UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

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

Research Topic ID: XL – 1.6

Proponent: Prof. Michela Clerici

Project Title: Exploring the influence of chromatin structure and remodelling on DNA repair in *Saccharomyces cerevisiae*

Scientific background and 'open issues'

The genome of living organisms is continuously exposed to DNA damage, which can result in the loss of genetic information and/or genetic instability if not properly repaired. Eukaryotic cells counteract DNA lesions by activating a DNA damage response (DDR), capable of detecting the lesions and promoting their proper and efficient repair. Hereditary defects in the DDR cause a variety of human diseases associated with cancer predisposition [1].

DNA insults elicit both DNA repair and a DNA damage checkpoint, which couples DNA repair with cell cycle progression. Furthermore, specialized repair mechanisms are activated for proper repair of the damage, depending on the type of DNA lesion and the phase of the cell cycle in which the damage is generated [2]. The protein kinases ATM and ATR (Tel1 and Mec1 in *Saccharomyces cerevisiae*, respectively) recognize aberrant DNA structures and phosphorylate a plethora of targets in both repair and checkpoint pathways, thus orchestrating the DDR [1,2].

DNA damage recognition and repair occur in the context of chromatin, a highly organized structure where DNA is wrapped around an octamer of four core histones: H2A, H2B, H3, and H4, forming the nucleosome [3,4,5]. Nucleosomes impose a barrier to the repair machinery, and nucleosomal organization around a DNA lesion must be disrupted to allow its repair [4,6]. This is achieved both by removing entire nucleosomes thanks to the action of chromatin remodelling factors and/or by modifying histones, thus increasing chromatin flexibility and accessibility of repair enzymes to damaged sites [4,5]. Histones are subjected to a vast array of post-translational modifications (PTMs) such as phosphorylation, acetylation, methylation, and ubiquitylation [7]. Histone PTMs either directly influence the overall structure of chromatin or regulate (positively or negatively) the binding of effector molecules, which specifically interact with modified histones via specific domains [6,7].

Extensive work has been carried out to define the role of histone dynamics in the DDR in both the model organism *S. cerevisiae* and in mammalian cells. However, a comprehensive view of histone PTMs in the vicinity of DNA lesions and their effects on the choice of DNA repair pathway is still incomplete.

Objectives

Despite extensive efforts, our knowledge of DNA damage-induced chromatin modifications and their role in modulating DNA repair pathway choice and coordinating checkpoint and

repair mechanisms is still fragmented. A comprehensive understanding of histone PTMs in the vicinity of DNA lesions and their effects on the DDR is necessary to elucidate the contribution of histone modifications in controlling DNA metabolism machineries and to anticipate the effects of targeting histone modifiers in cancer therapy.
By using the yeast *Saccharomyces cerevisiae* as a eukaryotic model, we aim to define both the role of histone PTMs in the DDR and the chromatin landscape at DNA lesions.

Methodologies
In both yeast and mammals, histone PTMs participate in the DDR by acting at different levels and supporting various pathways for DNA repair. However, a comprehensive understanding of chromatin modifications occurring in the vicinity of a DNA lesion and the contribution of histone PTMs to DNA repair, DNA repair pathway choice, and the coordination of checkpoint and repair in the DDR, is still elusive.
We propose to investigate the role of histone PTMs in the DDR using the budding yeast *S. cerevisiae* as a eukaryotic model organism. Yeast combines precise genetic manipulability with the ability to perform genome-wide high-throughput genetic screens, along with high levels of conservation of DDR proteins and histone modifiers. Additionally, histone-modifying enzymes are well-conserved but less redundant in yeast compared to mammals, simplifying the investigation of the effects caused by histone modifier inactivation.
We will employ a combination of genetic, molecular biology, and biochemical studies to:
i) Identify novel interactions among histone PTMs and DNA repair factors;
ii) Contribute to building a comprehensive landscape of histone PTMs around a DNA lesion.
These experimental approaches could be either systematic or focused on a specific genomic locus where DNA damage can be induced in a regulated manner. Additionally, specific protein modifications will be investigated.

Collaboration / Co-tutoring opportunities
An active external collaboration with Rodolfo Negri's Group at the Dipartimento di Biologia e Biotecnologie "Charles Darwin," Università di Rome-La Sapienza, could be highly relevant to the project. This is due to the extensive experience of R. Negri in studying the influence of chromatin structure on biological processes and the cellular response to ionizing radiation in both yeast and mammalian cells.

Project's Sustainability & Mobility
The proposed project aligns seamlessly with the expertise of the proponent's laboratory in DNA damage response (DDR) and yeast genetics, including the generation of mutants, conducting genome-wide genetic screenings, molecular analyses of DNA damage checkpoint and repair pathways, and detection of protein localization and histone modifications at DNA damage sites. The group is based on a laboratory is fully equipped with the necessary small instruments for conducting genetic and molecular biology experiments, and has the access to state-of-the-art instruments, including a high-throughput array pinning robot (Rotor HDA Singer) for Synthetic Genetic Array (SGA) analysis, BD FacsMelody and Cell Sorter, as well as a Thunder Image Live Cell microscopy system.

Pertinent research articles published by the proposer:

- Casari E, Gobbini E, Gnugnoli M, Mangiagalli M, Clerici M, Longhese MP. 2021. Dpb4 promotes resection of DNA double-strand breaks and checkpoint activation by acting in two different protein complexes. Nat Commun. 12:4750. doi: 10.1038/s41467-021-25090-9.
- Colombo CV, Menin L, Ranieri R, Bonetti D, Clerici M, Longhese MP. 2019. Uncoupling Sae2 Functions in Downregulation of Tel1 and Rad53 Signaling Activities. Genetics. 211:515-530. doi: 10.1534/genetics.118.301830.

Putative foreign institutions for achieving the required ordinary mobility:

- Francisca Lottersberger's group, Department of Biomedical and Clinical Sciences (BKV), Linkoping University, Linkopin (Sweden), for the expertise in advanced imaging techniques and the study of chromatin mobility in the DDR.

References

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[2] Bonetti D, Colombo CV, Clerici M and Longhese MP. 2018. Front Genet 9:390
[3] Casari E, Rinaldi C, Marsella A, Gnugnoli M, Colombo CV et al. 2019. Front Mol Biosci 6:43.
[4] Frigerio C, Di Nisio E, Galli M, Colombo CV, Negri R, Clerici M. 2023. Int J Mol Sci. 24:3248.
[5] Hauer MH and Gasser SM. 2017. Genes Dev 31:2204-21
[6] Karl LA, Peritore M, Galanti L and Pfander B. 2022. Front Genet 12:821543
[7] Bannister AJ and Kouzarides T. 2011. Cell Res 21:381-95