Nuclear Organization and Genome Function

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Annu. Rev. Cell Dev. Biol. 2012. 28:163–87

First published online as a Review in Advance on August 17, 2012

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

This article's doi:
10.1146/annurev-cellbio-101011-155824

1081-0706/12/1110-0163$20.00

Keywords

CTCF, polycomb, TFIIIC, cohesin, epigenetics

Abstract

Long-range interactions between transcription regulatory elements play an important role in gene activation, epigenetic silencing, and chromatin organization. Transcriptional activation or repression of developmentally regulated genes is often accomplished through tissue-specific chromatin architecture and dynamic localization between active transcription factories and repressive Polycomb bodies. However, the mechanisms underlying the structural organization of chromatin and the coordination of physical interactions are not fully understood. Insulators and Polycomb group proteins form highly conserved multiprotein complexes that mediate functional long-range interactions and have proposed roles in nuclear organization. In this review, we explore recent findings that have broadened our understanding of the function of these proteins and provide an integrative model for the roles of insulators in nuclear organization.

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INTRODUCTION

Eukaryotic genomes are packaged into higher-order chromatin structures and ultimately organized in a manner that functionally relates to gene expression. Understanding the mechanisms and molecular players involved in genome organization is therefore essential to fully comprehend the fundamental relationship between nuclear organization and genome function. Insulators are multiprotein DNA complexes proposed to underlie nuclear architecture on the basis of their ability to facilitate long-range physical interactions, to interact with nuclear substructures, and to cluster into nuclear foci termed insulator bodies. However, spatiotemporal expression and repression of genes pertinent to development and cell-type specification involve the function of additional regulatory elements, such as enhancers and Polycomb response elements (PREs), which suggests additional factors may also play a role in genome organization. It is then possible that the 3D arrangement of chromatin in the nuclear space is in part a consequence of function, i.e., the interaction of regulatory sequences with gene promoters, and in part due to structural elements whose role is to establish interactions between specific sequences to effect particular patterns of gene expression. The recent development of unbiased, high-throughput methods for mapping protein binding sites and genome-wide interactions has allowed an unprecedented look into the inner workings of genome biology, and new studies have provided valuable insights into the roles of insulators and chromatin structure in nuclear organization. In this review we highlight the relationship between nuclear organization and genome function and emphasize the dynamic interplay among chromatin insulators, transcription activation, and Polycomb (Pc)-mediated repression in creating and/or maintaining a 3D arrangement of the chromatin that is conducive to the establishment of patterns of gene expression required for cell-type specification.

NUCLEAR ORGANIZATION AND GENOME FUNCTION

Eukaryotic cells are tasked with packaging the genome several thousandfold into the confines of the cell nucleus while maintaining gene accessibility and chromatin structure that accommodates highly dynamic processes, including gene transcription, replication, and DNA repair. Interphase chromosomes are organized into discrete territories that are distributed nonrandomly with respect to the nucleus and with respect to other chromosomes, and whose placement can influence the potential for *trans* interactions and dictate whether a genomic locus is in an active or repressive nuclear environment (Cremer & Cremer 2010, Fraser & Bickmore 2007). The nucleus also harbors several discrete subnuclear foci, termed nuclear bodies, which are dynamically regulated structures that facilitate greater efficiency of

many nuclear processes (Mao et al. 2011). For example, active genes can relocate from chromosome territories (Branco & Pombo 2006, Chambeyron & Bickmore 2004) and cluster into subnuclear foci termed transcription factories for gene expression (Chakalova & Fraser 2010). Gene silencing is also accomplished through recruitment to repressive nuclear structures, and most biological processes are similarly compartmentalized into analogous nuclear bodies, which indicates an important relationship between nuclear organization and genome function.

Differentiation, Replication, and Genome Stability

The nonrandom order and significance of genome organization are perhaps best highlighted by its relationship to cellular differentiation, replication, and genome stability. The pathway from pluripotency to differentiated tissues is accompanied by changes in epigenomic landscapes, genome compaction, and some degree of chromosomal reorganization (Ahmed et al. 2010, Mikkelsen et al. 2007, Vastenhouw et al. 2010, Wiblin et al. 2005). Developmental genes are differentially targeted to transcriptionally active or transcriptionally repressive nuclear substructures, and differentiation is associated with restructuring of interactions between chromatin and the nuclear lamina (Peric-Hupkes et al. 2010). An increase in genome compaction may accommodate the organization of nuclear foci associated with transcription, DNA repair, replication, and splicing while restricting the complexity of genome function by concealing irrelevant transcription factor binding sites (Meister et al. 2011). Nuclear organization also plays a critical role in organizing DNA replication into discrete subnuclear compartments (Berezney et al. 2000) and in maintaining genome integrity. DNA damage gives rise to the accumulation of repair and DNA damage checkpoint proteins concomitant to increased chromatin accessibility (Kruhlak et al. 2006, Lisby et al. 2003), and studies in both yeast and human cells demonstrate an important relationship between nuclear organization, DNA repeat stability, and telomere protection (for reviews see Mekhail & Moazed 2010, Nagai et al. 2011). Characterizing the mechanisms involved in 3D genome organization is therefore essential for understanding the apparent fundamental relationship between nuclear organization and cellular function.

Genomic Strategies

Microscopy studies have been invaluable in revealing insights into the distribution and organization of chromosomes in individual cells and their relationship with gene regulation. However, to break down the 3D organization of chromosomes and the relationship between nuclear organization and underlying chromatin proteins, new techniques were required that exceeded the resolution and throughput limits imposed by traditional light microscopy (**Table 1**). The advent of the chromosome conformation capture (3C) technique described by Dekker et al. (2002) marked the first approach to effectively map physical chromosomal interactions across the genome. Although 3C has been useful in identifying locus-specific interactions between regulatory elements and target genes (Dekker et al. 2002, Tolhuis et al. 2002), derivations of the 3C technique have been introduced to extend the approach in an unbiased, high-throughput, genome-wide fashion. For example, the Hi-C method integrates an extended 3C protocol with massively parallel DNA sequencing, thereby capturing all genome-wide interactions at a resolution limited by the depth of sequencing (Lieberman-Aiden et al. 2009). Initial Hi-C analyses in a human lymphoblastoid cell line provided valuable insight into chromatin organization, supporting the fractal globule model in which chromosomes self-organize into a hierarchy of crumples, or series of globules governed by topological constraints (Grosberg et al. 1988, Lieberman-Aiden et al. 2009, Mirny 2011). Subsequent computational modeling supports the existence of chromosome territories and transcriptional foci and revealed

Table 1 Genomic tools for assaying chromatin occupancy, structure, and organization

Method	Description
ChIP-PCR	Identify binding status of chromatin-associated protein at selected genomic loci; PCR against genomic region of interest in DNA fragments obtained by chromatin immunoprecipitation (ChIP) against protein of interest
ChIP-chip	Identify the genome-wide binding profile of a chromatin-associated protein; microarray hybridization of protein-associated DNA fragments enriched for by ChIP
ChIP-seq	Identify the genome-wide binding profile for chromatin-associated protein; high-throughput sequencing of protein-associated DNA regions enriched for by ChIP
3C	Measure the interaction frequency between two selected genomic loci; quantitative PCR against ligated restriction fragments of interest
4C	Map physical interactions between a selected locus and the entire genome; microarray hybridization or high-throughput sequencing of interacting sequences captured by inverse PCR
5C	Map physical interactions between genomic loci within a targeted locus; ligation-mediated amplification and quantitation of interacting DNA fragments
Hi-C	Measure genome-wide interaction frequencies between all genomic loci; high-throughput sequencing of interacting sequences obtained by purification of biotin-marked ligation junctions
ChIA-PET	Map genome-wide interactions bound by chromatin-associated protein; high-throughput sequencing of ligated fragments enriched for by ChIP against protein of interest

new insights into the relationship between chromatin organization and CCCTC-binding factor (CTCF) as well as the nuclear lamina (Yaffe & Tanay 2011). However, further conclusions about the chromosome topology and nuclear organization of chromatin in human cells will require higher resolution, which is likely to be obtained in the near future with greater sequencing depth.

Mediators of Nuclear Organization

Determination of how interphase chromosomes are anchored within the nuclear space and which proteins mediate structural arrangements conducive to gene regulation and locus plasticity remains a critical hurdle to understanding the mechanisms that regulate genome function. Fortunately, genome-wide mapping of chromatin-associated proteins has increased at an extraordinary pace during the past few years thanks to the ENCyclopedia of DNA Elements (ENCODE) Projects (Birney et al. 2007, Celniker et al. 2009) and the increasingly affordable option of high-throughput sequencing. Analyses combining the 3D organization of interphase chromosomes—with genome-wide binding profiles of chromatin-associated factors—may ultimately establish which proteins functionally mediate nuclear organization. Information from microscopy and biochemical studies, combined with recent genome-wide mapping, has implicated multiple factors, including chromatin insulators, Pc complexes, and noncoding RNAs, as having roles in domain formation and chromatin organization that we consider in this review.

INSULATORS

Chromatin insulators originally were defined as regulatory elements that recruit proteins to establish boundaries between adjacent chromatin domains. Insulators were also shown to block the communication between enhancers and nearby promoters in an orientation-dependent manner, which led to intuitive models in which insulators limit the promiscuity of enhancers. However, further characterization of these sequences from yeast to humans has revealed

that insulators mediate long-range intra- and interchromosomal interactions, colocalize to subnuclear foci called insulator bodies as well as transcription factories, and preferentially cluster in *trans*, as revealed by Hi-C computational modeling in both yeast and humans. These findings suggest that insulators serve a greater purpose in chromatin organization.

Composition and Evolution

Studies of chromatin insulators in mammals have long been restricted to the highly conserved insulator protein CTCF, which until recently was the only characterized protein capable of insulator activity in humans. However, insulator activity in yeast involves tRNA genes and the transcription factor TFIIIC, and this role now appears to be conserved in mammals (Ebersole et al. 2011, Raab et al. 2012). Concurrent research on insulators in yeast, *Drosophila*, and mammalian systems therefore suggests that these elements serve an evolutionarily conserved role in gene regulation and nuclear organization.

Insulator protein CTCF. CTCF contains a central domain composed of 11 zinc fingers and is ubiquitously expressed (Klenova et al. 1993). Early biochemical studies demonstrated >93% amino acid identity between human and chicken CTCF proteins (Filippova et al. 1996), and studies in *Drosophila* later identified an orthologous CTCF factor with a similar domain structure and conserved insulator function (Moon et al. 2005), which suggests this zinc-finger protein plays a vital, highly conserved role in nuclear biology. Remarkably, CTCF primarily targets a highly similar core consensus sequence from *Drosophila* to humans, despite its ability to bind a variety of DNA sequences (Holohan et al. 2007). The proteins with which CTCF associates and the variant sequences it is able to bind have been suggested to underlie the numerous roles in which CTCF has been implicated (Weth & Renkawitz 2011, Zlatanova & Caiafa 2009), such as X-chromosome inactivation, V(D)J rearrangement, and chromatin insulation. Many CTCF binding sites recruit the cohesin complex (**Figure 1**), which is required for functional CTCF insulator activity (Nativio et al. 2009, Wendt et al. 2008). The cohesin complex forms a ring-shaped structure and mediates cohesion between sister chromatids from S-phase until mitosis, which suggests that cohesin may specifically stabilize chromatin loops arranged by CTCF through a similar mechanism. CTCF also interacts with Yin and yang 1 (YY1), a transcription factor involved in X-chromosome inactivation (Donohoe et al. 2007) capable of recruiting Pc complexes (Wilkinson et al. 2006), and CTCF-mediated insulator activity at the *H19/Igf2* requires the SNF2-like chromodomain helicase protein CHD8 (**Figure 1**) (Ishihara et al. 2006), the DEAD-box RNA helicase p68, and its associated RNA (SRA) (Yao et al. 2010).

