Regulation of Selectivity and Translocation of Aquaporins: an Update

(aquaporins / vasopressin / PKA / Rho GTPase)

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Abstract. All living beings need to solve the problem of controlled transport of water. To this purpose, a special group of integral membrane proteins called aquaporins has evolved. There are 13 known members of this family that act as channels for water and small solutes, such as glycerol and urea. Although they allow large flux of water, they successfully prevent passage of protons. Here, we present the review of the data from the literature on the selectivity mechanism of aquaporins. The regulation of aquaporin activity occurs through regulation of expression of their genes, changing the localization of the already existing proteins in the cells and direct regulation of the activity *in situ*. We present the review of new data on the mechanisms of direct regulation. Special emphasis is on the advances in comprehension of aquaporin-2 translocation in collecting tubule cells of the kidney. Four elements of this process are described: 1) the role of protein kinase A and phosphorylation of serine 256 on aquaporin-2, 2) the transport of vesicles along the microtubules toward the apical membrane, 3), the removal of cytoskeletal subapical obstruction and the role of Rho GTPase and ezrin-radixin-moesin proteins in this, and 4) elevation of the cytosolic Ca^{2+} concentration, the fusion of the vesicle with the apical membrane and the role of SNARE proteins in exocytosis.

Introduction

All terrestrial organisms need to solve the problem of controlled elimination, i.e. preservation of water in the body. In addition, the problem is complicated by the existence of the structures on the body surface that must remain wet, although water constantly evaporates from them (cornea and conjunctiva, as examples of the visible ones). To this purpose, a large group of membrane proteins called major intrinsic proteins has evolved in all groups of organisms to control the transport of water across the cellular membranes (Zardoya, 2005). In this way, these proteins become involved not only in the exchange of water with the environment, but also in processes that determine the distribution of water between the intracellular and the extracellular compartment in various tissues. Specific distribution of different protein subtypes on the adjacent membranes allows them to complement each other's function. Therefore, the disruption of these mechanisms can bear serious consequences to water homeostasis.

All major intrinsic proteins can be divided into two large groups, according to "substrate" specificity: water-selective aquaporins and aquaglyceroporins that allow passage of small solutes, such as glycerol and urea. So far, there are 13 members of the family in animals. The classic water-selective aquaporins are AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8 (King et al., 2004). The second group consists of AQP3, AQP7, AQP9 and AQP10. AQP11 cannot be classified as either, because no transport function was shown (Gorelick et al., 2006). In addition, AQP12 is intracellular and its function could not be tested (Ishibashi, 2006). The largest share of aquaporin research goes in four directions: definition of their structure, determination of their spatial and temporal distribution, role in different conditions and diseases and, only recently, regulation of their activity (Agre et al., 2002; Hedfalk, et al. 2006). The last area, well defined in ion channels, still remains largely unknown in aquaporins. In relation to this is the unpleasant fact that no laboratory, let alone therapeutically applicable regulators of their activity exist, although there are substances that can poison them. We will describe the structural properties of aquaporins and their relation to water permeability.

Received September 20, 2006. Accepted October 19, 2006

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Abbreviations: ADH – antidiuretic hormone, vasopressin; AKAP – A-kinase anchoring protein; AQP – aquaporin; cAMP – 3'-5'-cyclic adenosine monophosphate; ERM – ezrin-radixin-moesin proteins; NDI – nephrogenic diabetes insipidus; NPA – asparagin-proline-alanine; NSAIDs – non-steroidal anti-inflammatory drugs; PKA – protein kinase A; RK – Rho-associated kinase; RyR – ryanodine receptors; SNARE – SNAP receptors.

General part and structure

The first aquaporin to be discovered was AQP1, which has 269 amino acid residues (Preston and Agre 1991). The structure of aquaporins is described by the "hour-glass" model, which was initially assumed indirectly and later confirmed by visualization of their structure using high resolution x-ray diffraction (Ren et al., 2001). Based on hydrophobicity plot, it was concluded that the protein traverses the membrane six times, so both amino and carboxy terminal are, therefore, at the same, intracellular side. The transmembrane segments are not normal to the surface of the membrane. Instead, the angle between them and the membrane is unusual (almost 30°), thus forming the hour-glass shape (Fujiyoshi et al., 2002). Aquaporins have a symmetric structure (the first three transmembrane domains and the connecting loops are symmetric to the other half, as if rotated by 180°). Interesting reorientation occurs during AQP1 synthesis in endoplasmic reticulum: after the synthesis of transmembrane segments 4–6, transmembrane segment 3 undergoes retrograde translocation that allows the protein to assume its predicted six-transmembrane segment topology (Lu et al., 2000), otherwise it would have less segments. The loop connecting the second and third (loop B) and the loop connecting the fifth and sixth transmembrane segments (loop E) have three amino acids that in all aquaporins are the same, namely asparagin-proline-alanine (the so-called NPA motif). Electron crystallography analysis of AQP1 isolated in nearly physiological conditions (in ice, whereas in other studies aqua(glycero)porins were isolated in the presence of sugars) showed how the channel looks like. It is approximately 0.18 nm long, widening at both orifices and having the narrowest region exactly halfway. At this point, the channel bends for 25°: it corresponds to the location of NPA motifs (Ren et al., 2001).

