Visualizing Chromatin Dynamics in Interphase Nuclei

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Real-time fluorescence microscopy has emerged as a powerful tool for examining chromatin dynamics. The initial lesson is that much of the genome, particularly in yeast, is highly dynamic. Its movement within the interphase nucleus is correlated with metabolic activity. Nonetheless, the nucleus is an organelle with conserved rules of organization. Determining the distribution and regulation of mobile domains in interphase chromosomes, and characterizing sites of anchorage, will undoubtedly shed new light on the function of nuclear order.

The visualization of chromatin in living cells has long challenged cell biologists. In contrast to its successful application to metaphase chromosome structure, electron microscopy (EM) revealed little about the functional organization of interphase chromatin. However, thanks to a bacterial repressor/operator interaction and naturally fluorescing proteins [e.g., green fluorescent protein (GFP)], recent breakthroughs in high-resolution analysis of nuclear organization have been possible. The genomic integration of *lac* operator arrays in eukaryotic cells that express a GFP-*lac* repressor fusion permits the tracking of specific chromosomal sites by real-time fluorescence microscopy (*1*). The integrated binding sites can cover as little as 2 kb in a yeast chromosome (*2*), and both nuclease and functional analyses suggest that the inserts do not substantially alter local chromatin structure (*3*). By combining these chromosomal tags with a GFP-labeled nuclear pore protein, one can obtain high-resolution information on the movement of a given locus in relation to the nuclear envelope.

Interphase Chromatin Is Highly Dynamic

The initial surprise from this approach was the extremely dynamic behavior that chromatin exhibits in both yeast and *Drosophila* nuclei (*4–6*). The characterization of these rapid movements required high-speed image acquisition by either charge-coupled device–based deconvolution or scanning confocal microscopy. In the model systems explored to date, fluorescence and transmission imaging were combined to characterize the tagged cell with respect to its division cycle or differentiation pathway. In the first example of high-resolution tracking, a tagged site inserted near the telomere of the X chromosome was examined in *Drosophila* spermatocytes (*5*). These nuclei expanded markedly in size as the cells progressed through a prolonged G_2 phase, before the first prophase of their meiotic division. A second system made use of mitotically dividing budding yeast, in which multiple loci (including a centromere, a telomere, and two internal chromatin sites) were tagged in different strains (*6*). In yeast, large rapid movements (>0.5 μm in a 10-s interval) were observed for the internal chromosomal loci in G_1- and S-phase nuclei. Smaller, saltatory movements (<0.2 μm) occur throughout interphase in both yeast and flies, whereas the large rapid movements are most frequent in the G_1 phase, occurring on average once per minute in yeast (*6*). Given that the size of the GFP signal and the focal resolution of fluorescence microscopy both range from 0.2 to 0.3 μm, these shorter distances are difficult to characterize by light microscopy. In contrast, the half-micron movements were striking, representing movement across half the radius of a yeast nucleus, a distance equivalent to roughly 100 kb of folded chromatin [based on a linear compaction ratio of ~70-fold (*7*)].

What is the importance of this unexpected chromatin mobility? Is the motion a result of an active process—energy-dependent or motor-driven? Does it reflect the impact of the cytoskeleton or other cytoplasmic structures on the nucleus? Are the movements directed, or is the DNA on a random, diffusive walk? Most importantly, can such motion be linked to specific nuclear activities? As summarized below, the initial studies have provided nearly as many questions as answers and reveal the need for new models for nuclear organization that accommodate rapid and large-scale chromatin dynamics.

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Chromatin Movements Reflect the Metabolic State of the Cell

By monitoring the direction and length of each sequential movement of GFP-tagged foci in yeast and flies (see Fig. 1), one finds little support for the hypothesis of directed movement (*5*, *6*). Nonetheless, interphase chromatin movements were not completely random. Individual movement lengths have a roughly Gaussian distribution in the studies performed on *Drosophila* spermatocytes, although the direction of the motion changes with every two to four movements (*5*). In other words, a large movement in one direction was often followed by a leap back, indicating that chromatin moves in a limited space, modeled as a random walk on a chain (*5*, *6*). Tracking of a single site for 5 min in yeast shows that a chromosomal locus may "sample" a fairly large fraction of the nucleus within this period (Fig. 1D), yet show no sign of directed motion (*6*). This is in striking contrast to the poleward movement of chromosomes in mitosis, which is driven by microtubule-dependent motors.

