Chromatin Architecture Post UVC damage

July 26, 2015

0.1 Experimental Settings and Main 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 μm);
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. To gain 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 signal in the center of the damage area, 15 minutes post UVC, was accompanied by an increase of density at its boundaries, balancing the loss;

17. 20% loss of DNA signal in the damage region, accompanied by an expansion of the region was observed 15 minutes post UVC;

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 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.

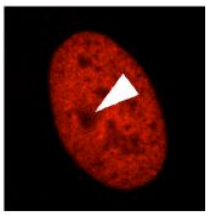

Figure 1: white triangle represents the UVC damage site, Histones are marked with red

0.2.2 The Simulation Domain

We set our simulation in a spherical domain with reflecting boundaries. This d-dimensional sphere, $\Omega_d(\rho)$ of radius ρ, will represent a region around the damage site, rather than the entire nucleus. To keep the polymer dense inside this region, we set $\rho = (b\sqrt{N/6})/2$. The actual value of this parameter is yet to be justified. The reflecting boundaries mimic the condensation barrier of the DNA with surrounding chromosomal chain, unaffected by the UV damage.

0.2.3 The UV beam

UV beam has 3 μm^2 section, yielding a radius of $r_{uv} = \sqrt{\frac{3}{\pi}} \approx 1\mu m$. In simulations we set the beam radius to be proportional to the polymer's radius of gyration, such that the simulation domain is 3 times the radius of the UVC beam. We therefore set the UVC beam radius, ρ_B, to be $\rho_B = \rho/3 = (b\sqrt{N/6})/6$.

0.2.4 The region of interest (ROI)

The area of chromatin expanded after UVC occupies $10\mu m^2$ which gives 3 times the UVC beam radius, i.e. $3\mu m$. In practice, we define the ROI as a rectangular region with diagonal ρ_R, proportional to the UCV beam's radius. The ROI's vertexes lay on the boundary of $\Omega(\rho)$, allowing room for monomers outside the ROI after expansion.

0.2.5 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.

0.2.6 DNA density

to be determined

0.2.7 Distribution of damage sites

to be determined

0.2.8 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, which we vary during simulations to approximate the observed experimental behavior. Before UVC beam is shot the polymer dynamics is governed by spring forces only, whereas after UVC the affected monomers of the chain along with their nearest neighbors are assigned additional bending elasticity forces.

Thermal diffusion fluctuation, results 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

$$F_e(t) = -\gamma_e \frac{dk_B T}{2b^2}\frac{\partial}{\partial R_n}\sum_{n-1}^{N-1}(|R_n(t) - R_{n+1}(t)| - l_0)^2$$

with $\gamma_e > 0$ spring constant,d is the dimension, b- the standard deviation of the distance between monomers, $R_n(t)$ is the 3D position of the n^{th} monomer at time t, and l_0 is the minimal allowed distance between neighboring monomers.

Bending force on the n^{th} monomer is defined in terms of the angles θ_i between three adjacent monomers of the chain, $n, n+1, n+2$;

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

The differential equation describing of motion of the chain is thus

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

0.2.9 Model Parameters

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

The parameter b

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

0.2.10 The minimal distance between monomers -l_0

We set l_0 to be $\sqrt{d}$ to match b and allow fast relaxation of the chain configuration before UVC beam shot, after diffusion is turned off.

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

The bending constant was observed to affect the rate at which the polymer assumes new conformation after UVC damage. We set it to be proportional to the bending constant, as $\gamma_b frac 3 K_b T b^2$. later we will adjust this constant according to the rate of chromatin expansion seen in experimental data.

0.2.11 Affected monomers after UVC

Affected monomers are those located within the UVC beam at initiation of beam around the center-of-mass of the polymer at that time. Because the polymer assumes random conformation just before beam initiation, it was observed that in many cases there were affected monomers for which there were no neighboring affected monomers. This phenomena causes peculiarities in the behavior of the chain, and a resting position could not be found for those monomers. We, therefore, assign bending force to the affected monomers' nearest neighbors to prevent these phenomenas and to form a more continuous segments affected by bending.

