Published in final edited form as:

Sci Signal. ; 3(125): pe20. doi:10.1126/scisignal.3125pe20.

Eradicating the Mediators of Neuronal Death with a Fine-Tooth Comb

R. Suzanne Zukin

Department of Neuroscience, Rose F. Kennedy Center, Room 610, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA. suzanne.zukin@einstein.yu.edu

Abstract

Ischemic tolerance is an evolutionarily conserved form of cerebral plasticity in which a brief period of cerebral ischemia (called ischemic preconditioning) confers transient tolerance to a subsequent ischemic challenge in the brain. Polycomb group proteins are gene-silencing factors that are abundant and widely distributed during embryogenesis and are essential to epigenetic cellular memory, pluripotency, and stem cell self-renewal. New insight into the molecular mechanisms underlying ischemic tolerance is highlighted by the finding that ischemic preconditioning activates polycomb proteins in mature neurons. Polycomb proteins act through epigenetic gene silencing to eradicate potential mediators of neuronal death and promote cellular arrest, enabling mature neurons to survive ischemic stroke.

In ischemic tolerance, a brief period of cerebral ischemia (called ischemic preconditioning) confers transient tolerance to a subsequent ischemic challenge to the brain (1–4). Tolerance is an evolutionarily conserved form of cerebral plasticity that occurs in invertebrates and vertebrates, including humans, and can be induced by diverse triggers such as brief ischemic insults, hypoxia, cortical spreading depression, and seizures. Ischemic preconditioning has attracted intense interest for decades in the hopes that it might offer insights into molecular mechanisms underlying endogenous neuroprotection and accelerate the development of therapeutic approaches for stroke. A paper by Zhou, Simon, and colleagues (5) provides new insight into how neurons acquire ischemic tolerance. This work casts light on an emerging role for polycomb group proteins and epigenetic gene silencing in brain function and neuronal survival and on the potential for epigenetic dysregulation to contribute to neurologic disorders and disease.

Polycomb group (PcG) proteins form large complexes of gene-silencing factors that are broadly distributed during embryogenesis and play a strategic role in patterning and differentiation (6–8). First discovered for their role in specifying the proper spatial expression of homeotic genes in *Drosophila*, PcG proteins serve as global enforcers of epigenetically repressed states. In pluripotent stem cells, PcG proteins actively repress genes important to embryonic development and cell fate decisions. Unbiased epigenome-wide analysis has identified >500 putative PcG targets (9).

A fundamental mechanism by which PcG proteins orchestrate gene silencing is through epigenetic remodeling of target genes (6–8). Epigenetic modifications, such as DNA methylation or histone modifications, reflect environmental influences that are not “hard-wired” into the DNA sequence (10–12). This phenomenon is critical to transcriptional regulation during brain development, tissue-specific gene expression, and global gene silencing. Changes in the epigenome have been postulated to be an underlying mechanism for long-lasting transcriptional changes in adult neurons. Epigenetic dysregulation has been implicated in the progressive neurodegeneration associated with neuropsychiatric disorders and diseases (13).

Polycomb proteins form large several-multimeric complexes known as polycomb repressive complex 1 (PRC1) and PRC2 (Fig. 1) (6–8). These complexes are recruited to repressed regions of the genome, where they posttranslationally modify histone proteins and silence target genes by altering local, higher-order chromatin structure (6). PRC2 is involved in the initiation of gene repression and contains four core proteins: the catalytic subunit enhancer of zeste homolog 1 or 2 (EZH1 or 2) [mammalian homologs of *Drosophila* E(z)], and three noncatalytic subunits: suppressor of zeste 12 [SU(Z)12, the mammalian homolog of *Drosophila* Suz12]; embryonic ectoderm development (EED; the homolog of *Drosophila* extra sex combs (Esc)); and retinoblastoma binding protein p46 or p48 (RbAp46 or RbAp48; homologs of *Drosophila* nucleosome remodeling factor 55 [NURF55; also known as p55 or chromatin assembly factor (CAF1)]. The catalytically active component of PRC2, EZH1 or EZH2, is a histone methyltransferase that confers a trimethylation mark on Lys²⁷ of histone H3 (H3K27me3), a mark of gene repression (6–8). In addition to EZH1 or EZH2, SU(Z)12 and EED are also critical for histone methyltransferase activity (8). H3K27me3 is crucial to PcG-mediated transcriptional repression and to recruitment and stabilization of its partner PRC1 to the genome. The other components of the PRC2 complex stimulate EZH2 enzymatic activity and mediate binding of the complex to histones and nucleosomes (6–8).