Insulators in *Drosophila melanogaster*. *Drosophila* insulator elements and their associated proteins have been particularly well characterized thanks to in vivo insulator activity reporter assays made easy by the genetic manipulations available in the fly model system. In addition to the *Drosophila* homolog of CTCF (dCTCF), several insulator proteins have been identified, including Suppressor of Hairy wing [Su(Hw)], Boundary Element Associated Factor (BEAF-32), and GAGA factor (GAF) (**Figure 1**) (Gurudatta & Corces 2009). Contrary to what happens in vertebrates, the *Drosophila* cohesin complex localizes to transcriptionally active genes independently of dCTCF (Misulovin et al. 2008). Instead of cohesin, *Drosophila* insulator activity relies on fly-specific insulator proteins Centrosomal Protein 190 kDa (CP190) and Modifier of Modg4 [Mod(mdg4)], both of which contain BTB/POZ domains and are capable of forming stable multimers in vitro (Bonchuk et al. 2011, Gerasimova et al. 2007, Ghosh et al. 2001). Genome-wide localization studies suggest that dCTCF tandemly aligns with Su(Hw), BEAF-32, and CP190 at many sites throughout the genome, perhaps representing a unifying and synergistic role in facilitating

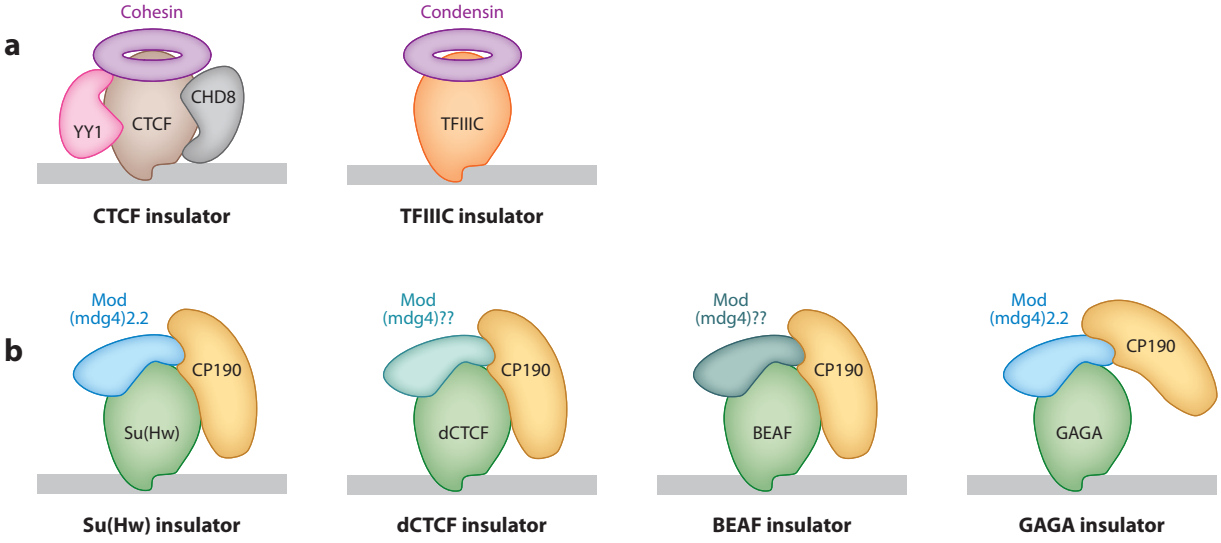

Figure 1

Diagram showing the structure of different vertebrate and *Drosophila* insulators. (*a*) Structure of the vertebrate CCCTC-binding factor (CTCF) and TFIIIC insulators. Indicated are factors associated with CTCF, such as cohesin, CHD8, and YY1, and with TFIIIC. (*b*) Each *Drosophila* insulator subclass contains a different binding protein that may define the specific function of the corresponding subclass. All insulators share the common protein Centrosomal Protein 190 kDa (CP190), although the role of this protein in the function of the GAGA insulator has not been demonstrated experimentally. In addition, all subclasses may also have one Modifier of mdg4 [Mod(mdg4)] isoform. The gypsy/Suppressor of Hairy wing [Su(Hw)] insulator contains Mod(mdg4)2.2. The dCTCF and Boundary Element Associated Factor (BEAF) insulators lack this isoform but contain a different variant of Mod(mdg4). GAGA has been shown to interact with Mod(mdg4)2.2.

chromosomal interactions and genome organization (Negre et al. 2010, Van Bortle et al. 2012). Interestingly, insulator activity in *Drosophila* also relies on components of the RNA interference (RNAi) machinery (Lei & Corces 2006, Moshkovich et al. 2011), though the mechanistic relationship remains poorly understood.

tDNA and TFIIIC. tRNA genes were first demonstrated to function as boundary elements flanking the repressed *HMR* locus in *Saccharomyces cerevisiae* and subsequently at the pericentromeric regions of *Schizosaccharomyces pombe* (Donze et al. 1999, Scott et al. 2006). The conservation of tDNA as an insulator element has been further extended by the demonstration that tRNA genes function as barrier and enhancer-blocking insulators in transgenic reporter assays in human cells (Raab et al. 2012). Analysis of the *mat* locus in *S. pombe* revealed that a repeat of B-box elements, which are highly conserved intragenic promoter elements in tRNA genes that recruit RNA polymerase III (RNAPIII) transcription initiation factor TFIIIC, were responsible for barrier activity (**Figure 1**) (Noma et al. 2006). Mutations in TFIIIC and TFIIIB, but not RNAPIII, affected insulator activity in *S. cerevisiae*, which suggests that insulator activity occurs independent of RNAPIII transcription (Donze & Kamakaka 2001). Although TFIIIC is sufficient for gene insulation alone, insulator activity is strengthened by TFIIIB and utilizes chromatin remodelers, possibly to evict histones at tDNA insulators (Valenzuela et al. 2009). Interestingly, in yeast and humans, TFIIIC binds many regions devoid of RNAPIII, called Extra TFIIIC (ETC) loci (Moqtaderi & Struhl 2004), and these sites are associated with the cohesin complex and

localize near CTCF-binding sites in mouse embryonic stem cells and humans (Carriere et al. 2012, Moqtaderi et al. 2010). TFIIIC sites and tRNA genes also function as loading sites for the highly conserved condensin complex in yeast, which suggests insulators serve an important role in chromatin architecture during both interphase and mitosis (D'Ambrosio et al. 2008).

Distribution and Chromatin Structure

Genome-wide localization studies by ChIP-chip and ChIP-seq have revealed that insulators are dispersed throughout eukaryotic genomes. CTCF is bound to thousands of independent sites in *Drosophila* (Bushey et al. 2009, Holohan et al. 2007, Negre et al. 2010) and tens of thousands of sites in human cell lines (Barski et al. 2007, Cuddapah et al. 2009, Kim et al. 2007), consistent with a global role in genome function. ChIP-chip analysis further showed that CTCF colocalizes with cohesin at more than half of its sites (Parelho et al. 2008, Rubio et al. 2008, Wendt et al. 2008), which suggests many CTCF sites are likely capable of functional insulator activity. Meanwhile, recent genome-wide localization of TFIIIC occupancy in mouse embryonic stem cells revealed that although all three TFIIIC subunits co-occupy <300 tRNA genes, as many as 2,200 independent TFIIIC-bound ETC loci are dispersed throughout the mouse genome (Carriere et al. 2012). Remarkably, as many as 85% of ETC loci lie within 20 kb of CTCF-binding sites, and cohesin subunits Smc1A and Smc3 were enriched at the ETC loci specifically, which suggests CTCF and TFIIIC distribution and insulator activity may be intimately associated. Evidence that suggests insulators may indeed collaborate comes from recent mapping of insulator proteins in *D. melanogaster* (Schwartz et al. 2012, Van Bortle et al. 2012). CTCF tandemly aligns with other classes of *Drosophila* insulators, including Su(Hw) and BEAF-32, and these multi-insulator complexes then become enriched for CP190, Mod(mdg4), and additional co-factors (Van Bortle et al. 2012). Alignment of insulators suggests CTCF may cluster with other distinct insulator proteins to efficiently recruit essential cofactors important for establishing a robust insulator complex and perhaps capable of maintaining stable, long-range interactions. Future studies may uncover a similar relationship between CTCF and TFIIIC in humans.

The distribution of CTCF, tDNA, and aligned insulators correlates with recent mapping of physical domain borders in both *Drosophila* and mammals. For example, Cavalli and colleagues recently utilized an independent, high-throughput 3C derivative (3C-seq) to explore the 3D folding and functional organization principles of the *Drosophila* genome (Sexton et al. 2012). Their data suggest eukaryotic genomes are partitioned into physical domains that can be clustered on the basis of strong statistical association with linear epigenomic profiles. Physical domains were categorized as active, which correlates with active histone marks; null, which comprises large transcriptionally repressive regions lacking silent chromatin marks; Pc domains associated with histone H3 K27 trimethylation (H3K27me3) repression; or HP1/centromeric domains associated with classical heterochromatin. Physical domains are demarcated sharply, and contacts within domains abruptly decay at positions corresponding to physical domain edges. Remarkably, insulators are highly enriched at domain borders, and hierarchical clustering revealed recurrent combinations of insulators and active histone marks that are present at all combinations of physical boundaries (e.g., even between two similarly annotated physical domains, such as null–null). Analogous mapping of physical domains reveals similar partitioning of the human genome as well as enrichment for CTCF and tRNA genes at chromatin domain borders (Dixon et al. 2012, Nora et al. 2012).

The enrichment of insulator proteins at all combinations of physical domain borders begs the question of what role insulators play in delimiting discrete chromatin domains. Earlier correlations for CTCF and tDNAs at the borders of repressed chromatin domains,

typically in the form of H3K27me3 (Cuddapah et al. 2009, Negre et al. 2010), have led many to believe insulators function as chromatin boundaries, where they simply serve to prevent the spread of heterochromatin into flanking chromatin domains (Bartkuhn et al. 2009). Recent compounding evidence strongly suggests that despite this correlation, endogenous insulator proteins are not important for restricting the spread of silencing chromatin. For example, RNAi depletion of CTCF in *D. melanogaster* does not lead to significant alterations in chromatin structure or gene activity (Schwartz et al. 2012, Van Bortle et al. 2012). Similar findings are reported for depletion of other insulator proteins (Van Bortle et al. 2012), and mutations in *Drosophila* insulator protein Su(Hw) have little consequence on several regions tested for gene activity (Soshnev et al. 2012). Meanwhile, CTCF is not required for barrier activity at the well-characterized *β-globin* locus (Barkess & West 2012, Recillas-Targa et al. 2002, Yao et al. 2003), together suggesting endogenous insulator proteins are not involved in heterochromatin barrier formation. The discovery that insulators are present at all combinations of physical domain borders rather than just repressive H3K27me3 domains perhaps reinforces the notion that chromatin insulators are playing a paramount role, beyond the scope of barrier activities observed in transgenic assays that remove insulators from their natural genomic context. Instead, the ability to facilitate long-range interactions appears to be the defining feature of insulators, underlying their role in nuclear organization and genome function.

