OM&P

Epigenetic Programming of Neural Circuits in the Brain

*Yue Yang and Azad Bonni**

Department of Neuroscience, Washington University School of Medicine, St. Louis, MO 63110, USA.
* Corresponding e-mail: bonni@wustl.edu

Abstract. The assembly of neural circuits during development endows the brain with the ability to perceive the environment, control motor output, and perform higher cognitive functions. Failure to assemble proper neural circuits may result in neurodevelopmental disorders including intellectual disability and autism spectrum disorders. Epigenetic mechanisms, and in particular chromatin remodeling, are potent regulators of neuronal connectivity. Here, we review recent studies highlighting the essential role of the ATP- dependent nucleosomal remodeling and deacetylase (NuRD) complex in epigenetic programming of neurons to drive neural circuit assembly and organism behavior.

Introduction

How are newly-born neurons programmed to assemble into circuits endowing organisms with complex behavior? Neuronal integration into early networks is thought to be largely genetically hardwired. Large numbers of genes turn on and off in temporally and spatially precise patterns in the developing mammalian brain (Hanchate et al., 2015; Telley et al., 2016) to create an intricate program of neuronal differentiation, from neuronal polarization to synaptogenesis and refinement (de la Torre-Ubieta and Bonni, 2011).

Besides transcription factors, chromatin enzymes are powerful regulators of gene expression (Ho and Crabtree, 2010; Narlikar et al., 2013). Chromatin enzymes interact with and modify nucleosomes, which organize genomic DNA to fit into a cell nucleus. Each nucleosome is comprised of a histone octamer and can be remodeled or covalently modified at histone tails to control gene expression. Nucleosome remodeling encompasses a wide range of activities including alterations of positioning of nucleosomes relative to each other and relationship of DNA to histone proteins as well as the exchange of histone subunit variants at promoters, enhancers, and gene bodies (Maze et al., 2014; Rhee et al., 2014; Weber and Henikoff, 2014).

The mechanisms and functions of epigenetic control of neural circuit assembly and coding are just beginning to be understood. In two recent studies, Yamada et al. and Yang et al. have discovered chromatin mechanisms that are critical for the assembly and refinement of neural circuits and hence learning and memory in the brain (Yamada et al., 2014; Yang et al., 2016).

The NuRD Complex Deploys Distinct Chromatin Mechanisms to Regulate Gene Expression

Among chromatin enzymes, the nucleosome remodeling and deacetylase (NuRD) complex uniquely harbors two enzymatic activities in vitro, the removal of acetyl groups from histones at lysine residues and remodeling of nucleosomes in an ATP-dependent manner (Xue et al., 1998). However, the *in vivo* function of the NuRD complex at the tens of thousands of genes encoded in the genome has remained a mystery.

Because the NuRD complex is highly expressed in the brain (Yamada et al., 2014), Yang et al. characterized the occupancy of the core NuRD subunit Chd4 across the genome in the mouse cerebellum (Yang et al., 2016). The NuRD complex occupies 25,000 genomic loci representing promoters and enhancers of actively transcribed genes in the brain (Yang et al., 2016). Changes in the epigenome and transcriptome have been profiled upon knockout of Chd4 in the cerebellum of mice using 72 datasets generated in these studies. These datasets include chromatin immunoprecipitation followed by massive parallel sequencing (ChIP-Seq) of histone modifications, histone proteins, and chromatin enzymes, genome-wide profiling of transcripts (RNA-Seq), and purification of translating mRNAs from synchronously developing neurons (Sync-TRAP-Seq) (Yamada et al., 2014; Yang et al., 2016).

Strikingly, intersection of these datasets has revealed that the NuRD complex regulates two distinct classes of genes via different chromatin mechanisms. The NuRD complex decommissions promoters and enhancers of a small group of ~200 developmentally regulated genes by removing the active histone modifications H3 lysine 9, 14, and 27 acetylation (Yamada et al., 2014; Yang et al., 2016). These histone tail alterations lead to the chronic silencing of developmentally regulated genes, including the transcription factor Nhlh1 and RNA-binding protein Elavl2 in the cerebellum (Figure 1, panel A, right). These findings are consistent with the functions of the NuRD complex in other developmental roles in mammals including the control of embryonic stem (ES) cell differentiation, somatic cell reprogramming, and immune cell fate specification (Fujita et al., 2004; Kaji et al., 2006; Rais et al., 2013; Whyte et al., 2012; Yildirim et al., 2011; Yoshida et al., 2008).

