

Close encounters between active genes in the nucleus

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Abstract

A recent paper demonstrates that coregulated genes on different chromosomes show surprisingly high frequencies of colocalization within the nucleus; this complements similar results found previously for genes localized tens of megabases apart on a single chromosome. Colocalization could be related to the earlier observation of active genes associating with foci where RNA polymerase II is concentrated.

Nuclear compartmentalization and its study using the 3C method

The nucleus is still not infrequently perceived as a microscopic test tube, in which activities such as transcription, replication, and recombination are performed on a template of randomly coiled chromatin bathed in a homogeneous nucleoplasm containing soluble enzymes and cofactors. Yet in recent years there has been a growing appreciation that the nucleus is in fact highly organized. In interphase chromosomes, DNA is compacted by varying amounts, from hundreds to thousands of times more compact than simple B-form DNA [1], and the chromosomes form distinct, largely non-overlapping 'territories' [2] that are non-randomly arranged within the nucleus. As well as chromosome territories, a plethora of other nuclear compartments and bodies have been identified, and many of the cofactors and enzymes mediating the processing of DNA and RNA are enriched in particular compartments or bodies [3]. The functional significance of this considerable nuclear compartmentalization remains unclear given that most nuclear proteins are quite dynamic, equilibrating rapidly between specific compartments and a soluble nucleoplasmic pool. But a 2004 paper [4] that examined the colocalization of active genes on the same chromosome arm fueled speculation about the possible functional significance of nuclear compartmentalization. A new report [5] now ignites interest in nuclear compartmentalization by extending this work to coregulated foci on different

chromosomes. Both papers break new ground by combining the recently developed 'chromosome conformation capture' (3C) method - a molecular method for assaying chromosome proximity - with more established fluorescent *in situ* hybridization (FISH) and immunocytochemistry techniques.

The 3C technique provides a powerful tool for dissecting the spatial organization of chromosomes within nuclei [6]. The 3C method identifies DNA sequences that are in close molecular proximity by detecting indirect linkage between them, mediated through formaldehyde-induced DNA-protein and protein-protein cross-links. Following treatment of intact cells with formaldehyde, isolated DNA-protein complexes are subjected to restriction-enzyme digestion. DNA fragments held together via cross-linked DNA-protein complexes have a higher probability than soluble DNA fragments of being ligated together at low DNA concentrations. Reversal of the formaldehyde cross-links is followed by detection using PCR of the relative cross-linking frequency of two DNA fragments, which is assumed to be proportional to their spatial proximity in the nucleus.

Association of linked active genes with 'transcription factories'

Using a combination of bromouridine (BrUTP) incorporation to label nascent transcripts and immunodetection of

RNA polymerase II (Pol II), previous work on mammalian cells [7,8] has typically revealed a few thousand visible foci per nucleus in mammalian cell lines. There are estimated to be tens of thousands of active genes per nucleus and fewer than one RNA polymerase per active gene; on the basis of the smaller number of foci than active genes, the authors [7,8] proposed that the observed Pol II foci are 'transcription factories', each containing several clustered active genes. But the fact that the number of foci is still in the thousands and that they are small in size, combined with the possibility that transcription could be intermittent and active genes could be packed compactly in interphase chromatin, has suggested the more trivial possibility that the foci arise from non-uniform spacing of active genes along a linear DNA template.

