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Perspective

# A Brief History of Neuronal Gene Expression: Regulatory Mechanisms and Cellular Consequences

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A central goal of cellular and molecular neuroscience is to explain the development and function of the nervous system in terms of the function of genes and proteins. Models of gene regulation have evolved from being focused on transcriptional and translational control to include a variety of regulatory mechanisms such as epigenetic control, mRNA splicing, microRNAs, and local translation. Here we discuss how developments in molecular biology influenced the study of neuronal gene expression, and how this has shaped our understanding of neuronal development and function.

## Introduction

The enormous influence of molecular biology on our understanding of nervous system function is reflected in the fact that in virtually all areas of neuroscience research we tend to describe mechanisms in terms of genes and proteins. This has been true in developmental neurobiology for a long time and is increasingly true in other areas of neuroscience, such as cellular physiology and neurological disease. We routinely describe developmental events in terms of transcription factors and ligand-receptor interactions. Notch and Delta, NGF and Trk, and Sonic Hedgehog and Patched are part of this new vocabulary. The phenomenon is not restricted to developmental neurobiology. It is now common to describe electrophysiological phenomena in terms of regulation of AMPA and NMDA receptors, PDZ proteins, TARPs, and CaM kinases. We are in an era where we gauge our understanding of the brain based on our ability to explain neurobiological phenomena in terms of the role of the underlying genes and proteins. One could very well describe the state of affairs as a "*Neuron* Effect," as a molecular approach to understanding the nervous system has been a hallmark of papers published in the journal over the past two decades. But it would be a mistake to think that this is a view of neuroscience that has been pushed by *Neuron*; instead, the founders of the journal and first *Neuron* editorial team from UCSF recognized the incredible impact that molecular biology was having on neuroscience and created a venue for publication of the most exciting work in the field. The experiment has been an unqualified success.

It is useful to look back at some of the early discoveries that made molecular biology of the nervous system an area of such great fascination. The discovery of Nerve Growth Factor by Rita Levi-Montalcini and Stanley Cohen was a transforming event and highlighted the great power of understanding developmental events in terms of ligand-receptor interactions. The work from Sydney Brenner, Seymour Benzer, and colleagues illustrated the power of genetics to get to the molecular basis of neuronal function. The discovery of sensory transduction pathways, first for vision and then for other sensory systems, allowed us to understand how we perceive the external world. The purification of proteins and cloning of genes involved in synaptic vesicle release and ion channels transformed the study of cellular physiology.

Whereas many of the early discoveries on the molecular basis of neuronal function had their roots in biochemistry, the rapid pace of discovery in molecular biology and the accompanying understanding of gene regulation has driven many of the advances in the past two decades. The central dogma had taught us that genes are encoded in DNA, that DNA was transcribed into mRNA, and that mRNA was translated into protein. Molecular investigations of gene regulation revealed a host of regulatory mechanisms that dramatically expand the ways in which a cell can regulate its protein composition. Not only is the transcription of many genes tightly regulated, but splicing, trafficking, and translation of mRNA can also be exquisitely controlled, which allows for incredibly precise control over protein levels and localization. In a cell as complex as a neuron, these gene regulatory mechanisms are widely used to facilitate proper development and function of the nervous system and allow the nervous system to adapt to changes in the environment. In this Perspective we discuss a few examples to illustrate how the discovery of gene regulatory mechanisms over the past 20 years has been closely linked to the emergence of major ideas regarding brain development and function.

### *Stimulus-Dependent Transcription and Neuronal Adaptive Responses*

Although the relationship between genes and proteins was described in the 1940s, the first study to show that extracellular signals could acutely regulate eukaryotic gene expression was a 1984 paper by Greenberg and Ziff where they reported that the proto-oncogene c-*fos* was rapidly induced by growth factor stimulation of 3T3 cells (Greenberg and Ziff, 1984). This study had a major impact, since it changed the concept of gene regulation from an autonomous property of cells to a process that was an integral part of a cell's response to changes in the environment. Greenberg, Greene, and Ziff, as well as Curran and Morgan, went on to show that NGF stimulation of PC12 cells also led to the rapid induction of c-*fos* expression, which suggested that such dynamic regulation of gene expression might be a feature of the nervous system (Greenberg et al., 1985; Curran and Morgan, 1985). Shortly thereafter, Greenberg and colleagues reported that stimulation of PC12 cells with Acetylcholine led to c-*fos* expression and that this required calcium influx via

voltage-sensitive calcium channels (VSCC) (Greenberg et al., 1986). This was a critical study, as it showed that neurotransmitter-induced calcium influx, which had previously thought only to exert acute effects, could lead to a rapid and robust transcriptional response. Thus, in a short period of 2 years, the concept of activity-dependent regulation of gene expression became established, which had major implications for activity-dependent development and function of the nervous system.

