Transcribing the connectome: Roles for transcription factors and chromatin regulators in activity-dependent synapse development

Liang-Fu Chen, Allen S. Zhou, and Anne E. West

Dept. of Neurobiology, Duke University, Durham, NC 27710

Corresponding author:

Anne E. West
DUMC Box 3209
Bryan Research 301D
311 Research Dr.
Durham, NC 27710
west@neuro.duke.edu
919-681-1909


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Abstract

The wiring of synaptic connections in the developing mammalian brain is shaped by both intrinsic and extrinsic signals. One point where these regulatory pathways converge is via the sensory experience-dependent regulation of new gene transcription. Recent studies have elucidated a number of molecular mechanisms that allow nuclear transcription factors and chromatin regulatory proteins to encode aspects of specificity in experience-dependent synapse development. Here we review the evidence for the transcriptional mechanisms that sculpt activity-dependent aspects of synaptic connectivity during postnatal development and we discuss how disruption of these processes is associated with aberrant brain development in autism and intellectual disability.
Introduction

The mammalian brain is a paragon of complexity. The average human brain contains tens of billions of neurons and hundreds of trillions of synaptic connections (Bargmann and Marder 2013; DeFelipe 2010). Yet every day, legions of new brains are assembled from scratch in developing embryos, and for the most part they work as intended. As is true for all developmental programs, the fundamental instructions for wiring the brain are encoded in DNA. Those plans are executed in concert with signals from the environment that guide and refine the developmental process. Importantly, during the prenatal period both the intrinsic and extrinsic regulatory mechanisms that drive brain development are highly robust, such that - barring mutations in key regulatory genes - the total number of neurons, their relative position in the brain, and their primary connections are grossly similar between individual members of a species.

By contrast, in the period after birth a new variable comes into play in the form of sensory experience, which adds dynamic range to the final state of any given connectome. Activation of sensory neurons drives patterned neural activity in the cortex that initiates critical period stages of synapse development and maturation (Katz and Shatz 1996; Wiesel 1982). Neural activity regulates excitatory and inhibitory synapse development in this time window in large part by converging on the regulation of environmentally-sensitive transcription factors that coordinate the expression of gene products required for synapse formation, elimination, and plasticity (Majdan and Shatz 2006; West and Greenberg 2011). Depending on the environment into which any given animal is born, the nature, timing, and extent of this sensory experience will differ and thus the experience-dependent development of synapses will vary between these
organisms as well. A likely evolutionary advantage of introducing uncertainty to the late
stage of brain development is allowing adaptations in brain function to match an
unpredictable outside world. However, the tradeoff is the possibility that the process
could go very wrong, leading to neurological or psychiatric disease.

A large number of transcription factors that promote both prenatal stages of brain
patterning and postnatal stages of synapse development have been identified through
gene expression profiling and molecular genetic studies (Chedotal and Richards 2010;
Polleux et al. 2007; Ross et al. 2003). The identity and molecular genetic function of
these early developmental transcription factors have been extensively reviewed elsewhere
and will not be further detailed here. Instead here we focus on three areas of substantial
recent growth in our understanding of the role of gene transcription in the experience-
dependent development of synapses in the postnatal mammalian brain. In each case
though we profile only a subset of the many transcriptional regulators that contribute to
synapse development, we use these stories to demonstrate major principles of the
transcriptional mechanisms at play. First we highlight new insights into the conundrum of
synapse specificity, by reviewing studies that elucidate how transcriptional processes
occurring in the nucleus of the neuron can promote the development of specific subsets of
synapses in the cell periphery. Next we discuss a series of recent studies showing how
chromatin regulatory factors can serve as modulators of the postnatal experience-
dependent stages of synapse development. Finally we review emerging evidence that
synapse dysfunction due to dysregulation of these transcriptional regulatory processes
can lead to autism and intellectual disability.
Transcription Factors and Synapse Specificity

Transcription factors reside predominantly in the nucleus, where they are well-poised to integrate signaling information originating anywhere within the cell. This integrative capacity of stimulus-regulated transcription factors allows them to transduce reception of tiny amounts of growth factors into binary cell survival/cell death decisions (Brunet et al. 2001), and to drive homeostatic plasticity by summatting neural activity levels into global synaptic scaling responses (Ibata et al. 2008). However the central location of transcription factors raises the question of how they can have a meaningful impact on local synaptic connectivity, where the specificity of connections is essential to brain function. Here we consider three examples of transcription factors that exemplify aspects of this specificity. These examples show that although nuclear transcription factors are unlikely to directly instruct the formation or elimination of individual synapses, they do encode specificity at the level of synapse type (e.g. excitatory, inhibitory), and subcellular synapse location (e.g. somatic, dendritic). Furthermore by interacting functionally with local translational machinery they can have regulatory control over local activity-dependent synaptic plasticity.

CaRF – A context-specific regulator of Bdnf transcription and GABAergic synapse formation

Calcium-response factor (CaRF) was initially discovered as a transcriptional activator of Brain-Derived Neurotrophic Factor (Bdnf), which it directly induces by binding to a 10-bp calcium response element (CaRE1) in the proximal region of Bdnf promoter IV (McDowell et al. 2010; Tao et al. 2002) (Fig. 1A). Expression of Exon IV-
containing forms of *Bdnf* is significantly reduced in the cortex of CaRF knockout mice demonstrating that CaRF contributes to transcriptional activation of *Bdnf* promoter IV in vivo (McDowell et al. 2010). Surprisingly although the CaRE1 element is required for transcriptional induction of *Bdnf* upon membrane depolarization, and the transcriptional activity of CaRF is induced by the subsequent calcium influx, CaRF is not required for the induction of *Bdnf* transcription that is driven by this stimulus (McDowell et al. 2010; Tao et al. 2002). Instead membrane depolarization-mediated activation of *Bdnf* promoter IV requires the transcription factor MEF2C, which binds CaRE1 independently of CaRF and acts together with CREB to drive the calcium-inducible component of *Bdnf* transcription (Hong et al. 2008; Lyons et al. 2012).

Given this context, it is intriguing that CaRF has recently been found to inhibit the induction of *Bdnf* promoter IV following activation of NMDA-type glutamate receptors (Lyons et al. 2016). Membrane depolarization promotes the activation of *Bdnf* transcription via the opening of L-type voltage-gated calcium channels (Tao et al. 1998). By contrast, treatment of neurons with the sodium channel inhibitor tetrodotoxin (TTX) silences action potentials and induces homeostatic synaptic plasticity such that upon TTX withdrawal there is rebound excitation, release of synaptic glutamate, activation of synaptic glutamate receptors, and the NMDA receptor-dependent induction of transcription from genes including *Bdnf* promoter IV (Ghiretti et al. 2014; Turrigiano and Nelson 2004). Following TTX withdrawal-induced activation of NMDARs, neurons lacking CaRF show significantly higher induction of Exon IV-containing forms of *Bdnf* mRNA and BDNF protein compared with CaRF-expressing control neurons (Lyons et al., 2016). Unlike the direct activation of *Bdnf* transcription by CaRF, which requires binding
of CaRF to *Bdnf* promoter IV, the CaRF-dependent inhibition of *Bdnf* transcription is proposed to occur through an indirect mechanism that involves CaRF-dependent induction of the unusual NMDA receptor subunit GluN3A. Incorporation of GluN3A into functional NMDARs with GluN1 and GluN2 subunits reduces receptor currents, including the influx of calcium (Das et al. 1998; Kehoe et al. 2013). Levels of GluN3A are reduced in the brains of CaRF knockout mice and restoration of GluN3A expression in CaRF knockdown neurons restores *Bdnf* inducibility to wildtype levels (Lyons et al., 2016). Thus although CaRF is a direct activator of basal *Bdnf* transcription via its binding to *Bdnf* promoter elements, it acts as an indirect inhibitor of NMDAR-inducible *Bdnf* transcription by its ability to modulate the subunit composition of neuronal NMDARs (Fig. 1A).