Nonetheless, these interphase dynamics were shown in yeast to be exquisitely sensitive to adenosine triphosphate (ATP) depletion and to changes in metabolic status in the cell (*6*). This correlation makes it unlikely that the movement results from simple diffusion. Because the motion of noncentromeric sites was not affected by microtubule-depolymerizing drugs, it seems that neither nuclear nor cytoplasmic microtubules are involved. Instead, it was proposed that the movement reflects the action of large ATP-dependent enzymes involved in transcription or chromatin remodeling. This hypothesis is consistent with the lack of mobility detected in stationary phase cells where transcriptional activity drops substantially (*8*).

The Extent of Chromatin Wandering

When thousands of measurements for a GFP-tagged locus are compared over time, either as a relative movement between two spots in a diploid, or as the movement of one locus relative to the nuclear center, an estimation of the spatial constraints imposed on the movement can be calculated by plotting the mean squared displacement over fixed intervals of time (*4*). Such plots permit one to reliably compare the mobilities of different chromosomal domains at

different points in the cell cycle, and they even allow cross-species comparisons of the confinement imposed on different loci. Except at very short time intervals, the chromatin movements in flies and yeast show confinement in restricted volumes that are much smaller than the nuclear volume (*4*–*6*), and for each locus a characteristic radius of confinement could be determined. Not all loci were the same; yeast centromeres and telomeres have confinement radii ≤0.3 μm, whereas nonspecialized internal chromosomal sites moved within zones at least twice that size (*4*, *5*). For loci on the X chromosome in *Drosophila* spermatocytes, short-range movement was confined to a zone with a radius of 0.5 μm, although over longer time periods an additional longer-range movement could be detected in early G_2 nuclei (*5*).

