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Moving chromatin within the interphase nucleus- controlled transitions?

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Abstract

The past decade has seen an increasing appreciation for nuclear compartmentalization as an underlying determinant of interphase chromosome nuclear organization. To date, attention has focused primarily on describing differential localization of particular genes or chromosome regions as a function of differentiation, cell cycle position, and/or transcriptional activity. The question of how exactly interphase chromosome compartmentalization is established and in particular how interphase chromosomes might move during changes in nuclear compartmentalization, has received less attention. Here we review what is known concerning chromatin mobility in relationship to physiologically regulated changes in nuclear interphase chromosome organization.

Keywords

interphase chromosome; chromatin mobility; nuclear organization

1. Overview of Interphase Chromosome Organization

Perhaps the most striking element of nuclear organization of interphase chromosomes is their partitioning into distinct “chromosome territories” (CTs) [1–3]. First inferred from the chromosomal distribution of DNA repair sites when a small region of the nucleus was irradiated by UV light [4], CTs are now best visualized by in situ hybridization using chromosome-specific probe sets [5]. Analysis using fluorescence in situ hybridization initially suggested the absence of overlap between CTs or even subchromosomal regions. However, higher resolution analysis has revealed significant, local chromosome intermingling between chromosome territories [6]. While much of this intermingling may be non-specific, new molecular methods such as “3C” (chromosome conformation capture) are beginning to identify examples of specific interactions between distant gene loci separated by a number of Mbp on the same chromosome or even on different chromosomes [7].

Superimposed on the organization of chromosomes into separate CTs, is the nonrandom location and orientation of individual CTs within interphase nuclei. In a number of species, including *Drosophila* [8] and yeast [9], chromosomes assume a Rabl conformation, at least in some tissue cell types, with centromeres clustered in a chromocenter at one end of the nucleus and telomeres at the other side. Interestingly, in *Drosophila* epithelial cells the chromocenter is located at the apical end of the nucleus. Even in cells without a Rabl conformation, centromeres and telomeres often localize at characteristic locations relative to the nuclear

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periphery, nucleolus, and nuclear interior depending on the cell type and cell cycle stage [10–12], with centromeres again forming chromocenters in some cell types. Blocks of heterochromatin also frequently localize to the nuclear or nucleolar periphery.

Early replicating chromosome regions tend to be localized more towards the nuclear interior versus late replicating regions [13,14]. Gene rich chromosomes are localized more towards the nuclear interior versus gene poor chromosomes [15]. In mammalian cells at least, a similar polarization is observed for gene rich versus gene poor chromosome regions [14]. In some cell types, these gene rich genomic regions associate closely with SC-35 domains which correlate with interchromatin granule clusters (IGCs) [16]; in other cell types, active genes are found clustered near foci of RNA polymerase II staining, called “transcription factories” [17]. A change in intranuclear positioning associated with transcriptional activation away from the nuclear periphery or chromocenters and towards the nuclear interior also has been described for a number of individual genes [18,19]. This includes examples of movement of specific genes towards IGCs [20] or transcription factories [17,21]. In some cases, particularly for clusters of active genes, a looping out from the main CT occurs [22–25], which may be associated with targeting to specific nuclear bodies. Conversely, a repositioning of genes to the nuclear periphery or chromocenters has been observed after transcriptional repression [26–28]. In contrast, in budding yeast many genes move to the nuclear periphery and become associated with nuclear pores upon gene activation [29].

Whereas most attention to date has focused on documenting these examples of nonrandom interphase chromosome nuclear positioning, what has not yet been examined carefully is how this highly nonrandom nuclear organization is established. Do gene loci move in the nucleus through random diffusion or directed movements? Is this organization established largely as nuclei reform after mitosis or do chromosomes move at all times during the cell cycle? In this review we will focus on different types of chromatin movements observed in interphase nuclei, their physiological context, and how they might be regulated.

