at the Drosophila neuromuscular junction. Current Biology, 16, 625–635. [PubMed: 16581507]

Dupont S, Mamidi A, Cordenonsi M, Montagner M, Zacchigna L, Adorno M, et al. (2009). FAM/USP9x, a deubiquitinating enzyme essential for TGFbeta signaling, controls Smad4 monoubiquitination. Cell, 136, 123–135. [PubMed: 19135894]

Eaton BA, & Davis GW (2005). LIM Kinase1 controls synaptic stability downstream of the type II BMP receptor. Neuron, 47, 695–708. [PubMed: 16129399]

Ehrlich M, Gutman O, Knaus P, & Henis YI (2012). Oligomeric interactions of TGF-beta and BMP receptors. FEBS Letters, 586, 1885–1896. [PubMed: 22293501]

Ellis JE, Parker L, Cho J, & Arora K. (2010). Activin signaling functions upstream of Gbb to regulate synaptic growth at the Drosophila neuromuscular junction. Developmental Biology, 342, 121–133. [PubMed: 20346940]

Eom DS, Amarnath S, Fogel JL, & Agarwala S. (2011). Bone morphogenetic proteins regulate neural tube closure by interacting with the apicobasal polarity pathway. Development, 138, 3179–3188. [PubMed: 21750029]

Eom DS, Amarnath S, Fogel JL, & Agarwala S. (2012). Bone morphogenetic proteins regulate hinge point formation during neural tube closure by dynamic modulation of apicobasal polarity. Birth Defects Research. Part A, Clinical and Molecular Teratology, 94, 804–816. [PubMed: 22865775]

Featherstone DE, Rushton E, Rohrbough J, Liebl F, Karr J, Sheng Q, et al. (2005). An essential Drosophila glutamate receptor subunit that functions in both central neuropil and neuromuscular junction. The Journal of Neuroscience, 25, 3199–3208. [PubMed: 15788777]

Feng XH, & Derynck R. (2005). Specificity and versatility in tgf-beta signaling through Smads. Annual Review of Cell and Developmental Biology, 21, 659–693.

Foletta VC, Lim MA, Soosairajah J, Kelly AP, Stanley EG, Shannon M, et al. (2003). Direct signaling by the BMP type II receptor via the cytoskeletal regulator LIMK1. The Journal of Cell Biology, 162, 1089–1098. [PubMed: 12963706]

Fouquet W, Owald D, Wichmann C, Mertel S, Depner H, Dyba M, et al. (2009). Maturation of active zone assembly by Drosophila Bruchpilot. The Journal of Cell Biology, 186, 129–145. [PubMed: 19596851]

Frank CA (2014). Homeostatic plasticity at the Drosophila neuromuscular junction. Neuropharmacology, 78, 63–74. [PubMed: 23806804]

Fuentes-Medel Y, Ashley J, Barria R, Maloney R, Freeman M, & Budnik V. (2012). Integration of a retrograde signal during synapse formation by glia-secreted TGF-beta Ligand. Current Biology, 22, 1831–1838. [PubMed: 22959350]

Fukui A, Inaki M, Tonoe G, Hamatani H, Homma M, Morimoto T, et al. (2012). Lola regulates glutamate receptor expression at the Drosophila neuromuscular junction. Biology Open, 1, 362–375. [PubMed: 23213426]

Ganesan S, Karr JE, & Featherstone DE (2011). Drosophila glutamate receptor mRNA expression and mRNP particles. RNA Biology, 8, 771–781. [PubMed: 21743295]

Giagtzoglou N, Ly CV, & Bellen HJ (2009). Cell adhesion, the backbone of the synapse: “Vertebrate” and “invertebrate” perspectives. Cold Spring Harbor Perspectives in Biology, 1, a003079.

Goldman DC, Hackenmiller R, Nakayama T, Sopory S, Wong C, Kulessa H, et al. (2006). Mutation of an upstream cleavage site in the BMP4 prodomain leads to tissue-specific loss of activity. Development, 133, 1933–1942. [PubMed: 16624858]

Gratz SJ, Goel P, Bruckner JJ, Hernandez RX, Khateeb K, Macleod GT, et al. (2019). Endogenous tagging reveals differential regulation of Ca^{2+} channels at single AZs during presynaptic homeostatic potentiation and depression. The Journal of Neuroscience, 39(13), 2416–2429. [PubMed: 30692227]

Greenwald J, Groppe J, Gray P, Wiater E, Kwiatkowski W, Vale W, et al. (2003). The BMP7/ActRII extracellular domain complex provides new insights into the cooperative nature of receptor assembly. Molecular Cell, 11, 605–617. [PubMed: 12667445]

Gui J, Huang Y, & Shimmi O. (2016). Scribbled optimizes BMP signaling through its receptor internalization to the Rab5 endosome and promote robust epithelial morphogenesis. PLoS Genetics, 12, e1006424.

Han TH, Dharkar P, Mayer ML, & Serpe M. (2015). Functional reconstitution of Drosophila melanogaster NMJ glutamate receptors. Proceedings of the National Academy of Sciences of the United States of America, 112, 6182–6187. [PubMed: 25918369]

Harris KP, & Littleton JT (2015). Transmission, development, and plasticity of synapses. Genetics, 201, 345–375. [PubMed: 26447126]

Harris RE, Pargett M, Sutcliffe C, Umulis D, & Ashe HL (2011). Brat promotes stem cell differentiation via control of a bistable switch that restricts BMP signaling. Developmental Cell, 20, 72–83. [PubMed: 21238926]

He L, Sun J, Gao Y, Li B, Wang Y, Dong Y, et al. (2021). Kainate receptor modulation by NETO2. Nature, 599, 325–329. [PubMed: 34552241]

Heckmann M, & Dudel J. (1997). Desensitization and resensitization kinetics of glutamate receptor channels from Drosophila larval muscle. Biophysical Journal, 72, 2160–2169. [PubMed: 9129818]

Hill CS (2016). Transcriptional control by the SMADs. Cold Spring Harbor Perspectives in Biology, 8(10), a022079.

Hogan BL (1996). Bone morphogenetic proteins in development. Current Opinion in Genetics & Development, 6, 432–438. [PubMed: 8791534]

Huang H, Liu S, & Kornberg TB (2019). Glutamate signaling at cytoneme synapses. Science, 363, 948–955. [PubMed: 30819957]

Jackson AC, & Nicoll RA (2011). The expanding social network of ionotropic glutamate receptors: TARPs and other transmembrane auxiliary subunits. Neuron, 70, 178–199. [PubMed: 21521608]

James RE, & Broihier HT (2011). Crimpy inhibits the BMP homolog Gbb in moto-neurons to enable proper growth control at the Drosophila neuromuscular junction. Development, 138, 3273–3286. [PubMed: 21750037]

James RE, Hoover KM, Bulgari D, McLaughlin CN, Wilson CG, Wharton KA, et al. (2014). Crimpy enables discrimination of presynaptic and postsynaptic pools of a BMP at the Drosophila neuromuscular junction. Developmental Cell, 31(5), 586–598. [PubMed: 25453556]

Jan LY, & Jan YN (1976a). L-glutamate as an excitatory transmitter at the Drosophila larval neuromuscular junction. The Journal of Physiology, 262, 215–236. [PubMed: 186587]

Jan LY, & Jan YN (1976b). Properties of the larval neuromuscular junction in Drosophila melanogaster. The Journal of Physiology, 262, 189–214. [PubMed: 11339]

Karr J, Vagin V, Chen K, Ganesan S, Olenkina O, Gvozdev V, et al. (2009). Regulation of glutamate receptor subunit availability by microRNAs. The Journal of Cell Biology, 185, 685–697. [PubMed: 19433455]

Kashima R, Roy S, Ascano M, Martinez-Cerdeno V, Ariza-Torres J, Kim S, et al. (2016). Augmented noncanonical BMP type II receptor signaling mediates the synaptic abnormality of fragile X syndrome. Science Signaling, 9, ra58.

