DYNAMIC RECRUITMENT OF LICENSING FACTOR CDT1 TO SITES OF DNA DAMAGE

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SUPPLEMENTARY INFORMATION

Modeling FRAP recoveries

Mean fluorescence intensities in the FRAP region and across the entire nucleus (including the FRAP region) were calculated and background subtracted. The bleached region and whole nucleus profiles were then individually normalized to their prebleach values (obtained from averaging the 10 images immediately preceding the bleaching). The final recovery profile was then obtained by dividing these two normalized profiles (FRAP region divided by whole nucleus). This final division removes the global decrease in fluorescence upon bleaching as well as any later gradual bleaching that occurred during the recovery (the latter was always less than a few percent). An incomplete recovery (to a value less than 1) indicates the presence of an immobile fraction.

Recoveries in undamaged and damaged nuclei were interpreted using an analytic Laplace-transform-based solution of the full reaction-diffusion equations in a rectangular geometry (see S1 for a similar approach in a circular geometry). For the undamaged nuclei, all recoveries were complete and consistent with "effective diffusion". The recoveries were therefore governed by two parameters, the asymptotic recovery value and the effective diffusion constant, which were fit by minimizing chi squared to individual nuclear recoveries. The effective diffusion constants were used, along with other information from the pre-

images and the asymptotic recovery value, to predict and/or fit the recoveries in the damaged nuclei.

For FRAP in undamaged nuclei, recoveries were monitored following bleaching within a narrow stripe (1.29 μm width) along the nuclear midpoint. All values were normalized to the instantaneous integrated nuclear intensity to correct for the reduction of total nuclear fluorescence following the bleaching. All recoveries were complete and consistent with a "diffusive" recovery, which was specified in the model by an effective diffusion constant, D_{eff} ($D_{\text{eff}} = fD$, where D is the actual diffusion constant and f is the instantaneous fraction of protein that is free to diffuse). Nuclear lengths were obtained from the images. Joint least-squares minimization of D_{eff} and an overall scaling factor (representative cell fits shown on the lower panel of Fig. 6C) led to the values for D_{eff} listed in Fig. 6C (upper panel, mean ± s.d. of eight separate cells). We note that the narrow stripe used for bleaching and the relatively long bleaching step are not optimal for truly accurate estimation of D_{eff} (S2, S3), therefore the D_{eff} values shown in Fig. 6C should be considered as only rough estimates.

In cells with damaged nuclei the obtained for the undamaged nuclei average values for D_{eff} (Fig. 6C) were assumed. The model implicitly accounted for uniform binding sites scattered throughout the nucleus (through D_{eff}), but explicitly specified the additional binding sites introduced by the damage (see Suppl. Fig. 7). The assumed model parameters listed in Fig. 6C correspond to the contrast β (brightness of the stripe compared to the nucleoplasm), the immobile fraction f_{imm} in the stripe, and the residence half-life $t_{1/2}^{f}$ for rapid-turnover binding sites in the stripe (superscript "f" denotes "fast").

FRAP recovery in undamaged nuclei

To model the recovery curves, we used a Laplace transform approach in the context of a 1D rectangular model. A centered 1D coordinate system with nuclear length defined as $L = 2l$ and the extent of the centered FRAP region defined as $W = 2w$ was assumed (Suppl. Figs. 7A,B). The diffusion equation for the fluorescent protein is simply:

$$\frac{\partial F(x,t)}{\partial t} = D_{\text{eff}} \nabla^2 F(x,t), \tag{1}$$

where

$$D_{\text{eff}} = \frac{1}{1 + \frac{k_{\text{on}} B}{k_{\text{on}}}} D \tag{2}$$

is the effective diffusion constant. The effective diffusion constant is the normal diffusion constant multiplied by the instantaneous freely-diffusing fraction of molecules, specified by k_{on}, k_{off}, and the concentration of *free* binding sites B (S1, S4). This approximation (see also S1 and S4) is valid for all of the proteins we observe in undamaged nuclei whose recoveries are well fit by a purely diffusive recovery. Due to symmetry, we can restrict our analysis to only the right half of the nucleus (from $0 \le x \le l$ in Suppl. Fig. 7A). For the FRAP assay, a fraction of the fluorescence is bleached in the FRAP region (the efficiency of the bleaching is given by ε, with $\varepsilon = 1$ denoting complete bleaching):

