Organogenesis 6:1, 37-47; January/February/March; © 2010 Landes Bioscience

Regulation of cellular chromatin state
Insights from quiescence and differentiation

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Key words: hox, polycomb, trithorax, quiescence, myogenesis, satellite cells, regeneration, chromatin state

Abbreviations: PcG, polycomb group; trxG, trithorax group; MLL, mixed lineage leukemia; PRE, polycomb response element; PRC, polycomb repressive complex; ChIP, chromatin immunoprecipitation; ESC, embryonic stem cell

The identity and functionality of eukaryotic cells is defined not just by their genomic sequence which remains constant between cell types, but by their gene expression profiles governed by epigenetic mechanisms. Epigenetic controls maintain and change the chromatin state throughout development, as exemplified by the setting up of cellular memory for the regulation and maintenance of homeotic genes in proliferating progenitors during embryonic development. Higher order chromatin structure in reversibly arrested adult stem cells also involves epigenetic regulation and in this review we highlight common trends governing chromatin states, focusing on quiescence and differentiation during myogenesis. Together, these diverse developmental modules reveal the dynamic nature of chromatin regulation providing fresh insights into the role of epigenetic mechanisms in potentiating development and differentiation.

Introduction

Over the course of the last decade, epigenetic regulation has emerged as a key contributor to all genomic processes, controlling and defining gene expression profiles that contribute to the functional identity of a cell. Essentially, the packaging of eukaryotic DNA into higher order chromatin structure controls its accessibility to the nuclear proteins that read, interpret and replicate the genetic material. Epigenetic mechanisms broadly act to regulate transcription factor binding by establishing and maintaining 'chromatin states' that enhance transcriptional activation or suppression. Specific changes in gene expression at every stage of development are tightly governed by epigenetic switches that include methylation of the DNA itself,[1] post-translational modification of the histones that package DNA into chromatin[2] and energy-dependent remodeling of the chromatin to alter access to the transcriptional machinery.[3] Altered chromatin states thus underlie the progression of embryogenesis and organogenesis, where the developmental path of a terminally differentiated cell from a progenitor/stem cell involves a series of increasingly committed stages of specialization. During development, genes that are required at a late stage of commitment are maintained in a transiently repressed state; this temporary silencing has to be appropriately reversed to initiate tissue-specific gene expression as a function of differentiation. Genes important for proliferation and/or maintaining plasticity and multipotency on the other hand, are maintained in an accessible chromatin conformation in progenitors and need to be stably silenced to allow initiation of differentiation.[4]

Such flexibility implies that the genome of a cell is not a constant entity; rather, it exists in different chromatin states during development. It was observed very early on that the determined state of a cell type is heritable through mitosis and becomes a part of what is now understood as the cellular memory that ensures cell type commitment.[5,6] It is now known that cycling cells maintain their identity through multiple generations such that the patterns of gene expression that define their identity remain constant unless actively changed by a developmental switch. Cells that are not cycling might move into a maintenance mode that may be characterized by a genome-wide chromatin state and defined by specific combinations of chromatin marks. It is now emerging that patterns of DNA methylation and histone modifications distinguish these states and can heritably govern cell fates and lineage commitment/maintenance.[7]

Developmental progression is governed by the interplay of extrinsic signals with intrinsic transcriptional programs. The insight that subtle differences in cellular microenvironment can lead closely juxtaposed and identical stem cells to form different cell lineages[8] has an intracellular parallel where the same set of genes are subtly distinguished by epigenetic mechanisms that eventually confer different phenotypes in different cell types. Here, we focus on the regulation of two separate developmental modules that have provided paradigms at different levels for understanding the development of form: at the genomic level (the Hox gene cluster controls the generation of embryonic form) and at the cellular level (muscle stem cells regenerate form in damaged muscle tissue in the adult). The coordinated expression of the sequentially arranged homeobox or Hox genes in preset patterns provides evidence of hierarchical regulation at the

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Submitted: 12/20/09; Revised: 01/19/10; Accepted: 01/29/10
Previously published online:
www.landesbioscience.com/journals/organogenesis/article/11337

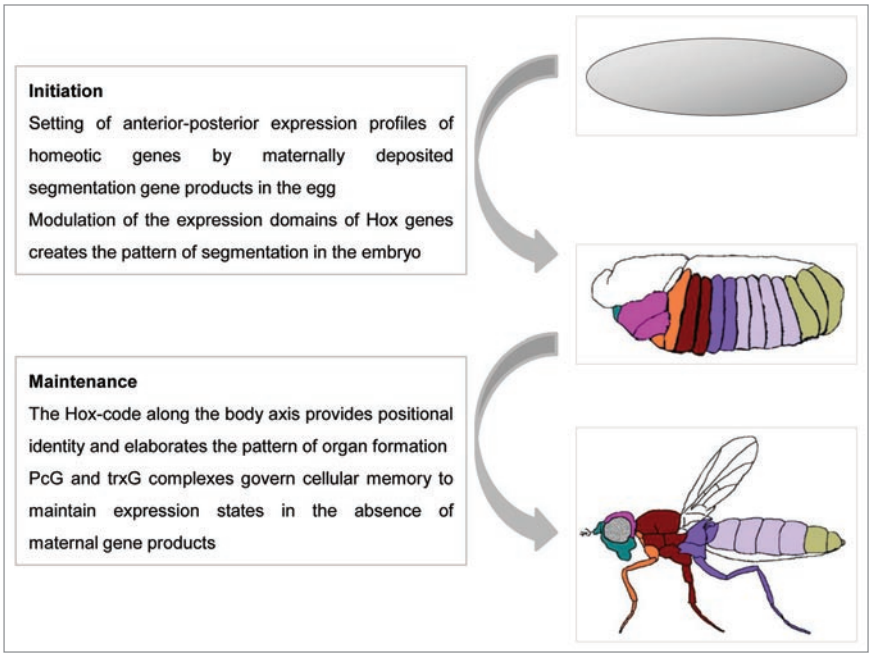

Figure 1. Hox genes set and maintain body plan and identity during development. Embryogenesis initiates with the developmental cues provided by maternal gene products that set off zygotic transcription in predetermined patterns. Coordinated expression of the Hox genes (depicted as colored patterns defining expression domains) establishes positional and segmental identity and is maintained throughout organogenesis. The domains of Hox expression profiles are tightly regulated by the PcG and trxG complexes and define the developmental plan of the adult (adapted from ref. 130).

