The Journal of Experimental Biology 212, 435-445
Published by The Company of Biologists 2009
doi:10.1242/jeb.024224

# Review

# Insights into the Malpighian tubule from functional genomics

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Accepted 20 October 2008

**Summary**

**Classical physiological study of the Malpighian tubule has led to a detailed understanding of fluid transport and its control across several species. With the sequencing of the *Drosophila* genome, and the concurrent development of post-genomic technologies such as microarrays, proteomics, metabolomics and systems biology, completely unexpected roles for the insect Malpighian tubule have emerged. As the insect body plan is simpler than that of mammals, tasks analogous to those performed by multiple mammalian organ systems must be shared out among insect tissues. As well as the classical roles in osmoregulation, the Malpighian tubule is highly specialized for organic solute transport, and for metabolism and detoxification. In *Drosophila*, the adult Malpighian tubule is the key tissue for defence against insecticides such as DDT; and it can also detect and mount an autonomous defence against bacterial invasion. While it is vital to continue to set insights obtained in *Drosophila* into the context of work in other species, the combination of post-genomic technologies and physiological validation can provide insights that might not otherwise have been apparent for many years.**

Key words: *Drosophila melanogaster*, Malpighian tubule, ion transport, cell signalling, detoxification, FlyAtlas.

## The Malpighian tubule

Although the insect renal tubule was first described by Malpighi in the seventeenth century, it was not until the twentieth that significant further progress was made. The pioneering studies of Wigglesworth and Ramsay demonstrated that the Malpighian tubule was experimentally accessible and worthy of study; and Maddrell provided a detailed explanation of solute and water flux, and its neuroendocrine control. These results led to an explosion of work around the world, which has resulted in a detailed understanding of fluid transport and its control across several species.

Insects, by virtue of their small size, have a large surface to volume ratio, and so live their lives under continual osmotic stress. For terrestrial insects, defence against instant desiccation is provided by behavioural and physical means. In particular, an impermeable cuticle limits evaporative water loss, while the excretory system (composed of Malpighian tubules and hindgut) dynamically balances primary urine generation and secondary reabsorption, so achieving a compromise between osmoregulation and excretion (Berridge and Oschman, 1969; Maddrell, 1971). Clearly, an understanding of osmoregulation is integral to the understanding of the success of the class Insecta.

## Advantages of *Drosophila melanogaster* as an experimental organism

*Drosophila melanogaster* is itself an unremarkable, but nonetheless highly representative, Dipteran insect. The special qualities which it brings are largely the result of insightful scientific exploitation over a century.

(1) Its short lifecycle, fecundity and ease of rearing led it to be selected as a genetic model by Morgan, Bridges and Sturtevant (Rubin and Lewis, 2000). The discovery of mutants, and of predictable patterns of recombination between them, led to mutant stocks being maintained in stock centres for future use.

(2) The discovery of polytene chromosomes in nuclei of epithelial cells such as the salivary gland (the tubules are actually used for this purpose in some other insects) allowed the phenomenon of chromosome puffing in response to environmental stressors (famously, heat shock or ecdysone) to be identified (Ashburner, 1971). This in turn allowed the physical mapping of mutants and genes against the chromosomes.

(3) The recent arrival of the P-element transposon in *D. melanogaster* led to an asymmetric sterility phenomenon called hybrid dysgenesis (Kidwell et al., 1977). Once this was found to be due to mobile genetic elements (Bingham et al., 1982), P-elements were extensively engineered as tools for both transgenesis and mutagenesis (Rubin and Spradling, 1983).

(4) The urge to tinker, common to geneticists and physiologists, has led to some powerful resources, such as luminescent and fluorescent reporters for cytoplasmic calcium (Rosay et al., 1997; Terhzaz et al., 2006), and more recently to calcium measurements in mitochondria (Terhzaz et al., 2006). Similar reporters have now been generated for cAMP (Shafer et al., 2008), and cGMP reporters are likely to follow soon. Together with the powerful GAL4/UAS binary expression system (Brand and Perrimon, 1993), it is now possible to record real-time responses to neuropeptides or other stimuli in intact tissues (Radford et al., 2002; Rosay et al., 1997). These advances are reviewed in this issue by Davies and Terhzaz (Davies and Terhzaz, 2009).

(5) The adoption of *Drosophila* as a candidate for genomic sequencing allowed existing mutant resources and their physical map to be tied down to a genetic (sequence) map. Indeed, the bioinformatic annotation of the *Drosophila* genome (at FlyBase; http://flybase.org) is exemplary among metazoan genome projects (Ashburner and Drysdale, 1994; Drysdale, 2008).

(6) In turn, the availability of the genomic sequence allowed the generation of comprehensive microarrays, enabling global gene

expression differences between life stages, genetic backgrounds or tissues to be identified quickly (albeit expensively).

(7) The discovery that RNA interference (RNAi) was capable of knocking down gene expression relatively selectively allowed the possibility that comprehensive mutant resources would be generated for *Drosophila*. So now, in addition to the classical mutant resources and several thousand P-element lines generated as part of genome-wide mutagenesis screens (Bellen et al., 2004; Spradling et al., 1995), there are stock centres carrying transgenic flies with RNAi constructs directed against nearly every gene in the genome (Dietzl et al., 2007).

Taken together, these technologies mean that it is possible to conceive a line of research, identify the resources required and design potent physiological experiments before getting up from one's desk. This is a hugely exciting opportunity for those physiologists who can adapt to such revolutionary techniques, and we have argued that *Drosophila* deserves 'Krogh' status alongside *Rhodnius prolixus* as an animal in which insect osmoregulation is best studied (Dow, 2007).

## New insights

Over the years, a detailed understanding of fluid secretion and its control has emerged; and other reviews previously (Beyenbach, 2003; Coast et al., 2002; Dow and Davies, 2001; Dow and Davies, 2003; O'Donnell and Spring, 2000) and in this volume cover this eloquently. Studies in *Drosophila* have helped because of the sequenced genome and the powerful genetic tools, and because of the availability of comprehensive microarray chips. This allows the genes underlying different processes to be identified quickly and easily (Wang et al., 2004). Subsequently, this approach has been validated in several ways; for example, the V-ATPase genes identified as abundantly expressed in Malpighian tubules are the same as those identified in other epithelia, suggesting that a single plasma membrane isoform energizes all insect epithelia (Allan et al., 2005).

However, the key value of post-genomic approaches like microarrays is that they can also identify new lines of investigation that might not have occurred in a 'linear' research programme. Some examples are outlined below; together, they broaden our understanding of tubule function.

## Using the GAL4-UAS system for physiology

Armed with a set of GAL4 'driver' lines appropriate to the tissue in question, and a basic skill in molecular biology, it is possible to design some highly informative experiments. At the very least, the major functional boundaries in the tissue can be mapped from a panel of GAL4 enhancer trap lines, and these boundaries can be visualized in the intact tissue using UAS-GFP (Fig. 1) (Sözen et al., 1997).

