A Novel Fluorescence Assay for DNA Synapsis and Ligation

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DNA double-strand breaks (DSBs) are amoung the most serious forms of cellular damage. Unrepaired or misrepaired breaks can lead to cell death, chromosome translocations and genomic instability. In human cells, DNA DSBs are repaired by two pathways, namely, homology-directed recombination (HDR) and non-homologous end joining (NHEJ).). In HDR, genetic information in the undamaged sister chromatid is used as a template to repair accurately the damaged doubled-stranded (ds) DNA. In NHEJ, the broken DNA ends are re-joined without the need for a DNA template. Genetic and biochemical studies have implicated several key proteins in the NHEJ pathway, including Ku70 and Ku80 (known as the Ku heterodimer), and the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs). Magnesium-ATP and the protein kinase activity of DNA-PKcs are essential for DNA double-strand break repair.

As more DNA-PK autophosphorylation sites are identified it will be critical to develop an assay that can examine the role of these sites in DNA-PK-DNA binding and synapsis, versus kinase activity and complex remodeling for ligation. Moreover, this assay should discriminate between the two and quantitate the degree of DNA synapsis and/or ligation in a standard reaction mixture with a minimum of effort.

In principle, two-photon excitation fluorescence cross-correlation spectroscopy (TPE-XCS) is perfectly suited to such an assay. Briefly, TPE-XCS uses a type of fluctuation analysis which selectively examines the making and breaking of linkages between species bearing different fluorescent labels. TPE-XCS provides information on the concentrations of linked and unlinked species and on their hydrodynamic sizes. TPE-XCS measures fluorescence that is separated by wavelength and then collected simultaneously in two detection channels. The time-dependent instantaneous fluorescence intensity from each channel is then cross-correlated in steps of time, τ. The time dependence (decay) of the XCS signal indicates the loss of the complex from the excitation volume, either by diffusion or dissociation. Thus, it is possible to use TPE-XCS to follow the association of oligonucleotides (labelled with different fluorophores) directly in the same reaction mixture that can subsequently be analyzed with standard gel-shift techniques.

Employing this novel assay, we demonstrate that both wild-type and A6 mutant DNA-PKcs are capable of synapsing multiple DNA ends, producing large multi-DNA complexes in solution. Moreover, this assay also shows DNA ligation in the presence of T4 ligase.