

Labeling of the Intestinal Lumen of *Caenorhabditis elegans* by Texas Red-dextran Feeding

Keiko Saegusa and Ken Sato*

Laboratory of Molecular Traffic, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan

*For correspondence: sato-ken@gunma-u.ac.jp

[Abstract] In this method, the intestinal lumen of *Caenorhabditis elegans* (*C. elegans*) is labeled with a fluorescent fluid-phase marker, Texas Red-dextran. Since dextran conjugates are membrane impermeable, animals fed with it show a red fluorescent signal in the lumen of the intestine. Texas Red-dextran in the lumen is not efficiently endocytosed by intestinal cells and is not effectively transported to the body cavity paracellularly. It is useful to determine whether round-shaped membrane structures are invaginations from the apical membrane or cytoplasmic vesicles. If the barrier function of the intestinal epithelium is impaired, Texas Red-dextran can leak from the intestinal lumen to the body cavity. Therefore, this method can be used to visualize apical membrane morphology in intestinal cells and to investigate the barrier properties of the intestinal epithelium.

Materials and Reagents

1. *C. elegans* strain
GK70 *unc-119 (ed3)* III; *dkIs37[Pact-5-GFP-pgp-1;unc-119(+)]*
2. *Escherichia coli* OP50 strain (obtained from the Caenorhabditis Genetics Center)
3. Texas Red-dextran (40,000 MW) (Life Technologies, Molecular Probes[®], catalog number: D-1829)
4. Levamisole hydrochloride (Sigma-Aldrich, catalog number: L9756)
5. Agarose (Nacalai Tesque, catalog number: 01158-85)
6. Egg buffer (see Recipes)
7. M9 buffer (see Recipes)
8. Agarose pads (see Recipes)

Equipment

1. Siliconized tubes (1.7 ml) (National Scientific Supply, catalog number: 20172-945)
2. Tabletop centrifuge (Hitachi Koki, model: CT15RE)

3. Confocal laser scanning microscopy system (Olympus, model: FV1000)
4. Four clean glass slides and a coverslip

Procedure

1. Place synchronized L1 larvae on an *Escherichia coli* (*E. coli*) OP50-seeded plate and incubate them for 3 days at 20 °C.
2. Pick approximately 30-40 young adult animals from an OP50-seeded plate and transfer them into 500 µl of egg buffer in 1.7 ml siliconized tubes.
3. Allow the animals to settle with gravity for a few minutes. Remove the supernatant and wash with 500 µl of egg buffer 3 times.
4. Remove most of the supernatant, leaving approximately 10-20 µl of worm suspension and add 100 µl of egg buffer containing 1 mg/ml Texas Red-dextran.
5. Incubate the animals for 90 min at room temperature with occasional gentle agitation every 30 min.
6. Collect the animals by centrifuging at 400 x g for 2 min and discard the supernatant. Wash with 1 ml of egg buffer at least 3 times.
Note: Remove as much Texas Red-dextran in the worm suspension as possible.
7. Add 1 M levamisole hydrochloride to the worm suspension to a final concentration 10~20 mM.
8. Transfer the worm suspension to a 1.5% agarose pad on a glass slide and cover it with a coverslip.
9. Observe the animals with confocal laser scanning microscopy (see Figure 1).

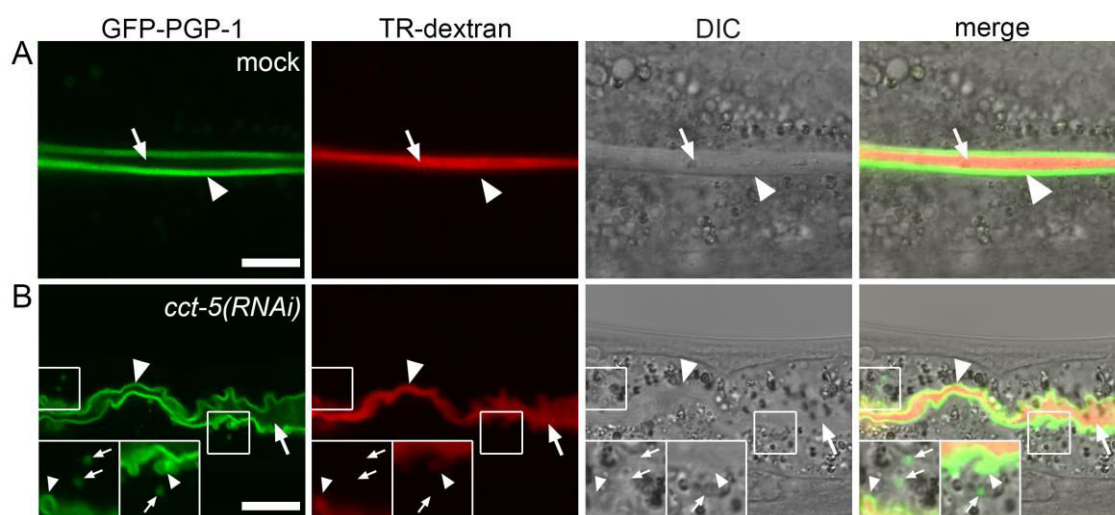


Figure 1. Animals expressing GFP-PGP-1 were fed Texas Red-dextran. GFP-PGP-1 and Texas Red-dextran labeled the intestinal apical membrane (large arrowheads) and the