The PRC1 complex recognizes the H3K27me3 mark conferred by PRC2 and also contains four core proteins: chromobox homolog 2 (CBX2), 4, or 8 [the mammalian homologs of *Drosophila* polycomb]; polyhomeotic-like protein (PHC) 1 (the mammalian homolog of *Drosophila* sex comb on midleg or polyhomeotic); BMI1, MEL18, or PcG ring finger protein (PCGF) (the mammalian homologs of posterior sex combs); and RING1A or RING1B (the mammalian homologs of dRING, which was named for its RING-type zinc finger) (6–8). PRC1 proteins contain chromodomains, which mediate binding to H3K27me3. Pc contains an extra chromodomain and binds with highest affinity to H3K27me3 (6–8). Recognition of H3K27me3 by PRC1 is thought to target, anchor, and stabilize PRC1 complexes to their specific loci and, more generally, to repressed regions of the genome (6–8). RING1A and RING1B are E3 ubiquitin ligases that monoubiquitinate histone H2A at Lys¹¹⁹ (H2AK119ub), an additional posttranslational modification that is critical to polycomb-dependent gene silencing. This modification, together with H3K27me3, is an epigenetic signature of polycomb-mediated gene silencing (6–8). A third polycomb repressive complex, pleiohomeotic repressive complex (PHORC), possesses sequence-specific DNA binding activity and is thought to mediate long-range interactions between locally bound PcG proteins and highly methylated regions of DNA in the flanking chromatin (6–8). PcG proteins are counterbalanced by trithorax group (TrxG) proteins, which maintain active gene expression patterns in the appropriate spatial domains. In the mammalian genome, TrxG proteins are often present together with PcG proteins at target loci, constituting what have been termed bivalent chromatin domains (14). In embryonic stem (ES) cells, bivalent chromatin domains, marked by overlapping repressive (H3K27me3) and activating (H3K4me3) histone modifications, are present at the promoters of more than 2000 genes (14).

Growing evidence suggests a role for polycomb proteins in cell survival. For example, the polycomb protein BMI1 is a cofactor for a E3 ubiquitin ligase that compacts polynucleosomes and promotes neural stem cell renewal and cell survival by repressing p16 and p19, cell cycle inhibitor genes (8). Furthermore, BMI1 protects epithelial cells against chemical stress-induced cell death by altering cell cycle regulatory protein abundance and inhibiting apoptosis (15) and boosts antioxidant defenses in neurons by repressing p53 pro-oxidant activity (16).

A prevailing view was that ischemic preconditioning affords protection by increasing the activity of endogenous pathways that increase resistance to injury (17). An earlier paper by

some of the same authors challenged this view (18). Genomic profiling showed that ischemic preconditioning, injurious ischemia, and preconditioning followed by injurious ischemia elicited unique patterns of alterations in gene expression, with little overlap among the three conditions. Whereas injurious ischemia in the absence of preconditioning increased the expression of genes involved in defense and repair, the ischemic-tolerant brain exhibited an overall pattern of gene repression (18), which was likened to mammalian hibernation, an evolutionarily conserved response to reduced availability of oxygen and nutrients (19, 20). During hibernation, cellular homeostasis is preserved in the face of oxygen deprivation through the controlled arrest of cellular functions or metabolic depression (tolerance of hypoxia). Examples include the fish that inhabit the hypoxic waters of the Amazon basin and Chinese bar-headed geese, which migrate over the summit of Mount Everest (>29,000 feet altitude) (20). An attractive scenario posited by the authors is that controlled cellular arrest associated with hibernation might underlie the neuroprotection afforded by ischemic tolerance.

The work of Stapels *et al.* (5) advances the previous work by providing the first direct link between PcG-mediated gene silencing and neuronal survival. Proteomics profiling and bioinformatics analysis revealed that the abundance of transcriptional repressors, particularly polycomb proteins, was increased in the ischemiatolerant brain. These included histone H2A and H2B variants and the polycomb proteins BMI1 and SCMH1 [the mammalian homolog of the *Drosophila* protein sex comb on midleg (21)]. The authors suggested that preconditioning epigenetically “reprograms” the ischemic-tolerant brain.

To determine whether the activity of PcG proteins was causally related to neuroprotection, the authors undertook a series of loss-of-function and gain-of-function strategies. Forced expression of the PcG proteins SCMH1 or BMI1 elicited tolerance to ischemia, even in the absence of ischemic preconditioning, and RNA interference (RNAi)-mediated depletion of PcG proteins prevented the induction of ischemic tolerance. Stapels *et al.* (5) also identified potential targets of PcG proteins critical to neuronal survival and showed that PcG proteins were recruited to the promoter regions of target genes, including those encoding two potassium channels whose abundance is decreased in ischemic tolerant brains. Expression of PcG proteins decreased the abundance of two potassium channels, and RNAi-mediated depletion of the potassium channels elicited tolerance in the absence of ischemic preconditioning. These findings were backed by physiological studies, which demonstrated attenuation of channel function in the tolerant brain (which may be similar to the channel arrest observed in hibernating organisms). Although illuminating, potassium channel genes may be just the tip of the iceberg, because additional polycomb targets remain unexplored. Future studies are warranted to pursue these targets in the hopes of identifying new effectors of neuroprotection.