$$F(x,0) = (1-\varepsilon) F_{\text{eq}}, \qquad 0 \le x \le w \tag{3}$$

$$F(x,0) = F_{\text{eq}}, \qquad w < x \le l. \tag{4}$$

In this simple case, we can separately consider the recovery of only the bleached fraction by subtracting off the initial, postbleach fluorescence in the FRAP region:

$$F_{\text{rec}}(x,t) = F(x,t) - (1-\varepsilon) F_{\text{eq}}\ . \tag{5}$$

The initial conditions are thereby simplified:

$$F_{\text{rec}}(x,0) = 0, \qquad 0 \le x \le w \tag{6}$$

$$F_{\text{rec}}(x,0) = \varepsilon F_{\text{eq}}, \qquad w < x \le l, \tag{7}$$

without affecting the form of the diffusion equation:

$$\frac{\partial F_{\text{rec}}(x,t)}{\partial t} = D_{\text{eff}} \nabla^2 F_{\text{rec}}(x,t). \tag{8}$$

Taking the Laplace transform of the diffusion equation gives the following:

$$p\overline{F}_{\text{rec}}(x,p) - F_{\text{rec}}(x,0) = D_{\text{eff}} \nabla^2 \overline{F}_{\text{rec}}(x,p), \tag{9}$$

where the bar denotes the Laplace-transformed version of the function:

$$\bar{f}(p) = \int_0^\infty f(t) \mathrm{e}^{-pt} dt. \tag{10}$$

After FRAP bleaching, the equation for each zone is then:

$$D_{\text{eff}} \nabla^2 \overline{F}_{\text{rec}}(x,p) - p\overline{F}_{\text{rec}}(x,p) = 0, \qquad 0 \le x \le w \tag{11}$$

$$D_{\text{eff}} \nabla^2 \overline{F}_{\text{rec}}(x,p) - p\overline{F}_{\text{rec}}(x,p) = -\varepsilon F_{\text{eq}}, \qquad w < x \le l. \tag{12}$$

In terms of the general solution, the solution in each region is just

$$\overline{F}_{\text{rec}}(x,p) = A\cosh\sqrt{\tfrac{p}{D_{\text{eff}}}}x + B\sinh\sqrt{\tfrac{p}{D_{\text{eff}}}}x, \qquad 0 \le x \le w \tag{13}$$

$$\overline{F}_{\text{rec}}(x,p) = C\cosh\sqrt{\tfrac{p}{D_{\text{eff}}}}x + D\sinh\sqrt{\tfrac{p}{D_{\text{eff}}}}x + \varepsilon F_{\text{eq}}/p, \quad w < x \le l. \tag{14}$$

Taking into account the zero-slope, Neumann boundary conditions at $x = 0$ (due to no source/sink there and symmetry) and $x = l$ ("hard wall" boundary), along with satisfying continuity and smoothness at $x = w$ gives the following form for the solution in the FRAP region:

$$\overline{F}_{\text{rec}}(x,p) = \frac{\varepsilon F_{\text{eq}}}{p} \frac{1}{\coth\left(\sqrt{\tfrac{p}{D_{\text{eff}}}}w\right) + \coth\left(\sqrt{\tfrac{p}{D_{\text{eff}}}}(l-w)\right)} \frac{\cosh\left(\sqrt{\tfrac{p}{D_{\text{eff}}}}x\right)}{\sinh\left(\sqrt{\tfrac{p}{D_{\text{eff}}}}w\right)}. \tag{15}$$

Integrating over the FRAP region, dividing by the total bleached fluorescence there ($\varepsilon F_{\mathrm{eq}} w$), and taking into account the decreased total nuclear fluorescence due to the bleaching (to obtain a recovery from 0 to 1) gives:

$$\bar{f}_{\mathrm{rec}}(p) = \frac{1}{p}\frac{1}{1-\frac{w}{l}}\frac{1}{w}\sqrt{\frac{D_{\mathrm{eff}}}{p}}\frac{1}{\coth\left(\sqrt{\frac{p}{D_{\mathrm{eff}}}}w\right)+\coth\left(\sqrt{\frac{p}{D_{\mathrm{eff}}}}(l-w)\right)}, \qquad (16)$$

