Supplemental Materials

*Molecular Biology of the Cell*

Kamnev *et al.*
Supplementary Methods:

Mathematical model of protein exchange following partial bleaching of the AMR in presence of finite cytosolic recovery pool.

Since diffusion within the cytoplasm should be fast compared to the recovery time of FRAP, a compartment model is justified for modelling of the distribution of an AMR component in the cell. We have a cytoplasmic compartment and two AMR compartments, split into the portion of the AMR that is bleached during the FRAP experiment, and the unbleached portion of the AMR. Additionally, the target protein in the AMR can exist in either a mobile state \( (m) \), which undergoes exchange with the cytoplasmic pool, or an immobile state \( (i) \), which undergoes much slower exchange with the mobile fraction. The target protein thus could be described as moving between cytoplasmic \( (C_c) \) and AMR-bound populations in the mobile and immobile fractions of the bleached and unbleached portions of the AMR \( (C_{b,m} \text{ and } C_{b,i}, \text{ and } C_{u,m} \text{ and } C_{u,i} \text{ respectively}) \). This is illustrated as follows:

The key assumption of the model is that the exchange between the mobile and immobile fractions is slow enough that it can be ignored on the timescale of the FRAP experiments. A fast exchange would in effect reduce the model to a single mobile compartment. We also assume that the cytoplasmic concentration of the target protein is approximately uniform throughout the cell (fast diffusion), and that there is no appreciable diffusion or transport of the protein within/along the AMR on the timescale of FRAP experiments.

With these assumptions, we can write down a differential equation model that describes the exchange of unbleached molecules of the AMR component between its various pools, as shown above:

\[
\begin{align*}
\frac{dC_{u,i}}{dt} & = 0, \\
\frac{dC_{u,m}}{dt} & = k_{on} C_c - k_{off} C_{u,m}, \\
\frac{dC_{b,m}}{dt} & = k_{on} C_c - k_{off} C_{b,m}, \\
\frac{dC_c}{dt} & = \left[ k_{off}(L_u C_{u,m} + L_b C_{b,m}) - k_{on} C_c(L_u + L_b) \right] / V.
\end{align*}
\]
Here, \( C_{u,i} \) and \( C_{u,m} \) \((C_{b,i} \) and \( C_{b,m} \)) are linear densities of the amount of unbleached target protein in the unbleached (bleached) portion of the AMR, in the immobile and mobile fractions, respectively. \( C_c \) is the concentration of the AMR protein in the cytoplasm, whilst \( k_{on} \) and \( k_{off} \) are the binding and unbinding rates of the protein to the AMR. Finally, \( L_u \) and \( L_b \) are the arc lengths of the unbleached and bleached portions of the AMR, respectively, whilst \( V \) is the cytoplasmic volume of the cell. Recovery then corresponds to binding of the AMR protein from the cytoplasmic pool to the bleached part of the AMR, and reequilibration of the unbleached part, specifically loss of the unbleached AMR protein from the unbleached part of the AMR to the cytoplasm.

As equations 2 – 4 are a set of coupled 1st order differential equations, the solution can be found by first rewriting equations 2 – 4 into their matrix form

\[
\frac{d}{dt} \vec{c} = \vec{A} \vec{c},
\]

where \( \vec{c} \) is a column vector of \( C_{u,m}, C_{b,m} \) and \( C_c \), while \( \vec{A} \) is a 3×3 matrix containing the coefficients in equations 2 - 4. Then, in order to solve equation 5 we can make the substitutions,

\[
\vec{c} = \vec{x} e^{\lambda t},
\]

\[
\frac{d}{dt} \vec{c} = \lambda \vec{x} e^{\lambda t}.
\]

Inserting these into equation 5, and rearranging, gives

\[
(\vec{A} - \lambda \vec{I})\vec{x} e^{\lambda t} = \vec{0},
\]

where \( \vec{I} \) is the 3×3 identity matrix. Then, in order to find non-trivial solutions for our model, equation 8 must be solved to find its eigenvalues (\( \lambda \)) and corresponding eigenvectors (\( \vec{x} \)). This is achieved by solving the equation

\[
\det(\vec{A} - \lambda \vec{I}) = 0,
\]

Doing so reveals that there are 2 non-zero eigenvalues, the 3rd is zero because the total amount of the AMR protein is assumed to remain the same during the FRAP experiment. The general solution is then given by:
Here, \( A, B \) and \( C \) are constants which are determined by the initial conditions of the model, and \( t \) is the time after the photobleaching.