Osborne and colleagues [4] showed that a number of differentiated mouse cell types contain significantly fewer Pol II foci per nucleus than observed previously in certain mammalian cell lines [7,8]: only 100-300 were seen, corresponding to one transcription factory per 20-60 megabase-pairs (Mbp) in a typical G₁-phase nucleus. The observed foci were also considerably larger, ranging in diameter from several hundred to one thousand nanometers. Five transcriptionally active genes distributed within a 40 Mbp chromosome region were examined, including the genes encoding the β -like hemoglobin *Hbb-b1* and the α -hemoglobin-stabilizing protein *Eraf* (Figure 1a,b). The authors [4] found colocalization of *Hbb-b1* with one of the other four genes in 40-60% of erythroid cells, seemingly an extraordinarily high percentage for random intrachromosome folding. The close proximity between several of these genes in the nucleus was confirmed using the 3C method. If it is assumed that most active genes are associated with transcription factories, these colocalization percentages are consistent with an estimated one factory per 20-60 Mbp. Indeed, the authors found that a very high percentage of active genes were associated with the factories, with a significant fraction of colocalizing genes sharing a single transcription factory.

Spatial association of coregulated genes on different chromosomes

These results [4,7,8] suggest that gene regulation is tied to the localization of genes to specific nuclear bodies. A natural question is whether coregulated genes located on different chromosomes might colocalize within the nucleus, which would imply a still higher degree of nuclear spatial organization. Osborne *et al.* [4] reported a 7% colocalization between *Hbb-b1* and the coregulated α -globin gene (*Hba*) located on a different chromosome. This percentage is much higher than the predicted 0.3-1% colocalization expected for a completely random intranuclear chromosome arrangement and the observed number of transcription factories.

The question has now been tackled further by Spilianakis *et al.* [5], who document interchromosomal interactions

