CONFOCAL SINGLE MOLECULE ANALYSIS OF NUCLEOTIDE EXCISION REPAIR COMPLEXES

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Scanning confocal fluorescence microscopy is used to study the Nucleotide Excision Repair process on a single molecule level. A selection of the 30 proteins, known to be involved in this complicated DNA repair system, has been fluorescently labeled with mutants of the Green Fluorescent Protein. Complex formation is accomplished by combining NER-GFP fusion proteins with fluorescently labeled model DNA substrates. Samples for confocal microscopy are prepared by introducing the reaction mixture into an agarose matrix at single molecule concentration. During confocal scanning uncomplexed DNA shows rapid diffusion within the gel matrix while NER-GFP proteins appear as immobilized single molecules. As a consequence DNA molecules are immobilized only because of binding with NER proteins. It follows that colocalization of the GFP-label on the NER-protein with the DNA-label is a representation of complex formation. This colocalization is demonstrated by performing simultaneous dual colour excitation together with emission detection in two spectrally separated channels during confocal scanning. From analysis of confocal images we were able to determine the DNA-bound fraction of NER proteins under equilibrium conditions. So far, the DNA binding properties of the XPA, RPA, ERCC1/XPF and XPC/hHR23B proteins have been studied on the single molecule level. To study the DNA binding specificity of the proteins we determined the equilibrium dissociation constants for binding to different DNA substrates. The diffusion of the DNA substrates within agarose matrices was also studied. Additional confocal single-molecule fluorescence results obtained in relation to the NER subject will be presented too. Our single molecule results are in good correspondence with results from more conventional biochemical methods, indicating the potential of single-molecule confocal fluorescence microscopy in the study of complicated biological systems.

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[1] Segers-Nolten, G.M.J., Wyman, C., Wijgers, N., Vermeulen, W., Lenferink, A.T.M., Hoeijmakers, J.H.J., Greve, J., and Otto, C. (2002) Scanning confocal fluorescence microscopy for single molecule analysis of nucleotide excision repair complexes. *Nucl. Acids Res.*, 30, 4720-4727
[2] Hoeijmakers, J.H.J. (2001) Genome maintenance mechanisms for preventing cancer. *Nature*, 411, 366-374