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

DIPARTIMENTO DI BIOSCIENZE

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ABSTRACT

Both in yeast and mammals, the scaffold protein SLX4/FANCP has been implicated in late steps of homologous recombination DNA repair, delivering the structure specific nucleases MUS81, SLX1 and XPF/RAD1 onto DNA repair intermediates (such as joint molecules and 3' non homologous DNA flap). Working with the model organism *S. cerevisiae*, we showed that Slx4 competes with the 53BP1-ortholog Rad9 for DSB end binding, favoring DNA end resection and homologous recombination repair. To investigate a possible conservation of the pathway, we exploited the AsiSI restriction enzyme and Cas9-based systems to study SLX4 role 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 foci 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 XPF.

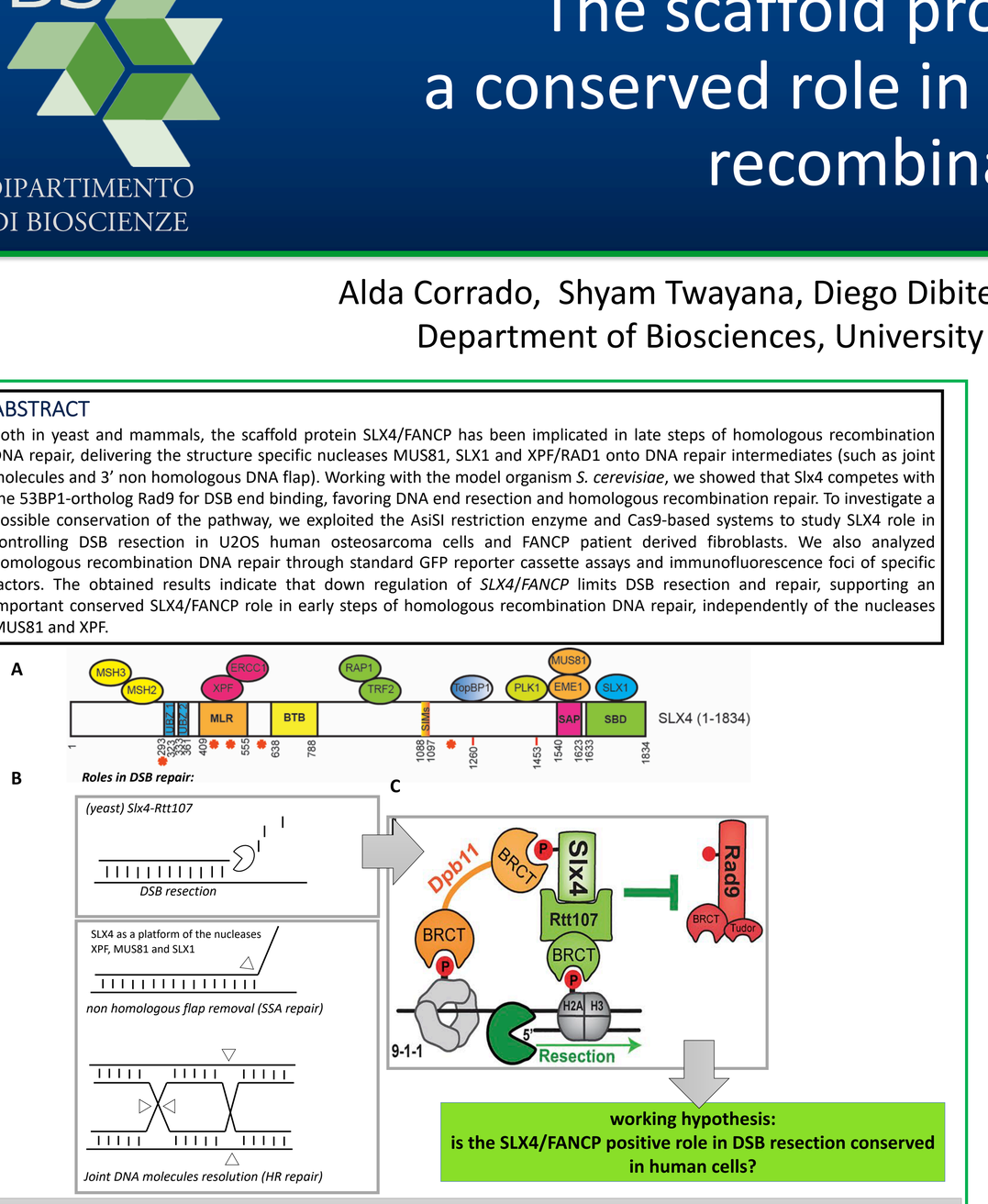


FIGURE 1_**SLX4/FANCP protein & its roles in DSB repair**

A) Scheme of the domain structure of SLX4/FANCP and its interacting partners. UBZ: ubiquitin-binding zinc finger motif; MLR: MEI9, XPF interaction Like Region; BTB: Broad-complex, Tramtrack and Bric a brac domain; SIM: SUMO-Interacting Motif; SAP: SAF-A/B, Acinus and PIAS domain; SBD: SLX1 Binding Domain. Red asterisks indicate mutated aminoacids that were recently identified in aplastic anemia patients.

B) Schematic representation of the proposed roles for SLX4/FANCP in DSB repair.

C) A working model for the role of Slx4-Rtt107complex counteracting the Dpb11-mediated recruitment of Rad9 to promote DSB resection in yeast (Dibitetto et al 2016. *Nucleic Acids Res*. 44(2): p669-82; Liu et al. 2017 *J. Cell Biol*. 216(3): p623-39)

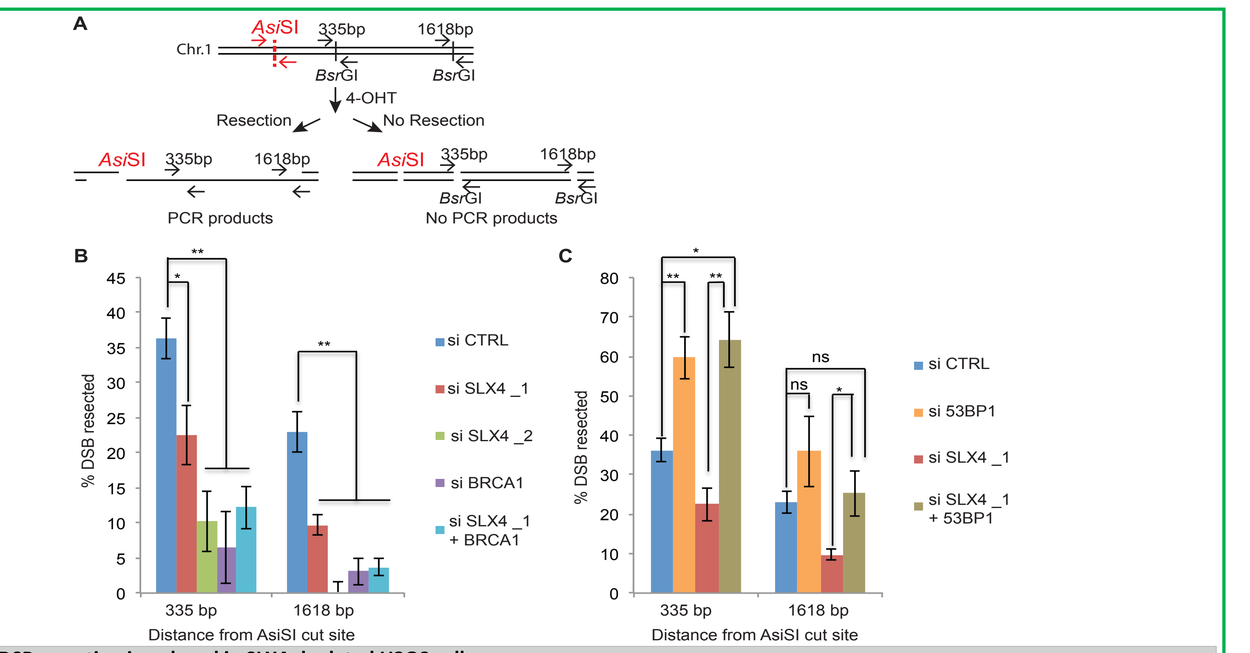
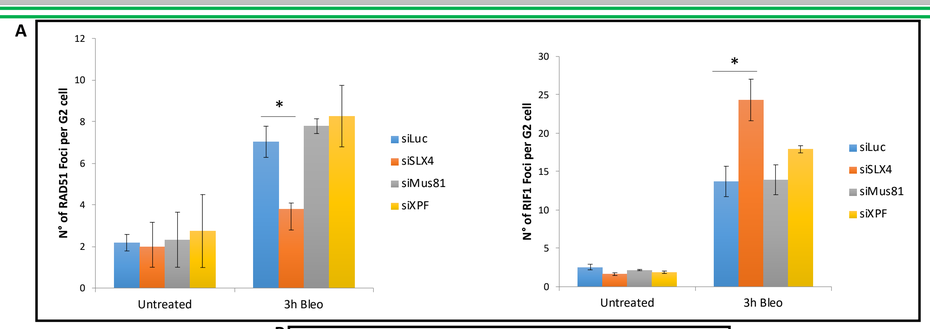


