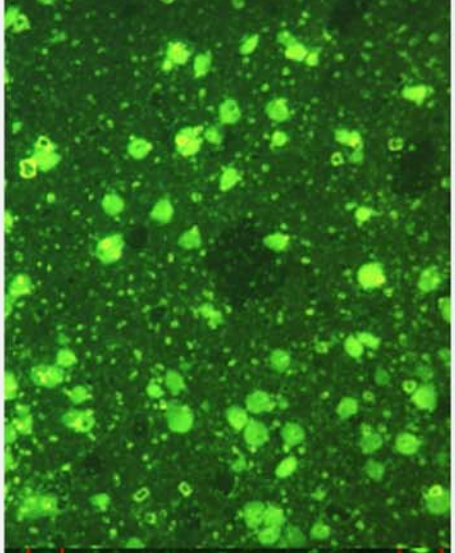
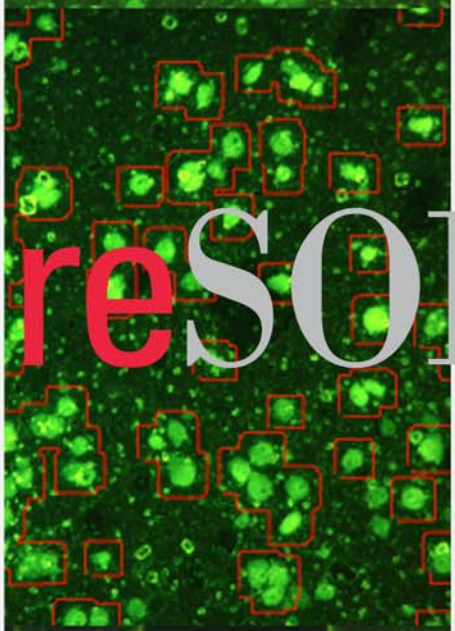
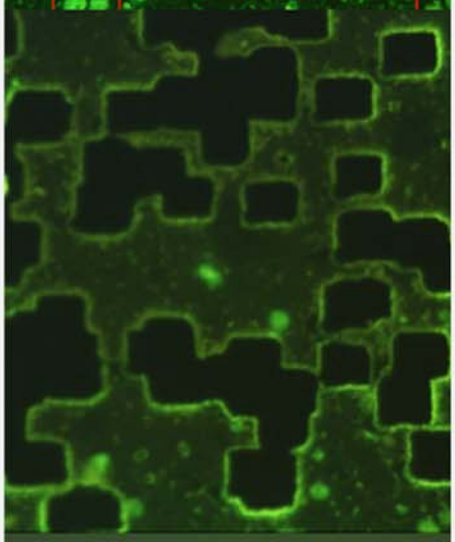






