Novel mammalian ribosomal system: probing the biogenesis and functional hot spots of the ribosome

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Keywords: Nucleolus, Ribosome, Translation

Assembly of ribosomes is a fundamental and highly complex process present in all living cells [1] and requires orchestration of transcription by different polymerases operating in different compartments within the nucleus [2-4]. Ribosome biogenesis occurs within the nucleolus and requires coordinated expression of four rRNAs: 18S, 5.8S, and 28S, which arise from a 47S precursor, 5S rRNA, and ≈80 ribosomal proteins (r-proteins). 47S pre-rRNA is transcribed in the nucleolus by polymerase I (Pol I), 5S RNA is transcribed at the nucleolar periphery by Pol III and then imported into the nucleolus, and r-protein mRNAs are transcribed by Pol II. Ribosome biogenesis has been extensively studied in yeast. In mammalian cells however, these processes are largely unknown. We can now start to address some of these fundamental questions and in particular the Pol I and Pol II promoter specialization using novel mammalian ribosomal system.

We have used laser scanning confocal microscope (LSCM) in combination with fluorescence in situ hybridization (FISH) technique to follow the biogenesis of ribosomal subunits. Figure-1 shows 3D-projection overlay of mouse neuroblastoma cells (N2a) transfected with a plasmid that encodes 18S ribosomal gene. The recombinant 18S (40S subunit) ribosomal gene was engineered with a Tag-sequence that allows its detection using a fluorescently labelled Cy3-DNA probe (Red) whereas the native 28S (60S subunit) is detected with a Cy5-lebelled DNA probe (Green). The nuclear compartment is shown in blue using DAPI staining. The overlay in figure-1 clearly shows two cells on the right hand side that express the recombinant 18S rRNA with intense red staining whereas the two cells on the left hand side are not transfected therefore show no red staining. An important observation is the strong red signal within specific areas of the nuclei from cells that express the recombinant 18S rRNA. The red nuclear staining in figure-1 reveals the nucleoli where the ribosomal genes are being transcribed and processed. Detailed analysis inside the nucleus reveals that the recombinant 18S rRNA gene is transcribed within the existing nucleoli and thus appears to co-localize with the same biosynthetic machinery that transcribes and processes the native ribosomal genes (figure-2).

We have carried out extensive analysis to quantify the data generated from the LSCM-FISH approach. This is crucial information because the recombinant 18S once processed has to be incorporated into a mature and functional 40S subunit that is exported into the cytoplasm where it is supposed to carry out protein translation together with the 60S subunit as an 80S complex. Our analysis reveals strong correlation between the signal that detects the recombinant 18S (40S subunit) and the signal from 28S (60S subunit). Importantly, a comparable correlation was seen between signals from endogenous 18S and 28S rRNA in control cells that are not transfected with recombinant 18S rDNA. Our data suggest that the homeostasis of ribsosome biogenesis is not disturbed by the transfection process that forces the cell to make specialized recombinant ribosomes.

Finally, we have used the ribosomal mammalian system and carried out targeted mutational analysis to probe hot spots within the 18S rRNA and their effects on both 40S ribosomal subunit biogenesis and translational activity. Data from this analysis will be presented.

References

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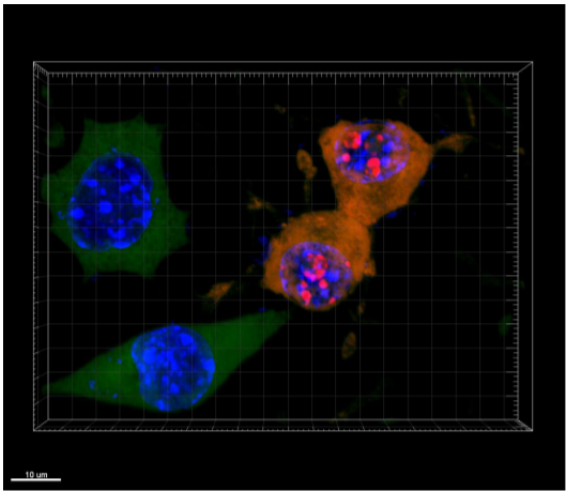

Figure-1: *Maximum intensity 3D-projection overlay of mouse neuroblastoma cells (N2a) transfected with a plasmid that encodes 18S ribosomal gene. The recombinant 18S (40S subunit) ribosomal gene was engineered with a Tag-sequence that allows its detection using a fluorescently labelled Cy3-DNA probe (Red) whereas the native 28S (60S subunit) is detected with a Cy5-lebelled DNA probe (Green). The nuclear compartment is shown in blue using DAPI staining. The image was collected using Leica TCS SP5 microscope and the 3D-projection overlay was generated by IMARIS software.*

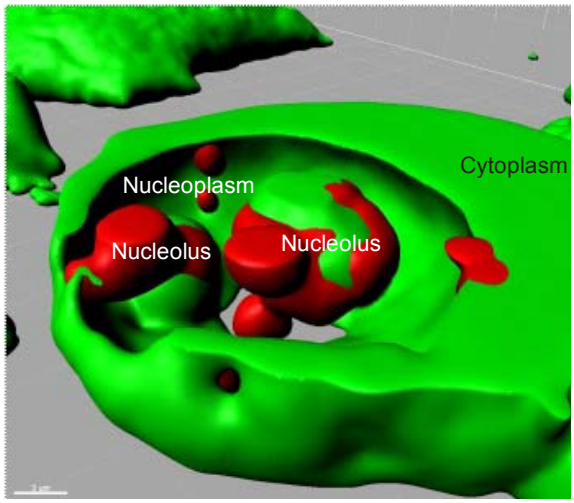

Figure-2: *IMARIS software was used to create surface representation and 3D segmentation of N2a cell transfected with recombinant 18S rDNA construct. To create the green surface we used a smoothing factor of 0.238 microns. For the segmentation we used all green pixels that had an intensity value higher than 10.8 absolute intensity units in order to exclude from the segmentation any background signal. Mean intensity value for the green surfaces is 18.85 + 4.88.*
For the segmentation of the red channel we followed a similar protocol as for the green channel. In this case we chose a smaller smoothing factor of 0.17. For the segmentation we selected all the pixels with an absolute intensity value higher than 31.36. Mean intensity value for the red surfaces is 55.32 + 15.48.