Protein identification during meiosis DSB homologous repair

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Introduction & Objective

- Cells induce programmed double-strand breaks (DSBs) during meiosis to promote genetic material exchange and accurate homolog segregation.
- DSB repair by homologous recombination (HR) is a highly regulated pathway which involves several proteins.
- High conservation of proteins involved in HR between budding yeast and mammals.

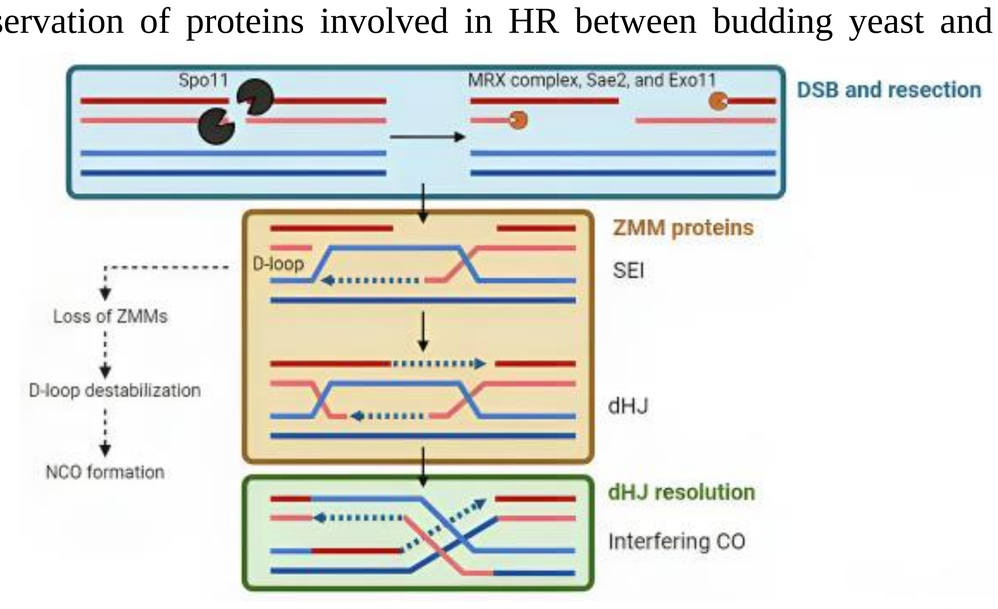

Figure 1. Schematic representation of the double Holliday junction pathway resolution ending with an interfering crossover (CO). ZMM proteins are involved in the stabilization and formation of the intermediates of this pathway: SEI (single-end invasion) and dHJ (double Holliday junction). dHJ resolution is promoted by the complexes MutSγ and MutLγ (modified from Pyatniskaya 2019).

Materials and Methods

iPOND methodology was chosen to identify the actors involved in the DNA synthesis during meiotic homologous recombination in *S. cerevisiae*.