earliest stages of development, defining the entire body plan of the developing embryo.[9] The regeneration of muscle tissue on the other hand involves resetting of gene expression profiles to allow developmental processes to reinitiate apparently at the end of the differentiation path, from the 'fully formed' functional adult state.[10] An examination of the progression of the genetic programs involved in both cases reveals that striking similarities exist in their epigenetic regulation with similar players directing the expression profiles of these distinctly different sets of genes. Together, these examples provide overlapping insights into the nature and role of the chromatin state in directing cellular memory throughout development—in proliferating progenitor cells of the early embryo as well as in quiescent adult stem cells poised at the threshold of differentiation.

Chromatin State in Early Development—Insights from Regulation of the Hox Cluster

Uncommitted embryonic cells need to acquire their positional identity and differentiate into different tissues at appropriate locations in the developing embryo. Maternal gene products localized in the early embryo initiate the activation of master regulatory genes known as homeotic selector or Hox genes that are expressed along the anterior-posterior body axis and specify positional identity throughout development[11] (**Fig. 1**). Displaying unique spatial and temporal patterns of expression, homeotic gene products determine the developmental paths of groups of early progenitor cells and govern tissue and organ identity in multiple ways. As development proceeds, progenitor cells maintain their determined state by using different mechanisms that keep combinations of the homeotic genes active through repeated cell divisions which is critical for providing body segmental identity.[12,13] Further, during morphogenesis of organs, these transcription factors also activate cell type-specific behaviors that allow proliferation and structural patterning besides directly or indirectly regulating large numbers of terminal differentiation genes called realizator genes.[14] Finally, the selector genes themselves are also kept stably inactive in a selective manner since their ectopic expression can completely alter the determined state of the cell.[15] The intricate networks governed by the homeotic genes are vast and here we focus on highlighting the memory mechanisms that define and preserve the discrete expression profiles of these genes, translating into the generation of different cell lineages during embryogenesis.

Polycomb and Trithorax Proteins and Cellular Memory

The control of cellular positional identity by the Hox family is exemplified by studies in *Drosophila melanogaster* where homeotic mutations were first identified.[16] Spatial restriction of expression of the two clusters of Hox genes, the antennapedia complex and the bithorax complex, is defined by sharp anatomical boundaries that are maintained by two other groups of chromatin regulatory genes, the Polycomb group (PcG) and the trithorax group (trxG).[17] Classical genetic analysis revealed that PcG genes generally contribute to the maintenance of the repressed state through the concerted action of polycomb repressive complexes like PRC1 and PRC2, while trxG gene products antagonize PcG mediated silencing and coordinate the active state.[18] Recent studies have begun to reveal the mechanisms by which these regulators function across the genome, though Hox genes remain their best understood targets. In the early embryo, complexes composed of specific sets of PcG or trxG proteins are believed to assist in structuring chromatin by interpreting the silent or active chromatin state. Indeed, these proteins have been shown to bind to promoters of Drosophila Hox genes before their final expression patterns are attained, indicating a model of 'preprogramming' of the transcriptional memory of these genes to respond appropriately to cell fate decisions.[19,20] DNA regulatory elements called Polycomb response elements (PREs) serve as targets to recruit distinct PcG

complexes that maintain the repressive state.[21-23] Similarly elements that recruit trxG proteins (TREs) have been studied, and interestingly often overlap with the PREs to regulate chromatin state.[21,24] Chromatin regulation by remodeling includes post-translational modification of the histone tails whose extent of association with DNA controls accessibility to the transcriptional machinery. These histone modifications become the targets of the next set of PcG/trxG factors, permitting the process of silencing or activation to extend over long distances by linear spreading or looping or a combination of the two mechanisms.[25] Long-range repression of Hox genes is mediated by members of PcG complexes through mechanisms involving PRC2 mediated trimethylation of lysine 27 of histone H3 (H3K27me3) along the entire Hox domain, and subsequent silencing by PRC1 and repressive factors. TrxG, and their vertebrate homologues, the MLL (mixed lineage leukemia) proteins, prevent this repression and catalyze trimethylation of lysine 4 of histone H3 (H3K4me3), associated with active transcription,[26] and their continued association with chromatin throughout development may be required for preventing inappropriate PcG mediated silencing of the Hox genes.[27]

Although initially examined in the context of cellular memory mechanisms regulating Hox genes in Drosophila, PcG/trxG complexes also regulate transcription across the genome especially of genes involved in cell fate determination and differentiation.[28,29] The cooperative binding of multiple DNA binding proteins at PRE sites likely defines platforms for recruitment of PcG and/or trxG complexes at these target genes (**Fig. 2**) which need not be mutually exclusive but assembled in a developmental stage specific manner.[30,31] The recruitment of different core remodeling complexes via interactions with DNA binding proteins has been demonstrated in Drosophila, especially for the PcG protein, pleiohomeotic (PHO).[31-35] Finally, the elucidation of their genome-wide distribution has led to the understanding that PcG and trxG complexes are not exclusive homeotic gene repressors/activators and points to their more dynamic role in gene regulation at a large number of loci.[36-38]