A separate line of investigation from Goodman, Montminy, and colleagues, who were studying cAMP regulation of gene expression, led to the identification of CREB, a key transcription factor that mediates stimulus-dependent transcription. In 1986 they reported that cAMP regulated somatostatin mRNA levels and identified a cAMP-responsive element (CRE), which was sufficient to confer cAMP responsiveness (Montminy et al., 1986a, 1986b). Montminy and colleagues isolated the transcription factor that binds to the CRE and named it cAMP-responsive element binding protein (CREB) (Montminy and Bilezikjian, 1987). They showed that elevation in cAMP led to phosphorylation of CREB at Ser-133, and that this modification was required for transcription activation by CREB (Gonzalez and Montminy, 1989). In the meantime, Greenberg and colleagues showed that calcium-dependent induction of c-*fos* expression was mediated by a calcium-responsive element that also bound CREB (Sheng et al., 1991). Thus CREB was identified as a key mediator of cAMP- and calcium-dependent transcription in neurons.

### The Role of CREB

A series of observations in the 1990s implicated CREB-mediated transcription as a critical mediator of adaptive responses in the nervous system. One area of active investigation was the potential role of CREB in memory. A study from the Benzer lab had implicated cAMP signaling in learning and memory (Dudai et al., 1976), and Kandel and colleagues had shown that synaptic plasticity in *Aplysia* required cAMP signaling, but it was not clear how cAMP signaling might be connected with memory. An intriguing possibility was that cAMP might exert its effects by regulating gene expression, which was supported by pharmacological studies from the 1960s and 1970s from the Flexner, Agranoff, and Barondes labs that suggested that gene expression and protein synthesis were required for the retention of memory (reviewed in Davis and Squire, 1984). Following the identification of the CRE by Montiminy and colleagues, Kandel's group showed that injection of a CRE-containing DNA fragment impaired long-term plasticity in *Aplysia* (Dash et al., 1990), which suggested that CRE-mediated gene expression was likely to be important for long-term memory.

Kandel and colleagues continued to investigate the role of cAMP signaling in plasticity and reported that cAMP stimulation of hippocampal slices mimics the late phase of long-term potentiation (LTP) (Frey et al., 1993). Shortly thereafter, Tully and colleagues reported that a dominant-negative form of CREB blocks long-term memory in *Drosophila*, and Silva and colleagues reported that mice carrying a mutation in CREB had deficient long-term memory (Yin et al., 1994; Bourtchuladze et al., 1994). While these studies built support for the idea that CREB might play an important role in memory, it was difficult to know if this pathway had a specific role in memory or whether these molecules played a more general role in mediating neuronal responses to environmental changes.

Investigation of the role of CREB in other systems suggested that CREB was unlikely to be selectively involved in memory, but rather was likely to be generally involved in mediating long-term neuronal responses to external stimuli. An important set of observations came from Eric Nestler and his colleagues, who examined the role of CREB in addiction. They showed that morphine administration reduces CREB phosphorylation in the rat locus coeruleus, and that opiate receptor antagonists increased CREB phosphorylation (Guitart et al., 1992). Subsequent work from the Nestler group showed that modulation of CREB could regulate the response to cocaine (Carlezon et al., 1998), and Malenka and colleagues showed that CREB regulates excitability of nucleus accumbens neurons, another structure implicated in cocaine addiction (Dong et al., 2006).

In separate studies Ginty, Greenberg, and colleagues showed that CREB phosphorylation in the suprachiasmatic nucleus was regulated by light, and Ginty and colleagues showed that NGF-induced signaling to CREB was important for the cell survival effects of NGF (Ginty et al., 1993; Riccio et al., 1997, 1999). Ghosh and colleagues showed that CREB was involved in activity-dependent dendritic growth (Redmond et al., 2002), and work from the Malenka group showed that CREB activity could regulate the number of silent synapses (Marie et al., 2005). These observations indicated that CREB-mediated transcription was likely to be involved in regulating a diverse set of neuronal responses.

While much of the early investigation of calcium-dependent transcription and its consequences was focused on CREB, it is now clear that calcium signaling targets a number of different transcription factors that mediate different cellular effects of calcium signaling. The Lipton, Greenberg, and Bonni labs identified MEF2 as a calcium-regulated transcription factor in neurons and showed that MEF2 was involved in mediating activity-dependent survival and in regulating excitatory synapse number (Leifer et al., 1993; Mao et al., 1999; Flavell et al., 2006; Shalizi et al., 2006). In an effort to identify novel calcium-dependent transcription factors, Ghosh and colleagues developed a new screen called Transactivator Trap and identified a set of new calcium-regulated transactivators (Aizawa et al., 2004). The first of these factors was CREST, which was shown to be involved in mediating activity-dependent dendritic growth (Aizawa et al., 2004). Two other factors cloned in this screen were NeuroD2 and LMO4, both of which are involved in barrel cortex development (Kashani et al., 2006; Ince-Dunn et al., 2006). It now appears that changes in neuronal activity in response to extracellular signals can lead to the activation of a large number of transcription factors. Some of them, such as CREB, may have a general role in neuronal adaptive responses, whereas others may have more specific roles in mediating specific aspects of activity-dependent development and plasticity.

