Entomology, Ornithology & Herpetology

Cohen, Entomol Ornithol Herpetol 2012, S1
DOI: 10.4172/2161-0983.S1-001

Review Article Open Access

Roles of Aquaporins in Osmoregulation, Desiccation and Cold Hardiness in Insects

Ephraim Cohen*

Department of Entomology, The Hebrew University of Jerusalem, Israel

Abstract

To maintain water homeostasis and osmoregulation, and to facilitate cryoprotection or overcome desiccation challenges, organisms across all taxa have utilized special and elaborate transmembrane channels that mediate the transport of water molecules (aquaporins, AQPs) as well as uncharged low molecular-weight solutes, such as glycerol (aquaglyceroporins). Nevertheless, some channels like Big Brain (BIB) in Drosophila play different roles that are involved in neural signal transduction, cell migration and cell to cell adhesion. Sanguinivorous insects and ticks, or insects feeding on plant sap are challenged with large volumes of ingested solutes that must be rapidly and effectively voided. AQP genes encoding functional transmembrane channel proteins were cloned from various ticks and mosquitoes species, as well as from xylem- (green leafhoppers) and phloem-sap feeders (whiteflies and aphids). AQPs are largely and abundantly expressed in organs associated with water balance such as the Malpighian tubules and the alimentary canal. In particular, abundance of AQP channels were detected in gut bypasses like the filter chamber in plant-sap feeders. AQPs and mobilization of cryoprotectants or dehydration protectants like glycerol and other polyols were studied in insects that overcome extreme environmental conditions such as sub-zero temperatures or severe desiccation. Hormone-mediated transcriptional regulation of AQP genes was demonstrated in insect and vertebrate systems. Post-translational control, related to trafficking of AQP-containing intracellular membrane vesicles to appropriate location in cell membrane domains, their docking on and fusion with the plasma membrane compartment, recycling and degradation as well as gating their pore entrance, was largely associated with specific kinase-mediated phosphorylation steps. Most of the above research was conducted with mammalian systems, yet several solid lines of evidence show that the same mechanisms apply to insects. As AQPs are tightly linked to human pathophysiological conditions, they have become promising targets for developing pharmaceutical drugs. Likewise, since AQPs are important for the survival of blood and plant-sap feeding arthropods, they have been suggested as attractive target sites to be tapped for developing effective pest control agents.

Keywords: Aquaporins; Aquaglyceroporins; Cryoprotection; Osmoregulation; Water homeostasis

Abbreviations: AQP: Aquaporin; ar/R: Aromatic/Arginine; BIB: Big Brain; MIP: Major Intrinsic Protein; DRIP: *Drosophila* Intrinsic Protein; NPA: Aspargine-Proline-Alanine; PIP: Plasma membrane Intrinsic Protein; PKA: Protein Kinase A; PKC: Protein Kinase C; TEA: Tetraethylammonium; TM: Transmembrane

Introduction

Plasma membranes serve as selective barriers regulating passage of water, solutes, ions and larger molecules via a variety of ingenious devices like pumps, carriers, transporters, exchangers and ion and water channels. In particular free and rapid water permeation among cells and their internal and external compartments is of paramount importance in maintaining cell volume and in providing the liquid ambience for imperative metabolic and catabolic cellular activities as well as in protection against stressful environmental conditions such as desiccation and freezing. Such rapid passage, driven by osmotic gradients, is facilitated by selective transmembrane channels belonging to a family of major intrinsic proteins (MIPs) that allow efficient bidirectional passive flow of water molecules. Uncharged solutes, mainly glycerol, but also other low molecular weight polyols, urea, hydrogen peroxide, dissolved gases (CO_2, NO, NH_3) as well as metalloids such as arsenite and antimonite are transported by similar mechanisms [1-8]. The transmembrane channel-forming MIPs are ubiquitous and highly conserved throughout all taxa (vertebrates, invertebrates, plants, fungi and bacteria). Furthermore, a functional water channel was even encoded by a *Chlorovirus* infecting *Chlorella* cells [9]. In brief, the channels are characterized by a homotetrameric structure where each functional unit is composed of 6 transmembrane helices and two conserved aspargine-proline-alanine (NPA) triplet motifs. The aligned NPA motifs from both sides of the plasma membrane forms a constriction pore half way into the lipid bilayer allowing the bidirectional passage of water and small neutral molecules. In the case of water-specific channels, no protons or charged molecules will permeate through the pore due to a filtering region of charged amino acid near the pore region. The suggestion that proteins of this MIP family stemmed from gene duplication of an apparent prokaryotic ancestor was based on the identical two-fold motif repeat and on sequence similarity of the N- and C-terminal segments of the channel protein [10-12]. Members of this protein family are involved not only in transport of water and neutral small solutes, but are also implicated in a number of unrelated physiological processes and functions such as lipid metabolism, cell migration, cell adhesion, epidermal biology and neural signal transduction [13]. Essentially, MIPs are classified into three major groups – a) aquaporins (AQP) of the water-specific

***Corresponding author:** Ephraim Cohen, The Morris and Helen Mauerberger Chair in Agriculture Entomology, Department of Entomology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel, Tel: 972-8-9489718; Fax: 972-8-9466768; E-mail: ecohen@agri.huji.ac.il

Received December 20, 2011; **Accepted** January 26, 2012; **Published** March 21, 2012

Citation: Cohen E (2012) Roles of Aquaporins in Osmoregulation, Desiccation and Cold Hardiness in Insects. Entomol Ornithol Herpetol S1:001. doi:10.4172/2161-0983.S1-001

channels; b) aquaglyceroporins that facilitate mainly flux of glycerol and water, but also solutes such as polyols, urea and gases; and c) a rare subcellular AQP group associated with intracellular organelles. The latter AQP proteins have rather limited sequence homology to the previous channel proteins, and function largely as water channels [14-16].

A substantial number of AQPs, which have been characterized beyond their DNA sequences, provides vital information regarding their physiological functions, properties and the molecular mechanism of solute transport. Functional studies consist of sensitivity to mercuric ions, inhibitory or modified effects using RNA interference (knockdown) techniques, knockout (AQP-deficient) organisms and site-directed mutagenesis experiments yielding mutated proteins and mutant organisms. In addition, functional system assays like AQPs expressed in *Xenopus laevis* oocytes [17], in yeast secretory vesicles [18] and in protoplasts of transformed yeast cells [19], or purified AQPs reconstituted into liposomes have been used for measuring permeability of water and solutes and its inhibition [20,21].

Expanding knowledge and impressive progress related to multifaceted aspects concerning transmembrane MIP channels that include isolation and sequencing of genes, analysis of crystal structures, molecular mechanism of water and other solute movement, physiological functionality of various channels, regulation of AQP synthesis and activity, and important roles of non-conducting AQPs have been extensively and thoroughly reviewed focusing largely on vertebrate, plant and bacterial systems [22-26]. Thirteen human MIPs (AQP0-AQP12) were isolated and divided into water-selective AQPs and aquaglyceroporins that play a role in epidermal hydration, cell volume or fat accumulation in adipose tissues [27]. A large number of plant AQPs were isolated and studied [28-30], and their pivotal physiological role in maintaining sensitive water homeostasis has been well documented [31,32]. AQPs were shown to be involved in hydration and buoyancy of fish eggs [33] and in adaptation of ducks to hyperosmotic salt solutions [34].

AQPs in diverse tissues are involved in multiple non-conducting functions such as cell migration, cell adhesion [13,35-37] and neural signal transduction [38,39]. AQPs were also implicated in a number of pathophysiological [22], neurophysiological disorders [37] and cancer [40]. Molecular insights of solutes' permeation as well as mechanisms of AQP trafficking, targeting and gating have been realized based on several high resolution crystal structures of AQPs from diverse species including mammalian (7), bacterial (2), archaeal (1), yeast (1), plant (1) and a microbial parasite (1) [41].

Phylogenetic analysis revealed 72 MIPs from 17 insect species, yet only a very few have been functionally characterized [2]. Seven AQPs were identified in *Drosophila melanogaster* genome [43], and 8 functional AQPs were isolated in the nematode *Caenorhabditis elegans*, none of which however is essential for regulating or maintaining osmotic homeostasis [44].

Insects (and other arthropods) are characterized by their phenomenal adaptations to survive extreme environmental conditions (freezing temperatures and desiccation) and to cope with excessively liquid source of food (blood, phloem- and xylem-sap) [45,46]. It is not surprising though that water-specific aquaporins play fundamental roles in such adaptations. Those transmembrane channels, which are largely crucial for water homeostasis and cryoprotection, were detected in organs and tissues such as the digestive track and its associated Malpighian tubules, but also in salivary glands and silk glands. Since

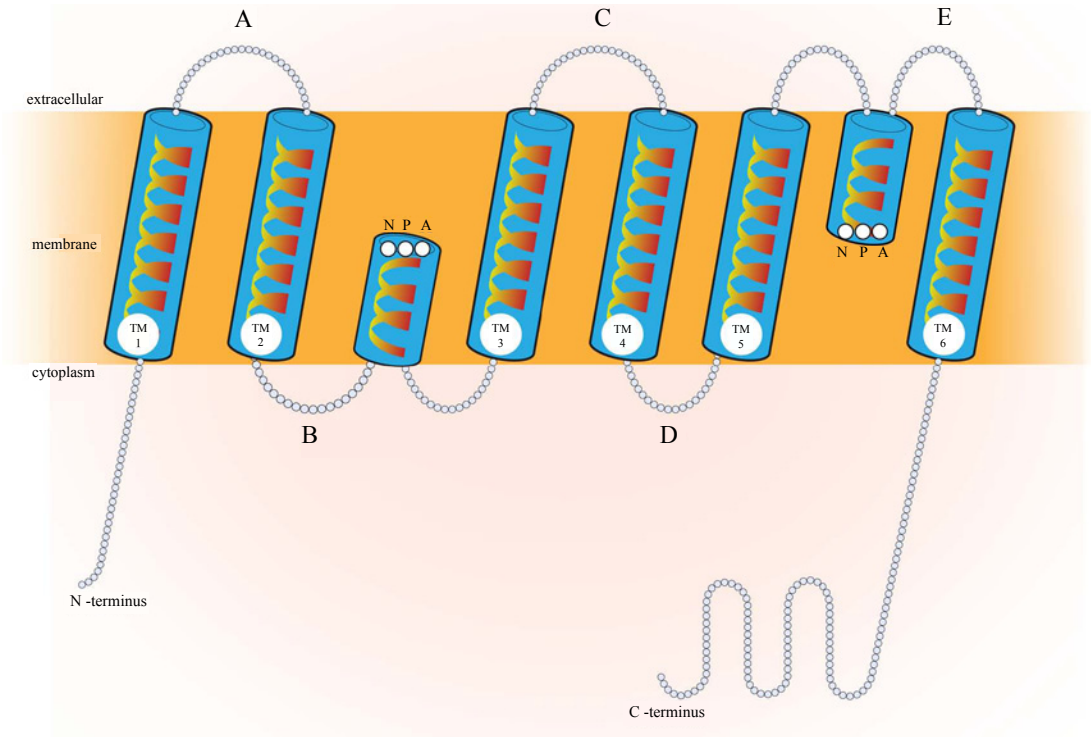

Figure 1: A schematic membrane topology model of a typical aquaporin subunit. The model depicts the six transmembrane (TM) domains 1-6, the five intracellular and extracellular loops A-E, the conserved NPA motifs on loops B and E half way into the membrane lipid bilayer, and the cytoplasmic C- and N-termini of the channel protein.

at present no AQP crystal structure of insects or any other arthropod species is available, mechanisms involved in permeation of water and solutes, trafficking of AQPs or their apparent gating are largely based on and extrapolated from advanced studies conducted with selected AQPs of vertebrate, plant and bacterial origin.

The scope of the present review covers diverse aspects related to structure, function and regulation of aquaporins with a focus on insect systems. It begins by providing a brief, yet necessary, background on the architectural configuration of aquaporin and aquaglyceroporin as based on several available mammalian and bacterial high-resolution atomic structures. Hence, the basics of intricate mechanisms underlying transportation of water and solutes across cell membraners were elucidated. The next section portrays and characterizes in great detail the functional AQPs in a number of insect (and a few arthropod) species with emphasis on haematophagous and plant-sap feeding organisms. The role played by AQPs in insects surviving environmental sub-zero temperatures and desiccation conditions is the subject of the following chapter. The next part overviews transcriptional and post translational (related mainly to trafficking and gating) regulation of AQPs in response to environmental conditions and/or physiological requirements. Finally, AQPs as target of chemical inhibition is described and the prospects of developing pest control agents and pharmaceuticals are discussed.

Architecture of Aquaporins

Water-selective channels

The specific structural architecture and characteristics of water-selective channel proteins are based on analyses of high resolution atomic structures of AQP crystals [35,47-52]. Studies with human AQP1 revealed that a filtering mechanism is involved in the bi-directional flux of water molecules alongside with the concomitant exclusion of charged molecules and protons [53]. The impressive magnitude of water permeation via mammalian AQP1 was calculated to be ~3.10^9 molecules per second per individual channel [54]. The capacity of water permeation is, however, determined by the type and density of a given AQP in cell membranes. AQPs share the same homotetrameric structure that is stabilized by interacting helices of adjacent monomers inside and outside plasma membranes [23]. Each monomer (~28kDa) consists of 6 tilted α-helical transmembrane segments (TM1-6) connected by 5 loops (A-E); loops B and D and the N- and C-termini are cytoplasmic (Figure 1). The hydrophobic loops B (cytoplasmic) and E (extracellular) fold from opposite directions and form a pore located half way deep into the lipid bilayer. The inverted symmetry of the conserved aspargine-proline-alanine (NPA) amino acid residue motifs on loops B and E creates a channel (~20Å long) and an aqueous pore (~2.8Å in diameter), the size of a water molecule. A molecular-dynamic simulation study of AQP1 confirmed that size exclusion is primarily responsible for water selectivity [55]. An "hourglass" model configuration (Figure 2) was evoked as the narrow channel is flanked by conically-shaped external and cytoplasmic vestibules [56]. Water molecules are reoriented and enter the pore as a single file by transient disruption of the hydrogen bonds between their oxygen atoms and adjacent hydrogens. Such disruption occurs as oxygen atoms of water molecules form hydrogen bonds with the amino groups of aspargine residues of the NPA motif that are extended into the pore. The reorientation of water molecules enables their passage through the pore with minimal energy barrier. This layout of water molecules permeating the pore precludes passage of protons as hydronium ions ($H3O^+$). Hydrogen bonds among water molecules are formed again as they pass through both sides of the pore entrance. In addition to the latter filtering system, another barrier/filter located at the extracellular site of the channel is the highly conserved aromatic and arginine amino acid residues (ar/R) constriction region around the NPA motifs. Their positive charges repel protons, charged molecules and ions (like Na^+, K^+) from traversing the pore [57-59]. Arginine and histidine amino acid residues at the respective extracellular and cytoplasmic sides of the AQP1 pore act to fend off protons and serve as a barrier to permeation of charged molecules. The major role of the ar/R region as an electrostatic proton barrier was demonstrated by functional analysis of three point mutations of rat AQP1 [60]. Enlarging the ar/R constriction diameter by certain amino acid replacement enabled glycerol, urea, ammonia and proton permeation [60]. Most AQPs are sensitive to mercurial compounds due to an accessible cysteine residue located near the NPA motif [61,62]. Using freeze-fracture electron microscopic method facilitated the identification of orthogonal arrangements of some AQPs in tissues of certain vertebrate and insect species. AQP4 orthogonal arrays were validated in native tissues as well as in transfected Chinese hamster ovary cells [63-68] and the absence of such configuration was confirmed in AQP4 knockout mice [64,69]. Such unique configurational pattern was also observed in the hemipteran *Cicadella viridis* AQP (AQPcic) [70,71], in the hagfish AQP4 [72], in calf lens fiber AQP0 [73] and in water channels detected in neuromuscular, epithelial and glandular rat tissues [74].