Water transport through this type of aquaporins is reversibly inhibited by $HgCl_2$, and mercapto-compounds recover the channel activity (Jung et al., 1994). Of the four cysteines in AQP1, three of them (residues 87, 102, and 152) may be replaced without obvious changes in water permeability. On the other hand, a mutant in which the fourth cysteine was replaced, C189S AQP1, could no longer be inhibited by mercury. Therefore, Cys-189 is the amino acid that conveys this mercury sensibility, as a part of loop E, which immediately precedes the NPA motif. Those aquaporins that don't have cysteine at that position will not be sensitive to mercury.

The aquaporins are organized as tetramers. It was assumed that previously mentioned cysteines may be used to create disulfide bonds among adjacent monomers (Preston and Agre 1991). However, the electrophoretic mobility does not change under the influence of reducing agents, so the monomers are not interconnected (Denker et al., 1988). Each monomer is functional by itself (Shi et al., 1994). Opposite to all other channels known so far, in which the subunits make just their portion of the pore, here every monomer has its own, functional, water channel.

"Thee shall not pass"

Although the molecule has symmetry, its channel does not (Kong and Ma, 2001). At the entrance, the channel widens, being tiled with hydrophobic amino acids. For this reason, the bonds between water molecules themselves are stronger than possible interactions with the channel wall, and water molecules are easily directed towards the channel constriction. However, it is wide enough to allow passage of molecules only one by one, in a single file.

On the way through the channel, the pore narrows to 0.3 nm. The walls of the pore are made of hydrophobic amino acids of the transmembrane segments 1, 2, 4 and 5. On the site of the largest constriction, there are polar amino acids Asn-76 and Asn-192 (the latter is the one from the NPA motif). A part of both loops B and E acts as a dipole, with their positive ends directed to the inner constriction site. Above it is another, 0.28 nm wide constriction surrounded by residues Phe-56, His-180, Cys-189 (the mercury inhibition site), and Arg-195. It is called ar/R constriction (de Groot, et al. 2001). Arg-195 (which is positively charged at physiological pH) is almost absolutely preserved in all aquaporins. In addition, His-180 is neutral at physiological pH, but when pH is decreased, His-180 becomes positive, thus further adding more positive charge to the site of obstruction.

Proton exclusion

The interesting issue was how aquaporins have such enormous water flux, while at the same time they are absolutely able to exclude its ionized form, H_3O^+. If the protons were allowed to do this along the water molecules on their way through the aquaporin channel, this would be highly detrimental to the cell homeostasis.

In the free solution, water molecules are connected by hydrogen bonds. Simultaneously, a small share of molecules is in the form of H_3O^+ ions. Here, it is important to emphasize that it is not simply a proton, but a molecule with added charge. The protons from hydronium ions can switch between adjacent water molecules, but only if they are connected by hydrogen bonds, which is called a Grotthuss or "proton wire" mechanism (Pomes and Roux 1996), analogous to a bucket brigade (Kozono et al., 2002).

There are several explanations for H_3O^+ exclusion. In one study, molecular dynamics simulations showed that the water molecule rotates for 180° when it passes the NPA motif. This brings it in such position that it cannot make hydrogen bonds with both water molecules ahead and behind it. Therefore, the line ("bucket brigade") is broken and protons cannot "jump" through

the channel (Tajkhorshid et al., 2002). However, as much as this concept of well-ordered gymnastic activity of water molecules seems attractive, it was recently challenged by a model that provides a more simplified view (de Groot et al., 2003; Chakrabarti et al., 2004).

The consensus conclusion is that electrostatic interactions, rather than proton wire interruption effects, are the dominant mechanism of proton exclusion in aquaporins. First, the size of the pore makes it impossible for larger molecules to reach the channel. Aquaglyceroporins have slightly less bulky amino acids here, so the diameter of their pore is slightly larger, allowing glycerol and other small solutes to pass (Fujiyoshi et al., 2002).

Second, the proton barrier is created by electrostatic field generated by the protein matrix within the NPA region, the point at which the positive N-terminal ends of loop B and E dipoles converge (de Groot et al., 2003); even if the "proton wire" were uninterrupted, the protons could not 'climb' the high energy barrier. Importantly, above the NPA motif is another constriction composed of amino acids Phe-56, His-180, Cys-189, and Arg-195. Mutations that eliminate positive charges of Arg-195 and His-180 bring about the cation current proportional to pH gradient, i.e. permeability to protons (Beitz et al., 2006). Apparently, Phe-56 serves to block larger molecules, because when it is mutated, glycerol and urea pass through the channel. Therefore, it seems that this site rather serves to negatively select other molecules than to positively select water. As the quantitative immunoblots indicate that each erythrocyte contains 120,000-160,000 copies of AQP1, this high selectivity is more than necessary (Denker et al., 1988).

Regulation of AQP2 membrane expression

Although long-term regulation of aquaporins (Terris et al., 1996; Marples et al., 1999) and specifically long-term regulation of AQP2 by ADH exists (Hasler et al., 2002; Kang et al., 2004; van Balkom et al., 2004), it is not the subject of this review.

We shall present the review of the data from the literature about the mechanism by which translocation of the already present AQP2 occurs, from the intracellular sites to the apical membrane of the collecting duct cells. This occurs because ADH acts on the collecting duct cells through binding to the V_2 receptor (Birnbaumer et al., 1992). There are at least four events that occur as a consequence of this: phosphorylation of the AQP2 molecules, translocation of the vesicles to the apical membrane, removal of the cytoskeleton obstruction below the apical membrane, and exocytosis of the vesicles. In addition, there is a process of removal of AQP2, which has its own independent regulation, i.e. is not simply a reversal of ADH-initiated events. The final amount of apical AQP2 will depend on the equilibrium between membrane insertion by exo- and removal by endocytosis.

