Endocytosis in Plant Protoplasts: Visualization and Quantitation of Fluid-Phase Endocytosis Using Silver-Enhanced Bovine Serum Albumin-Gold

Abstract

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Endocytosis in Plant Protoplasts: Visualization and Quantitation of Fluid-Phase Endocytosis Using Silver-Enhanced Bovine Serum Albumin-Gold [Abstract]

Fluid-phase endocytosis of a colloidal gold conjugate of bovine serum albumin by soybean protoplasts is characterized using en bloc silver enhancement of internalized gold. Uptake is biochemically quantified by spotting intact protoplasts which have taken up BSA-gold on nitrocellulose and silver enhancement of the gold. Gold uptake by the protoplasts is then measured using densitometric video analysis. Uptake is imaged by confocal epipolarization laser scanning microscopy combined with optical sectioning using Nomarski optics. At short times of uptake, the probe is found in the cortical cytoplasm associated with internal vesicles (endosomes). With longer times endosomes are found in the cortical cytoplasm and in the perinuclear region. In contrast to previous reports of fluid-phase endocytosis, long incubation times do not lead to gold label in the vacuole. The technique used in this work is more reliable than others previously used and can be extended to studies on absorptive and receptor-mediated endocytosis in plant protoplasts.