Chromatin Architecture Post UVC damage- Summary of Results

Ofir Shukron, David Holcman

September 15, 2015

Main findings are summarized in Section 0.1. Short explanations regarding each result are provided in Section 0.2.

0.1 Main Findings

- We have produced a conceptual 1D model in which sliding and pushing causes loss in different proportions in a ROI (Subsection 0.2.1 Figures 1, 2);
- We have found a formula for calculating the contribution of sliding to the radius of expansion, (Subsection 0.2.2 ,equation **??**);
- We have found a formula to calculate the ratio of chromatin length to the ROI radius, (Subsection 0.2.3 equation 2);
- We have presented a method to estimate the contribution of sliding to the expansion of the damage zone based on the analysis of the patch data and preservation of signal in the ROI (equation 1);
- We find that histone sliding is accounted to between 0.38 to 0.46 μm of expansion;
- We find that the ratio of DNA length in the damage zone to the ROI radius is between 3 to 5 (Figure **??**).
- Expansion and loss of 20% DNA was successfully simulated with a polymer having 80% additional linkers, and radius of exclusion around damaged monomers of 0.6 (Section 0.2.5).
- Measure of similarity between the polymer's organization before UV and at the end of repair- not fully simulated;
- histone and DNA loss dose dependency- not fully simulated.

0.2 Results explained

A short derivation accompanied by figures of the main results are presented in this section

0.2.1 Histone sliding model

The imbalance between histone and DNA loss is explained by a 1D histone sliding model. Histones and DNA are pushed out of the ROI by two sub-mechanisms, the first if repair mechanism crowding, and the second is histone sliding. We note that crowding is continuously active throughout histone sliding, but not conversely.

We find DNA loss fraction

$$d = \frac{\beta - L}{\beta}$$

Histone loss fraction

$$h = \frac{(\beta - L)(\beta + \alpha)}{\beta l}$$

with β the end radius of ROI, l the length of DNA in the damage zone, α the ratio of the length of a nucleosome to the DNA wrapped around a histone,

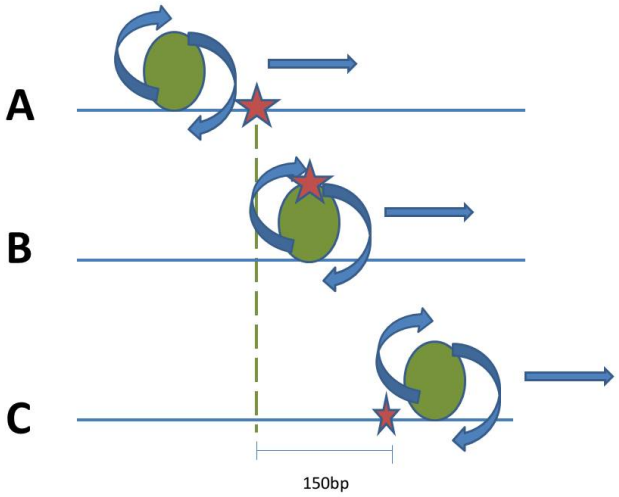

Figure 1: **Three time points during a displacement of damage site (red star) caused by histone sliding. The displacement of the damage site is equivalent to the length of DNA wrapped on a histone. A displacement is measured in aerial distance from a reference point, and does not refer to an actual motion of the point on the DNA**

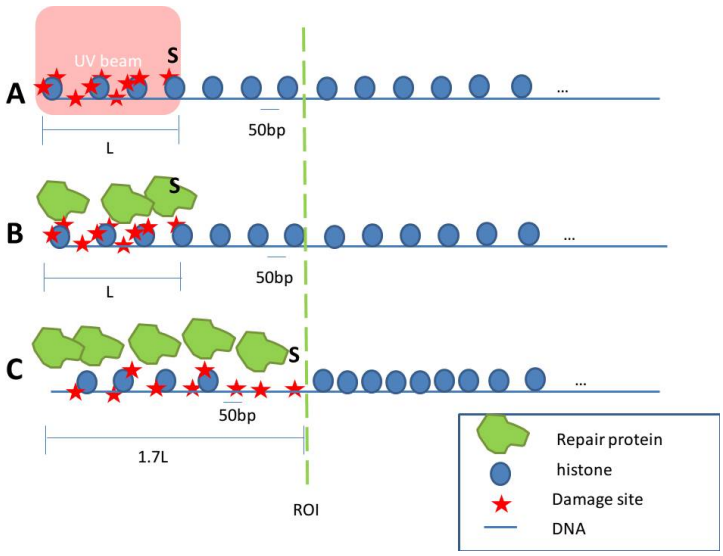

Figure 2: **Expansion of the ROI due to nucleusome sliding. A. A UV beam (transparent red) damages DNA, with the point S being the rightmost damage site B. Repair proteins (green polygons) are recruited to the damage site and expose the DNA in order the repair damages. C. Sliding the nucleosomes to the right in order to expose damage sites, translates the point S from S to β. Repair proteins follow the damage point to its new location. The presence of repair proteins in β mark the ROI's boundary (vertical dashed green line). All DNA and histones to the right of S are lost**

