**Nucleolar chromatin - a microscopy-based approach**

Schöfer, C.[1], Weipoltshammer, K.[1,2] and Snyers, L.[1,2]

[1] Dept. for Cell and Developmental Biology, Austria, [2] Medical University of Vienna, Austria

Chromatin is composed of DNA wrapped around histone proteins, which not only structure the genome but are also crucial regulatory elements of DNA-dependent functions such as transcriptional activity. Ribosomal genes are organized in tandem repeats (rDNA) of which several hundred exist in normal human genome. Part of these repeats can be found within nucleoli and are transcriptionally active, whereas silent genes can be found adjacent to it. The differential localization of active and inactive rDNA with respect to the nucleolar structure requires high-resolution approaches to study possible differences in histone occupancy related to transcriptional activity of rDNA.

We took a correlative approach to study the histone occupancy of rDNA. To this end, we expressed different isoforms of histones tagged with fluorescent molecules in human cells and spatially correlated these signals with rDNA, visualized by in situ hybridization. Samples were examined using confocal microscopy, correlative light-electron microscopy (CLEM), structured-illumination imaging (SIM) and Expansion Microscopy approaches. We found that in interphase the interior of the nucleolus was devoid of significant signal for all histones studied suggesting that the transcriptionally active genes are largely nucleosome-free. In contrast, (inactive) ribosomal genes located at the periphery of nucleoli contained histone proteins. Blocking rDNA transcription revealed an overall higher occupancy of rDNA by histones. Therefore, it appears that histone occupancy of ribosomal genes vary according to their different states of transcriptional activity. Our data support the view that highly transcribed ribosomal genes lack histones, which may enable high occupancy of the transcription machinery at each active ribosomal gene. In contrast, silent genes associate with histones which is thought to be required for initiation and maintenance of transcriptional silencing and which may also play a role in rDNA compaction. Our data furthermore indicate that the fraction of rDNA bound to histones increases when shutting down transcriptional activity. This process is correlated with a shift in the position of ribosomal genes from the "active-position" within the nucleolus to the "inactive-sites" adjacent to the nucleolus.

Acknowledgement: this study was supported by the Austrian Science Foundation and Herzfeldersche Familienstiftung (grant P 30642-b28 to C.S.)