Moving along the tracks

It has been shown that microtubules have fast-growing plus ends and slow-growing minus ends. The point from which a microtubule grows is its nucleation site, and the plus end grows away from it. This gives a hint to molecular motors that pull organelles along microtubules of what the correct polarity of the microtubule is (Buendia et al., 1990). In epithelial cells, they are aligned connecting the apex and the base of the cell, but in the way that the minus end is oriented towards the apical domain (Bacallao et al., 1989). This asymmetry of microtubule organization in epithelial cells presents a unique opportunity for efficient polarized vesicle targeting (Musch, 2004). By association with minus end-directed motors, post-Golgi exocytic and postendocytic vesicles would be directed towards the microtubular minus ends facing the cell apex. Dyneins are the molecular motors that pull their cargo to the minus end of the microtubule. Principal cells of collecting tubules contain components of the dynein holoenzyme and they are co-localized with AQP2-containing vesicles, supporting the idea that the energy for vesicle pulling in collecting duct cells is provided by them (Marples et al., 1998).

Phosphorylation

Human AQP2 contains one phosphorylation consensus sequence for PKA (Ser-256) (van Balkom et al., 2002). However, a basal level of phosphorylated AQP2 exists; even without ADH stimulation both AQP2 molecules at the apical membrane and those in vesicles were phosphorylated in the resting state (Christensen, Zelenina et al. 2000), but this resting level of phosphorylation by itself was not sufficient to cause apical insertion of the channel (Fushimi et al., 1997). This shows that phosphorylation is a necessary, but not sufficient step, i.e. that ADH has other actions that help transfer the AQP2. This short-term control, therefore, only re-localizes AQP2, and PKA phosphorylation has only permissive or facilitatory action on the primed vesicles. AQP2 is a homotetramer. In order to be transferred to the cell surface, three or all four of its subunits need to be phosphorylated (Kamsteeg et al., 2000).

Although phosphorylation is an obligatory step in translocation of the vesicles to the membrane, dephosphorylation is not necessary for the endocytosis that follows (Noda and Sasaki, 2005). PGE2 stimulates elimination of AQP2 from the membrane without changing its phosphorylation state (Zelenina et al., 2000).

PKA and AKAPs

A recurring property of complex signal transduction systems is that signalling molecules are closely associated (Hunter, 1995) and the signalling mechanism of

AQP2 translocation is no exception. It is believed that PKA is anchored to the cellular membrane by A-kinase anchoring proteins (AKAPs), which both raises the local concentration of the enzyme and localizes it in the close proximity of its targets (Johnson et al., 1994), therefore acting as efficient means to control the spatiotemporal resolution of cAMP-responsive phosphorylation events (Gao et al., 1997).

In order to bring PKA close to the AQP2 phosphorylation site, AKAPs are used. Lately, a new isoform of AKAP18, AKAP18δ was isolated in principal cells (Henn et al., 2004). AQP2 and AKAP18δ co-localize in vesicles of principal cells and co-translocate to the plasma membrane in response to ADH stimulation.

Exocytosis

The release of the vesicle containing the protein cargo is generally similar in different tissues, but is best described in neural tissue to explain exocytosis of neurotransmitter-containing vesicles (Pfeffer, 1999).

The role of SNARE proteins in exocytosis of AQP2-containing vesicles was shown: VAMP-2 and AQP2 co-localize in their membrane (Nielsen et al., 1995). This was shown in principal, but not in intercalated cells of collecting tubules. To show that AQP2 exocytosis depends on SNARE proteins, the tetanus toxin was used to cleave VAMP-2. Its application almost totally abolishes the effects of cAMP, but the resting level of apical AQP2 is not influenced. This indicates that the constitutive delivery of AQP2 may be VAMP-2 independent (Gouraud et al., 2002).

The second component of the system is syntaxin-4. Opposite to its action on AQP2, ADH does not influence distribution of syntaxin-4 between low-speed and high-speed fractions, i.e. syntaxin-4 is always in the apical cellular membrane (Mandon et al., 1996). In addition, the presence of the third component necessary for the whole system to function, SNAP-23, was shown. However, it was present not only in the apical membrane, as expected, but in the vesicle membrane as well (Inoue et al., 1998).

Therefore, all components of the SNARE system are present, and their functional involvement in vesicle fusion was proved, but how does V_2 receptor activation present the final trigger, the rise in cytosolic $[Ca^{2+}]$?

Elevation of cytosolic Ca^{2+} concentration by ADH

For a long time, it was well-known that vasopressin V_2 receptor activation is linked to both adenylyl cyclase activation and calcium mobilization (Champigneulle et al., 1993). Importantly, the calcium rise does not occur by a phosphatidylinositol pathway (Chou et al., 1998).

Increase in $[Ca^{2+}]$ in inner medullary collecting duct cells occurs in the absence of extracellular Ca^{2+} and is inhibited by ryanodine. Therefore, it is believed that vasopressin activates ryanodine receptors (RyR) on endoplasmic reticulum of these cells. It is RyR1 that is located predominantly in the apical region of the medullary collecting duct cells (Chou et al., 2000). This could explain how the V_2 activation produces the final assembly of SNARE proteins and vesicle exocytosis.