0.2.12 The ROI

The circular region of interest (ROI), in which we calculate monomer density gain and loss, is determined according to the expansion of the damaged monomers. We set the percentage of included damaged monomers to 95%. The ROI is calculated from the polymer's center of mass, such that 95% of the damaged monomers are included within it. The ROI is calculated at the end of the beam phase, after expansion has thought to reach saturation [How is saturation determined?]. The radius of the ROI is then used to back-calculate the densities within it relative to the center of mass of the polymer at any time step starting from the beam shot time.

0.3 Simulations

0.3.1 Simulations' settings

Simulations were ran *numRelaxationSteps* up to relaxation, after which the diffusion force was set to 0 and recording for *numRecordingSteps*. Following, the UV beam was shot through the center of the polymers mass. All

beads falling within the UV beam area were assigned bending force. To prevent out-of-the-ordinary monomer behavior, we assign bending force to the affected monomers' nearest neighbor along the chain (see subsection 0.2.11). Nearest neighbors were not counted as affected beads. Simulation then ran for additional *numBeamSteps* with the bending force active for the affected beads.

Simulation were placed in a spherical environment with reflecting boundaries. Measurement of density were performed on a rectangular region, which its center was dynamically placed at the polymer's center of mass. Sizes of the containing sphere (circle in 2d) and the measurement region were proportional to the radius of gyration, $\sqrt{N/6}b$.

0.3.2 3D Simulations

The number of monomers in the ROI as a function of the bending constant

In this experiment we examine the affect of the value of bending/spring constants on the number of monomers left at the ROI post UVC. For this end we examine 5 different type of chains, with number of monomers,N varying as $N = 100, 200, 400, 800, 1600$. For each N we keep the spring constant,$\gamma_s = 1$, and increase the bending constant $\gamma_b = 1, 2, 3, 4, 5$. For this simple experiment we perform only one realization per chain per bending constant, hence the results presented in the following table should be interpreted with care. Parmeter used in simulations:

- *numRelaxationSteps* = 2000
- numRecordingSteps = 1000
- numBeamSteps = 3000
- numBeads = [100 200 400 800 1600]
- dt = 0.1
- D = 1;
- b = $\sqrt{3}$
- opening angle $\theta_0 = \pi$

- bending constant = $[dD/b^2, 2dD/b^2, 3dD/b^2, 4dD/b^2, 5dD/b^2]$
- springConstant = dD/b^2
- beamRadius = $\sqrt{numBeads/6}b/6$
- containingSphereRadius = $0.5\sqrt{numBeads/6}b$
- regionOfInterestWidth = $2(\sqrt{numBeads/6}b)/6$
- regionOfInterestHeight = $2(\sqrt{numBeads/6}b)/6$
- regionOfInterestCenter = polymer center of mass

	Lost (mean) (%)[min max]				
	bending const. multiplier. γ_b				
N	1	2	3	4	5
100	31 (58%) [28,38]	35 (16%)[-3 17]	14 (37%) [11 22]	36 (61%)[26 43]	10 (26%)[2 26]
200	17 (21%) [2, 30]	58 (57%)[49 71]	54 (56%)[48 62]	86 (72%) [82 92]	33 (45%)[28 32]
400	22 (16%) [2 39]	100 (53%) [82 106]	73 (39%)[60 89]	92 (%)[72 100]	117 (66%)[74 120]
800	0 (0%) [-20 18]	0 (0%) [-39 29]	72 (20%)[4 133]	122 (33%)[29 196]	120 (31%)[0 180]
1600	0 (0%)[-23 21]	0 (0 %) [-47 25]	54 (9%)[0 104]	43 (3%)[-17 110]	67 (12%)[0 132]

2D simulations

0.3.3 Adding Lennard-Jones force

After UVC, several monomers are hit and together with their nearest-neighbors are assigned bending elasticity with an opening angle of a certain value. Usually, a region of consecutive affected monomers is enclosed by non-affected monomers. Therefore, the result of activating the bending elasticity force for the affected monomers is the formation of horse-show type of loops structures. Affected monomers are located at the center of the polymer and expand usually outwards (although the direction of expansion cannot be controlled). The problem is that the expanded region of the chain exit the ROI and keep expanding passed the layer of non-affected monomers, that are usually located outside the ROI.

