Supplementary Materials

Motion and dissolution of protein aggregates in budding yeast

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Supplementary Figure Legends:

Figure S1. Analysis of F-actin structures in LatA-treated cells (Related to Figure 3).

LatA treatment disrupted visible actin structures in wild-type cells. Yeast cells with and without 10 min treatment with 100μM LatA were stained with FITC-phalloidin.

Figure S2. STICS analysis of aggregate motility (Related to Figure 3).

A. Following background subtraction (see Extended Experimental Procedures), the decay of the spatial-temporal correlation function as a function of time shift was calculated. A single value was obtained per field of view of cells. Shown are the spatial correlation related to x and y shift as a function of time shift.

B. The spatial correlations were circularly averaged to generate plots of $G(\rho)$ over radial shift. These plots were fit to Guassians to quantify the increase in width of the spatial-temporal correlation function over time shift, as the motion of the particles decreases the spatial correlation over time. This increase in width was used to calculate MSD for the particles (see Extended Experimental Procedures). Initial amplitude of $G(\rho)$ is normalized here to emphasize the increase in width over time shift.

C. MSD was calculated from the increase in width as shown in (B) (see Extended Experimental Procedures). Representative MSD plots of STICS results for a single field of view are shown for wild type (wt), wt + LatA, and *hsp104*^{Y662A} (30 min heat shock).

D. Diffusion coefficients calculated from STICS analysis of different strains and conditions. Y662A*: *hsp104* ^{Y662A} mutant with 3.5min heat shock. All other strains and conditions were with 30 min heat shock. Each plot shows the mean and SEM of diffusion coefficient calculated from MSD plots from a minimum of 3 fields of view of cells, with each field of view consisting of 20 to 40 cells.

Supplemental movies:

We recommend all movies to be viewed using QuickTime player.

Movie S1. A field of wild-type cells with randomly moving Hsp104-GFP aggregates (Related to Figure 1)

Shown is a representative movie of maximum projections in 61 frames 20 seconds apart (A) and 1min apart (B). Time-lapse imaging in (A) started after 20min additional recovery time at 23 ^{o}C (also see Experimental Procedures).

Movie S2. Examples of movements of aggregates between mother and bud (Related to Figure 1)

A. An example of an aggregate (yellow arrow) exhibiting bud to mother movement in a wild-type cell. Shown is a representative movie of maximum projections in 61 frames 1 min apart.

B. An example of an aggregate (red arrow) exhibiting mother to bud movement in a wild-type cell. Arrowheads point to aggregates that undergo dissolution. Shown is a representative movie of maximum projections in 61 frames 1 min apart.

Movie S3. Trajectory of aggregates dynamics (Related to Figure 2)

A representative movie of aggregates in a field of wild-type cells moving along tracked trajectories. The movie is composed of maximum projections in 41 frames 1 min apart.

Movie S4. A field of cells with randomly moving Hsp104-GFP aggregates in the same wild-type strain (YBD401) as used in Liu et al (2010) (Related to Figure 3)

Shown is a representative movie of maximum projections in 72 frames 1 min apart.

Movie S5. Aggregates undergo dissolution (Related to Figure 4)

The movie corresponding to Figure 4A showing dissolution of aggregates in wild-type cells. Yellow arrow points to an aggregate that dissolved and red arrow heads point to two aggregates that fused and then dissolved.

Movie S6. Aggregates dynamics in *hsp104*^{Y662A} cells (Related to Figure 4)

A. Dynamics of Hsp104^{Y662A} -GFP aggregates produced after a 30 min heat shock. Shown is a representative movie of maximum projections in 61 frames 3 min apart. Arrowhead point to the cells showed in Fig.4C. Lower laser power was applied in this movie to avoid saturation at the end of the movie.

B. Dynamics of Hsp104^{Y662A} -GFP aggregates produced after a 3.5 min heat shock. Shown is a representative movie of maximum projections in frames 1 min apart.

Movie S7. Hsp104 aggregate dynamics in LatA-treated cells (Related to Figure 3)

Shown is a representative movie of maximum projections in 61 frames 1 min apart.

Movie S8. Aggregates dynamics in *bni1Δ* and *bnr1Δ* cells (Related to Figure 5)

A. Hsp104 aggregate dynamics in *bni1Δ* cells. Shown is a representative movie of maximum projections in 61 frames 1 min apart.

B. Hsp104 aggregate dynamics in *bnr1Δ* cells. Shown is a representative movie of maximum projections in 61 frames 1 min apart.