Equations 10 and 11 describe the amount of AMR-bound protein in the mobile fraction. However, in our experiments we are only able to measure the total amount of fluorescence from each portion of the AMR \((C_u, \text{ and } C_b \text{ for unbleached and bleached regions, respectively})\). These are given by:

\[
C_u = C_{u,i} + C_{u,m} = (1 - f_m)(1 - F_m)C_u(0) + C_{u,m},
\]

\[
C_b = C_{b,i} + C_{b,m} = (1 - f_m)(1 - F_m)C_b(0) + C_{b,m}.
\]

Here, \( F_m \) is the mobile fraction of AMR protein in the AMR, and \( C_u(0) \) and \( C_b(0) \) are the initial values of \( C_u \) and \( C_b \) immediately after the photobleaching. We are able to write equations 13 and 14 as they are shown because we have made the assumption that \( C_{u,i} \) and \( C_{b,i} \) do not change on the timescale of the FRAP experiment. By substituting equations 10 and 11 into equations 13 and 14, we obtain:

\[
C_u = (1 - f_m)(1 - F_m)C_{ini} + A + Be^{-k_{off}t} + Ce^{-(k_{off} + k_{on}(L_u + L_b)/\nu)t},
\]

\[
C_b = A - B \frac{k_{off}}{L_b} e^{-k_{off}t} + Ce^{-(k_{off} + k_{on}(L_u + L_b)/\nu)t}.
\]

For equation 16, we have made the assumption that \( C_b(0) = 0 \), i.e. that the bleached portion of the AMR is completely bleached at the start of the FRAP experiment, and for equation 15 we have assumed that \( C_u(0) = C_{ini} \), where \( C_{ini} \) is the concentration of the target protein in the AMR directly before photobleaching (i.e. we assume that the ‘unbleached’ portion of the AMR is not affected by the initial bleaching in the FRAP experiment). Thus, equations 12, 15 and 16 provide the general solutions for the density of fluorescent AMR protein located in the cytoplasm, unbleached portion of the AMR, and bleached portion of the AMR, respectively.
Finally, we wish to derive a method to measure $F_m$. As this only appears in one of the time-independent terms in equation 15, we can investigate the steady state form of our general solutions, i.e. when $t \to \infty$, which causes the terms multiplied by B and C to disappear. Then, subtracting the steady state form of equation 16 from the steady state form of equation 15, and rearranging for $F_m$, we find that

$$F_m = 1 - \frac{C_u(\infty) - C_b(\infty)}{C_{ini}}. \quad (17)$$

Therefore, in order to measure $F_m$, we only need to measure the steady state values of $C_u$ and $C_b$, and the initial value of $C_u$. Thus, using FLIP ($F_{loss} = (1 - C_u(\infty)/C_{ini})$) and FRAP ($F'_m = (C_b(\infty)/C_{ini})$) values for the true mobile fraction $F_m$ to be estimated as:

$$F_m = F'_m + F_{loss} \quad (18)$$

**Estimation of AMR age based on spindle length and AMR diameter.**

By co-visualising mitotic spindle and the AMR in a cell, the time $t$ can be calculated by either measuring the length of its spindle ($L'$) for cells with a growing spindle (Anaphase A and B, Formula 1), or the diameter of the AMR ($D'$) for cells with a contracting AMR (spindle already broken down, Formula 2):

$$t = -1 + \frac{L'}{L^0} \quad (19)$$

$$t = 1 - \frac{D'}{D^0} \quad (20)$$

Here $L^0$ is the average maximum spindle length and $D^0$ the average maximum AMR diameter for population.

**Measurement of protein mobile fraction:**

1- **Data normalization.** Using custom written Matlab script, first, for every individual measurement (bleached and postbleach intensities for both bleached and unbleached ROIs) background value was subtracted (as mean value in the bleached region in the first frame following the bleaching event), resulting values were normalized to a mean value of the first three time points (prebleach intensity). Next, the mean value and SD was calculated for each time point for respective groups (bleached ROI, unbleached ROI, age of the AMR).

2- **Correction for imaging-induced photobleaching.** First, we extracted normalized values for intensity of each of the the AMR in the imaging-induced bleaching control group (Supplementary Figure 3). Next we attempted to find an appropriate model expressing decay of AMR intensity over time. Empirically we found that two-term exponential model
showed the best result. Next, for each protein in analysis we found most appropriate values for the coefficients a,b,c and d using curve fitting toolbox in Matlab (exp2 function). Using two-term exponential model we could calculate expected loss of intensity ($Int_{loss}$) at each time-point ($t$) as

$$Int(t)_{loss} = 1 - Int(t)$$

Finally, in order to correct for imaging-induced photobleaching we added expected loss of intensity ($Int_{loss}$) to the observed FRAP data (mean normalized intensity values for each AMR) for each time point ($t$):

$$FRAP(t) = FRAP(t)_{observed} + Int(t)_{loss}$$

On average imaging-induced photobleaching accounted for 5~10% loss of the AMR intensity.

3- **Measurement of protein recovery and loss after bleaching.** Recovery of fluorescence at the bleached side of the AMR ($F'_{m}$) was measured as the mean value of the ROI intensity (normalized & compensated for imaging-induced photobleaching) in last 40-100 frames of the time-sequence at least 3 recovery half-lives after the bleaching event (determined for each protein individually). The remaining fluorescence at the unbleached side of the AMR ($F_{flip}$) was extracted in the same way as $F'_m$ and used to calculate FLIP ($F_{loss}$) as $1 - F_{flip}$. Finally, the corrected mobile fraction was calculated as $F'_m + F_{loss}$. The final plots are for the mean value per AMR age group per group (with SD).
**Supplementary Figure legends**

**Figure S1.** A. Changes of spindle length (blue) and diameter of the AMR (orange) in dividing *S. pombe* cells (N = 4). Data shown as mean of normalized value ± SD. B. Distribution of the diameters of wild-type *S. pombe* cells. (Left panel) Boxplot (raw data values shown as grey circles, N=42). (Right panel) Gaussian fit into frequency distribution of the raw data. C. Example of partial AMR bleaching in mitotic cells expressing GFP-Myo2. 30~50% of labelled AMR is bleached after a laser pulse along 1~2 um line ROI drawn at the midplane of the cell.

