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How to Study Protein Recruitment to DNA Lesions by a Combination of UV Laser and White Light Laser

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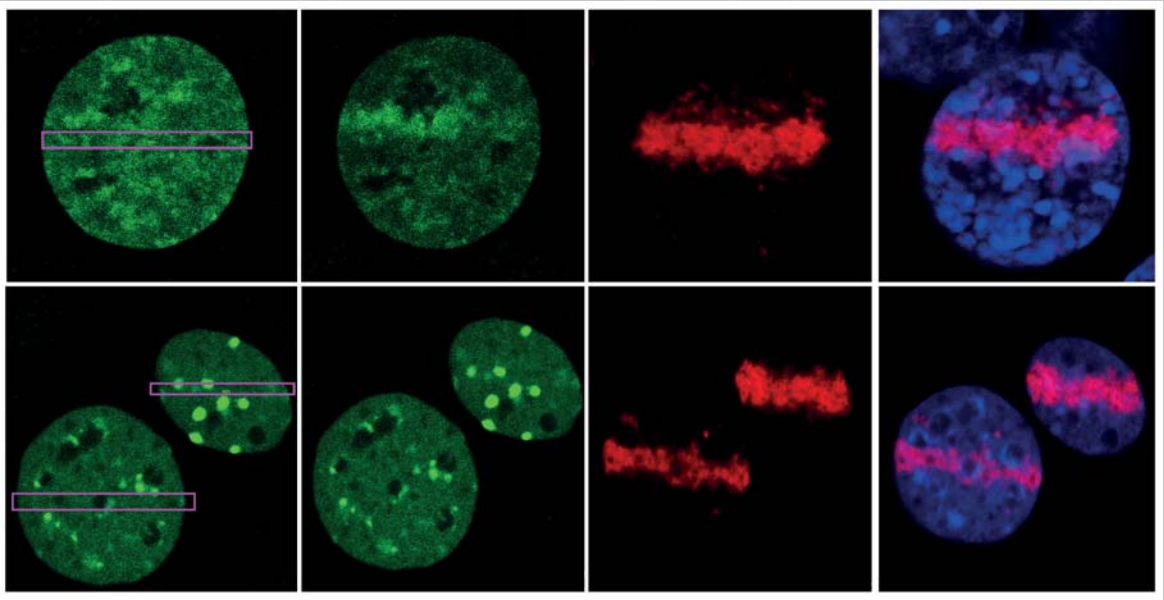

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Introduction

Understanding how DNA lesions are optimally repaired is of functional significance, especially from the view of genome karyotype stability. DNA lesions are recognized by several proteins in a hierarchical manner: First, an appearance of double strand breaks (DSBs) initiate recruitment of primary protein complexes, such as MRE11-RAD50-NBS1, which are responsible for the activation of a DNA damage-related serine/threonine protein kinase called ataxia telangiectasia mutated (ATM; summarized by Misteli and Soutoglou, 2009 or Larsen et al., 2010). MRE11-RAD50-NBS1 binding and ATM activation lead to the phosphorylation of histone H2AX (γH2AX), which is an early event in the repair of DNA lesions (Rogakou et al., 1998; Celeste et al., 2003). This is accompanied by activation of the mediator protein MDC1, which recruits chromatin remodeling factors, including 53BP1 and BRCA1. DNA lesions are additionally recognized by other chromatin related proteins, such as heterochromatin protein 1 (HP1) or the Polycomb group proteins BMI1 and Mel18 (Ayoub et al., 2008; Luijsterburg et al., 2009; Chou et al., 2010).