The Rouse polymer permits bonds to cross each other, and therefore the affected monomers do not stop at the non affected monomer layer. to try to confine the affected monomers to the region of the damage, we assign volume exclusion, Lennard-Jones potential, to the chain. The non-affected monomers will hopefully counter-balance the expansion of the affected monomers, keeping them from passing the non-affected layer outside the damage zone.

In the experiments explained below we ran the simulation to see if the radius of expansion of the affected monomers can be limited by the Lennard-Jones fores. Simulations are performed in two-dimensional spherical environment, the polymer includes 500 monomers.

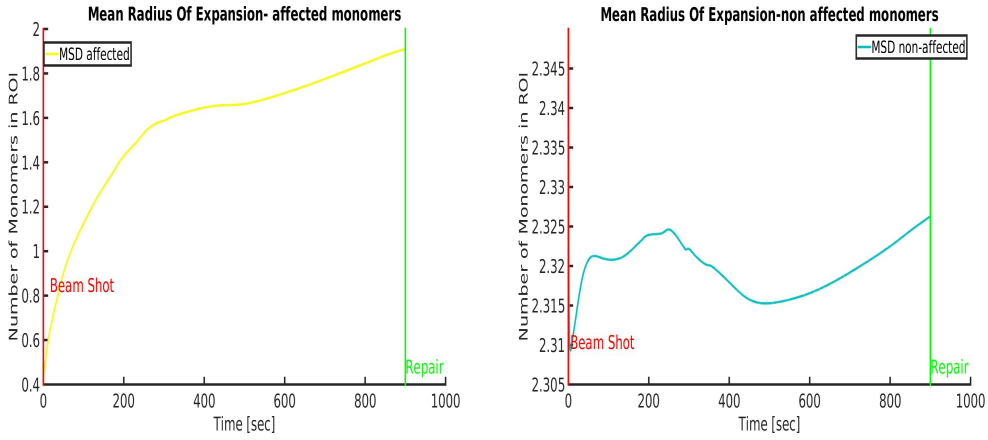

Figure 2: Radius of expansion relative to the monomers' center-of-mass, for the affected (left) and non-affected (right) monomers of the chain.(Mean of 5 experiments of 9000 steps each, polymers contain 500 monomers)

From figure 2 we can see that the Lennard Jones is not sufficient to stop the monomers from expanding. The affected monomers MSD keeps increasing until about time 300 sec, where it reaches a plateau. at this point many beads are at the same line as the non affected ones and probably the expansion comes into a halt. Once the bending force overcomes the resistance by the Lennard Jones force, the expansion continues. The affected beads' radius of expansion doesn't reach the value of the non affected. However, we suspect that it will given enough time. Moreover, the expansion is measured relative to the center of mass of either one of the two groups, affected and non-affected, and so it is not clear where are those two groups relative to each other.

Changing the radius of expansion to be calculated relatively to the beam center does not change the size of expansion. the affected monomers keep on expanding.

0.3.4 Cross-linking the polymer

Instead of the linear chain we add random connectors between different parts of the chain. The measure for connectivity is a percentage of connected monomers, nearest neighbors additional connectors being excluded. At simulation initiation, we let the polymer relax to a new state in which the cross-linked monomers are brought close to one another.

