

# CONDENSATION OF CHROMATIN AND DYNAMICS OF HISTONES IN LIVING CELLS EXPOSED TO DNA-INTERCALATING ANTIBIOTIC DAUNOMYCIN

Jurek W.Dobrucki, Mirosław Zarebski, Krzysztof Wojcik

Laboratory of Confocal Microscopy and Image Analysis, Department of Biophysics,  
Faculty of Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków,  
Poland; dobrucki@mol.uj.edu.pl

**Key words:** chromatin condensation, histone exchange, DNA intercalation, daunomycin

The goal of this study was to investigate the effects of a DNA-intercalating drug, daunomycin, on nuclear and chromatin structure. Daunomycin is an anthracycline antibiotic which intercalates into DNA and causes helix unwinding. Daunomycin is extensively used as antitumour drug, however, the mechanism of cytotoxic action of this agent is still not fully understood [1]. Cytotoxicity may derive from DNA binding and alkylation, DNA-crosslinking, and interference with DNA unwinding and strand separation.

The action of daunomycin was studied in transfected human fibroblasts and HeLa cells, with histones tagged with eGFP. Daunomycin at clinically relevant doses (25 - 1000 nM, 0.5-24h) caused various degrees of condensation of chromatin. Fluorescence images of viable cells *in vitro* treated with daunomycin revealed penetration of a drug into cells, accumulation in cytoplasm and nucleus, and apparent gradual change in nuclear structure leading to a state, where chromatin distribution resembled that of a prophase condensation pattern. Concentration of histones in whole nuclei and in selected areas of nucleus as well as mobility of histones was studied by flow cytometry, and confocal microscopy, including FRAP and FLIP techniques. Following exposure to daunomycin a fraction of linker histones appeared to be detached from DNA and degraded. Another fraction remained bound to DNA in condensed foci and was still undergoing exchange between different chromatin regions. The core histone H2B was not detached from condensing chromatin and did not exchange.

It has been shown previously (by flow cytometry and confocal microscopy) that sensitivity of DNA *in situ* to denaturation correlates with chromatin condensation and varies during cell cycle and apoptosis [2]. DNA denaturation was detected using a metachromatic dye acridine orange, which differentially stains single stranded and double stranded DNA sections. Using this approach we have demonstrated now that chromatin, which condensed as a result of exposure to daunomycin, exhibited greater sensitivity to denaturation than chromatin in untreated cells.

These preliminary data indicate that daunomycin used at clinically-relevant concentrations disrupts high-order structure in long stretches of chromatin in living cells and renders DNA more sensitive to denaturation. We postulate that the mechanism of this phenomenon involves drug intercalation, helix unwinding, increase in DNA torsional stress, and subsequent detachment of linker histones, but no detectable damage to nucleosomes.

## References:

1. D.A. Gewirtz, A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochem Pharmacol* **57**(7), 727-41 (1999)
2. J.W.Dobrucki J, Z. Darzynkiewicz, Chromatin condensation and sensitivity of DNA *in situ* to denaturation during cell cycle and apoptosis - a confocal microscopy study. *Micron* **32**(7), 645-52 (2001)

*Supported by The Wellcome Trust and Polish State Committee for Scientific Research.*