Much of the genetic and molecular analysis of PcG/trxG mediated mechanisms has been carried out in Drosophila, but multiple lines of evidence suggest that many of the key aspects are conserved in mammals. The mechanisms by which PcG/trxG complexes are recruited and mediate their effects in mammalian systems are not entirely clear but recent evidence for PRE-like activity in vertebrates[39,40] indicate greater parallels with Drosophila than previously predicted. Vertebrate factors with specific DNA binding capability may recruit PcG complexes that facilitate remodeling of chromatin to mediate transcriptional regulation, as has been demonstrated for Yin Yang1 (YY1), the functional homolog of Drosophila DNA binding PcG protein PHO.[41] PcG and trxG homologues in vertebrates have also been found to coordinate dynamic developmental gene regulation[20,42] and are not only conserved but have gone through expansion and subtle variations, indicating a key role of epigenetic mechanisms in the evolution of complexity.[43]

One of the most interesting questions to emerge is how the multiple homologues of these genes might provide an enhanced epigenetic tool kit. The existence of multiple copies of genes encoding individual subunits of these complexes opens up the possibility of alternative subunit compositions conferring distinct functions and contexts for chromatin remodeling.[44] While it is relatively easy to imagine the establishment of a transcriptionally 'off' or 'on' state and the chromatin structure that can support these states, it is not immediately obvious how intermediate levels of expression are established, apparently by a balance between antagonistic chromatin marks[45,46] (**Fig. 2**). Initially identified in embryonic stem cells (ESCs), domains containing both the H3K4me3 (activating) and H3K27me3 (repressive) chromatin marks are associated with many loci including the Hox gene clusters, suggesting the intriguing possibility that critical developmental genes are maintained in a special chromatin state (known as bivalent domains) poised for transcription in the event of developmental initiation.[47,48] Bivalent promoters of Hox genes and other developmental regulators have been shown to be associated with a high degree of chromatin state conservation in both human and mouse ESCs with stable retention of PRC2/PRC1 mediated repression.[49] In the mouse embryo, increased activating modifications combined with decreased repressive modifications mark a directional transition in chromatin status correlating with temporal activation of successive Hox genes.[50] Studies such as these indicate that during early development, cellular memory mechanisms direct an initially repressed state of chromatin at the Hox cluster to progressively convert to a state marked open for activation, followed by chromatin de-condensation for transcription.[11,51]

An intriguing question is how these states are maintained through early development during cell division, when access of DNA to the replication machinery necessitates the local disruption of DNA-protein complexes. Studies aimed at understanding chromatin states during cell cycle progression and exit are only now emerging, and seem to invoke mechanisms by which chromatin regulatory proteins like the polycomb complexes may remain bound to replicating DNA at sites of specific histone marks, enabling their perpetuation onto nucleosomes that package the newly synthesized strand.[52,53] Continued association with members of the trithorax family is required for the activity of particular sets of Hox genes and fluctuations in the occupancy of the trxG homologue, MLL1, as a function of the Hox cluster expression profile have been documented by ChIP (chromatin immunoprecipitation) analysis.[54] These studies provide some clues to the mechanisms by which PcG/trxG complexes perpetuate the epigenetic profiles of the Hox genes and help mediate their roles in proliferating and differentiating cells, even after the decay of the early acting transcription factors.

Chromatin State in Non-Proliferating Cells—Insights from Quiescence

Although PcG/trxG-mediated chromatin memory was defined in the developing embryo as a mechanism for maintaining body segmental identity by freezing patterns of expression of homeotic genes over multiple cell divisions, it was realized that it is not unique to a particular developmental stage or gene or cell type. The formation and maintenance of tissues and organs depends

