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Curr Opin Insect Sci. Author manuscript; available in PMC 2022 October 01.

Published in final edited form as:

Curr Opin Insect Sci. 2021 October ; 47: 7–11. doi:10.1016/j.cois.2021.02.001.

Molecular basis for epithelial morphogenesis and ion transport in the Malpighian tubule

Sima Jonusaite[1,2], **Aylin R. Rodan**[1,3,*]

[1]Department of Internal Medicine, Division of Nephrology and Hypertension, and Molecular Medicine Program, University of Utah, Salt Lake City, UT 84112, USA

[2]Department of Integrative Biology, University of Guelph, Guelph, Ontario NIG 2W1, Canada

[3]Medical Service, Veterans Affairs Salt Lake City Health Care System, Salt Lake City, UT, USA

Abstract

During development, the insect Malpighian tubule undergoes several programmed morphogenetic events that give rise to the tubule's ability to transport ions and water at unparalleled speed. Studies in Diptera, in particular, have greatly increased our understanding of the molecular pathways underlying embryonic tubule development. In this review, we discuss recent work that has revealed new insights into the molecular players required for the development and maintenance of structurally and functionally intact adult Malpighian tubules. We highlight the contribution of the smooth septate junction (sSJ) proteins to the morphogenesis and transport function of the epithelial cells of the *Drosophila melanogaster* Malpighian tubule and also discuss new findings on the role of the GATAe transcription factor. We also consider the roles of sSJ proteins in the fly midgut, as compared to the Malpighian tubule, and the importance of cellular context for the functions of these proteins.

Keywords

Septate junction; Mesh; Tetraspanin 2A; GATAe; Malpighian tubule; paracellular transport; epithelial ion transport; Malpighian tubule development; Drosophila melanogaster; ionoregulation; osmoregulation; midgut

[*]Corresponding Author Contact Information: Aylin Rodan, MD, PhD, Department of Internal Medicine, Division of Nephrology and Hypertension University of Utah Molecular Medicine Program 15 North 2030 East, Bldg. 533, Room 2420A Salt Lake City, UT 84112, USA Phone: 801-587-7929, Fax: 801-585-0701 Aylin.rodan@hsc.utah.edu.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of interest

None.

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Introduction

To take on its shape and function as the fastest fluid-transporting epithelium observed in biology [1,2], the insect Malpighian tubule undergoes a series of programmed morphogenetic events. Understanding the molecular mechanisms involved is critical, because the loss of tubule integrity and transport function can lead to organismal lethality.

In Diptera, such as flies and mosquitoes, the development of embryonic Malpighian tubules is complete by the end of embryogenesis, and the tubules become fully functional at the time of larval hatching, when feeding begins. The tubules grow in size by cell growth during larval stages. Unlike many other organs, tubules persist through metamorphosis and into adulthood. Embryonic tubule development has been extensively studied, particularly in *Drosophila melanogaster*, as detailed in multiple excellent reviews [e.g. 3,4,5]. However, less is known about the genetic mechanisms that underlie post-embryonic maturation and maintenance of structurally and functionally intact tubule epithelia.

An adult fly has two pairs of tubules, which join the junction of the midgut and hindgut through common ureters. The tubules are further divided into functionally and morphologically distinct regions, consisting of the initial, transitional, main and lower segments and the ureters [5]. The major cell types are principal and stellate cells, and the lower segments and ureter also have renal stem cells [6,7]. In the urine-generating main segment, fluid secretion is energized by the apical V-type H^+-ATPase in the principal cell, which secretes cations [5,8]. The stellate cell secretes chloride ions and is the primary transcellular pathway for water secretion [9*] (Figure 1). Like all insect epithelial cells, tubule cells share intercellular occluding septate junctions (SJs), which form circumferential belts around the apicolateral regions of the plasma membranes and are thought to define the barrier and permselectivity properties of the paracellular (i.e. between cells) pathway [10]. Despite their ectodermal and mesodermal origins, the tubules develop smooth SJs (sSJs) generally found in endodermal epithelia such as the midgut, but not the pleated SJs (pSJs) characteristic of all other ectodermal epithelia [11]. Studies in *Drosophila* have allowed the discovery of more than twenty pSJ-associated proteins and have revealed that in addition to forming paracellular barriers, a large group of these proteins play roles in essential developmental events during and post-embryogenesis, including epithelial growth, morphogenesis and polarization [10, 12,13,14,15]. Unlike pSJs, the identity and functions of sSJ-specific components are less understood. However, studies focused on *Drosophila* midgut have uncovered important roles for these proteins in maintaining epithelial integrity, barrier function and homeostasis [16,17,18,19*,20*,21*].