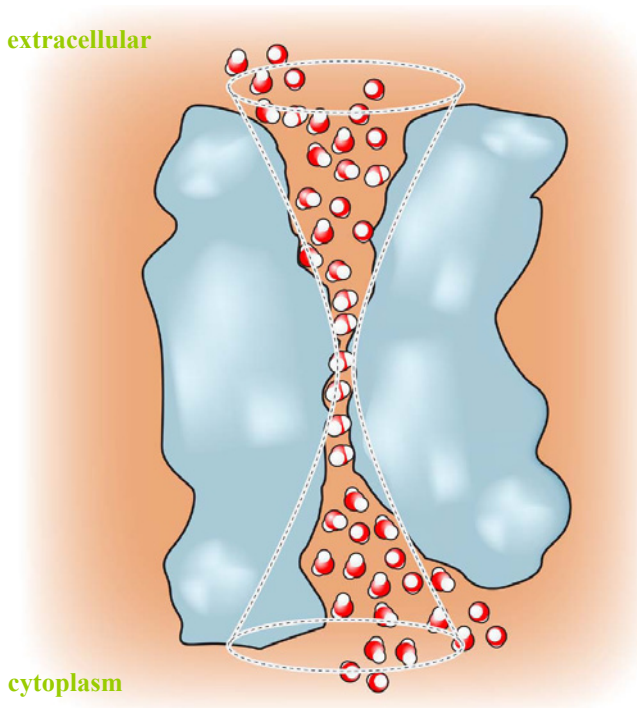

Figure 2: "Hourglass" model of AQP (side view). Circles represent water molecules inside the narrow channel and in the extracellular and cytoplasmic vestibules.

It was suggested that the tetrameric organization of AQPs is involved in the conductance of ions and gases. Certain AQPs like AQP1 [75,76], AQP6 [77], AQP0 [73,78] and *Drosophila* BIB [79] apparently conduct ions through the central pore of the tetramers [76]. These aspects of AQP-mediated ion permeation are beyond the scope of the present review.

Aquaglyceroporins

Aquaglyceroporins are transmembrane channels that conduct small neutral solutes like glycerol in addition to polar water molecules. For example, a number of vertebrate AQPs (AQP0, AQP1, AQP2, AQP4, AQP5; AQP6, AQP8) are water- specific channels [80,81]. Channels AQP3, AQP7 and AQP9 transport in addition to water also non-ionic low molecular-weight solutes such as glycerol and urea [16,27,82,83]. Anion permeation, specifically of nitrate by AQP6, was reported by Ikeda et al. [84], while the less selective hepatic AQP9 permeates *inter alia* polyols, urea, purines, pyrimidines and carbamides [16,85,86]. Similarly, the malaria parasites *Plasmodium berghei* AQP (PbAQP) [87], *P. falciparum* AQP (pfAQP) [88,89], and the archaeal AQP (AqpM) [90] are able to conduct both water and glycerol. The yeast aquaglyceroporin (Fps1) is implicated in the regulation of intracellular glycerol concentration in response to changes in external osmolarity [91,92]. Although not functionally identified, a possible role of aquaglyceroporins as chemosensors by mediating neural signal transduction for water detection in taste chemosensillae of a blowfly species (*Protophormia terraenovae*), has been speculated [93].

An *Escherichia coli* gene, *GlpF*, encodes for an inner membrane protein channel that facilitates glycerol permeation was demonstrated by either the *Xenopus* functional test system [94] or by reconstituted proteoliposomes [95]. Inhibition of glycerol conductance by mercuric ions was alleviated by thiol-reactive compounds such as dithiothreitol [95] and β-mercaptoethanol [94]). Crystal structure of *E. coli* glycerol facilitator *GlpF* channel protein at 2.2Å resolution shed light of the aquaglyceroporin specific architecture [96,97]. In comparison to water-selective AQPs, the relatively more hydrophobic and larger pore size facilitate the accommodation and permeation of larger neutral molecules like glycerol. The "hourglass" model structure consists of an external vestibule (~15Å in width) narrowing to about 3.8Å constricted pore size. This constriction leads into a long glycerol-selective channel (~28Å long) that widens to form the cytoplasmic vestibule. A switch of channel permeability from water to glycerol was shown in a double mutant of an insect AQP (AQPcic) where tyrosine and tryptophan were substituted in the 6th transmembrane helix by proline and leucine, respectively [98]. According to *E. coli GlpF* crystal structure, histidine residue in water-selective AQPs is replaced by a smaller glycine residue whereby the constriction region is enlarged. Isoleucine replaces histidine in an intermediate size archaeal AqpM channel which is permeable to both water and glycerol molecules [90]. Superposition of X-ray crystallographic structures of *GlpF* and its *E. coli* AqpZ water-selective counterpart, and functional analysis of site-directed channel mutants divulged the difference in selectivity [99]. The selectivity is determined mainly by the larger pore size of *GlpF* channel, although perhaps other structural elements might be indirectly involved.

Insect MIPs

Insects are characterized by and endowed with remarkable physiological adaptations to survive in extreme environmental conditions like freezing temperatures and desiccation or subsist on excessively liquid source of food such as blood and phloem and xylem saps. Part of the above adaptation is facilitated by transmembrane protein channels detected in organs and tissues with physiological functions related to bidirectional movements of water, glycerol and other small neutral solutes. The digestive tract and associated Malpighian tubules (Figure 3A) as well as salivary and silk glands play important roles largely in water homeostasis and cryptobiosis. Phylogenic trees constructed from public data by Campbell et al. [45] consist of 39 full-length AQPs, while Kambara et al. [42] reported 72 insect MIP sequences forming 4 major clusters that include diverse dipteran, lepidopteran, coleopteran, hymenopteran, hemipteran and orthopteran species. However, only a very small fraction of insect AQPs were functionally characterized.

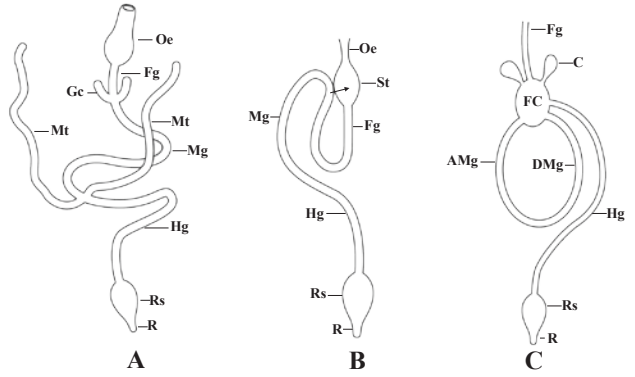

Figure 3: Schemes of insect digestive tracts: A – typical insect; B – aphid; C – whitefly. AMg – ascending midgut, C – coecum; DMg – descending midgut; Fc – filter chamber, Fg – foregut; Gc- gastric coecum; Hg – hindgut; Mg – midgut; Mt – Malpighian tubule; Oe – oesophagus; R – rectum; Rc – rectal sac; St - stomach. Anatomical gut structures associated with water-shunting in aphids and whiteflies are depicted in B and C, respectively.

The Aquaporin Family in Dipteran Species

BIB

Big Brain channel protein (BIB) is a MIP with sequence similarity to AQP family. However, it lacks the characteristic functional propery of AQPs related to permeation of molecules across cell membranes. BIB was detected in the neurogenic region of *Drosophila* embryos [100,101] as well as in other embryonic tissues requiring neurogenic gene activity [102]. The almost identical *D. melanogaster* and *D. virilis* BIBs are characterized by uniquely large intracellular C- and N-termini that most likely are implicated in the channel function [101]. The neurogenic BIB in *D. melanogaster* was suggested to be involved in embryonic neurogenesis by mediating intercellular interactions [100], and functions in intimate coordination with other neurogenic genes like *Notch* and *Delta* [102]. The diverse functions of BIB are illustrated by its apparent roles in endosomal maturation, trafficking and acidification as related to *Notch* [103]. It is noteworthy that mammalian AQP4, known for its cell adhesion properties [36], has high sequence similarity to *Drosophila* BIB protein [104]. Interestingly, BIB expressed in *Xenopus* oocytes displayed non-selective passage of Na^+ and K^+ cations [79]. It was, thus, hypothesized that a transmembrane voltage signaling mediated by cation passage through BIB channel is *inter alia* vital in *Drosophila* neurogenesis. Later however, using the L cells aggregation assay it was convincingly demonstrated that cell-cell adhesion rather than ion conductance is mediated by BIB, indicating that this function is most likely essential for *Drosophila* neurogenesis and normal brain development [104]. BIB is, however, also expressed in non-neurogenic tissues in *Drosophila* 3rd instar imaginal discs and in the hindgut of adults [102,105]. According to mere sequence similarity *Drosophila bib* homologues are also present in other insect species such as the mosquito *Aedes aegypti*, the flour beetle *Tribolium castaneum*, the honey bee *Apis mellifera* and the parasitoid wasp *Nasonia vitripennis* [45].

Water-selective aquaporins

Malpighian tubules, which play a significant role in fluid secretion and in water homeostasis in arthropods, are composed of two functionally different cell types [106]. The principal brush border cells engaged in cation passage while the scattered, much smaller in size and number, stellate cells, operate in water and anion (Cl^-) movement [107]. The Malpighian tubules in *Drosophila* as model insect were obvious candidates to explore channel proteins involved in water transport and water balance. Unlike BIB, a highly water-selective AQP named DRIP (for *Drosophila* integral protein) was detected in stellate cells of embryonic and adult Malpighian tubules [43,108]. DRIP, which is homologous to other water-specific vertebrate AQPs (44% amino acid sequence identity to human AQP4), is also expressed in other organs related to water transport like the pharynx, gut and posterior spiracles [43]. Functional analysis by DRIP mRNA expression in *Xenopus* oocytes and in yeast secretory vesicle membranes exhibited a high rate of water permeability [43]. Experiments using temperature-sensitive *Drosophila* mutants indicated that 20-hydroxyecdysone regulates the expression of DRIP in stellate cells via possible interaction with ecdysone receptor isoform, EcR-B2 [109]. A DRIP-like gene (*AqpBF1*) displaying high sequence homology was cloned and sequenced from the haematophagous adult buffalo fly, *Haematobia irritans exigua* [110].

The mechanism, by which larvae of the sleeping chironomid *Polypedilum vanderplanki* regulate water flux to induce anhydrobiosis, is apparently dependent on transmembrane water channels [111]. Two cDNAs encoding water-selective and mercury sensitive AQPs (*PvAQP1 and PvAQP2*) were isolated from *P. vanderplanki* larvae, and the expressed AQPs in *Xenopus* oocytes functional system facilitated permeation of water but not glycerol. Interaction of mercury ions with cysteine residues located near the NPA motif of the chironomid AQPs, most likely leads to the occlusion of the pore entrance. It was suggested that one AQP (PvAQP2), which is restricted to the fat body, controls water homeostasis in this tissue throughout normal conditions. Its AQP counterpart (*PvAQP1*), which is expressed in various tissues like epidermis, fat body, midgut and muscle, is involved in water removal during the onset of the dehydration-induced periods.

A single water-selective AQP (BaAQP1) with high sequence similarity to *PvAQP1* was cloned from a related chironomid species, the Antarctic midge, *Belgica antarctica* [112]. The mercury-sensitive BaAQP1 is mainly expressed in salivary glands, foregut, midgut and Malpighian tubule of larvae, but is weakly expressed in the fat body. Unlike *PvAQP1*, which is dehydration-inducible [111], the expression levels of BaAQP1 were not affected in response to dehydration or rehydration, and it was suggested to be constitutively expressed [112]. By using specific antibodies, additional AQPs such as *Drosophila* DRIP-like aquaporin and mammalian-like channel proteins such as AQP2 and AQP3 are present and immuno-localized in nearly all tissues of *B. antartica* larvae with the notable exception of Malpighian tubules [113]. The role of such proteins in bestowing cold hardiness and preventing desiccation is indicated by the increased levels of AQP2-like protein in larvae in response to dehydration, rehydration and freezing, and by enhanced levels of DRIP-like, AQP2-like and AQP3-like proteins in adults during dehydration.

The AQP detected in larvae of the goldenrod gall fly, *Eurosta solidaginis* (EsAQP1) is phylogenetically close to *PvAQP1* (52.3% sequence identity), and the position of amino acids forming the ar/R selective filter of both AQPs is almost identical [114]. EsAQP1 is highly abundant in the nervous system of overwintering larvae in comparison with tissues like gut, Malpighian tubules and salivary glands, and it is apparently absent in the fat body. Functional characterization using the *Xenopus* oocytes assay revealed a mercurial sensitive, highly water-selective AQP that is impermeable to glycerol and urea. Since seasonal adaptation to subzero temperatures and acquisition of cold-hardiness by the gall fly larvae was associated with accumulation of glycerol as the cryoprotectant, the existence of aquaglyceroporin channel(s) was speculated [114].

In contrast to *Drosophila*, a water-selective AQP in Malpighian tubules of the yellow fever mosquito *Aedes aegypti* females (AeaAQP) was solely associated with the tracheolar system therein [115]. It was noted that the mosquito AQP functioning in the respiratory system, and the rapid flow of water are firmly related to the physiology of the Malpighian tubules-associated tracheolar system in times of great need for oxygen supply during diuresis following blood meal, and after adult emergence. It was even speculated that AeaAQP may serve as an osmosensor for this tissue [115]. Additional studies evidently immunolocalized this AQP channel within the tracheoles; the *Xenopus* swelling assay established the water-specific property of this AQP, and inhibition of water permeability by mercury ions apparently involves the Cys^{79} near the NPA conserved motif [116]. In addition, freeze-fracture electron microscopy images of the *Aedes* AQP expressed in *Xenopus* oocytes revealed the tendency of this channel protein to form orthogonal arrays [116]. A recent bioinformatics study by Drake et al. [117] identified 6 putative AQPs in *A. aegypti* genome that are similar to vertebrate water-selective channels. Based on RNAi-mediated gene silencing experiments, three of those AQPs (AaAQP1, AQP4 and AQP5), which were intensely expressed in female Malpighian tubules, are the main channels that possibly play a functional role in diuresis following blood meals.