The link between CaRF and GluN3A is important because it suggests a potential mechanism by which CaRF could regulate the timing of synapse development. Both CaRF and GluN3A are most highly expressed during the first two postnatal weeks of brain development, after which time their expression declines (McDowell et al. 2010; Sucher et al. 1995). This decline parallels the onset of the critical period for sensory-dependent synapse development in the cortex, and notably, prolonging the expression of GluN3A inhibits critical period maturation of synapses in visual cortex, suggesting an essential function for the changing NMDAR composition in this process (Roberts et al. 2009). BDNF expression is strongly induced in sensory cortex during the critical period and it drives critical period closure by promoting the formation of inhibitory GABAergic synapses (Huang et al. 1999). Consistent with a role for CaRF-dependent repression of NMDAR-inducible *Bdnf* expression in the regulation of GABAergic synapse
development in vivo, adult CaRF knockout mice show substantially increased synaptic expression of GABAergic synapse markers (McDowell et al., 2010). This appears to be a cell autonomous effect of CaRF because single cell knockdown of CaRF in cultured mouse hippocampal neurons significantly enhances the formation of GABAergic synapses onto that neuron in a manner that can be rescued by simultaneous knockdown of BDNF (Lyons et al., 2016). Taken together these data establish CaRF as a novel regulator of the formation of GABAergic synapses in the developing brain and suggest that, via its regulation of GluN3A, it could contribute to the timing of the synaptic changes that underlie critical period closure.

Npas4: An activity-dependent, cell-type specific master regulator of inhibitory/excitatory balance

Npas4 belongs to the basic helix-loop-helix-Per-Arnt-Sim (bHLH-PAS) transcription factor family (Ooe et al. 2004), whose members share a bHLH DNA-binding domain, dual PAS domains, and a C-terminal activation domain (Partch and Gardner 2010). These transcription regulators dimerize or form complexes with transcriptional coactivators through their bHLH and PAS domains to regulate diverse functions including development, circadian rhythms and cellular responses to hypoxia and environmental toxins (Gu et al. 2000; Kewley et al. 2004; Partch and Gardner 2010). Among the bHLH-PAS family, Npas4 is unique for its restricted expression in neurons and the fact that its expression is selectively induced in neurons following membrane depolarization-induced calcium influx (Lin et al. 2008). Indeed even compared with other immediate-early gene transcription factors such as Fos, Npas4 is far more selective for
calcium signaling cascades, as its expression is induced only by calcium influx through L-type voltage gated calcium channels or NMDA receptors, but it is insensitive to the elevation of intracellular cAMP or the application of growth or neurotrophic factors (Lin et al. 2008). The mechanisms that confer this specificity upon Npas4 induction are not completely understood, though Npas4 seems to be a direct target of the activity-inducible transcription factor SRF (Kim et al. 2010; Kuzniewska et al. 2016).

Npas4 has been shown to play a prominent role in linking synaptic activity to inhibitory/excitatory synapse balance in a cell-type and subcellular specific manner during brain development. The calcium-dependent induction of Npas4 was first discovered in excitatory glutamatergic neurons, and interestingly, in these cells Npas4 was found to selectively promote the development of GABAergic synapses (Lin et al. 2008). Reduced numbers of GABAergic synapses were found in cultured hippocampal neurons when Npas4 was knocked down, whereas increased numbers of GABAergic synapses were found when Npas4 was overexpressed. In contrast, these manipulations did not affect glutamatergic synapses, indicating a selective role for Npas4 in inhibitory synapse development in these neurons. In addition to regulating the total number of inhibitory connections made during synapse formation, Npas4 appears to play an important role in the activity-dependent plasticity of GABAergic synapses in mature neural circuits. Npas4 knockout mice show decreased CA1 hippocampal neuron mIPSC frequency after exposure to an enriched environment compared to their wildtype littermates (Bloodgood et al. 2013), and Npas4 has also been found to underlie the activity-dependent increase in inhibition on newborn excitatory granule cells of the dentate gyrus as they integrate into the hippocampal circuitry (Sim et al. 2013).
studies together support a role for Npas4 in selectively upregulating inhibitory GABAergic synapse development in excitatory neurons following neuronal activity, suggesting that Npas4 diminishes future excitation and maintains homeostatic balance between inhibition and excitation.

Perhaps the most interesting and unexpected finding regarding the Npas4-dependent regulation of GABAergic synapses onto excitatory neurons is that there is subcellular specificity with respect to the effects of Npas4 manipulation on the synapses on the postsynaptic cell (Fig. 1B). Bloodgood et al. (2013) used the spatial organization of synaptic inputs onto distinct regions of pyramidal neurons in the hippocampus to reveal this specificity in vivo. The authors found that exposing mice to an enriched environment induces expression of Npas4 in the hippocampus. Following this exposure, when a stimulating electrode was placed in the pyramidal layer to activate somatic inhibitory synapses onto nearby pyramidal neurons, the authors saw relatively smaller evoked inhibitory currents in \( \text{Npas4} \) knockout neurons compared with \( \text{Npas4} \) wildtype neurons, which is consistent with the culture experiments showing Npas4 promotes inhibitory synapse formation. By contrast when the authors stimulated stratum radiatum to activate inhibitory synapses on the distal apical dendrites of these pyramidal neurons they found significantly larger evoked inhibitory currents in the knockout neuron compared with wildtype, suggesting that Npas4 actually inhibits the formation of distal inhibitory synapses presumably in the same neurons where it promotes somatic inhibition (Bloodgood et al. 2013). Given that different classes of inhibitory interneurons are known to synapse onto different subcellular domains of hippocampal pyramidal neurons (Freund...
and Buzsaki 1996), having distinct Npas4 regulation of these events is likely to be important for fine tuning circuit plasticity in the hippocampus.

Excitatory neurons comprise the large majority of cells in the culture systems that are most often used to study activity-inducible transcription, thus until recently very little was known about the identity of activity-inducible genes in inhibitory neurons. To fill this gap in knowledge, Spiegel et al. (2014) cultured neurons from the embryonic medial ganglionic eminence, which gives rise to the GABAergic interneurons that populate the forebrain. Using this system, they discovered that Npas4 expression was indeed induced in inhibitory neurons by membrane depolarization and subsequent calcium influx, similar to its regulation in excitatory neurons. However when the authors studied the effects of knocking out \textit{Npas4} on synapse formation, they found that rather than affecting GABAergic synapses, the loss of Npas4 reduced glutamatergic input to inhibitory neurons, while having no effect on the GABAergic synapses made onto these neurons (Spiegel et al. 2014). Thus, in contrast to its effects in excitatory neurons, activity-inducible Npas4 expression appears to up-regulate glutamatergic synapses in inhibitory neurons to increase their inhibitory output. This suggests a more complex role than previously thought for Npas4 in mediating neuronal inhibition/excitation balance across multiple cell types in the heterogeneous neural circuits that comprise the cortex. Overall these studies suggest that Npas4 functions as a key homeostatic factor in the cortex, depressing network excitability following neuronal activity via a negative feedback mechanism that is spread across multiple cell types and synapses.

