

Dissecting the role of the Ku complex in the DNA damage response

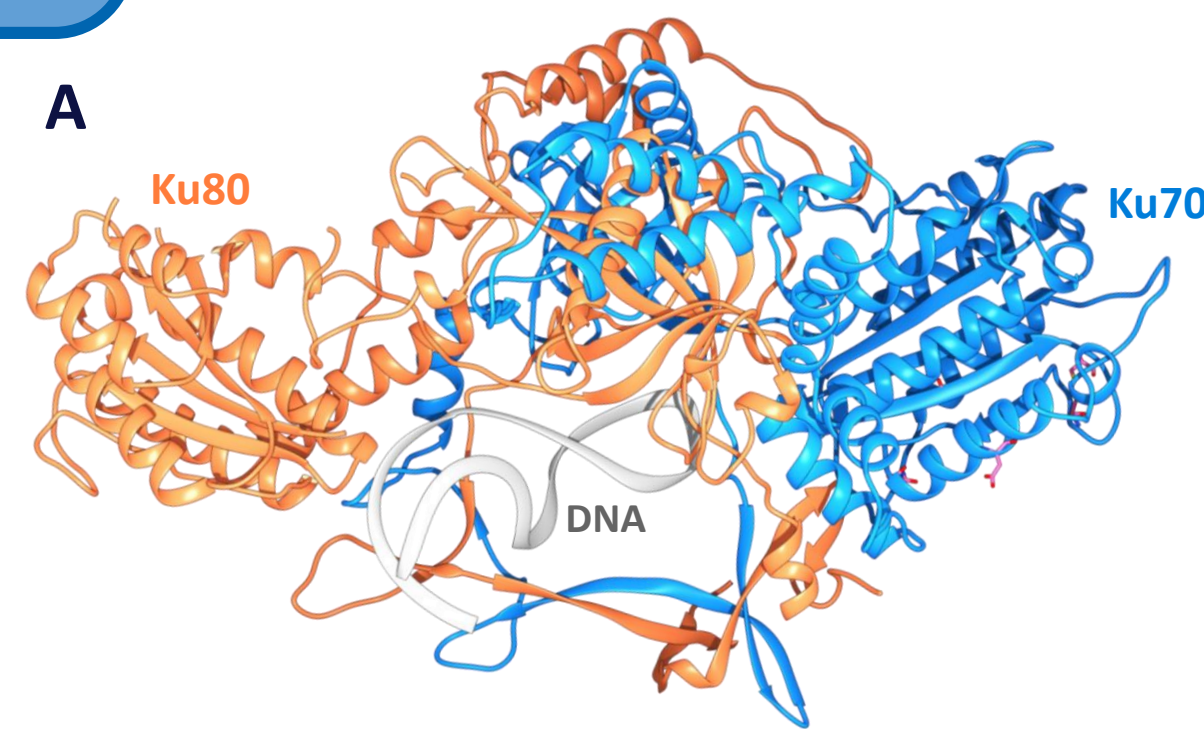
Carlo Rinaldi and Maria Pia Longhese

Introduction

DNA double-strand breaks (DSBs) are highly cytotoxic lesions that can form accidentally during DNA replication or upon exposure to genotoxic agents. DSBs must be repaired to ensure genomic stability avoiding loss of genetic information or chromosome rearrangements. Eukaryotic cells can repair DSBs by two main mechanisms: non-homologous end-joining (NHEJ) or homologous recombination (HR). The initial processing of DSB ends determines which pathways is used to repair the DNA lesion. While NHEJ requires little or no DNA end processing, HR is initiated by nucleolytic degradation of the terminated strands at both DNA ends in a process termed DNA end resection. The Sae2 protein is required for the first step of DNA end resection because it stimulates the endonuclease activity of Mre11, a component of the Mre11-Rad50-Xrs2 complex that recognizes, signals and initiates repair of DSBs. The Ku70-80 heterodimer is also involved in DSB repair. In fact, the Ku complex is rapidly recruited to DNA ends and protects them from degradation caused by resection process. To better understand the role of Ku complex in inhibition of DSB resection, we performed a genetic screening to search for *ku* mutants.

