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Fanconi anemia pathway: an unexpected player in the error-free repair of Cas9-induced DNA double strand breaks

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Abstract

When using the CRISPR-Cas9 system for genome editing, Cas9 introduces a DNA double strand break (DSB) at a specific locus in the genome, which can be repaired by two distinct pathways: error-prone non-homologous end-joining (NHEJ) or error-free homology-directed repair (HDR). To improve the efficiency of introducing precise modifications into the genome, the balance has to be shifted from NHEJ to HDR after Cas9-induced DSB-introduction.

Surprisingly, results from recently performed CRISPR-inhibition screens imply that the Fanconi anemia pathway, which is known to be responsible for the repair of interstrand crosslinks, is involved in HDR of Cas9-induced breaks (Figure 1)[1,2]. In a current model explaining the involvement of the Fanconi anemia pathway in Cas9-gene editing, the Fanconi anemia repair pathway acts as a "traffic signal" that directs the repair outcomes towards homology-directed repair, thereby shifting the balance from NHEJ to error-free repair[1]. This would make components of the Fanconi anemia repair pathway ideal candidates for manipulating the balance between error-prone and error-free repair.

In addition to resolving interstrand crosslinks, several Fanconi anemia proteins have been recently shown to play a role in the metabolism of R-loops, DNA:RNA hybrids that are generated upon hybridization of an RNA-molecule with double-stranded DNA[3–6]. Intriguingly, there are several reports describing that the structure of Cas9 together with gRNA, target DNA and repair template closely resembles the structure of an R-loop[7–10]. In this project, we would like to learn more about the involvement of Fanconi anemia proteins in the repair of Cas9-induced lesions and potentially find mechanisms how to use this information to increase HDR-efficiency in CRISPR-Cas9 genome editing.

