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Current Opinion in Genetics & Development

The transcriptional interactome: gene expression in 3D

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Transcription in the eukaryotic nucleus has long been thought of as conforming to a model in which RNA polymerase complexes are recruited to and track along isolated templates. However, a more dynamic role for chromatin in transcriptional regulation is materializing: enhancer elements interact with promoters forming loops that often bridge considerable distances and genomic loci, even located on different chromosomes, undergo chromosomal associations. These associations amass to form an extensive 'transcriptional interactome', enacted at functional subnuclear compartments, to which genes dynamically relocate. The emerging view is that long-range chromosomal associations between genomic regions, and their repositioning in the three-dimensional space of the nucleus, are key contributors to the regulation of gene expression.

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Current Opinion in Genetics & Development 2010, **20**:127–133

This review comes from a themed issue on
Chromosomes and expression mechanisms
Edited by Renato Paro and Jeannie T. Lee

Available online 6th March 2010

0959-437X/$ – see front matter

DOI 10.1016/j.gde.2010.02.002

Introduction

Almost two centuries after its discovery, it is clear that the eukaryotic nucleus is a highly organized organelle, with more than ten specialized subnuclear compartments described [1]. Chromatin itself is organized in a dynamic continuum of structuring that scales from chromosome territories [2,3] through higher order folding of chromatin domains [4,5] to accessibility of the chromatin fibre [6,7]. Chromosome territories do not possess rigid boundaries, and neighboring chromosomes can intermingle [8], offering the possibility for long-range regulatory contacts and functional compartmentalization among distal or unlinked genomic regions [9]. A combination of chromosome conformation capture (3C) technologies [10] and microscopy have catalyzed the discovery of long-range chromosomal interactions in a variety of cellular processes, including transcription [11–14,15•,16•,17•], recombination [18,19], Polycomb mediated gene silencing [20,21,22], and X chromosome inactivation [23,24]. These findings suggest that functional intrachromosomal and interchromosomal associations are at the heart of many genome functions. In this review, we focus on long-range intrachromosomal and interchromosomal interactions and associations involved in RNA polymerase (RNAP) II transcription, which form the 'transcriptional interactome'.

From a distance: long-range enhancer-promoter interactions

Regulatory DNA elements such as enhancers or locus control regions (LCRs) can act over considerable genomic distances. The *Hbb* LCR is found in close spatial proximity to its target genes in erythroid cells, causing the intervening 50 kb of DNA sequences to loop out [4,5]. Similar, tissue-specific chromosomal associations between genes and regulatory elements have been detected at many loci in the genome, including the *Kit* [25], *H19/Igf2* [26,27], and T helper 2 (T_H2) cytokine loci [28,29]. Genomic distance does not appear to be an obstacle, as the Sonic hedgehog (Shh) limb bud-specific enhancer has been shown to interact with its target promoter one megabase away [30•]. These examples are likely to be the tip of the iceberg: Genome-wide association studies have identified many disease-linked single nucleotide polymorphisms (SNPs) that map, often some distance, outside of annotated genes, indicating potential regulatory function [31]. For example, the SNP rs6983267, associated with increased risk of colorectal cancer, is located in a gene desert at human chromosome 8q24 [32]. The region surrounding rs6983267 acts as an enhancer in reporter gene assays [33] and interacts with the promoter of the *Myc* oncogene, located ~330 kb away [34].

Increasing numbers of examples suggest that regulatory DNA elements also seem capable of undergoing functional contacts with genes located on other chromosomes. In naïve T lymphocytes, the T_H2 LCR, located on chromosome 11, interacts with the interferon-γ gene on chromosome 10 [12]. In sensory neurons, the H enhancer element contacts multiple olfactory receptor genes on different chromosomes, and its interaction with a single gene in a given sensory neuron has been proposed to determine the choice of olfactory receptor gene expression [35]. However, deletion of the H element does not affect the expression of odorant receptor genes in *trans* [36]. While these conflicting findings may be reconciled by the existence of redundant H-like enhancer elements, further analysis is clearly required. The imprinting control region upstream of the *H19* gene also engages in long-range contacts, although there is considerable discrepancy about the number of interacting loci, ranging from three

[37] to over one hundred [38]. Importantly, deletions or point mutations introduced into regulatory elements affect the expression of interacting genes on different chromosomes [12,38]. As a whole, this evidence points to a functional crosstalk between distal chromosomal regions, potentially expanding the regulatory capacity of the genome to a great extent.

