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Hybrid Detectors Improved Time-Lapse Confocal Microscopy of PML and 53BP1 Nuclear Body Colocalization in DNA Lesions

Veronika Foltánková[1], Pavel Matula[2], Dmitry Sorokin[2], Barbora Malyšková[1], Stanislav Kozubek[1], Eva Bártová[1]

[1] Institute of Biophysics , Academy of Sciences of the Czech Republic, v.v.i., Královopolská 135, CZ-612 65, Brno, Czech Republic
[2] Faculty of Informatics, Masaryk University, Brno, Botanická 68a, 602 00, Brno, Czech Republic

Abstract

We used hybrid detectors (HyDs) to monitor the trajectories and interactions of promyelocytic leukemia (GFP-PML) nuclear bodies (NBs) and mCherry-53BP1-positive DNA lesions. 53BP1 protein accumulates in NBs that occur spontaneously in the genome or in γ-irradiation-induces foci. When we induced local DNA damage by ultraviolet irradiation, we also observed accumulation of 53BP1 proteins into discrete bodies, instead of the expected dispersed pattern. In comparison with photomultiplier tubes, which are used for standard analysis by confocal laser scanning microscopy, HyDs significantly eliminated photobleaching of fluorochromes during image acquisition. The low laser intensities used for HyD-based confocal analysis enabled us to observe NBs for the longer time periods, necessary for studies of the trajectories and interactions of PML and 53BP1 NBs. To further characterize protein interactions, we used resonance scanning and a novel bioinformatics approach to register and analyze the movement of individual PML and 53BP1 NBs. The combination of improved HyD-based confocal microscopy with a tailored bioinformatics approach enabled us to reveal damage-specific properties of PML and 53BP1 NBs, which co-localization limited localized movement of these nuclear bodies, as it was revealed by single particle tracking analysis.

Materials and Methods

1) In vitro cultivation of U2OS cells in DMEM supplemented by 10% fetal bovine serum and transfection by GFP-PML or mCherry-BP1-2 plasmid DNA using METAFECTENE™ PRO (Biontex Laboratories GmbH, Planegg, Germany).
2) Induction of DNA lesions by γ-irradiation (^{60}Co, dose 5 Gy) or microirradiation by UV laser (355 nm) 16-18h after sensitization by 5-bromo-2'-deoxy-uridine.
3) Analysis of trajectories of 53BP1 and PML NBs in spontaneous DNA lesions, UV-induced lesions and irradiation-induced foci (IRIF) was performed by using algorithm for single-particle tracking.
4) Kinetics of 53BP1 NBs were studied by FRAP.

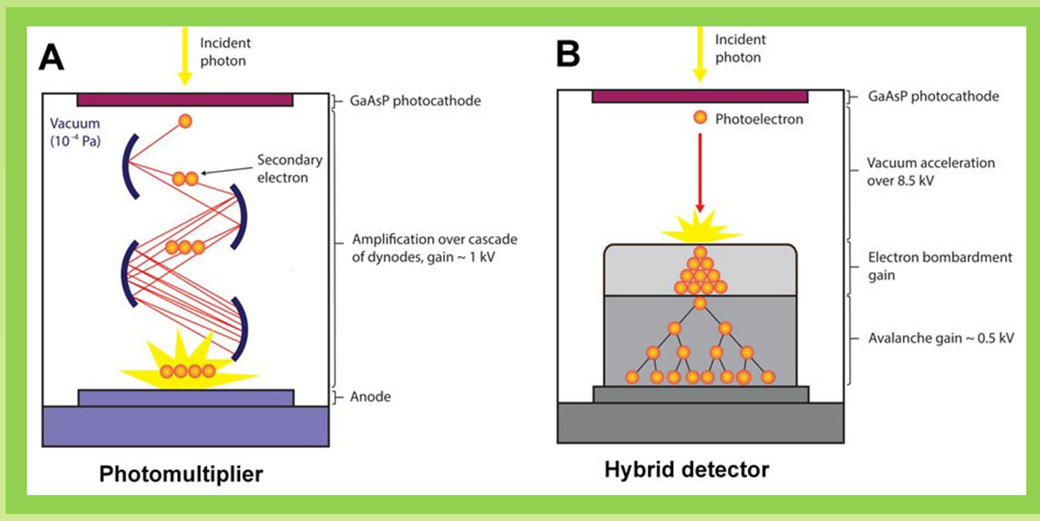

Figure 1. Comparison of PMTs and HyDs. In both cases, light is converted into electrons by gallium-arsenide-phosphide photocathode. **(A)** PMT enables amplification of secondary electrons through cascade of dynodes. **(B)** HyD contains silicon avalanche diode, which is composed of semiconductor layers, including a thin layer of heavily doped region that faces the photocathode and is connected to the thick silicon substrate.

