Cell nucleus: **Chromosome dynamics in nuclei of living cells**

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Unraveling chromosome movements *in vivo* is indispensable for understanding the functional architecture of the nucleus and its relationship to the functional state of the cell. New experimental approaches have now made it possible to monitor chromosome dynamics within the nuclei of living cells.

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The functional architecture of the cell nucleus has become a subject of great interest, as it has become obvious that cell nuclei are compartmentalized structures [1,2]. During interphase and in non-cycling cells, each chromosome occupies a distinct territory within the nucleus [3–7]. Nuclear proteins and RNAs are also compartmentalized, displaying characteristic spatial and temporal patterns [8–10]. It has been suggested that the positioning of specific DNA sequences, in relation to either their chromosomal context (or territory) or nuclear compartment, is involved in the control of nuclear functions, including gene expression [7,11–14]. The specific, dynamic positioning of DNA sequences and/or the corresponding chromosome territories may therefore be linked to the characteristic biological features of a given cell type.

Many studies on fixed cells have demonstrated a characteristic dynamic positioning of chromosomes and/or specific chromosome subregions associated with various functional aspects of the cell's state, such as cell-cycle progression, physiological changes, differentiation, gene expression changes, genetic imprinting and pathological states (reviewed in [15,16]). In this context, a central question emerges: how is the specific dynamic positioning of chromosomes, or chromosome subregions, achieved and regulated according to the functional state of the cell? To answer this question, the kinetics of chromosome movements have to be carefully analyzed in living cells; studies on fixed cells allow only rough and indirect conclusions by statistical analyses.

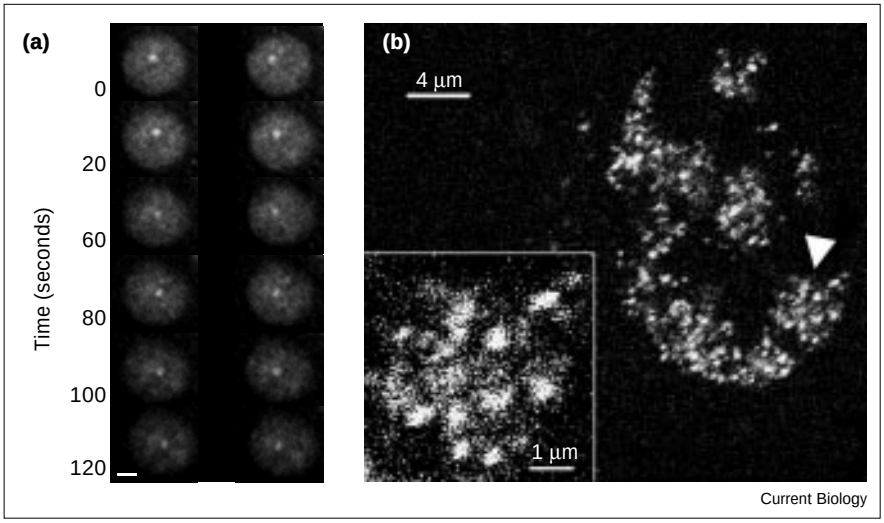
Recent technical developments [17–21] have made it possible to study chromosome dynamics in living cells of various types and from a broad spectrum of organisms. Such broad applicability is important, as dynamic positioning at the cellular level is influenced by a variety of different factors, and the organization of chromosome positioning is likely to vary between taxa. For example, many cell types in *Drosophila* exhibit features, such as polytenization or the pairing of homologous chromosomes [4,22], that are rarely observed in mammals [15,16]. Studies in different taxa therefore have to be carefully compared with one another, and extrapolations are in general not possible. We shall focus on the most recent developments; chromatin dynamics observed earlier, such as nuclear rotation [16], will not be considered.

Two different approaches are now available for visualizing single chromosomes, or chromosomal loci, in nuclei of living cells. One approach involves visualizing proteins that bind specific chromosomal loci or subregions [17–20]. (There are no proteins known to bind specifically to entire individual chromosomes.) Visualization is based on microinjection of fluorochrome-labeled proteins [20] or antibodies [19], or on the use of fusion proteins involving the green fluorescent protein (GFP) [17,18,20]. The proteins are targeted either to endogenous binding sites [18–20] or to introduced exogenous binding sites [17,20]. The visualization of proteins binding specific chromosomal sites has been successful in yeast [17,20], *Drosophila* [19,20] and mammalian cells [17,18]. The advantage of this approach is that it allows single loci (Figure 1a), or artificially introduced subregions, to be visualized.

