Aquaporins at a glance

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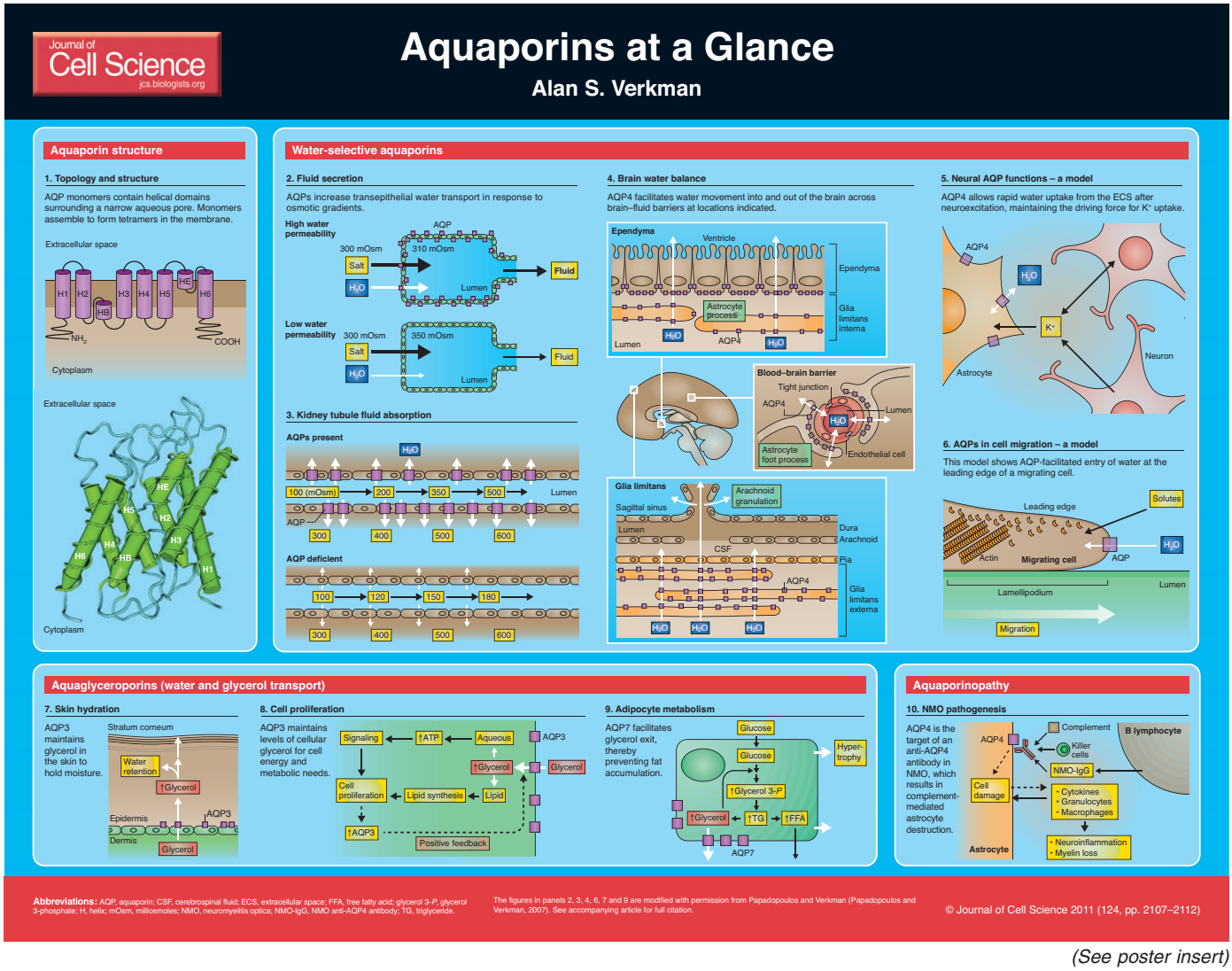
With a concentration of 55,000 mM, water is by far the most prevalent molecule in biological systems. For many years it was assumed that biological membranes are freely water permeable, so that the existence of a family of water channels would not have been predicted. However, biophysical studies in erythrocytes and kidney tubules in the 1960s through the 1980s revealed that some membranes are more water permeable than others and have certain biophysical properties, such as weakly temperature-dependent water transport and an enhanced osmotic compared with diffusional water permeability, which suggested water movement through a pore-like pathway.