More ambitious experiments are also feasible. Given that the control of the Malpighian tubule relies on all of the second messengers, cAMP, cGMP and calcium, a panel of transgenic fly lines was generated that would allow these second messengers to be manipulated with ligands that are normally inactive on the tubule. By placing different receptors [rat atrial natriuretic peptide (ANP) receptor and *Drosophila* serotonin receptors 5HT(Dro7) and 5HT(Dro1A)] under UAS control, they could be targeted to arbitrary defined populations of cells in any tissue of the fly, and second messenger levels could be manipulated simply by adding the natural ligand. These lines confirmed previous findings that raising cAMP, cGMP or calcium in the

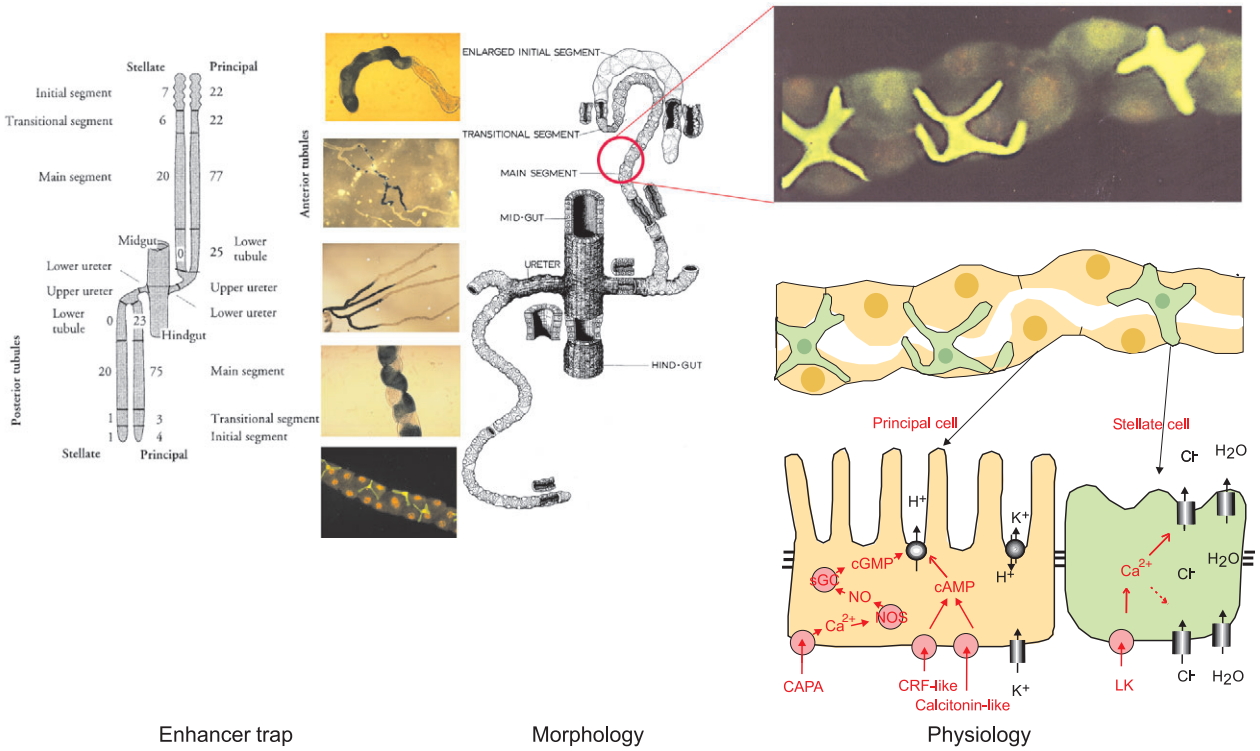


Fig. 1. Three views of the *Drosophila* tubule. Centre: the classical morphological view (Wessing and Eichelberg, 1978). Left, a genetically derived enhancer trap view, with some representative enhancer trap expression patterns (Sözen et al., 1997). Right, a summary of the physiology of the tubule (Dow and Davies, 2003): the stellate cells in the micrograph at the top are in fact lit with UAS-GFP, driven by the stellate cell-specific GAL4 line c724. CRF, corticotropin releasing factor; LK, leucokinin. [From Kerr et al. (Kerr et al. 2004).]

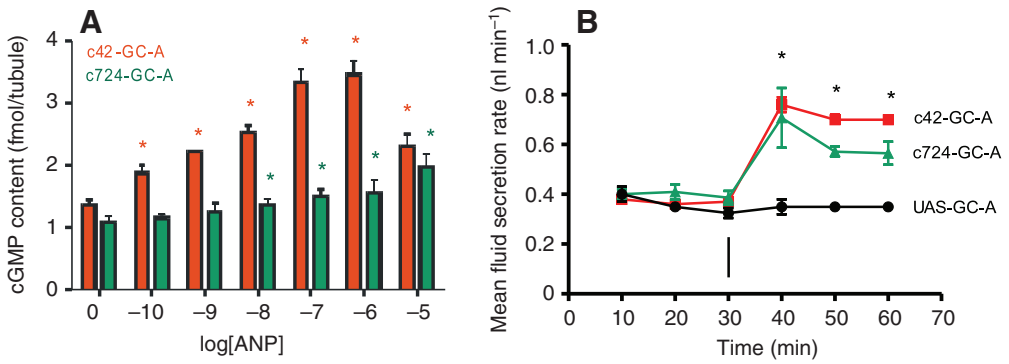


Fig. 2. Manipulation of cGMP levels in the Malpighian tubule by cell-specific transgenic expression of a rat atrial natriuretic peptide (ANP) receptor. The results refer to flies in which expression of UAS-GC-A was driven ubiquitously by heat-shock GAL4, in tubule principal cells by GAL4 line c42, or in tubule stellate cells by GAL4 line c724. (A) Effects on whole-tubule cGMP. The receptor can drive increases in cGMP in either principal or stellate cells. (B) Fluid secretion. Elevation of cGMP in either principal or stellate cells elicits increased fluid secretion. Asterisks denote significant difference from corresponding control values (Student's *t*-test, $P<0.05$). [From Kerr et al. (Kerr et al., 2004).]

principal cells stimulates fluid secretion by the tubule. However, although raising calcium in the stellate cells also stimulated fluid secretion (as expected), so did both cAMP and cGMP (Fig. 2). This unexpected finding demonstrates the existence of signalling pathways in stellate cells for which endogenous ligands are still to be identified (Kerr et al., 2004), although tyramine may be a candidate (Blumenthal, 2003).