Stand by me: co-associations of active genes at shared transcription factories

Co-associations of active genes at shared subnuclear compartments, such as transcription factories, may represent another class of chromosomal interactions. In this case, rather than DNA elements engaging directly in intrachromosomal and interchromosomal associations, it appears likely that the genomic loci simply co-associate with shared specialized subnuclear microenvironments to take advantage of, and potentially contribute to, increased local concentrations of specific factors required for gene expression. Transcription factories are highly enriched in the active, hyper-phosphorylated forms of RNAPII [39,40]. RNA FISH studies have shown that transcription of individual alleles occurs almost exclusively in association with transcription factories [11,14,41]. By contrast, temporarily inactive alleles are positioned away from transcription factories, suggesting that genes migrate to these subnuclear sites in order to be transcribed [42]. Crucially, the number of transcription factories per cell is severely limited compared to the number of expressed genes, compelling genes to share the same transcription factory [11]. A genome-wide screen for sequences that share transcription factories with the transcriptionally active mouse alpha-globin and beta-globin genes revealed preferential associations with hundreds of other transcribed loci, identifying extensive intrachromosomal and interchromosomal transcription networks [43••]. Among the globin-interacting loci, genes regulated by the erythroid transcription factor Klf1 were overrepresented. Further investigation revealed Klf1-regulated genes preferentially clustered at a limited number of transcription factories containing high levels of Klf1, suggesting that individual factories could become specialized hotspots for the transcription of a preferential network of genes (Figure 1a). These results support the finding that episomal reporter constructs with similar promoters have a greater tendency to cluster at shared transcription factories than constructs with heterologous promoters [44]. At present it is not known whether three-dimensional clustering of similarly regulated genes at transcription factories is a cause or consequence of the specific transcription factor-enriched microenvironment. However it appears that these specialized factories are optimized sites with increased probability of transcriptional initiation and re-initiation of preferential networks of genes [43••].

