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1 **Transcribing the connectome: Roles for transcription factors and chromatin**

2 **regulators in activity-dependent synapse development**

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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

52 organisms as well. A likely evolutionary advantage of introducing uncertainty to the late
53 stage of brain development is allowing adaptations in brain function to match an
54 unpredictable outside world. However, the tradeoff is the possibility that the process
55 could go very wrong, leading to neurological or psychiatric disease.

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

74

75 **Transcription Factors and Synapse Specificity**

76 Transcription factors reside predominantly in the nucleus, where they are well-poised to
77 integrate signaling information originating anywhere within the cell. This integrative
78 capacity of stimulus-regulated transcription factors allows them to transduce reception of
79 tiny amounts of growth factors into binary cell survival/cell death decisions (Brunet et al.
80 2001), and to drive homeostatic plasticity by summating neural activity levels into global
81 synaptic scaling responses (Ibata et al. 2008). However the central location of
82 transcription factors raises the question of how they can have a meaningful impact on
83 local synaptic connectivity, where the specificity of connections is essential to brain
84 function. Here we consider three examples of transcription factors that exemplify aspects
85 of this specificity. These examples show that although nuclear transcription factors are
86 unlikely to directly instruct the formation or elimination of individual synapses, they do
87 encode specificity at the level of synapse type (e.g. excitatory, inhibitory), and subcellular
88 synapse location (e.g. somatic, dendritic). Furthermore by interacting functionally with
89 local translational machinery they can have regulatory control over local activity-
90 dependent synaptic plasticity.

91

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

94 Calcium-response factor (CaRF) was initially discovered as a transcriptional
95 activator of *Brain-Derived Neurotrophic Factor* (*Bdnf)*, which it directly induces by
96 binding to a 10-bp calcium response element (CaRE1) in the proximal region of *Bdnf*
97 promoter IV (McDowell et al. 2010; Tao et al. 2002) (**Fig. 1A**). Expression of Exon IV-

98 containing forms of *Bdnf* is significantly reduced in the cortex of CaRF knockout mice
99 demonstrating that CaRF contributes to transcriptional activation of *Bdnf* promoter IV in
100 vivo (McDowell et al. 2010). Surprisingly although the CaRE1 element is required for
101 transcriptional induction of *Bdnf* upon membrane depolarization, and the transcriptional
102 activity of CaRF is induced by the subsequent calcium influx, CaRF is not required for
103 the induction of *Bdnf* transcription that is driven by this stimulus (McDowell et al. 2010;
104 Tao et al. 2002). Instead membrane depolarization-mediated activation of *Bdnf* promoter
105 IV requires the transcription factor MEF2C, which binds CaRE1 independently of CaRF
106 and acts together with CREB to drive the calcium-inducible component of *Bdnf*
107 transcription (Hong et al. 2008; Lyons et al. 2012).

108 Given this context, it is intriguing that CaRF has recently been found to inhibit the
109 induction of *Bdnf* promoter IV following activation of NMDA-type glutamate receptors
110 (Lyons et al. 2016). Membrane depolarization promotes the activation of *Bdnf*
111 transcription via the opening of L-type voltage-gated calcium channels (Tao et al. 1998).
112 By contrast, treatment of neurons with the sodium channel inhibitor tetrodoxin (TTX)
113 silences action potentials and induces homeostatic synaptic plasticity such that upon TTX
114 withdrawal there is rebound excitation, release of synaptic glutamate, activation of
115 synaptic glutamate receptors, and the NMDA receptor-dependent induction of
116 transcription from genes including *Bdnf* promoter IV (Ghiretti et al. 2014; Turrigiano and
117 Nelson 2004). Following TTX withdrawal-induced activation of NMDARs, neurons
118 lacking CaRF show significantly higher induction of Exon IV-containing forms of *Bdnf*
119 mRNA and BDNF protein compared with CaRF-expressing control neurons (Lyons et al.,
120 2016). Unlike the direct activation of *Bdnf* transcription by CaRF, which requires binding

121 of CaRF to *Bdnf* promoter IV, the CaRF-dependent inhibition of *Bdnf* transcription is
122 proposed to occur through an indirect mechanism that involves CaRF-dependent
123 induction of the unusual NMDA receptor subunit GluN3A. Incorporation of GluN3A into
124 functional NMDARs with GluN1 and GluN2 subunits reduces receptor currents,
125 including the influx of calcium (Das et al. 1998; Kehoe et al. 2013). Levels of GluN3A
126 are reduced in the brains of CaRF knockout mice and restoration of GluN3A expression
127 in CaRF knockdown neurons restores *Bdnf* inducibility to wildtype levels (Lyons et al.,
128 2016). Thus although CaRF is a direct activator of basal *Bdnf* transcription via its binding
129 to *Bdnf* promoter elements, it acts as an indirect inhibitor of NMDAR-inducible *Bdnf*
130 transcription by its ability to modulate the subunit composition of neuronal NMDARs
131 (**Fig. 1A**).
132 The link between CaRF and GluN3A is important because it suggests a potential
133 mechanism by which CaRF could regulate the timing of synapse development. Both
134 CaRF and GluN3A are most highly expressed during the first two postnatal weeks of
135 brain development, after which time their expression declines (McDowell et al. 2010;
136 Sucher et al. 1995). This decline parallels the onset of the critical period for sensory-
137 dependent synapse development in the cortex, and notably, prolonging the expression of
138 GluN3A inhibits critical period maturation of synapses in visual cortex, suggesting an
139 essential function for the changing NMDAR composition in this process (Roberts et al.
140 2009). BDNF expression is strongly induced in sensory cortex during the critical period
141 and it drives critical period closure by promoting the formation of inhibitory GABAergic
142 synapses (Huang et al. 1999). Consistent with a role for CaRF-dependent repression of
143 NMDAR-inducible *Bdnf* expression in the regulation of GABAergic synapse

144 development in vivo, adult CaRF knockout mice show substantially increased synaptic
145 expression of GABAergic synapse markers (McDowell et al., 2010). This appears to be a
146 cell autonomous effect of CaRF because single cell knockdown of CaRF in cultured
147 mouse hippocampal neurons significantly enhances the formation of GABAergic
148 synapses onto that neuron in a manner that can be rescued by simultaneous knockdown of
149 BDNF (Lyons et al., 2016). Taken together these data establish CaRF as a novel regulator
150 of the formation of GABAergic synapses in the developing brain and suggest that, via its
151 regulation of GluN3A, it could contribute to the timing of the synaptic changes that
152 underlie critical period closure.
153
154 *Npas4: An activity-dependent, cell-type specific master regulator of inhibitory/excitatory*
155 *balance*
156 Npas4 belongs to the basic helix-loop-helix-Per-Arnt-Sim (bHLH-PAS)
157 transcription factor family (Ooe et al. 2004), whose members share a bHLH DNA-
158 binding domain, dual PAS domains, and a C-terminal activation domain (Partch and
159 Gardner 2010). These transcription regulators dimerize or form complexes with
160 transcriptional coactivators through their bHLH and PAS domains to regulate diverse
161 functions including development, circadian rhythms and cellular responses to hypoxia
162 and environmental toxins (Gu et al. 2000; Kewley et al. 2004; Partch and Gardner 2010).
163 Among the bHLH-PAS family, Npas4 is unique for its restricted expression in neurons
164 and the fact that its expression is selectively induced in neurons following membrane
165 depolarization-induced calcium influx (Lin et al. 2008). Indeed even compared with other
166 immediate-early gene transcription factors such as Fos, Npas4 is far more selective for

167 calcium signaling cascades, as its expression is induced only by calcium influx through
168 L-type voltage gated calcium channels or NMDA receptors, but it is insensitive to the
169 elevation of intracellular cAMP or the application of growth or neurotrophic factors (Lin
170 et al. 2008). The mechanisms that confer this specificity upon Npas4 induction are not
171 completely understood, though Npas4 seems to be a direct target of the activity-inducible
172 transcription factor SRF (Kim et al. 2010; Kuzniewska et al. 2016).

