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# Where Shall We Meet? A Role for Genome Organisation and Nuclear Sub-Compartments in Mediating Interchromosomal Interactions

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**Abstract** A recent spate of examples of specific interactions between loci on separate chromosomes in mammalian nuclei has illuminated another layer of complexity in gene regulation. As the specifics of the cross-talk between interacting loci are worked out, it is also important to consider exactly how, when and where loci can ever reliably find each other within such an intricate environment. Answers may lie in how the genome is organised in relation to itself and to specialised nuclear sub-compartments. Here, we discuss how such specialised nuclear bodies may have the potential to specifically sequester loci and provide a context where interchromosomal communications can occur. J. Cell. Biochem. 104: 1553–1561, 2008. © 2008 Wiley-Liss, Inc.

**Key words:** interchromosomal interactions; genome organisation; nuclear sub-compartments

Several years ago, innovative methodologies applied to the mouse beta-globin gene locus provided the first evidence that genes and their distal regulatory elements physically associate, suggesting that interactions between regulatory elements are not constrained by the linearity of DNA [Carter et al., 2002; Tolhuis et al., 2002]. In particular, the chromosome conformation capture (3C) technique, and its adaptations, has released the field from a technical bottleneck, resulting in a rapid advance in our understanding of spatial aspects of gene regulation. There is now a strong appreciation for the importance of specific structural conformation at many loci. Still, it is not clear mechanistically how distal regulatory elements form these contacts, yet the constrained movement of two elements in *cis* relative to each other will certainly facilitate contact.

Recently, a handful of genetic interactions between sequences positioned on separate chromosomes have been uncovered, expanding the horizons of long-range interactions. These interchromosomal interactions have been implicated in processes such as choice of odorant receptor gene expression in sensory neurons [Lomvardas et al., 2006], T lymphocyte differentiation [Spilianakis et al., 2005], genomic imprinting [Ling et al., 2006] and X inactivation [Bacher et al., 2006; Xu et al., 2006]. These cases, which will undoubtedly be followed soon by dozens more examples, are discussed in some excellent reviews [de Laat and Grosveld, 2007; Schneider and Grosschedl, 2007].

If it is difficult to imagine how distal regulatory elements in *cis* manage to loop across to contact each other, the challenges are compounded when interactions in *trans* are considered. Free from the physical constraints of genes in *cis*, how could two unlinked genetic elements reliably find each other in the complexities of the nucleus? Answers may lie in how the nucleus is functionally compartmentalised into regions that specialise in processes such as gene transcription, ribosome biogenesis and DNA repair. In this essay, we explore the contributions that genome organisation and specialised nuclear sub-compartments may play in sequestering widely separated loci, to provide a platform where interchromosomal interactions can occur.

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## GENOME ORGANISATION

The position that each chromosome territory adopts in the mammalian interphase nucleus appears to be non-randomly determined. In relation to the dimensions of the nucleus, some chromosomes tend to be located towards the centre, while others are positioned more peripherally. Gene density appears to correlate with these differences; gene-rich chromosomes, such as human chromosome 19 are usually located centrally, whereas the similarly sized chromosome 18, which has a low gene density, tends to be more often associated with the nuclear membrane [Croft et al., 1999]. However, the rules determining these distributions are also influenced by nuclear shape, since in cells with flattened, ellipsoid nuclei, such as fibroblasts, distributions seem to correlate better with chromosome size, where smaller chromosomes are positioned more internally than larger ones [Bolzer et al., 2005]. It is notable too that nuclear volumes vary in different cell types, which may influence the distributions of chromosomes within.

Variations in chromosome radial positioning in different cell types have been uncovered, which highlights a context-dependent genome organisation. Interestingly, the radial positioning of chromosomes change during adipocyte differentiation [Kuroda et al., 2004] and spermatogenesis [Foster et al., 2005], emphasising that nuclear organisation appears responsive to functional inputs. In addition to preferred radial arrangements, chromosomes also appear to be arranged non-randomly in relation to each other. Certain heterologous chromosomes are juxtaposed more frequently than others, and again, this is cell-type specific [Parada et al., 2002, 2004]. While the gross physical properties of size, gene density and GC content will not vary between cell types, differences in gene expression may affect chromosomal positioning through differential chromatin structures and gene recruitment to specific nuclear sub-compartments, although these effects are mostly unexplored. Clearly, how the chromosomes are differentially arranged in the nucleus in relation to each other can act to either promote or hinder interactions between their resident genes.