Material and Methods

Cell cultivation

Human osteosarcoma U2OS cells stably expressing GFP-BMI1 originated from the laboratory of Prof. Maarten Van Lohuizen, The Netherlands Cancer Institute, Antoni van Leeuwenhoek Ziekenhuis, Amsterdam, The Netherlands. We obtained these cells from the laboratory of Assoc. Prof. Dušan Cmarko, Charles University in Prague. Mouse 3T3 cells were a generous gift from Dr. Paul Verbruggen from Swammerdam Institute for Life Sciences University of Amsterdam, The Netherlands. U2OS and 3T3 cells were cultivated in D-MEM medium (Dulbecco's Modified Eagle Medium, PAN Biotech, GmbH, Germany) containing 10% fetal calf serum. Cultivation proceeded at 37°C in a humidified atmosphere containing 5% CO_2.

Induction of DNA double stand breaks

U2OS cells stably expressing GFP-BMI1 and 3T3 cells stably expressing EGFP-HP1β were cultivated under standard conditions. For micro-irradiation, the cells were sensitized with 10 μM 5-bromo-2'-deoxy-uridine (BrdU), 16-18 h before local irradiation. Control and TSA-treated U2OS and 3T3 cells were stained using BrdU Labeling (Roche, #11296736001). BrdU-sensitized cells were irradiated by UV laser (355 nm). We irradiated half of the nuclei or defined strips by 80% laser output, not reduced at acousto-optic tunable filter (AOTF). The following settings were used: format 512 × 512 pixels, 400 Hz, bidirectional mode, 64 lines, zoom >5-10x. After irradiation, the cells were fixed in 4% paraformaldehyde, and phosphorylated histone H2A.X (γH2AX) was detected by immunofluorescence methods with rabbit polyclonal antibody against γH2A.X (phospho S139; Abcam, #ab2893). For visualization of locally micro-irradiated live cells and fixed cells after immunofluorescence, we used confocal microscopy. These analyses were performed using white light laser (WLL, 470-670 nm in 1 nm increments) at 554 nm connected to the confocal microscope Leica TCS SP5 X. For visualization of biological objects we used a magnification of 64× and numerical aperture N.A.=1.4.

Results

Here, DNA lesions were induced by 355 nm UV laser. After irradiation, live cells were monitored by White Light Laser (WLL) that significantly eliminated photobleaching during time lapse microscopy (Fig. 1). The presence of phosphorylated H2AX (γH2AX) was detected as a marker of chromatin with double strand breaks (DSBs) (Fig. 1, red strips). In micro-irradiated U2OS cells, we have observed pronounced accumulation of Polycomb group-related BMI1 protein (Fig. 1a). However, when the cells were treated with HDAC inhibitor, SAHA, no increased accumulation (nor a complete absence) of BMI1 at DNA lesions was detected (Fig. 1b). These experiments point to acetylation events as important players in the recognition of UV-damaged chromatin.