In this review, we highlight recent discoveries of the roles of sSJ proteins in the post-embryonic morphogenesis of the *Drosophila* Malpighian tubule epithelium and maintenance of its function in adult flies. We also discuss the importance of the cellular context for the functions of sSJ proteins and point out other molecular and cellular factors essential for tubule growth and maintenance, such as the transcription factor GATAe.

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New molecular players in post-embryonic Malpighian tubule morphogenesis and transepithelial transport

Seminal studies in *Drosophila* identified three integral membrane proteins, Snakeskin (Ssk), Mesh and Tetraspanin 2A (Tsp2A), that localize to the sSJs of larval and adult Malpighian tubules [16,17,18] (Figure 1). However, the role of these sSJ proteins in Malpighian tubule development and function was unknown. Two recent papers demonstrate that principal cell-specific knockdown of either *mesh* or *Tsp2* A beginning in late embryogenesis leads to profound morphological defects and a lack of fluid and ion secretion in the tubules of newly eclosed adult flies, which become edematous and die in early adulthood [22**,23**]. Developmental *mesh* or *Tsp2A* knockdown tubules appear distended, especially in the distal regions, and the epithelial cells of the urine-generating main segment are often devoid of mitochondria and apical membrane brush borders and basal membrane infoldings, which serve to increase the membrane surface area available to the active transport machinery [22**,23**]. Consistent with these phenotypes, expression of the principal cell Na^+/K^+-ATPase appears reduced, although localization to the basolateral membrane is maintained. Activity of the principal cell vacuolar H^+-ATPase is also reduced, and as a consequence tubules lack the lumen-positive transepithelial voltage generated by this ATPase [22**,23**]. The characteristic morphological differences between principal and stellate cells are also lost, as observed by electron microscopy, and light microscopy reveals that the main segment stellate cells are cuboidal rather than star-shaped [22**,23**]. However, known stellate cell markers, such as the nuclear transcription factor *teashirt (tsh)* and the chloride channel *secCl*, are retained [22** 24,25]. This suggests that at least some aspects of the stellate cell differentiation program are maintained. Consistent with these stellate cell morphologic abnormalities, both *mesh* and *Tsp2A* knockdown tubules fail to respond to the kinin diuretic neurohormone, which activates stellate cell ion and water transport [22**,23**,9*,26].

Developmental loss of *mesh* or *Tsp2A* also disrupts tubule sSJ molecular and structural organization, *mesh* or *Tsp2A* knockdown tubules from newly emerged flies exhibit mislocalization of all other integral sSJ proteins as well as the cytoplasmic scaffold protein Discs large (Dlg) [22**,23**]. Mutant tubules have reduced number of septa within the SJs, less parallel plasma membranes of adjacent cells and frequent intercellular gaps between them [22**,23**]. Together, these findings imply that Mesh and Tsp2A work together to assemble and maintain the sSJ complex in developing fly tubules. The third sSJ integral membrane protein, Ssk, likely also plays a role, but was not examined in detail in these studies.