Long-Range Interactions

Insulators have also been defined by their ability to impede the interaction between promoters and distal enhancers in a direction-dependent manner. Meanwhile, observations at numerous genomic loci across species suggest that insulators influence chromatin structure by establishing chromatin loops through physical interactions. Concurrent models therefore proposed that insulators evolved to ensure the fidelity of enhancers and their target promoters *in vivo* by establishing chromatin loops and thereby dictating the potential for enhancer-promoter interactions. However, accumulating data suggest that insulators facilitate long-range inter- and intrachromosomal interactions across the genome, including bridging connections between distant enhancers and target promoters, which suggests that local determination of enhancer-promoter interaction represents only part of a more significant role in chromosome organization.

The enhancer-blocking activities of CTCF have been best characterized at the chicken *β-globin* locus and the murine *H19/Igf2* imprinted locus, both of which have been extensively reviewed (Phillips & Corces 2009). These loci provide ideal scenarios for studying the role of insulators in allele-specific and developmental cell-type-specific gene regulation and chromatin architecture, and 3C experiments suggest CTCF underlies chromatin contacts at both genomic loci (Kurukuti et al. 2006, Splinter et al. 2006). Recent application of 3C has revealed that CTCF also underlies developmental higher-order architecture at the conserved homeobox gene A (*HOXA*) locus in mouse and humans (Kim et al. 2011). Specifically, CTCF and cohesin contribute to reorganization and selective gene activation at *HOXA* by partitioning silenced genes through chromatin loop formation upon differentiation. Furthermore, pluripotency factor OCT4 antagonizes cohesin loading at the CTCF binding site, thereby demonstrating developmental regulation of insulator activity and gene expression by inhibiting chromosome loop formation. Studies in *D. melanogaster* suggest insulators have a conserved role in developmental coordination of gene expression and chromatin structure. For example, genome-wide mapping revealed that dCTCF and *Drosophila*-specific insulator proteins are regulated through DNA binding and recruitment of CP190 during the ecdysone hormone response in Kc cells (Wood et al. 2011). In addition, 3C analysis at the ecdysone-induced *Eip75B* gene further revealed a developmentally regulated dCTCF site, wherein recruitment of

CP190 and enhanced chromatin looping upon ecdysone stimulation suggested alterations in *Eip57B* locus chromatin organization.

Numerous studies have focused on the role of insulators in locus-specific gene regulation and chromatin architecture, but the emerging picture of insulators in genome-wide nuclear organization requires global analyses of chromatin interactions. The first genome-wide map of CTCF-mediated functional interactions has been obtained by combining ChIP with high-throughput sequencing of enriched chromatin interactions (Handoko et al. 2011). The authors identified ~1,500 *cis* and ~330 *trans* interactions facilitated by CTCF in mouse embryonic stem cells and classified them into five categories on the basis of distinct epigenetic patterns. CTCF interactions harbor chromatin loops enriched for active or repressive chromatin signatures, which suggests that CTCF harnesses clusters of genes with coordinated expression (**Figure 2**). CTCF interactions also create chromatin hubs conducive to enhancer and promoter activities, in support of recent evidence suggesting that CTCF and cohesin underlie enhancer-promoter interactions at the *INFG* and *MHC-II* loci (Hadjur et al. 2009, Majumder & Boss 2011). The authors therefore speculate that insulators may instead facilitate cell-type specific enhancer-promoter interactions. Ultimately, Handoko et al. (2011) provide a genome-wide interrogation of CTCF interactions and reveal several modes through which CTCF functionally organizes the genome.

Genome-wide interrogations of intra- and interchromosomal interactions in yeast and humans independently demonstrate that tDNA insulators also underlie long-range genomic interactions. Noble and colleagues recently employed a high-throughput 3C-based technique to query the 3D organization of the *S. cerevisiae* genome (Duan et al. 2010). tRNA genes were significantly enriched for

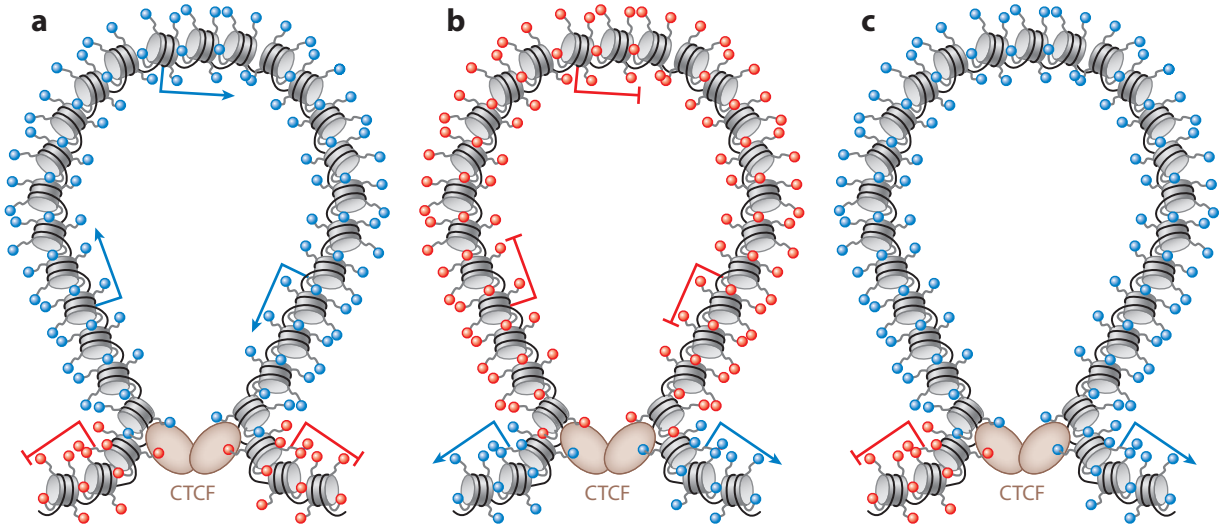

Figure 2

Structure of some of the domains created by interactions between CCCTC-binding factor (CTCF) insulators in mouse embryonic stem cells. Actively transcribed genes are represented by a blue arrow and silenced genes by a red inhibition line. Nucleosomes and the histone tails are represented in gray; active histone modifications are indicated as blue spheres and repressive modifications as red spheres. DNA is represented in black and CTCF as brown ovals. (*a*) CTCF forms a loop to separate a domain containing active histone modifications and transcribed genes from repressive marks and silenced genes. (*b*) CTCF forms a loop to separate a domain containing repressive histone modifications and silenced genes from active marks and transcribed genes. (*c*) CTCF forms a loop containing nucleosomes enriched in mono- and dimethylated H3K4, and trimethylated H3K4 at the boundaries of the loops, whereas the active transcription modification H3K36me3 and repressive H3K27me3 marks are observed outside the loops on opposite sides.

interactions with other tRNA genes, which suggests that insulator-to-insulator interactions are a conserved feature of eukaryotic genome organization. Hierarchical clustering revealed two clusters of colocalizing tRNA genes, one consistent with previously described nucleolar localization (Thompson et al. 2003) and another with centromeres. Similar mapping of interactions at a tDNA insulator in humans revealed analogous long-range interactions between tDNAs as well as ETC loci, which suggests tRNA genes and TFIIIC play a conserved role in genome organization. Recent findings indicate that TFIIIC binding sites facilitate condensin binding in *S. cerevisiae* and *S. pombe* (D'Ambrosio et al. 2008) and colocalize with cohesin in mammals (Carriere et al. 2012), which suggests TFIIIC recruitment of condensin and cohesin complexes may underlie chromosomal interactions in yeast and humans analogous to those with CTCF.

Role in Nuclear Organization

Microscopy-based analyses of the physical and functional organization of eukaryotic nuclei have led to the identification of several discrete subnuclear organelles called nuclear bodies, which play host to a variety of nuclear processes, including transcription, splicing, processing, and epigenetic regulation (Mao et al. 2011). Nuclear staining of insulator proteins has shown a clear propensity for insulators to concentrate into distinct nuclear foci, a feature that is conserved in yeast (Noma et al. 2006), *Drosophila* (Gerasimova et al. 2000), and mammals (MacPherson et al. 2009). Insulators also interact with and localize to nuclear substructures, including the nuclear and nucleolar peripheries, which suggests insulators tether associated chromatin to defined nuclear compartments (Gerasimova et al. 2000, Yusufzai et al. 2004). These findings, combined with knowledge about insulator-mediated functional interactions, have led to models proposing that insulators ultimately interact to partition chromatin into structural and functional domains that are physically organized through insulator bodies. Although tantalizing, the functional importance and molecular underpinnings of insulator bodies remain poorly characterized.

Nuclear organization clearly involves spatial arrangement of chromosomes whose position with respect to the nuclear periphery correlates with chromatin structure and gene expression. Chromatin interactions at the nuclear periphery recently have been mapped in both *Drosophila* (Pickersgill et al. 2006) and humans (Guelen et al. 2008), revealing large, sharply defined lamina-associated domains (LADs) that correlate with low gene density and transcriptional repression. The borders of LADs in humans are enriched for CTCF (Guelen et al. 2008, Zullo et al. 2012), which suggests this protein may separate chromatin environments at the nuclear periphery. Mapping of insulators in *D. melanogaster* with respect to the nuclear lamina has also revealed a significant enrichment for the *Drosophila*-specific Su(Hw) insulator at the borders of LADs (van Bemmel et al. 2010). Handoko et al. (2011) independently identified the apparent relationship between lamina and the CTCF interaction network in mouse embryonic stem cells. Specifically, CTCF loops were depleted within LADs but enriched at LAD borders, which supports a model in which CTCF orchestrates genome organization with respect to the nuclear lamina. An analogous role for TFIIIC in nuclear organization in yeast has been proposed on the basis of perinuclear staining of insulator bodies in *S. pombe* (Noma et al. 2006). In support of this model, perinuclear localization and silencing of the *HMR* locus in *S. cerevisiae* was recently shown to rely on nuclear pore proteins that localize to a tDNA barrier insulator (Ruben et al. 2011).

POLYCOMB

Genome plasticity and selective expression are essential features of multicellular development, and several early regulatory factors involved in body patterning and segmentation need to be strictly regulated to facilitate appropriate developmental decisions. Pc group (PcG) proteins are evolutionarily conserved epigenetic

transcriptional repressors that play an important role in establishing and maintaining cell fate by influencing the expression status of pertinent genes. PcG proteins specifically mediate the repression of numerous developmental genes through posttranslational modification of histone proteins, and recent studies demonstrate that Pc activity is involved in a broad scope of cellular processes, including differentiation, cell cycle regulation, X-inactivation, and cell signaling (Sawarkar & Paro 2010). Microscopy studies have demonstrated that PcG proteins concentrate into nuclear foci, called Pc bodies, which suggests PcG proteins may also mediate the nuclear organization of their target genes. Here we review recent progress in determining the relationship between Pc and nuclear organization.

Composition and Evolution

The PcG genes were first discovered as chromatin repressors that maintain silencing of the homeotic regulatory genes throughout *Drosophila* development (Paro 1990). Further studies in the fruit fly demonstrated that PcG proteins are recruited through *cis*-regulatory elements called PREs (Simon et al. 1993), and a recently identified element at the *HoxD* locus in humans facilitating Pc-dependent transcriptional repression throughout cell differentiation suggests the mechanism of Pc recruitment may be conserved (Woo et al. 2010). However, PREs are not easily broken into obvious DNA consensus sequences as described for insulator proteins, and the functional mechanism of Pc targeting remains unclear, though it appears to involve numerous players including DNA-binding proteins, histone posttranslational modification binding proteins, RNAi machinery proteins, and noncoding RNAs (Beisel & Paro 2011).

