An Emerging Consensus on Aquaporin Trafficking

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Abstract

Water can pass through the cell membrane relatively slowly by diffusion. However, in order to maintain water homeostasis, the rapid and specific regulation of cellular water flow is mediated by the aquaporin (AQP) family of membrane protein water channels. Thirteen human AQPs have been identified to date and the majority are highly specific for water while others show selectivity for water, glycerol and other small solutes. The wide range of tissues that are known to express AQPs is reflected by their involvement in many physiological processes and diseases. Receptor mediated translocation, via hormone activation, is an established method of AQP regulation, especially for AQP2. There is now an emerging consensus that the rapid and reversible translocation of other AQPs from intracellular vesicles to the plasma membrane, triggered by a range of stimuli, can confer increased membrane permeability. This review examines new advances that have identified molecular mechanisms of AQP regulation; these include the role of cytoskeletal proteins, kinases, calcium and retention or localisation signals as well as specific triggers of AQP translocation. This article reviews current knowledge of AQP regulation with a focus on rapid and dynamic regulation of sub-cellular AQP translocation in response to a specific trigger.

Introduction

Over 50% of total body water content is found inside cells. The water content of different tissues is diverse and ranges from about 10% in adipocytes to 75% in muscle tissue (Martini, 2009). Consequently, the homeostasis of the intracellular fluid (the cytosol) and the extracellular fluid (the interstitial fluid, plasma and other body fluids) is physiologically essential (Martini, 2009). In order to maintain this physiological homeostasis, the control of cellular water flow must be tightly regulated. In mammals, water can pass into and out of cells by diffusion through the plasma membrane and may also be co-transported passively with other ions or solutes (Loo et al., 1996). Neither of these routes permits the rapid, regulated and selective water permeability that is evident in tissues such as kidney and secretory glands. Rather, cellular water flow is mediated by aquaporin water channels (AQPs), which were first discovered in 1993 (Agre et al., 1993). Since then, 13 members of this membrane protein family have been identified in humans (AQP0-12), which are distributed throughout a wide range of tissues. For example, AQP1 has been shown to be highly expressed in red blood cells, kidney proximal tubule cells and many other tissues (Magni et al., 2006), while AQP11 has been found at low levels in smooth muscle or cells of the immune system (Ishibashi, 2009). A number of studies have also shown that AQPs play a role in various disorders from renal disease to cerebral oedema (Benarroch, 2007, Schrier and Cadnapaphornchai, 2003) and even cancer (Yin et al., 2008, Chae et al., 2008). Consequently AQPs have been highlighted as key drug targets (Huber et al., 2012, Wang et al., 2006) although drugs that target AQPs for clinical use remain to be discovered. This diverse tissue distribution and involvement in many physiological and pathological processes, is summarised in Table 1.

Structure and function of AQPs

The high resolution structures of several AQPs have been resolved (see Tornroth-Horsefield et al., 2010 for review) and these include the structures of human AQP1, 4 and 5 (Murata et

al., 2000, Ho et al., 2009, Horsefield et al., 2008). (Harries et al., 2004, Ho et al., 2009, Horsefield et al., 2008, Murata et al., 2000) revealing that the family shares a conserved architecture (Figure 1). AQPs exist as homotetramers with four AQP subunits per functional AQP tetramer; selective transport of water molecules, glycerol and/or ions occurs through each of the four pores. The carboxyl and amino termini of an AQP monomer is orientated towards the cytoplasm with six transmembrane α-helices linked by alternating extracellular and intracellular loops (designated A-E). The six α-helices are arranged in a right-handed bundle forming the highly water selective central pore, which resembles an hour-glass (Scheuring et al., 2000).

The pore of mammalian AQPs is widely acknowledged to be constitutively open and highly specific (Walz et al., 2009). Pore specificity involves two selection stages (de Groot and Grubmuller, 2001); the first is due to the fact that intracellular loop B and extracellular loop E fold into the transmembrane pore, each forming a helical region containing a highly conserved Asn-Pro-Ala (NPA) motif. Consequently, passage of the majority of ions, such as Na^+ is prohibited (Wu et al., 2009). The second stage is due to the aromatic/arginine (ar/R) constriction site region and facilitates water selectivity through re-positioning of water molecules and the exclusion of protons (de Groot et al., 2001).

AQPs 0, 1, 2, 4, 5 and 8 are thought to be permeable only to water, whilst AQPs 3, 7, 9 and 10 are known as aquaglyceroporins because they are permeable to both water and small non-ionic molecules such as glycerol, urea and ammonia (de Groot et al., 2003b, de Groot and Grubmuller, 2001, Litman et al., 2009). AQP6 is highly specific for water but may also be permeable to ionic molecules (Beitz et al., 2006). AQPs 11 and 12 are thought to function as water channels but have yet to be fully characterised (Ishibashi, 2009). Notably, the sequence

of the NPA motifs within AQP11 and 12 deviates from the conserved NPA of the other AQPs (Ikeda et al., 2010, Itoh et al., 2005).

