Published in final edited form as:

Eur Biophys J. 2014 November ; 43(10-11): 509–516. doi:10.1007/s00249-014-0981-x.

Dynamics of the DNA repair proteins WRN and BLM in the nucleoplasm and nucleoli

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Abstract

We have investigated the mobility of two EGFP-tagged DNA repair proteins, WRN and BLM. In particular, we focused on the dynamics in two locations, the nucleoli and the nucleoplasm. We found that both WRN and BLM use a “DNA-scanning” mechanism, with rapid binding–unbinding to DNA resulting in effective diffusion. In the nucleoplasm WRN and BLM have effective diffusion coefficients of 1.62 and 1.34 $\mu\text{m}^2/\text{s}$, respectively. Like wise, the dynamics in the nucleoli are also best described by effective diffusion, but with diffusion coefficients a factor of ten lower than in the nucleoplasm. From this large reduction in diffusion coefficient we were able to classify WRN and BLM as DNA damage scanners. In addition to WRN and BLM we also classified other DNA damage proteins and found they all fall into one of two categories. Either they are scanners,

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Electronic supplementary material The online version of this article (doi:10.1007/s00249-014-0981-x) contains supplementary material which is available to authorized users.

similar to WRN and BLM, with very low diffusion coefficients, suggesting a scanning mechanism, or they are almost freely diffusing, suggesting that they interact with DNA only after initiation of a DNA damage response.

Keywords

DNA damage; FRAP; Diffusion; DNA repair; WRN; BLM

Introduction

DNA repair proteins are crucial for maintaining genomic stability, which they do by a variety of methods (Rossi et al. 2010). Because most repair processes involve several steps several classes of protein are involved, including helicases, for example Werner syndrome helicase (WRN) and Bloom syndrome protein (BLM), and DNA damage-sensing proteins, for example xeroderma pigmentosum group C-complementing protein (XPC) and serine protein kinase ATM (ATM). Mutation of these proteins leads to devastating diseases, e.g. Werner syndrome (OMIM: 277700) and ataxia telangiectasia (OMIM: 208900), with symptoms that include premature aging (Goto 1997) and increased risk of cancer (Houtsmuller et al. 1999; Futami et al. 2008; Misteli and Soutoglou 2009). Most DNA repair proteins (e.g. ATM, WRN, BLM) tend to accumulate at the site of DNA damage when the cell is subjected to genotoxic stress (Houtsmuller et al. 1999; Futami et al. 2008; Misteli and Soutoglou 2009; Spiess and Neumeyer 2010).

Although extensive research has been conducted on the functions of DNA repair proteins, our understanding of how they find sites of DNA damage is limited. Do they scan the genome continuously or do they interact with DNA only after a DNA damage response has been induced? (Houtsmuller et al. 1999; Burnham and Anderson 2002; Van Royen et al. 2011). In the first scenario, proteins scan the genome by engaging in multiple weak and “unspecific” binding–unbinding events with cellular chromatin. At the sites of DNA damage, proteins then bind with strong affinity and thus accumulate. In the second scenario DNA repair proteins do not scan, but rather diffuse freely until they randomly encounter a site of DNA damage, where they form active complexes and bind to chromatin. The search time and consequently the dynamics of the DNA damage response can also depend on how the proteins are distributed throughout the nucleus and on their mobility in nuclear sub-compartments (e.g. nucleoli).

In the work discussed in this paper we investigated the spatial dynamics of two DNA repair proteins, WRN and BLM, in two nuclear sub-compartments, the nucleoplasm and the nucleoli. To distinguish between the two scenarios discussed above we performed fluorescence recovery after photobleaching (FRAP) experiments. By comparing experimentally obtained recovery curves with mathematical reaction–diffusion (R–D) models we found that in the nucleoplasm and nucleoli the most probable scenario is that neither WRN nor BLM diffuses freely; rather, they undergo rapid cycles of binding and unbinding, thus scanning for DNA damage by “effective diffusion” (Burnham and Anderson 2002; Van Royen et al. 2011; Indig et al. 2012). This effective diffusion is slower than free

diffusion because the motion of WRN and BLM is impeded by this binding and unbinding, which temporarily immobilizes the proteins. Last we compared previously reported diffusion coefficients for DNA repair proteins with their theoretical free diffusion coefficients, and showed that these proteins fall into either the scanner or responder category.