The second approach involves labeling the DNA directly, as in a set of experiments that we and our colleagues reported recently [21]. In these experiments, stable DNA labeling was achieved by incorporating fluorochrome-labeled nucleotides during replication. Semiconservative replication of the entire genomic DNA results in labeling of the two chromatids derived from each replicated chromosome. To obtain cells in which just one or a few interphase chromosomes — or chromosomal territories — are labeled, the cells were grown for several further cell cycles. During this additional growth period, DNA replication necessarily yields an increasing number of unlabeled DNA strands, while the number of originally labeled DNA strands is maintained.

At the second mitosis after the *in vivo* labeling, there is one labeled and one unlabeled chromatid for each chromosome. After several additional cell cycles, the random mitotic segregation of labeled and unlabeled chromatids results in living cells with nuclei containing a small number of fluorescently labeled chromosomes (or chromosome territories). While this approach is of limited usefulness for visualizing specific chromosomal

Figure 1



The two basically different approaches that have been taken to visualize single chromosomal loci or chromosome territories in nuclei of living cells. (**a**) An example of the visualization of specific loci by binding of a labeled protein. Rhodamine-labeled topoisomerase II, which detects a short repeat sequence on the X chromosome, was injected into *Drosophila* embryos; the embryos were imaged *in vivo* and the resulting time series of stereo pairs is shown. Times corresponding to each stereo pair are given in seconds; the bar represents 2 μm. (Reproduced from [20].) (**b**) An example of the visualization of chromosome territories by DNA labeling [21]. One optical section through the nucleus of a living human neuroblastoma cell is depicted. Single labeled chromosome territories are visible. One territory (arrowhead) is shown as an enlargement (inset). Subdivision of the territory into smaller subchromosomal regions with a diameter of approximately 400–800 nm is clearly visible. These regions are called subchromosomal foci, and display permanent refoldings and extensions of their surface (not shown). (Reproduced with permission from [21].)

subregions, it is well suited to studies of entire chromosomal territories. The two approaches of protein and DNA labeling are thus complementary (Figure 1).

What kinds of dynamics have been observed so far using these two methods? Marshall *et al.* [20] have recently reported their instructive study of the dynamics of single chromosomal loci in yeast and *Drosophila* nuclei. The loci were visualized, in yeast cells by the binding of Lac repressor–GFP fusion proteins to introduced Lac operator sequences, and in *Drosophila* embryo cells by the binding of fluorescently labeled topoisomerase II to a specific endogenous locus. Elaborate quantitative analyses of the resulting three-dimensional time series gave comparable results for the yeast and *Drosophila* loci. The observed dynamics are best explained by Brownian motions confined to a region with a radius of 0.3 μm in yeast nuclei and 0.9 μm in *Drosophila* embryo nuclei. It was shown that the movements are not dependent on active metabolism, and that microtubules are either directly or indirectly involved in constraining the movements.

Marshall *et al.* [20] suggested the following model to explain their results. Each chromosome is fixed by a series of attachment points distributed along its length to an immobile superstructure, such as the nuclear envelope or nuclear matrix. The chromosome is thus partitioned into a set of domains, each a region of confined movement. This suggestion that the observed confinement of diffusion reflects the tethering of discrete chromosome sites to an immobile structure is supported by the observation that loci on plasmids in yeast nuclei do not diffuse faster than loci on whole chromosomes. The size independence of the diffusion rate can be explained if the two structures, despite their different sizes, are tethered in the same way. This study shows that careful quantitative image analysis is indispensable in attempts to identify the driving force behind nuclear dynamics, for example, whether they reflect Brownian or motor-protein-driven motions.

Other studies on living *Drosophila* and mammalian cells, however, have indicated that there are additional types of chromosome motion within the nucleus, although these were not supported by elaborate quantitative analyses, as performed by Marshall *et al.* [20]. Working with *Drosophila* embryos, Buchenau *et al.* [19] visualized a single chromosomal locus (93D) by the specific binding of the Hrb57A protein after heat shock. Time series analysis revealed selective movements of locus 93D within the polyploid nuclei of an embryo. When several 93D loci were visualized within the same nucleus, some were found to move with a considerable speed — more than 3 μm in 10 minutes — whereas others in the same nucleus showed saltatory motion about an average position — referred to as 'jitter' — or random walk behavior intermediate in speed.

Shelby *et al.* [18] observed selective movements of α-satellite domains in human interphase cells, visualized with fusion proteins between GFP and CENP-B, a protein that binds specifically to the centromeric α-satellite sequences. Within the same nucleus, some α-satellite domains moved 7–10 μm per hour — corresponding to

1–2 μm in 10 minutes — whereas others were positionally stable. Selective movements of single loci may be motor-protein driven, but the present state of quantitative analysis does not unequivocally exclude Brownian motions as their origin.