PcG proteins are present in two major complexes, Polycomb repressive complex 1 (PRC1) and 2 (PRC2), whose core components are largely conserved from flies to humans. The PcG family also includes several additional proteins that allow for the formation of diverse Pc chromatin-binding complexes with variable enzymatic activities (Simon & Kingston 2009). PRC2 catalyzes H3K27 di- and trimethylation (H3K27me3, which is associated with transcriptional repression) through SET domain–containing subunit EZH1, as well as EZH2 depending on cellular context (Beck et al. 2010, Margueron & Reinberg 2011). PRC2 recruitment and activity is regulated by core components and ancillary subunits, such as PHF1, JARID2, and AEBP2, which stimulate PRC2 enzymatic activity (Beck et al. 2010, Kim et al. 2009, Li et al. 2010, Sarma et al. 2008). PRC1 subunits RING1B and BMI1 form a stable heterodimer capable of catalyzing H2AK119 ubiquitylation (H2AK199Ub1) (Cao et al. 2005), which likely underlies PRC1-mediated Pc silencing (Wang et al. 2004). There is also recent evidence that histone modification–binding proteins containing malignant brain tumor (MBT) modules contribute to Pc function. For example, L3MBTL2 interacts with and is required for Pc-mediated repression by a PRC1-like complex in human cells (Trojer et al. 2011). Interestingly, the *Drosophila* MBT protein L(3)mbt localizes specifically to chromatin insulators (Richter et al. 2011), and as we discuss below, recent evidence suggests insulator activity may play an important role in Pc repression.

Distribution and Chromatin Structure

The genome-wide localization of PcG proteins has been studied in several independent ChIP-chip and ChIP-seq experiments in both *Drosophila* and mammals. Pc complexes localize to putative PREs in *Drosophila* and correlate with broad repressive H3K27me3 domains that encompass genes involved in major developmental pathways (Schwartz et al. 2006, Tolhuis et al. 2006). Most PREs are co-occupied by the major Pc complexes PRC1 and PRC2, and the occupancy landscape of PcG proteins changes during development, consistent with its role in mediating cell fate restriction by differential gene silencing (Negre et al. 2006, Oktaba et al. 2008). Mapping of PRC1 and PRC2 complexes in mammals shows similar co-occupation of

developmental pathway genes and displacement of PcG proteins during gene activation (Boyer et al. 2006, Bracken et al. 2006, Lee et al. 2006), which suggests Pc repression is a highly conserved feature of multicellular development. However, PcG proteins also localize to bivalent domains characterized by overlapping H3K27me3 and H3K4me3 encompassing genes poised for activation or repression upon cellular differentiation in mammals, a feature that is largely absent in fly embryos (Schuettengruber et al. 2009).

Genome-wide mapping of trithorax group (trxG) proteins, which catalyze H3K4 methylation and antagonize Pc repression through transcriptional activation, suggests a dynamic interplay between Pc repression and Trx activation dependent on overlapping recruitment proteins and the relative levels of Pc- and Trx-associated factors (Kwong et al. 2008, Schuettengruber et al. 2009, Schwartz et al. 2010). Interestingly, PRC2 also functionally associates with numerous noncoding RNAs (ncRNAs) (Zhao et al. 2010). For example, the 2.2-kb long ncRNA *HOTAIR* serves as a scaffold for both PRC2 and H3K4 demethylase LSD1 complexes, and thereby coordinates targeting of Pc to chromatin while coupling H3K27 trimethylation and H3K4 demethylation activities for epigenetic repression (Tsai et al. 2010). Mapping of the genome-wide occupancy of *HOTAIR* revealed >800 focal, transcription factor–like binding sites that co-occupied the genomic binding profiles for the PRC2 subunits EZH2, SUZ12, and H3K27me3. *HOTAIR* occupancy was maintained upon EZH2 depletion, which suggests *HOTAIR* actively binds chromatin and may underlie the nucleation of PRC2 domains (Chu et al. 2011).

Long-Range Interactions

PcG proteins have long been shown to concentrate into nuclear foci called Pc bodies, whose number and size change upon cellular differentiation (Ficz et al. 2005, Grimaud et al. 2006), which suggests that Pc facilitates genome-wide interactions that are further compartmentalized within the nuclear space. Accumulating data suggest that PcG proteins are indeed involved in long-range interactions essential for Pc repression. Combinatorial use of 3C and fluorescence in situ hybridization (FISH) showed that PRE-PRE interaction occurs between the *bxd* and *Fab-7* elements separated by ~130 kb in the *Drosophila* bithorax complex (BX-C) and that colocalization occurs specifically in embryonic tissues and cell lines in which both the *AbdA* and *Ubx* genes are corepressed (Lanzuolo et al. 2007). Long-range associations dependent on PRC2 core component EZH2 at the mammalian *GATA-4* locus, which is silenced in undifferentiated human TERA-2 cells, have also been observed (Tiwari et al. 2008b). Tiwari et al. (2008a) subsequently devised a 3C-based approach analogous to chromatin interaction analysis with paired-end tag sequencing (ChIA-PET) and demonstrated a limited number of intra- and interchromosomal interactions in human TERA-2 cells dependent on EZH2, which suggests that PRC2 is involved in mediating long-range interactions in mammals.

The formation of Pc bodies and identification of long-range PRE interactions support a global role in genome function, but understanding the role of Pc complexes in multigene regulation and nuclear organization requires genome-wide exploration of genomic interactions. To this end, van Steensel and colleagues recently adapted chromosome conformation capture on chip (4C), a 3C derivative that determines genome-wide interactions of a given locus (Simonis et al. 2006), to explore where Pc domains are able to interact within the *Drosophila* genome (Tolhuis et al. 2011). The authors demonstrate long-range interactions between Pc target genes and independent Pc and/or H3K27me3 domains in larval brain tissue, and further show that Pc interactions are topologically constrained to a single chromosome arm. Accordingly, chromosome inversion dramatically altered the interaction profiles revealed by 4C, but global gene expression patterns were relatively unchanged. Although specific interaction partners were altered, Pc

target genes nevertheless continue to interact with independent Pc domains, which suggests that Pc interactions are flexibly amenable to new partners for gene repression. Genome-wide mapping of chromosomal interactions by Sexton et al. (2012) independently identified 30 significant pairs of long-range interactions between Pc domains, thus supporting the role of PcG proteins in mediating specific long-range associations. However, the mechanisms by which these interactions are established and maintained and whether PcG proteins are directly responsible for mediating physical interactions are not clear.

Role in Nuclear Organization

The correlation of long-range chromatin interactions with Pc domains provides compelling evidence in support of the possibility that Pc complexes orchestrate the nuclear organization of repressed genes, but whether PcG proteins are required for long-range interactions has been called into question recently. Co-staining for insulator protein CTCF and PcG protein Pc2 shows clear colocalization of Pc and insulator bodies in HeLa cells (MacPherson et al. 2009), and accumulating evidence suggests that insulators underlie the colocalization and nuclear organization of Pc domains (Pirrotta & Li 2011).

The best-studied examples of physical interactions involving Pc targets occur between elements within *Drosophila Hox* gene clusters that have been shown to harbor insulator activity, and genome-wide localization studies have characterized numerous dCTCF insulator sites within the Antennapedia (Antp) complex and BX-C (Holohan et al. 2007). Only recently have Pirrotta and colleagues shown that interactions between copies of the BX-C *Fab-7* or *Mcp* elements are not dependent on PREs (Li et al. 2011). Importantly, both *Fab-7* and *Mcp* elements have been shown to harbor enhancer-blocking insulator activities (Gruzdeva et al. 2005, Zhou et al. 1996), and Li et al. (2011) now demonstrate that interactions depend on insulators flanking the *Mcp* and *Fab-7* PREs. A concurrent study independently revealed that the Su(Hw) insulator is capable of dictating PRE-target interactions through chromatin looping and topological constraint in *D. melanogaster* (Comet et al. 2011), consistent with earlier findings showing that the Su(Hw) insulator facilitates PRE-PRE contacts in *trans* (Sigrist & Pirrotta 1997). Interestingly, the maintenance of long-range interactions between copies of the *Fab-7* element also requires components of the RNAi machinery (Grimaud et al. 2006), including those required for insulator activity (Lei & Corces 2006), and recent mapping of RNAi component Argonaute2 (AGO2) shows specific colocalization with insulator proteins dCTCF and CP190 throughout the BX-C (Moshkovich et al. 2011).

Although insulators likely play an important role in previously described Pc interactions, Li et al. (2011) speculate that Pc complexes may contribute to the stability of physical interaction. In support of this theory, mutations in PcG proteins significantly reduce the level of *Antp* and *Abd-B* colocalization at Pc bodies (Bantignies et al. 2011). Meanwhile, current models suggest Pc mechanistically facilitates repression through PRC1-mediated chromatin compaction (Grau et al. 2011), and mapping of genome accessibility in *D. melanogaster* reveals that H3K27me3 domains are indeed the most inaccessible (Bell et al. 2010). Together, these findings suggest that insulators mediate the interactions between Pc targets and that PRCs likely strengthen the association and repression of Pc domains through histone modifications and chromatin compaction. In support of this model, RNAi depletion of CTCF and other *Drosophila* insulator proteins results in loss of H3K27me3 within associated Pc domains (Van Bortle et al. 2012), which suggests that insulators are important for maintaining appropriate chromatin architecture within these domains.

The interrogation of physical interactions between distant *Hox* loci has provided new and important insight into the regulation of long-range interactions during development. In *D. melanogaster*, the homeotic genes *Antp* and *Abd-B*, which are corepressed and colocalize

to Pc bodies in *Drosophila* embryo heads, are recruited to separate nuclear compartments when one gene becomes activated (Bantignies et al. 2011). In mammals, the *HoxD* cluster forms a single interaction domain in murine embryonic tissues in which all genes are inactive and transitions to a bimodal state in embryonic tissues in which *HoxD* genes are differentially expressed, with active genes segregated into an active domain (Noordermeer et al. 2011b). This suggests that developmentally regulated genes are dynamically targeted to specific nuclear subcompartments, such as Pc bodies and transcription factories, for transcriptional repression or activation. The apparent role of insulators in Pc contacts suggests that insulators are likely involved in gene localization to both transcriptionally repressive and transcriptionally permissive environments.

TRANSCRIPTION FACTORIES

The organization of transcription within eukaryotic nuclei is far more complex than traditional textbook models of polymerase recruitment and gene tracking. Instead, transcription is spatially organized into discernable nuclear structures in which multiple RNA polymerases and active genes dynamically localize into nuclear bodies termed transcription factories. The formation of transcriptionally active subcompartments presumably allows for more efficient transcription by concentrating the molecular players, reactants, and DNA substrates within a confined nuclear volume. Recent evidence suggests that transcription factories are highly conserved features of nuclear organization, that long-range chromosomal interactions are a hallmark of gene expression, and that insulators likely play an important role at transcription factories.

