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## Doxorubicin induces large-scale histone redistribution in live cells

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Anthracyclines are widely used anti-cancer drugs exhibiting pleiotropic effects. At the chromatin level, their mechanism of action includes topoisomerase inhibition, DNA intercalation and histone eviction. Anthracyclines also bind histones thus modulating their DNA binding properties, turnover and intra-nuclear localization. Doxorubicin is an anthracycline derivative frequently used in clinical practice. However, it is challenging to overcome its most common side-effect, cardiotoxicity. It has also been reported previously that anthracyclines cause chromatin aggregation. We used a laser scanning microscopy based assay to detect antibody labeled histones in doxorubicin treated and triton-permeabilized Jurkat cells. Doxorubicin was applied in a concentration range between 1-36  $\mu$ M and was detected by immunofluorescence and increment in the average nuclear amount of histones in the case of H1 and H2A after two hours of treatment in a sub population of the cells; these cells did not show the symptoms of apoptosis. The increase was already observed at the concentration of 1-2  $\mu$ M corresponding to the usual peak plasma concentrations of the drug reached upon intravenous infusion. At the same time, a marked decrease was observed in the case of total H3 and the levels of H2B were not affected. These results were reproduced using different antibodies, but not with GFP-tagged histones all of which exhibited a variable, minor decrease. The above changes can be readily interpreted in terms of differential release from the permeabilized nuclei of the histones exhibiting different degree of aggregation. By confocal microscopy, we detected H1 accumulation in nucleoli after doxorubicin treatment. At the same time, H2A filled up the space between the trabecular, aggregated chromatin. We observed a marked intra-nuclear decrease and cytoplasmic increase in antibody labeled H2B histone levels, a phenomenon undetectable using GFP-tagged H2B. The inhibition of de novo protein synthesis had no effect on the latter phenomenon, so we propose that doxorubicin bound H2B histone levels become diminished in the nuclei with one of the following mechanisms: either the drug-bound H2B gets ubiquitinated for proteasomal targeting or H2B is transported out of the nucleus using nuclear export. Experiments are in progress to investigate these alternatives. The data on H1 are in line with those on H2A and H2B were unexpected based on the previous studies.

### Recent Publications:

1. Imre L and Szabo G (2017) Nucleosome stability measured *in situ* by automated quantitative imaging. Scientific Reports 7(1):12734.
2. Pang B and Neefjes J (2013) Drug-induced histone eviction from open chromatin contributes to the chemotherapeutic effects of doxorubicin. Nature Communications 4:1908.
3. Wójcik K and Dobrucki J W (2013) Daunomycin, an antitumor DNA intercalator, influences histone-DNA interactions. Cancer Biology & Therapy 14(9):823-32.

### Biography

Peter Nanasi graduated at University of Debrecen in 2012 as a medical doctor. After 2012 he works at the Department of Biophysics and Cell Biology first as a PhD student then as a junior research fellow. Area of research focused on chromatin architecture and nucleosome stability, which includes, studying the role of single-strand DNA breaks in connection with higher order chromatin assembly, investigating the effect of different histone posttranslational modifications to nucleosome stability using a salt and intercalator based assay. Laser Scanning Cytometry and Confocal Microscopy is freely accessible in our department. Article published: Imre L and Szabo G (2017) Nucleosome stability measured *in situ* by automated quantitative imaging. Scientific Reports 7(1):12734.