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 particular region of the cell;
5. Changed to the red fluorescence signal were measured in the entire volume of the cell, post UVC;
6. images where 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;
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

$$F_d = \sqrt{2D}\dot{w}$$

with D the diffusion constant, defined by $\frac{k_B T}{\psi}$, k_B- the Boltzmann constant, T- the absolute temperature in Kelvin, and ψ-the friction coefficient.

The harmonic potential of springs connecting neighboring monomers is given by

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

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 two adjacent links of the chain, and the opening angle θ_0

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

with $\theta_n(t)$ the angle between the chain links formed by monomer $n, n+1, n+2$. The forces acting on the n^{th} monomer at each time is calculated by $\frac{\partial U_b}{\partial R_n}$

The differential equation describing of motion of the chain is thus

$$\frac{dR_n}{dt} = \frac{\partial U_b}{\partial R_n} + \frac{\partial U_e}{\partial R_n} + \sqrt{2D}\dot{w}$$

0.2.2 Parameters

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

0.2.3 defining the region of interest

Following the article by Polo et al. we define our region of interest (ROI) 15 minutes post UVC.

0.2.4 The UV beam

0.3 Findings