REPAIR OF DNA SINGLE-STRAND BREAKS AS A FUNCTION OF CELL CYCLE PHASE, OXYGEN LEVEL, AND THE NUMBER OF BREAKS

Oskar Szelest, Damian Kurleto, Izabela Dąbrowska, Mirek Zarębski, Jurek Dobrucki
Department of Cell Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow
Email: szelest.oskar@gmail.com

Background. DNA single-strand breaks (SSBs) are one of the most common types of DNA damage. Potentially harmless when present in low number, can led to serious genome mutations and cell death when accumulated. We studied repair of SSBs induced by a beam of visible light and imaged accumulation of XRCC1 (X-ray Repair Cross Complementing Protein 1) and PCNA (Proliferating Cell Nuclear Antigen). These factors are involved in short and long patch SSB repair pathways.

Aims. This research was focused on: (1) investigating which of the SSBs repair pathways, short-patch (SP, XRCC1 dependant) or long-patch (LP, PCNA dependant) are activated in replicating (early-S, middle-S and late-S phase) and non-replicating cells, (2) detecting the process of saturation of a capacity to repair of increasing numbers of SSBs, and (3) ability to activate repair under conditions of low oxygen tension.

Methods. Local DNA damage (SSBs) was induced by exposing a region of the cell nucleus to a focused beam of laser light [1]. Live cells expressing GFP-PCNA and RFP-XRCC1, or cells stained by immunofluorescence, were imaged using fluorescence confocal microscopy.

Results. XRCC1 was recruited to DNA lesions induced in all phases of the cell cycle. In contrast, in middle and late S-phase PCNA was recruited to only some lesions, and was not recruited in early S phase. When SSBs were induced at short time intervals (seconds) in several locations in the cell nucleus, XRCC1 was recruited to only a few of them. Recruitment of PCNA was limited to an even lower number of damage spots. When DNA lesions were induced in close succession, the amount of the recruited XRCC1 was the highest in the first spot, and lower in each subsequent location. A significant mobile pool of XRCC1 and PCNA always remained in the cell nucleus regardless of the number of induced DNA lesions. Recruitment of XRCC1 to SSBs still occurred at low oxygen tensions, but was abolished by hypoxia. Studies of live cells expressing fluorescent fusion proteins were confirmed by immunofluorescence in wild type cells.

Hypothesis and Conclusions. SSBs in G1/G0 cells are often repaired by LP pathway but involvement of PCNA in DNA replication results in switching to SP pathway in early-S phase. Also probability of activating LP pathway in middle- and late-S phase is low. Cellular ability to repair single-strand DNA breaks that were generated in different locations, in succession of each other, is limited to a just few initial SSBs spots. The cell appears to recruit very large number of copies of XRCC1 to the first detected lesion rather than spread the resources over all the lesions. Low oxygen has no impact on the recruitment process of XRCC1, but hypoxia prevents XRCC1 recruitment to SSBs.

[1]. *Solarczyk KJ, Zarębski M, Dobrucki JW. Inducing local DNA damage by visible light to study chromatin repair. DNA Repair (Amst). 2012 Dec 1;11(12):996-1002*

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