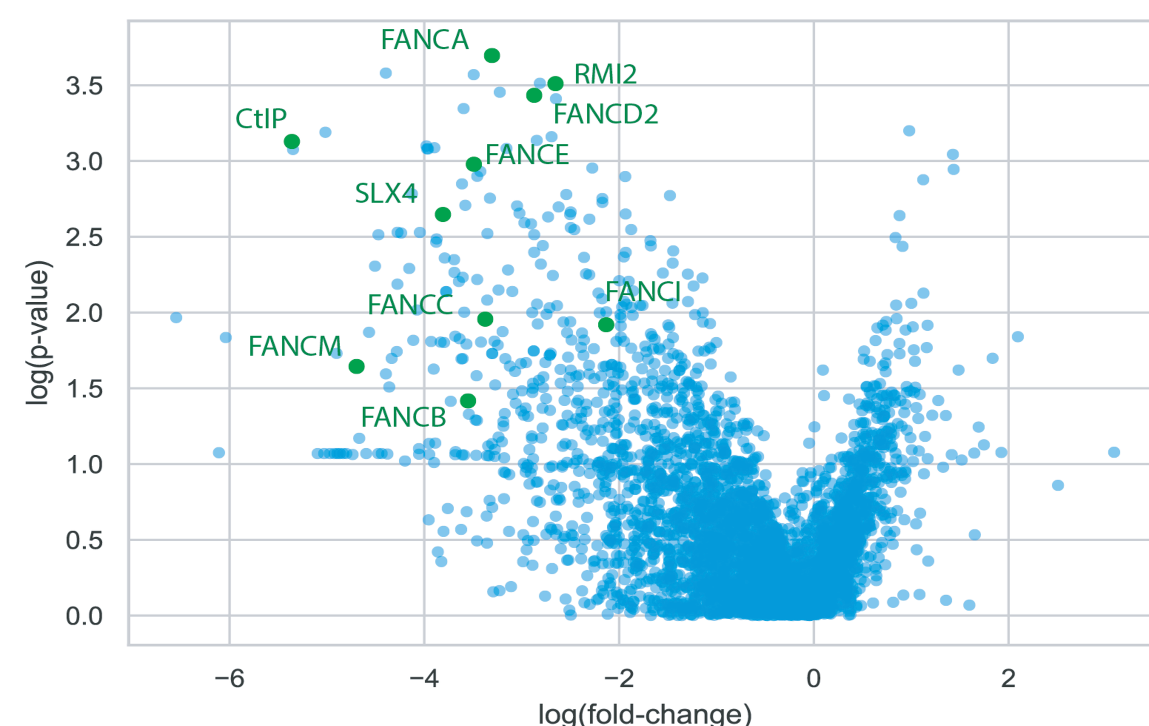


Figure 1: **Homology-directed repair (HDR) efficiency is decreased upon knock-down of Fanconi anemia genes.** Adapted from Richardson et