cGMP signalling in a transporting epithelium

S.-A. Davies1 and J.P. Day

Division of Molecular Genetics, Institute of Biomedical and Life Sciences, University of Glasgow, Dumbarton Road, Glasgow G11 6NU, U.K.

Abstract

The biochemical aspects of cGMP signalling are well known, although in vivo roles of cGMP have only been recently discovered through work in genetic model organisms. The Drosophila melanogaster Malpighian (renal) tubule has been used to address the roles of cGMP in epithelial function. Here, we describe some of this work and outline recent progress in understanding the organotypic function of novel phosphodiesterases encoded by the D. melanogaster genome.

Introduction

Signalling via cGMP provides important regulation for an increasing number of diverse cellular and physiological processes. Downstream cellular targets and/or effectors of cGMP include CNG (cyclic nucleotide-gated) channels [1] and cGKs (cGMP-dependent protein kinases) [2]. Furthermore, a family of PDEs (phosphodiesterases), some of which are themselves regulated by cGMP [3], control intracellular cGMP content.

While the elements of cGMP signalling have been discovered using in vitro and cell-based systems, the recent use of genetic model organisms such as the mouse has been instrumental in the discovery of critical roles for cGMP signalling in physiology [4]. However, genetic models such as Drosophila melanogaster have much greater scope for targeted expression and transgenesis [5]. This genetic power of Drosophila has enabled sophisticated studies of gene function to be performed for many years, although it has been difficult to achieve the range of physiological studies compared with that using vertebrate models. However, the development of the Drosophila Malpighian (renal) tubule as a genetic model for transporting epithelia has enabled physiological studies of cyclic nucleotide signalling in vivo [6]. The conservation of genes associated with renal disease in humans and flies [6], and the close structural and functional similarity in cell signalling pathways, also suggests the utility of such studies in the Drosophila tubule.

The cGMP pathway in the Drosophila Malpighian tubule

The Malpighian tubule is a fluid-transporting osmoregulatory epithelium which is critical for detoxification, ion homeostasis and immune responses in the fly [7]. Work from this laboratory has established that tubule function is modulated by cGMP [8], primarily in the principal cell (Figure 1, yellow), which contains the large complexes of proton pumps required for ion transport.

Generation of cGMP in the Drosophila tubule can occur via autocrine stimulation of nitric oxide synthase and soluble guanylate cyclase in the principal cell [8]. Tubules also express several receptor guanylate cyclases [8], although their specific ligands have yet to be identified.

Tubules express all four CNG channel genes encoded by the Drosophila genome [8]; and cAMP- and cGMP-stimulated calcium transients occur in only principal cells in the intact tubule [9]. Thus cyclic nucleotide-stimulated calcium signals modulate tubule function via activation of cell-specific calcium signalling events.

The major effector of cGMP signalling, cGK(s), is encoded in Drosophila by two genes, dg1 and dg2 (foraging, for) [8]. Tubules express both dg1 and all multiple transcripts for dg2. Recent work from this laboratory has shown that the dg2 transcripts, dg2P1 and dg2P2, encode isoforms of bona fide cGKs, with K_m values in the sub-micromolar range [10]. Transfection studies in Drosophila S2 cells showed that DG1 was localized to the cytoplasm, while dg2-encoded DG2 isoforms localized at the plasma membrane. Targeted expression of these cGKs in tubule principal cells in vivo further showed that differential localization of the DG2 isoforms occurred: DG2P1 at the apical membrane; and DG2P2 expression at both apical and basolateral membranes. The functional consequence of this is startling: DG2P2, but not DG2P1, stimulates fluid transport in response to capa-1, via activation of the capa receptor, which is possibly localized at the basolateral membrane. In contrast, DG1 significantly modulates fluid transport in response to exogenous cGMP, taken up into the cytosol via a cGMP transporter. Thus this suggests the existence of cGMP pools in tubule cells, and the transduction of specific cGMP signals via particular cGKs at the site of cGMP generation.

Maintenance of cGMP levels:
PDEs in Drosophila

In vertebrates, hydrolysis of cGMP is carried out by cyclic nucleotide PDEs, including PDE1, PDE5, PDE6, PDE9,
Figure 1 | *Drosophila* tubules contain two main cell types, principal (yellow) and stellate (green) cells
Capa-1 and -2 are neuropeptides that stimulate cGMP signalling in principal cells. Reproduced from [8] with permission from Elsevier.