Movie S9. Examples of aggregates retention during budding in *bni1Δ* and *bnr1Δ* cells (Related to Figure 5)

A. Examples showing the retention of protein aggregates in a budding *bni1Δ* cell. Arrowhead points to the cell in which aggregates were fully retained in the mother during bud growth.

B. Examples showing the retention of protein aggregates in a budding *bnr1Δ* cell. Arrowhead points to the cell in which aggregates were fully retained in the mother during bud growth.

Movie S10. Examples of retention and leakage of aggregates during budding in wild type cells (Related to Figure 5)

A. An example showing the retention of protein aggregates in a budding wild-type cell. Arrowhead points to the cell as shown in Figure 5A, where aggregates were fully retained in the mother during bud growth.

B. An example showing the leakage of protein aggregates from mother to bud cell in a budding wild-type cell. Arrowhead points to the cell as shown in Figure 5B. Red arrow points to an aggregate that escaped the mother into the bud but subsequently moved back to mother; yellow arrow points to an aggregate that escaped the mother into the bud but subsequently dissolved. The movie is composed of maximum projections in 91 frames 1 min apart.

Movie S11. Dynamics of natural Hsp104-GFP-associated aggregates in aged wild-type cells (Related to Figure 6)

The movie is composed of maximum projections in 56 frames 1 min apart.

Extended Experimental Procedures

List of yeast strains

Strain	Genotype (*)	Source and comment
RLY4392	*his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Steffen et al., Cell 2008.
RLY7022	*HSP104-GFP:HIS3 pSPC42-SPC42-mCHERRY(pRS315LEU2)*	This study
RLY7110	*HSP104-GFP:HIS3*	Huh et al.,Nature 2003.
RLY7169	*hsp104Δ::kanR*	This study
RLY7200	*hsp104^{Y662A}-GFP:HIS3*	This study
RLY7315	*bni1Δ::kanR HSP104-GFP:HIS3*	This study
RLY7316	*bnr1Δ::kanR HSP104-GFP:HIS3*	This study
YBD401	*HSP104-GFP:HIS3*	Liu et al, 2010; Invitrogen;

(*) All the strains used in this study are derivatives of the S288c background (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*)

Hsp104-GFP aggregates clearance assay

Triplicate mid-log cultures grow in 30 ^{o}C (10ml) were heat shocked at 42 ^{o}C for 30min and then shifted back to growth at 30 ^{o}C. Samples were taken every 10 min and fixed in 4% formaldehyde for 25min. Fixation was terminated by washing cells three times with PBS. Imaging was performed on the same day. Aggregates clearance efficiency was calculated as the percentage of buds without aggregates at each time point.

Actin staining and LatA treatment

Cells were fixed with 4% formaldehyde for 2 hr at room temperature with gentle shake. The cells were washed 3 times with PBS and 1 time with PBS+0.2%TX-100. Cells were resuspended in 80µl PBS+0.2%TX-100 containing 1.5ul FITC-phalloidin for 40min in dark.

In LatA experiments, mid-log cells were heat shocked and recovered as described in main text. Then 100µM LatA (Invitrogen) or the same amount of DMSO was applied

to the cells for 10min. 3D time-lapse imaging was performed with present of 100μM LatA (or DMSO as control).

Isolation of old cells

Cell populations with advanced replicative age were obtained by magnetic sorting (Chen et al., 2003). Exponentially growing cells were collected and washed twice with ice cold PBS (pH 8.0). $2.5X10^7$ cells were labeled with 0.5mg/ml Sulfo-NHS-LC Biotin (Pierce) at room temperature for 30 min with gentle shaking. These cells were used as M-cells. The cells were then washed twice with 1ml cold PBS to get rid of free biotin and grown in 500ml YPD for 14-15 hr (OD_{600} was not allowed to exceed 1). The separation of M-cell was carried out by incubating $2x10^9$ cells with 60μ anti-biotin microbeads (MACS) in 30ml PBS pH7.2 for 1 hr. Unbounded beads were removed by washing twice with PBS. M-cells were isolated with a magnetic sorter. Successful acquisition of cells with advanced replicative age was verified by staining of buds scars with calcaflour (main text Fig. 6A).