### Activity-Regulated Genes

Ever since c-*fos* was identified as a calcium-regulated gene, there has been an interest in identifying genes whose expression is regulated by neuronal activity. The earliest in vivo evidence of activity-dependent regulation of gene expression came from Morgan and colleagues, who showed that c-*fos* and other

immediate-early genes were induced in the hippocampus after seizures (Morgan et al., 1987). The development of strategies to clone differentially expressed genes facilitated various screens to clone activity-regulated genes. Worley, Nedivi, and colleagues identified a number of seizure-induced genes using these strategies (Nedivi et al., 1993; Cole et al., 1989). One of the genes identified in the Worley screen was called Arc, which was subsequently shown to be involved in regulating AMPA receptor-mediated transmission and AMPA receptor internalization (Lyford et al., 1995; Chowdhury et al., 2006; Shepherd et al., 2006; Rial Verde et al., 2006).

One of the most intensely studied activity-regulated genes is Brain-derived neurotrophic factor (BDNF) (Ernfors et al., 1991; Isackson et al., 1991). BDNF was originally identified as a survival factor for peripheral neurons but was subsequently shown to be involved in regulating a number of attributes of neurons, including axonal and dendritic growth, the efficacy of synaptic transmission, and synaptic plasticity (Lohof et al., 1993; Kang and Schuman, 1995; Figurov et al., 1996). Moreover, it was shown that BDNF expression was regulated by CREB-dependent transcription (Shieh et al., 1998; Tao et al., 1998), which suggests a mechanism by which activity-dependent gene expression might affect the organization and function of the brain. The development of methods for investigating genome-wide changes in mRNA levels in response to various stimuli should facilitate efforts to identify genes that are selectively induced in response to certain kinds of stimuli.

### *Epigenetic Control*

Research on transcriptional regulation in the last 10 years has been characterized by a shift from the study of sequence-specific transcription factors to an investigation of mechanisms that regulate chromatin. This was driven by new discoveries on the role of histone modifications, DNA methylation, and chromatin remodeling in transcriptional regulation. In 1995, Hecht and colleagues showed that histones were not just structural proteins but instead could interact with transcription regulatory factors to regulate gene expression (Hecht et al., 1995). Shortly thereafter, the Allis and Schreiber labs showed that histone acetyltransferases and histone deacetylases could regulate transcription (Brownell et al., 1996; Taunton et al., 1996). During this period, Goodman and colleagues used an ingenious strategy to identify proteins that interact with phosphorylated CREB and identified a protein called CREB-binding protein (CBP) (Chrivia et al., 1993; Kwok et al., 1994), which turned out to be a histone acetyltransferase (Ogryzko et al., 1996). The Bading and Ghosh labs showed that CBP-mediated transcription could be regulated by calcium and CaM kinase IV signaling (Chawla et al., 1998; Hu et al., 1999), suggesting that neuronal activity might regulate gene expression by regulating histone modification. Recent studies from the Mayford and Kandel groups support a role for CBP in memory (Alarcon et al., 2004; Korzus et al., 2004). It will be interesting to know whether CBP is also involved in mediating other adaptive responses in the nervous system.

Another area of epigenetic regulation that has received a great deal of attention is DNA methylation. DNA methylation has long been recognized as a mechanism to repress transcription. It is generally thought that transcriptional repression is mediated by recruitment of histone deacetylases to the methylated DNA via methyl CpG-binding proteins. Renewed interest in DNA methylation has been driven in part by the discovery that MeCP2, a methyl-CpG-binding protein, is mutated in the childhood neurological disorder Rett syndrome (Nan et al., 1997; Amir et al., 1999). It was recently shown that MeCP2 phosphorylation is regulated by calcium signaling and appears to regulate methylation of the BDNF promoter (Chen et al., 2003; Martinowich et al., 2003). This raises the exciting possibility that DNA methylation might be rapidly regulated by extracellular stimuli, but this still needs to be confirmed. There has been a flurry of papers that have linked MeCP2 to various aspects of neuronal function. Nelson and colleagues reported that cortical activity is reduced in MeCP2 null mice, and the Rosenmund lab reported that MeCP2 regulates synapse number (Dani et al., 2005; Chao et al., 2007). Zoghbi and colleagues have linked MeCP2 to feeding behavior and aggression (Fyffe et al., 2008). Identifying the targets of MeCP2 that mediate these effects should be an important goal of future studies.

A final area of investigation on epigenetic regulation has focused on chromatin remodeling complexes. These complexes generally include the core chromatin remodeling protein BRG1, which modifies chromatin by using energy from ATP hydrolysis. It is thought that remodeling of chromatin influences gene expression by affecting access of transcription-regulatory factors to DNA. The Crabtree group analyzed various BRG1 complexes in neurons and discovered that BRG1 complexes change during development and can influence dendritic development (Wu et al., 2007). Both the Crabtree and Ghosh labs found that CREST, which had previously been implicated in activity-dependent dendritic growth, is present in a complex with BRG1, suggesting that the function of the BRG1 complex can be regulated by activity (Wu et al., 2007; Qiu and Ghosh, 2008). In support of this possibility, the Ghosh group recently found that calcium influx leads to a release of a repressor from BRG1-CREST complex and recruitment to CBP to activate gene expression (Qiu and Ghosh, 2008). While CREST has been implicated in activity-dependent dendritic growth, the role of the CREST-BRG1 complex in neuronal development and plasticity needs to be rigorously tested.