Definitions of chromosome territories are based on what is visualised using whole chromosome probes. These experiments often show minimal overlap with adjacent territories, which might suggest little scope for interchromosomal interactions. However, other evidence suggests that a certain degree of intermingling between adjacent chromosomes does occur. Some gene loci have been shown to reside well outside their chromosome territories, extending over a micrometer beyond the densely stained territorial mass [Volpi et al., 2000; Mahy et al., 2002]. Gene positioning relative to the territories can change in conjunction with gene activation, as shown by the *Hox A9* gene, which loops away from its territory when it is expressed [Chambeyron and Bickmore, 2004]. However, extra-territorial positioning is not necessarily a requirement of transcription, which also can occur within the territory [Verschure et al., 1999]. Branco and Pombo [2006] have examined thin cryo-sections of the interface between territories by high resolution microscopy, and found considerable intermingling between chromatin loops of adjacent chromosomes. Preferred neighbouring and blurred edges of chromosome territories may be a prerequisite for interchromosomal interactions, yet to bring about an intimate juxtaposition to allow cross-talk, loci likely must be specifically sequestered to nuclear sub-compartments.

## NUCLEOLUS

The nucleolus, the site of ribosome biogenesis, is a paradigm for nuclear compartmentalisation; it is a well-known example of interchromosomal interactions, where loci separated onto different chromosomes are brought together into highly organised regions of specialised function. Following mitosis, nucleolar organising regions (NORs), which are tandem head to tail oriented ribosomal genes located on several chromosomes, coalesce into one to four structures, and recruit RNA polymerase I (RNAP I) machinery. Not every NOR is transcriptionally active in every cell, and those that are quiescent are excluded from nucleoli. Functional NORs are bound by upstream binding factor (UBF), which binds to the promoter region and the body of the rDNA gene itself [Dousset et al., 2000]. UBF is thought to be central in recruiting both the rDNA genes and the protein components, and may represent a major architectural unit of nucleolar structure. Transcription appears also important to the integrity of nucleolar organisation, as evidenced by inhibition of RNAP I function by actinomycin D as the cells exit mitosis, resulting

in disruption of normal nucleolus formation [Dousset et al., 2000]. The importance of transcription in the organisation of nucleoli is further highlighted by experiments in which artificial NORs were inserted into random location of the human genome [Mais et al., 2005]. These "pseudo-NORs" are capable of recruiting UBF and RNA Pol I, however they were transcriptionally silent, and excluded from nucleoli. Hence, the presence of transcriptional machinery is not enough to promote interchromosomal interactions, but active transcription also appears to be required for these interactions to occur.

## TRANSCRIPTION FACTORIES

Similar to the compartmentalised RNAP I transcription, RNA polymerase II (RNAP II)-driven transcription is centralised at discrete sub-compartments positioned throughout the nucleus, termed transcription factories, where most of the hyperphosphorylated, elongating fraction of RNAP II is concentrated [Kimura et al., 1999]. Fluorescence detection of RNAP II immuno-staining combined with bromo-uridine incorporation into nascent transcripts reveals a strong co-incidence between transcription sites and RNAP II foci [Grande et al., 1997]. A more highly resolved view of transcription sites has been achieved by immuno-staining of nascent transcription using gold particles, detected by electron microscopy, and suggests that factories are small, less than 100 nm in diameter [Iborra et al., 1996]. Such limited dimensions would mean that any two transcription units engaged at the same factory would place them in close proximity. Quantitation of transcription sites in HeLa cell nuclei indicate that there are several thousand factories dedicated to transcription of RNAP II genes [Iborra et al., 1996; Pombo et al., 1999]. The RNAP II immuno-staining of a variety of cells isolated from mice suggests a considerably lower number of RNAP II factories in ex vivo cells, where only a few hundred discrete RNAP II foci are detected. This observed discrepancy could be due to differences in detection methods (EM detection of pulse-labelled transcription sites vs. fluorescence detection of immuno-stained RNAP II foci), nucleus shape (ellipsoid vs. spherical), nuclear volume or cell source (cultured vs. ex vivo).

Although the existence of a nuclear scaffold or matrix remains controversial, some evidence suggests that transcription factories are tethered to a nuclear ultrastructure. Jackson and Cook [1985] found that the majority of chromatin could be digested by endonucleases, and eluted from agarose-embedded nuclei without removing the bulk of transcriptional activity. Recently, Mitchell and Fraser [2008] have demonstrated that under heat shock conditions that inhibits all transcription, factory numbers and distribution patterns are preserved, even though genes appear to be dissociated from these sites, suggesting that transcription factories represent *bona fide* nuclear sub-compartments, and not simply a coalescence of transcribing genes. Together, this evidence suggests chromosomes are tethered to a nuclear ultra-structure through their transcription units. This might imply that chromosomes form transient attachments to a nuclear scaffold, which might be expected to be affected by changes in gene expression patterns. Transcriptional elongation by polymerase generates a considerable amount of pulling power, at least twofold higher than conventional mechano-enzymes, kinesin and myosin [Yin et al., 1995]. With at least several hundred active genes on each chromosome, such combined forces generated by each transcribed transcription unit may be ample to influence nuclear positioning of the chromosomes.