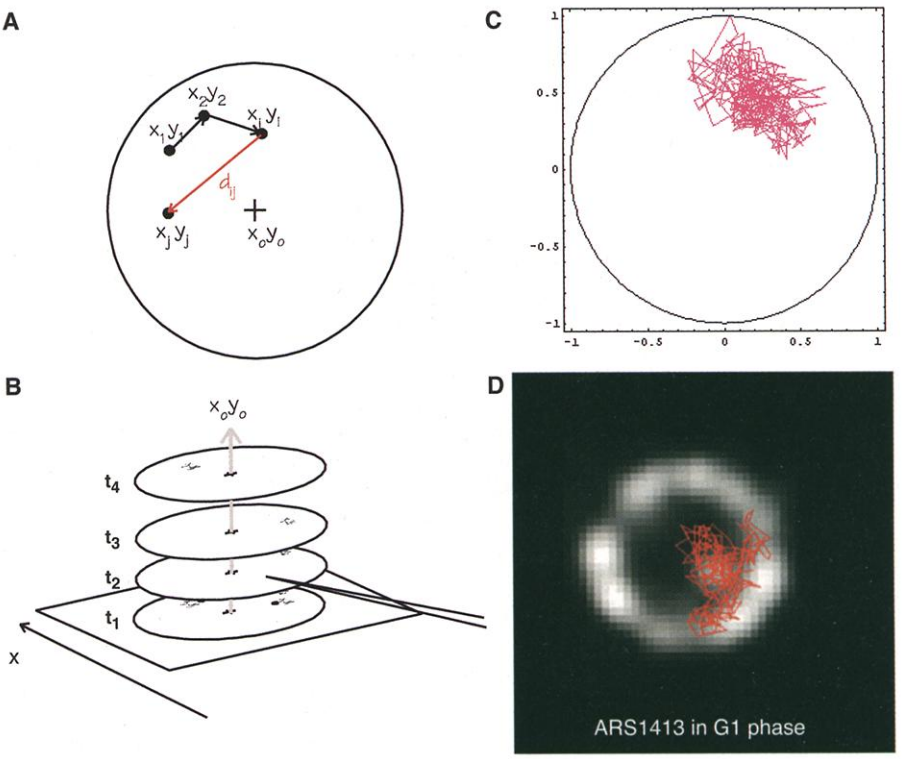

Fig. 1. Movement of a GFP-*lac*^{OP}–tagged yeast chromosomal site in a G_1-phase nucleus. The path of a GFP-tagged yeast chromosomal domain during 200 consecutive images taken at 1.5-s intervals is shown in red, superimposed on a single nuclear section (**D**). The experiment [described in detail in (*6*)] makes use of a haploid *Saccharomyces cerevisiae* cell in G_1 carrying a series of *lac* repressor–binding sites near an origin of replication on chromosome 14 (ARS1413). The strain expresses both a GFP-*lac* repressor fusion and GFP-Nup49, for visualization of the chromosomal locus and nuclear pores, respectively. The method of monitoring movement in sequential images (t_1–t_4) is shown in (**A** to **C**). For tracking analysis, the peripheral ring (nuclear pore signal) was aligned within the time series (B), and the position of the GFP-tagged chromosomal region is tracked frame by frame and projected onto one idealized nuclear plane (C). Time lapse movies of this can be accessed online at www.sciencemag.org/cgi/content/full/294/5549/2181/DC1.

Observing this degree of mobility for multiple loci, one is prompted to reconsider the hypothesis that each eukaryotic chromosome is assigned to a discrete subnuclear territory, surrounded by interchromatin channels that facilitate macromolecular movement (*9*). Whereas the data on chromatin dynamics do not refute the notion of nuclear compartmentation (*9*), this latter must accommodate a substantial degree of intermingling of mobile domains from one chromosome to the next (Fig. 2). Moreover, it is unlikely that macromolecular movement is restricted to interchromatin channels, because chromatin itself shows a high degree of motility. A chromosome's invasion into its neighbor's space seems to be particularly pronounced in yeast, which have a nuclear diameter of ~2 μm and calculated confinement radii for individual loci of ~0.5 μm. The unusual efficiency of homologous recombination events in yeast may in part reflect this mobility. In any case, the rapid local motion and long-range migrations discussed below require changes in our concept of chromosome compartmentization even in mammalian cells.

Centromeres, Telomeres, and Replication Sites Tether Yeast Chromosomes

Rather than thinking of entire chromosomes as having fixed locations, it is perhaps more useful to propose that periodic sites of tethering keep uninemic chromatin in a general subnuclear zone. For example, in yeast it was shown that telomeres and centromeres are markedly more constrained in their movements than other tagged loci along the chromosomal arm (*6*). Knowing that telomeres are tethered to the nuclear envelope (*10*) and that centromeres are tethered near the spindle pole body (SPB) (*3*), one might propose that the forces on the yeast chromatin fiber are more or less universal, but that movement is restricted by periodic sites at which a physical "drag" is imposed. This anchoring can be envisioned as a reversible binding to elements of a nuclear structure, or even as interaction with a cluster of similar domains (Fig. 2). The tether on the yeast centromere appears to involve microtubules that associate with the SPB, a membrane-embedded equivalent to the centrosome (*4*, *6*), whereas telomeres interact with the nuclear envelope through the yeast Ku heterodimer (*10*). Consistently, GFP-tagged telomeres regain considerable mobility in yku mutant strains (*11*).

Constraints on yeast centromeres and telomeres persist through the G_1 and S phases. But an additional S phase–specific drop in mobility is observed for internal yeast chromosomal loci (*6*). This stage-specific constraint correlates with the presence of active replication forks and can be modulated either by drugs or mutations that impair replication efficiency. The movement of both late-replicating and early-replicating loci becomes restricted in early S phase, which suggests that it is not the proximity of a replication fork, but the overall amount of replication in the cell, that imposes constraint (*6*). Whether this reflects a physiological change in chromatin remodeling activities, or in the sheer bulk of clustered replication complexes, is unknown. It will be important to compare chromatin mobility in the G_1 and S phases of *Drosophila* cells to see how universal this phenomenon is.