### Insights from microarray analysis

Virtually all of the tissues in our body contain an identical copy of our genome; the difference between, say, a liver or a kidney cell exists because they express different mRNAs and thus proteins. Classically, the measurement of mRNA expression levels required the tedious gene-by-gene approach of northern blotting. By contrast, microarrays allow all (or at least most) of

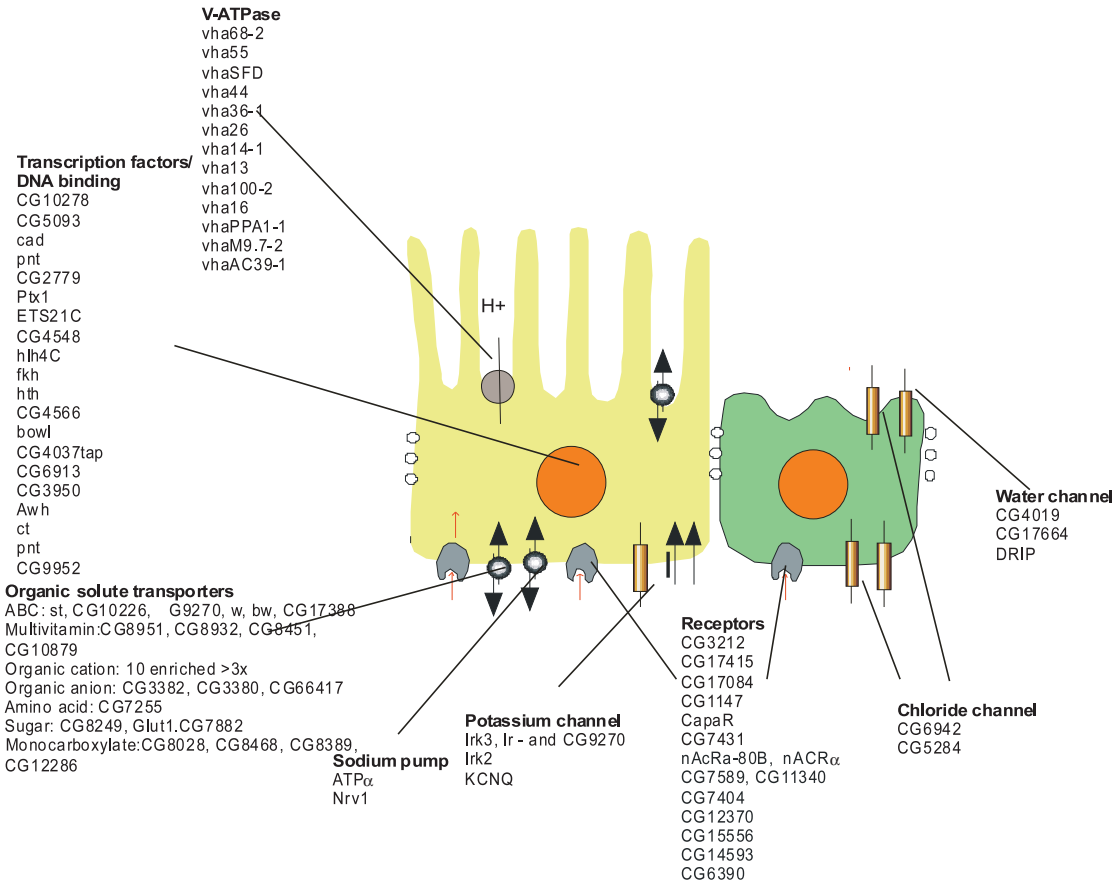


Fig. 3. Genes corresponding to different functional classes found to be enriched or highly abundant in adult *Drosophila* Malpighian tubule (Wang et al., 2004).

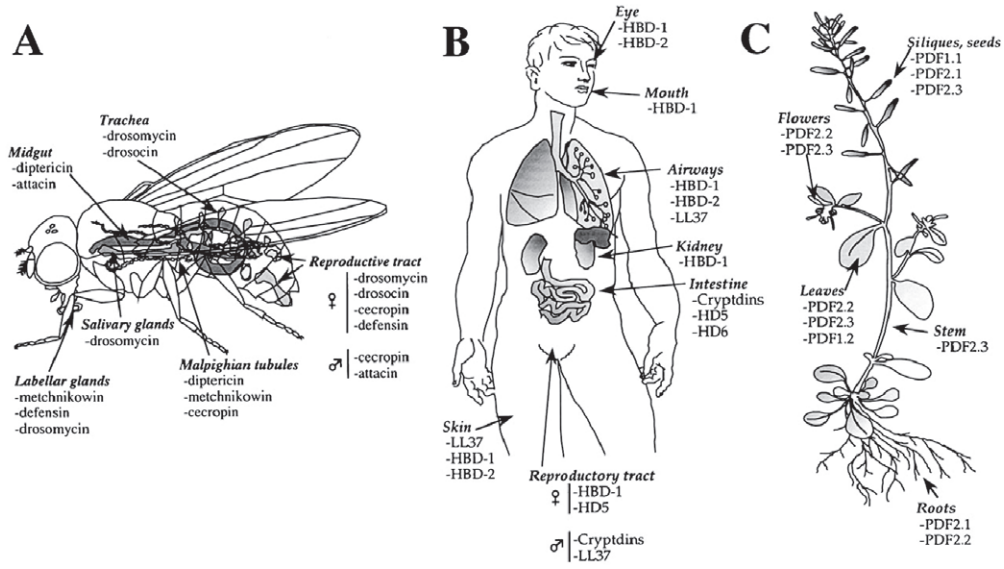


Fig. 4. Innate immunity by tissue-specific expression of antimicrobial peptides is conserved across insects, mammals and plants (from Tzou et al., 2000).

the mRNAs potentially encoded by the genome to be measured in a single experiment; the extra cost of the technology must thus be set against the enormous amount of extra information that it generates. The first insect epithelia to be analysed by microarray were the midgut and Malpighian tubules of *Aedes aegypti*; as this pre-dated the release of the *Aedes* genome, only 1778 cDNAs were arrayed on the chip, providing expression levels for around 10% of genes (Sanders et al., 2003). The *Drosophila* field had things rather easier, and a comprehensive (13,500 probeset) Affymetrix array became available within a year of the genome being sequenced. These arrays were used to characterize genes that were either abundant or enriched in adult Malpighian tubule, compared with whole adult fly, and the results reconciled with known function (Wang et al., 2004). In the manner of reading a book, it thus became possible to identify likely genes for all the functional properties previously ascribed to the tubule (Fig. 3).

Perhaps even more excitingly, entirely new functions such as immune response or neglected functions such as detoxification or organic solute transport were also highlighted by the data (Wang et al., 2004). Functional genomic techniques, such as microarrays, can thus produce results that are highly informative, novel and relevant to functional biologists, even if they themselves do not have the interest or resources to perform their own experiments.

## Innate immunity

In humans, attention is focused on the adaptive immune system, whereas the insects are seen to display a 'primitive' innate immunity, centred on the release of short antimicrobial peptides such as diptericin and attacin (Hoffmann, 1995). However, just as in other areas, findings in insects presaged those in 'higher' organisms, and it is clear that innate immunity is widely distributed (Fig. 4). Furthermore, there is increasing awareness that dysregulation of the human innate immune response may underlie several inflammatory conditions, such as bronchial asthma and Crohn's disease (Rosenstiel et al., 2008). The ease with which gene expression can be manipulated in flies has allowed the innate immune signalling pathways to be dissected in detail, and the model of signalling through the Toll and imd pathways has since been shown to be closely conserved even in humans.

