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Detection of GFP-labeled Proteins by Electron Microscopy

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Reliable and quantifiable high-resolution protein localization is critical for understanding protein function. Green fluorescent protein (GFP) has revolutionized the detection of proteins by light microscopy. Similarly, new genetic tags for electron microscopy (EM) [1] have great potential for EM visualization of proteins in cells and, potentially, in whole organisms.

We have sought to develop systems which allow EM detection of GFP-tagged proteins. We have pioneered the use of a processing method involving fast freezing, freeze substitution, and resin embedding that preserves GFP fluorescence and facilitates correlative light and electron microscopy (CLEM) in cells and tissues [2,3,4]. We show that in the absence of primary chemical fixation, excellent ultra-structure, preservation of GFP fluorescence, immunogold labelling and electron tomography can be obtained using a single technique involving high-pressure freezing and embedding in Lowicryl resins at low temperature. This method has been used to great effect to correlate dynamic membrane trafficking events in living cells with electron microscopic 3D ultrastructure of the same events [5]. This processing scheme has been further modified to involve ultra-rapid freeze substitution providing a simple fast method to correlate GFP fluorescence in sections with EM ultrastructure (Figs. 1 & 2).

More recently we have developed a system for detection of GFP-tagged proteins in cells and tissues. This method significantly reduces the time required for the determination of subcellular protein distribution. We demonstrate that this technique can allow localization of numerous GFP-tagged proteins of unknown cellular distribution at high-resolution within a few days and is compatible with both electron tomography and serial blockface scanning electron microscopy. We also demonstrate that we can readily apply this method to the localization of GFP-tagged proteins to EM-resolution *in vivo*. These new methods can be used to complement light microscopic studies as a simple way to provide ultrastructural information on protein localization in a high throughput manner.

References

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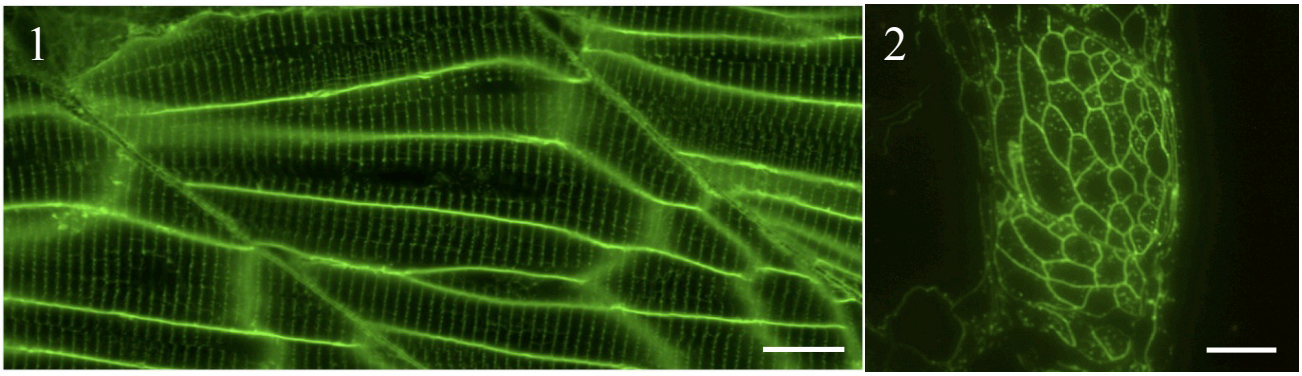

Figure Legends. Figure 1 shows GFP fluorescence in the muscle of a 3-day zebrafish embryo expressing a plasma membrane protein, caveolin-3, fused to GFP. GFP fluorescence is associated with the muscle surface and the T-tubule system. Figure 2 shows a Lowicryl section from the same zebrafish embryo after fast freezing, rapid freeze substitution, and embedding in resin at low temperature. Note the excellent preservation of the GFP fluorescent signal after EM processing. Bars 10μm.

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