2. The null hypothesis and local chromatin mobility

Conceptually, one can imagine a range of mechanisms accounting for the establishment of nonrandom intranuclear positioning of interphase chromosomes. At one extreme is the “null hypothesis” in which nonrandom interphase chromosome positioning would be established entirely through regulated attachment / detachment of chromosome loci from specific nuclear compartments and/or other loci. Random diffusion would account for chromosome repositioning between different nuclear locations. At the other extreme, interphase chromosomes would move by directed movements from one location within the nucleus to another. These models are not mutually exclusive, and intermediate models can be proposed as well, including for instance randomly directed mobility driven by energy dependent processes. In the null hypothesis model, chromatin mobility must be sufficiently fast and long-range to account for observed changes in interphase nuclear positioning. Moreover, this random motion should be energy independent and diffusive in nature. For efficient chromosome repositioning, chromosomes must be able to move significantly faster, over the relevant time period for the observed interphase chromosome repositioning, than the net distance change during the chromosome movements in order to allow sampling of different nuclear compartments. Conversely, if random chromosome mobility is slow or constrained relative to the time and distance scale of observed interphase chromosome repositioning, then active mechanisms underlying interphase chromosome movements must be considered.

3. Local chromatin mobility versus chromosome repositioning in *Saccharomyces cerevisiae*

In the budding yeast *Saccharomyces cerevisiae*, measurements of local chromatin mobility indicate rapid, localized movements which, although constrained spatially, are fast enough to allow interphase chromosomes to explore a large nuclear subvolume over a biologically meaningful time scale. By tagging a locus near centromere III with GFP-lac repressor bound to a lac operator direct repeat, Marshall et al. [30] were able to track chromatin motion during G1 in diploid cells by measuring the distance between two homologous loci. The mean square change in distance (MSD) versus time interval increased linearly over short time intervals before reaching a plateau at time intervals greater than 100–200 seconds. The dependence of MSD versus time is commonly used to analyze particle motion. A linear increase over time interval is observed for free diffusion, whereas a linear increase followed by a plateau is observed for particle diffusion constrained within a limited volume. The inferred radius of confinement from these observations was $\sim 0.3 \mu\text{m}$. A “constrained diffusion” model was proposed in which local diffusion of chromatin results in rapid movements constrained by tethering of adjacent chromosome sites to nuclear substructure. In budding yeast nuclei, clustering of centromeres near the spindle pole body is eliminated by a mutation in a kinetochore protein or by nocodazole treatment [31]. Interestingly, nocodazole treatment significantly increased the observed radius of constraint to $\sim 0.7 \mu\text{m}$, suggesting that microtubules mediate tethering of interphase centromeres in yeast.

Later experiments systematically examined variations in chromosome mobility for different chromosomal locations [32]. Measurements of chromosome IV centromere mobility were quite close to the previously published values for chromosome III, and the mobility of a telomere on chromosome VI was even lower. However, two interstitial chromosome locations, one near an early replicating origin of replication and another in a late replicating chromosome region, showed a several fold increased mobility in G1 nuclei, with more than a doubling of the radius of confinement observed for centromeres. Over a 10 min period, trajectories for these interstitial chromosome locations extended over $\sim 1/3$ of the haploid nuclear volume. Moreover, examination of individual trajectories revealed occasional rapid jumps in chromosome position, defined by movements exceeding $0.5 \mu\text{m}$ within a 10 second interval, occurring on average more than once per minute.

4. Local chromatin mobility in *Drosophila melanogaster* and mammalian cells

Measurements of chromatin mobility in *Drosophila* precellular blastoderm, embryonic nuclei similarly suggest a constrained diffusion pattern, with a linear increase in MSD over a 10–100 second time interval range, followed by a plateau at times greater than ~ 200 seconds [30]. The estimated diffusion rate was several fold higher than that observed for internal yeast chromosome sites and the radius of confinement corresponded to $\sim 0.9 \mu\text{m}$, close to that observed for interstitial chromosome sites in yeast G1 nuclei.