The orthodoxy in the field was that induction of antimicrobial peptide expression in the fat body was the key response to bacterial or fungal infection (Hoffmann, 1995). However, it was also found that several antimicrobial peptide genes were quite widely expressed, particularly in barrier epithelia such as the gut; and that this expression could be upregulated by bacterial challenge (Tzou et al., 2000). This phenomenon, 'epithelial immunity', was not thought to challenge the primacy of the fat body. However, in *Drosophila* it was possible to show that the Malpighian tubules play a critical role in the immune response (McGettigan et al., 2005). Excised tubules were able to kill *E. coli in vitro* by upregulating expression of diptericin; this means that the tubules are autonomous immune tissues, capable of sensing insults and mounting an effective response entirely independent of the fat body. Furthermore, overexpression of nitric oxide synthase in just the tubule principal cells (using the GAL4/UAS system) resulted in enhanced survival of the whole fly upon *E. coli* challenge. Again, this argues that the tubules are key players in the immune response (McGettigan et al., 2005).

## Detoxification

The microarray dataset (Wang et al., 2004) highlighted an abundance of solute transporters in the Malpighian tubule, as a reminder that ion and water transport are only a part of the tubule's role in the whole animal. Even more remarkable, the tubule was also highly enriched for mRNAs for detoxification genes, notably cytochrome P450s and glutathione S-transferases. In particular, *cyp6g1*, which is probably consistently the most overexpressed gene in resistant insects (Daborn et al., 2002), is ninefold enriched in the Malpighian tubule compared with the whole fly (Yang et al., 2007). Previously, there was little work on the tissue specificity of such processes; perhaps it was assumed that such enzyme activities were relatively ubiquitous, or that they were nervous system specific (most insecticides target the CNS), or that the fat body was the insect 'liver'. However, these microarray data suggest that the tubule may be playing a major role in insecticide (and other xenobiotic) handling.

To test this model, the ideal experiment would be to manipulate levels of *cyp6g1* in just the Malpighian tubules, and expose the flies to different concentrations of DDT: if the tubule

was indeed important in xenobiotic handling, then survival of the whole fly upon DDT challenge would be correlated with *cyp6g1* expression in the tubule. With the GAL4/UAS system, exactly this experiment was possible (see Fig. 5). Using the c42 tubule principal cell-specific driver, RNAi against *cyp6g1* increased sensitivity of the whole fly to DDT, and overexpression of *cyp6g1* improved survival. This suggests that, for topical exposure of the adult fly, the Malpighian tubules are not just important but the key tissues (and Cyp6g1 the limiting enzyme) for normal handling of xenobiotics such as DDT (Yang, 2007).

This is of great interest to tubule physiologists, because it suggests an entirely new line of investigation. A detailed understanding of how tubules handle DDT also confers a detailed understanding of how the insect handles it. Although DDT use is now decried outside malarial areas, it is well known that DDT-resistant insects tend to show cross-resistance to other insecticide classes, so the Malpighian tubule is likely to be important for insecticide handling in general.

Once xenobiotics have been metabolized or otherwise rendered soluble, the Malpighian tubule may transport them onwards. There has been a large amount of literature on the handling of dyes, plant secondary metabolites and similar molecules by the tubule (Gaertner and Morris, 1999; Gaertner et al., 1998; Linton and O'Donnell, 2000; Maddrell et al., 1974; Meredith et al., 1984; Quinlan and O'Donnell, 1998; Torrie et al., 2004). Indeed, the use of low levels of amaranth or phenol red to help render the bathing droplets more visible is a widespread experimental trick, although the interaction of these sulphonates with transporters for cyclic nucleotides and sulphonylureas (Evans et al., 2005; Quinlan and O'Donnell, 1998) makes this practice risky.

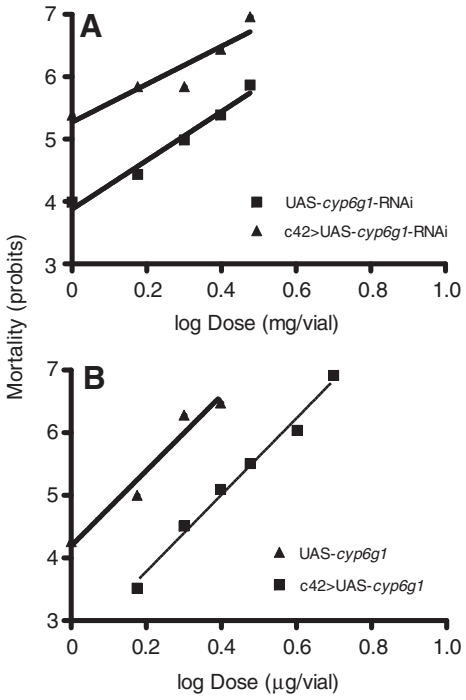


Fig. 5. The Malpighian tubule is limiting in xenobiotic handling by adult *Drosophila*. These probit curves show 24 h mortality of flies exposed to given amounts of DDT. Flies in which *cyp6g1* is either (A) knocked down by RNAi or (B) over-expressed, in just the tubule principal cells by the c42 GAL4 driver, are compared with their respective parents. The RNAi lines are over two times as sensitive and the over-expressing lines are about half as sensitive as their parental control lines, confirming that the tubule is limiting in the handling of this xenobiotic. [Adapted from Yang et al. (Yang et al., 2007).]

Recently, this has been taken forward in other species, using particularly the cross-sensitivity of ion-selective electrodes, for example the response of $K^+$-selective electrodes to tetraethylammonium and tridodecylmethylammonium (TDMA)-based anion-selective electrodes to salicylate (O'Donnell and Rheault, 2005; Rheault et al., 2006; Ruiz-Sanchez et al., 2007).

The identification of the transporters that underlie the excretion of organic solutes is clearly of great interest. By analogy with vertebrates, attention has focused on the multiple drug resistance (mdr)/P-glycoprotein ABC transporter. However, one clear result from the microarray study is that nearly every subclass of the huge ABC transporter gene family, as well as the OAT, OATP, sugar, multivitamin and amino acid transporter families, are very highly expressed in the Malpighian tubule (Wang et al., 2004). Thus the three genes annotated as *mdr* homologues in *Drosophila* may be only minority players in a vast array of more or less promiscuous organic solute transporters in the Malpighian tubule.

The impact of active organic solute transporters on experimental tubule pharmacology can also be significant and unexpected. As described previously, sulphonates like amaranth and Phenol Red can block the actions of externally applied cAMP and cGMP (Evans et al., 2008; Quinlan and O'Donnell, 1998). In *Drosophila*, cGMP (but not cAMP) is carried by the ABC transporter white, which was the very first mutant locus to be identified in *Drosophila* (Evans et al., 2008). This transport depends on the presence of di- or tri-carboxylates. White is the most common visible marker for transgenes in *Drosophila*, so care needs to be taken for certain experiments in this area.