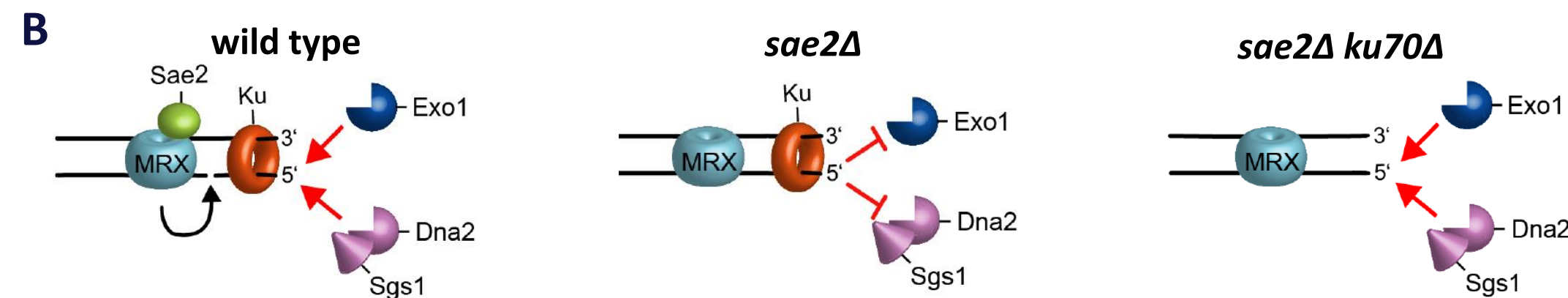
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Structure and role of the Ku complex



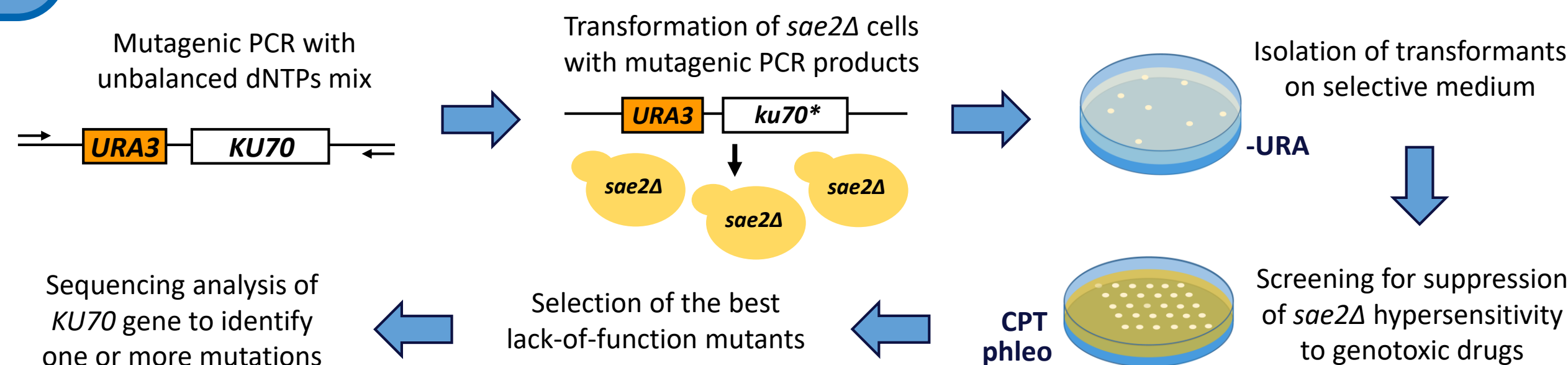
(A) The Ku heterodimer is a conserved DNA end-binding protein complex composed by the Ku70 and Ku80 subunits. The associated subunits form a symmetrical ring-like structure that encircles DNA ends.

(B) MRX, Sae2 and Ku are rapidly recruited to DNA ends. Ku inhibits Exo1 access to DNA ends and protects them from degradation caused by resection process. The lack of Ku complex partially restores DNA damage resistance in *sae2Δ* cells, indicating the Ku bound to the DSB ends acts as a protein block to resection.



