Q&A

CELL BIOLOGY

Chromosome territories

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The natural habitat of eukaryotic genomes is the cell nucleus, where each chromosome is confined to a discrete region, referred to as a chromosome territory. This spatial organization is emerging as a crucial aspect of gene regulation and genome stability in health and disease.

What do chromosome territories look like?

The word 'chromosome' usually conjures up striking images of the dense, X-shaped entities seen during cell division. It is easy to forget that for most of the time chromosomes exist as unravelled structures and their arrangement is confined by the boundaries of the cell nucleus. We now know that each chromosome maintains its individuality during the cell cycle and occupies a spatially limited volume, known as a chromosome territory. Using fluorescent tags, these can be seen *in vivo* as roughly spherical domains of about 2 micrometres in diameter (Fig. 1a, b; Box 1, overleaf).

Do all cells have them?

Chromosome territories can only form in cells that have a nucleus (eukaryotic cells), and most higher eukaryotes are thought to have them. But some lower eukaryotes, such as the yeast *Saccharomyces cerevisiae*, lack chromosome territories and their chromosomes seem to be more loosely arranged.

Do the territories have an internal structure?

The interiors of chromosome territories are permeated by highly branched, interconnected networks of channels. These make the genome sequences deep inside accessible to regulatory factors such as gene activators and inhibitors (Fig. 1c). In addition, the structure of the DNA within chromosome territories is nonrandom, as the chromosome arms are mostly kept apart from each other and gene-rich chromosome regions are separated from gene-poor regions. This arrangement probably contributes to the structural organization of the chromosome, and might also help in regulating particular sets of genes in a coordinated manner.

So how does a chromosome fold up into this form?

This is not yet clear. Observations in plants suggest that the chromatin fibre — comprising the DNA and its associated proteins — forms large loops that are anchored to each other at their base (Fig. 1d). However, in higher eukaryotes such as mammals the fibre seems rather to be folded into distinct megabase-sized domains that are linked to one another (Fig. 1e). Each of these domains might represent a functional unit, because their replication is coordinated and maintained in consecutive cell cycles. A hybrid model in which smaller loops emanate from a central chromosome core has also been suggested (Fig. 1f). In addition, there is debate about how chromatin fibres are organized at the surface of the chromosome territory. Some observations suggest that giant chromatin loops protrude from the chromosome territory and intermingle extensively with fibres from neighbouring chromosomes, whereas other studies seem to show that abutting chromosomes don't mix much. Emerging high-resolution light-microscopy methods should settle this issue soon.

Are the territories arranged in particular patterns within the nucleus?

Remarkably, yes. In lower eukaryotes such as plants and flies chromosomes tend to be polarized, with the ends of the arms (telomeres) on one side of the cell nucleus and the point at which the two arms meet (the centromere) on the opposite side. In mammalian cells, however, chromosome arrangement is more complex. Even so, each chromosome can be assigned a preferential position relative to the nuclear centre, with particular chromosomes tending to be at the nuclear interior and others at the edge (Fig. 2a, overleaf). This preferential radial arrangement also, of course, gives rise to preferred clusters of neighbouring chromosomes.