Even more strikingly, the OATP class of organic solute transporter can explain the 'ouabain paradox' for tubules. This is the observation that insect epithelia can express high levels of $Na^+$,$K^+$-ATPase (which can be shown biochemically to be ouabain sensitive), but are themselves relatively insensitive to ouabain (Torrie et al., 2004). Ouabain transport has been documented in some members of the OATP gene family in mammals (Noe et al., 1997), and this family is abundantly expressed in the Malpighian tubule. It was possible to show that ouabain sensitivity could be unmasked by competition with classical OATP substrates, and that ouabain was actively transported across the tubule by one member of the family (Torrie et al., 2004). By using the GAL4/UAS system to target RNAi against this gene to just the tubule principal cells (the cells shown to transport ouabain), it was possible to knock down ouabain transport, so confirming the other observations (Torrie et al., 2004). It is thus a salutary lesson in pharmacology that simply co-locating two transporters in an epithelial context produces an emergent property (ouabain insensitivity) that is misleading at a macroscopic scale.

### The Malpighian tubule as a model for human renal disease

The rapid rate of fluid secretion by the tubule suggests that it is an excellent model for basic epithelial physiology, and the demonstration of its importance in insecticide handling makes it a tissue of great applied interest. However, there is also the potential to exploit similarities between the insect and human renal systems. Across over 400 million years of divergent evolution, one might expect very little in common between renal tubules; and at first sight, the use of an epithelial V-ATPase to energize fluid secretion – in marked contrast to the $Na^+$,$K^+$-ATPase-dominated picture in mammals – might seem to emphasize differences more than similarities. However, it is now

Table 1. Some organotypic human disease candidates in *Drosophila*

| Gene | Brain | Head | Midgut | Hindgut | Tubule | Ovary | Testis | Accessory gland | Human gene | OMIM entry |
|---|---|---|---|---|---|---|---|---|---|---|
| Brain | | | | | | | | | | |
| *kek2* | 156 | 19 | 4 | 5 | 8 | 2 | 3 | 5 | *SLITRK1* (KIAA1910) | Tourette syndrome (137580) |
| *CG5594* | 2267 | 867 | 441 | 142 | 35 | 133 | 53 | 124 | *Slc12a6* | Agenesis of the corpus callosum with peripheral neuropathy (218000) |
| *CG1909* | 1265 | 306 | 2 | 24 | 2 | 6 | 0 | 9 | (*RAPSYN*) | Congenital myasthenic syndrome associated with AChR deficiency (608931) |
| *Lcch3* | 748 | 173 | 2 | 3 | 3 | 2 | 8 | 5 | GABA-A receptor γ-2 | Myoclonic epilepsy, severe, of infancy (607208) |
| *CG7971* | 1722 | 437 | 117 | 62 | 70 | 31 | 23 | 171 | *NIPBL* (delangin) | Cornelia de Lange syndrome (122470) |
| Midgut | | | | | | | | | | |
| *CG6295* | 3 | 21 | 5461 | 4 | 2 | 1 | 1 | 2 | *LIPI* (*PRED 5*) | Hypertriglyceridemia, susceptibility to (145750) |
| *CG31636* | 2 | 19 | 159 | 2 | 1 | 1 | 7 | 1 | *c17orf79* (*T TP1*) | Ataxia with isolated vitamin E deficiency (277460) |
| *eTry* | 2 | 5 | 5967 | 6 | 6 | 3 | 5 | 3 | Proenterokinase | Enterokinase deficiency (226200) |
| Tubule | | | | | | | | | | |
| *CG3762* | 441 | 1213 | 4665 | 5497 | 6242 | 1906 | 354 | 1170 | Vacuolar proton pump | Renal tubular acidosis with deafness (267300) |
| *ry* | 80 | 142 | 97 | 49 | 770 | 2 | 18 | 7 | Xanthine oxidase | Xanthinuria type I (278300) |
| *Irk3* | 328 | 123 | 4 | 48 | 4932 | 1 | 1 | 11 | Renal outer-medullary potassium channel; ROMK | Bartter syndrome, antenatal, type 2 (241200) |
| *CG5284* | 568 | 451 | 292 | 361 | 1334 | 540 | 75 | 357 | Chloride channel *CLCN5* | Dent disease 1, nephrolithiasis, X-linked (30008) |
| *CG17752* | 1 | 1 | 2 | 1 | 6341 | 1 | 2 | 3 | *SLC22A12* (*URAT1*) | Hypouricemia, renal (220150) |
| Ovary | | | | | | | | | | |
| *Pi3K92E* | 283 | 171 | 182 | 188 | 108 | 395 | 92 | 185 | PI3 kinase-α | Ovarian cancer (604370) |
| Testis | | | | | | | | | | |
| *bol* | 104 | 19 | 1 | 9 | 6 | 1 | 1778 | 82 | *DAZL* (*SPYGLA*) | Spermatogenic failure, susceptibility to (601486) |
| *Ubp64E* | 294 | 221 | 238 | 514 | 334 | 196 | 752 | 367 | *Drosophila* fat facets related | Azoospermia (415000) |
| *CG17150* | 2 | 2 | 5 | 3 | 5 | 2 | 391 | 6 | *ITLN1* (*HL1*) | Kartagener syndrome (244400) |

AchR, acetyl choline receptor; OMIM, online Mendelian inheritance in man database.
Data from Chintapalli et al., 2007.

clear that $Na^+$,$K^+$-ATPase also plays a major role in insect tubule function (Ianowski and O'Donnell, 2004; Linton and O'Donnell, 1999; Maddrell and Overton, 1988; Torrie et al., 2004; Xu and Marshall, 1999). Conversely, multiple vertebrate epithelia express high levels of plasma membrane V-ATPase (Harvey and Wieczorek, 1997; Wieczorek et al., 1999; Wieczorek and Harvey, 1995). Indeed, the discovery of a Malpighian tubule phenotype in the first animal knockout of a V-ATPase, in *Drosophila* (Davies et al., 1996), presaged the discovery of a human renal phenotype in the first human mutation to be discovered (Karet et al., 1999). Furthermore, the demonstration that mutations in any of the epithelial subunits can produce a similar renal phenotype has been made in humans, but more comprehensively in *Drosophila* (Allan et al., 2005).

Can we extend this argument? Is the insect tubule a good model for diseases other than tubular acidosis? There are 13,500 genes in *Drosophila*, and a slightly larger number in humans. Rather than a laborious many-to-many comparison, Wang and colleagues compared Homophila, a database of human genetic disease cognates in *Drosophila* (Chien et al., 2002), with the enrichment of the *Drosophila* homologues in the Malpighian tubule (Wang et al., 2004). This produced a list of tubule-specific (or at least enriched) genes which were highly similar to human disease genes. Remarkably, the list was also enriched for classical human renal disease loci (Wang et al., 2004), implying far greater commonality between the two systems than previously supposed. Some examples are given below.

