DNA binding modes influence Rap1 activity in the regulation of telomere length and MRX functions at DNA ends

Rinaldi C.*1, Pizzul P.1, Vertemara J.1, Zampella G.1, Tisi R.1, Longhese M.P.1, Bonetti D.1
*(lead presenter) c.rinaldi4@campus.unimib.it

1 University of Milano-Bicocca, Dept of Biotechnology and Biosciences, Milan, Italy

Keywords (DNA damage, telomeres, MRX, Rif2, genetic screening, molecular dynamics):

Each cell in our body receives about $10^5$ DNA lesions each day. Failure to properly recognize and repair this damage can result in genome instability, one of the main driving forces towards cancer development. DNA double-strand break (DSB) is the most dangerous and cytotoxic lesion that can occur to DNA. The cellular response to this lesion is initiated by the MRN complex, called MRX in yeast *S. cerevisiae* (Mre11-Rad50-Xrs2) that has both structural and catalytic functions and that is essential for DNA repair. MRX association at DSBs and telomeres is counteracted by Rif2, which is known to interact at telomeres with Rap1, a protein that directly binds DNA through two tandem Myb-like domains. However, whether and how Rap1 also acts at DSBs is unknown.

We have demonstrated that Rif2 inhibits MRX association to DSBs in a manner dependent on Rap1, which itself binds to DSBs and promotes Rif2 association to them. Moreover, Rap1 can negatively control both MRX association at DSBs and telomere length also in a Rif2-independent manner. In fact, by taking advantage of Rap1 mutant variants and by combining *in vivo, in vitro* and *in silico* approaches, we have demonstrated that Rap1 binding to DNA through both Myb-like domains results in formation of Rap1-DNA complexes that control MRX functions at DSBs and telomere length primarily through Rif2. By contrast, Rap1 bound to DNA through a single Myb-like domain acts at DNA ends mostly in a Rif2-independent manner. Altogether these findings indicate that the DNA binding modes of Rap1 influence its functional properties, highlighting the structural plasticity of this protein.