Biol. Cell (2015) **00,** 1–15 DOI: 10.1111/boc.201500050

Research Article

Distinct kinetics of DNA repair protein accumulation at DNA lesions and cell cycle-dependent formation of γH2AX- and NBS1-positive repair foci

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Background Information. The DNA damage response is a fundamental, well-regulated process that occurs in the genome to recognise DNA lesions. Here, we studied kinetics of proteins involved in DNA repair pathways and their recruitment to DNA lesions during the cell cycle. In non-irradiated and irradiated cells, we analysed the distribution pattern and spatiotemporal dynamics of γH2AX, 53BP1, BMI1, MDC1, NBS1, PCNA, coilin and BRCA1 proteins.

Results. We observed that spontaneous and irradiation-induced foci (IRIF) demonstrated a high abundance of phosphorylated H2AX, which was consistent with 53BP1 and BMI1 protein accumulation. However, NBS1 and MDC1 proteins were recruited to nuclear bodies (NBs) to a lesser extent. Irradiation by γ-rays significantly increased the number of 53BP1- and γH2AX-positive IRIF, but cell cycle-dependent differences were only observed for γH2AX-positive foci in both non-irradiated and γ-irradiated cells. In non-irradiated cells, the G2 phase was characterised by an increased number of spontaneous γH2AX-foci; this increase was more pronounced after γ-irradiation. Cells in G2 phase had the highest number of γH2AX-positive foci. Similarly, γ-irradiation increased the number of NBS1-positive NBs only in G2 phase. Moreover, NBS1 accumulated in nucleoli after γ-irradiation showed the slowest recovery after photobleaching. Analysis of protein accumulation kinetics at locally induced DNA lesions showed that in HeLa cells, BMI1, PCNA and coilin were rapidly recruited to the lesions, 10–15 s after UVA-irradiation, whereas among the other proteins studied, BRCA1 demonstrated the slowest recruitment: BRCA1 appeared at the lesion 20 min after local micro-irradiation by UVA laser.

Conclusion. We show that the kinetics of the accumulation of selected DNA repair-related proteins is protein specific at locally induced DNA lesions, and that the formation of γH2AX- and NBS1-positive foci, but not 53BP1-positive NBs, is cell cycle dependent in HeLa cells. Moreover, γH2AX is the most striking protein present not only at DNA lesions, but also spreading out in their vicinity. Our conclusions highlight the significant role of the spatiotemporal dynamics of DNA repair-related proteins and their specific assembly/disassembly at DNA lesions, which can be cell type- and cell cycle dependent.

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Key words: DNA repair, γ-Irradiation, Living cell studies, Cell cycle, Interphase, Micro-irradiation.

Abbreviations used: CPDs, cyclobutane pyrimidine dimers; DDR, DNA damage response; DSBs, double-strand breaks; FRAP, fluorescence recovery after photobleaching; HR, homologous recombination; IRIF, irradiation-induced foci; NB, nuclear body; NER, nucleotide excision repair; NHEJ, non-homologous end joining; ROI, region of interest.