(1) Bartter's syndrome embraces three distinct severe renal salt-wasting diseases of neonates (Rodriguez-Soriano, 1998), due to mutations in the $Na^+/K^+/2Cl^-$-cotransporter (NKCC2), in the inward rectifying $K^+$ channel ROMK, or in the epithelial chloride channel ClC-Kb. Although *NKCC* homologues are widely expressed in *Drosophila*, the *ROMK* homologues *ir* and *irk3* are highly tubule specific, as is *CG31116*, the *Drosophila* gene most similar to *ClC-Kb*.

(2) Xanthinuria type I is a peroxisomal disorder, in which the metabolism of purines through xanthine, uric acid and urea is blocked by a mutation of xanthine oxidase, the enzyme that converts xanthine to urate. It is characterized by xanthine calculi in the kidney, and can be fatal unless diagnosed in time to impose a low-purine diet with plenty of water (Dent and Philpot, 1954). Remarkably the same disease had been described around 1916 in *Drosophila*! The second mutant locus to be identified, *rosy*, encodes xanthine oxidase, and produces similar xanthine calculi that can block the Malpighian tubule, causing it to bloat (Hilliker et al., 1992; McCarron et al., 1979). In *Drosophila*, *rosy* expression is massively enriched in the Malpighian tubule compared with other tissues.

(3) Kidney stones (renal calculi) are so painful that they underlie nearly half a million emergency room admissions annually, in the USA alone (Worcester and Coe, 2008). Kidney stones can be composed of many different minerals: xanthine stones are relatively rare, but uric acid calculi are relatively common. Uric acid stones are attributed to high levels of urate and low urinary pH (Moe, 2006), and treatment emphasizes alkalinization of the urine to keep urate in solution. Remarkably, this can be modelled in insects, because their uricotelic excretory system normally generates insoluble uric acid crystals, so reducing urinary volume. In mutants of any of the 13 genes encoding the subunits of the plasma membrane V-ATPase, uric acid deposition in the *Drosophila* Malpighian tubule is reduced or abolished (Allan et al., 2005). Insects (and other uricotelic animals) thus provide an inversion of

Table 2. Where are the Wieczorek exchanger candidates expressed?

| | Mean array expression signal | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gene (probeset) | Brain | Head | Thoracico-abdominal ganglion | Crop | Midgut | Tubule | Hindgut | Ovary | Testis | Accessory gland | Carcass | Larval tubule | Larval fat body | Whole fly |
| CPA1 exchangers | | | | | | | | | | | | | | |
| *nhe1* (1635858_s_at) | **201** | **105** | **251** | **211** | **244** | **521** | **260** | **206** | **136** | **557** | **134** | **442** | 63 | **120** |
| *nhe2* (1635858_s_at) | 54 | 13 | 53 | 33 | 36 | 6 | 14 | 6 | 12 | 15 | 14 | 52 | 63 | 10 |
| *nhe3* (1635858_s_at) | **558** | **222** | **612** | **121** | 33 | 28 | **377** | 81 | 16 | 49 | **108** | 87 | 43 | 81 |
| CPA2 exchangers | | | | | | | | | | | | | | |
| *CG10806* (1635858_s_at) | 8 | 30 | 13 | 9 | **288** | **155** | **3064** | 41 | 13 | 2 | 8 | 38 | 4 | 99 |
| *CG31052 / tango12* (1635858_s_at) | 10 | 99 | 9 | 45 | 19 | **875** | **1654** | 4 | 8 | 13 | **133** | **376** | 46 | 46 |
| Plasma membrane V-ATPase | | | | | | | | | | | | | | |
| *Vha68-2* (1637000_at) | 441 | 1213 | 623 | 1956 | **4665** | **6242** | **5497** | 1906 | 354 | 1171 | 961 | **4898** | 1420 | 1905 |

Data were mined from FlyAtlas (http://flyatlas.org). For ease of viewing, expression levels in excess of 100 are in bold. For comparison, a plasma membrane-validated V-ATPase subunit (Allan et al., 2005) is also shown. Because of the higher general levels of V-ATPase expression, signals above 2000 are in bold. For clarity, errors are omitted; these are typically of the order of 10%, and easily obtained from FlyAtlas. (Reproduced from Day et al., 2008.)

Searching for URATE OXIDASE through 18771 annotations produced 1 hits

**Urate oxidase (Uro) CG7171;** FBgn0003961; 1625436_at
Accessions: NP_476779.1; NM_057431;
GeneOntology: 0006144 / purine base metabolic process ; 0019428 / allantoin biosynthetic process / traceable author statement
BioGrid reports that CG7171 interacts with CG9269 CG9036 .

| Tissue | mRNA Signal | Present Call | Enrichment | Affy Call |
|---|---|---|---|---|
| Brain | 3 ± 1 | 0 of 4 | 0.00 | Down |
| Head | 4 ± 1 | 0 of 4 | 0.00 | Down |
| Thoracicoabdominal ganglion | 4 ± 1 | 0 of 4 | 0.00 | Down |
| Salivary gland | 17 ± 5 | 1 of 4 | 0.09 | Down |
| Crop | 6 ± 3 | 0 of 4 | 0.00 | Down |
| Midgut | 2 ± 0 | 0 of 4 | 0.00 | Down |
| Tubule | 6590 ± 555 | 4 of 4 | 35.80 | Up |
| Hindgut | 4 ± 0 | 0 of 4 | 0.00 | Down |
| Ovary | 2 ± 1 | 0 of 4 | 0.00 | Down |
| Testis | 2 ± 0 | 0 of 4 | 0.00 | Down |
| Male accessory glands | 11 ± 3 | 2 of 4 | 0.10 | Down |
| Adult carcass | 6 ± 3 | 1 of 4 | 0.00 | Down |
| Larval tubule | 2892 ± 400 | 4 of 4 | 15.70 | Up |
| Larval fat body | 1 ± 0 | 0 of 4 | 0.00 | Down |
| Whole fly | 183 ± 43 | 4 of 4 | | |

Fig. 6. Typical FlyAtlas (http://flyatlas.org) output. In this case, the search was for 'urate oxidase'. Even though this dataset is based on highly technical microarray data, it is easy to deduce that this is effectively a tubule-specific gene.

the mammalian condition, as gene dysfunction reduces uric acid deposition. The principle, however, remains the same; low luminal pH causes deposition, and higher pH keeps urate in solution.

So although *Drosophila* and humans diverged over 450 million years ago, there are intriguing parallels between kidney function across even this phylogenetic divide. Given the relative ease, speed and low cost with which *Drosophila* mutants can be obtained and studied (compared with mouse), the *Drosophila* Malpighian tubule system may prove highly informative in biomedical research.