Chromatin mobility was observed to drop significantly during some examples of cell differentiation. In *Drosophila* early G2 spermatocyte nuclei, the estimated diffusion rate was nearly the same as that observed in embryonic nuclei but dropped roughly 20 fold in late G2 spermatocyte nuclei [33]. The actual drop in mobility is likely even larger, as the local mobility is overestimated because of changes in locus separation produced by nuclear shape changes which occur in these late G2 nuclei. Equally dramatic was the change in the estimated radius of confinement from $\sim 3 \mu\text{m}$ in early G2 to $\leq 0.3 \mu\text{m}$ in late G2 spermatocyte nuclei. The decreased mobility observed in late G2 spermatocyte was attributed to a developmentally, cell cycle regulated increased local tethering of chromatin to nuclear substructure. Because tagged loci on sister chromatids in late G2 nuclei could show within the same nuclei a range of mobility

from high values typical of early G2 nuclei to essentially immobile behavior, this tethering would appear to vary stochastically.

In larval eye imaginal disk nuclei, the estimated local chromatin diffusion rate was intermediate between that observed in embryonic versus late spermatocyte nuclei, and did not change during differentiation [34]. However, the motion became significantly more constrained, with the radius of constraint falling from $\sim 1 \mu\text{m}$ to $\sim 0.2 \mu\text{m}$ in cells with a nuclear radius of $\sim 2 \mu\text{m}$ [34,35].

The mobility in embryonic and early G2 spermatocyte nuclei would allow a given chromosome site to move throughout its chromosome territory in 30–60 min. However, the limited mobility in late G2 spermatocyte and differentiated imaginal disk nuclei would restrict a locus to a volume appreciably smaller than a chromosome territory, although it would only take ~ 10 seconds for a chromosome locus to move a distance equal to the observed $\sim 0.3 \mu\text{m}$ radius of constraint.

Local chromatin mobility in human fibrosarcoma HT1080 cells [36] also can be fit to a constrained diffusion model with estimated diffusion rates very close to that observed in yeast and *Drosophila* late spermatocyte G2 nuclei, several fold lower than in *Drosophila* imaginal disk nuclei, and 10–20 times slower than *Drosophila* embryonic and early G2 spermatocyte nuclei. The estimated radius of confinement was $\sim 0.5 \mu\text{m}$. Significantly, similar mobility was observed for loci in either a G or R band, suggesting an independence of mobility parameters on location in gene rich versus gene poor regions.

Similar to the situation in yeast, a reduced mobility was observed for human chromosome loci associated with the nuclear periphery or nucleoli [36] and, in *Drosophila*, for a heterochromatin block associated with centromeric heterochromatin [35].

5. What do local mobility measurements tell us about physiological changes in intranuclear chromosome positioning?

Most analysis of physiologically regulated changes in intranuclear chromosome positioning in *Saccharomyces cerevisiae* has focused on transitions between nuclear periphery and interior localization. Telomeres are clustered on the nuclear periphery [9,37,38] and the silenced mating type loci are associated with the nuclear periphery [39]. Whereas artificial tethering of an acidic activator, VP16, to a peripherally located site in yeast results in loss of nuclear periphery association [40], a large subset of inducible yeast genes move to the nuclear interior to the periphery [29], including galactose and alpha factor [41] induced genes. This relocation has been proposed alternatively to occur through an association of transcriptional activators with nuclear pore components facilitating transcriptional activation [42,43], or the interaction of nascent transcripts and the mRNA processing / export machinery with nuclear pores [40, 44].

In the case of galactose induced genes, mRNA induction occurs within 5–10 minutes. The time course after transcriptional induction of nuclear repositioning has not been examined for these genes. However, assuming that nuclear repositioning parallels gene activation, the observed mobility of interphase chromosome loci could easily account, at least qualitatively, for such repositioning; in G1 nuclei, $0.5 \mu\text{m}$ jumps occur 1–2 times per minute and loci move over $\sim 1/3$ the haploid nuclear volume within 5–10 minute intervals.

In *Drosophila* and mammalian cells the observed local chromatin mobility has roughly similar magnitude to that observed in yeast but nuclear size is much larger. The radius of constrained chromatin mobility provides a useful parameter for classifying intranuclear chromosome

repositioning. We can define local repositioning as that occurring over distances comparable to the radius of constrained mobility, and long-range repositioning as that occurring over distances several fold larger than the radius of constrained mobility.

