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Chromosome crosstalk in three dimensions

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The genome forms extensive and dynamic physical interactions with itself in the form of chromosome loops and bridges, thus exploring the three-dimensional space of the nucleus. It is now possible to examine these interactions at the molecular level, and we have gained glimpses of their functional implications. Chromosomal interactions can contribute to the silencing and activation of genes within the three-dimensional context of the nuclear architecture. Technical advances in detecting these interactions contribute to our understanding of the functional organization of the genome, as well as its adaptive plasticity in response to environmental changes during development and disease.

The complexity of chromosome architecture has been known about since the end of the nineteenth century, when chromatin loops were first visualized[1]. Subsequently, the early *Drosophila* geneticists perceived the importance of interactions along the chromatin fibre, when they observed that inactivation of genes could spread over huge distances along the chromosome in *cis*, and variably from cell to cell, to give rise to variegated gene-silencing effects. These geneticists also had a highly developed understanding of the *trans* effects of chromatin interactions[2]. For example, in 1954, the term 'transvection' was applied to the complementation seen when two alleles of the bithorax complex were paired. When the alleles were separated, this complementation was lost, thereby providing evidence that one allele had to somehow sense its partner allele in order to be active[2].

In subsequent decades, exploring communication among chromatin fibres remained largely outside the mainstream of chromatin research, which focused on understanding the structure of the chromatin fibre itself. This work uncovered important features, such as the smallest chromatin unit (the nucleosome) and how the primary chromatin fibre is organized into nucleosome arrays. However, the pioneering work by *Drosophila* geneticists eventually led to efforts to explore higher-order chromatin organization within the architecture of the nucleus. Today, it is clear that highly sophisticated but poorly understood processes organize higher-order chromatin structures. These structures in turn contribute to the regulation of transcriptional programs, as well as replication patterns, in the context of the three-dimensional space of the nucleus[3].

The nucleus displays an immensely complex architecture that in many instances can be visualized only by using specific antibodies[4]. With the exception of the nuclear lamina, there are no membranes surrounding subcompartments, such as the nucleolus. These structural and functional hallmarks, probably organized stochastically by self-assembling factors[5], provide key environments for chromatin interactions, as exemplified by active ribosomal RNA gene clusters driving the formation of the nucleoli[6]. Furthermore, large heterochromatic regions of chromosomes assemble at the nuclear lamina in a cell-type-specific manner[7,8], whereas transcriptionally active regions tend to loop out into the interior of the nucleus[7,8]. The simultaneous juxtaposition of active transcriptional units in transcription factories[9–12] and replicons in replication factories[13–15] provides yet more levels of organization.

Recent progress in this research area has been facilitated by the development of new methods that allow genome-wide screens of chromosomal interactions. Here, with a focus on mammalian cells, we discuss the current understanding of how chromatin communicates with itself — which we term chromatin crosstalk — and how this functionally relates to biological processes. We start with an overview of important principles governing chromatin crosstalk in *cis* (loops) and in *trans* (bridges).

Constraints on chromatin crosstalk

Chromatin loops bring distal elements of the chromosome into close physical proximity, with potential consequences for gene expression and/or propagation of the genome. The loops can be visualized when two or more portions of a chromatin fibre interact in *cis*. To enable loop formation, the chromatin fibres must physically encounter each other. A growing body of evidence suggests that stochastic movements of chromatin fibres provide such opportunities by bringing physical neighbours together[16], with the frequency of interactions largely dictated by their proximities to, and affinities for, each other. The physical interactions of chromatin fibres can be measured by using techniques based on chromosome conformation capture (3C) and the related techniques circular chromosome conformation capture (4C) and chromosome conformation capture carbon copy (5C) (Box 1). Analyses of the regulatory regions of the *H19* and β-globin gene loci by using 4C and 5C[17,18] have revealed large domains of interacting chromatin fibres. Depending on the resolution of the technique, domains encompassing between 100 kilobases (kb) and more than 10^6 kb have been observed to be in physical proximity.