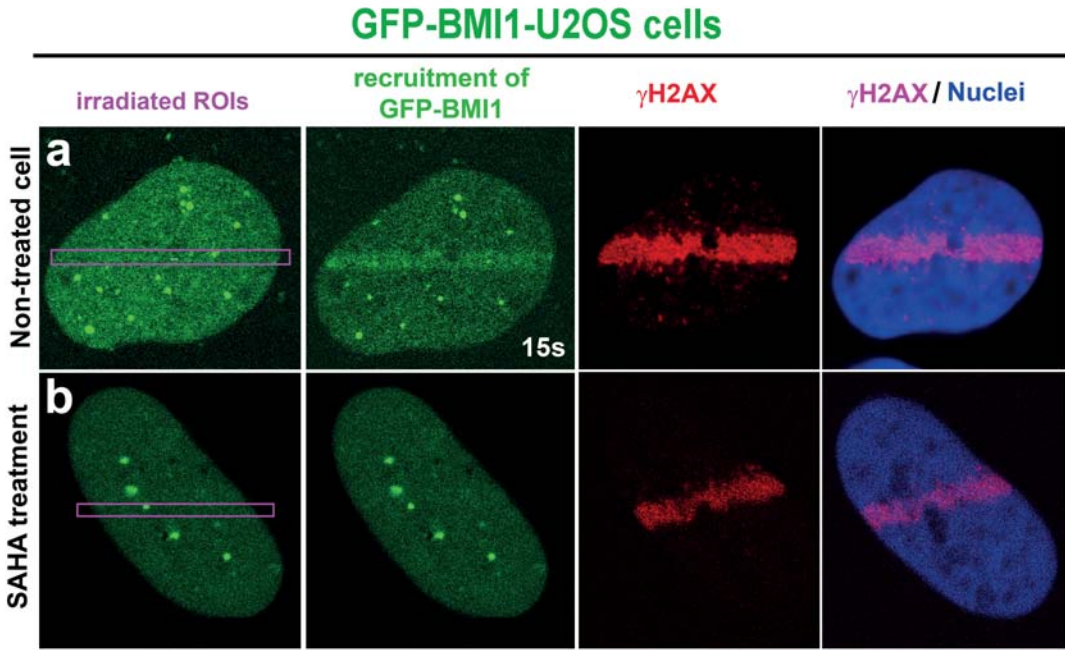

Figure 1: Acetylation-dependent recruitment of BMI1 protein to UV-damaged chromatin. (a) Live control GFP-BMI1-U2OS cells were monitored for 100 s after irradiation, and BMI1 protein accumulation at DNA lesions was detected within ~15 s. UV-irradiated regions were γH2AX positive (red), nuclei were stained by DAPI (blue). (b) SAHA (an inhibitor of histone deacetylases) prevented increased BMI1 accumulation at UV-damaged chromatin.

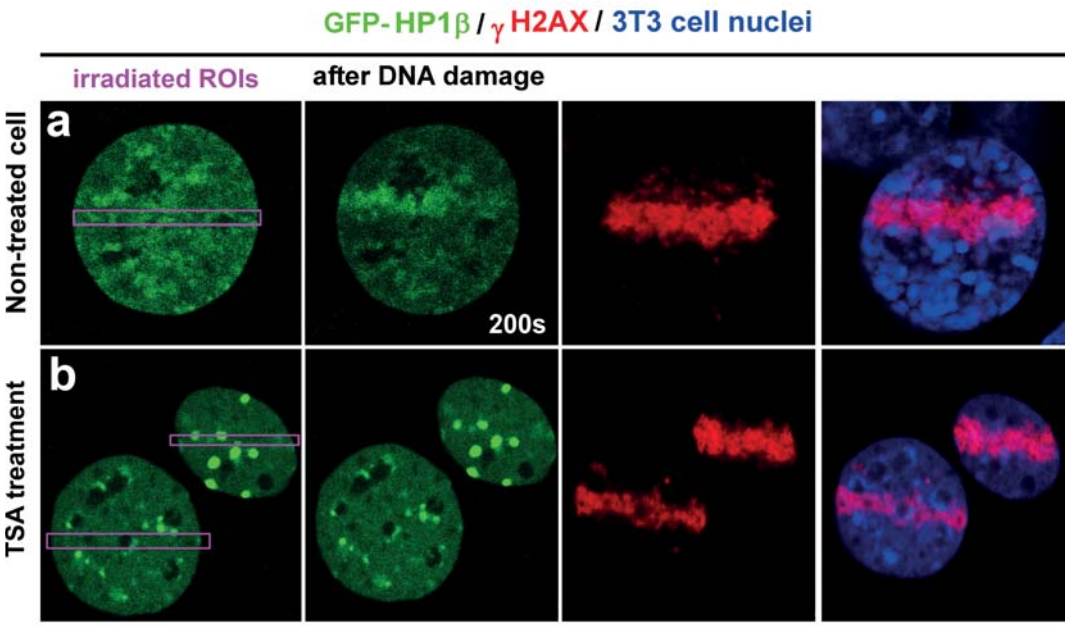

Figure 2: Recruitment of HP1β to UV-damaged chromatin is influenced by the histone acetylation state. (a) Live control GFP-HP1β-3T3 cells were micro-irradiated, and increased accumulation of HP1β at damaged chromatin was detected within ~200 s. (b) TSA (an inhibitor of histone deacetylases) prevented HP1β recruitment to damaged chromatin, however, HP1β was not absent at micro-irradiated regions of TSA treated cells.