STICS measurements of particle motion

As a second, independent method to quantify dynamics of protein aggregates, a variant of STICS and kICS was used (Berland et al., 1995; Hebert et al., 2005; Kolin et al., 2006). In STICS analysis, the spatio-temporal correlation function is measured from the raw image data as follows:

$$G(\rho,\tau) = \frac{\left\langle \delta I(t+\tau, r+\rho) - \delta I(t,r) \right\rangle_{t,r}}{\left\langle I \right\rangle_{t,r}^{2}}. \qquad (1)$$

Here ρ and τ are the spatial and temporal shifts of the correlation function. This function can be quickly calculated using spatial fourier transforms (Berland et al., 1995; Hebert et al., 2005). For randomly diffusing diffraction limited particles, the spatio-temporal correlation function is described as follows:

$$G(\rho,\tau) = G(\tau)\exp\left(\frac{-\rho^2}{\omega_0^2 + 4D\tau}\right) = G(\tau)\exp\left(\frac{-\rho^2}{\omega_0^2 + MSD(\tau)}\right). \quad (2)$$

Here the G(τ) term is the traditional correlation function from fluorescence correlation spectroscopy and describes the amplitude of the spatial correlation, but not its spatial extent. The rest of the equation is a Gaussian function essentially identical to the spatial probability function for diffusion convolved with the microscopic point spread function PSF. Here ω_0 is the "beam waist" of the PSF or two times the spatial standard deviation of the PSF. It is easy to see that the above equation can be extended to anomalous forms of diffusion (Schwille et al., 1999). If the spatial variance of the above equation is denoted as $\sigma^2(\tau)$ and the variance at τ = 0 is denoted as $\sigma^2(0)$, then the mean squared displacement is given as:

$$MSD(\tau) = 2\left(\sigma^2(\tau) - \sigma^2(0)\right). \quad (3)$$

In order to reduce any effect of changes in Hsp104 expression level over time on the STICS map, the time-series were first detrended by dividing each time point by its average. A weak threshold was applied to remove signal from the cytosol. These two processes left only aggregates and their motion as the dominating factor in the spread of the spatial cross-correlation over time. A single STICS map was calculated per 256x256 pixel field of view containing approximately between 20 and 40 cells. Each line

(corresponding to each time shift) of a circular average of the STICS map was fit to a Gaussian to obtain the variance. The points at $\rho = 0$ were not included in the Gaussian fit so as to avoid the contribution from noise and overall intensity changes. Then equation 3 was used to determine the MSD. Similarly to the tracking data, the first 5 points of the MSD plot were fit to $MSD(\tau) = 4D\tau^{\alpha}$ (see Supplemental Figure 2).

3D numerical simulation of aggregate partitioning between mother and bud.

Simulations of particle diffusion through a bud neck were performed using a spherical cell model where "mother" and "bud" spheres were separated by a barrier except at the position of the neck opening. The mother and bud diameters were set to 5 and 4.5 μm respectively and the neck diameter was adjusted as described in the text. 80000 particles were distributed randomly in three dimensions within the mother box before the start of the simulation. At each time step of the simulation, particles were moved from their previous position in each dimension by a distance given by a Gaussian random number with standard deviation $\sqrt{2Dt}$ where D is the diffusion coefficient and t is the time step. The diffusion coefficient was set to 0.001 $\mu m^2/s$ for consistency with the diffusion coefficient in aged cells and the time step was set to 5 seconds. This value was chosen because it is small enough to prevent large steps outside of the cell boundary but large enough to allow reasonable simulation time (2000 minutes). Diffusion steps occurring outside of the spheres were reflected back into the spheres. The barrier between mother and bud was enforced by reflecting any diffusion steps that crossed the neck and did not land within the cylinder delineated by the neck opening. Images were created from the simulations by creating a 160 x 160 nm pixel square corresponding to each particle that was within 1.25 μm of the middle z plane of the simulation. 1D

probability density functions for particle position were then created using custom written kymograph tools in ImageJ (NIH, Bethesda, MD). For 2D probability density functions, a sum projection of all particles was created.

Simulation of random walk with confinement and transport components.