Anchorage Sites Along Chromosome Arms

A periodic attachment of chromatin to the nuclear periphery seems to be a conserved mechanism for positioning chromosomes, although such tethering does not necessarily occur through telomeric sequences. Three-dimensional (3D) deconvolution microscopy on fixed cells has provided clear evidence for anchorage sites along *Drosophila* chromosomal arms (Fig. 2B) (*12*). Rapid time-lapse microscopy of such sites tagged with GFP has not yet been performed; thus it can only be inferred from their reproducible positions that these sites will show constrained mobility. Whether

this involves short inserts of repetitive DNA or a clustering of unlinked or distant sites, as observed for yeast telomeres (*3*), is not yet known.

The mobility of a large cluster of a 359-bp satellite repeat on the *Drosophila* X chromosome was visualized through its affinity for a fluorescently modified topoisomerase II and was tracked by 3D time-lapse microscopy of stage-12 embryos (*12*). This repetitive, nontranscribed DNA showed constrained dynamics and a confinement radius of 0.9 μm, which is much larger than that monitored for nonrepetitive DNA in the spermatocyte nucleus (*5*). It is not known whether these differences are a reflection of cell type or the particular locus examined.

There is evidence, however, that nuclear size and, as a result, free nuclear volume may influence chromatin dynamics. In early G_1-phase yeast cells, daughter nuclei have 40% less volume than do mother cell nuclei, and chromatin movements greater than 0.5 μm are five times less frequent than in mother cells of the same culture (*6*). This correlation remains to be generalized to other species, yet differences in nuclear size may well be at the root of the differences in confinement radii detected between embryonic and spermatocyte nuclei of flies. In this context, it is interesting to note that terminal differentiation of tissues often correlates with a reduction in nuclear volume (Fig. 3).

A Role for Repetitive DNA?

The analysis of fluorescent probes for the large satellite repeats found at higher eukaryotic centromeres has shown that these domains, like the clusters of simple repeat DNA that are found dispersed along the chromosomal arms, move little in interphase nuclei (*13*). A more direct study of repetitive DNA, on the other hand, concerns inserts of *lac*^{op} sites in mammalian cells, which contain from 10 to 1000 copies of a 14-kb unit. A study reported by the Belmont group indicates that this region assumes the characteristics of heterochromatin, being late replicating and tightly condensed (*14*). The insert showed little short-range mobility in G_1-phase nuclei, although it moved inward at mid-S and decondensed for replication (*14*). When transcription was induced on similar constructs by targeting a strong activator to a co-amplified promoter, the chromatin was seen to expand, occupying a larger volume at a position less peripheral in G_1-phase nuclei than it did at the silent locus (*15*). The expansion or unfolding of multimerized inserts has been observed in several instances, yet there are as yet no data published correlating the fine movements of a single-tagged gene in a mammalian nucleus with its expression. Ideally, movement would be monitored in relation to the nuclear lamina, to allow an analysis of local chromatin dynamics in mammalian nuclei.

Although the data available suggest that heterochromatin-like inserts in mammalian nuclei are less dynamic than tagged sites in yeast or flies, the studies of protein diffusion coefficients based on photobleaching assays suggest that DNA bound proteins in mammalian cells can be highly mobile [reviewed in (*16*)]. Intriguingly, certain inserted *lac*^{op} arrays were found to associate with promyleocytic leukemia (PML) bodies, which themselves show energy-dependent movements within the nucleus (*17*). Thus, the simple idea that DNA bound proteins or compartments like PML bodies intrinsically impede nuclear mobility needs to be modified.

It remains to be seen whether large blocks of simple repetitive DNA, like centromeric satellite repeats, form zones that are relatively immobile (*13*). Clustered in AT-rich isochores of mammalian chromosomes are retrotransposon-like long interspersed noncoding elements (LINEs), which constitute about 35% of the chromosomal sequence. These nontranscribed islands may stabilize the interphase chromosome position in the nucleus, possibly through proteins that tether several such elements together. Clearly, these models for nuclear organization can be tested with live imaging techniques.