FIGURE 4_**DSB resection is reduced in SLX4-depleted U2OS cells**

A) Scheme for the resection quantification at AsiSI site in U2OS-AsiSI-ER cells (according to *Zhou et al* (2014). *Nucleic Acids Res.* 42, e19, with some modifications). **B-C)** Cells silenced with the indicated siRNAs and treated with 4OHT for 6hr were used to extract genomic DNA and calculate % DSB resected (see *Ferrari et al*., *Methods Molecular Biology* (2017), Vol. 1672, Genome Instability, Methods and Protocols, Springer Protocols -ISBN 978-1-4939-7306-4). Data are shown as mean ± S.E.M from at least three independent experiments. Two-tailed unpaired student's T test was performed for statistical analysis (** P<0.01, * P<0.05, ns p>0.05).



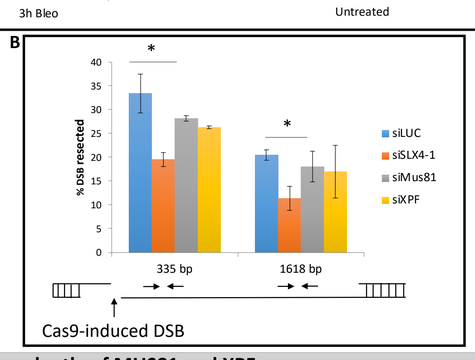


FIGURE 5_**SLX4 promotes resection independently of MUS81 and XPF**

A) Quantification of RAD51 and RIF1 foci in *SLX4*, *MUS81*, *XPF* or control silenced cells, either untreated or after treatment with Bleomycin 20 μg/ml for 3hrs. Data are shown as mean ± SDfrom at least three independent experiments. Two-tailed unpaired student's T test was performed for statistical analysis (* P<0.05). **B)** U-2OS-SEC cells (stably expressing Cas9,under the tetracycline-inducible element and generously provided by John Rouse, MRC, Dundee, UK) were silenced with indicated siRNAs for 48hrs and then doxycycline was added for 24hrs to induce Cas9 expression. Genomic DNA was extracted and the % of DSB resected was calculated with Droplet Digital PCR, according to *Dibitetto et al*. (DNA Repair 68 (2018) 68–74) . The means ± SD of the % DSB resected of two independent experiments are plotted. Two-tailed unpaired student's T test was performed for statistical analysis (* P<0.05).