Shorter-range interactions are restricted by the physical properties of chromatin, with a minimal estimated length of 10 kb for uninterrupted chromatin fibres and 0.5 kb for naked DNA[18]. By extrapolation, nucleosome-free regions at promoters and enhancers, for example, provide potential 'hinges', which could increase the mobility of the flanking chromatin fibres, thereby facilitating the formation of shorter-range chromatin loops (Fig. 1). Additionally, as histone-acetylation states regulate chromatin flexibility[19], the formation of chromatin loops may be facilitated by transcription factors cooperating with chromatin-remodelling complexes. Associated helicases, such as CHD8, might help to release torsional stress that hampers the formation of, or results from, chromatin fibre interactions[20]. For longer-range interactions in *cis*, the chromosome might transiently display particular conformations, facilitated by increased mobility, that bring distal elements into sufficient proximity to promote direct interaction. Patterns of intrachromosomal chromatin folding may therefore be influenced by the position the chromosome occupies in the nucleus and, hence, its neighbourhood.

Whereas chromatin loops describe short-range and long-range interactions in *cis*, chromatin bridges depict long-range interactions in

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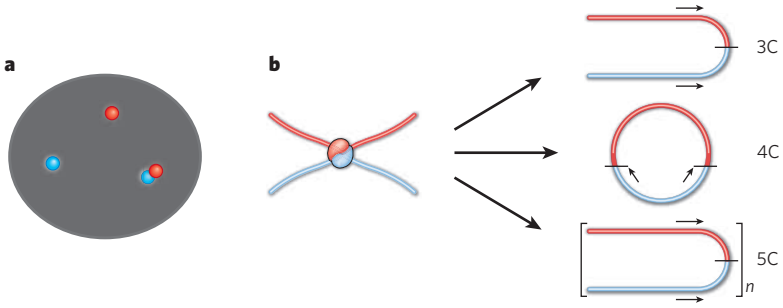
Box 1 | Chromosome conformation capture methods



The 3C method was invented to address the folding of chromosomes and how the chromatin fibre can establish both intrachromosomal and interchromosomal interactions[38]. Its resolution is higher than that of DNA fluorescence *in situ* hybridization (FISH) analysis by two orders of magnitude but, in contrast to DNA FISH, it does not provide a quantitative assessment of frequencies of physical juxtapositions. Colour-coded DNA FISH analysis visualizing the proximity between alleles of two different loci is shown in panel **a** of the figure (one locus in red, the other in blue). The 3C method has proved useful for determining the close physical proximity of sequences (with a resolution of a few kilobase pairs) from remote interchromosomal or intrachromosomal locations[38]. Briefly, formaldehyde-crosslinked chromatin is solubilized by detergents, digested with restriction enzymes of choice, and then ligated under very dilute conditions, which favour intramolecular ligation events. Subsequently, interacting chromatin fibres can be identified on reverse-crosslinked ligated DNA by using PCR primers representing both sequences (see figure, panel **b**; small arrows depict PCR primers). The 3C method is, however, less suitable for screening interactions without prior knowledge or expectation of their existence.

To deal with this shortcoming, several laboratories developed alternative methods based on the 3C approach; these are collectively known as 4C methods. These techniques differ from the 3C method in various ways, such as the inclusion of a circularization step that allows the identification of interacting sequences by using primers positioned on the bait (that is, the known sequence of interest) but close to the junction between the bait and interacting sequence[25,47] (see figure, panel **b**). This allows high-throughput screening of physical interactions between chromosomes without a preconceived idea of the interacting partners. An interesting variant of 4C methods, termed 5C, is based on analysis of all potential interactions within a limited region and is basically an extended 3C approach (see figure, panel **b**; *n* denotes numerous interactions)[17]. However, the 3C, 4C and 5C methods allow at best a semiquantitative estimate of genome-wide patterns of interactions from particular baits. Moreover, their inability to assess readily the frequency of patterns of interactions necessitates complementing the 4C screens with DNA FISH analysis. The present low sensitivity of the 4C technology (which usually requires at least a million cells) offers only snapshots of accumulated interactions. Thus, it should be expected that the number of interacting elements is limited at any given time and that dynamic on–off patterns of interactions generate a wide range of interacting elements in large cell populations. In this regard, rather than scoring all possible chromatin interactions in the entire genome, which presents both financial and logistical problems, a more prudent strategy is to filter the information with respect to a particular chromatin factor by combining a 3C, 4C or 5C technique with chromatin immunoprecipitation.

