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REVIEW

Interchromosomal interactions: A genomic love story of kissing chromosomes

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Nuclei require a precise three- and four-dimensional organization of DNA to establish cell-specific gene-expression programs. Underscoring the importance of DNA topology, alterations to the nuclear architecture can perturb gene expression and result in disease states. More recently, it has become clear that not only intrachromosomal interactions, but also interchromosomal interactions, a less studied feature of chromosomes, are required for proper physiological gene-expression programs. Here, we review recent studies with emerging insights into where and why cross-chromosomal communication is relevant. Specifically, we discuss how long noncoding RNAs (lncRNAs) and three-dimensional gene positioning are involved in genome organization and how low-throughput (live-cell imaging) and high-throughput (Hi-C and SPRITE) techniques contribute to understand the fundamental properties of interchromosomal interactions.

Introduction

How DNA is folded and organized in the nucleus is a critical aspect of gene regulation and in turn of cell-fate determinations. Recent technical advances, such as molecular (e.g., chromosome conformation capture) and microscopic imaging approaches have provided important insight into where and how DNA is differentially packaged in normal and disease states. The nucleus is organized into chromosomal territories: each chromosome occupies its own distinct territory in the nuclear space (Cremer and Cremer, 2010). Many recent studies have resolved the fundamental properties of how chromosomes "fold" intra-molecularly into highly organized structures, such as topologically associated domains (TADs) and gene-enhancer looping in cis, which are readily detectable with molecular and microscopic approaches (Barutcu et al., 2018; Maass et al., 2018b). On a molecular level, these topological features are guided by a molecular toolkit consisting of lncRNAs, CTCF, cohesin, and other chromatin-associated protein complexes (Parelho et al., 2008; Rinn and Chang, 2012; Rinn and Guttman, 2014; Vance and Ponting, 2014; Dekker and Mirny, 2016; Engreitz et al., 2016). Despite the progress in understanding and mapping of intrachromosomal interactions, the fundamentals of interchromosomal organization remain poorly characterized. Therefore, elucidating the underlying mechanisms that form interchromosomal interactions is critical to understand the formation of nuclear bodies, as well as the crosstalk between chromosomal territories and DNA elements that regulate gene expression and are part of the 3D genome organization.

More than a decade ago, "intermingling" or "kissing" chromosomes were observed by microscopy approaches as overlapping regions between chromosomal territories (Branco and Pombo, 2006; Cremer and Cremer, 2010). We will refer to these interchromosomal interactions as non-homologous chromosomal contacts (NHCCs). The best known NHCC formation is the preassembly of the ribosomes by the coalescence of ribosomal RNA genes, encoded across five different human chromosomes, to form the nucleolus, the largest subnuclear compartment (McStay, 2016). Similarly, olfactory receptor (OR) genes are located across several different chromosomes and undergo a complex choreography to conglomerate in the same nuclear space ("olfactosome") to regulate their expression (Lomvardas et al., 2006; Monahan et al., 2017, 2018).

In addition to the NHCCs forming the nucleolus and clusters of OR genes, NHCCs have been identified between defined genomic regions. For example, gene regulatory regions from one chromosome can activate a gene on another chromosome via NHCCs (Spilianakis et al., 2005). Moreover, lncRNA loci form NHCCs that affect gene-regulatory mechanisms in healthy and disease states (Maass et al., 2012; Hacisuleyman et al., 2014). In this paper, we will review studies identifying NHCCs in the context of a fundamental biological question: why and how do

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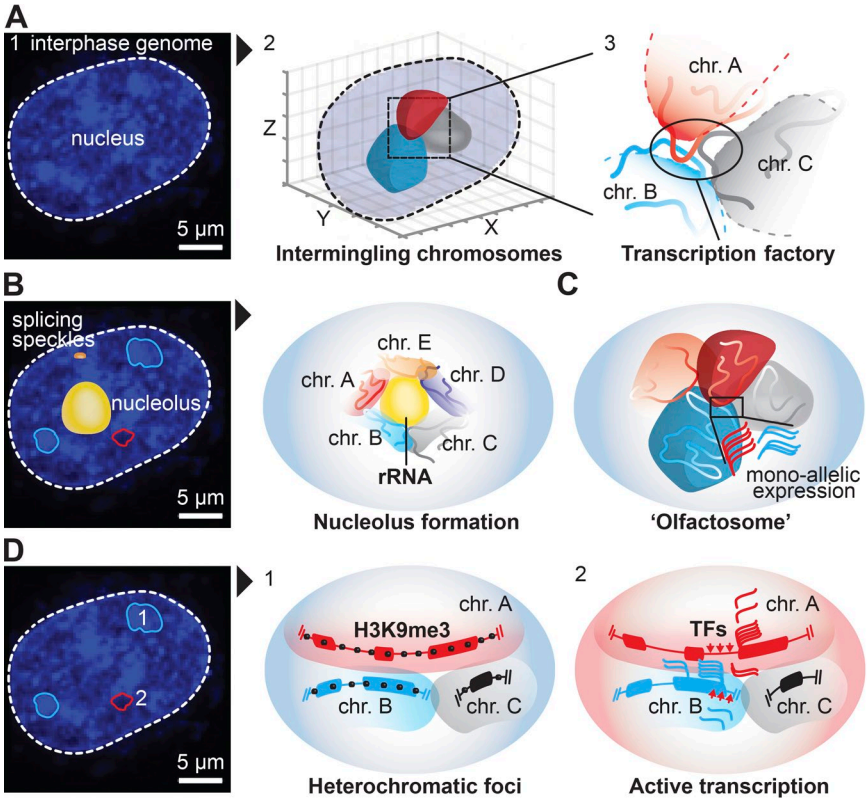

