

Results & Material and methods

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1 Result section

To estimate the nucleosome reorganization following DNA damages, we have constructed a mathematical model, where redistribution can be due either to chromatin de-compaction or nucleosome sliding along the chromatin or both of them. We have used the model to assess the relative contribution of these two processes to the total DNA and nucleosome signal loss from a region of interest (ROI), a quantity which is inaccessible experimentally.

The model (presented in Material and methods) follows the DNA, $D(u)$, and nucleosome, $H(u)$, fraction of signal loss from the ROI as a function of the UV dose, u . We have used the measured H3.3 and DNA signal loss to calibrate parameters of nucleosome and DNA models respectively. Using the calibrated models we have found that the relative contribution of nucleosome sliding to the total signal loss in the ROI is monotonically decreasing from 62% to 54% for nucleosomes and 27% to 20% for DNA loss, as the UV dose increases from 0 to 100 msec. The remaining percentages are attributed to chromatin expansion and de-compaction (see Figure 1).

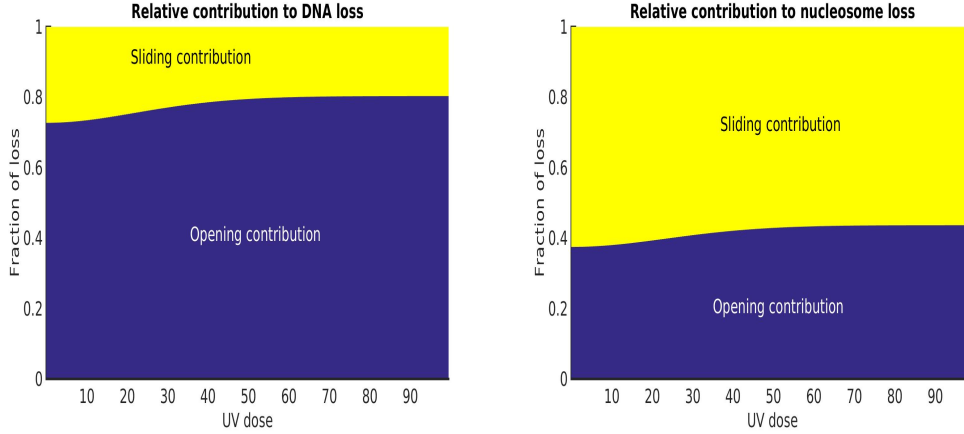


Figure 1: **Relative contribution of chromatin opening and nucleosome sliding to DNA (left) and histone (right) loss.** Sliding contribution is monotonically decreasing with UV dose for both DNA and nucleosome signals. The increase in chromatin opening contribution is due to the increase in chromatin reorganization following high dosages of UV, which is responsible for the majority of signal loss.

2 Material and methods

We present here a model for nucleosomes and DNA re-organization following UV damages. The cascade of events leading to tagged DNA and nucleosomes' redistribution, results in signal extrusion from a region of interest (ROI) up to a maximal loss, measured 15 minutes post UV-C.

2.1 The experimental procedure

Following the experimental protocol (see Figure NNN), a two-dimensional circular initial damage region (DR), induced by the UV-C laser, is centered around the focal point (origin of the coordinates). Following laser induction with a UV exposure u [msec], the damaged region expands radially outward and reaches its maximal area after 15 minutes. At the end of expansion, the circular region is defined as the ROI, in which DNA and nucleosome signals are measured and compared to signals measured at the ROI just prior to UV induction. The fraction of signal loss is calculated as the difference between the two measurements relative to time 0.

2.2 Modeling nucleosomes and DNA redistribution following UV damages

We assume that the loss of DNA and nucleosome signals post UV-C is due to two mechanisms: the first is chromatin expansion, and the second is nucleosomes sliding along the chromatin. For chromatin expansion, recruitment of repair and chromatin remodeling factors to bind to damaged DNA causes chromatin de-compaction and cross-links break [3] to facilitate efficient repair [2]. Accumulation of repair factors in the DR will generate a pushing force surrounding chromatin in a radial outward direction [1]. As a result, DNA and nucleosome outside the DR will be extruded from the ROI in equal proportions.

An additional proportion of nucleosome signal loss is caused by the mechanism of nucleosome sliding. Repair proteins slide nucleosomes wrapped by damaged DNA away from high concentration of DNA damages (see Figure NNN) to expose damaged DNA [2]. As a result the chromatin in the DR will loosen and remodeling and repair proteins will bind to the exposed damaged sites. This further contribute to the pushing of undamaged DNA outside the DR while retaining damaged DNA within it.

In-line with the description above, we construct a model representing signal loss 15 minutes post UV-C. We do not take into account the mechanism of signal loss in time and only present equations representing the maximal loss of signal 15 minutes post UV-C.

2.3 Dynamics of DNA and nucleosome loss from the DR

The DR and the ROI are considered to be two-dimensional concentric circular regions, characterized by an area A_0 and $A(u)$, respectively. We assume an initial uniform distribution of DNA in the DR and its vicinity, such that the amount of DNA in $A(u)$ is $c_d A(u)$, with c_d in units of $bp/\mu m^2$.

We restrict DNA damages to be inflicted only in A_0 immediately after UV induction. We set $T(u)$ to represent the amount of damaged DNA left in $A(u)$ 15 minutes post UV-C, while the undamaged DNA is assumed to be extruded. Therefore, the fraction of DNA signal loss, $D(u)$, is calculated as

the ratio of extruded DNA to the total amount of DNA in the ROI, $c_d A(u)$.

$$D(u) = \frac{c_d A(u) - T(u)}{c_d A(u)} \quad (1)$$

We set the number of nucleosomes in a $A(u)$ to be $c_n A(u)$, with c_n a constant in units of *nucleosomes*/ μm^2 . The total fraction of nucleosome extruded from the ROI, $H(u)$, is calculated as the sum of nucleosomes pushed with undamaged DNA and the ones sliding out.

$$H(u) = D(u) + \frac{N_S(u)}{c_n A(u)} \quad (2)$$

In order to evaluate the fractions in (1) and (2), we shall first formulate a model for the damaged DNA in the DR, $T(u)$ and derive $A(u)$ and $N_S(u)$ based on it.

2.4 DNA damages in the DR as a function of the UV dose

We assume a uniform illumination of the UV beam in A_0 such that the average number of damages per μm^2 is constant for a fixed u . As the uv exposure time increases, we assume that the average number of damages per μm^2 increases as $k_t u$, with k_t in units of *bp*/*(msec* \times μm^2). Because damages cannot occur on the same position on the DNA, the rate of accumulation of DNA damages with UV dose can be written as

$$\frac{dT(u)}{du} = \lambda(u) A_0 (c_d A_0 - T(u)) \quad (3)$$

Using the initial condition $T(0) = 0$, the solution to equation (3) is

$$T(u) = c_d A_0 (1 - \exp(-k_t u^2)) \quad (4)$$

2.5 The number of nucleosomes sliding out of the DR

We now turn to construct a model for the number of nucleosomes $N_S(u)$ sliding out of the DR and subsequently out of the ROI as a function of the UV dose. Although the exact mechanism by which nucleosome are lost is not known, we propose that the rate of nucleosomes sliding out is proportional

to the fraction of nucleosomes affected by increase of UV dose in A_0 . In the first-order approximation, the dynamics of nucleosomes sliding is given by

$$\frac{dN_S(u)}{du} = k_s (c_n A_0 - N_S(u)) \frac{dT(u)}{du} \quad (5)$$

with k_s a constant of units $1/bp$. Using the initial condition $N_S(0) = 0$, the solution to equation (5) is

$$N_S(u) = c_n A_0 (1 - \exp(-k_s T(u))) \quad (6)$$

with $T(u)$ defined in equation (4).

2.6 A model for ROI expansion

Next, we model the dynamics of the ROI area $A(u)$ with increasing UV dose. For this end, we consider the ROI area to expand as a result of the de-compaction of the damaged DNA, and by the effect of nucleosome sliding.

$$\frac{dA(u)}{du} = k_a \frac{dN_S(u)}{du} + k_b \frac{dT(u)}{du} \quad (7)$$

where k_a is a constant of units $\mu m/nucleosomes$ and k_b of units $\mu m/bp$. Using the initial condition $A(0) = A_0$, the solution to equation (7) is

$$A(u) = A_0 + k_a c_n A_0 (1 - \exp(-k_s T(u))) + k_b T(u) \quad (8)$$

with A_0 representing the size of the DR even in the absence of UV irradiation.

2.7 Final expression for $D(u)$ and $H(u)$

We can now substitute the functions (4), (6), (8) into the equations (1) and (2) to get the expressions for $D(u)$ and $H(u)$. We present the result in terms of $T(u)$

$$D(u) = 1 - \frac{1}{1 + k_a c_n (1 - \exp(-k_s T(u))) + k_b T(u)/A_0} \quad (9)$$

$$H(u) = 1 - \frac{\exp(-k_n T(u))}{1 + k_a c_n (1 - \exp(-k_s T(u))) + k_b T(u)/A_0} \quad (10)$$

2.8 Parameter fit for $D(u)$ and $H(u)$

We now fit equations (9) and (10) to the experimental data. We simultaneously fit equations (9) and (10) to the H3.3 and DNA loss data, with the goal of maximizing the R^2 of both equations. Excluding the measurement at 5 msec, and using classical fitting procedure, we find

$$k_t A_0 = 8 \times 10^{-4}, \quad k_s c_d A_0 = 0.31, \quad k_a c_n = 0.29, \quad k_b c_d = 0.24$$

with $R^2 = 0.94$ and $R^2 = 0.955$ for DNA and nucleosome loss fit respectively.