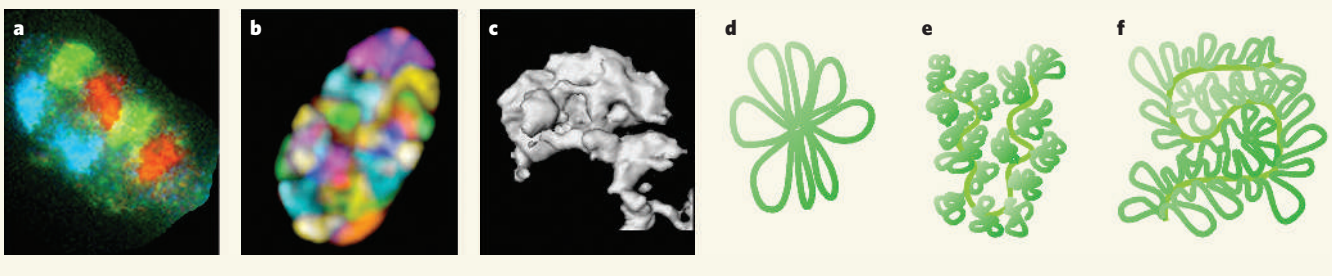
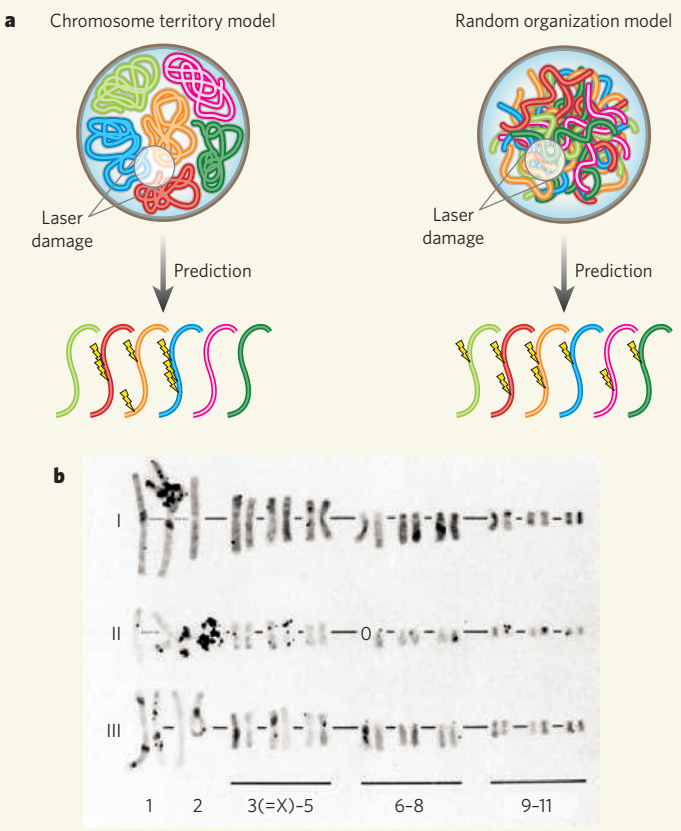


Figure 1 | Spatial organization in the nucleus. **a**, Territories can be visualized using chromosome-specific fluorescent probes. In this mouse liver nucleus, chromosome 12 (red), chromosome 14 (green) and chromosome 15 (blue) were painted. **b**, Modern imaging techniques allow all chromosomes in a cell to be visualized simultaneously, as seen here in a human fibroblast. **c**, Chromosome territories are not solid entities, and their interior is permeated by a network of nucleoplasmic channels. **d**–**f**, Higher-order organization of chromosome territories. In plants, the chromatin fibre forms a rosette-like structure (**d**), whereas in higher eukaryotes chromatin forms interconnected megabase-sized domains (**e**). Other models suggest looping of the fibre from a central backbone (**f**). (Panel **a** courtesy of L. Parada; panels **b** and **c** courtesy of T. Cremer.)

Box 1 | The discovery of chromosome territories



At the turn of the twentieth century, Carl Rabl and Theodor Boveri proposed that each chromosome maintains its individuality during the cell cycle, and Boveri explained this behaviour in terms of 'chromosome territories'.

The existence of chromosome territories was demonstrated experimentally during the early 1980s in pioneering microlaser experiments by the brothers Thomas and Christoph Cremer. They used a microlaser to induce local genome damage, and predicted that inflicting DNA damage within a small volume of the nucleus would yield different results depending on how chromosomes were arranged. If chromosomes occupied distinct territories (**a**, left panel), localized damage would affect only a small subset of chromosomes, whereas if the chromatin fibres of each chromosome were randomly distributed throughout the nucleus (**a**, right panel), many chromosomes would be damaged. **b**, Three sets (I–III) of hamster chromosomes after laser damage. Only a subset of the chromosomes was damaged, as indicated by the black grains of radioactivity most prominently seen on chromosomes 1 and 2. This demonstrates the existence of chromosome territories. (Panel **b** was reprinted with permission from C. Zorn *et al. Exp. Cell Res.* **124,** 111–119; 1979.) **K.J.M. & T.M.**

Is the arrangement always the same, then?