Figure 1. **Interchromosomal interactions are a substantial part of genome organization and biological processes. (A)** The interphase genome (1; Hoechst staining) consists of chromosomal territories (2) that share intermingling chromosomal regions in the 3D space of the nucleus. (3) Chromatin strands can loop out of their chromosomal territory and facilitate the formation of transcription factories. **(B)** The nucleus is organized in subnuclear compartments (e.g., speckles and nucleolus). In humans, five chromosomes are positioned around the nucleolus. **(C)** Intermingling chromosomal territories with highly specific NHCCs form the olfactosome to drive exclusive monogenic and mono-allelic OR gene expression. **(D)** Left: Heterochromatic foci appear as dense regions in the interphase genome, while dark (less dense) spots indicate less condensed chromatin. (1) Heterochromatic foci with H3K9me3 histone marks and silent gene loci can colocalize in the nucleus. (2) Transcription factors (TFs) facilitate the regulation of gene expression in tissue-specific transcription factories, where intermingling loci are in close spatial proximity.

nonhomologous chromosomes communicate? In particular, we will provide a historical background and discuss the most recent findings on how NHCCs of coding and noncoding loci add information to the genome organization and to the control of gene regulation.

Principles of chromosomal structure and nuclear organization

Chromosomes are nonrandomly organized in defined territories within the nucleus, and these territories exist across different taxonomic orders: yeast, plants, and mammals (Fig. 1 A; Abranches et al., 2000; Parada et al., 2002, 2004; Bolzer et al., 2005; Branco and Pombo, 2006; Noma et al., 2006; Cremer and Cremer, 2010; Fritz et al., 2014; Sehgal et al., 2016; Maass et al., 2018c). For example, FISH experiments in various cells revealed that chromosomal territory arrangements are conserved in different primates (Old and New World monkeys) and in humans, suggesting a functional relevance for the spatial organization of the higher-order chromosomal architecture (Tanabe et al., 2002, 2005; Bolzer et al., 2005). However, chromosome and locus positioning can vary within the same organism, as has been described for different mouse cell types (Mayer et al., 2005; Hepperger et al., 2008).

Chromosomes are further organized into A- and B-type genomic compartments that represent active and inactive chromatin domains, respectively. The A-type compartment is associated with higher gene density and early replication—vice versa for the B-type compartment (Lieberman-Aiden et al., 2009; Pope et al., 2014). Each of these compartment types can consist of multiple subcompartments defined by their differential histone marks (Rao et al., 2014), and the changes in genomic compartmentalization are associated with changes in transcriptional activity (Barutcu et al., 2015; Dixon et al., 2015). Interactions between the compartments may be the driving force for the large-scale genomic organization, such as the nuclear speckles (Chen et al., 2018) or NHCCs.

A well-studied aspect of the nuclear architecture includes TADs and the intrachromosomal interactions (i.e., between enhancers and promoters) within them (Dixon et al., 2012; Nora et al., 2012; Sanyal et al., 2012; Terakawa et al., 2017; Ganji et al., 2018). TADs are clusters of genomic interactions 100 kb to 1 Mb in size in mammalian genomes (Dixon et al., 2012; Nora et al., 2012). The intra-TAD interactions are highly variable between cell types, but previous studies have shown that TADs are highly invariant between cell types and species (Dixon et al., 2012, 2015). More recently, single-cell studies determined that these structures are highly variable within individual nuclei (Nagano et al., 2013, 2017; Ramani et al., 2017; Stevens et al., 2017). Studying structural genomic variations, such as the deletion of boundaries between two TADs, revealed that this can result in disease phenotypes in some cases (Lupiáñez et al., 2015; Franke et al., 2016; Taberlay et al., 2016). However, in other cases, these perturbations do not lead to phenotypic changes with biological significance (Barutcu et al., 2018), unless larger regions between 200 and 400 kb of DNA are deleted (Nora et al., 2012; Rodríguez-Carballo et al., 2017). The genomic organization and its interactions within the TAD structure are explained by a model called "loop-extrusion" that proposes how intrachromosomal interactions bring otherwise distal, regulatory regions (e.g., enhancers) into 3D proximity to target genes (Fudenberg et al., 2016). This model suggests that two cohesin/condensin molecules slide toward each other

while extruding the intervening DNA until two convergent CTCF sites are reached. The loop extrusion model has been shown to mediate intrachromosomal looping interactions and to form the majority of TADs (Fudenberg et al., 2016; Goloborodko et al., 2016; Ganji et al., 2018). A recent study has highlighted the possibility that large-scale genomic compartments can also be affected by the loop extrusion mechanism (Nuebler et al., 2018).

More recently, an emerging theme that explains the formation of cellular substructures via liquid-liquid phase separation has been proposed. Both DNA and RNA interact with proteins that harbor low-complexity regions (van der Lee et al., 2014; Protter et al., 2018) and form liquid, gel, or solid aggregates that may shape and compartmentalize the genome (Erdel and Rippe, 2018; Langdon et al., 2018; Maharana et al., 2018).

While great progress in understanding the intramolecular dynamics of chromosomal structure on a genomic scale have been made, there are several outstanding questions. What are the mechanisms of intrachromosomal interactions and, more importantly, of NHCCs? Are there common or distinctive molecular features between these two different classes of genomic interactions? We are beginning to understand the molecular mechanisms governing the principles of genome architecture, especially of intrachromosomal interactions, at an unprecedented pace. However, our understanding about the formation and the function of NHCCs is still in its infancy compared with other aspects in chromatin biology. Indeed, several studies have shown examples of NHCCs, as they are detectable in many contexts (Bolzer et al., 2005; Spilianakis et al., 2005; Branco and Pombo, 2006; Lomvardas et al., 2006; Maass et al., 2012, 2018b,c; Hacisuleyman et al., 2014; Horta et al., 2018; Quinodoz et al., 2018), yet their function remains elusive.

