

GRAB

We have developed a simple correlative photo-oxidation method that allows for the direct ultrastructural visualization of the green fluorescence protein (GFP) upon illumination.

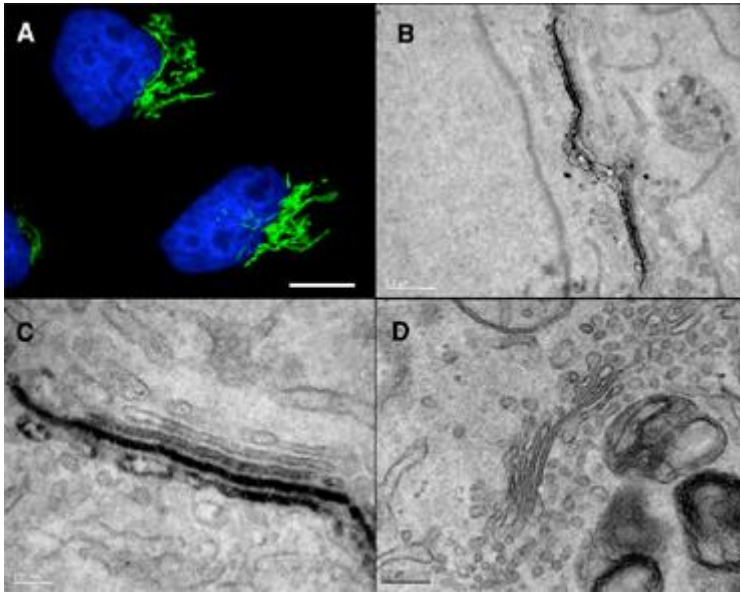


Figure 1

The method, termed GRAB for "GFP Recognition After Bleaching", uses oxygen radicals generated during the GFP bleaching process to photooxidize diaminobenzidine (DAB) into an electron dense precipitate that can be readily visualized by routine electron microscopy.

The DAB product produced by the GRAB method appears linear to the initial fluorescence and is of sufficient quality to reveal detailed spatial information. This is exemplified by the observed intra-Golgi stack and intra-cisternal distribution of a Golgi resident glycosylation enzyme, N-acetylgalactosaminyltransferase-2 fused either to enhanced GFP (Figure 1) or CFP (Figure 2).

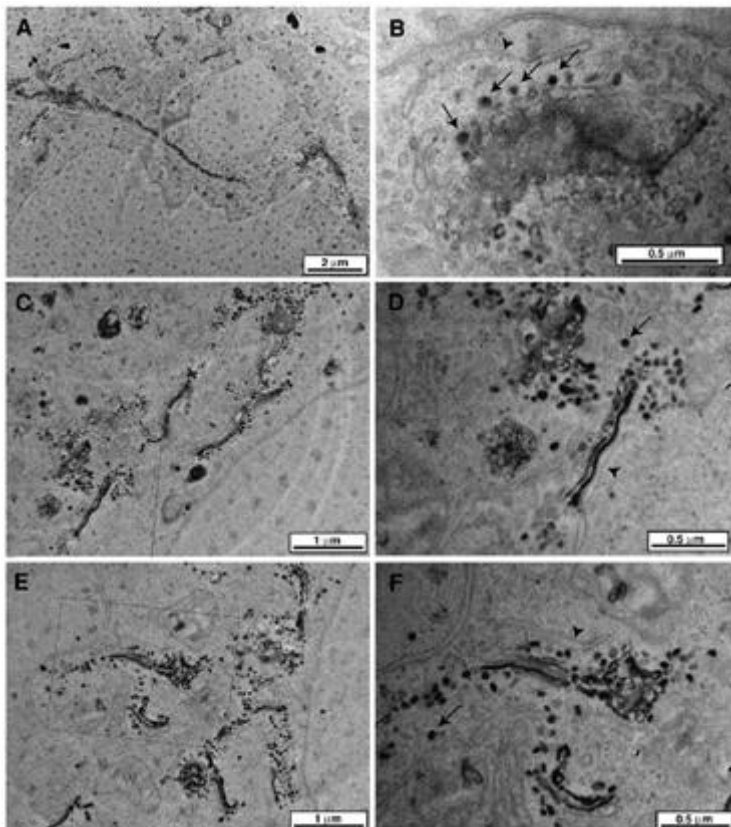


Figure 2

Ref: Grabenbauer M, Geerts WJ, Fernandez-Rodriguez J, Hoenger A, Koster AJ, Nilsson T. "Correlative microscopy and electron tomography of GFP through photooxidation." Nat Methods. 2005 Nov; 2(11):857-62.