Nucleolus in Motion: Contraction of Intra- and Perinucleolar Condensed Chromatin Induces Relocalization of FC/DFC Assembly during the Selective Inhibition of rRNA Synthesis

P.Tchelidze[1,2], L. Lucas[3], H. Bobichon[3], L. Rusishvili[1,2], G. Mosidze[1,2], N. Lalun[3], C. Terrin[3], D. Ploton[3]

[1)] Faculty of Exact and Natural Sciences, Department of Biology, Division of Morphology, Iv. Javakhishvili Tbilisi State University;

[2)] Institute of fundamental research of bioeffective technologies, Iv. Javakhishvili Tbilisi State University;

[3)] URCA (University of Reims Champagne-Ardenne, France), Phaculty of Pharmacy, Laboratory MeDIC (Matrix Extracellulaire et Dinamique Intracellulaire)

To demonstrate 4D dynamics of FC together with juxtaposed DFC (FC/DFC assembly) and Nucleolus Associated Chromatin (NAC) we studied the redistribution of UBF and fibrillarin under the selectiveblock of RNA polymerase I. For this we used KB cells transiently tagged with GFP/DSred-UBF and GFP/CFP-fibrillarin and treated by a low dose (0.05µg/ml) of Actinomycine D (AmD) during 8 hours. We found that the treatment with AmD leads to a gradual coalescence and fusion of individual FCs on one side and DFCs on another side (on the other side – если всего две стороны)followed by their displacement to the nucleolar periphery to form caps. The reorganization of NAC induced by AmD treatment was studied by 4D confocal microscopy in the chosen HeLa cells stably expressing H2B-GFP. At different time points (for example 1h) the same cells were fixed, immunolabeled for UBF, imaged for simultaneous localizationof chromatin and UBF and finally processed for EM. By using a precise positioning and orientation system, we were able to find exactly the same cells and image them by TEM. By merging images of corresponding areas identified on EM and fluorescent images we identified the intra-nucleolar H2B-GFP label to the filamentous meshwork of Intra-nucleolar Condensed Chromatin (ICC) that links FCs to Peri-nucleolar Condensed Chromatin (PCC) shell. In the course of treatment we observed a gradual condensation of filamentous structure of ICC into coarse clumps and their shift from the nucleolar interior towards the PCC. We conclude that fusion events are largely involved into the mechanisms of nucleolar segregation and capping. Presumably, the concerted condensation of NAC constituents induces forces that pull FC/DFC assemblies to the PCC shell.