classification, 210 genes were identified as candidates for driving differentiation of neurons. These corresponded to genes that are active during development and that become hypermethylated and silenced in adults. Several of these encode TFs that were known to drive cell-type-specific neuronal differentiation. Other large cell-type-specific hypo-DMRs are enriched downstream of promoters with histone modifications characteristic of enhancers. Thus, neuronal methylomes can provide an epigenetic trace of neural development, whereby methylation gains within DMVs reflect past gene expression and differential methylation of large intragenic hypo-DMRs reflect the cell-type-specific regulation of current gene expression.

This demonstration that DNA methylation and ATAC-seq in specific cell types can be used to follow neuronal differentiation suggests an attractive general strategy for epigenomic studies, which are presently dominated by ChIP-based mapping. ChIP is limited by the need for highly specific antibodies and large amounts of material and can be hampered by artifacts including epitope masking and "hyper-chippable" regions (Zentner and Henikoff, 2014). In contrast, purification of DNA for mapping DNA methylation and for ATAC-seq is simple and efficient and, as this study shows, provides sensitive discrimination of regulatory elements without the need for ChIP. This approach is especially attractive when coupled with INTACT, which requires no specialized equipment or expertise. The decreasing cost of short-read sequencing, and the availability of lines engineered for INTACT, provides a practical and affordable general platform for developmental epigenomics on both large and small scales.

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# Inhibitory Synapses Get Madd for Neuroligin

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**The mechanisms mediating the appropriate clustering of neurotransmitter receptors opposite release sites are poorly understood. Two studies in this issue of *Neuron*, Maro et al. (2015) and Tu et al. (2015), identify a new extracellular effector for neuroligin in GABAergic postsynaptic differentiation.**

Synapses are the basic units of communication in the nervous system. Synaptic function relies on the precise apposition of chemically matched neurotransmitter receptors and associated scaffolding and signaling molecules in the postsynaptic cell to vesicle release and recycling machinery in the axon. Hundreds of molecules each function at presynaptic and postsynaptic specializations to mediate finely tuned neurotransmission. Uncovering the processes mediating the development of such complex junctions spanning two cells and their interiors remains a challenge. Significant progress in understanding how synapses develop was made through the identification of secreted and cell surface synaptic organizing proteins that can locally trigger postsynaptic and presynaptic differentiation (Siddiqui and Craig, 2011; Südhof, 2008).

The first identified synaptic organizing protein was agrin, a large protein secreted from mammalian motoneurons. Agrin binds low-density lipoprotein receptor-related protein 4 (LRP4) to activate muscle-specific kinase (MuSK) to stabilize acetylcholine receptors (AChRs) at the mammalian neuromuscular junction (NMJ) (Wu et al., 2010). Perhaps the most extensively studied synaptic organizing molecules are the presynaptic transmembrane proteins neurexins, which function at mammalian glutamatergic and GABAergic synapses (Südhof, 2008). Agrin and neurexins share limited structural similarity with key interactions mediated by LNS (laminin, neurexin, sex hormone binding protein) domains and regulated by alternative splicing. Importantly, neurexins organize synapses