On a much larger group of over 1000 genes, the NuRD complex deposits the histone variant H2A.z at promoters, thus altering the composition of nucleosomes (Yang et al., 2016). However, the NuRD complex fails to alter histone modifications at these genes. This group of NuRD-regulated genes is enriched for stimuli-responsive genes that are expressed in the developing and adult cerebellum. When neurons are stimulated, H2A.z is loaded at the promoters of neuronal activity-dependent genes after cessation of stimulation. The NuRD complex

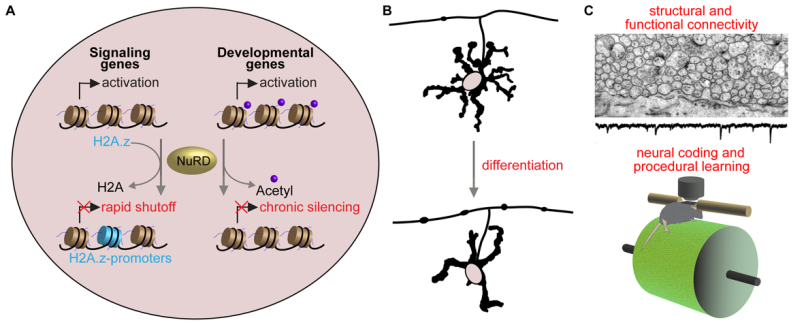

Figure 1. *(A) The NuRD complex deploys distinct epigenetic mechanisms to inhibit gene expression in the brain in vivo. (B) Epigenetic silencing of gene expression drives granule neuron dendrite pruning and presynaptic differentiation. (C) Proper structural and functional connectivity is necessary for sensorimotor coding in the cerebellum and procedural learning in mice.*

specifically deposits H2A.z at promoters upon stimuli shutoff to rapidly inactivate activity-dependent genes (Figure 1, panel A, left). Notably, H2A.z eviction at immediate early gene promoters has also been observed in response to sensory experience in the hippocampus (Zovkic et al., 2014). Although the mechanisms that mediate the induction of activity-dependent genes have been the subject of intense scrutiny (Ebert et al., 2013; Madabhushi et al., 2015; Qiu and Ghosh, 2008; West and Greenberg, 2011), the molecular underpinnings of the shutoff of activity-dependent transcription had remained largely unexplored. The elucidation of the NuRD/H2A.z chromatin remodeling pathway suggests that epigenetic mechanisms play an active role in the shutoff of gene expression in the brain following neuronal activity and that shutoff is not simply achieved by turning off transcriptional activation mechanisms. It will be important in the future to determine the role of the NuRD complex in shutoff of actively transcribed genes in response to other extrinsic cues besides neuronal activity in the brain as well as outside the nervous system.

The identification of a novel link between the NuRD complex and histone variant H2A.z sheds light on the roles and mechanisms of these two major epigenetic factors. An important question that remains to be addressed is how the NuRD complex deposits H2A.z at the promoters of actively transcribed genes in the brain. The SWR and INO80 chromatin remodeling enzymes have been implicated in the deposition and eviction of H2A.z in chromatin in yeast and embryonic stem cells (Mizuguchi et al., 2004; Papamichos-Chronakis et al., 2011; Weber and Henikoff, 2014), though their roles in the brain remain poorly understood. It will be interesting to assess whether the NuRD complex coordinates its H2A.z depositing activity with the SWR or INO80 complex in the control of signal-dependent transcription.

The NuRD Complex Controls Neural Coding and Procedural Learning

NuRD repression of genes implicated in neuronal signaling and development suggests that this chromatin remodeling complex may shape neural circuit architecture. Granule neurons of the rodent cerebellum represent an ideal system for the study of neuronal integration into circuits (Kim et al., 2009; Konishi et al., 2004; Shalizi et al., 2006; Yang et al., 2009). Newly born granule neurons transiently form exuberant dendrites and then prune them until ~4 dendrites remain (Yang et al., 2016). At the other end of granule neurons, presynaptic boutons form along axons throughout development (Yamada et al., 2014). Conditional knockout of the NuRD complex in granule neurons impairs presynaptic bouton formation as well as elimination of excess dendrites (Figure 1, panel B) (Yamada et al., 2014; Yang et al., 2016). An RNA interference (RNAi) screen identified the NuRD developmentally-silenced genes Nhlh1 and Elavl2 as playing a critical role downstream of the NuRD complex in the control of presynaptic differentiation. By contrast, expression of the NuRD-regulated activity-dependent genes c-Fos, Fosl2, and Nr4a1 phenocopies the NuRD knockout-dependent dendrite pruning phenotype. Thus, the NuRD complex controls the structural maturation of granule neuron afferent and efferent connections via activity-dependent genes and developmentally-regulated genes, respectively.