Beyond the presence of high concentrations of hyperphosphorylated RNAP II, there is little known about other components that reside in transcription factories. Many transcription factors display punctate staining patterns by immuno-staining or fluorescent tagging that is similar in appearance to the RNAP II foci. Some transcription factors, such as Oct1, E2F, GR, ER, Sp1 and Sp3 show little or no overlap with RNAP II foci [Grande et al., 1997; Stenoien et al., 2000; He et al., 2005]. Others such as BRG1, TFIIH, AML-1B, p53 and dioxin receptor exhibit partial overlap to varying degrees [Grande et al., 1997; Zeng et al., 1998; Rubbi and Milner, 2000; Elbi et al., 2002]. The roles of non-overlapping transcription factor foci are uncertain, although they may represent storage sites.

In light of the pattern of partial overlap of specific transcription factors, it is attractive to ponder the existence of specialised transcription factories, which are dedicated to the transcription of specific subsets of genes, although

this concept needs further validation. Notably, TFIIH shows only partial co-localisation with RNAP II foci [Grande et al., 1997], yet one might have predicted it to be present at all transcription factories, as part of the general transcription machinery. It is important to consider the detection limitations of immuno-fluorescence; the quantities of a specific transcription factor that are localised in a factory may be well under the detection thresholds by immuno-fluorescence. In addition to studying distribution patterns of transcription factors, an approach to be used in parallel is to study how frequently genes regulated by the same transcription factors cluster at a shared transcription factory.

Interestingly, topoisomerase II beta-induced double strand breaks are required for efficient transcription of PARP-1 responsive genes [Ju et al., 2006], which emphasises that a tight coordination of transcription and DNA break repair is required. Consistent with this concept, DNA repair factors Ku70/80 show a striking degree of overlap with the factories [Mo and Dynan, 2002]. This seems logical in light of such programmed DNA damage, in addition to any consequential breaks that might occur as a result of the torsional stress that is generated during transcription. Compounded by the extreme proximity of transcribing genes, one would imagine that double strand break repair must need to be extremely efficient to prevent chromosomal translocations, as discussed below.

The transcription of active genes is not a steady and continuous process, but instead occurs in bursts that are interspersed by periods of inactivity. Studies employing highly sensitive single-molecule detection and live-cell imaging show that expression of individual genes is stochastic, alternating through irregular on/off cycles [Cai et al., 2006; Chubb et al., 2006; Raj et al., 2006; Kaufmann et al., 2007]. This evidence is supported by transcription analysis by RNA FISH, which shows that in a population of cells, only a subset of alleles exhibit a transcription signal at any given time. Virtually all transcription occurs at the transcription factory [Osborne et al., 2004, 2007; Ragoczy et al., 2006]. However, the percentage of alleles within a cell population that have a transcription signal varies from gene to gene and correlates with how often the gene is associated with a transcription factory [Osborne et al., 2004, 2007]. This observation infers that alleles that are not transcribing are positioned away from the factories implying that for an inactive allele to undergo a new cycle of transcription, it must relocate to a transcription factory. There are indications that this can be a highly responsive and dynamic event. Stimulation of cells to activate immediate early gene transcription leads to a sharp increase, within a few minutes, in the percentage of alleles associated with a factory, and a comparable fold increase in the levels of transcription [Osborne et al., 2007].

Transcription factories are by far outnumbered by active genes, let alone the abundance of other, non-coding transcribed sequences [Cheng et al., 2005]. This supply and demand imbalance seems to be in part rectified by transcribing multiple genes in the same factory. It is unknown how many genes can be accommodated in a single factory concurrently. Limits will probably be set by the availability of transcriptional machinery, and spatial constraints. Cook and colleagues have suggested that a range of 6–17 elongating polymerases are typically present in each factory [Faro-Trindade and Cook, 2006], however it is possible that some of these may be engaged with the same transcription unit. Genes in *cis* that are separated by tens of megabases are co-associated at shared factories between 30% and 60% of the time [Osborne et al., 2004, 2007]. Even genes that reside on separate chromosomes co-localise, albeit at reduced frequencies, typically ranging from 1% to 12% [Osborne et al., 2004, 2007]. A remarkable exception is the *trans* co-associations of the *Myc* and *Igh* genes in B lymphocytes, which co-localise approximately 25% of the time, when both genes are active [Osborne et al., 2007]. This example is highly poignant due to the involvement of these genes in chromosomal translocations associated with Burkitt's lymphoma in humans and plasmacytoma in mice, and suggests that the co-association of genes at factories may provide a platform and context where cancer-causing chromosomal translocations may occur. The incidence of other translocations involved in plasmacytoma correlates loosely with the preponderance of these genes co-associate at factories, which emphasises a possible direct impact of transcriptional organisation on cancer rates.