A water-selective channel protein (*AgAQP1*), sensitive to $HgCl_2$ and with high expression in several tissues including Malpighian tubules, alimentary canal and ovaries has been recently detected and characterized in the malaria vector mosquito, *Anopheles gambiae* [118]. Sequence alignment showed a 35-38% similarity with DRIP and AeaAQP dipteran orthologs. However, unlike *A. aegypti* tracheolar AQP, the *AgAQP1* protein was identified in the Malpighian tubules *per se*, and immunofluorescence studies localized the channel protein only in stellate cells known to be involved in water permeation. *AgAQP1* in gut and Malpighian tubules is most likely essential to maintain water homeostasis in immature stages dwelling in aquatic systems as well as in females following blood meal ingestion. It was revealed that the channel protein expression is higher in adults in comparison to larval and pupal stages, and is higher in females than in males. Expression of the mosquito channel protein, which is vital for water balance, is apparently also essential for reproduction. It addition to in the gut and the Malpighian tubules, it is up-regulated in the ovaries following blood meals. *AgAQP1* expression in female mosquitoes is elevated at high relative humidity, and RNA interference studies demonstrated that compared to controls, insects survived longer periods of severe desiccating conditions when expression of the channel protein was reduced [118]. This reduction is in sharp contrast to the expression level of certain AQPs in the Antarctic midge adults that are elevated under dehydration conditions [113].

Aquaporins in haematophagous (non-dipteran) arthropods

Water homeostasis in blood-feeding arthropods is tightly related to dual physiological requirements. On one hand, the large water volume in the ingested blood meal must be voided, while on the other hand, water conservation is critical during irregular off-host non-feeding periods. Sanguinivorous arthropods like ticks, for example,

endure enormous osmoregulatory stress following blood ingestion that increases their initial body weight by up to 100 fold [119]. Salivary glands play a major dual role in water management of ticks as they help to absorb atmospheric water vapor via secretion of hygroscopic droplets [120,121] at periods between meals, while returning ingested blood-meal water (~75%) back to the host via the saliva [122]. A water-selective AQP gene (*RsAQP1*), which was cloned and sequenced from the salivary gland cDNA library of the dog tick, *Rhipicephalus sanguineus*, was abundantly expressed in these glands but was also detected in the gut and Malpighian tubules [123]. The water-selectivity of the tick channel was demonstrated by the routine *Xenopus* oocytes system, and the inhibition of water flux by mercury ions was attributed to a cysteine residue located near the NPA motif. In the sheep tick *Ixodes ricinus*, the water-specific AQP (*IrAQP1*) was detected by *in situ* hybridization in tissues solely involved in massive water transport like the gut, rectal sacs and particularly in salivary glands [119]. The blocked dopamine-stimulated secretion of *I. ricinus* ligated salivary duct by mercury ions was reversed upon exposure to β-mercaptoethanol [121], and silencing of *IrAQP1* in knockdown experiments using isolated salivary glands demonstrated that the dopamine-stimulated secretion was blocked [119]. Body-weight gain was reduced and haemolymph osmolarity was increased in knockdown *I. ricinus* female ticks injected with dsRNA. The reduced volume of ingested blood meal was attributed to impaired water flux from gut to the salivary glands. The physiological role played by a putative aquaglyceroporin with sequence similarity to the mammalian AQP9 and which is primarily expressed in ovaries of the American dog tick, *Dermacentor variabilis*, is still elusive [124].

An AQP (*Rp-MIT*) cDNA from Malpighian tubules, which is intensely engaged in diuresis in the triatomid bug *Rhodnius prolixus*, was cloned and sequenced and its transcellular water permeability was indicated by the *Xenopus* oocytes expression assay [125,126]. *Rp-MIP* transcripts were detected in the proximal and distal regions of the Malpighian tubules and the protein channel expressed in the *Xenopus* oocyets system were partially inhibited by mercurial agents. The amino acid sequence of Rp-MIP contains sites for potential phosphorylation and N-glycosylation that might play regulatory roles in fluid excretion. *RP-MIT* transcripts are up-regulated in Malpighian tubules after blood ingestion or after exposure of isolated Malpighian tubules to the diuretic 5-hydroxytryptamine or to c-AMP [126].

Aquaporins in plant sap-feeding insects

Hemipteran insects, divided into xylem and phloem feeders, are challenged with large volumes of ingested plant sap that must be voided in addition to osmotic pressures that have to be managed and alleviated. The alimentary canals of those insects (apart from aphids) have a characteristic water-conducting complex called the filter chamber (Fig 3C) that bypass part of the digestive tract and functions to shunt rapidly and directly large volumes of fluids between the foregut and midgut [127,128]. A freeze-fracture electron-microscopic study of the green leafhopper, *C. viridis* and the common froghopper *Philaenus spumarius* filter-chambers revealed cell infoldings and detected intramembrane particles extending into the cytoplasm [127]. Later studies, focusing on the filter chamber of *C. viridis*, disclosed the first water-specific AQP (AQPcic) in this xylem-sap feeding insect [129]. AQP-like proteins were also detected in filter chambers of a number of other hemipteran species – the froghopper *Cecropis sanguinolenta*, the European alder spittlebug *Aphrophora alni*, the leafhopper *Euscelidius variegatus* and the American grapevine leafhopper *Scaphoideus titanus* [130]. A 765bp cDNA encoding a 26kDa protein was cloned from *C. viridis* filter-chamber cDNA library. The AQPcic protein expressed in *Xenopus* oocytes following injection of cRNA isolated from the filter chamber epithelium instigated a 15-fold increase in membrane water permeability, and this increase in water flux, which is similar to that of human AQP1, was inhibited by $HgCl_2$, albeit to a lesser extent [131]. Inhibition of water flow by mercury was also demonstrated using AQPcic-integrated yeast reconstituted proteoliposomes, and by using AQPcic mutants, it was established that Cys^{82} near the NPA box in the B loop is crucial for such inhibition as its substitution by a serine residue eliminated completely this mercurial sensitivity [132]. The apparent reduced accessibility of mercurial compounds to Cys^{82} located deep within the pore may explain the reduced sensitivity of AQPcic to mercurials as compared to AQP1 in which the thiol group is positioned in the peripheral loop E region [132]. The water-specific AQPcic (impermeable to glycerol, urea and ions), which displays 40% and 43% sequence identity with vertebrate AQP1, AQP2, AQP4 and AQP 5, was detected in the filter-chamber but not in the midgut [131]. Freeze-fracture electron microscopy of *C. viridis* AQPcic expressed in *Xenopus oocytes* revealed the tetrameric organization of the channel protein and a structural pattern of packaged particles in orthogonal arrays [71] similar to the configuration observed in native *C. viridis* filter-chamber membranes [70,127]. It is noteworthy that a comparable AQP orthogonal conformation was reported for mammalian AQP4 [66-68,73].

Aphids feeding on phloem sap face a dual challenge. The insects have to deal with stressfully large quantities of ingested fluid similar to the xylem-sap feeder *C. viridis*, while in addition, they must overcome the high osmotic pressure of the ingested sap that contains high levels of sucrose. Osmoregulation is partly maintained due to the transglycosylation process by which the disaccharide is enzymatically cleaved along with subsequent oligomerization of glucose that consequently reduces the osmotic pressure in the gut lumen [133,134]. Anatomical features of aphids' gut (Fig 3B) may explain water cycling to dilute the high sucrose content of the ingested phloem sap. There is a close proximity between distal and proximal regions of the midgut [135] that functionally resembles the *C. viridis* filter-chamber described before. A highly water-specific aquaporin (*ApAQP1*) was indentified in the pea aphid, *Acyrthosiphon pisum* and its transcripts were immumolocalized in the stomach and the adjacent loop of the distal intestine as well as in the gut and salivary glands of mature embryos [136]. This specific presence is suggestive for the function of ApAQP in upholding water shunting. *In silico* structural analysis by superposition of the pea aphid AQP model onto bovine AQP1 indicated a similarity in amino acid residues at the ar/R domain that determine water-selectivity. Expression of the pea aphid gene in the *Xenopus oocytes* system demonstrated a substantial increase (by 18-fold) of water translocation across membranes that is inhibited by mercury ions, a significant reduction in activation energy of water flux through the plasma membrane, and no glycerol permeation. A corroborating functional analysis study of knocking down the *ApAQP1* gene expression by oral application of dsRNA, demonstrated a significant rise in the osmotic pressure of the haemolymph [136].

Recently, a water-specific and mercury-sensitive aquaporin channel protein was identified and characterized in the sweetpotato whitefly, *Bemisia tabaci* [137]. Phylogenetic analysis showed that BtAQP1 has sequence identity of 54% with the corresponding hemipterans *C. viridis*; and; 41%-43% with two spliced variants of *A. pisum*; 53% with the termite *C. formosanus* and 40% with human AQP-4. Presumably associated with feeding behavior, BtAQP1 was highly expressed in 2^{nd} instar nymphs, but was less abundant in 4^{th} instar juveniles and in pupae that are characterized by periods of discontinued feeding. BtAQP1 was

primarily located in the filter chamber and the anterior ileum part of the hindgut of *Bemisia* adults (Fig 3C). Depending on the osmotic potential, the organ may have physiological functions related to voiding excess dietary water and alleviating osmotic stress due to high sucrose-containing phloem-sap ingesta.

Aquaporins in other insects

Unlike plant sap-feeder and haematophagous insects, in which extensive diuresis to void large quantities of fluid is mandatory, most terrestrial insects being exposed to arid ambiences must conserve water. Accordingly, the dry feces produced by lepidopteran larvae indicate that water in ingested food is strictly preserved. The paramount physiological importance of maintaining water homeostasis in *Bombyx mori* is mediated by AQPs that are present in water-transporting epithelia like the midgut, hindgut, Malpighian tubules and silk glands [138]. Two similar AQP cDNA sequences, which are differently expressed in *B. mori* larval tissues and share ~42% sequence identity, were cloned and characterized. Functional studies using the *Xenopus* oocyte diagnostic system revealed a water-specific AQP (AQP-Bom1) with widespread yet uneven tissue distribution. Immunohistochemical experiments detected abundant AQP-Bom1 in the apical surfaces of the colon and rectal epithelia in comparison to other tissues like midgut, Malpighian tubules and silk glands. Translocation of water from the haemolymph to the silk glands in conjunction with V-ATPase activity as the pH-regulating proton pump [139] are believed to stabilize the liquid silk in the glandular lumen. The minor second AQP isoform (AQP-Bom2), which is characterized as an aquaglyceroporin, is able to increase uptake of glycerol and urea in addition to water permeation [138]. AQP-Bom2 is mostly expressed in the Malpighian tubules and the posterior region of the midgut, and is absent in the colon and the anterior silk gland regions [138]. The physiological function of AQP-Bom2 is unclear and it was speculated to be involved in urea transportation. Interestingly, this AQP is abundantly expressed in the silk glands during the feeding phase and decreased in spinning larvae [140]. Two similar AQP types with functional properties and ~70% and ~40% sequence identity with AQP-Bom1 and AQP-Bom2, respectively, were detected in the oriental fruit moth, *Grapholita molesta* [141]. Data concerning additional AQPs from several insect species are presented in Table 1.

Cryptobiosis and Role of Aquaporins

Cold hardiness and cryoprotectants

Animals develop adaptation strategies like freeze tolerance (ability to survive extracellular ice formation) and freeze avoidance (adaptation of mechanisms that prevent freezing) to overcome damaging cold climate sub-zero temperatures [144-146]. Freeze tolerance strategy involves generation of various cryoprotectants such as antifreeze proteins and glycoproteins, heat shock proteins, low-molecular weight polyols like glycerol and sorbitol, saccharides like trehalose and sucrose, and free amino acids [145,147,148]. Accumulation of diverse polyols (sorbitol, ribitol, mannitol and arabinitol) in diapausing adults of the firebug *Pyrrhocoris apterus* coincides with a drop of temperature [149], and high level of trehalose as a cryoprotectant was detected during winter in dampwood termites [150]. Urea retention triggered elevated blood osmolarity in a turtle species [151], and its accumulation in terrestrially hibernating amphibians, functions as a cryoprotectant that bestows cold hardiness [152]. Glycerol as a common cryoprotectant derived from degradation of stored glycogen in insects [153] is non-toxic at high concentrations, and confers cold hardiness in insects adapted to survive in sub-zero temperature regions. Accumulation of high levels of glycerol is associated with cold resistance in diapausing larvae of the rice stem borer *Chilo suppressalis* [154], and similar glycerol buildup was reported for *Diplolepis* gall wasps overwintering on roses [155] and for the midge *Belgica antarctica* [156]. Artificially induced stress by applying sub-zero temperatures initiated glycerol accumulation in non-diapausing larvae of the flesh fly *Sarcophaga bullata* [157]. Overwintering diapausing larvae of the codling moth *Cydia pomonella* accumulate trehalose and such buildup was correlated with cold hardiness [158], and a similar correlation between cold tolerance and higher levels of trehalose was demonstrated in coleopteran species [159] and in an arctic springtail species [160]. Overwintering third instar larvae of the goldenrod gall fly *Eurosta solidaginis* generate sequentially high levels of glycerol, sorbitol and trehalose to acquire gradual freeze tolerance [161]. Dehydration and subzero conditions impose similar osmotic stress on insects, and similar molecules may serve as mechanism of protection. Trehalose levels in *B. antarctica* larvae were elevated during dehydration [162], and large amounts of this sugar were accumulated in larvae of the cryptobiotic sleeping chironomid *P. vanderplanki* under desiccating conditions [163,164] that induced a trehalose transporter gene [165].

Aquaglyceroporins in insects

As mentioned in the previous section, glycerol is the main cryoprotectant that facilitates physiological adaptation to cold hardiness in insects. Since subzero temperatures trigger cell dehydration, glycerol may be involved in desiccation tolerance as well. The gall fly *E. solidaginis* withstands extreme winter temperatures reaching levels of -80°C [144,166] and tolerates desiccation conditions inside the gall in the dry autumn [167]. Using mammalian antibodies to detect insect AQPs is an indirect approach that should be treated with caution. Nevertheless, aquaporin homologues of mammalian AQP2, AQP3

Insect	AQP name	Tissue distribution	Remarks*	Ref.
Silkmoth, *Bombyx mori*	AQP-Bom1	Hindgut, Malpighian tubules, mid-gut, silk glands	Aquaporin	[138]
	AQP-Bom2	Malpighian tubules, midgut	Aquaglyceroporin	[138]
Oriental fruit moth, *Graphiolita molesta*	AQP-Gra1	Hindgut	Aquaporin	[141]
	AQP-Gra2	Midgut	Aquaglyceroporin	
Formosan subterranean termite, *Coptotermes formosanus*	CfAQP	Digestive tract, Malpighian tubules, labial gland reservoir, epidermis, antennae	Aquaporin	[2]
Korean firefly *Pyrocoelia rufa*	NS	Most body tissues	Aquaporin	[142]
House cricket, *Acheta domesticus*	NS	Malpighian tubules (distal regions)	*Drosophila* DRIP-like aquaporin	[143]

*Aquaporin - water-specific channel; NS – not specified.

Table 1: AQP channels of certain insect species.

(an aquaglyceroporin) and AQP4 were detected in *E solidaginis* and survival of frozen tissues like fat body and midguts, but not salivary glands was reduced in the presence of mercury ions [168]. It was reported that glycerol is accumulated in the gall fly through autumn with concomitant increase in profusion of cellular aquaporin and aquaglyceroporin-like proteins [166]. This dual abundance, which was suggested to be regulated by seasonal changes, facilitates the acquiring of freeze hardiness via the countercurrent loss of water and influx of glycerol. Nevethrless, it is noteworthy and rather puzzling that so far no aquaglyceroporin channel has been identified in dipteran species [117]. It was reported that survival of *C. suppressalis* diapausing larvae exposed to freezing temperatures is managed by replacing water with glycerol in fat body cells [169]. The involvement of AQPs is construed from inhibition of the above solutes' flux by mercury ions. The role played by AQPs (AQP Bom2 and AQP Gra2) in glycerol transport has been demonstrated in the lepidopteran species *B. mori* [138] and *G. molesta* [139]. Although functional analysis using the *Xenopus oocytes* swelling assay demonstrated its involvement in permeation of water, glycerol and urea, the exact physiological role played by AQP-Bom2 and AQP-Gra2 is still unclear.