Kissing chromosomes: NHCCs

The first notion of NHCCs in the nucleus may have come from Carl Rabl, who, based on studies in sea urchins, proposed in 1885 that chromosomes occupy defined volumes at defined positions, and that they interact with neighboring chromosomes. Between 1902 and 1904, the married biologists Theodor Boveri and Marcella O'Grady Boveri studied the equine round worms *Parascaris univalens* and *Parascaris equorum* and postulated that chromosomal territories are stably arranged during interphase (Satzinger, 2008; Strickfaden et al., 2010). Their findings were validated by laser-UV-microbeam experiments in the 1980s (Cremer et al., 1982) and by radiosensitive and fluorescent replication labeling of neighboring chromosomal subdomains (Zink et al., 1998; Visser and Aten, 1999). Furthermore, it was shown that chromatin regions with high gene density and expression levels can extend from their chromosomal territory to colocalize at the interchromosomal space (Mahy et al., 2002); likewise, a mathematical model describes the probability that individual chromatin strands can pass through one another, thereby validating that direct interactions between different chromosomes are mathematically possible (Blackstone et al., 2011).

Among various subnuclear structures identified in the nucleus (Misteli et al., 1997; Lamond and Earnshaw, 1998; Pederson, 2002, 2011; Cremer and Cremer, 2010; Spector and Lamond, 2011), one of the most well-known and large-scale phenomena of NHCCs is the formation of the nucleolus. In human nuclei, ∼300 ribosomal genes located on five different acrocentric chromosomes (six in mouse) come into physical proximity to build the ribosomal preassembly in the nucleus (Fig. 1 B; Németh et al., 2010; Pliss et al., 2015; McStay, 2016). This spatial formation of the nucleolus is a conserved phenomenon and validates that nonhomologous chromosomes can intermingle in a nonrandom manner in all nuclei.

A structure equally as fascinating is the OR gene cluster, in which individual NHCCs allow the expression of single ORs in each cell to create a diverse repertoire of OR expression at the tissue level. At any given time, only a few of the ∼1,400 OR genes located on 18 different chromosomes converge in the same interchromosomal space (Horta et al., 2018). The regulation of OR genes is orchestrated by binding of Ldb1, Lhx2, and Ebf transcription factors to highly similar transcription factor motifs of multiple enhancers on different chromosomes, thereby leading to nondeterministic mono-allelic OR gene expression (Lomvardas et al., 2006; Markenscoff-Papadimitriou et al., 2014; Monahan and Lomvardas, 2015; Monahan et al., 2017, 2018). Remarkably, the monogenic and mono-allelic gene expression of OR genes is explained by the spatial clustering of inactive genes to the same heterochromatic foci in the olfactosome (Fig. 1, C and D; Clowney et al., 2012). Recent in situ Hi-C experiments of FACS-sorted, differentiated olfactory sensory neurons determined that, at very large scales (i.e., 500-kb resolution), NHCCs between OR genes are highly specific and frequent, and that they consist of multiple different chromosomes to regulate selectively and specifically the transcription of each individual OR gene (Horta et al., 2018).

NHCCs affect distinct transcriptional programs of biological pathways

At higher resolution, NHCCs have been observed between specific enhancer and gene targets. For example, the formation of an NHCC results in expression of cytokines in T cell types and also *IFNγ* expression in response to viral infection (Spilianakis et al., 2005; Apostolou and Thanos, 2008). Specifically, the promoter region of *IFNγ* on chromosome 10 is in physical proximity to regulatory regions of the Th2 cytokine locus on chromosome 11 to coordinate gene expression in a cell type–specific dynamic manner (Spilianakis et al., 2005). Taken together, these and other findings that we will discuss indicate that nonrandom NHCCs occur in homogenous cell populations to contribute to transcriptional regulation. For example, the formation of interchromosomal gene-specific regulatory events leads to the ability to smell or results in cytokine expression, underscoring the importance of NHCCs in diverse biological pathways. One common feature of these biological pathways, where NHCCs are involved, is that they occur in systems of variegated gene expression and seem to occur mostly in a cell type–specific fashion.

Furthermore, genomic interactions appear to be influenced by chromosomal location and transcription (Gandhi et al., 2012; Krueger et al., 2012). Human chromosomes share similar positions in interphase and prophase of the cell cycle (Chen et al., 2017), and the spatial positioning of genes and NHCCs in the 3D nucleus can be important for their transcriptional regulation (Kosak and Groudine, 2004; Maass et al., 2012). For instance,

genes that are either actively transcribed or silent, are spatially recruited to subnuclear hubs, such as transcription factories and splicing speckles, consistent with the observation that experimental repositioning of chromosomes leads to gene expression changes (Finlan et al., 2008).

Gene positioning and NHCCs are also important during larval development in *Drosophila melanogaster*, where heterochromatin-mediated transcriptional silencing is due to discrete spatial proximity in the nucleus (Dernburg et al., 1996). In addition, mitotic processes, such as chromatin decondensation, and transcriptional activation can influence the genomic architecture and cause gene repositioning (Therizols et al., 2014). Indeed, the formation of NHCCs between early histone genes was accompanied by repositioning of gene loci toward the interior nucleus during the highest transcriptional activity in sea urchins (Matsushita et al., 2017). Similarly, a correlation between transcriptional activity and chromosomal intermingling occurs during differentiation of mouse embryonic stem cells (mESCs; Maharana et al., 2016). Evidence that stochasticity is involved in NHCC formation was described in the *Drosophila* eye, where the photoreceptor choice is driven by DNA elements that control allelic expression via NHCCs (Johnston and Desplan, 2014). Also, during myogenic differentiation, cell type–specific genes on different chromosomes have been shown to cluster in the nuclear space (Harada et al., 2015), and recently, a sequential FISH approach showed that sites of active transcription tend to interact more with other chromosomes than with sites along the same chromosome, supporting the idea that NHCCs are more frequent than previously appreciated (Shah et al., 2018). Collectively, these examples demonstrate a broad repertoire of interplay across chromosomes to establish cell-specific expression programs.