Post-embryonic *Drosophila* tubule development also requires cell-specific activity of the transcription factor GATAe, which is expressed in the principal cells of larval tubules and in principal cells, stellate cells and renal stem cells of the adult tubules [27**] (Figure 1). Knockdown of *GATAe* in the principal cells of late larval tubules disrupts tubule architecture in emerged adult flies, which exhibit bloated abdomens and reduced lifespan [27**]. The tubules of these flies are shorter and thicker, appearing tumorous, and have reduced number of cells [27**]. In addition, these tubules display increased proliferation of the renal stem

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cells and have altered expression of cancer-related genes, suggesting that GATAe might function as a tumor suppressor in the principal cells of fly tubules [27**]. GATAe is also required for stellate cell survival through metamorphosis, as their number and localization are markedly decreased in adult tubules with silenced stellate cell *GATAe* expression [27**]. The shape of the remaining stellate cells of these tubules is unaltered but the tubules have reduced fluid secretion in response to kinin stimulation [27**]. Lastly, GATAe is required in the renal stem cells for their migration from the midgut into the ureter during metamorphosis [27**,28].

Morphological and functional maintenance of adult Malpighian tubules

Recent studies in *Drosophila* have also provided new insights into the role of *mesh* in adult tubules [22**]. Similar to developmental mesh silencing, tubules from flies subjected to 14-day principal cell *mesh* knockdown post-eclosion have mislocalized expression of Tsp2A, Ssk, and Dlg [22**]. However, these tubules appear normal, with no apparent defects in the sSJ, or principal or stellate cell ultrastructure. Since stellate cell Mesh expression persists, and *mesh* was knocked down (not knocked out) in principal cells, it is possible residual Mesh activity is sufficient for the maintenance of sSJ and epithelial morphology [22**]. Nevertheless, despite the normal appearance of these tubules, function is impaired, with reduced lumen-positive transepithelial voltage in the main segment, and a proportional decrease in fluid and ion secretion, although kinin responsiveness is preserved. This suggests an impairment in principal cell function with the loss of adult *mesh* expression [22**]. There is also reduced paracellular flux of 4 kDa dextran, suggesting that Mesh plays a role in the epithelial leak pathway of the adult fly tubule [22**]. Interestingly, unlike tubules of other insects, in which paracellular permeability to macromolecules is modulated by kinins, drosokinin has no effect on dextran permeability in the fly tubule, suggesting species-specific roles for this hormone [22**,29,30].

Roles for GATAe and the renal stem cells in the maintenance of adult *Drosophila* tubules have also been uncovered. *GATAe* knockdown causes cell shape defects and disruption of the tubule architecture, including abnormal renal stem cell proliferation, indicating that GATAe expression in the principal cells is essential for maintaining proper cellular morphology and integrity of the tubules during adulthood [27**]. The renal stem cells, which are found together with principal cells in the ureter and lower segment of the fly tubule, are unipotent cells that normally remain quiescent [7,31*]. These cells, however, become activated and differentiate into replacement principal cells in the ureter and lower segment in response to damage or loss of nearby principal cells [31*]. Interestingly, it has been reported that renal stem cell proliferation is also under the control of the tumour suppressor Scribble, which localizes to the sSJs within the ureter and lower tubules [32].

Tissue-specific functions of sSJ proteins

Studies on the sSJ proteins have provided some evidence to suggest that these proteins might have different functions depending on the cellular context. For example, adult-onset reduction of either *mesh*, *Tsp2A* or *Ssk* in the absorptive enterocytes of the *Drosophila* midgut causes defects in epithelial architecture, including hypertrophy and accumulation