How it is possible for Npas4 to promote opposite types of synapses in two different classes of neurons? The expression of distinct cell-type specific programs of
Npas4-regulated genes in excitatory and inhibitory neurons may underlie the differential
effects of Npas4 in these two neuron types (Spiegel et al. 2014). Using a genetic method
to identify activity-regulated genes that are selectively induced in inhibitory versus
excitatory neurons, Spiegel et al. (2014) identified a set of inhibitory neuron-specific
genes whose induction is impaired in the absence of Npas4. These Npas4-regulated
inhibitory neuron genes include *Kcnal, Frmpd3,* and *Nptx2*. These gene products have
been reported previously to be postsynaptic at glutamatergic synapses and therefore may
contribute to the Npas4-dependent enhancement of glutamatergic synapse formation in
inhibitory neurons. With respect to the Npas4-regulated genes that drive GABAergic
synapse formation in excitatory neurons, one possible target is *Bdnf*. *Bdnf* is selectively
induced by activity only in excitatory but not inhibitory neurons and is well known to
promote GABAergic synapse development in the postnatal cortex. Npas4 binds to three
regulatory elements within the *Bdnf* gene, and knocking down *Bdnf* partly rescues the
effects of Npas4 overexpression on increased GABAergic synapse number and function
in hippocampal neurons (Lin et al. 2008). Interestingly, BDNF expression is reduced in
the hippocampus of *Npas4* KO mice, and disruption of BDNF function prevents Npas4-
mediated increases in inhibition at the soma, but does not affect Npas4’s effects at the
dendrites, thus providing a potential explanation for the subcellular specificity of Npas4-
mediated inhibitory synapse formation (Bloodgood et al. 2013). Taken together, these
data support the idea that Npas4’s differential effects in these two cell types are due to
distinct cell-type specific programs of Npas4-regulated gene expression. Further
understanding of the distribution of Npas4 binding sites in different cell types by
chromatin immunoprecipitation will help to elucidate our understanding of how broadly-
expressed transcription factors can generate cell-type specific biological consequences.

MEF2: activation, repression, transcription, translation – MEF2 does it all.

Initially identified for their role in muscle cell differentiation (Gossett et al. 1989),
the myocyte enhancer factor 2 (MEF2) family of transcription factors is comprised of
four members, MEF2A-D, that are now understood to have essential functions in multiple
tissues including the CNS (Dietrich 2013; Pon and Marra 2016). In the brain the MEF2A,
C, and D proteins are highly expressed in distinct, yet overlapping brain regions, both
during neuronal development and in the adult (Leifer et al. 1993; Lyons et al. 1995).
MEF2B is found at much lower levels and will not be discussed further here. Importantly,
the MEF2s are known to be targets of activity-dependent calcium signaling in neurons
where they have been studied for their roles in activity-dependent neuronal survival (Mao
et al. 1999; Okamoto et al. 2000) as well as stimulus-regulated glutamatergic synapse
formation and elimination (Flavell et al. 2006). The MEF2 proteins are subject to
multiple, stimulus-regulated post-translational modifications that are thought to regulate
MEF2’s dual function as both a repressor and activator (Fig. 2) (Lyons and West 2011;
McKinsey et al. 2002). In resting neurons, the MEF2s are sumoylated and they function
primarily as transcriptional repressors (Shalizi et al. 2006). Following neuronal activity,
calcium influx through NMDA receptors and L-type voltage gated calcium channels
results in calcineurin-mediated dephosphorylation, MAP kinase-dependent
phosphorylation, and a switch from sumoylation to acetylation at specific resides on the
MEF2s, all of which are associated with functional MEF2 activation (Flavell et al. 2006;
Shalizi et al. 2006). Thus MEF2 transcription factors are ideally poised to be important regulators of stimulus-inducible effects on neuronal development and function.

Both the transcriptional repressor and activator functions of the MEF2s have been implicated in synapse formation, with the accumulated evidence suggesting that the repressor forms regulate synapse formation and the activator forms promote synapse elimination. The repressor functions of the MEF2s have been studied in both the cerebellum and cortex, and repression by sumoylated MEF2A is particularly well characterized for its role in early cerebellar synapse development (Shalizi et al. 2006). In cerebellar granule neurons, sumoylated MEF2A enhances the formation of the mature claw morphology of granule neuron dendrites, and knocking down MEF2A in cultured rat cerebellar slices impairs this dendritic differentiation. In cortical excitatory neurons, the repressor functions of MEF2C have similarly been shown to function in a cell-autonomous manner to regulate the development of glutamatergic synapses. Knocking out MEF2C in cortical neurons results in a mildly decreased frequency of miniature excitatory synaptic currents and significantly decreased dendritic spine density (Harrington et al. 2016). Spine density in the MEF2C knockout neurons was rescued by expression of a fusion protein that links the DNA binding domain of MEF2C to the repressor domain of Engrailed (MEF2-En), indicating that the synapse promoting effects of MEF2C in this context are mediated by its function as a transcriptional repressor. Interestingly, MEF2C also has a cell autonomous effect on GABAergic synapses made onto cortical glutamatergic neurons, such that single cell knockout of MEF2C is associated with increased amplitude and frequency of miniature inhibitory synaptic
currents, and an increased number of GABAergic synapses, and this effect is again rescued by overexpression of MEF2C-En.

By contrast the transcriptional activator functions of the MEF2 transcription factors have been implicated in the activity-dependent elimination of glutamatergic synapses in the hippocampus. Knocking down/out MEF2A, MEF2C or MEF2D in hippocampal neurons was found to increase excitatory synapse number, which first suggested a role for these factors in restricting synapse number (Barbosa et al. 2008; Flavell et al. 2006). The increase in glutamatergic synapses seen in MEF2A/D knockdown neurons can be reversed by expressing a fusion protein that combines the DNA binding domain of MEF2 and the transcriptional activation domain of the viral transcription factor VP16 (MEF2-VP16) implicating the transcriptional activator function of MEF2 in this negative-regulatory effect on synapses.

A further level of MEF2-driven synapse specificity was revealed in a recent paper that showed MEF2C differentially regulates local versus long-range excitatory synaptic inputs onto neocortical neurons (Rajkovich et al. 2017). The authors found that knocking out Meff2c in a sparse population of layer 2/3 neurons in mouse barrel cortex weakened local excitatory synaptic input while enhancing long-range trans-colossal excitatory inputs onto the same cells. Thus even for the same type of synapses (excitatory) on a single type of neuron (also excitatory), MEF2C shows functional specificity in the direction of its regulatory effect depending on the source of the presynaptic input.

As sequence-specific, DNA-binding transcription factors, a key focus of investigation has been identifying MEF2 targets by a combination of gene expression profiling and MEF2 chromatin binding analyses. In RNA-seq data comparing
transcriptional profiles from MEF2C conditional knockout and control cortex, over one thousand differentially expressed genes (DEGs) were identified (Harrington et al. 2016). Consistent with MEF2’s function as both an activator and a repressor, these were nearly evenly split between those that are upregulated and downregulated in the absence of MEF2C, and they were enriched for genes involved in neuron differentiation and development and synaptic transmission. As expected from their post-translational regulation, the MEF2s also play an important role in the regulation of activity-inducible programs of gene expression, and a set of activity-inducible genes that are direct targets of MEF2 were identified in hippocampal neurons by coordinate microarray analysis and chromatin immunoprecipitation for MEF2D (Flavell et al. 2008). Interestingly ChIP-Seq experiments in primary mouse cortical neurons have found that MEF2A and MEF2C bind not only gene promoters but also distal enhancer regions for genes involved in glutamatergic synapse transmission, drug addiction, axon guidance and MAPK signaling pathways (Telese et al. 2015).