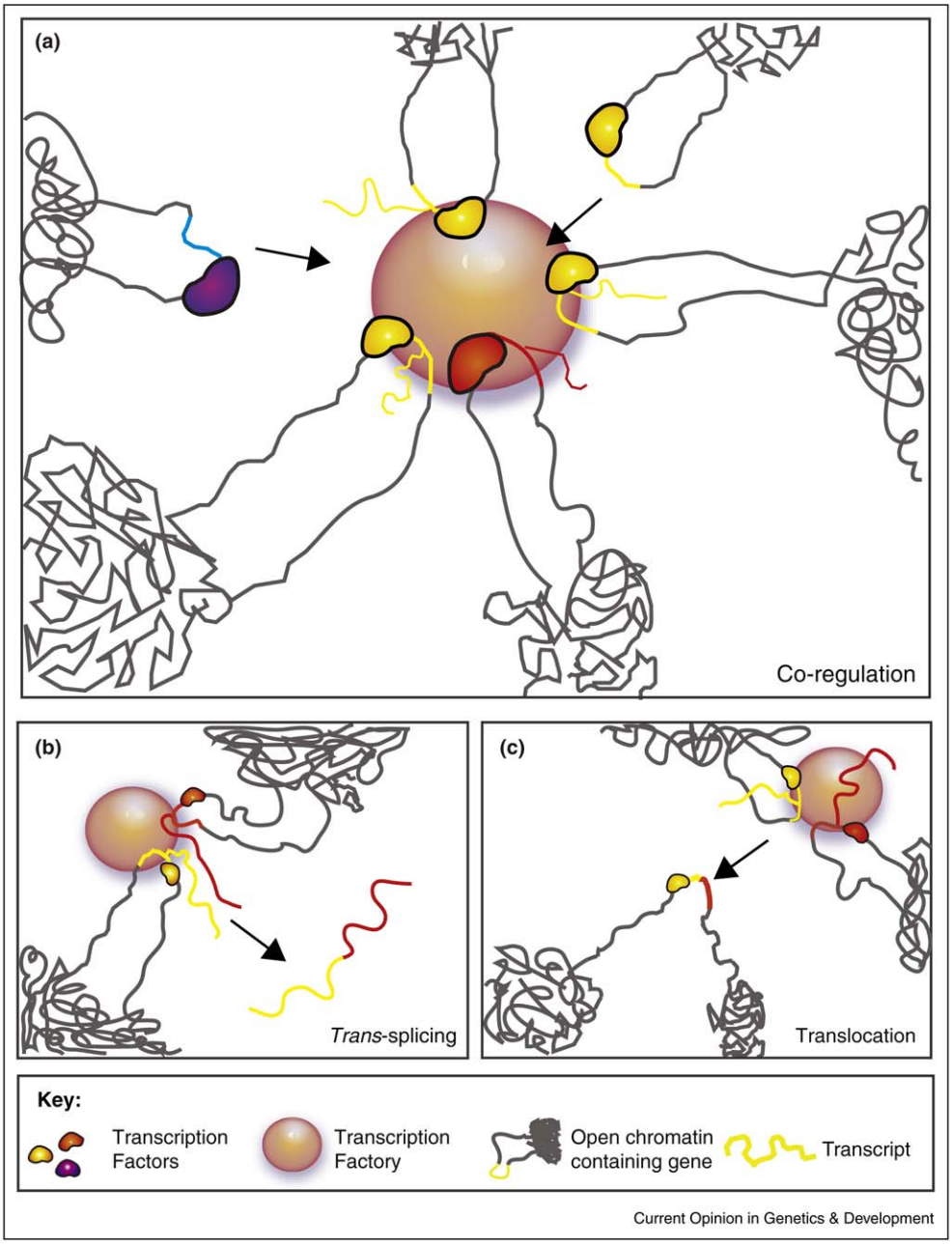
Juxtaposition of active genes has also been observed at nuclear speckles [45,46], large subnuclear domains marked by the splicing factor Sc-35. As transcription and splicing are not only temporarily, but also spatially tightly linked [47,48], it is conceivable that these associations are a consequence of transcriptional co-associations between active genes at transcription factories. Currently, experimental evidence to support a functional role for Sc-35 speckles in gene co-associations, such as genetic ablation or RNAi knockdown of Sc-35, or *in vivo* disassembly of nuclear speckles [49], is missing. By contrast, accumulating data support the concept of transcription-factor mediated associations between active genes (see below).

Hold me close: protein factors required for long-range chromatin interactions

Several studies have implicated transcription factors in the establishment of three-dimensional active chromatin conformations, thus expanding their classical textbook function. For example, the erythroid transcription factors Klf1 [50] and GATA-1 [51] are required for the tissue-specific active chromatin conformation at the *Hbb* LCR. GATA-2 fulfills a related function at the *Kit* locus [25], and contacts between the T_H2 LCR and promoters of protein-coding genes in the locus require the transcription factors GATA3 and STAT6 [28]. Notably, transcription-factor mediated interactions are not confined to the establishment of 'local' chromatin associations required for gene activation. Estrogen receptor α (ERα) bound genomic regions form a chromatin 'interactome' of primarily intrachromosomal interactions [17•], but ERα also mediates interchromosomal interactions [16•]. Similarly, genomic loci bound by the androgen receptor (AR) undergo intrachromosomal and interchromosomal associations [52••]. In response to viral infection, specific interactions between NF-kB bound genomic sites have been observed [15•]. Finally, intrachromosomal and interchromosomal associations between Klf1-regulated genes at transcription factories are specifically disrupted in erythroid cells lacking Klf1 [43••]. Thus, accumulating evidence suggests that transcription factors influence the establishment of local active chromatin conformations as well as the three-dimensional positioning of active genes and their chromosomal associations in the nucleus.

