Developmental Biology 329 (2009) 201–211

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Lipocalin signaling controls unicellular tube development in the *Caenorhabditis elegans* excretory system

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ARTICLE INFO

Article history:
Received for publication 17 February 2009
Accepted 18 February 2009
Available online 6 March 2009

Keywords:
Lipocalin
Tubulogenesis
Intercellular signaling
lpr-1
Excretory

ABSTRACT

Unicellular tubes or capillaries composed of individual cells with a hollow lumen perform important physiological functions including fluid or gas transport and exchange. These tubes are thought to build intracellular lumina by polarized trafficking of apical membrane components, but the molecular signals that promote luminal growth and luminal connectivity between cells are poorly understood. Here we show that the lipocalin LPR-1 is required for luminal connectivity between two unicellular tubes in the *Caenorhabditis elegans* excretory (renal) system, the excretory duct cell and pore cell. Lipocalins are a large family of secreted proteins that transport lipophilic cargos and participate in intercellular signaling. *lpr-1* is required at a time of rapid luminal growth, it is expressed by the duct, pore and surrounding cells, and it can function cell non-autonomously. These results reveal a novel signaling mechanism that controls unicellular tube formation, and provide a genetic model system for dissecting lipocalin signaling pathways.

Introduction

Tubes are an essential component of many organs such as the kidney, lungs, and vasculature (Lubarsky and Krasnow, 2003). These tubes can vary widely in size and structure, but all have an apical surface lining a hollow lumen through which vital liquids or gases can be transported. Although tubes can be many cells in diameter, the smallest tubes are unicellular. Examples of such unicellular tubes include many capillaries found in the terminal vascular bed of mammalian organs such as the kidney, duodenum and cerebral cortex (Bar et al., 1984), specialized tip cells in the trachea of *Drosophila* (Samakovlis et al., 1996), and a variety of epithelial, glial and mesodermal cells in the nematode *C. elegans* (Buechner et al., 1999; Nelson et al., 1983; Perens and Shaham, 2005; Rasmussen et al., 2008; Ward et al., 1975).

Unicellular tubes can form by either wrapping or hollowing mechanisms. During wrapping, the cell body folds around and forms an adherens junction with itself (termed an autocellular junction), and the resulting lumen is formed from a previously extracellular space (Rasmussen et al., 2008; Ribeiro et al., 2004). In contrast, during hollowing, vesicular structures within a cell's cytoplasm coalesce and grow to form an eventual interior lumen (Berry et al., 2003; Buechner, 2002; Davis and Camarillo, 1996; Folkman and Haudenschild, 1980; Kamei et al., 2006). Such tubes are termed "seamless" because they lack autocellular junctions (Bar et al., 1984). Recently, it has been shown that cells that form unicellular tubes by wrapping also can lose their autocellular junctions and become seamless through a self-fusion event (Rasmussen et al., 2008). Whether formed initially by wrapping or hollowing, the lumen of a unicellular tube often grows extensively during development and must connect with that of other tubes to generate a functional conduit. The molecular signals that control lumen formation, growth, and connectivity are poorly understood.

The *C. elegans* excretory (renal) system is a simple model system for studying unicellular tube formation, since it consists of only three connected unicellular tubes (Nelson et al., 1983) (Fig. 1). Two of these tubes (the excretory canal cell and duct cell) are seamless; the canal cell forms a tube by a hollowing mechanism (Berry et al., 2003; Buechner, 2002), while the duct cell appears to form a tube through wrapping and self-fusion (see Results). The third tube (the pore) has an autocellular junction and thus forms by wrapping. The largest of these tube cells is the excretory canal cell, whose cell body is located in the head of the animal, but which extends two hollow tubules, termed canals, anteriorly and posteriorly along the entire length of the animal, forming a large H-shape (Buechner, 2002). The canal cell has an apical cytoskeleton that stabilizes the luminal structure and maintains its uniform size and shape (Buechner, 2002; Gobel et al., 2004). The canals, which are closed at their termini, collect and transport fluid to the excretory cell body for subsequent expulsion through the duct and pore cells (Nelson et al., 1983; Nelson and Riddle, 1984). The canals join at the excretory sinus within the excretory canal cell body, and the sinus forms an apical junction (termed the "secretory junction") to connect with the excretory duct cell (Nelson et al., 1983). The duct cell

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doi:10.1016/j.ydbio.2009.02.030