Kawabata M, Inoue H, Hanyu A, Imamura T, & Miyazono K. (1998). Smad proteins exist as monomers in vivo and undergo homo- and hetero-oligomerization upon activation by serine/threonine kinase receptors. The EMBO Journal, 17, 4056–4065. [PubMed: 9670020]

Keshishian H, Broadie K, Chiba A, & Bate M. (1996). The drosophila neuromuscular junction: A model system for studying synaptic development and function. Annual Review of Neuroscience, 19, 545–575.

Kim YJ, Bao H, Bonanno L, Zhang B, & Serpe M. (2012). Drosophila Neto is essential for clustering glutamate receptors at the neuromuscular junction. Genes & Development, 26, 974–987. [PubMed: 22499592]

Kim YJ, Igiesuorobo O, Ramos CI, Bao H, Zhang B, & Serpe M. (2015). Prodomain removal enables neto to stabilize glutamate receptors at the Drosophila neuromuscular junction. PLoS Genetics, 11, e1004988.

Kim N, Kim S, Nahm M, Kopke D, Kim J, Cho E, et al. (2019). BMP-dependent synaptic development requires Abi-Abl-Rac signaling of BMP receptor macropinocytosis. Nature Communications, 10, 684.

Kim MJ, & O'Connor MB (2014). Anterograde activin signaling regulates postsynaptic membrane potential and GluRIIA/B abundance at the Drosophila neuromuscular junction. PLoS One, 9, e107443.

Kim YJ, & Serpe M. (2013). Building a synapse: A complex matter. Fly, 7(3), 146–152. [PubMed: 23680998]

Kim N, Stiegler AL, Cameron TO, Hallock PT, Gomez AM, Huang JH, et al. (2008). Lrp4 is a receptor for Agrin and forms a complex with MuSK. Cell, 135, 334–342. [PubMed: 18848351]

Kiragasi B, Wondolowski J, Li Y, & Dickman DK (2017). A presynaptic glutamate receptor subunit confers robustness to neurotransmission and homeostatic potentiation. Cell Reports, 19, 2694–2706. [PubMed: 28658618]

Koch I, Schwarz H, Beuchle D, Goellner B, Langegger M, & Aberle H. (2008). Drosophila ankyrin 2 is required for synaptic stability. Neuron, 58, 210–222. [PubMed: 18439406]

Koh TW, Verstreken P, & Bellen HJ (2004). Dap160/intersectin acts as a stabilizing scaffold required for synaptic development and vesicle endocytosis. Neuron, 43, 193–205. [PubMed: 15260956]

Lee HX, Mendes FA, Plouhinec JL, & De Robertis EM (2009). Enzymatic regulation of pattern: BMP4 binds CUB domains of Tolloids and inhibits proteinase activity. Genes & Development, 23, 2551–2562. [PubMed: 19884260]

Lee G, & Schwarz TL (2016). Filamin, a synaptic organizer in Drosophila, determines glutamate receptor composition and membrane growth. eLife, 5, e19991.

Li J, Ashley J, Budnik V, & Bhat MA (2007). Crucial role of Drosophila neurexin in proper active zone apposition to postsynaptic densities, synaptic growth, and synaptic transmission. Neuron, 55, 741–755. [PubMed: 17785181]

Li Y, Dharkar P, Han TH, Serpe M, Lee CH, & Mayer ML (2016). Novel functional properties of Drosophila CNS glutamate receptors. Neuron, 92, 1036–1048. [PubMed: 27889096]

Li H, Janssens J, Waegeneer MD, Kolluru SS, Davie K, Gardeux V, et al. (2022). Fly cell Atlas: A single-nucleus transcriptomic atlas of the adult fruit fly. Science, 375, eabk2432.

Li W, Yao A, Zhi H, Kaur K, Zhu YC, Jia M, et al. (2016). Angelman syndrome protein Ube3a regulates synaptic growth and endocytosis by inhibiting BMP signaling in Drosophila. PLoS Genetics, 12, e1006062.

Liebl FL, & Featherstone DE (2005). Genes involved in Drosophila glutamate receptor expression and localization. BMC Neuroscience, 6, 44. [PubMed: 15985179]

Liebl FL, & Featherstone DE (2008). Identification and investigation of Drosophila postsynaptic density homologs. Bioinformatics and Biology Insights, 2, 375–387.

Liebl FL, Werner KM, Sheng Q, Karr JE, McCabe BD, & Featherstone DE (2006). Genome-wide P-element screen for Drosophila synaptogenesis mutants. Journal of Neurobiology, 66, 332–347. [PubMed: 16408305]

Ljaschenko D, Ehmann N, & Kittel RJ (2013). Hebbian plasticity guides maturation of glutamate receptor fields in vivo. Cell Reports, 3, 1407–1413. [PubMed: 23643532]

Mace PD, Cutfield JF, & Cutfield SM (2006). High resolution structures of the bone morphogenetic protein type II receptor in two crystal forms: Implications for ligand binding. Biochemical and Biophysical Research Communications, 351, 831–838. [PubMed: 17094948]

Marie B, Sweeney ST, Poskanzer KE, Roos J, Kelly RB, & Davis GW (2004). Dap160/intersectin scaffolds the periactive zone to achieve high-fidelity endocytosis and normal synaptic growth. Neuron, 43, 207–219. [PubMed: 15260957]

Marmion RA, Jevtic M, Springhorn A, Pyrowolakis G, & Yakoby N. (2013). The Drosophila BMPRII, wishful thinking, is required for eggshell patterning. Developmental Biology, 375, 45–53. [PubMed: 23274688]

Marom B, Heining E, Knaus P, & Henis YI (2011). Formation of stable homomeric and transient heteromeric bone morphogenetic protein (BMP) receptor complexes regulates Smad protein signaling. The Journal of Biological Chemistry, 286, 19287–19296. [PubMed: 21471205]

Marques G, Haerry TE, Crotty ML, Xue M, Zhang B, & O'Connor MB (2003). Retrograde Gbb signaling through the Bmp type 2 receptor wishful thinking regulates systemic FMRFa expression in Drosophila. Development, 130, 5457–5470. [PubMed: 14507784]

Marques G, & Zhang B. (2006). Retrograde signaling that regulates synaptic development and function at the Drosophila neuromuscular junction. International Review of Neurobiology, 75, 267–285. [PubMed: 17137932]

Marrus SB, & DiAntonio A. (2004). Preferential localization of glutamate receptors opposite sites of high presynaptic release. Current Biology, 14, 924–931. [PubMed: 15182665]

Marrus SB, Portman SL, Allen MJ, Moffat KG, & DiAntonio A. (2004). Differential localization of glutamate receptor subunits at the Drosophila neuromuscular junction. The Journal of Neuroscience, 24, 1406–1415. [PubMed: 14960613]

Massague J. (1990). The transforming growth factor-beta family. Annual Review of Cell Biology, 6, 597–641.

Mayer ML (2021). Structural biology of kainate receptors. Neuropharmacology, 190, 108511.