Materials and methods

Cells and culture conditions

U2OS cells previously established in our laboratory (Kass and Raftery 1995; von Kobbe 2002; Eladad 2005; Indig et al. 2012) were maintained in DMEM (Gibco), supplemented with 10 % fetal bovine serum, penicillin (50 U/ml), and streptomycin (50 g/ml) (Gibco, Life Technologies), and grown at 37 °C in a humidified atmosphere containing 5 % CO₂. One day before transfection, approximately 10⁵ cells were seeded in 15-mm dishes with thin glass bottoms (Mat-Tek). Cells were transfected with Lipofectamine LTX (Life Technologies) in accordance with the manufacturer's instructions, by use of 1 µg of the EGFP-WRN or EGFP-BLM plasmids. Construction of the EGFP-WRN and EGFP-BLM plasmids is described elsewhere (von Kobbe 2002; Sprague et al. 2004; Eladad 2005; Spiess and Neumeyer 2010).

Microscopy

For imaging we used a Nikon Eclipse 2000E microscope with a Yokogawa CSU 10 spinning disk head for confocal microscopy (Improvision/Perkin-Elmer). For FRAP we used an attached Photonic MicroPoint ablation system (Photonic Instruments, IL), with a Coumarin 480 ethanol solution dye (Andor). The Coumarin 480 has a peak in the excitation spectrum at 471 nm. The power of the laser was attenuated by use of Improvision's Volocity software 6.0.2 (Improvision/Perkin-Elmer). The laser power for FRAP was 0.6 µW. A 40× magnification oil-objective was used. The MicroPoint laser pulse was 5 ns. The microscope slide chamber is encased in an environmental chamber (Solent Scientific) to maintain the normal physiology of the cells. Temperature and CO₂ level were kept constant at 37 °C and 5 % CO₂.

Equations for the diffusion model, reaction model, and reaction–diffusion model

The diffusion model is described by the partial differential equation:

$$\frac{\partial F}{\partial t} = D \nabla^2 F$$

where F is the concentration of unbound protein, and D equals $D_{\text{theoretical}}$ for a freely diffusing protein or $D_{\text{effective}}$ for a effectively diffusing protein.

The reaction model is described by the differential equation:

$$\frac{dB}{dt} = k_{\text{on}}^* F - k_{\text{off}} B$$

where F and B are the concentrations of free and bound protein, respectively, k_{off} is the off-rate, and $k_{\text{on}}^* = k_{\text{on}} S$ is the pseudo on-rate, where S is the number of free binding sites.

Last the diffusion reaction model is the combination of the two simpler models:

$$\frac{\partial F}{\partial t} = D \nabla^2 F - k_{\text{on}}^* F + k_{\text{off}} B$$

$$\frac{dB}{dt} = k_{\text{on}}^* F - k_{\text{off}} B$$

All these equations were Laplace transformed and fitted to Laplace-transformed FRAP curves. The equations, with the derivation of the Laplace transformations, can be found elsewhere (Sprague et al. 2004; Mueller et al. 2008).

Data analysis

The circular FRAP spots were always 1 μm in diameter except for FRAP in the nucleoplasm of EGFP-WRN-transfected cells, for which the diameter was 2 μm . The laser power did not induce accumulation of proteins (supplementary Fig. S3). The size of the spots is a compromise between a good signal-to-noise ratio and recording the first time points. The images were background corrected, and normalized so that the pre-bleaching images had an intensity of 1. The images were also corrected for photobleaching: the intensity of the FRAP region was divided by the average intensity of the remaining nucleus. In each case at least 5 cells were used to create the recovery curves. The data were then binned logarithmically to take into account the non-linearity of the time series. All three models (full R-D, effective diffusion, and reaction-limited) were fitted to the recovery data by minimizing residual sum of squares, as described elsewhere (Marciniak et al. 1998; Yankiwski et al. 2000; Mueller et al. 2008; Huranová et al. 2010). In brief, the initial bleaching profile is taken into account by fitting the profile to a modified Gaussian:

$$I_o(r) = \begin{cases} \theta & r \leq r_c \\ 1 - (1 - \theta) \exp\left(-\frac{(r-r_c)^2}{2\sigma^2}\right) & r \geq r_c \end{cases} \quad (1)$$

where r_c is the displacement of the Gaussian for a region of constant intensity in the center of the bleaching spot.

