

PCNA is recruited to irradiated chromatin in late S-phase and is most pronounced in G2 phase of the cell cycle

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Received: 21 June 2016 / Accepted: 9 January 2017
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Abstract DNA repair is a complex process that prevents genomic instability. Many proteins play fundamental roles in regulating the optimal repair of DNA lesions. Proliferating cell nuclear antigen (PCNA) is a key factor that initiates recombination-associated DNA synthesis after injury. Here, in very early S-phase, we show that the fluorescence intensity of mCherry-tagged PCNA after local micro-irradiation was less than the fluorescence intensity of non-irradiated mCherry-PCNA-positive replication foci. However, PCNA protein accumulated at locally irradiated chromatin in very late S-phase of the cell cycle, and this effect was more pronounced in the following G2 phase. In comparison to the dispersed form of PCNA, a reduced mobile fraction appeared in PCNA-positive replication foci during S-phase, and we observed similar recovery time after photobleaching at locally induced DNA lesions. This diffusion of mCherry-PCNA in micro-irradiated regions was not affected by cell cycle phases. We also studied the link between function of PCNA and A-type lamins in late S-phase. We found that the accumulation of PCNA at

micro-irradiated chromatin is identical in wild-type and A-type lamin-deficient cells. Only micro-irradiation of the nuclear interior, and thus the irradiation of internal A-type lamins, caused the fluorescence intensity of mCherry-tagged PCNA to increase. In summary, we showed that PCNA begins to play a role in DNA repair in late S-phase and that PCNA function in repair is maintained during the G2 phase of the cell cycle. However, PCNA mobility is reduced after local micro-irradiation regardless of the cell cycle phase.

Keywords PCNA · DNA repair · Micro-irradiation · rDNA · Lamins · S/G2 phases