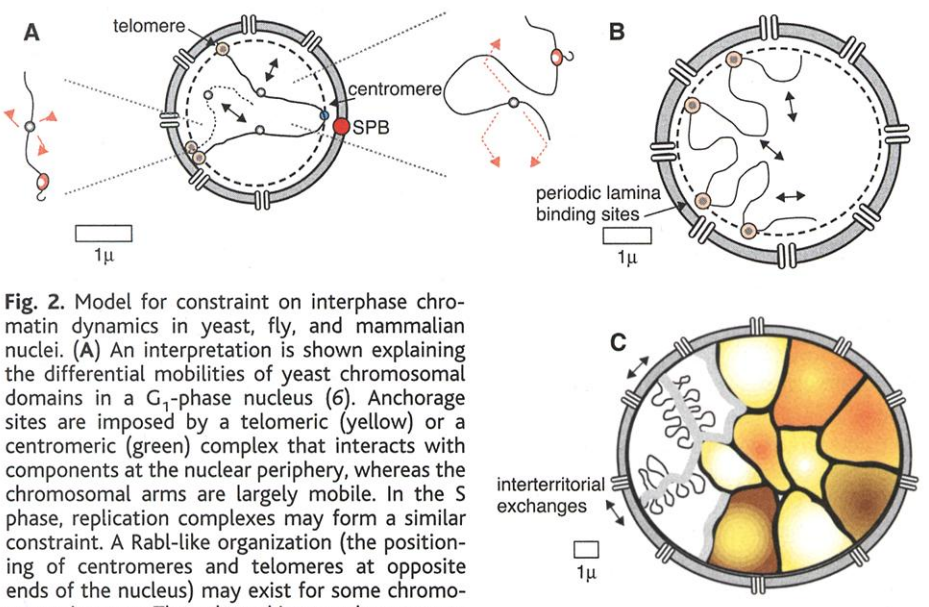

Fig. 2. Model for constraint on interphase chromatin dynamics in yeast, fly, and mammalian nuclei. (**A**) An interpretation is shown explaining the differential mobilities of yeast chromosomal domains in a G_1-phase nucleus (*6*). Anchorage sites are imposed by a telomeric (yellow) or a centromeric (green) complex that interacts with components at the nuclear periphery, whereas the chromosomal arms are largely mobile. In the S phase, replication complexes may form a similar constraint. A Rabl-like organization (the positioning of centromeres and telomeres at opposite ends of the nucleus) may exist for some chromosomes in yeast. The enlarged images demonstrate the reduced mobility (red arrows) of a tagged locus due to its proximity to an anchorage site (hooked oval). (**B**) Multiple anchorage sites have been mapped along the *Drosophila* chromosome, as detected by FISH for embryonic cells (*12*). It is possible that periodic chromosomal sites interact with the nuclear lamina, constraining chromosome dynamics (black arrows). In these *Drosophila* embryonic cells, centromeres and telomeres are also polarized. (**C**) In cultured mammalian cell nuclei, FISH analysis using whole-chromosome probes suggests that the bulk of a given chromosome tends to occupy a fairly well defined territory (*9*), indicated here in different shades. The short-range movements monitored in *Drosophila* and yeast (*4–6*), and long-range movements observed in *Drosophila* and mammals, suggest that chromatin can readily invade adjacent territories and even migrate across the nucleus. In the left-hand part of the nucleus, we suggest a "grey zone" of dynamic chromatin that has the potential to migrate both inward and outward from its "territory." Mammalian somatic cell nuclei generally do not maintain a Rabl configuration of chromosomes, although it is not excluded that in particular cell types this might exist.