As in all cases where a transcription factor regulates synapses, it is natural to wonder how gene transcription in the nucleus of the neuron can be transduced into distal changes in synapse number in the periphery. Intriguingly, insights gleaned from studying a mouse model of Fragile X Syndrome (FXS), a genetic form of autism and mental retardation that occurs due to loss-of-function mutations in the \textit{Fmr1} gene (Abrahams and Geschwind 2008; Bassell and Warren 2008), have suggested that the Fragile X Mental Retardation Protein (FMRP) encoded by \textit{Fmr1} may link MEF2 in the nucleus and its effects at synapses (\textbf{Fig. 1C}). FMRP is an RNA binding protein that mediates RNA trafficking from the nucleus to synapses and regulates local mRNA translation at
synapses. FMRP is required for MEF2-dependent synapse elimination because MEF2-
VP16 overexpression fails to drive excitatory synapse elimination in the absence of
FMRP expression (Pfeiffer et al. 2010).

An explanation for the requirement for FMRP in MEF2-dependent synaptic changes may be that FMRP regulates translation of MEF2-induced transcripts at synapses. In hippocampal neurons, MEF2 promotes excitatory synapse elimination by inducing degradation of postsynaptic density protein 95 (PSD-95) at the dendrites, a process requiring Mdm2-dependent ubiquitination and Pcdh10-dependent degradation of PSD-95. *Pcdh10* is a target of both MEF2- and activity-dependent transcription and FMRP-dependent translational repression (Morrow et al. 2008; Tsai et al. 2012) and inhibition of Pcdh10 function inhibits MEF2-dependent synapse elimination. MEF2 and FMRP also cooperate to regulate the synaptic localization and activation of Mdm2. As a result of this dual regulatory circuit, even though Pcdh10 levels are elevated in *Fmr1* knockout neurons, MEF2-dependent degradation of PSD-95 is blocked in the absence of FMRP due to a failure of PSD95 at synapses to be ubiquitinated. Dendritic FMRP is also known to regulate the transport and translation of specific mRNAs in response to metabotropic glutamate receptor activation, and blocking dendritic mGluR5 activity also results in loss of MEF2-induced glutamatergic synapse elimination in cultured hippocampal neurons (Wilkerson et al. 2014). One potential mediator of synapse elimination in this context is the cytoskeletal protein Arc, which plays important roles in dendritic spine plasticity and glutamate receptor trafficking (Korb and Finkbeiner 2011). *Arc* transcription is induced in an activity- and MEF2-dependent manner, and it is required for MEF2 and dendritic mGluR5-induced synapse elimination (Wilkerson et al.
Dendritic mGluR5 activation promotes local translation of Arc mRNA, and Arc is also a known translational target of FMRP in dendrites (Park et al. 2008). Thus through coordinate transcriptional and translational control of a program of synapse regulatory gene products, MEF2 and FMRP work together to shape synaptic activity-dependent changes in glutamatergic synapses.

**Chromatin Regulation of Synapse Development**

Sequence-specific transcription factors are the primary determinants of gene expression programs in cells because they directly mediate the recruitment of RNA polymerase II onto gene promoters. However there are many more potential binding sites across the genome for any given transcription factor than are used, and it is the biochemical state and physical structure of DNA and its associated histone proteins, a complex called chromatin, that determine which of the potential sites are available to be bound (Sheffield et al. 2013). In this manner chromatin state functions to establish the potential range of transcription factor action in any given cell. Though chromatin structure is one step upstream from transcription factor binding, loss-of-function phenotypes for several chromatin regulatory factors have revealed important and specific roles for many of these proteins in synapse development. Here we review how studies of three different classes of chromatin regulatory factors have advanced insights into the distinct mechanisms by which chromatin can regulate synapses in the developing brain.

*Methyl-CpG Binding Protein 2: non-CpG methylation matters too*
Methyl-CpG-binding protein 2 (MeCP2) was first characterized as a factor purified from the brain that has the ability to bind single methylated CpG dinucleotides in DNA (Lewis et al. 1992). MeCP2 is the founding member of the eleven-member methyl-DNA binding (MDB) domain family (Du et al. 2015), and it is ubiquitously expressed in both neuronal and non-neuronal tissues. However expression levels of MeCP2 are higher in neurons than they are in other cell types even within the brain (Skene et al. 2010), and mutations that result in loss of MeCP2 function have a predominant effect on the function of neurons, causing the neurodevelopmental disorder Rett syndrome (RTT) (Amir et al. 1999). These data raise the possibility that understanding how MeCP2 regulates transcription may offer insights into how a global chromatin regulatory process like DNA methylation can selectively impact neuronal functions.

MeCP2 levels are low in neural precursors and rise as neurons mature, peaking around the time of synaptogenesis (Cohen et al. 2003). The biological requirement for MeCP2 tracks with the postnatal increase in expression, such that individuals affected by RTT exhibit largely normal development for the first 6 to 18 months of life followed by a period of developmental regression when motor and cognitive milestones are lost (Hagberg et al. 1983). RTT patients exhibit acquired microcephaly, meaning they are born with brain sizes in the normal range but fall below the normal brain growth curve as they age (Hagberg et al. 2001). Neuropathological studies in brains from RTT patients found no evidence of neuronal death, but they did show a global reduction in the size of neurons and decreased dendritic spine density in the cortex and hippocampus, suggesting that the impaired postnatal brain growth in RTT patients was due to a failure of MeCP2-dependent synapse development (Armstrong 2005; Chapleau et al. 2009). Indeed the
findings of reduced neuronal size and decreased dendritic spine density are recapitulated in *Mecp2* knockout neurons (Belichenko et al. 2009; Chen et al. 2001). Furthermore hippocampal neurons from *Mecp2* knockout mice show a reduced excitatory synaptic response whereas neurons from mice overexpressing MeCP2 display an enhanced synaptic response, and these differences in neurotransmission are primarily due to changes in the number of glutamatergic synapses (Chao et al. 2007). Taken together, these studies indicate that MeCP2 plays a critical role in synapse development and function and establish *Mecp2* knockout mice as a model for discovering the molecular mechanisms of MeCP2-dependent effects on synapse development.

Because MeCP2 was isolated based on its ability to bind methylated DNA and because methylation of gene promoters is associated with transcriptional repression, the first models of MeCP2 function in neurons proposed that it acted primarily at gene promoters to repress expression of specific genes (Chen et al. 2003; Martinowich et al. 2003). Indeed MeCP2 has been found to recruit a multiprotein corepressor complex to gene promoters that includes histone deacetylases and methyltransferases to silence gene transcription (Fuks et al. 2003; Jones et al. 1998; Lyst et al. 2013; Nan et al. 1998). However a subsequent microarray study of gene expression in the hypothalamus from *Mecp2* knockout mice revealed that there were nearly as many genes downregulated in the absence of MeCP2 as were upregulated suggesting that MeCP2 might also act as a transcriptional activator (Chahrour et al. 2008). Furthermore once genome-scale analyses of MeCP2 binding profiles emerged they revealed widespread binding of MeCP2 across the genome, suggesting that MeCP2 might act to modulate the global chromatin state...
rather than functioning as a gene-specific regulator of either transcriptional activation or repression (Baker et al. 2013; Cohen et al. 2011; Skene et al. 2010).