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of morphologically aberrant enterocytes, and increased midgut-to-hemolymph leak of the 800-Da Brilliant Blue FCF, suggestive of midgut barrier dysfunction [19*,20*,21*]. As discussed above, depletion of *mesh* in the principal cells of adult *Drosophila* Malpighian tubules decreases main segment macromolecule permeability with no effect on epithelial integrity. Tissue-specific roles for Mesh and Ssk have also been suggested in the Malpighian tubules and midgut of larval mosquito *Aedes aegypti* [33]. The tissue-specific roles of sSJ proteins might reflect the differences in the functions of the epithelia in which they are found. These include water and ion absorption by the midgut vs. secretion in the tubule, and varied environmental exposures, for example to microbes, requiring differences in epithelial paracellular permeability. Alternatively, the differing impacts of sSJ component depletion in adulthood on epithelial structure may be due to the higher rates of turnover compared to the Malpighian tubules [34]. Mesh, Ssk and Tsp2A functions in these two epithelia might be further modulated by their interactions with different proteins within the sSJ complex, since a number of midgut sSJ components, such as Coracle, Fasciclin III, Lethal giant larva, and Big Bang, have not been reported in the tubule sSJs [35,36]. This may reflect the distinct developmental origin of these epithelia [34].

Conclusions and perspectives

Studies performed in *Drosophila* over the last decade have greatly advanced our knowledge of the molecular mechanisms orchestrating the development of functionally competent Malpighian tubules, which in Diptera are established by the end of embryogenesis. It is becoming more evident that the sSJs, thought to function primarily as regulators of paracellular permeability, play an equally important role in tubule epithelial organization and maintenance throughout adulthood. Future studies will no doubt answer open questions regarding sSJ protein architecture, interactome and contribution to tubule cell biology and transport. Recent discoveries of the presence of sSJ integral components - Mesh, Ssk or Tsp2A - within the sSJ complex of the tubules of non-Dipteran species [29,37] also provide opportunities for the comparative understanding of the molecular machinery regulating insect Malpighian tubule structure and function.

Acknowledgments

This work was supported by the National Institutes of Health (National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-110358 to A. R. Rodan).

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** of outstanding interest

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Highlights

- Malpighian tubule structure and function are highly coordinated throughout insect life
- Transmembrane proteins associated with smooth septate junctions (sSJs) found in the midgut and Malpighian tubules are essential for post-embryonic morphogenesis and transport function of *Drosophila* tubules
- sSJ proteins maintain ion and water transport in adult fly tubules
- Late fly tubule development and maintenance in adulthood also relies on GATAe transcription factor
- sSJ proteins may have specific roles associated with insect midgut and tubule functions

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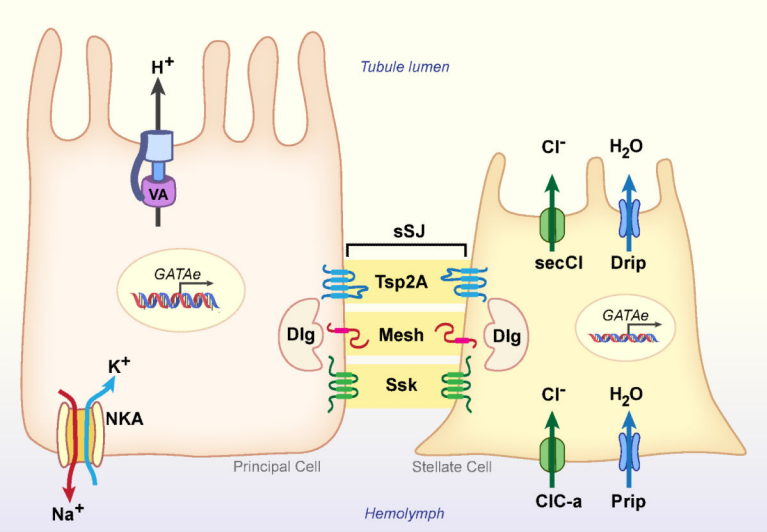

Figure 1.

Summary of the major proteins highlighted in this review that play essential roles in the morphogenesis and ion and water transport in the urine-generating main segment epithelium of *Drosophila* Malpighian tubule. sSJ, smooth septate junction; VA, H^+-ATPase; NKA, Na^+/K^+-ATPase; Dlg, Discs large; Ssk, Snakeskin; Tsp2A, Tetraspanin 2A.