Cryo-EM technique avoids chemical fixation and by the way its inherent artifacts allowing the visualization and analysis of samples in a close to native state. This technique has been coupled with fluorescence imaging (cryo-CLEM). We developed correlative cryo-analytical STEM, for mapping water by dark field imaging and ions by energy dispersive X-Ray spectrometry (EDXS) within compartment previously identified by GFP tagged proteins.

Stable transfected HeLa cells expressing H2B-GFP (allowing the identification of chromatin in the nucleus) are vitrified by rapid plunging into liquid ethane without cryoprotectant. In classical cryo-CLEM methods, the grid is first placed in a dedicated cryo-setup mounted on the stage of a fluorescence microscope for recording fluorescent images then transferred in the cryo-holder of the electron microscope for imaging. However, this method suffers from an important drawback due to the transfer of the grid which can induce: i) mechanical or thermal damages, ii) grid bending, contamination or even loss of the sample during transfer. We developed an original technical protocol whereby cryo-ultrathin sections (80nm) are placed on a formvar-carbon coated indexed grid, directly and definitely mounted on the Gatan EM cryo-holder, and successively imaged by cryo-fluorescence microscopy and cryo-electron microscopy (1).

In order to demonstrate the potentiality of our method we studied effects of a cytotoxic chemotherapy drug at 2 exposure levels:

*Actinomycin D at 50 ng/mL in order to induce a nucleolar stress to inhibit RNA synthesis. We map at the ultrastructural level water and elements. Cryo-CLEM allows us to measure them specifically in rich chromatin areas that are undistinguishable by ultrstructural morphology in STEM (Figure 1). By the way we have shown that nucleolar stress induce an increase of water and a large decrease of elemental contents in all cell compartments (2).

*Actinomycin D at 500 ng/mL in order to induce an apoptosis state. Live confocal microscopy experiments allowed to define six chronological steps for apoptosis. These steps, which are not always identified by ultrastructural morphology, are fortunately easy to recognize by fluorescence microscopy. So correlative cryo-analytical STEM allows us, for the first time, to correlate water and ions content evolutions in the different cell compartments as a function of apoptosis states.

Our original process has several advantages: i) it is universal ie valid for all labeled proteins and ii) it allows the targeted nanoanalysis of water and ions in connection with fluorescent labeling.

(2) Nolin et al., CMLS 70(13) (2013) 2383-2394.

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Fig. 1: Targeted nano analysis. A) Fluorescence image of a HeLa H2B-GFP cell cryosection. B) STEM DF image of the same cell after freeze-drying. C) Merge between the strongest fluorescence areas (red) identifying clumps of condensed chromatin and the STEM image. So we identify clumps of chromatin on STEM image and we are able to target them for EDXS (D).