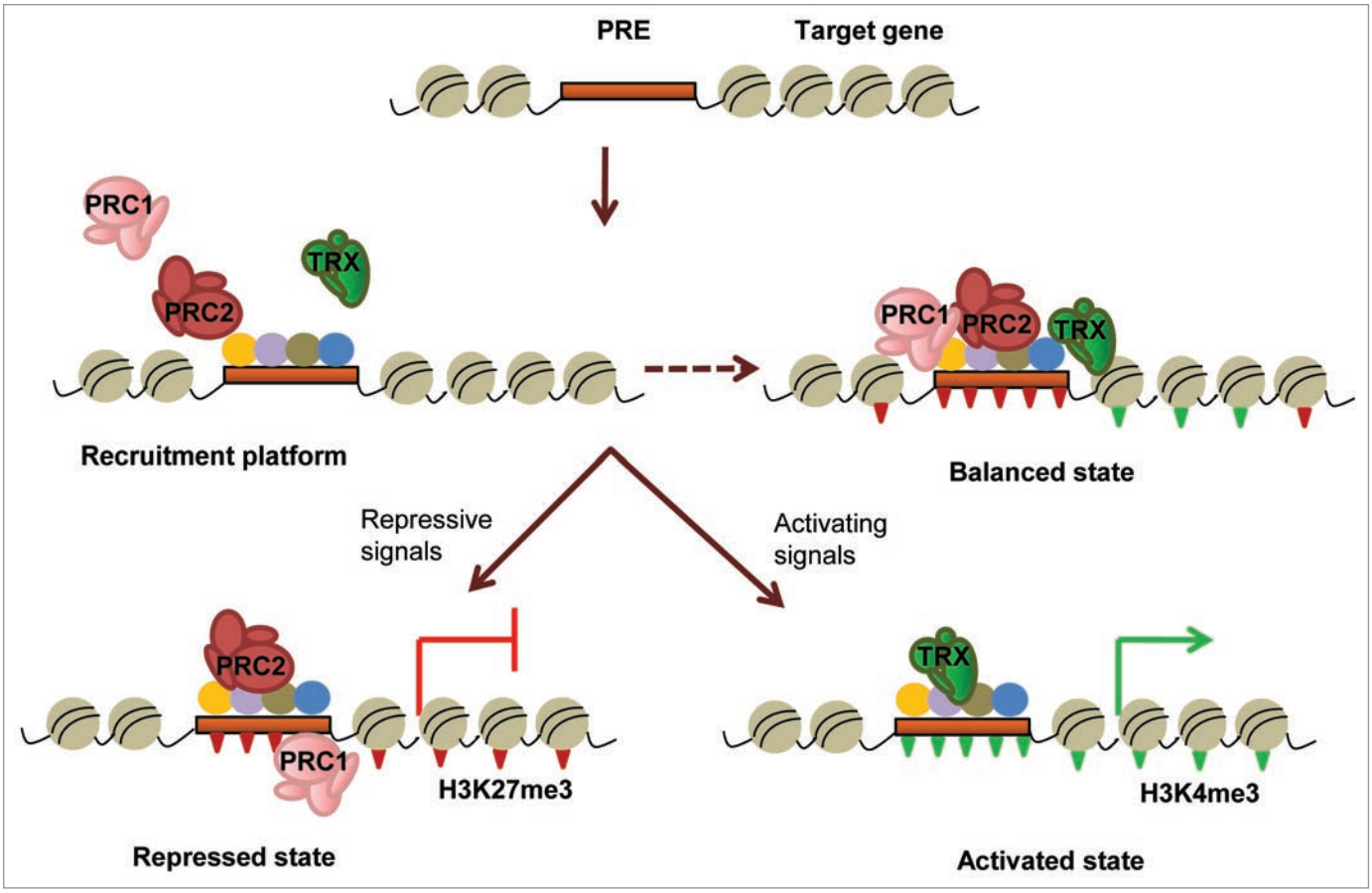

Figure 2. Schematic representation of the action of PcG and trxG complexes on chromatin. Multiple DNA binding proteins (colored circles) interact cooperatively with each other and with the PREs (brown box) located within or near the promoters of Polycomb target genes, setting the appropriate context for assembly of polycomb (PRC1/PRC2) or trithorax (TRX) remodeling activities. Balanced presence of both activating and repressive complexes can create a 'poised' state in some genes (dashed arrow) till appropriate differentiation signals are received. Depending on the type of developmental cues, the transcriptional status of the gene is resolved differently. PRC2 causes trimethylation of lysine 27 of histone H3 (H3K27me3; inverted red triangles) leading to recruitment of PRC1 and other modification factors that create a repressed chromatin conformation to keep the gene inactive. Binding of trithorax complexes causes trimethylation of lysine 4 (H3K4me3; inverted green triangles) and recruitment of transcription factors and activators that create a cascade of activating modifications leading to gene transcription.

not only on the generation of embryonic architecture but also on achieving the right balance of differentiated cells and progenitor cells as development proceeds. Cells in both these states are non-dividing in normal adult tissues but reach the arrested state by different means, which are governed by developmental programs that either co-ordinate cellular differentiation with cell cycle exit, or uncouple these two programs. Thus, unlike the permanently arrested differentiated cells (often referred to as 'post-mitotic'), adult progenitor/stem cells are reversibly arrested and respond to regenerative cues such as injury by returning temporarily to a proliferative state, generating new cells for tissue repair. While perpetuating the chromatin state in dividing cells is important for maintaining cell identity, cell memory mechanisms are also required in these temporarily non-dividing cells which may re-enter the cell cycle for tissue homeostasis and repair. In the following sections, we examine recent evidence that points to a role for higher order chromatin mechanisms in regulating this critical distinction that has far-reaching implications for the generation and regeneration of tissue form and function.

The vast majority of cells in the adult body do not cycle. Cell cycle exit is critical for normal cell physiology and is generally permanent as in the case of terminally differentiated, 'functional' cells which make up the bulk of adult tissues. Here, epigenetic mechanisms that stably silence tracts of the genome by the generation of heterochromatin operate, along with feedback and feed-forward transcriptional loops that induce and stably maintain tissue-specific genes. However, adult tissues also contain reversibly arrested cells (stem/progenitors) that are maintained in a temporarily quiescent state. Quiescence is often defined operationally as the state generated in culture by growth factor deprivation, contact inhibition, or loss of anchorage.[55] Cell cycle exit from G_1 into quiescent G_0 phase is characterized by lower rates of transcription, translation and metabolic activity as well as reduced cell size and a heterochromatic nucleus. Quiescence is completely reversible and quiescent cells are viable, readily re-entering the cell cycle when activating conditions prevail (**Box 1**). It is increasingly becoming clear that quiescence is not a just a passive state of cell cycle arrest characterized merely by a depressed macromolecular

metabolism, but an actively regulated one maintained by a typical transcriptional profile that includes induction of a large number of genes.[56-58] The transcription factors that promote quiescence are distinct from factors that determine irreversible cell differentiation decisions and, in fact, hold the key to many cell fate decisions. However, the mechanisms regulating these genes and their activation (and repression) are not yet well understood.