### *Regulation of mRNA: Splicing, Local Translation, and MicroRNAs*

One of the most active areas of research in the past decade has been the investigation of mRNAs. There are several mechanisms that can regulate the production of proteins from mRNAs. These include mRNA splicing, regulation of mRNA abundance by microRNAs, and control of translation. Recent studies implicate each of these mechanisms in the developing and mature nervous system.

### *mRNA Splicing and Neuronal Connectivity*

mRNA splicing represents a powerful mechanism for generating a diverse set of proteins from one genetic locus. There are two particularly striking examples of mRNA splicing in neurons that may bear upon the problem of neuronal connectivity. The first is the protocadherin cluster in vertebrates, identified by Maniatis and colleagues (Wu and Maniatis, 1999). They described a cluster of 52 cadherin-like genes with an unusual genomic organization. The N-terminal extracellular domains of these proteins are

encoded by separate exons, organized in three clusters (alpha, beta, and gamma) arrayed in tandem. These exons are spliced with one of three C-terminal exons to generate a family of cadherin-like proteins. The extraordinary diversity of protocadherins suggests that they may be involved in highly specific protein-protein interactions. Sanes and colleagues examined the consequences of deleting 22 genes in the gamma cluster and reported that these genes collectively are involved in both cell survival and synapse formation (Wang et al., 2002; Weiner et al., 2005). Whether the diversity of protocadherin genes plays a role in synaptic specificity remains to be determined.

Another impressive example of alternative splicing in the nervous system involves the DSCAM gene in flies. The Zipursky lab identified DSCAM in flies as a protein that interacts with the adaptor protein Dock. Remarkably, through alternative splicing, the DSCAM gene is predicted to encode as many as 38,016 isoforms (Schmucker et al., 2000). DSCAM isoforms show exquisite binding specificity such that each isoform binds itself but not closely related isoforms (Wojtowicz et al., 2004). Work from the Zipursky, Schmucker, and other groups has shown that DSCAM proteins play a critical role in patterning of axons and dendrites in flies as well as vertebrates (reviewed in Schmucker, 2007; Hattori et al., 2008).

### Local Translation: Axon Guidance and Synaptic Function

There is growing evidence that local translation of mRNAs is an important regulatory mechanism in neuronal development and plasticity. A role for local protein synthesis in axon guidance was suggested by Holt and colleagues, who showed that retinal axons lose their responsiveness to nertrin-1 and Sema-3a when translation is inhibited (Campbell and Holt, 2001). Recently they showed that β-actin mRNA was localized to growth cones, where it binds to the RNA binding protein Vg1RBP. Netrin-1 stimulation leads to movement of Vg1RBP granules into filopodia and activates the translation regulator eIF4E-binding protein to regulate β-actin translation (Leung et al., 2006). In a related study, Flanagan and colleagues reported axonal translation of EphA2 in the spinal cord, suggesting that local translation may be broadly involved in regulating axon guidance (Brittis et al., 2002).

Interest in the role of local translation in synaptic function was sparked by the observation by Steward and Levy that polyribosomes were present in dendrites (Steward and Levy, 1982) and that synaptic stimulation led to a rapid increase in dendritic mRNAs (Steward et al., 1998). Schuman and colleagues reported that the effect of BDNF on synaptic plasticity required local protein synthesis (Kang and Schuman, 1996). More recently they have shown that protein synthesis in dendrites can be regulated by miniature synaptic events (Sutton et al., 2004).

One of the mRNAs that have been shown to be targeted to dendrites is αCaMKII, a kinase implicated in synaptic plasticity. Richter and colleagues reported that CPEB, a protein that binds to the polyadenylation tail of mRNAs, binds to the αCaMKII mRNA and regulates αCaMKII translation (Wu et al., 1998). Kandel and colleagues have reported that CPEB is required for local protein synthesis and synaptic plasticity in *Aplysia* (Si et al., 2003).

Another gene involved in regulating protein synthesis in dendrites is Fmr1, the gene mutated in Fragile X syndrome (Bagni and Greenough, 2005). Mutations in Fmr1 lead to defects in spine morphogenesis, and Bear and colleagues have reported that synaptic plasticity mediated by metabotropic glutamate receptors depends on the Fmr1 gene (Bear et al., 2004).

### MicroRNAs and Synapse Morphology

One of the most exciting recent discoveries regarding control of mRNA levels is that microRNAs exert a major influence on mRNA abundance (reviewed in Ruvkun et al., 2004; Lee et al., 2004; Klein et al., 2005). It now appears that much of the genome is transcribed, and that many of the noncoding RNAs represent microRNAs that are processed to generate 20–30 nucleotide long fragments that can recognize and degrade endogenous mRNAs. Investigation of microRNAs in the nervous system is just beginning, and there have already been some exciting advances. Impey and colleagues reported that the CREB-regulated microRNA miR132 can affect neuronal morphogenesis (Vo et al., 2005), and Klein and colleagues reported that the same microRNA can regulate MeCP2 expression (Klein et al., 2007). Schratt and colleagues have reported that miR134 can influence spine morphogenesis (Schratt et al., 2006). These findings show that neuronal activity can influence the abundance of specific mRNAs by regulating microRNA expression. It will be of great interest to identify the microRNAs that are expressed in different cell populations, determine their targets, and explore how microRNA regulation might contribute to neuronal development and function.