The molecular identity of the first water channel, aquaporin 1 (AQP1), was reported in 1992 (Preston et al., 1992). AQP1 had little similarity to other known proteins, except for the major integral protein (MIP) of lens fiber, which was subsequently renamed AQP0. Soon thereafter, additional AQP family members were identified from animal, yeast, bacterial and plant sources, with at least 13 AQPs in humans.

This Cell Science at a Glance article aims to highlight the biological roles of mammalian AQPs. Although some roles of AQPs, such as their function in the concentration of urine and the secretion of gland fluid, were predictable, other roles, such as those in cell migration and neural signaling, were unexpected. Following a brief review of AQP structure, function and regulation, this article will focus on the physiological roles of AQPs, with an emphasis on current knowledge of the cellular mechanisms. In addition, aquaporinopathies – AQP-related human diseases – and possibilities for AQP-based therapeutics, will be discussed.

AQP structure, function and regulation

Compared with ion channels and solute transporters, AQPs are relatively simple proteins with regard to their structure and function. High-resolution X-ray crystal structures have been determined for several mammalian AQPs. Each ~30-kDa AQP monomer is made up of six membrane-spanning helical domains (H1–H6) and two short helical segments (HB and HE) that surround cytoplasmic and extracellular vestibules, respectively. These vestibules are connected by a narrow aqueous pore of ~25 Å (1 Å=0.1 nm) in length (reviewed by Fujiyoshi et al., 2002; Walz et al., 2009) (poster panel 1). Four AQP monomers assemble to form tetramers in the plasma membrane. Tetramers of one of



(See poster insert)

the AQPs, AQP4, further assemble into supramolecular square arrays – called orthogonal arrays of particles – which are maintained by inter-tetrameric N-terminal interactions involving specific residues (Crane and Verkman, 2009).

Structural data on AQPs, together with mutagenesis and molecular dynamics simulations, have indicated that single-file transport occurs through a narrow pore in each monomer, where water selectivity is conferred by electrostatic and steric factors (Hub et al., 2009; Khalali-Araghi et al., 2009). Glycerol-transporting AQPs, called aquaglyceroporins, have a less-constricted pore compared with that of water-selective AQPs (diameter of 3.4 Å compared with 2.8 Å, respectively), with relatively more hydrophobic residues lining the pore.

Many mammalian AQPs, including AQP1, AQP2, AQP4, AQP5 and AQP8, function primarily as bidirectional water-selective transporters. Cells expressing AQPs on their plasma membrane have an ~5- to 50-fold higher osmotic water permeability than membranes that do not (Verkman and Mitra, 2000). Water transport through single-file pores poses a biophysical limitation on the efficiency with which AQPs can transport water, so that AQPs must be present in the membrane at a high density to increase membrane water permeability substantially (Yang and Verkman, 1997). AQP-expressing cells generally contain several thousands, or more, AQPs per μm^2 of membrane, as compared with ten or fewer ion channels per μm^2 of membrane.

A subset of the AQPs, the aquaglyceroporins AQP3, AQP7 and AQP9, transport both water and glycerol, and studies have suggested that AQP9 can also transport other small polar solutes, including amino acids, sugars and even arsenite (Tsukaguchi et al., 1998; Wu and Beitz, 2007; Carbrey et al., 2009). AQP6 has been proposed to function as an intracellular chloride channel (Yasui et al., 1999). The functions and cellular localization of AQPs 10–13 are not clear (Ishibashi, 2009). In addition to their well-established water- and glycerol-transporting functions, AQPs have also been proposed to transport other small molecules and gases, including carbon dioxide, ammonia, nitric oxide and hydrogen peroxide (Musa-Aziz et al., 2009; Miller et al., 2010; Wang and Tajkhorshid, 2010). Although some gases are theoretically small enough to pass through the aqueous pore of the AQP, compelling evidence is lacking for physiologically relevant gas transport, partly because the intrinsic lipid-mediated membrane permeability to most gases is high (Yang et al., 2000; Missner et al., 2008; Tornroth-Horsefield et al., 2010). Current evidence suggests that most, if not all, significant biological functions of the mammalian AQPs, including those described here, can be attributed to AQP-facilitated water and/or glycerol transport.