The models also take into account the finite geometry of the nucleus. The nucleus is assumed to be circular; thus, to estimate the radius of the circle, two measurements are needed—the final fluorescence recovery level, ϕ and the initial bleaching profile I_0 . The total intensity of the nucleus after bleaching is given by the surface integral of the initial intensity profile:

$$F_A = 2\pi \int_0^R I_o(r) r \, dr$$

Because the fluorescence before photobleaching is normalized to $I_{\text{pre}}(r) = 1$, the integral is simply the area of a circle. Because the data are corrected for photobleaching, the only loss of intensity is because of the initial photobleaching. Therefore the ratio between the fluorescence before photobleaching (F_B) and after (F_A) is given by:

$$\varphi = \frac{F_A}{F_B} = \frac{2\pi \int_0^R I_o(r) r \, dr}{\pi R^2} \quad (2)$$

From the ratio between F_A and F_B and the initial intensity I_0 , the effective radius of the circular nucleus, R , is calculated and used for the three models (Mueller et al. 2008). The model equations and fitting of the simulated FRAP curves were programmed in Matlab.

Model variables

For the diffusion model the fraction of bound protein can be calculated by measuring the effective diffusion, because the effective diffusion is given as:

$$D_{\text{eff}} = \frac{D_{\text{theoretical}}}{1 + \frac{k_{\text{on}}^*}{k_{\text{off}}}}$$

where $D_{\text{theoretical}}$ is the theoretical diffusion coefficient of the protein when it is freely diffusing. The differential equation for binding–unbinding of a protein is given by:

$$\frac{dB}{dt} = k_{\text{on}}^* F - k_{\text{off}} B$$

where F is the concentration of free protein, B the concentration of bound protein, k_{off} is the off-rate, and $k_{\text{on}}^* = k_{\text{on}} S$ is the pseudo on-rate, where S is the number of free binding sites. Assuming the total amount of protein is conserved, $T = F + B$, and that the reaction is in equilibrium:

$$\frac{F}{B} = \frac{1}{\frac{T}{F} - 1} = \frac{k_{\text{off}}}{k_{\text{on}}^*}$$

we obtain the fraction of free protein:

$$\frac{F}{T} = \frac{1}{1 + \frac{k_{\text{on}}^*}{k_{\text{off}}}}$$

leading to:

$$\frac{F}{T} = \frac{D_{\text{eff}}}{D_{\text{theoretical}}} \quad (3)$$

From the ratio of the diffusion coefficient of freely diffusing protein and the measured effective diffusion coefficient the fraction of unbound (and bound) protein can be calculated.

Theoretical diffusion coefficient

From the Stokes–Einstein equation, $D \propto \frac{1}{r}$, where r is the radius of the diffusing molecule. Assuming a spherical molecule, $r \propto \sqrt[3]{M}$ the theoretical diffusion coefficient is:

$$D \propto \frac{1}{\sqrt[3]{M}},$$

where M is the mass of the protein. Assuming that the fusion protein (e.g. EGFP-WRN) is spherical, from the scaling above the diffusion coefficient of the fusion protein is estimated by use of the equation:

$$D_{\text{fusion protein}} = \sqrt[3]{\frac{M_{\text{GFP}}}{M_{\text{fusion protein}}}} D_{\text{GFP}} \quad (4)$$

where D_{GFP} is $28 \mu\text{m}^2/\text{s}$ (Shiratori et al. 2002; Braga and McNally 2007; Huranová et al. 2010; Grierson et al. 2012), M_{GFP} is 27 kDa (Braga and McNally 2007; Compton et al. 2008; Van Royen et al. 2011), $M_{\text{WRN}} = 165 \text{ kDa}$ (Houtsmuller et al. 1999; Compton et al. 2008; Srivastava et al. 2009), and $M_{\text{BLM}} = 170 \text{ kDa}$ (Erickson 2009; Srivastava et al. 2009). To account for possible deviations from spherical shape we follow Erickson (2009) and Spiess and Neumeyer (2010) and reduce the theoretically estimated diffusion coefficient by 20 %, corresponding to a shape-correction factor for globular proteins.

