Materials and Methods for measuring movement in yeast (Neumann et al., 2012)

Yeast strains and growth conditions for microscopy experiments

Genotypes of the yeast strains and plasmids used can be found in Table S1 and S2, respectively. For the microscopy experiments, we grew yeast in minimal synthetic medium with the appropriate selection for the plasmids used. Experiments were always performed with exponentially growing cell cultures from freshly transformed cells to avoid potential long term effects of LexA fusions. To inhibit RNA polymerase II, we added 6-Azauracil (6-AU) dissolved in DMSO to a final concentration used at a 500 μM 30 to 45min before imaging.

Microscopy

Time-lapse fluorescence imaging was performed as described (Meister et al. 2010) unless otherwise stated, using either LSM point scanning confocal or a spinning disc confocal (see below). Cells were mounted in a Ludin chamber (Life Imaging Services, Reinach (BL) Switzerland) filled with the appropriate minimal medium. For scanning confocal capture we used a Zeiss LSM510 Axiovert 200M, equipped with a Zeiss Plan-Apochromat 100x/ NA=1.4 oil immersion objective to image the cells. The stage of the microscope was equipped with a hyperfine motor HRZ 200 and temperature was maintained at 25°C, during acquisition using a temperature-controlled box that surrounded the microscope. The 488 nm laser was used at a tube current of 4.7 amp and 25% output. We used an Lp 505 filter and acquired the images with a pinhole radius of 1 to 1.2 airy units. The detector gain was 930 to 999, the amplifier gain 1-1.5. We used the amplifier offset at levels of 0.2V–0.1 V and the AOTF was set between 0.1 and 2%. Other conditions were as follows: a dwell time of 1.28 μs/pixel in a 8-bit format with one scanning direction, 4 average/Mean/Line and a restricted region of interest to smaller than 40x38 pixels with a zoom of 1.8 to yield a pixel size of 100 nm in XY.

The spinning disc confocal (SDC) is Olympus IX81 hardware mounted with a Yokogawa CSU-X1 scan head and equipped with an ASI MS-2000 Z-piezo. We excited the GFP-LacI and NUP49-GFP with a 491nm laser set at 30% of the total power. A Semrock Di01-T488/568-13x15x0.5 dichroic and a Semrock FF01-525/40-25 filter along with a PlanApo 100x/1.45 TIRFM oil objective were used. A 512x512 EM-CCD Cascade II camera (Photometrics) acquired the signal, leading to a pixel size of 94 nm. With the LSM microscope, we took optical slices over 7 or 8 focal steps of 300μm every 1.5s for 5 to 7.5

min at a speed of less than 200 ms/slice, while the scanning disc microscope was used to scan 4 μm at 300 μm steps with 30 ms exposure for each optical slice. To control for potential phototoxicity, we monitored cell cycle progression by rebudding of wild-type cells with phase contrast imaging for 4 hours after fluorescence acquisition. The rate of cell cycle progression was comparable to unirradiated cells. The tracking of the I-SceI-induced double-strand break was performed on the SPC in a similar manner, except that in the case of cleavage Rad52-YFP was scored and compared with the CFP-LacI tracking of the uncut locus. Rad52 and LacI foci exactly colocalize. Details of the constructs are in (Dion et al. submitted).

Movie tracking and analysis

To project the movies onto the xy plane, we used the LSM software (Zeiss) and exported the resulting data as 8-bit TIFF images for the movies acquired with the LSCM, while we used the Huygens Remote Manager (Ponti et al. 2007) (http://huygens-rm.org) to deconvolve the images acquired with the SDC and Imaris to project them. We used the SpotTracker plugin for ImageJ (Sage et al. 2005) to track the moving GFP-LacI signal. This plugin corrects for translational movement of the nucleus by defining the center of the nucleus in each frame based on background nuclear fluorescence or GFP-Nup49. Then, it determines the relative position of the spot compared to that of the center of the nucleus frame by frame. We used the following settings: Cone Aperture: 5; Normalization Factor: 80; Center Constraint: 20-25; Movement Constraint: 20; active subpixel resolution. A custom-tailored Excel macro was used to calculate the MSD, large steps (defined as displacements larger than 500 nm in 10.5 s normalized to 10 min movies), the R_C and D. We used the slope (m) of the first 5 time intervals (1.5 s to 7.5 s) to determine the 3D diffusion coefficient (D) knowing that D = m/2*d, where d is the number of dimensions (see Supplementary material). For practical reason, the radius of constraint (R_C) was derived from the maximum MSD value within the first 150s. For 2D time lapse movies, the plateau of the MSD equals 4/5 R_C^2 (see Supplementary Material).

Random walk simulations and calculations

Simulations of random walks in a spherical volume were programmed in C++ using reflective boundary conditions. The radius of the sphere was 1000 times bigger than the step size of the walk. To compare the simulation data to experiments, the step length and the time step of the simulated random walks were normalized as follows: first, the size of confinement

was chosen such that the plateau of the MSD curve matched the experimentally measured MSD plateau (see also Supplementary Methods). This also determined the step length of the simulated random walks. Second, the time step of the walks was chosen such that the mean square displacement of the simulated walks during 1.5 s matched the quadratically averaged step size of the measured trajectories. The simulated MSD curves were each calculated from 750000 independent simulations.