Because most AQPs are constitutively expressed at the cell plasma membrane, regulation of their function occurs mainly at the level of transcription. There are numerous descriptive studies of up- and down-regulation by various AQPs in response to various stresses, such as AQP4 upregulation in brain trauma and inflammation (Aoki-Yoshino et al., 2005; Tomura et al., 2011). With the exception of AQP2, the biological significance of transcriptional regulation of AQP expression is unclear. Transcription and post-translational regulation of AQP2 expression are particularly important for the mechanism of urine concentration (Nedvetsky et al., 2009; Noda et al., 2010). Similar to the process of GLUT4 translocation to the membrane in response to insulin, targeting of AQP2 to the apical plasma membrane in the collecting duct of the kidney is controlled by the antidiuretic hormone vasopressin through a mechanism involving regulated vesicular trafficking of AQP2-containing endosomes. In addition to the wealth of information described above about AQP structure, function and regulation, there is an abundance of information about the roles of AQPs in cell and organ physiology, which will be discussed in the following sections.

AQPs in epithelial fluid transport

A major role of epithelial cells is the transport of fluid across tissue barriers. Therefore, the involvement of AQPs in epithelial fluid transport is not unexpected. Certain epithelia carry out what has been called 'active near-isosmolar' fluid transport, such as fluid absorption by kidney proximal tubule and fluid secretion by salivary gland acinar epithelium. Active transepithelial fluid transport involves the generation of an osmotic gradient by active solute transport, which drives osmotic water transport (poster panel 2). The high transepithelial water permeability conferred by AQPs, increases net fluid transport.

Mice that lack AQP1, which is expressed in the kidney proximal tubule, show defective fluid absorption (Schnermann et al., 1998). Mice lacking AQP5, which is expressed in salivary and airway submucosal glands, show defective secretion of saliva (Ma et al., 1999) and airway mucus (Song and Verkman, 2001). AQP-facilitated fluid secretion has also been found in the ocular ciliary epithelium (Zhang et al., 2002; Verkman et al., 2008b), which produces aqueous fluid, and in the brain choroid plexus (Oshio et al., 2005), which produces cerebrospinal fluid (CSF). There is a caveat, however: AQPs appear to be required for active transepithelial fluid transport only when fluid transport rate, normalized to epithelial surface area, is very high, as it is in the proximal tubule and salivary gland. By contrast, in the lung alveolus, deletion of AQP5 in mice does not impair active fluid absorption, although it is expressed in alveolar epithelial cells and is responsible for ~90% of osmotically driven water transport (Ma et al., 2000a). This is because the area-normalized rate of fluid absorption by the alveolus is more than 100-fold lower than that in the proximal tubule. The basal (AQP independent) water permeability of the alveolar epithelium is sufficient to support relatively slow fluid absorption.

In addition to their role in active near-isosmolar fluid absorption and secretion, AQPs also facilitate passive transepithelial water transport in response to sustained large osmotic gradients, as exemplified in the thin descending limb of Henle and the collecting duct in the kidney (poster panel 3). Osmotic water transport by the thin descending limb of Henle, where AQP1 is highly expressed, is required for the countercurrent multiplication mechanism to generate concentrated urine (Chou et al., 1999). Water transport across the collecting duct epithelium involves vasopressin-regulated AQP2 trafficking to the apical plasma membrane, as mentioned above, with constitutively high water permeability of the basolateral membrane conferred by the constitutive expression of AQP3 and AQP4 (Verkman, 2008; Noda et al., 2010).

To concentrate the urine, the high water permeability of the kidney collecting duct facilitates osmotic water transport from the tubule lumen to the hypertonic renal interstitium (generated by countercurrent multiplication). Mice lacking one of the AQPs 1–4 manifest defects in urinary concentrating ability (Ma et al., 1997; Ma et al., 1998; Ma et al., 2000b; Yang et al., 2009). In humans, loss-of-function mutations in AQP2 result in the rare genetic disease nephrogenic diabetes insipidus (NDI) (Deen et al., 1994), which is characterized by polyuria and urinary hypo-osmolality. Mice lacking AQP11 manifest an interesting polycystic kidney disease phenotype (Morishita et al., 2005), although it remains unclear how loss of AQP11 produces renal cysts. AQP-facilitated water transport in various epithelia is thus crucial to a wide range of physiological processes.