For the common mammalian cell lines that have been examined, local repositioning would occur within a $\sim 0.5 \mu\text{m}$ radius and the estimated time for local repositioning would range from tens of seconds to minutes. Long-range enhancer – promoter interactions, the associations of multiple cis-regulatory regions over a large gene locus as in for example the association of DNase I hypersensitive sites from the LCR and gene enhancer / promoter regions within a beta-globin “hub” [45–47], and the coalescence of neighboring insulator sequences, as observed for suppressor of Hairy-wing binding sites in *Drosophila* [48] and CTCF binding sites in mammalian cells [49], would all fall into this local repositioning category.

At the other extreme would be long-range changes in chromosome positioning or associations involving movements several fold larger than the radius of constrained local mobility. Here examples would include interactions in trans between chromosome loci on different chromosomes, for instance the CTCF mediated interchromosomal interaction between Wdb1/ NF1 on chromosome 11 and Igf2/H19 on chromosome 7 in mouse fibroblasts [50], the colocalization of the IFN- γ gene located on chromosome 10 and the IL-4 gene on chromosome 11 in naive CD4+ T cells [51], and the α Hba and β (Hbb-b1) globin genes located on different chromosomes [17]. Other examples would include long-range movement of centromeres and telomeres relative to the nuclear interior or periphery [52], the sorting of early and late replicating chromatin to the nuclear interior versus periphery, respectively [14], the looping of distal heterochromatin blocks back to the chromocenter in *Drosophila* nuclei [53,54], and movement of entire chromosomes relative to the nuclear interior or periphery or between the nuclear periphery and centrally located nucleolus [55–58].

Intermediate between local and long range movements would be a number of interesting specific examples involving change in gene location as a function of transcriptional activation or repression. For instance, transcription factories, defined as local accumulations of RNA polymerase II, vary in numbers from thousands to 100–300 as depending on mammalian cell type [17]. A tight correlation between gene activity / inactivity with association / disassociation of gene loci with transcription factories was demonstrated in a cell type containing just 100–300 transcription factories implying gene repositioning towards or away from these bodies shortly before or after changes gene activation or repression [17]. Dividing the nuclear radius by the cube root of the number of factories provides a rough estimate of the maximum distance a chromosome locus would need to move to associate with the nearest factory. For a nuclear radius of $5 \mu\text{m}$ and 200 transcription factories we get a maximum distance of $0.85 \mu\text{m}$, $\sim 70\%$ higher than the expected radius of constrained diffusion.

A similar repositioning of active gene loci to SC-35 domains has been described [20,59], including the muscle specific genes beta-cardiac myosin heavy chain and myogenin during muscle differentiation and the movement of the hsp70 gene after heat shock. With ~ 20 – 40 SC35 stained regions per nucleus, the estimated maximum distance to the nearest SC35 island is $\sim 1.6 \mu\text{m}$, roughly three times the expected radius of constrained diffusion. Similarly, the observed movement of genes away from chromocenters into the nuclear interior with gene activation [60], and towards chromocenters upon gene silencing [26], falls into this intermediate regime, given variations in chromocenter number (e.g. 10 to 20) during cell differentiation [61].

In these intermediate examples, the observed chromosome locus repositioning is on the borderline of what can be expected from observed local chromatin mobility parameters,

particularly in cases where chromosome locus repositioning would occur over short time periods, as might be observed for rapidly induced genes.

6. How can we explain long-range motions?

The null hypothesis of regulated attachment / detachment with diffusion of chromosome loci between target nuclear sites appears to break down in the case of long-range chromosome associations or changes in position where the distances are several fold greater than the observed radius of constraint for default chromatin mobility. There are several ways conceptually to explain these long-range interactions.