Simulations of random diffusion for alpha value distribution analysis and individual trajectory visualization were performed similarly to those described above. Here particle displacements were restricted to two dimensions for consistency with the projected 2D tracking that was performed experimentally. Experimental trajectories are observed for a variable length of time, either due to loss of tracking or disappearance of the particle. We found that the trajectory length distribution was exponential with an average trajectory length of 9.3 frames. In order to match the experimental noise characteristics, our simulated trajectories had lengths specified by an exponential random number with an average of 9.3 frames. Particle positions were updated at each time point as before, but the time between frames was set to 60 seconds, and the diffusion coefficient was set to 0.0005 $\mu m^2/s$ for similarity with that for heat-induced aggregates. In order to simulate restricted motion (sub-diffusion), 30% of the aggregates were not allowed to diffuse more than 0.5 μm from their starting position. Displacements outside of this region were reflected back into the region. In order to simulate transport (super-diffusion), particles were given a 10% probability of transitioning into a "transport" state at each time point. Once in the transport state, the particles moved along a random vector with a linear speed of 0.005 μm/s while still diffusing with a 100 fold reduced diffusion coefficient compared to the non-transport species. Once in the transport state particles

were given a 25% chance at each time point of transitioning back out of the transport state.

1D analytical model of aggregate partitioning between mother and bud

Statement of Problem. Dynamics of the aggregates in the yeast cells were cosidered as a constrained random walk, similar to the episomal DNA motion (Gehlen et al., 2011). Simulations were repeated several hundred thousand times and the result was averaged to obtain the distribution of the particles in a region of prescribed geometry. This approach requires long computations and is justified when applied to the problem with complex geometry. When the geometry is simple enough a simpler approximate approach can be used based on numerical and/or analytical solution of the well-known diffusion equation. This approach is justified in an assumption about well-mixing dynamics, i.e., when the averaging of many random trajectories of small number of particles can be effectively replaced by a distribution of very large number of moving particles.

Consider a problem of aggregates dynamics in the budding yeast cell. The geometry is represented by an union of two intersecting spheres of the radii R_1 of mother cell and R_2 of the bud, where $R_1 > R_2$. For simplicity assume that at $t = 0$ all aggregates concentrated in the mother cell and the bud is free of them, so that the initial aggregates distribution $C(r, t = 0)$ is uniform in the mother compartment and it is zero in the bud.

The dynamics of the particles density is given by the diffusion equation

$$\frac{\partial C}{\partial t} = D\Delta C, \tag{S1}$$

where D denotes the diffusion coefficient and Δ is the Laplacian second order differential operator that in the Cartesian coordinates reads $\Delta = \partial^2/\partial x^2 + \partial^2/\partial y^2 + \partial^2/\partial z^2$.

Assume that the mother-bud axis coincides with the x axis, and the center of the mother compartment is in the origin. The region corresponding to the mother compartment is defined as $|r|<R_1$, the corresponding region for the bud compartment reads $|r-R_{20}|<R_2$, where $R_{20}=\{R_1+R_2-\varepsilon,0,0\}$; here the maximal size of the overlapping regions ε determines the neck size $d\approx 4R_1^2\varepsilon/(R_1+R_2)$. The boundary conditions assume no flux of concentration at the cell membrane and reads $n\cdot\nabla C=0$, where n denotes the normal to the boundary and ∇C is the concentration gradient.

Reduction to 1D Problem. Analysis of 3D numerical solution leads to reduction of the original problem to the 1D version presented below. The equation (S1) converts into

$$\frac{\partial C}{\partial t}=D\frac{\partial^2 C}{\partial x^2}, \tag{S2}$$

that should be solved in the region $-R_1\le x\le R_1+2R_2-\varepsilon$ subject to the boundary and initial conditions

$$\frac{\partial C}{\partial x}|_{x=-R_1}=\frac{\partial C}{\partial x}|_{x=R1+2R_2-\varepsilon}=0, \tag{S3}$$

$$\begin{aligned} &C(x,0)=1,-R_1\le x\le R_1,\\ &C(x,0)=0,R_1\le x\le R_1+2R_2-\varepsilon. \end{aligned} \tag{S4}$$

Redefining the spatial variable $x\to x-R_1$ and introducing the parameters $L_1=2R_1$ and $L_2=2R_2-\varepsilon$ we arrive at the updated boundary and initial conditions

$$\frac{\partial C}{\partial x}|_{x=-L_1}=\frac{\partial C}{\partial x}|_{x=L_2}=0, \tag{S5}$$

$$C(x,0) = 1, -L_1 \le x \le 0,$$
$$C(x,0) = 0, 0 \le x \le L_2. \tag{S6}$$

Analytical Solution in 1D. The problem (S2, S5, S6) is solved using the separation of variables method (Tikhonov et al., 1990) in which the spatial and temporal dependencies in the solution are separated. As the result the linear partial differential equation (S2) splits into two ordinary linear differential equations.