Leica Laser Microdissection
Application Note

reSOLUTION

Local DNA Damage and Repair in Live Cells

Nov
2006

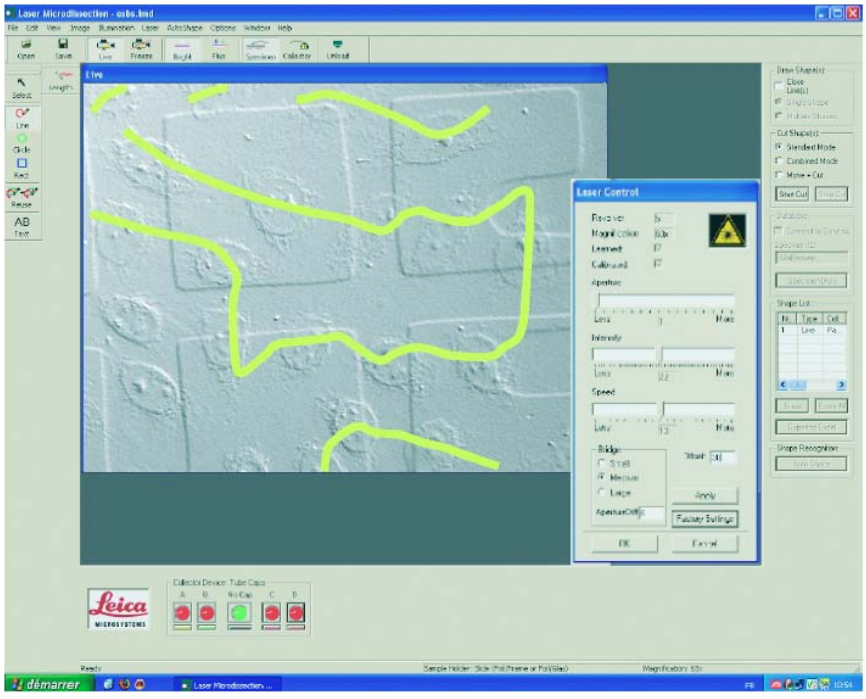
Leica
MICROSYSTEMS

Local DNA Damage and Repair in Live Cells Using the Leica LMD

Poly(ADP-ribose) (PAR) that is synthesized by poly (ADP-ribose) polymerases (PARPs) in response to DNA-strand breaks is a DNA-damage signalling molecule that contributes to cell survival. Here, the Leica Laser Microdissection System has been used to induce locally DNA strand-breaks in sub-nuclear volumes of cultured cells to follow the cellular distribution of endogenous XRCC1, an essential factor of the Single-Strand Break Repair (SSBR) pathway. We visualize the immediate PAR-dependent recruitment of XRCC1 to the sites of breakage marked by the local synthesis of poly(ADP-ribose).

1. Laser induced DNA strandbreaks and DNA-damage dependent PAR synthesis

HeLa cells were grown onto 55 µm square size CEL-Locate coverslips (Eppendorf, Hamburg, Germany). The cells were incubated with 10 µg/ml Hoechst dye 33258 in phosphate-buffered saline (PBS) or DMEM medium for 20 min at room temperature. Laser micro-irradiation was performed with a Leica LMD microscope (Leica Microsystems, Wetzlar Germany, Version 4.4) fitted with a 337.1 nm laser focused through a 40x or a 63x objective. The peak performance was 7.2 mW at the maximum pulse repeat rate of 30 Hz. The laser was guided over cell nuclei by a joystick used to draw the path of the laser (yellow). The irradiation was performed using the following features of the laser: aperture 1, power 22, speed 10, off-set 30.



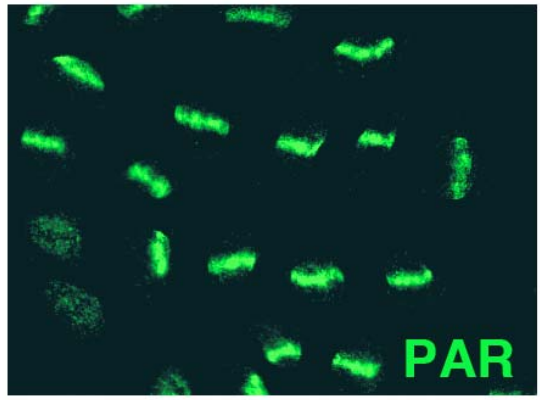


fig.1

Cell positioning on CELLocate coverslips was recorded by phase contrast imaging of irradiated cells (fig.1) After irradiation, cells were fixed in ice cold methanol/acetone (50:50, v/v) for 10 min at 4 °C. washed three times with phosphate-buffered saline supplemented with Triton X-100 0.1% (V/V), blocked with 0.2% non fat milk or 1% BSA in PBS/Triton X-100 0.1% and incubated overnight at 4°C with primary antibodies. Here (fig. 2) a monoclonal anti-PAR antibody and a secondary goat anti-mouse (Alexa fluor 488, green) were used to immunostain the DNA-damage dependent synthesis of PAR.

2. Laser induced DNA strand-breaks triggers the PAR-dependent recruitment of XRCC1.

Following laser irradiation cells were fixed and immunostained with a monoclonal anti-PAR antibody (green) revealed by a polyclonal anti-XRCC1 antibody (red). Figure 2 shows the colocalisation of the robust PAR response with the accumulated XRCC1 along the laser path. No recruitment of XRCC1 was observed in the presence of various PARP inhibitors (not shown).

3. Fast recruitment of GFP-tagged XRCC1 at DNA strand-breaks in live cells

Figure 3 shows HeLa cells expressing GFP-tagged XRCC1. Time lapse experiments were performed and images were taken every 5 sec. Within 15 sec, a very fast accumulation of GFP-XRCC1 was already observed along the laser path (fig. 3). No XRCC1 accumulation was observed using HeLa cells expressing the empty vector or in the presence of a PARP inhibitor (data not shown).

