Unveiling the DNA Strand Transfer-mechanism of Relaxase TrwC

Sivia Russi^a, M. Lucas^b, A.Guasch^a, R. Boer^a, R. Pérez-Luque^a, M. Cabezas^b, F. De la Cruz^b, M. Coll^a, ^aInstitut de Biologia Molecular de Barcelona, CSIC, Barcelona-Spain. ^bDepartamento de Biología Molecular, Universidad de Cantabria, Santander-Spain. E-mail: srucri@ibmb.csic.es

The three-dimensional crystal structure of the relaxase domain of TrwC in complex with DNA (25-mer oligonucleotide), recently reported [1], showed that the protein has a metal binding site at the active site, in which, three histidine residues (His150, His161 and His163) and a water molecule coordinate a metal cation. The nature and role of this metal in the strand transfer-mechanism it is not clear. It was suggested that it could play an important role polarizing the scissile phosphate or stabilizing the transition state. Further structural information is needed to understand its function and unveil the enzymatic mechanism.

In the present work we discuss the results obtained with TrwC-DNA crystals, soaked with different metals: Cu^{+2} , Ni^{+2} , Mg^{+2} and Mn^{+2} . We also report the successful cocrystallization and structure determination of the protein with longer oligonucleotide sequences, that include the scissile bound (27 and 29-mer oligonucleotides), achieved by introducing a mutation in the catalytic residue Tyr18 (Y18F).

[1] Guasch A., Lucas M., Moncalián G., Cabezas M., Pérez-Luque R., Gomis-Rüth F.X., De la Cruz F., Coll M., *Nat. Struct. Biol.*, 2003, **10**, **12**, 1002. **Keywords: DNA-binding protein, metal bound-structure, DNA**strand transfer-mechanism