EVALUATION OF THE REDOX POTENTIAL OF THE GOLGI OF CHO CELLS

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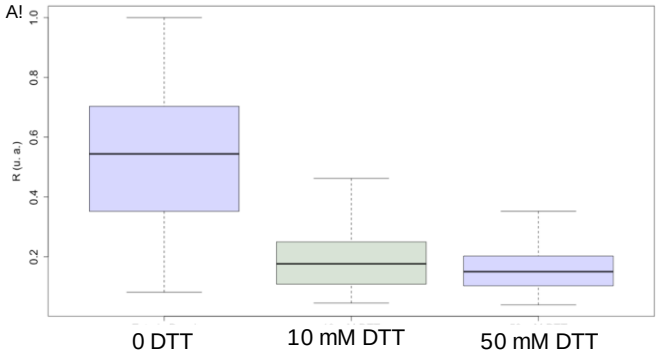
The redox potential of cellular organelles is tightly regulated. However, under stressful conditions or nutrient limitation, it may change and intracellular processes may be compromised. To measure the redox potential in the Golgi, the roGFP2 redox sensing protein was fused to the transmembrane domain of the hamster a 2,3 sialyltransferase, in order to direct it to the trans Golgi. First, roGFP was calibrated to determine the range of redox potentials it can measure. Then, CHO cells were transduced with a recombinant baculovirus containing the a 2,3 SialT'roGFP2 gene. The localization of roGFP in the Golgi was confirmed by fluorescence confocal microscopy by colocalization with Bodipy TR. Then, high resolution confocal microscopy was used to determine for each cell the redox potential. It was found that the redox potential of the Golgi is -240 mVolts. Addition of DTT or peroxide changed the Golgi redox potential (see figure A). The effect of relevant biological stressful conditions in the redox potential of the Golgi will be presented.



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