First, there could be special times within the cell cycle or development in which local chromatin mobility increases significantly, allowing longer-range changes in chromosome positioning. Second, within a given cell cycle or developmental stage, there could be a localized increase in local chromatin mobility. This could occur through an increased radius of constraint through a loss of nearby tethers to nuclear substructure, perhaps associated with changes in chromatin structure across a large domain. Changes in local chromatin structure might also change diffusion rates. This would not change the confinement radius but would decrease the time for a chromosome locus to move within the constrained volume. Third, there could in fact be an active mechanism for interphase chromatin mobility. This could produce a randomly directed increased mobility, with the possibility of breaking local chromatin tethers to nearby nuclear substructure. Or there could be a mechanism producing long-range, directed chromatin movements within interphase nuclei.

7. Global changes in chromatin mobility at specific cell cycle and developmental stages

In mammalian tissue culture cells, chromatin mobility is in fact much higher during the first 1–2 hours of G1 than through the remainder of interphase. This conclusion comes from several experimental sources. Ultrastructural analysis of chromosome decondensation during early G1 revealed a dramatic redistribution of chromatin from a largely peripheral localization to a homogenous distribution throughout the nuclear interior between 1 and 2 hrs after mitosis in CHO cells [62]. The characteristic differential segregation of early versus late replicating chromosome regions relative to the nuclear interior and periphery also was observed to occur during the first 1–2 hours in G1 [14]. Direct observations using live cell microscopy confirmed higher chromosome mobility during early G1 [63]. Individual chromosome territories containing fluorescently labeled replication domains were tracked over several hours in cells at different cell cycle stages. Maximum changes in distances between different chromosome territories ranged from 0.5–4 μm for early G1 nuclei, with ~40% exceeding 2 μm , versus 0.3–2 μm for subsequent interphase stages, with only 1/45 examples showing a maximum distance change greater than 2 μm . A selective photobleaching approach in which large regions of interphase nuclei were bleached also revealed little overall long-range movements of chromosomes in mammalian cells [64]. The higher and longer-range chromosome mobility observed during nuclear reformation in early G1 suggests changes in long-range chromosome positioning could occur during nuclear reformation after passage through mitosis, with chromosome positioning essentially becoming locked in place later in G1 for the remainder of the cell cycle.

One documented change in chromosome positioning meets this description. In proliferating human fibroblasts, the gene poor chromosome 18 shows a preferential association with the nuclear periphery versus the similarly sized gene rich chromosome 19 which is located more interiorly [15]. However, in senescent or G0 arrested cells, this peripheral localization of chromosome 18 is lost. After serum stimulation the peripheral localization of chromosome 18

does not reappear until the first few hours after the first mitosis [55]. During differentiation of *Drosophila* eye imaginal disk cells, a similar global decrease in chromatin mobility was observed. The brown dominant allele, located distally on chromosome 2, contains an 1.6 Mbp heterochromatin insertion. Looping back of this chromosome arm and association of this heterochromatin block with the centromere is associated with gene silencing and variegated expression [53,54]. Examination of the kinetics for this looping interaction indicates that the frequency of association of the brown dominant allele with the centromere increases as a function of time after mitosis in undifferentiated cells. However, after differentiation the frequency of looping remains constant, apparently as a consequence of the radius of constrained chromatin mobility dropping from ~ 1 to $\sim 0.2 \mu\text{m}$ [34,35] and locking down global chromosome positions.

8. Examples of long-range interphase movements without an intervening mitosis or change in differentiation

Most documented examples of long-range gene or chromosome repositioning occur within the context of differentiating cells undergoing ongoing cell division. However, several documented examples occur without an intervening mitosis. The inactive X chromosome was observed to move from the nucleolus well into the nuclear interior in post-mitotic cat neuronal cells following 8 hrs continuous electrical stimulation. This change in location appeared several days after electrical stimulation and required ~ 2 weeks for reversal [56]. In human neurons both the active and inactive X chromosome moved from the nuclear periphery in control cells to the nuclear interior in neuronal cells located within epileptic foci [57]. During cell cycle progression between G1 and G2 in lymphocytes, centromeres assumed a more interior distribution and telomeres a more peripheral distribution in mouse lymphocytes [52]. And very recently, the inactive X chromosome in mouse cells has been observed to show an increased association (80–90%) with the nucleolus during mid to late S phase [58].