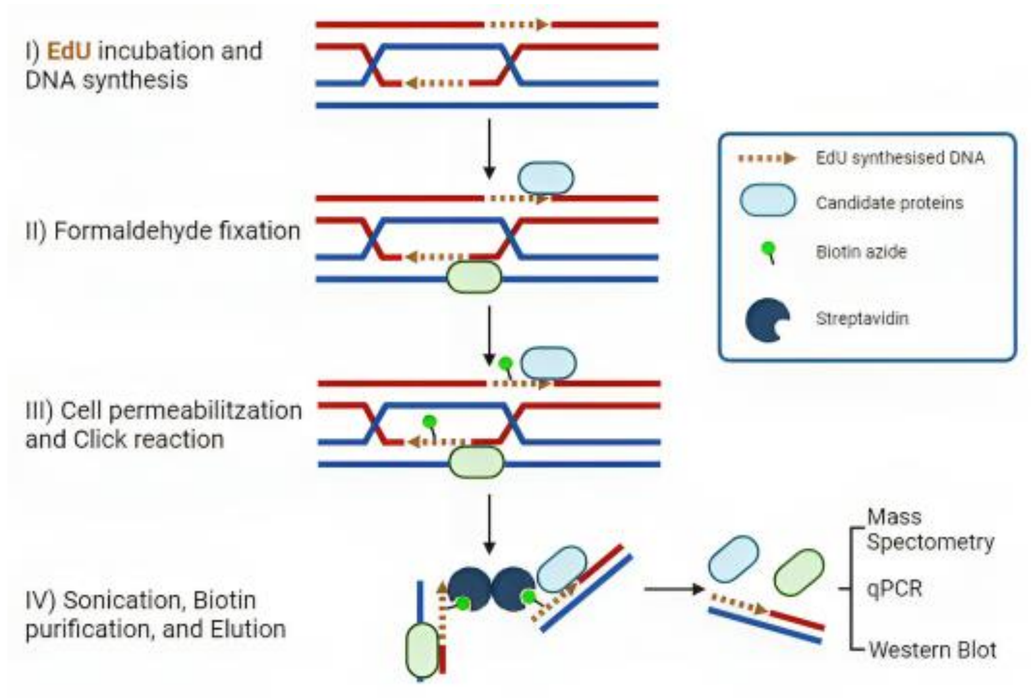

Figure 2. Schematic representation of the main steps involved in the iPOND purification system. I-III steps are intracellular. IV is an extracellular step.

Our aim is to identify and characterise the proteins involved in DNA synthesis during meiotic HR.

Results

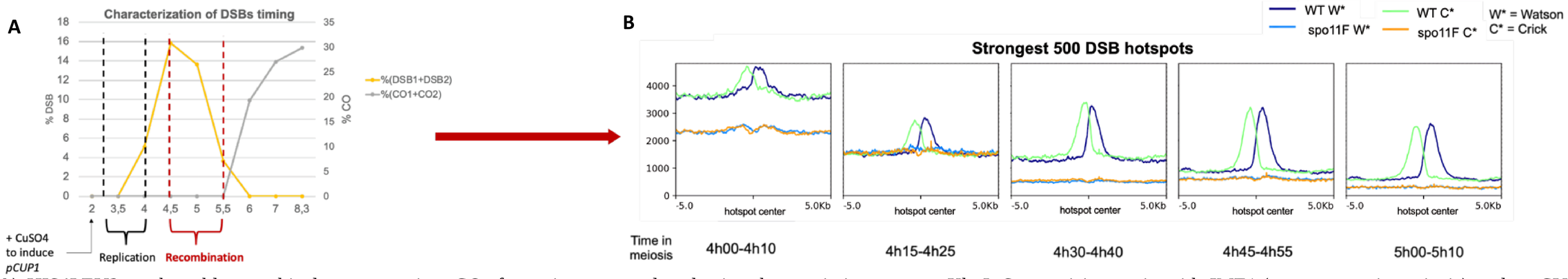

Figure 3. A) *HIS4LEU2* southern blot graphical representation. COs formation was analysed using the restriction enzyme XhoI. *S. cerevisiae* strain with *IME1* (master gene in meiosis) under pCUP1 promoter was used to synchronise meiosis events. **B)** EdU-seq at different time points during meiotic recombination of the strongest 500 DSB sites in *S. cerevisiae*. Time points represent the incubation time with EdU. Watson and Crick refer to each of the DNA strands.

