

Measuring Limits of Telomere Movement on Nuclear Envelope

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ABSTRACT The dynamic behavior of the decondensed chromatin can be monitored by real-time fluorescence confocal microscopy. It can be observed that different chromosomal sites enjoy different degrees of freedom during a certain period, exploring larger or smaller portions of nuclear volume. Here we measure the accessible surface for two chromosomal sites (yeast telomeres Tel3R and Tel6R) that both exhibit strong preferential association with the nuclear membrane in galactose-containing media, but differ significantly in gene activity. Telomere Tel6R, which harbors an inducible gene with high levels of transcription, explores a much larger surface than the telomere Tel3R, which is adjacent to inactive chromatin. Thus, our results distinguish two perinuclear movements characteristic of different transcriptional state, allowing for a better understanding of the correlation between activity of genes and chromatin dynamics.

Received for publication 17 November 2005 and in final form 23 November 2005.

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The movements of specific chromosomal regions within living cells can be monitored using real-time fluorescence confocal microscopy (1,2). Such studies reveal that in healthy interphase nuclei the decondensed chromosomes show a dynamic behavior that is, however, remarkably different from movements of extra-chromosomal DNA constructs. Although the latter can freely diffuse and explore the entire nuclear volume, the chromosomal sites are confined to much smaller available volumes within the nucleus (3). Interestingly, different chromosomal sites enjoy different degrees of freedom during a certain period; they can explore larger or smaller portions of nuclear volume. In addition, in yeast, some specific sites are free to move in three-dimension (3D) whereas others move preferentially along the inner surface of the nuclear membrane (2,4). Several earlier studies measured the volume accessible to freely diffusing chromosomal sites (2–5), but for chromosomal sites that show preferential attachment there has been no attempt to measure the surface of the nuclear membrane along which they can freely slide. Here we measure the accessible surface for two chromosomal sites (yeast telomeres Tel3R and Tel6R) that both exhibit strong preferential association with the nuclear membrane in galactose-containing media, but differ significantly in gene activity. To rule out differences in mobility during cell cycle, this study is based on data acquired in G1-phase of the cell cycle only.

Our analysis is based on confocal fluorescence microscopy studies of *Saccharomyces cerevisiae* cells. The telomeres Tel3R and Tel6R were fluorescently tagged by association with naturally fluorescent proteins (6,7). To determine whether a given chromosomal locus is preferentially attached to the

nuclear membrane we analyzed 3D data obtained by taking a series of thin optical slices through the entire heights of hundreds of cell nuclei. Taking the slice in which the tagged chromosomal region appears, it was determined how frequently it localized within the external zone occupying 33% of the area of each individual optical cross section. Let us remark that at the upper and lower extremities of the sphere, some points on the membrane may appear far from the periphery of the equatorial plane, occupying the middle zone of a given optical slice. Despite this case, Tel3R and Tel6R were both found in the peripheral zone most in 85–90% of randomly chosen cells from a growing population in galactose-containing media, indicating that both of these telomeres are likely to have interaction with components of the nuclear membrane (3).

We then asked how much of the membrane surface a moving telomere may contact. Time-lapse 3D imaging was performed over 5–7.5 min at 1.5-s intervals. To analyze movement at each time point, a series of six partially overlapping optical sections spaced by 450 nm are projected onto a single equatorial plane. The principle of monitoring chromosomal movement is shown in Fig. 1 (cf. Gartenberg et al. (3), for more detail).

Because the measured z coordinates are prone to higher error than measurements in x and y , calculations generally use only chromosomal positions projected onto an imagined equatorial plane of the nucleus. The data are thus a sequence of points (x_i, y_i) , where $i = 1, \dots, N \approx 300$ is the number of measurements, which are acquired at intervals $\Delta t = 1.5$ s.

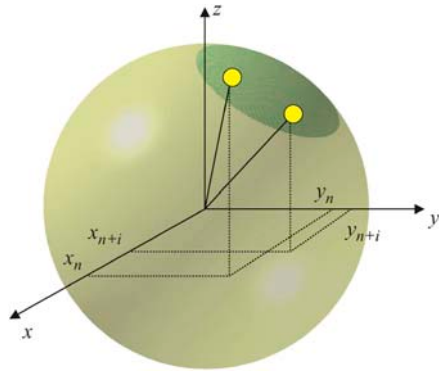


FIGURE 1 Schematic representation of confinement of a tagged chromosomal site (yellow dot) to a simple spherical cap on the nuclear membrane. Due to diffusive motion, the dot occupies different locations at different data acquisition times. Only x and y coordinates can be reliably measured.

The mean square displacement $\langle \Delta d^2 \rangle(t)$ (2,8), defined as $\langle \Delta d^2 \rangle(n\Delta t) = 1/(N-n) \sum_{i=1}^{N-n} [(x_{n+i} - x_i)^2 + (y_{n+i} - y_i)^2]$ is a measure of how far on average the projected spot moves in a specified time $t = n\Delta t$. Observation over a period of several minutes reveals that initially the projected points tend to spread, but then stabilize within a zone smaller than the entire equatorial plane.

This behavior is consistent with the plots of $\langle \Delta d^2 \rangle(t)$ shown in Fig. 2 a, which for large t exhibit plateaus at much

smaller heights than the value of $1.08 \mu\text{m}^2$ that can be calculated explicitly as the limiting value when the tag explores the entire surface of a yeast nucleus (radius = $0.9 \mu\text{m}$). Moreover, experimental observation suggests that for large times, the projection (x_i, y_i) systematically explores the constrained zone (2,6,9). Therefore, the limit of $\langle \Delta d^2 \rangle(t)$, namely $\langle \Delta d^2 \rangle(\infty)$, can be approximated as an ensemble average over a stationary probability distribution defined on the confinement region.

