

Visualization of Endosome to Vacuole Pathway in BY-2 or Arabidopsis

Root

The endosome is responsible for the sequestration of plasma membrane to vacuole. The method is easy way to observe endocytotic pathway.

FM4-64 stock solution

1 mg/ml of FM4-64 [*N*-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino) phenyl) hexatrienyl) pyridinium dibromide] (Molecular Probes) in DMSO. Stock it at -20 °C.

Procedure

1. BY-2

-Pick up 1-3 ml of cell culture in 50ml Falcon tube. (We usually use 4-day-old cells.)

-Add FM4-64 [*N*-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino) phenyl) hexatrienyl) pyridinium dibromide] at final concentration, 8 μ M.

-Keep shaking on rotator for several hours (150 rpm, 25°C).

-Observe with fluorescent microscopy, rhodamine range.

-0 h, plasma membrane. ~3h, endosome (small punctate structure in cytoplasm). 20 h, vacuolar membrane (tonoplast).

2. Arabidopsis root

-Aseptically grow Arabidopsis plants in plastic plates ($\phi = 9$ cm).

-Add 3 ml of 8 μ M FM4-64 in distilled water to the plate.

-Observe with fluorescent microscopy, rhodamine range.

Reference

Yamada K, Fuji K, Shimada T, Nishimura M, Hara-Nishimura I.

Endosomal proteases facilitate the fusion of endosomes with vacuoles at the final step of the endocytotic pathway. *Plant J.* (2005) 41, 888-898.