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insulator (Xiao et al., 2011), suggesting that they may contribute to the formation of CTCF-mediated DNA loops (Dorsett, 2011). The formation of such loops enclosing the *Wnt4* locus may be the basis for the functional chromatin domain established by Wt1. Wt1-mediated chromatin- and gene-expression regulation is limited to the region bound by the two CTCF sites and does not affect the neighboring *Zbtb40* and *Cdc42* genes that are located on either side of the domain. Indeed, if the boundaries of the domain are disrupted through the reduction of CTCF and/or cohesin, the transcription of these two neighboring genes is induced (Figure 1). This observation suggests that, in the absence of CTCF, the active chromatin domain created by Wt1 in kidney cells spreads outside of its normal boundaries and alters the transcription of neighboring genes, with perhaps important consequences for the viability or differentiation of the affected cells. The first CTCF insulator analyzed in vertebrates, the chicken HS4 insulator element located in the β-globin locus, shows only enhancer-blocking insulator function (Bell et al., 1999). The findings of Essafi et al. (2011) now convincingly demonstrate that CTCF can also have barrier insulator activity. Analysis of the basis for these two different behaviors of CTCF in different genomic and cell-type contexts should give insights into the mechanisms by which this protein affects nuclear organization and gene expression.

Recently, a high-resolution CTCF chromatin interactome map in mouse embryonic stem cells has identified 1480 *cis*- and 336 *trans*-interacting loci (Handoko et al., 2011). Up to 23% of all loops formed by these CTCF-mediated interactions appear to separate domains of active or repressive chromatin modifications. The nature and function of the genes located in these domains has not yet been analyzed. It will be interesting to examine in future studies whether domain-specific regulation of chromatin structure, as shown by Essafi et al. (2011) for epicardial and kidney mesenchymal cells, is a general mechanism broadly used during cell differentiation. Such mechanism would ensure coregulation of genes present in a specific CTCF-delimited domain.

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Redirecting Traffic in the Nucleus

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Nuclear spatial organization of genes has emerged as an important determinant of their transcriptional activity. In this issue, Wang et al. (2011) show that the Msx1 homeoprotein induces a dramatic redistribution of Ezh2 and H3K27me3 to the nuclear periphery of muscle progenitor cells to repress transcription of developmentally regulated genes.

It is now established that mammalian genomes take on a nonrandom spatial organization in the nucleus that is both dynamic and cell-type specific. Chromosomes, segments of the chromatin, or individual gene loci adopt a highly organized structure and move between spatially distinct chromosome territories (Rajapakse and Groudine, 2011). Among these chromosome territories, the nuclear lamina (or nuclear periphery) regroups chromatin enriched for repressed genes and represents ~40% of the genome (Peric-Hupkes et al., 2010), whereas RNA Polymerase II-rich transcription factories in the lumen are associated with highly expressed genes (Eskiw et al., 2010). However, it should be noted that genes on the nuclear periphery are not always repressed nor are genes in the lumen always expressed (Meister et al., 2011).

Like chromatin, the distribution of histone marks has also been observed to change with cell identity. The transcriptionally repressive lysine 27 trimethylation mark on histone H3 (H3K27me3) is established by the Ezh2 subunit of the PRC2

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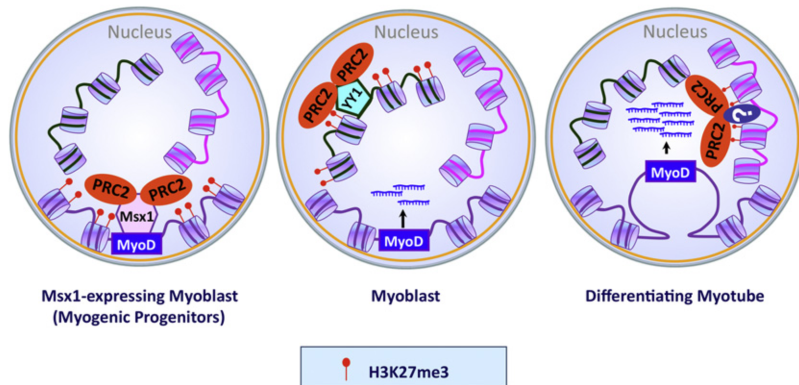