Composition and Evolution

Transcription is a fundamental cellular process carried out by highly conserved multisubunit RNA polymerases that share a high degree of homology in bacteria, archaea, and eukaryotes (Werner & Grohmann 2011). RNA polymerase I transcription of ribosomal genes is organized into a strongly conserved and highly organized nuclear substructure called the nucleolus (Thiry & Lafontaine 2005), which represents the classical example of transcriptional clustering into a factory structure. Meanwhile, most protein-coding genes are transcribed by RNA polymerase II (RNAPII), and several findings now support models proposing that RNAPII transcription occurs at analogous factories. The localization of transcription into discrete sites was initially identified by detection of nascent transcripts and by RNAPII staining, which revealed a limited number of foci unable to account for the number of active genes in human nuclei (Iborra et al. 1996). Subsequent studies further revealed that transcription factories are large and relatively immobile proteinaceous structures whose numbers vary by cell type and nuclear morphology (Chakalova & Fraser 2010) and that active genes dynamically localize to factories for expression in a transcription-dependent manner (Osborne et al. 2004). Recent genome-wide interaction assays described in this review also provide supporting evidence for the existence of transcription factories and further demonstrate that clustering of active genes is a highly conserved phenomenon. Hi-C modeling of chromosomal contacts reveals preferential clustering and interactions among actively transcribed genes and active chromatin domains in *S. pombe*, *Drosophila*, and humans (Sexton et al. 2012, Tanizawa et al. 2010, Yaffe & Tanay 2011).

How transcription factories are physically organized and how genes are dynamically targeted to them remains poorly understood. To this end, Cook and colleagues recently isolated transcription complexes from human nuclei and identified the proteome of RNAPI, II, and III factories by mass spectrometry (Melnik et al. 2011). Each complex was shown to harbor a characteristic set of unique proteins, though several proteins involved in DNA or RNA metabolism were shared. Whereas most proteins isolated from RNAPI complexes overlap those characteristic of nucleoli, RNAPII factories contain general transcription factors and

CTCF, consistent with the finding that CTCF underlies organization of coregulated genes in mammals. Interestingly, RNAPII factories also contain repressive histone methyltransferases, including PRC2-core component EZH2, which suggests that the transition from epigenetic repression to gene activation may not require the ejection of PcG proteins. In support of this possibility, Pc2 was recently shown to relocate from Pc bodies to transcription factories dependent on demethylase KDM4C (Yang et al. 2011). Targeting of Pc2 relies on ncRNAs, including *TUG1*, which interacts with PRC2 and acts as a scaffold at PcG bodies, and *NEAT2*, which associates with epigenetic regulators involved in gene activation. Taken together, genes likely are directed to repressive Pc bodies or active transcription factories through dynamic interplay of PcG and trxG proteins, whose long-range interactions require the function of ncRNAs and chromatin insulators. This model, recently highlighted by Pirrotta & Li (2011), is consistent with early findings in *D. melanogaster*, wherein mutations in both PcG and trxG genes were shown to modulate the activity and nuclear organization mediated by insulators (Gerasimova & Corces 1998).

Enhancer-Promoter Interactions

As the name suggests, enhancers are regulatory elements functionally defined by their ability to activate transcription and to do so regardless of their location, distance, or orientation with respect to gene promoters (Banerji et al. 1981). Enhancers underlie complex spatiotemporal regulation of tissue-specific gene expression and have been characterized by chromatin and transcription factor signatures in mammalian cells (Barski et al. 2007, Heintzman et al. 2007, Wang et al. 2008). Enhancers are commonly separated by large genomic distances from their associated promoters, which makes accurate assignments of enhancer-promoter relationships difficult and suggests that long-range interactions are a defining feature of gene regulation. Numerous 3C-based interaction studies have supported enhancer looping models wherein enhancers directly contact gene promoters for activation, and recent models suggest chromatin signatures at enhancers may act as epigenetic signals for transcriptional activation (Ong & Corces 2011). Interestingly, a distinct class of enhancer elements is also capable of recruiting RNAPII and is transcribed into enhancer-derived RNAs (Kim et al. 2010, Wang et al. 2011). The transcriptional output of neuronal activity-regulated enhancers positively correlates with the expression levels of associated genes (Kim et al. 2010), and alternative models of enhancer function have been proposed (Bulger & Groudine 2011), including ones in which enhancers and their associated promoters colocalize by virtue of recruitment to transcription factories.

Enhancer-promoter interactions and transcriptional clustering of active genes consistent with transcription factory models are further supported by ChIA-PET analyses enriching for RNAPII-based chromosomal interactions (Li et al. 2012). As many as 65% of RNAPII binding sites have been shown to be involved in a complex network of physical interactions in human cell lines. RNAPII interactions were intergenic (e.g., promoter-promoter), and extragenic (e.g., promoter-enhancer), with most contacts aggregated into ~1,500 interaction complexes. Multigene complexes typically consisted of related and coregulated genes, which suggests gene families functionally associate for cotranscription, whereas single-gene complexes tended to associate with tissue-specific or developmentally regulated genes and cell-type-specific enhancer-promoter interactions. The authors ultimately reveal a complex organization of transcription reflecting the importance of long-range chromatin interactions between coregulated promoters and between enhancers and promoters, possibly at transcription factories.

Nevertheless, the role of long-range enhancer-promoter interactions in eukaryotic gene activation and how these interactions are organized is not fully understood. Traditional models propose that enhancers underlie recruitment and assembly of the transcription

machinery at core promoters (Maston et al. 2006). However, in the case of the human *FOSL1* gene, histone cross talk between an enhancer and promoter triggers transcription elongation (Zippo et al. 2009), which suggests some enhancers may function by releasing RNAPII from promoter-proximal pausing. Meanwhile, recent characterization of chromatin-associated proteins at the human *α-globin* genes and upstream MCS-R2 enhancer suggest distal enhancers stimulate gene expression by reversing PcG activities (Vernimmen et al. 2011). The MCS-R2 enhancer is required for recruitment of H3K27 demethylase JMJD3, although active chromatin marks indicative of Trx activity (H3K4me3) were present in the absence of the enhancer. This supports a model wherein PcG and Trx complexes dynamically associate with target genes and enhancers promote gene induction by favoring Trx activity. This is consistent with ChIA-PET mapping of RNAPII contacts, which found high enrichment of active chromatin marks H3K4me1 and H3K4me3 coupled with a lack of repressive marks at RNAPII interaction sites (Li et al. 2012).

Recent studies have also provided new insight into how enhancers interact with distant target promoters to induce gene transcription and have suggested roles for transcription factors, chromatin insulators, and a unique cohesin complex in enhancer-promoter organization. In the case of the well characterized *β-globin* locus, whose expression levels are developmentally regulated by a distal upstream locus control region (LCR), long-range enhancer-promoter interactions require transcription factors EKLF and GATA-1 (Drissen et al. 2004, Vakoc et al. 2005), and genes coregulated by EKLF preferentially cluster into shared transcription factories (Schoenfelder et al. 2010). Ectopically integrated human LCR on a discrete chromosome in transgenic mice preferentially interacts in *trans* with EKLF- and GATA-1-regulated genes (Noordermeer et al. 2011a), which suggests transcription factors coordinate the specificity and organization of enhancer-promoter interactions. Through the powerful combination of 4C and FISH, Noordermeer et al. (2011a) also demonstrate that interchromosomal interactions between the LCR and *β-globin* genes are limited to specific "jackpot" cells actively transcribing *β-globin* genes, which suggests interactions are cell specific and reflect genome conformations that are conducive to enhancer-promoter association. In other words, enhancers preferentially interact with genes through shared transcription factors, but they do so stochastically in a restricted nuclear space that varies from cell to cell.

Insulators also play an important role in facilitating cell-type-specific chromatin organization conducive to enhancer-promoter interactions, and recent mapping of CTCF interactions in pluripotent cells supports this possibility (Handoko et al. 2011). CTCF-mediated tissue-specific chromatin architecture has been characterized at the apolipoprotein gene cluster; the imprinted *IGF2-H19* locus; and the developmentally regulated *IFNG*, *β-globin*, *MHC-II*, and *CFTR* loci (Gillen & Harris 2011, Ong & Corces 2011). However, recent findings suggest that cohesin complexes, which are recruited by CTCF, are also capable of stabilizing enhancer-promoter chromatin looping in the absence of CTCF. For example, cell-type-specific enhancer-promoter interactions in murine embryonic stem cells require a unique cohesin complex, including the transcriptional coactivator Mediator and cohesin loading factor nipped B-like protein (NIPBL) (Kagey et al. 2010). Cohesin and NIPBL were also recently shown to mediate interactions between the *β-globin* genes and upstream LCR, and whereas CTCF-coordinated organization at the locus does not directly influence gene expression (Splinter et al. 2006), the cohesin-mediated LCR interaction regulates globin gene expression in vivo and in vitro (Chien et al. 2011).

PERSPECTIVES

The power of chromatin profiling and 3C-based genomic strategies for exploring genome-wide interactions has led to substantial progress in our understanding of nuclear

organization over the past few years. Meanwhile, the recent identification of tDNA insulator activity in humans (Raab et al. 2012), mapping of CTCF-mediated chromosomal interactions in mammals (Handoko et al. 2011), and identification of genome-folding principles in human cells (Dixon et al. 2012, Lieberman-Aiden et al. 2009, Nora et al. 2012, Yaffe & Tanay 2011) and *Drosophila* embryos (Sexton et al. 2012) have significantly expanded our knowledge of insulator function and the highly conserved role of insulators in genome organization. The requirement for insulators in mediating long-range interactions essential for Pc repression (Li et al. 2011) and their localization to transcription factories (Melnik et al. 2011) suggest insulators underlie the dynamic interplay between epigenetic gene repression and gene induction associated with developmental gene regulation. These observations can be used to derive a comprehensive model of the role of insulators and chromatin structure in nuclear organization (**Figure 3**), with emphasis on the evolutionarily conserved role of insulators in yeast, *Drosophila*, and mammals; the molecular players involved in each; and their role in gene localization to PcG bodies and transcription factories. Despite substantial progress, several important questions remain, including how chromosomal associations and underlying insulator activities are regulated. The discovery of tDNA insulator activity in mammals raises the question of whether the highly conserved tDNA and CTCF insulators functionally cooperate. Finally, studies have only begun to characterize the new roles for ncRNAs in gene regulation and nuclear organization.

Interactions mediated by insulators, PcG proteins, and enhancer/promoter-bound factors result in the creation of a 3D arrangement