### Conclusions

Advances in the molecular biology of gene regulation over the past 20 years have driven investigation of these mechanisms in the nervous system. Although we have focused on extracellular control of gene expression, it is important to note that an equally important area of investigation in the past two decades has been developmental regulation of gene expression and its role in cell fate specification. This is perhaps best illustrated in the developing spinal cord, where Jessell, Pfaff, and colleagues have shown that the specification of these cell types is regulated by gradients of extracellular signals, and subtypes of spinal neurons are uniquely defined by combination of transcription factors (reviewed in Tanabe and Jessell, 1996; Dessaud et al., 2008). The mechanism by which morphogen gradients regulate expression of specific sets of transcription factors is an area of active investigation. It is important to recognize that in many cases where there is an association between a gene or protein and neuronal response, our understanding of how the protein affects the response is still quite limited. The problem is more tractable in instances where the cellular response can be tightly linked to a molecular change but is difficult to address when the outcome is complex, as in the case of memory retention.

One of the major challenges in understanding the role of gene regulatory mechanisms in adaptive responses in vivo has been a lack of methods to achieve spatially and temporally controlled inactivation of the gene or protein being studied. For example, the lack of precise spatial and temporal control has made it difficult to assess the role of genes such as CREB or BDNF in learning and memory. Thus it is not yet clear whether defects associated with loss of these genes reflects an ongoing requirement for the function of the gene or whether they are required during a critical period of memory consolidation. Similarly, lack of effective ways to inactivate specific RNAs in restricted axonal and dendritic compartments has made it difficult to causally relate local

translational events with cellular outcomes. Resolution of these questions will require the development of molecular and genetic approaches to target molecular events with greater precision.

The rapid progress in identification of mechanisms that control the production and targeting of mRNAs and proteins, together with the development of tools to manipulate genes and proteins with spatial and temporal precision, should facilitate efforts to understand the molecular basis of neural development and plasticity. Looking ahead, we expect that some of the most important advances in identifying signaling networks that regulate gene expression over the next decade will come from studies in systems biology. The sequencing of the human and mouse genomes ushered in a new era in cell and molecular biology, and the traditional approach of linking individual genes to specific phenotypes may well be on its way out. Increasingly technological advances are providing a much more comprehensive view into the mRNAs that are present in specific cell types and cell compartments, gene networks that are activated by extracellular signals, and the proteomes of cells and organelles. The tools to analyze such data sets are rapidly being developed, and the emerging views of gene and protein function will likely redefine our understanding of the molecular basis of neuronal function.

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## REFERENCES

Aizawa, H., Hu, S.C., Bobb, K., Balakrishnan, K., Ince, G., Gurevich, I., Cowan, M., and Ghosh, A. (2004). Dendrite development regulated by CREST, a calcium-regulated transcriptional activator. Science *303*, 197–202.

Alarcon, J.M., Malleret, G., Touzani, K., Vronskaya, S., Ishii, S., Kandel, E.R., and Barco, A. (2004). Chromatin acetylation, memory, and LTP are impaired in CBP+/− mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. Neuron *42*, 947–959.

Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat. Genet. *23*, 185–188.

Bagni, C., and Greenough, W.T. (2005). From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. Nat. Rev. Neurosci. *6*, 376–387.

Bear, M.F., Huber, K.M., and Warren, S.T. (2004). The mGluR theory of fragile X mental retardation. Trends Neurosci. *27*, 370–377.

Bourtchuladze, R., Frenguelli, B., Blendy, J., Cioffi, D., Schutz, G., and Silva, A.J. (1994). Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. Cell *79*, 59–68.

Brittis, P.A., Lu, Q., and Flanagan, J.G. (2002). Axonal protein synthesis provides a mechanism for localized regulation at an intermediate target. Cell *110*, 223–235.

Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y., and Allis, C.D. (1996). Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell *84*, 843–851.

Campbell, D.S., and Holt, C.E. (2001). Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation. Neuron *32*, 1013–1026.

Carlezon, W.A., Jr., Thome, J., Olson, V.G., Lane-Ladd, S.B., Brodkin, E.S., Hiroi, N., Duman, R.S., Neve, R.L., and Nestler, E.J. (1998). Regulation of cocaine reward by CREB. Science *282*, 2272–2275.

Chao, H.T., Zoghbi, H.Y., and Rosenmund, C. (2007). MeCP2 controls excitatory synaptic strength by regulating glutamatergic synapse number. Neuron *56*, 58–65.

Chawla, S., Hardingham, G.E., Quinn, D.R., and Bading, H. (1998). CBP: a signal-regulated transcriptional coactivator controlled by nuclear calcium and CaM kinase IV. Science *281*, 1505–1509.

Chen, W.G., Chang, Q., Lin, Y., Meissner, A., West, A.E., Griffith, E.C., Jaenisch, R., and Greenberg, M.E. (2003). Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science *302*, 885–889.

Chowdhury, S., Shepherd, J.D., Okuno, H., Lyford, G., Petralia, R.S., Plath, N., Kuhl, D., Huganir, R.L., and Worley, P.F. (2006). Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. Neuron *52*, 445–459.