Granule neurons receive sensorimotor inputs from mossy fibers and relay this neural code to Purkinje cells. Mature granule neurons have 4 dendrites, which is optimal for lossless, sparse encoding of sensorimotor information (Billings et al., 2014). To determine how NuRD control of dendrite pruning impacts the neural coding of granule neurons, a large population of over 700 granule neurons were subjected to *in vivo* two-photon calcium imaging in awake, behaving mice. Remarkably, upon Chd4 knockout, an increased number of granule neurons are responsive to sensorimotor stimuli (Figure 1, panel C) (Yang et al., 2016). These findings suggest that the NuRD complex ensures sparse coding in the cerebellum. In other experiments, Chd4 conditional knockout mice also have reduced synaptic neurotransmission between granule neurons and Purkinje cells, consistent with the decrease in presynaptic boutons along granule neuron axons (Figure 1, panel C) (Yamada et al., 2014). Together, these studies reveal that the NuRD complex drives granule neuron maturation and proper integration into neural circuits in the developing cerebellum.

How do these impairments in cerebellar circuitry

 doi:10.20388/omp2016.003.0037

impact organism behavior? The cerebellum performs computations necessary for motor coordination and subconscious, "procedural" learning such as playing a piano. Strikingly, Chd4 conditional knockout mice have profound defects in procedural learning tasks including delay eyeblink conditioning, a classic associative learning paradigm, and the accelerating rotarod assay, a test of motor skill learning (Yang et al., 2016). These deficits are specific to learning and memory, because Chd4 conditional knockout mice have little or no effect on motor coordination (Yang et al., 2016).

These studies have uncovered novel epigenetic mechanisms that mediate the rapid shutoff of activity genes and the chronic silencing of developmental genes in the brain to thereby control neural circuit architecture and procedural learning in mice (Figure 1). These findings bear significant implications for our understanding of the role of gene regulation in brain circuitry. Although it has been long appreciated that activity-induced genes undergo rapid and robust shutoff, the biological consequences of the shutoff of activity-dependent genes remained unknown. Studies of the NuRD chromatin remodeling complex suggest that the epigenetic-dependent shutoff of activity-dependent transcription is essential for the maturation of granule neuron dendrite arbors in the developing cerebellum and consequently in the control of neural circuit activity in response to sensorimotor signals. It will be interesting in future studies to determine the role of shutoff of activity-dependent transcription in circuit assembly and function in other regions of the developing brain as well as in neuronal plasticity in the adult brain.

Deregulation of chromatin remodeling enzyme function may contribute to the pathogenesis of diverse diseases including neurodevelopmental disorders of cognition and epilepsy in the nervous system (Neale et al., 2012; O'Roak et al., 2012; Ronan et al., 2013; Talkowski et al., 2012). Mutations of NuRD complex subunits have been found in intellectual disability and autism (Cukier et al., 2010; Hamdan et al., 2014; Weiss et al., 2016; Willemsen et al., 2013). It will be interesting in future studies to determine whether impairment of NuRD-dependent shutoff of activity genes and chronic silencing of developmental genes and consequent deregulation of circuit architecture and sensorimotor encoding influences the pathogenesis of brain disorders of cognition.

Acknowledgements

Supported by NIH grant NS041021 (A.B.) and the Mathers Foundation (A.B.).

References

Billings, G., Piasini, E., Lorincz, A., Nusser, Z., and Silver, R.A. (2014). Network structure within the cerebellar input layer enables lossless sparse encoding. Neuron 83, 960-974.

Cukier, H.N., Rabionet, R., Konidari, I., Rayner-Evans, M.Y., Baltos, M.L., Wright, H.H., Abramson, R.K., Martin, E.R., Cuccaro, M.L., Pericak-Vance, M.A., et al. (2010). Novel variants identified in methyl-CpG-binding domain genes in autistic individuals. Neurogenetics 11, 291-303.

De la Torre-Ubieta, L., and Bonni, A. (2011). Transcriptional regulation of neuronal polarity and morphogenesis in the mammalian brain. Neuron 72, 22-40.

