

Nucleolar 3D Organization of Active Ribosomal Chromatin: A Multifolded Loop Model as Revealed by Electron Tomography of UBF

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The nucleolus is the largest and densest (therefore sharply outlined) nuclear compartment, where the cascade of molecular and structural events related to ribosome biogenesis is properly organized and tightly coordinated. A continuous rDNA strand of “Christmas tree” appearance (reaching millimeters or even centimeters in length) has been known for long to provide the structure-functional basis of the nucleolus. A huge amount of structural and regulatory proteins specifically linked to rRNA genes (r-genes) are engaged in the highly dense package of the molecular constituents that compose the nucleolus. Genes of rRNA are transcribed by the rDNA-dependent RNA-polymerase I (RNAP I) in co-operation with a variety of accessory proteins (...). Hence, the nucleolus emerges as a result of compact folding of active r-genes, functioning in association with specific multiprotein assemblies, defined as transcription and rRNA processing machineries. The obligatory members of rDNA transcription machinery are known as RNAP I associated factors (PAFs), whereas the transcriptionally competent set is referred to as transcription complex (TC). An exclusively important PAF for regulation of nucleolar transcription is the architectural transcription factor UBF whose ability to activate rDNA promoter and mediate protein-protein interactions is crucial for the assembling of TC.

The transcriptional factor UBF seems to have a unique function in the formation of rDNA loops. In addition, UBF appears to be consistently associated with the entire r-gene segment. Such an extensive binding to rDNA along the full-length of the repeated unit suggests a structural function of UBF as architectural RNAP I transcription factor. Definitely, this protein could be seen as an equivalent of a structural protein of r-genes chromatin due to the ability to compete with the histones for binding to the rDNA. It is commonly accepted that main function of UBF consists in maintaining of the non-nucleosomal (or under-condensed) structure of r-chromatin. During the intra-nucleolar package the rRNA synthesis and processing machineries acquire the strict territorial pattern which is clearly recognizable in the form of three regular sub-compartments or nucleolar components (NCs). Resulting tripartite nucleolar structure consists of: (i) the interphase derivate of mitotic NORs, known as *fibrillar centers* (FCs); in fact, sites of localization and transcription of r-genes that comprise non-nucleosomal rDNA loops; (ii) *dense fibrillar component* (DFC), where early processing of nascent transcripts or pre-rRNA takes place and (iii) *granular component* (GC) believed as a late processing and pre-ribosome assembling territory. Each NC contains the specific set of resident proteins therefore the GFP- or immuno-detection of their essential members is particularly important in studies dedicated to localization and structure of corresponding machineries.

Up to now, the nucleolus represents the best-investigated nuclear structure. Nevertheless very little is known about how nucleolar r-chromatin is structurally and functionally organized at rDNA transcription sites. Presumably, the transcriptionally active r-chromatin consists of non-nucleosomal CDs while the specific folding of their transcribing sites generates the structure of FC. However, the mode of r-genes package within the limited volume of FC remains undetermined. The size and clear definition of FC can ensure the depiction of the 3D structure of active r-chromatin. In this context the electron microscope (EM) 3D imaging using GFP-/immuno-labeling of essential members of RNAP I transcription machinery, looks like particularly promising for the depiction of active r-genes conformation that generates FC. Thus, the 3D organization of active r-genes

within FCs has been successfully identified in medium voltage scanning-transmission EM (MV STEM) study, using immuno-labeled RNAP I. Then, it could be well assumed that labeling of UBF may appear to be approach ensuring precise localization and 3D ultrastructure of r-chromatin fibrils in under-condensed state.

In this study we have designed a reliable detection technique based on medium-voltage STEM tomography and applied it to high-resolution 3-D mapping of GFP-tagged UBF within interphase FC's and mitotic NORs of KB human cancer cell lines after they were transfected with UBF-GFP. For this we used pre-embedding protocol to reach appropriate labeling of r-chromatin fibers within the whole volume of FC. Localization of UBF molecules was found to be strongly restricted to FCs and mitotic NORs where they are organized in 10-25 nm fibrils (chains) folded in a loop-like manner. The spatial folding of UBF-positive fibers within the FC's did not correlate with the distribution of Pol I molecules (Cheutin et al., 2002; Tchelidze et al., 2008). Selective inhibition rRNA synthesis by Actinomycin D does not provoke disassembling of the loop-like organization of UBF with the progression of nucleolar segregation. Thus a multifold loop model was postulated for both, active as well as artificially and naturally inactivated FCs and NORs. To the best of our knowledge, these findings present the first direct experimental evidence of structural organization of UBF and their re-arrangement under the conditions of rRNA synthesis blockage.

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