

A NEW METHOD OF INFLICTING LOCAL OXIDATIVE DAMAGE IN CHROMATIN, USING PHOTSENSITISER AND FOCUSED GREEN LIGHT, REVEALS INVOLVEMENT OF HETEROCHROMATIN PROTEIN 1 IN DNA DAMAGE RESPONSE

Mirosław Zarębski, Elżbieta Wiernasz, Jerzy Dobrucki

**Division of Cell Biophysics
Faculty of Biochemistry, Biophysics and Biotechnology
Jagiellonian University, 30-387 Kraków, Poland
jerzy.dobrucki@uj.edu.pl**

Recruitment of DNA repair proteins to damage can be detected and studied in situ, by microscopy methods, after inflicting local, sublethal damage in nuclei of live cells. Several methods of inflicting local chromatin damage have been proposed, including local UV damage, creating alpha-particle tracks, three-photon excitation of components of nucleic acids, etc.

Ideally, a method of inflicting local damage should not only allow control over the area of the nucleus which is affected, but most of all - control over the type of the primary lesion (ss or ds DNA break, base oxidation, pyrimidine dimer, etc.) and the extent of damage (sublethal vs lethal and extensive). We have tested several compounds, which are capable of entering live cells, and acting as photosensitizers. We examined their ability to photooxidize DNA after local illumination by visible light of a selected wavelength.

We will discuss the conditions required to generate local sublethal oxidative damage using several photosensitizers. Further, we will describe how local oxidative damage, which is caused by exciting DNA-intercalated ethidium with 514 nm light triggers recruitment of epigenetic regulator heterochromatic protein 1 (HP1) to this type of damage.