al, 2018[1]. Fanconi anemia genes are highlighted in green.

The Fanconi anemia repair pathway

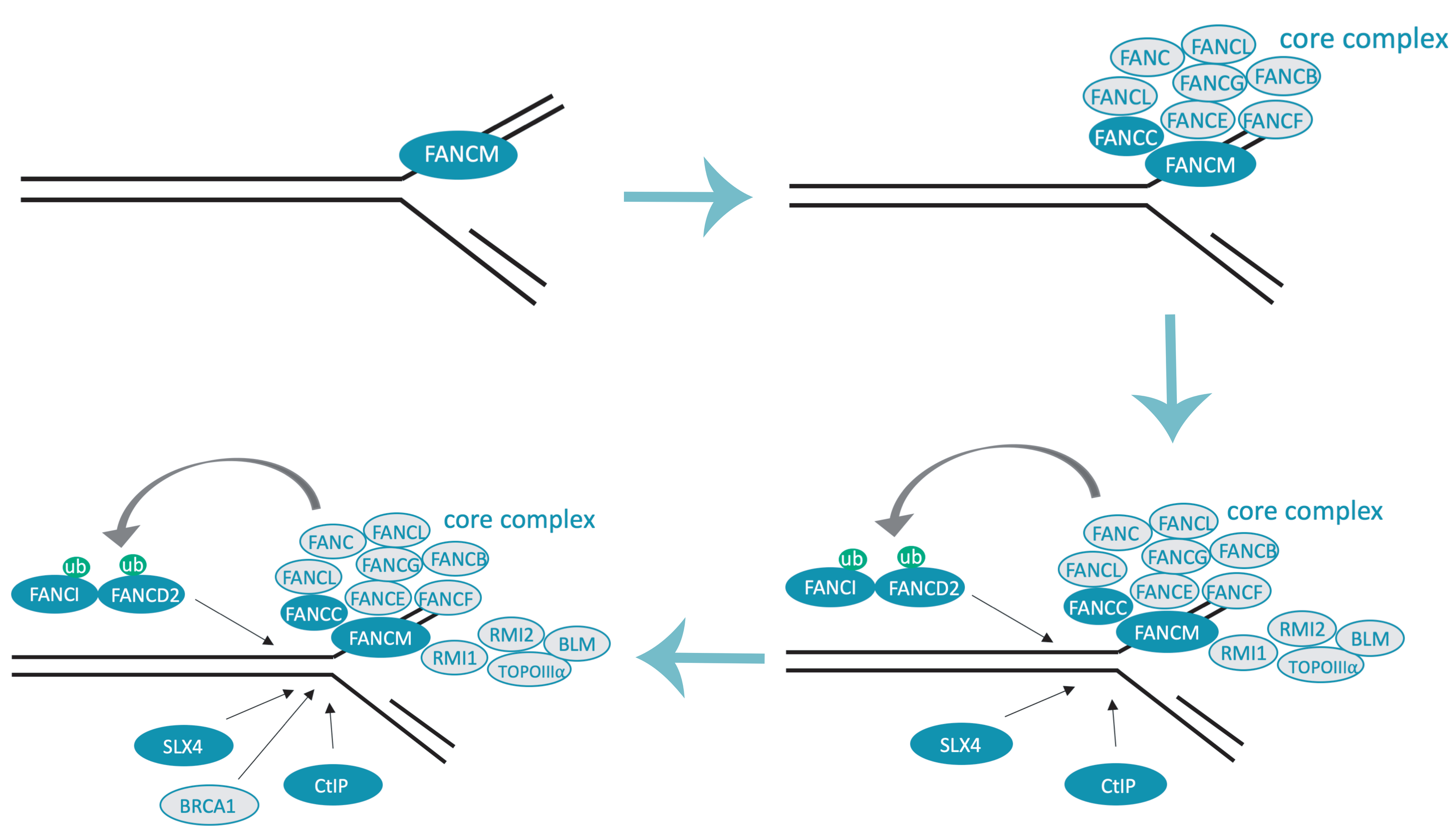
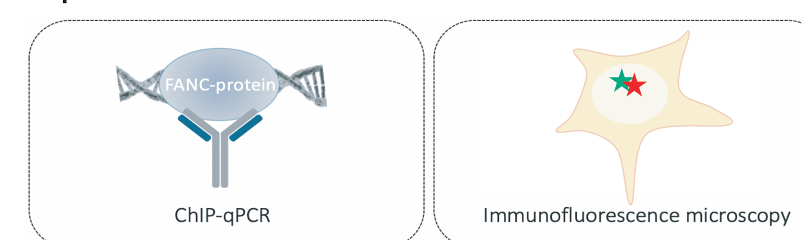


