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MOLECULAR GENETIC ANALYSIS OF V-ATPase FUNCTION IN *DROSOPHILA MELANOGASTER*

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Summary

V-ATPases are phylogenetically widespread, highly conserved, multisubunit proton pumps. Originally characterised in endomembranes, they have been found to energise transport across plasma membranes in a range of animal cells and particularly in certain epithelia. While yeast is the model of choice for the rapid generation and identification of V-ATPase mutants, it does not allow their analysis in a plasma membrane context. For such purposes, *Drosophila melanogaster* is a uniquely suitable model. Accordingly, we have cloned and characterised genes encoding several V-ATPase subunits in *D. melanogaster* and, using P-element technology, we have succeeded in generating multiple new alleles. Reporter gene constructs reveal ubiquitous expression, but at particularly high levels in those epithelia thought to be energised by V-ATPases, and several of the alleles have lethal recessive phenotypes characterised by epithelial dysfunction. These results, while providing the first gene knockouts of V-ATPases in animals, also illustrate the general utility of *D. melanogaster* as a model for the genetic analysis of ion transport and its control in epithelia.

Key words: V-ATPase, *Drosophila melanogaster*, genetic analysis, *Polycomb*, gap junction, ion transport, vacuolar ATPase.

V-ATPases

Ion-transporting ATPases fall into three main classes (Pedersen and Carafoli, 1987*a*,*b*): F-type, multisubunit H^+-motive pumps found on membranes of mitochondria, chloroplasts and bacteria, and normally driven in reverse as ATP synthases; P-type, cation pumps with single catalytic subunits (and frequently with accessory subunits) found on plasma membranes and some endomembranes, and typified by the Na^+/K^+-ATPase; and V-type, or 'vacuolar' ATPases, which transport H^+ across eukaryotic endomembranes and which, more recently, have been found to energise ion transport across plasma membranes (reviewed in Gluck, 1992).

V-ATPases in insects

Insects are conspicuous exemplars of higher organisms in which epithelial function is energised by a proton, rather than a Na^+, gradient (Harvey, 1992*b*; Wieczorek and Harvey, 1995). The evidence assembled is based on the failure to purify a primary electrogenic K^+-ATPase from any of the many insect epithelia which displayed active transport of K^+; on the demonstration that K^+-transporting activity was concentrated in the apical membranes of the goblet cavity of larval lepidopteran midgut (Dow *et al.* 1984; Dow and Peacock, 1989); on the biochemical purification of this membrane (Harvey *et al.* 1983*a*); on the demonstration that the major ATPase of this membrane was in fact an H^+-ATPase (Wieczorek *et al.* 1991); and on the subsequent demonstration that apical plasma membranes of all those epithelia in which the classical 'K^+ pump' had been invoked contained V-ATPases, by immunocytochemistry, by direct protein biochemistry or by enzymology (Klein *et al.* 1991; Wieczorek, 1992). These results led to the development of the 'Wieczorek model' for insect epithelia, that active transport of alkali metal cations (i.e. Na^+ or K^+) is the product of a primary apical plasma membrane H^+-motive V-ATPase that drives one or more alkali metal/H^+ exchangers (Wieczorek, 1992). This view is now accepted as orthodoxy, because insect epithelia are found to be exquisitely sensitive to both bafilomycin (a specific H^+ V-ATPase inhibitor) and amiloride (a blocker of Na^+ channels or exchangers) (Bertram *et al.* 1991; Dow *et al.* 1994*a*; Maddrell and O'Donnell, 1992). Additionally, interference with the alkali metal transport pathway (by amiloride application, Ba^{2+} blockade of basal K^+ entry or depletion of K^+ from the bathing medium) unmasks the apical H^+-ATPase by inducing an apical acidification (Bertram *et al.* 1991; Dow, 1992; Dow *et al.* 1994*a*; Maddrell and O'Donnell, 1992; Weltens *et al.* 1992). The concept that epithelia can be energised by a proton-motive, rather than a Na^+-motive, force can be extended to several vertebrate epithelia, such as the

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kidney (Gluck and Nelson, 1992) and frog skin (Harvey, 1992*a*).