trans. One or both partners of chromatin bridges must reach beyond the confines of its chromosome territory (that is, the space occupied by a chromosome in an interphase nucleus) for interactions to be possible[21]. (The potential scenarios for both short-range and long-range interactions are summarized in Fig. 2.) For example, interactions dependent on ligand-activated oestrogen receptors occur at the edges of the relevant chromosome territories. Moreover, the occurrence of such interactions is frequently accompanied by the reorganization of the chromosome territory neighbourhood and depends on β-actin polymerization[22].

These observations raise an important unresolved issue, namely that of how, from numerous potential combinations of interactions, it is possible for a locus to select another locus situated on a separate chromosome to interact with specifically. We propose that for precision to be achieved in the interaction, the process occurs in several steps that gradually increase the specificity of the communication between chromatin fibres. The initial step may depend on more general features of larger domains of chromatin, perhaps involving the whole chromosome territory to establish an interaction that is sufficiently stable to promote additional and more specific interactions within the formed complex. Although evidence in support of this idea is scant, chromatin features at individual repeat elements may synergize to create particular constellations of higher-order chromatin conformations and provide a three-dimensional platform for interacting chromatin fibres. In line with this hypothesis, it has been observed that the interchromosomal complex impinging on the interferon-β gene has Alu elements as a common feature[23]. Furthermore, several imprinted domains, which can be predicted from specific constellations of surrounding repeat elements[24], interact with the imprinted *H19* gene locus[25].

We conclude that the recognition of key chromatin motifs during chromosomal interactions involves a combination of chromatin movements, chromatin fibre collisions and the stabilization of these interactions as a result of specific DNA–protein complexes and epigenetic marks. It will be important to address the functional effects of such chromatin loops and bridges and how these features are regulated during pivotal biological processes.

Chromatin crosstalk and transcriptional activation

It has been proposed that transcriptional activation is associated with subcompartments termed transcription factories. First postulated in ref. 10, transcription factories are thought to support the simultaneous transcription of many genes, thus providing opportunities for chromatin crosstalk both in *cis* and in *trans*[12]. The suggestion that transcription factories that are visualized by using antibodies directed against the active RNA polymerase II may not be functionally homogeneous[26] is gaining support. For example, minichromosomes containing different sets of transcriptional units uncovered at least five kinds of specialized transcription factory, according to the promoter type, the presence of introns and the type of transcribing polymerase[27].

How transcription factories in general, and these specialized transcription factories in particular, are formed is not known. One possibility is that transcriptional units poised for transcription attain increased mobility to explore nuclear microenvironments, eventually leading to recognition and association with a subset of previously formed transcription factories[12]. If these are equipped with a key factor, an initial encounter may stabilize the interaction, eventually triggering transcription. It is also possible, however, that transcription factories are formed only after the clustering of genes. For example, the interleukin 4, 5 and 13 genes cluster in type-2 helper T cells (T_H2 cells), physically juxtaposing regulatory elements before transcriptional activation coordinated by the T_H2-cell locus control region (LCR)[28]. Transcriptional activation could then be initiated by the formation of transcription factories on such clustered genes.

The enhancers are likely to play a major part in these scenarios by driving the physical clustering of genes[28]. Such complexes might involve direct communication between enhancer and promoter regions in *cis* and in *trans* to prepare for transcription by modifying chromatin marks along

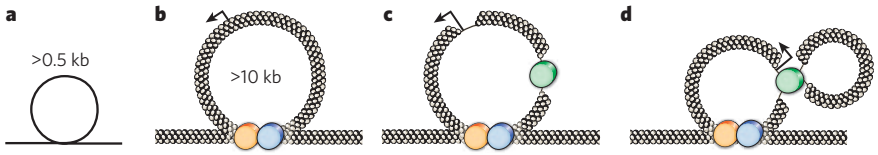


Figure 1 | Structural constraints of DNA/chromatin loop formation. A loop containing only DNA must be larger than 0.5 kb (**a**), whereas a chromatin loop needs to be more than 10 kb in length (**b**) to form[18]. Upon chromatin remodelling and eviction of nucleosomes at promoter (arrow) and/or enhancer (green sphere) regions (**c**), the naked DNA could provide a 'hinge' region, and thus opportunities for creating chromatin loops smaller than 10 kb (**d**) may arise. The orange and blue spheres represent protein complexes that organize the basic loop structure.