which can be rewritten as:

$$\bar{f}_{\mathrm{rec}}(p) = \frac{l^2}{D_{\mathrm{eff}}}\bar{g}_{\mathrm{rec}}(\alpha;\phi), \qquad (17)$$

with

$$\bar{g}_{\mathrm{rec}}(\alpha;\phi) = \frac{1}{1-\alpha}\frac{1}{\phi}\frac{1/\left(\sqrt{\phi}\alpha\right)}{\coth\left(\sqrt{\phi}\alpha\right)+\coth\left(\sqrt{\phi}(1-\alpha)\right)}, \qquad (18)$$

where $\phi = \frac{l^2}{D_{\mathrm{eff}}}p$ and $\alpha = \frac{w}{l}$. Taking the inverse Laplace transform then gives:

$$f_{\mathrm{rec}}(t) = \frac{1}{2\pi i}\int_{\mu-i\infty}^{\mu+i\infty}\bar{f}_{\mathrm{rec}}(p)\mathrm{e}^{pt}dp = \frac{1}{2\pi i}\int_{\nu-i\infty}^{\nu+i\infty}\bar{g}_{\mathrm{rec}}(\alpha;\phi)\mathrm{e}^{\phi\tau}d\phi = g_{\mathrm{rec}}(\alpha;\tau), \qquad (19)$$

with dimensionless time $\tau = \frac{D_{\mathrm{eff}}}{l^2}t$. The *shape* of the recovery (as a function of τ) is therefore dependent only on α. The actual recovery in normal time units can be obtained from $g_{\mathrm{rec}}(\alpha;\tau)$ by simply scaling the τ-axis by l^2/D_{eff}. Again, due to the symmetry condition, $g_{\mathrm{rec}}(\alpha;\tau)$ applies equally well to a centered geometry with nuclear length $L = 2l$ and centered FRAP region of extent $W = 2w$ (as considered in this work), or to a FRAP region of extent w flush against one side of a nucleus of length l. To calculate the inverse Laplace transform, we use the algorithms described in S5-S7.

The fluorescence profiles before, immediately after, and long after FRAP bleaching are shown in Suppl. Fig. 7B. The renormalization applied above involves setting the lowest value of the bleaching (red, dashed line) to zero and the recovery value of one to the

predicted asymptotic value (blue line). The effects of changes in D_{eff} or FRAP region width are shown in Suppl. Figs. 7C and D, respectively.

Assuming a FRAP region of size $W = 2w = 1.29$ μm for all nuclei and a nuclear length of $L = 2l$ estimated on a cell-by-cell basis from analyzing the images (average length was roughly 13 μm), we obtained the average effective diffusion constants for all labeled proteins (Figs. 6C). All profiles recovered completely (to a few percent) within seconds, and a significant immobile fraction was not detected.

If a uniform immobile fraction η had been present, the final recovery would have instead been to:

$$f_{\text{rec}}(t \to \infty) = 1 - \eta \frac{\varepsilon(1-\alpha)}{\varepsilon(1-\alpha)+1-\varepsilon} \le 1. \qquad (20)$$

FRAP at the damage site

For analyzing FRAP in the damaged region, we again employ a 1D Laplace transform approach. The following equations describe the concentration of a freely diffusing fluorescent protein, $F(x,t)$; unoccupied binding sites, $B(x,t)$; and binding sites occupied by fluorescent protein, $C(x,t)$:

$$\frac{\partial F(x,t)}{\partial t} = D\nabla^2 F(x,t) - k_{\text{on}} B(x,t) F(x,t) + k_{\text{off}} C(x,t) \qquad (21)$$

$$\frac{\partial B(x,t)}{\partial t} = -k_{\text{on}} B(x,t) F(x,t) + k_{\text{off}} C(x,t) \qquad (22)$$

$$\frac{\partial C(x,t)}{\partial t} = k_{\text{on}} B(x,t) F(x,t) - k_{\text{off}} C(x,t). \qquad (23)$$

