Exploring the relationship between interphase gene positioning, transcriptional regulation and the nuclear matrix

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Abstract
Since the advent of FISH (fluorescence in situ hybridization), there have been major advances in our understanding of how the genome is organized in interphase nuclei. Indeed, this organization is found to be non-random and individual chromosomes occupy discrete regions known as chromosome territories. Interestingly, this spatial arrangement of chromosomes appears to alter both on differentiation [1] and exit from the cell cycle [2]. Studies have shown that individual chromosomes have preferential neighbours in nuclei [3]; an organization which appears to be cell-type-specific [4].

A correlation between gene density and chromosome organization exists; generally, gene-rich chromosomes are positioned within the nuclear centre, whereas gene-poorer chromosomes are located towards the nuclear periphery [5]. Within chromosomes, transcriptionally inactive alleles are embedded in the heart of the territory, while active alleles tend to be positioned either towards the territory edge or outside the territory altogether [6]. Indeed, gene-rich chromatin loops radiating away from chromosome territories have been reported [7,8]. Recent research suggests that both GC content and replication timing could also be important for positioning chromatin domains within nuclear space [9,10].

Nuclear periphery: an area of repression?
Historically, the nuclear edge has been regarded as an area of transcriptional inactivity. Constitutive heterochromatin is consistently found to associate with both the nuclear periphery and nucleoli, while the early replicating, transcriptionally active, euchromatin occupies a more internal position. This observation conurs with the apparent gene-density-dependent radial positioning of chromosomes revealed by FISH (fluorescence in situ hybridization) studies [5]. Unfortunately, however, the picture is not as straightforward as perhaps once imagined; work is continually being published which makes the transcriptional distinction between the nuclear edge and interior increasingly less certain.

Research shows that the nuclear interior is unquestionably a hot bed of transcriptional activity. RIDGEs (regions of increased gene expression) are found located within the nuclear interior, while anti-RIDGEs, transcriptionally inactive genomic regions, are often observed at the periphery [11–14]. Indeed, Kosak et al. [15] report that the majority of transcribed genes are positioned within the nuclear interior. In line with this, transcriptionally active alleles tend to cluster within the nuclear interior, unlike their inactive counterparts [16].

Is the nuclear periphery in mammals, however, incompatible with transcription? By tracking nascent RNA production, it is evident that transcription occurs throughout the nucleus, both within the nuclear interior and at the nuclear edge [17]. Work performed using mice demonstrates that the IFNγ (interferon γ) locus is located at the nuclear periphery regardless of whether the gene is active or inactive [18]. A study involving HIV further strengthens the opinion that the nuclear edge is not unsuited to gene expression [19]. Recently, three groups have focused on the transcriptional consequences of artificially tethering regions of the genome to the nuclear edge in mammalian cells. Reddy et al. [20] reported that while cxcl5 and cxcl1 were significantly down-regulated as a result of such anchorage to...
the nuclear periphery, no noteworthy changes were observed
in any of the other genes examined. Conclusions from the
studies of Bickmore and co-workers [21] and Kumaran
and Spector [22] appear to confirm this, in that targeting
to the nuclear edge represses some, but not all, genes. To
further complicate matters, it is emerging that NPCs
(nuclear pore complexes) are in fact areas of high
transcriptional activity; this view is, in part, formed by
observing events occurring in both yeast and Drosophila
[23]. This chimes with Blobel’s early gene-gating hypothesis
where he suggested that high levels of gene transcription at
NPCs would logically facilitate mRNA export [24].

What is the functional relevance of gene
repositioning?
Specific genes are found to be repositioned from the
nuclear periphery to the interior during their activation
[25–27]. Other studies demonstrate that when expression
is increased, loci move towards the nuclear interior, while
when repressed they preferentially associate with the
nuclear edge [28–31]. However, it is becoming apparent that
rather than nuclear repositioning dictating transcriptional
activity, it is expression that drives repositioning. Indeed,
purely relocating the Mash1 locus from the periphery
to a more interior position was not sufficient to activate
transcription [32]. Furthermore, the looping out of a gene
from its chromosome territory, into a more interior position,
was insufficient to up-regulate gene expression [6]. Taken
together, these conclusions infer that an internal nuclear
position is more important for attaining high transcriptional
activity, than for the actual activation event itself [33].

Gene positioning and transcription
factories
It is generally accepted that transcription occurs in discrete
foci known as transcription factories, which are found to be
distributed throughout the nucleus, attached to an underlying
nucleoskeleton. These structures were originally identified
by tracking the incorporation of BrdU (bromodeoxyuridine)
to nascent transcripts. The number of these factories
per nucleus is still debatable, with reported values ranging
from hundreds to thousands. They are also very small; on
average, factories are 40–100 nm in diameter. Current models
suggest that gene foci migrate towards these protein-rich
factories, where they are fed through the immobilized
RNA polymerase II complex and transcribed (see Figure 1).
Transcription in these foci is catalysed by one of three
distinct RNA polymerases; in general, RNA polymerase I is
restricted to the nucleolus, while RNA polymerases II and
III are nucleoplasmic (for a review, see [34]).

Contrary to popular belief, mRNA is generated from
active genes during infrequent bursts rather than continual
periods of transcription [35]. The majority of active genes
reside within transcription factories, while inactive loci are
found outside these structures [36]. There is some evidence
to indicate that certain genes preferentially co-transcribe.
Using RNA FISH, Osborne et al. [36] demonstrated
that in mouse B lymphocytes, the proto-oncogene, Myc,
preferentially shares the same transcription factory as the highly transcribed Igh [36].