McCabe BD, Hom S, Aberle H, Fetter RD, Marques G, Haerry TE, et al. (2004). Highwire regulates presynaptic BMP signaling essential for synaptic growth. Neuron, 41, 891–905. [PubMed: 15046722]

McCabe BD, Marques G, Haghighi AP, Fetter RD, Crotty ML, Haerry TE, et al. (2003). The BMP homolog Gbb provides a retrograde signal that regulates synaptic growth at the Drosophila neuromuscular junction. Neuron, 39, 241–254. [PubMed: 12873382]

McMahan UJ (1990). The agrin hypothesis. Cold Spring Harbor Symposia on Quantitative Biology, 55, 407–418. [PubMed: 1966767]

Menon KP, Andrews S, Murthy M, Gavis ER, & Zinn K. (2009). The translational repressors Nanos and Pumilio have divergent effects on presynaptic terminal growth and postsynaptic glutamate receptor subunit composition. The Journal of Neuroscience, 29, 5558–5572. [PubMed: 19403823]

Menon KP, Carrillo RA, & Zinn K. (2013). Development and plasticity of the Drosophila larval neuromuscular junction. Wiley Interdisciplinary Reviews: Developmental Biology, 2, 647–670. [PubMed: 24014452]

Menon KP, Sanyal S, Habara Y, Sanchez R, Wharton RP, Ramaswami M, et al. (2004). The translational repressor Pumilio regulates presynaptic morphology and controls postsynaptic accumulation of translation factor eIF-4E. Neuron, 44, 663–676. [PubMed: 15541314]

Merino C, Penney J, Gonzalez M, Tsurudome K, Moujahidine M, O'Connor MB, et al. (2009). Nemo kinase interacts with Mad to coordinate synaptic growth at the Drosophila neuromuscular junction. The Journal of Cell Biology, 185, 713–725. [PubMed: 19451277]

Metwally E, Zhao G, Li W, Wang Q, & Zhang YQ (2019). Calcium-activated calpain specifically cleaves glutamate receptor IIA But Not IIB at the *Drosophila* neuromuscular junction. The Journal of Neuroscience, 39, 2776–2791. [PubMed: 30705102]

Meyerson JR, Chittori S, Merk A, Rao P, Han TH, Serpe M, et al. (2016). Structural basis of kainate subtype glutamate receptor desensitization. Nature, 537, 567–571. [PubMed: 27580033]

Meyerson JR, Kumar J, Chittori S, Rao P, Pierson J, Bartesaghi A, et al. (2014). Structural mechanism of glutamate receptor activation and desensitization. Nature, 514, 328–334. [PubMed: 25119039]

Morimoto T, Nobechi M, Komatsu A, Miyakawa H, & Nose A. (2009). Subunit-specific and homeostatic regulation of glutamate receptor localization by CaMKII in Drosophila neuromuscular junctions. Neuroscience, 165, 1284–1292. [PubMed: 19961909]

Mosca TJ, Hong W, Dani VS, Favaloro V, & Luo L. (2012). Trans-synaptic Teneurin signalling in neuromuscular synapse organization and target choice. Nature, 484, 237–241. [PubMed: 22426000]

Moustakas A, & Heldin CH (2005). Non-Smad TGF-beta signals. Journal of Cell Science, 118, 3573–3584. [PubMed: 16105881]

Newman ZL, Hoagland A, Aghi K, Worden K, Levy SL, Son JH, et al. (2017). Input-specific plasticity and homeostasis at the Drosophila larval neuromuscular junction. Neuron, 93(1388–1404), e1310.

Ng D, Pitcher GM, Szilard RK, Sertie A, Kanisek M, Clapcote SJ, et al. (2009). Neto1 is a novel CUB-domain NMDA receptor-interacting protein required for synaptic plasticity and learning. PLoS Biology, 7, e41. [PubMed: 19243221]

Nguyen TH, Han TH, Newfeld SJ, & Serpe M. (2020). Selective disruption of synaptic BMP signaling by a Smad mutation adjacent to the highly conserved H2 helix. Genetics, 216(1), 159–175. [PubMed: 32737119]

Nicoll RA (2017). A brief history of long-term potentiation. Neuron, 93, 281–290. [PubMed: 28103477]

O'Connor-Giles KM, Ho LL, & Ganetzky B. (2008). Nervous wreck interacts with thickveins and the endocytic machinery to attenuate retrograde BMP signaling during synaptic growth. Neuron, 58, 507–518. [PubMed: 18498733]

Owald D, Khorramshahi O, Gupta VK, Banovic D, Depner H, Fouquet W, et al. (2012). Cooperation of Syd-1 with Neurexin synchronizes pre- with postsynaptic assembly. Nature Neuroscience, 15, 1219–1226. [PubMed: 22864612]

Ozdamar B, Bose R, Barrios-Rodiles M, Wang HR, Zhang Y, & Wrana JL (2005). Regulation of the polarity protein Par6 by TGFbeta receptors controls epithelial cell plasticity. Science, 307, 1603–1609. [PubMed: 15761148]

Park H, & Poo MM (2013). Neurotrophin regulation of neural circuit development and function. Nature Reviews. Neuroscience, 14, 7–23.

Parnas D, Haghighi AP, Fetter RD, Kim SW, & Goodman CS (2001). Regulation of postsynaptic structure and protein localization by the Rho-type guanine nucleotide exchange factor dPix. Neuron, 32, 415–424. [PubMed: 11709153]

Petersen SA, Fetter RD, Noordermeer JN, Goodman CS, & DiAntonio A. (1997). Genetic analysis of glutamate receptors in Drosophila reveals a retrograde signal regulating presynaptic transmitter release. Neuron, 19, 1237–1248. [PubMed: 9427247]

Petzoldt AG, Lee YH, Khorramshahi O, Reynolds E, Plested AJ, Herzel H, et al. (2014). Gating characteristics control glutamate receptor distribution and trafficking in vivo. Current Biology, 24, 2059–2065. [PubMed: 25131677]

Piccioli ZD, & Littleton JT (2014). Retrograde BMP signaling modulates rapid activity-dependent synaptic growth via presynaptic LIM kinase regulation of cofilin. The Journal of Neuroscience, 34, 4371–4381. [PubMed: 24647957]

Qin G, Schwarz T, Kittel RJ, Schmid A, Rasse TM, Kappei D, et al. (2005). Four different subunits are essential for expressing the synaptic glutamate receptor at neuromuscular junctions of Drosophila. The Journal of Neuroscience, 25, 3209–3218. [PubMed: 15788778]

Ramesh N, Escher MJF, Mampell MM, Bohme MA, Gotz TWB, Goel P, et al. (2021). Antagonistic interactions between two Neuroligins coordinate pre- and postsynaptic assembly. Current Biology, 31(1711–1725), e1715.

Ramos CI, Igiesuorobo O, Wang Q, & Serpe M. (2015). Neto-mediated intracellular interactions shape postsynaptic composition at the Drosophila neuromuscular junction. PLoS Genetics, 11, e1005191.