Why the mouse *Igh* and *Myc* genes should co-localise so frequently is unclear. The chromosomes on which these genes reside, chromosomes 12 and 15, respectively, are frequently juxtaposed in lymphocytes [Roix et al., 2003; Parada et al., 2004]. Therefore, these genes are often found within the same 'nuclear neighbourhood', so would be expected to co-associate more often. However, relative positioning of chromosomes cannot fully account for such high co-association frequencies. We have found that the *Eif3s6* gene, a highly expressed translation factor located 20 Mb from the *Myc* gene co-localises with *Igh* at less than half the frequency of *Myc*, indicating that not all gene co-associations are equal. Differences could be due in part to gene position with respect to the chromosome territory; a gene embedded within its territory may have fewer opportunities for *trans* associations than a gene that is either at the periphery or looped out from the territory. Transcriptional activation of the *HoxB1* gene, which involves its repositioning to a location outside of the chromosome territory is accompanied by an increase in interchromosomal interactions [Wurtele and Chartrand, 2006].

Perhaps a more provocative explanation for differential co-localisation frequencies is that genes may be preferentially sequestered to specialised factories that contain their particular cocktail of transcription factors. Comprehensive examinations of all other genes that commonly co-associate with specific genes of interest are needed to fully understand the molecular basis for preferences in co-associations. Global 3C methodologies that have the potential to identify all genomic sequences that are closely associated with a locus of interest [Ling et al., 2006; Simonis et al., 2006; Wurtele and Chartrand, 2006; Zhao et al., 2006], may be useful at identifying genes that commonly co-associate at transcription factories. While initial applications of this technology may not as yet illuminated any preferred transcription networks, or indeed many *trans* interactions, refinements to the technique to improve its sensitivity may bring these to the surface.

Transcription factories themselves may be specialised, with specific transcription factors present as stable components. Alternatively, specialisation may solely depend upon the genes that are resident; the presence of one transcribing gene at a factory, along with all its unique transcription factors, may be sufficient to trap other commonly regulated genes. If this is true, then one might predict that the specialty of one factory could change over time as some genes move out and others move in.

One can envision how the functional compartmentalisation of transcription could influence genome organisation. However some preferred co-associations may not always be predictable. One must also consider that genes may be subject to a bystander effect, where genes in *cis* may be influenced by the co-association tendencies of each other.

## OTHER NUCLEAR BODIES

There are numerous other nuclear substructures that could participate in genome organisation. PML bodies (PB) are positionally stable structures that have been implicated in a variety of cellular processes including cell cycle regulation, DNA repair and apoptosis. In addition to the PML protein, over fifty other factors have been suggested to associate at PBs, including the tumour-suppressor, p53. It is tempting to speculate that during the rapid induction of p53 gene expression upon cellular stress, PBs may also organise p53 responsive genes. Interestingly, the p53 gene, which up regulates its own expression during periods of cellular stress, is localised to the surface of PBs [Sun et al., 2003]. Indeed, PBs make extensive contacts with the local chromatin environment it occupies [Eskiw et al., 2003, 2004], indicating that in general, PBs may actively recruit genes to their surfaces. Other studies have also documented a preferential localisation of transcriptionally active loci around PBs [Wang et al., 2004]. In particular, the actively transcribing MHC class I locus found on chromosome 6, frequently localised to the surface of PBs. When the MHC class I locus is artificially inserted onto chromosome 18 and transcriptionally stimulated, a new association with PBs is seen [Shiels et al., 2001]. PB's may work in concert with factors such as SATB1, to organise chromatin loops within the local environment to promote or inhibit gene expression, as is seen with IFN$\gamma$ stimulation of the MHC class I locus [Kumar et al., 2007]. It is unclear, however, if these PBs play an active role in transcription (e.g. physical interaction with polymerase), or if PBs only promote

association by recruiting both genes and proteins required for transcription.