Rho GTPases and cytoskeleton remodelling

All cells, and epithelial cells in particular, have a cytoskeleton that provides their shape and polarity. Actin fibres in the subapical cytoskeleton negatively regulate AQP2 targeting, forming a physical barrier that must be removed for vesicular fusion to occur (Tamma et al., 2005). It has been shown that substances that disrupt actin polymerization, such as latrunculin or cytochalasin D, prevent the vasopressin effect (Tamma et al., 2001; Chou et al., 2004). However, it was also shown that vasopressin itself depolymerizes actin fibres (Simon et al., 1993). This could seem confusing, but then it was shown that the vasopressin effect is restricted only to the subapical region (Valenti et al. 2000; Klussmann et al., 2001), even leading to remodelling of the cell's shape, thus opening way to the AQP-2-containing vesicles.

Rho proteins belong to the family of small GTPases. Normally, the action of Rho is regulated by its binding of GTP, which activates them, followed by its hydrolysis (due to Rho's innate GTPase activity) and release (Van Aelst and D'Souza-Schorey, 1997). After cAMP-induced PKA activation, Rho is phosphorylated at Ser-188 (Lang et al., 1996). This inhibits Rho, by interfering with its association with downstream effectors (Dransart et al., 2005). Downstream action of Rho is mediated by Rho-associated kinase that phosphorylates target proteins.

Rho is involved in the regulation of F-actin polymerization: when Rho and its associated downstream effector proteins are active, polymerization is stimulated (Van Aelst and D'Souza-Schorey, 1997). Some of the toxins synthesized by clostridial bacteria act by inhibiting Rho GTPases (Voth and Ballard, 2005). More specifically, *Clostridium limosum*-derived C3-fusion toxin specifically inhibits Rho (Barth et al., 1998). In the cells transfected with AQP2, inhibition of the small GTPase system by this bacterial toxin results in recruitment of AQP2 to the apical cell membranes in the absence of ADH or other cAMP-elevating agents (Tamma et al., 2001). In addition, when it is phosphorylated by PKA in the presence of cAMP-inducing agents, Rho binds less tightly to Rho-associated kinase (Dong et al., 1998). However, it is still unknown what connects these events to cytoskeleton rearrangement.

Members of the ezrin-radixin-moesin (ERM) family of proteins are involved in functional and

structural organization of cytoskeletal elements (actin filaments) and membrane adhesion proteins. This is manifested by their structure, because their COOH terminus contains an actin-binding domain, whereas the NH_2 terminus contains domains for interaction with integral membrane proteins (Tsukita et al., 1997). They exist in two states: soluble (inactive) and membrane-bound (active). In the inactive state (either as monomers or oligomers), these proteins have conformation in which the COOH and NH_2 termini are folded, thus preventing interaction with regulatory molecules (Gary and Bretscher, 1995). This auto-inhibition is relieved upon change in conformation and translocation to the cellular membrane. This requires phosphorylation (of Thr-558 on moesin) by different kinases (Gautreau et al., 2000), among them Rho-activated kinase (Yonemura et al., 2002).

A peptide that is similar to one of the ERM proteins, moesin, has been synthesized. Importantly, this peptide contains the equivalent of Thr-558 of moesin. Application of this peptide competitively presents one more phosphorylation site to endogenous kinases that would otherwise phosphorylate moesin. Application of this peptide caused the same events as the application of cAMP-inducing agent forskolin: reduction of phosphorylated moesin, its translocation and actin depolymerization (Tamma et al., 2005).

Therefore, cAMP-inducing agents have antagonistic effects on Rho in regulating cellular morphology, and this branch of ADH-initiated events could be summarized as follows: after V_2 receptor activation and cAMP increase, PKA phosphorylates Ser-188 of Rho to inhibit the association of Rho with Rho-activated kinase. This decreases phosphorylation of Thr-558 on moesin (and probably corresponding consensus sites at other members of the ERM family as well), thus reverting them to inactive state. When inactivated, moesin no longer preserves actin in polymerized state below the apical membrane of the collecting duct cell. When the actin fibres are removed, the approach of AQP2-bearing vesicles to the membrane is no longer obstructed and they can fuse with it by exocytosis, regulated by another branch of V_2-regulated event. The process of vesicle transport and release is summarized in Fig. 1.

Concluding remarks

The information about AQP regulation continues to accumulate. In the last few years, we have witnessed major advances in identification of new members of this large family, their visualization in more and more

