The scaffold protein SLX4/FANCP plays a conserved role in early steps of homologous recombination DNA repair

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ABSTRACT

Both in yeast and mammals, the scaffold protein SLX4/FANCP has been implicated in key steps of homologous recombination DNA repair, delivering the structure specific nucleases MUS81, Sae3 and SSAH1 onto DNA repair intermediates such as joint molecules and 3’-normal homologous DNA nicks. Working with the model organism C. elegans, we showed that the minimal component of the SSAH1 orthologs Rad51 for DSB end binding, locating DNA ends and homologous recombination repair is independently required in controlling DSB resection in U2OS human osteosarcoma cells and FANCP patient derived fibroblasts. We also analyzed homologous recombination DNA repair through standard GFP reporter cassette assays and immunofluorescence blot of specific factors. The obtained results indicate that down regulation of SLX4/FANCP limits DSB resection and repair, supporting an important conserved SLX4/FANCP role in early steps of homologous recombination DNA repair, independently of the nucleases MUS81 and SSAH1.

A

B

C

D

working hypothesis: is the SLX4/FANCP positive role in DSB resection conserved in human cells?

FIGURE 1 SLX4/FANCP protein B & its roles in DSB repair
(5) Scheme of the domain structure of SLX4/FANCP and its interacting partners: A20—amphiphilic helix; NLS—200-1800, RPF interaction-like region; RPF: RNA helicase, Tom40 and hHS1 a b c d e f g h i j k l m n o p q r s t u v w x y z
(A) Working model for the size of SLX4/FANCP complex containing the RPF mediated recruitment of Rad51 to generate DSB resection in yeast [Dietlein et al. 2016, Nuclear Acid Res. 44, 21]; and (B) Cartilaginous (C) Various DSB resection models (A) SLX4/FANCP role in DSB resection. (B) SLX4/FANCP role in DSB resection. (C) SLX4/FANCP role in DSB resection.

FIGURE 2 SLX4/FANCP Filaments are sensitive to DSB inducing agents and are defective in end resection.
(A) Schematic of control (C) and DSB induced (I-SceI) conditions used in this study (not to scale). (B) Transmission electron microscopy (TEM) images of control and I-SceI generated DSBs in HeLa cells. Micrographs were imaged 2 hrs after transfection with pBS/PLB/TOPO. Magnification: 20,000x.

FIGURE 3 DBCAL, RAD51, SSBP1 and RIF1 recruitment to DSB in SLX4-depleted U2OS cells
Quantification of SLX4-depleted (U2OS) DBCAL, RAD51, SSBP1 and RIF1 recruitment to DSB in nuclear extracts. Cells were treated with Bleomycin 200U/ml for 2 hrs (A) or incusated with 200U/ml Bleomycin for 2 hrs, then fixed with 2% paraformaldehyde (B). 2 hrs post treatment with Bleomycin was used to isolate DNA repair intermediates such as joint molecules and 3’-normal homologous DNA nicks. Working with the model organism C. elegans, we showed that the minimal component of the SSAH1 orthologs Rad51 for DSB end binding, locating DNA ends and homologous recombination repair is independently required in controlling DSB resection in U2OS human osteosarcoma cells and FANCP patient derived fibroblasts. We also analyzed homologous recombination DNA repair through standard GFP reporter cassette assays and immunofluorescence blot of specific factors. The obtained results indicate that down regulation of SLX4/FANCP limits DSB resection and repair, supporting an important conserved SLX4/FANCP role in early steps of homologous recombination DNA repair, independently of the nucleases MUS81 and SSAH1.