Rasse TM, Fouquet W, Schmid A, Kittel RJ, Mertel S, Sigrist CB, et al. (2005). Glutamate receptor dynamics organizing synapse formation in vivo. Nature Neuroscience, 8, 898–905. [PubMed: 16136672]

Rawson JM, Lee M, Kennedy EL, & Selleck SB (2003). Drosophila neuromuscular synapse assembly and function require the TGF-beta type I receptor saxophone and the transcription factor Mad. Journal of Neurobiology, 55, 134–150. [PubMed: 12672013]

Reichardt LF (2006). Neurotrophin-regulated signalling pathways. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences, 361, 1545–1564. [PubMed: 16939974]

Reist NE, Werle MJ, & McMahan UJ (1992). Agrin released by motor neurons induces the aggregation of acetylcholine receptors at neuromuscular junctions. Neuron, 8, 865–868. [PubMed: 1316763]

Roche JP, Packard MC, Moeckel-Cole S, & Budnik V. (2002). Regulation of synaptic plasticity and synaptic vesicle dynamics by the PDZ protein Scribble. The Journal of Neuroscience, 22, 6471–6479. [PubMed: 12151526]

Rodal AA, Blunk AD, Akbergenova Y, Jorquera RA, Buhl LK, & Littleton JT (2011). A presynaptic endosomal trafficking pathway controls synaptic growth signaling. The Journal of Cell Biology, 193, 201–217. [PubMed: 21464232]

Ruiz-Canada C, Ashley J, Moeckel-Cole S, Drier E, Yin J, & Budnik V. (2004). New synaptic bouton formation is disrupted by misregulation of microtubule stability in aPKC mutants. Neuron, 42, 567–580. [PubMed: 15157419]

Schmid A, Hallermann S, Kittel RJ, Khorramshahi O, Frolich AM, Quentin C, et al. (2008). Activity-dependent site-specific changes of glutamate receptor composition in vivo. Nature Neuroscience, 11, 659–666. [PubMed: 18469810]

Schmid A, Qin G, Wichmann C, Kittel RJ, Mertel S, Fouquet W, et al. (2006). Non-NMDA-type glutamate receptors are essential for maturation but not for initial assembly of synapses at Drosophila neuromuscular junctions. The Journal of Neuroscience, 26, 11267–11277. [PubMed: 17079654]

Schmierer B, & Hill CS (2007). TGFbeta-SMAD signal transduction: Molecular specificity and functional flexibility. Nature Reviews Molecular Cell Biology, 8, 970–982. [PubMed: 18000526]

Shi W, Chen Y, Gan G, Wang D, Ren J, Wang Q, et al. (2013). Brain tumor regulates neuromuscular synapse growth and endocytosis in Drosophila by suppressing mad expression. The Journal of Neuroscience, 33, 12352–12363. [PubMed: 23884941]

Sigrist SJ, Reiff DF, Thiel PR, Steinert JR, & Schuster CM (2003). Experience-dependent strengthening of Drosophila neuromuscular junctions. The Journal of Neuroscience, 23, 6546–6556. [PubMed: 12878696]

Smith RB, Machamer JB, Kim NC, Hays TS, & Marques G. (2012). Relay of retrograde synaptogenic signals through axonal transport of BMP receptors. Journal of Cell Science, 125, 3752–3764. [PubMed: 22573823]

Sobolevsky AI, Rosconi MP, & Gouaux E. (2009). X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. Nature, 462, 745–756. [PubMed: 19946266]

Spinner MA, Pinter K, Drerup CM, & Herman TG (2020). A conserved role for vezatin proteins in cargo-specific regulation of retrograde axonal transport. Genetics, 216, 431–445. [PubMed: 32788307]

Stephan R, Goellner B, Moreno E, Frank CA, Hugenschmidt T, Genoud C, et al. (2015). Hierarchical microtubule organization controls axon caliber and transport and determines synaptic structure and stability. Developmental Cell, 33, 5–21. [PubMed: 25800091]

Stinchfield MJ, Takaesu NT, Quijano JC, Castillo AM, Tiusanen N, Shimmi O, et al. (2012). Fat facets deubiquitylation of Medea/Smad4 modulates interpretation of a Dpp morphogen gradient. Development, 139, 2721–2729. [PubMed: 22745309]

Sudhof TC (2012). The presynaptic active zone. Neuron, 75, 11–25. [PubMed: 22794257]

Sulkowski MJ, Han TH, Ott C, Wang Q, Verheyen EM, Lippincott-Schwartz J, et al. (2016). A novel, noncanonical BMP pathway modulates synapse maturation at the drosophila neuromuscular junction. PLoS Genetics, 12, e1005810.

Sulkowski M, Kim YJ, & Serpe M. (2014). Postsynaptic glutamate receptors regulate local BMP signaling at the Drosophila neuromuscular junction. Development, 141, 436–447. [PubMed: 24353060]

Sun K, Westholm JO, Tsurudome K, Hagen JW, Lu Y, Kohwi M, et al. (2012). Neurophysiological defects and neuronal gene deregulation in Drosophila mir-124 mutants. PLoS Genetics, 8, e1002515.

Sweeney ST, & Davis GW (2002). Unrestricted synaptic growth in spinster-a late endosomal protein implicated in TGF-beta-mediated synaptic growth regulation. Neuron, 36, 403–416. [PubMed: 12408844]

Tang AH, Chen H, Li TP, Metzbower SR, MacGillavry HD, & Blanpied TA (2016). A trans-synaptic nanocolumn aligns neurotransmitter release to receptors. Nature, 536, 210–214. [PubMed: 27462810]

Thoenen H, & Barde YA (1980). Physiology of nerve growth factor. Physiological Reviews, 60, 1284–1335. [PubMed: 6159658]

Thomas U, & Sigrist SJ (2012). Glutamate receptors in synaptic assembly and plasticity: Case studies on fly NMJs. Advances in Experimental Medicine and Biology, 970, 3–28. [PubMed: 22351049]

Tomita S. (2010). Regulation of ionotropic glutamate receptors by their auxiliary subunits. Physiology (Bethesda), 25, 41–49. [PubMed: 20134027]

Tomita S, & Castillo PE (2012). Neto1 and Neto2: Auxiliary subunits that determine key properties of native kainate receptors. The Journal of Physiology, 590, 2217–2223. [PubMed: 22431337]

Tsang HT, Edwards TL, Wang X, Connell JW, Davies RJ, Durrington HJ, et al. (2009). The hereditary spastic paraplegia proteins NIPA1, spastin and spartin are inhibitors of mammalian BMP signalling. Human Molecular Genetics, 18, 3805–3821. [PubMed: 19620182]

Urist MR (1965). Bone: Formation by autoinduction. Science, 150, 893–899. [PubMed: 5319761]

Van Vactor D, & Sigrist SJ (2017). Presynaptic morphogenesis, active zone organization and structural plasticity in Drosophila. Current Opinion in Neurobiology, 43, 119–129. [PubMed: 28388491]

Vanlandingham PA, Fore TR, Chastain LR, Royer SM, Bao H, Reist NE, et al. (2013). Epsin 1 promotes synaptic growth by enhancing BMP signal levels in moto-neuron nuclei. PLoS One, 8, e65997.

Wang Q, Han TH, Nguyen P, Jarnik M, & Serpe M. (2018). Tenectin recruits integrin to stabilize bouton architecture and regulate vesicle release at the Drosophila neuromuscular junction. eLife, 7, e35518.