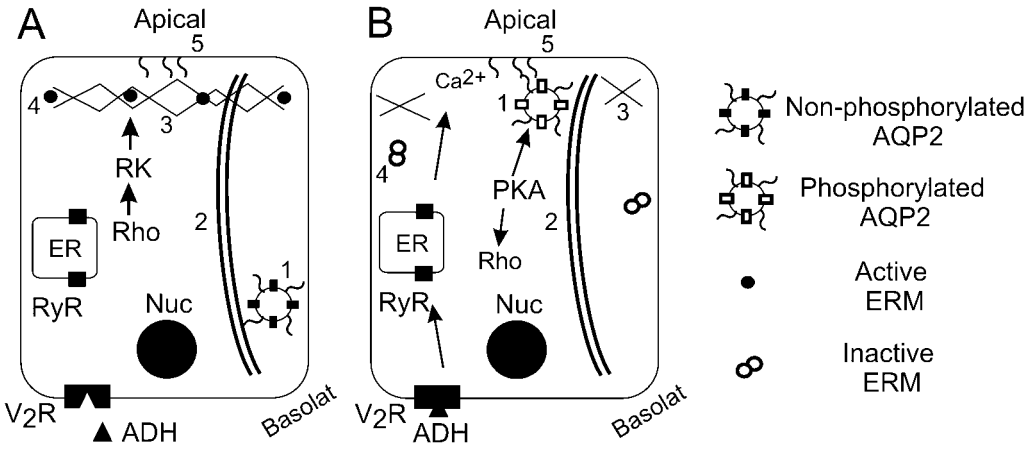

Fig. 1. A) In non-stimulated state, when vasopressin (ADH) is not bound to V_2 receptor (V2R), vesicles containing non-phosphorylated AQP2 (1) are located within the cell. They do not move along microtubules (2) to reach the apical membrane, because they are blocked by subapical actin meshwork (3). These fibres are held in place by interaction with active ERM proteins (4). What keeps them active is Rho GTPase (Rho), which stimulates Rho-associated kinase (RK). In turn, RK phosphorylates ERM proteins. Therefore, AQP2-bearing vesicles cannot release their content by exocytosis, although SNARE proteins (5) are ready to start the process. B) After stimulation, ADH initiates two signalling cascades. In the first cascade, activation of V_2R is coupled to activation of adenylyl cyclase that synthesizes cAMP (not shown in the figure). This leads to activation of PKA that phosphorylates AQP2 and Rho GTPase. Phosphorylation of AQP2 is a permissive step in its release, because it is necessary, but not sufficient to allow fusion of the vesicles with the apical membrane. The latter event, phosphorylation of Rho GTPase, leads to its dissociation from Rho-associated kinase. After that, ERM proteins (4), which previously acted to preserve the subapical actin meshwork, are no longer active, and the actin fibres (3) start to depolymerize. This allows unobstructed translocation of the AQP2-containing vesicles to the apical membrane. The second cascade leads to the activation of ryanodine receptors (RyR) located on the membrane of endoplasmic reticulum, producing the rise in cytosolic [$Ca2^+$]. This triggers the terminal step in vesicle fusion during exocytosis (5), mediated by appropriate assembly of SNARE proteins (not shown in the figure, described in the text in more detail).

detail, regulation of their genes, the mechanisms of their intracellular movement and, particularly, of regulation of their activity. The deepening knowledge in this area could, in foreseeable future, indicate the steps in control of aquaporin activity that could be therapeutically employed. In addition, the genetic analyses are an extremely useful diagnostic tool in early diagnosis of diseases caused by innate mutations in aquaporin structure, such as those leading to diabetes insipidus. More and more mutations causing various forms of NDI are identified in families throughout the world (Pasel et al., 2000; Lin et al., 2002). By deciphering the problem of AQP2 synthesis and/or expression, the point of obstruction could be overcome and functionality restored. Indeed, this was shown to occur, indicating a potential therapeutic use (Tamarappoo and Verkman, 1998). Another example for the application of this newly acquired knowledge is the advance in the treatment of heart failure. Overactivity in V_2R signalling can lead to inappropriate volume expansion that adversely affects ventricular remodelling (Goldsmith, 2006). Therefore, there is a therapeutic rationale in trying to reverse these events. In addition, knowledge about another branch of the family, aquaglyceroporins, had equally rapid development, indicating their importance in a big problem of modern societies: obesity, particularly when it is related to non-insulin-dependent diabetes mellitus (Kishida et al., 2001). This is because, by regulating the entry and release of glycerol, aquaglyceroporins become involved in the regulation of adipocyte size and, therefore, the total amount of fat tissue (Hara-Chikuma et al., 2005). Hopefully, in the years to come we shall see the practical application of these recent advances.

References

Agre, P., King, L. S., Yasui, M., Guggino, W. B., Ottersen, O. P., Fujiyoshi, Y., Engel, A., Nielsen, S. (2002) Aquaporin water channels – from atomic structure to clinical medicine. *J. Physiol. (Lond.)* **542**, 3-16.

Bacallao, R., Antony, C., Dotti, C., Karsenti, E., Stelzer, E., Simons, K. (1989) The subcellular organization of Madin-Darby canine kidney cells during the formation of a polarized epithelium *J. Cell Biol.* **109**, 2817-2832.

Barth, H., Hofmann, F., Olenik, C., Just, I., Aktories, K. (1998) The N-terminal part of the enzyme component (C2I) of the binary Clostridium botulinum C2 toxin interacts with the binding component C2II and functions as a carrier system for a Rho ADP-ribosylating C3-like fusion toxin. *Infect. Immun.* **66**, 1364-1369.

Beitz, E., Wu, B., Holm, L. M., Schultz, J. E., 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. USA* **103**, 269-274.

Birnbaumer, M., Seibold, A., Gilbert, S., Ishido, M., Barberis, C., Antaramian, A., Brabet, P., Rosenthal, W. (1992) Molecular cloning of the receptor for human antidiuretic hormone. *Nature* **357**, 333-335.

Buendia, B., Bre, M., Griffiths, G., Karsenti, E. (1990) Cytoskeletal control of centrioles movement during the establishment of polarity in Madin-Darby canine kidney cells. *J. Cell Biol.* **110**, 1123-1135.

