Chromatin Architecture Post UVC damage

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0.1 Experimental Settings and Findings

1. Cell type used: U20S, 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 tits 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 redestribution 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 μm^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.

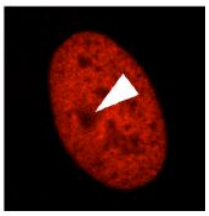

Figure 1: white triangle represents the UVC damage site, Histones are marked with red

0.2.2 The UV beam

UV beam has 3 μm^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 and in the damage zone. There are 3×10^6 histones marked, which makes their density $3 \times 10^6/(4\pi 7.25^3) \approx 630$ histones/μm^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 raiuds 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, ξ-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 θ_i between three adjacent monomers of the chain, $n, n+1, n+2$, and the opening angle θ_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 Model Parameters

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

b

we set $b = \sqrt{3} \times 100nm$,

number of monomers

The number of monomers, N, is determined by setting the polymer's radius of gyration to covers the ROI. The radius of gyration is given by $\sqrt{N/6}b$, equating it to $3\mu m$ we get $N \approx 1800$

the Spring constant

We set the spring constant $\gamma_s = 1$

The bending constant

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

0.3 Simulations

0.3.1 3D

0.3.2 2D