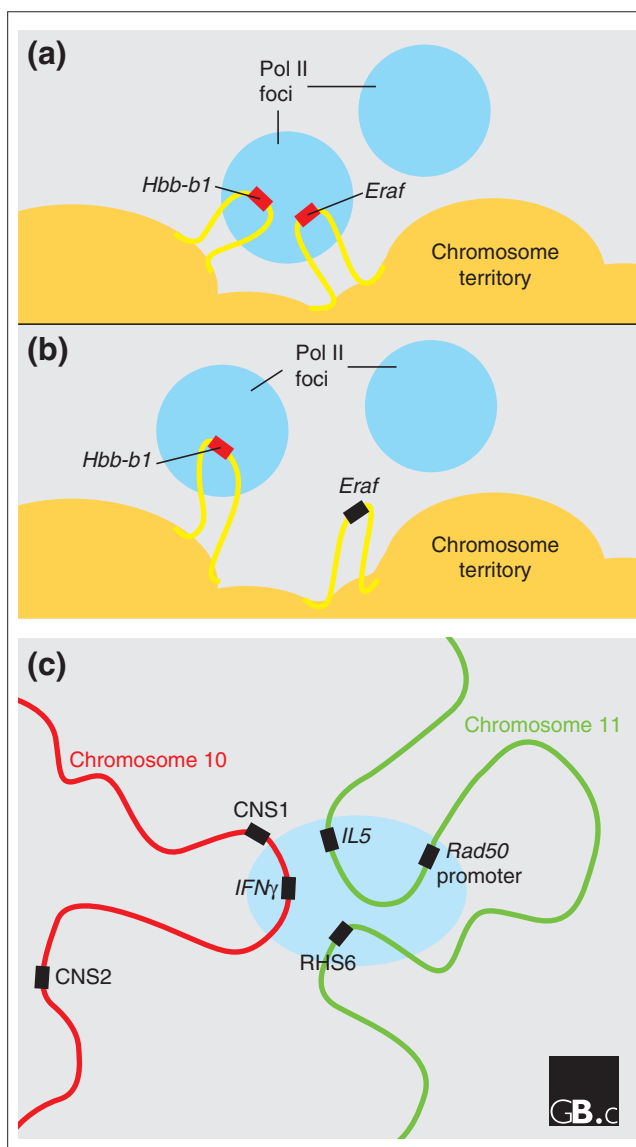


Figure 1
Specific localization of genes on the same and different chromosomes, as detected using a combination of fluorescent *in situ* hybridization (FISH) and 3C technology. **(a,b)** A representation of part of a mouse erythroid-cell nucleus, showing the edge of the territory of one chromosome and two active genes *Hbb-b1* and *Eraf* looping out from the territory. **(a)** Osborne *et al.* [4] found that colocalization within the nucleus of these two genes, together with other active genes (not shown) distributed over the same 40 Mbp region of the chromosome, appears to be driven at least in part through the shared colocalization with the same focal concentration of RNA polymerase II (Pol II), in a 'transcription factory' (circles). **(b)** When the genes are not localized to Pol II foci, for example, when *Eraf* is inactive (black), they are not colocalized. **(c)** Association in naive mouse CD4⁺ T cells between the gene encoding the cytokine interferon γ (*IFN γ*) and specific sequences in the *T μ 2* locus, including the genes encoding interleukin 5 (*IL5*) and the DNA-repair protein *Rad50* as well as a DNase I hypersensitive site called *RHS6* [5]. In this cell type, both gene loci are poised for rapid induction of low levels of expression. *CNS1* and *CNS2* indicate conserved noncoding sequences near the *IFN γ* gene on chromosome 10. The genes may be associated with a shared nuclear body represented by the oval, for instance a 'transcription factory', but this has not been demonstrated [5].

between two coordinately regulated gene loci. The genes encoding the interleukins (cytokines) 3, 4 and 5 (IL3, IL4, and IL5) all lie within the approximately 100 kilobase-pair (kbp) T_H2 locus on mouse chromosome 11, and the gene encoding the cytokine interferon γ ($IFN\gamma$) is located on chromosome 10. In naive (uncommitted) T cells that bear the CD4 cell-surface marker ($CD4^+$), both IL4 and $IFN\gamma$ can be transcriptionally activated to low levels within several hours of exposure to activating conditions, causing differentiation into either of the two types of T helper cells, T_H1 or T_H2 . After this activation, the naive $CD4^+$ T cells then differentiate over several days into T_H1 T-helper cells, which express IL3, IL4 and IL5 at high levels but do not express $IFN\gamma$, or - under different conditions - into T_H2 T-helper cells, which express $IFN\gamma$ but not IL3, IL4 or IL5.

Using the 3C method, Spilianakis *et al.* [5] found a striking colocalization of the $IFN\gamma$ gene with the unlinked T_H2 cytokine locus in naive $CD4^+$ T cells (Figure 1c). Three regulatory regions within the T_H2 locus showed several-fold higher cross-linking frequencies to the $IFN\gamma$ gene compared with the cross-linking frequency between intrachromosomal fragments separated by several kilobase-pairs within the *Gapd* gene. The colocalization found using the 3C method was largely lost after differentiation into T_H1 or T_H2 cells. The authors [5] confirmed the colocalization results using FISH: at least one $IFN\gamma$ allele colocalized with the T_H2 locus in 37% of naive $CD4^+$ T cells but in only 10-13% of T_H1 or T_H2 cells.

Spilianakis *et al.* [5] propose that the potential of naive $CD4^+$ T cells rapidly to induce both $IFN\gamma$ and the T_H2 cytokines at low levels within hours of activation is related to the close interchromosomal interactions between the two loci. To test the functional significance of this interaction, cells were examined from mice containing a homozygous deletion of a DNase I hypersensitive site (RHS7) within the locus control region of the T_H2 locus, an element that is essential for expression of T_H2 -specific cytokines. Previous work had shown that deletion of RHS7 resulted in loss of intrachromosomal interactions between another hypersensitive site - RHS6 - and other *cis* elements within the T_H2 locus. As anticipated, deletion of RHS7 also eliminated the interchromosomal interaction between RHS6 and the $IFN\gamma$ gene and the T_H2 locus, as detected by 3C analysis. Moreover, the rapid induction of $IFN\gamma$ on chromosome 10 was delayed from 3 to 12 hours in naive $CD4^+$ T cells lacking RHS7.