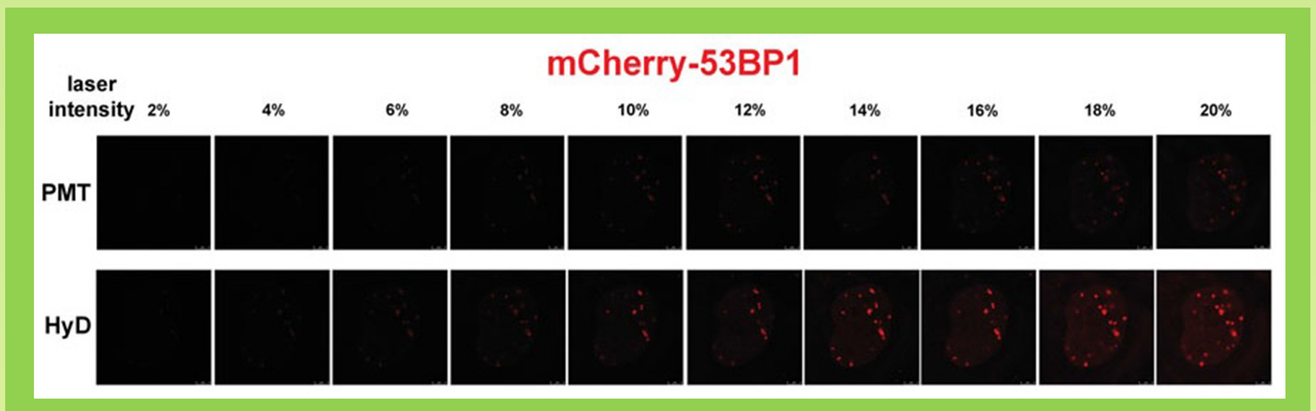

Figure 2. Comparison of the confocal scanning of mCherry-tagged 53BP1 NBs in living cells using different laser intensities. Detection was performed by PMTs or HyDs.

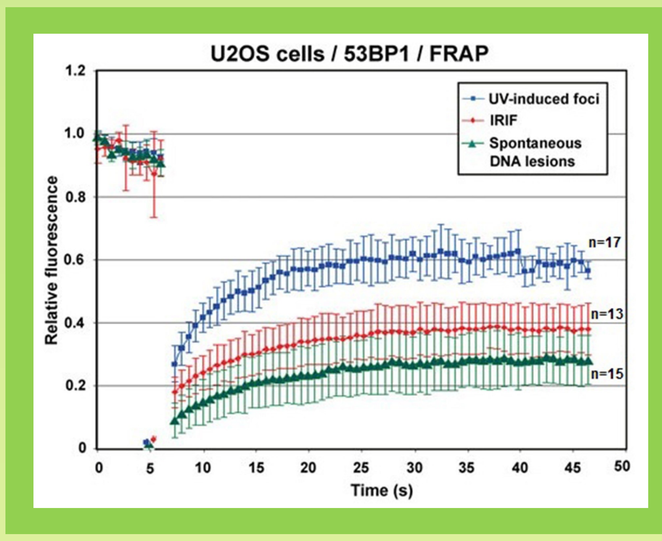

Figure 3. Kinetics of 53BP1 NBs studied by FRAP in spontaneously occuring lesions, UV-induced lesions, and IRIF. Blue line shows 53BP1 NBs in UV-irradiated ROIs (number of nuclei analyzed: n=17), green line represents spontaneously occuring DNA lesions (n=13), and red line shows recovery of 53BP1 after γ-irradiation (n=15).