First of all, the patterns are probabilistic, rather than absolute; so although a chromosome may have a preferred average position in a cell population, the location of the chromosome in individual cells within that population can vary greatly. Even the two copies of the same chromosome within the same nucleus often occupy distinct positions and have different immediate neighbours.

Chromosome arrangements are also specific to the cell and tissue type, and can change during processes such as differentiation and development. For example, during differentiation of immune T cells, mouse chromosome 6 moves from an internal position to the nuclear periphery.

The precise physiological relevance of chromosome positioning is currently unclear. However, its significance is hinted at by the fact that there is similarity in chromosome-position patterns among cell types that share common developmental pathways and by the observation that chromosome positions in a given cell type are evolutionarily conserved. For example, in human lymphocyte cells, chromosomes 18 and 19 tend to occupy a peripheral and an internal position, respectively — as does the corresponding genetic material in Old World monkeys.

Why have all this organization?

The nonrandom organization of the genome allows functional compartmentalization of the nuclear space. At the simplest level, active and inactive genome regions can be separated from each other, possibly to enhance the efficiency of gene expression or repression. Such compartmentalization might also act in more subtle ways to bring co-regulated genes into physical proximity to coordinate their activities. For instance, in eukaryotes, the genes encoding ribosomal RNAs tend to cluster together in an organelle inside the nucleus known as the nucleolus. In addition, observations made in blood cells suggest that during differentiation co-regulated genes are recruited to shared regions of gene expression upon activation.

So, how do chromosomes find their place in the nucleus?

We don't know. Chromosomes are physically separated during cell division, but they tend to settle back into similar relative positions in the daughter cells, and then they remain stable throughout most of the cell cycle. So there must be some molecular mechanism that establishes and maintains the chromosomes' positions. The radial positioning of chromosomes has been related to either the chromosome gene density or the amount of DNA they contain, depending on cell type and proliferation status. But these cannot be the only factors involved, because the arrangement changes during differentiation and proliferation, when gene density and chromosome size remain constant.

What are the mechanisms of chromosome positioning?

There are two fundamentally different possibilities. It may be that chromosome positions are determined through their association with immobile nuclear elements — possibly a nuclear scaffold similar to the molecular structures that support and organize the cell's cytoplasm. Although such anchoring may explain chromosome immobility and stability during the cell cycle, it cannot account for nonrandom positioning unless there is some sort of tethering mechanism that is specific to each chromosome and also encodes positioning information.

An attractive alternative is a self-organization model in which the position of each chromosome is largely determined by the overall activity of all of its genes; that is, the number and pattern of active and silent genes on a given chromosome. The idea here is that the expression status of a genome region affects local chromatin structure, with inactive regions being more condensed (heterochromatin) and highly active ones decondensed (euchromatin). Depending on the degree of genome activity and the linear distribution of active and

inactive regions on a chromosome, different chromosomes are expected to have distinct overall physical properties. These might, in turn, determine their likelihood of interacting with each other and so affect their relative arrangement. This model explains the observed clustering of functionally equivalent chromosomal regions, such as the heterochromatic centromeres. It also explains the documented tissue-specificity of chromosome patterns, because chromosomes in different tissues express distinct subsets of genes.

Does nuclear position matter as far as individual genes are concerned?

The position within the nucleus of several genes has been linked to their activity. In yeast, certain genes relocate to the nuclear periphery after activation. In mammalian cells, some genes relocate from the periphery towards the interior once they have been switched on. Similarly, a number of highly transcribed gene-dense regions — for example the MHC class II locus — are expelled from the chromosome territory once they are activated by formation of a large chromatin loop. On the other hand, many other genes either do not change position upon activation or move in the opposite direction, and active genes are found both at the surface of chromosome territories and deeply buried within the territory.

So there isn't a simple rule that applies to all genes?

No. It seems that the actual position of a gene in the cell nucleus is not essential to its function. It is more likely that positioning contributes to optimizing gene activity. Indeed, in yeast the association between a gene and the nuclear periphery does not determine whether or not the gene is active *per se*, but seems instead merely to modulate a gene's expression to optimize it.

What about the position of genes relative to each other — does that affect how they function?