The solution is written as infinite series

$$C(x,t) = A_0 + \sum_{n=1}^{\infty} A_n \cos\frac{\pi n(x+L_1)}{L_1+L_2} \exp\left(-\frac{\pi^2 n^2 Dt}{(L_1+L_2)^2}\right), \tag{S7}$$

where the coefficients A_n are given by

$$A_0 = \frac{L_1}{L_1+L_2}, \quad A_n = \frac{2}{\pi n}\sin\frac{\pi n L_1}{L_1+L_2}, (n>0).$$

Qualitative analysis of the solution shows that at large times $Dt >> 1$ the contribution of the summation terms in (S7) rapidly goes to zero for $n > 1$, so that one can use an approximate solution retaining only the first term in the sum

$$C(x,t) \approx \frac{L_1}{L_1+L_2} + \frac{2}{\pi}\sin\frac{\pi L_1}{L_1+L_2}\cos\frac{\pi(x+L_1)}{L_1+L_2}\exp\left(-\frac{\pi^2 Dt}{(L_1+L_2)^2}\right). \tag{S8}$$

A comparison of the first 20 terms in (S7) and the 3D experimental simulation for a bud neck diameter of 4.25 μm is shown in Fig. 7C at the 90 minute time point. The 3D simulation distribution resembles the 1D analytical solution. A comparison in Fig. 7A shows that the 1.25 μm girth constrains diffusion to a great extent. Therefore, the 1D solution overestimates the rate at which particles equilibrate between the mother and bud.

Retention of Particles in the Mother Compartment. Having the exact (S7) and approximate (S8) solution in analytical form we compute the fraction of particles retained in the mother compartment as a function of time. Using the assumption that 3D distribution $C(x,y,z,t)$ is approximated by the 1D solution $C(x,y,z,t) = C(x,t)$ we compute the total number N_m of particles in the mother as integral

$$N_m(t) = \pi \int_{-L_1}^{0} r^2 C(x,t)\, dx, \tag{S9}$$

where r denotes the radius of the circular cross section orthogonal to the mother-bud axis at position x. The square of the radius is found from geometry as $r^2 = -x(x+L_1)$. The initial particle number $N_m(0)$ equals to the mother compartment volume $N_m(0) = \pi L_1^3/6$. The fraction f of the mother retained particles reads $f(t) = N_m(t)/N_m(0)$ and is computed as

$$f(t) = -\frac{6}{L_1^3} \int_{-L_1}^{0} x(x+L_1)\, C(x,t)\, dx. \tag{S10}$$

Substitution of the exact solution (S7) into (S9) produces the explicit expression

$$f(t) = \nu + \frac{12}{\pi} \sum_{n=1}^{\infty} \frac{\sin a_n}{n a_n^3} \left[2 \sin a_n - a_n (1 - \cos a_n)\right] e^{-a_n^2 Dt/L_1^2}, \tag{S11}$$

where the parameter a_n is defined as $a_n = n\pi\nu$, and $\nu = L_1/(L_1 + L_2)$.

Retaining the first term in the sum in (S10) we find an approximate solution

$$f(t) = \nu + \frac{12}{\pi^4 \nu^3} \exp\left(-\frac{\pi^2 \nu^2 Dt}{L_1^2}\right) \sin \pi\nu \cdot \left[2 \sin \pi\nu - \pi\nu (1 + \cos \pi\nu)\right]. \tag{S12}$$

It follows from the solution (S11) that the minimal fraction of the particles in the mother compartment equals to $f_{min} = \nu$ and it is reached at equilibration time t_* roughly estimated as $t_* = (L_1 + L_2)^2/(\pi D)$.

Qualitative analysis of the particle dynamics and retention implies that the particles exchange between the two compartments leads to leveling off the concentration distribution at the characteristic time t_* inversely proportional to the particles diffusivity and proportional to square of the cell size. The negligible exchange may be assigned to two reasons: initial state is close to the dynamical equilibrium, or the diffusion coefficient is small enough to prevent transfer of aggregates between the compartments.

We analyzed the data on fraction f dynamics for the WT yeast cells and found the value $f = 0.8$ at the time scale of 90 min (Fig. 7B). The estimate of the diffusion coefficient D based on the aggregates trajectory analysis gives $D = 1.0 \cdot 10^{-3}\ \mu m^2/s$. The characteristic cell size is in the range $2(R_1 + R_2) = 8 - 10\ \mu m$. The estimate of complete relaxation time produces $t_* = 600\ min$. This means that the random walk motion does not affect the aggregate distribution between the mother and the bud.

Supplemental References:

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