Ebert, D.H., Gabel, H.W., Robinson, N.D., Kastan, N.R., Hu, L.S., Cohen, S., Navarro, A.J., Lyst, M.J., Ekiert, R., Bird, A.P., et al. (2013). Activity-dependent phosphorylation of MeCP2 threonine 308 regulates interaction with NCoR. Nature 499, 341-345.

Fujita, N., Jaye, D.L., Geigerman, C., Akyildiz, A., Mooney, M.R., Boss, J.M., and Wade, P.A. (2004). MTA3 and the Mi-2/NuRD complex regulate cell fate during B lymphocyte differentiation. Cell 119, 75-86.

Hamdan, F.F., Srour, M., Capo-Chichi, J.M., Daoud, H., Nassif, C., Patry, L., Massicotte, C., Ambalavanan, A., Spiegelman, D., Diallo, O., et al. (2014). *De novo* mutations in moderate or severe intellectual disability. PLoS Genet 10, e1004772.

Hanchate, N.K., Kondoh, K., Lu, Z., Kuang, D., Ye, X., Qiu, X., Pachter, L., Trapnell, C., and Buck, L.B. (2015). Single-cell transcriptomics reveals receptor transformations during olfactory neurogenesis. Science 350, 1251-1255.

Ho, L., and Crabtree, G.R. (2010). Chromatin remodelling during development. Nature 463, 474-484.

Kaji, K., Caballero, I.M., MacLeod, R., Nichols, J., Wilson, V.A., and Hendrich, B. (2006). The NuRD component Mbd3 is required for pluripotency of embryonic stem cells. Nat Cell Biol 8, 285-292.

Kim, A.H., Puram, S.V., Bilimoria, P.M., Ikeuchi, Y., Keough, S., Wong, M., Rowitch, D., and Bonni, A. (2009). A centrosomal Cdc20-APC pathway controls dendrite morphogenesis in postmitotic neurons. Cell 136, 322-336.

Konishi, Y., Stegmuller, J., Matsuda, T., Bonni, S., and Bonni, A. (2004). Cdh1-APC controls axonal growth and patterning in the mammalian brain. Science 303, 1026-1030.

Madabhushi, R., Gao, F., Pfenning, A.R., Pan, L., Yamakawa, S., Seo, J., Rueda, R., Phan, T.X., Yamakawa, H., Pao, P.C., et al. (2015). Activity-Induced DNA Breaks Govern the Expression of Neuronal Early-Response Genes. Cell 161, 1592-1605.

Maze, I., Noh, K.M., Soshnev, A.A., and Allis, C.D. (2014). Every amino acid matters: essential contributions of histone variants to mammalian development and disease. Nat Rev Genet 15, 259-271.

Mizuguchi, G., Shen, X., Landry, J., Wu, W.H., Sen, S., and Wu, C. (2004). ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science 303, 343-348.

Narlikar, G.J., Sundaramoorthy, R., and Owen-Hughes, T. (2013). Mechanisms and functions of ATP-dependent chromatin-remodeling enzymes. Cell 154, 490-503.

Neale, B.M., Kou, Y., Liu, L., Ma'ayan, A., Samocha, K.E., Sabo, A., Lin, C.F., Stevens, C., Wang, L.S., Makarov, V., et al. (2012). Patterns and rates of exonic *de novo* mutations in autism spectrum disorders. Nature 485, 242-245.

O'Roak, B.J., Vives, L., Fu, W., Egertson, J.D., Stanaway, I.B., Phelps, I.G., Carvill, G., Kumar, A., Lee, C., Ankenman, K., et al. (2012). Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science 338, 1619- 1622.

Papamichos-Chronakis, M., Watanabe, S., Rando, O.J., and Peterson, C.L. (2011). Global regulation of H2A.Z localization by the INO80 chromatin-remodeling enzyme is essential for genome integrity. Cell 144, 200-213.

Qiu, Z., and Ghosh, A. (2008). A brief history of neuronal gene expression: regulatory mechanisms and cellular consequences. Neuron 60, 449-455.

Rais, Y., Zviran, A., Geula, S., Gafni, O., Chomsky, E., Viukov, S., Mansour, A.A., Caspi, I., Krupalnik, V., Zerbib, M., et al. (2013). Deterministic direct reprogramming of somatic cells to pluripotency. Nature 502, 65-70.