the chromatin fibres from the enhancer[29] and simultaneously increasing their mobility[16,22]. The complexity of this process is highlighted by the demonstration that one enhancer can stochastically communicate with multiple promoters[30]; multiple enhancers can also crosstalk with a single promoter[31]. Not mutually exclusive with this possibility is that enhancers might also operate by anchoring the transcriptional unit to a transcription factory to trigger transcriptional activation. Moreover, it is not known whether the enhancers and promoters engage in crosstalk at the time the locus is functionally incorporated into a transcription factory to modulate the efficiency of transcription. However, an analogous principle has been used to explain the efficiency of the rRNA transcription process. The promoter and terminator regions of the rRNA gene physically interact to facilitate reinitiation of transcription[32]. This principle ensures efficient transcription by keeping the polymerase complex in the loop, thus contributing to the fact that more than half of all RNAs in most living cells are made up of ribosomal transcripts.

Chromatin crosstalk and transcriptional silencing

The separation of euchromatic (active) and heterochromatic (inactive) domains is a common theme throughout evolution. Apart from maintaining constitutive heterochromatin at functionally essential regions, such as centromeres, this mechanism ensures stable inheritance of the lineage-specific gene expression patterns that specify various cell types. Thus, chromatin crosstalk must not traverse these boundaries unless it involves a dynamic change in transcriptional potential[14]. This separation can be achieved through the establishment of chromatin insulators that prevent enhancer functions from leaking inappropriately into neighbouring domains, and through the formation of chromatin barriers that prevent the silencing features of heterochromatin from inappropriately spreading into neighbouring active domains[33]. The 11-zinc-finger protein CTCF is currently the only known insulator protein in mammals[34] and has chromatin barrier properties[35]. CTCF-binding sites follow the density of genes[36] and flank nuclear-lamina-associated heterochromatic regions[8]. As CTCF also forms complexes with proteins that may relocate chromatin fibres to nuclear subcompartments, such as the nucleolus[37], CTCF emerges as a key component in the functional organization of the mammalian nuclear architecture.

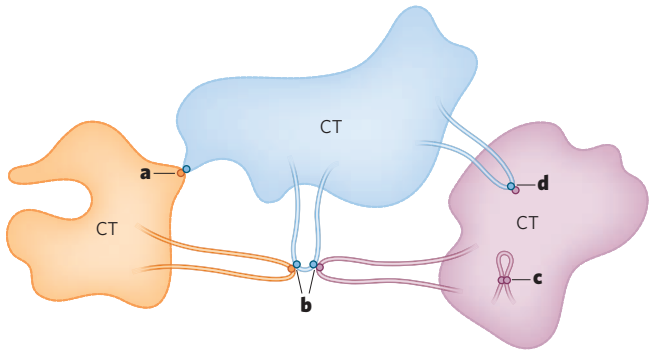


Figure 2 | Intrachromosomal and interchromosomal interactions in relation to chromosome territories. **a**, An interchromosomal interaction between loci at the edge of the associated chromosome territories (CTs). **b**, Multiple long-range interactions with the interacting loci looping out of their CTs. This scenario is tentatively supported by the identification of up to five chromosomes simultaneously impinging on each other, as determined by using the 4C technique[25] (Box 1). **c**, An intrachromosomal interaction occurring within the CT. **d**, An interaction in which one locus loops into the CT of another chromosome to find its partner. The illustration does not take into consideration the dynamics of CTs. Moreover, the long-range loops and bridges may not be based on single chromatin fibres but might instead consist of thin extensions of the CT itself to reduce the potential for DNA breaks.