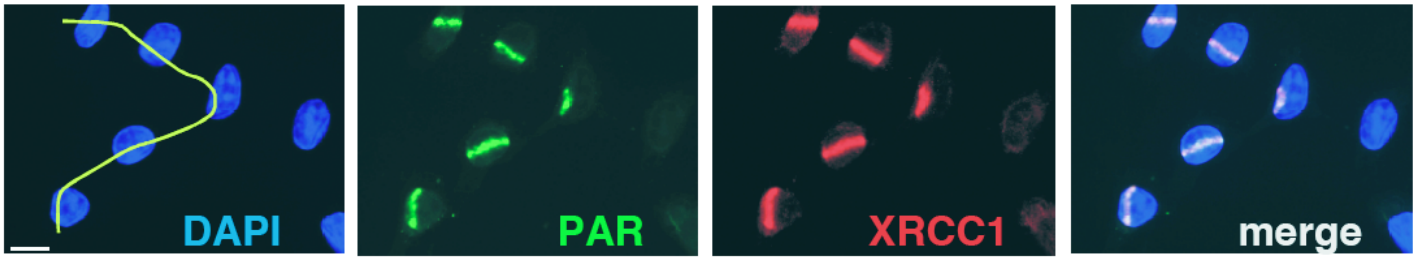


fig.2

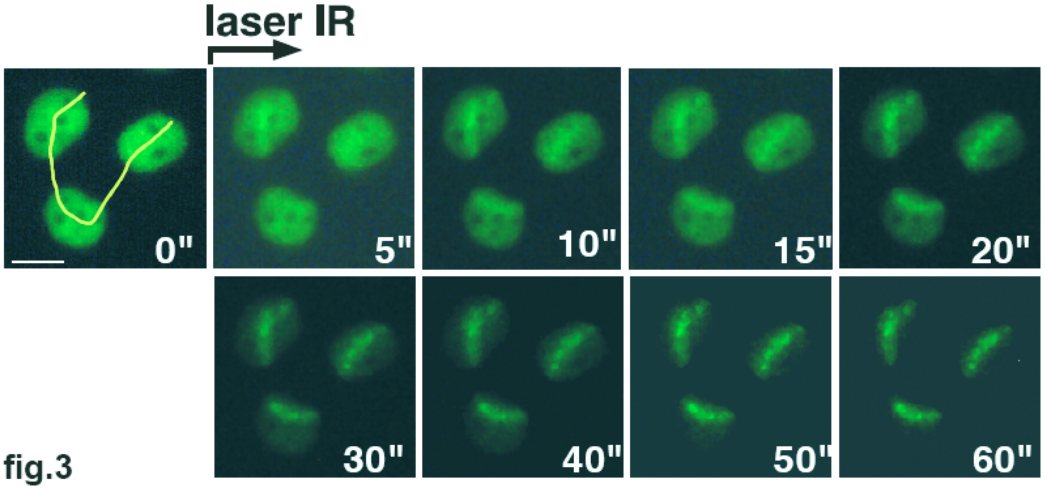


fig.3

4. H2AX phosphorylation at Ser139: a posttranslational mark on histone H2AX induced by DNA double-strand breaks

Laser microirradiation also induces double-strand breaks in DNA that in turn triggers the ATM-dependent phosphorylation of the H2AX histone variant at Ser139 (red). Following laser irradiation cells were fixed and immunostained with a polyclonal anti-Phospho Ser139-H2AX revealed by a secondary goat anti-rabbit antibody (Alexa 568) (fig. 4).

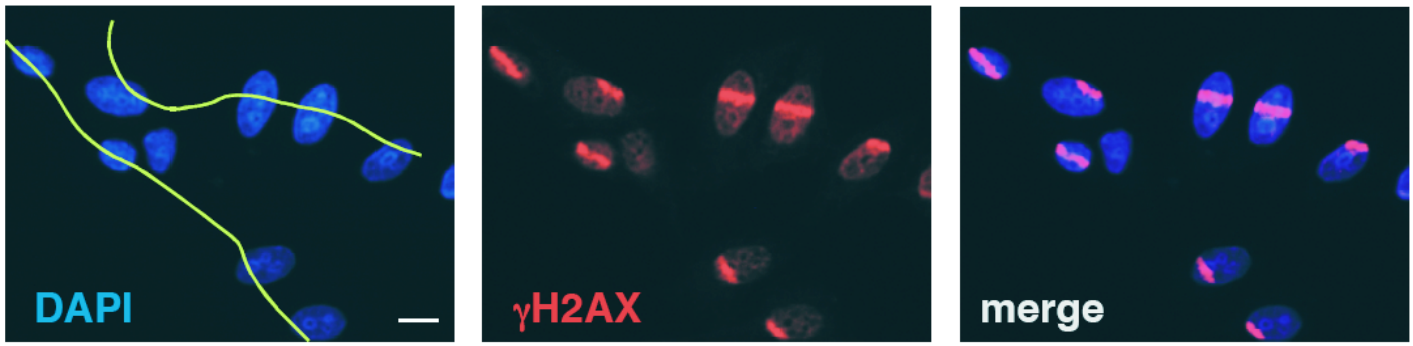


fig.4

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