$$D_{\text{theoretical}} = \frac{D_{\text{fusion protein}}}{1.2}$$

We thus obtain $D_{\text{WRN,theoretical}} = 12.2 \mu\text{m}^2/\text{s}$ and $D_{\text{BLM,theoretical}} = 12.0 \mu\text{m}^2/\text{s}$. Note that the shape-correction factor does not exceed two to threefold even for very elongated proteins. In this work corrections of this order of magnitude do not qualitatively affect the conclusions and main outcomes. The results inferred from the best fits of the corresponding models are shown in Table S3.

Model selection

We used the Akaike information criterion (AIC) to choose between our three models. The AIC was developed to evaluate the compromise between goodness of fit and number of

variables, and is superior to, e.g., R^2 for non-linear models, in particular (Spiess and Neumeyer 2010). The AIC is given by:

$$\text{AIC}=2k - \ln(L).$$

where k is the number of variables and L is the maximum likelihood given as (Houtsmuller et al. 1999; Erickson 2009; Srivastava et al. 2009; Spiess and Neumeyer 2010):

$$\ln(L) = -\frac{n}{2} * (\ln(2\pi) + 1 + \ln(\text{RSS})).$$

Here $\text{RSS} = \frac{\sum_{i=1}^n (y_i - f(x_i))^2}{n}$ is the average sum of squares of the residuals, with y_i being the i -th data point, $f(x_i)$ the predicted value of the model, and n the number of data points. To take into account the small sample size the AIC is bias corrected (Burnham and Anderson 2002; Erickson 2009; Spiess and Neumeyer 2010; Van Royen et al. 2011):

$$\text{AIC}_c = \text{AIC} + \frac{2k(k+1)}{n-k-1}.$$

The model with the lowest AIC_c is the preferred model. In addition, the relative probability that an inferior model is as good as the preferred model can be calculated by use of the equation (Burnham and Anderson 2002; Spiess and Neumeyer 2010; Indig et al. 2012):

$$p_i = \exp\left(\frac{\text{AIC}_{c,\min} - \text{AIC}_{c,i}}{2}\right) \quad (5)$$

It should be noted that the AIC is only a method for selecting among models, and does not give an absolute estimate of how well each of the models fits the data. To take this into account we also calculate the average residuals sum of squares (Supplementary Table S2). Another commonly used model-selection criterion is the Bayesian information criterion (BIC) (Burnham 2004):

$$\text{BIC} = k \ln(n) - 2 \ln(L).$$

Use of either of these two model-selection criteria led to the same conclusions. Because, as previously observed (Kass and Raftery 1995), the BIC penalizes the more complex models more severely, it favored the pure diffusion model slightly more than did the AIC, as is apparent from the likelihood ratios in Supplementary Table S2.

Results

The spatiotemporal dynamics of proteins are often well described by reaction–diffusion (R–D) models. By fitting solutions of R–D models to fluorescent recovery curves one can

determine whether there are distinct rate-limiting steps in the dynamics of interest. One can also distinguish whether the rate is limited by diffusion of the protein or by binding–unbinding reactions.

