Contribution ID: **246** Type: **oral (invited speaker)**

# Investigating DNA repair centers in mammalian cells and their impact on dose-response linearity

*Tuesday, 16 October 2012 13:30 (30 minutes)*

We recently showed in non-malignant human breast cells (MCF10A) that at doses of X-ray above or equal to 1 Gy, radiation-induced foci (RIF) do not increase linearly with dose. RIFs were characterized by the local recruitment of DNA damage sensing proteins p53 binding protein (53BP1). Using a mathematical model of RIF kinetics we also showed that RIF induction rate increased with increasing radiation dose, whereas the rate at which RIFs disappear decreased. In addition, live cell imaging showed that RIF do not all appear synchronously after exposure, clearly demonstrating that counting RIF at a given time point only reflects a net number of damages. By integrating RIF kinetics over time one can infer the total number of RIF generated at a given dose. Mathematically correcting for the dose dependence of induction/resolution rates, we observed an absolute RIF yield that was surprisingly much smaller at higher doses: 15 RIF/Gy after 2 Gy exposure compared to ~64 RIF/Gy, after 0.1 Gy. Cumulative RIF counts from time lapse of 53BP1-GFP in human breast cells confirmed these results. We hypothesized that DNA damage was clustering into common regions of the nucleus more suited for DNA repair. Data collected so far suggest these 'repair centers'would be located into euchromatic regions of the nucleus with an average spacing of 1 to 2 µm. In this new work, we are now presenting RIF dose-kinetics from other mammalian cell lines (primary lines from humans and mice, immortal lines grown in 3D) to evaluate the universality of DNA damage clustering. We also introduce a new microfluidics approach integrated with an X-ray milli-beam on a fully automated fluorescent microscopy platform to accelerate RIF dose-kinetics quantification in an unbiased manner. Since mice cells have much better-defined heterochromatic domains, they are used primarily to test the impact of heterochromatin on DNA damage clustering. Cell-type response specificity is tested by having human breast cells grown on various extra-cellular matrix substrates, leading to specific cell differentiation. Our overarching goal is to characterize for low and high doses, RIF kinetics across species and within species and correlate RIF kinetics parameters and RIF frequencies to persistent DNA damage.

**Primary author:** Dr COSTES, Sylvain V. (Lawrence Berkeley National Laboratory, Berkeley, USA)

**Co-authors:** Dr LO, Alvin (Lawrence Berkeley National Laboratory, Berkeley, USA); Dr SNIJDER, Antoine (Lawrence Berkeley National Laboratory, Berkeley, USA); Dr TANG, Jonathan (Lawrence Berkeley National Laboratory, Berkeley, USA); Dr MORA-BLANCO, Lorena (Lawrence Berkeley National Laboratory, Berkeley, USA); Dr LABARGE, Mark (Lawrence Berkeley National Laboratory, Berkeley, USA); Dr BISSEL, Mina (Lawrence Berkeley National Laboratory, Berkeley, USA)

**Presenter:** Dr COSTES, Sylvain V. (Lawrence Berkeley National Laboratory, Berkeley, USA)

**Session Classification:** DNA Damage and Repair

**Track Classification:** DNA Damage and Repair