While this model is widely accepted, some problems remain. The exchanger remains enigmatic, largely due to the failure to clone any insect gene with homology to the classical vertebrate Na^+/H^+ exchanger. There may be a single promiscuous exchanger that can accept either Na^+ or K^+, or there may be separate exchangers for each metal ion species; the latter possibility would allow a straightforward way of explaining the apparent differences in alkali metal ion specificity of different insect epithelia. The Wieczorek model proposes that, in midgut, the exchanger may be electrophoretic (Azuma *et al.* 1995), and this may directly explain the remarkably high pH values encountered in larval lepidopteran gut lumen (Dow, 1984, 1992); however, there is evidence in other insect epithelia that any exchanger is electroneutral (van Kerkhove, 1994), and the existence of any exchanger has been challenged even in midgut (Küppers and Bunse, 1996).

A heretical alternative explanation, based on the insensitivity of electrical measurements of the trichogen sensilla of insects to amiloride or harmaline, is that there is no exchanger and that the apical V-ATPase is primarily an H^+-ATPase, but with the additional ability to transport alkali metal cations (Küppers and Bunse, 1996). Given that the intracellular pH is 7, and that intracellular $[K^+]$ is approximately $100\,mmol\,l^{-1}$, even if the pump were 10^5:1 selective in favour of H^+ over K^+, under normal conditions the two ion species would be transported at nearly equal rates. Classically, the assignment of both F-ATP synthases and V-ATPases as purely H^+-motive would argue against so radical a model; however, recent evidence suggests that, at least in some species, both F-ATP synthases and V-ATPases may have significant alkali metal transporting ability (Takase *et al.* 1994), and that the ion-specificity of the ATPase may be switched by mutagenesis of the proteolipid subunit (Zhang and Fillingame, 1995). If this were true, then, by selective targetting of appropriate subunits, an organism might have both a proton-motive endosomal V-ATPase and a cation-motive plasma membrane V-ATPase in its epithelia. Against this, however, it should be noted that the proteolipid subunits of both *Manduca sexta* and *Drosophila melanogaster* show no divergence from those of other species in which the identity of the transported ion is not in doubt (Dow *et al.* 1992; Meagher *et al.* 1990), and so it is unlikely that these subunits could support a significant flux of ions other than protons.

The big questions

From the preceding arguments, it can be seen that there are several interesting questions which need to be addressed, both in insects and more generally: are the plasma membrane V-ATPases different from those of endomembranes, in terms of expression levels, transcript choice or functional properties, and are such differences functionally important?

Reverse genetics

How can the roles of plasma membrane be dissociated from those of endomembranes? To study such problems, we require a system in which we can selectively manipulate gene expression in the intact organism. Although yeast is the preferred model for straightforward structure/function analysis of V-ATPases (Nelson and Nelson, 1990), it is not a suitable model for analysis of a plasma membrane function. Clearly, a transgenic animal model is required. There are three species for which genetics is sufficiently advanced; the mouse, the zebrafish and *D. melanogaster*. The mouse model allows elegant and precise disruption of a gene by homologous recombination, but the ability to intervene in a particular tissue, or at a particular phase of the life-cycle, remains poorly developed. It is thus a poor model for reverse genetics of most of the genes of interest in transport physiology, precisely because of their ubiquity: we can predict that many of these genes will be essential for organismal function, and so the phenotype of disruptant homozygotes will be lethal at a stage too early to permit detailed physiological analysis. Although the zebrafish holds considerable promise for the future, the genetics of the system is still underdeveloped and there is very little transport physiology to permit a phenotypic assay. By contrast, there is a wealth of transport physiological data for insects, although *D. melanogaster* ranks as one of the smallest yet studied.

Drosophila melanogaster as a model system

D. melanogaster is the higher organism with perhaps the best balance of structural complexity and genetic tractability available at present (Rubin, 1988). Indeed, the power of this model in producing information of general significance is illustrated by two famous examples; the developmental (Lawrence, 1993) and behavioural (Davis and Dauwalder, 1991; Tully, 1991) phenotypes. The advantages of *D. melanogaster* are as follows: (i) a short generation time and high fecundity, allowing crosses to be made and analysed within 2 weeks; (ii) the ease and relatively low cost of rearing, which permit large-scale genetic experimentation; (iii) the relatively small genome, such that a given recombination interval corresponds to a far smaller (and thus more tractable) piece of DNA than would be the case in mouse or human; (iv) the existence of a huge body of classical genetics, which gives an excellent density of mapped, visible markers (such as eye colour and bristle length) for use in experimentation; (v) the reconciliation of these data into a genome databank, which allows new data to be reconciled easily with previously existing data; (vi) the development of synthetic transposons (particularly those based on the P-element), which allow targetted mutagenesis of desired genes, the incorporation of transgenes into the genome at high efficiency and the identification of potentially interesting loci by enhancer-trapping (Kaiser, 1993; Kaiser and Goodwin, 1990; Rubin and Spradling, 1982).

In the face of such overwhelming advantages, it should be noted that there are deficiencies in *D. melanogaster* as a physiological model for the study of transport genes such as

V-ATPases. These are (i) the lack of a pre-existing phenotype for studies of mutants, (ii) the extremely small size of the organism, which makes it difficult to perform such physiological analysis, and (iii) the different body architecture of invertebrates compared with vertebrates, which requires experiments to be carefully designed if they are to yield results of general significance. Nonetheless, we have been able to develop in the last few years a model which allows the detailed analysis of transport or cell signalling genes in a physiological context that is strongly reminiscent of several vertebrate epithelia.

The *Drosophila melanogaster* Malpighian (renal) tubule

The insect Malpighian tubule performs a function loosely analogous to that of the vertebrate kidney tubule. Despite its small size (a linear array of just 121 principal cells), the *D. melanogaster* tubule is remarkably robust and provides a valuable physiological phenotype (Dow *et al.* 1994*a*). Although originally developed as a screen for V-ATPase mutations because of the known sensitivity of insect tubules to bafilomycin (Dow *et al.* 1994*a*), it has also proved a valuable prototype for an epithelium controlled by nitric oxide and cyclic GMP (Dow *et al.* 1994*b*) and for the study of neuropeptides such as the leucokinins and CAP_{2b} (Davies *et al.* 1995; O'Donnell *et al.* 1996). Potentially, then, the *D. melanogaster* Malpighian tubule may prove a useful tool for the study of plasma membrane V-ATPase function.

The *Drosophila melanogaster* V-ATPase family

We have embarked on a characterisation of V-ATPase genes in *D. melanogaster*. In addition to those cloned explicitly by our group, two further subunits have been cloned serendipitously, one from an enhancer-trap study (Harvie and Bryant, 1996) and one from a yeast two-hybrid study of cytoskeletal proteins (He and Kramer, 1996). This means that, in terms of molecular characterisation, *D. melanogaster* V-ATPase approaches that of human, yeast and *Manduca sexta* in the number of subunits characterised (Table 1).

V-ATPase mutants in *Drosophila melanogaster*

In principle, the identification of a chromosomal localisation for a gene in *D. melanogaster* unlocks a wealth of published data, as effectively all known mutations have been mapped by recombination and documented as part of a visionary genome project that has run from the early decades of this century. It is thus possible that, when a novel genetic locus is characterised, there may be existing mutations in the region that merit further analysis as candidate alleles of the gene under study. Over the last few years, the probability of such findings has been increased greatly by the systematic physical mapping of the genome, the production of comprehensive panels of thousands of lines carrying lethal P-element insertions, which must presumably have inactivated a large number of essential genes (Török *et al.* 1993), and the development of economical screening strategies that allow the easy identification of candidate lines for a particular genes (Guo *et al.* 1996*a*).

Our V-ATPase work has been greatly assisted by this integration of work from the *D. melanogaster* community, and we now have a panel of flies in which V-ATPase subunits have been inactivated (Table 1). Although the discovery of a V-ATPase C-subunit mutant was serendipitous (Harvie and Bryant, 1996), we have identified mutant alleles of the A, B, c and E subunit by screening lethal P-element insertions which had been mapped previously to the same genetic interval by *in situ* hybridisation (Davies *et al.* 1996; Guo *et al.* 1996*a*). Although

Table 1. *Characterisation of* Drosophila melanogaster *genes encoding V-ATPase subunits*

Subunit	Gene location	Copy number	Transcript size(s) (kbp)	Deduced peptide size (kDa)	Deduced peptide identity (%)		P-element lethal?	Homozygous phenotype	Reference
					Human	*Manduca sexta*			
A	34B	2+	2.6	68, 68	91	91	Yes	Embryonic, lethal to viable	Y. Guo, personal communication
B	87C	1	2.8, 2.3, 2.1, 1.8	55	93 (brain) 89 (kidney)	97	Yes	Embryonic, lethal to viable	Davies et al. (1996)
C		1	1.8		66		Yes	Second instar to pupal lethal	Harvie and Bryant (1996)
D									He and Kramer (1996)
E	83B	1	2.3	26	63	77	Yes	Embryonic, lethal	Guo et al. (1996*c*); Y. Guo, personal communication
F	52B	1	0.65	14	71	90	No		Guo et al. (1996*b*)
c	42B	1	1, 1.2	16	87	93	Yes	Third instar, lethal	Meagher et al. (1990); S. Graham (unpublished)

such mutations might lie anywhere within approximately 200 kb of the target gene, we have been fortunate in identifying at least one line for each subunit in which a P-element is situated either within an intron, or directly 5′ to the start of the gene.

In one case, the B-subunit, this lethal insertion has proved invaluable in demonstrating that *vha55*, the gene encoding the subunit, is synonymous with *l(3)87Ca* (Davies *et al.* 1996), a lethal locus whose detailed characterisation (Gausz *et al.* 1979) predates the discovery of V-ATPases by a decade. It is thus the first knockout of a V-ATPase in an animal.

What phenotype could be expected for V-ATPase mutations?

Our working model is that there are two distinct roles for V-ATPases in animals: the classical endomembrane role first characterised in plants and fungi, and a plasma membrane role in specialised cell types and epithelia. There may be differences in subunit composition or splice site choices in at least some subunits of the holoenzyme, and there is evidence in vertebrates that B-subunit transcript choice influences the choice of other subunits. There is also a more general difference; while only a few V-ATPase holoenzymes may suffice to acidify a given endomembrane compartment to the desired level, V-ATPase activity is the primary driving force, and thus the rate-limiting factor, in epithelial function. We thus expect to find a far higher holoenzyme level on plasma membranes of epithelia energised by V-ATPases than on any endomembrane; and indeed this is the case. V-ATPases form effectively close-packed arrays of 'portasomes', as seen by electron microscopy of most insect epithelia (Harvey *et al.* 1983*b*). This property is also shared by the plasma membrane of kidney intercalated cells (Brown *et al.* 1992).

Given that V-ATPase levels are critical to epithelial function, it is reasonable to suppose that an epithelial phenotype might be one of the earlier manifestations of a V-ATPase null phenotype.

The SzA *tubule phenotype*

Although there is no detectable heterozygous phenotype of any V-ATPase mutation we have so far characterised in *D. melanogaster*, there is a homozygous phenotype manifest in tubules. This was first described by Gausz *et al.* (1979) in some alleles of the *SzA*, or *l(3)87Ca*, lethal complementation group at 87C, which we have subsequently shown to be synonymous with *vha55* (Davies *et al.* 1996). Classically, those mutant homozygotes that survived to late embryonic or early larval stages showed transparent Malpighian tubules, without the luminal white material observed in healthy larvae. The phenotype was variable, and not all lethal alleles displayed it. If this phenotype is indeed associated with a defective V-ATPase B-subunit, then it is reasonable to suppose that defects in other V-ATPase subunits would be equally effective in eliciting the phenotype. Indeed, we have been able to replicate this phenotype with other alleles of *vha68-2*, one of the genes encoding the A-subunit (Fig. 1). Taken together, these results suggest that genetic intervention, not just in V-ATPase subunit genes but in any gene essential for plasma membrane V-ATPase function, is likely to show this characteristic phenotype, which may thus constitute a general screen for such genes.

We interpret this phenotype as a failure to acidify the tubule luminal contents, thus impeding the precipitation either of uric acid or of the mineral concretions that accumulate in the tubule lumen.

Expression mapping

An important line of evidence for differences between plasma membrane and endomembrane V-ATPases is the study of cell-specific expression patterns. As described above, it has already been established by immunocytochemistry, in both insects and vertebrates, that plasma membranes of epithelia energised by V-ATPases contain far higher levels of protein than endosomal membranes of cells in general (Klein, 1992; Nelson *et al.* 1992). Is this upregulation in epithelia through higher levels of expression of the gene, enhanced stability of the mRNA or enhanced stability of the protein product? There

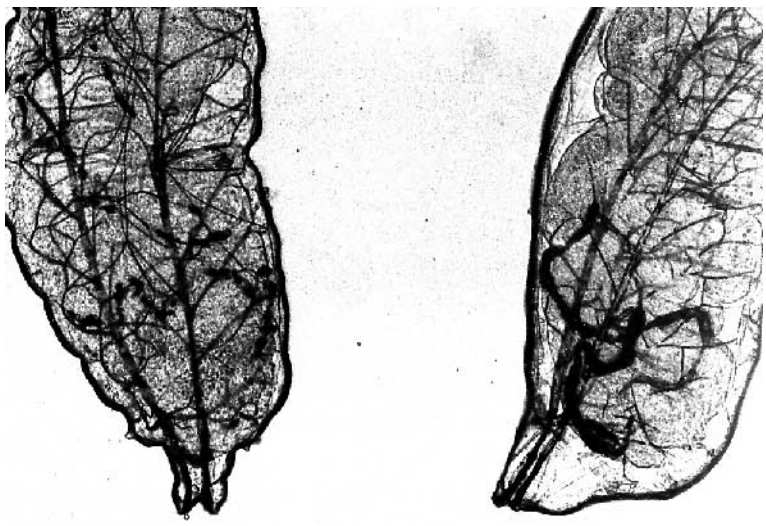

Fig. 1. The *SzA* tubule phenotype is replicated by a lethal allele of *vha68-2*, a gene encoding an A-subunit of V-ATPase. The *vha68-2*$^{-}$ homozygote is shown on the left; a heterozygous sib is shown on the right. In transmitted light, the relatively poor development of luminal insoluble material in the mutant tubules is clear.

is now evidence to support the first of these models. Synthetic P-elements contain the *lacZ* reporter gene downstream of a very weak,'permissive' promoter. A P-element inserted near a given gene (they have a tendency to insert non-randomly either upstream or in early introns) will thus fall under the influence of the control elements (promoters and enhancers) that control expression of that gene. *lacZ* expression can thus be taken as at least an approximate estimate of the rate of transcription of the gene; and as it is an exogenous gene, its expression will not be affected by post-transcriptional tissue-specific effects that might modulate the longevity of the neighbouring mRNA. Such reporter expression patterns can thus distinguish between the first two possibilities outlined above.

P-element insertions in either *vha68-2*, one of at least two genes encoding A-subunits (Y. Guo, in preparation), or in *vha55*, the single gene encoding a B-subunit (Davies *et al.* 1996), both show that transcription is selectively upregulated in epithelial tissues known to be energised by V-ATPases (Fig. 2). Enhanced expression is first detected in embryos, in line with the embryonic lethal phenotype elicited by severe disruptants (Davies *et al.* 1996).

Expression is also detected in epithelia known to be

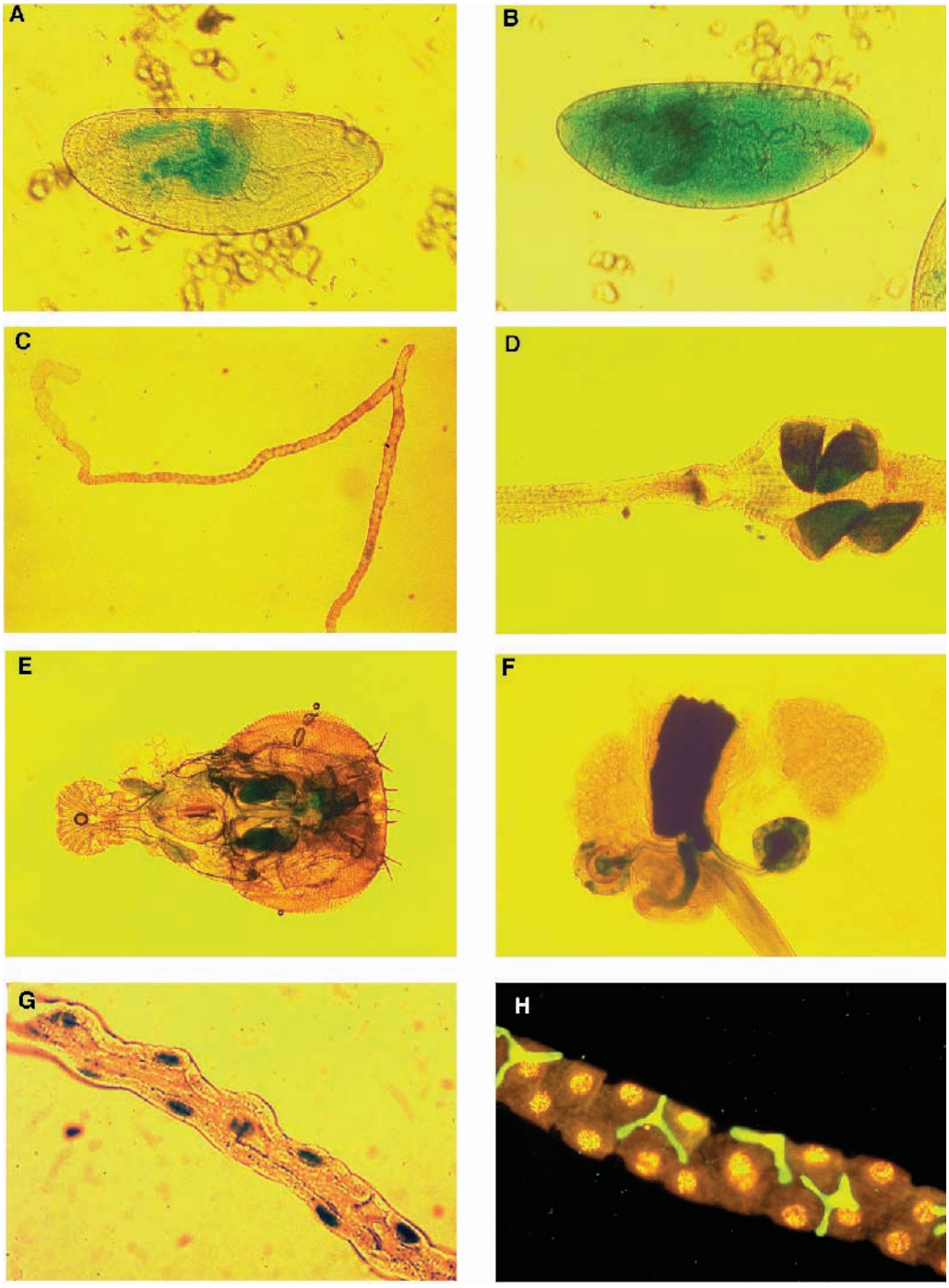

Fig. 2. *lacZ* reporter gene expression in a P-element insertional mutant of *vha55*. (A) Early embryo; (B) later embryo; (C) Malpighian tubules; (D) rectum; (E) antennal palps; (F) oviduct. (G) Higher-power view of Malpighian tubule main segment reporter expression, showing that expression is confined to the larger principal cell nuclei. (H) Enhancer trap line c724 stained with fluorescent anti-β-galactosidase to reveal stellate cells and counterstained with ethidium bromide to reveal nuclei. A–F are reproduced from Davies *et al.* (1996) and G–H are reproduced from Sozen, M. A., Armstrong, J. D., Yang, M.-Y., Kaiser, K. and Dow, J. A. T., in preparation.

energised by V-ATPases, the Malpighian tubules, the antennal palps (rich in tormogen cells) and the rectum (Fig. 2). Expression is also elevated in the female oviduct and accessory glands (Fig. 2F), not a tissue in which V-ATPase action had previously been implicated: although plasma membrane V-ATPases have been shown to play an important part in regulating the pH of the male reproductive tract, and thus of sperm motility, in vertebrates (Breton *et al.* 1996).

Recently, physiological analysis of the Malpighian tubules of *D. melanogaster* has shown that they are stimulated by each of the second messengers cyclic AMP, cyclic GMP and Ca^{2+} (Davies *et al.* 1995; Dow *et al.* 1994*a*,*b*). These act independently, with cyclic nucleotides stimulating an apical V-ATPase, whereas Ca^{2+} acts to elevate Cl^- permeability. This separate control of cation and anion transport led to the speculation that these transport processes might also be separately located in the different cell subtypes of the tubule (O'Donnell *et al.* 1996). The enhancer detector mapping of V-ATPase expression levels described above (Davies *et al.* 1996) has recently provided the first demonstration of such segregation; elevated V-ATPase expression is unique to the principal cells of the main segment, and not to the stellate, secondary cells (Sozen *et al.* 1996). Cation transport is thus confined to a genetically defined population of principal cells in this epithelium, which would allow the stellate cells, in turn, to be specialised for transport of anions and perhaps water (Dow *et al.* 1995).

Do V-ATPases modulate *Polycomb*?

An intriguing byproduct of the *Drosophila* genetic approach as that a gene may map to the same position as some, apparently quite unrelated, known locus. This proves to be the case for *vha55*, the gene encoding the B-subunit, which maps to the same interval (87C on chromosome 3) as the far more fashionable gene *Polycomb*. Products of the *Polycomb* (*Pc*) group of genes are thought to act cooperatively to elicit transcriptional silencing of genomic DNA in response to the initial transcription levels of homeotic genes in the embryo by binding untranscribed DNA and packing it into heterochromatin (Moehrle and Paro, 1994). They thus help to preserve the 'memory' of the homeotic selector genes by shielding inactivated genes from later access by transcription factors that might otherwise tend to activate them ectopically. The mutant phenotypes of any of the *Pc* group (ectopic expression of sex combs on T2 and T3 legs of males) thus resemble a homeotic misassignment of T2 and T3 legs to a T1 fate. This model for transcriptional silencing may have general significance for the long-term inactivation of genes during development. A previous screen for dosage-dependent suppressors or enhancers of *Pc* had identified a number of homeotic genes, together with several that were mapped but unidentified (Kennison and Tamkun, 1988). One of the powerful suppressing loci was shown to be allelic to *l(3)87Ca* (Kennison and Tamkun, 1988), a locus that we have now demonstrated to be *vha55* (Davies *et al.* 1996). This implies that V-ATPases might play a role in transcriptional silencing. This finding could reflect some specific but uninformative interaction between these two mutant proteins, it could reflect a general sensitivity of *Pc* to the V-ATPase status of the insect, or it could reflect some unusual genetic event involving 87C, unrelated to the role of *l(3)87Ca* as a gene encoding a V-ATPase subunit. For example, most of the loci previously identified as modulating *Pc* were known homeotic genes (Kennison and Tamkun, 1988), and at 87C, *vha55* is placed between the two major homeotic clusters on chromosome 3. It is thus conceivable that some alleles that inactivate the V-ATPase might also destroy some exceptionally long-range control element of a homeotic gene in one of the neighbouring clusters, although the distance makes this unlikely.

Are V-ATPases multi-purpose proteins?

The finding that the effects of mutations in *Polycomb* can be modulated by hemizygosity for a gene encoding a proton pump subunit is quite unexpected and raises the general question of whether the V-ATPase holoenzyme or particular subunits may serve multiple roles, in addition to straightforward endosomal acidification. This proves to be the case, and an increasing number of cellular processes are being shown to be dependent on V-ATPase function.

V-ATPase subunits may affect holoenzyme assembly either directly or by interaction with the cytoskeleton. The B-subunit can be found preferentially attached to early endosomal vesicles of kidney collecting duct, in the absence of other major V-ATPase subunits, suggesting an independent role in vesicle anchoring or trafficking (Sabolic *et al.* 1992). Similarly, preliminary data from a yeast two-hybrid screen implicates the *D. melanogaster* D-subunit in binding to the cytoskeletal protein hook (He and Kramer, 1996). If the V-ATPase interacts directly with the cytoskeleton, it could play a major active role in vesicle trafficking and in the sorting processes that govern the relative acidity of different endosomal compartments.