We again take a symmetric, centered coordinate system as shown in Suppl. Fig. 7E. Here, we assume that the FRAP region is exactly the same as the damage region. Again, due to symmetry, we need only consider the solution in one half of the nucleus. The FRAP process

will not change the concentration of unoccupied binding sites, which is $B(x) = B_{\text{eq}}$ in the damage/FRAP region ($0 \le x \le w$) and 0 outside the damage/FRAP region ($w < x \le l$). The relevant equations are then

$$\frac{\partial F(x,t)}{\partial t} = D_{\text{eff}} \nabla^2 F(x,t) - k_{\text{on}} B(x) F(x,t) + k_{\text{off}} C(x,t) \tag{24}$$

$$\frac{\partial C(x,t)}{\partial t} = k_{\text{on}} B(x) F(x,t) - k_{\text{off}} C(x,t). \tag{25}$$

In the damage region at steady-state,

$$C_{\text{eq}} = \frac{k_{\text{on}} B_{\text{eq}}}{k_{\text{off}}} F_{\text{eq}}. \tag{26}$$

Laplace transforming the differential equations gives:

$$p\overline{F}(x,p) - F(x,0) = D_{\text{eff}} \nabla^2 \overline{F}(x,p) - k_{\text{on}} B(x) \overline{F}(x,p) + k_{\text{off}} \overline{C}(x,p) \tag{27}$$

$$p\overline{C}(x,p) - C(x,0) = k_{\text{on}} B(x) \overline{F}(x,p) - k_{\text{off}} \overline{C}(x,p). \tag{28}$$

The second equation gives:

$$\overline{C}(x,p) = \frac{k_{\text{on}} B(x) \overline{F}(x,p) + C(x,0)}{p + k_{\text{off}}}. \tag{29}$$

Substituting this into the first equation gives:

$$D_{\text{eff}} \nabla^2 \overline{F}(x,p) - p\left(1 + \frac{k_{\text{on}} B(x)}{p + k_{\text{off}}}\right) \overline{F}(x,p) = -F(x,0) - \frac{k_{\text{off}}}{p + k_{\text{off}}} C(x,0). \tag{30}$$

The bleaching process removes a fraction ε of the fluorescence of the freely diffusing and bound proteins, giving:

$$F(x,0) = (1 - \varepsilon) F_{\text{eq}} \tag{31}$$

$$C(x,0) = (1 - \varepsilon) \frac{k_{\text{on}} B_{\text{eq}}}{k_{\text{off}}} F_{\text{eq}}. \tag{32}$$

Plugging these in gives the final equations for both inside and outside the damage/FRAP region:

$$D_{\text{eff}}\nabla^2\overline{F}(x,p) - p\left(1+\frac{k_{\text{on}}B_{\text{eq}}}{p+k_{\text{off}}}\right)\overline{F}(x,p) = -(1-\varepsilon)F_{\text{eq}}\left(1+\frac{k_{\text{on}}B_{\text{eq}}}{p+k_{\text{off}}}\right), \qquad 0 \le x \le w \quad (33)$$

$$D_{\text{eff}}\nabla^2\overline{F}(x,p) - p\overline{F}(x,p) = -F_{\text{eq}}, \qquad w < x \le l \quad (34)$$

The general solution in each region is then:

$$\overline{F}(x,p) = A\cosh\left(\sqrt{1+\tfrac{\beta}{1+p/k_{\text{off}}}}\sqrt{\tfrac{p}{D_{\text{eff}}}}x\right) + B\sinh\left(\sqrt{1+\tfrac{\beta}{1+p/k_{\text{off}}}}\sqrt{\tfrac{p}{D_{\text{eff}}}}x\right) + (1-\varepsilon)\tfrac{F_{\text{eq}}}{p},$$

$$0 \le x \le w \qquad (35)$$

$$\overline{F}(x,p) = C\cosh\sqrt{\tfrac{p}{D_{\text{eff}}}}x + D\sinh\sqrt{\tfrac{p}{D_{\text{eff}}}}x + F_{\text{eq}}/p, \qquad w < x \le l \qquad (36)$$

where $\beta = k_{\text{on}}B_{\text{eq}}/k_{\text{off}}$ is the dimensionless ratio of the intensity in the binding sites to the intensity in the *effectively* diffusing fraction. Taking into account the zero-slope conditions at $x = 0$ and $x = l$, and continuity and smoothness at $x = w$ (see previous section) yields:

$$\overline{F}(x,p) = \varepsilon\frac{F_{\text{eq}}}{p}\frac{1}{\coth\left(\sqrt{1+\tfrac{\beta}{1+p/k_{\text{off}}}}\sqrt{\tfrac{p}{D_{\text{eff}}}}w\right) + \sqrt{1+\tfrac{\beta}{1+p/k_{\text{off}}}}\coth\left(\sqrt{\tfrac{p}{D_{\text{eff}}}}(l-w)\right)} \times \frac{\cosh\left(\sqrt{1+\tfrac{\beta}{1+p/k_{\text{off}}}}\sqrt{\tfrac{p}{D_{\text{eff}}}}x\right)}{\sinh\left(\sqrt{1+\tfrac{\beta}{1+p/k_{\text{off}}}}\sqrt{\tfrac{p}{D_{\text{eff}}}}w\right)} + (1-\varepsilon)\frac{F_{\text{eq}}}{p}. \quad (37)$$

Integrating over the FRAP region and dividing by αF_{eq} gives:

$$\bar{f}(p) = \frac{l^2}{D_{\text{eff}}}\frac{1}{\phi}\left(\varepsilon\frac{1}{1+\tfrac{\beta}{1+\gamma\phi}}\frac{1/\left(\sqrt{\phi}\alpha\right)}{\coth\left(\sqrt{1+\tfrac{\beta}{1+\gamma\phi}}\sqrt{\phi}\alpha\right)/\sqrt{1+\tfrac{\beta}{1+\gamma\phi}} + \coth\left(\sqrt{\phi}(1-\alpha)\right)} + (1-\varepsilon)\right), \quad (38)$$

where $\phi = \frac{l^2}{D_{\text{eff}}}p$, $\alpha = \frac{w}{l}$ and $\gamma = \frac{D_{\text{eff}}}{l^2}/k_{\text{off}}$. The bound fraction from above is again just:

$$\overline{C}(x,p) = \frac{k_{\text{on}}B(x)\overline{F}(x,p) + C(x,0)}{p+k_{\text{off}}} \quad (39)$$

Integrating over the FRAP region and dividing by αF_{eq} gives:

$$\bar{c}(p) = \frac{\beta}{1+\gamma\phi}\bar{f}(p) + \frac{l^2}{D_{\text{eff}}}(1-\varepsilon)\beta\gamma\frac{1}{1+\gamma\phi}. \tag{40}$$

The total recovery is then simply:

$$\bar{r}(p) = \frac{(1+\beta)\alpha+1-\alpha}{(1-\varepsilon)(1+\beta)\alpha+1-\alpha}\frac{\bar{f}(p)+\bar{c}(p)}{1+\beta}, \tag{41}$$

where we have divided by $1+\beta$ and normalized to take into account the reduction in total fluorescence upon FRAP bleaching, yielding a recovery to 1 (if no additional immobile fractions are present). The inverse Laplace transform is then:

$$\bar{r}(p) = \frac{l^2}{D_{\text{eff}}}\bar{r}(\alpha,\beta,\gamma,\varepsilon;\phi), \tag{42}$$

with

$$\bar{r}(\alpha,\beta,\gamma,\varepsilon;\phi) = \frac{(1+\beta)\alpha+1-\alpha}{(1-\varepsilon)(1+\beta)\alpha+1-\alpha}\times\frac{1}{1+\beta}\times$$
$$\frac{1}{\phi}\left[\varepsilon\frac{1/\left(\sqrt{\phi}\,\alpha\right)}{\coth\left(\sqrt{1+\frac{\beta}{1+\gamma\phi}}\sqrt{\phi}\,\alpha\right)/\sqrt{1+\frac{\beta}{1+\gamma\phi}}+\coth\left(\sqrt{\phi}(1-\alpha)\right)}+(1-\varepsilon)\left(1+\beta\frac{\gamma\phi}{1+\gamma\phi}\right)\right]. \tag{43}$$

The inverse transform then gives the recovery:

$$r(t) = \tfrac{1}{2\pi i}\int_{\mu-i\infty}^{\mu+i\infty}\bar{r}(\alpha,\beta,\gamma,\varepsilon;\phi)\mathrm{e}^{\phi\tau}d\phi. \tag{44}$$

