UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XLII CICLO

Research Topic ID: XLII – 1.10

Project Tutor: Maria Pia Longhese

Project Supervisor: Diego Bonetti

Project Title: DNA damage processing and checkpoint control at DNA double-strand breaks

Scientific background & Objectives

DNA double-strand breaks (DSBs) are highly cytotoxic lesions that, if unrepaired or misrepaired, cause genome instability and chromosomal rearrangements. In eukaryotes, DSBs are repaired mainly by non-homologous end joining, which directly ligates DNA ends, or by homologous recombination, which uses an intact homologous template. A key prerequisite for HR is 5′-3′ DNA end resection, which generates 3′ single-stranded DNA tails that promote strand invasion. In budding yeast, resection occurs in two steps: the Mre11-Rad50-Xrs2 (MRX) complex with Sae2 initiates processing by clipping the 5′-terminated strands near the break, while long-range resection is extended by the nucleases Exo1 and Dna2. DSB repair is coordinated with cell-cycle progression through checkpoint activation by Tel1/ATM and Mec1/ATR kinases. This project aims to define how DSB processing is regulated and how resection is mechanistically linked to checkpoint signaling in *Saccharomyces cerevisiae*.

Project's Networks, Sustainability & Mobility

a) the coherence of the suggested project with competences/tools of the hosting lab
b) intradepartmental or external collaborations
c) at least one pertinent research article published by the proposer/s
d) 1 (or more) putative foreign institutions for achieving the required ordinary mobility (6 months)

a) This project is fully coherent with the host lab's long-standing expertise in the DNA damage response in *Saccharomyces cerevisiae*, combining yeast genetics, molecular biology, and checkpoint/resection assays to dissect mechanisms linking DSB processing and signaling.

b) Collaborations are active and/or will be strengthened with groups focusing on DNA damage signaling, genome stability, and replication stress. Potential partners include Marco Muzi-Falconi (University of Milan), Marco Foiani (IFOM/University of Milan/CNR), and Fabrizio d'Adda di Fagagna (IFOM). These interactions will provide complementary expertise and access to specialized approaches, supporting mechanistic analyses of checkpoint activation/attenuation and quantitative DNA end resection readouts, and broadening the experimental toolkit for the proposed aims.

c)

Colombo CV, Casari E, Gnugnoli M, Corallo F, Tisi R, Longhese MP. Functional and molecular insights into the role of Sae2 C-terminus in the activation of MRX endonuclease. Nucleic Acids Res. 2024 52:13849-13864. doi: 10.1093/nar/gkae1049.

Casari E, Gnugnoli M, Pizzul P, Tisi R, Longhese MP. Sae2 integrates CDK and checkpoint phosphorylation to coordinate MRX cleavage with checkpoint attenuation. Commun Biol. 2025 9:144. doi: 10.1038/s42003-025-09424-7.

Casari E, Corallo F, Milani LE, Tisi R, Longhese MP. Stn1 supports Mec1 function in protecting stalled replication forks from degradation. PLoS Genet. 2025 21:e1011917. doi: 10.1371/journal.pgen.1011917.

d) Potential host laboratories include Francisca Lottersberger (Sweden), Petr Cejka (Switzerland), and Corrado Santocanale (Ireland). The final choice will be made based on the best alignment with the specific experimental needs emerging from the results.