173 Npas4 has been shown to play a prominent role in linking synaptic activity to
174 inhibitory/excitatory synapse balance in a cell-type and subcellular specific manner
175 during brain development. The calcium-dependent induction of Npas4 was first
176 discovered in excitatory glutamatergic neurons, and interestingly, in these cells Npas4
177 was found to selectively promote the development of GABAergic synapses (Lin et al.
178 2008). Reduced numbers of GABAergic synapses were found in cultured hippocampal
179 neurons when Npas4 was knocked down, whereas increased numbers of GABAergic
180 synapses were found when Npas4 was overexpressed. In contrast, these manipulations
181 did not affect glutamatergic synapses, indicating a selective role for Npas4 in inhibitory
182 synapse development in these neurons. In addition to regulating the total number of
183 inhibitory connections made during synapse formation, Npas4 appears to play an
184 important role in the activity-dependent plasticity of GABAergic synapses in mature
185 neural circuits. *Npas4* knockout mice show decreased CA1 hippocampal neuron mIPSC
186 frequency after exposure to an enriched environment compared to their wildtype
187 littermates (Bloodgood et al. 2013), and Npas4 has also been found to underlie the
188 activity-dependent increase in inhibition on newborn excitatory granule cells of the
189 dentate gyrus as they integrate into the hippocampal circuitry (Sim et al. 2013). These

190 studies together support a role for Npas4 in selectively upregulating inhibitory
191 GABAergic synapse development in excitatory neurons following neuronal activity,
192 suggesting that Npas4 diminishes future excitation and maintains homeostatic balance
193 between inhibition and excitation.

194 Perhaps the most interesting and unexpected finding regarding the Npas4-
195 dependent regulation of GABAergic synapses onto excitatory neurons is that there is
196 subcellular specificity with respect to the effects of Npas4 manipulation on the synapses
197 on the postsynaptic cell (**Fig. 1B**). Bloodgood et al. (2013) used the spatial organization
198 of synaptic inputs onto distinct regions of pyramidal neurons in the hippocampus to
199 reveal this specificity in vivo. The authors found that exposing mice to an enriched
200 environment induces expression of Npas4 in the hippocampus. Following this exposure,
201 when a stimulating electrode was placed in the pyramidal layer to activate somatic
202 inhibitory synapses onto nearby pyramidal neurons, the authors saw relatively smaller
203 evoked inhibitory currents in *Npas4* knockout neurons compared with *Npas4* wildtype
204 neurons, which is consistent with the culture experiments showing Npas4 promotes
205 inhibitory synapse formation. By contrast when the authors stimulated stratum radiatum
206 to activate inhibitory synapses on the distal apical dendrites of these pyramidal neurons
207 they found significantly larger evoked inhibitory currents in the knockout neuron
208 compared with wildtype, suggesting that Npas4 actually inhibits the formation of distal
209 inhibitory synapses presumably in the same neurons where it promotes somatic inhibition
210 (Bloodgood et al. 2013). Given that different classes of inhibitory interneurons are known
211 to synapse onto different subcellular domains of hippocampal pyramidal neurons (Freund

212 and Buzsaki 1996), having distinct Npas4 regulation of these events is likely to be
213 important for fine tuning circuit plasticity in the hippocampus.
214 Excitatory neurons comprise the large majority of cells in the culture systems that
215 are most often used to study activity-inducible transcription, thus until recently very little
216 was known about the identity of activity-inducible genes in inhibitory neurons. To fill this
217 gap in knowledge, Spiegel et al. (2014) cultured neurons from the embryonic medial
218 ganglionic eminence, which gives rise to the GABAergic interneurons that populate the
219 forebrain. Using this system, they discovered that Npas4 expression was indeed induced
220 in inhibitory neurons by membrane depolarization and subsequent calcium influx, similar
221 to its regulation in excitatory neurons. However when the authors studied the effects of
222 knocking out *Npas4* on synapse formation, they found that rather than affecting
223 GABAergic synapses, the loss of Npas4 reduced glutamatergic input to inhibitory
224 neurons, while having no effect on the GABAergic synapses made onto these neurons
225 (Spiegel et al. 2014). Thus, in contrast to its effects in excitatory neurons, activity-
226 inducible Npas4 expression appears to up-regulate glutamatergic synapses in inhibitory
227 neurons to increase their inhibitory output. This suggests a more complex role than
228 previously thought for Npas4 in mediating neuronal inhibition/excitation balance across
229 multiple cell types in the heterogeneous neural circuits that comprise the cortex. Overall
230 these studies suggest that Npas4 functions as a key homeostatic factor in the cortex,
231 depressing network excitability following neuronal activity via a negative feedback
232 mechanism that is spread across multiple cell types and synapses.
233 How it is possible for Npas4 to promote opposite types of synapses in two
234 different classes of neurons? The expression of distinct cell-type specific programs of

235 Npas4-regulated genes in excitatory and inhibitory neurons may underlie the differential
236 effects of Npas4 in these two neuron types (Spiegel et al. 2014). Using a genetic method
237 to identify activity-regulated genes that are selectively induced in inhibitory versus
238 excitatory neurons, Spiegel et al. (2014) identified a set of inhibitory neuron-specific
239 genes whose induction is impaired in the absence of Npas4. These Npas4-regulated
240 inhibitory neuron genes include *Kcna1, Frmpd3,* and *Nptx2*. These gene products have
241 been reported previously to be postsynaptic at glutamatergic synapses and therefore may
242 contribute to the Npas4-dependent enhancement of glutamatergic synapse formation in
243 inhibitory neurons. With respect to the Npas4-regulated genes that drive GABAergic
244 synapse formation in excitatory neurons, one possible target is *Bdnf*. *Bdnf* is selectively
245 induced by activity only in excitatory but not inhibitory neurons and is well known to
246 promote GABAergic synapse development in the postnatal cortex. Npas4 binds to three
247 regulatory elements within the *Bdnf* gene, and knocking down *Bdnf* partly rescues the
248 effects of Npas4 overexpression on increased GABAergic synapse number and function
249 in hippocampal neurons (Lin et al. 2008). Interestingly, BDNF expression is reduced in
250 the hippocampus of *Npas4* KO mice, and disruption of BDNF function prevents Npas4-
251 mediated increases in inhibition at the soma, but does not affect Npas4's effects at the
252 dendrites, thus providing a potential explanation for the subcellular specificity of Npas4-
253 mediated inhibitory synapse formation (Bloodgood et al. 2013). Taken together, these
254 data support the idea that Npas4's differential effects in these two cell types are due to
255 distinct cell-type specific programs of Npas4-regulated gene expression. Further
256 understanding of the distribution of Npas4 binding sites in different cell types by

257 chromatin immunoprecipitation will help to elucidate our understanding of how broadly-
258 expressed transcription factors can generate cell-type specific biological consequences.
259

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

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

280 Shalizi et al. 2006). Thus MEF2 transcription factors are ideally poised to be important
281 regulators of stimulus-inducible effects on neuronal development and function.
282 Both the transcriptional repressor and activator functions of the MEF2s have been
283 implicated in synapse formation, with the accumulated evidence suggesting that the
284 repressor forms regulate synapse formation and the activator forms promote synapse
285 elimination. The repressor functions of the MEF2s have been studied in both the
286 cerebellum and cortex, and repression by sumoylated MEF2A is particularly well
287 characterized for its role in early cerebellar synapse development (Shalizi et al. 2006). In
288 cerebellar granule neurons, sumoylated MEF2A enhances the formation of the mature
289 claw morphology of granule neuron dendrites, and knocking down MEF2A in cultured
290 rat cerebellar slices impairs this dendritic differentiation. In cortical excitatory neurons,
291 the repressor functions of MEF2C have similarly been shown to function in a cell-
292 autonomous manner to regulate the development of glutamatergic synapses. Knocking
293 out MEF2C in cortical neurons results in a mildly decreased frequency of miniature
294 excitatory synaptic currents and significantly decreased dendritic spine density
295 (Harrington et al. 2016). Spine density in the MEF2C knockout neurons was rescued by
296 expression of a fusion protein that links the DNA binding domain of MEF2C to the
297 repressor domain of Engrailed (MEF2-En), indicating that the synapse promoting effects
298 of MEF2C in this context are mediated by its function as a transcriptional repressor.
299 Interestingly, MEF2C also has a cell autonomous effect on GABAergic synapses made
300 onto cortical glutamatergic neurons, such that single cell knockout of MEF2C is
301 associated with increased amplitude and frequency of miniature inhibitory synaptic

302 currents, and an increased number of GABAergic synapses, and this effect is again
303 rescued by overexpression of MEF2C-En.