Diffusion and reaction models are simpler cases of the full R–D model in which diffusion (or reaction) is set to be rate-limiting. If binding–unbinding reactions are much faster than diffusion, diffusion is the rate-limiting step. Thus, the diffusion model will fit the data better than the reaction model, and nearly as well as the full R–D model. If, on the other hand, binding–unbinding reactions are much slower than diffusion, the reaction is a rate-limiting step and only a fit of the reaction model will be comparable with that of the full R–D model. To establish the nature of the rate-limiting step we compared the quality of the fits for the three different mathematical models: a full R–D model, a diffusion model, and a reaction model. Because these models have different numbers of variables, we selected the “preferred model” by use of the Akaike Information criterion (AIC). The AIC is based on an information-theoretical approach and reflects the relative quality of the fit by rewarding for goodness of the fit and penalizing for an increasing number of variables (von Kobbe 2002; Burnham and Anderson 2002; Sprague et al. 2004; Spiess and Neumeyer 2010). Note that the AIC is used to determine the preferred model, rather than simply the best fit, and does not give an absolute measure of how well each model fits the data.

Each of the two simpler models provides additional information about the spatio-temporal dynamics:

Diffusion model

When the data can be reliably fitted with a diffusion model we can estimate the “effective” diffusion coefficient that comprises the limiting factor. This effective diffusion coefficient,

$$D_{\text{eff}} = \frac{D_{\text{theoretical}}}{1 + \frac{k_{\text{on}}^*}{k_{\text{off}}}}$$

(discussed in the “Materials and methods” section), will be lower than the theoretical free diffusion coefficient we can calculate from the size and shape of the protein, because diffusion is impeded by binding and unbinding (even while these are not rate-limiting). The two variables describing these events are a pseudo binding rate (k_{on}^*), which is the binding rate multiplied by the concentration of binding sites, and an unbinding rate (k_{off}). The ratio of these rates enables us to estimate the fraction of bound proteins at a given time (Eq. 3).

Reaction model

When the reaction model fits the data the limiting factor is the unbinding rate (k_{off}), which can be calculated directly from the fit.

The variables obtained from fitting can be used to differentiate between “DNA scanning” and free diffusion. The latter only occurs when there is no binding, or the binding is

negligibly weak ($\frac{k_{\text{on}}^*}{k_{\text{off}}} \ll 1$), and the data are well described by a diffusion model. Conversely, data described by use of the diffusion model but with non-negligible binding, or data best described by the reaction or R–D model, support the “DNA scanning” mechanism.

When diffusing in the nucleoplasm WRN and BLM undergo fast binding–unbinding cycles, with approximately 90 % of the proteins bound to chromatin at any time.

Our analysis of WRN helicase mobility in the nucleoplasm is illustrated in Fig. 1. We bleach a spot indicated by the arrow in Fig. 1a. To simulate FRAP recovery curves in the three models we first identify the initial bleaching profile $I_0(r)$ (Eqs. 1, 2 in the section “Materials and methods”). $I_0(r)$ is found by fitting the modified Gaussian profile in Eq. 1 to the intensity loss as a function of the distance from the center of the bleached region, as shown in Fig. 1b. In all cases the data are in good agreement with the fit, which thus describes our bleached volume.

The Akaike model selection criteria showed that the diffusion model was the superior model (i.e. model with the smallest AIC_c; Supplementary Table S2). We use the AIC probability, p (Eq. 5), to quantify how significantly better the preferred model describes the data. From the AIC probabilities reported in Table S2 it is apparent the reaction model is much inferior: the probability the reaction model describes the data as efficiently as the diffusion model is $p = 4.1 \times 10^{-4}$. The diffusion model is also superior to the full R–D model, because the probability that R–D models describe the data equally efficiently is only $p = 0.08$.

From the fits in Fig. 1c we found that the experimentally measured WRN diffusion is a factor of eight slower (diffusion coefficient $D_{\text{WRN-eff}} = 1.62 \mu\text{m}^2/\text{s}$) than the theoretical diffusion ($D_{\text{WRN,theoretical}} = 12.2 \mu\text{m}^2/\text{s}$). Sprague et al. (2004), Mueller et al. (2008), and Spiess and Neumeyer (2010) showed that such cases can occur when proteins engage in rapid binding–unbinding reactions with chromatin and can be thought of as “effective

