2020

Molecular Mechanisms of the DYRK1A-regulated DNA Repair

Polina Bukina
Varsha Ananthapadmanabhan

Follow this and additional works at: https://scholarscompass.vcu.edu/uresposters

© The Author(s)

Downloaded from
https://scholarscompass.vcu.edu/uresposters/371

This Book is brought to you for free and open access by the Undergraduate Research Opportunities Program at VCU Scholars Compass. It has been accepted for inclusion in Undergraduate Research Posters by an authorized administrator of VCU Scholars Compass. For more information, please contact libcompass@vcu.edu.
Molecular Mechanisms of the DYRK1A-regulated DNA Repair

Polina Bukina¹, Varsha Ananthapadmanabhan², Larisa Litovchick²

¹VCU Honors College, Richmond, VA
²Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA

The functions of human Dual-specificity tyrosine (Y)-Regulated Kinase 1A, or DYRK1A, include cell cycle control and cell differentiation, for which the optimum dosage of DYRK1A is critical. DYRK1A is required for assembly of the DREAM complex (Dimerization Partner, Retinoblastoma-like, E2F, and MuvB) and repression of the cell cycle-dependent genes, such as BRCA1 and RAD51, during quiescence. Our laboratory previously reported that overexpression of DYRK1A inhibits the accumulation of a DNA repair protein 53BP1, at the DNA double-stranded breaks (DSB). Accumulation of 53BP1 is attributed to repair by the non-homologous end joining (NHEJ) as opposed to homologous recombination (HRR). The function of 53BP1 is opposed by RNF169, a ubiquitin-binding protein that also accumulates at the DSB sites and promotes HRR over NHEJ. It was found that DYRK1A interacts with RNF169 to regulate the displacement of 53BP1 from the DSB sites. The molecular mechanism of this regulation is not fully understood therefore the current study focuses specifically on RNF169 in order to better understand the role of DYRK1A in the DNA damage response pathway. Here, we used the Multi-Dimensional Protein Identification Technology (MudPIT) proteomic analysis to identify RNF169-interacting proteins. Human cancer U-2 OS cells stably expressing HA-tagged RNF169, as well as the control (parental) cells were used for immunoprecipitation with anti-HA antibody agarose beads. The sample quality was assessed by protein gel electrophoresis followed by silver staining and Western blot to confirm the pulldown and elution of RNF169. The samples were then sent to Stowers Institute for Medical Research for MudPIT proteomic analysis. Three repeats of this experiment were performed to identify high confidence RNF169 interacting proteins for further validation. This study is now in progress. In order to further understand the regulation of DNA repair by DYRK1A, the RNA sequencing dataset was obtained and analyzed out as part of other studies in the laboratory. The expression of the mRNA for repair factors RAD51 and BRCA1 was found to be regulated by DYRK1A. To determine the significance of this finding, an experiment was designed to assess BRCA1 and RAD51 protein levels in the normal U-2 OS cells and in the cells lacking DYRK1A (U-2 OS DYRK1A knockout cells) after inducing DNA damage by gamma irradiation. It was found that the levels of RAD51, BRCA1, and 53BP1 levels were increased with DYRK1A knockout. These results were consistent with the finding that DNA repair efficiency is increased with DYRK1A knockout. Further studies can help to understand the whether these effects are mediated by DYRK1A-regulated DNA damage repair pathway. Overall, our studies characterize DYRK1A as a multi-functional protein kinase involved in DNA repair and other processes.

**Future directions:** Previously, it was determined that DYRK1A loss could increase HRR, but the mechanism was unknown (Manon et al., 2019). From this data, it can be seen that RAD51, BRCA1, and 53BP1 levels increase with DYRK1A knockout but further studies can help understand the unknown mechanisms by which these proteins are affected.

**References:**

Manon, Vijay R et al. “DYRK1A regulates the recruitment of 53BP1 to the sites of DNA damage in part through interaction with RAD19.” Cell Cycle (Georgetown, Tex.) Volume 18, Issue 51, Pages 531-551. doi:10.1080/15384101.2019.1577525


**Acknowledgements:** We thank S. Swanson, M. Washburn and L. Florens from the proteomics core at Stowers Institute for MudPIT proteomics core. We also thank W. Becker for DYRK1A constructs and S. Gruszec for technical support.