0.2.2 Expansion attributed to sliding and pushing sub-mechanisms

The relative contribution of each sub-mechanism to the total expansion is estimated by dividing the expansion process into two: pushing and then pushing+sliding. The composition and order of events will be neglected in this description.

We consider the chromatin to be coiled in the ROI before UVC. After UVC, repair mechanism push and straighten the chromatin to create expansion, marked by repair protein presence. If due to pushing up to a distance of $0 < L' < \beta$ we have lost a fraction of k of both histones and DNA. if the total chromatin length after pushing is $l + r$, in which l is the length of chromatin in the UVC region and r is the reminder.

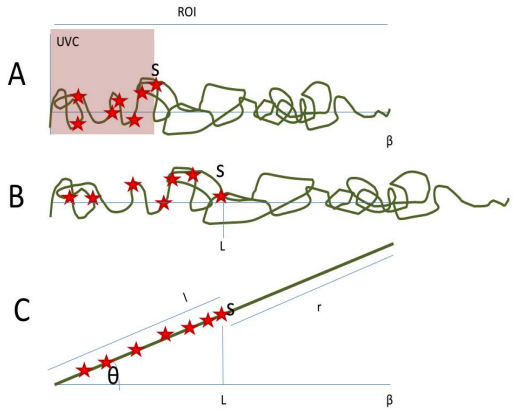

Figure 3: **1D representation of 2D chromatin arrangement for sliding. A. The chromatin is coiled and arranged in a ROI of length β. UVC damages a portion of the chromain, in which the right most damage point is marked s. B. due to crowding some of the chromatin is lost from the ROI, the rightmost damage point is pushed toward β at a distance L', C. the additional histone and DNA signal loss is attributed to sliding, in which the expansion is modeled as the projectio of the chromatin of length l onto the horozontal 1D axes.**

We have for the total fraction of DNA and histone loss

$$\begin{aligned} d &= k + \frac{\beta - L'}{\beta}(1-k) \\ h &= k + \frac{(\beta - L')(1+\alpha/\cos(\theta))}{\beta l}(1-k) = k + \frac{(\beta - L')(L' + \alpha l)}{\beta L'}(1-k) \end{aligned}$$

(see notation in Figure 3 and in previous subsection 0.2.1).

We extract k from both equations and equate them to have

$$\frac{L + \beta(h-1) + \alpha l}{L + \alpha(l-\beta)} = \frac{L + \beta(d-1)}{L}$$

which results in radius for which pushing is responsible

$$L' = (\beta - l)(1-d)$$

0.2.3 Estimation of the contribution of sliding to the expansion from the data

We have no direct access to the length of the chromatin expanded in the ROI. We estimate it indirectly from mass conservation considerations during

expansion of the illuminated patch and Assuming uniform density of histones before UVC.

Measurement of the expansion of the patch shows 25-30% increase in radius (Figure 4)

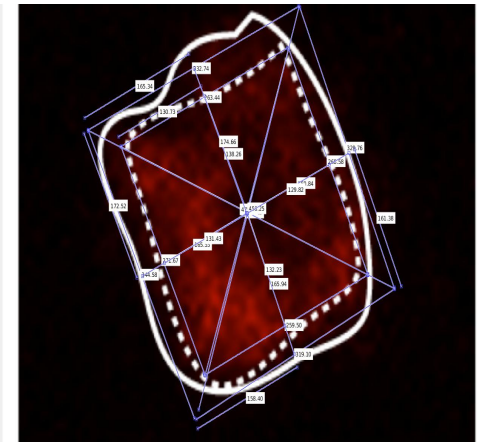

Figure 4: **measurement of patch expansion from time 0 just before UVC (dashed) to 15 minutes post UVC (full line) the patch grows by 25-30% of its initial radius, from 2.52 to the range [3.15, 3.28]**