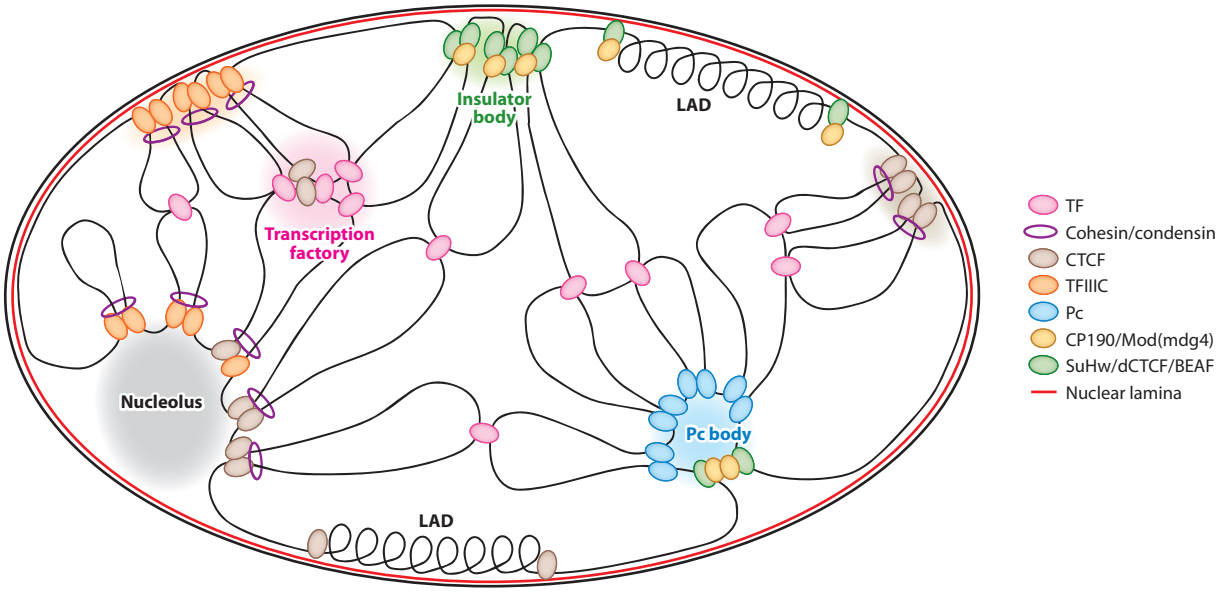

Figure 3

Comprehensive model for the highly conserved role of insulators in nuclear organization. Insulators in yeast (TFIIIC, *orange*), *Drosophila* [Su(Hw), dCTCF, BEAF, *green*; CP190, Mod(mdg4), *yellow*], and mammals (CTCF, brown; TFIIIC, *orange*) mediate long-range inter- and intrachromosomal interactions important for gene regulation and cluster into subnuclear foci called insulator bodies. Insulators underlie interactions necessary for Polycomb (Pc) body repression (*blue*) and localize with general transcription factors (TF, *pink*) to transcription factories. Insulators localize to subnuclear structures, including the nuclear lamina (*red*), where they are enriched at the borders of lamina-associated domains (LADs) and the nucleolus (*gray*). CTCF insulator activity in mammals requires cohesin (*purple*), and TFIIIC insulator sites are associated with both cohesin and condensin (*purple*). Insulator activity in *Drosophila* relies on recruitment of fly-specific proteins CP190 and Mod(mdg4).

of the DNA that must represent a fingerprint of the functional status of the nucleus. Therefore, a detailed understanding of all inter- and intrachromosomal interactions in the nucleus together with information on the nature and function of the interacting loci can lead to the establishment of structure-based functional maps of nuclear output that are a representation of cell identity. Some of these interactions may be a consequence of genome function, whereas others may be established during cell differentiation to elicit specific patterns of gene expression. As a consequence, the 3D architecture of the genetic material may carry epigenetic information in addition to that written into the 10-nm chromatin fiber. Understanding how this information is maintained during the cell cycle and how the 3D arrangement of chromosomes during interphase relates to their structure during mitosis remains a major challenge for the near future.

SUMMARY POINTS

1. Long-range interactions between regulatory elements often govern transcriptional regulation, in the form of both gene activation and repression, and are therefore a fundamental process underlying nuclear organization and genome function.
2. Chromatin insulators, including tRNA genes and transcription factor TFIIIC, appear to be highly conserved features involved in the nuclear organization of all eukaryotic genomes, from yeast to humans.
3. Insulators have outgrown the enhancer-blocking and barrier activities for which they were defined. Endogenous insulators facilitate long-range interactions that both mediate functional contacts between regulatory elements, including enhancer-promoter interactions, and correlate with topological domains enriched for coregulated genes. Insulators do not appear to be necessary to prevent the spread of heterochromatin, as proposed previously.
4. Colocalization of genes targeted by Pc group proteins for repression and maintenance of H3K27me3 within Pc domains relies on insulators. Insulator proteins also localize to transcription factories, which suggests a role in directing the localization of target genes to nuclear substructures for regulation.
5. Transcription factors and insulator-independent complexes also contribute to the organization of coregulated genes for coordinated expression, which suggests that nuclear organization and appropriate genome function depend on numerous additional factors.
6. Preliminary findings suggest that insulators can collaborate, perhaps to establish robust complexes capable of facilitating stable long-range interactions. Insulators also appear to be developmentally regulated by recruitment of both DNA-binding insulator proteins and additional cofactors.
7. Understanding how regulatory elements contribute to cell type–specific nuclear architecture, and how this information is maintained throughout the cell cycle, remains a major challenge for the near future.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Work in the author's lab is supported by US Public Health Service Award GM35463 from the National Institutes of Health.

LITERATURE CITED

Ahmed K, Dehghani H, Rugg-Gunn P, Fussner E, Rossant J, Bazett-Jones DP. 2010. Global chromatin architecture reflects pluripotency and lineage commitment in the early mouse embryo. *PLoS ONE* 5:e10531

Banerji J, Rusconi S, Schaffner W. 1981. Expression of a β-globin gene is enhanced by remote SV40 DNA sequences. *Cell* 27:299–308

Bantignies F, Roure V, Comet I, Leblanc B, Schuettengruber B, et al. 2011. Polycomb-dependent regulatory contacts between distant Hox loci in *Drosophila*. *Cell* 144:214–26

Barkess G, West AG. 2012. Chromatin insulator elements: establishing barriers to set heterochromatin boundaries. *Epigenomics* 4:67–80

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, et al. 2007. High-resolution profiling of histone methylations in the human genome. *Cell* 129:823–37

Bartkuhn M, Straub T, Herold M, Herrmann M, Rathke C, et al. 2009. Active promoters and insulators are marked by the centrosomal protein 190. *EMBO J.* 28:877–88

Beck DB, Bonasio R, Kaneko S, Li G, Margueron R, et al. 2010. Chromatin in the nuclear landscape. *Cold Spring Harb. Symp. Quant. Biol.* 75:11–22

Beisel C, Paro R. 2011. Silencing chromatin: comparing modes and mechanisms. *Nat. Rev. Genet.* 12:123–35

Bell O, Schwaiger M, Oakeley EJ, Lienert F, Beisel C, et al. 2010. Accessibility of the *Drosophila* genome discriminates PcG repression, H4K16 acetylation and replication timing. *Nat. Struct. Mol. Biol.* 17:894–900

Berezney R, Dubey DD, Huberman JA. 2000. Heterogeneity of eukaryotic replicons, replicon clusters, and replication foci. *Chromosoma* 108:471–84

Birney E, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeras TR, et al. 2007. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* 447:799–816

Bonchuk A, Denisov S, Georgiev P, Maksimenko O. 2011. *Drosophila* BTB/POZ domains of "ttk group" can form multimers and selectively interact with each other. *J. Mol. Biol.* 412:423–36

Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, et al. 2006. Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* 441:349–53

Bracken AP, Dietrich N, Pasini D, Hansen KH, Helin K. 2006. Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. *Genes Dev.* 20:1123–36

Branco MR, Pombo A. 2006. Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. *PLoS Biol.* 4:e138

Bulger M, Groudine M. 2011. Functional and mechanistic diversity of distal transcription enhancers. *Cell* 144:327–39

Bushey AM, Ramos E, Corces VG. 2009. Three subclasses of a *Drosophila* insulator show distinct and cell type-specific genomic distributions. *Genes Dev.* 23:1338–50

Cao R, Tsukada Y, Zhang Y. 2005. Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene silencing. *Mol. Cell* 20:845–54

Carriere L, Graziani S, Alibert O, Ghavi-Helm Y, Boussouar F, et al. 2012. Genomic binding of Pol III transcription machinery and relationship with TFIIS transcription factor distribution in mouse embryonic stem cells. *Nucleic Acids Res.* 40:270–83

Celniker SE, Dillon LA, Gerstein MB, Gunsalus KC, Henikoff S, et al. 2009. Unlocking the secrets of the genome. *Nature* 459:927–30

Chakalova L, Fraser P. 2010. Organization of transcription. *Cold Spring Harb. Perspect. Biol.* 2:a000729

Chambeyron S, Bickmore WA. 2004. Chromatin decondensation and nuclear reorganization of the HoxB locus upon induction of transcription. *Genes Dev.* 18:1119–30

Chien R, Zeng W, Kawauchi S, Bender MA, Santos R, et al. 2011. Cohesin mediates chromatin interactions that regulate mammalian β-globin expression. *J. Biol. Chem.* 286:17870–78

Chu C, Qu K, Zhong FL, Artandi SE, Chang HY. 2011. Genomic maps of long noncoding RNA occupancy reveal principles of RNA-chromatin interactions. *Mol. Cell* 44:667–78

Comet I, Schuettengruber B, Sexton T, Cavalli G. 2011. A chromatin insulator driving three-dimensional Polycomb response element (PRE) contacts and Polycomb association with the chromatin fiber. *Proc. Natl. Acad. Sci. USA* 108:2294–99

Cremer T, Cremer M. 2010. Chromosome territories. *Cold Spring Harb. Perspect. Biol.* 2:a003889

Cuddapah S, Jothi R, Schones DE, Roh TY, Cui K, Zhao K. 2009. Global analysis of the insulator binding protein CTCF in chromatin barrier regions reveals demarcation of active and repressive domains. *Genome Res.* 19:24–32

D'Ambrosio C, Schmidt CK, Katou Y, Kelly G, Itoh T, et al. 2008. Identification of *cis*-acting sites for condensin loading onto budding yeast chromosomes. *Genes Dev.* 22:2215–27

Dekker J, Rippe K, Dekker M, Kleckner N. 2002. Capturing chromosome conformation. *Science* 295:1306–11

Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, et al. 2012. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 485:376–80

Donohoe ME, Zhang LF, Xu N, Shi Y, Lee JT. 2007. Identification of a Ctcf cofactor, Yy1, for the X chromosome binary switch. *Mol. Cell* 25:43–56

Donze D, Adams CR, Rine J, Kamakaka RT. 1999. The boundaries of the silenced *HMR* domain in *Saccharomyces cerevisiae*. *Genes Dev.* 13:698–708

Donze D, Kamakaka RT. 2001. RNA polymerase III and RNA polymerase II promoter complexes are heterochromatin barriers in *Saccharomyces cerevisiae*. *EMBO J.* 20:520–31

Drissen R, Palstra RJ, Gillemans N, Splinter E, Grosveld F, et al. 2004. The active spatial organization of the β-globin locus requires the transcription factor EKLF. *Genes Dev.* 18:2485–90

Duan Z, Andronescu M, Schutz K, McIlwain S, Kim YJ, et al. 2010. A three-dimensional model of the yeast genome. *Nature* 465:363–67

Ebersole T, Kim JH, Samoshkin A, Kouprina N, Pavlicek A, et al. 2011. tRNA genes protect a reporter gene from epigenetic silencing in mouse cells. *Cell Cycle* 10:2779–91

Essafi A, Webb A, Berry RL, Slight J, Burn SF, et al. 2011. A Wt1-controlled chromatin switching mechanism underpins tissue-specific *Wnt4* activation and repression. *Dev. Cell* 21:559–74

Ficz G, Heintzmann R, Arndt-Jovin DJ. 2005. Polycomb group protein complexes exchange rapidly in living *Drosophila*. *Development* 132:3963–76

Filippova GN, Fagerlie S, Klenova EM, Myers C, Dehner Y, et al. 1996. An exceptionally conserved transcriptional repressor, CTCF, employs different combinations of zinc fingers to bind diverged promoter sequences of avian and mammalian c-*myc* oncogenes. *Mol. Cell. Biol.* 16:2802–13

Fraser P, Bickmore W. 2007. Nuclear organization of the genome and the potential for gene regulation. *Nature* 447:413–17

Gerasimova TI, Byrd K, Corces VG. 2000. A chromatin insulator determines the nuclear localization of DNA. *Mol. Cell* 6:1025–35

Gerasimova TI, Corces VG. 1998. Polycomb and trithorax group proteins mediate the function of a chromatin insulator. *Cell* 92:511–21

Gerasimova TI, Lei EP, Bushey AM, Corces VG. 2007. Coordinated control of dCTCF and *gypsy* chromatin insulators in *Drosophila*. *Mol. Cell* 28:761–72

