In search of the components of nuclear skeleton and their binding partners in a nucleolus

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At the end of mitosis, nucleoli form around the tandemly repeated clusters of ribosomal DNA (rDNA) genes. This results in creating a subnuclear compartment that sequesters the transcription and processing machineries that are responsible for generating ribosomal subunits. The complex processes that are involved in the formation of ribosomal subunits occur in morphologically/functionally distinct parts of the nucleoli enriched with various nucleolar factors. These areas are termed fibrillar centres (FCs), dense fibrillar component (DFC), and granular component (GC) [1]. Actively transcribed rDNA is attached through RNA polymerase complexes to the fibrillar centers, embedded into the nucleoskeleton. The FCs provide structural support for nucleolar transcription and are probably the primary elements in forming the nucleolar architecture [2]. In a search for structural components of the nucleolus, we concentrated on phosphatidylinositol(4,5)-bisphosphate (PIP 2), UBF, RNA polymerase I (Pol I), and fibrillarin, and followed their dynamics during early nucleolar formation. PIP 2 is envisaged as a structural interface between some of the splicing factors (SC35, Sm), U1-U6 snRNAs, Pol II, and nuclear skeletal proteins [3]. UBF and Pol I are the components of the rDNA transcription machinery, and fibrillarin participates in the very early steps of pre-rRNA processing when the nascent transcripts are synthesized [4]. We have showed that from prophase to anaphase NORs contain Pol I and PIP 2 as well UBF and PIP 2 colocalized. During anaphase and telophase, the presence of PIP 2 is usually observed in NORs with low presence of Pol I. From the other hand, the recruitment of Pol I to NORs parallels a release of PIP 2 from NORs (**Figure 1**). These results evoked the question what is the first step: the beginning of rDNA transcription or PIP 2 dissociation from NORs? We have demonstrated that in anaphase, NORs begin to accumulate fibrillarin, while PIP 2 is still present in NORs. Moreover, fibrillarin is recruiting only to NORs that contain PIP 2. This process is continuing till telophase, when PIP 2 leaves NORs (**Figure 2**). Hence we can hypothesize, that pre-rRNA synthesis begins while PIP 2 still localizes in NORs, and the onset of rDNA transcription can be connected with the dissociation of PIP 2 from NORs. However, the functional significance of these results remains to be further clarified with molecular approaches.

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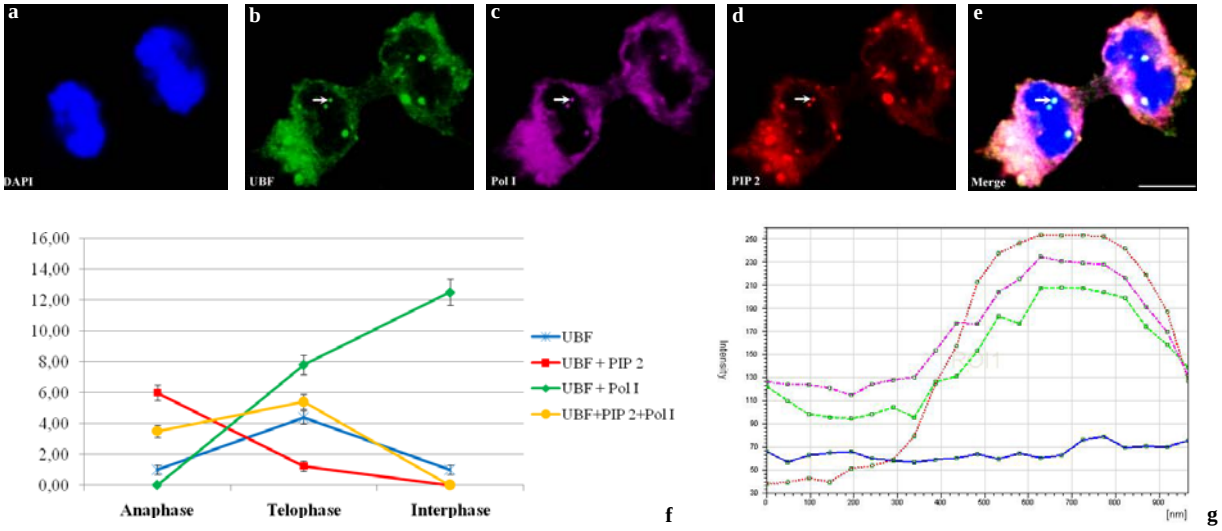