Wang Y, Lobb-Rabe M, Ashley J, Anand V, & Carrillo RA (2021). Structural and functional synaptic plasticity induced by convergent synapse loss in the Drosophila neuromuscular circuit. The Journal of Neuroscience, 41, 1401–1417. [PubMed: 33402422]

Wang R, Mellem JE, Jensen M, Brockie PJ, Walker CS, Hoerndli FJ, et al. (2012). The SOL-2/Neto auxiliary protein modulates the function of AMPA-subtype ionotropic glutamate receptors. Neuron, 75, 838–850. [PubMed: 22958824]

Xing G, Gan G, Chen D, Sun M, Yi J, Lv H, et al. (2014). Drosophila neuroligin3 regulates neuromuscular junction development and synaptic differentiation. The Journal of Biological Chemistry, 289, 31867–31877. [PubMed: 25228693]

Zeng YA, Rahnama M, Wang S, Sosu-Sedzorme W, & Verheyen EM (2007). Drosophila Nemo antagonizes BMP signaling by phosphorylation of Mad and inhibition of its nuclear accumulation. Development, 134, 2061–2071. [PubMed: 17507407]

Zhai RG, & Bellen HJ (2004). The architecture of the active zone in the presynaptic nerve terminal. Physiology (Bethesda), 19, 262–270. [PubMed: 15381754]

Zhang YE (2009). Non-Smad pathways in TGF-beta signaling. Cell Research, 19, 128–139. [PubMed: 19114990]

Zhang YQ, Bailey AM, Matthies HJ, Renden RB, Smith MA, Speese SD, et al. (2001). Drosophila fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. Cell, 107, 591–603. [PubMed: 11733059]

Zhang Y, Feng X, We R, & Derynck R. (1996). Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. Nature, 383, 168–172. [PubMed: 8774881]

Zhang B, Luo S, Wang Q, Suzuki T, Xiong WC, & Mei L. (2008). LRP4 serves as a coreceptor of agrin. Neuron, 60, 285–297. [PubMed: 18957220]

Zhang W, St-Gelais F, Grabner CP, Trinidad JC, Sumioka A, Morimoto-Tomita M, et al. (2009). A transmembrane accessory subunit that modulates kainate-type glutamate receptors. Neuron, 61, 385–396. [PubMed: 19217376]

Zhao K, Hong H, Zhao L, Huang S, Gao Y, Metwally E, et al. (2020). Postsynaptic cAMP signalling regulates the antagonistic balance of Drosophila glutamate receptor subtypes. Development, 147(24), dev191874.

Zhao G, Wu Y, Du L, Li W, Xiong Y, Yao A, et al. (2015). Drosophila S6 kinase like inhibits neuromuscular junction growth by downregulating the BMP receptor thickveins. PLoS Genetics, 11, e1004984.

Zhu H, Kavsak P, Abdollah S, Wrana JL, & Thomsen GH (1999). A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. Nature, 400, 687–693. [PubMed: 10458166]

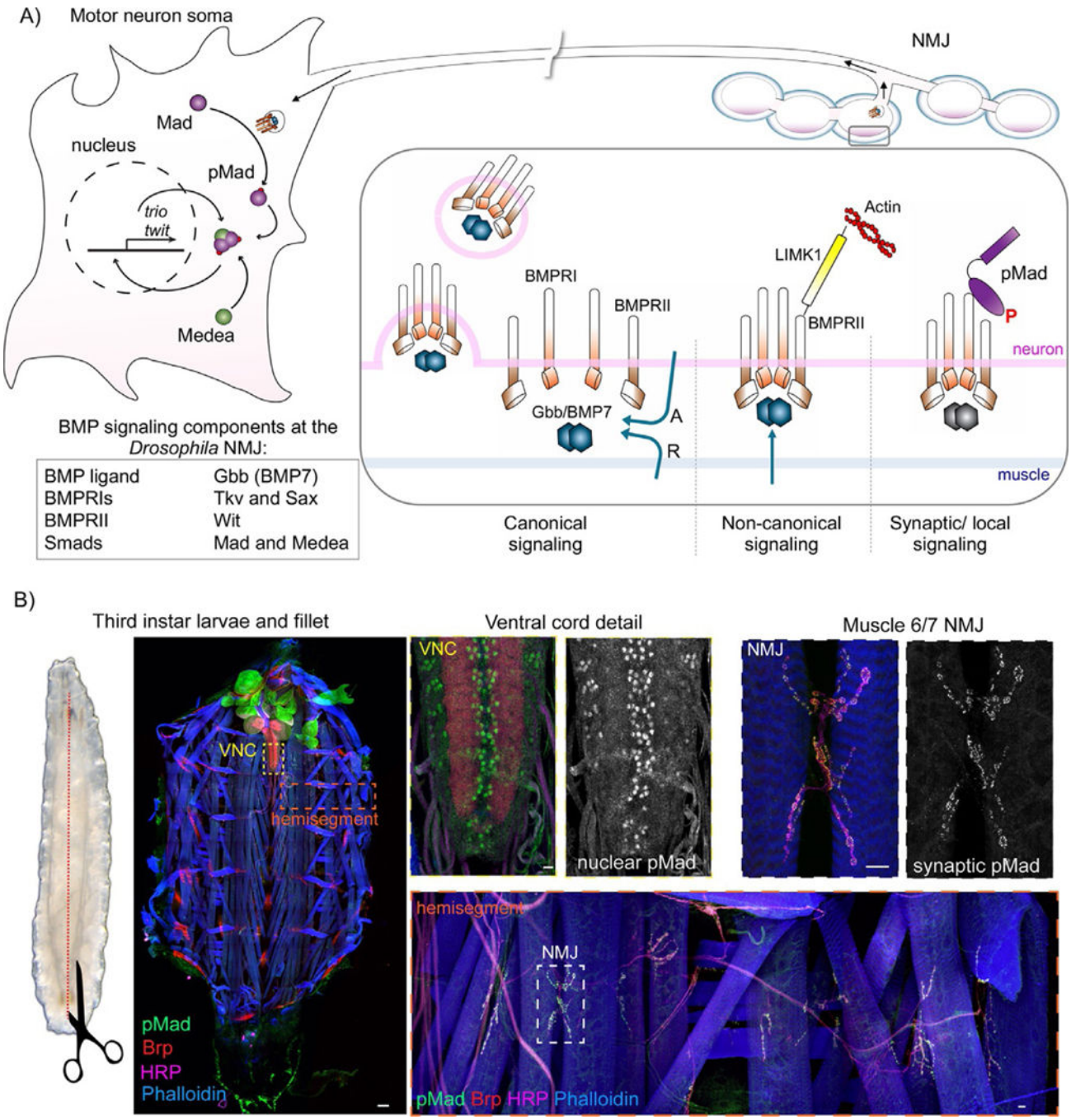

Fig. 1.

Fly motor neurons receive and integrate multiple BMP signaling pathways. (A) Diagram of various BMP pathways signaling to fly motor neurons. At synaptic terminals, Gbb/BMP7 binding to BMP receptors on the motor neuron membrane triggers canonical and non-canonical BMP signaling. Gbb is secreted from both muscle (retrograde signaling—marked here “R”) and motor neurons (autocrine signaling—labeled “A”). During canonical BMP signaling, the high-order BMP/BMPR (Gbb/Wit/Tkv/Sax) complexes are endocytosed and transported to the motor neuron soma where they phosphorylate Mad to regulate various

transcriptional programs required for NMJ growth and function. During non-canonical signaling, Wit, the BMPRII, signals through LIMK1 to regulate synapse stability. This pathway does not involve Mad or Medea. In addition, the BMPRs Wit, Tkv and Sax, but not Gbb, enable the synaptic/local BMP signaling. (B) Dissection of a third instar larva along the dorsal side exposes the highly stereotyped body wall muscles labeled with phalloidin (blue) and imaged by confocal microscopy. The anti-horseradish peroxidase antibodies (HRP, magenta) label neuronal membranes. Bruchpilot (Brp, red) is a synaptic scaffold which marks the active zones. In response to canonical BMP signaling, pMad (green) accumulates in the motor neuron nuclei within the ventral nerve cord (VNC), the fly equivalent of mammalian spinal cord. In addition, pMad also accumulates in motor neuron synaptic terminals (right upper panels—muscle 6/7 NMJ). Synaptic/junctional pMad forms discrete puncta that co-localize with the active zone scaffold, Brp. Scale bars: 100 μm (larval fillet), 10 μm (others).

