Recruitment of HRDC domain of WRN and BLM to the sites of DNA damage induced by mitomycin C and methyl methanesulfonate

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Figure S1  Protein expression of the different pEGFP-WRN and pEGFP-BLM constructs

Exponentially growing HeLa cells were transfected with (A) pEGFPC1 vector, (B) GFP-tagged full-length WRN and WRN-HRDC and (C) GFP-tagged full-length BLM and BLM-HRDC. Equal amounts of protein from EGFP-WRN-construct transfected cell lysates were loaded in each lane. The immunoblots were then reprobed against β-actin (Cell Signaling). The asterisks correspond to the cross reactivity of the anti-GFP antibody (Abcam). Arrows show the expression of the GFP fusion protein.

SUPPLEMENTARY ONLINE DATA

Abbreviations: BER, base excision repair; BLMp, Bloom protein; dsb, double-strand break; EGFP, enhanced green fluorescent protein; FBS, fetal bovine serum; GFP, green fluorescent protein; HR, homologous recombination; HRDC, helicase and RNase D C-terminal; LP-BER, long-patch BER; MMC, methyl mitomycin C; MMS, methyl methanesulfonate; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide; PCNA, proliferating-cell nuclear antigen, RQC, C-terminal domain of RecQ; WRNp, Werner protein.

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Figure S2  Intracellular localization of PCNA after DNA damage induced by MMC and MMS
HeLa cells were treated with (A) MMC (0.5 μg/ml) for 16 h and (B) MMS (1 mM) for 1 h, washed once with PBS and then subjected to immunostaining reaction. Cells were fixed and then probed with anti-PCNA antibody (Upstate) and then analysed by fluorescence microscopy in ×100 magnification. Representative images of PCNA foci were captured just after withdrawal of treatment (0 h).

Figure S3  Intracellular localization of endogenous BLM and WRN after DNA damage induced by MMC and MMS
HeLa cells were treated with (A) MMC (0.5 μg/ml) for 16 h and (B) MMS (1 mM) for 1 h, washed once with PBS and then allowed to grow for another 24 h in fresh damage-free medium. Cells were fixed and then probed with anti-WRN and anti-BLM antibody (Santa Cruz) respectively and then analysed by fluorescence microscopy in ×100 magnification. Representative images of WRN and BLM foci were captured just after withdrawal of treatment (0 h) and after recovery (24 h).
Figure S4  Intracellular localization of PCNA after DNA damage induced by H$_2$O$_2$ (250 μM, 30 min)
Localisation of PCNA and GFP-tagged full-length WRN and WRN-HRDC (A), BLM and BLM-HRDC (B) after H$_2$O$_2$ induced DNA damage in HeLa cells overexpressed with the wild-type full-WRN (1–1432), WRN-HRDC (1150–1432), BLM (1–1417) and BLM-HRDC (1212–1417). Cells were treated with 250 μM H$_2$O$_2$, washed once with PBS and then subjected to immunostaining reaction. Overexpressed cells were fixed and then probed with anti-PCNA antibody (Upstate) and then analysed by fluorescence microscopy in × 100 magnification. Representative images of PCNA foci were captured just after withdrawal of treatment (0 h).