It is clear that the structural features of the AQP family and the mechanism of selective water passage through the AQP pore are now established (de Groot et al., 2003a, de Groot and Grubmuller, 2001). However, less information is available describing how AQPs regulate the flow of water into and out of cells in order to meet the constant and rapid changes in local cellular environment that challenge them.

What regulates AQP function?

Eukaryotes have evolved to fine-tune water transport through AQPs by three main regulatory mechanisms: (i) at the transcriptional/translational level (Matsumura et al., 1997); (ii) by conformational change or “gating” (Walz et al., 2009); and (iii) by translocation to the membrane in response to a trigger (Nedvetsky et al., 2009). Regulation by AQP gene expression can be achieved over a timescale fromminutes to days (Gunnarson et al., 2004, Zelenina, 2010). However, a gene expression model for regulation of membrane permeability is contingent upon constitutive membrane expression, which does not account for the established receptor-mediated regulation of AQP2 sub-cellular localisation (Nedvetsky et al., 2009) or the dynamic control of AQPs that may be necessary to rapidly alter membrane water permeability.

Water permeability through the AQP pore can be inhibited by non-specific, usually mercury-based compounds (Savage and Stroud, 2007). Alterations in pH and calcium concentration, through interaction with specific histidine residues within the AQP pore have also been shown to cause different effects on water permeability in bovine and fish AQPs, despite

sharing approximately 70% of their sequence (Nemeth-Cahalan et al., 2004). Regulation via gating mechanisms, which allow conformationally distinct open and closed states, has specifically been reported for plant and microbial AQPs (Tornroth-Horsefield et al., 2006). Structures of gated AQPs have revealed the molecular details of gating by phosphorylation, pH and Ca^{2+} for the spinach aquaporin, SoPIP2;1 (Johansson et al., 1996), and mechanosensivity for the yeast aquaporin, AQY1 (Fischer et al., 2009). Direct phosphorylation has also been suggested to cause an increase in water permeability of AQP1 (Han and Patil, 2000), whereas the water permeability of phosphorylated AQP5 has been suggested to be indistinguishable from that of non-phosphorylated AQP5 (Sidhaye et al., 2005, Woo et al., 2008). The role of gating in the regulation of mammalian AQP function therefore remains to be conclusively established, but it is not a widely-accepted regulatory mechanism for water homeostasis in mammalian AQPs (Wang and Tajkhorshid, 2007).

In contrast to regulation by AQP gene expression, and in the absence of an established gating mechanism, a more dynamic, rapid and selective means of regulation could be achieved by translocation of AQPs: the regulation of membrane permeability is possible by altering AQP abundance in response to a specific trigger. Tables 2 and 3 summarise current knowledge of triggers and mechanisms of post-translational AQP translocation. It is possible that the regulation of basal membrane expression following translation and Golgi processing of nascent AQP proteins shares mechanisms with such post-translational, induced subcellular localisation.

What triggers AQP translocation?

The most well established trigger of post-translational AQP translocation is the G protein-coupled receptor (GPCR) agonist, vasopressin, which induces translocation of AQP2 to

promote water reabsorption in the kidney (Table 2). AQP2 is retained in sub-cellular compartments and translocation occurs as a result of vasopressin binding and activating the vasopressin V_2 GPCR. Subsequently, the G protein, Gs, initiates adenylate cyclase activation leading to increases in cyclic AMP which mediates activation of protein kinase A (PKA) and results in phosphorylation of serine 256 on the C-terminus of AQP2. This facilitates AQP2 translocation to the plasma membrane and water reabsorption in order to maintain osmotic homeostasis (see (Valenti et al., 2005) for review).

A number of other GPCR agonists are also thought to regulate AQP translocation events (Table 2). For example, early studies demonstrated that secretin, a hormone involved in osmoregulation, promotes osmotic water movement in cholangiocytes by inducing exocytic insertion of AQP1 into the cell membrane (Marinelli et al., 1997). Quantitative immunoblotting assays revealed an inverse correlation between the presence of AQP1 at the plasma membrane and within the microsomal intracellular compartment in response to secretin. In the case of AQP3, the beta-adrenergic receptor agonist, isoprenaline, and the adrenergic receptor agonist, adrenaline, have been shown to trigger its translocation in human adipocytes and Caco-2 cells, respectively (Rodriguez et al., 2011b, Yasui et al., 2008). A decrease in cell water permeability was discovered to be linked to the internalisation of AQP4 from the cell surface following histamine stimulation (Carmosino et al., 2007). Recently, it was determined that short term regulation of AQP4 is also mediated by vasopressin; (Moeller et al., 2009) in the presence of vasopressin, a reduction in water permeability was detected. The water permeability of astrocytes has also been shown to be affected by glutamate activation of metabotropic glutamate receptors that may mediate AQP4 translocation (Gunnarson et al., 2008). Understanding how induced translocation affects water permeability of a cell is also likely to be crucial in physiological processes such as maintenance of the