Chrivia, J.C., Kwok, R.P., Lamb, N., Hagiwara, M., Montminy, M.R., and Goodman, R.H. (1993). Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature *365*, 855–859.

Cole, A.J., Saffen, D.W., Baraban, J.M., and Worley, P.F. (1989). Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. Nature *340*, 474–476.

Curran, T., and Morgan, J.I. (1985). Superinduction of c-fos by nerve growth factor in the presence of peripherally active benzodiazepines. Science *229*, 1265–1268.

Dani, V.S., Chang, Q., Maffei, A., Turrigiano, G.G., Jaenisch, R., and Nelson, S.B. (2005). Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. Proc. Natl. Acad. Sci. USA *102*, 12560–12565.

Dash, P.K., Hochner, B., and Kandel, E.R. (1990). Injection of the cAMP-responsive element into the nucleus of Aplysia sensory neurons blocks long-term facilitation. Nature *345*, 718–721.

Davis, H.P., and Squire, L.R. (1984). Protein synthesis and memory: a review. Psychol. Bull. *96*, 518–559.

Dessaud, E., McMahon, A.P., and Briscoe, J. (2008). Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen-regulated transcriptional network. Development *135*, 2489–2503.

Dong, Y., Green, T., Saal, D., Marie, H., Neve, R., Nestler, E.J., and Malenka, R.C. (2006). CREB modulates excitability of nucleus accumbens neurons. Nat. Neurosci. *9*, 475–477.

Dudai, Y., Jan, Y.N., Byers, D., Quinn, W.G., and Benzer, S. (1976). dunce, a mutant of Drosophila deficient in learning. Proc. Natl. Acad. Sci. USA *73*, 1684–1688.

Ernfors, P., Bengzon, J., Kokaia, Z., Persson, H., and Lindvall, O. (1991). Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. Neuron *7*, 165–176.

Figurov, A., Pozzo-Miller, L.D., Olafsson, P., Wang, T., and Lu, B. (1996). Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature *381*, 706–709.

Flavell, S.W., Cowan, C.W., Kim, T.K., Greer, P.L., Lin, Y., Paradis, S., Griffith, E.C., Hu, L.S., Chen, C., and Greenberg, M.E. (2006). Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. Science *311*, 1008–1012.

Frey, U., Huang, Y.Y., and Kandel, E.R. (1993). Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. Science *260*, 1661–1664.

Fyffe, S.L., Neul, J.L., Samaco, R.C., Chao, H.T., Ben-Shachar, S., Moretti, P., McGill, B.E., Goulding, E.H., Sullivan, E., Tecott, L.H., and Zoghbi, H.Y. (2008). Deletion of Mecp2 in Sim1-expressing neurons reveals a critical role for MeCP2 in feeding behavior, aggression, and the response to stress. Neuron *59*, 947–958.

Ginty, D.D., Kornhauser, J.M., Thompson, M.A., Bading, H., Mayo, K.E., Takahashi, J.S., and Greenberg, M.E. (1993). Regulation of CREB phosphorylation in the suprachiasmatic nucleus by light and a circadian clock. Science *260*, 238–241.

Gonzalez, G.A., and Montminy, M.R. (1989). Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell *59*, 675–680.

Greenberg, M.E., and Ziff, E.B. (1984). Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. Nature *311*, 433–438.

Greenberg, M.E., Greene, L.A., and Ziff, E.B. (1985). Nerve growth factor and epidermal growth factor induce rapid transient changes in proto-oncogene transcription in PC12 cells. J. Biol. Chem. *260*, 14101–14110.

Greenberg, M.E., Ziff, E.B., and Greene, L.A. (1986). Stimulation of neuronal acetylcholine receptors induces rapid gene transcription. Science *234*, 80–83.

Guitart, X., Thompson, M.A., Mirante, C.K., Greenberg, M.E., and Nestler, E.J. (1992). Regulation of cyclic AMP response element-binding protein (CREB) phosphorylation by acute and chronic morphine in the rat locus coeruleus. J. Neurochem. *58*, 1168–1171.

Hattori, D., Millard, S.S., Wojtowicz, W.M., and Zipursky, S.L. (2008). Dscam-mediated cell recognition regulates neural circuit formation. Annu. Rev. Cell Dev. Biol. *24*, 597–620.

Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S.M., and Grunstein, M. (1995). Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. Cell *80*, 583–592.

Hu, S.C., Chrivia, J., and Ghosh, A. (1999). Regulation of CBP-mediated transcription by neuronal calcium signaling. Neuron *22*, 799–808.

Ince-Dunn, G., Hall, B.J., Hu, S.C., Ripley, B., Huganir, R.L., Olson, J.M., Tapscott, S.J., and Ghosh, A. (2006). Regulation of thalamocortical patterning and synaptic maturation by NeuroD2. Neuron *49*, 683–695.

Isackson, P.J., Huntsman, M.M., Murray, K.D., and Gall, C.M. (1991). BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: temporal patterns of induction distinct from NGF. Neuron *6*, 937–948.

Kang, H., and Schuman, E.M. (1995). Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science *267*, 1658–1662.

