

# Chromatin Architecture Post UVC damage

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## 0.1 Experimental Settings and Findings

1. Cell type used: U2OS, which are human osteosarcoma cells;
2. H3.3 histones are tagged 48 hours before experiments using the SNAP-tag method, tag color is red;
3. Repair factors XFP are labeled with GFP;
4. a region of  $20\mu m^2$  was photo-activated 8-10 hours before UVC;
5. UVC damage is induced in a region of the cell using a 266 nm laser ( $0.266\mu m$ );
6. Changed to the red fluorescence signal were measured in the entire volume of the cell, post UVC;
7. Images were acquired using confocal microscopy, with an auto-focus module on, to acquire images from the best focal plane;
8. Fluorescence intensity were normalized against values measured in undamaged nucleus;
9. Fluorescence loss at irradiated sites was determined by dividing the intensity in the illuminated area by the intensity of the entire nucleus after background subtraction;
10. Illuminated area was defined 15 minutes post UVC based on GFP labeled repair factors and was kept similar throughout measurements;
11. Fluorescent recovery was measured relative to previous illumination starting from the frame with the minimal fluorescent values;
12. 2D projection of the 3D images were obtained by *maximal intensity z projection*
13. For sensitivity, most of the cell H3.3 fluorescence was photo-bleached, aside from the region of UVC illumination;
14. 20% loss of H3.3 signal from the *entire nucleus* was detected after photo-bleaching the fluorescence patch;

15. However, using UVC in the fluorescent patch led to 40% loss of parental H3.3 signal, while no detectable loss was seen in the entire nucleus;
16. The depletion of fluorescence in the center of the damage area, 15 minutes post UVC, was accompanied by an increase of density at its boundary, balancing the loss;
17. 20% loss of DNA signal in the damage region, accompanied by an expansion of the region was observed 15 minutes postUVC;
18. The expansion of the damage region depends on the dose of repair-factor;
19. The early repair factor DDB2 recruits histone chaperons HIRA, which promotes the deposition of newly synthesized histones at UVC sites;
20. newly synthesized histones are detectable in the repair region only 45 minutes post UVC;
21. Histone chaperons do not participate in histone redistribution after UVC irradiation;

## 0.2 Model and Parameter Estimation

For reasons of convenience we will work in units of  $100nm$ . Parameter values calculated in the subsection below will be converted to this measure when simulated.

### 0.2.1 Nucleus Size

Cells' cross-section are  $240 \mu m^2$  in the  $x - y$  plane and  $11\mu m$  in height, giving an average radius of  $r_c = 7.25\mu m$ . 1), The red fluorescence represent the histones.

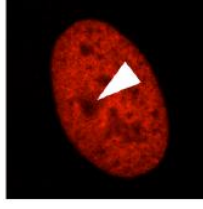


Figure 1: white triangle represents the UVC damage site, Histones are marked with red

### 0.2.2 The UV beam

UV beam has  $3 \mu m^2$  section, yielding a radius of  $r_{uv} = \sqrt{\frac{3}{\pi}} \approx 1 \mu m$ .

### 0.2.3 Histone Density

We consider histones to be uniformly distributed in the nucleus. There are  $3 \times 10^6$  histones marked, which makes their density  $3 \times 10^6 / (4\pi 7.25^3) \approx 630$  histones/ $\mu m^3$ . The expected number of histones in the UV beam region is  $14.5\pi \times 630 \approx 30,000$  histones, assuming the beam is shot through the center of the sphere.

### 0.2.4 DNA density

to be determined

### 0.2.5 Distribution of damage sites

to be determined

### 0.2.6 Defining the region of interest

We model the experiment in a region around the damage zone rather than the entire nucleus. We define a region of interest (ROI) in terms of radii of the UVC beam radius. The area expanded after UVC occupies  $10 \mu m^2$  which gives 3 times the UVC beam radius. We therefore set the radius of the ROI  $r_{roi} = 3 \mu m$ .

### 0.2.7 The chromatin

The chromatin is modeled as a Rouse polymer of  $N$  monomers connected by harmonic springs. The dynamics of the polymer is governed by 3 forces: thermal fluctuations, spring force, and bending force. Thermal diffusion fluctuation, resulting from the random collision of the polymer with the particles of its surrounding, and is given by

$$F_d(t) = \sqrt{2D} \frac{dw}{dt}$$

with  $D$  the diffusion constant, defined by  $\frac{k_B T}{\xi}$ ,  $k_B$ - the Boltzmann constant,  $T$ - the absolute temperature in Kelvin,  $\xi$ -the friction coefficient, and  $w$  is a standard white Gaussian noise.

The spring force, derived from an harmonic potential of springs connecting neighboring monomers, is given by

$$F_e(t) = -\gamma_e \frac{3k_B T}{2b^2} \frac{\partial}{\partial R_n} \sum_{n=1}^{N-1} (R_n(t) - R_{n+1}(t))^2$$

with  $\gamma_e > 0$  spring constant,  $b$ - the standard deviation of the distance between monomers, and  $R_n(t)$  is the 3D position of the  $n^{th}$  monomer.

Bending force on the  $n^{th}$  monomer is defined in terms of the angles  $\theta_i$  between three adjacent monomers of the chain,  $n, n+1, n+2$ , and the opening angle  $\theta_0$

$$F_b(R_n) = -\gamma_b \frac{3k_B T}{2b^2} \frac{\partial}{\partial R_n} \sum_{i=1}^{N-2} (\cos(\theta_i(t)) - \cos(\theta_0))^2$$

The differential equation describing of motion of the chain is thus

$$\frac{dR_n(t)}{dt} = F_e + F_b + \sqrt{2D} \frac{dw}{dt}$$

### 0.2.8 the Spring constant

We set the spring constant  $\gamma_s = 1$

### 0.2.9 The bending constant

bending constant is defined according to the opening of the chromatin up to 15 minutes post UVC.

### 0.2.10 Model parameters

we set  $b = \sqrt{3} \times 100nm$ , the number of monomers,  $N$ , is given by calculating the radius of gyration such that it covers the ROI. The radius of gyration is given by  $\sqrt{N/6}b$ , equating this to  $3\mu m$  we get  $N \approx 1800$

### 0.2.11 Model Parameters

Parameters used in simulations are set proportional to of the quantity  $\frac{3K_bT}{b^2}$ , which we fix to be 1 by setting the friction factor  $\xi = 1$ .

## 0.3 Simulations

### 0.3.1 3D

### 0.3.2 2D