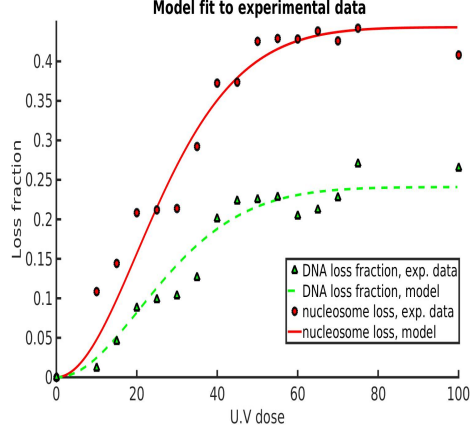


Figure 2: **Histone (red) and DNA (green) loss: experimental data for nucleosomes (circle) and DNA (triangles) versus model curves (continuous and dashed, respectively).** Parameter values are obtained by simultaneously fitting equations(9) and (10) to the experimental data with the goal of maximizing the R^2 . The resulting curves show $R^2 = 0.94$ and $R^2 = 0.955$ for DNA and nucleosome loss, respectively.

2.9 Relative contribution of opening and sliding to DNA and nucleosome signal loss

Using the calibrated model in equation equations (9) and (10), we now calculate the relative contribution of chromatin opening and nucleosome sliding to the total loss of DNA and nucleosomes. The sliding contribution refers to all loss caused by either directly sliding nucleosome out of the DR or as the effect nucleosome sliding has on pushing chromatin out of the DR.

Chromatin opening contribution refers to all signal loss caused by chromatin remodeling.

We start by partitioning equation (8), describing the ROI into the two sub-mechanisms contributing to its expansion

$$A(u) = A_P(u) + A_S(u)$$

with $A_P(u) = A_0 + k_b T(u)$, the area attributed to chromatin opening, and $A_S(u) = k_a N_S(u)$ to nucleosome sliding.

The fraction of DNA signal loss attributed to chromatin opening is calculated as the ratio

$$D_P(u) = \frac{c_d A_P(u) - c_d A_0}{c_d A(u)}$$

The relative contribution of chromatin opening to the total DNA signal loss is therefore,

$$\frac{D_P(u)}{D(u)} = \frac{A_P(u)/A_0 - 1}{A(u)/A_0 - 1} \quad (11)$$

The relative contribution of nucleosome sliding to the total DNA signal loss is

$$\frac{D_S(u)}{D(u)} = 1 - \frac{D_P(u)}{D(u)} \quad (12)$$

The relative contribution of chromatin opening and nucleosome sliding to the total nucleosome signal loss is given respectively by

$$\frac{H_P(u)}{H(u)} = \frac{D_P(u)}{H(u)}, \quad \frac{H_S(u)}{H(u)} = 1 - \frac{H_P(u)}{H(u)} \quad (13)$$

where here we have used the fact that $H_P(u) = D_P(u)$, i.e fraction of nucleosomes pushed with undamaged DNA out of the ROI. Graphs of equations (11) and (13) are presented in Figure 1.

References

- [1] Christoffel Dinant, Martijn de Jager, Jeroen Essers, Wiggert A van Cappellen, Roland Kanaar, Adriaan B Houtsmuller, and Wim Vermeulen. Activation of multiple dna repair pathways by sub-nuclear damage induction methods. *Journal of cell science*, 120(15):2731–2740, 2007.

- [2] Hélène Gaillard, Daniel J Fitzgerald, Corey L Smith, Craig L Peterson, Timothy J Richmond, and Fritz Thoma. Chromatin remodeling activities act on uv-damaged nucleosomes and modulate dna damage accessibility to photolyase. *Journal of Biological Chemistry*, 278(20):17655–17663, 2003.
- [3] Martijn S Luijsterburg, Michael Lindh, Klara Acs, Mischa G Vrouwe, Alex Pines, Haico van Attikum, Leon H Mullenders, and Nico P Dantuma. Ddb2 promotes chromatin decondensation at uv-induced dna damage. *The Journal of cell biology*, 197(2):267–281, 2012.