Regulation of Aquaporins

Functioning aquaporins are the end result of a dynamic sequential cascade of cellular events that start from generation of transcripts, their translation and post-translational modifications, followed by recruitment as vesicular cargo that is transported to appropriate plasma membrane domains, and finally ending in precise docking on and fusion with the cell membranes. *In situ* functional AQPs may be inhibited or modulated by gating the channels or possibly undergo dynamic recycling by internalization in endosomal compartments for later mobilization or breakdown. Regulation of the above processes plays a fundamental role in water homeostasis, tolerance to subzero temperatures and resistance to desiccation conditions.

Expression at the transcriptional level

Control of AQPs is divided into long-term transcriptional regulation and short-term regulation such as AQP trafficking and gating that is required to manage acute osmoregulation needs. Up-regulation of various AQP transcripts to address the requirement for increased solute permeation is well documented. For example, *AgAQP1* expression in mosquito females is up-regulated after ingesting a blood meal, and is down-regulated when insects are exposed to desiccating conditions [118]. Atmospheric humidity *per se* influenced *AgAQP1* transcription as mRNA levels were increased in insects exposed to relative humidity of 80% in comparison to a lesser level at 42%. The marked increase of transcript levels detected in ovaries for several days after blood meal indicates the vital role of water uptake during development of oocytes [118]. Up-regulation of *Rp-MIT* mRNA of the triatomid bug, which follows a blood meal, is probably triggered by cAMP-mediated 5-hydroxytryptamine [126]. The selective up-regulation of AQP2 and AQP3 expression levels in the cortex and medulla of the rat renal collecting duct is affected by water deprivation and directly by arginine-vasopressin treatment [170,171], and the transcriptional up-regulation of AQP2 in the mouse renal C4 collecting duct cultured cells is mediated by a cAMP–responsive element (CRE) in the promoter region of this gene [172]. AQP2, which is under-expressed in the inner medulla collecting renal duct of senescent rats, was upregulated by water deprivation [173]. Proliferation of cultured astrocytes was reduced by dopamine, which also downregulated the expression of AQP4 transcripts [174]. Several transcription factors, which play a role in AQP transcriptional regulation, were identified. Up-regulation of rat brain AQP4 and AQP9 [175] and rat spinal cord AQP1 and AQP4 [176] is induced by HIF-1α transcription factor following traumatic brain injury and spinal cord injury. Transcriptional regulation involving a *cis*-regulatory element was identified in the regulatory upstream region of *C. elegans* AQP gene (*aqp-8*) expressed in excretory cells [177]. Promoter analysis of upstream this gene identified a transcription factor (CEH-6) exclusively expressed in excretory cells of the nematode. A DNA replication-related element factor (DREF) and its binding site (DRE) play essential roles in the regulatory expression of *bib* gene, largely in proliferating cells of imaginal discs of *Drosophila* larvae and in hindgut epithelium of *Drosophila* adults [105]. As DRE sequence was found in the 5'-flanking regions of both *bib* gene and its human AQP1 homologue, a similar mechanism that regulates transcriptional expression of insect and mammalians AQPs via the DREF/DRE system was suggested [105].

Trafficking of aquaporins

Intracellular protein transport, its intricate signaling and elaborate mechanisms are of pivotal relevance in cell biology [178]. Studies related to packaging, trafficking, docking and recycling of AQP proteins, the involvement of cytoskeletal elements and calcium in AQP mobilization as well as hormonal regulation triggered by kinase(s)-mediated phosphorylation of accessible serine or threonine residues, have been intensively studied in mammalian AQPs. However, despite the impressive number of such studies, they are far from being coherent, and it is still premature and unclear whether the described signaling and intracellular triggered mechanisms, which are associated with AQP transport, may apply to other protein homologues. A brief account of some of the studies with mammalian systems is presented below.

A number of mammalian hormones play roles in regulating or modulating expression and trafficking of various AQPs in a variety of organs and cultured cell systems. Trafficking of mammalian AQP2 [179,180] and AQP2-like anuran [181] proteins to plasma membrane compartments is regulated by antidiuretic vasopressin or a vassopresin-like analog, respectively. AQP4 was internalized in response to vasopressin in glial cells [182], aldostereone modulates AQP2 expression in cultured renal collecting duct cells [183], while testosterone and estrogen up-regulate expression of AQP4 in cultured astrocytes [184] and AQP2 expression in mouse uterus [185], respectively. The hormones secretin and oxytocin are involved in regulating AQP2 expression and translocation in kidney cells [186], and glucagon regulates AQP8 trafficking in rat hepatocytes, [187]. Hormone PYY increased the AQP4 expression in mouse gastric pariental cells [188], the vasoactive intestinal polypeptide increased the amount of AQP5 protein in rat duodenum cells [189], and epinephrine is involved in trafficking of AQP3 [190].

The cascade of events triggered by vasopressin, involves a G protein-coupled receptor that activates adenylyl cyclase, and in turn, the produced cAMP activates protein kinase A (PKA) that phosphorylates the AQP2 proteins and triggers the mobilization of the water channel protein to apical domains in plasma membranes [191-209]. A different kinase, protein kinase C (PKC), is involved in trafficking of AQP1 [210,211] and AQP4 [212,213]. Vasopressin removal and PKC activation regulate the ubiquitination of AQP2 that initiates and mediates its endocytosis and ensuing degradation [214,215]. The C-terminus of AQP4 in cultured astrocytes' cells contains two signal motifs for plasma membrane targeting [216]. One of the signal sequences regulates trafficking to the basolateral membrane domains, while the other motif directs clathrin-dependent endocytosis of basolateral membrane-

containing AQP4, and targets the protein for lysosomal degradation following its phosphorylation by casein kinase II [217].

A number of reports indicate that cytoskeletal microtubules and calcium are involved and facilitate the movement and translocation of intracellular phosphorylated AQPs such as AQP1 [211,218-222], AQP2 [223], AQP4 [224], AQP5 [225-226] and AQP8 [187,227]. Complex of motor protein molecules which transport cellular cargo along cytoskeletal microtubules, were present in association with intracellular vesicles containing AQP2 and AQP1in rat kidney cells [208,228] and in rat cholangiocytes [229], respectively.

In comparison to the considerable number of investigations conducted with mammalian AQP systems, information regarding intracellular translocation of channel proteins in insects is rather scarce. By and large, evidence clearly indicates the central role of phosphorylation as a mechanism facilitating AQP mobility in insects. Fast diuresis is vitally required for insects ingesting high volumes of blood or plant-sap fluids. To excrete excess fluids after a blood meal

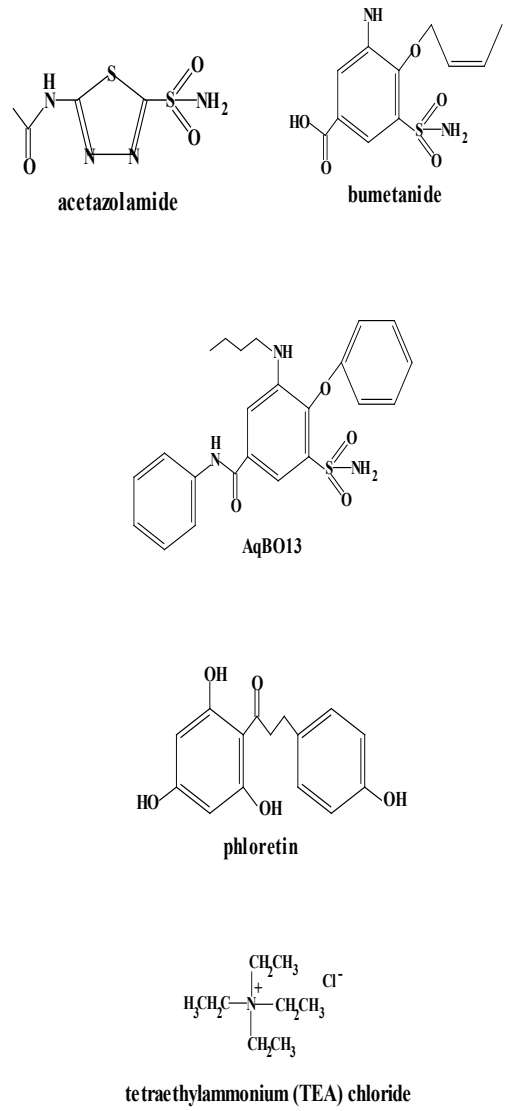

Figure 4: Chemical structures of several aquaporin inhibitors.

the up-regulated expression of AQP-like water channels in Malpighian tubules of the bug *R. prolixus* is increased [126]. Based on a study using isolated Malpighian tubules it was suggested that this increase is mediated by the cAMP-dependent pathway that is triggered via exposure to 5-hydroxytryptamine. This regulation is clearly reminiscent of the activity triggered by vasopressin in mammalian renal AQP2 [193]. Salivation in ticks is induced by the dopaminergic system that was implicated in activation of adenylyl cyclase by which cAMP levels were increased [121,230]. It was speculated that dopamine-dependent phosphorylation of the brown dog tick *RsAQP1* at several phosphorylation sites, which is mediated via PKC activity, is involved in this AQP translocation [123]. Since transcript levels of the chironomid *PvAQP1* increased 6 hours following onset of desiccation, it was speculated that regulation of AQP trafficking or gating might involve PKC-mediated phosphorylation of serine residues [111]. The plasma membrane targeting of *Bemisia* BtAQP1 was speculated to involve possible phosphorylation sites in the protein C-terminus [137].

Gating of aquaporins

Gating of AQP channel entrance, which was extensively studied in plants, is a major mechanism by which flow rates of solutes across plasma membranes are regulated. Studies with PIP plant AQP subfamily have shown that drought conditions, changes in cytoplasmic pH (due to flooding) and mechanical stress induce gating that is triggered via phosphorylation or calcium binding [231-233]. Crystal structure studies of the spinach AQP (SoPIP2;1) demonstrated that phosphorylation and dephosphorylation of two conserved serine residues in loop D are involved in the open and close states of the channel protein, respectively. Gating the plant SoPIP2;1 is attained by conformational reorientation and folding of a cytoplasmic loop D that interacts with the C terminus and caps the cytoplasmic entrance of the channel via pushing the conserved Leu^{197} residue in the loop to block water flow [234]. Calcium-mediated anchoring of loop D with the N-terminus, as observed by dynamic simulation studies, was also implicated in gating of plant AQPs [235]. The relatively large cytoplasmic N-terminus of the yeast *Pichia pastoris* is involved in gating its water-selective AQP (Aqy1) [236]. The crystal structure of Aqy1 at a strikingly high 1.15Å resolution, mutational studies and molecular dynamic simulations revealed rearrangement and folding of the N-terminus, whereby a Tyr^{31} residue forms hydrogen bonds with a water molecule inside the pore, and by which the cytoplasmic entrance of the channel is occluded [236]. In contrast to the above large-scale capping configuration, gating in the mammalian AQP0 and the bacterial AqpZ is apparently achieved by movement of a few amino acids at the ar/R constriction domain [237]. Recently, it has been established that Arg^{189} residue, attached the conserved NPA motif, plays a functional role in AqpZ gating [238].

Both N and C-termini of the yeast, *Sacharomyce cerevisiae*, aquaglyceroporin channel (Fps1p) were implicated in regulating glycerol transport [239,240]. Water flux was increased in AQP0 expressed in *Xenopus oocytes* system at lower pH value and low levels of $Ca2^{+}$ [241], and this regulation was attributed to external histidine residues in loops A and C and to a separated calcium binding protein at the C-terminus on the opposite entrance of the channel, respectively [242]. Regulation of water permeability by pH was suggested to be associated with altered single file orientation of water molecules entering the pore. However, the above postulated mechanism of pH-related gating at values higher than 6.5 was not supported by the crystallographic studies of AQP0 [35]. In another study it was shown that gating of glycerol and water transport via AQP3 is strongly and reversibly dependent on pH, and

that conformational changes are apparently not involved in such effect [243].

Aquaporins as Target Sites for Interference

As aquaporins are involved in a plethora of vital biological processes, largely water homeostasis, they have naturally become promising targets for discovery of pharmaceutical drugs and pest control agents [119,244-246]. Several screening systems such as *in silico* simulations [247,248], the common *in vitro* water conductance assays and a high throughput yeast-based phenotype method using transformed cells [249] have been available to examine potent AQP inhibitory compounds. Nevertheless, what is still lacking are potent (high affinity) and selective compounds acting as AQP blocker or modulator candidates with potential to serve as lead compounds.

Metals like mercury, silver, gold, copper and zinc are effective inhibitors of plant, yeast and human AQPs [19,62, 250-253]. They interact with cysteine sulfhydryl groups close to the NPA motif, block the constriction region and consequently inhibit water flux. Unlike mercurials, copper and zinc, inhibition by silver or gold is not reversed by sulfhydryl reactive compounds like β-mercaptoethanol suggesting a different mode of action. Since the activity of the above metals is physiologically non-specific and toxic, their practical use as AQP inhibitors is unlikely.

By using the oocytes swelling system it was demonstrated that water and solutes permeability via AQP9 was inhibited by the dihydroxychalcone, phloretin (Figure 4) [85,254], whereas AQP4 was practically unaffected [255]. The quaternary ammonium compound, tetraethylammonium (TEA) chloride (Figure 4), which is a known blocker of the voltage-activated potassium channel [256], also inhibits water transportation via mammalian AQP1, AQP2 and AQP4 in native channel as well as in channel proteins heterologously expressed in the *Xenopus oocytes* system[174, 257-260]. Molecular docking and molecular dynamic simulations confirmed the inhibitory effect of TEA vis-à-vis human AQP1 and stressed the complexity of the putative binding site that largely involves the A-loop [261]. AQP1 and AQP4-mediated inhibition apparently involves an interaction with tyrosine residues in the external loop E domain [257,259]. In contrast to the above AQPs, AQP3 and AQP5 which lack the corresponding tyrosine residue (Tyr^{186} in bovine AQP1), are insensitive to TEA [259]. A corresponding Tyr^{185} residue in the *Anopheles* AgAQP1 channel protein is involved in TEA binding as its replacement by a Phe residue eliminated its sensitivity to the inhibitor [118]. It is noteworthy that the inhibitory effects of TEA is controversial as for instance other studies showed that water conductance mediated via mammalian AQP1 [258,262] or insect BtAQP1 [137] was not affected by TEA.

Acetazolamide (Figure 4), an arylsulfonamide carbonic anhydrase inhibitor, downregulated AQP1 expression in rabbit myocardium following myocardial angiogenesis [263] and also decreased AQP1 expression in endothelial cells of mice Lewis-lung-carcinoma [264]. However, acetazolamide, which reversibly inhibited water permeability via rat AQP4, had no effect on human AQP1, and a similar arylsulfonamide (methazolamide) failed to inhibit water flux via both AQPs reconstituted in proteoliposomes [265].