Proteins involved in chromatin architecture have also been implicated in mediating interactions between chromosomal regions. For example, at the mouse T_H2 cytokine locus, SATB1 mediates associations between regions in *cis* to generate a three-dimensional, active chromatin configuration [29]. The *H19* imprinting control region associates with multiple genomic loci, mainly via its maternal allele that binds the chromatin insulator protein CTCF [38]. Interestingly, recent studies have revealed that binding of CTCF and cohesin, a protein complex previously known for its essential role in sister chromatid cohesion [53], overlap at many sites across the human and mouse genomes [54,55]. Cohesin and CTCF cooperate to

Figure 1



Proximity of active genes in a shared transcription factory. (**a**) Co-regulated genes cluster in a specialized transcription factory. Transcription factors (yellow, red, and blue) bind their target genes while probing their nuclear environment. Upon relocation to a transcription factory, potentiated genes initiate transcription (nascent transcripts depicted in yellow and red). Dynamically bound transcription factors may dissociate from their target genes, freeing transcription factors for use by other co-regulated genes in close proximity. Thus, genes in a factory with other co-regulated genes may have a higher probability of re-initiation in that factory through dynamic exchange of transcription factors, stabilizing their presence there. By contrast, genes transcribing in the absence of other network partners (genes regulated by red and blue factors) may be more likely to dissociate from the factory after an initial burst of transcription. Repetition of factor dissociation and binding cycles would result in a transcription site highly enriched in specific binding sites and factors, seemingly specialized to preferentially transcribe a subset of co-regulated genes. (**b**) Close proximity between transcripts generated in a transcription factory may allow specific exons to be joined by *trans*-splicing. (**c**) Juxtaposition of active genes in a shared transcription factory may also increase the probability of translocations between loci.

mediate chromatin interactions in *cis* at the human *IFNG*, *APO A1/C3/A4/A5* and *H19/Igf2* gene loci [56•,57•,58]. It is tempting to speculate that cohesin utilizes its ability to hold chromosomal regions together for the establishment and/or maintenance of other intrachromosomal, and potentially interchromosomal, associations.

Do the loco-motion: movement of chromosomal loci in the nucleus

How do genomic regions 'find' each other and/or nuclear compartments in the complex nuclear environment, in order to establish chromosomal associations? In general, chromatin motion is regionally constrained in the nucleus [59]. However, this does not exclude the possibility that genomic regions probe their nuclear environment by Brownian motion over relatively short distances, with subsequent stabilization of preferred associations. Active, directed long-range chromatin movements have also been reported. Targeting of a transcriptional activator to a transgene array resulted in relocation from the nuclear periphery to the interior, over distances of up to 5 μm [60]. Upon transcriptional induction, movements over 2–3 μm toward a Cajal body were observed for an U2 snRNA transgene array [61]. Interestingly, actin [60,61] and myosin [60] have been implicated in these chromatin movements. Similarly, the interchromosomal association between the estrogen-regulated *TFF1* and *GREB1* genes depends on actin, nuclear myosin I, and the dynein light chain-1 (DLC1) [16•], and interference with actin polymerization or nuclear myosin I function abolished interchromosomal interactions between the androgen receptor bound *TMPRSS2* and *ETV1* genes [52••]. Numerous studies have observed a role for nuclear actin and myosin in transcription [62], but how the actin/myosin system is mechanistically involved in the relocation of genes and transcription is presently unclear. Treatment with drugs that inhibit actin polymerization or depolymerization interfere with interchromosomal associations between nuclear receptor bound genes [16•,52••], and overexpression of a nonpolymerizable actin mutant abolished the interaction between Cajal bodies and the U2 array [61], indicating that actin filaments might be involved in these movements. Long actin filaments, comparable to those found in the cytoplasm, have not been detected in mammalian nuclei. This does not, however, exclude the existence of relatively short, highly dynamic actin filaments upon which nuclear myosin could act to promote directed gene movements.

Too close for comfort: translocations and *trans*-splicing

Juxtaposition of active genes may maximize transcriptional output or allow their co-regulation, but is not without risks for the cell. For example, translocation prone gene loci are often found in close spatial proximity in the nucleus [63]. *Myc* and *Igh*, frequent translocation partners in Burkitt's lymphoma and mouse plasmacytoma, preferentially associate at a shared transcription factory in mouse B lymphocytes [14]. In prostate cancer cells, transcriptional activation of androgen receptor bound genes induces not only their intrachromosomal [52••,64] and interchromosomal [52••] co-localization, but also, upon treatment with agents that cause DNA double strand breaks, translocation events between these loci. Furthermore, interfering with the association between *TMPRSS2* and *ETV1* inhibited translocation between the two loci [52••]. Together, these findings suggest that co-localization of transcribed genes provides an opportunity for chromosomal translocations.

