

High throughput chromatin motion tracking in living yeast reveals the flexibility of the fiber throughout the genome

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Supplementary material contains: 1supplementary text, 5 figures, and 1 video

Supplementary Material

1- Model for step distribution analysis

We use the method developed by Guérin *et al.* to derive the step distribution function.

We first define the Rouse modes for an end-tethered polymer chain, which requires to diagonalize the Hessian matrix. This matrix of size N^2 relates the elastic forces acting on each monomer, and it is tridiagonal:

$$\begin{pmatrix} 2 & -1 & 0 & \cdots & \cdots & \cdots \\ -1 & 2 & -1 & 0 & \cdots & \cdots \\ 0 & -1 & 2 & -1 & 0 & \cdots \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & -1 & 2 & -1 \\ \cdots & \cdots & \cdots & 0 & -1 & 2 \end{pmatrix} \quad (\text{S1})$$

Note that this matrix is slightly different from that described in (Guérin et al. 2012) to account for the difference in boundary conditions. Its eigenvalues are:

$$\lambda_j = 2 \left\{ 1 - \cos \left(j \frac{\pi}{N+1} \right) \right\} \quad (\text{S2})$$

Its eigenvectors are:

$$Q_{ij} = \sqrt{\frac{2}{N}} \sin \left(ij \frac{\pi}{N+1} \right) \quad (\text{S3})$$

Using Supplementary Eq. A29-A30 of the work of Guérin *et al.*, we deduce the temporal evolution of a diffusive observable X :

$$\psi(t) = 2 \sum_{j=1}^N \frac{b_j^2 (1 - e^{-\lambda_j t})}{\lambda_j} \quad (\text{S4})$$

$$P(X, t) = \frac{1}{\sqrt{2\pi\psi}} e^{-\frac{X^2}{2\psi}} \quad (\text{S5})$$

In our case, we focus on the dynamics of a segment located at position n along the chromosome, and the terms b_j are defined by the following equations:

$$b_j = Q_{nj} = \sqrt{\frac{2}{N}} \sin \left(nj \frac{\pi}{N+1} \right) \quad (\text{S6})$$

These solutions are obtained with dimensionless variables, and we have to define the useful units of time and length:

$$\tau = \frac{\zeta b^2}{k_B T} \text{ and } l = b \quad (\text{S7})$$

Using the set of equations (S2,4,5,6,7), one can derive equation (5) and (6) given in the main text.

Supplementary Table 1: Strain list

Supplementary Figures

Figure S1: *High-throughput tracking and segmentation.* Starting from the raw data (upper left image), we automatically define whether the locus has a central or peripheral localization.

For this the contour of the nucleus is extracted, and adjusted by a circle to evaluate its radius

(upper right image), and the distance of the locus to the nuclear center and to the edge of the nucleus is automatically measured. In the middle panel, the segmented nucleus and the locus trajectory are represented (left image), and the nuclear center is represented by a green outline (right image). In the bottom panel, we show the segmented nucleus as derived from our automatic software analysis (Albert et al., 2013).

Figure S2: Fluorescence micrographs of the different yeast strains used for this study (inter-frame intervals are mentioned in the inset).

Figure S3: *Comparison of 2D vs. 3D particle tracking.* (A) We placed yeast cells tagged at position 194 kb on chromosome III on our microfabricated device for 3D microscopy based on tilted micromirrors (Hajjoul et al. 2009), and we monitored the movements of the locus in 2D and in 3D using one and two side views of the sample, respectively (see image in inset). MSD curves were fitted to the Rouse model, showing an expected difference of $2/3$ in spatial fluctuations between the 2D and 3D response (see insets on the graph). (B) Comparison of MSD response in living and fixed yeast cells (blue and black datasets, respectively) in linear and logarithmic scales (right and left plots, respectively).

Figure S4: (A) *Analytical model of the segmental dynamics of an isolated end-tethered chain.* The left panel represents the temporal evolution of the MSD for a segment located half-, fifth, or tenth-way through an end-tethered polymer (blue, green, and red curves, respectively). The plot in the right shows the MSD maximal amplitude vs. the genomic position of the locus (blue, green, and red data points are color-coded as in the left graph). Note that equation is consistent with this picture: when we consider the long time regime ($\tau \gg \tau_R$), the exponential term in equation (3) vanishes, and the discrete sum admits an analytical solution:

$$MSD_n(\tau \gg \tau_R) \sim 2Lb \left(\left(\frac{n}{N} \right)^2 - \frac{n}{N} \right) \quad (\text{S8})$$

The amplitude of spatial fluctuations is thus greater for a monomer located halfway between the chain *vs.* close to the tethering ends, as expected from the folding of the polymer in an arch-shape.

(B) *Visualization of the tube in molecular dynamics simulations.* The four images are snapshots representing the density of chromosome segments around chromosome III (continuous blue fiber). Segments of all other chromosomes, which are distant by less than 30, 50, 80, 150 nm (clockwise from upper left to lower left images, respectively), are color-coded using the same convention as in (Duan et al. 2010), illustrating the effect of volume exclusion between chromosomes.

Figure S5: *Chromosome contacts and slowed down MSD responses.* (A) Representation of a chromosome arm with two contacts that restrict the motion of one locus tagged in yellow. (B) The motion of the locus is dictated by the Rouse response in the small time regime, and anchors define a plateau of the MSD curve, which is dependent on the distance to the anchor, as described in Fig. S4A. The anchor is transient, and upon release, MSD increases to follow the Rouse model until reaching the next plateau determined by more distant contacts on the chromosome. (C) Dynamic and random contacts shift the average MSD response, plotted in logarithmic scale, along the temporal axis. Note that the response in the short time limit is representative of contact-free dynamics, because chromosome loci do not sense any restriction in motion.

Video S1: *Time lapse imaging of chromosome loci and the associated tracking analysis.* The left panel represents fluorescence micrographs of a yeast strain tagged on chromosome XII

(position 630). The inter-frame interval is set to 200 ms. The right panel shows the output of our tracking software, which can be downloaded as an executable file at <ftp://intermtt:MTTinterface@ftp.laas.fr/>.