The *Drosophila* Nephrocyte: Back on Stage

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Drosophila nephrocytes have been an object of study for more than a century. They were named by Bruntz, based on observations of cells around the heart, the central nervous system, and in the sternal area in scorpions.[1] Kowalevsky suggested that they function as a storage kidney, because they can absorb ammonia carmine from the hemolymph.[2] Despite decades of study, however, the precise function of the nephrocyte has remained elusive. Two papers in the current issue of *JASN* now extend their function, making these remarkable cells still more surprising.

The adult *Drosophila* nephrocytes are categorized as thoracic nephrocytes or abdominal nephrocytes.[3] They derive from larval nephrocytes, among the largest cells in the larval body, which are classified as garland cells or pericardial cells based on their location. Garland cells lie close to the esophagus or between the salivary glands.[4,5] Pericardial nephrocytes develop from the cardiogenic mesoderm by the late embryo/early larval stage[6,7]; they arrange into two rows of 20–25 flanking each side of the heart from the first to the sixth segment.

Using transmission electron microscopy of adult *Drosophila* nephrocytes, Mills and King found that the plasma membrane of the pericardial nephrocyte is invaginated to form elaborate sheets and tubules along with organelles that resemble lysosomes.[8] Functional lysosomes were confirmed by histochemical methods[9,10] and were presumed to act as a garbage disposal system. By studying the third-instar larva of *Calliphora*, Crossley documented the ultrastructure of pericardial cells and demonstrated a desmosome-like structure, very similar to the podocyte slit diaphragm that is required for proper filtration within the vertebrate kidney.[4]

Although Schwinck observed that pericardial cells of *Panorpa* synthesize proteins and export them to hemolymph,[11,12] more attention has been paid to the role of nephrocytes in taking up materials from the hemolymph. Pericardial nephrocytes were demonstrated to absorb foreign materials from the hemolymph, providing selection by size and charge.[13] Based on dye injections, Hollande proposed that the *Lepidopteran* pericardial cell absorbs and stores toxic compounds from the hemolymph, hydrolyzing them to soluble, nontoxic molecules that are released into the hemolymph, perhaps to be excreted by the Malpighian tubules.[14] This hypothesis was later supported by Wigglesworth's observation in bloodsucking insects that hemoglobin protein constituents consumed in a blood meal are broken down into biliverdin and stored in the nephrocytes, while the iron is released to the hemolymph.[15]

Recently, *Drosophila* nephrocytes have returned to center stage. Weavers *et al.* and Zhuang *et al.*[16,17] independently showed that the molecular structure of the nephrocyte diaphragm is similar to that of the podocyte slit diaphragm. Reducing key components of the nephrocyte diaphragm, the Nephrin or Neph1 orthologs SNS and Kirre/Duf, respectively, led to structural defects. This strongly suggests that nephrocytes act in a manner analogous to our podocytes, the fly nephrocyte diaphragm presumably functioning similar to the mammalian slit diaphragm to regulate filtration.

Weavers *et al.* therefore proposed that the nephrocyte diaphragm functions as a filter to exclude large hemolymph constituents from the labyrinthine channels, and that this filtration depends on the proper functioning of SNS/Nephrin and Kirre/Neph1. This led to the attractive hypothesis that the nephrocyte acts primarily as a fly podocyte (filtration), perhaps without the need for an associated renal proximal tubule (reabsorption). This work broadened interest in nephrocytes as a useful model for understanding kidney filtration.

The charge and size-selectivity of nephrocytes is reminiscent of podocytes, a crucial component of the mammalian glomerular filtration barrier. Genetic mutations that affect constituent proteins within the slit diaphragm lead to severe proteinuria and kidney failure in humans.[18,19] Proteinuria can also be caused by defects in the re-absorption machinery in the proximal tubule, involving the major proteins in the endocytic receptor complex (e.g., megalin, cubilin, and amnionless).[20,21]

In a pair of articles in the current issue of *JASN*, Zhang *et al.* use genetic approaches to further explore the functions of the *Drosophila* nephrocytes.[22,23] Establishing a stable transgenic fly line producing a secreted fluorescently tagged protein (ANF-RFP) that accumulates in nephrocytes—reminiscent of the secreted GFP system described by Ferrandon *et al.*,[24]—they assessed the ability of loci to regulate protein uptake. For example, they demonstrated that mutating Sns/Nephrin, Kirre/Neph1, or *Drosophila* podocin abolished secreted protein accumulation in nephrocytes. Using an unbiased screen, they then identified >70 genes required for nephrocyte function. These provide an important resource for exploring nephrocyte function.

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Their screen identified the *Drosophila* orthologs of mammalian cubilin and amnionless as required for nephrocyte function. Reducing cubilin or amnionless function in the nephrocyte abolished both toxin and ANF-RFP uptake, whereas overexpression of amnionless increased protein uptake. Cubilin and amnionless are both specifically expressed in nephrocytes. Mammalian amnionless does not bind ligands directly, and the authors postulate that amnionless acts to enrich cubilin at the nephrocyte membrane, leading to increased protein uptake. Evidence from transmission electron microscopy further suggests that a cubilin/amnionless receptor complex is required for maintaining endocytic trafficking machinery within the nephrocyte. Of note, overexpressing human amnionless rescued ANF-RFP uptake in amnionless-knockdown flies, demonstrating that a cubilin/amnionless receptor complex is conserved between flies and humans.