diffusion” described by the coefficient $D_{\text{eff}} = \frac{D_{\text{theoretical}}}{1 + \frac{k_{\text{on}}^*}{k_{\text{off}}}}$. Furthermore, under these conditions we can calculate (Eq. 3) how much of the protein is bound to the chromatin and how much is diffusing freely. Remarkably, we find that only approximately 10 % of WRN and BLM is in the free unbound form. This means that, although binding and unbinding occurs very rapidly, at any moment in time the vast majority of WRN (~90 %) is in the bound state. It is important to note that although WRN is known to form several complexes, the factor of eight decrease in diffusion speed cannot be explained by a complex’s higher molecular weight, because it would imply a complex of 1,000 proteins. (Complex size is

based on Eq. (4): $M_{\text{complex}} = \left(\frac{D_{\text{complex}}}{D_{\text{WRN,theoretical}}} \right)^3 M_{\text{GFP-WRN}} = 10^3 M_{\text{GFP-WRN}}$)

The results for BLM (Fig. S1) are equivalent; the effective diffusion coefficient is $D_{\text{BLM-eff}} = 1.34 \mu\text{m}^2/\text{s}$.

Effective diffusion in nucleoli is a factor of 13 slower than in the nucleoplasm

Nucleoli are sub-compartments of the nucleus wherein ribosomal RNA (rRNA) is transcribed. In Fig. 2a we show that the fusion protein EGFP-WRN is located in nucleoli. This is unlikely to result from overexpression, because immunostaining of the endogenous protein leads to similar results (Fig. S4). Furthermore, it has been reported elsewhere that under non-stressed conditions WRN and BLM are distributed unequally throughout the

nucleus, tending to be predominantly located in the nucleoli (Marciniak et al. 1998; Yankiwski et al. 2000; Sprague et al. 2004; Huranová et al. 2010). Interestingly, the nucleolar location of both BLM and WRN depends on ongoing rRNA transcription, and both bind to RNA polymerase I (Pol I) and facilitate Pol I-mediated rRNA transcription (Marciniak et al. 1998; Yankiwski et al. 2000; Shiratori et al. 2002; Braga and McNally 2007; Grierson et al. 2012). It is thus interesting to investigate the nature of the nucleolar reactions of WRN and BLM, i.e. whether helicases engage in a few slow reactions (e.g. reaction-limited behavior) or, as in the nucleoplasm, undergo many rapid binding–unbinding cycles (so that their mobility in nucleoli is diffusion-limited).

As shown in Fig. 2c and by the AIC_c (Table S2), the diffusion model is also the preferred model in nucleoli (with AIC probabilities $p = 0.07$ and $p = 3 \times 10^{-5}$, respectively for the R–D and reaction models). We find that although mobility is lower than in nucleoplasm, the processes are still limited by diffusion and not by reactions. The effective diffusion ($D_{\text{WRN-eff}} = 12.2 \mu\text{m}^2/\text{s}$) is a factor of 100 slower than the theoretically estimated value, which suggests that only 1 % of the WRN is in a free diffusing form at any time. The results are similar for BLM (Supplementary Table S3 and Fig. S2).

DNA repair proteins fall into two groups: scanners and responders

To put our results into a wider perspective, we compared them with previously reported diffusion constants for other DNA repair proteins (Table S1; Fig. 3). We used the ratio of theoretical to measured effective diffusion constants to estimate the degree of interaction with DNA (chromatin). We define two regions (Fig. 3):

1. $\frac{D_{\text{effective}}}{D_{\text{theoretical}}} \lesssim 0.2$ (shaded red in Fig. 3). In this region it is likely the slowdown results from interaction with chromatin and/or other static species. Complex formation cannot explain this region, because slowdown by a factor of five would require at least 100 proteins in a complex.
2. $\frac{D_{\text{effective}}}{D_{\text{theoretical}}} \gtrsim 0.4$ (shaded blue in Fig. 3). Here the slowdown could feasibly result from complex formation (~15 proteins) or imperfect assumptions for the theoretical calculation.