intestinal lumen (large arrows), respectively. Texas Red-dextran that is taken up by feeding is restricted to the intestinal lumen when the junctional seals of intestinal epithelium cells are intact (Carberry *et al.*, 2012). A. RNAi-mediated knockdown of *C. elegans* chaperonin subunit (CCT-5) disorganized the microvillus actin filament and microtubules, leading to aberrant morphology of the intestinal apical membrane (Saegusa *et al.*, 2014). B. In such animals, the apical membrane, labeled by GFP-PGP-1 (*C. elegans* P-glycoprotein homolog), formed bubble-like membrane structures invaginated from the apical membrane (B, small arrows). Such structures contain Texas Red-dextran (B, small arrows). By contrast, GFP-PGP-1-containing vesicles underneath the apical membrane (B, small arrowheads) did not contain Texas Red-dextran, indicating that these are exocytic vesicles. Images were obtained using an FV1000 confocal microscopy system. GFP-PGP-1; excitation: 473 nm, detection: 490-540 nm. Texas-Red dextran; excitation: 559 nm, detection: 575 to 675 nm. The scale bars indicate 10 μ m.

Notes

1. Although Texas Red-dextran is not efficiently taken up by the intestinal cells in this feeding method, some other apical endocytic markers such as rhodamine-dextran (M_r 40K) and Texas Red BSA are reported to be available to visualize endocytic vesicles (Grant *et al.*, 2001).
2. Depletion of tight junction components such as claudins (CLC-1-4 in *C. elegans*) causes the infiltration of dextran conjugates from the intestinal lumen into the body cavity (Asano *et al.*, 2003).

Recipes

1. Egg buffer
 - 118 mM NaCl
 - 48 mM KCl
 - 2 mM CaCl_2
 - 2 mM MgCl_2
 - 25 mM HEPES
 - pH 7.3

Note: This solution is more physiologically isotonic than M9 for C. elegans tissues (Grant et al., 2001).

2. M9 buffer
 - 42 mM Na_2HPO_4

22 mM KH₂PO₄

86 mM NaCl

1 mM MgSO₄

3. Agarose pads (prepare beforehand)
 - a. Melt 1.5% agarose in M9 buffer in a microwave oven and transfer it to a glass tube.
 - b. Keep the agarose in a molten state by placing the tube in a heat block at 65 °C.
 - c. Place a clean glass slide between two glass slides sealed with pieces of labeling tape over both ends.
 - d. Drop the 1.5% agarose onto the center glass slide and place another clean slide on top, perpendicular to the other three slides.
 - e. Gently remove the top perpendicular slide after the agarose solidifies.
 - f. Use the agarose before it dries up.

Acknowledgments

This work has been supported by the JSPS KAKENHI Grant Number 26291036, the Sumitomo Foundation, the Naito Foundation, and the Mochida Memorial Foundation for Medical and Pharmaceutical Research (to Ken Sato). The protocol has been adapted from Saegusa *et al.* (2014).

References

1. Asano, A., Asano, K., Sasaki, H., Furuse, M. and Tsukita, S. (2003). [Claudins in *Caenorhabditis elegans*: their distribution and barrier function in the epithelium.](#) *Curr Biol* 13(12): 1042-1046.
2. Carberry, K., Wiesenfahrt, T., Geisler, F., Stöcker, S., Gerhardus, H., Überbach, D., Davis, W., Jorgensen, E., Leube, R. E. and Bossinger, O. (2012). [The novel intestinal filament organizer IFO-1 contributes to epithelial integrity in concert with ERM-1 and DLG-1.](#) *Development* 139(10): 1851-1862.
3. Grant, B., Zhang, Y., Paupard, M. C., Lin, S. X., Hall, D. H. and Hirsh, D. (2001). [Evidence that RME-1, a conserved *C. elegans* EH-domain protein, functions in endocytic recycling.](#) *Nat. Cell Biol.* 3(6): 573-579.
4. Saegusa, K., Sato, M., Sato, K., Nakajima-Shimada, J. and Harada, A. and Sato, K. (2014). [Caenorhabditis elegans chaperonin CCT/TRiC is required for actin and tubulin biogenesis and microvillus formation in intestinal epithelial cells.](#) *Mol. Biol. Cell* 25(20): 3095-3104.