CHANGES IN STRUCTURE AND CHEMICAL CONTENT INDUCED BY NUCLEAR/NUCLEOLAR STRESS: NEW C.L.E.M. AND *CRYO*-C.L.E.M. NANO IMAGING APPROACH

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The cell is a crowded volume, with estimated mean mass percentage of macromolecules and of water ranging from 7.5 to 45 % and 55 to 92.5 %, respectively. However, the concentrations of macromolecules and water at the nanoscale, within the various cell compartments, are unknown. We recently developed a new approach, correlative cryo-analytical scanning transmission electron microscopy, for mapping the quantity of water within compartments previously shown to display GFP-tagged protein fluorescence on the same ultrathin cryosection. Using energy- dispersive X-ray spectrometry (EDXS), we then identified various elements (C, N, O, P, S, K, Cl, Mg) in these compartments and quantified them in mmol/l. Here, we used this new approach to quantify water and elements in the cytosol, mitochondria, condensed chromatin, nucleoplasm and nucleolar components of control and stressed cancerous cells. The water content of control cells was between 60 and 83 % (in the mitochondria and nucleolar fibrillar centers, respectively). Potassium was present at concentrations of 128 to 462 mmol/l in nucleolar fibrillar centers and condensed chromatin, respectively. The induction of nucleolar stress by treatment with a low dose of actinomycin-D to inhibit rRNA synthesis resulted in both an increase in water content and a decrease in the elements content in all cell compartments. We generated a nanoscale map of water and elements within the cell compartments, providing insight into their changes induced by nucleolar stress. Studying nucleus/nucleolus in motion (time-laps LCM or 4-D observations) we recognized that nuclear/nucleolar stress causes joint displacement of nucleosomal and non-nucleosomal intranucleolar chromatin domains with following coalescence with perinucleolar condensed chromatin shell.