NHCCs and nuclear bodies

It has been proposed that a possible common feature of NHCCs can be the formation of subnuclear structures, such as the nucleolus. These subnuclear compartments can present transcriptional factories where many active genes from multiple chromosomes are brought into proximity to maintain their activated states (Sutherland and Bickmore, 2009; Ferrai et al., 2010; Edelman and Fraser, 2012; Papantonis and Cook, 2013). These transcription factories can take place between expression-dependent loci in cis (intrachromosomal; Tolhuis et al., 2002) or between NHCCs where transcriptionally active loci preferentially contact active rather than silent loci (Fig. 1; Brown et al., 1997; Osborne et al., 2004, 2007; Spilianakis et al., 2005; Ling et al., 2006; Lomvardas et al., 2006; Simonis et al., 2006; Zhao et al., 2006; Maass et al., 2012, 2018b; Hacisuleyman et al., 2014). Another well-studied example of a transcription factory is when active globin genes cooperate with hundreds of other transcribed genes both intrachromosomally and interchromosomally for efficient and coordinated transcriptional control (Schoenfelder et al., 2010). Molecular mechanisms are actively involved in forming NHCCs: for example, the genome organizer CTCF can generate a mono-allelic NHCC between the imprinted H19 locus/*Igf2* and *Wsb1/Nf1* (Ling et al., 2006).

Therefore, from transcription factories of related gene types to finer resolution of specific gene loci that are in physical proximity within the 3D space of the nucleus, it is becoming clear that NHCCs represent important regulatory interactions.

Interchromosomal contacts between homologous chromosomes (transvection)

Similar to contacts between nonhomologous chromosomes, nonmeiotic transvection between homologous chromosomes is another layer of epigenetic regulation to activate or repress genes. Transvection was microscopically observed in 1908 (Stevens, 1908), and the well-established example of interacting bithorax complexes in *Drosophila* (Lewis, 1954) is studied in the current era by live imaging (Lim et al., 2018). These homologous chromosomal contacts occur at the sites of DNA double-strand breaks (Gandhi et al., 2012). It was also shown in mESCs that DNA elements at the *Oct4* promoter/enhancer locus mediate pairing of the *Oct4* alleles, and perturbation of the Oct4/Sox2 binding sites at these elements leads to the disappearance of allele pairing (Hogan et al., 2015). Also, X-chromosome inactivation is accompanied by a transient physical interaction of both of the X-inactivation centers (Bacher et al., 2006), and the well-studied lncRNA *XIST* participates in the homologous chromosome pairing during X-chromosome inactivation (Marahrens, 1999).

However, the principle mechanisms of transvection and their impact on gene regulation and genome organization are not well understood and are out of the scope of this review about nonhomologous chromosomal contacts.

Toward identifying NHCCs with molecular techniques

The current understanding of nuclear organization derives from many technologies that have been developed to investigate the organizational features of chromatin and DNA packaging on a genomic scale. Multiple methods in both imaging (e.g., FISH and CRISPR live cell imaging [CLING]) and molecular (e.g., Hi-C and SPRITE) approaches probed the properties of chromosomal structure (Lieberman-Aiden et al., 2009; Maass et al., 2018a,b; Quinodoz et al., 2018). The advantage of imaging approaches is that they reveal cell-to-cell chromatin conformations, while the molecular methods assess the genome structure across a cell population.

The advent of chromatin conformation capture (3C) methods and their next-generation sequencing approaches (Dekker et al., 2002; Dekker, 2006; Ea et al., 2015; Barutcu et al., 2016), single-molecule imaging, and polymer simulations (Barbieri et al., 2013; Fudenberg and Imakaev, 2017) has rapidly expanded research in the genome organization field and resulted in a deeper understanding of genomic interactions. A plethora of advanced 3C-based methods, such as 4C (Simonis et al., 2006; Würtele and Chartrand, 2006; Zhao et al., 2006), 5C (Dostie et al., 2006), Hi-C (Lieberman-Aiden et al., 2009), ChIA-PET (Fullwood et al., 2009), capture Hi-C (Ma et al., 2015b), and the more recent genome architecture mapping, a ligation-free method to probe genomic interactions (Beagrie et al., 2017), resolve DNA–DNA interactions on a genome scale to unseal the entire intrachromosomal folding properties of each chromosome (Bickmore, 2013; Dekker and Misteli, 2015; Fraser et al., 2015; Barutcu et al., 2016; Bonev and Cavalli, 2016). More recently, improved Hi-C techniques measured interaction dynamics across individual nuclei

within single nuclei (Nagano et al., 2013; Ramani et al., 2017; Stevens et al., 2017).

Surprisingly, many of the known NHCCs such as the nucleolus are not readily identified in many of the currently used 3C-based technical approaches. Apart from cell-to-cell variability, one reason for the limited evidence of functional NHCCs is most likely a bias of capturing predominantly intrachromosomal interactions rather than interchromosomal contacts in genome-wide 3C-based techniques (e.g., Hi-C; Maass et al., 2018b). This bias has been further compounded by modifying the existing 3C methodology. As the majority of genomic interactions arise from the insoluble fraction of the 3C material (Gavrilov et al., 2013), two independent groups have devised the in situ ligation protocol for 3C/Hi-C, which further enriches for intrachromosomal interactions over interchromosomal interactions (Rao et al., 2014; Nagano et al., 2015; Allahyar et al., 2018).