Kang, H., and Schuman, E.M. (1996). A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. Science *273*, 1402–1406.

Kashani, A.H., Qiu, Z., Jurata, L., Lee, S.K., Pfaff, S., Goebbels, S., Nave, K.A., and Ghosh, A. (2006). Calcium activation of the LMO4 transcription complex and its role in the patterning of thalamocortical connections. J. Neurosci. *26*, 8398–8408.

Klein, M.E., Impey, S., and Goodman, R.H. (2005). Role reversal: the regulation of neuronal gene expression by microRNAs. Curr. Opin. Neurobiol. *15*, 507–513.

Klein, M.E., Lioy, D.T., Ma, L., Impey, S., Mandel, G., and Goodman, R.H. (2007). Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. Nat. Neurosci. *10*, 1513–1514.

Korzus, E., Rosenfeld, M.G., and Mayford, M. (2004). CBP histone acetyltransferase activity is a critical component of memory consolidation. Neuron *42*, 961–972.

Kwok, R.P., Lundblad, J.R., Chrivia, J.C., Richards, J.P., Bachinger, H.P., Brennan, R.G., Roberts, S.G., Green, M.R., and Goodman, R.H. (1994). Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature *370*, 223–226.

Lee, R., Feinbaum, R., and Ambros, V. (2004). A short history of a short RNA. Cell *S116*, S89–S92.

Leifer, D., Krainc, D., Yu, Y.T., McDermott, J., Breitbart, R.E., Heng, J., Neve, R.L., Kosofsky, B., Nadal-Ginard, B., and Lipton, S.A. (1993). MEF2C, a MADS/MEF2-family transcription factor expressed in a laminar distribution in cerebral cortex. Proc. Natl. Acad. Sci. USA *90*, 1546–1550.

Leung, K.M., van Horck, F.P., Lin, A.C., Allison, R., Standart, N., and Holt, C.E. (2006). Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1. Nat. Neurosci. *9*, 1247–1256.

Lohof, A.M., Ip, N.Y., and Poo, M.M. (1993). Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. Nature *363*, 350–353.

Lyford, G.L., Yamagata, K., Kaufmann, W.E., Barnes, C.A., Sanders, L.K., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Lanahan, A.A., and Worley, P.F. (1995). Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron *14*, 433–445.

Mao, Z., Bonni, A., Xia, F., Nadal-Vicens, M., and Greenberg, M.E. (1999). Neuronal activity-dependent cell survival mediated by transcription factor MEF2. Science *286*, 785–790.

Marie, H., Morishita, W., Yu, X., Calakos, N., and Malenka, R.C. (2005). Generation of silent synapses by acute in vivo expression of CaMKIV and CREB. Neuron *45*, 741–752.

Martinowich, K., Hattori, D., Wu, H., Fouse, S., He, F., Hu, Y., Fan, G., and Sun, Y.E. (2003). DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science *302*, 890–893.

Montminy, M.R., Low, M.J., Tapia-Arancibia, L., Reichlin, S., Mandel, G., and Goodman, R.H. (1986a). Cyclic AMP regulates somatostatin mRNA accumulation in primary diencephalic cultures and in transfected fibroblast cells. J. Neurosci. *6*, 1171–1176.

Montminy, M.R., Sevarino, K.A., Wagner, J.A., Mandel, G., and Goodman, R.H. (1986b). Identification of a cyclic-AMP-responsive element within the rat somatostatin gene. Proc. Natl. Acad. Sci. USA *83*, 6682–6686.

Montminy, M.R., and Bilezikjian, L.M. (1987). Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. Nature *328*, 175–178.

Morgan, J.I., Cohen, D.R., Hempstead, J.L., and Curran, T. (1987). Mapping patterns of c-fos expression in the central nervous system after seizure. Science *237*, 192–197.

Nan, X., Campoy, F.J., and Bird, A. (1997). MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell *88*, 471–481.

Nedivi, E., Hevroni, D., Naot, D., Israeli, D., and Citri, Y. (1993). Numerous candidate plasticity-related genes revealed by differential cDNA cloning. Nature *363*, 718–722.

Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B.H., and Nakatani, Y. (1996). The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell *87*, 953–959.

Qiu, Z., and Ghosh, A. (2008). A calcium-dependent switch in CREST-BRG1 complex regulates activity-dependent gene expression. Neuron, in press.

Redmond, L., Kashani, A.H., and Ghosh, A. (2002). Calcium regulation of dendritic growth via CaM kinase IV and CREB-mediated transcription. Neuron *34*, 999–1010.

Rial Verde, E.M., Lee-Osbourne, J., Worley, P.F., Malinow, R., and Cline, H.T. (2006). Increased expression of the immediate-early gene arc/arg3.1 reduces AMPA receptor-mediated synaptic transmission. Neuron *52*, 461–474.

Riccio, A., Pierchala, B.A., Ciarallo, C.L., and Ginty, D.D. (1997). An NGF-TrkA-mediated retrograde signal to transcription factor CREB in sympathetic neurons. Science *277*, 1097–1100.