There is accumulating evidence that the quiescent state actively confers resistance to a variety of differentiation signals. For instance, reversibly arrested cultures of human fibroblasts are resistant to MyoD-induced muscle differentiation while proliferating fibroblasts are readily converted to muscle cells when MyoD is overexpressed and a differentiation-associated cell cycle exit is induced.[55] In muscle, Rb is essential for the proliferation block that is required for differentiation to occur. Proliferation associated genes such as E2F are unresponsive to growth inducing signals in terminally differentiated muscle cells (myotubes). The mechanism of this 'irreversible' repression is likely to be distinct from that employed in cycling cells.[64] Thus, beyond preservation of replicative capacity, quiescence—and its associated epigenetic state—may serve as a blockade to irreversible terminal differentiation thereby maintaining developmental potency, although much is not known in this regard so far.

Marking Chromatin Containing Newly Synthesized DNA: A Role for PcG Proteins

Somatic cells re-entering the cell cycle from quiescence phosphorylate pRb at the G_1-S transition causing de-repression of the E2F target genes and subsequent recruitment of PRC2/PRC1 polycomb complexes.[60] As DNA is replicated, freshly generated histones are incorporated along with 'old' histones from the parent strand. During this window, epigenetic marks on histones associated with the parental strand (H3K27me3) may recruit the remodeling machinery (PRC2) to mark neighboring, newly incorporated histones, thereby faithfully maintaining the chromatin state and gene expression profiles of the progeny cells.[52] The dynamic association of MLL1 and H3K4me3 at chromatin associated with Hox gene regulation provides evidence for the role of trxG proteins in chromatin dynamics through cell cycle progression.[54]

These models indicate the need to examine quiescence from the point of view of changes in the epigenome rather than just the transcriptome. The ability of quiescent cells to actively maintain the option of cell cycle re-entry may in part be governed by the transcriptional repressor Hairy and Enhancer of Split1 (HES1), which is associated with a chromatin-modifying complex.[65,66] A global reorganization of the epigenetic chromatin state including heterochromatin associated markers and core histone modifications has also been correlated with reactivation from quiescence.[67] A more comprehensive analysis of chromatin states associated with quiescence in mammalian cells should prove to be informative in delineating its phases (entry—maintenance—exit). Given that primitive adult stem cells usually exist in a slow cycling or quiescent compartment, it is conceivable that the chromatin state of quiescence may be instrumental in achieving the stem cell state. Indeed, mechanisms regulated by quiescence-induced chromatin regulators such as the tumor suppressor MLL5, specifically repress S phase promoting genes in a model of quiescent satellite stem cells of the myogenic lineage.[68]

Box 1. A brief summary of cell cycle control in quiescence

Progression through the cell cycle involves the coordinated action of several cyclin-dependent kinases (cdk) governed by cyclins (positive regulators) and their inhibitors (CKIs; negative regulators).[59] The quiescent state is associated with very low levels of most cyclins. Cell cycle activation and progression occurs when the synthesis of G_1 phase cyclins sets off a cascade of events involving cdk activation, phosphorylation of the retinoblastoma protein (pRb) and consequent derepression of E2F target promoters in conjunction with histone modifiers and chromatin remodeling to activate genes leading to the transition into S-phase.[60] E2F proteins may directly repress cell cycle target genes, thereby actively maintaining the quiescent state.[61] Recent ChIP studies indicate that E2F-pRB complexes interact in different ways with chromatin modifying machinery[62] and can also enable transcriptional regulation driving cell proliferation, by interactions with MLL family members that lead to the activation of the cyclins.[63]

Chromatin State in Differentiation—Insights from Myogenesis

Skeletal muscle tissue has emerged as an excellent model for studying the distinction between irreversible and reversible arrest, since this readily accessible tissue contains terminally differentiated muscle fibers as well as a small population of quiescent stem cells which are responsible for a robust regenerative capacity following injury.[69] Importantly, cell culture systems are available to model each of these states, permitting an analysis of their molecular/regulatory distinctions[70,71] and substantial information exists on the transcriptional networks and chromatin regulators of skeletal muscle.[72]

Myogenesis provides a precise model of differentiation and is a relatively easy system to study chromatin state change and function. Mesodermal precursors expressing the paired box transcription factor Pax3 initiate the skeletal muscle program via activation of muscle regulatory factors (MRFs)—Myf5, MyoD, myogenin and MRF4—upon receiving appropriate regulatory signals within the developing embryo.[73] Myf5 and MyoD are essential for lineage determination and mice mutant for both genes lack skeletal muscle and myoblasts,[74] while the late MRFs (in particular myogenin) regulate morphological differentiation into functional muscle fibers. The MRFs interact with ubiquitously expressed E-proteins to form heterodimers that bind to and regulate expression from the E-box present in muscle regulatory regions by functional interaction with transcriptional regulators and myocyte enhancer factor2 (Mef2) proteins[75] and recruitment of chromatin remodelers for transcriptional activation. Indeed, the demonstration of MyoD-directed chromatin remodeling of muscle specific genes such as myogenin under the regulation of growth factors established the mechanism for lineage determination during myogenesis.[76] This process involves a multitude of epigenetic interactions directing chromatin remodeling by