Some resolution of these disparate findings has recently begun to emerge from the recent discovery that MeCP2 also binds non-CpG dinucleotides (primarily methyl-CpA) in neuronal DNA (Chen et al. 2015; Gabel et al. 2015; Guo et al. 2014). As an epigenetic mark on genomic DNA, cytosine methylation at non-CpG dinucleotides (i.e. CpA, CpC and CpT, collectively called CpH) exhibits distinct regulation and function as compared with the more widely studied CpG methylation (Guo et al. 2014; Lister et al. 2013). Of particular relevance for understanding MeCP2 function in neurons, it was found that global methyl-CpG levels are stable throughout the lifespan in both neurons and glial cells, whereas methyl-CpH is very low in the fetal brain and then increases rapidly and selectively in neurons after birth (Lister et al. 2013). Methyl-CpH levels are then actively maintained by the action of DNA methyltransferase 3A (DNMT3A) in mature neurons and the distribution of this mark over gene bodies is inversely proportional to the abundance of the associated mRNA transcript, suggesting methyl-CpH inhibits transcription of expressed genes (Guo et al. 2014).

The timing of the onset of neurological symptoms in RTT parallels the timecourse of methyl-CpH accumulation in neurons, suggesting that MeCP2-dependent regulation of the genes marked by methyl-CpH in neurons might explain the synaptic developmental defects in RTT (Fig. 3A). Genes that are upregulated in Mecp2 knockout mice have higher MeCP2 binding and higher methyl-CpH levels over their gene bodies than unaffected genes (Chen et al. 2015; Gabel et al. 2015). Furthermore the genes that rely most on MeCP2 and methyl-CpH for their proper regulation tend to be long genes
 (>100kb) (Gabel et al. 2015; Sugino et al. 2014). These long genes contain the highest levels of methyl-CpA in neurons and are most sensitive to disruption of DNMT3A expression (Gabel et al. 2015). Interestingly long genes are enriched for neuronal and synaptic functions, many are selectively expressed in the brain, and a number of these genes are found among the set of high-likelihood candidates for causing autism spectrum disorders (King et al. 2013). Taken together these data suggest that although MeCP2 likely has many functions in transcriptional regulation including CpG-mediated gene repression and global effects on chromatin structure, its impact on synapse development may be most closely related to its ability to modulate methyl-CpA dependent repression of long genes in maturing neurons of the postnatal brain. This model awaits in vivo testing in MeCP2 knockout mice to determine the degree to which correcting long gene expression can rescue synaptic and behavioral phenotypes of MeCP2 deficiency.

ATP dependent chromatin-remodelers

ATP dependent chromatin remodelers use the energy of ATP hydrolysis to alter nucleosome positions along genomic DNA or to exchange nucleosomes on chromatin. By moving nucleosomes into or out of transcription factor binding sites and clearing chromatin of nucleosomes marked with regulatory histone modifications, chromatin remodelers have the ability to modulate gene transcription. There are four families of ATP-dependent chromatin remodeling complexes defined by the ATPase they contain: 1) BAF, 2)INO80/SWR1, 3) ISWI, and 4) CHD (Hargreaves and Crabtree 2011). Recently several specific members of the BAF and CHD families have been shown to play important roles in synapse development and those factors are reviewed here.
BAF complexes: specificity of function through subunit variation

Brg1/Brm-associated factor (BAF) complexes are mammalian SWI/SNF-like, ATP-dependent chromatin-remodeling complexes that are comprised of an assembly of at least 15 subunits encoded by 29 genes. The core ATPase subunit of these ~2MDa protein complexes can be either Brg1 (also known as SmarcA4) or Brm (also known as SmarcA2) (Kadoch and Crabtree 2015; Ronan et al. 2013; Vogel-Ciernia and Wood 2014). Some accessory subunits of the BAF complexes are tissue specific, and the particular subunit composition of a given BAF complex affects both its genome targeting and functions. For example, in neural progenitors, BAF complexes contain primarily BAF45a and BAF53a, and in these cells BAF complexes are required for cell proliferation. By contrast when progenitors exit the cell cycle to become postmitotic neurons, BAF45a and BAF53a are replaced by BAF45b, BAF45c, and BAF53b, and blocking this subunit switch inhibits neural differentiation (Lessard et al. 2007). Studying the functions and protein interactions of neural-specific subunits of the BAF complex has helped explain how these chromatin remodeling complexes regulate neuronal and synaptic development.

In postmitotic neurons, the BAF complexes have been implicated as regulators of both dendrite outgrowth and synapse maturation. For example, neurons lacking Brm show increased numbers of mature, mushroom-shaped spines (Loe-Mie, 2010) whereas neurons lacking Brg1 show increased numbers of immature, thin spines (Zhang, 2016), and Brg1 is required for activity-dependent dendritic outgrowth (Wu et al., 2007). Although BAF complexes can still assemble without the neural specific subunit BAF53b,
hippocampal neurons lacking BAF53b show similar defects to neurons lacking Brg1 including reduced activity-dependent dendritic outgrowth (Wu et al., 2007). Profiling gene expression in mice with heterozygous knockout of BAF53b revealed altered expression of genes involved in actin cytoskeletal remodeling and the postsynaptic density, suggesting the BA53b-mediated recruitment of the BAF complex to these genes may play an important role in regulation of synapse functions (Vogel-Ciernia et al. 2013). Chromatin immunoprecipitation studies also show that BAF53b is required for the recruitment of BAF complexes to the promoter region of genes involved in dendritic outgrowth (Wu et al. 2007). Taken together, these studies indicate that BAF complexes regulate genes that are important for synaptic functions and suggest that neural-specific subunits are important for targeting the complexes to their genes.

How do distinct BAF complexes achieve functional biological specificity? It is well established that BAF complexes are recruited to target genes via their interaction with sequence-specific transcription factors, and if these interactions differ between cell types or conditions, then the function of the BAF complex will differ as well (Cosma et al. 1999). For example, under basal conditions in neural progenitors, Brg1 interacts with the transcription factor Gli3 and functions as a transcriptional repressor to suppress the expression of Shh target genes (Zhan et al. 2011). However following exposure to Shh, Brg1 interacts with the transcription factor Gli1 and is required for Shh-induced gene activation. In postmitotic neurons, BAF complex subunit-specific interactions with transcription factors and other chromatin regulators may define the specific function of BAF complexes in synapse development (Fig. 3B). For example, the neural specific BAF subunit CREST has been shown to regulate dendrite development via its interactions with
the transcriptional co-activator and histone acetyltransferase CBP (Aizawa et al. 2004). BAF complexes containing CREST are found bound to the promoters of genes related to synapse functions including \textit{Arc} and \textit{Grin2b} where CREST is required to mediate their expression (Qiu and Ghosh 2008). One key transcription factor that has been shown to link BAF complexes to synapse development is MEF2C. Gene expression profiling from a recent study revealed a significant overlap between neuronal activity-induced genes that are disrupted in the absence of Brg1 with genes that are targets of regulation by MEF2. Knocking out MEF2C was shown to impair the activity-dependent recruitment of Brg1 to gene promoters, and conversely knocking out Brg1 impaired the ability of MEF2C overexpression to drive synapse elimination (Zhang et al. 2016). These data suggest that Brg1 functions as a coactivator of the MEF2C-dependent transcriptional program that mediates excitatory synapse elimination. Taken together these studies suggest that identifying and selectively disrupting specific BAF subunit-transcription factor interactions offers a strong opportunity to dissect the many roles of BAF complexes in synapse development.