Large-Scale Migration of Chromosomes Within Interphase Nuclei

In addition to the rapid short-range dynamics of chromatin, longer-range chromosome movements have also been documented in mammalian and *Drosophila* cells [reviewed in (*16*)]. Using both whole-chromosome and satellite-specific fluorescence in situ hybridization (FISH) probes and GFP fusions, chromosomal domains have been observed to migrate from the nuclear periphery to the interior, and vice versa, often reflecting changes in the cell cycle or in transcriptional activity (*18–20*). In some cases, these movements reflect the establishment of interchromosomal heterochromatin contacts (*20*), or their subsequent disruption in the S phase. Two other instances of long-range movement have been reported in living cells. GFP-labeled human centromeres revealed a slow but directional movement of individual or small groups of centromeres (*13*), and the *lac*^{op}-tagged domain on the *Drosophila* X chromosome was shown to undergo directed migra-

tion in spermatocyte nuclei, in addition to the rapid motion discussed above (*5*). This longer-range movement occurred in early, but not late, G_2 phase, possibly reflecting a step in the spermatocyte's progression toward meiosis.

Long-range relocalization from a random position to an internal site has also been documented for the highly transcribed human chromosome 19, when cultured dermal fibroblasts recover from serum starvation and reenter the cell cycle (*18*). The more internal positioning of the active chromosome 19 was again altered when RNA polymerase II (Pol II) transcription was inhibited (*19*). This mechanism is reminiscent of movements described above, in which an amplified *lac*^{op} insertion moved from the nuclear periphery inward when stimulated by a highly potent acidic transactivation domain (*21*). To what extent these longer-range movements reflect or depend on the short rapid movements is unknown. Nonetheless, long-range movements are likely to require a certain degree of local mobility.

chromatin mobility would correlate with its genetic plasticity, whereas increased anchorage would correlate with fixed patterns of gene expression, including genes that are stably on and others that are stably off (Fig. 3). Whether this involves attachment to the nuclear envelope or interactions between chromosomes through proteins like the Polycomb group (*24*) remains to be seen. The imposition of artificial anchorage sites might test this model.

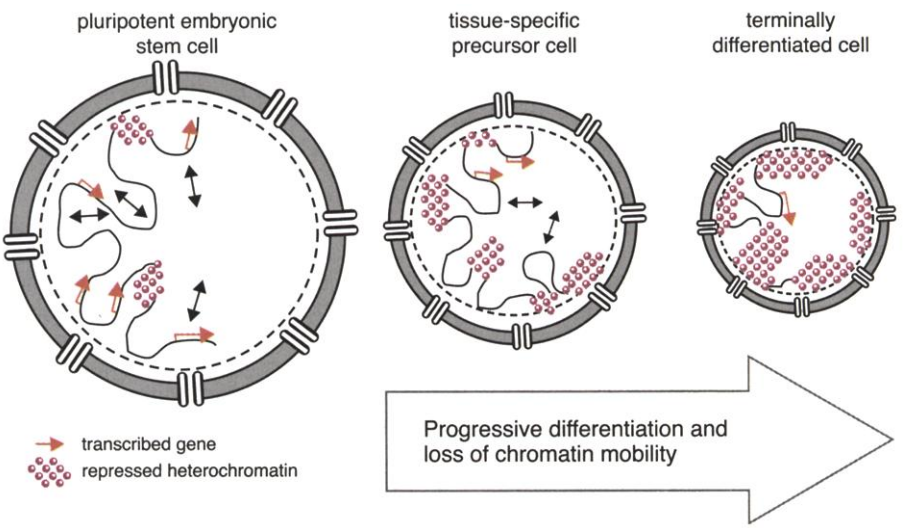

Fig. 3. Model for nuclear organization during the restriction of genomic expression profiles. This speculative model predicts that progressive differentiation within a multicellular organism cell will restrict both the expression profile and the dynamics of the cell's interphase chromatin. Totipotent embryonic nuclei are large and may have fully dynamic chromatin, like that of a yeast cell. We propose that the mobility reflects the potential to be either transcribed or modified into an inactive chromatin domain, accounting for most genes in an embryonic stem cell. As transcription patterns become increasingly defined, nuclei are generally smaller and less of the chromatin remains mobile. Reversal of this may occur during meiosis and oncogenic transformation.

