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The ins and outs of gene regulation and chromosome territory organisation

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The establishment and maintenance of differential patterns of gene expression lie at the heart of development. How the precision of developmental gene regulation is achieved, despite the highly repetitive and complex nature of the mammalian genome, remains an important question. It is becoming increasingly clear that genetic regulation must be considered not only in the context of short- and long-range regulatory sequences and local chromatin structure, but also at the level of position within the nucleus. Recent studies have addressed the extent to which the location of a gene relative to its interphase chromosome territory affects its regulation or its capacity to be expressed. Two model systems have emphasized the role of this level of nuclear organization during development. Hox gene clusters have provided important insights into the dynamic repositioning of a locus relative to its chromosome territory during spatial and temporal patterning of gene expression. The inactive X chromosome has also become a useful paradigm for studying the differential chromatin status and chromosomal organization of the two X's within the same nucleus. Recent work suggests that chromosome territory reorganisation can be an important step in the gene silencing process.

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Introduction

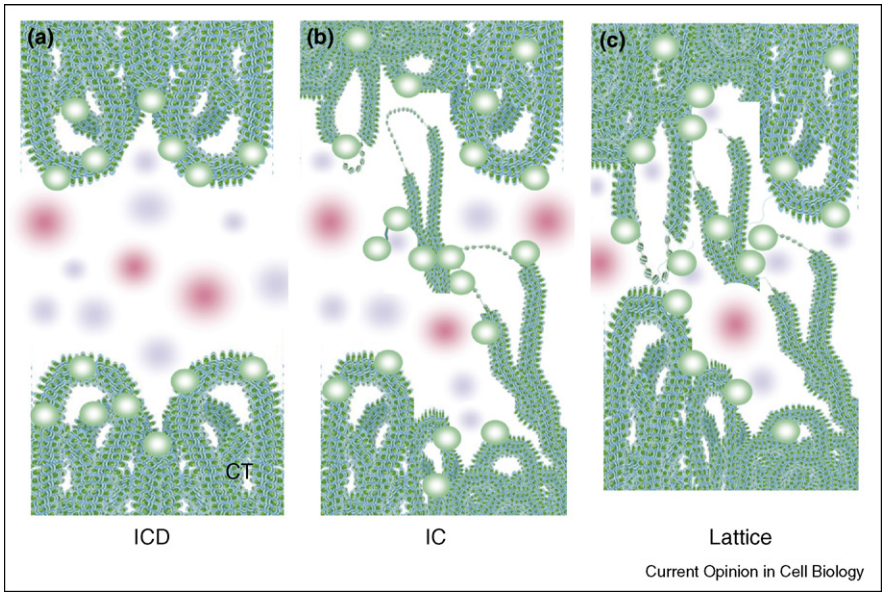
In the nucleus individual chromosomes are organized into chromosome territories (CTs), and the idea that the conformation of these CTs has functional significance for the regulation of gene expression has gained ground in recent years. This has been partly driven by improvements in RNA and DNA fluorescence *in situ* hybridisation (FISH) techniques, light microscopy, and image acquisition and analysis. However, even before this, the ability to visualize the heteropycnotic (cytologically condensed) Barr body of the inactive X (Xi) — originally identified by Barr in 1949 — in the nuclei of female cells using simple histochemical stains, such as Giemsa or DAPI, suggested that the configuration of CTs correlates to gene expression. Throughout this review, we will show how the study of the Xi continues to inform and drive our understanding of this area. In particular, recent FISH analyses have provided an interesting new perspective on the molecular content of the Barr body.

Various models have provided a framework within which to consider the relationship between the organization of CTs and the distribution of the gene expression machinery in the nucleus (Figure 1). Initial models suggested that there might be a rather distinct boundary between the surface of a CT and an interchromosome domain (ICD) containing the transcriptional machinery. A modification of this suggested that the CT has a much more invaginated structure in which a chromatin-free interchromatin compartment (IC) forms channels that penetrate within the CT [1]. However, an appreciation of the dynamic properties of chromatin [2], the visualization of genes at sites apparently outside CTs, and a reassessment of how much intermingling of chromatin occurs between CTs [3••,4••] have led to a more plastic view of CTs in which a lattice of chromatin fibres from the periphery of adjacent CTs are interdigitated, and in which the functional properties of the chromatin can drive CT organization as well as respond to it [5].