Figure 3 is indicative of a wide range of diffusion dynamics for nuclear DNA repair proteins. Whereas some diffuse almost freely, others seem to be engaged in chromatin scanning to different degrees. Interestingly, most DNA repair proteins can be classified into two major categories:

1. WRN, BLM, NSB1, MDC1, and Ku-proteins are “scanners” (Shiratori et al. 2002; Compton et al. 2008; Van Royen et al. 2011; Grierson et al. 2012) with low effective diffusion coefficients because of constant scanning of DNA for damage; and
2. PCNA and RAD 51, 52, and 54 are “responders” (Houtsmuller et al. 1999; Srivastava et al. 2009; Van Royen et al. 2011) which only bind to DNA after a DNA damage response has been activated.

This division might be useful for characterizing repair proteins when effective diffusion coefficients can be calculated.

Conclusion

Our experimental data and theoretical analysis revealed several interesting aspects of the dynamics of WRN and BLM. In both the nucleoplasm and nucleoli, an “effective” diffusion process describes both WRN and BLM. They undergo rapid cycles of binding and unbinding, with only a low percentage of the proteins diffusing freely at any time. This enables continuous “scanning” of the genome for possible damage sites. We measured their effective diffusion coefficients in the nucleoplasm to be $D_{\text{WRN-eff}} = 1.62 \mu\text{m}^2/\text{s}$, and $D_{\text{BLM-eff}} = 1.34 \mu\text{m}^2/\text{s}$. In the nucleoli we measured the diffusion coefficients to be $D_{\text{WRN-eff}} = 0.12 \mu\text{m}^2/\text{s}$ and $D_{\text{BLM-eff}} = 0.13 \mu\text{m}^2/\text{s}$. We also found that DNA repair proteins fall into either the scanner or response category.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank members of Center for Models of Life and the Bohr Laboratory for discussions. AT is supported by a Steno fellowship granted by the Danish Council for Independent research (DFF), KMB is supported by CMOL through the Danish National Research Foundation (DG), and MBJ is supported by the Center for Healthy Aging and an Elite Research Scholarship from the Danish Ministry of Science. This work was supported by funds from the intramural program of the National Institute of Aging, National Institutes of Health, USA.

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Fig. 1.

In the nucleoplasm the dynamics of WRN are governed by effective diffusion with $D_{\text{eff}} = 1.62 \mu\text{m}^2/\text{s}$. **a** In cells expressing EGFP-WRN, a $2 \mu\text{m}$ spot is bleached in the nucleoplasm, as indicated by the *orange arrow*. **b** To identify the shape of the initial loss of intensity, $I_0(r)$, a Gaussian profile (*blue line*) is fitted to measurements of the initial intensity at a given radius from the center of the bleached region (*circles*). **c** Fluorescence recovery curves (*circles*) and the corresponding best fits obtained by using: reaction (*solid green line*), diffusion (*dashed blue line*), and reaction–diffusion (*solid black line*) models. The *inset* is a magnification for the first five seconds. *Data points* show averages for five cells; *error bars* represent standard deviations

Fig. 2.

In the nucleoli the dynamics of WRN are governed by effective diffusion, with $D_{\text{eff}} = 0.12 \mu\text{m}^2/\text{s}$. **a** In cells expressing EGFP-WRN, a $1 \mu\text{m}$ spot is bleached in the nucleoli, as indicated by the *orange arrow*. **b** To identify the shape of the initial loss of intensity, $I_0(r)$, a Gaussian profile (*blue line*) is fitted to measurements of the initial intensity at a given radius from the center of the bleached region (*circles*). **c** Fluorescence recovery curves (*circles*) and the corresponding best fits using the reaction (*solid green line*), diffusion (*dashed blue line*), and reaction–diffusion (*solid black line*) models. The *inset* is a magnification of the first five seconds. *Data points* show averages for five cells; *error bars* represent standard deviations

Fig. 3.

DNA repair proteins can be classified into two major categories: scanners and responders. Plotted are the theoretical, $D_{\text{theoretical}}$, and measured effective, $D_{\text{effective}}$, diffusion coefficients for DNA repair proteins (for exact values and references are given in Table S1). When proteins do not bind to chromatin or other static cellular components, the ratio of the theoretical diffusion coefficient based on mass and shape ($D_{\text{theoretical}}$) to the effective diffusion coefficient determined experimentally ($D_{\text{effective}}$) approaches 1