Cajal bodies (CBs), which are hypothesised to have role in RNA transcription and processing, may also be involved in the organisation of specialised genes. CBs associate frequently with histone gene clusters on human chromosomes 1 and 6, as well as the U1 and U2 small nuclear RNA genes [Frey et al., 1999; Schul et al., 1999]. The association with the histone gene clusters is only seen during S-phase, when histone genes are transcribed. In addition, live-cell imaging shows that tagged U2 gene arrays, inserted at ectopic sites, move 1–3 μm at the start of S-phase to form contacts with the relatively immobile CBs [Dundr et al., 2007].

Other nuclear bodies may also be involved in recruiting specific sequences. The OPT (Oct/PTF/transcription) domain, which function is as yet unclear, associates preferentially with human chromosomes 6 and 7 at G1 phase [Pombo et al., 1998]. SC35 domains, which are thought to be primarily involved in splicing factor storage, have been suggested to participate in preferred associations between the alpha-globin and beta-globin genes in human erythroid cells [Brown et al., 2006]. However, not all transcribing genes are found in associations with SC35 domains; these associations are perhaps limited to only highly transcribed genes. Transcriptional inhibition by DRB treatment results in dramatic morphological changes in SC35 domains, and a loss of contact with the surrounding chromatin [Kruhlak et al., 2000], which may suggest a critical role of transcription in driving contact between genes and SC35 domains.

## CONCLUDING REMARKS

Clearly, co-association of genes at nuclear sub-compartments could potentially provide the intimate juxtaposition to allow a specific interchromosomal interaction, and assuming that the genes have the correct 'post code', may facilitate the genes ending up in the same place at the same time. Information will emerge about the specific *trans*-acting factors that are involved in mediating the cross-talk between loci. Already, CTCF has been implicated in interactions between the two X inactivation centres [Xu et al., 2007], and in interactions between imprinted loci [Ling et al., 2006], and may form a bridge between loci to facilitate communication. Still, there is as yet no indication of the environment in which CTCF acts; it may carry out its function within transcription factories, or alternatively, at discrete insulator bodies [Gerasimova et al., 2007].

Whether in the context of a transcription factory, or each of the nuclear sub-compartments discussed in this review, in many cases active transcription appears to be a common requirement in bringing loci together. The forces generated by elongating polymerases may ensure that a specifically recruited locus remains tethered to the sub-compartment, thereby providing the stability to allow interchromosomal interactions to occur.

Although it is apparent that nucleoli are able to recruit NORs from different chromosomes into a single structure to promote ribosome biogenesis, it is not clear if other nuclear bodies either have the ability to recruit specific loci to specialised regions of the nucleus, and if they do, are they as efficient as nucleoli at promoting intra- or interchromosomal interactions. CBs actively recruit histone gene clusters at the onset of S-phase. Is this the only role they play, or are other subsets of genes recruited to CBs, at different phases of the cell cycle, for specific regulation? Regardless, the movement of chromatin to the surface of CBs demonstrated the potential for nuclear bodies to promote genome organisation. It is obvious from the localisation of proteins to PBs that these structures aid in the establishment of local nuclear environments. It is logical to speculate that, in such cases as p53 mediated gene expression, the genes responsive to those proteins localised to PBs would also be recruited. Even if PBs do not themselves contain polymerase, they may bring loci into close physical contact promoting long range interactions between distal genes, and those loci on different chromosomes. Future experiments using immuno-FISH for candidate genes may prove vital in demonstrating preferential genes interacting with the surface of these and other nuclear bodies.

Questions remain as to whether sequestering at a nuclear sub-compartment would actually be enough to achieve spatial and temporal coordination. The many layers of independent stochastic events that occur in the nucleus mean that the same genes do not always end up at the same transcription factory. If an interacting partners transcribe at a shared transcription

factory a quarter of the time, it implies that 75% of the time, these genes are transcribed apart. When they are positioned far apart, would they ever be able to find each other? Some specific interactions appear to occur only transiently, and given enough time, interacting partners may eventually enter the same location. However, large-scale chromatin movements appear to be minimalised following early G1 phase of the cell cycle [Thomson et al., 2004], which may hinder the hunt for an interacting partner. A plausible hypothesis poised by de Laat and Grosveld [2007] suggests that specific inter-chromosomal interactions may occur in cells very rarely, resulting in the survival of these few cells that have passed this restriction point. Uncovering the preferred interaction networks, and visualisation of the dynamics by live-cell imaging of fluorescently tagged genes, transcription and nuclear compartment should provide an indication of how interactions are achieved.

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