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**IT-13-P-5975 LIFE CELL IMMUNOGOLD LABELLING OF RNA POLYMERASE II visualized by Focused Ion Beam Scanning Electron Microscopy (SEM-FIB)**

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The intracellular localization and dynamics of proteins involved in cellular processes are often studied in living cells at light microscopy resolution by monitoring proteins fused to fluorescent tags. These methods have nevertheless intrinsic drawbacks. First, the level of expressed fusion proteins is difficult to match with endogenous levels and since the endogenous protein is generally not extinct, the fusion protein acts on top of its native counterpart. The second restriction comes from the limited spatial resolution of light microscopy which, despite the spectacular development of super-resolution light microscope modalities, does not attain molecular dimensions.
Electron microscopy is an invaluable method to improve spatial resolution and to describe the cellular context of proteins of interest but relies on electron dense probes or reagents to detect the labeled macromolecule. The most successful and widely used method consists in conjugating primary or secondary antibodies to gold particles (Faulk and Taylor, 1971) which have a high electron scattering power and create an easily recognizable highly contrasted round shape.
Here we exploited the ability of cells to internalize macromolecules with a method named “live cell immunogold labeling” which takes advantage of lipid-based protein delivery agents compatible with cell viability to internalize the probes (Futami et al., 2012, Freund et al., 2013).
We used this method to label RNA polymerase II with electron dense labels suitable for EM localization studies and demonstrate for the first time that antibodies coupled to 0.8 nm ultrasmall gold particles (Van de Plas P. and Leunissen J.L., 1993) can enter the nucleus and be detected after amplification. Cells grew normally for more than 8 hours after probe uptake. The label was detected, after silver enhancement, by transmission electron microscopy and by scanning electron microscopy coupled to Focussed Ion Beam slicing (SEM-FIB)(Schroeder-Reiter E.et al.,2009). These methods open the new possibility to label nuclear or cytoplasmic antigens in living cells, and to immunolocate them in the whole cell volume using the SEM-FIB technology.

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