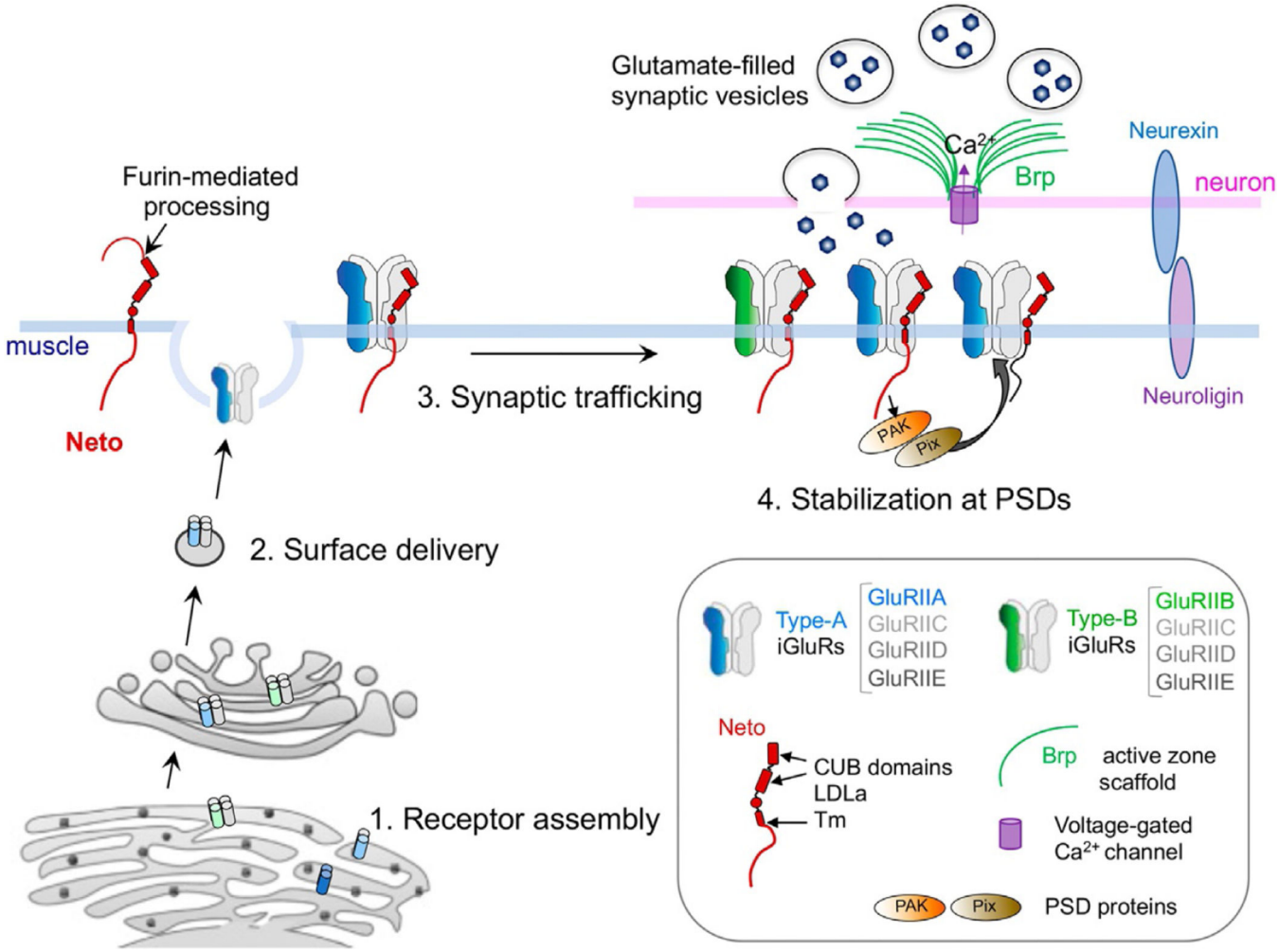

Fig. 2.

Synaptic recruitment marks the end of a long journey for iGluRs. The hetero-tetrameric iGluR NMJ complexes must assemble in ER before they could be delivered to muscle surface. Once on the membrane, iGluRs form complexes with Neto and together bind to scaffolds and motors which ensure trafficking to postsynaptic densities, juxtaposing the active zones. Synaptic iGluRs form large aggregates that are further stabilized through interactions with postsynaptic density components. Additional trans-synaptic interactions (such as Neurexin-Neuroligin) keep active zones and postsynaptic densities in register, further minimizing the distance that the neurotransmitter must travel to reach the postsynaptic receptors.

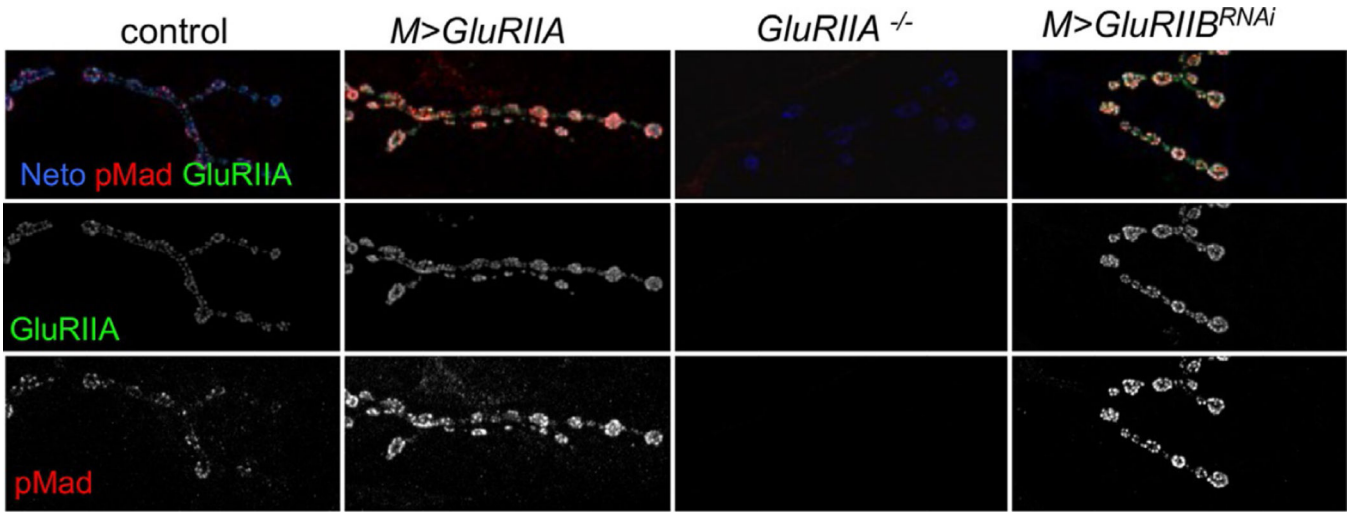

Fig. 3.

pMad mirrors the postsynaptic type-A receptors. Confocal images of NMJ boutons from third instar larvae of the indicated genotypes labeled for the obligatory auxiliary subunit Neto (blue), which marks both type-A and type-B glutamate receptors, GluRIIA (green), the glutamate receptor subunit specific for type-A receptors, and pMad (red). Synaptic pMad follows the distribution and intensity of GluRIIA-positive signals: It increases with the muscle overexpression of *GluRIIA* and becomes undetectable at *GluRIIA* mutant NMJs. Depletion of GluRIIB in the postsynaptic muscle triggers an increase of synaptic GluRIIA levels and therefore increased synaptic pMad.