blood brain barrier (Saadoun and Papadopoulos, 2009). Furthermore, the membrane-localisation of AQP4 in astrocytes is a physiological requirement for the formation of cytotoxic brain oedema following stroke, blunt-force trauma or meningitis (Papadopoulos and Verkman, 2007). Finally, AQP5, which has a major role in the generation of saliva, tears and pulmonary secretions, has been shown to translocate to the plasma membrane of rat parotid cells upon stimulation with the M3 muscarinic GPCR agonist, acetylcholine, or the adrenergic receptor agonist, adrenaline (Ishikawa et al., 1999). Internalisation of AQP5 was observed following prolonged exposure to acetylcholine (Ishikawa et al., 1998a, Ishikawa et al., 2002). A 4 h stimulation with the agonist, isoprenaline, was also shown to mediate the translocation of AQP7 from the cytoplasm compartment to the plasma membrane (Rodriguez et al., 2011b). Vasoactive intestinal polypeptide and lipopolysacharide has been shown to trigger the translocation of AQP5 to the plasma membrane of duodenal Brunner's gland cells (Parvin et al., 2005) and mouse lung epithelial cells (Ohinata et al., 2005), respectively.

As AQPs are water channels, it is not surprising that water itself might act as a trigger for AQP translocation: a recent study described how immunofluorescent staining revealed cytoplasmic localisation of AQP3 and its translocation to the cell membrane of cultured, human keratinocytes following a 2 h osmotic stress (Garcia et al., 2011). Hypertonicity has also been shown to trigger the translocation of AQPs 1, 2, 3, 4, 5 and 9 (Arima et al., 2003, Hasler et al., 2005, Hoffert et al., 2000, Loitto et al., 2007, Matsuzaki et al., 2001) whilst hypotonicity has been shown to be involved in translocation of AQPs 1, 3 and 8 (Conner et al., 2012, Conner et al., 2010, Garcia et al., 2011, Qi et al., 2009) (Table 2).

What are the molecular components of triggered translocation mechanisms?

Cytoskeletal proteins

Following transcription and translation, several mechanisms are thought to mediate AQP trafficking. AQPs may traffic within vesicles (Garcia et al., 2001), often via a microtubule network and sometimes with the involvement of the actin cytoskeleton, whereby re-arrangement of the actin network assists in AQP membrane integration (Riethmuller et al., 2008). Once fully integrated into the membrane, newly synthesised AQPs exhibit certain chemical properties. The most noticeable is the clustering of positively charged lysine and arginine residues at the cytoplasmic side of the channel, over the external region. This 'positive inside rule' assists in facilitating insertion into the membrane (Lerch-Bader et al., 2008).

Elements of the mechanisms of post-translational translocation of AQPs may be different from constitutive AQP membrane expression mechanisms, although both actin and microtubules have been implicated in the regulation of the latter. Colchicine-induced disruption of microtubules suggested secretin-induced AQP1 translocation was mediated by the microtubule network (Marinelli et al., 1997). The microtubule network has been further implicated in the induced translocation of AQP1 from intracellular compartments to the cell membrane following diverse stimuli (Conner et al., 2010, Tietz et al., 2006). Studies have determined which proteins and mechanisms regulate AQP1 trafficking in cholangiocytes. Following addition of secretin or activation of messenger cAMP, AQP1 was shown to co-localise with cytoskeletal motor proteins; dynein and kinesin in cholangiocytes. Partial reorganisation of actin upon vesicular contact at the apical membrane was thought to facilitate fusion with the cell membrane (Tietz et al., 2006).

Similarly to studies on AQPs 1 and 2, inhibitors of the microtubule network and actin microfilaments implicated cytoskeletal proteins in the mechanism of AQP5 trafficking to the

plasma membrane (Tada et al., 1999). However, trafficking of AQP5 via microtubules (but not actin filaments) has been reported in Madin-Darby canine kidney cells (Karabasil et al., 2009). Depolymerisation of the F-actin cytoskeleton using cytochalasin-D has also been shown to impair AQP4 plasma membrane localisation (Nicchia et al., 2008). Application of microtubule inhibitor colchicine was discovered to be sufficient to prevent cAMP stimulated translocation, emphasising reliance of AQP8 translocation on a fully functional microtubule network (Koyama et al., 1998).

Calcium

Calcium is ubiquitous in the mechanisms regulating cell signalling and function, so it is not surprising that calcium has been shown to be essential in the mechanism of translocation of AQPs. The role of calcium in trafficking of vesicles containing AQP5 has been demonstrated in human salivary gland cells in which thapsigargin and calcium ionophores induced AQP5 trafficking (Ishikawa et al., 1998a). A putative calmodulin binding site at the N-terminus of AQP6 has also been identified (Rabaud et al., 2009). Calmodulin binding may have a role in the translocation of AQP6 to the cell surface; it is likely that trafficking of the majority of AQPs are stimulated by calcium elevations (Rabaud et al., 2009).

Extracellular calcium influx and calmodulin have been shown to be involved in the hypotonicity-induced translocation of AQP1 to the plama membrane of human embryo kidey cells (HEK) and interestingly, there is a high degree of sequence homology between the AQP1 C-terminus and EF-hands from Ca^{2+}-binding proteins belonging to the calmodulin superfamily (Fotiadis et al., 2002).

