Results & Material and methods

Ofir Shukron & David Holcman

January 5, 2016

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 (Fig. 3A-D). 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 75% to 70% for nucleosomes and 51% to 40% for DNA loss, as the UV dose increases from 5 to 100 msec. The remaining percentages are attributed to chromatin expansion and de-compaction (see Figure 1).

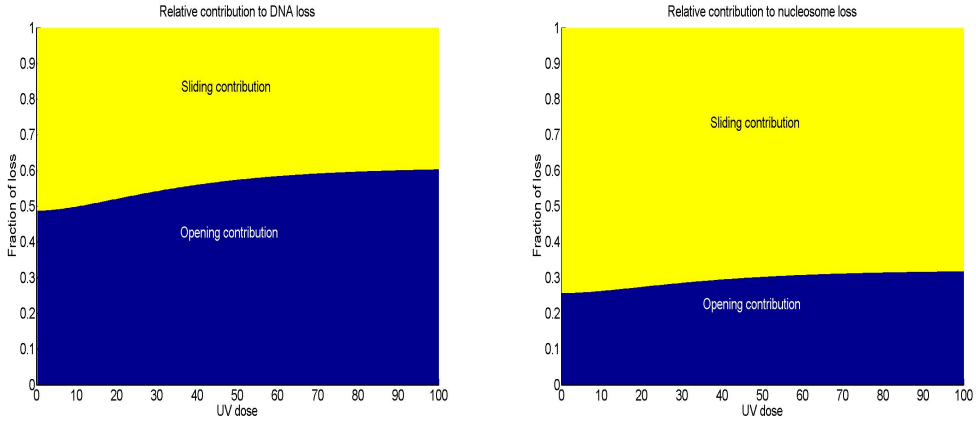

Figure 1: **Relative contribution of chromatin opening and nucleosome sliding to DNA (left) and histone (right) loss**. Sliding contribution is monotonically decreasing with UV dose for both DNA and nucleosome signals. The increase in chromatin opening contribution is due to the increase in chromatin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

2 Material and methods: Modeling nucleosomes and DNA redistribution following UV damages

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 Dynamics of nucleosomes following UV damages in the region of interest

Following the experimental protocol, a two-dimensional circular initial damage region (IDR), induced by the UV-C laser, is centered around the focal point (origin of the coordinates) with a fixed area of A_0. Following laser induction with a UV dose u $[msec]$, the damaged region expands radially

outward and reaches its maximal area of $A(u)$ after 15 minutes. At the end of expansion, the circular region is defined as the ROI, in which DNA and nucleosome signals are measured at time 0 and 15 minutes. The fraction of signal loss is calculated for DNA and nucleosomes as

$$\frac{signal(0) - signal(15)}{signal(0)}$$

where $signal(0)$ and $signal(15)$ are the DNA or nucleosome signals at time 0 and 15 minutes respectively.

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 enable repair factor access to damages [2]. Because the majority of damages are inflicted around the laser's focal point, the accumulation of repair factors there [1] will generate a pushing force on chromatin outside the DR in a radial outward direction. 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 facilitate efficient repair [2]. Sliding nucleosomes out of the DR loosens the chromatin in the DR and exposes damaged DNA. Remodeling and repair proteins, binding to the exposed damaged position, 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 specifically take into account the mechanism of signal loss in time and only present equations representing the maximal loss of signal as a function of the UV dose.

2.2 Dynamics of DNA and nucleosome loss from the DR

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

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

Similarly to the DNA, we assume that the number of nucleosome in a $A(u)$ is $c_n A(u)$, with c_n a constant in units of $nucleosomes/\mu m^2$. 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.

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

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.

2.3 DNA damages in the IDR as a function of the UV dose

We restrict all DNA damages to lie entirely in A_0. We assume that the probability of a bp to be damaged is an increasing function of the UV exposure time. We further assume that the UV illumination is uniform in A_0. Thus, the rate of accumulation of DNA damages in the A_0 with increasing UV dose is proportional to the undamaged DNA in A_0. Therefore,

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

with k_t a constant. Using the initial condition $T(0) = 0$, the solution to equation (3) is

$$T(u) = c_d A_0(1 - \exp(-k_t u)) \tag{4}$$

2.4 Deriving the functions describing loss of nucleosome from the IDR and the ROI expansion

We now turn to construct a model for the number of nucleosomes $N(u)$ extruded from 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 will describe the loss by that contribution of chromatin expansion and nucleosome sliding out of the DR.