Figure 1. Msx1 Establishes Repression of the MyoD Gene through the Redistribution of Ezh2-Containing PRC2 Complexes to the Nuclear Periphery

Spatial positioning of *MyoD* (and its transcriptional status) is shown in the nuclei of Msx1-expressing myoblasts (left), normal myoblasts (center), and differentiating myotubes (right). In the nucleus of Msx1-expressing myoblasts (left), Msx1 represses the transcription of its target genes (*MyoD* is shown in purple as an example) by recruiting the PRC2 complex, leading to an enrichment of H3K27me3 at the nuclear periphery. In normal myoblasts (center), *MyoD* is transcribed at a moderate rate but remains localized to the nuclear periphery. However, in the absence of conditions favorable to myogenesis, the cells do not differentiate as YY1 recruits the PRC2 to muscle differentiation genes (dark green) to mediate their repression via H3K27 trimethylation (H3K27me3). Finally, under cellular conditions that are favorable to myogenesis (right), *MyoD* moves toward the center of nucleus and becomes more heavily transcribed. This leads to MyoD-dependent activation of muscle differentiation genes (dark green) through a displacement of PRC2. At the same time, the PRC2 complex is then relocated to another set of genes (pink), such as those involved in cell cycle progression, to repress the expression of genes that are no longer required for muscle development.

Polycomb Group (PcG) complex, proteins that are involved in the maintenance of transcriptional repression at developmentally regulated genes (Margueron and Reinberg, 2011). Mouse embryonic stem (ES) cells show an enrichment for the H3K27me3 at the nuclear periphery, whereas differentiated cells display a more diffuse pattern of H3K27me3 throughout the nucleus (Luo et al., 2009). These findings suggest that the spatial organization of H3K27me3 within the nucleus during differentiation has functional significance, though the mechanism by which H3K27me3 marks are redistributed in the nuclear space remains unclear.

In this issue, Wang et al. (2011) provide insight into the mechanism through which H3K27me3 becomes enriched at the nuclear lamina. Analyzing skeletal muscle myoblasts and cells in murine limb buds, the authors demonstrate that the expression of the homeoprotein Msx1 is sufficient to induce a dramatic redistribution of both Ezh2 and H3K27me3 to the nuclear periphery. This redistribution of H3K27me3 leads to repression of developmentally regulated Msx1 target genes that are localized at the lamina. Interestingly, the important accumulation of H3K27me3 at the nuclear periphery is not a result of global changes in H3K27me3 levels. Instead, the enrichment of H3K27me3 in the nuclear periphery occurs through a redistribution of Ezh2-induced H3K27me3 marks that would normally localize to the nuclear lumen in the absence of Msx1. The authors also demonstrate that this redistribution of H3K27me3 requires an interaction between PRC2 and Msx1, as the absence of either Ezh2 or Msx1 in the cell prevents relocalization of Ezh2, H3K27me3, or Msx1 to the nuclear periphery. This finding suggests a mechanism of co-operative binding between Msx1 and Ezh2 at target genes. Interestingly, Wang and colleagues (2011) find that this ability to redistribute H3K27me3 marks to the nuclear periphery is unique to Msx1 among homeoproteins when examined in the muscle cell context. However, their data also show that cells of the neural tube (which express endogenous Msx1 and Ezh2) do not display a nuclear periphery organization of H3K27me3, Msx1, or Ezh2. This suggests the co-operative binding leading to this redistribution also involves other factors. It remains to be seen whether other transcriptional repressors might possess a similar ability to redistribute Ezh2 and H3K27me3 to the nuclear periphery when provided with a proper cellular context.

While current models for spatial organization in the nucleus propose that genes move between chromosomal territories, the findings of Wang et al. (2011) suggest a mechanism in which Msx1/Ezh2 proteins establish a H3K27me3-rich domain at genes that are already present in the nuclear periphery, rather than Msx1 displacing its target genes to a pre-existing Ezh2/H3K27me3-rich nuclear chromosome territory. To provide further evidence for this, the authors examined the master regulator gene MyoD, which is expressed from the nuclear periphery in myoblasts and moves to the lumen upon differentiation (Yao et al., 2011). They show that expression of Msx1 in myoblasts does not affect localization of the MyoD gene to the nuclear periphery (Figure 1). Instead, Msx1 acts to repress MyoD expression through the establishment of the H3K27me3 mark in an Ezh2-dependent manner, supporting the notion that Msx1 promotes formation of an H3K27me3-enriched compartment at specific genes.