Chakrabarti, N., Tajkhorshid, E., Roux, B., Pomes, R. (2004) Molecular basis of proton blockage in aquaporins. *Structure* **12**, 65-74.

Champigneulle, A., Siga, E., Vassent, G., Imbert-Teboul, M. (1993)V_2-like vasopressin receptor mobilizes intracellular Ca^{2+} in rat medullary collecting tubules. *Am. J. Physiol. Renal Physiol.* **265**, F35-45.

Chou, C.-L., Rapko, S. I., Knepper, M. A. (1998) Phosphoinositide signaling in rat inner medullary collecting duct. *Am. J. Physiol. Renal Physiol.* **274**, F564-572.

Chou, C.-L., Yip, K.-P., Michea, L., Kador, K., Ferraris, J. D., Wade, J. B., Knepper, M. A. (2000). Regulation of aquaporin-2 trafficking by vasopressin in the renal collecting duct. Roles of ryanodine-sensitive Ca^{2+} stores and calmodulin. *J. Biol. Chem.* **275**, 36839-36846.

Chou, C.-L., Christensen, B. M., Frische, S., Vorum, H., Desai, R. A., Hoffert, J. D., de Lanerolle, P., Nielsen, S., Knepper, M. A. (2004) Non-muscle myosin II and myosin light chain kinase are downstream targets for vasopressin signaling in the renal collecting duct. *J. Biol. Chem*. **279**, 49026-49035.

Christensen, B. M., Zelenina, M., Aperia, A., Nielsen, S. (2000) Localization and regulation of PKA-phosphorylated AQP2 in response to V_2-receptor agonist/antagonist treatment. *Am. J. Physiol. Renal Physiol.* **278**, F29-42.

de Groot, B. L., Engel, A., Grubmuller, H. (2001) A refined structure of human aquaporin-1. *FEBS Lett.* **504**, 206-211.

de Groot, B. L., Frigato, T., Helms, V., Grubmuller, H. (2003) The mechanism of proton exclusion in the aquaporin-1 water channel. *J. Mol. Biol.* **333**, 279-293.

Denker, B., Smith, B., Kuhajda, F., Agre, P. (1988) Identification, purification, and partial characterization of a novel Mr 28,000 integral membrane protein from erythrocytes and renal tubules. *J. Biol. Chem.* **263**, 15634-15642.

Dong, J.-M., Leung, T., Manser, E., Lim, L. (1998) cAMP-induced morphological changes are counteracted by the activated RhoA small GTPase and the Rho kinase ROKα. *J. Biol. Chem.* **273**, 22554-22562.

Dransart, E., Olofsson, B., Cherfils, J. (2005) RhoGDIs revisited: novel roles in Rho regulation. *Traffic* **6**, 957-966.

Fujiyoshi, Y., Mitsuoka, K., de Groot, B. L., Philippsen, A., Grubmuller, H., Agre, P., Engel, A. (2002) Structure and function of water channels. *Curr. Opin. Struct. Biol.* **12**, 509-515.

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.

Gao, T., Yatani, A., Dell'Acqua, M. L., Sako, H., Green, S. A., Dascal, N., Scott, J. D., Hosey, M. M. (1997) cAMP-dependent regulation of cardiac L-type Ca^{2+} channels requires membrane targeting of PKA and phosphorylation of channel subunits. *Neuron* **19**, 185-196.

Gary, R., Bretscher, A. (1995) Ezrin self-association involves binding of an N-terminal domain to a normally masked C-terminal domain that includes the F-actin binding site. *Mol. Biol. Cell* **6**, 1061-1075.

Gautreau, A., Louvard, D., Arpin, M. (2000) Morphogenic effects of ezrin require a phosphorylation-induced transition from oligomers to monomers at the plasma membrane. *J. Cell Biol.* **150**, 193-204.

Goldsmith, S. R. (2006) Is there a cardiovascular rationale for the use of combined vasopressin V1a/V2 receptor antagonists? *Am. J. Med*. **119**, S93-S96.

Gorelick, D. A., Praetorius, J., Tsunenari, T., Nielsen, S., Agre, P. (2006) Aquaporin-11: a channel protein lacking apparent transport function expressed in brain. *BMC Biochem.* **7,** 14.

Gouraud, S., Laera, A., Calamita, G., Carmosino, M., Procino, G., Rossetto, O., Mannucci, R., Rosenthal, W., Svelto, M., Valenti, G. (2002) Functional involvement of VAMP/synaptobrevin-2 in cAMP-stimulated aquaporin 2 translocation in renal collecting duct cells. *J. Cell Sci.* **115**, 3667-3674.

Hara-Chikuma, M., Sohara, E., Rai, T., Ikawa, M., Okabe, M., Sasaki, S., Uchida, S., Verkman, A.S. (2005) Progressive adipocyte hypertrophy in aquaporin-7-deficient mice: adipocyte glycerol permeability as a novel regulator of fat accumulation. *J. Biol. Chem.* **280**, 15493-15496.

Hasler, U., Mordasini, D., Bens, M., Bianchi, M., Cluzeaud, F., Rousselot, M.,. Vandewalle, A, Feraille, E., Martin, P.-Y. (2002) Long term regulation of aquaporin-2 expression in vasopressin-responsive renal collecting duct principal cells. *J. Biol. Chem.* **277**, 10379-10386.