\textit{CHD4: timing transcriptional repression}

Whereas the BAF complexes illustrate how subunit specificity can allow a chromatin remodeler to differentially control distinct stages in the process of synapse formation, recent findings about the CHD family chromatin remodeler CHD4 have provided new insights into regulation of gene expression on distinct time scales during synapse development.
The ATPases of the CHD family are characterized by the presence of a chromodomain in addition to the conserved DEAD/H-related ATPase domain. CHD4 assembles with DNA binding proteins and histone deacetylases into a large protein complex called the nucleosome-remodeling and histone deacetylase (NuRD) complex (Hargreaves and Crabtree 2011). NuRD functions as a transcriptional repressor and has canonically been suggested to play an opposing role to the gene activation mediated by BAF complexes in regulation of gene transcription (Ho and Crabtree 2010). Although NuRD is well established to play a critical role in the differentiation of embryonic stem cells, far less is known about functions of NuRD in postmitotic tissue. However a recent study showed that knocking out CHD4 in granule neurons in the cerebellar cortex results in a reduction in the density of synapses and neurotransmission between parallel fibers and Purkinje cell dendrites, suggesting that the NuRD complex promotes synaptogenesis (Yamada et al. 2014). Genome-wide chromatin state profiling in the cerebellum of mice lacking CHD4 revealed that in the absence of NuRD, a number of gene promoters fail to undergo developmentally-regulated, NuRD-dependent repression. Expression of these NuRD target genes, which include Nhlh1, Elavl2, and Cplx3 is highly developmental regulated in wildtype mice, such that expression peaks at postnatal day 6 and then decreases to very low levels in the adult brain. In the absence of CHD4, expression of the NuRD target genes remains high. Because in vivo RNAi knockdown reveals that many of these genes suppress presynaptic differentiation in the developing cerebellar cortex, these finding suggest that NuRD-dependent gene repression mediates the timing of proper synapse development by releasing the suppression of presynaptic differentiation in granule neurons during neural development (Fig. 3C).
Interestingly, in addition to its role in gene repression on a developmental time scale, CHD4-dependent repression has also been found to act much more rapidly to regulate the dynamics of neural activity-dependent gene expression in neurons (Yang et al. 2016). Surprisingly, given its known functions in gene repression, genome-wide CHD4 binding and chromatin state profiling revealed that CHD4 occupies the promoters of most actively transcribed genes in the mouse cerebellum including the classic activity-inducible genes Bdnf, Fos, and Npas4. Conditional knockout mice lacking CHD4 in cerebellar granule neurons show a prolonged time course of expression after the induction of activity-regulated genes is triggered, and this gene expression change is associated with defective activity-dependent granule neuron dendrite pruning. A clue to the mechanism of CHD4’s action in neuronal activity-regulated gene transcription came when the authors observed that binding of the histone variant H2A.Z at the promoters of activity-regulated genes was reduced in CHD4 knockout granule neurons. In yeast the homolog of H2A.Z is enriched at the promoters of genes when they are in their inactive state, but is rapidly lost from gene promoters when transcription is induced which suggested that H2A.Z might poise genes in the off state for their rapid activation (Zhang et al. 2005). However in the absence of CHD4, H2A.Z is depleted from activity-inducible gene promoters, and rather than affecting induction, knockdown of H2A.Z phenocopies the prolonged time course of activity-induced transcription seen in CHD4 knockout neurons. (Yang et al. 2016) Thus, these data suggest a new model in which CHD4-dependent recruitment of H2A.Z to activity-inducible gene promoters in neurons controls the timing of transcriptional inactivation perhaps by serving to facilitate recruitment of repressors to promoters as has been observed for H2A.Z in ES cells (Hu et al. 2013). In
sum, these data reveal two distinct function for CHD4 in cerebellar synapse regulation –
one of which relies on the classic function of the NuRD complex in developmental gene
silencing and the other of which is mediated by interactions between CHD4 and the
histone variant H2A.Z to control the inactivation kinetics of stimulus-inducible genes.
Future understanding of the CHD4-dependent mechanisms that differentiate its function
in these two processes will enhance our understanding of the time scales of gene
transcription that contribute to synapse formation and refinement in the developing CNS.

Transcriptional Dysregulation of Synapses in Autism and Intellectual Disability

The human genome project was key to discovering single-gene mutations that cause brain
disorders, such as those in CHD7 in CHARGE syndrome, TCF4 in Pitt–Hopkins
syndrome, MECP2 in Rett syndrome, GTF2I in Williams syndrome, and PHF6 in
Börjeson-Forssman-Lehman syndrome (Amir et al. 1999; Jahani-Asl et al. 2016; Morris
et al. 2003; Zentner et al. 2010; Zweier et al. 2007). Now substantial improvements in
genetic methodologies, including increases in the speed and decreases in the cost of
genome sequencing, are driving a revolution in our understanding of the genetic
underpinnings of neurodevelopmental disorders by giving a more nuanced understanding
of the types of gene variants that may predispose individuals to complex disorders
including intellectual disability (ID) and autism spectrum disorders (ASDs) (Sanders
2015). By compiling the data derived from multiple large patient cohorts and building
statistical tools to mine these data comprehensively, there has been a significant
expansion in our knowledge of the genes that can be linked to these brain disorders. Not
surprisingly one major category of gene function linked to neurodevelopmental disorders
including both ASDs and ID are genes involved in synapse function, supporting the well-established idea that it is dysregulation of brain wiring that underlies the nature of these diseases. However it is perhaps somewhat surprising that a second major category of genes implicated in ASDs and ID are those involved in transcriptional regulation (Bourgeron 2015; De Rubeis et al. 2014; Najmabadi et al. 2011; Ronan et al. 2013). Though a comprehensive understanding of how transcriptional dysregulation contributes to ASDs and ID remains elusive, the sheer number of genetic variants in transcriptional regulators that have been associated with neurodevelopmental disorders in recent large sequencing studies (summarized in Table 1) suggests the physiological relevance of studying this process (Deciphering Developmental Disorders 2017; Stessman et al. 2017; Yuen et al. 2017). Given the evidence linking transcription factors and chromatin regulators with synapse development, it is likely that mechanistic insights into the pathophysiology of these disorders will come from studying how disease-associated mutations in transcriptional regulators affects the programs of gene transcription required for synapse formation. Here we review two recent examples of these findings and discuss both the advances and remaining challenges for understanding the links between transcription and synapse abnormalities in disease.