Some of these limitations have been mitigated by a more recent technique termed engineered DNA-binding molecule-mediated chromatin immunoprecipitation (enChIP) in combination with next-generation sequencing (enChIP-Seq; Fujita et al., 2016). enChIP leverages locus-specific CRISPR targeting to probe for nearby DNA interactions. For example, applying enChIP-Seq to target the globin genes during erythroid differentiation revealed multiple NHCCs occurring in close physical proximity. Interestingly, a majority of these NHCCs contained transcriptionally active genes (Fujita et al., 2017). Another recent advancement for high-throughput identification of NHCCs is the split-pool recognition of interactions by tag extension (SPRITE) approach. SPRITE facilitates the detection of higher-order interactions occurring within the same nucleus at a new dimension (Quinodoz et al., 2018). Importantly, SPRITE robustly detects DNA–DNA as well as DNA–RNA NHCCs that form the nucleolus. Furthermore, it demonstrates that the nuclear bodies (splicing speckles, nucleolus, etc.) act as organizational hubs and scaffolds, where especially NHCCs are critical to shape the 3D chromatin folding and transcriptional programs. Furthermore, SPRITE also offers the opportunity to probe loci-specific interactions relative to different nuclear bodies inside the nucleus. Together, these recent technological advances are beginning to provide a genome-wide map of NHCCs.

Location matters for NHCCs in health and in disease

In addition to a role for NHCCs in coordinating nuclear organization in healthy states, where genomic loci and their interactions (intrachromosomal and NHCCs) reside in the nucleus has important implications for disease; for example, the ability to confidently phenotype different cancer subtypes based on changes in genome organization (Meaburn et al., 2009). Moreover, proper locus positioning and the occurrence of frequent functional NHCCs can be drawn from the detection of recurrent balanced or unbalanced chromosomal translocations that occur often in rare disease and in ∼40% of all cancers (Shaffer and Pandolfi, 2006). These translocations shed light on the genomic organization and NHCCs, since spatial proximity of chromosomal territories is associated with tissue-specific translocation prevalence (Parada et al., 2004; Branco and Pombo, 2006).

Deciphering where and when intrachromosomal and interchromosomal rearrangements appear can be tracked by induced DNA double-strand breaks in cell populations with high-throughput approaches. Although intrachromosomal translocations were found to be enriched in these studies, several hotspots for NHCCs suggest that 3D proximity of NHCCs in the nucleus can cause interchromosomal rearrangements, such as translocations, between nonhomologous chromosomes (Chiarle et al., 2011; Klein et al., 2011). Thus, the preexisting spatial proximity between chromosomal territories in the 3D nucleus is also related to recurrent translocations (Zhang et al., 2012; Sklyar et al., 2016). Finally, the direct correlation of cancer-associated translocations with Hi-C contacts (Engreitz et al., 2012), the reduction of NHCCs of gene-rich chromosomes in breast cancer cells compared with normal cells (Barutcu et al., 2015), and altered NHCCs due to a disease-associated deletion of *HDAC4* (Maass et al., 2018c) indicate that rearrangements of the 3D genome architecture directly affect biological and pathogenic pathways. Moreover, anchors of long-range DNA loops have been associated with increased torsional stress that can lead to chromosomal breaks (Canela et al., 2017). As a whole, these examples suggest that the subnuclear positioning of chromosomal subdomains is critical to support specific gene expression programs for developmental and disease processes.

LncRNAs are involved in the 3D organization of NHCCs

While increasing evidence points to a pivotal role of noncoding RNAs (ncRNAs) in nuclear organization (Rinn and Chang, 2012; Rinn and Guttman, 2014; Vance and Ponting, 2014), the functional contribution of different species of ncRNAs in the assembly and regulation of long-range chromatin contacts, both intrachromosomal and of NHCCs, still remains poorly understood. So far, several lncRNAs have been determined to actively establish the nuclear architecture. Specifically, splicing speckle formation is associated with functional lncRNAs as mediators of nuclear organization. For example, the lncRNA *NEAT1*, in conjunction with the lncRNA *MALAT1*, can assemble a splicing speckle, which itself does not contain DNA, rather several DNA loci come in close proximity to process newly transcribed RNA (Ferrai et al., 2010; Spector and Lamond, 2011; Vera and Singer, 2014). ncRNAs can bind to multiple molecules and act as molecular scaffolds to shape the genome (Zappulla and Cech, 2006). Additionally, they can regulate looping interactions by binding to several RNA-binding proteins (RBPs) and/or chromatin modifiers (Hendrickson et al., 2016), thereby providing scaffolds for RNA–protein complexes that interact with and shape DNA organization (Santos-Pereira and Aguilera, 2015).