Using CHO cell lines containing chromosomes tagged with lac operator repeats, three examples of chromosome long-range intranuclear position changes occurring over just a several hour time period without an intervening mitosis have been documented. In A03 cells containing an ~ 90 Mbp heterochromatic, amplified chromosome arm, this amplified region changes from a largely peripheral to interior localization, frequently associated with the nucleolus, between 4 and 6 hours into S phase [65]. Interestingly, the mid to late S phase timing of the movement of this heterochromatic, engineered chromosome arm from the nuclear periphery to the nucleolus, matches the timing of the similar movement of the heterochromatic inactive X chromosome [58]. In C6 cells, a multi-copy plasmid insertion moves from a largely peripheral distribution to the nuclear interior during S phase progression from an early replication pattern I to an early replication pattern II [66].

In these same C6 cells, tethering the VP16 acidic transcriptional activation domain also resulted in a redistribution of this locus from the nuclear periphery to the interior [66]. This differential distribution is established within 1–2 hours after mitosis in cells stably expressing either GFP-lac repressor or GFP-lac repressor-VP16. However, using an inducible tethering system, this redistribution occurred in log phase cells between 1–2 hours after VP16 targeting [67]. Live cell imaging revealed an average displacement of $1.1 \mu\text{m}$ and a maximum movement of $2.5 \mu\text{m}$ between 0 and 2 hours after VP16 targeting versus just a few tenths of a micron average displacement in the controls. Significantly, the 0–2 hours displacement observed in nearly all cells after VP16 targeting exceeded the maximum displacement observed in control cells during this same time period.

9. Regulated changes in local chromatin mobility?

The above examples suggest the possibility of a regulated change in intranuclear position for specific chromosome regions at specific times during the cell cycle or in response to physiological stimuli. In all these examples the change in position relates to the movement away or towards an identifiable nuclear or chromosomal structure, and thus it is reasonable to infer a regulated molecular interaction that mediates attachment / detachment from this structure. But as discussed earlier the molecular explanation for long-range movements must extend beyond regulation of attachment / detachment. Detailed measurements of changes in local chromatin mobility for specific chromosome loci undergoing physiologically triggered changes in nuclear localization are limited. Interestingly, though, for one example results converge from systems as diverse as budding yeast and mammalian cells. Targeting of the acidic activator VP16 in both systems lead to both an increased radius of constrained motion and an increased apparent diffusion rate [40,67]. The increased radius of constrained motion could simply reflect the loss of tethering to the nuclear periphery or other nuclear substructure, but the increased apparent diffusion constant implies a second, regulated change in mobility. This increased diffusion might be explained simply by a VP16 induced change in chromatin structure. However, in the case of the C6 CHO cell line, no change in the condensed dot like appearance of the transgene array is observed after VP16 targeting, although a change in the structure of flanking chromosome regions cannot be excluded. Alternatively, an active, regulated mechanism might be involved in this increased “diffusion” rate.

10. Evidence for an energy-dependent mechanism contributing to local chromatin mobility

In the first study examining chromatin mobility in yeast, treatment with azide to reduce cell ATP pools resulted in no change in apparent chromatin mobility near the tethered chromosome III centromere [30]. However, a later analysis of chromatin mobility at other, less constrained chromosome locations concluded that a component of the observed, rapid but localized chromatin mobility was energy-dependent [32]. Temporal analysis of tagged loci revealed near constant fluctuations in position of less than 0.2 μm , with occasional much larger jumps exceeding 0.5 μm . While distinguishing non-random, non-diffusive motion during a single random walk is quite difficult, the number of these larger jumps per time interval together with the overall chromatin mobility was significantly reduced by experimental treatments which did not change the smaller, oscillatory movements. This included dramatic drops in large amplitude jump frequency after treatment with azide or carbonyl cyanide chlorophenyl hydrazone (CCCH) to reduce ATP levels, and several fold reductions in this jump frequency after changes in metabolic state induced by increased cell density or reduction in glucose levels. From the published results it is not entirely clear to what degree this reduced mobility reflects change in the actual mobility versus tethering as CCCH treatment is associated with a greatly increased association with the nuclear envelope. A later analysis in *Drosophila* temporal resolution in the 1 second range also observed anomalous single particle mobility that could not be explained by simple constrained diffusion models [33]. In a simple constrained diffusion model, the linear regime of the MSD versus time interval plot should extrapolate to zero displacement at zero time interval. Instead a significantly faster mobility was observed at short time intervals less than 4 seconds. A full description of this faster mobility was limited by the experimental time resolution.