AQP4, which was linked to a variety of brain diseases and disorders, was subjected to *in vitro* oocytes swelling assays and *in silico* docking studies using a number of sulfanilamide antiepileptic drugs (Figure 4) [220,246,266,267]. Dose-response relationship analysis of inhibitory activity demonstrated that acetazolamide is the most efficient inhibitor. Yet in contrast, Yang et al. [268] were unable to find inhibition of water permeation via AQP4 by a large series of antiepileptic drugs in transfected rat thyroid cell line and in primary glial cells. A series of bumetanide compounds were screened for water flux inhibition in AQP1 and AQP4 using the *Xenopus oocytes* swelling assay [249], and although the 4-aminopyrimidine carboxamide analog, AqBO13 (Figure 4), was the most active one, it displayed only a mild inhibitory effect (IC_{50} ~20μM). It was postulated that the binding site of the compound is located in the intracellular loop D gating domain [248,269].

Conclusions and Perspectives

The paramount physiological importance of MIPs-forming transmembrane channels (aquaporins) in maintaining water homeostasis has been well documented and recognized. Modified structural design of channel proteins (mainly aquaglycerporins) facilitates flux of other small solutes notably glycerol. Challenging osmotic stress and extreme environmental conditions like subzero temperatures and desiccation states are alleviated by manipulating the permeations of water and some cryoprotectant solutes. A large body of evidence has established roles for transmembrane channels in non-transporting functions like cell migration, cell to cell adhesion or neural transduction signals via unclear interactions with membrane and cytoplasmic proteins. Nevertheless, overall information related to the dynamics of posttranslational events related to AQP trafficking, docking on and integration with target domains in plasma membranes, gating of channel proteins and their recycling is still fragmentary. There are still gaps in knowledge for understanding the intricate and the complex biophysical spectacle of AQP channel function.

Currently, only a relatively small number of insect AQPs were cloned and functionally characterized. As more genomic sequences of diverse insect species become available, insights regarding structures and physiological functions of their respective AQPs will be gained. It will facilitate *inter alia* studies related to intricate mechanisms of water homeostasis and osmoregulation which are critical to insect species feeding on blood and plant-sap, or insects surviving in extremely salty, cold, or hot and dry environments.

High-resolution structures of AQPs are essential for the mechanistic elucidation of water and solute transport across transmembrane channels. Furthermore, water homeostasis and osmoregulation are attractive targets to alleviate human AQP-related pathophysiological disorders as well as to control insect pests. At present, the unfortunate limited number AQP inhibitors are non-selective with an overall disappointing potency. Nevertheless, the available crystal structures combined with throughput functional assays might facilitate a rational structure-based design and synthesis of new and powerful inhibitors. They are indispensible in research aimed at finding molecular leads for the development of future commercial pharmaceutical drugs along with pest control agents.

In addition to possible chemical interference, viral-based systems may be used as an efficient vehicle that facilitae the introduction of dsRNA into plants. Such a genetic interversion, aimed at knocking down essential AQPs, should be considered as a future approach to control insect pests.

Acknowledgements

I thank Professors Hans Merzendorfer and Subbarathan Muthukrishnan for carefully reading the manuscript and for their useful comments. The help of Ora Tzur from the Computer Unit in constructing the AQP schematic model is highly appreciated, and I gratefully acknowledge the creative illustrations of Yossi Maoz.

References

1. Sanders OI, Rensing C, Kuroda M, Mitra B, Rosen BP (1997) Antimonite is accumulated by the glycerol facilitator GlpF in Escherichia coli. J Bacteriol 179: 3365-3367.
2. Liu Z, Shen J, Carbrey JM, Mukhopadhyay R, Agre P, et al. (2002) Arsenite transport by mammalian aquaglyceroporins AQP7 and AQP9. Proc Natl Acad Sci U S A 99: 6053-6058.
3. Bienert GP, Møller AL, Kristiansen KA, Schulz A, Møller IM, et al. (2007) Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem 282: 1183-1192.
4. Saparov SM, Liu K, Agre P, Pohl P (2007) Fast and selective ammonia transport by aquaporin-8. J Biol Chem 282: 5296-5301.
5. Uehlein N, Otto B, Hanson DT, Fischer M, McDowell N, et al. (2008) Function of *Nicotiana tabacum* aquaporins as chloroplast gas pores challenges the concept of membrane CO2 permeability. Plant Cell 20: 648-657.
6. Hamdi M, Sanchez MA, Beene LC, Liu Q, Landfear SM, et al. (2009) Arsenic transport by zebrafish aquaglyceroporins. BMC Mol Biol 10: 104.
7. Chen LM, Zhao J, Musa-Aziz R, Pelletier MF, Drummond IA, et al. (2010) Cloning and characterization of a zebrafish homologue of human AQP1: a bifunctional water and gas channel. Am J Physiol Regul Integr Comp Physiol 299: R1163-R1174.
8. McDermott JR, Jiang X, Beene LC, Rosen BP, Liu Z (2010) Pentavalent methylated arsenicals are substrates of human AQP9. Biometals 23: 119-127.
9. Gazzarrini S, Kang M, Epimashko S, Van Etten JL, Dainty J, et al. (2006) Chlorella virus MT325 encodes water and potassium channels that interact synergistically. Proc Natl Acad Sci USA 103: 5355-5360.
10. Pao GM, Wu LF, Johnson KD, Höfte H, Chrispeels MJ, et al. (1991) Evolution of the MIP family of integral membrane transport proteins. Mol Microbiol 5: 33-37.
11. Wistow GJ, Pisano MM, Chepelinsky AB (1991) Tandem sequence repeats in transmembrane channel proteins. Trends Biochem Sci 16: 170-171.
12. Reizer J, Reizer A, Saier MH Jr (1993) The MIP family of integral membrane channel proteins: sequence comparisons, evolutionary relationships, reconstructed pathway of evolution, and proposed functional differentiation of the two repeated halves of the proteins. Crit Rev Biochem Mol Biol 28: 235-257.
13. Verkman AS (2005) More than just water channels: unexpected cellular roles of aquaporins. J Cell Sci 118: 3225-3232.
14. Ishibashi K (2006) Aquaporin subfamily with unusual NPA boxes. Biochim Biophys Acta 1758: 989-993.
15. Nozaki K, Ishii D, Ishibashi K (2008) Intracellular aquaporins: clues for intracellular water transport? Pflugers Arch Eur J Physiol 456: 701-707.
16. Rojek A, Praetorius J, Frøkiaer J, Nielsen S, Fenton RA (2008) A current view of the mammalian aquaglyceroporins. Annu Rev Physiol 70: 301-327.
17. Zhang RB, Verkman AS (1991) Water and urea permeability properties of Xenopus oocytes: expression of mRNA from toad urinary bladder. Am J Physiol 260: C26-34.
18. Coury LA, Zeidel ML, Brodsky JL (1999) Use of yeast *sec*6 mutant for purification of vesicles containing recombinant membrane proteins. Methods Enzymol 306: 169-186.
19. Pettersson N, Filipsson C, Becit E, Brive L, Hohmann S (2005) Aquaporins in yeasts and filamentous fungi. Biol Cell 97: 487-500.
20. Yakata K, Hiroaki Y, Ishibashi K, Sohara E, Sasaki S, et al. (2007) Aquaporin-11 containing a divergent NPA motif has normal water channel activity. Biochim Biophys Acta 1768: 688-693.
21. Hovijitra NT, Wuu JJ, Peaker B, Swartz JR (2009) Cell-free synthesis of functional aquaporin Z in synthetic liposomes. Biotechnol Bioeng 104: 40-49.
22. Agre P (2006) The aquaporin water channels. Proc Am Thorac Soc 3: 5-13.
23. Gonen T, Walz T (2006) The structure of aquaporins. Q Rev Biophys 39: 361-396.
24. Krane CM, Goldstein DL (2007) Comparative functional analysis of aquaporins/glyceroporins in mammals and anurans. Mamm Genome 18: 452-462.
25. Benga G (2009) Water channel proteins (later called aquaporins) and relatives: past, present, and future. IUBMB Life 61: 112-133.
26. Gomes D, Agasse A, Thiébaud P, Delrot S, Gerós H, et al. (2009) Aquaporins are multifunctional water and solute transporters highly divergent in living organisms. Biochim Biophys Acta 1788: 1213-1228.
27. Frühbeck G (2005) Obesity: aquaporin enters the picture. Nature 438: 436-437.
28. Maurel C, Javot H, Lauvergeat V, Gerbeau P, Tournaire C, et al. (2002) Molecular physiology of aquaporins in plants. Int Rev Cytol 215: 105-148.
29. Kaldenhoff R, Fischer M (2006) Functional aquaporin diversity in plants. Biochim Biophys Acta 1758: 1134-1141.
30. Maurel C, Verdoucq L, Luu DT, Santoni V (2008) Plant aquaporins: membrane channels with multiple integrated functions. Annu Rev Plant Biol 59: 595-624.
31. Kaldenhoff R, Ribas-Carbo M, Sans JF, Lovisolo C, Heckwolf M, et al. (2008) Aquaporins and plant water balance. Plant Cell Environ 31: 658-666.
32. Maurel C, Santoni V, Luu DT, Wudick MM, Verdoucq L (2009) The cellular dynamics of plant aquaporin expression and functions. Curr Opin Plant Biol 12: 690-698.
33. Fabra M, Raldúa D, Power DM, Deen PM, Cerdà J (2005) Marine fish egg hydration is aquaporin-mediated. Science 307: 545.
34. Müller C, Sendler M, Hildebrandt JP (2006) Downregulation of aquaporins 1 and 5 in nasal gland by osmotic stress in ducklings, *Anas platyrhynchos:* implications for the production of hypertonic fluid. J Exp Biol 209: 4067-4076.
35. Harries WE, Akhavan D, Miercke LJ, Khademi S, Stroud RM (2004) The channel architecture of aquaporin 0 at a 2.2-Å resolution. Proc Natl Acad Sci U S A 101: 14045-14050.
36. Hiroaki Y, Tani K, Kamegawa A, Gyobu N, Nishikawa K, et al. (2006) Implications of the aquaporin-4 structure on array formation and cell adhesion. J Mol Biol 355: 628-639.
37. Yool AJ (2007) Functional domains of aquaporin-1: keys to physiology, and targets for drug discovery. Curr Pharm Des 13: 3212-3221.
38. Verkman AS, Binder DK, Bloch O, Auguste K, Papadopoulos MC (2006) Three distinct roles of aquaporin-4 in brain function revealed by knockout mice. Biochim Biophys Acta 1758: 1085-1093.
39. Buffoli B (2010) Aquaporin biology and nervous system. Curr Neuropharmacol 8: 97-104.
40. Verkman AS, Hara-Chikuma M, Papadopoulos MC (2008) Aquaporins--new players in cancer biology. J Mol Med (Berl) 86: 523-529.
41. Törnroth-Horsefield S, Hedfalk K, Fischer G, Lindkvist-Petersson K, Neutze R (2010) Structural insights into eukaryotic aquaporin regulation. FEBS Lett 584: 2580-2588.
42. Kambara K, Takematsu Y, Azuma M, Kobayashi J (2009) cDNA cloning gene expressed in the digestive track of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera; Rhinotermitidae). Appl Entomol Zool 44: 315-321.
43. Kaufmann N, Mathai JC, Hill WG, Dow JA, Zeidel ML, et al. (2005) Developmental expression and biophysical characterization of a *Drosophila melanogaster* aquaporin. Am J Physiol Cell Physiol 289: C397-C407.
44. Huang CG, Lamitina T, Agre P, Strange K (2007) Functional analysis of the aquaporin gene family in *Caenorhabditis elegans*. Am J Physiol Cell Physiol 292: C1867-C1873.
45. Campbell EM, Ball A, Hoppler S, Bowman AS (2008) Invertebrate aquaporins: a review. J Comp Physiol B 178: 935-955.
46. Spring JH, Robichaux SR, Hamlin JA (2009) The role of aquaporins in excretion in insects. J Exp Biol 212: 358-362.
47. Ren G, Reddy VS, Cheng A, Melnyk P, Mitra AK (2001) Visualization of a water-selective pore by electron crystallography in vitreous ice. Proc Natl Acad Sci USA 98: 1398-1403.
48. Savage DF, Egea PF, Robles-Colmenares Y, O'Connell JD 3rd, Stroud RM (2003) Architecture and selectivity in aquaporins: 2.5 Å X-ray structure of aquaporin Z. PLoS Biol 1: E72.
49. Gonen T, Sliz P, Kistler J, Cheng Y, Walz T (2004) Aquaporin-0 membrane junctions reveal the structure of a closed water pore. Nature 429: 193-197.

50. Wang YF, Fan ZK, Cao Y, Yu DS, Zhang YQ, et al. (2011) 2-Methoxyestradiol inhibits the up-regulation of AQP4 and AQP1 expression after spinal cord injury. Brain Res 1370: 220-226.

51. Horsefield R, Nordén K, Fellert M, Backmark A, Törnroth-Horsefield S, et al. (2008) High-resolution x-ray structure of human aquaporin 5. Proc Natl Acad Sci U S A 105: 13327-13332.

52. Ho JD, Yeh R, Sandstrom A, Chorny I, Harries WE, et al. (2009) Crystal structure of human aquaporin 4 at 1.8 Å and its mechanism of conductance. Proc Natl Acad Sci U S A 106: 7437-7442.

53. Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, et al. (2000) Structural determinants of water permeation through aquaporin-1. Nature 407: 599-605.

54. Borgnia M, Nielsen S, Engel A, Agre P (1999) Cellular and molecular biology of the aquaporin water channels. Annu Rev Biochem 68: 425-458.

55. Kong Y, Ma J (2001) Dynamic mechanisms of the membrane water channel aquaporin-1 (AQP1). Proc Natl Acad Sci U S A 98: 14345-14349.

56. Sui H, Han BG, Lee JK, Walian P, Jap BK (2001) Structural basis of water-specific transport through the AQP1 water channel. Nature 414: 872-878.

57. de Groot BL, Grubmüller H (2001) Water permeation across biological membranes: mechanism and dynamics of aquaporin-1 and GlpF. Science 294: 2353-2357.

58. Fujiyoshi Y, Mitsuoka K, de Groot BL, Philippsen A, Grubmüller H, et al. (2002) Structure and function of water channels. Curr Opin Struct Biol 12: 509-515.

59. de Groot BL, Frigato T, Helms V, Grubmüller H (2003) The mechanism of proton exclusion in the aquaporin-1 water channel. J Mol Biol 333: 279-293.

60. Beitz E, Wu B, Holm LM, Schultz JE, Zeuthen T (2006) Point mutations in the aromatic/arginine region in aquaporin 1 allow passage of urea, glycerol, ammonia, and protons. Proc Natl Acad Sci U S A 103: 269-274.

61. Preston GM, Jung JS, Guggino WB, Agre P (1993) The mercury-sensitive residue at cysteine 189 in the CHIP28 water channel. J Biol Chem 268: 17-20.

62. Savage DF, Stroud RM (2007) Structural basis of aquaporin inhibition by mercury. J Mol Biol 368: 607-617.

63. Yang B, Brown D, Verkman AS (1996) The mercurial insensitive water channel (AQP-4) forms orthogonal arrays in stably transfected Chinese hamster ovary cells. J Biol Chem 271: 4577-4580.

64. Verbavatz JM, Ma T, Gobin R, Verkman AS (1997) Absence of orthogonal arrays in kidney, brain and muscle from transgenic knockout mice lacking water channel aquaporin-4. J Cell Sci 110: 2855-2860.

65. Rash JE, Yasumura T, Hudson CS, Agre P, Nielsen S (1998) Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. Proc Natl Acad Sci U S A 95: 11981-11986.

66. van Hoek AN, Yang B, Kirmiz S, Brown D (1998) Freeze-fracture analysis of plasma membranes of CHO cells stably expressing aquaporins 1-5. J Membr Biol 165: 243-254.

67. Nicchia GP, Mastrototaro M, Rossi A, Pisani F, Tortorella C, et al. (2009) Aquaporin-4 orthogonal arrays of particles are the target for neuromyelitis optica autoantibodies. Glia 57: 1363-1373.

68. Crane JM, Verkman AS (2009) Determinants of aquaporin-4 assembly in orthogonal arrays revealed by live-cell single-molecule fluorescence imaging. J Cell Sci 122: 813-821.

69. Van Hoek AN, Bouley R, Lu Y, Silberstein C, Brown D, et al. (2009) Vasopressin-induced differential stimulation of AQP4 splice variants regulates the in-membrane assembly of orthogonal arrays. Am J Physiol Renal Physiol 296: F1396-F1404.

70. Beuron F, Le Cahe'rec F, Guillam M-T, Cavalier A, Garret A, et al. (1995) Structural analysis of a MIP family protein from the digestive track of *Cicadella viridis*. J Biol Chem 270: 17414-17422.

71. Bron P, Lagrée V, Froger A, Rolland JP, Hubert JF, et al. (1999) Oligomerization state of MIP proteins expressed in *Xenopus* oocytes as revealed by freeze-fracture electron-microscopy analysis. J Struct Biol 128: 287-296.

72. Nishimoto G, Sasaki G, Yaoita E, Nameta M, Li H, et al. (2007) Molecular characterization of water-selective AQP (EbAQP4) in hagfish: insight into ancestral origin of AQP4. Am J Physiol Regul Integr Comp Physiol 292: R644-R651.

73. Ehring GR, Zampighi G, Horwitz J, Bok D, Hall JE (1990) Properties of channels reconstituted from the major intrinsic protein of lens fiber membranes. J Gen Physiol 96: 631-664.

74. Frigeri A, Gropper MA, Umenishi F, Kawashima M, Brown D, et al. (1995) Localization of MIWC and GLIP water channel homologs in neuromuscular, epithelial and glandular tissues. J Cell Sci 108: 2993-3002.

75. Yool AJ, Weinstein AM (2002) New roles for old holes: ion channel function in aquaporin-1. News Physiol Sci 17: 68-72.

76. Boassa D, Stamer WD, Yool AJ (2006) Ion channel function of aquaporin-1 natively expressed in choroid plexus. J Neurosci 26: 7811-7819.

77. Liu K, Kozono D, Kato Y, Agre P, Hazama A, et al. (2005) Conversion of aquaporin 6 from an anion channel to a water-selective channel by a single amino acid substitution. Proc Natl Acad Sci U S A 102: 2192-2197.

78. Zampighi GA, Hall JE, Kreman M (1985) Purified lens junctional protein forms channels in planar lipid films. Proc Natl Acad Sci U S A 82: 8468-8472.

79. Yanochko GM, Yool AJ (2002) Regulated cationic channel function in *Xenopus* oocytes expressing *Drosophila* Big Brain. J Neurosci 22: 2530-2540.

80. King LS, Kozono D, Agre P (2004) From structure to disease: the evolving tale of aquaporin biology. Nat Rev Mol Cell Biol 5: 687-698.

81. Ishibashi K, Hara S, Kondo S (2009) Aquaporin water channels in mammals. Clin Exp Nephrol 13: 107-117.

82. Ishibashi K, Sasaki S, Fushimi K, Uchida S, Kuwahara M, et al. (1994) Molecular cloning and expression of a member of the aquaporin family with permeability to glycerol and urea in addition to water expressed at the basolateral membrane of kidney collecting duct cells. Proc Natl Acad Sci USA 91: 6269-6273.

83. Ishibashi K, Kuwahara M, Gu Y, Kageyama Y, Tohsaka A, et al. (1997) Cloning and functional expression of a new water channel abundantly expressed in the testis permeable to water, glycerol, and urea. J Biol Chem 272: 20782-20786.

84. Ikeda M, Beitz E, Kozono D, Guggino WB, Agre P, et al. (2002) Characterization of aquaporin-6 as a nitrate channel in mammalian cells. Requirement of pore-lining residue threonine 63. J Biol Chem 277: 39873-39879.

85. Tsukaguchi H, Shayakul C, Berger UV, Mackenzie B, Devidas S, et al. (1998) Molecular characterization of a broad selectivity neutral solute channel. J Biol Chem 38: 24737-24743.

86. Rojek AM, Skowronski MT, Füchtbauer EM, Füchtbauer AC, Fenton RA, et al. (2007) Defective glycerol metabolism in aquaporin 9 (AQP9) knockout mice. Proc Natl Acad Sci U S A 104: 3609-3614.

87. Promeneur D, Liu Y, Maciel J, Agre P, King LS, et al. (2007) Aquaglyceroporin PbAQP during intraerythrocytic development of the malaria parasite *Plasmodium berghei*. Proc Natl Acad Sci U S A 104: 2211-2216.

88. Beitz E, Pavlovic-Djuranovic S, Yasui M, Agre P, Schultz JE (2004) Molecular dissection of water and glycerol permeability of the aquaglyceroporin from *Plasmodium falciparum* by mutational analysis. Proc Natl Acad Sci U S A 101: 1153-1158.

89. Newby ZE, O'Connell J 3rd, Robles-Colmenares Y, Khademi S, Miercke LJ, et al. (2008) Crystal structure of the aquaglyceroporin PfAQP from the malarial parasite *Plasmodium falciparum*. Nat Struct Mol Biol 15: 619-625.

90. Lee JK, Kozono D, Remis J, Kitagawa Y, Agre P, et al. (2005) Structural basis for conductance by the archaeal aquaporin AqpM at 1.68 A. Proc Natl Acad Sci U S A 102: 18932-18937.

91. Luyten K, Albertyn J, Skibbe WF, Prior BA, Ramos J, et al. (1995) Fps1, a yeast member of the MIP family of channel proteins, is a facilitator for glycerol uptake and efflux and is inactive under osmotic stress. EMBO J 14: 1360-1371.

92. Beese SE, Negishi T, Levin DE (2009) Identification of positive regulators of the yeast FPS1 glycerol channel. PLoS Genet 5: e1000738.

93. Solari P, Masala C, Falchi AM, Sollai G, Liscia A (2010) The sense of water in the blowfly *Protophormia terraenovae*. J Insect Physiol 56: 1825-1833.

94. Maurel C, Reizer J, Schroeder JI, Chrispeels MJ, Saier MH Jr (1994) Functional characterization of the *Escherichia coli* glycerol facilitator, GlpF, in *Xenopus* oocytes. J Biol Chem 269: 11869-11872.

95. Borgnia MJ, Agre P (2001) Reconstitution and functional comparison of purified

GlpF and AqpZ, the glycerol and water channels from *Escherichia coli*. Proc Natl Acad Sci U S A 98: 2888-2893.

96. Fu D, Libson A, Miercke LJ, Weitzman C, Nollert P, et al. (2000) Structure of a glycerol-conducting channel and the basis for its selectivity. Science 290: 481-486.

97. Nollert P, Harries WE, Fu D, Miercke LJ, Stroud RM (2001) Atomic structure of a glycerol channel and implications for substrate permeation in aqua(glycero) porins. FEBS Lett 504: 112-117.

98. Lagrée V, Froger A, Deschamps S, Hubert JF, Delamarche C, et al. (1999) Switch from an aquaporin to a glycerol channel by two amino acids substitution. J Biol Chem 274: 6817-6819.

99. Savage DF, O'Connell JD 3rd, Miercke LJ, Finer-Moore J, Stroud RM (2010) Structural context shapes the aquaporin selectivity filter. Proc Natl Acad Sci U S A 107: 17164-17169.

100. Rao Y, Jan LY, Jan YN (1990) Similarity of the product of the *Drosophila* neurogenic gene *big brain* to transmembrane channel proteins. Nature 345: 163-167.

101. Burris PA, Zhang Y, Rusconi JC, Corbin V (1998) The pore-forming and cytoplasmic domains of the neurogenic gene product, BIG BRAIN, are conserved between *Drosophila virilis* and *Drosophila melanogaster*. Gene 206: 69-76.

102. Doherty D, Jan LY, Jan YN (1997) The Drosophila neurogenic gene *big brain*, which encodes a membrane-associated protein, acts cell autonomously and can act synergistically with *Notch* and *Delta*. Development 124: 3881-3893.

103. Kanwar R, Fortini ME (2008) The big brain aquaporin is required for endosome maturation and notch receptor trafficking. Cell 133: 852-863.

104. Tatsumi K, Tsuji S, Miwa H, Morisaku T, Nuriya M, et al. (2009) *Drosophila big brain* does not act as a water channel, but mediates cell adhesion. FEBS Lett 583: 2077-2082.

105. Park JS, Kim SR, Park SY, Yang DJ, Lee SH, et al. (2008) *Big brain*, a *Drosophila* homologue of mammalian aquaporin, is regulated by the DRE/DREF system. Biochim Biophys Acta 1779: 789-796.

106. O'Donnell ME (2008) Insect excretory mechanisms. Adv Insect Physiol 35: 1-122.

107. Beyenbach KW, Skaer H, Dow JAT (2010) The developmental, molecular, and transport biology of Malpighian tubules. Annu Rev Entomol 55: 351-374.

108. Dow AT, Kell DC, Davies SA, Maddrell SHP, Brown D (1995) A novel member of the major intrinsic protein family in *Drosophila* are aquaporins involved in insect Malpighian (renal) tubule fluid secretion. J Physiol 489: 110-111.

109. Gautam NK, Tapadia MG (2010) Ecdysone signaling is required for proper organization and fluid secretion of stellate cells in the Malpighian tubules of *Drosophila melanogaster*. Int J Dev Biol 54: 635-642.

110. Elvin CM, Bunch R, Liyou NE, Pearson RD, Gough J, et al. (1999) Molecular cloning and expression in Escherichia coli of an aquaporin-like gene from adult buffalo fly *(Haematobia irritans exigua*). Insect Mol Biol 8: 369-380.

111. Kikawada T, Saito A, Kanamori Y, Fujita M, Snigórska K, et al. (2008) Dehydration-inducible changes in expression of two aquaporins in the sleeping chironomid, *Polypedilum vanderplanki*. Biochim Biophys Acta 1778: 514-520.

112. Goto SG, Philip BN, Teets NM, Kawarasaki Y, Lee Jr RE, et al. (2011) Functional characterization of an aquaporin in the Antarctic midge *Belgica antarctica*. J Insect Physiol 57: 1106-1114.

113. Yi SX, Benoit JB, Elnitsky MA, Kaufmann N, Brodsky JL, et al. (2011) Function and immuno-localization of aquaporins in the Antarctic midge *Belgica antarctica*. J Insect Physiol 57: 1096-1105.

114. Philip BN, Kiss AJ, Lee RE Jr (2011) The protective role of aquaporins in the freeze-tolerant insect *Eurosta solidaginis*: functional characterization and tissue abundance of EsAQP1. J Exp Biol 214: 848-857.

115. Pietrantonio PV, Jagge C, Keeley LL, Ross LS (2000) Cloning of an aquaporin-like cDNA and in situ hybridization in adults of the mosquito *Aedes aegypti* (Diptera: Culicidae). Insect Mol Biol 9: 407-418.

116. Duchesne L, Hubert JF, Verbavatz JM, Thomas D, Pietrantonio PV (2003) Mosquito (*Aedes aegypti*) aquaporin, present in tracheolar cells, transports water, not glycerol, and forms orthogonal arrays in *Xenopus* oocyte membranes. Eur J Biochem 270: 422-429.

117. Drake LL, Boudko DY, Marinotti O, Carpenter VK, Dawe AL, et al. (2010) The Aquaporin gene family of the yellow fever mosquito, *Aedes aegypti*. PLoS One 5: e15578.

118. Liu K, Tsujimoto H, Cha SJ, Agre P, Rasgon JL (2011) Aquaporin water channel AgAQP1 in the malaria vector mosquito *Anopheles gambiae* during blood feeding and humidity adaptation. Proc Natl Acad Sci U S A 108: 6062-6066.

119. Campbell EM, Burdin M, Hoppler S, Bowman AS (2010) Role of an aquaporin in the sheep tick *Ixodes ricinus*: assessment as a potential control target. Int J Parasitol 40: 15-23.

120. Gaede K, Knüle W (1997) On the mechanism of water vapour sorption from unsaturated atmospheres by ticks J Exp Biol 200: 1491-1498.

121. Bowman AS, Sauer JR (2004) Tick salivary glands: function, physiology and future. Parasitology 129 Suppl: S67-S81.

122. Kaufman WR, Phillips JE (1973) Ion and water balance in the ixodid tick *Dermacentor andersoni*. I Routes of ion and water excretion. J Exp Biol 58: 523-536.

123. Ball A, Campbell EM, Jacob J, Hoppler S, Bowman AS (2009) Identification, functional characterization and expression patterns of a water-specific aquaporin in the brown dog tick, *Rhipicephalus sanguineus*. Insect Biochem Mol Biol 39: 105-112.

124. Holmes SP, Li D, Ceraul SM, Azad AF (2008) An aquaporin-like protein from the ovaries and gut of American dog tick (Acari: Ixodidae). J Med Entomol 45: 68-74.

125. Echevarría M, Ramírez-Lorca R, Hernández CS, Gutiérrez A, Méndez-Ferrer S, et al. (2001) Identification of a new water channel (Rp-MIP) in the Malpighian tubules of the insect *Rhodnius prolixus*. Pflugers Arch Eur J Physiol 442: 27-34.

126. Martini SV, Goldenberg RC, Fortes FSA, Campos-de-Carvalho AC, Falkenstein D, et al. (2004) *Rhodnius prolixus* Malpighian tubule's aquaporin expression is modulated by 5-hydroxytryptamine. Arch Insect Biochem Physiol 57: 133-141.

127. Hubert JF, Thomas D, Cavalier A, Gouranton J (1989) Structural and biochemical observations on specialized membranes of the "filter chamber", a water-shunting complex in sap-sucking homopteran insects. Biol Cell 66: 155-163.

128. Cicero JM, Brown JK, Roberts PD, Stansly PA (2009) The digestive system of *Diaphorina citri* and *Bactericera cockerelli* (Hemiptera: Psyllidae (Hemiptera: Psyllidae). Ann Entomol Soc Am 102: 650-665.

129. Guillam MT, Beuron F, Grandin N, Hubert JF, Boisseau C, et al. (1992) Expression of RNA isolated from the water-shunting complex of a sap-sucking insect increases the membrane permeability for water in *Xenopus* oocytes. Exp Cell Res 200: 301-305.

130. Le Caherec F, Guillam MT, Beuron F, Cavalier A, Thomas D, et al. (1997) Aquaporin-related proteins in the filter chamber of homopteran insects. Cell Tissue Res 290: 143-151.

131. Le Cahérec F, Bron P, Verbavatz JM, Garret A, Morel G, et al. (1996) Incorporation of proteins into (Xenopus) oocytes by proteoliposome microinjection: functional characterization of a novel aquaporin. J Cell Sci 109 : 1285-1295.