The 3C method[38] (Box 1) revealed that the CTCF-dependent insulator sites at the HS4 site at the 5′ boundary of the β-globin gene[39], as well as at the *H19* imprinting control region (ICR)[40], form transient interactions with the chromatin fibres of the neighbouring transcriptional units. Deleting or mutating the CTCF-binding sites at the *H19* ICR led to *de novo* DNA methylation not only at the ICR but also at a key regulatory element located in *cis*[40]. Similarly, targeted deletion of CTCF-binding sites within the β-globin gene locus not only disrupted long-range chromatin loops but also induced local loss of histone acetylation and gain of histone methylation[39]. The mechanism underlying the deposition of epigenetic marks established by such chromatin crosstalk is still poorly understood. One possibility is that an interaction between CTCF and SUZ12 juxtaposes the polycomb repressive complex 2 (PRC2) with chromatin fibres interacting with the *H19* ICR to establish repressive H3K27me3 (histone H3 trimethylated at Lys 27) marks[41]. Interestingly, the distribution of the cohesin complex on the chromatin fibre extensively overlaps with that of CTCF-binding sites, suggesting that cohesin might contribute to the stability of such chromatin loops[42–45], perhaps guiding PRC2 to other CTCF-binding sites[46].

Derivatives of the 3C method that allow the identification of unknown sequences interacting with known sequences (Box 1) have revealed extensive crosstalk between the *H19* ICR[25] or the β-globin gene LCR[47] and the rest of the genome. At least in the case of the *H19* ICR, this 'chromosome interactome' seems to have an effect on the expression of several participating members. Maternal inheritance of mutations in the CTCF-binding sites of the *H19* ICR not only disrupted its interactions with the *Wsb1–Nf1* domain[48] and the *Osbpl1a–Impact* imprinted domain[25] but also led to changes in the expression levels of these loci. The widely assumed function of chromatin insulators and barriers (to partition expression domains or prevent crosstalk in *cis* between euchromatin and heterochromatin) should thus be extended to include their ability to fine-tune gene expression in *trans* by means of chromatin crosstalk.

This function extends to the regulation of the X-chromosome inactivation process. Thus, CTCF-mediated interaction between the two X chromosomes in female mammals seems to be an essential part of the counting phase and inactivation process[49,50]. X-chromosome inactivation is subsequently manifested by the creation of a repressive pocket that lacks transcription factors on the future inactive X chromosome, a process mediated by a non-coding transcript termed *Xist*[51]. The inactivation process is tightly linked with the recruitment of most X-linked genes into this pocket, although the cause-and-effect relationships of these events are currently not known. The repressive pocket is likely to depend on H3K27me3 marks, which are laid down by PRC2, because EZH2, a component of PRC2, interacts directly with the *Xist* RNA[52].

There is increasing evidence that chromatin loops are also involved in polycomb-mediated gene silencing. In *Drosophila*, polycomb-mediated silencing seems to be enhanced by interactions between DNA sequences

containing polycomb-repressor-binding elements. Indeed, all major polycomb-bound elements at the bithorax complex multigene locus display extensive chromatin loops[53], implying that the three-dimensional structure of chromatin plays a role in the maintenance of cellular identity. Similarly, polycomb proteins organize dynamic chromatin loops to keep the *GATA4* gene inactive in human embryonic carcinoma cells[54]. However, such polycomb-dependent chromatin loops may not provide a generally applicable explanation for inactive chromatin hubs. For example, whereas the α-globin gene cluster is associated with polycomb when inactive, the β-globin gene cluster is not[55]. This distinction might reflect the fact that the β-globin cluster is in a relatively gene-poor region, whereas the α-globin cluster resides in a gene-rich domain, thus demonstrating the context-dependent principles for the organization of inactive domains.

