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Endostatin Is a *Trans*-Synaptic Signal for Homeostatic Synaptic Plasticity

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SUMMARY

At synapses in organisms ranging from fly to human, a decrease in postsynaptic neurotransmitter receptor function elicits a homeostatic increase in presynaptic release that restores baseline synaptic efficacy. This process, termed presynaptic homeostasis, requires a retrograde, *trans*-synaptic signal of unknown identity. In a forward genetic screen for homeostatic plasticity genes, we identified *multiplexin*. Multiplexin is the *Drosophila* homolog of Collagen XV/XVIII, a matrix protein that can be proteolytically cleaved to release Endostatin, an antiangiogenesis signaling factor. Here we demonstrate that Multiplexin is required for normal calcium channel abundance, presynaptic calcium influx, and neurotransmitter release. Remarkably, Endostatin has a specific activity, independent of baseline synapse development, that is required for the homeostatic modulation of presynaptic calcium influx and neurotransmitter release. Our data support a model in which proteolytic release of Endostatin signals *trans*-synaptically, acting in concert with the presynaptic CaV2.1 calcium channel, to promote presynaptic homeostasis.

INTRODUCTION

The nervous system is continually modified by experience. Given the tremendous complexity of the nervous system, it is astounding that robust and reproducible neural function can be sustained throughout life. It is now apparent that homeostatic signaling systems stabilize the excitable properties of nerve and muscle and, thereby, constrain how the nervous system can be altered by experience or crippled by disease. The *Drosophila* neuromuscular junction (NMJ) has emerged as a powerful model system to dissect the underlying mechanisms that achieve the homeostatic modulation of presynaptic neurotransmitter release. At the *Drosophila* NMJ, inhibition of postsynaptic glutamate receptor function causes a homeostatic increase in presynaptic neurotransmitter release that precisely restores muscle excitation to baseline levels. This phenomenon is conserved from fly to human (Cull-Candy et al., 1980; Plomp et al., 1992). Importantly, presynaptic homeostasis has also been observed at mammalian central synapses in vitro in response to differences in target innervation (Liu and Tsien, 1995) and altered postsynaptic excitability (Burrone et al., 2002) and following chronic inhibition of neural activity (Kim and Ryan, 2010; Zhao et al., 2011).

Despite progress in identifying presynaptic effector proteins that are required for the expression of presynaptic homeostasis (Davis, 2013), the identity of the retrograde signaling system remains unknown. Numerous neurotrophic factors, such as nerve growth factor; brain-derived neurotrophic factor (BDNF); and glia-derived neurotrophic factor, as well as nitric oxide, endocannabinoids, and adhesion molecules, are identified as retrograde signals that regulate presynaptic cell survival, differentiation, and biophysical properties in an activity-dependent manner (Gottmann, 2008; Harrington and Ginty, 2013; Iremonger et al., 2013). Among these molecules, BDNF has been implicated in the *trans*-synaptic control of presynaptic release in cultured hippocampal neurons (Jakawich et al., 2010). It was previously demonstrated that a bone morphogenetic protein (BMP) ligand (Glass bottom boat) is released from muscle, activates a type II BMP receptor at the presynaptic terminal, and is required for the growth of the presynaptic nerve terminal (McCabe et al., 2003). This BMP signaling system is also necessary for presynaptic homeostasis. However, the BMP signaling system is a permissive signal that acts at the motoneuron cell body (Goold and Davis, 2007).

