

## USING LOCAL PHOTODAMAGE TO STUDY CELLULAR REPAIR MECHANISMS - POTENTIAL PITFALLS

**Dominika Żurek, Dominika O.Trembecka, Mirosław Zarębski, Katarzyna Kędziora,  
Mateusz Kuzak, Jurek W.Dobrucki**  
**Division of Cell Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology,  
Gronostajowa 7, Jagiellonian University, Krakow, Poland**  
E-mail: jerzy.dobrucki@uj.edu.pl

**KEY WORDS:** photodamage, heterochromatin protein 1 (HP1), oxidative damage, DNA repair, DNA damage

Photodamage can be inflicted by a focused laser beam in a selected small area of a cell. If the damage is sublethal, the cell activates repair mechanisms, therefore this approach has been used successfully to study repair of DNA or plasma membranes [1,2]. We inflicted local DNA damage by exposing cells to ethidium anion and focused green light, in order to study the repair processes. We noticed that, in addition to known repair factors, heterochromatin protein 1 (HP1) was also recruited to the damaged area, indicating that HP1 is involved in DNA damage response (DDR) [1,2,3]. We now demonstrate that this experimental approach, although elegant and useful in studies of DNA repair mechanisms, can also lead to a false negative. If the concentration of a photosensitizer and the intensity of exciting light is sufficiently high (more than approx. 1 mW) the expected recruitment of HP1 to damage is abolished. Moreover, the total intensity of fluorescence of GFP-HP1 in the whole nucleus diminishes following damage, indicating that the concentration of GFP tagged HP1 protein decreases. It is yet unclear if this process is due to protein degradation or other mechanisms. We will discuss two potential reasons for a failure of the repair factor to accumulate at a damage site - impairing of the repair mechanism itself, and extensive scattering of exciting light [4], which leads to damage in a large area, beyond the directly illuminated region. This widespread damage results in no obvious recruitment of the repair factors to any specific nuclear region. The possibility of encountering a false negative in this type of experiment can lead to problems with data interpretation. For instance, while under some conditions HP1 recruitment was reported [3], other investigators observed dissociation of HP1 from damage [5]. We conclude that experimental conditions of inflicting local chromatin lesions must be carefully optimised in order to permit the recruitment of a repair factor to damage.

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