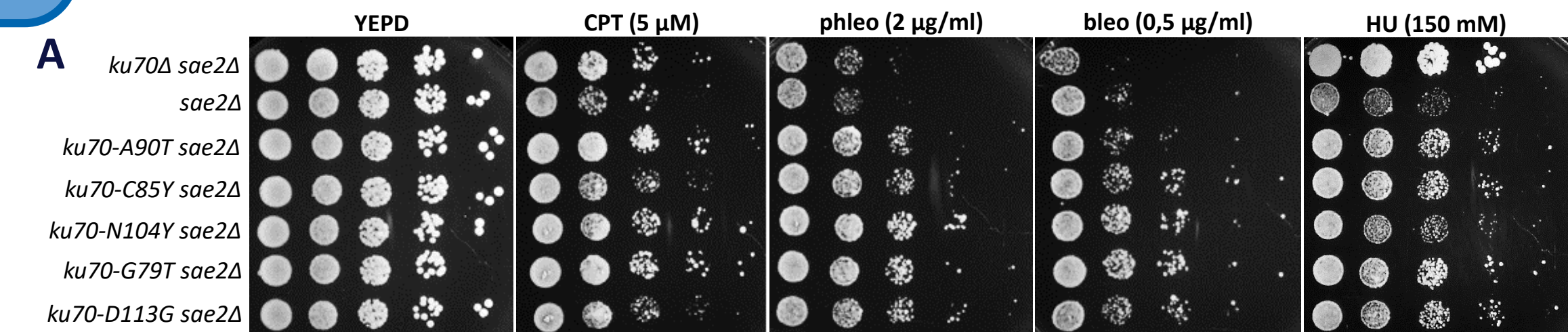
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Genetic screening of *ku70* mutants in *S. cerevisiae*



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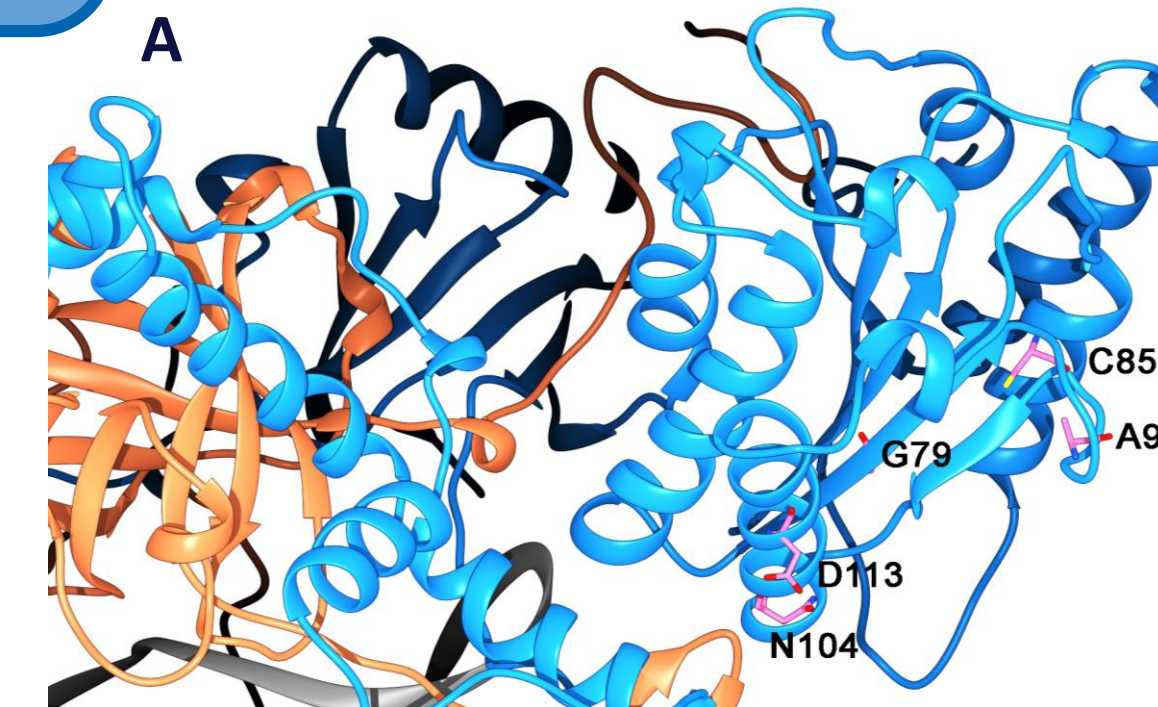
ku70 alleles that suppress the DNA damage sensitivity of *sae2Δ* cells



(A) Exponentially growing cells with the indicated genotypes were serially diluted (1:10) and each dilution was spotted out onto YEPD plates with or without camptothecin (CPT), phleomycin (pleo), bleomycin (bleo) or hydroxyurea (HU).

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Position of the mutations within the Ku70 structure



(A) Sequence analysis revealed that all the *ku* alleles suppressing DNA damage sensitivity of *sae2Δ* cells carry mutations in the N-terminus of the Ku70 protein.

These mutations are located on the outer face of Ku70.

We suppose that the suppression is due to loss of interaction of Ku70 with an unknown protein.

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Future plans

- Study of the suppression mechanisms by genetic and molecular biology approaches
- Investigation of the genetic interactions
- Analysis of telomere length, checkpoint activation and resection mechanisms
- Identification of supposed interactor