Riccio, A., Ahn, S., Davenport, C.M., Blendy, J.A., and Ginty, D.D. (1999). Mediation by a CREB family transcription factor of NGF-dependent survival of sympathetic neurons. Science *286*, 2358–2361.

Ruvkun, G., Wightman, B., and Ha, I. (2004). The 20 years it took to recognize the importance of tiny RNAs. Cell *S116*, S93–S96.

Schratt, G.M., Tuebing, F., Nigh, E.A., Kane, C.G., Sabatini, M.E., Kiebler, M., and Greenberg, M.E. (2006). A brain-specific microRNA regulates dendritic spine development. Nature *439*, 283–289.

Schmucker, D. (2007). Molecular diversity of Dscam: recognition of molecular identity in neuronal wiring. Nat. Rev. Neurosci. *8*, 915–920.

Schmucker, D., Clemens, J.C., Shu, H., Worby, C.A., Xiao, J., Muda, M., Dixon, J.E., and Zipursky, S.L. (2000). *Drosophila* Dscam is an axon guidance receptor exhibiting extraordinary molecular diversity. Cell *101*, 671–684.

Shalizi, A., Gaudilliere, B., Yuan, Z., Stegmuller, J., Shirogane, T., Ge, Q., Tan, Y., Schulman, B., Harper, J.W., and Bonni, A. (2006). A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation. Science *311*, 1012–1017.

Sheng, M., Thompson, M.A., and Greenberg, M.E. (1991). CREB: a Ca(2+)-regulated transcription factor phosphorylated by calmodulin-dependent kinases. Science *252*, 1427–1430.

Shepherd, J.D., Rumbaugh, G., Wu, J., Chowdhury, S., Plath, N., Kuhl, D., Huganir, R.L., and Worley, P.F. (2006). Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors. Neuron *52*, 475–484.

Shieh, P.B., Hu, S.C., Bobb, K., Timmusk, T., and Ghosh, A. (1998). Identification of a signaling pathway involved in calcium regulation of BDNF expression. Neuron *20*, 727–740.

Si, K., Giustetto, M., Etkin, A., Hsu, R., Janisiewicz, A.M., Miniaci, M.C., Kim, J.H., Zhu, H., and Kandel, E.R. (2003). A neuronal isoform of CPEB regulates local protein synthesis and stabilizes synapse-specific long-term facilitation in aplysia. Cell *115*, 893–904.

Steward, O., and Levy, W.B. (1982). Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. J. Neurosci. *2*, 284–291.

Steward, O., Wallace, C.S., Lyford, G.L., and Worley, P.F. (1998). Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. Neuron *21*, 741–751.

Sutton, M.A., Wall, N.R., Aakalu, G.N., and Schuman, E.M. (2004). Regulation of dendritic protein synthesis by miniature synaptic events. Science *304*, 1979–1983.

Tanabe, Y., and Jessell, T.M. (1996). Diversity and pattern in the developing spinal cord. Science *274*, 1115–1123.

Tao, X., Finkbeiner, S., Arnold, D.B., Shaywitz, A.J., and Greenberg, M.E. (1998). Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron *20*, 709–726.

Taunton, J., Hassig, C.A., and Schreiber, S.L. (1996). A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science *272*, 408–411.

Vo, N., Klein, M.E., Varlamova, O., Keller, D.M., Yamamoto, T., Goodman, R.H., and Impey, S. (2005). A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. Proc. Natl. Acad. Sci. USA *102*, 16426–16431.

Wang, X., Weiner, J.A., Levi, S., Craig, A.M., Bradley, A., and Sanes, J.R. (2002). Gamma protocadherins are required for survival of spinal interneurons. Neuron *36*, 843–854.

Weiner, J.A., Wang, X., Tapia, J.C., and Sanes, J.R. (2005). Gamma protocadherins are required for synaptic development in the spinal cord. Proc. Natl. Acad. Sci. USA *102*, 8–14.

Wojtowicz, W.M., Flanagan, J.J., Millard, S.S., Zipursky, S.L., and Clemens, J.C. (2004). Alternative splicing of *Drosophila* Dscam generates axon guidance receptors that exhibit isoform-specific homophilic binding. Cell *118*, 619–633.

Wu, Q., and Maniatis, T. (1999). A striking organization of a large family of human neural cadherin-like cell adhesion genes. Cell *97*, 779–790.

Wu, L., Wells, D., Tay, J., Mendis, D., Abbott, M.A., Barnitt, A., Quinlan, E., Heynen, A., Fallon, J.R., and Richter, J.D. (1998). CPEB-mediated cytoplasmic polyadenylation and the regulation of experience-dependent translation of alpha-CaMKII mRNA at synapses. Neuron *21*, 1129–1139.

Wu, J.I., Lessard, J., Olave, I.A., Qiu, Z., Ghosh, A., Graef, I.A., and Crabtree, G.R. (2007). Regulation of dendritic development by neuron-specific chromatin remodeling complexes. Neuron *56*, 94–108.

Yin, J.C., Wallach, J.S., Del Vecchio, M., Wilder, E.L., Zhou, H., Quinn, W.G., and Tully, T. (1994). Induction of a dominant negative CREB transgene specifically blocks long-term memory in *Drosophila*. Cell *79*, 49–58.