Organization within the territory

The original ICD model envisaged the CT as a structure that was impenetrable to the transcription machinery and predicted that active genes would locate at the CT surface [6] (Figure 1). However the observation that sites of nascent transcription are scattered throughout CTs [7,8] and that active genes can be found inside CTs [9] was incompatible with this idea. However, recent evidence suggests that the transcription machinery can be physically excluded from one particular CT — that of the Xi — and that this may be an important early step in the silencing of gene expression from this chromosome [10••]. The exclusion of RNA polymerase II (RNAPII) from a compartment containing the repetitive bulk of the future Xi is a consequence of Xist RNA coating the chromosome (Figure 2). Xist RNA, or molecules associated with it, may form some kind of physical barrier that inhibits access to transcription factors. The accumulation of stably bound scaffold attachment factor A (SAF-A)/hnRNP U on the Xi, in an RNA-dependent manner, lends support to this idea [11,12]. Other proteins with RNA-binding activity have also been reported to

Figure 1



Models of the chromosome territory. **(a)** In the interchromosome domain (ICD) model, there is a boundary between the surface of a CT and a compartment that contains the gene expression machinery (foci of RNAPII in blue, splicing factor enriched speckles in pink). This model predicts that all active genes (green circles) are located at the surface of CTs. **(b)** In the interchromatin compartment (IC) model, the surface of the CT is invaginated so that the gene expression machinery can better penetrate within the CT [1]. Loops of decondensed chromatin containing active genes may loop out into this compartment. **(c)** In the lattice model there is extensive intermingling of chromatin fibres from the periphery of adjacent CTs [5]. In both (b) and (c), genes from different CTs can co-localize in the nucleus together with transcription factories (blue) or splicing-factor-enriched speckles. Adapted with permission from [31].

concentrate on the Xi [13]. Alternatively, the silencing of the repetitive core of the X chromosome by Xist RNA could be sufficient of itself to generate a biochemically silent compartment, which then participates in the subsequent inactivation of X-linked genes.

Even though the structure of most CTs is permeable to the transcription machinery, the organization of genes and DNA within CTs is not random. Gene density and base composition are co-segregating properties in the mammalian genome, and it is generally the most gene-poor and AT-rich regions that are located within the CT interior [14–16]. The inactive X chromosome is an extreme example of this, given its organization as a repeat-rich core with a gene-rich outer rim in both human and mouse cells [10••,17•]. Indeed, it is very likely that the Barr body in fact corresponds mainly to the repetitive core of the inactive X. A picture is emerging of the programme of X-chromosome inactivation (XCI) in which repeat-rich/gene-poor regions within the interior of the Xi CT become silenced first, and that other genes are then moved closer to, or within, this repetitive core as they progressively become subject to inactivation. This model is consistent with the differential CT position of X-linked genes between the active and inactive Xs, and between genes on Xi that are subject to, or escape from, XCI [18] (Figure 2).

The distinctive folding of gene-rich and gene-poor domains within the CT has been explored in detail recently. Using FISH, the four gene-rich regions, and the intervening gene deserts, within a 4.3 Mb stretch of mouse chromosome 14 (Mmu14) were found to adopt four probabilistic conformations: a linear arrangement that largely reflects the organization of the primary genome sequence; a 'zig-zag' arrangement that segregates the gene-rich regions from the gene-poor; a clustering of the gene-rich regions into one 'hub'; and a final category with mixed properties [19•] (Figure 3). These observations suggest that like tends to associate with like, especially for gene-rich domains, and, although the distributions of these higher-order configurations within the CT were not analysed in these experiments, it seems likely that the clustering of gene-rich regions occurs mainly at the periphery of CTs.

This FISH study is backed up by newly developed 4C approaches, which allow the physical interactions between a locus and the rest of the genome to be captured by formaldehyde fixation and then identified by either large-scale sequencing [20•,21•] or hybridisation to microarrays [4••]. The first conclusion from these 4C analyses is that active gene regions tend to interact with other active regions [4••]. Conversely, a silent locus (β-globin in brain) tends to associate with other silent regions (the olfactory