Figure 2: **A schematic of the Fanconi anemia repair pathway.** To date, 22 Fanconi anemia proteins have been described. FANCM is the first Fanconi anemia protein that recognizes the interstrand crosslink and recruits the Fanconi anemia core complex. Binding of the core complex triggers ubiquitination of the FANCID2-heterodimer, a central event of the Fanconi anemia pathway. After removing the interstrand crosslink, the resulting DSB is repaired by HDR. Adapted from Deans et al, 2011[11]. Fanconi anemia proteins shown in blue will be analyzed in the following points.

Aim 1: Confirm the recruitment of Fanconi anemia proteins to the DSB and analyze their influence on DSB repair pathway choice

a. Are Fanconi anemia proteins localized to Cas9-induced DNA double-strand breaks?



b. Is homology-directed repair (HDR) efficiency altered upon knock-out of Fanconi genes?

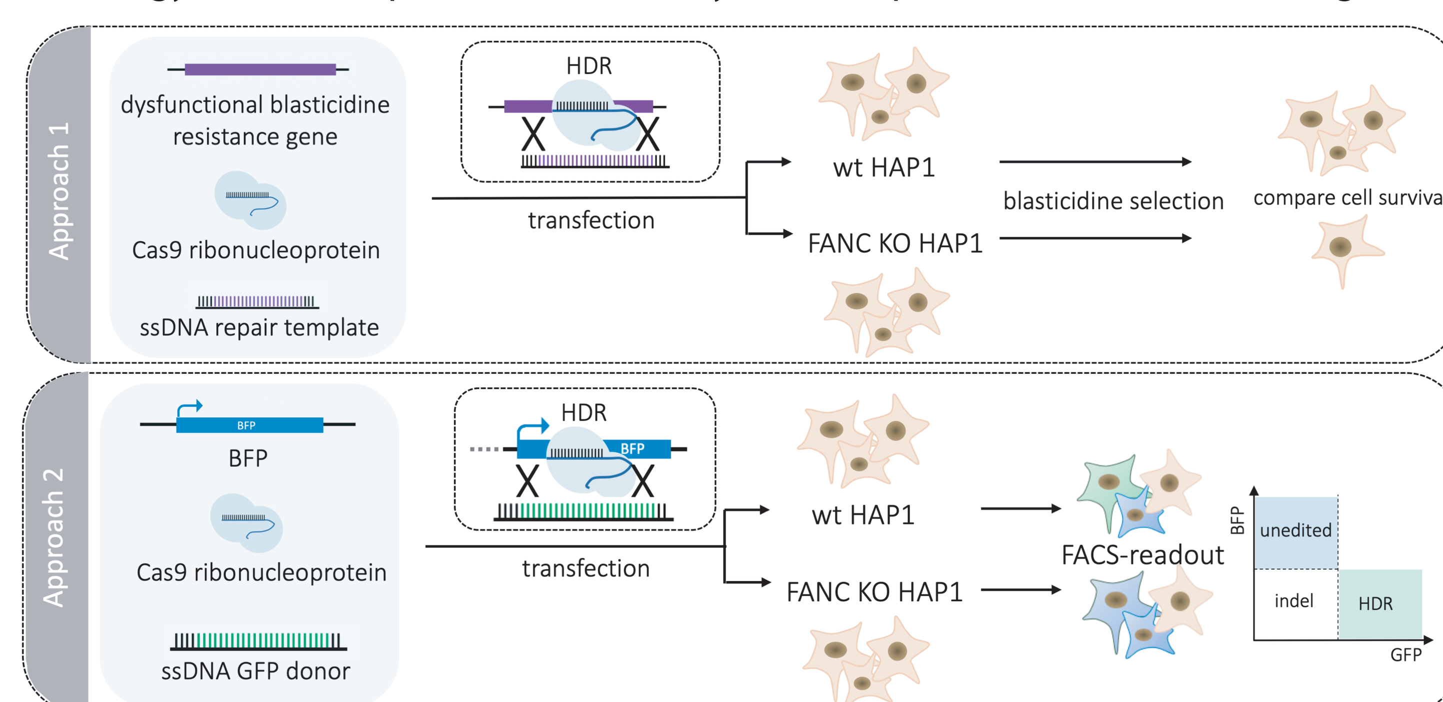


Figure 3: **Planned experimental procedures to confirm the recruiment of Fanconi anemia proteins to the DSB and influence their influence on DSB repair pathway choice.** a. We will determine whether Fanconi anemia proteins are localized to Cas9-induced DSBs by two complementary methods chromatin-immunoprecipitation (ChIP)-qPCR and immunofluorescence microscopy using antibodies against Fanconi anemia proteins and proteins known to localize to DSBs (e.g. γH2AX). b. Using two complementary approaches, we will ask how knock-out of indiviual Fanconi anemia genes affects HDR of Cas9-induced DSBs.

Aim 2: Dissect the involvement of Fanconi anemia proteins in the repair of Cas9-induced DSBs