Protein kinase A

Protein kinases are key regulators of vesicle and protein trafficking mechanisms (Pearce et al.). The involvement of PKA phosphorylation in the vasopressin-triggered translocation of AQP2 is well documented (Noda and Sasaki, 2006, Procino et al., 2003) and the AGC group of protein kinases is also involved in the regulation of other AQPs (Table 3). For example putative phosphorylation sites in the C-terminus of AQP0 have been implicated in membrane translocation. Studies involving oocyte swelling assays revealed that truncation of the AQP0 C-terminus at residue 243 resulted in a 15% decrease in water permeability compared to full length AQP0, while surface protein expression analysis showed that impaired water permeability was a result of less efficient membrane trafficking. Truncation of the C-terminus at residue 234 or 238 resulted in completely impaired trafficking but interestingly, mutation of seine 235 to alanine had little effect on water permeability (Ball et al., 2003). Serine 235 in the C-terminus of AQP0 has been shown to be located in a PKA consensus sequence. Nuclear magnetic resonance data demonstrated that serine 235 phosphorylation resulted in inhibition of calmodulin binding to AQP0, and this calcium-dependent calmodulin binding to AQP0 resulted in decreased membrane water permeability (Reichow and Gonen, 2008).

AQP4 is particularly highly expressed in the astrocytic glial cells of the brain with a role in maintaining osmotic potential across the blood brain barrier (Pasantes-Morales and Cruz-Rangel, 2010). Crucially, AQP4 knock-out models are protected from cytotoxic brain oedema (Manley et al., 2000) and AQP4 antibodies are thought to be key to the onset of *neuromyelitis optica* (Chan et al., 2012). Fluorescence localisation studies on histamine-mediated AQP4 internalisation have shown AQP4 co-localisation with the mannose-6-phosphate receptor, a protein that assists in intracellular trafficking of lysosomal digestive enzymes from the Golgi sorting apparatus to the lysosome cell surface. During the internalisation period, AQP4 underwent enhanced, specific phosphorylation via PKA; this was thought to be essential for

preventing redirection of AQP4 to the lysosomes. Removal of the stimulus enabled free AQP4 translocation from late endosomes back to the basolateral cell surface after a brief time delay (Carmosino et al., 2007). To confirm whether a vasopressin-triggered decrease in water permeability (Moeller et al., 2009) was due to alterations in a putative gating mechanism or endocytosis of the channel from the plasma membrane, measurements of capacitive currents were performed using a voltage-clamp technique on *Xenopus* oocytes. The gating possibility was rejected and verification of AQP4 endocytosis was obtained via immunoblotting in combination with immunofluorescence analyses. Contrary to the situation for AQP2, vasopressin was found not to mediate translocation of AQP4 from internal stores to the plasma membrane, but to induce internalisation of AQP4 from the cell surface. It was also discovered that vasopressin regulation could be mediated by protein kinase C (PKC), which phosphorylates serine 180 of loop D. This is believed to be the critical residue required for internalisation of AQP4. Interestingly, a second serine residue at position 111 was discovered which antagonised the actions of serine 180 as it serves to increase water permeability when activated by phosphorylation (Gunnarson et al., 2008).

Using calcium ionophores to stimulate AQP5 plasma membrane localisation, it was deduced that activation of M3 muscarinic receptors by acetylcholine, leads to a signalling cascade occurring via intermediates, such as inositol 1,4,5-trisphosphate, triggering release of calcium from intracellular stores (Ishikawa et al., 1998b). Dephosphorylation of a PKA consensus sequence starting at serine residue 152 increased translocation of AQP5 to the membrane of Madin-Darby canine kidney cells (Karabasil et al., 2009). Although serine 156 of AQP5 has been shown to be phosphorylated in human bronchial epithelial cells, AQP5 targeting to the membrane may require additional mechanisms besides cAMP-dependent PKA phosphorylation (Woo et al., 2008). Constitutive membrane localisation of an AQP5-C-

terminal GFP chimera in Madin-Darby canine kidney cells has also been observed, whereas an AQP5-N-terminal GFP chimera was localised in intracellular vesicles. Translocation of the AQP5-N-terminal GFP chimera in response to PKA activation was independent of phosphorylation of the putative PKA phosphorylation residue, threonine 259 (Kosugi-Tanaka et al., 2006). This suggests that the PKA phosphorylation of the C-terminus may not be involved in AQP5 translocation, however AQP5 has shown to be translocated to the plasma membrane in a PKA-dependent fashion in duodenal Brunner's gland tissue (Parvin et al., 2005). The PKA pathway has also been shown to be involved in AQP5 regulation through cAMP induced AQP5 translocation to the plasma membrane of murine lung epithelial cells (Yang et al., 2003).