Using a novel particle tracking methodology with ~20 nm spatial resolution and 30 millisecond time resolution, further deviations from simple diffusive models for local chromatin mobility were observed in mammalian cells [68]. Tracking a GFP- lac repressor labeled chromosome site in a CHO cell line, MSD versus time interval plots clearly showed a bilinear behavior, with an estimated fast diffusion rate for time intervals under 1 second ~10 fold higher than the

diffusion rate over time intervals of ~2–15 seconds. Spot trajectories consisted of long periods of local mobility, consistent with constrained diffusion, connected through sudden, rectilinear jumps. The jump step size varied from 50–400 nm with a mode of 150nm, and occurred over an estimated 0.3 to 2 second time interval. Periods of intervening constrained motion averaged 56 seconds. A high percentage of the high-velocity motion (above the median velocity) was found during these jumps, with the spot moving 4 times faster on average than during times between jumps. In contrast, trajectory measurements of latex beads diffusing through agarose showed that the mean velocity in the pore regions between regions of constrained diffusion did not differ significantly from the mean velocity of the overall trajectory. These jumps were blocked by ATP depletion and decreased at lower incubation temperature, suggesting an energy-driven mechanism.

11. Visualization of directed long-range interphase chromosome motion- one example

Examination of long-range chromatin movement may provide the best chance of revealing the existence of an active mechanism for interphase chromatin mobility. In *Drosophila* embryonic mitotic cycle 14 nuclei, during which time cellularization occurs, cell cycle progression is tightly correlated with a stereotypical coordinated increase in nuclear length and cell membrane invagination and elongation down from the embryo surface. By measuring the length of the invaginated membrane, the timing from mitosis 13 can be determined. Fluorescence in situ hybridization detection of specific genes positioned along the length of the chromosome arms revealed a specific pattern of gene localization relative to the elongating nuclear axis [69]. Assuming a Rabl chromosome conformation, specific gene loci were expected to maintain a constant relative position along the nuclear axis. Instead gene loci located near the centromeric chromosome end showed stereotypical movements over a period of minutes relative to the apical nuclear pole. Two nearby centromere-proximal genes moved over 5–10 μm distances at inferred velocities of 0.5 - 0.7 μm / minute. The stereotyped nature of these movements and the magnitude of the distances traveled and velocities involved suggest an active transport mechanism. Two genes near each other on the same chromosome showed independent movements. However, in these studies the actual trajectory of a chromosome locus was never visualized but only inferred from analysis of fixed samples. Analysis of centromere movement in live mammalian interphase nuclei showed most centromeres were relatively immobile over a 2 hour observation period [70]. In one published example, movements of several centromeres persisted in a uniform direction for over 1 μm at speeds of ~ 0.1–0.2 μm per minute for ~10 minutes. Similarly, live cell imaging of labeled heat shock loci in *Drosophila* revealed several examples of long-range separation of two loci [71]. In both of these studies, however, this long-range motion was not systematically examined and could not be distinguished from the expected occasional observations of linear trajectories expected from a random walk, or from apparent movements caused by elastic nuclear deformation and shape changes.