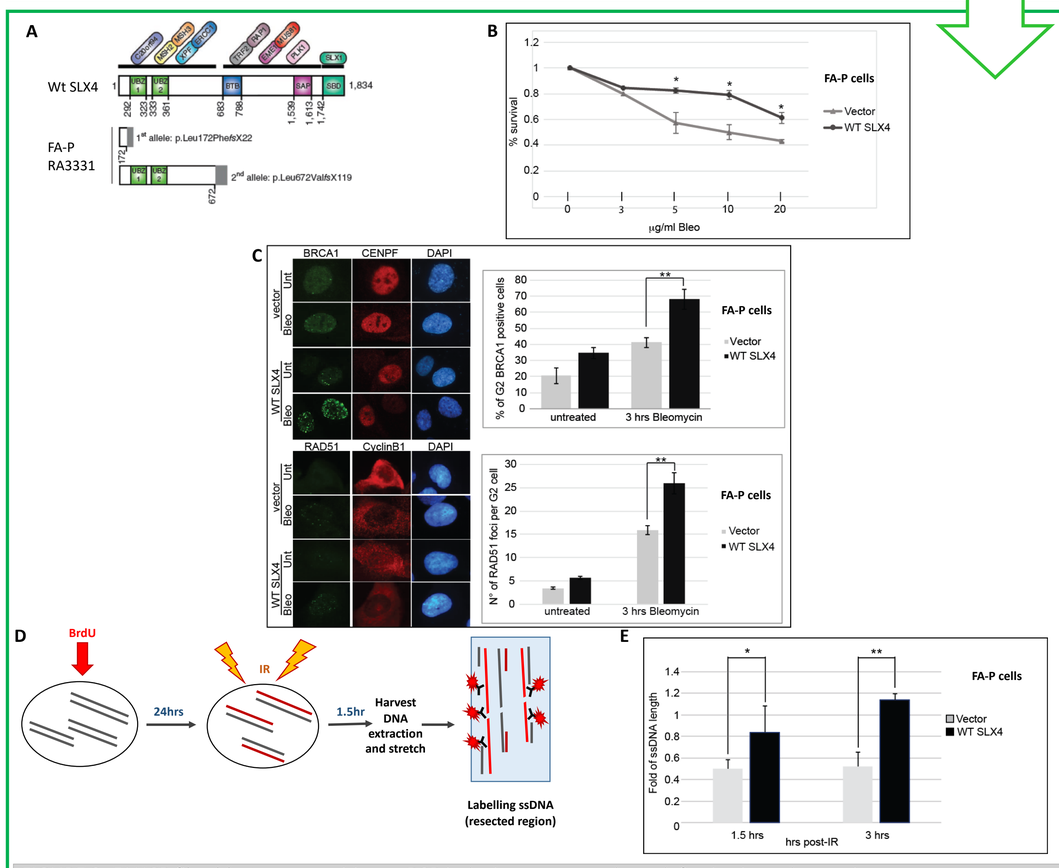


FIGURE 2_FANCP fibroblasts are sensitive to DSB inducing agents and are defective in end resection.

A) Schematic of SLX4 and the predicted protein effect of *SLX4/FANCP* mutations in FA-P RA3331 fibroblasts (generously provided by A. Smogorzewska, Rockefeller University of NY, *Kim et al* 2011, *Nature Genet* 43(2): p142-46) **B)** Bleomycin sensitivity of FA-P cells either transformed with an empty vector or complemented with wtSLX4 **C)** Immunodetection (left) and quantification (right) of BRCA1 and RAD51 foci in FA-P and FA-P complemented cells, either untreated of after 3hrs treatment with Bleomycin 20 μg/ml. Data are shown as mean ± SD from at least three independent experiments. **D)** Schematic workflow of the SMART (Single-Molecule Analysis of Resected Tracks), according to Cruz-Garcia et al 2014. *Cell Reports* 9, p451-59. **E)** The FA-P cells were incubated with BrdU for 24 hrs, then irradiated (10Gy) and after 1.5 and 3 hrs SMART was performed by S. Aliprandi and FM Navarro in Huertas's lab (CABIMER, Sevilla). For each sample 300 ssDNA fibers were measured, then the medium of the fibers lenght was calculated and each medium of treated sample was divided for the median of the untreated sample (** P<0.01, * P<0.05).