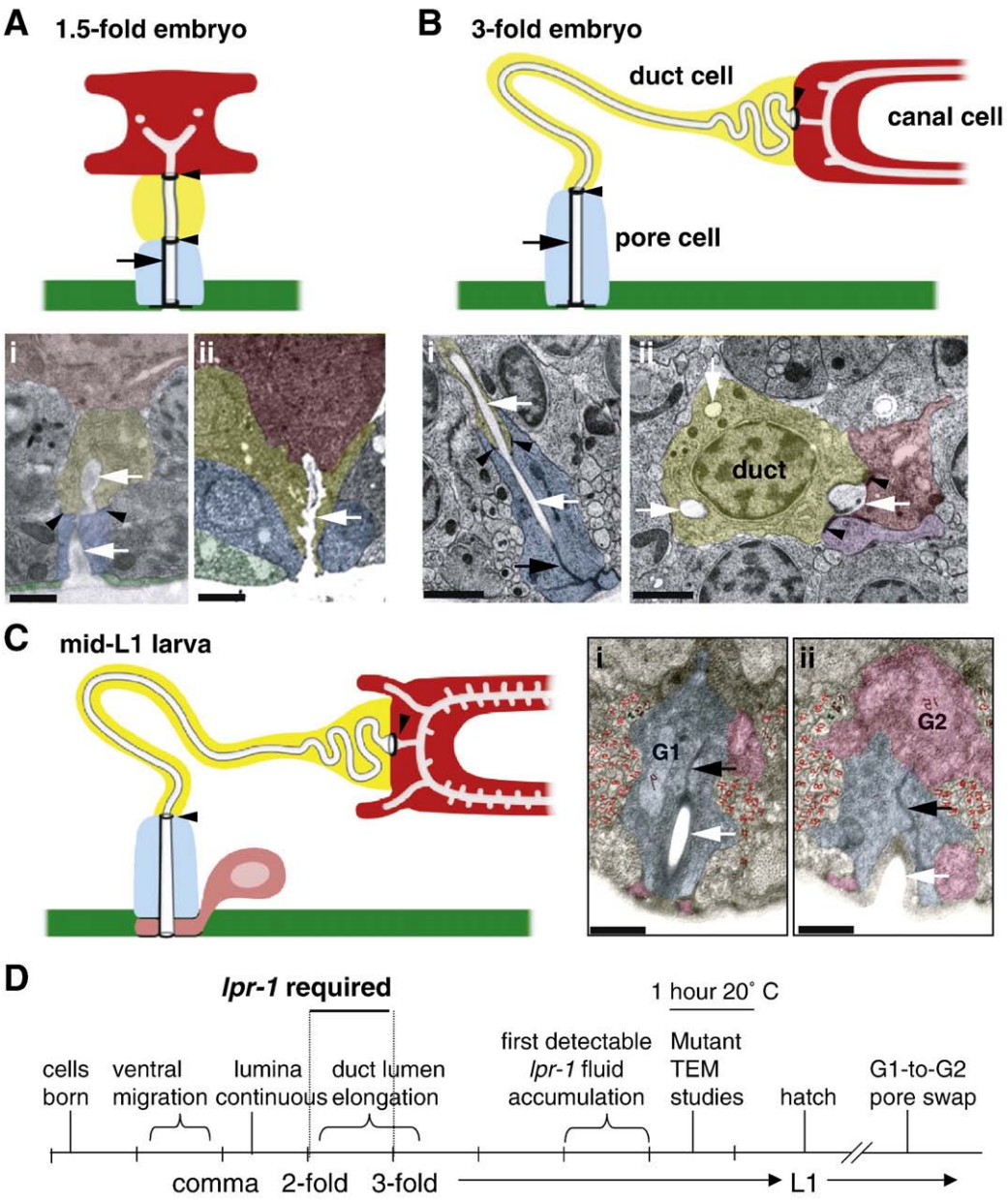

Fig. 1. Timecourse of excretory system development in wild-type. (A–C) Transmission electron micrographs (TEMs) and corresponding schematics of the excretory system in wild-type animals at different stages. TEMs have been false-colored for clarity. Colored regions represent cell cytoplasm of the canal cell (red), duct cell (yellow), pore cell (blue), or ventral epithelium (green) and uncolored areas represent lumen (indicated by white arrows in TEMs). Thick black circles and lines in schematics indicate *C. elegans* apical junctions, which include the canal/duct and duct/pore intercellular junctions (black arrowheads) and the pore autocellular junction (black arrow). The canal/duct junction has also been termed the "secretory junction" (Nelson et al., 1983). In all images, ventral is on the bottom. In lateral views, anterior is to left. Scale bars, 1 μm. A) 1.5-fold embryo, anterior view. The cells are compact, and the duct has a short, linear lumen. i) Embryo "N2E6B" TEM kindly provided by Shai Shaham (Rockefeller U.). Anterior view. ii) Embryo "N2 egg" kindly provided by John Sulston (MRC). Lateral view. B) Late 3-fold embryo, lateral view. The tube cells and lumina have expanded in length. For simplicity, only a portion of the canal cell is shown in the schematic. The duct and pore lumina are lined with cuticle (indicated by grey outline), whereas the canal cell lumen is not. i, ii) Two TEM sections from the same embryo, showing i) duct/pore junction, and ii) duct nucleus and cell body (with multiple cross-sections of lumen) and canal/duct junction. These TEM sections are separated by 3–4 μm. Purple color in ii) indicates an excretory gland cell, which also connects to the canal and duct lumina at their junction (Nelson et al., 1983). *lin-17(n677)* embryo kindly provided by Richard Fetter and Cornelia Bargmann (Rockefeller U.). Anterior view. C) Mid-L1 larva, lateral view. i, ii) Adjacent TEM sections show G2 (pink) beginning to take over the ventral-most portion of the pore channel. "L1C" TEMs kindly provided by John Sulston (MRC). Anterior view. D) Timeline of excretory system development, based on (Sulston et al., 1983) and TEM data above.

connects to the pore cell through another apical junction, and together the duct and pore lumina (which are both lined with cuticle, unlike the canal cell lumen) form a continuous channel that opens out of the body, allowing fluid to be excreted (Nelson et al., 1983; Nelson and Riddle, 1984).

The excretory system functions as the primitive renal system of the worm and has an essential role in osmoregulation. Larvae in which any of the three tubular cells are ablated accumulate fluid within the body cavity and die with a characteristic rod-like morphology (Nelson and Riddle, 1984). Mutants that lack the excretory duct cell (Yochem et al., 1997) or mutants that have physiological defects in excretory system function (Liegeois et al., 2007), have a similar rod-like lethal phenotype, arresting at an early larval stage. This distinctive phenotype can be used to identify gene products important for various steps of excretory system development or function.

Here we describe several interesting aspects of excretory duct and pore development and identify *lpr-1*as a gene important for connection between these two unicellular tubes.