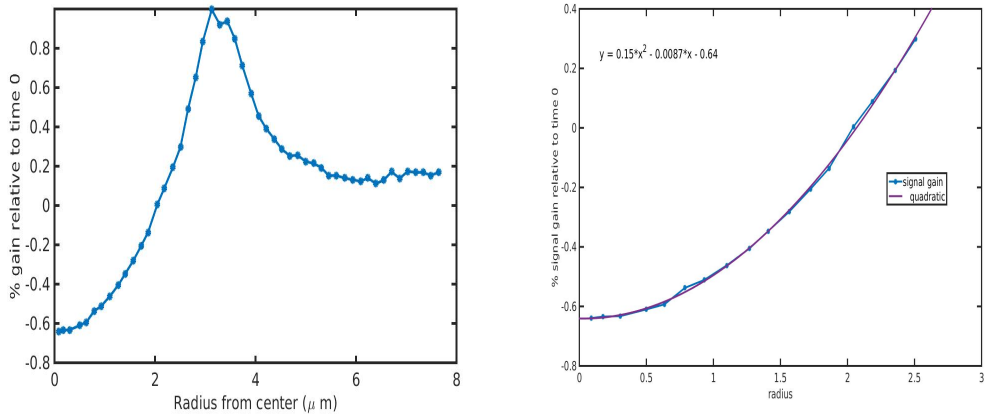

Figure 5: **The relative gain 15 minutes post UVC relative to 0 minutes (left). A fit of the gain function up to the boundary of the patch (right) by a quadratic polynomial**

Using

$$\frac{\int_0^R F(r)(y(r)+1)dr}{\int_0^R F(r)dr} = \gamma \tag{1}$$

with $F(r)$ the amount of histones in concentric ring between r and $r + dr$, $y(r)$ the quadratic fit to the gain function, and γ is the fraction of histones in a region of radius R. We find that the expansion radius attributed to sliding is in the range 0.34 to 0.46 μm.

0.2.4 Geometrical implications

Normally we do not have access to the value of l, the length of the chromatin in the damage zone. We can bypass this demand by experimentally measure the expansion attributed to sliding by the procedure described in subsection 0.2.2. The expansion radius, L, can then be used to solve for l/β in equation **??** to arrive at

$$\frac{l}{\beta} = \frac{L\pi(d-h)}{(\beta - L)(d-1)} \tag{2}$$

0.2.5 Simulation of expansion and repair

To examine the organization of the chromatin after repair, we perform 2D simulations of the damage process and expansion. Expansion of 1.7 is obtained for a Rouse polymer having 500 monomers, with 80% additional cross-links. After UVC, cross-links from damaged monomers are removed. We simulate crowding around damage zones by employing an harmonic potential around damaged monomers having a radius of exclusion between 0.6-0.65.

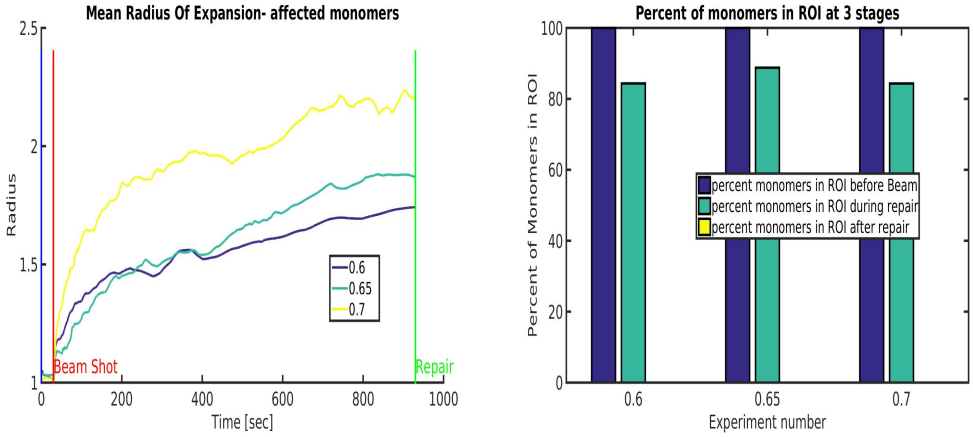
Mean Radius Of Expansion- affected monomers
Radius
Time [sec]
Beam Shot
Repair
0.6
0.65
0.7
Percent of monomers in ROI at 3 stages
Percent of Monomers in ROI
Experiment number
percent monomers in ROI before Beam
percent monomers in ROI during repair
percent monomers in ROI after repair

Figure 6: