Damage recognition process in DNA repair pathway: cryo-EM-based analysis of the UvrA-UvrB complex in Mycobacterium tuberculosis

SpT-01-1

M. Genta[I], G. Ferrara[I], M. Bolognesi[II], F. Rossi[I], M. Rizzi[I], A. Chaves *[II], R. Miggiano *[I]

[I]University of Piemonte Orientale, Department of Pharmaceutical Sciences, Via Bovio 6, Novara, Italy, [II]University of Milan, Department of Biosciences, Via Celoria 26, Milan, Italy

Nucleotide Excision Repair (NER) pathway represents one of the major molecular machineries that control chromosome stability in all living species. In Eubacteria, this pathway includes the three components of the UvrABC excinuclease complex, namely the UvrA, UvrB and UvrC proteins. These proteins act in a multi-step pathway in which the dynamic assembling of protein complexes is required for the lesion sensing and removal activities in an ATP-dependent manner[1]. Specifically, UvrA and UvrB are the first actors of NER and they have been reported as interacting proteins for the recognition of the damage across the DNA double helix. Interestingly, there is evidence in the literature of the formation of UvrA-UvrB complex[2], but both the stoichiometry and the function of the complex is still under debate and many molecular aspects of this process remain unsolved.

NER pathway is extremely essential for Mycobacterium tuberculosis (MTB), that, being an intracellular pathogen, must face toxic agents and oxidative stress, altering its genomic stability[3].

Here we present a Cryo-EM-based structural investigation of the recombinant UvrAUvrB complex from MTB, as well as of the UvrA dimer, both in complex with damaged DNA. Our analyses reveal new insights in the DNA binding mode of UvrA, with an alternative conformation of some crucial regions involved in DNA coordination. Moreover, at supramolecular level, we obtained a structural snapshot of the different oligomers which alternate during the early stages of the damage recognition process, adding details to the scientific debate regarding the stoichiometry of the protein assemblies that lead the system to the formation of the UvrB-DNA pre-incision complex and to the repairing of DNA damages.

1 Goosen N et al. (2001) Research in microbiology 152(3-4):401-9.

2 Pakotiprapha D et al. (2012) Nature Structural & Molecular Biology 19, 291–298.

3 Miggiano R et al. (2020) Molecules 25(5):1205.

* The authors marked with an asterisk equally contributed to the work.