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

314 A further level of MEF2-driven synapse specificity was revealed in a recent paper
315 that showed MEF2C differentially regulates local versus long-range excitatory synaptic
316 inputs onto neocortical neurons (Rajkovich et al. 2017). The authors found that knocking
317 out *Mef2c* in a sparse population of layer 2/3 neurons in mouse barrel cortex weakened
318 local excitatory synaptic input while enhancing long-range trans-colossal excitatory
319 inputs onto the same cells. Thus even for the same type of synapses (excitatory) on a
320 single type of neuron (also excitatory), MEF2C shows functional specificity in the
321 direction of its regulatory effect depending on the source of the presynaptic input.

322 As sequence-specific, DNA-binding transcription factors, a key focus of
323 investigation has been identifying MEF2 targets by a combination of gene expression
324 profiling and MEF2 chromatin binding analyses. In RNA-seq data comparing

325 transcriptional profiles from MEF2C conditional knockout and control cortex, over one
326 thousand differentially expressed genes (DEGs) were identified (Harrington et al. 2016).
327 Consistent with MEF2's function as both an activator and a repressor, these were nearly
328 evenly split between those that are upregulated and downregulated in the absence of
329 MEF2C, and they were enriched for genes involved in neuron differentiation and
330 development and synaptic transmission. As expected from their post-translational
331 regulation, the MEF2s also play an important role in the regulation of activity-inducible
332 programs of gene expression, and a set of activity-inducible genes that are direct targets
333 of MEF2 were identified in hippocampal neurons by coordinate microarray analysis and
334 chromatin immunoprecipitation for MEF2D (Flavell et al. 2008). Interestingly ChIP-Seq
335 experiments in primary mouse cortical neurons have found that MEF2A and MEF2C bind
336 not only gene promoters but also distal enhancer regions for genes involved in
337 glutamatergic synapse transmission, drug addiction, axon guidance and MAPK signaling
338 pathways (Telese et al. 2015).

339 As in all cases where a transcription factor regulates synapses, it is natural to
340 wonder how gene transcription in the nucleus of the neuron can be transduced into distal
341 changes in synapse number in the periphery. Intriguingly, insights gleaned from studying
342 a mouse model of Fragile X Syndrome (FXS), a genetic form of autism and mental
343 retardation that occurs due to loss-of-function mutations in the *Fmr1* gene (Abrahams and
344 Geschwind 2008; Bassell and Warren 2008), have suggested that the Fragile X Mental
345 Retardation Protein (FMRP) encoded by *Fmr1* may link MEF2 in the nucleus and its
346 effects at synapses (**Fig. 1C**). FMRP is an RNA binding protein that mediates RNA
347 trafficking from the nucleus to synapses and regulates local mRNA translation at

348 synapses. FMRP is required for MEF2-dependent synapse elimination because MEF2-
349 VP16 overexpression fails to drive excitatory synapse elimination in the absence of
350 FMRP expression (Pfeiffer et al. 2010).

351 An explanation for the requirement for FMRP in MEF2-dependent synaptic
352 changes may be that FMRP regulates translation of MEF2-induced transcripts at
353 synapses. In hippocampal neurons, MEF2 promotes excitatory synapse elimination by
354 inducing degradation of postsynaptic density protein 95 (PSD-95) at the dendrites, a
355 process requiring Mdm2-dependent ubiquitination and Pcdh10-dependent degradation of
356 PSD-95. *Pcdh10* is a target of both MEF2- and activity-dependent transcription and
357 FMRP-dependent translational repression (Morrow et al. 2008; Tsai et al. 2012) and
358 inhibition of Pcdh10 function inhibits MEF2-dependent synapse elimination. MEF2 and
359 FMRP also cooperate to regulate the synaptic localization and activation of Mdm2. As a
360 result of this dual regulatory circuit, even though Pcdh10 levels are elevated in *Fmr1*
361 knockout neurons, MEF2-dependent degradation of PSD-95 is blocked in the absence of
362 FMRP due to a failure of PSD95 at synapses to be ubiquitinated. Dendritic FMRP is also
363 known to regulate the transport and translation of specific mRNAs in response to
364 metabotropic glutamate receptor activation, and blocking dendritic mGluR5 activity also
365 results in loss of MEF2-induced glutamatergic synapse elimination in cultured
366 hippocampal neurons (Wilkerson et al. 2014). One potential mediator of synapse
367 elimination in this context is the cytoskeletal protein Arc, which plays important roles in
368 dendritic spine plasticity and glutamate receptor trafficking (Korb and Finkbeiner 2011).
369 *Arc* transcription is induced in an activity- and MEF2-dependent manner, and it is
370 required for MEF2 and dendritic mGluR5-induced synapse elimination (Wilkerson et al.

371 2014). Dendritic mGluR5 activation promotes local translation of Arc mRNA, and Arc is
372 also a known translational target of FMRP in dendrites (Park et al. 2008). Thus through
373 coordinate transcriptional and translational control of a program of synapse regulatory
374 gene products, MEF2 and FMRP work together to shape synaptic activity-dependent
375 changes in glutamatergic synapses.
376

377 **Chromatin Regulation of Synapse Development**

378 Sequence-specific transcription factors are the primary determinants of gene
379 expression programs in cells because they directly mediate the recruitment of RNA
380 polymerase II onto gene promoters. However there are many more potential binding sites
381 across the genome for any given transcription factor than are used, and it is the
382 biochemical state and physical structure of DNA and its associated histone proteins, a
383 complex called chromatin, that determine which of the potential sites are available to be
384 bound (Sheffield et al. 2013). In this manner chromatin state functions to establish the
385 potential range of transcription factor action in any given cell. Though chromatin
386 structure is one step upstream from transcription factor binding, loss-of-function
387 phenotypes for several chromatin regulatory factors have revealed important and specific
388 roles for many of these proteins in synapse development. Here we review how studies of
389 three different classes of chromatin regulatory factors have advanced insights into the
390 distinct mechanisms by which chromatin can regulate synapses in the developing brain.
391

392 *Methyl-CpG Binding Protein 2: non-CpG methylation matters too*

393 Methyl-CpG-binding protein 2 (MeCP2) was first characterized as a factor
394 purified from the brain that has the ability to bind single methylated CpG dinucleotides in
395 DNA (Lewis et al. 1992). MeCP2 is the founding member of the eleven-member methyl-
396 DNA binding (MDB) domain family (Du et al. 2015), and it is ubiquitously expressed in
397 both neuronal and non-neuronal tissues. However expression levels of MeCP2 are higher
398 in neurons than they are in other cell types even within the brain (Skene et al. 2010), and
399 mutations that result in loss of MeCP2 function have a predominant effect on the function
400 of neurons, causing the neurodevelopmental disorder Rett syndrome (RTT) (Amir et al.
401 1999). These data raise the possibility that understanding how MeCP2 regulates
402 transcription may offer insights into how a global chromatin regulatory process like DNA
403 methylation can selectively impact neuronal functions.
404 MeCP2 levels are low in neural precursors and rise as neurons mature, peaking
405 around the time of synaptogenesis (Cohen et al. 2003). The biological requirement for
406 MeCP2 tracks with the postnatal increase in expression, such that individuals affected by
407 RTT exhibit largely normal development for the first 6 to 18 months of life followed by a
408 period of developmental regression when motor and cognitive milestones are lost
409 (Hagberg et al. 1983). RTT patients exhibit acquired microcephaly, meaning they are
410 born with brain sizes in the normal range but fall below the normal brain growth curve as
411 they age (Hagberg et al. 2001). Neuropathological studies in brains from RTT patients
412 found no evidence of neuronal death, but they did show a global reduction in the size of
413 neurons and decreased dendritic spine density in the cortex and hippocampus, suggesting
414 that the impaired postnatal brain growth in RTT patients was due to a failure of MeCP2-
415 dependent synapse development (Armstrong 2005; Chapleau et al. 2009). Indeed the