Dissecting the NHCCs of the lncRNA loci *Firre* and *CISTR-ACT* (Maass et al., 2012; Hacisuleyman et al., 2014), has provided insights into the involvement of lncRNAs in 3D chromosomal intermingling. The functional intergenic repeating RNA element (*Firre*, also known as *linc-RAP-1*) is a lncRNA locus that was detected in a loss-of-function screen as being required for proper adipogenesis (Sun et al., 2013). *Firre* is encoded on chromosome X, but it escapes X chromosome inactivation and interacts with the nuclear matrix factor hnRNPU (also known as SAF-A), a known mediator of genome organization (Nozawa et al., 2017; Fan et al., 2018). The *Firre* locus has an interesting property of forming NHCCs with several loci on nonhomologous mouse

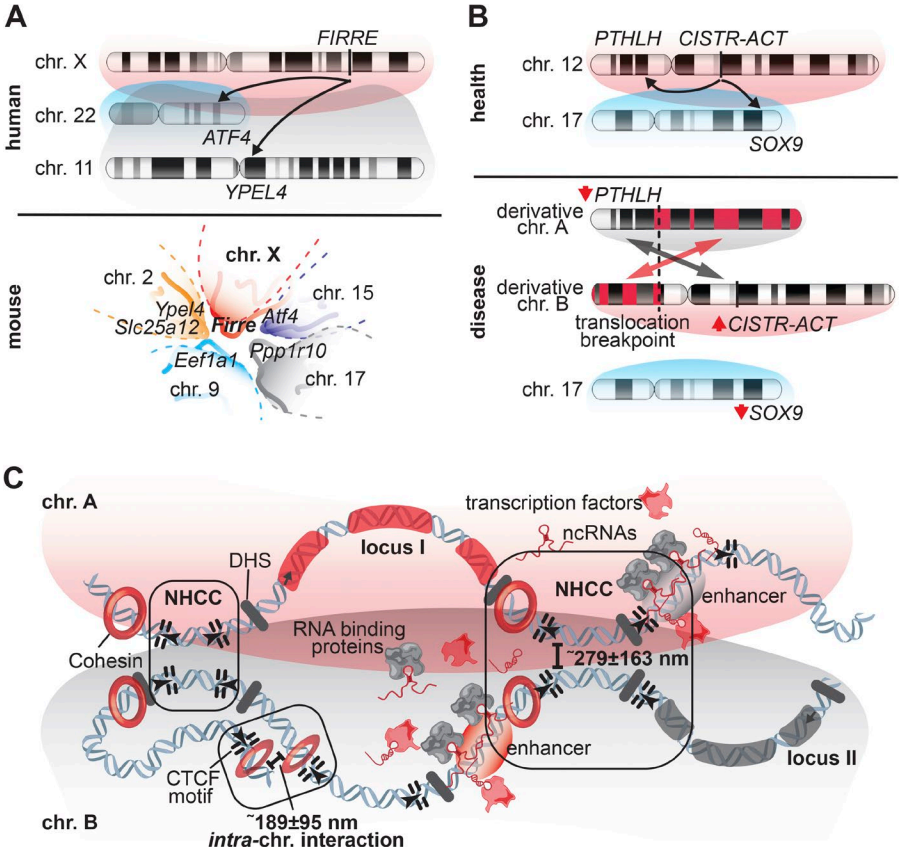

Figure 2. **LncRNA loci and their NHCCs, and mechanistic principles of NHCCs. (A)** The lncRNA locus *FIRRE* interacts with *ATF4* and *YPEL4* in human cells. This 3D organization is conserved in the mouse genome, where *Firre* also interacts with *Slc25a12*, *Eef1a1*, and *Ppp1r10*. **(B)** The regulatory lncRNA locus *CISTR-ACT* facilitates 3D proximity to *PTHLH*, and the NHCC with *SOX9*, in normal cells. When balanced translocations disrupt this interaction and misplace *CISTR-ACT* onto a derivative chromosome, the positional effect leads to down-regulated *PTHLH* and *SOX9* and up-regulated *CISTR-ACT*. **(C)** CLING determined that NHCCs have an average proximity of ∼279 ± 163 nm, while intrachromosomal interactions were 189 ± 95 nm apart. DNase I hypersensitivity sites (DHS) and convergent CTCF motifs are features of gene regulatory regions (loop-extrusion model) that are in spatial proximity to cooperate with tissue-specific transcription factors, ncRNAs, and RBPs to regulate the expression of genes that are located on different chromosomes (see transcription factory in Fig. 1 A).

chromosomes; moreover, *Firre* RNA is bound at different chromosomes, identified by RNA affinity purification and RNA-FISH in mESCs (Hacisuleyman et al., 2014). More recently, the human *FIRRE* locus was found to make conserved NHCCs, with at least two of these NHCCs in human and mouse (Fig. 2 A; Maass et al., 2018b). The *Firre* locus is also implicated in shaping nuclear architecture, by anchoring the inactive X chromosome to the perinucleolar space (Yang et al., 2015). In addition to a possible role of *FIRRE* in forming NHCCs and tethering them to the nucleolar periphery is the interesting notion that the *FIRRE* locus harbors one of the highest densities of conserved CTCF motifs at its locus (Barutcu et al., 2018). Deleting this CTCF-rich *Firre* locus preserves its TAD boundary structure. Nevertheless, neither the *Firre* DNA sequence, promoter, nor CTCF motifs are required for this TAD boundary structure, as these elements may comprise a molecular toolkit of RNA and protein to establish and/or maintain NHCCs (Barutcu et al., 2018).

The cis- and trans-acting lncRNA locus *CISTR-ACT* is encoded on human chromosome 12 and forms a specific NHCC with the loci of the chondrogenic morphogenesis gene *PTHLH* on the same chromosome (cis) and with the transcription factor *SOX9* on chromosome 17 (trans) to regulate chondrogenic gene expression (Fig. 2 B; Maass et al., 2012, 2018c). The physical disruption of this regulatory landscape by balanced translocations misplaces the *CISTR-ACT* locus to a derivative translocation chromosome, causing dysregulation of *CISTR-ACT*, *PTHLH*, and *SOX9*, thereby leading to the congenital cartilage malformation chondrodysplasia brachydactyly type E (shortened fingers and extremities; Maass et al., 2012). Labeling the entire chromosomes 12 and 17, as well as the individual *CISTR-ACT* and *SOX9* loci, revealed that these two chromosomal territories frequently interact in a recurrent pattern, and although these chromosomal patterns are stable across different cell types, tissue-specific NHCCs occurred at the level of the individual gene loci (Maass et al., 2018c). These results support the concept that defined genomic loci come into 3D proximity to drive gene regulation in a highly specific manner. Any physical disruption of a tissue-specific transcription factory (Melnik et al., 2011) causes local reorganization of the genome, resulting in altered transcriptional programs that affect developmental programs and may cause disease (Fig. 2 B; Maass et al., 2018c). These findings indicate that the positioning of individual lncRNA loci may be a specific nonrandom feature in the 3D nuclear space, required to fulfill important functions.