Our objective is to estimate the size of the confinement region from a comparison of experimentally observed values for $\langle \Delta d^2 \rangle(\infty)$ (cf. Fig. 2 b) and numerically computed values for spherical caps of various sizes (cf. Fig. 2 c). Specifically, we idealize the nuclear membrane as a sphere with the confinement region being a spherical cap of varying size, random orientation, and a uniform stationary probability distribution. To calculate the expected values of $\langle \Delta d^2 \rangle(\infty)$ as a function of spherical cap size, we do the following: 1), fix the dimensions of the cap, measured as a percentage of the entire spherical surface; 2), generate a random orientation of the cap; 3), generate a randomly sampled uniform distribution of points within this cap; 4), calculate $\langle \Delta d^2 \rangle(\infty)$ for this distribution; and finally, 5), average over 1000 different cap orientations. Step 5 is necessary because the same size spherical cap can project very differently depending upon its orientation. We then repeat the whole procedure for different cap sizes to obtain the dotted curve shown in Fig. 2 c. We next

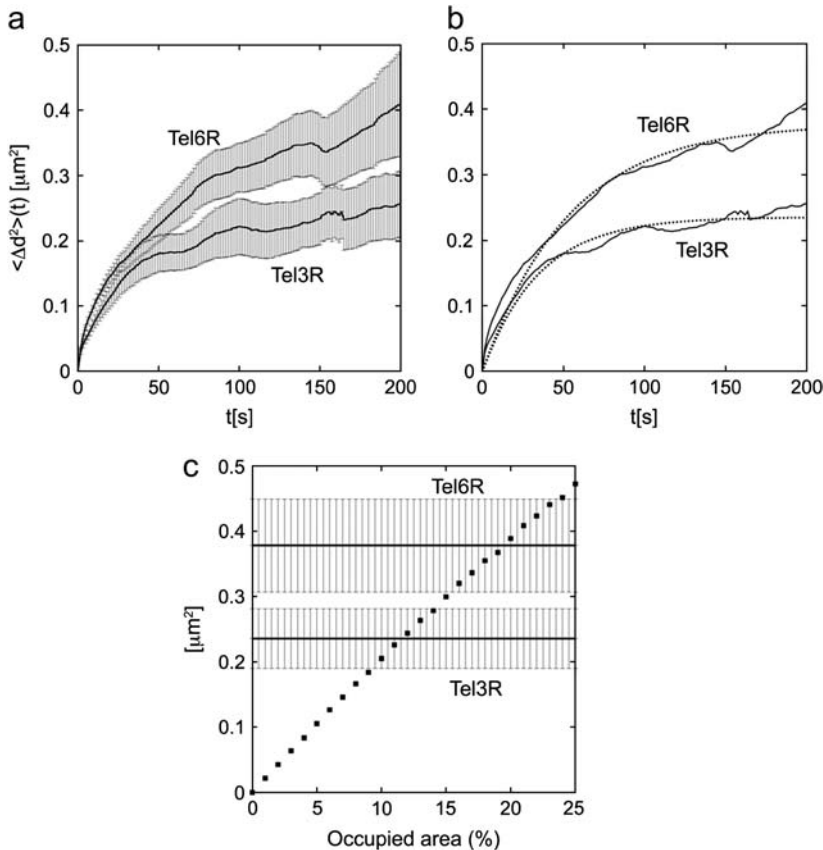


FIGURE 2 (a) The mean square displacements $\langle \Delta d^2 \rangle(t)$ observed experimentally for Tel3R and Tel6R (black curves, averaged over, respectively, 11 and 10 independent 300-frame series) in cells growing on galactose. The vertical bars represent statistical errors. (b) The main curves as in panel a (solid curves) and the fits of the function $a(1 - \exp(-t/\tau))$ (dotted curves), with $a = 0.38 \mu\text{m}^2$ for Tel6R (nonlinear regression test $R^2 = 0.97$) and $a = 0.24 \mu\text{m}^2$ for Tel3R ($R^2 = 0.95$) being the respective limiting values $\langle \Delta d^2 \rangle(\infty)$. (c) The dots are calculated values of $\langle \Delta d^2 \rangle(\infty)$ for spherical caps of increasing area. The intersection with the mean experimental observed values of $\langle \Delta d^2 \rangle(\infty)$ (horizontal solid line) suggests that Tel3R explores a spherical cap that covers $\sim 10\%$ of nucleus, whereas the cap for Tel6R is nearly twice as large. The vertical bars represent statistical errors.

compare the experimental saturation values of $\langle \Delta d^2 \rangle(\infty)$ for the two telomeres Tel3R and Tel6R with the calculated curve (Fig. 2 c). The intersection of the experimental plateau values with the curve suggests that the area of confinement of Tel3R corresponds to $\sim 10\%$ of the total area of the nuclear envelope, whereas that of Tel6R is $\sim 20\%$. It is known that the chromatin adjacent to Tel3R is inactive, whereas on Tel6R there is a gene (*HXK1*) that is induced by galactose and therefore has a high level of transcriptional activity under the conditions used for imaging. Moreover, recent studies suggest that transcriptionally active chromatin is less restricted than heterochromatin in mammalian and yeast cells (2,9,10). Although further chromosomal sites that preferentially attach to the membrane will need to be analyzed, we suggest here a correlation between the dynamics of a chromosomal region and its gene activity.

ACKNOWLEDGMENTS

This research was partially sponsored by grants No. 3152-68151.02 and No. 3100A0-103962 of the Swiss National Science Foundation.

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