416 findings of reduced neuronal size and decreased dendritic spine density are recapitulated
417 in *Mecp2* knockout neurons (Belichenko et al. 2009; Chen et al. 2001). Furthermore
418 hippocampal neurons from *Mecp2* knockout mice show a reduced excitatory synaptic
419 response whereas neurons from mice overexpressing MeCP2 display an enhanced
420 synaptic response, and these differences in neurotransmission are primarily due to
421 changes in the number of glutamatergic synapses (Chao et al. 2007). Taken together,
422 these studies indicate that MeCP2 plays a critical role in synapse development and
423 function and establish *Mecp2* knockout mice as a model for discovering the molecular
424 mechanisms of MeCP2-dependent effects on synapse development.
425 Because MeCP2 was isolated based on its ability to bind methylated DNA and
426 because methylation of gene promoters is associated with transcriptional repression, the
427 first models of MeCP2 function in neurons proposed that it acted primarily at gene
428 promoters to repress expression of specific genes (Chen et al. 2003; Martinowich et al.
429 2003). Indeed MeCP2 has been found to recruit a multiprotein corepressor complex to
430 gene promoters that includes histone deacetylases and methyltransferases to silence gene
431 transcription (Fuks et al. 2003; Jones et al. 1998; Lyst et al. 2013; Nan et al. 1998).
432 However a subsequent microarray study of gene expression in the hypothalamus from
433 *Mecp2* knockout mice revealed that there were nearly as many genes downregulated in
434 the absence of MeCP2 as were upregulated suggesting that MeCP2 might also act as a
435 transcriptional activator (Chahrour et al. 2008). Furthermore once genome-scale analyses
436 of MeCP2 binding profiles emerged they revealed widespread binding of MeCP2 across
437 the genome, suggesting that MeCP2 might act to modulate the global chromatin state

438 rather than functioning as a gene-specific regulator of either transcriptional activation or
439 repression (Baker et al. 2013; Cohen et al. 2011; Skene et al. 2010).
440 Some resolution of these disparate findings has recently begun to emerge from the
441 recent discovery that MeCP2 also binds non-CpG dinucleotides (primarily methyl-CpA)
442 in neuronal DNA (Chen et al. 2015; Gabel et al. 2015; Guo et al. 2014). As an epigenetic
443 mark on genomic DNA, cytosine methylation at non-CpG dinucleotides (i.e. CpA, CpC
444 and CpT, collectively called CpH) exhibits distinct regulation and function as compared
445 with the more widely studied CpG methylation (Guo et al. 2014; Lister et al. 2013). Of
446 particular relevance for understanding MeCP2 function in neurons, it was found that
447 global methyl-CpG levels are stable throughout the lifespan in both neurons and glial
448 cells, whereas methyl-CpH is very low in the fetal brain and then increases rapidly and
449 selectively in neurons after birth (Lister et al. 2013). Methyl-CpH levels are then actively
450 maintained by the action of DNA methyltransferase 3A (DNMT3A) in mature neurons
451 and the distribution of this mark over gene bodies is inversely proportional to the
452 abundance of the associated mRNA transcript, suggesting methyl-CpH inhibits
453 transcription of expressed genes (Guo et al. 2014).
454 The timing of the onset of neurological symptoms in RTT parallels the timecourse
455 of methyl-CpH accumulation in neurons, suggesting that MeCP2-dependent regulation of
456 the genes marked by methyl-CpH in neurons might explain the synaptic developmental
457 defects in RTT (**Fig. 3A**). Genes that are upregulated in *Mecp2* knockout mice have
458 higher MeCP2 binding and higher methyl-CpH levels over their gene bodies than
459 unaffected genes (Chen et al. 2015; Gabel et al. 2015). Furthermore the genes that rely
460 most on MeCP2 and methyl-CpH for their proper regulation tend to be long genes

461 (>100kb) (Gabel et al. 2015; Sugino et al. 2014). These long genes contain the highest
462 levels of methyl-CpA in neurons and are most sensitive to disruption of DNMT3A
463 expression (Gabel et al. 2015). Interestingly long genes are enriched for neuronal and
464 synaptic functions, many are selectively expressed in the brain, and a number of these
465 genes are found among the set of high-likelihood candidates for causing autism spectrum
466 disorders (King et al. 2013). Taken together these data suggest that although MeCP2
467 likely has many functions in transcriptional regulation including CpG-mediated gene
468 repression and global effects on chromatin structure, its impact on synapse development
469 may be most closely related to its ability to modulate methyl-CpA dependent repression
470 of long genes in maturing neurons of the postnatal brain. This model awaits in vivo
471 testing in *Mecp2* knockout mice to determine the degree to which correcting long gene
472 expression can rescue synaptic and behavioral phenotypes of MeCP2 deficiency.
473
474 *ATP dependent chromatin-remodelers*
475 ATP dependent chromatin remodelers use the energy of ATP hydrolysis to alter
476 nucleosome positions along genomic DNA or to exchange nucleosomes on chromatin. By
477 moving nucleosomes into or out of transcription factor binding sites and clearing
478 chromatin of nucleosomes marked with regulatory histone modifications, chromatin
479 remodelers have the ability to modulate gene transcription. There are four families of
480 ATP-dependent chromatin remodeling complexes defined by the ATPase they contain: 1)
481 BAF, 2)INO80/SWR1, 3) ISWI, and 4) CHD (Hargreaves and Crabtree 2011). Recently
482 several specific members of the BAF and CHD families have been shown to play
483 important roles in synapse development and those factors are reviewed here.

484

485 *BAF complexes: specificity of function through subunit variation*

486 Brg1/Brm-associated factor (BAF) complexes are mammalian SWI/SNF-like,
487 ATP-dependent chromatin-remodeling complexes that are comprised of an assembly of at
488 least 15 subunits encoded by 29 genes. The core ATPase subunit of these ~2MDa protein
489 complexes can be either Brg1 (also known as SmarcA4) or Brm (also known as
490 SmarcA2) (Kadoch and Crabtree 2015; Ronan et al. 2013; Vogel-Ciernia and Wood
491 2014). Some accessory subunits of the BAF complexes are tissue specific, and the
492 particular subunit composition of a given BAF complex affects both its genome targeting
493 and functions. For example, in neural progenitors, BAF complexes contain primarily
494 BAF45a and BAF53a, and in these cells BAF complexes are required for cell
495 proliferation. By contrast when progenitors exit the cell cycle to become postmitotic
496 neurons, BAF45a and BAF53a are replaced by BAF45b, BAF45c, and BAF53b, and
497 blocking this subunit switch inhibits neural differentiation (Lessard et al. 2007). Studying
498 the functions and protein interactions of neural-specific subunits of the BAF complex has
499 helped explain how these chromatin remodeling complexes regulate neuronal and
500 synaptic development.

501 In postmitotic neurons, the BAF complexes have been implicated as regulators of
502 both dendrite outgrowth and synapse maturation. For example, neurons lacking Brm
503 show increased numbers of mature, mushroom-shaped spines (Loe-Mie, 2010) whereas
504 neurons lacking Brg1 show increased numbers of immature, thin spines (Zhang, 2016),
505 and Brg1 is required for activity-dependent dendritic outgrowth (Wu et al., 2007).
506 Although BAF complexes can still assemble without the neural specific subunit BAF53b,

507 hippocampal neurons lacking BAF53b show similar defects to neurons lacking Brg1
508 including reduced activity-dependent dendritic outgrowth (Wu et al., 2007). Profiling
509 gene expression in mice with heterozygous knockout of BAF53b revealed altered
510 expression of genes involved in actin cytoskeletal remodeling and the postsynaptic
511 density, suggesting the BA53b-mediated recruitment of the BAF complex to these genes
512 may play an important role in regulation of synapse functions (Vogel-Ciernia et al. 2013).
513 Chromatin immunoprecipitation studies also show that BAF53b is required for the
514 recruitment of BAF complexes to the promoter region of genes involved in dendritic
515 outgrowth (Wu et al. 2007). Taken together, these studies indicate that BAF complexes
516 regulate genes that are important for synaptic functions and suggest that neural-specific
517 subunits are important for targeting the complexes to their genes.