A surprising twist to these experiments, however, was that the deletion of RHS7 nearly doubled the percentage of $IFN\gamma$ and T_H2 loci that colocalized as measured by FISH. Despite this, however, the mean separation between the apparently colocalized loci increased from 1.9 pixels in wild-type cells to 4.5 pixels in cells with RHS7 deleted, and overlapping signals (indicating that the two loci are separated by less than one pixel) decreased from 41% to 13%. One way to reconcile the

3C and FISH results would be to postulate that the observed interchromosomal interactions between the $IFN\gamma$ and T_H2 loci depend on two separate phenomena: a non-random positioning of chromosome 10 and 11 to the same nuclear subcompartment, together with a molecular interaction between specific DNA sequences within the two loci that is dependent on the locus control region. In this model the non-random chromosome positioning would be independent of the function of the locus control region, and perhaps even independent of the gene activity status of the T_H2 and $IFN\gamma$ loci. By bringing these two loci into relatively close proximity, however, establishment of non-random chromosome positioning would then facilitate their close molecular interaction, which would be dependent on the $IFN\gamma$ locus control region. Loss of the $IFN\gamma$ locus control region and gene activity might paradoxically lead to increased percentages of chromosome colocalization, owing to a reduced association of the active $IFN\gamma$ locus with other, competing nuclear structures - distant transcription factories for instance.

The functional significance of gene colocalization

The intrachromosomal interactions demonstrated by Osborne *et al.* [4] appear to be established, at least in part, by the shared attachment of *cis*-linked active genes to large foci enriched in Pol II (transcription factories). The authors [4] propose the tantalizing hypothesis that gene activation requires association with these Pol II foci. What they have actually shown is that a very high fraction of active genes, as detected by RNA FISH, colocalize with these transcription factories in the mouse cell types examined. At the very least, these results show that in their study most of the genes examined become associated with these foci when transcription is on and disassociated when transcription is off. Formal testing of their hypothesis awaits experiments that directly measure a correlation between the onset or cessation of transcription with either the association or the disassociation of gene loci and transcription factories, respectively. Certainly, in fibroblast-like cell types such large transcription factories do not exist [4], and in engineered, artificial systems transcriptional activation is observed along the lengths of large-scale chromatin fibers [9,10]. Yet it is striking that fibroblasts appear to be the exception among differentiated cell types in lacking large Pol II foci. Interestingly, in typical mammalian tissue-culture cell lines that do not show large Pol II foci, a significant fraction of active genes were previously found to be associated with interchromatin granule clusters (IGCs), with multiple active genes colocalizing to a single IGC [11]. The relationship between the transcription factories described in the differentiated primary cells [4] with the interchromatin granule clusters described in other studies is not yet clear.

The origin of the interchromosomal interactions described by Spilianakis *et al.* [5] is even less certain. Given that the

long-range mobility of chromatin within interphase nuclei is generally observed to be low, it is difficult to imagine how two cytokine loci on different chromosomes would find each other within the nucleus if the interchromosomal interaction was driven solely by interactions *in trans* between DNA sequences in the two loci (Figure 2a,b). But if an independent mechanism meant that the territories of chromosomes 10 and 11 were preferentially localized to the same nuclear compartment, the association of cytokine loci on the two chromosomes would be facilitated (Figure 2c,d). This could occur through a direct, *trans* interaction or via association with a shared nuclear body such as a transcription factory.

A major uncertainty involved in understanding the functional significance of the observed colocalization [4,5] is intrinsic to the 3C methodology itself. Does an elevated cross-linking frequency reflect a stable, close molecular interaction between two DNA fragments in a significant fraction of cells? In this case the effects on gene regulation affected by colocalization of coregulated loci could resemble transvection effects in *Drosophila*. Transvection is a phenomenon in which gene

regulation is altered by the interaction of two alleles *in trans*, for instance allowing complementation between two different mutations. Typically, transvection is dependent on the close pairing of homologous chromosomes present in *Drosophila*. Recent data have shown interactions *in trans* between regulatory regions on homologous chromosomes [12]. In mammalian cells, where homologous pairing is generally not observed, similar interactions *in trans* might be facilitated through colocalization to a shared nuclear compartment. Alternatively, does the elevated cross-linking frequency instead reflect a transient molecular interaction, such as collision, that is present within a very small fraction of cells?