Hedfalk, K., Tornroth-Horsefield, S., Nyblom, M., Johanson, U., Kjellbom, P., Neutze, R. (2006) Aquaporin gating. *Curr. Opin. Struct. Biol. Memb. Eng. Des.* **16**, 447-456.

Henn, V., Edemir, B., Stefan, E., Wiesner, B., Lorenz, D., Theilig, F., Schmitt, R., Vossebein, L., Tamma, G., Beyermann, M., Krause, E., Herberg, F. W., Valenti, G., Bachmann, S., Rosenthal, W., Klussmann, E. (2004) Identification of a novel A-kinase anchoring protein 18 isoform and evidence for its role in the vasopressin-induced aquaporin-2 shuttle in renal principal cells. *J. Biol. Chem.* **279**, 26654-26665.

Hunter, T. (1995) Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell* **80**, 225-236.

Inoue, T., Nielsen, S., Mandon, B., Terris, J., Kishore, B. K., M. A. Knepper, M. A., (1998) SNAP-23 in rat kidney: colocalization with aquaporin-2 in collecting duct vesicles. *Am. J. Physiol. Renal Physiol.* **275**, F752-760.

Ishibashi, K. (2006) Aquaporin subfamily with unusual NPA boxes. *Biochim. Biophys. Acta* **1758**, 989-993.

Johnson, B. D., Scheuer, T., Catterall, W. A. (1994) Voltage-dependent potentiation of L-type Ca^{2+} channels in skeletal muscle cells requires anchored cAMP-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* **91**, 11492-11496.

Jung, J., Preston, G., Smith, B., Guggino, W., Agre, P. (1994) Molecular structure of the water channel through aquaporin CHIP. The hourglass model. *J. Biol. Chem.* **269**, 14648-14654.

Kamsteeg, E. J., Heijnen, I., van Os, C. H., Deen, P. M. T. (2000) The subcellular localization of an aquaporin-2 tetramer depends on the stoichiometry of phosphorylated and nonphosphorylated monomers. *J. Cell Biol.* **151**, 919-930.

Kang, D.-Y., Park, J.-I., Cho, W.-S., Jeong, M.-H., Cho, G.-W., Park, H.-T., Bae, H.-R. (2004) Identification of vasopressin-induced genes in AQP2-transfected MDCK cells by suppression subtractive hybridization. *Biochem. Biophys. Res. Commun.* **324**, 1234-1241.

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

Kishida, K., Shimomura, I., Kondo, H., Kuriyama, H., Makino, Y., Nishizawa, H., Maeda, N., Matsuda, M., Ouchi, N., Kihara, S., Kurachi, Y., Funahashi, T., Matsuzawa, Y. (2001) Genomic structure and insulin-mediated repression of the aquaporin adipose (AQPap), adipose-specific glycerol channel. *J. Biol. Chem.* **276**, 36251-36260.

Klussmann, E., Tamma, G., Lorenz, D.,. Wiesner, B., Maric, K., Hofmann, F., Aktories, K., Valenti, G., Rosenthal, W. (2001) An inhibitory role of Rho in the vasopressin-mediated translocation of aquaporin-2 into cell membranes of renal principal cells. *J. Biol. Chem.* **276**, 20451-20457.

Kong, Y., Ma J. (2001) Dynamic mechanisms of the membrane water channel aquaporin-1 (AQP1). *Proc. Natl. Acad. Sci. USA* **98**, 14345-14349.

Kozono, D., Yasui, M., King, L. S., Agre, P. (2002) Aquaporin water channels: atomic structure molecular dynamics meet clinical medicine. *J. Clin. Invest.* **109**, 1395-1399.

Lang, P., Gesbert, F., Delespine-Carmagnat, M., Stancou, R., Pouchelet, M., Bertoglio, J. (1996) Protein kinase A phosphorylation of RhoA mediates the morphological and functional effects of cyclic AMP in cytotoxic lymphocytes. *EMBO J.* **15**, 510-519.

Lin, S.-H., D. Bichet, G., Sasaki, S., Kuwahara, M., Arthus, M.F., Lonergan, M., Lin, Y.F. (2002) Two novel aquaporin-2 mutations responsible for congenital nephrogenic diabetes insipidus in Chinese families. *J. Clin. Endocrinol. Metab.* **87**, 2694-2700.

Lu, Y.,. Turnbull, I. R, Bragin, A., Carveth, K., Verkman, A. S., Skach, W. R. (2000) Reorientation of aquaporin-1 topology during maturation in the endoplasmic reticulum. *Mol. Biol. Cell* **11**, 2973-2985.

Mandon, B., Chou, C.-L., Nielsen, S., Knepper, M. A. (1996) Syntaxin-4 is localized to the apical plasma membrane of rat renal collecting duct cells: possible role in aquaporin-2 trafficking. *J. Clin. Invest.* **98**, 906-913.

Marples, D., Schroer, T. A., Ahrens, N., Taylor, A., Knepper, M. A., Nielsen, S. (1998) Dynein and dynactin colocalize with AQP2 water channels in intracellular vesicles from kidney collecting duct. *Am. J. Physiol. Renal Physiol.* **274**, F384-394.

Marples, D., Frokiaer, J., Nielsen, S. (1999) Long-term regulation of aquaporins in the kidney. *Am. J. Physiol. Renal Physiol.* **276**, F331-339.

Musch, A. (2004). Microtubule organization and function in epithelial cells. *Traffic* **5**, 1-9.