518 How do distinct BAF complexes achieve functional biological specificity? It is
519 well established that BAF complexes are recruited to target genes via their interaction
520 with sequence-specific transcription factors, and if these interactions differ between cell
521 types or conditions, then the function of the BAF complex will differ as well (Cosma et
522 al. 1999). For example, under basal conditions in neural progenitors, Brg1 interacts with
523 the transcription factor Gli3 and functions as a transcriptional repressor to suppress the
524 expression of Shh target genes (Zhan et al. 2011). However following exposure to Shh,
525 Brg1 interacts with the transcription factor Gli1 and is required for Shh-induced gene
526 activation. In postmitotic neurons, BAF complex subunit-specific interactions with
527 transcription factors and other chromatin regulators may define the specific function of
528 BAF complexes in synapse development (**Fig. 3B**). For example, the neural specific BAF
529 subunit CREST has been shown to regulate dendrite development via its interactions with

530 the transcriptional co-activator and histone acetyltransferase CBP (Aizawa et al. 2004).
531 BAF complexes containing CREST are found bound to the promoters of genes related to
532 synapse functions including *Arc* and *Grin2b* where CREST is required to mediate their
533 expression (Qiu and Ghosh 2008). One key transcription factor that has been shown to
534 link BAF complexes to synapse development is MEF2C. Gene expression profiling from
535 a recent study revealed a significant overlap between neuronal activity-induced genes that
536 are disrupted in the absence of Brg1 with genes that are targets of regulation by MEF2.
537 Knocking out MEF2C was shown to impair the activity-dependent recruitment of Brg1 to
538 gene promoters, and conversely knocking out Brg1 impaired the ability of MEF2C
539 overexpression to drive synapse elimination (Zhang et al. 2016). These data suggest that
540 Brg1 functions as a coactivator of the MEF2C-dependent transcriptional program that
541 mediates excitatory synapse elimination. Taken together these studies suggest that
542 identifying and selectively disrupting specific BAF subunit-transcription factor
543 interactions offers a strong opportunity to dissect the many roles of BAF complexes in
544 synapse development.
545
546 *CHD4: timing transcriptional repression*
547 Whereas the BAF complexes illustrate how subunit specificity can allow a
548 chromatin remodeler to differentially control distinct stages in the process of synapse
549 formation, recent findings about the CHD family chromatin remodeler CHD4 have
550 provided new insights into regulation of gene expression on distinct time scales during
551 synapse development.

552 The ATPases of the CHD family are characterized by the presence of a
553 chromodomain in addition to the conserved DEAD/H-related ATPase domain. CHD4
554 assembles with DNA binding proteins and histone deacetylases into a large protein
555 complex called the nucleosome-remodeling and histone deacetylase (NuRD) complex
556 (Hargreaves and Crabtree 2011). NuRD functions as a transcriptional repressor and has
557 canonically been suggested to play an opposing role to the gene activation mediated by
558 BAF complexes in regulation of gene transcription (Ho and Crabtree 2010). Although
559 NuRD is well established to play a critical role in the differentiation of embryonic stem
560 cells, far less is known about functions of NuRD in postmitotic tissue. However a recent
561 study showed that knocking out CHD4 in granule neurons in the cerebellar cortex results
562 in a reduction in the density of synapses and neurotransmission between parallel fibers
563 and Purkinje cell dendrites, suggesting that the NuRD complex promotes synaptogenesis
564 (Yamada et al. 2014). Genome-wide chromatin state profiling in the cerebellum of mice
565 lacking CHD4 revealed that in the absence of NuRD, a number of gene promoters fail to
566 undergo developmentally-regulated, NuRD-dependent repression. Expression of these
567 NuRD target genes, which include *Nhlh1, Elavl2,* and *Cplx3* is highly developmental
568 regulated in wildtype mice, such that expression peaks at postnatal day 6 and then
569 decreases to very low levels in the adult brain. In the absence of CHD4, expression of the
570 NuRD target genes remains high. Because in vivo RNAi knockdown reveals that many of
571 these genes suppress presynaptic differentiation in the developing cerebellar cortex, these
572 finding suggest that NuRD-dependent gene repression mediates the timing of proper
573 synapse development by releasing the suppression of presynaptic differentiation in
574 granule neurons during neural development (**Fig. 3C**).

575 Interestingly, in addition to its role in gene repression on a developmental time
576 scale, CHD4-dependent repression has also been found to act much more rapidly to
577 regulate the dynamics of neural activity-dependent gene expression in neurons (Yang et
578 al. 2016). Surprisingly, given its known functions in gene repression, genome-wide
579 CHD4 binding and chromatin state profiling revealed that CHD4 occupies the promoters
580 of most actively transcribed genes in the mouse cerebellum including the classic activity-
581 inducible genes *Bdnf*, *Fos*, and *Npas4*. Conditional knockout mice lacking CHD4 in
582 cerebellar granule neurons show a prolonged time course of expression after the
583 induction of activity-regulated genes is triggered, and this gene expression change is
584 associated with defective activity-dependent granule neuron dendrite pruning. A clue to
585 the mechanism of CHD4's action in neuronal activity-regulated gene transcription came
586 when the authors observed that binding of the histone variant H2A.Z at the promoters of
587 activity-regulated genes was reduced in CHD4 knockout granule neurons. In yeast the
588 homolog of H2A.Z is enriched at the promoters of genes when they are in their inactive
589 state, but is rapidly lost from gene promoters when transcription is induced which
590 suggested that H2A.Z might poise genes in the off state for their rapid activation (Zhang
591 et al. 2005). However in the absence of CHD4, H2A.Z is depleted from activity-inducible
592 gene promoters, and rather than affecting induction, knockdown of H2A.Z phenocopies
593 the prolonged time course of activity-induced transcription seen in CHD4 knockout
594 neurons. (Yang et al. 2016) Thus, these data suggest a new model in which CHD4-
595 dependent recruitment of H2A.Z to activity-inducible gene promoters in neurons controls
596 the timing of transcriptional inactivation perhaps by serving to facilitate recruitment of
597 repressors to promoters as has been observed for H2A.Z in ES cells (Hu et al. 2013). In

598 sum, these data reveal two distinct function for CHD4 in cerebellar synapse regulation –
599 one of which relies on the classic function of the NuRD complex in developmental gene
600 silencing and the other of which is mediated by interactions between CHD4 and the
601 histone variant H2A.Z to control the inactivation kinetics of stimulus-inducible genes.
602 Future understanding of the CHD4-dependent mechanisms that differentiate its function
603 in these two processes will enhance our understanding of the time scales of gene
604 transcription that contribute to synapse formation and refinement in the developing CNS.
605

606 **Transcriptional Dysregulation of Synapses in Autism and Intellectual Disability**

607 The human genome project was key to discovering single-gene mutations that cause brain
608 disorders, such as those in *CHD7* in CHARGE syndrome, *TCF4* in Pitt–Hopkins
609 syndrome, *MECP2* in Rett syndrome, *GTF2I* in Williams syndrome, and PHF6 in
610 Börjeson-Forssman-Lehman syndrome (Amir et al. 1999; Jahani-Asl et al. 2016; Morris
611 et al. 2003; Zentner et al. 2010; Zweier et al. 2007). Now substantial improvements in
612 genetic methodologies, including increases in the speed and decreases in the cost of
613 genome sequencing, are driving a revolution in our understanding of the genetic
614 underpinnings of neurodevelopmental disorders by giving a more nuanced understanding
615 of the types of gene variants that may predispose individuals to complex disorders
616 including intellectual disability (ID) and autism spectrum disorders (ASDs) (Sanders
617 2015). By compiling the data derived from multiple large patient cohorts and building
618 statistical tools to mine these data comprehensively, there has been a significant
619 expansion in our knowledge of the genes that can be linked to these brain disorders. Not
620 surprisingly one major category of gene function linked to neurodevelopmental disorders

621 including both ASDs and ID are genes involved in synapse function, supporting the well-
622 established idea that it is dysregulation of brain wiring that underlies the nature of these
623 diseases. However it is perhaps somewhat surprising that a second major category of
624 genes implicated in ASDs and ID are those involved in transcriptional regulation
625 (Bourgeron 2015; De Rubeis et al. 2014; Najmabadi et al. 2011; Ronan et al. 2013).
626 Though a comprehensive understanding of how transcriptional dysregulation contributes
627 to ASDs and ID remains elusive, the sheer number of genetic variants in transcriptional
628 regulators that have been associated with neurodevelopmental disorders in recent large
629 sequencing studies (summarized in **Table 1**) suggests the physiological relevance of
630 studying this process (Deciphering Developmental Disorders 2017; Stessman et al. 2017;
631 Yuen et al. 2017).

632 Given the evidence linking transcription factors and chromatin regulators with
633 synapse development, it is likely that mechanistic insights into the pathophysiology of
634 these disorders will come from studying how disease-associated mutations in
635 transcriptional regulators affects the programs of gene transcription required for synapse
636 formation. Here we review two recent examples of these findings and discuss both the
637 advances and remaining challenges for understanding the links between transcription and
638 synapse abnormalities in disease.

639

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

641 The most reproducible genetic association with transcriptional regulation to arise
642 from autism exome sequencing studies to date is the finding that de novo mutations in the
643 chromodomain family nucleosome remodeling protein *CHD8* are associated with

644 increased risk for sporadic ASDs (Iossifov et al. 2014; Neale et al. 2012; O'Roak et al.