For $\gamma \to 0$ ($k_{\text{off}} >> \frac{D_{\text{eff}}}{l^2}$):

$$\lim_{\gamma\to 0}\bar{r}(\alpha,\beta,\gamma,\varepsilon;\phi) = \frac{(1+\beta)\alpha+1-\alpha}{(1-\varepsilon)(1+\beta)\alpha+1-\alpha}\times\frac{1}{1+\beta}\times$$
$$\frac{1}{\phi}\left[\varepsilon\frac{1/\left(\sqrt{\phi}\,\alpha\right)}{\coth\left(\sqrt{1+\beta}\sqrt{\phi}\,\alpha\right)/\sqrt{1+\beta}+\coth\left(\sqrt{\phi}(1-\alpha)\right)}+(1-\varepsilon)\right], \tag{45}$$

which corresponds to a recovery in the *effectively* diffusing fraction and at the binding sites that is only diffusion-limited.

For $\gamma \to \infty$ ($k_{\text{off}} << \frac{D_{\text{eff}}}{l^2}$):

$$\lim_{\gamma\to\infty}\bar{r}(\alpha,\beta,\gamma,\varepsilon;\phi)=\frac{(1+\beta)\alpha+1-\alpha}{(1-\varepsilon)(1+\beta)\alpha+1-\alpha}\times\frac{1}{1+\beta}\times \\ \frac{1}{\phi}\left[\varepsilon\frac{1/\left(\sqrt{\phi}\,\alpha\right)}{\coth\left(\sqrt{\phi}\,\alpha\right)+\coth\left(\sqrt{\phi}(1-\alpha)\right)}+(1-\varepsilon)(1+\beta)\right], \tag{46}$$

which corresponds to a diffusive recovery for the *effectively* diffusing fraction but no recovery at the completely immobile binding sites.

The effects on the recovery of different β (ratio of total bound to free protein in the nucleus) and γ (proportional to the binding site residence time $t_{1/2}^{f}$) are displayed in Suppl. Fig. 7E.

For completeness, we give the general solution for the Laplace transform of the recovery in the presence of different types of binding sites within the FRAP region ($\beta_k^{\text{in}},\gamma_k^{\text{in}}$) and outside of the FRAP region ($\beta_k^{\text{out}},\gamma_k^{\text{out}}$):

$$\bar{r}(p)=\frac{l^2}{D}\bar{r}(\alpha,\beta_j^{\text{in}},\gamma_j^{\text{in}},\beta_k^{\text{out}},\gamma_k^{\text{out}},\varepsilon;\phi), \tag{47}$$

with

$$\bar{r}(\alpha,\beta_j^{\text{in}},\gamma_j^{\text{in}},\beta_k^{\text{out}},\gamma_k^{\text{out}},\varepsilon;\phi)=\frac{\left(1+\sum_j\beta_j^{\text{in}}\right)\alpha+\left(1+\sum_k\beta_k^{\text{out}}\right)(1-\alpha)}{(1-\varepsilon)\left(1+\sum_j\beta_j^{\text{in}}\right)\alpha+\left(1+\sum_k\beta_k^{\text{out}}\right)(1-\alpha)}\frac{1}{1+\sum_j\beta_j^{\text{in}}}\times \\ \frac{1}{\phi}\left(\varepsilon\frac{\dfrac{1}{\sqrt{\phi}\,\alpha}}{\dfrac{\coth\left(\sqrt{1+\sum_j\frac{\beta_j^{\text{in}}}{1+\gamma_j^{\text{in}}\phi}}\sqrt{\phi}\,\alpha\right)}{\sqrt{1+\sum_j\frac{\beta_j^{\text{in}}}{1+\gamma_j^{\text{in}}\phi}}}+\dfrac{\coth\left(\sqrt{1+\sum_k\frac{\beta_k^{\text{out}}}{1+\gamma_k^{\text{out}}\phi}}\sqrt{\phi}(1-\alpha)\right)}{\sqrt{1+\sum_k\frac{\beta_k^{\text{out}}}{1+\gamma_k^{\text{out}}\phi}}}}+(1-\varepsilon)\left(1+\sum_j\beta_j^{\text{in}}\frac{\gamma_j^{\text{in}}\phi}{1+\gamma_j^{\text{in}}\phi}\right)\right). \tag{48}$$

In the above, we use the actual diffusion constant, *D*, assuming that all binding interactions have already been accounted for in $\bar{r}(\alpha,\beta_j^{\text{in}},\gamma_j^{\text{in}},\beta_k^{\text{out}},\gamma_k^{\text{out}},\varepsilon;\phi)$.

