

## *A pathway for unicellular tube extension depending on the lymphatic vessel determinant Prox1 and on osmoregulation.*

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Biological tubes formation and extension is a universal, yet still poorly understood process. Excretory canal in nematode *C. elegans* is a unicellular tube that runs alongside the body. It was previously assigned an osmoregulatory role and served as a model for kidney. Using genetics, light and electron microscopy we characterized and identified several steps in excretory canal growth and lumen extension. In model we propose the basal and apical extensions of canal membranes that grow sequentially: basal is followed by apical, fed by cytoplasmic vesicles (canaliculi) fusion to the central lumen. The process of basal process extension resembles the paradigm of the axon growth cone. The internal lumen of the canal, as indicated by our results extends as a result of an osmoregulatory activity that triggers the fusion of peri-apical vesicles. Intermediate filaments and actin crosslinking proteins in the apical cytoskeletal web provide straight lumen growth.

Characterization of *rdy-3 /ceh-26/pros-1* mutant by fluorescent and electron microscopy revealed that this gene is essential for excretory canal formation. Expression of several genes encoding proteins mediating excretory lumen extension, such as the osmoregulatory STE20-like kinase GCK-3 and the intermediate filament IFB-1, is regulated by *pros-1*. PROS-1 is homologous to vertebrate Prox1, a transcription factor controlling lymphatic vessel growth. These findings have potential evolutionary implications for the origin of fluid-collecting organs, and provide a reference for lymphangiogenesis.