Live Cell Imaging of Rab5 Localization During Mitosis

The small GTPase Rab5 has a well established role in endocytosis during interphase. There is, however, some evidence to suggest that Rab5 may also have a function at the mitotic spindle. In this study, researchers set out to investigate a putative role for Rab5 in mitosis.

The localization of Rab5 throughout mitosis was examined using time lapse imaging of human osteosarcoma (U2OS) cells stably expressing YFP-Rab5A, and mRFP-α-tubulin to label microtubules. For live cell imaging, researchers needed high speed image capture and maximum sample protection. For this reason, the UltraVIEW® VoX 3D Live Cell Imaging System, with Volocity® 3D Image Analysis Software, was chosen. A 60X oil-immersion objective was used and a step size of 0.6 µm was selected.

Researchers observed a clustering of Rab5+ vesicles around the spindle poles at the beginning of mitosis. At prometaphase and metaphase, the number of vesicles close to the poles decreased and only increased again at late anaphase (Figure 1). Some Rab5+ vesicles were observed moving within the spindle during metaphase (Figure 2), supporting the idea of a role for Rab5 at the mitotic spindle.

The researchers then employed siRNA mediated silencing to investigate the functional involvement of Rab5 during mitosis. Taken together with the live cell imaging results, they revealed an unexpected role for Rab5 in chromosome congression and segregation. They went on to demonstrate that in mitotic cells Rab5 forms a complex with centromere protein F (CENP-F), a kinetochore component which is required for chromosome congression. It was proposed that Rab5 regulates the kinetics of release of CENP-F and its accumulation on kinetochores.

In conclusion, the findings suggest that Rab5 plays an important role in ensuring the correct regulation of mitotic programs, thus protecting against chromosomal instability, an important hallmark of cancer.[2]]

Figure 1 (left): Rab5 dynamics during mitosis. Frames taken from a movie in which a mitotic U2OS cell expressing YFP-Rab5A (green) and RFP-α-tubulin (red) was observed from prophase to cytokinesis. Images were captured every 5 min. T=0 was defined as the point at which the spindle starts to assemble. The movie can be viewed in the supplementary material associated with this paper (movie S1).

Figure 2 (above): Rab5 vesicles move within the spindle at metaphase. Single frame from a movie (movie S2) showing a U2OS cell expressing YFP-Rab5A and RFP-α-tubulin at metaphase. Images of a single Z section taken in the equatorial region of the spindle were captured every 600 ms. The frame shown was taken at 22 sec.