We propose that the rate of nucleosomes leaving the DR by sliding 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, $N_S(u)$, is given by

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

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

$$N_S(u) = c_n A_0 \left(1 - \exp\left(-k_s T(u)\right)\right) \tag{6}$$

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

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

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

$$A(u) = k_a c_n A_0 \left(1 - \exp\left(-k_s T(u)\right)\right) + k_b c_d A_0 \left(1 - \exp\left(-k_t u\right)\right) \tag{8}$$

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 functions in terms of $T(u)$

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

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

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

We now use equations (9) and (10) to fit 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

$$k_t = 0.037, \quad k_s c_d A_0 = 0.35, \quad k_a c_n = 0.24, \quad k_b c_d = 0.46$$

with $R^2 = 0.93$ and $R^2 = 0.96$ for DNA and nucleosome loss fit respectively.

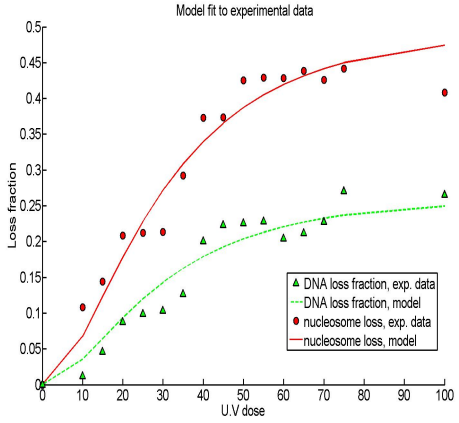

Figure 2: **Histone (red) and DNA (green) loss: experimental data for nucleosomes (circle) and DNA (triangles) versus model curves (continuous and dashed, respectively)**. Parameter values are obtained by simultaneously fitting equations(9) and (10) to the experimental data with the goal of maximizing the R^2. The resulting curves show $R^2 = 0.96$ and $R^2 = 0.93$ for nucleosome and DNA loss, respectively.

2.6 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 by

operations of repair factors. Chromatin opening contribution refers to all signal loss caused by chromatin remodeling, which causes expansion of the DR and subsequent loss of signal from the ROI.

We start by dividing the equation describing the ROI expansion into the two sub-mechanisms of signal loss

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

with $A_P(u)$ the area attributed to chromatin opening, and $A_S(u)$ to nucleosome sliding. Subtracting the sliding contribution from the ROI expansion in equation (**??**), we arrive at

$$A_P(u) = A_0 + k_a N_P(u)$$

The DNA loss attributed to chromatin opening and de-compaction is thus

$$D(u)_{opening} = \frac{A_P(u)/A_0 - 1 + D_P(u)/A_0}{A_P(u)/A_0}$$

The fraction attributed to chromatin opening and sliding out of the total DNA loss are given respectively by

$$\frac{D(u)_{opening}}{D(u)}, \quad \frac{D(u)_{sliding}}{D(u)} = 1 - \frac{D(u)_{opening}}{D(u)} \tag{11}$$

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

$$\frac{H(u)_{opening}}{H(u)} = \frac{D(u)_{opening}}{H(u)}, \quad \frac{H(u)_{sliding}}{H(u)} = 1 - \frac{H(u)_{opening}}{H(u)} \tag{12}$$

where here we have used the fact that $H(u)_{opening} = D(u)_{opening}$. Graphs of equations (11) and (12) are presented in Figure 1.

2.7 Nucleosome sliding out of the IDR

The fraction of nucleosomes lost by sliding out of the total nucleosomes in the IDR (and eventually pushed out of the ROI) is found to be an increasing function of the UV dose

$$N_S(u) = \frac{H(u) - D(u)}{1 - D(u)}$$

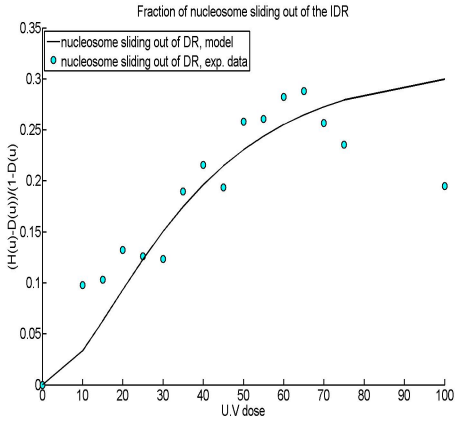

Figure 3: **Fraction of nucleosomes sliding out of the IDR. The plot against the experimental data resulted in** $R^2 = 0.75$

Substituting the parameter values in subsection 2.5 and plugging (9) and (10) into the expression above, we obtain the results in Figure 3, where the model in equation (**??**) is plotted against the experimental data ($R^2 = 0.75$).

The parameters values found in subsection 2.5, can further be used to estimate the fraction of nucleosome loss from the IDR attributed to sliding and chromatin opening. These contributions are given by the leading coefficients in the equations (**??**) and (**??**) for $N_P(u)$ and $N_S(u)$, respectively. We have, for chromatin opening and sliding, respectively

$$N_P(u) = 0.3N_T(u), \quad N_S(u) = 0.7N_T(u)$$

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.