**CHD8: De novo mutations confer risk to sporadic ASDs.**

The most reproducible genetic association with transcriptional regulation to arise from autism exome sequencing studies to date is the finding that de novo mutations in the chromodomain family nucleosome remodeling protein \( \text{CHD8} \) are associated with
increased risk for sporadic ASDs (Iossifov et al. 2014; Neale et al. 2012; O’Roak et al. 2012b; Talkowski et al. 2012). Following its identification as a high confidence hit in two large GWAS studies, focused rescreening of additional patient samples led to the estimation that CHD8 is one of a set of just six genes (CHD8, DYRK1A, GRIN2B, TBR1, PTEN, and TBL1XR1) that contribute to as many as 1% of sporadic ASDs (Iossifov et al. 2014; O’Roak et al. 2012a). Phenotypically, those with CHD8 mutations were found to have significant larger head circumference relative to patients lacking these mutations (O’Roak et al. 2012a). Interestingly in the same cohort, patients with DYRK1A mutations had significantly reduced head circumference, suggesting that distinct genetics underlie the range of ASD presentations. The association between CHD8 mutations and macrocephaly was validated in a second targeted resequencing study that focused on a cohort of patients with a broader range of developmental delay phenotypes (Iossifov et al. 2014). In addition to increased head circumference, this study broadened the putative CHD8 phenotype to include distinct facial characteristics and gastrointestinal complaints. Importantly in a zebrafish model, chd8 knockdown during development was shown to cause both macrocephaly and impaired GI motility due to reduced colonization of the GI tract by enteric neurons (Iossifov et al. 2014), suggesting a causative association between loss of CHD8 and these phenotypic features.

To identify gene targets of CHD8 that may explain its function in brain development, several groups have conducted genome-wide chromatin immunoprecipitation (ChIP) and/or transcriptome sequencing studies from CHD8 mutant cells in culture (Cotney et al. 2015; Sugathan et al. 2014; Wang et al. 2015). In human iPSC-derived neural progenitor cells, CHD8 was found to be broadly distributed across
the genome and bound near ~5,000 genes that were enriched for functions in chromatin 
modifications and transcriptional regulation. Reducing CHD8 expression led to both up 
and down regulation of a large set of genes, with the downregulated set enriched for 
neuronal annotations such as cell adhesion, synapse, and axon guidance (Sugathan et al. 
2014). Most of the genes that showed differential regulation in the CHD8 knockdown 
cells were not direct targets of CHD8 binding, leading the authors to suggest a model in 
which CHD8 sits atop a program of transcriptional regulation that subsequently feeds 
forward to coordinate neuronal differentiation.

New insights into the basic biology of CHD8 have come from the analysis of 
CHD8 loss-of-function mouse models (Durak et al. 2016; Katayama et al. 2016; Platt et 
al. 2017). Durak et al. (2016) focused on the functions of CHD8 in neural progenitors by 
knocking down expression of CHD8 in vivo using in utero electroporation. This 
controlled, cell-type specific approach allowed the authors to demonstrate that loss of 
CHD8 in progenitors leads to precocious neurogenesis due to loss of a cell-type specific 
action of CHD8 as an activator of genes in the Wnt signaling pathway. By contrast, 
Katayama et al. (2016) and Platt et al. (2017) mimicked the messier but more 
physiologically relevant haploinsufficiency of human CHD8 mutations by generating 
constitutive heterozygous CHD8 knockout mice. All three studies found that reducing 
expression of CHD8 in the developing brain was associated with ASD-like behaviors, 
such as impaired sociability and social novelty in three chamber interaction tasks. Both 
approaches also revealed disrupted expression of neuronal differentiation genes, 
validating the function of CHD8 as a modulator of neuronal development in vivo. 
However only the haploinsufficient models mirrored the macrocephaly that is seen in
human patients with CHD8 mutations (Katayama et al. 2016). Thus despite the gross behavioral similarities between the models, these distinct observations raise the possibility that the ultimate phenotype of CHD8 mutation may depend on both cell autonomous and cell non-autonomous effects on brain function, and they highlight the importance of using multiple kinds of loss-of-function models for gaining insight into the complex etiologies of disorders like ASDs and ID.

**MEF2C haploinsufficiency: a human-specific requirement for MEF2C in brain development?**

MEF2C haploinsufficiency was relatively recently recognized as a cause of syndromic mental retardation that is characterized by severe intellectual disability, lack of speech, hypotonia, stereotypic movements, and epilepsy. Using comparative genomic hybridization to genome oligonucleotide arrays, two clinical groups discovered overlapping microdeletions on chromosome 5q14.3 in small cohorts of unrelated patients (Le Meur et al. 2010; Nowakowska et al. 2010). Though the microdeletions in these patients ranged from ~150kb to 8.8Mb, the minimal common deleted region encompassed only **MEF2C** suggesting loss of MEF2C function as the common cause of the consistent phenotype. A number of additional patients have been reported with deletions that disrupt MEF2C who show highly similar presentations, suggesting that MEF2C haploinsufficiency is a discrete clinical entity (Novara et al. 2010; Paciorkowski et al. 2013; Rocha et al. 2016). Finally in the strongest case for the causative association of MEF2 haploinsufficiency with the clinical syndrome, one patient with a similar
phenotype has been characterized to have a de novo point mutation within exon 8 of the 
*MEF2C* gene that is predicted to result in a premature stop codon (Le Meur et al. 2010).

Despite the common clinical phenotype, the underlying brain pathophysiology of 
MEF2 haploinsufficiency syndrome remains unknown. Patients with 5q14.3 have 
variable morphological abnormalities of the brain that include mild thinning of the corpus 
callosum and delay of white matter myelination, but no detailed pathological findings 
have been reported from patients. Often, as we described above for CHD8, hypotheses of 
pathophysiological mechanism emerge from studies of model organisms. As discussed 
above, neuron- or brain-specific conditional MEF2C knockout mice show changes in 
synapse formation (Barbosa et al. 2008; Harrington et al. 2016). Furthermore these 
mouse models show deficits in learning and memory (Barbosa et al. 2008), cortical 
development (Li et al. 2008) and deficits in behaviors relevant to ASDs and ID such as 
changes in ultrasonic vocalizations, deficits in social novelty, reduced sucrose preference, 
locomotor hyperactivity, and impaired fear conditioning (Harrington et al. 2016). 
However heterozygous conditional deletion of MEF2C either in excitatory forebrain 
neurons (Harrington et al. 2016) or throughout the brain (Barbosa et al. 2008) fails to 
disrupt performance on cognitive and social tasks compared with wildtype littermates. A 
more faithful model of the human genetic condition is found in mice heterozygous for 
germline deletion of *Mef2c*, and though these were described upon generation as showing 
no discernable phenotype (Lin et al. 1997), they have not yet been subjected to detailed 
neural and behavioral phenotyping. Thus the relevance of the synaptic mechanisms 
underlying behavioral abnormalities in the brains of conditional MEF2C knockout mice 
for understanding the human haploinsufficiency syndrome remain to be fully determined.
A further point of interest with respect to understanding MEF2C haploinsufficiency syndrome is a recent paper that suggests MEF2C may have human-specific targets. Specifically, MEF2C was discovered to bind to an evolutionarily acquired MEF2-responsive element regulating the gene encoding the secreted protein osteocrin (OSTN) by ChIP from primary fetal human neurons (Ataman et al. 2016). This MEF2C binding enhancer element emerged in the primate lineage, where it is required for the primate-specific neuronal activity-inducible transcription of OSTN. Whether osteocrin expression is affected in patients with MEF2C haploinsufficiency syndrome is not known, however knockdown of osteocrin disturbs dendrite development in cultured human iPSC-derived neurons suggesting that it could be part of a human-specific mechanism of synapse development. These data raise the possibility that MEF2C could play species-specific roles in synapse formation by virtue of its differential recruitment to evolutionarily adapted enhancers. Further development of methods for examining transcription factor function in human neurons will advance our understanding of this and other similar effects of regulatory element variation on species-specific aspects of brain function and dysfunction.