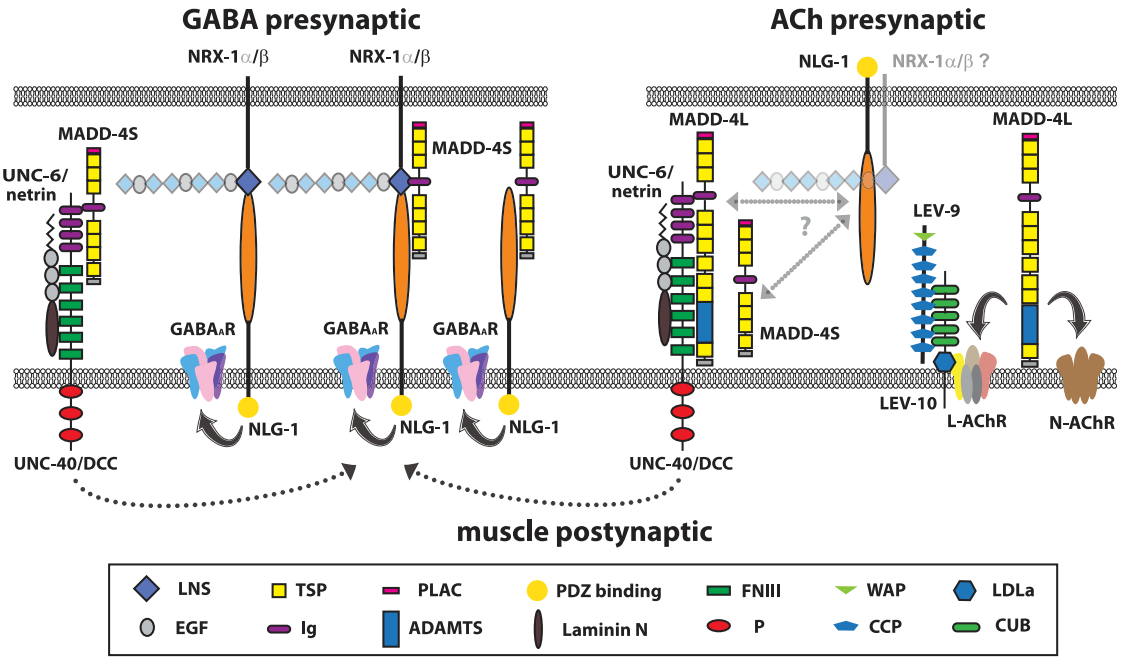


**Figure 1. Model for the Role of MADD-4/Ce-Punctin in Postsynaptic Differentiation at *C. elegans* Neuromuscular Junctions**
At GABAergic NMJs (left), binding of axon-derived MADD-4S, neurexin NRX-1, or both, to neuroligin NLG-1 on muscle triggers postsynaptic clustering of $GABA_AR$s in a PDZ binding motif-dependent manner (solid arrows). MADD-4S or MADD-4L binding to UNC-40/DCC on muscle also stabilizes $GABA_AR$s through an unknown signaling pathway (dashed arrows). At cholinergic NMJs (right), MADD-4L is required for the proper synaptic localization of N-AChRs and of an L-AChR-LEV9-LEV10 complex (solid arrows). There may be additional interactions between MADD-4 and presynaptic NLG-1 (gray arrows) and roles for NRX-1 if it is also expressed at cholinergic presynaptic sites.

through interactions with diverse postsynaptic partners including neuroligins, leucine-rich repeat transmembrane proteins (LRRTMs), and a cerebellin-GluRδ complex (Siddiqui and Craig, 2011). Neurexins and each of these postsynaptic partners can bidirectionally mediate synapse organization, recruiting neurotransmitter receptors and receptive machinery and synaptic vesicles and release apparatus.

Whereas neurexins act through multiple families of postsynaptic partners to organize synapses, neuroligins were thought to act only through neurexins—until now. In this issue of *Neuron*, two groups, Maro et al. (2015) and Tu et al. (2015), identify MADD-4/Ce-punctin as a novel axon-derived partner that binds to postsynaptic neuroligin to organize GABAergic NMJ synapses in *C. elegans*. Each body wall muscle in *C. elegans* clusters inhibitory GABA ionotropic receptors ($GABA_AR$s) opposite GABAergic inputs and excitatory levamisole-sensitive (L-AChR) and levamisole-insensitive (N-AChR) acetylcholine receptors opposite cholinergic inputs (Figure 1). MADD-4, a disintegrin and metalloprotease with thrombospondin repeat-like (ADAMTSL) secreted extracellular matrix protein that lacks protease activity, was recently identified from a screen for mislocalized L-AChRs (Pinan-Lucarré et al., 2014).

Maro et al. (2015) and Tu et al. (2015) both show that MADD-4 derived from GABAergic motoneurons binds to the single *C. elegans* neuroligin NLG-1 on muscle to cluster postsynaptic $GABA_AR$s. NLG-1 on muscle targets only to GABAergic, not to cholinergic postsynaptic sites. Whereas *Nlg-1* mutants show strongly disrupted clustering of $GABA_AR$s, but normal localization of MADD-4, *madd-4* mutants show partially disrupted clustering of both NLG-1 and $GABA_AR$s. These results place MADD-4 upstream of NLG-1 in a molecular pathway toward GABAergic postsynaptic differentiation, and indicate the existence of an additional anterograde signal to NLG-1. A corresponding functional deficit, reduced mIPSC frequency, was observed in these mutants. However, perhaps related to their analyses of different alleles, significant differences were observed between the two groups in the severity of the *madd-4* mutant phenotypes. The more subtle phenotype of the *madd-4* mutant studied by Maro et al. (2015) allowed them to uncover functions for neurexin in this system.

Surprisingly, mutations in the single *C. elegans* neurexin *nrx-1*, even a newly generated mutation deleting the transmembrane and intracellular domains, had no effect on the synaptic localization of NLG-1 or $GABA_AR$ and no effect on mIPSCs (Maro et al., 2015). Previous studies of *nrx-1* mutants revealed only subtle differences in mEPSC kinetics and retrograde homeostatic signaling at cholinergic NMJs (Hu et al., 2012) and altered locomotion including hyperreversal, reduced exploration, and anomalous sinusoidal motion (Calahorro and Ruiz-Rubio, 2013). These observations contrast with the central role of neurexins at synapses in mice: even deletion of a subset of neurexins, the longer α-neurexins, markedly reduces synaptic transmission and is perinatal lethal (Südhof, 2008).