AQP6 is thought to be retained within intracellular vesicles and recent studies have focused on identifying the mechanism and function of AQP6 retention in intracellular stores (Beitz et al., 2006). Localisation studies on AQP6-GFP chimeras have revealed that residues at the N-terminal sequence of the protein are necessary and sufficient for cytosolic retention. Substitution of the N-terminal sequence on AQP1 for that of AQP6 resulted in intracellular retention of the AQP1/AQP6-N-terminus chimera. A putative AQP6 C-terminal PKA phosphorylation site was also identified but activation studies failed to demonstrate a significant role of this site in AQP6 localisation. Interestingly, continuous expression of AQP6 at the cell surface led to apoptosis of the cell (Beitz et al., 2006).

AQP8 is expressed in a number of tissues but noted for its role in the colon (Liu et al., 2011). The expression and distribution of AQP8 in amnion epithelial cells were regulated by osmotic loads, suggesting a role for AQP8 in intramembranous water transport and the balance of amniotic fluid (Qi et al., 2009). AQP8 expression may also be stimulated to increase at the

cell surface by cAMP in hepatocytes; cells that normally exhibit low water permeability in their resting state due to intracellular sequestration of AQP8 (Garcia et al., 2001). Evidence for this was provided through labelling experiments, which showed an inverse correlation between AQP8 numbers located within microsomes and those on the plasma membrane, with an increase in the latter following stimulation by cAMP (Koyama et al., 1998).

Although AQP8 shares conserved NPA sequences and 6 transmembrane architecture with other members of the aquaporin family (Koyama et al., 1998), it is interesting to note that despite cAMP-induced activation, AQP8 lacked the conserved regions required for phosphorylation via a PKA- or PKC-mediated mechanism. The significance of this points towards the possibility of an intermediate protein that is independent of the PKA pathway, being involved in phosphorylation of AQP8.

Protein kinase C

AQP1 was the first AQP identified (Agre et al., 1993) and has a diverse tissue distribution; including expression in red blood cells, kidney proximal tubule cells and the bile duct. Early studies suggested that AQP1 was constitutively expressed in cell membranes in the kidney (Knepper et al., 1996), however recent studies have shown that AQP1 is expressed in both the cytoplasm and in the membrane and can be induced to undergo rapid and reversible translocation to the plasma membrane upon hypotonic stimulation mediated by PKC and microtubules (Conner et al., 2010). This dynamic mechanism of AQP1 translocation in human embryo kidney cells involves extracellular calcium influx through stretch activated transient receptor potential channels and calmodulin-mediated PKC phosphorylation of two specific threonine residues at positions 157 and 239 (Conner et al., 2012). These residues of AQP1 have previously been shown to be phosphorylated by PKC. Furthermore, stimulation of

PKC resulted in an increase of AQP1-dependent water permeability and this was reduced in AQP1 mutants lacking either threonine 157 or threonine 239 and abolished in an AQP1 mutant lacking both threonine residues (Zhang et al., 2007).

AQP0 is the major protein in the membrane of the lens and has a role in cataract formation. Inhibition of PKC or mutation of serine 235 to alanine resulted in retention of AQP0, but not AQP1, in the cytoplasm suggesting that PKC phosphorylation of serine 235, following translation and ER/Golgi sorting, mediates specific AQP0 targeting to the cell surface (Golestaneh et al., 2008).

The biochemical analysis of epinephrine-induced AQP3-translocation after 60 min in Caco-2 colonic epithelia cells has been described. The use of inhibitors and activators for phospholipase C and PKC has suggested involvement of the PKC pathway (Yasui et al., 2008). Threonine at position 514 in PKC was found to be phosphorylated with time upon the treatment of the cells with epinephrine while the phosphorylation of serine 660 in PKC was shown significantly following 60 min of the treatment. This suggests epinephrine mediated post-translational modification of PKC threonine 514 may act as a signal for translocation of AQP3 to the basolateral domain, and that perhaps phosphorylation of PKC serine 660 activates the process of AQP3 recycling.

AQP9 has greater sequence similarity to AQP3 and AQP7 than other AQPs and it has been suggested that in leukocytes AQP9 may be selective for water and urea but not glycerol (Ishibashi et al., 1998). It is thought to be involved in glycerol metabolism in the liver and adipocytes (Maeda et al., 2009). Potential AQP9 PKC binding or phosphorylation sites have been identified as serine residues 11 and 222 (Loitto et al., 2007). AQP9 has also been shown

to be phosphorylated in human neutrophils. Expression of AQP9 or a phosphomimetic mutation of the putative PKC phosphorylation site, serine 11, to aspartate dynamically localised to the plasma membrane and changed cell volume regulation as a response to hyperosmotic changes. However when serine 11 was mutated to alanine to create a phosphorylation deficient mutant, AQP9 failed to localise to the plasma membrane. AQP9 translocation to the membrane was also shown to be regulated by the G protein Rac1 (Karlsson et al., 2011).

Protein kinase G

The use of specific inhibitors has revealed that the mechanism of acetylcholine-induced AQP5 translocation is mediated by cGMP, calmodulin and protein kinase G (Ishikawa et al., 2002).