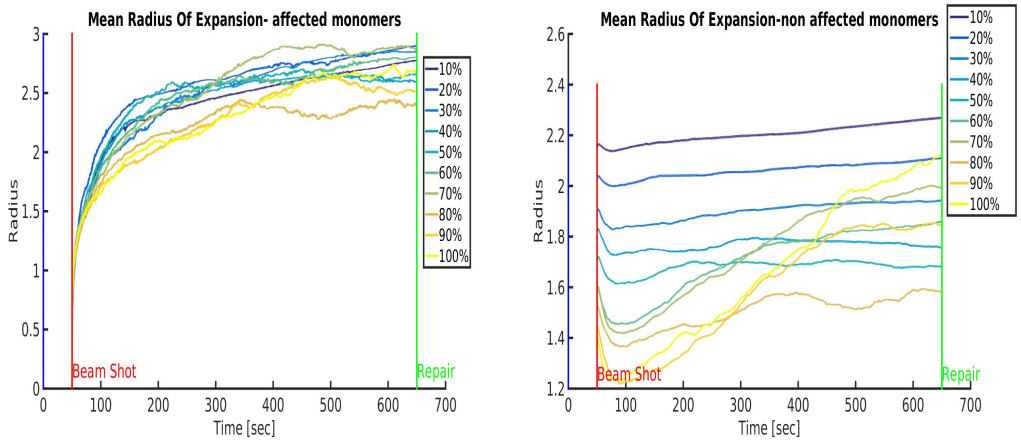

Figure 3: Radius of expansion for the affected (left) and non affected (right) monomers, when the polymer is cross-linked by varying percentages. Expansion of the affected monomers is not stopped and goes beyond the non-affected expansion radius. (500 monomers)

As can be seen from Figure 3 even with cross-links, and the given parameter set, expansion of the affected monomers is not stopped. Because the region of interest to measure density in is determined post-prior to the expansion, this will pose a problem, since the ROI will include also the non-affected monomers, which do not seem to expand much in low percentages of cross-linking.

This brings us to believe that high level of connectivity is needed, where both the affected and non-affected radius of expansion increases (see, for example, the curves for 80-100% connectivity in Figure 3). In that scenario, the ROI, which is determined at the end of the beam steps will more likely represent the expanded area seen in experiments, in which we see 20% loss of material.

Two monomers of a cross-linked polymer are more likely to be found in the center. In a cross-linked polymer, after we shut-down diffusion, there is a convergence toward the center because all cross-links are pulling toward one another. This creates the situation that the affected beads do not have to expand much to pass the cross-linked layer of the non-affected monomers.

0.3.5 Bending elasticity for the non-affected monomers

The result of the previous subsection brought us to think that the expansion should not occur in the affected monomers but rather in the non-affected ones. In this manner, the simulation captures the process as follows:

1. cross-linked polymer is being shot by UVC;
2. cross-links to and from the damaged monomers are released;
3. repair mechanism pushes the non damaged monomers out of the way, this causes bending elasticity for the non-damaged monomers.

an alternative to step 3 is to assign bending elasticity potential to monomers in the UVC beam but those that are undamaged. The two alternative will be examined. We start with the first option. No lennard-Jones force.

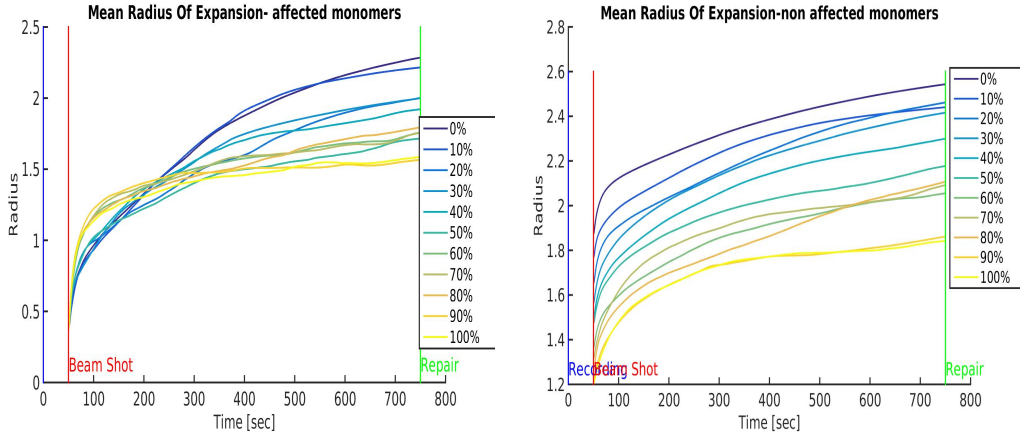

Figure 4: expansion of the damaged (left) and non-damaged (right) monomers, after the links were broken for damaged monomers and bending elasticity was assigned to non damaged monomers. (polymer with 500 monomers)