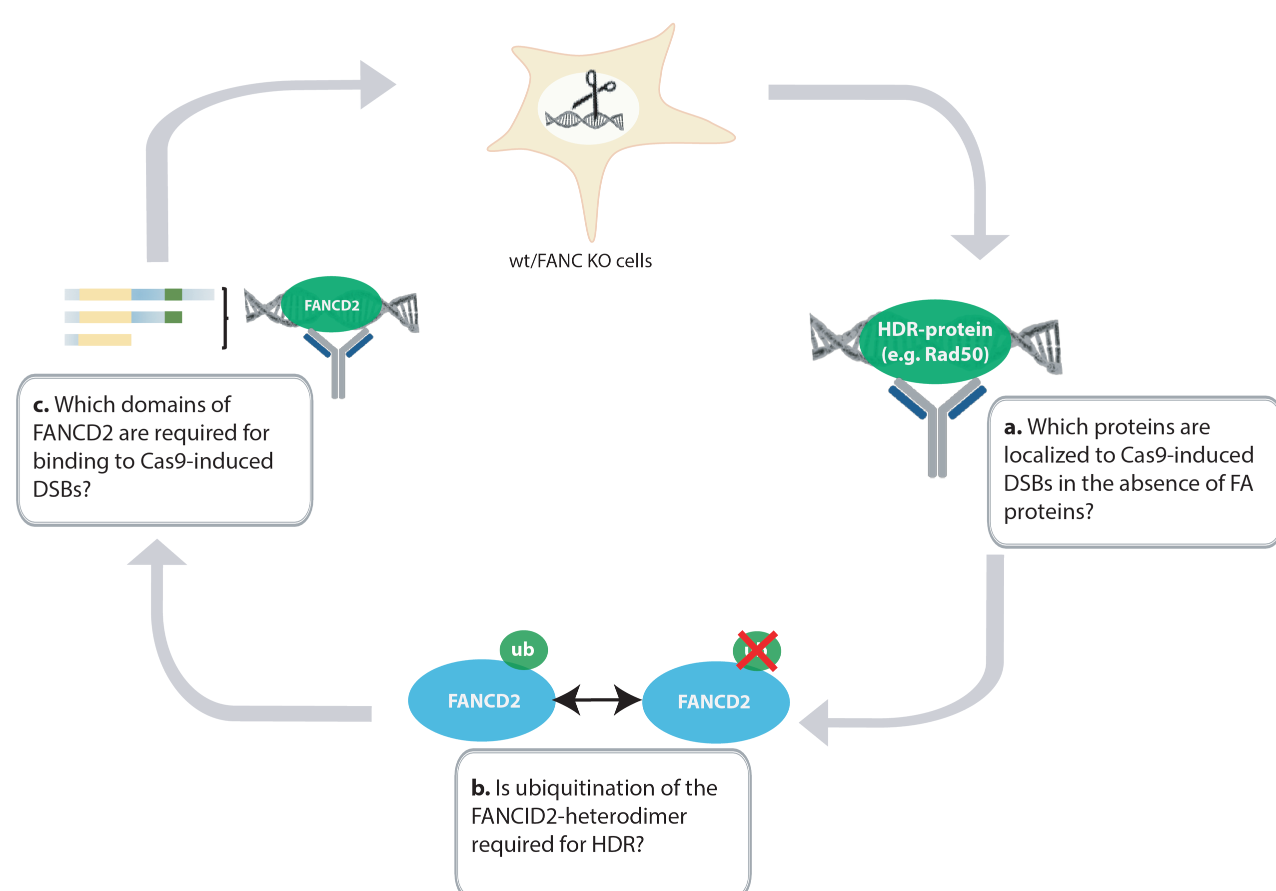
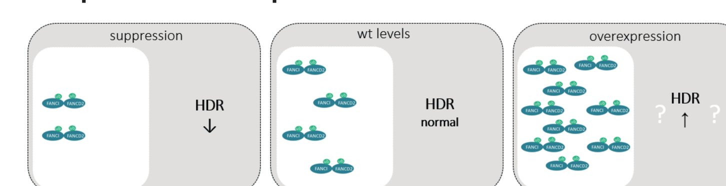


Figure 4: **Planned experimental procedures to dissect the involvement of Fanconi anemia proteins in the repair of Cas9-induced DSBs.** a. Using ChIP-qPCR, we will ask which repair proteins are localized to Cas9-induced DSBs in Fanconi anemia knock-out (KO) cell lines. b. By comparing HDR-efficiency between FANCD2 KO cell lines reconstituted with wt FANCD2 and FANCD2 KO cell lines reconstituted with monoubiquitination-dead FANCD2, we will ask whether ubiquitination of the FANCID2-heterodimer is required for HDR. c. By expressing different domains of FANCD2 in a FANCD2 KO background, we will ask which domains of FANCD2 are required for binding to Cas9-induced DSBs.

Aim 3: Using the Fanconi anemia pathway as a tool to increase HDR-efficiency after CRISPR-Cas9 editing

a. Is HDR-efficiency increased upon overexpression of FANCI and FANCD2?



b. What is the signal that recruits Fanconi anemia proteins to Cas9-induced DSBs and can we optimize repair template design to increase HDR-efficiency?