Phosphatidylinositol 3-kinase

The aquaglyceroporin, AQP7, is responsible (with AQP3) for the majority of glycerol transport across the membranes of adipose tissue (Rodriguez et al., 2011a) and signalling pathways, including phosphatidylinositol 3-kinase, have been associated with the regulation of aquaglyceroporin transcription (Kishida et al., 2001). Evidence for negative feedback regulation in lipolytic states to restrict glycerol release from fat cells by restricting AQP7 expression comes from treatment of cells with leptin, isoprenaline and carboxymethyl chitin (Rodriguez et al., 2011a). It is also thought that insulin regulates AQP7 levels, but the evidence is contradictory and there may be divergent effects of insulin on the regulation of all aquaglyceroporins in humans compared to rodents (Kishida et al., 2001, Rodriguez et al., 2011b). Whilst it has been proposed that PKA may be involved in the translocation of AQP7 (Kishida et al., 2000), experimental evidence for such a process is currently lacking.

Localisation and retention signals

The AQP0 mouse model Cat^{Fr} synthesises dysfunctional AQP0; AQP0-LTR in which LTR is translated into a peptide and causes retention of AQP0-LTR in sub-cellular compartments of lens epithelial cells producing cataracts (Kalman et al., 2006).

AQP3 is a water/glycerol transporter, generally thought to be expressed in the basolateral membrane of cells. This has been demonstrated for the AQP3-rich, polarised epithelial (Zhang et al.) and kidney collecting duct cells (Langaa et al., 2012). Analysis of the AQP3 protein sequence revealed the presence of a conserved localisation Tyr-Arg-Leu-Leu (YRLL) motif at the N-terminus. Mutations within this YRLL motif of either tyrosine or the di-leucine residues alone led to partial disruption of the protein translocation mechanism *in vitro* in polarised Madin-Darby canine kidney type II cells, whereas a complete mutation of the YRLL motif was presented with the complete absence of protein trafficking. The combined presence of the tyrosine, together with the di-leucine residues is essential for proper localisation at the basolateral domain (Rai et al., 2006). Chimeric proteins involving substitution of the AQP2 N-terminus for that of the AQP3 was able to re-direct localisation of AQP2 from its original site at the apical membrane to its new site at the basolateral membrane. Interestingly, despite this clear amino acid-sorting motif, membrane expression of AQP3 is not polarised in red blood cells (Roudier et al., 1998) or in epidermal keratinocytes and in the epidermis "basal cells"; AQP3 may be predominantly intracellular (Sougrat et al., 2002).

Systematic deletion analyses of C-terminal residues of AQP4 identified a region necessary for correct localisation of AQP4 in epithelial cells (Madrid et al., 2001). Two separate localisation signals have been identified within the C-terminal tail; a specific sequence

containing tyrosine 277 and its adjacent glycine residue was associated with correct localisation of AQP4 to the basolateral membrane. The second region consisted of a combined acidic cluster Glu-Thr-Glu-Asp (ETED) and a Leu-Ile-Leu (LIL) sequence motif, causing reduced localisation at the apical domain. The tyrosine sorting signal Try-Met-Glu-Val (WMEV) doubles its function as a mediator of endocytosis via clathrin-coated vesicles.

AQP11 and 12 appear to lack the conserved NPA motifs, being NPC and NPT in AQPs 11 (Ikeda et al., 2010) and 12 (Itoh et al., 2005), respectively. Interestingly mutation of the alanine of the AQP4 NPA motif to threonine to mimic AQP12 resulted in retention within the endoplasmic reticulum of mammalian cell lines, whereas mutation of this alanine to cysteine, mimicking AQP11, expresses normally in plasma membrane (Guan et al., 2010).

Conclusions: implications for disease therapies.

Identification of the triggers and mechanisms of the dynamic regulation of AQP-containing vesicles from the cytoplasm to the membrane (Figure 2) is the focus of much interest. It is clear that the rapid, regulation of sub-cellular localisation of AQPs can be targeted to specific areas of the cell in order to increase water, ion or small molecule permeability. Manipulation of AQP abundance may therefore provide novel methods for treating specific diseases. For example, AQP4 knockout mice show an increased protection and reduced accumulation of water in the brain in models of ischemic stroke, cerebral injury and meningitis (Manley et al., 2000, Papadopoulos and Verkman, 2005). The opposite is the case for hydrocephalus and vasogenic oedema, where AQP4 null mice show greater water accumulation in the brain and a worse clinical outcome (Papadopoulos et al., 2004). AQP5 is expressed in the ciliated epithelia that line the upper airways and is involved in near-isomolar fluid secretion by the epithelium of airway submucosal glands (Verkman, 2003). Thus, the manipulation of AQP4

and AQP5 translocation and subsequent subcellular localisation appears to be a potential target for the development of novel drugs for cerebral oedema and cystic fibrosis (Song and Verkman, 2001, Zador et al., 2009). AQPs are an extremely important group of proteins and their role in diverse disease states is becoming more evident. Identification of the stimuli and mechanisms of dynamic induced translocation may offer a new avenue for drug targets and eventual disease therapy.