It has also been suggested that proximity of active genes at shared transcription factories may facilitate *trans*-splicing [65,66], a process in which exons from separate pre-mRNAs are joined to create chimeric RNAs. First discovered in trypanosomes [67], *trans*-splicing also exists in mammals, and can involve sequences from the same chromosome [68,69,70], or located on different chromosomes [71,72••]. For most *trans*-spliced products, evidence for a functional role is lacking. However, the ability of *trans*-splicing to complement genetic mutations [73] has been exploited in gene therapy strategies [74,75,76], and demonstrates it represents an essential mechanism for gene function. A recent report describes a striking correlation between translocations and *trans*-splicing [72••]. In human stromal cells, *trans*-splicing joins exons from the *JAZF1* and *JJAZ1* genes to produce a chimeric RNA, which is translated into a protein with anti-apoptotic function. Remarkably, the chimeric RNA and protein are identical to those generated by a translocation found in stromal tumor cells. One possible explanation for this finding is that *trans*-splicing might predispose genomic loci for chromosomal exchange [72••]. An alternative possibility is that spatial proximity between the two loci allows the production of chimeric RNAs by *trans*-splicing in normal stromal cells, whereas the juxtaposition becomes 'fixed' via translocation in some cells, allowing them to proliferate as cancer cells. In this scenario, the common denominator underlying the generation of chimeric *JAZF1-JJAZ1* RNA in normal and cancer stromal cells would be close proximity in nuclear space, possibly at a shared transcription factory (Figure 1b,c). It is puzzling that a genome conformation that increases the risk of potentially grave translocations can evolutionarily persist. We speculate that three-dimensional gene clustering of transcribed loci must elicit evolutionary advantages that outweigh the dangers of translocations.

Conclusions and outlook

Fuelled by the 3C assay [10] and its modifications, our understanding of genome structure and function has remarkably expanded over the past five years. Novel genome-wide proximity ligation assays such as Hi-C

[77••] and ChIA-PET [17•] now offer the possibility of mapping whole genome conformations. These 'anchor-free' assays have the potential to describe connectivity between all loci in the genome, albeit, compared to analyses of chromosomal associations focusing on specific bait loci [13,38,43••], this may currently come at the expense of a reduced resolution for specific interactions. Nevertheless, aided by the ever-increasing power and rapidly falling cost of high-throughput DNA sequencing, the characterization of the complete repertoire of chromosomal interactions within a cell type now seems an achievable goal. However, caution must be applied when using 3C approaches to study the dynamics of genome organization. Active genes are transcribed in non-synchronous bursts [78,79], and transcription factory associations between genes in a preferred network vary strongly from cell to cell [43••]. This suggests that the transcriptional interactome is inherently plastic and that a 'single solution' describing the complete spatial arrangement of the genome in a particular cell type does not exist. Thus, one inevitable caveat of 3C assays, because they describe the average conformation in a population of cells, is their failure to account for cell-to-cell heterogeneity.

We propose that spatial clustering between co-regulated genes is a widespread phenomenon. Three-dimensional gene clustering is not only limited to RNAPII transcription units, but has also been described for genes transcribed by RNAPIII [80] and RNAPI—in fact, the nucleolus can be regarded as the archetypical example of a specialized transcription factory [81]. Other types of specific or preferred interactions are thought to mediate transcriptional repression [20,21]. Furthermore, during immunoglobulin recombination in B cell development [19], and at the onset of X chromosome inactivation [23,24], transient interactions between homologous chromosomal regions are involved in establishing polar opposite states of transcriptional activity on homologous alleles, or indeed entire chromosomes. We predict these multiple dynamic chromosomal interactions will together drive higher order chromosome conformations, and tissue-specific chromosome positioning [82]. Alteration of gene expression programs during differentiation, development, and nuclear reprogramming [83] will probably be associated with and may require corresponding changes in the nuclear interactome. A major challenge will be to decipher the relation between these genome conformation changes and the numerous epigenetic alterations of the genome, allowing their integration into a comprehensive picture of the spatial and functional organization of the nucleus.

Acknowledgements

We thank all members of the Laboratory of Chromatin and Gene Expression for their help and advice, and especially thank N Cope for critical reading of the manuscript. PF is a Senior Fellow of the Medical Research Council. This work was supported by the Medical Research Council and the Biotechnology and Biological Sciences Research Council, UK.

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