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. UVC damage is induced in a region of the cell using a 266 nm laser ($0.266\ \mu m$);
5. Changed to the red fluorescence signal were measured in the entire volume of the cell, post UVC;
6. Images were acquired using confocal microscopy, with an auto-focus module on, to acquire images from the best focal plane;
7. Fluorescence intensity were normalized against values measured in undamaged nucleus;
8. 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;
9. Illuminated area was defined 15 minutes post UVC based on GFP labeled repair factors and was kept similar throughout measurements;
10. Fluorescent recovery was measured relative to previous illumination starting from the frame with the minimal fluorescent values;
11. 2D projection of the 3D images were obtained by *maximal intensity z projection*
12. For sensitivity, most of the cell H3.3 fluorescence was photo-bleached, aside from the region of UVC illumination;
13. 20% loss of H3.3 signal from the *entire nucleus* was detected after photo-bleaching the fluorescence patch;
14. 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;

15. 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;

16. 20% loss of DNA signal in the damage region, accompanied by an expansion of the region was observed 15 minutes postUVC;

17. The expansion of the damage region depends on the dose of repair-factor;

18. The early repair factor DDB2 recruits histone chaperons HIRA, which promotes the deposition of newly synthesized histones at UVC sites;

19. newly synthesized histones are detectable in the repair region only 45 minutes post UVC;

20. Histone chaperons do not participate in histone redestribution after UVC irradiation;

0.2 Simulation Setting

0.2.1 The chromatin

The chromatin is modeled as a Rouse chain of N monomers. The dynamics of the chain 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

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

0.2.2 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 $\xi = 1$.

0.2.3 defining the region of interest

We model the experiment in a region around the damage zone rather than in the entire cell. We define our region of interest (ROI) in terms of the expected number of monomers located at steady around the polymer's center of mass. We wish to include 50% of the monomers inside the region of interest, therefore, the radius of the ROI is set to be the median of the equilibrium distribution of the Rouse chain, which is a Gaussian. We therefore set it at the 0.75% quantile of the normal distribution, $s = 0.6745\sqrt{\frac{2}{3}}b$

0.2.4 The UV beam

0.3 Findings