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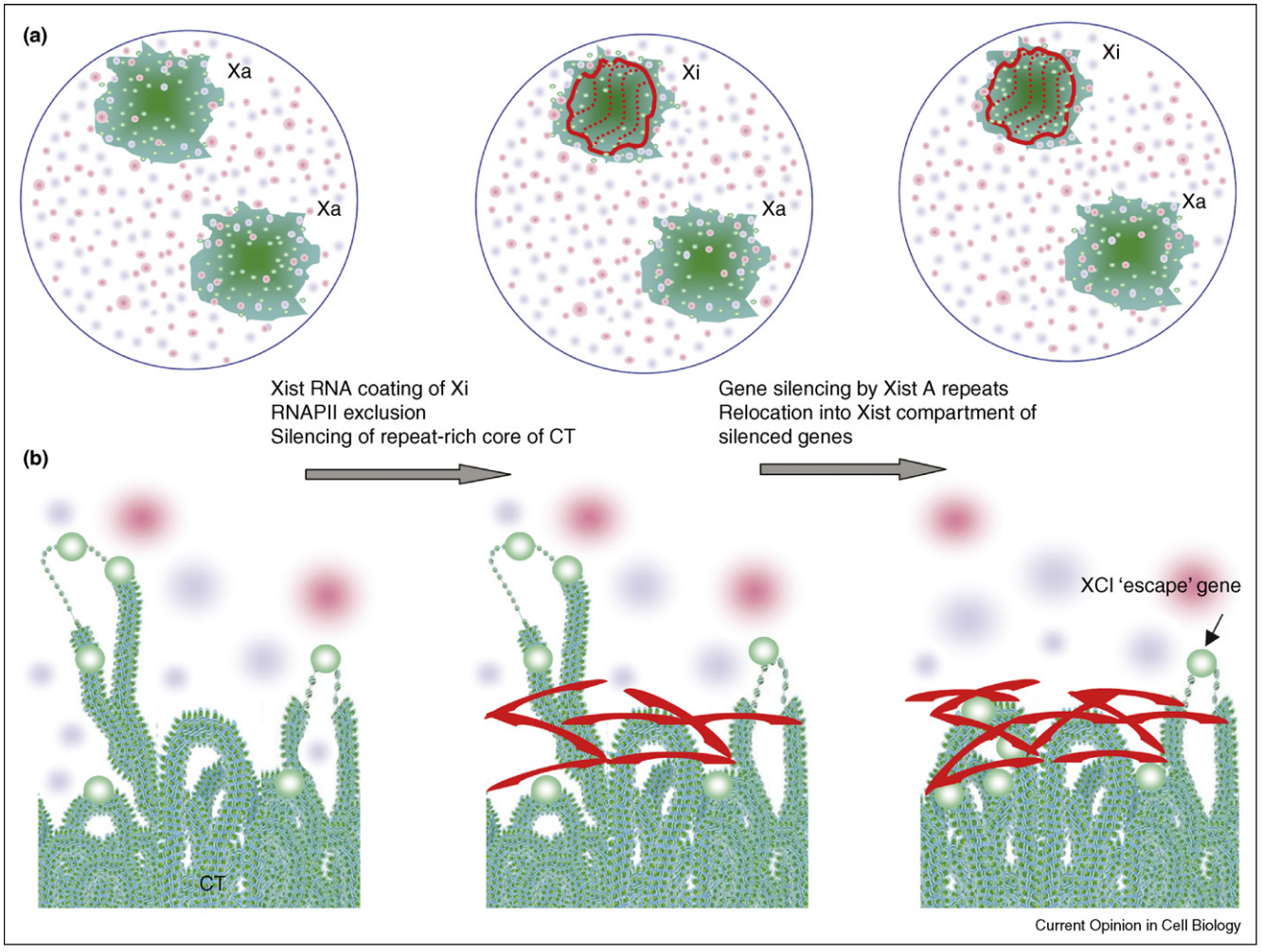
Figure 2



Chromosome territory re-organisation during X chromosome inactivation (XCI). **(a)**. Diagram of the nuclear organization of the two X chromosomes in the female nucleus and **(b)** close-up view of the surface of the Xi chromosome territory (CT) during the process of X-chromosome inactivation. As Xist RNA (red) begins to coat the territory of the future Xi, it excludes the transcription machinery (blue circles) from the repeat-rich core of the Xi CT. This independent of the A-repeats of Xist and may correspond to Barr body formations [10••]. Silencing of genes (green circles) is dependent on the Xist A-repeats and silenced genes are relocated to the inside of the Xist RNA compartment. Only genes that escape XCI remain outside the Xist RNA compartment.

receptor loci). This is reminiscent of the co-localisation of some silent genes (including β-globin) at sites of constitutive heterochromatin that has been seen by FISH [22–24]. Even though interactions *in trans* are detected by 4C, the predominant sequences captured by a given locus are generally from other regions of the same chromosome — even those many 10s of Mb away *in cis* [4••,20•]. This reinforces the significance of the 2D organization of the genome and the 3D organization of the CT in determining a gene's nuclear environment.

Blurring the edges of chromosome territories

If loci *in trans* can be captured by formaldehyde fixation, albeit at relatively low frequency compared to those *in cis*, this implies either that a locus from one chromosome can invade the territory of another and/or that loci can be located outside the confines of CTs. Evidence that the latter is often the case comes from comparing the 4C interactions that the murine *HoxB* locus makes during ES cell differentiation with the alterations in *HoxB* localisation relative to its CT. Activation of the *HoxB* gene cluster during differentiation is coincident with its relocation away from the visible limits of its CT [25]. A consequence of this is that the 'looped out' *HoxB* locus seems now to have increased interactions with regions from other chromosomes, as assayed by 4C [20•]. High-resolution cryo-FISH has also revealed extensive intermingling of DNA from different chromosomes at the boundary of, or just outside, CTs [3••]. This, together with the frequency

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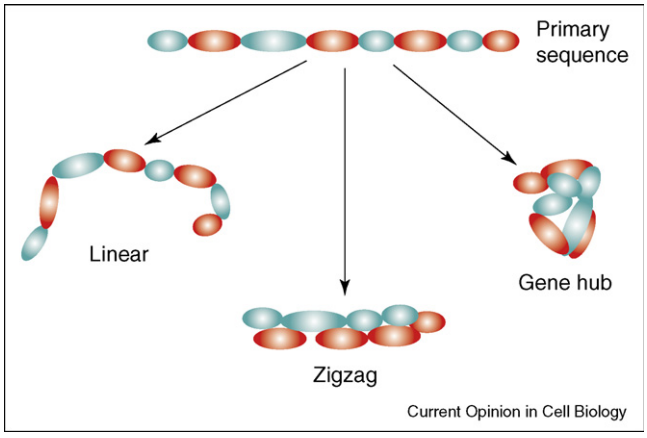
Figure 3



Interphase chromosome folding patterns. Folding patterns of a ~4 Mb region of primary sequence of the mouse genome containing four gene-rich (green) regions interspersed with four gene deserts (red). In the nucleus, FISH revealed three distinctive folding patterns: a linear arrangement that reflects the primary sequence, a zigzag arrangement that segregates the gene-rich and gene-poor regions from each other, and a 'gene-hub' in which the gene-rich regions cluster together [19•].

with which translocations between different chromosomes can be recovered, suggests some blurring at the edges of the idea that CTs are discrete [3••] (Figure 1).

Localisation outside chromosome territories and gene activity

Even though genes can be transcribed from within CTs [9], many active genomic loci have been seen outside their CTs. These tend to be either regions of constitutively high transcriptional activity [14,26], or loci where gene expression from a region is co-ordinately induced in a differentiation or developmental context [25,27,28,29•,30]. A key issue is whether this form of nuclear organization is just a consequence of transcriptional activation or whether it has a causative role. The reduced incidence of localisation outside CTs and chromosome intermingling upon the experimental inhibition of RNAPII [3••,14] suggests that relocalisation is, at least in part, driven by transcription itself. On the other hand, it cannot simply be an inevitable consequence of gene activation. The activation of *Hoxb* and *Hoxd* loci along the primary (anterior–posterior) axis of the developing mammalian embryo is accompanied by both chromatin decondensation and looping out from the CT of these loci [29•,30]. But in the limb bud, *Hoxd* activation and chromatin decondensation occur without relocalisation of this locus to the outside of its CT [29•]. It is suggested that this difference in nuclear behaviour is a reflection of the different regulatory pathways and cis-acting sequences that contribute to the activation of the locus in different developmental contexts [29•]. The temporal sequence of *Hoxd* relocalisation and gene activation during differentiation also suggests that movement to the exterior of the CT may be important in activating the transcriptional potential of the locus, but that it is not sufficient to activate the expression of all the genes. Presumably this may require additional chromatin modifications.