645 2012b; Talkowski et al. 2012). Following its identification as a high confidence hit in two

646 large GWAS studies, focused rescreening of additional patient samples led to the

647 estimation that *CHD8* is one of a set of just six genes (*CHD8, DYRK1A, GRIN2B, TBR1,*

648 *PTEN,* and *TBL1XR1*) that contribute to as many as 1% of sporadic ASDs (Iossifov et al.

649 2014; O'Roak et al. 2012a). Phenotypically, those with *CHD8* mutations were found to

650 have significant larger head circumference relative to patients lacking these mutations

651 (O'Roak et al. 2012a). Interestingly in the same cohort, patients with *DYRK1A* mutations

652 had significantly reduced head circumference, suggesting that distinct genetics underlie

653 the range of ASD presentations. The association between *CHD8* mutations and

654 macrocephaly was validated in a second targeted resequencing study that focused on a

655 cohort of patients with a broader range of developmental delay phenotypes (Iossifov et al.

656 2014). In addition to increased head circumference, this study broadened the putative

657 CHD8 phenotype to include distinct facial characteristics and gastrointestinal complaints.

658 Importantly in a zebrafish model, *chd8* knockdown during development was shown to

659 cause both macrocephaly and impaired GI motility due to reduced colonization of the GI

660 tract by enteric neurons (Iossifov et al. 2014), suggesting a causative association between

661 loss of CHD8 and these phenotypic features.

662 To identify gene targets of CHD8 that may explain its function in brain

663 development, several groups have conducted genome-wide chromatin

664 immunoprecipitation (ChIP) and/or transcriptome sequencing studies from CHD8 mutant

665 cells in culture (Cotney et al. 2015; Sugathan et al. 2014; Wang et al. 2015). In human

666 iPSC-derived neural progenitor cells, CHD8 was found to be broadly distributed across

667 the genome and bound near ~5,000 genes that were enriched for functions in chromatin
668 modifications and transcriptional regulation. Reducing CHD8 expression led to both up
669 and down regulation of a large set of genes, with the downregulated set enriched for
670 neuronal annotations such as cell adhesion, synapse, and axon guidance (Sugathan et al.
671 2014). Most of the genes that showed differential regulation in the CHD8 knockdown
672 cells were not direct targets of CHD8 binding, leading the authors to suggest a model in
673 which CHD8 sits atop a program of transcriptional regulation that subsequently feeds
674 forward to coordinate neuronal differentiation.

675 New insights into the basic biology of CHD8 have come from the analysis of
676 CHD8 loss-of-function mouse models (Durak et al. 2016; Katayama et al. 2016; Platt et
677 al. 2017). Durak et al. (2016) focused on the functions of CHD8 in neural progenitors by
678 knocking down expression of CHD8 in vivo using in utero electroporation. This
679 controlled, cell-type specific approach allowed the authors to demonstrate that loss of
680 CHD8 in progenitors leads to precocious neurogenesis due to loss of a cell-type specific
681 action of CHD8 as an activator of genes in the Wnt signaling pathway. By contrast,
682 Katayama et al. (2016) and Platt et al. (2017) mimicked the messier but more
683 physiologically relevant haploinsufficiency of human CHD8 mutations by generating
684 constitutive heterozygous CHD8 knockout mice. All three studies found that reducing
685 expression of CHD8 in the developing brain was associated with ASD-like behaviors,
686 such as impaired sociability and social novelty in three chamber interaction tasks. Both
687 approaches also revealed disrupted expression of neuronal differentiation genes,
688 validating the function of CHD8 as a modulator of neuronal development in vivo.
689 However only the haploinsufficient models mirrored the macrocephaly that is seen in

690 human patients with CHD8 mutations (Katayama et al. 2016). Thus despite the gross
691 behavioral similarities between the models, these distinct observations raise the
692 possibility that the ultimate phenotype of CHD8 mutation may depend on both cell
693 autonomous and cell non-autonomous effects on brain function, and they highlight the
694 importance of using multiple kinds of loss-of-function models for gaining insight into the
695 complex etiologies of disorders like ASDs and ID.
696
697 *MEF2C haploinsufficiency: a human-specific requirement for MEF2C in brain*
698 *development?*
699 MEF2C haploinsufficiency was relatively recently recognized as a cause of
700 syndromic mental retardation that is characterized by severe intellectual disability, lack of
701 speech, hypotonia, stereotypic movements, and epilepsy. Using comparative genomic
702 hybridization to genome oligonucleotide arrays, two clinical groups discovered
703 overlapping microdeletions on chromosome 5q14.3 in small cohorts of unrelated patients
704 (Le Meur et al. 2010; Nowakowska et al. 2010). Though the microdeletions in these
705 patients ranged from ~150kb to 8.8Mb, the minimal common deleted region
706 encompassed only *MEF2C* suggesting loss of MEF2C function as the common cause of
707 the consistent phenotype. A number of additional patients have been reported with
708 deletions that disrupt MEF2C who show highly similar presentations, suggesting that
709 MEF2C haploinsufficiency is a discrete clinical entity (Novara et al. 2010; Paciorkowski
710 et al. 2013; Rocha et al. 2016). Finally in the strongest case for the causative association
711 of MEF2 haploinsufficiency with the clinical syndrome, one patient with a similar

712 phenotype has been characterized to have a de novo point mutation within exon 8 of the
713 *MEF2C* gene that is predicted to result in a premature stop codon (Le Meur et al. 2010).
714 Despite the common clinical phenotype, the underlying brain pathophysiology of
715 MEF2 haploinsufficiency syndrome remains unknown. Patients with 5q14.3 have
716 variable morphological abnormalities of the brain that include mild thinning of the corpus
717 callosum and delay of white matter myelination, but no detailed pathological findings
718 have been reported from patients. Often, as we described above for CHD8, hypotheses of
719 pathophysiological mechanism emerge from studies of model organisms. As discussed
720 above, neuron- or brain-specific conditional MEF2C knockout mice show changes in
721 synapse formation (Barbosa et al. 2008; Harrington et al. 2016). Furthermore these
722 mouse models show deficits in learning and memory (Barbosa et al. 2008), cortical
723 development (Li et al. 2008) and deficits in behaviors relevant to ASDs and ID such as
724 changes in ultrasonic vocalizations, deficits in social novelty, reduced sucrose preference,
725 locomotor hyperactivity, and impaired fear conditioning (Harrington et al. 2016).
726 However heterozygous conditional deletion of MEF2C either in excitatory forebrain
727 neurons (Harrington et al. 2016) or throughout the brain (Barbosa et al. 2008) fails to
728 disrupt performance on cognitive and social tasks compared with wildtype littermates. A
729 more faithful model of the human genetic condition is found in mice heterozygous for
730 germline deletion of *Mef2c*, and though these were described upon generation as showing
731 no discernable phenotype (Lin et al. 1997), they have not yet been subjected to detailed
732 neural and behavioral phenotyping. Thus the relevance of the synaptic mechanisms
733 underlying behavioral abnormalities in the brains of conditional MEF2C knockout mice
734 for understanding the human haploinsufficiency syndrome remain to be fully determined.

735 A further point of interest with respect to understanding MEF2C
736 haploinsufficiency syndrome is a recent paper that suggests MEF2C may have human-
737 specific targets. Specifically, MEF2C was discovered to bind to an evolutionarily
738 acquired MEF2-responsive element regulating the gene encoding the secreted protein
739 osteocrin (*OSTN*) by ChIP from primary fetal human neurons (Ataman et al. 2016). This
740 MEF2C binding enhancer element emerged in the primate lineage, where it is required
741 for the primate-specific neuronal activity-inducible transcription of *OSTN*. Whether
742 osteocrin expression is affected in patients with MEF2C haploinsufficiency syndrome is
743 not known, however knockdown of osteocrin disturbs dendrite development in cultured
744 human iPSC-derived neurons suggesting that it could be part of a human-specific
745 mechanism of synapse development. These data raise the possibility that MEF2C could
746 play species-specific roles in synapse formation by virtue of its differential recruitment to
747 evolutionarily adapted enhancers. Further development of methods for examining
748 transcription factor function in human neurons will advance our understanding of this and
749 other similar effects of regulatory element variation on species-specific aspects of brain
750 function and dysfunction.
751
752 **Conclusions**
753 The data reviewed here reveal the degree to which transcription factors and
754 chromatin regulators can have complex and selective effects on synapse development.
755 The function of sequence-specific binding transcription factors is ultimately controlled by
756 the distribution and accessibility of their binding sites in the genome, which determines
757 their potential target genes. ChIP-seq and RNA-seq studies have begun to reveal the

758 expression profiles controlled by synapse regulatory transcription factors and chromatin
759 regulators, giving new insights into the molecular mechanisms of synapse formation,
760 elimination, and maintenance. Interestingly, evidence that mRNAs regulated by the
761 transcription factor MEF2 are transported to synapses by the RNA binding protein FMRP
762 and can be subject to local translation suggests a new model for how the action of a
763 transcription factor in the nucleus can be directed to specific sets of synapses in the
764 periphery. Understanding the extent to which other RNA trafficking proteins contribute to
765 the synaptic actions of transcription factors will be an interesting area of exploration for
766 the future.