**Figure S2.** Representative time lapse series, kymograph and quantification of Cdc15 FRAP/FLIP within AMR in cells at different stages of cytokinesis: few minutes after SPB separation (t = -0.75), towards the end of Anaphase B (t = -0.2), few minutes after onset of contraction (t = 0.15) and half way through contraction of the AMR (t = 0.5). Data is shown as mean intensity per each time-frame normalized against initial intensity before bleaching and background. B. Effect of imaging induced bleaching on AMR intensity during acquisition of FRAP data. Data is shown as mean normalized intensity per AMR (N = 10) for each time-frame.

**Figure S3.** A. Quantification of Myp2 density in the AMR during cytokinesis. Vertical axis indicates density of Myp2-mApple per unit of the AMR length (measured in A.U.). Horizontal axis displays ratio between cell and AMR diameters and indicates progression of the cytokinesis. B. ZY projection and kymograph of AMR in cells expressing chosen AMR components tagged to GFP or mNeonGreen. ZY projection reconstructed from dZ time-lapse series. Numbers on the top indicate number of frames before or after bleaching event. Dotted white line in the kymograph indicate border between bleached and not-bleached regions. For Cdc12 movement of Cdc12 clusters from unbleached to bleached zones of AMR is indicated.

**Figure S4.** Analysis of proliferation of *S. pombe* in Δclp1 and spg1-106 cells. A. Time lapse series of wt and Δclp1 *S. pombe* cells expressing mCherry tagged tubulin (green) and Cdc15 tagged with GFP (orange). Images shown are maximum-intensity projections of Z stacks. Scale bars represent 5 µm. B. Serial dilutions (10 fold) of wild-type and Δclp1 cells were spotted onto YEA plates and grown for 3 days at 24°C, 30°C and 36°C. C. Quantification of DAPI and anillin blue staining used to visualize the nucleus and septum of wt and Δclp1 cells. Phenotypes of the cells were categorized into two types: septa with two nuclei (S/2N; cells completed cytokinesis) and one or two nuclei without septa (NS/(1/2D); cells in S or M phase). D. Time lapse series of spg1-106 cell expressing mCherry tagged tubulin (green) and Cdc15 tagged with GFP (orange) at 30°C (top) and their schematic representation (bottom). Images shown are maximum-intensity projections of Z stacks. Scale bars represent 5 µm. E. Quantification of DAPI and anillin blue staining used to visualize the nucleus and septum of wild-type and spg1-106 cells at permissive (24°C) and restrictive temperature (30°C). Phenotypes of the cells were categorized into two types: septa with two nuclei (S/2N; cells completed cytokinesis), one or two nuclei without septa (NS/(1/2D); cells in S or M phase), multiple septa with 2 nuclei (MS/2D) and lysed cells with no septa and 2 nuclei (NS/2D LC). F. Serial dilutions (10 fold) of wt and spg1-106 cells were spotted onto YEA plates and grown for 3 days at 24°C, 30°C and 36°C. G. Quantification of cytokinetic phenotypes in spg1-106 (N = 26) and wt cells (N = 15). Bars are showing relative percentage of all three phenotypes found in each group.
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A

![Graph showing length (normalised) against time (min). The graph has two curves, one for Spindle and one for AMR.](image)

Mean = 3.7 ± 0.2 µm

B

![Bar plot showing diameter with 25%~75% range within 1.5IQR, median line, and mean. The Gauss fit is also displayed.](image)

| Model | Gauss |
|-------|-------|
| Equation | \( y = y_0 + (A/(w^{2/2}) \exp(-2(x-x_0)/w)^2) \) |
| Plot | Counts |
| y0 | 0.188 ± 1.81914 |
| xc | 3.65167 ± 0.03976 |
| w | 0.41063 ± 0.1039 |
| A | 8.09957 ± 2.40413 |
| Reduced Chi-Sqr | 8.4953 |
| R-Square (C OD) | 0.87905 |
| Adj. R-Square | 0.78838 |

C

![Images showing Middle Z-plane and ZY projection of the AMR before and after bleaching.](image)
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A

Equation: \( y = a + b \cdot x \)

Plot: Myp2 intensity/length

Weight: No Weighting

Intercept: 3640.6148 ± 221.0

Slope: -3746.0773 ± 403.2

Residual Sum of Squares: 9044359.52178

Pearson’s r: -0.83673

R-Square (COD): 0.70011

Adj. R-Square: 0.69201

B

-1  1  5  20  Kymograph

Ain1

Cdc12

Cdc15

Myo2

Myo51

Myp2

Rng2