132. Lagrée V, Pellerin I, Hubert JF, Tacnet F, Le Cahérec F, et al. (1998) A yeast recombinant aquaporin mutant that is not expressed or mistargeted in *Xenopus* oocyte can be functionally analyzed in reconstituted proteoliposomes. J Biol Chem 273: 12422-12426.

133. Karley AJ, Ashford DA, Minto LM, Pritchard J, Douglas AE (2005) The significance of gut sucrase activity for osmoregulation in the pea aphid, *Acyrthosiphon pisum*. J Insect Physiol 51: 1313-1319.

134. Douglas AE (2006) Phloem-sap feeding by animals: problems and solutions. J Exp Bot 57: 747-754.

135. Douglas AE (2003) The nutritional physiology of aphids. Adv Insect Physiol 31: 73-140.

136. Shakesby AJ, Wallace IS, Isaacs HV, Pritchard J, Roberts DM, et al. (2009) A water-specific aquaporin involved in aphid osmoregulation. Insect Biochem Mol Biol 39: 1-10.

137. Mathew LG, Campbell EM, Yool AJ, Fabrick JA (2011) Identification and characterization of functional aquaporin water channel protein from alimentary

tract of whitefly, *Bemisia tabac*i. Insect Biochem Mol Biol 41: 178-190.

138. Kataoka N, Miyake S, Azuma M (2009) Aquaporin and aquaglyceroporin in silkworms, differently expressed in the hindgut and midgut of *Bombyx mori*. Insect Mol Biol 18: 303-314.

139. Azuma M, Ohta Y (1998) Changes in H^+-translocating vacuolar-type ATPase in the anterior silk gland cell of *Bombyx mori* during metamorphosis. J Exp Biol 201: 479-486.

140. Miyaki S, Azuma M (2008) Developmental expression and the physiological role of aquaporin in the silk gland of *Bombyx mori*. J insect Biotechnol Sericol 77: 87-93.

141. Kataoka N, Miyake S, Azuma M (2009) Molecular characterization of aquaporin and aquaglyceroporin in the alimentary canal of *Grapholita molesta* (the oriental fruit moth) – comparison with *Bombyx mori* aquaporins. J Insect Biotechnol Sericol 72: 81-90.

142. Lee KS, Kim SR, Lee SM, Lee KR, Sohn HD, Jin BR (2001) Molecular cloning and expression of a cDNA encoding the aquaporin homologue from the firefly, *Pyrocoelia rufa*. Korean J Entomol 31: 269-279.

143. Spring JH, Robichaux SR, Kaufmann N, Brodsky JL (2007) Localization of a *Drosophila* DRIP-like aquaporin in the Malpighian tubules of the house cricket, *Acheta domesticus*. Comp Biochem Physiol A Mol Integr Physiol 148: 92-100.

144. Storey KB, Storey JM (1988) Freeze tolerance in animals. Physiol Rev 68: 27-84.

145. Duman JG, Wu DW, Xu L, Tursman D, Olsen TM (1991) Adaptation of insects to subzero temperatures. Quart Rev Biol 66: 387-410.

146. Storey KB (1997) Organic solutes in freezing tolerance. Comp Biochem Physiol A 117: 319-326.

147. Rinehart JP, Li A, Yocum GD, Robich RM, Hayward SA, et al. (2007) Up-regulation of heat shock proteins is essential for cold survival during insect diapause. Proc Natl Acad Sci U S A 104: 11130-11137.

148. Zhang G, Storey JM, Storey KB (2011) Chaperone proteins and winter survival by a freeze tolerant insect. J Insect Physiol 57: 1115-1122.

149. Kost'ál V, Slachta M, Simek P (2001) Cryoprotective role of polyols independent of the increase in supercooling capacity in diapausing adults of *Pyrrhocoris apterus* (Heteroptera: Insecta). Comp Biochem Physiol B Biochem Mol Biol 130: 365-374.

150. Lacey MJ, Lenz M, Evans TA (2010) Cryoprotection in dampwood termites (Termopsidae, Isoptera). J Insect Physiol 56: 1-7.

151. Costanzo JP, Litzgus JD, Iverson JB, Lee RE Jr (2000) Seasonal changes in physiology and development of cold hardiness in the hatchling painted turtle *Chrysemys picta*. J Exp Biol 203: 3459-3470.

152. Costanzo JP, Lee Jr RE (2005) Cryoprotection by urea in a terrestrially hibernating frog. J Exp Biol 208: 4079-4089.

153. Churchill TA, Storey KB (1989) Seasonal variation in the temperature-stimulated interconversion of glycogen and glycerol pools in a freeze avoiding moth larva. Cryo-Lett 10: 127-136.

154. Ishiguro S, Li Y, Nakano K, Tsumuki H, Goto M (2007) Seasonal changes in glycerol content and cold hardiness in two ecotypes of the rice stem borer, *Chilo suppressalis*, exposed to the environment in the Shonai district, Japan. J Insect Physiol 53: 392-397.

155. Williams JB, Shorthouse JD, Lee RE Jr (2002) Extreme resistance to desiccation and microclimate-related differences in cold-hardiness of gall wasps (Hymenoptera: Cynipidae) overwintering on roses in southern Canada. J Exp Biol 205: 2115-2124.

156. Michaud RM, Benoit JB, Lopez-Martinez G, Elnitsky MA, Lee RE Jr, et al. (2008) Metabolomics reveals unique and shared metabolic changes in response to heat shock, freezing and desiccation in the Antarctic midge, *Belgica antarctica*. J Insect Physiol 54: 645-655.

157. Yoder JA, Benoit JB, Denlinger, DL, Rivers DB (2006) Stress-induced accumulation of glycerol in the flesh fly, *Sarcophaga bullata*: Evidence indicating anti-desiccant and cryoprotectant functions of this polyol and a role for the brain in coordinating the response. J Insect Physiol 52: 202-214.

158. Khani A, Moharramipour S, Barzegar M (2007) Cold tolerance and trehalose accumulation in overwintering larvae of the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae). Eur J Entomol 104: 385-392.

159. Fields PG, Fleurat-Lessard F, Lavenseau L, Febvay G, Peypelut L, et al. (1998) The effect of cold acclimation and deacclimation on cold tolerance, trehalose and free amino acid levels in *Sitophilus granarius* and *Cryptolestes ferrugineus* (Coleoptera). J Insect Physiol 44: 955-965.

160. Clark MS, Thorne MAS, Purać J, Burns G, Hillyard G, et al. (2009) Surviving the cold: molecular analyses of insect cryoprotective dehydration in the Arctic springtail *Megaphorura arctica* (Tullberg). BMC Genom 10: 328.

161. Morrissey RE, Baust JG (1976) The ontogeny of cold tolerance in the gall fly, *Eurosta solidagensis*. J Insect Physiol 22: 431-437.

162. Benoit JB, Lopez-Martinez G, Elnitsky MA, Lee RE Jr, Denlinger DL (2009) Dehydration-induced cross tolerance of *Belgica antarctica* larvae to cold and heat is facilitated by trehalose accumulation. Comp Biochem Physiol A Mol Integr Physiol 152: 518-523.

163. Watanabe M, Kikawada T, Minagawa N, Yukuhiro F, Okuda T (2002) Mechanism allowing an insect to survive complete dehydration and extreme temperatures. J Exp Biol 205: 2799-2802.

164. Kikawada T, Minakawa N, Watanabe M, Okuda T (2005) Factors Inducing Successful Anhydrobiosis in the African Chironomid *Polypedilum vanderplanki:* Significance of the Larval Tubular Nest. Integr Comp Biol 45: 710-714.

165. Cornette R, Kanamori Y, Watanabe M, Nakahara Y, Gusev O, et al. (2010) Identification of anhydrobiosis-related genes from an expressed sequence tag database in the cryptobiotic midge *Polypedilum vanderplanki* (Diptera; Chironomidae). J Biol Chem 285: 35889-35899.

166. Philip BN, Lee RE Jr (2010) Changes in abundance of aquaporin-like proteins occurs concomitantly with seasonal acquisition of freeze tolerance in the goldenrod gall fly, *Eurosta solidaginis*. J Insect Physiol 56: 679-685.

167. Rojas RR, Lrr Jr RE, Baust JG, (1986) Relationship of environmental water content to glycerol accumulation in the freezing tolerant larvae of Eurosta *solidaginis* (Fitch). Cryo Lett 7: 234-245.

168. Philip BN, Yi SX, Elnitsky MA, Lee RE Jr (2008) Aquaporins play a role in desiccation and freeze tolerance in larvae of the goldenrod gall fly, *Eurosta solidaginis*. J Exp Biol 211: 1114-1119.

169. Izumi Y, Sonoda S, Yoshida H, Danks HV, Tsumuki H (2006) Role of membrane transport of water and glycerol in the freeze tolerance of the rice stem borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae). J Insect Physiol 52: 215-220.

170. Ma T, Hasegawa H, Skach WR, Frigeri A, Verkman AS (1994) Expression, functional analysis, and in situ hybridization of a cloned rat kidney collecting duct water channel. Am J Physiol Cell physiol 266: C189-C197.

171. Terris J, Ecelbarger CA, Nielsen S, Knepper MA (1996) Long-term regulation of four renal aquaporins in rats. Am J Physiol Fluid Electrolyte Physiol 271: F414-F422.

172. Matsumura Y, Uchida S, Rai T, Sasaki S, Marumo F (1997) Transcriptional regulation of aquaporin-2 water channel gene by cAMP. J Am Soc Nephrol 8: 861-867.

173. Combet S, Gouraud S, Gobin R, Berthonaud V, Geelen G, et al. (2008) Aquaporin-2 downregulation in kidney medulla of aging rats is posttranscriptional and is abolished by water deprivation. Am J Physiol Renal Physiol 294: F1408-F1414.

174. Küppers E, Gleiser C, Brito V, Wachter B, Pauly T, et al. (2008) AQP4 expression in striatal primary cultures is regulated by dopamine--implications for proliferation of astrocytes. Eur J Neurosci 28: 2173-2182.

175. Ding JY, Kreipke CW, Speirs SL, Schafer P, Schafer S, et al. (2009) Hypoxia-inducible factor-1α signaling aquaporin upregulation after traumatic brain injury. Neurosci Lett 453: 68-72.

176. Wang YF, Fan ZK, Cao Y, Yu DS, Zhang YQ, et al. (2011) 2-Methoxyestradiol inhibits the up-regulation of AQP4 and AQP1 expression after spinal cord injury. Brain Res 1370: 220-226.

177. Mah AK, Armstrong KR, Chew DS, Chu JS, Tu DK, et al. (2007) Transcriptional regulation of AQP-8, a *Caenorhabditis elegans* aquaporin exclusively expressed in the excretory system, by the POU homeobox transcription factor CEH-6. J Biol Chem 282: 28074-28086.

178. Rothman JE (1994) Mechanisms of intracellular protein transport. Nature 372: 55-63.

179. Nielsen S, Agre P (1995) The aquaporin family of water channels in kidney. Kidney Int 48: 1057-1068.

180. Gustafson CE, Levine S, Katsura T, McLaughlin M, Aleixo MD, et al. (1998) Vasopressin regulated trafficking of a green fluorescent protein-aquaporin 2 chimera in LLC-PK1 cells. Histochem Cell Biol 110: 377-386.

181. Suzuki M, Tanaka S (2010) Molecular diversity of vasopressin-dependent aquaporins closely associated with water adaptation strategy in anuran amphibians. J Neuroendocrinol 22: 407-412.

182. Moeller HB, Fenton RA, Zeuthen T, Macaulay N (2009) Vasopressin-dependent short-term regulation of aquaporin 4 expressed in *Xenopus* oocytes. Neuroscience 164: 1674-1684.

183. Hasler U, Mordasini D, Bianchi M, Vandewalle A, Fe'raille E, et al. (2003) Dual influence of aldosterone on AQP2 expression in cultured renal collecting duct principal cells. J Biol Chem 278: 21639-21648.

184. Gu F, Hata R, Toku K, Yang L, Ma Y-J, et al. (2003) Testosterone up-regulates aquaporin-4 expression in cultured astrocytes. J Neurosci Res 72: 709-715.

185. Jablonski EM, McConnell NA, Hughes FM Jr, Huet-Hudson YM (2003) Estrogen regulation of aquaporins in the mouse uterus: potential roles in uterine water movement. Biol Reprod 69: 1481-1487.

186. Cheng CYY, Chu JYS, Chow BKC (2009) Vasopressin-independent mechanisms in controlling water homeostasis. J Mol Endocrinol 43: 81-92.

187. Gradilone SA, García F, Huebert RC, Tietz PS, Larocca MC, et al. (2003) Glucagon induces the plasma membrane insertion of functional aquaporin-8 water channels in isolated rat hepatocytes. Hepatology 37: 1435-1441.

188. Carmosino M, Mazzone A, Laforenza U, Gastaldi G, Svelto M, et al. (2005) Altered expression of aquaporin 4 and H^+/K^+-ATPase in the stomachs of peptide YY (PYY) transgenic mice. Biol Cell 97: 735-742.

189. Parvin MN, Kurabuchi S, Murdiastuti K, Yao C, Kosugi-Tanaka C, et al. (2005) Subcellular redistribution of AQP5 by vasoactive intestinal polypeptide in the Brunner's gland of the rat duodenum. Am J Physiol Gast Liver Physiol 288: G1283-G1291.

190. Yasui H, Kubota M, Iguchi K, Usui S, Kiho T, et al. (2008) Membrane trafficking of aquaporin 3 induced by epinephrine. Biochem Biophys Res Commun 373: 613-617.

191. Klussmann E, Rosenthal W (2001) Role and identification of protein kinase A anchoring proteins in vasopressin-mediated aquaporin-2 translocation. Kidney Int 60: 446-449.

192. Katsura T, Gustafson CE, Ausiello DA, Brown D (1997) Protein kinase A phosphorylation is involved in regulated exocytosis of aquaporin-2 in transfected LLC-PK_1 cells. Am J Physiol 272: F817-F822.

193. Knepper MA, Inoue T (1997) Regulation of aquaporin-2 water channel trafficking by vasopressin. Curr Opin Cell Biol 9: 560-564.

194. Brown D, Katsura T, Gustafson CE (1998) Cellular mechanisms of aquaporin trafficking. Am J Physiol Renal Physiol 275: F328-F331.

195. van Balkom BWM, Savelkoul PJM, Markovich D, Hofman E, Nielsen S, et al. (2002) The role of putative phosphorylation sites in the targeting and shuttling of the aquaporin-2 water channel. J Biol Chem 277: 41473-41479.

196. Noda Y, Sasaki S (2004) Molecular mechanisms and drug development in aquaporin water channel diseases: molecular mechanism of water channel aquaporin-2 trafficking. J Pharmacol Sci 96: 249-254.

197. De mattia F, Savelkoul PJM, Kamsteeg E-J, Konings IBM, van der Sluijs P, et al. (2005) Lack of arginine vasopressin-induced phosphorylation of aquaporin-2 mutant AQP2-R254L explains dominant nephrogenic diabetes insipidus. J Am Soc Nephrol 16: 2872-2880.

198. Noda Y, Sasaki S (2005) Trafficking mechanism of water channel aquaporin-2. Biol Cell 97: 885-892.

199. McDill BW, Li SZ, Kovach PA, Ding L, Chen F (2006) Congenital progressive hydronephrosis (*cph*) is caused by an S256L mutation in aquaporin-2 that affects its phosphorylation and apical membrane accumulation. Proc Natl Acad Sci U S A 103: 6952-6957.

