

Interactions of Nucleotide Excision Repair Factors in living cells

Christoffel Dinant¹⁾, Wim Vermeulen¹⁾ and Adriaan B Houtsmuller²⁾ Departments of Genetics¹⁾ and Pathology²⁾, Erasmus MC Rotterdam

Nucleotide excision repair (NER) of UV-induced single strand DNA damage involves more than twenty proteins. Little is known about the dynamic interactions between different NER proteins required for proper repair function in the living cell. We have used fluorescence resonance energy transfer (FRET) between GFP and YFP to study real time interactions of NER factors. Recent developments including spectral confocal imaging and subsequent linear unmixing allow simultaneous imaging of GFP and YFP in live cell nuclei. The first protein-protein interaction we have studied is between ERCC1 and XPF. ERCC1 and XPF form a heterodimer, which is responsible for the 5' incision of the damaged strand during NER. Energy transfer occurred between ERCC1-GFP and XPF-YFP when transfected in ERCC1 mutant hamster cells. Interestingly the efficiency of this energy transfer decreased upon DNA damage-induction by UV-light. Whether this lower FRET signal was caused by a disruption of the heterodimer or by a conformational change is not yet clear.