The relative positioning of DNA sequence elements to one another and their physical interaction is emerging as highly significant for several cellular functions (Fig. 2b). In particular, changing the relative arrangement of genome regions can bring regulatory regions into proximity with otherwise distant genes to control their function. It has long been known from observations in the fruitfly that placing a gene near a block of heterochromatin represses expression of that gene. Recent observations suggest that gene loci may also be controlled in a similar manner by interactions with particular regulatory regions on other chromosomes. The most striking example of such regulation comes from odorant receptor genes. Each mouse olfactory neuron expresses only one of more than 1,000 odorant receptors. Which gene is expressed is determined when

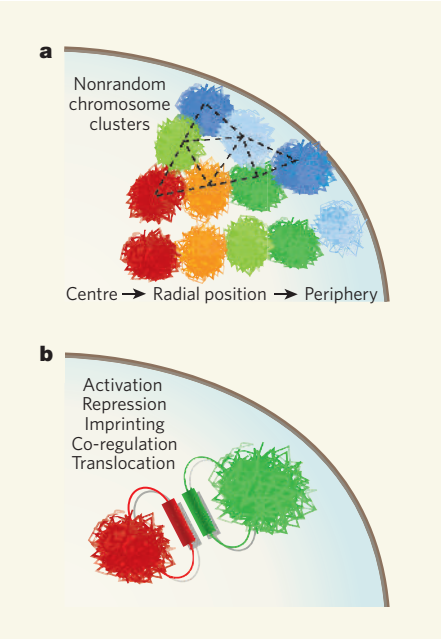


Figure 2 | Arrangement of chromosome territories. a, Chromosomes occupy nonrandom radial positions relative to the centre of the nucleus. Red and orange chromosomes are preferentially internal, green chromosomes intermediate and blue chromosomes peripheral. Positioning patterns are probabilistic, and preferential radial positions lead to nonrandom clusters of chromosomes. **b**, Changing the relative arrangement of genome regions (coloured rectangles) to bring them into close proximity is functionally relevant for gene activity, and for the formation of chromosomal translocations.

a regulatory element on one chromosome associates with an odorant receptor gene on a different chromosome to selectively activate it. Similar interchromosomal interactions may be involved in differentiation-specific gene activation in immune T cells and in gene imprinting — the process whereby the maternal or paternal copy of certain genes is permanently inactivated in a cell.

Are there any other consequences of the relative arrangement of genome regions?

Arrangement can affect genome stability, particularly the formation of cancer-promoting chromosome translocations. Such translocations occur when double-strand breaks in neighbouring chromosomes are not rapidly and correctly repaired, and the broken ends from different chromosomes are mistakenly joined. It turns out that chromosomes that are preferentially close to one another seem to undergo translocations more readily than those that are farther apart.

Is any of this of practical interest?

Apart from providing insight into genome function, it might be possible to exploit the positioning patterns of genes and chromosomes in the near future for diagnostic purposes. Any genome region that changes its nuclear position as a result of aberrant gene expression may be indicative of a disease process. For example, diseased cells could be identified by detecting changes in the positions of marker genes brought about by their misregulation. The advantage of such positional analysis would be the detection of aberrant cells in the context of intact tissue and in primary biopsy samples, without the need for cell culturing. In addition, because changes in positioning are an initial step in gene activation, relocalization of certain regions may permit early diagnosis of a disease. Such methods might be particularly useful for the analysis of solid tumours, which are mostly refractory to routine diagnostic chromosome analysis.

And what of the future?

This is a truly exciting field for many reasons. Identifying the rules and mechanisms that determine how genomes and chromosomes are spatially organized, and how their organization changes during physiological processes, is a logical continuation of our ongoing exploration of genome sequences. Without elucidating the cell biology of genomes, we will not understand how genomes function in intact living cells or how these functions go awry in disease.

So far, much of the work in this field has been descriptive and correlative. But these early mapping studies have provided the necessary framework to begin to design experiments that test the role of genome positioning in gene expression. The great aspiration for the field now is to identify the molecular mechanisms responsible for the repositioning of single genes, genome regions and whole chromosomes within the nuclear space, and to determine how these mechanisms respond and contribute to physiological cues, such as stimulation through signalling pathways. Such insight will contribute greatly to a full appreciation of how the one-dimensional DNA sequence gives rise to the multi-dimensional complexity of the gene-transcriptional networks that determine all aspects of life. ■

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