A large-scale, electrophysiology-based forward genetic screen for mutations that block presynaptic homeostasis (Dickman and Davis, 2009; Müller et al., 2011) identified *multiplexin* as a candidate homeostatic plasticity gene. *Drosophila* Multiplexin is the homolog of human Collagen XV and XVIII, matrix molecules that are expressed ubiquitously in various vascular and epithelial basement membranes throughout the body (Seppinen and Pihlajaniemi, 2011). Mutations in the human *COL18A1* gene cause Knobloch syndrome, characterized by retinal detachment, macular abnormalities, and occipital encephalocele (Passos-Bueno et al., 2006; Sertié et al., 2000; Suzuki et al., 2002). Patients with Knobloch syndrome are also predisposed to epilepsy (Suzuki et al., 2002), highlighting the critical function of Collagen XVIII in the central nervous system. Moreover, the C-terminal of Collagen XVIII, encoding an Endostatin domain, can be cleaved proteolytically (Chang et al., 2005; Felbor et al., 2000; Heljasvaara et al., 2005) and functions as an antiangiogenesis factor to inhibit tumor progression (Dhanabal et al., 1999; O'Reilly et al., 1997; Yamaguchi et al., 1999). Endostatin inhibits angiogenesis by interacting with various downstream signaling factors, including vascular endothelial growth factor receptors (Kim et al., 2002), integrins (Wickström et al., 2002), and Wnt