Together, the *FIRRE* and *CISTR-ACT* lncRNA loci exemplify the emerging concept that noncoding loci with distinct features—multispecies conservation, DNase I hypersensitivity sites, open chromatin marks, enrichment of CTCF motifs and transcription factor binding sites, and noncoding transcription—can shape nuclear organization by facilitating the colocalization of euchromatic features between multiple nonhomologous chromosomes (Fig. 2 C). *FIRRE* and *CISTR-ACT* form specific NHCCs, and the fact that the interchromosomal contacts of *FIRRE* are CTCF/cohesin-independent suggests that gene regulation by intra- versus interchromosomal interactions operates by distinct, yet potentially overlapping mechanisms. Interestingly, albeit different gene order and content of linear orthologous sequences in different species, a spatial conservation of some NHCCs seems to exist (Chambers et al., 2013). It remains to be determined how many

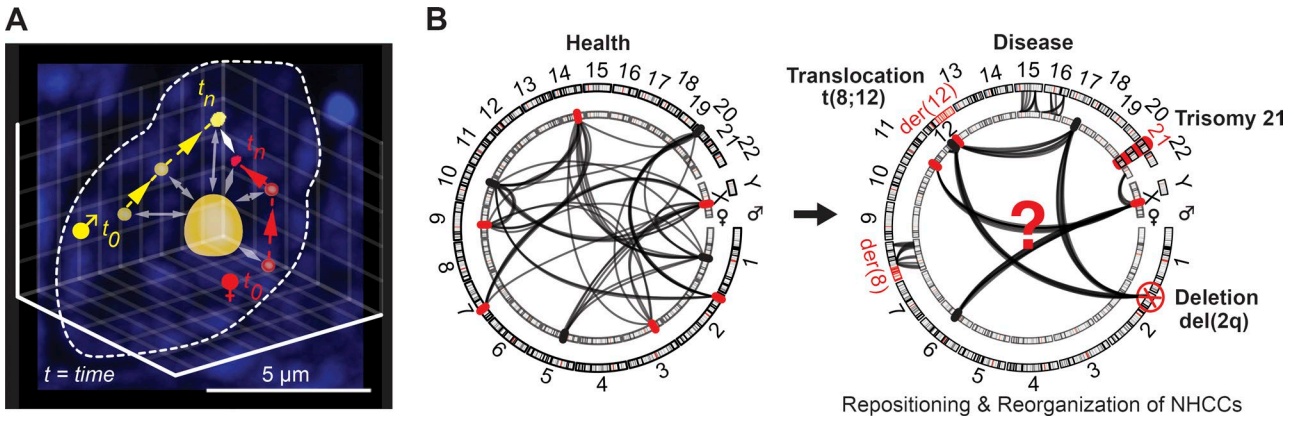

Figure 3. **Exploring NHCCs toward understanding their contribution to gene regulation and genome organization in health and disease. (A)** SNP-CLING enables the study of allele-specific locus positioning and spatiotemporal dynamics in living cells (depicted example: mESC with exemplified time-lapse imaging of a maternal and paternal allele and their distances to the nucleolus). **(B)** Left: Maternal and paternal alleles of the interphase genome are in physical proximity and intermingle to control tissue-specific gene regulation in the 3D space of the nucleus. LncRNAs, proteins (chromatin modifiers, transcription factors, etc.), biophysical properties of the chromatin, genome organization, and stochastics cooperate to contribute to variable, but specific, biological processes. Right: Structural (i.e., deletions, translocations, etc.) and numerical chromosomal aberrations (e.g., trisomies) can disrupt and reorganize the intricate network of NHCCs. These aberrations cause altered transcriptional programs, repositioning of genomic loci, and reorganization of tissue-specific gene regulation that can influence genetic and developmental processes. They comprise a variable layer of genome organization and are often associated with pathogenic pathways and disease.

other lncRNA loci are involved in NHCCs and contribute to the 3D organization of the genome. More studies on NHCCs, compared with intrachromosomal regulatory processes, will decipher if similar or different regulators (transcription factors, chromatin modifying complexes, and CTCF and cohesin, etc.) provide the platform for interchromosomal communication.

Watching kissing chromosomes in real time: live-cell imaging of NHCCs

Fixation-based in situ methods, such as FISH-related and 3C-based approaches, are limited in their capabilities (Hoffman et al., 2015), since they cannot address the spatiotemporal dynamics of intra- or interchromosomal interactions. Therefore, live-cell imaging techniques are highly advantageous to understand the spatiotemporal chromatin dynamics in non–cross-linked living cells and to explore the spatial dimensions of NHCCs. Toward this goal, numerous studies have proven the principle of live-cell imaging of DNA using CRISPR-Cas technologies (Chen et al., 2013; Deng et al., 2015; Ma et al., 2015a, 2016b,a; Shechner et al., 2015; Fu et al., 2016; Shao et al., 2016; Wang et al., 2016; Guan et al., 2017; Qin et al., 2017; Takei et al., 2017; Zhou et al., 2017; Maass et al., 2018a,b; Wu et al., 2018). Collectively, these studies have established the principle that modification to guide sequences can target and label specific DNA loci that can be monitored in living cells.