New evidence in favour of a functional consequence of localisation outside the CT comes from a study of the early events of XCI. The small number of X-linked genes that escape inactivation appear to be located at the edge of, or outside, the Xist RNA compartment, at least in mouse [10••] (Figure 2). Interestingly, if the A-repeat motif, which is required for silencing, is deleted from the Xist RNA, the RNAPII exclusion compartment and heteropycnotic Barr body still form, but genes normally scheduled for inactivation are not silenced and do not relocate to inside the Xist RNA compartment. The implication of this is that studies over the decades that have deduced the presence of an inactive X chromosome simply from the presence of a Barr body need to be reconsidered, as transcriptional activity of genes and the formation of a Barr body may be separable events. These results link the silencing mechanism with the physical relocation of X-linked genes within the CT [10••]. They also reveal a dual role for Xist RNA: the first being to silence the repetitive bulk of the X chromosome and create a repressive nuclear compartment independently of the Xist A-repeat domain, and second being to silence X-linked genes via the Xist A-repeats. Although the strict order of events is not known, an intriguing possibility is that the Xist A-repeats, and the factors that interact with them, capture X-linked genes and consign them to the silent interior of the X chromosome CT, eventually leading to epigenetic silencing. Whatever the underlying mechanism of gene recruitment to the silent repetitive core of the X chromosome, this relocation may be important to ensure the spread of epigenetic marks into genic sequences.

Where are genes that move out of chromosome territories going?

Genes might become located outside CTs just because they can — for example because chromatin decondensation that occurs in conjunction with transcriptional activation [25] relaxes constraints on chromatin mobility. However, the recent studies of the *Hoxd* locus have shown that chromatin decondensation and movement out of the CT can be uncoupled [29•]. Alternatively, loci outside CTs may be localising at, or interacting with, discrete nuclear sites [31]. RNA FISH and 3C have revealed that some actively transcribed genes that are separated from each other by 10s of Mb *in cis* or are even located on different chromosomes apparently co-localize at focal concentrations of RNAPII that have been termed 'transcription factories' [32] (Figure 1). Others have seen active genes congregating around accumulations of mRNA splicing factors (splicing-factor-enriched speckles)

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[26,33,34]. Many of these genes have a high frequency of looping out from their CTs. Thus, although the transcriptional machinery is not excluded from CTs (except for the Xi), there may be especially high focal concentrations of RNAPII and mRNA processing factors outside CTs that enhance the efficiency of transcription and processing of mature mRNAs. The idea of nuclear zones of active genes fits well with the recent 4C analyses of the β-globin gene in fetal liver, and the ubiquitously expressed *Rad23a* gene in liver and brain [4••]. Looping out of loci could also serve purposes other than transcriptional enhancement, of course. For example, gene silencing, or monoallelic gene regulation, could involve association of a locus with a silent nuclear compartment located away from a territory [31].

Conclusions and future directions

The structure and organization of the chromosome territory forms the most immediate nuclear environment of a gene, and can be important in steps of gene regulation. In the case of the Xi, genes become internalised into the Xist RNA-repeat-rich compartment during early steps of gene silencing and before the establishment of epigenetic chromatin marks that may then help to perpetuate the silent state. However, for the most part, the links between interphase chromatin structure and biochemical chromatin modifications have not been established. Cytological and biochemical studies of long-range chromatin structure need to be brought together to give a more unified view of how chromatin structures regulate gene expression. Similarly, looping out from territories to distant parts of the nucleus, such as transcription factories, may be crucial for gene regulation in many cases, and may help to maximise the efficiency of co-regulated gene expression. In the future it will be interesting to determine to what extent such active nuclear zones and transcription factories have a specific composition of transcription factors and/or a fixed nuclear address (e.g. relative to a particular CT for example), or to what extent they are mobile, self-nucleating structures. Live cell imaging of fluorescently tagged RNA polymerases, transcription factors or other proteins, combined with Lac/Tet operator tagging of chromosomal regions known to associate with transcription factories, should provide important insights into the relative dynamics of a locus and the transcriptional apparatus. In conclusion, the chromosome territory is turning out to be a very versatile entity, whose organization can participate in generating appropriate gene expression patterns during development, as it can interact dynamically with its surrounding nuclear landscape to ensure appropriate gene activity when required, but can also 'keep itself to itself' when silencing must prevail, as in the case of the inactive X.

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