

Supplementary Information for

Spatial telomere organization and clustering in yeast *S. cerevisiae* nucleus is generated by a random dynamics of aggregation-dissociation

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Polymer simulations

We modeled the chromosomes as semi-flexible Rouse polymers with a resting length l_0 (Doi and Edwards, 1988), where each monomer is driven by a Brownian component and a monomer-monomer interaction derived from a harmonic potential. The equations of the N monomers are

$$dX_n = F(X_{n-1}, X_n, X_{n+1})dt + \sqrt{2D}dW_n, \text{ where}$$
$$F(X_{n-1}, X_n, X_{n+1}) = -\nabla_{X_k} U(X_1, \dots, X_N)$$
$$U(X_1, \dots, X_N) = \sum_{k=2}^N k(|X_k - X_{k-1}| - l_0)^2$$

and $X_n(t)$ is the position of the n -th monomer at time t , W_n is a Brownian motion, k is the spring constant and D is the diffusion coefficient. Furthermore, the inner monomers (2 to $N - 1$) were constrained to the interior of the sphere (the nucleus), with a reflexive condition at the sphere surface, while telomere motion (1 and N) was restricted to the sphere surface. We simulated the telomere dynamics and extracted the distribution of the first arrival time of the telomere X_N to a small absorbing window located on the surface. We found that the arrival time distribution to a small target is well approximated by an exponential (Fig. 2B).

Numerical simulations of telomere dynamics using Markovian equations

We shall now present the Markovian equations to describe telomere dynamics. However due to the complexity of these equations, we will use stochastic simulations to compute any quantity of interest. We derive the Master equation for the probability

$P(n_1, \dots, n_K, t)$ of having a distribution of K clusters composed of n_1 particles, ..., n_K particles where clusters are ordered by size ($n_1 \geq \dots \geq n_K \geq 1$) at time t when the total

number is preserved $\sum_{i=1}^K n_i = \mathcal{N}$. The transition between time t and $t + \Delta t$ for the

probability P is obtained by summing over various probabilities. First is the probability of starting with a distribution of $K + 1$ clusters ($n_1, \dots, n_i - n_j, \dots, n_K, n_{K+1}$) and

the clusters of sizes $n_i - n_j$ and n_j associate. Second is the probabilities of starting with a distribution

($n_1, \dots, n_i + n_j, \dots, n_K$), and a cluster of size $n_i + n_j$ dissociates into two clusters of size n_i and n_j , and finally the probability of starting with the distribution

(n_1, \dots, n_K) and nothing happens. While the first probability is the product of the probability $P(n_1, \dots, n_i - n_K + 1, \dots, n_K + 1, t)$ by the transition rate $k_f \Delta t$, the second is the

product of $P(n_1, \dots, n_i + n_j, \dots, n_K, t)$ by the transition rate of $n_i + n_j$ to the clusters n_i and n_j . This dissociation transition rate is the product of the dissociation rate of a cluster of

size $n_i + n_j$, equal to $(n_i + n_j - 1)k_b \Delta t$, by the probability that the dissociation results effectively in the clusters n_i and n_j . Since the dissociation probability is uniform, it is

equal to $\frac{1}{2} \frac{1}{n_i + n_j - 1}$ if $n_i = n_j$, and $\frac{1}{n_i + n_j - 1}$ otherwise. The dissociation rate from

n_{i+j} to $n_i + n_j$ is thus equal to $1/2 k_b \Delta t$ if $n_i = n_j$ and $k_b \Delta t$ otherwise. Finally, the Master equation reads (Schuss, 2010; Matkowsky et al., 1984)

$$\frac{dP(n_1, \dots, n_K, t)}{dt} = \sum_{i=1}^K \sum_{k=1}^{n_i-1} P(n_1, \dots, n_i - k, \dots, k, \dots, n_K, t) k_f + \sum_{i,j=1, i \neq j}^K P(n_1, \dots, n_i + n_j, \dots, n_K, t) k_b - \left((\mathcal{N} - K) k_b + \frac{K(K-1)}{2} k_f \right) P(n_1, \dots, n_K, t).$$

Using a Gillespie's algorithm (Gillespie, 1976), we shall obtain various quantities of interest. At steady state, we compute the probability

$$\Pi(n_1, \dots, n_K) = \lim_{t \rightarrow \infty} P(n_1, \dots, n_K, t),$$

the mean, the variance, the size of clusters and the number of telomeres per cluster.