One recent study leveraged CLING to measure the fundamental NHCC properties of two lncRNA loci. Specifically, CLING was applied to the previously determined NHCCs mediated by the *FIRRE* and *CISTR-ACT* loci to explore NHCCs and mechanisms of genome organization in living cells (Dekker, 2016; Fudenberg and Imakaev, 2017; Maass et al., 2018b). This revealed several fundamental properties of NHCCs. (a) NHCCs occur in a majority of the cell populations (>50%); and (b) NHCCs are stable over time. One possible way NHCCs could be missed is if they were in constant flux of movement. However, this is not the case, as they remain in close proximity over a substantial time period of the cell cycle. (c) Consistent with their stable proximity, NHCCs exhibit less tortuosity or tumble more slowly in the nucleus. (d) A substantial difference between intra- and interchromosomal interactions is the distance at which they occur. Specifically, the spatial distances for intrachromosomal interactions were found in the range of 189 ± 95 nm, and for NHCCs (*FIRRE* and *CISTR-ACT*), these interactions occurred at a larger distance (279 ± 163 nm; Maass et al., 2018b). Thus, in living cells, the NHCCs are frequent, stable, less mobile, and similar to intra-molecular interactions.

A fundamental feature of NHCCs is that one allele interacts with another one (Johnston and Desplan, 2014; Monahan and Lomvardas, 2015). However, this does not necessarily mean that NHCCs always represent interactions between both alleles. More often, in CLING experiments, only one allele of two different chromosomes interacts (~40%), rather than both alleles forming NHCCs (~17%; Maass et al., 2018b). This raises the interesting question whether NHCCs are nonrandom with respect to parental origin. For example, are NHCCs formed between paternal–paternal, maternal–maternal, or paternal–maternal chromosomes? Or do they have a random combination, that is, based on a determined order of the genomic architecture (Nagele et al., 1995; Weise et al., 2016)? Addressing this question would require either a molecular or imaging technique that distinguishes between the contacts of parental alleles in an allele-specific manner.

A very recent study developed and proved the principle of an allele-specific CRISPR-Cas9–based imaging approach, termed single nucleotide polymorphism (SNP)–CLING. In short, this approach leverages SNPs that can be used to target CRISPR-Cas9 constructs specifically to either the maternal or the paternal allele (Fig. 3 A). Applying SNP-CLING to the NHCC of *Firre* and *Ypel4* revealed a slightly shifted NHCC distribution toward the maternal–paternal combination (Maass et al., 2018a). Thus, the possibility that 3D imprinting exists and contributes to cell

type–specific gene expression still remains unresolved. Overall, SNP-CLING is a powerful approach to understand how specific alleles of chromosomes are positioned relative to each other and with respect to nuclear subcompartments, such as the nucleolus. Furthermore, for the first time, SNP-CLING allows the study of heterozygous states of genetic disease in living cells by distinguishing between healthy and affected alleles.

Collectively, these results show that genomic loci of the noncoding genome are actively involved in the 3D formation of NHCCs. Several different loci on nonhomologous chromosomes may share the same spatial hub, which can be conserved across species to regulate transcriptional programs. Therefore, studying genomic noncoding regions (enhancer and ncRNAs) by molecular high-throughput methods and imaging approaches provides immense potential to understand the formation and significance of NHCCs.

Perspective

The coordinated regulation of multiple genes within specific transcriptional programs requires physical proximity between genomic loci, either on the same chromosome or across different chromosomes. To accomplish defined transcriptional regulation, transcriptional hubs or factories can be formed around NHCCs in distinct nuclear locations. Specifically, subnuclear domains of NHCCs could emerge from phase transitions. This is seemingly the case for two of the larger subnuclear structures, the nucleolus and the olfactosome. Perhaps lncRNA loci and other NHCCs are also phase-state transitions, comprised of lncRNAs, RBPs, CTCF, and many other factors coalescing in physical proximity. For example, cohesin, CTCF, and transcription factors that are bound to loci on different chromosomes through RNA–protein interactions are the bridging factors that keep these loci in physical proximity (Fig. 2 C). In this scenario, the loop extrusion complexes on two different chromosomes would extend the DNA in the same spatial hub, similarly to an intrachromosomal interaction, to retain the chromatin strands of two different chromosomes in proximity (Fig. 2 C).

As presented in this review, increasing evidence supports a role for NHCCs in the establishment of proper gene-regulatory networks. Yet our understanding of NHCC's impact on the nuclear architecture and of how repositioning of genomic loci and reorganization of tissue-specific gene regulation influences genetic processes and disease progression is still in its infancy (Fig. 3 B). Structural and numerical genomic aberrations add a variable dimension of genomic organization to both normal and disease states.

The reviewed results suggest that NHCCs could favor diverse, but recurrent and important, stochastic interactions that add an important informational layer to tissue-specific gene regulation. NHCCs, the biophysical properties of chromatin, and stochastics contribute to variable, but specific, gene regulation that supports monogenic and also mono-allelic expression of genes in a coordinated manner. Collectively, the examples covered here demonstrate that NHCCs account for a multitude of biological processes.

Moving forward, it is critical to elucidate the molecular mechanisms underlying NHCCs. Specifically, the role of regulatory noncoding DNA and lncRNA loci and the biophysical features underlying the regulation and formation of NHCCs will facilitate the identification of tissue-specific interchromosomal spatial hubs and broaden our current knowledge of gene regulation and of NHCCs. Exploring loci positioning and the spatial proximities of those loci interacting with regulatory DNA and RNA sequences will provide crucial insights into nuclear organization and etiologies therein.

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