Functions for Chromosome Tethering

It seems probable that sites of regulated anchorage are used by the cell nucleus to regulate patterned gene expression. One possibility is that anchoring is used to create boundaries between chromatin domains, sequestering DNA either from histone modifiers or from the action of polymerases. An example of this is the *Drosophila* Gypsy insulator element, which is thought to function by tethering its target DNA to perinuclear sites, where many Gipsy sites aggregate (*22*). Rather than targeting a precise subnuclear position, the act of anchoring may itself provide a boundary function, separating active and inactive domains (*23*).

A variation on this model suggests that local chromatin movement is a prerequisite for changing the transcriptional status of a gene, whether for repression or activation. Mobility may facilitate access for enzymes involved in histone modification, nucleosome remodeling, and the ensuing folding or unfolding of the domain. In this model, one should note, in this respect, that roughly 90% of the yeast genome is "open" or potentially transcribed chromatin, whereas the situation is reversed in mammalian cells, where 90% of the genomic DNA can be transcriptionally silent in a fully differentiated tissue. The random insertion of *lac*^{op} sites into the nucleus of a differentiated mammalian cell is thus more likely to reveal the behavior of an inactive locus than an active one. If the patterning of gene expression correlates with an increase in chromatin anchoring, it will be important to compare chromatin mobility between embryonic stem cells and differentiated tissues.

Does RNA Pol II Move Chromatin?

Is it possible that the rapid movements of interphase chromatin reflect RNA polymerase activity itself? Consistent with this view are data from Buchenau *et al.* (*25*), who monitored large and rapid movements (~1 μm in less than 20 s) of an actively transcribed heat-shock locus in *Drosophila* embryos, which was tracked by the injection of fluorescent antibodies against a heterogeneous nuclear RNA binding protein, Hrb57A. This protein binds specifically to the 93D heat-shock locus after heat shock and was seen to move in heat-shocked embryos very similarly to the *lac*^{op} inserts characterized in yeast. Both rapid "jittering" motion and leaps of >0.5 μm were monitored (*25*). Because, in this case, an RNA binding protein was followed, it is clear that the movement correlated with a transcribed gene.

On the other hand, EM analyses and careful calculations have suggested that a moderately transcribed gene (i.e., not a heat-shock locus) has, on average, only one engaged polymerase associated with it (*26*). Because the average size of a yeast transcript is roughly 2 kb, it requires a minute or two to be completed; one might predict that in yeast one would see 1 to 2 min of rapid movement, and then several minutes of immobility, as the RNA polymerase reinitiates. This has not yet been seen (*6*). In mammalian cells, with intron-containing transcripts having a mean length of 25 kb, the polymerase would be engaged for 20 to 30 min. If the elongation of an engaged RNA polymerase were the source of the interphase dynamics, we would expect a more sustained period of movement for a given transcription unit in a higher eukaryotic cell than we would in yeast. Moreover, differentiated mammalian cell nuclei should have fewer mobile domains than do pluripotent, undifferentiated cells. The possibility of imaging chromatin live at sites of induced transcription should allow one to test definitively whether RNA pol II transcription is a fixed or mobile event.

Conclusion

For over a century, the dynamic poleward movement of mitotic chromosomes was subject to analysis by light microscopy, yet similar techniques applied to interphase chromatin led to primarily static models of rigidly separated compartments, representing either individual chromosomes or clusters of heterochromatic repeats. The recent application of high-resolution time-lapse microscopy to distinct chromosomal loci opens a field of research rich with

promise—the spatial dynamics of interphase chromatin. It is not simply the relative position of domains within the nucleus but also the interactions between homologous and nonhomologous sites, and their role in the propagation of a 3D nuclear organization through mitotic division, that will be relevant to questions of genomic function. The characterization of gene regulation through their dynamic spatial properties must be accompanied by a precise determination of higher-order chromatin structure. Nonetheless, it is a safe bet that both sets of information will be essential for unlocking the mechanisms that control expression of the eukaryotic genome.

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