Although the work of Stapels *et al.* (5) sheds light on how polycomb proteins co-ordinate intricate changes in gene expression and effect a state of cellular arrest or hibernation (hypoxia-tolerant) and thus neuroprotection, it raises new questions. I consider two of these here. First, what recruits PcG proteins to their targets in mammalian neurons? In *Drosophila*, PcG complexes are recruited to DNA elements known as polycomb response elements (PREs). However, mammalian equivalents of these elements have yet to be identified (22). What, then, determines the specificity of interaction between PcG proteins and DNA? Bernstein and colleagues identified CpG islands enriched for PRC1 and PRC2 in mouse and human stem cells (14). By applying computational genomics, they found that polycomb proteins are enriched at repressor element-1 silencing transcription factor (REST) binding sites (14). REST [also known as neuron-restrictive silencing factor (NRSF)] is a gene-silencing factor and master transcriptional regulator that represses a wide array of neuron-specific coding and noncoding genes (23–25). REST is abundant and widely distributed

during embryogenesis and is essential for maintaining stem cell pluripotency and self-renewal (23–25). During the late stages of neuronal differentiation, loss of REST is critical to acquisition of the neuronal phenotype. These findings suggest the enticing possibility that REST may recruit PRCs to common sites in the genome. If so, REST and polycomb complexes might act in concert to silence target genes and actively maintain not only stem cell pluripotency but also a state of ischemic tolerance in mature neurons.

Second, is dysregulation of PcG proteins more broadly related to neurologic disorders and disease? Here, the plot thickens. In mature neurons under physiological conditions, REST is quiescent, but can be activated in hippocampal neurons most vulnerable to insults such as global ischemia (26, 27) and epileptic seizures (28) and aberrantly accumulates in the nucleus of selectively vulnerable striatal neurons in Huntington's disease (29). Dysregulation of REST and its target genes play a role in various diseases, including malignant brain tumors (30, 31), neuropathic pain associated with peripheral nerve injury (32), Down syndrome (33, 34), and SMCX-associated X-linked mental retardation (35). An attractive scenario is that, under pathologic conditions, REST acts in concert with polycomb-repressive proteins to promote the neurodegeneration associated with global ischemia, epilepsy, Huntington's disease, and axonal injury and to mediate the impairment of higher cognitive function associated with Down syndrome and SMCX.

Consistent with this notion, a recent paper implicates increased abundance of RING1 and ubiquitinated H2A in Angelman syndrome, a neurodevelopmental disorder that shares features in common with autism (36). Angelman syndrome can arise from a mutation in the *UBE3A* gene (37), which encodes an E6-associated protein (E6-AP) ubiquitin ligase that targets the polycomb protein RING1B for ubiquitination and degradation. Mice lacking *Ube3a* are a model of Angelman syndrome and show impaired hippocampal memory formation, most notably a deficit in a learning paradigm that involves hippocampus-dependent contextual fear conditioning (37). *Ube3a*^{-/-} mice display elevated abundance of Ring1B and ubiquitinated H2A in cerebellar Purkinje neurons and impaired synaptic plasticity in the CA1 area of the hippocampus. Undoubtedly, dysregulation of polycomb proteins will appear in other brain disorders.

In summary, Stapels *et al.* (5) have identified polycomb proteins, which once had a defined role in patterning and development of insect wings and legs, as critical mediators of ischemic tolerance and neuroprotection. This, in turn, adds neuroprotection to the growing list of biological functions of polycomb proteins. At the same time, the study by Stapels *et al.* implicates dysregulation of polycomb proteins in neuronal death caused by ischemic stroke. Understanding the role of polycomb proteins in ischemic tolerance and neuroprotection is likely to cast light on the molecular mechanisms involved in neuroprotection and might improve therapeutic strategies for patients with stroke or other forms of ischemic brain injury.

Acknowledgments

Funding: This work was supported by the NIH and by a generous grant from the F. M. Kirby Foundation. R. Suzanne Zukin is the F. M. Kirby Professor in Neural Repair and Protection.

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Fig. 1.

The PcG proteins may work in concert with the master transcriptional factor, REST, to repress genes encoding death-promoting factors and protect neurons (A) from ischemic stroke. PRC1 and PRC2 are enriched at binding sites for REST (14). This finding suggests a model (B and C) in which REST recruits PRCs to promoters in stem cells under physiological conditions. REST and PRCs act in concert to silence target genes and maintain pluripotency. Me indicates a methyl group; NRSE, neuron-restrictive silencing element. In mature neurons, REST and polycomb complexes are quiescent but can be reactivated by preconditioning stimuli. Upon activation, PRCs mediate ischemic tolerance, enabling neurons to survive in the face of injurious ischemia (5). [Image credit: O. Yizhar, Deisseroth Laboratory, Stanford University]