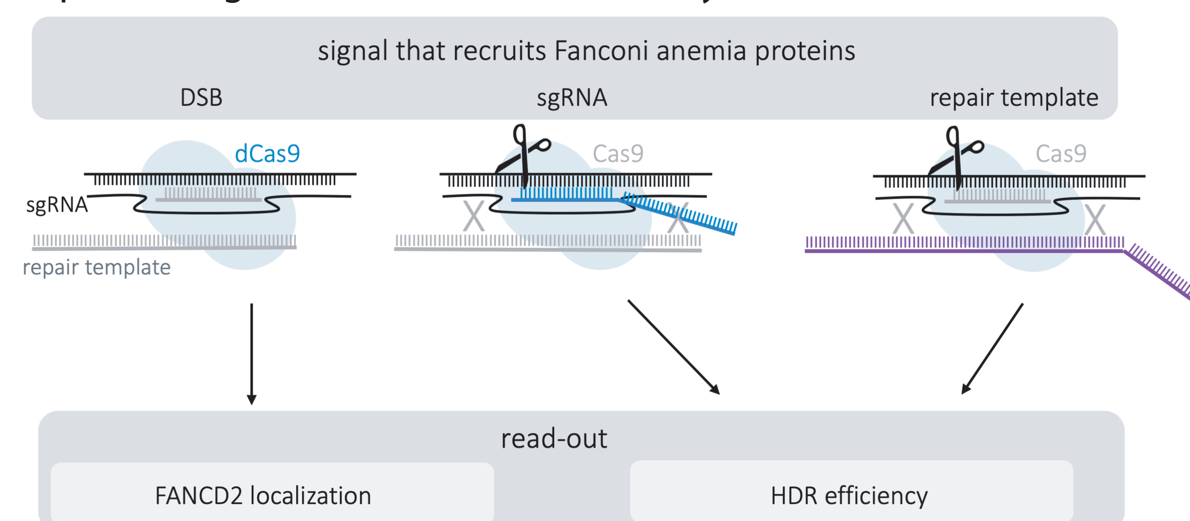


Figure 5: **Planned experimental procedures which will enable us to learn whether we can use the Fanconi anemia pathway as a tool to increase HDR-efficiency after CRISPR-Cas9 editing,.** a. We know that suppression of Fanconi anemia proteins results in decreased HDR-efficiency. We would like to know whether we can increase HDR-efficiency upon overexpression of FANCI and FANCD2. b. Fanconi anemia proteins could bind to the DSB itself, to the sgRNA or to the repair template. By assaying HDR-efficiency upon altering the set-up of the ribonucleoprotein in complex with target DNA and repair template, we will test these different models.

References. 1. Richardson, C. D. et al. CRISPR–Cas9 genome editing in human cells occurs via the Fanconi anemia pathway. Nat. Genet. 50, 1132–1139 (2018). 2. Wienert, B. et al. Timed inhibition of CDC7 increases CRISPR-Cas9 mediated templated repair. bioRxiv (2018). 3. García-Rubio, M. L. et al. The Fanconi Anemia Pathway Protects Genome Integrity from R-loops. PLoS Genet. 11, 1–17 (2015). 4. Hatchi, E. et al. BRCA1 recruitment to transcriptional pause sites is required for R-loop-driven DNA damage repair. Mol. Cell 57, 636–647 (2015). 5. Schwab, R. A. et al. The Fanconi Anemia Pathway Maintains Genome Stability by Coordinating Replication and Transcription. Mol. Cell 60, 351–361 (2015). 6. Liang, Z. et al. Binding of FANCI-FANCD2 Complex to RNA and R-Loops Stimulates Robust FANCD2 Monoubiquitination. Cell Rep. 26, 564–572.e5 (2019). 7. Thompson, A. J., Nogales, E. & Doudna, J. A. Structures of a CRISPR-Cas9 R-loop complex primed for DNA cleavage. Science (80-.). 351, 867–871 (2016). 8. Rutkauskas, M. et al. Directional R-loop formation by the CRISPR-cas surveillance complex cascade provides efficient off-target site rejection. Cell Rep. 10, 1534–1543 (2015). 9. Szczelkun, M. D. et al. Direct observation of R-loop formation by single RNA-guided Cas9 and Cascade effector complexes. Proc. Natl. Acad. Sci. 111, 9798–9803 (2014). 10. Xiao, Y. et al. Structure Basis for Directional R-loop Formation and Substrate Handover Mechanisms in Type I CRISPR-Cas System. Cell 170, 48–60.e11 (2017). 11. Deans, A. J. & West, S. C. DNA interstrand crosslink repair and cancer. Nat rev Cancer 11, 467–480 (2011).