

## FOCUSED LOW POWER VISIBLE LIGHT CAN INDUCE DNA DAMAGE IN LIVE CELLS

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Recent advances in microscopy techniques opened new possibilities to investigate biological processes in situ, in cellular environment. Fluorescent proteins are now routinely used to tag proteins of interest in order to investigate their behavior in living cells [1]. EGFP and other fluorescent proteins are generally quite photostable and appear to cause less phototoxic damage than low molecular weight dyes. This encourages experimenter to extend duration of microscopy experiments and increase the dose of exciting light in order to improve signal-to-noise ratio. Large doses of exciting light are also used in fluorescence recovery after photobleaching (FRAP) and related techniques (FLIP, iFRAP) [2].

We report evidence that illuminating cell nuclei with blue as well as green light can cause photodamage to DNA and activate repair processes. When a small region (square side 1.2 – 6  $\mu\text{m}$ ) of a cell nucleus containing eGFP-tagged XRCC1 (a protein involved in base excision repair pathway) was exposed to blue light (100  $\mu\text{W}$ , pixel dwell time 12  $\mu\text{s}$ , 15 scans, 512 x 512 px), this protein was recruited to the illuminated area, as usually observed in response to local DNA damage. We observed a similar recruitment of eGFP-tagged heterochromatin protein 1 (HP1) to a small illuminated region of the nucleus. HP1 not only plays a structural role in the nucleus, but was also recently shown to be involved in DNA damage response [3]. However GFP does not appear to be necessary to induce photodamage. Immunofluorescence studies indicate that HP1 protein is also recruited to illuminated regions in the nuclei of nontransfected HeLa cells. When a selected region of chromatin was illuminated with blue light, DNA breaks were formed in cells even without GFP presence. These DNA lesions were demonstrated by immunofluorescence detection of phosphorylated histone H2AX, which markers DNA breaks. Thus we conclude that following an exposure of a region of a cell nucleus to exciting light, DNA damage and recruitment of repair factors may occur. This process is, however, only an artifact resulting from phototoxic damage.

Photodamage caused by irradiation may complicate interpretation of several types of experiments. FRAP of repair proteins is expected to be altered by recruitment of these factors to the damage, which was caused by the bleaching light itself. Also, studies of gene activation may be obfuscated by recruitment of repair factors. Both types of experiments can be complicated by damage to potential binding sites for proteins under study. A possible way to verify if these adverse effects occur is to investigate if a number of DNA breaks in the illuminated area remains on the background level characteristic for unilluminated cells.

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