Recently, several emerging roles of V-ATPases in processes that may have developmental implications could offer additional explanations of this interaction. One is the high-affinity binding of oncoproteins, such as bovine papillomavirus E5 (Goldstein *et al.* 1991) or human lymphotrophic leukaemia virus type I P12 proteins (Koralnik *et al.* 1995), to the transmembrane proteolipid subunit of the V-ATPase (Koralnik *et al.* 1995). Binding may reduce the efficiency of dissociation of a growth factor (probably platelet-derived growth factor or epidermal growth factor) from its receptor in endosomes by inhibiting V-ATPases and thus raising endosomal pH. The growth factor receptor would then signal occupancy for longer than normal and so predispose a cell to inappropriate growth. The sensing of the gradients of *D. melanogaster* segmentation gene products may involve analogous ligand–receptor interactions. A slightly underactive endosomal compartment would then cause the position of the cell in such a gradient to be misread, resulting in subsequent mis-setting of homeotic selector genes, and thus explain an interaction with *Pc*.

V-ATPases have also been implicated in the regulation of cytoplasmic pH, a putative signalling system. Pharmacological blockade of V-ATPases in culture increases susceptibility to apoptosis (Nishihara *et al.* 1995), whereas granulocyte colony-stimulating factor delays apoptotic cell death in neutrophils by upregulating V-ATPase (Gottlieb *et al.* 1995), suggesting that downregulation of V-ATPases and concomitant cytoplasmic adicification may be an integral part of the apoptotic machinery. Inhibition of apoptotic processes in development could lead to survival of cells expressing inappropriate gene products.

V-ATPases have also been found to co-localise with calcineurin, an important Ca^{2+}-sensitive phosphatase; and, in yeast, mutations in calcineurin or other Ca^{2+} regulating genes and in V-ATPase have been shown to have synthetic lethality, suggesting an important role for V-ATPases in regulating intracellular Ca^{2+} (Garrettengele *et al.* 1995; Tanida *et al.* 1995).

Another possibility is that a slightly higher than normal endosomal pH would produce aberrant post-translational processing of genes not normally associated with ion transport; differential endocytic processing of wild-type and mutant β-amyloid is observed when V-ATPase activity is reduced by the toxin bafilomycin-A_1 (Haass *et al.* 1995).

It is also possible that a human genetic disease may be associated with haploabnormality for a V-ATPase gene. Recently, cat eye syndrome, a disease characterised by multifaceted developmental abnormalities, has been shown to be caused by a supernumerary minute ring chromosome, corresponding to the centromeric region of chromosome 22 which contains the gene encoding the V-ATPase subunit-E (Baud *et al.* 1994). The E-subunit probe is the most distal marker duplicated in all patients screened to date (Mears *et al.* 1995), suggesting that it might be involved in the pathogenesis of the syndrome.

The proteolipid c-subunit of the V-ATPase has also been argued to play an essential role in, if not form the pore of, gap junctions, at least insects (Finbow *et al.* 1995), a process with wide-ranging significance both in development and in adult function of all organisms, and in the 'mediatophore' (vesicle fusion apparatus) of *Torpedo marmorata* (Leroy and Meunier, 1995). Evidence for this proposal includes the purification of a 17 kDa integral membrane protein, with peptide sequence indistinguishable from V-ATPase proteolipid, from several invertebrate gap junctional preparations (Dow *et al.* 1992; Finbow *et al.* 1992; Finbow and Pitts, 1993). This view is heretical, but in its support is the patent failure to clone invertebrate homologues of connexins, despite their close conservation across other phyla. In rat embryos, proteolipid gene expression is transiently elevated at sites of mesenchymal/epithelial interactions, in a pattern resembling that of connexin 43 (Numata *et al.* 1995). Unusually, this model invokes membrane insertion in both possible orientations (reviewed elsewhere, Dow, 1994); however, it is prudent to allow the possibility that V-ATPases may not be passengers in membrane fusion, cycling and communication events, but essential participants.

Overview

The results presented above lead to the slightly suprising conclusion that, despite its small size, *D. melanogaster* may be the ideal model for the multidisciplinary investigation of V-ATPases and other transport- or signalling-related genes in an organismal context. The advanced genetics unique to *D. melanogaster* can be combined with a novel physiological phenotype to allow interesting experiments in integrative physiology to be designed. Taken together with the evidence presented here for a great richness in roles for the V-ATPase, both in *D. melanogaster* and in other organisms, there is great potential in this synthesis of experimental technologies.

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