Strikingly, Maro et al. (2015) found a major reduction in GABAergic postsynaptic protein clustering and inhibitory

transmission in *C. elegans nrx-1;madd-4* double mutants, more severe than in either mutant alone and similar to *nlg-1* mutants. Further, Maro et al. (2015) found that MADD-4 can bind to NLG-1, and the immunoglobulin domain of MADD-4 can directly bind to the LNS domain of NRX-1, leading to their model that MADD-4, NRX-1, and NLG-1 function synergistically in a tripartite complex. This tripartite model in which all components interact pairwise differs from the neurexin-cerebellin-GluRδ tripartite model in mammals in which secreted cerebellin is required to bridge neurexin and GluRδ (Siddiqui and Craig, 2011). However, the significance of the interactions of MADD-4, NRX-1, and NLG-1 and their precise roles warrant further study. The phenotypic evidence seems to favor a model in which NRX-1 and MADD-4 act as parallel, largely redundant, anterograde synaptic organizers for GABAergic postsynaptic differentiation both through NLG-1.

Tu et al. (2015) also performed a visual screen for mislocalization of $GABA_AR$s and isolated mutants in *unc-40/DCC* (Deleted in Colorectal Cancer), a receptor for UNC-6/netrin. Further, MADD-4 binds UNC-40 and localizes UNC-40 to postsynaptic sites (both GABAergic and cholinergic). The overall content of postsynaptic $GABA_AR$, but not of NLG-1, was dependent on UNC-40, which in turn was dependent on MADD-4 (Tu et al., 2015). A diffusely distributed membrane-associated constitutively active intracellular domain of UNC-40 rescued postsynaptic $GABA_AR$ levels. These data indicate that MADD-4 controls an UNC-40 signaling pathway to promote postsynaptic stabilization of $GABA_AR$s. There are several open questions remaining regarding the MADD-4/UNC-40 pathway. First, is there cross-talk between the MADD-4/NLG-1 and MADD-4/UNC-40 pathways? For example, it would be interesting to test whether this constitutively active UNC-40 could rescue the mislocalization of $GABA_AR$ in the absence of NLG-1. Alternately, UNC-40 might control the overall level of $GABA_AR$, rather than its postsynaptic clustering; one wonders whether response to GABA application in *unc-40* mutants is normal as shown for *nlg-1* mutants. Second, whether MADD-4 not only recruits and stabilizes UNC-40, but also activates its signaling pathway requires further investigation. Finally, a recent study reported roles for DCC and netrin-1 in glutamatergic synaptogenesis in cultured mouse cortical neurons (Goldman et al., 2013). Considering the new data from *C. elegans*, it may prove fruitful to study their roles in GABAergic synapse development.

Like most exciting experiments, these two studies raise several broad questions. How does the specificity arise, how do the appropriate neurotransmitter receptors cluster opposite the corresponding release sites? At the *C. elegans* NMJ, perhaps this question can be phrased more specifically as how does NLG-1 localize only to GABAergic, and not cholinergic, postsynaptic sites? NLG-1 localization was dependent only on its extracellular region and therefore should be dependent on its extracellular binding partners (Maro et al., 2015; Tu et al., 2015). MADD-4 is expressed in multiple isoforms: short forms (MADD-4S, or MADD-4B) are expressed at both GABAergic and cholinergic inputs, whereas long forms (MADD-4L, or MADD-4A and MADD-4C) are expressed only at cholinergic inputs. However, specific deletion of MADD-4L does not change $GABA_AR$ localization (Pinan-Lucarré et al., 2014), arguing that MADD-4 isoforms alone cannot specify the localization of NLG-1 and $GABA_AR$s. NRX-1 is expressed in most, if not all, neurons (Haklai-Topper et al., 2011), making it not a good candidate either. Interestingly, NLG-1 is not expressed in GABAergic neurons, but is expressed in cholinergic motoneurons where it can be presynaptic (Hu et al., 2012). It is tempting to speculate that NLG-1 in cholinergic inputs may sequester NRX-1 in *cis*, preventing it from binding muscle NLG-1 across the cleft.