Ghosh D, Gerasimova TI, Corces VG. 2001. Interactions between the Su(Hw) and Mod(mdg4) proteins required for gypsy insulator function. *EMBO J.* 20:2518–27

Gillen AE, Harris A. 2011. The role of CTCF in coordinating the expression of single gene loci. *Biochem. Cell Biol.* 89:489–94

Grau DJ, Chapman BA, Garlick JD, Borowsky M, Francis NJ, Kingston RE. 2011. Compaction of chromatin by diverse Polycomb group proteins requires localized regions of high charge. *Genes Dev.* 25:2210–21

Grimaud C, Bantignies F, Pal-Bhadra M, Ghana P, Bhadra U, Cavalli G. 2006. RNAi components are required for nuclear clustering of Polycomb group response elements. *Cell* 124:957–71

Grosberg A, Nechaev SK, Shakhnovich EI. 1988. The role of topological limitations in the kinetics of homopolymer collapse and self-assembly of biopolymers. *Biofizika* 33:247–53

Gruzdeva N, Kyrchanova O, Parshikov A, Kullyev A, Georgiev P. 2005. The Mcp element from the bithorax complex contains an insulator that is capable of pairwise interactions and can facilitate enhancer-promoter communication. *Mol. Cell. Biol.* 25:3682–89

Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, et al. 2008. Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. *Nature* 453:948–51

Gurudatta BV, Corces VG. 2009. Chromatin insulators: lessons from the fly. *Brief. Funct. Genomics Proteomics* 8:276–82

Hadjur S, Williams LM, Ryan NK, Cobb BS, Sexton T, et al. 2009. Cohesins form chromosomal *cis*-interactions at the developmentally regulated *IFNG* locus. *Nature* 460:410–13

Haldar D, Kamakaka RT. 2006. tRNA genes as chromatin barriers. *Nat. Struct. Mol. Biol.* 13:192–93

Handoko L, Xu H, Li G, Ngan CY, Chew E, et al. 2011. CTCF-mediated functional chromatin interactome in pluripotent cells. *Nat. Genet.* 43:630–38

Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, et al. 2007. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat. Genet.* 39:311–18

Holohan EE, Kwong C, Adryan B, Bartkuhn M, Herold M, et al. 2007. CTCF genomic binding sites in *Drosophila* and the organisation of the bithorax complex. *PLoS Genet.* 3:e112

Iborra FJ, Pombo A, Jackson DA, Cook PR. 1996. Active RNA polymerases are localized within discrete transcription "factories" in human nuclei. *J. Cell Sci.* 109(Pt. 6):1427–36

Ishihara K, Oshimura M, Nakao M. 2006. CTCF-dependent chromatin insulator is linked to epigenetic remodeling. *Mol. Cell* 23:733–42

Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, et al. 2010. Mediator and cohesin connect gene expression and chromatin architecture. *Nature* 467:430–35

Kim H, Kang K, Kim J. 2009. AEBP2 as a potential targeting protein for Polycomb Repression Complex PRC2. *Nucleic Acids Res.* 37:2940–50

Kim TH, Abdullaev ZK, Smith AD, Ching KA, Loukinov DI, et al. 2007. Analysis of the vertebrate insulator protein CTCF-binding sites in the human genome. *Cell* 128:1231–45

Kim TK, Hemberg M, Gray JM, Costa AM, Bear DM, et al. 2010. Widespread transcription at neuronal activity-regulated enhancers. *Nature* 465:182–87

Kim YJ, Cecchini KR, Kim TH. 2011. Conserved, developmentally regulated mechanism couples chromosomal looping and heterochromatin barrier activity at the homeobox gene A locus. *Proc. Natl. Acad. Sci. USA* 108:7391–96

Klenova EM, Nicolas RH, Paterson HF, Carne AF, Heath CM, et al. 1993. CTCF, a conserved nuclear factor required for optimal transcriptional activity of the chicken c-*myc* gene, is an 11-Zn-finger protein differentially expressed in multiple forms. *Mol. Cell. Biol.* 13:7612–24

Kruhlak MJ, Celeste A, Dellaire G, Fernandez-Capetillo O, Muller WG, et al. 2006. Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. *J. Cell Biol.* 172:823–34

Kurukuti S, Tiwari VK, Tavoosidana G, Pugacheva E, Murrell A, et al. 2006. CTCF binding at the *H19* imprinting control region mediates maternally inherited higher-order chromatin conformation to restrict enhancer access to *Igf2*. *Proc. Natl. Acad. Sci. USA* 103:10684–89

Kwong C, Adryan B, Bell I, Meadows L, Russell S, et al. 2008. Stability and dynamics of Polycomb target sites in *Drosophila* development. *PLoS Genet.* 4:e1000178

Lanzuolo C, Roure V, Dekker J, Bantignies F, Orlando V. 2007. Polycomb response elements mediate the formation of chromosome higher-order structures in the bithorax complex. *Nat. Cell Biol.* 9:1167–74

Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, et al. 2006. Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* 125:301–13

Lei EP, Corces VG. 2006. RNA interference machinery influences the nuclear organization of a chromatin insulator. *Nat. Genet.* 38:936–41

Li G, Margueron R, Ku M, Chambon P, Bernstein BE, Reinberg D. 2010. Jarid2 and PRC2, partners in regulating gene expression. *Genes Dev.* 24:368–80

Li G, Ruan X, Auerbach RK, Sandhu KS, Zheng M, et al. 2012. Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. *Cell* 148:84–98

Li HB, Muller M, Bahechar IA, Kyrchanova O, Ohno K, et al. 2011. Insulators, not Polycomb response elements, are required for long-range interactions between Polycomb targets in *Drosophila melanogaster*. *Mol. Cell. Biol.* 31:616–25

Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, et al. 2009. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326:289–93

Lisby M, Mortensen UH, Rothstein R. 2003. Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. *Nat. Cell Biol.* 5:572–77

MacPherson MJ, Beatty LG, Zhou W, Du M, Sadowski PD. 2009. The CTCF insulator protein is posttranslationally modified by SUMO. *Mol. Cell. Biol.* 29:714–25

Majumder P, Boss JM. 2011. Cohesin regulates MHC class II genes through interactions with MHC class II insulators. *J. Immunol.* 187:4236–44

Mao YS, Zhang B, Spector DL. 2011. Biogenesis and function of nuclear bodies. *Trends Genet.* 27:295–306

Margueron R, Reinberg D. 2011. The Polycomb complex PRC2 and its mark in life. *Nature* 469:343–49

Maston GA, Evans SK, Green MR. 2006. Transcriptional regulatory elements in the human genome. *Annu. Rev. Genomics Hum. Genet.* 7:29–59

Meister P, Mango SE, Gasser SM. 2011. Locking the genome: nuclear organization and cell fate. *Curr. Opin. Genet. Dev.* 21:167–74

Mekhail K, Moazed D. 2010. The nuclear envelope in genome organization, expression and stability. *Nat. Rev. Mol. Cell Biol.* 11:317–28

Melnik S, Deng B, Papantonis A, Baboo S, Carr IM, Cook PR. 2011. The proteomes of transcription factories containing RNA polymerases I, II or III. *Nat. Methods* 8:963–68

Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, et al. 2007. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* 448:553–60

Mirny LA. 2011. The fractal globule as a model of chromatin architecture in the cell. *Chromosome Res.* 19:37–51

Misulovin Z, Schwartz YB, Li XY, Kahn TG, Gause M, et al. 2008. Association of cohesin and Nipped-B with transcriptionally active regions of the *Drosophila melanogaster* genome. *Chromosoma* 117:89–102

Moon H, Filippova G, Loukinov D, Pugacheva E, Chen Q, et al. 2005. CTCF is conserved from *Drosophila* to humans and confers enhancer blocking of the Fab-8 insulator. *EMBO Rep.* 6:165–70

Moqtaderi Z, Struhl K. 2004. Genome-wide occupancy profile of the RNA polymerase III machinery in *Saccharomyces cerevisiae* reveals loci with incomplete transcription complexes. *Mol. Cell. Biol.* 24:4118–27

Moqtaderi Z, Wang J, Raha D, White RJ, Snyder M, et al. 2010. Genomic binding profiles of functionally distinct RNA polymerase III transcription complexes in human cells. *Nat. Struct. Mol. Biol.* 17:635–40

Moshkovich N, Nisha P, Boyle PJ, Thompson BA, Dale RK, Lei EP. 2011. RNAi-independent role for Argonaute2 in CTCF/CP190 chromatin insulator function. *Genes Dev.* 25:1686–701

Nagai S, Davoodi N, Gasser SM. 2011. Nuclear organization in genome stability: SUMO connections. *Cell Res.* 21:474–85

Nativio R, Wendt KS, Ito Y, Huddleston JE, Uribe-Lewis S, et al. 2009. Cohesin is required for higher-order chromatin conformation at the imprinted *IGF2-H19* locus. *PLoS Genet.* 5:e1000739

Negre N, Brown CD, Shah PK, Kheradpour P, Morrison CA, et al. 2010. A comprehensive map of insulator elements for the *Drosophila* genome. *PLoS Genet.* 6:e1000814

Negre N, Hennetin J, Sun LV, Lavrov S, Bellis M, et al. 2006. Chromosomal distribution of PcG proteins during *Drosophila* development. *PLoS Biol.* 4:e170

Noma K, Cam HP, Maraia RJ, Grewal SI. 2006. A role for TFIIIC transcription factor complex in genome organization. *Cell* 125:859–72

Noordermeer D, de Wit E, Klous P, van de Werken H, Simonis M, et al. 2011a. Variegated gene expression caused by cell-specific long-range DNA interactions. *Nat. Cell Biol.* 13:944–51

Noordermeer D, Leleu M, Splinter E, Rougemont J, De Laat W, Duboule D. 2011b. The dynamic architecture of *Hox* gene clusters. *Science* 334:222–25

Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, et al. 2012. Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* 485:381–85

Oktaba K, Gutierrez L, Gagneur J, Girardot C, Sengupta AK, et al. 2008. Dynamic regulation by Polycomb group protein complexes controls pattern formation and the cell cycle in *Drosophila*. *Dev. Cell* 15:877–89

Ong CT, Corces VG. 2011. Enhancer function: new insights into the regulation of tissue-specific gene expression. *Nat. Rev. Genet.* 12:283–93

Osborne CS, Chakalova L, Brown KE, Carter D, Horton A, et al. 2004. Active genes dynamically colocalize to shared sites of ongoing transcription. *Nat. Genet.* 36:1065–71

Parelho V, Hadjur S, Spivakov M, Leleu M, Sauer S, et al. 2008. Cohesins functionally associate with CTCF on mammalian chromosome arms. *Cell* 132:422–33

Paro R. 1990. Imprinting a determined state into the chromatin of *Drosophila*. *Trends Genet.* 6:416–21

Peric-Hupkes D, Meuleman W, Pagie L, Bruggeman SW, Solovei I, et al. 2010. Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. *Mol. Cell* 38:603–13

Phillips JE, Corces VG. 2009. CTCF: master weaver of the genome. *Cell* 137:1194–211

Pickersgill H, Kalverda B, de Wit E, Talhout W, Fornerod M, van Steensel B. 2006. Characterization of the *Drosophila melanogaster* genome at the nuclear lamina. *Nat. Genet.* 38:1005–14

Pirrotta V, Li HB. 2012. A view of nuclear Polycomb bodies. *Curr. Opin. Genet. Dev.* 22:101–9