In summary, Spilianakis *et al.* [5] have demonstrated a compelling example of interactions between two coregulated gene loci located on different chromosomes. Colocalization was demonstrated both by cytological methods (FISH), demonstrating proximity over a scale of several hundred nanometers to a micrometer, and by the 3C method, demonstrating proximity at a molecular level in an undetermined fraction of cells. These results parallel to a certain extent the previous demonstration of interactions between active genes linked *in cis* on the same chromosome but distributed over a large 40 Mbp region [4].

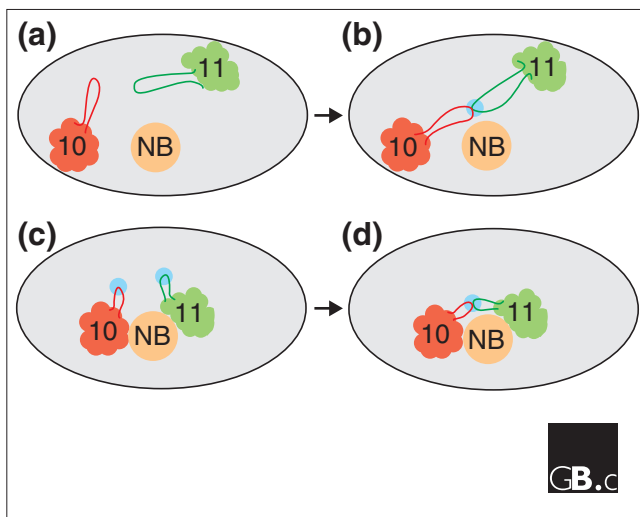


Figure 2

A model showing a way in which specific interchromosomal colocalization could be facilitated by associations with a common nuclear body. Each panel shows a mouse cell nucleus; the territories of chromosomes 10 and 11 are indicated, with loops representing gene loci on each chromosome. Small circles represent transcription factories and NB indicates another nuclear body. **(a,b)** In a one-step model there is no correlation between the positions of the two chromosome territories in the nucleus (a), but genes loop out from the chromosome territories and colocalize as a result of interacting with a shared nuclear body (NB), with or without an associated transcription factory (b). But it is difficult to imagine how loci on two different chromosomes can find each other with the high efficiency that is observed if their chromosome territories are randomly distributed. **(c,d)** A more plausible two-step model in which the two chromosome territories are brought into the same general vicinity of each other, perhaps through association with the same nuclear body (c). Specific colocalization of genes on the two chromosomes is then established (d), perhaps by association with the same nearby transcription factory.

Key questions for future investigation include the following. How general will the observation of interchromosomal interactions between coregulated genes prove to be? More specifically, will a large fraction of coregulated gene loci demonstrate such colocalization, or will it be restricted to a few select examples? Is interchromosomal interaction intrinsic to the interacting loci or does it require distant sequences on the two chromosomes? Experiments using transgene loci and/or chromosome translocations would address this issue. Is the interchromosomal interaction mediated by direct interactions *in trans* between the two loci, or is it facilitated by colocalization to a shared nuclear compartment, as discussed above and outlined in Figure 2? Finally, there is the fundamental question of the physiological consequences of these interactions. How transient or stable are they at both the cytological and the molecular level? What is the temporal correlation between the interchromosomal interactions and the initiation of transcription? What are the consequences of disrupting the interaction for transcriptional regulation *in trans*? Future advances in live-cell imaging should be invaluable in addressing the latter questions.

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