A question that remains unanswered is how Msx1 hijacks the bulk of cellular Ezh2 for its enrichment at the nuclear periphery. Indeed, the drastic relocalization of Ezh2 in myoblasts that is observed in the presence of Msx1 suggests the formation of a strong Ezh2/Msx1 interaction at the expense of interactions with other partners. Consistent with this, muscle-specific genes where Ezh2 is targeted by the protein YY1 in proliferating myoblasts (Caretti et al., 2004; Palacios et al., 2010) display a decreased enrichment of H3K27me3 upon exogenous Msx1 expression. One possible explanation for this observation is that the PRC2 complex has a stronger affinity for Msx1 compared to YY1. Alternatively, Msx1 expression may more directly result in the downregulation of YY1 expression. Finally, posttranslational modification of Ezh2 (or another PRC2 subunit) could modulate its ability to interact with alternative partners, as previously suggested (Palacios et al., 2010). Future studies are required to distinguish between these possibilities to resolve the mechanism of Ezh2 enrichment at the nuclear periphery.

This exciting study from Wang and colleagues has provided us with insight into the formation of the H3K27me3 mark at developmentally regulated genes. Of particular note, Msx1 is expressed in cells of the developing limb bud that have not yet committed to the skeletal muscle lineage. As H3K27me3 enrichment at the nuclear periphery has previously been observed in ES cells, it will be of interest to determine whether the remarkable spatial reorganization of this transcriptionally repressive mark is also present in other multi-potent cell types.

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Heart Development: Mitochondria in Command of Cardiomyocyte Differentiation

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Continuous developmental maturation of cardiomyocytes is essential to meet the functional and metabolic demands of the growing heart. A new study (Hom et al., 2011) reports that embryonic cardiomyocytes are influenced by mitochondrial maturation, such that closure of the mitochondrial permeability transition pore results in decreased levels of reactive oxygen species, thereby inducing differentiation.

Unraveling the molecular program that induces and establishes differentiation and maturation of cardiomyocytes during heart development is imperative for understanding the pathogenesis of pre- and postnatal cardiac disease as well as for therapeutic approaches aiming for regeneration of damaged myocardium. Although a multitude of transcription factors and small molecules have been identified that promote or inhibit cardiomyogenesis, their precise interaction is incompletely understood. This is complicated by the fact that cardiomyocyte differentiation is not only reflected by the assembly of a contractile apparatus but involves changes in cellular metabolism, cell-cell communication, and organelle structure. In this issue of *Developmental Cell*, Hom et al. (2011) add another level of complexity by reporting that cardiomyocyte differentiation in the embryonic heart is directly controlled by mitochondrial maturation. The authors show that mouse hearts at embryonic day (E) 9.5 contain relatively few and immature mitochondria, characterized by rare and disorganized cristae. In E13.5 hearts, in contrast, the mitochondrial mass increases substantially, accompanied by maturation of the organelle as indicated by abundant laminar cristae.

While these findings per se might be regarded as a consequence (rather than a cause) of cardiomyocyte differentiation and could just reflect the increasing contractile and therefore metabolic demands of the developing heart, the authors instead demonstrate that mitochondrial maturation induces cardiomyocyte differentiation. In vitro studies revealed that cardiomyocytes from E9.5 hearts exhibit a reduced mitochondrial membrane potential as well as increased levels of reactive oxygen species (ROS) when compared to E13.5 cardiomyocytes. These findings are characteristic for opening of the mitochondrial permeability transition pore (mPTP) within the inner mitochondrial membrane and suggest that the mPTP is open in early embryonic cardiomyocytes but closes upon developmental progression. Indeed, the authors could show that closing the mPTP in E9.5 cardiomyocytes by pharmacological or genetic manipulations results in functional as well as morphological maturation of mitochondria. Importantly, closing the mPTP induces differentiation of early (but not late) cardiomyocytes, which is evident as an increase in the number of sarcomeres per cell. This effect is dependent on intracellular ROS levels, which are high in E9.5 cardiomyocytes but drop upon physiological as well as induced closure of the mPTP. The authors could show that pharmacologically reducing ROS levels in early cardiomyocytes mimics the effect of mPTP closure and promotes