Conclusions

The data reviewed here reveal the degree to which transcription factors and chromatin regulators can have complex and selective effects on synapse development. The function of sequence-specific binding transcription factors is ultimately controlled by the distribution and accessibility of their binding sites in the genome, which determines their potential target genes. ChIP-seq and RNA-seq studies have begun to reveal the
expression profiles controlled by synapse regulatory transcription factors and chromatin regulators, giving new insights into the molecular mechanisms of synapse formation, elimination, and maintenance. Interestingly, evidence that mRNAs regulated by the transcription factor MEF2 are transported to synapses by the RNA binding protein FMRP and can be subject to local translation suggests a new model for how the action of a transcription factor in the nucleus can be directed to specific sets of synapses in the periphery. Understanding the extent to which other RNA trafficking proteins contribute to the synaptic actions of transcription factors will be an interesting area of exploration for the future.

The advances in next-generation sequencing methods that emerged from the completion of the human genome drove many of the recent advances in our understanding of transcription factors in brain development and disease. We are still feeling the impact of these methods – for example, exome sequencing is still in its relative infancy and the majority of cases of sporadic ASDs and ID are without a defined genetic cause. With the cost of sequencing continuing to fall and whole genome sequencing become an increasing reality at scale, it is reasonable to expect that new mutations including non-coding mutations will emerge that show association with brain disorders offering new clues to the etiology of these diseases. As we discussed above for CHD8 and MEF2C, one way the clarification of disease genetics can improve outcomes for patients is by offering diagnostic criteria for predicting the course of the clinical syndrome. Further, the development of model systems that permit investigations into the pathophysiology of the disorder can in ideal scenarios suggest potential treatment options. For example the loss-of-function mutations in MeCP2 that cause Rett Syndrome
in girls were found to disrupt expression of the neuronal growth factor IGF-1 in the developing brain of a mouse model (Tropea et al. 2009). Because IGF-1 was already FDA-approved for treatment of certain endocrine disorders, this rapidly led to a clinical trial for IGF-1 replacement therapy in Rett Syndrome (Khwaja et al. 2014). Though at the time of this writing the outcome of the larger trial remains unknown, the simple fact that a neurodevelopmental disorder could potentially be treatable offers great hope to the families of the children with these disorders. Finally in the future gene editing strategies using new developments in TALEs or Cas9/CRISPR (Long et al. 2016; Nelson et al. 2016; Tabebordbar et al. 2016) might have the potential to fully correct these genetic disorders or their transcriptional consequences, in the most desirable outcomes of all.

**Figure Legends**

**Figure 1: Mechanisms of specificity in the transcriptional regulation of synapses.**

A) Calcium-response factor (CaRF) binds to *Bdnf* IV promoter and activates basal *Bdnf* transcription. It also inhibits activity dependent *Bdnf* transcription via indirect activation of *Grin3a* expression, which encodes the NMDA-receptor subunit GluN3A. GluN3A inhibits NMDAR-induced *Bdnf* transcription without impairing the ability of L-type voltage gated calcium channels to activate *Bdnf* transcription. B) Npas4 expression is induced rapidly by neuronal activity. Npas4 target genes promote GABAergic synapse formation at the soma while also inhibiting GABAergic synapse formation at the apical dendritic spines. C) Neuronal activity induces MEF2 dependent gene transcription. FMRP traffics mRNAs from the nucleus to the synapses and represses local mRNA translation at the synapses until neuronal activation of mGluR5 receptors leads to target
gene mRNA dissociation from FMRP. The ability of constitutively active MEF2-VP16 to drive synapse elimination is blocked in FMRP knockout neurons suggesting that these two pathways converge to locally regulate synapse pruning.

**Figure 2: Domain organization and posttranslational regulatory modifications of MEF2 family members in neurons.** MEF2A, C, and D proteins all contain N-terminal MADS and MEF2 domains (gray and brown), which mediate DNA binding and dimerization, and a conserved transactivation domain (TAD, green). The α, β, and γ indicated alternatively spliced exons. In neurons the MEF2s predominantly contain the α splice variant and are β+. The γ-domain is constitutively present in MEF2A and MEF2D but alternatively spliced in MEF2C. The figure shows post-translational modifications (P-Phosphorylation; SUMO-sumoylation; Ac-Acetylation) and their residue locations in human MEF2A. Whether the modifications function to enhance or repress MEF2 activity is shown above the diagram.

**Figure 3: Chromatin regulatory factors in synapse development.** A) Neuronal specific accumulation of methyl-CpH leads to a distinct MeCP2 regulated gene program in mature neurons. Upper, during brain development methyl-CpH levels increase and accumulate selectively in mature neurons whereas methyl-CpG levels are constant across development in both neurons and glia. Bottom, because MeCP2 binds to both methyl-CpG and methyl-CpH the distinct high level of methyl-CpH in mature neurons leads to a neuron-specific developmental effect on MeCP2 regulated gene transcription. Diagrams are based on the data in (Lister et al. 2013) and (Chen et al. 2015). B) Tissue specific
BAF subunits interact with distinct transcription factors or transcriptional coactivators resulting in activation of different gene programs during neural development. BAF complexes regulate a group of synaptic genes and mediate synapse formation and maturation. In activated neurons, BAF complexes regulate Arc and Grin2b expression by interacting with CBP. BAF complexes also interact with MEF2C and are required for MEF2C mediated synapse elimination. C) NuRD controls the timing of gene expression by turning genes off both on a developmental time scale and in response to transient neuronal activity. During cerebellar development, NuRD turns off genes that suppress presynaptic differentiation by histone deacetylation and inactivates neuronal activity-dependent genes transcription by replacing H2A to H2A.Z.

Table 1: Genes encoding transcriptional regulatory proteins that have de novo mutations achieving statistical significance in large scale sequencing screens of ASDs and other developmental disorders (Deciphering Developmental Disorders 2017; Stessman et al. 2017; Yuen et al. 2017).

References


The neuron-specific chromatin regulatory subunit BAF53b is necessary for synaptic plasticity and memory. *Nat Neurosci* 16: 552-561, 2013.


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BDNF promotes GABAergic synapse formation

Inhibits GABAergic synapse formation at dendrite

Promotes GABAergic synapse formation at soma

Neuronal activity

L-type calcium channels

GluN3A lacking NMDARs

GluN3A containing NMDARs

Negative regulators of synapses

Neuronal activity

mGluR5
Effect on MEF2 activity

- Increase
- Decrease

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Figure: MADS-MEF2 protein domain structure with indicated phosphorylation sites (P) and sumoylation site (SUMO).
A. Brain development

Birth

Mature

Immature neuron & Glia

Mature neuron

methyl-CpH (Neuron)
methyl-CpG (Neuron & Glia)
methyl-CpH (Glia)

Abundance

B. Neural development

Activated neuron

Arc Grin2b

MEF2 regulated genes

BAFs

Brg1/Brm

Actin

TF

CREST

BAF53b

BAF45 b/c

MECP2

methyl-CpG

methyl-CpH

C. Cerebellum development

Developmental regulated genes

Genes that suppress presynaptic differentiation

Activity regulated genes

H2A

H2A.Z

NuRD complex

CHD4

Brg1/Brm

Actin

BAF53b

BAF45 b/c

MEF2

CREST

BAFs

Ac

Ac

Ac

TF

CBP

Arc

Grin2b

Developmental regulated genes