Conclusions

Single-particle tracking analysis revealed that 53BP1 and PML NBs undergo distinct localized movements in response to distinct types of DNA damage. Furthermore, we found that 53BP1 and PML NBs interact, especially in IRIF. Interaction between nuclear bodies also changed their localized movement. Specifically, the trajectories of 53BP1 NBs that colocalized with PML NBs were significantly shortened after UV-irradiation. Additionally, FRAP analysis revealed significantly enhanced fluorescence recovery after photobleaching of 53BP1 in UV-induced DNA lesions, especially in comparison with spontaneously occuring DNA damage-related foci.

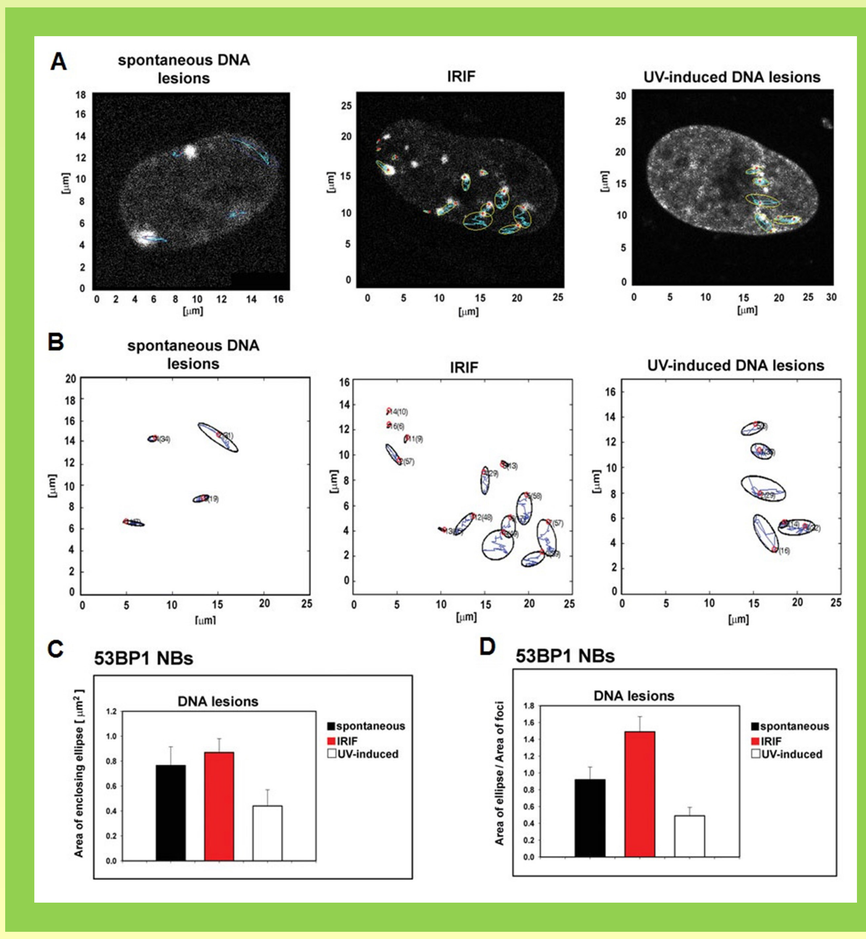

Figure 4. Trajectories of 53BP1 NBs in living cells. Trajectories were monitored for all 53BP1 bodies in U2OS cells in spontaneously occuring DNA lesions, IRIF, an UV-induced DNA lesions. Bioinformatics analysis was performed for particular 53BP1 NBs, whose trajectory is shown in the minimal enclosing ellipse. Period of observation was 1-2 h. **(A)** Minimal enclosing ellipses for individual DNA lesions. **(B)** Comparison of minimal enclosing ellipse (black) with individual tracks (blue). **(C)** Average area of enclosing ellipse in spontaneous, IRIF, and UV-induced DNA lesions. **(D)** Average area of enclosing ellipse normalized to the area of individual foci containing accumulated 53BP1 protein.