767 The advances in next-generation sequencing methods that emerged from the
768 completion of the human genome drove many of the recent advances in our
769 understanding of transcription factors in brain development and disease. We are still
770 feeling the impact of these methods – for example, exome sequencing is still in its
771 relative infancy and the majority of cases of sporadic ASDs and ID are without a defined
772 genetic cause. With the cost of sequencing continuing to fall and whole genome
773 sequencing become an increasing reality at scale, it is reasonable to expect that new
774 mutations including non-coding mutations will emerge that show association with brain
775 disorders offering new clues to the etiology of these diseases. As we discussed above for
776 CHD8 and MEF2C, one way the clarification of disease genetics can improve outcomes
777 for patients is by offering diagnostic criteria for predicting the course of the clinical
778 syndrome. Further, the development of model systems that permit investigations into the
779 pathophysiology of the disorder can in ideal scenarios suggest potential treatment
780 options. For example the loss-of-function mutations in MeCP2 that cause Rett Syndrome

781 in girls were found to disrupt expression of the neuronal growth factor IGF-1 in the
782 developing brain of a mouse model (Tropea et al. 2009). Because IGF-1 was already
783 FDA-approved for treatment of certain endocrine disorders, this rapidly led to a clinical
784 trial for IGF-1 replacement therapy in Rett Syndrome (Khwaja et al. 2014). Though at the
785 time of this writing the outcome of the larger trial remains unknown, the simple fact that
786 a neurodevelopmental disorder could potentially be treatable offers great hope to the
787 families of the children with these disorders. Finally in the future gene editing strategies
788 using new developments in TALEs or Cas9/CRISPR (Long et al. 2016; Nelson et al.
789 2016; Tabebordbar et al. 2016) might have the potential to fully correct these genetic
790 disorders or their transcriptional consequences, in the most desirable outcomes of all.
791

792 **Figure Legends**

793 **Figure 1: Mechanisms of specificity in the transcriptional regulation of synapses.** A)
794 Calcium-response factor (CaRF) binds to *Bdnf* IV promoter and activates basal *Bdnf*
795 transcription. It also inhibits activity dependent *Bdnf* transcription via indirect activation
796 of *Grin3a* expression, which encodes the NMDA-receptor subunit GluN3A. GluN3A
797 inhibits NMDAR-induced *Bdnf* transcription without impairing the ability of L-type
798 voltage gated calcium channels to activate *Bdnf* transcription. B) Npas4 expression is
799 induced rapidly by neuronal activity. Npas4 target genes promote GABAergic synapse
800 formation at the soma while also inhibiting GABAergic synapse formation at the apical
801 dendritic spines. C) Neuronal activity induces MEF2 dependent gene transcription.
802 FMRP traffics mRNAs from the nucleus to the synapses and represses local mRNA
803 translation at the synapses until neuronal activation of mGluR5 receptors leads to target

804 gene mRNA dissociation from FMRP. The ability of constitutively active MEF2-VP16 to
805 drive synapse elimination is blocked in FMRP knockout neurons suggesting that these
806 two pathways converge to locally regulate synapse pruning.
807
808 **Figure 2: Domain organization and posttranslational regulatory modifications of**
809 **MEF2 family members in neurons.** MEF2A, C, and D proteins all contain N-terminal
810 MADS and MEF2 domains (gray and brown), which mediate DNA binding and
811 dimerization, and a conserved transactivation domain (TAD, green). The α, β, and γ
812 indicate alternatively spliced exons. In neurons the MEF2s predominantly contain the α_1
813 splice variant and are β+. The γ-domain is constitutively present in MEF2A and MEF2D
814 but alternatively spliced in MEF2C. The figure shows post-translational modifications (P-
815 Phosphorylation; SUMO-sumoylation; Ac-Acetylation) and their residue locations in
816 human MEF2A. Whether the modifications function to enhance or repress MEF2 activity
817 is shown above the diagram.
818
819 **Figure 3: Chromatin regulatory factors in synapse development.** A) Neuronal
820 specific accumulation of methyl-CpH leads to a distinct MeCP2 regulated gene program
821 in mature neurons. Upper, during brain development methyl-CpH levels increase and
822 accumulate selectively in mature neurons whereas methyl-CpG levels are constant across
823 development in both neurons and glia. Bottom, because MeCP2 binds to both methyl-
824 CpG and methyl-CpH the distinct high level of methyl-CpH in mature neurons leads to a
825 neuron-specific developmental effect on MeCP2 regulated gene transcription. Diagrams
826 are based on the data in (Lister et al. 2013) and (Chen et al. 2015). B) Tissue specific