This work led the authors to propose the intriguing model that the *Drosophila* nephrocyte encompasses two functions in one cell: filtration and protein re-absorption. These would make nephrocytes analogous to podocyte plus proximal tubule. In mammals, the filtration system is composed of three layers: fenestrated endothelium, glomerular basement membrane, and podocyte foot processes. In *Drosophila*, the filtration system is composed of two: the nephrocyte basement membrane and nephrocyte diaphragm. Proteins, particles, or toxins enter the lacuna network by the filtration membrane, where they are degraded or reused. If correct, this model indicates that nephrocytes are a remarkably conserved yet parsimonious solution to the problem of blood filtration.

Emerging structural, molecular, and functional similarities between the *Drosophila* nephrocyte and the mammalian nephron emphasize its promise for dissecting the mechanisms that underlie proper and compromised kidney function. Given its powerful genetic and molecular tools, several fundamental questions may prove usefully addressed by the fly system. For example, cubilin and megalin lie within the endocytosis receptor complex and have many ligands including vitamin-binding proteins, enzymes, drugs, hormones, toxins, calcium, albumin, lipoproteins, and hemoglobin.[21] Identifying new ligands may help elucidate the mechanism of nutrient recycling in the proximal renal tubule, a process central to proteinuria. Flies can help with this task.

Megalin is an enormous protein that includes an intracellular region composed of a menagerie of domains including endocytic motifs (NPXY), a NPXY-like motif (NQNY), SH2 and SH3 recognition motifs, and consensus phosphorylation sites.[21] Flies provide a tool for understanding structure/function *in vivo*. Third, the *amn* locus yields five protein products, but the one that interacts with cubilin is unknown. Human amnionless is able to rescue loss of *Drosophila* amnionless, providing an interesting tool for exploring the details of cubilin/amnionless interactions as well as their downstream targets.

Kidney disease is the eighth leading cause of death in the United States.[25] Although they are useful, cell culture systems are limited in their ability to recapitulate the *in vivo* characteristics and functions of the podocyte.[26,27] As Han and colleagues demonstrate, insect nephrocytes continue to amaze, taking the stage yet again as a promising *in vivo* tool for studying podocyte function. We anticipate more surprises will be in store for our understanding of this remarkable cell.

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DISCLOSURES

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See related articles, "Cubilin and Amnionless Mediate Protein Reabsorption in *Drosophila* Nephrocytes," and "An *In Vivo* Functional Analysis System for Renal Gene Discovery in *Drosophila* Pericardial Nephrocytes," on pages 209–216 and 191–197, respectively.

Salt and Pepper Distribution of Cell Types in the Collecting Duct

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Many epithelial organs, such as the kidney, gastrointestinal tract, skin, lung, and brain, are segmented such that each region has its characteristic cell type. Close examination of these segments reveals that they are homogenous and contain only one epithelial cell type. With a bit of training, it is easy to distinguish colon from ileum or proximal tubule from thick ascending limb. Rare epithelia, however, exhibit what has come to be known as a salt and pepper type. These are composed of one major cell type, with others sprinkled throughout that clearly differ in structure and function. The classic example of this epithelium is the skin of fish, amphibians, and reptiles, in which so-called chloride cells are present in a distinctive pattern. In mammals, the collecting tubule of the kidney is such an epithelium; remarkably, the sprinkled cells (the intercalated cells) are similar to the chloride cells in structure and in function.

Development of organs can be considered a straightforward march starting with a multipotent progenitor that can produce all types of the cells of the organ, followed by stepwise differentiation with restricted potencies to produce only one or another segment. Within this context, the presence of a mosaic pattern in a single segment raises an interesting question: How do the minority cells develop? Do they invade from the interstitium, or does the progenitor cell type give rise to both? Because the distribution often seems random, how would the progenitor cell know where and when to stop making the majority cell and instead specify the minority cell?

When both cell types arise from the same progenitor, the key mechanism to cause the mosaic pattern is *lateral inhibition*. In this process one cell with a given developmental fate sends a direct signal to its neighbor, causing it to assume a different fate. The notch signaling pathway mediates the molecular mechanism whereby the *sending* cell expresses a notch ligand (Delta-like or Jagged in mammals) and the *receiving* cell expresses the notch receptor. Upon ligand binding, the extracellular domain of the notch receptor undergoes endocytosis within the signal-sending cell. In the signal-receiving cell, the notch intracellular domain (NICD) is generated by a series of proteolytic steps through γ-secretase. The NICD is then translocated to the nucleus of this cell and ultimately induces expression of several transcription factors of the HES family (*hairy* and *enhancer of split-1*) that are usually repressors. Recently, Jeong *et al.*[1] deleted one component of the notch signaling pathway in the collecting tubule and found an increase in percentage of intercalated cells and a concomitant decrease in that of principal cells. When NICD was expressed in these mutant mice in the collecting duct, all of the cells were found to be principal cells with no intercalated cells. Similar results were found in the *Xenopus* skin.[2] These studies suggest that active Notch signaling allows the intercalated cells to appear.

Not clear, however, were the identities of the sending and receiving cells because the inductions of the mutation in these two studies were performed without the use of cell-type–specific agents. Earlier studies showed that although the ureteric bud expresses three of the notch receptors, it does not express any of the ligands at embryonic day 15.5 (i.e., before the intercalated cell–specific proteins are expressed).[3] This observation suggests that expression of the notch ligand in the adjacent principal cell results in the suppression of the principal cell fate and the appearance of the intercalated cell

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