How do these synaptic organizing proteins recruit the neurotransmitter receptors? MADD-4 and NRX-1 recruit $GABA_AR$s through NLG-1, through a mechanism requiring its intracellular domain including its PDZ domain binding site (Maro et al., 2015; Tu et al., 2015). Thus, $GABA_AR$s may be recruited through a PDZ domain protein yet to be identified. Curiously, alternative splicing of NLG-1 regulates mainly intracellular regions, including a proline-rich potential SH3 binding region (Hunter et al., 2010). It remains to be determined whether development of GABAergic postsynaptic sites in *C. elegans* involves a mechanism similar to collybistin activation by neuroligin-2 found for mammalian GABAergic synapses (Poulopoulos et al., 2009). Both L-AChRs and N-AChRs were mislocalized in *madd-4*-null mutants (Pinan-Lucarré et al., 2014). Further work is needed to understand how MADD-4 links to pathways previously found to control clustering of AChRs (Figure 1).

Do MADD-4, NRX-1, NLG-1, and UNC-40 signal bidirectionally to control presynaptic as well as postsynaptic differentiation? At first glance, the answer appears to be no. Unlike mammalian synapses where most identified synaptic organizing complexes including neurexins-neuroligins mediate aspects of presynaptic and postsynaptic differentiation, localization of presynaptic markers was not perturbed in these mutants (Maro et al., 2015; Tu et al., 2015). Furthermore, presynaptic sites are ultrastructurally normal in *madd-4*-null mutants (Pinan-Lucarré et al., 2014). However, a careful assessment of presynaptic function may reveal more subtle deficits, and it would be interesting to test roles of MADD-4 and UNC-40 in the retrograde homeostatic pathway involving NRX-1 and NLG-1 (Hu et al., 2012).

Is this MADD-4 synaptic organizer conserved in mammals? The closest ortholog Punctin-1/ADAMTSL-1 is also expressed in long and short isoforms, but was only detected in muscle (Hirohata et al., 2002). However, Punctin-2/ADAMTSL-3 is expressed in brain (Pinan-Lucarré et al., 2014), and there are altogether seven ADAMTSL family members whose functions have not been well studied (Apte, 2009). Thrombospondin-1, which shares structural domains with MADD-4, binds neuroligins to accelerate excitatory synapse formation in cultured rat hippocampal neurons (Xu et al., 2010). Given the new data from *C. elegans*, we wonder whether thrombospondin might signal through neuroligins independently of neurexins, or whether neurexins are involved in this process.

Regardless of whether this MADD-4 synaptic pathway is conserved in vertebrates, these new studies raise intriguing possibilities. It came as a surprise to

many of us to discover that neurexins act through multiple postsynaptic partners; now it seems wise to be on the lookout for new presynaptic effectors for neuroligins. Given the overwhelming evidence linking the neurexin-neuroligin pathway to autism and schizophrenia (Südhof, 2008), these are key issues not only for fundamental neuroscience, but also for understanding and eventually developing treatments for neuropsychiatric disorders.

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# Rats Exert Executive Control

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**In this issue of *Neuron*, Duan et al. (2015) introduce a novel rodent model of executive control. Their neural recordings provide direct evidence for the task-set inertia theory and suggest a crucial role for the superior colliculus in executive control.**

After hearing a radio report of a traffic jam as you approach a familiar intersection on the drive to work, you might opt to turn right rather than make your usual left turn to take an alternate route to your destination. We depend on our ability to alter our response to the same sensory input, such as the view of that familiar intersection, as we receive new information or as context changes on a moment-to-moment basis. Humans are not the only animals to exhibit this sort of behavioral flexibility. In fact, non-human primates have traditionally provided a powerful model system for studying cortical activity at the level of individual neurons during controlled behavior requiring this type of executive control (Miller, 2000). Now in *Neuron*, Duan et al. (2015) devise a rodent model in which animals can be trained to display such behavior, opening the door to new types of experiments, at a scale not previously possible.

One well-established behavioral paradigm for the study of executive control is the so-called Pro-/Anti-saccade task (Munoz and Everling, 2004), in which a monkey is instructed at the start of each behavioral trial to respond by directing its gaze either toward ("Pro") or away from ("Anti") a peripheral visual stimulus that appears later in the trial (Figure 1A). Rodents are both practically and scientifically appealing, due to their low cost and the ease of working with them compared with primates, coupled with recent technological breakthroughs in monitoring and manipulating individual neurons in intact rodents. There has been a push to develop more sophisticated behavioral paradigms for rodents in order to take advantage of these benefits, but it has not been clear to what extent they can be trained to perform tasks that can probe complex cognitive behaviors such as this type of executive control.

However, in this issue of *Neuron*, Duan et al. (2015) introduce a novel rodent model of executive control analogous to the Pro-/Anti-saccade primate paradigm. In this new paradigm, rats learn two sets of sensorimotor associations — they respond to a visual stimulus that may