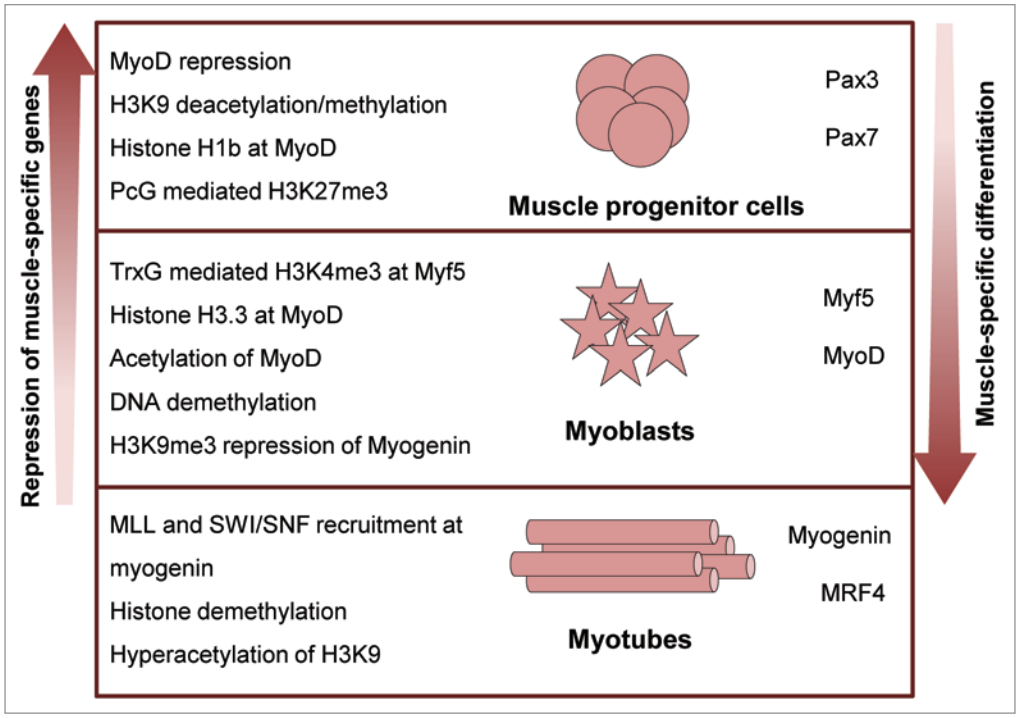

Figure 3. Epigenetic regulation of myogenic differentiation. Muscle myofibres are formed by the activation of the myogenic program in undifferentiated progenitor cells. Terminal differentiation into myotubes involves a series of chromatin remodeling and histone modifications (left) that lead to the derepression of myogenic regulators and the progressive activation of muscle-specific gene cascades (right).

recruitment of PcG/trxG and associated complexes and provides an example of the role of chromatin modulation in myogenic differentiation as encapsulated in **Figure 3**.

The discovery of MyoD as the dominant regulator of myogenic progenitor specification that can convert non muscle cell types to a myogenic fate is one of the earliest known examples of cellular reprogramming.[77] Expression of MyoD in a wide variety of cultured cells activates transfected reporter genes driven by skeletal muscle promoters and endogenous muscle specific genes.[78] Further, MyoD expression can convert ES cells to differentiated muscle under conditions of cell aggregation and nonproliferation, indicating that MyoD myogenic regulatory functions can initiate a complete differentiation program in the presence of appropriate environmental triggers.[79] Early evidence showed that the mere presence of MyoD binding sites is insufficient for activation via MyoD;[80] and though MyoD is expressed by undifferentiated progenitor myoblasts, it activates the cascade of muscle genes only upon receiving differentiation-promoting cues indicating tight regulation of the induction of myogenesis. The premature induction of muscle differentiation is prevented via repression of chromatin and associated MRFs by histone deacetylases (HDACs) and the dissociation of MyoD and deacetylase is a critical step for differentiation.[81] In conjunction with histone deacetylation, muscle specific genes are also associated with trimethylation of H3K27 by the interaction of the PcG protein YY1 with the Ezh2 subunit of PRC2; replacement of chromatin interaction of Ezh2, YY1 and HDAC1 by positive regulators MyoD and serum response factor (SRF) correlates with loss of H3K27, hyperacetylation and transcriptional activation.[82] Recruitment of Suv39 h1 histone methyltransferase (HMT), that methylates lysine 9 of histone H3 resulting in repression via association with heterochromatin protein HP1, is implicated in silencing MyoD- and MEF2-dependent myogenin activation in undifferentiated myoblasts, coupled with histone deacetylation.[83,84]

The positive regulation of muscle-specific gene transcription is promoted by the association of MyoD with HATs (histone acetyle transferases that relax chromatin by disruption of histone-DNA and histone-histone bonds) such as p/CAF in the presence of differentiation cues.[85] The downregulation of HDAC1 and its disassociation from MyoD, coincident with its association with the proliferation regulator, pRb, allows MyoD-mediated activation of myoblast differentiation.[86] Evidence for the direct role of TrxG proteins in establishing the myogenic expression state comes from studies demonstrating recruitment of Ash2L/MLL2 complexes via interaction with Mef2d at muscle-specific promoters in a differentiation stage specific manner.[87] Many studies have also indicated that the temporal regulation of myogenesis is regulated by the dynamic and selective recruitment of the ATP-dependent chromatin remodeling SWI/SNF enzymes by stage specific MRFs in association with Mef2 proteins, thereby creating an accessible conformation that is maintained by continued association with the activator complexes.[88-91] The link between the chromatin remodelers, MRFs and p38/MAP kinase signaling exemplify the mechanisms by which epigenetic modifications interact to regulate changes to the chromatin state under the influence of differentiation cues to effect muscle formation in a developmentally regulated manner.[91,92] Finally, a role for homeodomain proteins in establishing early myogenic potential

has been indicated by demonstrating that Pbx1 marks genes for activation by MyoD which sets off the epigenetic reprogramming cascades by promoting chromatin remodeling.[93] On the other hand, the homeoprotein Msx1 interaction with linker histone H1b in association with H3K9 methylation and deacetylation at the MyoD enhancer inhibits the initiation of myogenesis.[94]

