P29 Spatial dynamics of rRNAs within the cell nucleus

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It is well known that the formation of eukaryotic ribosomes occurs predominantly in the nucleolus but late maturation steps take place in the nucleoplasm and cytoplasm. Precise identification of nucleolar compartments containing pre-rRNA during their synthesis and processing was first addressed by using autoradiography procedure and photon or electron microscopy imaging. After a short pulse of tritiated uridine, ultrastructural autoradiography revealed that pre-rRNA are synthesised within the fibrillar component. The use of a short pulse followed with a chase further revealed that pre-rRNAs enter within granular component before reaching the nucleoplasm. However, the autoradiographic technique is limited by its low resolution and the impossibility to study the three-dimensional localisation of labelled rRNA within cellular compartments.
We recently developed a new technique using BrUTP as a precursor to label pre-rRNAs which allowed us to identify sites of pre-rRNA synthesis (short pulse) and their route towards the cytoplasm (pulse-chase experiment) within isolated nucleoli or entire cells (Thiry et al., 2000; Cheutin et al., 2002). The use of the same antibody, identified either with fluorescence or with gold particles, revealed the 3D organisation of sites containing labelled rRNA or their precise localisation by using confocal and ultrastructural microscopy respectively. Comparison of 3D reconstruction obtained from optical sections series and of ultrathin sections was extremely fruitful to describe topological and spatial dynamics of rRNAs from their synthesis site inside the nucleolus to the cytoplasm. The results of this work clearly indicated further that the nucleolar transport of rRNAs occurred neither randomly nor along tracks as for some mRNA but followed a volumic, well-defined pathway from the inner part of the nucleolus toward its periphery.
In the present study, we have focused on the routes of rRNA traffic from the nucleolus to the nuclear membrane for transport to the cytoplasm by using new software for 3D imaging. The results clearly indicate that rRNA molecules leave the mammalian cell nucleolus in two steps. A first exit occurs after a few minutes of chase whereas a second exit, more obvious, takes place later leading to the disappearance of the nucleolar signal. We also show that the rRNA molecules move out from the nucleolus in all the directions. These results will be discussed in the light of the recent biochemical data.