Recent work by Peter Cook’s group suggests that such
factories appear to specialize in the production of certain
transcript species. Minichromosomes were introduced into
cells and their transcription tracked using FISH. Interestingly,
only ∼20 transcription factories were employed to transcribe the 8000 minichromosomes present per cell; this indicates: (i) that factory specificity exists and (ii) that one factory can process multiple transcripts simultaneously. The study reported that this selectivity was driven by intronic status and promoter type [41]. This implies that specific sequences are transcribed by selected factories; thus, surely if this is the case, the distribution of different transcription factory types would influence the position of genes within the nucleus. Perhaps some genes are positioned within the vicinity of necessary factories, which contain the required transcription factors, while other genes need to migrate some distance in order to be expressed or repressed. This begs the question: which particular elements target gene sequences to specific transcription factories? Elucidating such factors will also aid in understanding the basis of genome organization.

What role does the NM (nuclear matrix) play in organizing the genome?

The NM or nucleoskeleton is proposed to be a permanent network of core filaments, underlying thicker fibres, present regardless of transcriptional activity. It is found to be both RNA and protein rich; indeed, treatment with RNase A destroys filament integrity. In addition to mediating the organization of entire chromosomes, the NM has also been demonstrated to tether telomeres via their TTAGGG repeats. Numerous proteins are found to associate with and form part of the NM; one such protein family is the lamins. It is apparent that these proteins partially mediate the structure's role in organizing the genome; both A- and B-type lamins bind MARs (matrix-attachment regions) as well as telomeric and repetitive DNA (for review see [42]).

Importantly, the NM provides the foundation on which transcription factories operate [43]. A fully functional NM is required to support transcription; if lamin proteins are missing or mutant, this process is perturbed. Spann et al. [44] reported that the presence of mutant lamin A inhibits the activity of RNA polymerase. Furthermore, depleting lamin B1 levels by interference technology significantly disrupts RNA synthesis [45].

Since the NM has a role to play in mediating the organization of both chromatin and transcription factories, it is logical to hypothesize that the structure is also indirectly responsible for the positioning of genes. It is possible to conceive a situation whereby MARs tether chromatin to the NM at particular points, thus influencing the global organization of the genome. If these anchorage points are disrupted, this could, in theory, perturb both global (chromosome) and local (gene) chromatin structure.

Perturbed genome organization in disease

Within the literature, evidence exists which suggests that genome organization is perturbed in disease. In several cancers, certain human chromosomes are found to be incorrectly positioned [46,47]. This also occurs in lamino-pathy patients whose cells harbour mutations in LMNA; both HSA (human chromosome) 13 and 18 are found to occupy a more interior position than controls [48].

However, what effect does this disorganization have on the behaviour of specific genes and how does it contribute to pathology? The repositioning of chromosomes or genes seen in disease could merely reflect the perturbation of nuclear architecture and the inability of certain structures, such as the NM, to organize the genome. Most interestingly, gene positioning could be used for diagnostics since specific genes in breast tumour tissue are consistently mispositioned compared with control tissue [49].

It is possible that if genes are incorrectly positioned, their normal local environment changes significantly, thus altering the type of transcription factories available in the vicinity. As a result of this, gene expression could be misregulated, with genes either not participating in transcription or being forced to use suboptimal factories. Indeed, it is evident that the relationship between the positioning of certain genes and their transcriptional activity needs to be studied more extensively before being fully understood.

What can evolution tell us?

Perhaps one of the most thought-provoking articles on genome organization in recent years has been that published by Solovei et al. [50]. The group investigated and compared the nuclear architecture of rod photoreceptor cells in both nocturnal and diurnal mammals. Significantly, the results questioned the widely accepted theory; that the heterochromatin-rich regions of the periphery are a staple in nuclear organization. The study discovered that, in rod photoreceptor cells of the mouse, this typical arrangement is in fact reversed; gene-dense regions decorate the nuclear edge, while gene-poor regions localize in the interior. In line with this, the majority of transcription machinery is also found peripherally. Interestingly, all genes, regardless of transcriptional activity, were located towards the nuclear edge. The authors reported that this ‘inverse’ pattern of organization in the retina was indeed associated with nocturnal vision; diurnal mammals have conventionally arranged rod photoreceptor nuclei. This finding has led to many questions; one of which centres around evolution. The demonstration that an ‘inverse’ version of the ‘conventional’ genome organization exists, suggests that each must confer an evolutionary advantage to the corresponding organisms. Solovei et al. [50] suggest that this inverse arrangement facilitates, in some way, the transmission of photons. Whether or not this is the sole reason, the study reveals the flexibility of the organization and indicates that it remains unchanged through evolution for a purpose. Evidently, this ‘purpose’ will be inextricably linked to transcriptional regulation; however, for the time being, the answer remains elusive. It is extremely important to identify those elements which encode the global organization of the genome.
Closing remarks
Over the last decade, there have been major advances in our understanding of how the genome is organized. It is now becoming increasingly apparent that the functional relevance of this organization is somewhat complex; indeed, the transcriptional distinction between the nuclear periphery and nuclear interior is not as straightforward as once imagined. The repositioning of certain genes correlates with changes in expression; however, with other loci, it does not. It is likely that gene repositioning is used in order to regulate the expression of specific genes; it is probably not representative of a blanket genomic response. Furthermore, the significance and role of transcription factors in mediating this organization should not be overlooked. As our understanding of these factories increases, so too will our comprehension of genome organization and the nuclear structures which dictate it.

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