4D Dynamics of the Nucleolus and its Subcompartments

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The nucleolus is the largest nuclear compartment, integrating molecular and structural factories of ribosome biogenesis. The nucleolus emerges because of compact folding of active r-chromatin and r-genes expression products. Corresponding synthesis, processing and assembling machineries acquire the strict territorial pattern — nucleolar components (NCs) such as fibrillar centers (FCs; specific marker UBTF), dense fibrillar component (DFC; specific marker fibrillarin) and granular component (GC). Nucleolus-associated DNA domains, integrating transcriptionally active r-genes, gain by folding into FCs the non-nucleosomal conformation. Meanwhile, its inactive part retains nucleosomal organization in form of intra- and peri-nucleolar condensed chromatin, linked to interstices or nucleolar vacuoles. DFC and GC correspond to early and late processing subcompartments.

In this research, we used our CLEM model system that correlate LSM 4D imaging with post-fixation immunolabeling and TEM performed on a single cell. Special regard was made to the intranucleolar distribution of FCs and DFC along with related marker factors of r-genes transcription and pre-rRNA early processing - UBTF, and fibrillarin, respectively. To combine dynamics of FC and DFC (associated in the form of FC/DFC assembly) with the changes in rRNA synthesis level we followed the redistribution of these proteins under action of nucleolar stress factor - actinomycin D (AmD). To study these NCs and linked factors by natural inactivation of r-genes, the folding of r-chromatin into the structure of mitotic NORs represents an intriguing model.

Displaying the dynamics of fibrillarin and UBTF during AmD-induced nucleolar stress and natural inactivation of r-genes in mitotic cells we observed their different behavior. Thus in contrast to UBTF, in interphase cells treated with AmD we did not find fibrillarin in "free" movement, because its engagement in the structure of DFC, where fibrillarin binds to nascent transcripts. Therefore, we propose that AmD does not disrupt early processing complex composing DFC, so that fibrillarin stay anchored to nascent transcripts as long as they are present. In metaphase cells, fibrillarin relocalizes to the chromosome surface due to transcriptional arrest followed by the depletion of nascent pre-rRNA and the release of protein molecules. Based on such data we postulate the new dynamic model of the nucleolus.