

IMMUNOFLUORESCENCE MICROSCOPY TO STUDY THE INTERACTION OF HOMOLOGOUS RECOMBINATION AND NON HOMOLOGOUS END JOINING OF UV-A INDUCED DNA DOUBLE STRAND BREAKS

Alexander Rapp, Paulius Grigaravicius and Karl Otto Greulich
Institute for Molecular Biotechnology Jena, Department of Single Cell and Single Molecule Techniques, Beutenbergstr. 11, 07745 Jena, Germany
E-mail: bar@imb-jena.de

KEY WORDS: DNA Damage, Homologous Recombination Repair, Non Homologous End Joining, UV-A, FRET

ABSTRACT:

In human cells it is known, that the most critical DNA damage for genomic integrity is the DNA double strand break (DSB). To handle such DNA alterations the cell has developed two independent repair mechanisms, which are known as Homologous Recombination (HR) and Non Homologous End Joining (NHEJ) [1]. The latter one is active throughout the cell cycle, where in contrast the HR is restricted to late S and G₂ phase, when two homologous sequences exist in close proximity [2]. DSBs can be labelled with an antibody against the phosphorylated histone H2AX termed γ -H2AX. This phosphorylation takes occurs in thousands of histones flanking the DSB. Thus individual DSBs can be visualized as foci.

In this work we studied the question whether the two DNA repair pathways cooperate or compete at an individual DSB, if the DSB is induced in G₂, when both systems are available. Since each repair pathway is represented by a set of unique proteins (e.g. Rad51, Rad52 and Rad54 for HR or DNA-PKcs, XRCC4 for NHEJ) and some proteins are shared between the two pathways (e.g. Mre11, Rad50). We quantified the interaction of the individual repair proteins using Laser Scanning Microscopy with optical colocalisation, FRET measurements as well as biochemical co-immuno-precipitation. From the pairwise analysis of nine proteins we have established a spatial interaction matrix as well as the temporal sequence of events at the sites of the DSB. From this data it can be summarized that in G₂, when both systems are available to the cell, HR and NHEJ can cooperate at least at a fraction of the DSB sites and that NHEJ precedes HR, thus HR has a potential proofreading function [2].

[1] J. H. Hoeijmakers, "Genome maintenance mechanisms for preventing cancer", *Nature* **411**, 366-374 (2001).

[2] K. Valerie, and L. F. Povirk, "Regulation and mechanisms of mammalian double-strand break repair". *Oncogene* **22**, 5792-5812 (2003).

[3] A. Rapp, and K.O. Greulich, "After double stands break induction by UV-A homologous recombination and non homologous end joining cooperate at the same DSB if both systems are available" *J.Cell Sci.* **117**, 4935-4945 (2004).