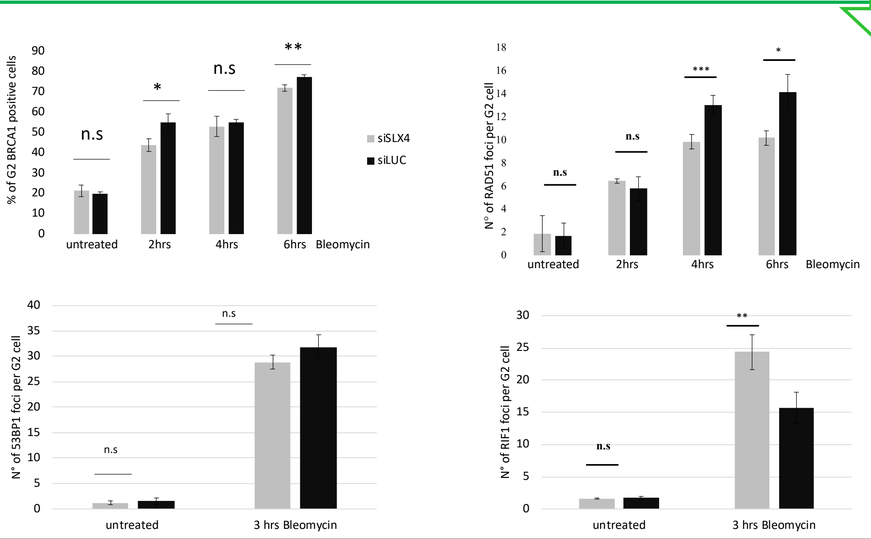


FIGURE 3_BRCA1, RAD51, 53BP1 and RIF1 recruitment to DSB in SLX4-depleted U2OS cells. Quantification of BRCA1, RAD51, 53BP1 and RIF1 foci in SLX4 or control silenced cells, after treatment with Bleomycin 20 μg/ml, for the indicated times. Data are shown as mean ± SD from at least three independent experiments. Two-tailed unpaired student's T test was performed for statistical analysis (***P<0.001, ** P<0.01, * P<0.05, ns p>0.05).

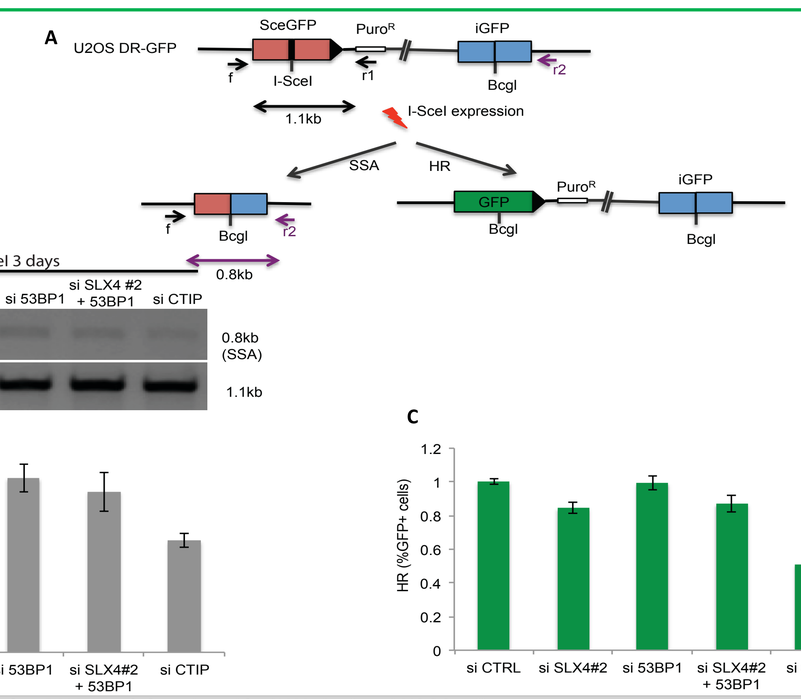
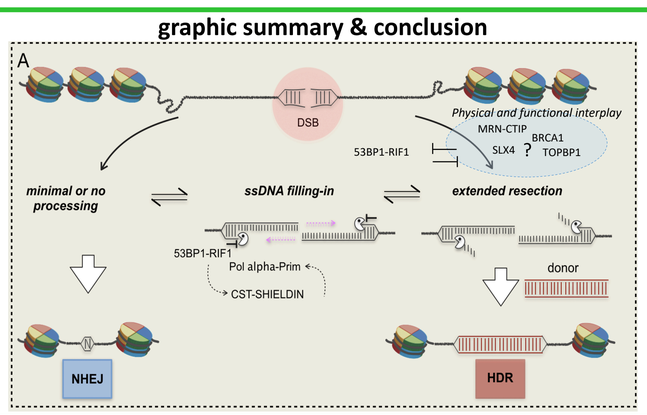


FIGURE 6_**Homologous recombination DNA repair is reduced in SLX4-depleted U2OS cells**

A) Schematic workflow of the SSA/GC report assay, according to Ochs et al . 2016. *Nat Struct Mol Biol* 23(8): p714-21.

B-C) Cells transfected with indicated siRNA and plasmid expressing I-SceI or empty vector were collected after three days. Genomic DNA was extracted and used as PCR template for two pairs of primers as indicated in A. After running PCR product on 1% gel, SSA bands (from f and r2) were quantified using Image J. Quantification of SSA bands is shown as gray bars, according to Ochs et al. Part of the cells were analysed by flow cytometry to measure GFP+ cells corresponding to HR, which are shown as green bars in C. SD calculated from three independent experiments is shown.

graphic summary & conclusion



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