Using engineered chromosome regions containing lac operator sites tagged with GFP-lac repressor, three examples of long-range chromosomal movements were observed as described earlier. However, attempts to directly visualize these movements were problematic due to phototoxicity effects. Rare examples of long range movements during live cell microscopy were obtained but normal statistics for movement, as deduced by analysis of fixed cells, were not reproduced [66,72]. Recently a systematic visualization of chromosome locus trajectories was carried out in the C6 CHO cell line during an inducible repositioning of a specific chromosome locus between the nuclear periphery and interior [67]. This systematic analysis allowed minimization of phototoxicity and visualization of chromosome movements under conditions reproducing normal statistics of chromosome redistribution as measured in the absence of light. Inducible tethering of the VP16 acidic activator resulted in long-range movement of a chromosome locus between 1–2 hours after VP16 targeting. Using 1 minute

time sampling over a restricted, 20 minute time period beginning at 1 hour after VP16 targeting, 1/3 of recorded cells showed curvilinear, long-range chromosome site movements over distances of 1–5 μm at velocities from 0.1–0.9 $\mu\text{m}/\text{min}$. These trajectories showed a pronounced radial bias with movements towards the nuclear interior frequently occurring near perpendicular to the nuclear envelope. Three-dimensional optical sectioning demonstrated that these curvilinear trajectories corresponded to actual chromosome movements as opposed to nuclear rotation. These results strongly indicate an active, directed movement of this engineered chromosome site after VP16 targeting. Such an active, directed movement suggests a possible involvement of a molecular motor. Actin has long been known to exist in the nucleus and is now known to function in chromatin remodeling and RNA polymerase complexes [73–75]. Proteins involved in regulating actin polymerization are also found in the interphase nucleus. A recent study using FRAP provided evidence for a fraction of this nuclear actin existing in a polymerized state [76]. Meanwhile, two types of nuclear myosin, nuclear myosin I and nuclear myosin VI, have been identified and implicated in RNA-polymerase II transcription [77–79]. In the C6 cell line, the inward movement of the chromosome spot after inducible VP16 targeting was dependent on actin and nuclear myosin I [67]. Overexpression and targeting to the nucleus of an actin mutant incapable of polymerization abolished translocation of the chromosome site. Also repositioning of the chromosome site was delayed for several hours by a nuclear myosin I mutant with severely attenuated motor function. In contrast, overexpression of a different actin mutant which stabilizes F-actin filaments accelerated the timing of this translocation by 30 minutes. These results suggest a direct or indirect involvement of actin / myosin in this long-range chromosome movement.

12. Summary and Conclusion

The differential localization of specific chromosome loci to different regions within the nucleus is now well documented. Beginning to emerge are specific long distance interactions between gene loci on different chromosomes. The functional impact of this nuclear compartmentalization remains largely unknown. In the case of changes in gene position as a function of transcription, rather than being required these positional changes instead may facilitate or strengthen gene activation or repression. However, a clear experimental demonstration of a functional relationship between nuclear compartmentalization and gene activity is still missing. Moreover these changes may not be related to transcription per se, but rather to the epigenetic modifications normally accompanying changes in the transcriptional state. More poorly understood are the mechanisms underlying these changes in intranuclear chromosome position. Local chromatin mobility appears to be constrained to varying degrees and can change significantly as a function of differentiation and cell cycle progression. The period of early G1 appears to be a period during which chromosomes show higher mobility which then becomes more constrained, in general, throughout the rest of the cell cycle. Similarly, increased constraints on chromatin mobility have been observed during differentiation in a few cases where this has been examined. However, if one looks at the right chromosome region at the right time in the cell cycle or during differentiation, then exceptions are seen where larger mobility is observed for specific chromosome regions or loci.

Regulated attachment / detachment of chromosome loci followed by random diffusion could explain many local changes in intranuclear chromosome position. Some evidence, however, points to this local mobility, although apparently random in direction, being driven at least in part through active, ATP dependent mechanisms whose molecular basis remains unknown. In many differentiated cell types, local tethering of chromatin would appear to prevent long-range changes in nuclear positioning observed for specific loci without the existence of an active, non-diffusive mechanism. In one case so far involving an engineered chromosome site an apparent long-range, directed chromosome movement has been visualized. In this artificial system, directed movement depended directly or indirectly on actin / myosin. The challenge

for the future will be choose the proper biological systems to test whether similar active, directed movements are responsible for nuclear repositioning of endogenous loci and to dissect the underlying molecular mechanisms responsible for these long range movements.

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