PDE10 and PDE11 [3]. PDE5, PDE6 and PDE9 are cG-PDEs (cGMP-specific PDEs), while the others are dual-specificity enzymes that hydrolyse both cAMP and cGMP. Until recently, the only characterized *Drosophila* PDE was that encoded by dunce, a PDE4 enzyme. Given that cGMP signalling is an important modulator of renal function in *Drosophila* and that PDE activity was known to modulate tubule function [11,12], novel PDEs encoded by the *Drosophila* genome were investigated. There are five novel PDEs encoded by the *Drosophila* genome, which are widely expressed throughout the fly. These PDEs, *Dm*PDE1 (*D. melanogaster* PDE1), *Dm*PDE6, *Dm*PDE8, *Dm*PDE9 and *Dm*PDE11, share close sequence identity and functional characteristics with their closest human homologues [13]. There are no homologues of PDE2, PDE3, PDE5, PDE7 and PDE10 encoded by the *Drosophila* genome. Biochemical characterization of some of the novel PDEs using an antibody-based approach has shown that *Dm*PDE1 and *Dm*PDE11 are dual-specificity enzymes, whereas *Dm*PDE6 is a cG-PDE, with a $K_m$ for cGMP in the micromolar range [13]. *Dm*PDE1, *Dm*PDE6 and *Dm*PDE11 are also sensitive to specific inhibitors of vertebrate cG-PDEs, Zaprinast and sildenafil [13].

The *Drosophila* tubule expresses all PDEs, including dunce. This means that an apparently simple transporting epithelium requires complex regulation of cyclic nucleotide content. *In vivo* studies using transgenics have helped in our understanding of the organotypic roles of PDEs.

Work using bovine PDE5A showed that the function and inhibitor-sensitivity of a vertebrate cG-PDE was conserved in *Drosophila* [12]. Furthermore, PDE5A significantly modulates tubule fluid transport when ectopically expressed in tubule principal cells. In contrast, specific targeting of *Dm*PDE6 to tubule principal cells does not result in modulation of fluid transport rates, in spite of substantially increased cG-PDE activity [14]. This may suggest then that
modulation of fluid transport rates may occur via PDE1, PDE9 or PDE11 (or a combination of these) in vivo.

Interestingly, targeted overexpression of DmPDE6 in principal cells inhibits active transport of cGMP, measured by cellular efflux. However, ectopic expression of bovine PDE5A in tubules [12] does not lead to this decrease in cGMP transport, suggesting that, in tubules, DmPDE6 is associated with a pool of cGMP distinct from that recognized by PDE5. Direct testing of the 'cGMP pool' hypotheses using new in vivo indicators for cGMP in intact tubules remains an intriguing possibility.

Confirmation of the direct role of DmPDE6 in active transport of cGMP was shown by genetic and pharmacological ablation of DmPDE6 in principal cells, using in vivo RNAi (RNA interference) and vertebrate inhibitors respectively. This allows reversal of the stimulated cGMP transport phenotype seen in DmPDE6 overexpressors, and also suggests that other endogenous PDEs do not contribute to this phenotype. It is likely that DmPDE6 activity results in modulation of cGMP content in the vicinity of a cGMP transporter. The tubule is highly enriched in several classes of broad-specificity transporters including OATs (organic anion transporters), ABC (ATP-binding cassette) multidrug resistance transporters [MRPs (multidrug resistance proteins)] and a multidrug efflux transporter [15]. Bioinformatic survey of the Drosophila MRP family, coupled with results from a tubule microarray screen, suggest that a potential candidate for a tubule cGMP transporter may be an MRP encoded by the gene CG9270 [14]. These studies using transgenic animals have ascribed a novel role to a cG-PDE in active transport of cGMP. In addition to the role of PDEs in cyclic nucleotide hydrolysis, it is clear that DmPDE6 also regulates cGMP content via cellular efflux. Given the close functional and structural homology of vertebrate and Drosophila PDEs [13], it is probable that specific cyclic nucleotide PDEs may provide important control mechanisms for regulating cellular efflux of cGMP in vertebrates.

These studies in the renal tubule of Drosophila have uncovered novel roles for cG-PDEs, as well as providing tantalizing evidence that cell-specific cGMP signals significantly modulate renal function. This may lead to further understanding of such processes in vertebrates.

Work in our laboratory is supported by the U.K. Biotechnology and Biological Sciences Research Council.

References

Received 13 March 2006