Raab JR, Chiu J, Zhu J, Katzman S, Kurukuti S, et al. 2012. Human tRNA genes function as chromatin insulators. *EMBO J.* 31:330–50

Recillas-Targa F, Pikaart MJ, Burgess-Beusse B, Bell AC, Litt MD, et al. 2002. Position-effect protection and enhancer blocking by the chicken β-globin insulator are separable activities. *Proc. Natl. Acad. Sci USA* 99:6883–88

Richter C, Oktaba K, Steinmann J, Muller J, Knoblich JA. 2011. The tumour suppressor L(3)mbt inhibits neuroepithelial proliferation and acts on insulator elements. *Nat. Cell Biol.* 13:1029–39

Ruben GJ, Kirkland JG, MacDonough T, Chen M, Dubey RN, et al. 2011. Nucleoporin mediated nuclear positioning and silencing of *HMR*. *PLoS ONE* 6:e21923

Rubio ED, Reiss DJ, Welcsh PL, Disteche CM, Filippova GN, et al. 2008. CTCF physically links cohesin to chromatin. *Proc. Natl. Acad. Sci. USA* 105:8309–14

Sarma K, Margueron R, Ivanov A, Pirrotta V, Reinberg D. 2008. Ezh2 requires PHF1 to efficiently catalyze H3 lysine 27 trimethylation in vivo. *Mol. Cell. Biol.* 28:2718–31

Sawarkar R, Paro R. 2010. Interpretation of developmental signaling at chromatin: the Polycomb perspective. *Dev. Cell* 19:651–61

Schoenfelder S, Sexton T, Chakalova L, Cope NF, Horton A, et al. 2010. Preferential associations between co-regulated genes reveal a transcriptional interactome in erythroid cells. *Nat. Genet.* 42:53–61

Schuettengruber B, Ganapathi M, Leblanc B, Portoso M, Jaschek R, et al. 2009. Functional anatomy of Polycomb and trithorax chromatin landscapes in *Drosophila* embryos. *PLoS Biol.* 7:e13

Schwartz YB, Kahn TG, Nix DA, Li XY, Bourgon R, et al. 2006. Genome-wide analysis of Polycomb targets in *Drosophila melanogaster*. *Nat. Genet.* 38:700–5

Schwartz YB, Kahn TG, Stenberg P, Ohno K, Bourgon R, Pirrotta V. 2010. Alternative epigenetic chromatin states of Polycomb target genes. *PLoS Genet.* 6:e1000805

Schwartz YB, Linder-Basso D, Kharchenko PV, Tolsorukov MY, Kim M, et al. 2012. Nature and function of insulator protein binding sites in the *Drosophila* genome. *Genome Res.* In press

Scott KC, Merrett SL, Willard HF. 2006. A heterochromatin barrier partitions the fission yeast centromere into discrete chromatin domains. *Curr. Biol.* 16:119–29

Sexton T, Yaffe E, Kenigsberg E, Bantignies F, Leblanc B, et al. 2012. Three-dimensional folding and functional organization principles of the *Drosophila* genome. *Cell* 148:458–72

Sigrist CJ, Pirrotta V. 1997. Chromatin insulator elements block the silencing of a target gene by the *Drosophila* Polycomb response element (PRE) but allow *trans* interactions between PREs on different chromosomes. *Genetics* 147:209–21

Simon J, Chiang A, Bender W, Shimell MJ, O'Connor M. 1993. Elements of the *Drosophila* bithorax complex that mediate repression by Polycomb group products. *Dev. Biol.* 158:131–44

Simon JA, Kingston RE. 2009. Mechanisms of Polycomb gene silencing: knowns and unknowns. *Nat. Rev. Mol. Cell Biol.* 10:697–708

Simonis M, Klous P, Splinter E, Moshkin Y, Willemsen R, et al. 2006. Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C). *Nat. Genet.* 38:1348–54

Song L, Zhang Z, Grasfeder LL, Boyle AP, Giresi PG, et al. 2011. Open chromatin defined by DNaseI and FAIRE identifies regulatory elements that shape cell-type identity. *Genome Res.* 21:1757–67
Soshnev AA, He B, Baxley RM, Jiang N, Hart CM, et al. 2012. Genome-wide studies of the multi-zinc finger *Drosophila* Suppressor of Hairy-wing protein in the ovary. *Nucleic Acid Res.* 40:5415–31
Splinter E, Heath H, Kooren J, Palstra RJ, Klous P, et al. 2006. CTCF mediates long-range chromatin looping and local histone modification in the β-globin locus. *Genes Dev.* 20:2349–54
Tanizawa H, Iwasaki O, Tanaka A, Capizzi JR, Wickramasinghe P, et al. 2010. Mapping of long-range associations throughout the fission yeast genome reveals global genome organization linked to transcriptional regulation. *Nucleic Acids Res.* 38:8164–77
Thiry M, Lafontaine DL. 2005. Birth of a nucleolus: the evolution of nucleolar compartments. *Trends Cell Biol.* 15:194–99
Thompson M, Haeusler RA, Good PD, Engelke DR. 2003. Nucleolar clustering of dispersed tRNA genes. *Science* 302:1399–401
Tiwari VK, Cope L, McGarvey KM, Ohm JE, Baylin SB. 2008a. A novel 6C assay uncovers Polycomb-mediated higher order chromatin conformations. *Genome Res.* 18:1171–79
Tiwari VK, McGarvey KM, Licchesi JD, Ohm JE, Herman JG, et al. 2008b. PcG proteins, DNA methylation, and gene repression by chromatin looping. *PLoS Biol.* 6:2911–27
Tolhuis B, Blom M, Kerkhoven RM, Pagie L, Teunissen H, et al. 2011. Interactions among Polycomb domains are guided by chromosome architecture. *PLoS Genet.* 7:e1001343
Tolhuis B, de Wit E, Muijrers I, Teunissen H, Talhout W, et al. 2006. Genome-wide profiling of PRC1 and PRC2 Polycomb chromatin binding in *Drosophila melanogaster*. *Nat. Genet.* 38:694–99
Tolhuis B, Palstra RJ, Splinter E, Grosveld F, de Laat W. 2002. Looping and interaction between hypersensitive sites in the active β-globin locus. *Mol. Cell* 10:1453–65
Trojer P, Cao AR, Gao Z, Li Y, Zhang J, et al. 2011. L3MBTL2 protein acts in concert with PcG protein-mediated monoubiquitination of H2A to establish a repressive chromatin structure. *Mol. Cell* 42:438–50
Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, et al. 2010. Long noncoding RNA as modular scaffold of histone modification complexes. *Science* 329:689–93
Vakoc CR, Letting DL, Gheldof N, Sawado T, Bender MA, et al. 2005. Proximity among distant regulatory elements at the β-globin locus requires GATA-1 and FOG-1. *Mol. Cell* 17:453–62
Valenzuela L, Dhillon N, Kamakaka RT. 2009. Transcription independent insulation at TFIIIC-dependent insulators. *Genetics* 183:131–48
van Bemmel JG, Pagie L, Braunschweig U, Brugman W, Meuleman W, et al. 2010. The insulator protein SU(HW) fine-tunes nuclear lamina interactions of the *Drosophila* genome. *PLoS One* 5:e15013
Van Bortle K, Ramos E, Takenaka N, Yang J, Wahi J, Corces V. 2012. *Drosophila* CTCF tandemly aligns with other insulator proteins at the borders of H3K27me3 domains. *Genome Res.* In press
Vastenhouw NL, Zhang Y, Woods IG, Imam F, Regev A, et al. 2010. Chromatin signature of embryonic pluripotency is established during genome activation. *Nature* 464:922–26
Vernimmen D, Lynch MD, De Gobbi M, Garrick D, Sharpe JA, et al. 2011. Polycomb eviction as a new distant enhancer function. *Genes Dev.* 25:1583–88
Wang D, Garcia-Bassets I, Benner C, Li W, Su X, et al. 2011. Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. *Nature* 474:390–94
Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, et al. 2004. Role of histone H2A ubiquitination in Polycomb silencing. *Nature* 431:873–78
Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, et al. 2008. Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat. Genet.* 40:897–903
Wendt KS, Yoshida K, Itoh T, Bando M, Koch B, et al. 2008. Cohesin mediates transcriptional insulation by CCCTC-binding factor. *Nature* 451:796–801
Werner F, Grohmann D. 2011. Evolution of multisubunit RNA polymerases in the three domains of life. *Nat. Rev. Microbiol.* 9:85–98
Weth O, Renkawitz R. 2011. CTCF function is modulated by neighboring DNA binding factors. *Biochem. Cell Biol.* 89:459–68
Wiblin AE, Cui W, Clark AJ, Bickmore WA. 2005. Distinctive nuclear organisation of centromeres and regions involved in pluripotency in human embryonic stem cells. *J. Cell Sci.* 118:3861–68

Wilkinson FH, Park K, Atchison ML. 2006. Polycomb recruitment to DNA in vivo by the YY1 REPO domain. *Proc. Natl. Acad. Sci. USA* 103:19296–301

Woo CJ, Kharchenko PV, Daheron L, Park PJ, Kingston RE. 2010. A region of the human HOXD cluster that confers Polycomb-group responsiveness. *Cell* 140:99–110

Wood AM, Van Bortle K, Ramos E, Takenaka N, Rohrbaugh M, et al. 2011. Regulation of chromatin organization and inducible gene expression by a *Drosophila* insulator. *Mol. Cell* 44:29–38

Yaffe E, Tanay A. 2011. Probabilistic modeling of Hi-C contact maps eliminates systematic biases to characterize global chromosomal architecture. *Nat. Genet.* 43:1059–65

Yang L, Lin C, Liu W, Zhang J, Ohgi KA, et al. 2011. ncRNA- and Pc2 methylation-dependent gene relocation between nuclear structures mediates gene activation programs. *Cell* 147:773–88

Yao H, Brick K, Evrard Y, Xiao T, Camerini-Otero RD, Felsenfeld G. 2010. Mediation of CTCF transcriptional insulation by DEAD-box RNA-binding protein p68 and steroid receptor RNA activator SRA. *Genes Dev.* 24:2543–55

Yao S, Osborne CS, Bharadwaj RR, Pasceri P, Sukonnik T, et al. 2003. Retrovirus silencer blocking by the cHS4 insulator is CTCF independent. *Nucleic Acids Res.* 31:5317–23

Yusufzai TM, Tagami H, Nakatani Y, Felsenfeld G. 2004. CTCF tethers an insulator to subnuclear sites, suggesting shared insulator mechanisms across species. *Mol. Cell* 13:291–98

Zhao J, Ohsumi TK, Kung JT, Ogawa Y, Grau DJ, et al. 2010. Genome-wide identification of Polycomb-associated RNAs by RIP-seq. *Mol. Cell* 40:939–53

Zhou J, Barolo S, Szymanski P, Levine M. 1996. The Fab-7 element of the bithorax complex attenuates enhancer-promoter interactions in the *Drosophila* embryo. *Genes Dev.* 10:3195–201

Zippo A, Serafini R, Rocchigiani M, Pennacchini S, Krepelova A, Oliviero S. 2009. Histone crosstalk between H3S10ph and H4K16ac generates a histone code that mediates transcription elongation. *Cell* 138:1122–36

Zlatanova J, Caiafa P. 2009. CTCF and its protein partners: divide and rule? *J. Cell Sci.* 122:1275–84

Zullo JM, Demarco IA, Pique-Regi R, Gaffney DJ, Epstein CB, et al. 2012. DNA sequence-dependent compartmentalization and silencing of chromatin at the nuclear lamina. *Cell* 149:1474–87

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