Chromatin crosstalk and nuclear architecture

The tendency towards spatial separation between active and inactive regions influences the organization of the genome within the nuclear architecture. Thus, gene-poor chromosomes are likely to be present at the nuclear periphery, whereas gene-rich chromosomes tend to occupy more internal positions. The same principle drives the organization of chromosome territories of individual chromosomes[21]. As a result, (G+C)-rich gene clusters generally displaying open chromatin structure localize preferentially in the nuclear interior, whereas (A+T)-rich constitutive heterochromatin is positioned towards the nuclear lamina and perinucleolar space[7] (Fig. 3). The chromatin loops or chromosome territory extensions contributing to this arrangement in the nucleus might therefore reflect the formation of specialized subcompartments for gene transcription and silencing, where high-level transcription is associated with a more internal position but is not totally excluded from the nuclear periphery. In fact, many of the gene–gene interactions determined by the 3C, 4C and FISH methods can be accounted for by their co-localization to special subnuclear compartments, such as transcription factories[9] and splicing speckles[56]. However, not all nuclei display this arrangement of active and silent compartments. The structural plasticity of nuclear architecture is illustrated by the remarkable observation that it can undergo a complete reorganization in some cells. For example, rod cells in the eyes of nocturnal mammals display 'inverted' architecture, in which all heterochromatic portions localize to the centre of the nucleus and genes map to the nuclear periphery irrespective of their transcriptional activity[57].

This observation raises the issue of how nuclear architecture can be stably maintained and yet simultaneously allow dynamic behaviour. It is possible that these features depend on the physical properties of the nuclear environment, which — being a system containing large amounts of polymers, such as nucleic acids and protein complexes — is a classic example of macromolecular crowding[58]. The physical laws operating in such systems influence the dynamics of chromatin structures and nuclear subcompartments. Non-local interactions strongly promote the compaction of chromosomes into chromosome territories without anchoring them to an immobile platform[58]. The same principles favour the segregation of macromolecules into aggregates, such as transcription factories, on the basis of their shapes and affinities for each other, without restricting the exchange of their contents with the diffusible pool of the nucleoplasm[58]. This raises the question of how the radial arrangement of chromosome territories is maintained in conventional nuclei and reorganized in inverted nuclei. One possibility is that gene-poor chromosomes are tethered to the nuclear lamina by an interaction between the nuclear lamina and chromatin. This scenario is supported by the observation that abrogation of lamin B1 expression in mouse embryonic fibroblasts leads to loss of chromosome-18 anchorage to the nuclear lamina[59]. Similarly, a mutation in the gene encoding lamin A compromises the ability of chromosome territories to reorganize themselves when the cells leave the cell cycle[60]. It will be interesting to see how inversion of nuclear architecture negotiates the interaction between lamins and heterochromatin and why the conventional architecture is more common than the inverted one. It has been suggested that the conventional architecture might have been selected for because it allows flexible chromosome arrangements and provides positional information about nuclear functions[57].

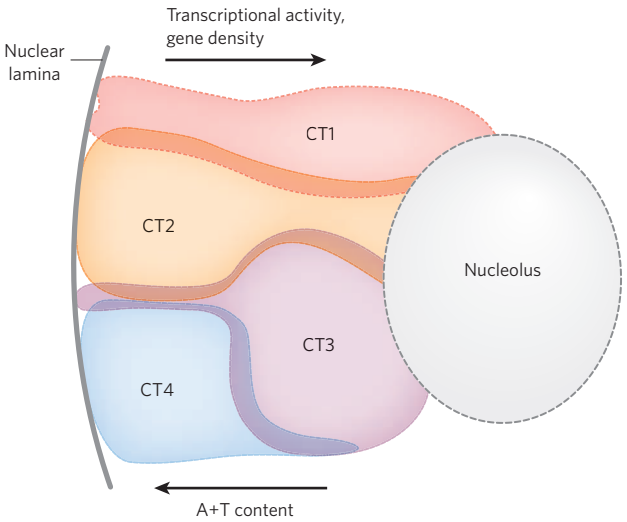


Figure 3 | Radial organization of chromosome territories within the nucleus regulates opportunities for chromatin crosstalk. The relative positions of chromosomes in an interphase nucleus depend on the proportion of genes and the A+T content. The opportunities for chromatin crosstalk between gene-rich and gene-poor regions are thus generally restricted by this organization. Hypothetical areas of chromatin communication are indicated by the patterns of overlapping CTs. Reorganization of CTs could provide new patterns of chromosomal interactions. The presence of the nucleolus and many other subnuclear compartments (not shown) may provide additional opportunities for the formation of chromatin loops and bridges.

