Localising Cytoskeletal Associated Proteins to Filamentous Networks within Plant and Animal Cells using Correlative Imaging Techniques

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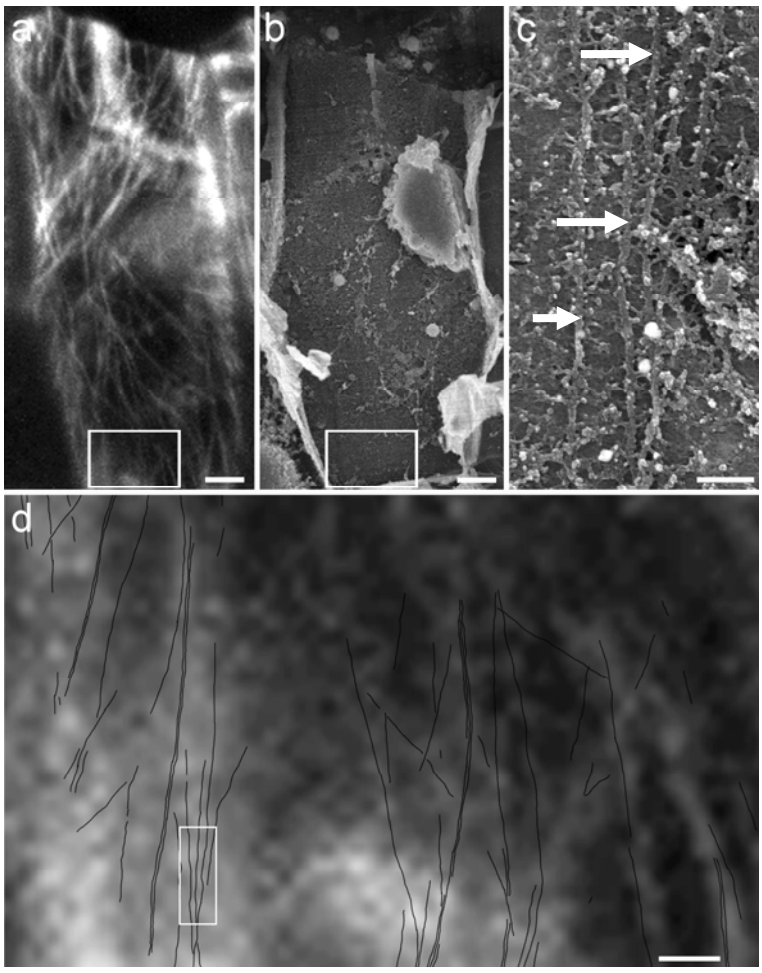
Background:
Imaging the distribution, abundance and interaction of cytoskeletal associated proteins within filamentous cytoskeletal networks is often a challenge as they are far smaller than the resolution limit of optical microscopes. When tagged with fluorescent markers the distribution of these proteins within cells can be determined using fluorescence or confocal microscopy. However, the precise localisation of these proteins to the filamentous networks often requires the use of electron microscopy instead. Correlative microscopy techniques, by combining both fluorescence and electron microscopy methods, can therefore provide essential information about both the widespread distribution and specific localisation of cytoskeletal associated proteins within cells.

Outcomes:
- the development of reliable, reproducible methods to image cytoskeletal associated proteins within single plant or animal cells using immunocytochemistry combined with confocal and electron microscopy
- critical evaluation of probes used for immunocytochemistry, such as FluoroNanogold or Quantum dot secondary antibodies.

Progress to date:
- successfully immunolabelled interphase microtubule arrays within the same plant cells and imaged them with both confocal microscopy and electron microscopy (see picture).

Things still to do:
- immunolabel plant and mammalian cells with antibodies against specific cytoskeletal associated proteins
- investigate the distribution of these proteins with confocal microscopy
- localize the specific interaction between the proteins and cytoskeletal filaments using either high resolution scanning electron microscopy or transmission electron microscopy.



A plant interphase microtubule array immunofluorescently labelled and imaged with a) confocal microscopy and b) high resolution scanning electron microscopy. c) Individual microtubules (arrows) of the regions boxed in d). The position of microtubules in the FESEM image was traced and positioned over the corresponding region in the confocal image (box in a). Scale bars: a, b = 5 μm, c = 200 nm d = 1 μm.