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Table 1: Tissue distribution and functional roles of the 13 human AQPs. The table summarises the distribution and function of AQPs in specific cells and tissues of the human body. Immunohistochemistry has revealed the diversity of AQP distribution within cells and tissues. For example, AQPs 1, 2, 3, 4, 6, 7, 8, 9, 10 and 11 have all been shown to be expressed in tissues of the kidney whereas AQP0 is almost exclusively expressed in lens epithelial cells. AQP11 expression in leucocytes may explain the immunohistochemical identification of AQP11 in other tissues.

AQP	Location	Putative Functional Roles
0	Lens fibre gap junctions	Water regulation, structural function
1	Kidney (proximal renal tubule), eye, CNS, lung, erythrocytes	Water homeostasis, fluid reabsorption
2	Kidney collecting duct	Water homeostasis, reabsorption from urine
3	Kidney, epithelial cells	Water homeostasis in renal collecting duct, glycerol/lipid metabolism
4	Glial cells, (astrocytes), renal collecting duct	Water regulation, cerebral oedema
5	Salivary glands, lung, eye	Facilitates isotonic secretions
6	Renal collecting duct	Intracellular water/ion regulation
7	Adipocytes, kidney, liver, testis	Glycerol/lipid metabolism
8	Liver, kidney, testis, pancreas, heart, colon, placenta	Water homeostasis
9	Kidney, liver, leucocytes, lung, spleen, brain, testis	Water/ small molecule homeostasis, glycerol metabolism
10	Small intestine (duodenum, jejunum), kidney	Water regulation
11	Kidney, testis, brain, pancreas, leucocytes, smooth muscle	Unknown
12	Pancreas	Unknown

Table 2: Triggers of aquaporin translocation to the plasma membrane (PM). The table summarises known stimuli of the dynamic translocation of AQPs, where possible avoiding observations solely linked to water permeability (WP) and/or transcription/translation. The experimental system used to determine the AQP translocation is included for each trigger.

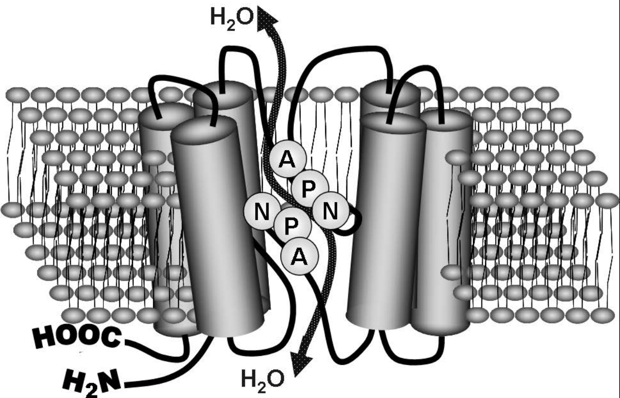
Trigger	AQP	Translocation	Experimental system	Reference
Vasopressin	AQP2	PM	Native kidney tissue	(Christensen et al., 2009)
	AQP4	WP	Xenopus oocytes	(Moeller et al., 2009)
Histamine	AQP4	PM	Human gastric cells	(Carmosino et al., 2007)
Isoprenaline	AQP3	PM	Human adipocytes	(Rodriguez et al., 2011b)
	AQP7	PM	Human adipocytes	(Rodriguez et al., 2011b)
Secretin	AQP1	PM	Cholangiocytes	(Marinelli et al., 1997)
Vasoactive intestinal polypeptide	AQP5	PM	Duodenum (Brunner's gland)	(Parvin et al., 2005)
Adrenaline	AQP3	PM	Caco-2 epithelial cells	(Yasui et al., 2008)
	AQP5	PM	Rat parotid cells	(Ishikawa et al., 1999)
Glutamate	AQP4	PM/WP	Astrocytes	(Gunnarson et al., 2008)
Acetylcholine	AQP5	PM	Rat parotid cells	(Ishikawa et al., 1998b)
Lipopolysaccharide	AQP5	PM	Lung epithelial cells	(Ohinata et al., 2005)
Hypotonicity	AQP1	PM	HEK293/astrocytes	(Conner et al., 2010)
	AQP3	PM	Keratinocytes	(Garcia et al., 2011)
	AQP8	PM	Amnion epithelial cells	(Qi et al., 2009)
Hypertonicity	AQP1	PM	Human neutrophils	(Loitto et al., 2007)
	AQP2	PM	Collecting duct cells	(Hasler et al., 2005)
	AQP3	PM	MDCK cells	(Matsuzaki et al., 2001)
	AQP4	PM	Rat astrocytes	(Arima et al., 2003)
	AQP5	PM	Lung epithelial cells	(Hoffert et al., 2000)
	AQP9	PM	Rat astrocytes	(Arima et al., 2003)

Table 3. Intracellular components involved in the translocation of specific aquaporins to the plasma membrane (PM); The table summarises the components of dynamic translocation of AQPs avoiding, where possible, observations solely linked to water permeability (WP) and/or transcription/translation. Arrows indicate the direction of the effect. The experimental system used to determine the AQP translocation is included for each trigger and the residue that the component acts upon is also included where known. *AQP2 is not added in more detail than PKA-linked as this has been very well reviewed elsewhere (Valenti et al., 2005).