Figure 1. NORs in HeLa cells during mitosis. UBF, Pol I and PIP 2 are colocalized in NORs in telophase (b-e, arrows). The diagram (f) represents the number of NORs/cell (Y axis) containing only UBF (blue line, arrow markers), or UBF and PIP 2 (red line, square markers), or UBF and Pol I (green line, rhomb markers), or UBF, Pol I and PIP 2 together (yellow line, round markers). The graph (g) represents the fluorescence intensities for UBF (green line), Pol I (pink line), and PIP 2 (red line) image channels in the NOR of interest (b-d, arrows). The fixed Hela cells have been triply immunolabeled and observed in confocal laser scanning microscope. The number of cells investigated was ten for each mitotic stage; $p<0,001$ for every two subsequent mitotic stages, except the case of triple colocalization of UBF, Pol I and PIP 2 (yellow line, round markers) in Anaphase vs Telophase comparison, where $p<0,01$. Bar, 5 µm.

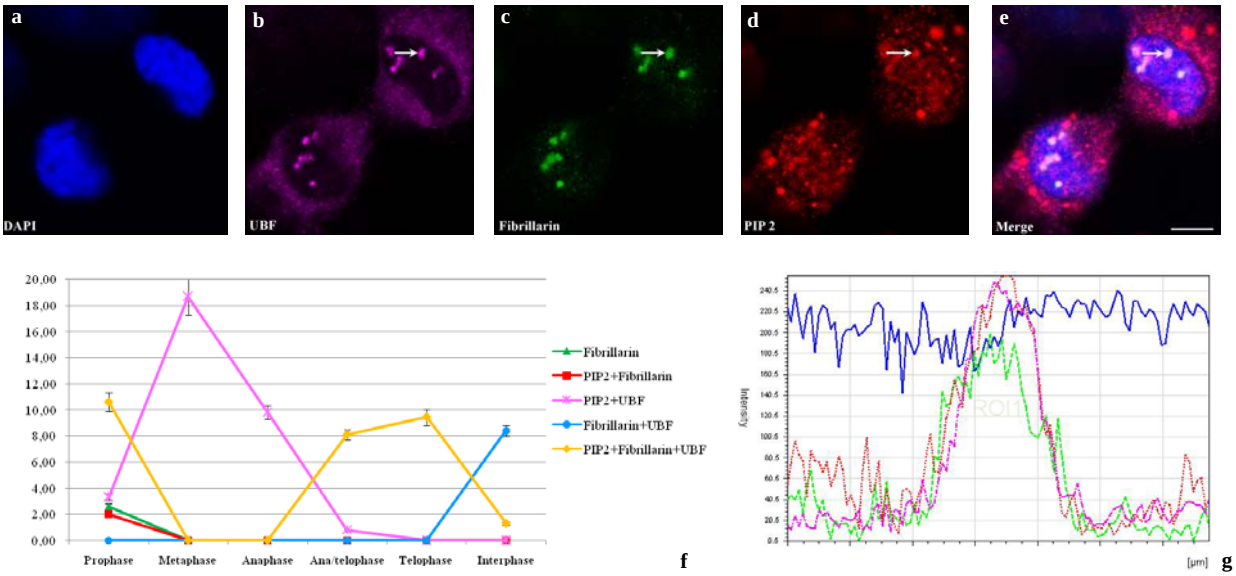

Figure 2. NORs in HeLa cells during mitosis. UBF, fibrillarin and PIP 2 are colocalized in NORs in telophase (b-e, arrows). The diagram (f) represents the number of NORs/cell (Y axis) containing only fibrillarin (green line, triangle markers), or PIP 2 and fibrillarin (red line, square markers), or PIP 2 and UBF (pink line, arrow markers), or fibrillarin and UBF (blue line, round markers), or PIP 2, fibrillarin and UBF together (yellow line, rhomb markers). The graph (g) represents the fluorescence intensities for UBF (pink line), fibrillarin (green line), and PIP 2 (red line) image channels in the NOR of interest (b-d, arrows). The fixed Hela cells have been triply immunolabeled and observed in confocal laser scanning microscope. The number of cells investigated was ten for each mitotic stage; $p<0,001$ for every two subsequent mitotic stages (of course, excluding the cases, where the number of NORs/cell in two subsequent mitotic stages is zero). Bar, 5 µm.

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