The stochastic simulations allow us to compute the steady state probability $\Pi(n_1, \dots, n_K)$ for a distribution (n_1, \dots, n_K) of clusters. We shall now describe our Markovian simulation procedure (Fig. S1). We shall further extract from this numerical computation the total average number of clusters

$$\langle M_\infty(a, M) \rangle = \sum_{(n_1, \dots, n_K)} K \Pi(n_1, \dots, n_K),$$

and the mean number of clusters of size k

$$\langle M_k(a, M) \rangle = \sum_{(n_1, \dots, n_K)} \#\{n_i = k\} \Pi(n_1, \dots, n_K).$$

In the Gillespie's algorithm, the transition rate constants between different cluster configurations are given as follows: for a distribution (n_1, \dots, n_K) of clusters, the transition probabilities to the neighboring states depend on two events: either two clusters (n_i, n_j) associate to form a new cluster of size $n_i' = n_i + n_j$ with an association rate k_f or a cluster of size n dissociates into two, with a rate $(n-1)k_b$ that depends on the number of bonds. The size of the resulting dissociated clusters is uniformly

distributed in the interval $[1, n-1]$. Since there are $\frac{K(K-1)}{2}$ pairs, the association rate

equals $\frac{K(K-1)}{2} k_f$, and the total fragmentation rate is the sum over all dissociation

rates $\sum_j (n_j - 1) k_b = (N - K) k_b$. The total transition rate from the state (n_1, \dots, n_K) to any

of the possible association and dissociation events is

$$a_0(n_1, \dots, n_K) = \sum_i a_i = \frac{K(K-1)}{2} k_f + (N-K) k_b.$$

Each iteration step of the algorithm uses the classical Poissonian random transition time $\tau = -\log(r_1)/a_0$, where r_1 is a uniform random variable in $[0,1]$ and each reaction event i has a probability a_i/a_0 to occur, and the chosen reaction i is sorted out using the criteria

$$\sum_{j=1}^{i-1} \frac{a_j}{a_0} < u \leq \sum_{j=1}^i \frac{a_j}{a_0}$$

where u is uniformly distributed in $[0, 1]$.

Number of clusters for various equilibrium parameter a

We use our model to predict the effect of changing the dissociation rate k_b and thus the equilibrium ratio $a = k_f/k_b$. In Fig. 7A, we simulated and plotted the number of observable clusters as a function of a . We found that the number of observable clusters goes from 1 for $a = \infty$ (no dissociation), to 0 when $a = 0$ (very fast dissociations). Between those two values, we obtain a maximum of 4.8 visible clusters for $a = 0.51$. We conclude that changing the dissociation rate modulates significantly cluster organization in yeast.

Influence of the chromosome arm length on the clustering dynamics

Because chromosome arms with a length below 300 kb are mainly located in a small region near the spindle pole body (SPB) (Therizols et al., 2010), while telomeres of longer chromosome arms exhibit motion near the nucleolus, we decided to integrate these constraints into the telomere dynamics (Fig. S2A). We distribute telomeres into

two classes based on the length of the chromosome arm (Therizols et al., 2010) and restricted 12 telomeres to a small region SS around the SPB (1/3 of the surface) and the other 20 are free to diffuse in a larger region SL, which excludes both the nucleolus and a small cap around the SPB (SL is 2/3 of the nucleus surface). In the common region $SS \cap SL$, both types of telomeres can meet to form mixed clusters. There are three possible classes of telomere clusters: clusters containing telomeres from long chromosome arms only (long), from short chromosome arms only (short) or from long and short chromosome arms (mixed), leading to six forward rates, accounting for the long-long, short-short, long-short, long-mixed, short-mixed and mixed-mixed interactions. We then ran our simulations to estimate the cluster distributions. We found similar results with our initial model where telomeres are undifferentiated.