Intracellular component	AQP	Effect	Experimental system	Residue	Reference
PKA	AQP0	↓WP	Collecting duct	S235	(Reichow and Gonen, 2008)
	AQP2*	↑PM	Kidney tissue	S256	(Christensen et al., 2000)
	AQP4	↓PM	Human gastric cells	-	(Carmosino et al., 2007)
	AQP5	↓PM	MDCK	S152	(Karabasil et al., 2009)
	AQP5	↑PM	Brunner's gland	-	(Parvin et al., 2005)
	AQP6	None	MDCK	-	(Beitz et al., 2006)
	AQP7	Putative	n/a	-	(Kishida et al., 2000)
PKC	AQP0	↑PM	RK13 epithelial cells	S235	(Golestaneh et al., 2008)
	AQP1	↑PM	HEK293 cells	T157/T239	(Conner et al., 2012)
	AQP3	↑PM	Caco-2 epithelial cells	T514	(Yasui et al., 2008)
	AQP3	↓PM	Caco-2 epithelial cells	S660	(Yasui et al., 2008)
	AQP4	↑PM	Astrocytes	S111	(Gunnarson et al., 2008)
	AQP4	↓PM	Xenopus oocytes	S180	(Moeller et al., 2009)
	AQP9[1]	↑PM	Human neutrophils	S11/S222	(Karlsson et al., 2011)
PKG	AQP5	↑PM	Rat parotid cells	-	(Ishikawa et al., 2002)
Actin	AQP1	↑PM	Cholangiocytes	-	(Tietz et al., 2006)
	AQP4	↑PM	Astrocytes	-	(Nicchia et al., 2008)
	AQP5	↑PM	Salivary gland cells	-	(Tada et al., 1999)
	AQP5	↑PM	Rat parotid	-	(Ishikawa et al., 1999)
Microtubule	AQP1	↑PM	HEK293 cells	-	(Conner et al., 2010)
	AQP5	↑PM	MDCK cells	-	(Karabasil et al., 2009)
+ cAMP	AQP8	↑PM	Hepatocytes	-	(Garcia et al., 2001)
	AQP5	↑PM	Lung epithelial cells	-	(Yang et al., 2003)
Ca2+/ Calmodulin	AQP1	↑PM	HEK293 cells	-	(Conner et al., 2012)
	AQP5	↑PM	Rat parotid cells	-	(Ishikawa et al., 1998a, Ishikawa et al., 1999,
	AQP6	↑PM	CHO-K1 cells	-	Rabaud et al., 2009)
TRP channels	AQP1	↑PM	HEK293 cells	-	(Conner et al., 2012)
RAC1	AQP9	↑PM	Human neutrophils	-	(Karlsson et al., 2011)
Dynein	AQP1	↑PM	Cholangiocytes	-	(Marinelli et al., 1997)
Kinesin	AQP1	↑PM	Cholangiocytes	-	(Marinelli et al., 1997)

Figure 1: Schematic diagram of the shared structural architecture of aquaporins. Six transmembrane α-helices are connected by alternating extracellular and intracellular loops with the carboxyl and amino termini orientated towards the cytoplasm. Two of these connecting loops (B and E) fold into the transmembrane pore each forming a helical region containing highly conserved Asn-Pro-Ala (NPA) motifs that facilitate selectivity of the pore.

Figure 2: Trigger-induced aquaporin translocation: a regulatory mechanism for cellular water flow. Some or all of the components shown may be involved in the translocation of different AQPs. For example, AQP1 translocation is known to be triggered by hypotonicity, which causes calcium influx through transient receptor potential (TRP) channels and subsequent calmodulinmediated PKC phosphorylation of specific AQP1 threonine residues, resulting in microtubule-dependent AQP1 translocation. Other protein kinases are thought to be involved in AQP translocation such as PKAmediated translocation of AQP2 and AQP5 following activation of vasopressin V_2 and M3 muscarinic GPCRs, respectively.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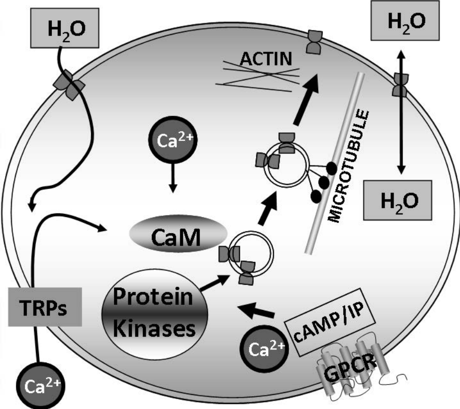
A
P
N
N
P
A
HOOC
H_2N
H_2O



H_2O
H_2O
ACTIN
Ca^{2+}
MICROTUBULE
H_2O
CaM
Protein
Kinases
TRPs
cAMP/IP
Ca^{2+}
GPCR
Ca^{2+}