Nielsen, S., Marples, D., Birn, H., Mohtashami, M., Dalby, N. O., Trimble, M., Knepper, M. (1995) Expression of VAMP-2-like protein in kidney collecting duct intracellular vesicles. Colocalization with Aquaporin-2 water channels. *J. Clin. Invest.* **96**, 1834-1844.

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

Pasel, K., Schulz, A., Timmermann, K., Linnemann, K., Hoeltzenbein, M., Jaaskelainen, J., Gruters, A., Filler, G., Schoneberg, T. (2000) Functional characterization of the molecular defects causing nephrogenic diabetes insipidus in eight families. *J. Clin. Endocrinol. Metab.* **85**, 1703-1710.

Pfeffer, S. R. (1999) Transport-vesicle targeting: tethers before SNAREs. *Nat. Cell. Biol.* **1**, E17-22.

Pomes, R., Roux, B. (1996) Structure and dynamics of a proton wire: a theoretical study of H+ translocation along the

single-file water chain in the gramicidin A channel. *Biophys. J.* **71**, 19-39.

Preston, G., Agre, P. (1991) Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: member of an ancient channel family. *Proc. Natl. Acad. Sci. USA* **88**, 11110-11114.

Ren, G., Reddy, V. S., Cheng, A., Melnyk, P., Mitra, A. K. (2001) Visualization of a water-selective pore by electron crystallography in vitreous ice. *Proc. Natl. Acad. Sci. USA* **98**, 1398-1403.

Shi, L., Skach, W., Verkman, A. (1994) Functional independence of monomeric CHIP28 water channels revealed by expression of wild-type mutant heterodimers. *J. Biol. Chem.* **269**, 10417-10422.

Simon, H., Gao, Y., Franki, N., Hays, R. M. (1993) Vasopressin depolymerizes apical F-actin in rat inner medullary collecting duct. *Am. J. Physiol. Cell Physiol.* **265**, C757-762.

Tajkhorshid, E., Nollert, P., Jensen, M. O., Miercke, L. J., O'Connell, J., Stroud, R.M., Schulten, K. (2002) Control of the selectivity of the aquaporin water channel family by global orientational tuning. *Science* **296**, 525-530.

Tamarappoo, B. K., A. S. Verkman (1998) Defective aquaporin-2 trafficking in nephrogenic diabetes insipidus and correction by chemical chaperones. *J. Clin. Invest.* **101**, 2257-2267.

Tamma, G., Klussmann, E., Maric, K., Aktories, K., Svelto, M., Rosenthal, W., Valenti, G. (2001) Rho inhibits cAMP-induced translocation of aquaporin-2 into the apical membrane of renal cells. *Am. J. Physiol. Renal Physiol.* **281**, F1092-1101.

Tamma, G., Klussmann, E., Oehlke, J., Krause, E., Rosenthal, W., Svelto, M., Valenti, G. (2005) Actin remodeling requires ERM function to facilitate AQP2 apical targeting. *J. Cell Sci.* **118**, 3623-3630.

Terris, J., Ecelbarger, C. A., Nielsen, S., Knepper, M. A. (1996) Long-term regulation of four renal aquaporins in rats. *Am. J. Physiol. Renal Physiol.* **271**, F414-422.

Tsukita, S., Yonemura, S., Tsukita, S. (1997) ERM (ezrin/radixin/moesin) family: from cytoskeleton to signal transduction. *Curr. Opin. Cell Biol.* **9**, 70-75.

Valenti, G., Procino, G., Carmosino, M., Frigeri, A., Mannucci, R., Nicoletti, I., Svelto, M. (2000) The phosphatase inhibitor okadaic acid induces AQP2 translocation independently from AQP2 phosphorylation in renal collecting duct cells. *J. Cell Sci.* **113,** 1985-1992.

Van Aelst, L., D'Souza-Schorey, C. (1997) Rho GTPases and signaling networks. *Genes Dev*. **11**, 2295-2322.

van Balkom, B. W. M., Savelkoul, P. J. M., Markovich, D., Hofman, E., Nielsen, S., van der Sluijs, P., Deen, P. M. T. (2002) The role of putative phosphorylation sites in the targeting and shuttling of the aquaporin-2 water channel. *J. Biol. Chem.* **277**, 41473-41479.

van Balkom, B. W. M., Hoffert, J. D., Chou, C.-L., Knepper, M. A. (2004) Proteomic analysis of long-term vasopressin action in the inner medullary collecting duct of the Brattleboro rat. *Am. J. Physiol. Renal Physiol.* **286**, F216-224.

Voth, D. E., Ballard, J. D. (2005) Clostridium difficile toxins: mechanism of action and role in disease. *Clin. Microbiol. Rev.* **18**, 247-263.

Yonemura, S., Matsui, T., Tsukita, S., Tsukita, S. (2002) Rho-dependent and -independent activation mechanisms of ezrin/radixin/moesin proteins: an essential role for polyphosphoinositides in vivo. *J. Cell Sci.* **115**, 2569-2580.

Zardoya, R. (2005) Phylogeny and evolution of the major intrinsic protein family. *Biol. Cell* **97**, 397-414.

Zelenina, M., Christensen, B. M., Palmer, J., Nairn, A. C., Nielsen, S., Aperia, A. (2000) Prostaglandin E2 interaction with AVP: effects on AQP2 phosphorylation and distribution. *Am. J. Physiol. Renal Physiol.* **278**, F388-394.