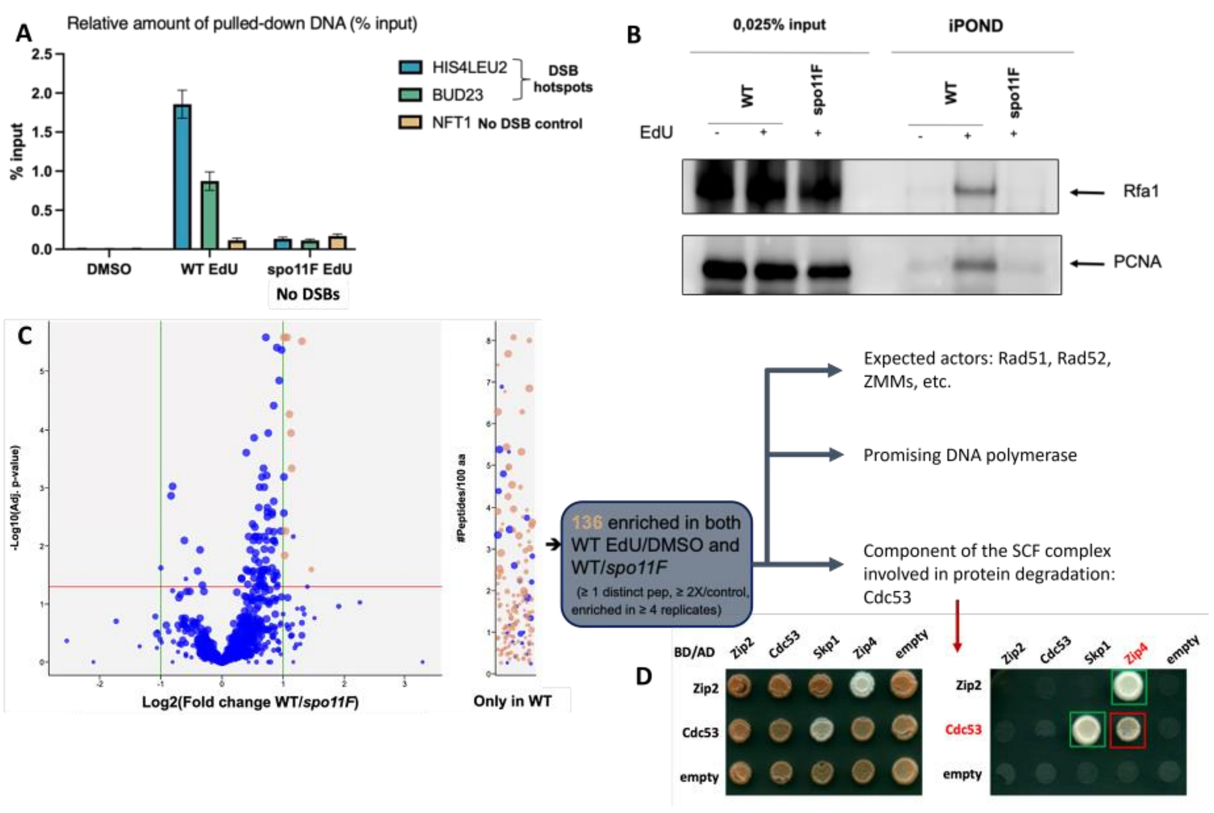

Figure 4. A) qPCR of the newly synthesised DNA. Different controls were used to assure that the DNA pulled-down belongs to the nascent DNA from the DSBs. **B**) Western blot, antibodies against DNA binding proteins (Rfa1 and PCNA) to determine the capacity to pull-down nascent DNA associated proteins. **C)** Identification of proteins associated with DSB homologous repair by mass spectrometry. Comparison between WT strains and strains not cultivated with EdU (DMSO) and strains not able to generate DSBs (*spo11F*). **D)** Two yeast hybrid assay, the SCF member Cdc53 and the meiotic crossover protein Zip4 interact. Skip1 and Cdc53 were used as control positive and negative, respectively for Cdc53.

Conclusions

- There is a temporal separation between meiotic replication and HR.
- iPOND methodology enables to purify proteins involved in the HR process in an unbiased way.
- Two promising candidates, found in iPOND during HR, will be under further analysis.

What comes next?

Characterization of the two candidates and their involvement in homologous recombination repair:

- Cdc53
  - Tag pull-down
  - Yeast double-hybrid
- Promising DNA polymerase
  - ChiP to validate its location to DSBs during homologous recombination in meiosis.
- Consequences of its conditional depletion for meiotic recombination.
  - *cdc53-AID*
  - *pol1-AID*

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The authors declare no conflict of interest