A few examples hint at the possibility that the interplay between nuclear subcompartmentalization and the formation of chromatin loops and bridges could indeed increase the sophistication of transcriptional regulation by diversifying transcriptional states and influencing the kinetics of gene transcription[22,61]. The ligand-induced physical clustering of a specific subset of transcriptional units bound to oestrogen receptor-α (ER-α; also known as ESR1) illustrates how the formation of interchromosomal interactions before transcription is likely to facilitate coordinated and efficient transcription[22]. Although both the ER-α-bound interacting units and the non-interacting ones display chromatin structure that is permissive for transcription, only the interacting loci become relocated — by the action of the histone lysine demethylase LSD1 (also known as KDM1) — to interchromosomal granules that contain transcription elongation and splicing factors[22].

Nuclear architecture could also be reflected in the replication process. Whether a region replicates during the early, middle or late S phase of the cell cycle strongly correlates with its position within the nuclear architecture, perhaps as a result of the various chromatin conformations and their availability to replication factors[62]. Replication is proposed to take place in replication factories that might harbour up to a dozen simultaneously replicating sequences. As large regions (domains of up to several million base pairs) need to be replicated within just a few hours, the coordination of origin firing over large distances is likely to involve chromatin crosstalk[63]. It has been proposed that, to achieve this, 'licensed' origins coalesce in *cis* before initiation of DNA replication. As a result of such interactions, licensed origins might be able to coordinate the firing and, hence, the timing of replication of large subchromosomal domains[15]. Because the timing of replication can influence the potential for transcription, interactions between licensed replication origins might govern the pattern of gene expression in the subsequent cell cycle, and thus link positional information within the nuclear architecture to either propagation or reprogramming of epigenetic states during cell division.

Noise and order in chromatin crosstalk

Although it is generally accepted that chromatin fibres interact with each other in mammalian cells, what this means in functional terms is much less clear. The large number of physical interactions captured by 3C, 4C and FISH methods, as well as evident from the diversity

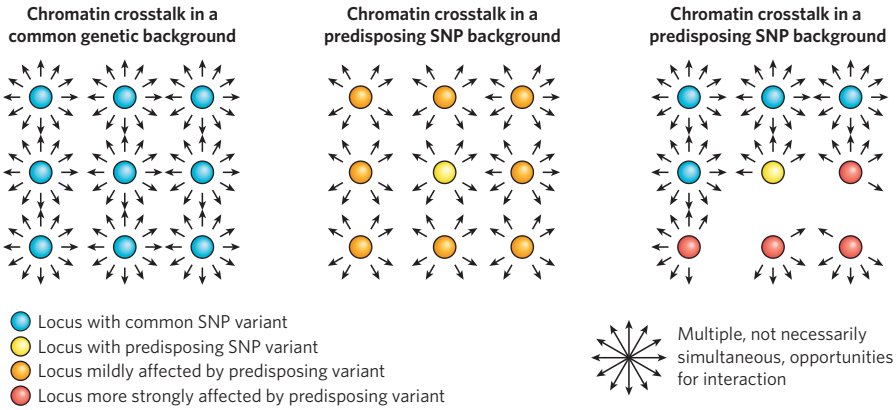


Figure 4 | Genetic background may influence the expressivity of the genome through chromosome loops and/or bridges. Chromosomal networks, defined by two or more nodes of interaction, may coordinate and fine-tune transcription (left panel). Allelic variants may stabilize or antagonize such networks, modulating gene expression patterns. The middle and right panels illustrate different scenarios of perturbed chromatin crosstalk (compared with the advantageous variant schematically depicted in the left panel) as potentially resulting from disease-predisposing SNPs. The severity and character of disease phenotype may depend on the number of genes affected, the extent of change in their expression and their function.

of chimaeric transcripts identified[64] (see page 206), implies that the nuclear architecture has a dynamic nature with a high level of stochastic collisions between chromatin fibres that do not necessarily influence genomic functions. Indeed, it is not always clear whether the juxtaposition of distal regulatory sequences and genes represents processes that are directly involved in gene regulation and are thus causal or, instead, merely represents consequences of such regulation. An example in which the cause-and-effect relationship has been elucidated is provided by the observation that a physical interaction between the interferon-γ gene promoter on one chromosome and the T_H2-cell LCR that coordinates the expression of interleukin genes on another chromosome fine-tunes the kinetics of transcriptional activation of the interferon-γ gene upon T-cell differentiation[61].