In Fig. 6C, analysis of the recoveries in representative cells for each protein are shown (using Eq. 44). For PCNA, the only recovery was due to bleaching of the free fraction, with all bound protein immobilized in the damaged region. However, for p21 and Cdt1, most bound protein was turned over rapidly (a significant immobile fraction cannot be ruled out). For Cdt2, robust evidence is found for two different types of Cdt2 binding sites: those with rapid Cdt2 turnover and those that immobilize Cdt2.

The displayed models for the damaged cells in Figs. 6C are not fits to the data with all parameters left unspecified. Rather, these models are predictions of the recovery based on parameters already obtained from image analysis (bound-to-free ratio β, nuclear length L, immobile fraction determined from asymptotic recovery) and the D_{eff} values measured in undamaged cells. The only parameter that might actually have required fitting was γ (proportional to $t_{1/2}^{f}$), but we were only able to obtain an upper limit (all model recovery curves below this upper limit were essentially the same, due to "effective-diffusion-limited" nature of all of the observed recoveries).

Turnover rate at binding sites

The amount of protein over a given duration of time that has been bound at least once in the damaged region can be approximated as follows. On timescales longer than seconds, diffusion rapidly mixes all proteins across the cell. This allows us to make the assumption that the exchange of protein at binding sites samples from the entire nuclear population, allowing us to neglect diffusion and leading to the following simple rate equation:

$$\frac{dX(t)}{dt} = -\alpha k_{\text{on}} B_{\text{eq}} \frac{X}{X_T} X, \qquad (49)$$

where X is the amount of protein that has not yet been bound and X_T is the total amount of protein in the nucleus. This can be rewritten in terms of the fractional amount that has not yet been bound, $\chi = X/X_T$:

$$\frac{d\chi(t)}{dt} = -\alpha k_{\text{on}} B_{\text{eq}} \chi^2 = -\alpha\beta k_{\text{off}} \chi^2 \tag{50}$$

The solution is simply:

$$\chi(t) = \chi_0 \frac{1}{1 + \alpha\beta\chi_0 k_{\text{off}} t}. \tag{51}$$

The amount of time needed before only a fraction χ_f is left (protein that has never been bound) is:

$$t_f = \frac{1}{k_{\text{off}}} \frac{1}{\alpha\beta}\left(\frac{1}{\chi_f} - \frac{1}{\chi_0}\right) = \frac{t_{1/2}^f}{\ln 2} \frac{1}{\alpha\beta}\left(\frac{1}{\chi_f} - \frac{1}{\chi_0}\right). \tag{52}$$

Taking $\alpha \approx 0.1$, $\chi_0 = 1$, and $\chi_f = 0.1$ (so that 90% of the protein has been bound at some point) gives $t_f \approx 87$ s for p21 ($\beta \approx 3$, $t_{1/2}^f = 2$ s) and $t_f < 130$ s for Cdt1 ($\beta \approx 1$, $t_{1/2}^f < 1$ s). So, within roughly 2 min, 90% of both p21 and Cdt1 have been bound at least once in the damaged region.

References

S1) B.L. Sprague, R.L. Pego, D.A. Stavreva, & J.G. McNally, Biophys J **86**, 3474-3495 (2004)

S2) J. Braga, J.M. Desterro, & M. Carmo-Fonseca, Mol Biol Cell **15**, 4749-4760 (2004)

S3) F. Mueller, D. Mazza, T.J. Stasevich, & J.G. McNally. 2010 Curr Opin Cell Biol **22**, 403-411 (2010)

S4) B.L. Sprague, F. Müller, R.L. Pego, P.M. Bungay, D.A. Stavreva, & J.G. McNally, Biophys J **91**, 1169-1191 (2006)

S5) J. Abate, P.P. Valkó, Int J Numer Meth Engng **60**, 979–993 (2004)

S6) http://library.wolfram.com/infocenter/MathSource/4738/

S7) http://library.wolfram.com/infocenter/MathSource/5026/