In the next step, we asked whether BMI1 interacts with other heterochromatin-related proteins such as HP1β at UV-damaged chromatin. We have observed that HP1β accumulated at UV-damaged regions slowly; 200 s after irradiation (Fig. 2a), while BMI1 recruits to UV-damaged chromatin immediately after UV-irradiation (Fig. 1a).

Inhibition of histone deacetylases by TSA also prevented the recruitment of HP1β proteins to UV-damaged chromatin, similarly as it was observed for BMI1 protein, when the cells were treated by TSA (compare Fig. 1b and 2b).

Summary

Taken together, we showed that PcG-related BMI1 protein is recruited to UV-damaged chromatin immediately after micro-irradiation, which is followed by pronounced recruitment of GFP-HP1β. However, we are aware that the physiological significance of UV micro-irradiation may be limited, as is the biological significance of exogenous BMI1 expression. Nevertheless, in this experimental system, the recognition of UV-damaged chromatin by BMI1 and HP1β was likely dependent on acetylation events, owing to the fact that histone hyperacetylation prevented pronounced recruitment of BMI1 and HP1β to UV-damaged chromatin (Fig. 1 and 2). Using independent experimental approaches and by using a combination of UV laser (355 nm) and WLL connected to the Leica TCS SP5 X confocal microscope, we showed the importance of acetylation status for the DNA repair processes. Thus, our results contribute to the understanding of how the specific histone signature can influence DNA repair-related events. Data shown in this short report were published by Šustáčková et al., J. Cell Physiol. (2011).

References

Ayoub N, Jeyasekharan AD, Bernal JA, Venkitaraman AR (2008) HP1-beta mobilization promotes chromatin changes that initiate the DNA damage response. Nature 453: 682–686.

Celeste A., Fernandez-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, Bonner RF, Bonner WM, Nussenzweig A (2003) Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. Nat Cell Biol 5: 675–679.

Chou DM, Adamson B, Dephoure NE, Tan X, Mottle AC, Budov KE, Gygi SP, Colaiácovo MP, Elledge SJ (2010) A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. Proc Natl Acad Sci USA 107: 18475–18480.

Larsen DH, Poinsignon C, Gudjonsson T, Dinant C, Payne MR, Hari FJ, Danielsen JM, Menard P, Sand JC, Stucki M, Lukas C, Bartek J, Andersen JS, Lukas J (2010) The chromatin-remodeling factor CHD4 coordinates signaling and repair after DNA damage. J Cell Biol 190:731–740.

Luijsterburg MS, Dinant C, Lans H, Stap J, Wiernasz E, Lagerwerf S, Warmerdam DO, Lindh M, Brink MC, Dobrucki JW, Aten JA, Fousteri MI, Jansen G, Dantuma NP, Vermeulen W, Mullenders LH, Houtsmuller AB, Verschure PJ, van Driel R (2009) Heterochromatin protein 1 is recruited to various types of DNA damage. J Cell Biol 185: 577–586.

Misteli T, Soutoglou E (2009) The emerging role of nuclear architecture in DNA repair and genome maintenance. Nat Rev Mol Cell Biol 10:243–254.

Rogakou EP, Pilch DR, Orr AH, Ivanova,VS, Bonner WM (1998) DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 273: 5858–5868.

Šustáčková G, Kozubek S, Stixová L, Legartová S, Matula P, Orlova D, Bártová E. (2011) Acetylation-dependent nuclear arrangement and recruitment of BMI1 protein to UV-damaged chromatin. J Cell Physiol. 2011 Jul 5. doi: 10.1002/jcp.22912.