The evidence from all the studies described provides an overview of the crucial role of epigenetic regulation in myogenic differentiation, governed by interactions with the MyoD family of regulatory factors. Further studies revealing as-yet unidentified interactions between different chromatin remodelers and their associated modifications should provide valuable insights into the integration of epigenetic networks in patterning muscle gene expression. Indeed, chromatin immunoprecipitation coupled with mouse promoter DNA microarray hybridization (ChIP-chip analysis) has enabled the identification of over 200 genes bound by MyoD family members, highlighting how key regulatory genes may govern a multitude of chromatin interactions to direct the expression state of the genome, leading to regulated development and differentiation.[95,96]

Muscle Regeneration: Balancing Proliferation and Differentiation

Adult skeletal muscle contains stem cells known as satellite cells because of their location at the periphery of multinucleated myofibers. Satellite cells are held in a quiescent state, sandwiched between the myofiber plasma membrane and the ensheathing basement membrane. During regeneration, in response to damage and death of myofibers, satellite cell nuclei exit quiescence, proliferate and eventually initiate terminal differentiation.[97] These transitions have been studied in cultured myoblasts, and appear to involve major changes to the chromatin landscape where proliferation-specific genes are maintained in transcriptionally active chromatin only in myoblasts and repressed with differentiation into myotubes. By contrast, muscle-specific genes are repressed in undifferentiated, proliferating myoblasts and acquire a chromatin conformation permissive for transcription at the onset of differentiation.[73] Proliferation and differentiation are thus generally mutually exclusive and terminal differentiation requires permanent exit from the cell cycle associated with drastic reprogramming of the chromatin state.

To maintain the satellite cell pool, and therefore the option for further regeneration, a small proportion of the activated cells exit the cell cycle and retreat into a quiescent state that remains permissive both for self-renewal and differentiation. Quiescent satellite cells remain committed to myogenesis, and lineage specification is in part achieved by Pax3, Pax7, MyoD and Myf5 using mechanisms that overlap to some extent with those regulating embryonic myogenesis.[98] Both lineage-determining MRFs, MyoD and Myf5, are cell cycle regulated.[71,99] While the muscle-specific Myf5 locus remains active during quiescence,[100] the expression of MyoD, regulated by histone acetylation,[101] recurs upon activation due to muscle injury and is critical for proliferation and regeneration.[102] The Pax genes contribute to satellite cell population expansion while maintaining commitment to the myogenic lineage.[103] Expression of Pax7 helps maintain survival and the undifferentiated proliferative phenotype; in its absence the satellite stem cell niche cannot be repopulated.[104] As described earlier, H3K9 methylation by Suv39 h at Rb/E2F target promoters may provide a mechanism governing the switch between exit from cell cycle by repression of S-phase promoters and entry into differentiation.[64] Studies using C2C12 myoblasts indicate that Pax7 induces H3K4 trimethylation at regulatory regions of the Myf5 locus through association with an Ash2L/MLL2 complex thereby mediating its transcriptional activation.[105] Differentiation cascades coincide with downregulation of Pax7,[106] and are set in place by the MRFs by their interaction with chromatin remodelers, with the expression of myogenin leading to differentiation and fusion into myotubes. The Ezh2 gene is expressed in proliferating satellite cells and is downregulated in terminally differentiated cells with loss of the H3K27me3 mark indicating a similar role for removal of PcG mediated repression for terminal differentiation as in embryonic myogenesis.[82] A recent study, however, issues a note of caution against extensive extrapolations from studies on embryonic myoblast regulation by demonstrating that the conditional inactivation of Pax7 or the related Pax3 in adult muscle does not adversely affect proliferation, differentiation or adult muscle regeneration;[107] the evidence that Pax7 dependence may actually only be critical during the transition of the early muscle progenitors to the quiescent adult stem cell state implies a highly developmental stage-specific role of these interactions. Nevertheless, taken together, the evidence from these models indicates that the path to developmental differentiation is regulated by similar cellular memory mechanisms directing epigenetic chromatin changes and common networks of players work together to establish transiently stable genomic expression states that can either be maintained as "flexible" or become "rigid" depending on the developmental state.

Perpetuation of the Chromatin State

As highlighted in the preceding sections, the identical genetic information in all cell types of an organism can be interpreted in a large number of ways to create discrete profiles of gene expression that define cell identity and function. Each cell type is characterized by a distinct pattern of chromatin organization that ensures its pattern of gene expression. Thus, the genome in each cell type exists in a distinct functional state—the cell type specific epigenome—such that though the genome of the organism remains the same, its packaging becomes cell type specific. However, if epigenetic features specify cell identity, it follows that chromatin structure must be propagated through cell division to maintain cell lineage fidelity. A proportion of histone proteins are believed to remain associated with DNA through replication[108] but if a cell is to recapitulate its epigenetic state after division, faithful recreation of precise histone modifications in association with chromatin remodeling would be necessary.[109] Conversely, a cell committed to differentiation would need to precisely repackage its genome to redirect patterns of gene expression towards a new functionality.

Box 2. Chromatin state features associated with 'stemness'

In embryonic stem (ES) cells, transcriptional programs regulating pluripotency are faithfully propagated between generations of cells and yet the cells retain the ability to efficiently respond to a wide variety of differentiation cues due to their unique epigenetic profile. The chromatin conformation of ES cells represents a ground state from which the entire spectrum of differentiated cells can be generated.[119] Some of the key chromatin features of stem/progenitor cells are summarized here.