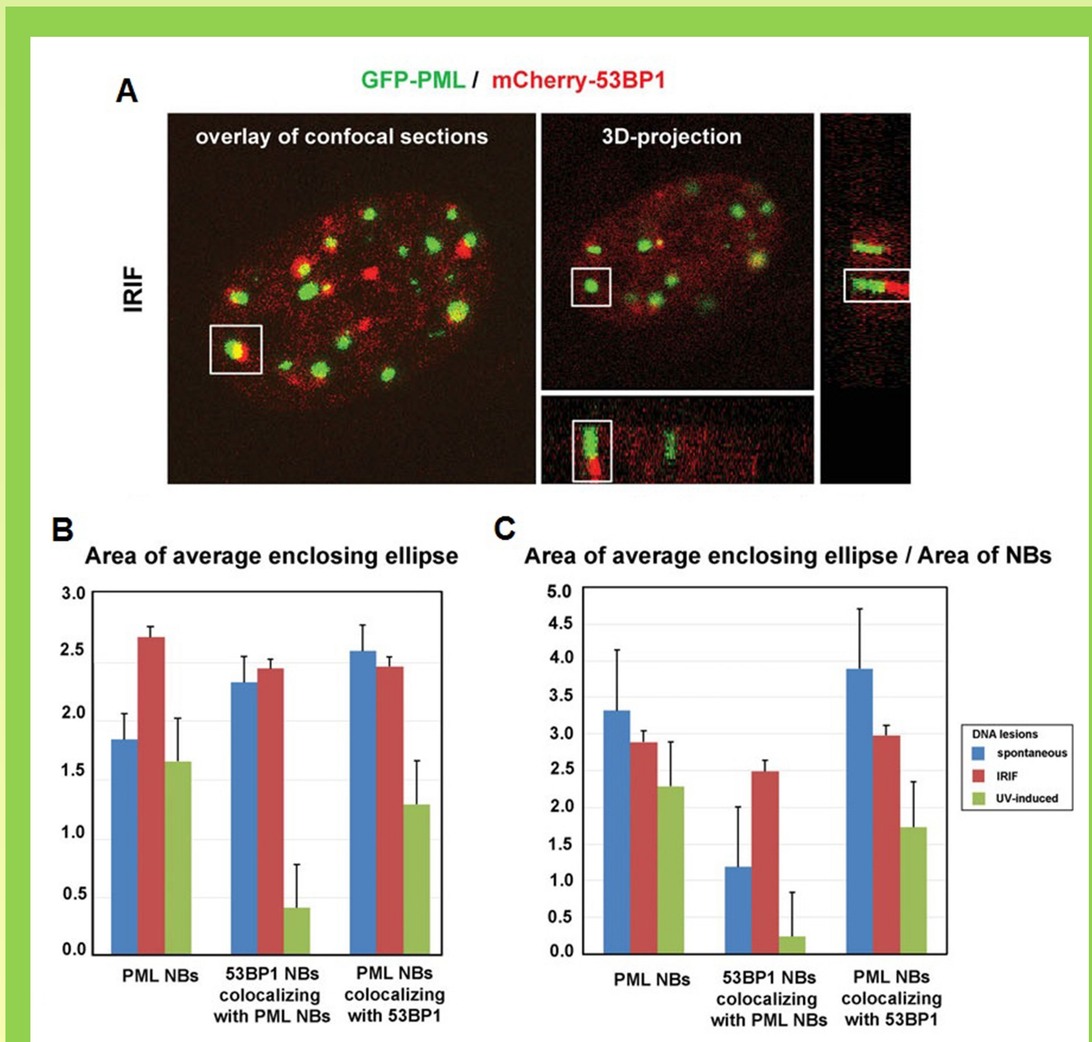

Figure 5. Comparison of minimal enclosing ellipses for PML and 53BP1 NBs in spontaneous DNA lesions, IRIF, and UV-induced DNA lesions. **(A)** Colocalization of PML and 53BP1 NBs shown as the 3D projection of confocal sections. Single particle tracking analysis was performed for particular PML NBs and compared with the position of the nearest 53BP1 body. **(B)** Average area of enclosing ellipse ± standard error (S.E.) is shown for individual PML NBs, 53BP1 NBs colocalizing with PML NBs, and PML NBs colocalizing with 53BP1 NBs. **(C)** Average area of enclosing ellipse was normalized to average area of individual NBs. This parameter was calculated for individual PML NBs, 53BP1 NBs colocalizing with PML NBs, and PML NBs colocalizing with 53BP1 NBs.

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