### Other tissues: FlyAtlas

Clearly, the microarray study of the *Drosophila* Malpighian tubule provided a very cost-effective insight into tubule function. Can this approach be extended to other tissues? Since late 2006, just such an online microarray resource has been available at FlyAtlas (Chintapalli et al., 2007). This resource provides an easily interpretable view on gene expression across multiple tissues in larval and adult *Drosophila*, based on nearly half a million microarray data points (Fig. 6).

As well as providing a useful hypothesis-building tool, together with the hyperlinks needed to order up the materials to test the hypotheses, it is possible to interrogate the dataset in novel ways. For example, it is possible to look up a 'top 50' of genes expressed specifically in any particular tissue.

Alternatively, it is possible to extend the search for organotypic disease models, usefully employed in the Malpighian tubule (Wang et al., 2004), to other tissues (Chintapalli et al., 2007). The Homophila database already lists a thousand *Drosophila* genes with close similarity to known human disease loci; but testing these genes to identify genes with enrichments in analogous tissues (like brain) in both species can help to identify particularly fruitful candidates for further study (Table 1).

Of course, that *Drosophila* is capable of informing human research is well known; indeed, a Nobel prize was awarded for *Drosophila* developmental studies. However, the extension of this principle to epithelial transport physiology would be most welcome in a research environment driven by ever-tighter funding constraints, and (increasingly) with heavy-handed strategic steering. If insect biology is to continue to excite and advance, it must continue to attract research funding worldwide!

### The elusive apical 'Wieczorek' exchanger

FlyAtlas has also helped in one of the great mysteries of insect physiology: the identity of the elusive 'Wieczorek' exchanger that partners the apical plasma membrane V-ATPase in insect epithelia. Since the discovery that the V-ATPase constituted the apical portasomes present at high abundance in insect epithelia (Berridge and Oschman, 1969; Harvey et al., 1983; Harvey et al., 1981; Schweikl et al., 1989), and the demonstration that it was indeed functioning as a proton-motive ATPase (Schweikl et al., 1989; Wieczorek et al., 1991), it has been necessary to infer one or more co-localized alkali-metal cation/proton exchangers (termed by this

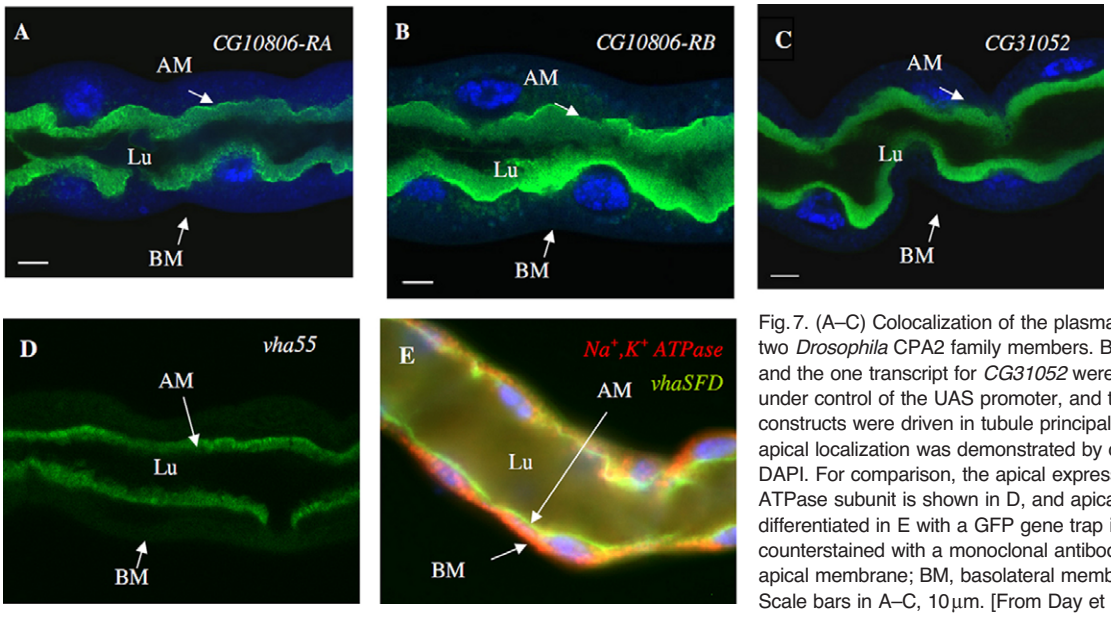


Fig. 7. (A–C) Colocalization of the plasma membrane V-ATPase with the two *Drosophila* CPA2 family members. Both transcripts for *CG10806* and the one transcript for *CG31052* were fused to eYFP and placed under control of the UAS promoter, and transgenic flies were made. The constructs were driven in tubule principal cells with c42-GAL4, and the apical localization was demonstrated by counterstaining nuclei with DAPI. For comparison, the apical expression of an eGFP-tagged V-ATPase subunit is shown in D, and apical and basolateral domains are differentiated in E with a GFP gene trap insertion in vhaSFD, counterstained with a monoclonal antibody to $Na^+,K^+$-ATPase. AM, apical membrane; BM, basolateral membrane; Lu, apical tubule lumen. Scale bars in A–C, 10 μm. [From Day et al. (Day et al., 2008).]