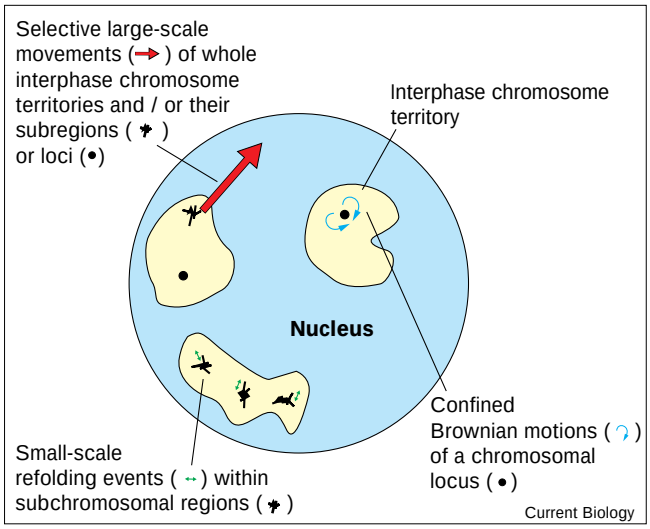
Constrained Brownian motions, such as those described by Marshall *et al.* [20], could be responsible for the jitter observed for otherwise positionally stable loci by Buchenau *et al.* [19]. The stationary loci described by Shelby *et al.* [18] show some oscillation around fixed positions, which could also be explained by constrained Brownian motions. In this sense, it appears that chromosome loci within interphase nuclei might display both types of dynamics: constrained Brownian motions in general and, in addition, selective large-scale movements (Figure 2). Selective dynamics are likely to be specific for the locus, cell type and state, and species. Such specificity might explain why they were not observed by Marshall *et al.* [20].

Marshall *et al.* [20] interpreted the constrained Brownian motion they observed as diffusive movements of chromosomal subregions within a positionally stable interphase chromosome territory. Selective large-scale movements of single loci, as observed in the other studies, either have to deform the underlying chromosome territory or have to be coupled to similar movements of the whole territory. A set of experiments that we and our colleagues recently reported [21] sheds light on the relationship between the dynamics of single loci and the dynamics of whole chromosomes. In this study, single interphase chromosome territories of living human cells were visualized using the DNA-labeling approach described above.

Three types of movement were observed. First, the repositioning of subchromosomal regions (subchromosomal foci; see Figure 1b) within positionally stable territories. Second, selective movements of single territories through the nucleus, while other territories within the same nucleus were positionally stable. The speed and selectivity of movements of entire chromosomes that we observed are in good agreement with the observations by Shelby *et al.* [18]. And third, small-scale refolding events within subchromosomal regions (subchromosomal foci; Figure 2). The first and second types of movement fit very well the picture outlined above, in which loci move either constrained within their chromosome territory or selectively and at larger distances accompanied by their entire territory (Figure 2).

In future studies, it will be helpful to evaluate the kinetics and energy dependence of the selective movements; this should open the way to answering the most intriguing questions about chromosome dynamics. Which molecules mediate specific recognition and positioning of chromosomes or subchromosomal regions? And how is this process regulated? In addition to specific recognition coupled to motor-protein-driven dynamics, selective tethering to an underlying immobile structure might also play a part. With regard to the motor proteins that could drive selective movements, a variety of candidates have been identified in interphase nuclei, including myosin (reviewed in [16]). Whether these proteins really have a role in chromatin dynamics remains to be established.

Figure 2



The different types of chromosome dynamics observed in interphase nuclei. Chromosomes occupy distinct territories within the cell nucleus during interphase. Chromosomal loci display confined Brownian motions, as observed in nuclei of budding yeast cells [20] and *Drosophila* embryos [20]. Selective large-scale movements of entire territories and chromosomal subregions or loci have been seen in nuclei of human cells [18,21] and *Drosophila* embryos [19]. Small-scale refolding of subchromosomal regions has been observed in nuclei of human cells [21].

In conclusion, while studies on nuclei of fixed cells revealed large-scale chromosomal rearrangements that are associated with changes in the functional state of the cell ([15,16] for example), recent studies on living cells have shown that chromosomal motions are generally constrained to a small nuclear volume, and large-scale movements are selective and relatively rare. These observations can be reconciled if the large-scale chromosomal movements occur, by and large, only when the functional state of the cell changes, as during differentiation, for example. If a cell has reached a particular state, its large-scale chromosome organization is maintained. No systematic studies on the possible relationship between major alterations of the functional state of a cell and intranuclear chromosome movements have been made yet in living cells.

If the large-scale organization of chromosomes and subchromosomal regions is indeed essentially fixed when

a cell is in a particular state, then the potential functional interactions of chromosome loci would be topologically restricted to their neighboring loci and compartments. These functional interactions could be mediated by constrained Brownian motions, and their fine tuning might be indicated by the highly dynamic refolding that has been observed for chromosomal subregions and other nuclear compartments [23]. The tools are now at hand to find out how chromosomes are organized in relation to the functional state of the cell.

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