Quantitative real-time PCR and β-galactosidase reporter assay

We extracted RNA from a 5 ml culture of exponentially growing cells using the RNeasy kit (Qiagen), and generated cDNA from 500 ng of total RNA using the Protoscript AMV First strand cDNA synthesis kit (New England Biolabs). We used a ABI 7500 Fast real-time thermocycler to quantify the cDNA produced essentially as described (Cobb et al. 2003). All experiments were performed in triplicate and normalized to the *ACT1* message (Schawalder et al. 2004). Table S3 lists the sequences of the primers and Taqman probes we used to quantify the message level at *ACT1*, *TRP1,* and *PES4.* The β-galactosidase assay was performed as described (Burke et al. 2000) using crude yeast extracts from strains transformed with pSH18-34 and the appropriate LexA fusion.

Ectopic recombination assay

We determined the frequencies of homologous recombination as described (Freedman and Jinks-Robertson 2002; Nagai et al. 2008). The lexA fusion proteins that are targeted to the *lys2* locus on Chr II are under control of the Tet-off promoter to ensure there is no detriment to growth by extended overexpression. The Tet-off plasmid was derived from pCM190 (Gari et al. 1997), which we subcloned into pRS415. We transformed GA3232 and GA3208 in the presence of doxycyline (expression off) with the appropriate inducible plasmid encoding LexA fusion proteins (Table S2). For each fusion tested, at least eight transformants were inoculated into 5 ml cultures and propagated for 3 days without doxycycline (expression ON). The cells were then plated onto SC-lysine to select recombinants. The recombination rate was determined using the median method and the FALCOR webtool (Hall et al. 2009).

Statistics

The p-values presented here are derived from one-tailed Student's T-tests. P-values of 0.05 or less were considered statistically significant.

Supplementary Methods, Tables, Figures, and references

Supplementary Methods

Mean Square Displacement and radius of constraint

If an object's movement is spatially confined the distance between two points on the trajectory cannot exceed the maximal extension of the confining volume. Moreover, with an increase of the MSD time window, the correlation of two points on the trajectory decreases and finally vanishes in the limit of infinite time windows. From these two properties, it follows that the MSD curve must reach a plateau at large time windows. One can expect that the value of this plateau contains information about the size of the confinement.

In the case of a spherical confinement (e.g. the yeast nucleus), the relationship between the plateau value and the radius of the confining sphere (called radius of constraint) can be calculated exactly (see below). For confining volumes which are not exactly spherical the radius of constraint can still be used to measure the size of the confinement. It gives the radius of a sphere which would lead to the same MSD plateau.

As mentioned above, the positions of the spot at different times lose their correlation with increasing time window. Therefore the value of the MSD plateau is the expected value of the squared distance of two independent points inside the confining ball. This expected value can be calculated by multiplying the probability for point 1 being at position $\mathbf{r_1}$ and point 2 being at position $\mathbf{r_2}$ with the squared distance $(\mathbf{r_2} - \mathbf{r_1})^2$ and integrating this product over the volumes V_1 and V_2, which both correspond to the whole spherical volume. Since the probability for one of the points being at a given position inside the ball is constant, the probability for residing inside a small volume dV is – independently of the position – dV/V, where $V = 4/3\pi R^3$ is the volume of the sphere. The desired mean squared distance can now be calculated as follows:

$$\left\langle r^2 \right\rangle = \frac{1}{V^2} \int_V \int_V \left[(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2 \right] dV_1 dV_2 \,.$$

The problem is symmetric in x, y, and z, and therefore the above integral can be written as:

$$\left\langle r^2 \right\rangle = \frac{3}{V^2} \int_V \int_V (x_2 - x_1)^2 \, dV_1 dV_2 \,.$$

Moreover, a projection of the trajectory onto the xy plane just means that z_1 and z_2 are equal to 0, and the third summand vanishes. In general, the projection of the trajectory onto n dimensions results in

$$\left\langle r^2 \right\rangle = \frac{n}{V^2} \int_V \int_V (x_2 - x_1)^2 \, dV_1 dV_2 \,.$$

This integral can be further simplified:

$$\left\langle r^2 \right\rangle = \frac{n}{V^2} \int_V \int_V \left(x_2^2 - 2x_1 x_2 + x_1^2 \right) dV_1 dV_2$$

$$\left\langle r^2 \right\rangle = \frac{n}{V^2} \left[V \int_V x_2^2 \, dV_2 + V \int_V x_1^2 \, dV_1 + 2 \int_V \int_V x_1 x_2 \, dV_1 dV_2 \right]$$

In the first two summands, one trivial integration was executed. The resulting integrals are again equal due to symmetry reasons. The third integral vanishes because the integration interval for x_1 is symmetric with respect to 0 (the same holds for x_2) and the function $x_1 x_2$ is antisymmetric in x_1. This results in:

$$\left\langle r^2 \right\rangle = \frac{2n}{V} \int_V x^2 dV .$$

The remaining integral is a standard integral with the value

$$\left\langle r^2 \right\rangle = \frac{2n}{5} R^2 .$$

It follows that the plateau of an MSD curve calculated from the 3D trajectory of an object has the value $6/5R^2$ where R is the radius of the confining sphere. If the trajectory is projected onto two dimensions, the plateau of the MSD curve has the value $4/5R^2$.

Initial slope and diffusion coefficient

For unconfined diffusion, the mean squared displacement of an object is proportional to time. The proportionality constant (i.e. the slope of the MSD curve) is 2dD where d is the number of spatial dimensions in which the movement takes place and D is the diffusion coefficient (Berg 1993).

Even if the movement is confined to a certain volume this should have only little effect on the displacement of an object on small time scales. Therefore the initial slope of the MSD curve can be used to measure the diffusion coefficient of the object: D = m/6 where m is the initial slope of the curve.

As shown in Figure 1, the MSD curve calculated from the projected trajectory of the object is accurately described by the 3D MSD curve multiplied with 2/3. Therefore, the diffusion coefficient of the object can be extracted from the slope m* of the projected MSD curve as D = m*/4.

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