While pluripotency genes are permanently repressed in differentiated cells by stable mechanisms like DNA cytosine methylation, key differentiation genes in pluripotent and lineage-committed cells are maintained in an inactive but transcriptionally poised state by means of functionally opposed methylation of H3K4 and H3K27 residues in association with polycomb complexes, the so called bivalent chromatin domains.[47-49,120] These domains resolve into transcriptionally repressive or permissive states and represent a general chromatin mechanism in conjunction with DNA methylation to regulate lineage commitment as well as terminal differentiation from progenitor states.[121]

Chromatin association with a high level of PcG-mediated H3K27 methylation at developmental loci may be crucial for avoiding premature differentiation of stem cells.[42,116]

Chromatin associated proteins, including histones, in pluripotent stem cells exhibit hyperdynamic interactions with the DNA and a very rapid rate of turnover.[122] Undifferentiated cells that are lineage committed do not display this loosely bound state indicating that one of the attributes of pluripotency may be the dynamic deposition and removal of different histone modifications at lineage governing loci, allowing differentiation based on environmental cues.

Stem cells exhibit comparatively decondensed chromatin with dispersed constitutive heterochromatin. This contributes to a more 'open' or globally accessible state than that of terminally differentiated cells. Indeed, in ES cells, global levels of histone modifications representing transcriptionally permissive marks are enriched while silenced tracts of chromatin are depleted compared to differentiated cells[123] and this correlates well with a study demonstrating that global transcriptional activity is a hallmark of pluripotency and is reduced upon lineage specification.[124]

Stem cells provide an excellent canvas for investigations of whether changes in chromatin structure are causal in driving differentiation. Stem cells are characterized by unique transcriptional and cell cycle regulatory features that distinguish them from somatic cells[110] and are associated with special chromatin states (**Box 2**). While a substantial body of evidence supports transcriptional networks of stem cell potency and self-renewal, the question of how chromatin states set during development are heritably maintained through replication has not been resolved. A stem cell can divide asymmetrically into an identical pluripotent daughter cell and another that differentiates. Following replication the parental genome can retain its epigenomic state while the newly synthesized one takes cues present during cell division to attain the epigenome of a derivative cell type.[111] In this manner pluripotent stem cells may give rise to progeny with increasingly restricted potency from which over 200 different cell types that make an organism are derived during morphogenesis. It is possible that similar mechanisms contribute to the plasticity of adult stem cells as well. It has already been shown that the satellite cells of muscle, when activated from quiescence, share the stem cell-like property of parental 'immortal' DNA strand retention[111,112] and satellite cell divisions have long been known to be asymmetric in growing muscle.[113] How is the epigenetic information segregated into two daughter cells committed to two different fates, where one returns to quiescence to replenish the satellite cell pool while the other proceeds towards terminal differentiation? PcG proteins are implicated in the maintenance of adult stem cell populations[114,115] and in dynamically repressing developmental regulators in ES cells for the controlled onset of differentiation.[42,116] The current model predicts that specific PcG proteins actively maintain the overall stem cell state in conjunction with a few key 'stemness' genes and cell fate determination requires relief from this repression of subsets of differentiation genes by the concerted action of trxG complexes with specific transcription factors.[117] Whether specific chromatin states govern different adult stem cells such as the muscle satellite cells during regeneration is as yet unclear. Chromatin mechanisms akin to the H3K4me3/H3K27me3 containing bivalent domains—which are believed to maintain key genes in a 'poised' state, amenable for lineage responsive transcription upon receipt of developmental cues (**Box 2**)—have not yet been demonstrated in these adult stem cells. In this regard, a study on muscle regeneration demonstrates that epigenetic interactions can switch between permissive and repressive chromatin states depending on the interplay of parallel signaling pathways.[118] In this context, the pre-activation marking of the promoters of early muscle genes and associated epigenetic marks[93] may be hypothesized to form a mechanism for rapid induction of these genes upon regulated activation of signaling pathways. The expression of the primed lineage determining genes may then be rapidly followed by the processive activation of downstream differentiation genes.

The application of genome-wide technologies to transcriptionally well defined systems promises to shed light on the regulatory mechanisms underlying signal-dependent distribution of epigenetic marks. Indeed, the last decade has seen numerous studies based on ChIP and microarray analysis elucidating transcription factor binding networks governing gene expression profiles in various populations of stem cells and their differentiated progeny as well as genome-wide profiling of histone modications.[125] Genome-wide studies have also identified a vast range of target genes of PcG/trxG complexes indicating their dynamic role as cells transition from stemness to differentiated states.[126,127] Regulation of the chromatin state in fact appears to be critical to maintaining the fate and identity of both stem cells as well as differentiated cells.[128]

The temporal relationship between transcription factor networks and epigenetic signatures that may be coordinated by developmental cues, however, remains to be clarified and requires the integration of cell fate studies from different developmental systems, of which the ones discussed in this review serve only as an example. Correlating the spatial information from genome-wide transcription factor binding and gene expression studies on quiescent and adult stem cells with epigenetic analysis would serve to shed some light on how the epigenome is managed for development and differentiation.[131] A final level of organization that may integrate both genome distribution and temporal networks

would be the ability to visualize or determine the 3-dimensional chromatin landscape for the entire genome. While we are far from this at present, genome-scale application of techniques such as chromosome conformation capture (3C)[129] in combination with advanced imaging techniques may eventually permit the construction of a topological 3D map whose rules and regulation may encapsulate cell identity.

Acknowledgements

Work in the Dhawan and Mishra labs is supported by the Government of India Council of Scientific and Industrial Research and grants from the Government of India Department of Biotechnology, Indo-Australia Biotechnology Fund (J.D. and R.K.M.) and the Wellcome Trust (UK) (J.D.). J.D. is an International Senior Research Fellow of the Wellcome Trust.

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