200. Takata K, Matsuzaki T, Tajika Y, Ablimit A, Hasegawa T (2008) Localization and trafficking of aquaporin 2 in the kidney. Histochem Cell Biol 130: 197-209.

201. Verkman AS (2009) Aquaporins: translating bench research to human disease. J Exp Biol 212: 1707-1715.

202. Kamsteeg EJ, Heijnen I, van Os CH, Deen PMT (2000) The subcellular localization of an aquaporin-2 tetramer depends on the stoichiometry of phosphorylated and nonphosphorylated monomers. J Cell Biol 151: 919-930.

203. Hoffert JD, Fenton RA, Moeller HB, Simons B, Tchapyjnikov D, et al. (2008) Vasopressin-stimulated increase in phosphorylation at Ser^{269} potentiates plasma membrane retention of aquaporin-2. J Biol Chem 283: 24617-24627.

204. Valenti G, Procino G, Tamma G, Carmosino M, Svelto M (2005) Minireview: aquaporin 2 trafficking. Endocrinology 146: 5063-5070.

205. Noda Y, Sasaki S (2006) Regulation of aquaporin-2 trafficking and its binding protein complex. Biochim Biophys Acta 1758: 1117-1125.

206. Szaszák M, Christian F, Rosenthal W, Klussmann E (2008) Compartmentalized cAMP signalling in regulated exocytic processes in non-neuronal cells. Cell Signal 20: 590-601.

207. Nedvetsky PI, Tamma G, Beulshausen S, Valenti G, Rosenthal W, et al. (2009) Regulation of aquaporin-2 trafficking. Handbook of Experimental Pharmacology (Beitz E ed), Spinger Verlag, Berlin 190: 133-157.

208. Marples D, Schroer TA, Ahrens N, Taylor A, Knepper MA, et al. (1998) Dynein and dynactin colocalize with AQP2 water channels in intracellular vesicles from kidney collecting duct. Am J Physiol Renal Physiol 274: F384-F394.

209. Fushimi K, Sasaki S, Marumo F (1997) Phosphorylation of serine 256 is required for cAMP-dependent regulatory exocytosis of the aquaporin-2 water channel. J Biol Chem 272: 14800-14804.

210. Zhang W, Zitron E, Hömme M, Kihm L, Morath C, et al. (2007) Aquaporin-1 channel function is positively regulated by protein kinase C. J Biol Chem 282: 20933-20940.

211. Conner MT, Conner AC, Brown JEP, Bill RM (2010) Membrane trafficking of aquaporin 1 is mediated by protein kinase C via microtubules and regulated by tonicity. Biochemistry 49: 821-823.

212. Han Z, Wax MB, Patil RV (1998) Regulation of aquaporin-4 water channels by phorbol ester-dependent protein phosphorylation. J Biol Chem 273: 6001-6004.

213. Zelenina M, Zelenin S, Bondar AA, Brismar H, Aperia A (2002) Water permeability of aquaporin-4 is decreased by protein kinase C and dopamine. Am J Physiol Renal Physiol 283: F309-F318.

214. Kamsteeg EJ, Hendriks G, Boone M, Konings IB, Oorschot V, et al. (2006) Short-chain ubiquitination mediates the regulated endocytosis of the aquaporin-2 water channel. Proc Natl Acad Sci U S A 103: 18344-18349.

215. Moeller HB, Praetorius J, Rützler MR, Fenton RA (2010) Phosphorylation of aquaporin-2 regulates its endocytosis and protein-protein interactions. Proc Natl Acad Sci U S A 107: 424-429.

216. Nakahama K, Fujioka A, Nagano M, Satoh S, Furukawa K, et al. (2002) A role of the C-terminus of aquaporin 4 in its membrane expression in cultured astrocytes. Genes Cells 7: 731-741.

217. Madrid R, Le Maout S, Barrault MB, Janvier K, Benichou S, et al. (2001) Polarized trafficking and surface expression of the AQP4 water channel are coordinated by serial and regulated interactions with different clathrin-adaptor complexes. EMBO J 20: 7008-7021.

218. Tietz PS, McNiven MA, Splinter PL, Huang BQ, Larusso NF (2006) Cytoskeletal and motor proteins facilitate trafficking of AQP1-containing vesicles in cholangiocytes. Biol Cell 98: 43-52.

219. Fotiadis D, Suda K, Tittmann P, Jenö P, Philippsen A, et al. (2002) Identification and structure of a putative Ca^{2+}-binding domain at the C terminus of AQP1. J Mol Biol 318: 1381-1394.

220. Baus M, Medjugorac-Popovski M, Brown D, Sabolic I (2000) In colchicine-treated rats, cellular distribution of AQP-1 in convoluted and straight proximal tubule segments is differently affected. Pflugers Arch Eur J Physiol 439: 321-330.

221. Marinelli RA, Pham L, Agre P, LaRusso NF (1997) Secretin promotes osmotic water transport in rat cholangiocytes by increasing aquaporin-1 water channels in plasma membrane. Evidence for a secretin-induced vesicular translocation of aquaporin-1. J Biol Chem 272: 12984-12988.

222. Elkjaer ML, Birn H, Agre P, Christensen EI, Nielsen S (1995) Effects of

microtubule disruption on endocytosis, membrane recycling and polarized distribution of Aquaporin-1 and gp330 in proximal tubule cells. Eur J Cell Biol 67: 57-72.

223. Sabolic I, Katsura T, Verbavatz JM, Brown D (1995) The AQP2 water channel: effect of vasopressin treatment, microtubule disruption, and distribution in neonatal rats. J Membr Biol 143: 165-175.

224. Nicchia GP, Rossi A, Mola MG, Procino G, Frigeri A, et al. (2008) Actin cytoskeleton remodeling governs aquaporin-4 localization in astrocytes. Glia 56: 1755-1766.

225. Tada J, Sawa T, Yamanaka N, Shono M, Akamatsu T, et al. (1999) Involvement of vesicle-cytoskeleton interaction in AQP5 trafficking in AQP5-gene-transfected HSG cells. Biochem Biophys Res Commun 266: 443-447.

226. Karabasil MR, Hasegawa T, Azlina A, Purwanti N, Yao C, et al. (2011) Effects of naturally occurring G103D point mutation of AQP5 on its water permeability, trafficking and cellular localization in the submandibular gland of rats. Biol Cell 103: 69-86.

227. García F, Kierbel A, Larocca MC, Gradilone SA, Splinter P, et al. (2001) The water channel aquaporin-8 is mainly intracellular in rat hepatocytes, and its plasma membrane insertion is stimulated by cyclic AMP. J Biol Chem 276: 12147-12152.

228. Noda Y, Horikawa S, Katayama Y, Sasaki S (2005) Identification of a multiprotein "motor" complex binding to water channel aquaporin-2. Biochem Biophys Res Commun 330: 1041-1047.

229. Tietz PS, McNiven MA, Splinter PL, Huang BQ, Larusso NF (2006) Cytoskeletal and motor proteins facilitate trafficking of AQP1-containing vesicles in cholangiocytes. Biol Cell 98: 43-52.

230. Sauer JR, Essenberg RC, Bowman AS (2000) Salivary glands in ixodid ticks: control and mechanism of secretion. J Insect Physiol 46: 1069-1078.

231. Johansson I, Karlsson M, Shukla VK, Chrispeels MJ, Larsson C, et al. (1998) Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. Plant Cell 10: 451-459.

232. Tournaire-Roux C, Sutka M, Javot H, Gout E, Gerbeau P, et al. (2003) Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. Nature 425: 393-397.

233. Alleva K, Niemietz CM, Sutka M, Maurel C, Parisi M, et al. (2006) Plasma membrane of *Beta vulgaris* storage root shows high water channel activity regulated by cytoplasmic pH and a dual range of calcium concentrations. J Exp Bot 57: 609-621.

234. Nyblom M, Frick A, Wang Y, Ekvall M, Hallgren K, et al. (2009) Structural and functional analysis of SoPIP2;1 mutants adds insight into plant aquaporin gating. J Mol Biol 387: 653-668.

235. Törnroth-Horsefield S, Wang Y, Hedfalk K, Johanson U, Karlsson M, et al. (2006) Structural mechanism of plant aquaporin gating. Nature 439: 688-694.

236. Fischer G, Kosinska-Eriksson U, Aponte-Santamaría C, Palmgren M, Geijer C, et al. (2009) Crystal structure of a yeast aquaporin at 1.15Å reveals a novel gating mechanism. PLoS Biol 7: e1000130.

237. Hedfalk K, Törnroth-Horsefield S, Nyblom M, Johanson U, Kjellbom P, et al. (2006) Aquaporin gating. Curr Opin Struct Biol 16: 447-456.

238. Xin L, Su H, Nielsen CH, Tang C, Torres J, et al. (2011) Water permeation dynamics of AqpZ: a tale of two states. Biochim Biophys Acta 1808: 1581-1586.

239. Tamás MJ, Karlgren S, Bill RM, Hedfalk K, Allegri L, et al. (2003) A short regulatory domain restricts glycerol transport through yeast Fps1p. J Biol Chem 278: 6337-6345.

240. Hedfalk K, Bill RM, Mullins JG, Karlgren S, Filipsson C, et al. (2004) A regulatory domain in the C-terminal extension of the yeast glycerol channel Fps1p. J Biol Chem 279: 14954-14960.

241. Németh-Cahalan KL, Hall JE (2000) pH and calcium regulate the water permeability of aquaporin 0. J Biol Chem 275: 6777-6782.

242. Németh-Cahalan KL, Kalman K, Hall JE (2004) Molecular basis of pH and Ca^{2+} regulation of aquaporin water permeability. J Gen Physiol 123: 573-580.

243. Zeuthen T, Klaerke DA (1999) Transport of water and glycerol in aquaporin 3 is gated by H^+. J Biol Chem 274: 21631-21636.

244. Castle NA (2005) Aquaporins as targets for drug discovery. Drug Discov Today 10: 485-493.

245. Wang F, Feng XC, Li YM, Yang H, Ma TH (2006) Aquaporins as potential drug targets. Acta Pharmacol Sin 27: 395-401.

246. Haddoub R, Rützler M, Robin A, Flitsch SL (2009) Design, synthesis and assaying of potential aquaporin inhibitors. Handbook of Experimental Pharmacology (Beitz E ed), Spinger Verlag, Berlin 190: 385-402.

247. Huber VJ, Tsujita M, Nakada T (2009b) Identification of aquaporin 4 inhibitors using in vitro and in silico methods. Bioorg Med Chem 17: 411-417.

248. Migliati E, Meurice N, DuBois P, Fang JS, Somasekharan S, et al. (2009) Inhibition of aquaporin-1 and aquaporin-4 water permeability by a derivative of the loop diuretic bumetanide acting at an internal pore-occluding binding site. Mol Pharmacol 76: 105-112.

249. Wu B, Altmann K, Barzel I, Krehan S, Beitz E (2008) A yeast-based phenotypic screen for aquaporin inhibitors. Pflugers Arch Eur J Physiol 456: 717-720.

250. Niemietz CM, Tyerman SD (2002) New potent inhibitors of aquaporins: silver and gold compounds inhibit aquaporins of plant and human origin. FEBS Lett 531: 443-447.

251. Zelenina M, Tritto S, Bondar AA, Zelenin S, Aperia A (2004) Copper inhibits the water and glycerol permeability of aquaporin-3. J Biol Chem 279: 51939-51943.

252. Yang B, Kim JK, Verkman AS (2006) Comparative efficacy of $HgCl_2$ with candidate aquaporin-1 inhibitors DMSO, gold, TEA^+ and acetazolamide. FEBS Lett 580: 6679-6684.

253. Yukutake Y, Hirano Y, Suematsu M, Yasui M (2009) Rapid and reversible inhibition of aquaporin-4 by zinc. Biochemistry 48: 12059-12061.

254. Cheung KH, Leung CT, Leung GPH, Wong PYD (2003) Synergistic effects of cystic fibrosis transmembrane conductance regulator and aquaporin-9 in the rat epididymis. Biol Reprod 68: 1505-1510.

255. Tanaka K, Koyama Y (2011) Endothelins decrease the expression of aquaporins and plasma membrane water permeability in cultured rat astrocytes. J Neurosci Res 89: 320-328.

256. MacKinnon R, Yellen G (1990) Mutations affecting TEA blockade and ion permeation in voltage-activated K^+ channels. Science 250: 276-279.

257. Brooks HL, Regan JW, Yool AJ (2000) Inhibition of aquaporin-1 water permeability by tetraethylammonium: involvement of the loop E pore region. Mol Pharmacol 57: 1021-1026.

258. Yool AJ, Brokl OH, Pannabecker TL, Dantzler WH, Stamer WD (2002) Tetraethylammonium block of water flux in Aquaporin-1 channels expressed in kidney thin limbs of Henle's loop and a kidney-derived cell line. BMC Physiol 2: 4.

259. Detmers FJ, de Groot BL, Müller EM, Hinton A, Konings IBM, et al. (2006) Quaternary ammonium compounds as water channel blockers. Specificity, potency, and site of action. J Biol Chem 281: 14207-14214.

260. Küppers E, Gleiser C, Brito V, Wachter B, Pauly T, et al. (2008) AQP4 expression in striatal primary cultures is regulated by dopamine--implications for proliferation of astrocytes. Eur J Neurosci 28: 2173-2182.

261. Müller EM, Hub JS, Grubmüller H, de Groot BL (2008) Is TEA an inhibitor for human aquaporin-1? Pflugers Arch Eur J Physiol 456: 663-669.

262. Søgaard R, Zeuthen T (2008) Test of blockers of AQP1 water permeability by a high-resolution method: no effects of tetraethylammonium ions or acetazolamide. Pflugers Arch Eur J Physiol 456: 285-292.

263. Ran X, Wang H, Chen Y, Zeng Z, Zhou Q, et al. (2010) Aquaporin-1 expression and angiogenesis in rabbit chronic myocardial ischemia is decreased by acetazolamide. Heart Vessels 25: 237-247.

264. Xiang Y, Ma B, Li T, Gao JW, Yu HM, et al. (2004) Acetazolamide inhibits aquaporin-1 protein expression and angiogenesis. Acta Pharmacol Sin 25: 812-816.

265. Tanimura Y, Hiroaki Y, Fujiyoshi Y (2009) Acetazolamide reversibly inhibits water conduction by aquaporin-4. J Struct Biol 166: 16-21.

266. Huber VJ, Tsujita M, Yamazaki M, Sakimura K, Nakada T (2007) Identification of arylsulfonamides as Aquaporin 4 inhibitors. Bioorg Med Chem Lett 17: 1270-1273.

267. Huber VJ, Tsujita M, Kwee IL, Nakada T (2009) Inhibition of aquaporin 4 by antiepileptic drugs. Bioorg Med Chem 17: 418-424.

268. Yang B, Zhang H, Verkman AS (2008) Lack of aquaporin-4 water transport inhibition by antiepileptics and arylsulfonamides. Bioorg Med Chem 16: 7489-7493.

269. Yool AJ, Brown EA, Flynn GA (2010) Roles for novel pharmacological blockers of aquaporins in the treatment of brain oedema and cancer. Clin Exp Pharmacol Physiol 37: 403-409.