Rhee, H.S., Bataille, A.R., Zhang, L., and Pugh, B.F. (2014). Subnucleosomal structures and nucleosome asymmetry across a genome. Cell 159, 1377-1388.

Ronan, J.L., Wu, W., and Crabtree, G.R. (2013). From neural development to cognition: unexpected roles for chromatin. Nat Rev Genet 14, 347-359.

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.

Talkowski, M.E., Rosenfeld, J.A., Blumenthal, I., Pillalamarri, V., Chiang, C., Heilbut, A., Ernst, C., Hanscom, C., Rossin, E., Lindgren, A.M., et al. (2012). Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. Cell 149, 525-537.

Telley, L., Govindan, S., Prados, J., Stevant, I., Nef, S., Dermitzakis, E., Dayer, A., and Jabaudon, D. (2016). Sequential transcriptional waves direct the differentiation of newborn neurons in the mouse neocortex. Science 351, 1443-1446.

Weber, C.M., and Henikoff, S. (2014). Histone variants: dynamic punctuation in transcription. Genes Dev 28, 672-682.

Weiss, K., Terhal, P.A., Cohen, L., Bruccoleri, M., Irving, M., Martinez, A.F., Rosenfeld, J.A., Machol, K., Yang, Y., Liu, P., et al. (2016). *De Novo* Mutations in CHD4, an ATPDependent Chromatin Remodeler Gene, Cause an Intellectual Disability Syndrome with Distinctive Dysmorphisms. Am J Hum Genet 99, 934-941.

West, A.E., and Greenberg, M.E. (2011). Neuronal activity-regulated gene transcription in synapse development and cognitive function. Cold Spring Harb Perspect Biol 3.

Whyte, W.A., Bilodeau, S., Orlando, D.A., Hoke, H.A., Frampton, G.M., Foster, C.T., Cowley, S.M., and Young, R.A. (2012). Enhancer decommissioning by LSD1 during embryonic stem cell differentiation. Nature 482, 221-225.

Willemsen, M.H., Nijhof, B., Fenckova, M., Nillesen, W.M., Bongers, E.M., Castells- Nobau, A., Asztalos, L., Viragh, E., van Bon, B.W., Tezel, E., et al. (2013). GATAD2B loss-of-function mutations cause a recognisable syndrome with intellectual disability and are associated with learning deficits and synaptic undergrowth in Drosophila. J Med Genet 50, 507-514.

Xue, Y., Wong, J., Moreno, G.T., Young, M.K., Cote, J., and Wang, W. (1998). NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. Mol Cell 2, 851-861.

Yamada, T., Yang, Y., Hemberg, M., Yoshida, T., Cho, H.Y., Murphy, J.P., Fioravante, D., Regehr, W.G., Gygi, S.P., Georgopoulos, K., et al. (2014). Promoter decommissioning by the NuRD chromatin remodeling complex triggers synaptic connectivity in the mammalian brain. Neuron 83, 122-134.

Yang, Y., Kim, A.H., Yamada, T., Wu, B., Bilimoria, P.M., Ikeuchi, Y., de la Iglesia, N., Shen, J., and Bonni, A. (2009). A Cdc20-APC ubiquitin signaling pathway regulates presynaptic differentiation. Science 326, 575-578.

Yang, Y., Yamada, T., Hill, K.K., Hemberg, M., Reddy, N.C., Cho, H.Y., Guthrie, A.N., Oldenborg, A., Heiney, S.A., Ohmae, S., et al. (2016). Chromatin remodeling inactivates activity genes and regulates neural coding. Science 353, 300-305.

Yildirim, O., Li, R., Hung, J.H., Chen, P.B., Dong, X., Ee, L.S., Weng, Z., Rando, O.J., and Fazzio, T.G. (2011). Mbd3/NURD complex regulates expression of 5-hydroxymethylcytosine marked genes in embryonic stem cells. Cell 147, 1498-1510.

Yoshida, T., Hazan, I., Zhang, J., Ng, S.Y., Naito, T., Snippert, H.J., Heller, E.J., Qi, X., Lawton, L.N., Williams, C.J., et al. (2008). The role of the chromatin remodeler Mi-2beta in hematopoietic stem cell self-renewal and multilineage differentiation. Genes Dev 22, 1174-1189.

Zovkic, I.B., Paulukaitis, B.S., Day, J.J., Etikala, D.M., and Sweatt, J.D. (2014). Histone H2A.Z subunit exchange controls consolidation of recent and remote memory. Nature 515, 582-586.