In addition, for two telomeres from the pool of long chromosome arms, we can estimate the recurrence time and we report that $T_R = 442$ s ($n = 1,000$) (Fig. S2B), shorter than the forward time $k_f^{-1}(1,1) \approx 500$ s. Thus, the interaction of telomeres from short chromosome arms with a cluster made of long ones will contribute to the confinement of the cluster to a smaller region of the nuclear periphery, which will consequently decrease the mean time for two telomeres to meet again.

The colocalization time T_C was similar for telomeres from Short-Short, Short-Long and Long-Long chromosome arms (≈ 21 s, versus 31 s for the dissociation time between two telomeres, $n = 1,000$), reflecting that clusters contain the same number of telomeres independently of their composition. Interestingly, the distribution of the times T_R and T_C seems to be Poissonian (Fig. S2B).

Finally, the equilibrium probability to find a given telomere in a visible cluster (containing more than 2) was $\Pr(S, S) = 0.06$, $\Pr(L, L) = 0.045$ and $\Pr(L, S) = 0.04$ (for

short-short, long-long and long-short arm interaction), confirming that the encounter rate for small telomeres is higher than for long ones, due to the smaller space they can explore. Our results are mainly consistent with (Therizols et al., 2010), where the probabilities for two telomeres to belong to the same focus are determined experimentally to be mostly in the range 0.04-0.09. The differences between these experimental data and our simulations might be due to specific interactions between telomere pairs, which we did not take into account. Indeed, (Schober et al., 2008) showed contact between telomeres on opposite chromatid arms of equal length is favored.

We next studied the colocalization and recurrence times in the cases of SIR3 overexpression. Interestingly, two telomeres of small chromosome arms have a recurrence time of about 9 minutes for GALS and 8 minutes for GAL1, much lower than the forward rate that defined the mean encounter time, showing that clustering reduces the time to meet and thus increases the encounter probabilities.

References

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Fig S1: Schematic representation of the Markov chain representing the cluster configurations.

Fig S2: Influence of long and short chromosome arms on clustering. (A) Decomposition of the nucleus in subdomains with telomeres from short and long chromosome arms. Both types of telomere can interact in a common region. (B) Histograms of the time two telomeres spend in a cluster before they separate T_C , and the time needed for two telomeres to meet again after they separated T_R .

Movie S1: Time-lapse imaging of Rap1-GFP clusters. yAT340 strain was grown in glucose complete medium. Z-stack images were acquired every 10 sec, 100 time points. A median filter of radius 0.5 pixel was applied to the images. The frame rate is 4 images per second. Scale bar 5 μm .

Movie S2: Time-lapse imaging of Rap1-GFP clusters. yAT1565 strain was grown in glucose complete medium. Z-stack images were acquired every 10 sec, 100 time points. A median filter of radius 0.5 pixel was applied to the images. The frame rate is 4 images per second. Scale bar 5 μm .

Table S1: Summary of results and parameters.

Table 1. **Summary of results and parameters.**

Parameter	Haploid	<i>GalSp</i>	<i>Gallp</i>
Equilibrium parameter a	0.083 ± 0.031	5.9	8.3
Dissociation time k_b^{-1}	34 s	109 min	154 min
Association time k_f^{-1} (min.)	9	18	18
Number of clusters (exp.)	3.4 ± 2.0	2.8 ± 1.0	2.6 ± 1.0
Number of clusters (sim.)	3.4 ± 1.4	2.9 ± 1.1	2.5 ± 1.0
Isolated telo./pairs (sim.)	10/4	0.3/0.3	0.2/0.2
T_C	23 s	423 s	713 s
T_R	480 s	543 s	506 s
P_2	0.05	0.44	0.58