827 BAF subunits interact with distinct transcription factors or transcriptional coactivators
828 resulting in activation of different gene programs during neural development. BAF
829 complexes regulate a group of synaptic genes and mediate synapse formation and
830 maturation. In activated neurons, BAF complexes regulate *Arc* and *Grin2b* expression by
831 interacting with CBP. BAF complexes also interact with MEF2C and are required for
832 MEF2C mediated synapse elimination. C) NuRD controls the timing of gene expression
833 by turning genes off both on a developmental time scale and in response to transient
834 neuronal activity. During cerebellar development, NuRD turns off genes that suppress
835 presynaptic differentiation by histone deacetylation and inactivates neuronal activity-
836 dependent genes transcription by replacing H2A to H2A.Z.
837
838 **Table 1**: Genes encoding transcriptional regulatory proteins that have de novo mutations
839 achieving statistical significance in large scale sequencing screens of ASDs and other
840 developmental disorders (Deciphering Developmental Disorders 2017; Stessman et al.
841 2017; Yuen et al. 2017).
842
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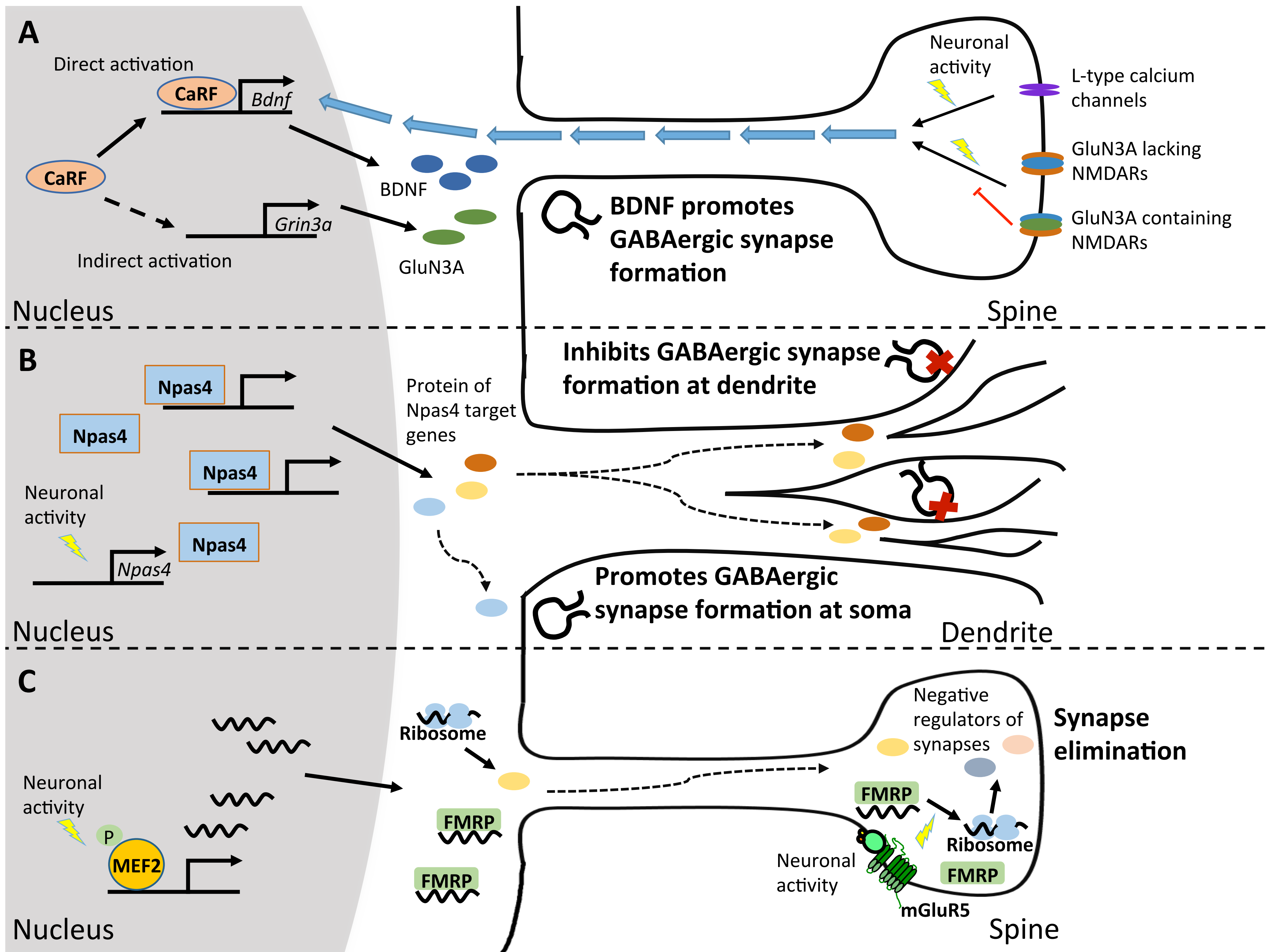
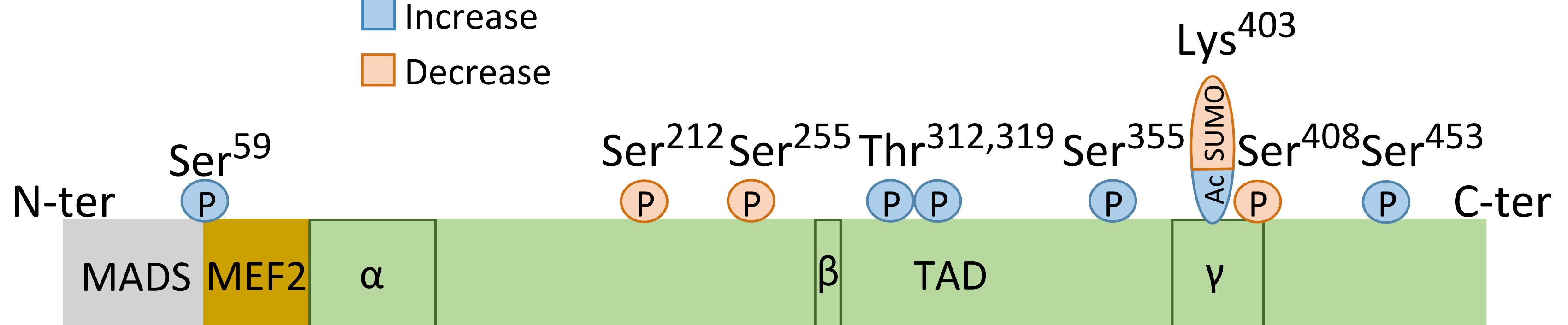
ADNP	*CHD4*	*FOXP1*	*KMT2C*	*NR3C2*	*SMARCA2*
AHDC1	*CHD8*	*GATAD2B*	*MECP2*	*NSD1*	*SMARCC2*
ARID1B	*CNOT3*	*HDAC8*	*MED13*	*PAX5*	*SMC1A*
ASH1L	*CREBBP*	*KANSL1*	*MED13L*	*PHF3*	*SUV420H1*
ASXL1	*CTCF*	*KAT6A*	*MEF2C*	*PURA*	*TBL1XR1*
ASXL3	*DNMT3A*	*KAT6B*	*MLL3*	*SATB2*	*TBR1*
AUTS2	*EHMT1*	*KDM5B*	*MSL3*	*SET*	*TCF20*
CHD2	*EP300*	*KDM6A*	*MYT1L*	*SETD5*	*TCF4*
CHD2	*FOXG1*	*KMT2A*	*NFIX*	*SMAD4*	*TCF7L2*



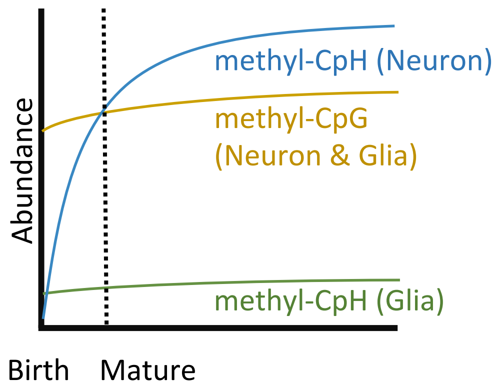
A
Direct activation
CaRF
Bdnf
CaRF
Grin3a
Indirect activation
BDNF
GluN3A
Nucleus
Neuronal activity
L-type calcium channels
GluN3A lacking NMDARs
GluN3A containing NMDARs
BDNF promotes GABAergic synapse formation
Spine
B
Npas4
Npas4
Npas4
Neuronal activity
Npas4
Npas4
Protein of Npas4 target genes
Inhibits GABAergic synapse formation at dendrite
Promotes GABAergic synapse formation at soma
Nucleus
Dendrite
C
Neuronal activity
P
MEF2
Ribosome
FMRP
FMRP
Negative regulators of synapses
Synapse elimination
FMRP
Ribosome
FMRP
Neuronal activity
mGluR5
Nucleus
Spine



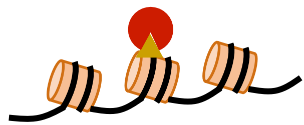
Effect on MEF2 activity
Increase
Decrease
Lys403
SUMO
Ac
Ser59
Ser212
Ser255
Thr312,319
Ser355
Ser408
Ser453
P
N-ter
C-ter
MADS
MEF2
α
β
TAD
γ

A

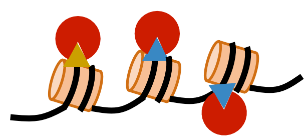
Brain development



Immature neuron & Glia



Mature neuron



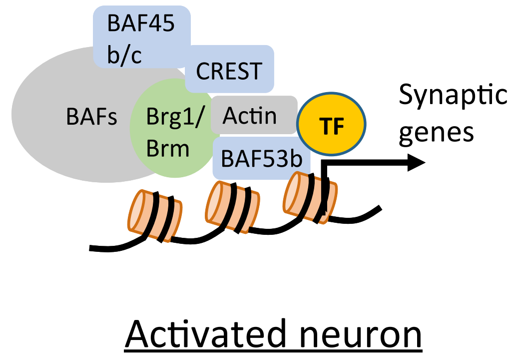
MECP2

methyl-CpG

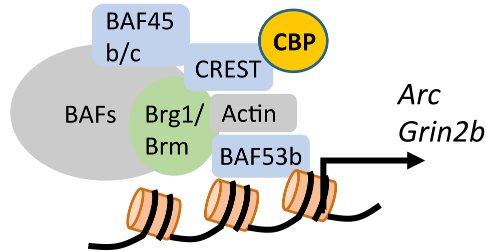
methyl-CpH

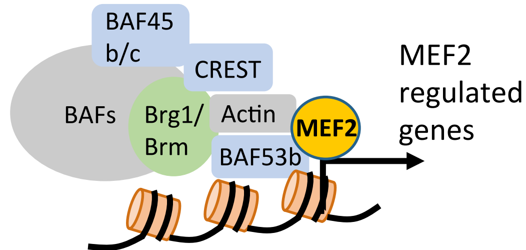
B

Neural development



Activated neuron



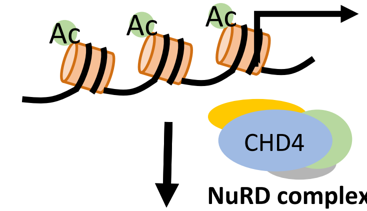


C

Cerebellum development

Developmental regulated genes

Genes that suppress presynaptic differentiation



Activity regulated genes

Activity regulated genes