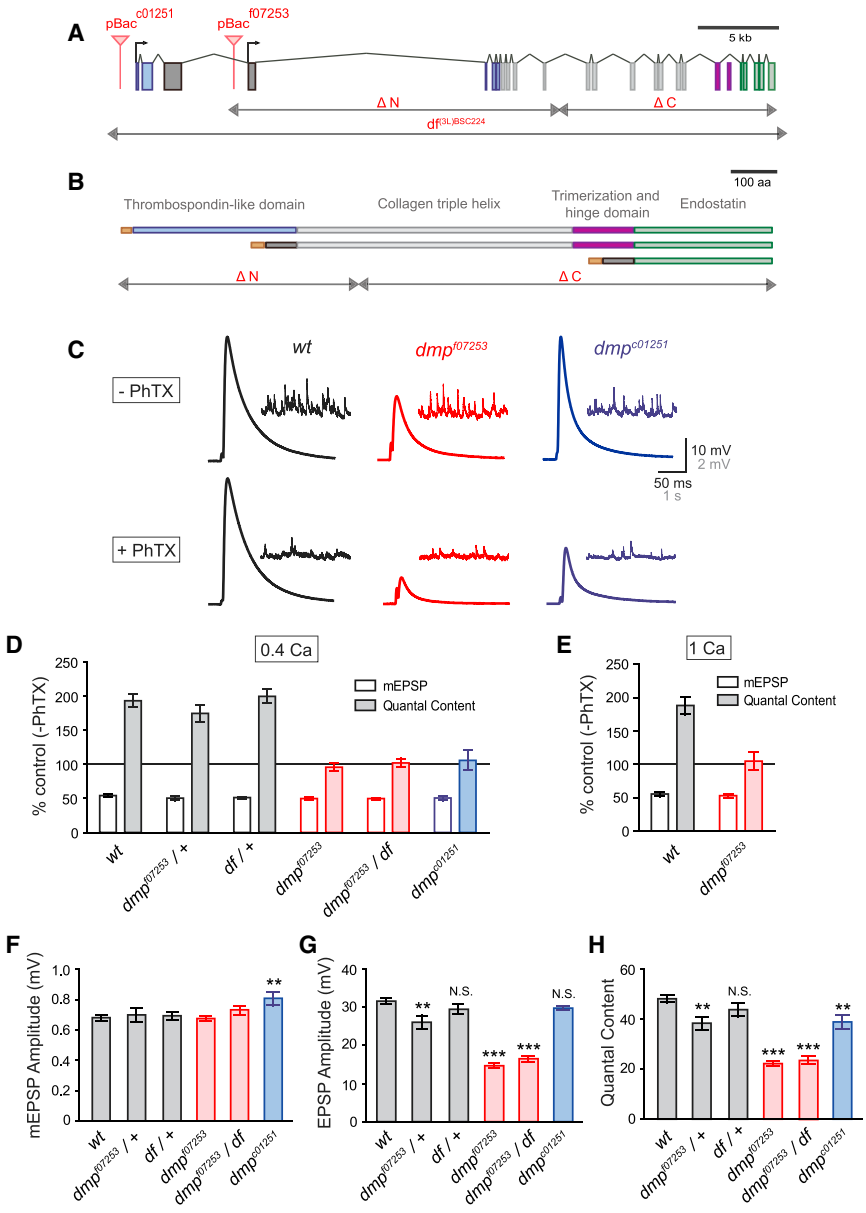

Figure 1. Mutations in *multiplexin* Block Presynaptic Homeostasis

(A) The *Drosophila multiplexin* gene locus. Exons are shown in filled color boxes indicating translated protein domains: blue, Thrombospondin-like domain; dark gray, alternative cap; gray, collagen triple helix domain; purple, trimerization and hinge domain; green, Endostatin domain. Transposon insertions *pBac c01251* and *pBac f07253* reside in introns (red triangles). Genomic deletions at the N-terminal exon 4-11 (ΔN), C-terminal exon 12-25 (ΔC) and deficiency $df^{(3L)BSC224}$ are indicated by gray arrows in the diagram.

(B) Multiplexin protein diagram. Three major protein isoforms are presented. Signal peptides are in brown, and other translated protein domains are presented as in (A). Deletions at the N-terminal exon 4-11 (ΔN) and C-terminal exon 12-25 (ΔC) are indicated by gray arrows in the diagram.

(C) Representative EPSP traces (scale: 10 mV, 50 ms) and spontaneous mEPSP traces (scale: 2 mV, 1 s) in the absence and presence of philanthotoxin (−PhTX, +PhTX; top and bottom, respectively) in wt (black) and two *multiplexin* mutants (dmp^{f07253}, red; dmp^{c01251}, blue).

(D) mEPSP amplitudes (open bars) and presynaptic release (quantal content, filled bars) in the presence of PhTX. Average mEPSP amplitude and quantal content are normalized to values in the absence of PhTX for each genotype. The following genotypes are presented: wt (gray bars), heterozygous dmp^{f07253} mutant (dmp^{f07253}/+, gray bars), heterozygous $df^{(3L)BSC224}$ mutant (*df*/+, gray bars), homozygous dmp^{f07253} mutant (dmp^{f07253}, red bars), dmp^{f07253}placed in *trans* to a deficiency $df^{(3L)BSC224}$ (dmp^{f07253}/*df*, red bars), and homozygous dmp^{c01251} mutant synapses (dmp^{c01251}, blue bars).

(E) mEPSP amplitude (open bars) and presynaptic release (quantal content, filled bars) in the presence of PhTX recorded at 1 mM calcium. Average mEPSP amplitude and quantal content are normalized to values in the absence of PhTX. wt (gray bars) and homozygous dmp^{f07253} mutant synapses (dmp^{f07253}, red bars) are presented.

(F–H) Baseline transmission for *multiplexin* mutants. Average mEPSP amplitude (F), EPSP amplitude (G), and presynaptic release (quantal content, [H]) in the absence of PhTX. Genotypes are presented the same as in (D). Mean ±SEM; **p < 0.005; ***p < 0.001. N.S., not significant; Student's t test.

signaling molecules (Hanai et al., 2002). Little is known regarding the function of *multiplexin* in the nervous system. Here, we provide evidence that Endostatin, a proteolytic cleavage product of *Drosophila* Multiplexin, functions as a *trans*-synaptic signaling molecule that is essential for the homeostatic modulation of presynaptic neurotransmitter release at the *Drosophila* NMJ.

RESULTS

Multiplexin is Required for the Rapid Induction of Synaptic Homeostasis

Application of philanthotoxin (PhTX) to the *Drosophila* NMJ, at sub-blocking concentrations, inhibits postsynaptic glutamate receptor function and leads to a homeostatic potentiation of presynaptic neurotransmitter release, termed presynaptic homeostasis (Frank et al., 2006). This assay is the basis for an ongoing, electrophysiology-based forward genetic screen for mutations that block presynaptic homeostasis (Dickman and Davis, 2009; Müller et al., 2011). This screen identified a transposon insertion ($pBac^{f07253}$) in the *Drosophila multiplexin* gene that blocks PhTX-induced presynaptic homeostasis (Figure 1A). The expression of the *multiplexin* gene is complex, with multiple splice variants and three major protein isoforms (Figure 1B) (Meyer and Moussian, 2009) (http://flybase.org/). The $pBac^{f07253}$ transposon insertion resides within an intron and has been reported to disrupt expression of the long and middle