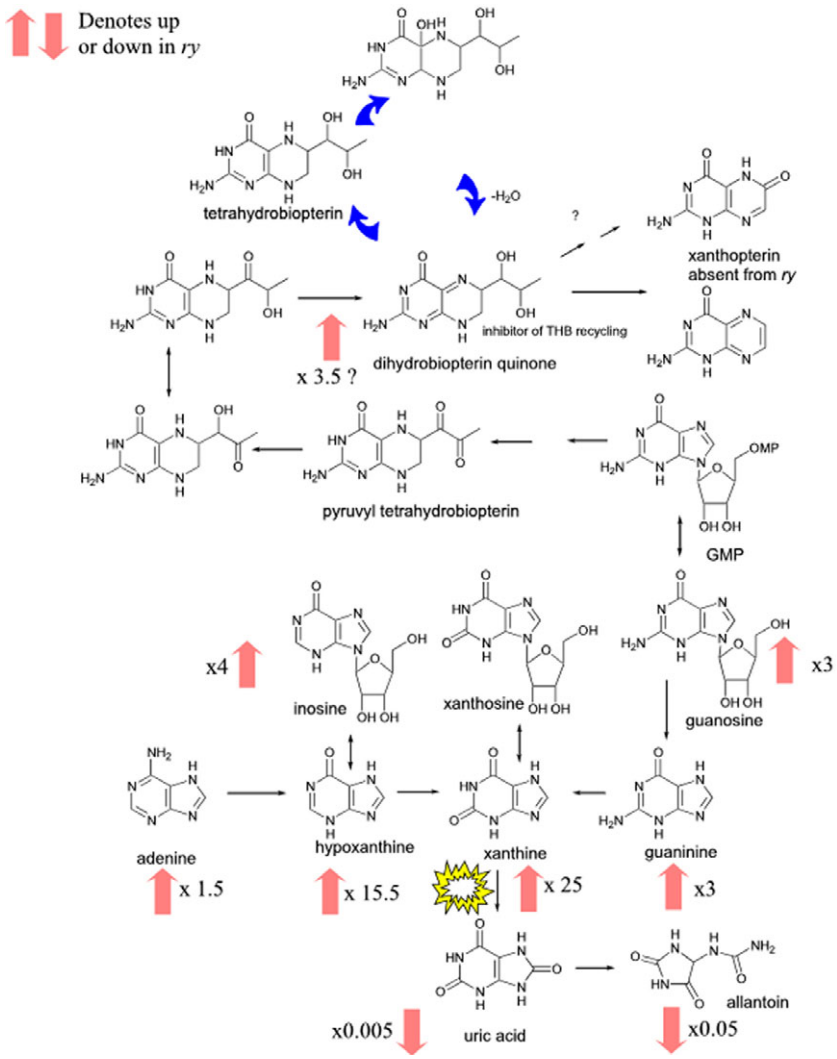


Fig. 8. Mapping the impact of the *rosy* mutation (*ry*). The molecular lesion affects xanthine oxidase, the enzyme which catalyses the conversion of xanthine to urate; however, the perturbations induced by the lesion can be traced much further away than previously possible by classical analytical biochemistry. [From Kamleh et al. (Kamleh et al., 2008).]

author 'Wieczorek' exchangers), in order to achieve a net transmembrane flux of sodium, potassium, or both (Azuma et al., 1995; Wieczorek et al., 1991). There have also been contrasting views on the nature of the exchanger(s); addition of the sodium ionophore gramicidin to *Rhodnius* Malpighian tubules or *Calliphora* salivary glands switches them from $K^+$- to $Na^+$-transporting mode (Maddrell and O'Donnell, 1993). Although this has been interpreted as implying that there is a single promiscuous apical exchanger, and that it is regulated by the differential permeability of the basolateral membrane to $Na^+$ and $K^+$, the data are not inconsistent with two apical exchangers with distinct ionic specificities. In *Manduca* midgut, it has been argued that the exchanger needs to be electrogenic ($2H^+/1K^+$) (Azuma et al., 1995) in order to generate the extraordinary high pH values observed in caterpillars (Dow, 1984).

The obvious place to seek such exchangers is in the $Na^+/H^+$ exchanger (NHE) family. *Drosophila* has three such genes (Giannakou and Dow, 2001), but they are widely expressed and their distribution is a poor match to the plasma membrane V-ATPase, at either mRNA or protein levels (Day et al., 2008), as can be seen from FlyAtlas (Table 2).

However, the NHE genes are a subfamily of the cation/proton exchanger (CPA) gene family, which also includes $K^+/H^+$ exchangers, originally identified in prokaryotes (Brett et al., 2005). NHEs sit in the CPA1 group, whereas the CPA2 group includes both $Na^+/H^+$ and $K^+/H^+$ exchangers, and members are found from bacteria through to humans (Brett et al., 2005). Not surprisingly, therefore, several groups have identified CPA2 genes as alternative candidate Wieczorek exchangers (Day et al., 2008; Rheault et al., 2007). In this case, the evidence is much stronger; the two *Drosophila* CPA2 members show a much better match in their expression patterns to the apical V-ATPase (Table 2), and (importantly) are the only CPAs localized to the apical membrane of principal cells (Fig. 7). An apical localization has also been reported in mosquito (Okech et al., 2008).

Functional data also support the assignment of the two CPA2 members to the role of Wieczorek exchanger. Both genes can rescue the salt sensitivity of exchanger-deficient yeast, though *CG10806* is better at protecting against high $K^+$, and *CG31052* against high $Na^+$ (Day et al., 2008). Transgenic interference with the CPA2s also impacts on tubule secretion by the fly: overexpression of CG10806 inhibits the stimulation of fluid secretion. Taken together, the data suggest that the two CPA2s are co-expressed with the apical V-ATPase in semi-redundant fashion, but that they have differing specificities for $Na^+$ and $K^+$ (Day et al., 2008). As these exchangers are visible across other sequenced insect genomes, it will be interesting to test the generality of this model.

## Metabolomics

The newest 'omic' science is metabolomics, the quantification of all the metabolites in a cell. The metabolome, because it is potentally downstream of both gene expression and protein function, has the potential to provide a multi-parameter snapshot of a cell's function. Such studies are powerfully helped by recent advances in separation and analysis technologies, particularly in mass spectrometry (Kell, 2004). Recently, we have applied metabolomics to *Drosophila* (Kamleh et al., 2008). Extracts from one or a few flies proved sufficient to resolve several hundred metabolites in a single run; the difficulty lies primarily in analysing the very large datasets, and deciding which peaks merit the detailed attention necessary for first identification.

The classical *rosy* mutation of *Drosophila* is widely known as an eye colour mutant, but FlyAtlas reveals that *rosy* is predominantly expressed in the Malpighian tubule, implying that xanthine metabolism is a tubule-specific task. *Rosy* mutants had previously been described as having high levels of hypoxanthine and xanthine, and low levels of urate and allantoin (Mitchell and Glassman, 1959). Metabolomic analysis, based on liquid chromatography and an Orbitrap spectrometer, were able to reproduce these effects, so validating the new technology as an alternative to painstaking analytical biochemistry. However, it was also able to demonstrate that significant changes could be detected at a far greater radius from the original lesion (Fig. 8).

In addition, intriguing effects were found in quite remote metabolites, particularly in the production of osmolytes like phosphocholine and phosphoethanolamine, suggesting new avenues of investigation (Kamleh et al., 2008). Although these experiments were performed on whole fly, the dominant expression of rosy in the Malpighian tubules suggests that the results are providing new insights into tubule function. We are presently studying the Malpighian tubule metabolome in more detail.

Of course, present metabolomics technologies cannot address all questions, or even detect all metabolites. However, the ability to sample many hundreds of compounds simultaneously and relatively easily makes it a promising tool in the high-throughput study of mutations in novel genes, for example in panels of novel mutants from stock centres.

## Conclusion

For a small tissue first described nearly 400 years ago, and researched intensively for the last half-century, it is exciting that so many new avenues for research into Malpighian tubules are being opened by post-genomic technologies. As well as confirming what we knew already, genomics, transcriptomics, proteomics and metabolomics all have the potential to highlight new genes, pathways or functions that we might not have deduced from existing knowledge. In the case of the Malpighian tubule, we find a tissue which performs not just like a kidney but also like a liver and an autonomous immune system. The prospects for the future are very exciting!

This work was generously supported by the UK Biotechnology and Biological Sciences Research Council (BBSRC). I am most grateful to Dr Shireen Davies for her help and support in this work, and to Prof. Simon Maddrell FRS, for his inspiration.

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