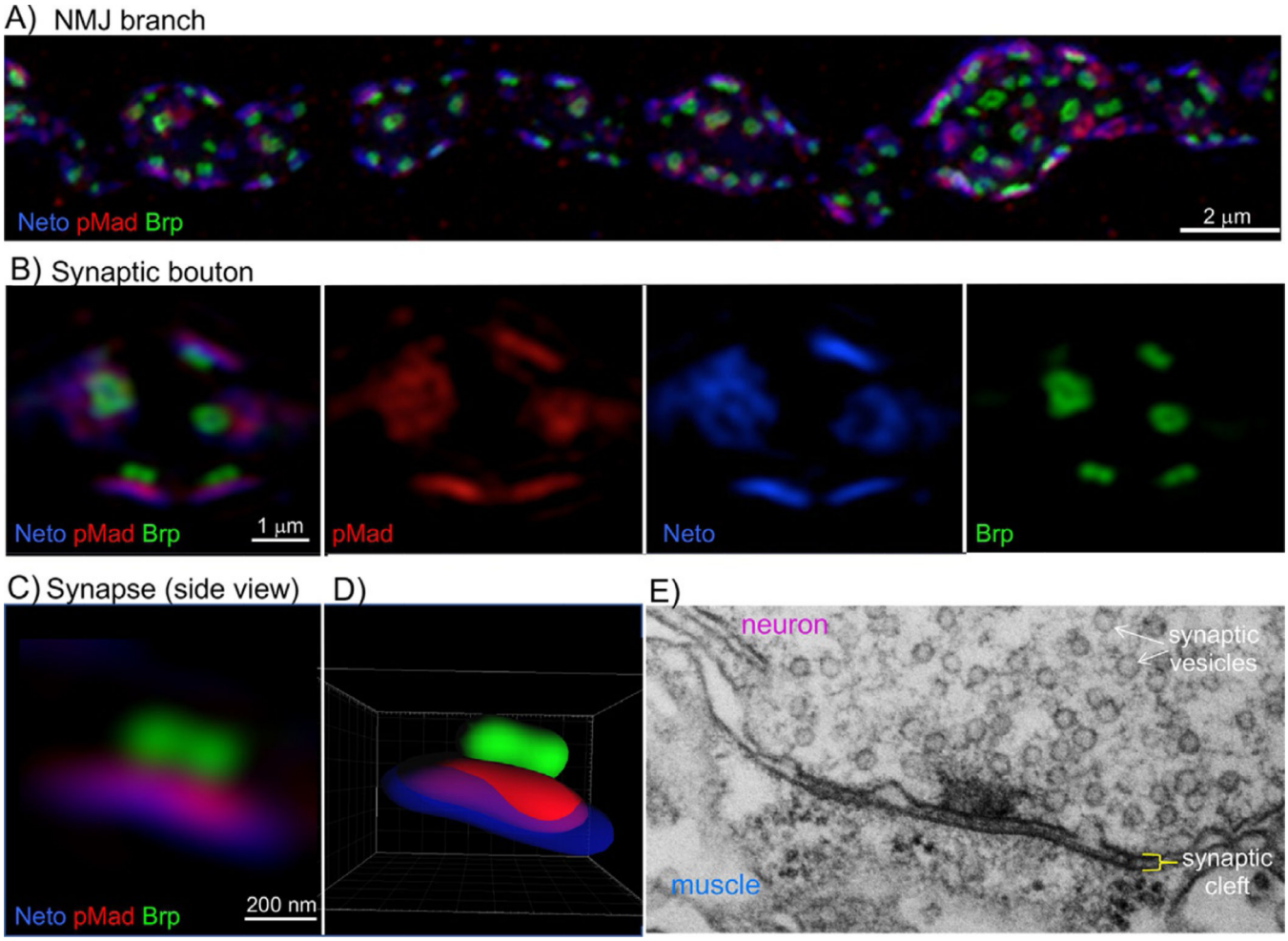

Fig. 4.

Synaptic pMad localizes at the active zone. (A, B) 3D structured illumination microscopy (3D-SIM) images of NMJ boutons from third instar larvae labeled for Brp- an active zone scaffold (green), pMad (red) and the obligatory auxiliary subunit Neto (blue). (C) High magnification view of a single synapse profile (from panel B). (D) Side view of a surface rendered volume of the synapse shown in panel (B). (E) Electron micrograph of a single synapse illustrating the characteristic T-bar structure juxtaposing the postsynaptic density. The anti-Brp monoclonal antibody recognizes the tip of the T-bar, hence the ring appearance. The anti-Neto antibodies recognize the extracellular CUB1 domain within the synaptic cleft and mark the postsynaptic densities. The pMad signals concentrate in between Brp and Neto, closer to Neto and form thin discs suggestive of a layer of pMad parallel to the presynaptic membrane.

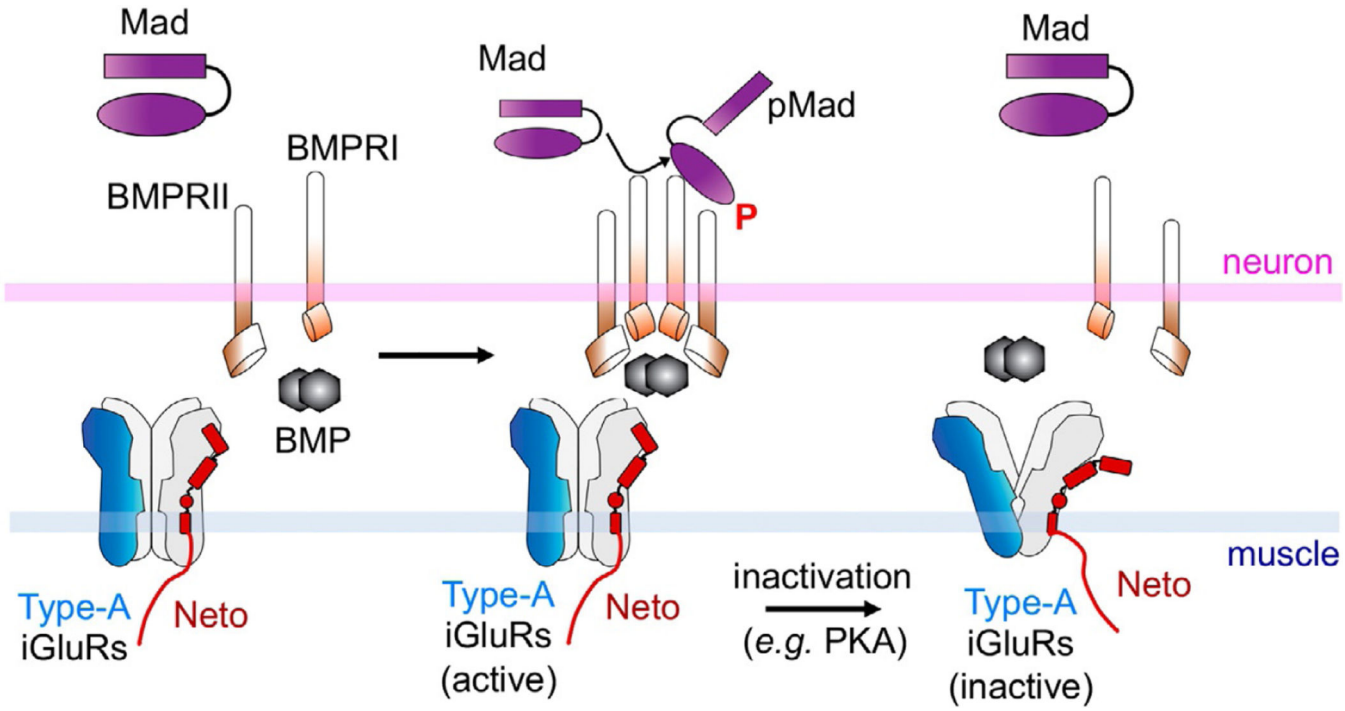

Fig. 5.