Another example is the restriction of interferon-β gene expression to a particular environmental context, for example viral infection. The stochastic allelic expression of the interferon-β gene requires nuclear factor-κB, which is a rate-limiting factor for the assembly of the interferon-β enhanceosome. Viral exposure triggers the juxtaposition of Alu repeat segments from different chromosomes with the interferon-β locus. As these Alu elements carry nuclear factor-κB, their interaction allows the formation of the enhanceosome and thus transcriptional activation of the interferon-β gene[23]. Despite the difficulties in proving cause and effect, these examples convincingly illustrate how chromatin crosstalk can functionally increase the adaptive plasticity of the cell exposed to the changing microenvironment.

Although noise in chromatin crosstalk would be expected to be largely non-functional, if stabilized it might contribute to phenotypic diversity, for example by establishing and/or maintaining stochastic patterns of monoallelic expression[65]. It is currently unknown, however, the way in which stable interactions between distant elements are orchestrated, especially in the context of the chromosome territories. At least part of the solution to this mystery may lie in the observation that the relative positions of the chromosome territories are subject to developmental regulation[66], influencing the probability of interactions in *trans* in cell-type-specific ways. Whether cohesin is the stabilizing factor, as was recently shown for chromatin fibre interactions in *cis*[45], remains to be determined. Irrespective of what factor or factors are involved, a key issue is how these interactions are specified, for example among thousands of binding sites in the instance of cohesin.

Perspectives

Without doubt, an emerging major challenge in chromatin biology is to unravel the mechanism(s) of interactions between chromosomes in three dimensions and to map and understand the influence of this interactome on the expressivity of the genome. Real progress in the field will depend on the development of new strategies and technologies that more precisely allow the definition of the cause-and-effect relationship between chromosomal interactions and genomic functions. Although real-time imaging of interactions between different loci is technically feasible and is essential for understanding how chromatin mobility is regulated in relation to a biological process, it has low resolution and cannot readily be used to screen genome-wide patterns of chromatin interactions. Other limitations apply to the 3C, 4C and 5C techniques, which require large populations of cells for analysis and hence do not readily advance our understanding of the dynamics of chromatin crosstalk. A new strategy is needed, therefore, to address the forces driving higher-order chromatin folding and to observe simultaneous co-localization events in relation to nuclear subcompartments at high resolution in individual cells. This would allow comparisons testing of a range of variables, such as how an interaction responds to the micro-environment, the three-dimensional position of the interaction, and when in the cell cycle the interaction occurs. Ideally, this should allow the identification of the molecular factors and chromatin marks participating in the interaction, enabling us to understand the phenotypic read-out effect of the interaction.

Addressing these issues may ultimately yield new perspectives on how chromatin crosstalk influences human diseases. For example, we may uncover why genome-wide association studies of complex diseases often map to gene deserts[67]. As chromatin loop formation has been documented to be sensitive to particular combinations of sequence polymorphisms[68], one possibility is that particular sets of single-nucleotide polymorphisms (SNPs) may influence communication between different parts of the genome by inducing or abolishing loop formation. Figure 4 shows two potential scenarios for how a particular combination of SNPs within a gene desert might generate pleiotropic changes in gene function elsewhere in the genome, either through the formation of disadvantageous chromosomal interactions or through the loss of advantageous patterns of interactions. Furthermore, chromatin crosstalk can be linked to misregulation of nuclear processes, as it may provide a platform for chromosomal translocation events between genes that are frequently transcribed in the same transcription factory[69,70]. For these and many other reasons, it may be useful to integrate the concept of chromosome interactomes when exploring the genetic and/or epigenetic background of complex diseases, including cancer. ■

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