Model for how Neto-mediated trans-synaptic interactions relay the status of postsynaptic type-A receptors to presynaptic BMP/BMPR complexes. Neto and iGluRs traffic together at synaptic locations. Neto also has two extracellular BMP-interacting CUB domains that may localize BMP activities and anchor the presynaptic BMP/BMPR complexes at active zones via trans-synaptic interactions. These complexes phosphorylate Mad locally and induce pMad accumulation at the synaptic junction. Inactivation of type-A receptors induces conformational changes and dissociation of these trans-synaptic complexes.

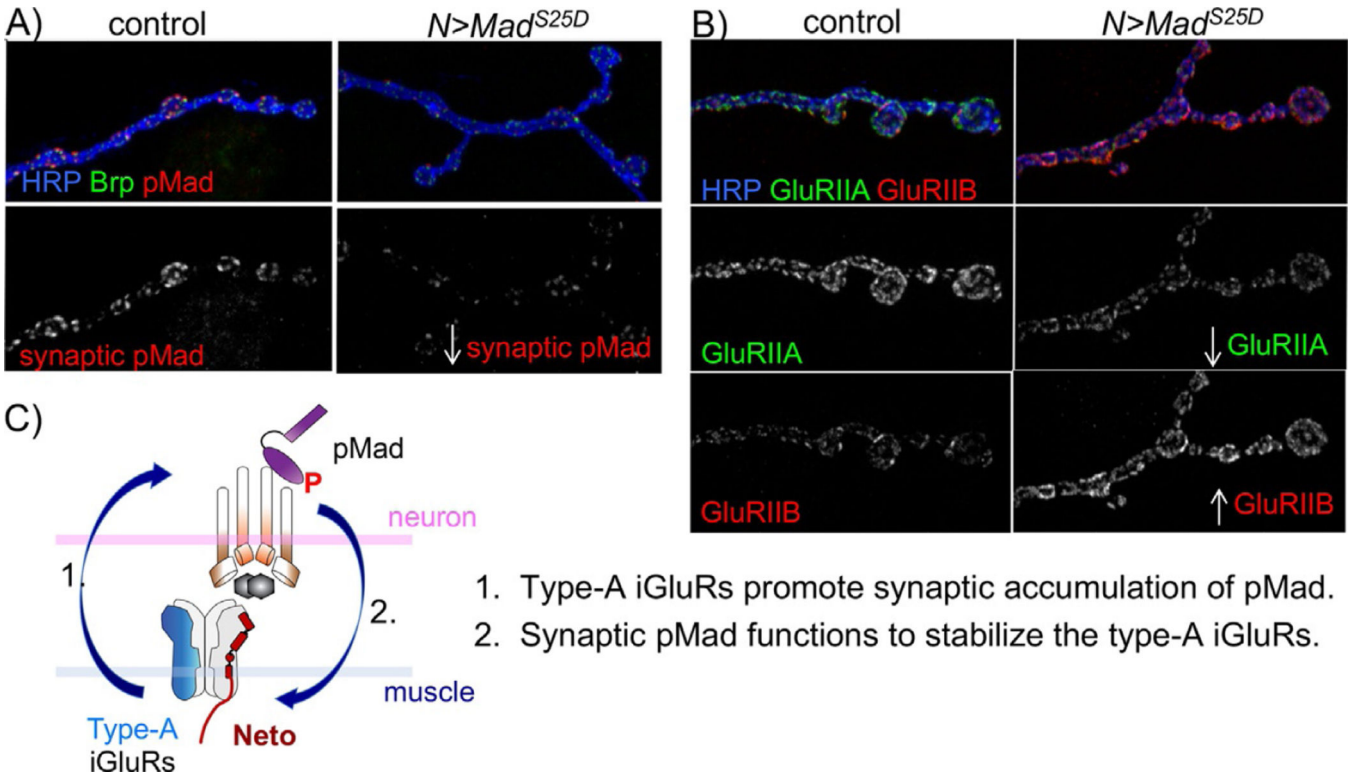

Fig. 6.

Disruption of presynaptic pMad reduces the levels of postsynaptic type-A receptors. (A, B) Confocal images of NMJ4 boutons from control and third instar larvae with a phosphomimetic Mad variant overexpressed in motor neurons (*N> Mad*^{S25D}). Neuronal expression of Mad^{S25D} reduces the accumulation of synaptic pMad and GluRIIA (type-A receptors) and increases the GluRIIB synaptic accumulation. The anti-horseradish peroxidase (HRP-blue) labels neuronal membranes. (C) The positive feedback loop model

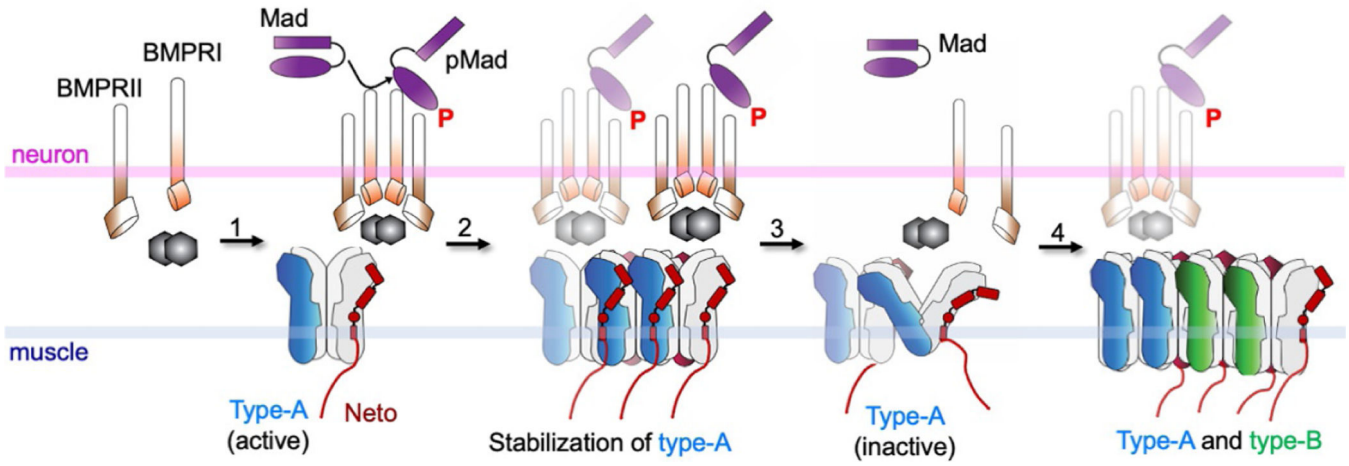

Fig. 7.

Model for type-A receptor stabilization via local BMP pathway. Neto in complexes with active type-A receptors localizes BMP activities, promoting the formation of presynaptic BMP/BMP receptor complexes (step 1). These complexes function as “accumulation centers” for stabilizing type-A receptors at nascent synapses (step 2). Dissociation of these local complexes terminates further incorporation of type-A receptors and allows for recruitment of type-B subtypes, which mark mature synapses (steps 3–4).