AQP4 in brain swelling

Water movement across barriers also occurs in the brain, but it is the astrocytes rather than epithelial cells that are involved. The AQP4 water channel is expressed in astrocytes

throughout the central nervous system, particularly at brain–fluid interfaces at the blood–brain and ependymal–CSF barriers (poster panel 4). In cytotoxic (cellular) brain edema, water moves into the brain through an intact blood–brain barrier in response to osmotic driving forces. For example, the acute serum hyponatremia that occurs during water intoxication causes brain swelling by a simple osmotic mechanism. Mice lacking AQP4 show improved outcome and reduced brain water accumulation compared with that in wild-type mice in models of cytotoxic brain edema, such as water intoxication, ischemic stroke and bacterial meningitis (Manley et al., 2000; Papadopoulos and Verkman, 2005).

In contrast to cytotoxic edema, vasogenic (leaky-vessel) brain edema involves water movement into the brain by a bulk-fluid flow mechanism, through a leaky blood–brain barrier, and exit from the brain through the AQP4-rich glia limitans, which lines the ventricles and the surface of the brain (poster panel 4). When these water exit routes are blocked, such as in obstructive hydrocephalus, water also moves out of the brain through microvessels at the blood–brain barrier. Mice lacking AQP4 have a worse clinical outcome and greater brain water accumulation in models of vasogenic brain edema, including intraparenchymal fluid infusion, cortical-freeze injury, brain tumor and brain abscess (Papadopouolos et al., 2004; Bloch et al., 2005), and in a model of obstructive hydrocephalus (Bloch et al., 2006). Therefore, as a bidirectional water channel, AQP4 facilitates brain water accumulation in cytotoxic edema and the clearance of excess brain water in vasogenic edema. AQP4 appears to have a similar role in the spinal cord, reducing cytotoxic swelling and improving the clinical outcome following spinal cord compression injury (Saadoun et al., 2008), whereas increasing vasogenic swelling following spinal cord contusion injury (Kimura et al., 2010).

AQP4 in neural signaling

AQPs are expressed in electrically excitable tissues in supportive cells adjacent to excitable cells (e.g. in astrocytes but not neurons in the brain, in Müller cells but not bipolar cells in the retina, in supportive cells but not hair cells in the inner ear, and in support cells but not olfactory receptor neurons in the olfactory epithelium). Electrophysiological measurements show impaired vision (Li et al., 2002), hearing (Li and Verkman, 2001) and olfaction (Lu et al., 2008) in *Aqp4*-null mice. In the brain, the seizure threshold is reduced and seizure duration prolonged by an AQP4 deficiency (Binder et al., 2006). Possible mechanisms for altered neuroexcitation in AQP4 deficiency include slower K^+ re-uptake into astrocytes following neuroexcitation (Padmawar et al., 2005; Binder et al., 2006) and mild expansion of the extracellular space (ECS) surrounding these cells (Yao et al., 2008; Zhang and Verkman, 2010b).

How AQP4 alters K^+ re-uptake following neuroexcitation is the subject of ongoing investigation. It had been postulated that the interaction between AQP4 and the inwardly rectifying K^+ channel Kir4.1 was responsible. However, patch-clamp analysis has indicated that AQP4 deficiency does not affect Kir4.1 K^+ channel function in brain astrocytes or retinal Müller cells (Ruiz-Ederra et al., 2007). We propose that AQP4-dependent water permeability enhances K^+ transport by a 'pseudo-solvent drag' mechanism (poster panel 5). Neuroexcitation involves ion buffering in the ECS, which is the small aqueous volume surrounding cells (~20% of the volume in brain). Excess K^+ released by neurons during excitation is taken up and 'siphoned' largely by astrocytes. We postulate that AQP4-facilitated water transport in astrocytes in the brain is an important determinant of both water and ion movement between cells and the ECS during neuroexcitation. Re-uptake of K^+ following neuroexcitation results in osmotic water influx into AQP4-expressing astrocytes and consequent ECS shrinkage, which maintains the electrochemical driving force for K^+ re-uptake (by maintaining elevated K^+ concentrations in the ECS). The diminished astrocyte water permeability that occurs in AQP4 deficiency would reduce ECS contraction and hence slow K^+ re-uptake. This hypothesis, although unproven as yet, is attractive because it relates neuroexcitation phenotypes to the water-transporting role of AQP4.

AQPs in cell migration

The involvement of AQPs in cell migration was discovered following the observation of impaired tumor angiogenesis in *Aqp1*-null mice and the characterization of endothelial cell cultures derived from wild-type and *Aqp1*-null mice (Saadoun et al., 2005a). Motivated by the high expression of AQP1 in tumor microvessels (Endo et al., 1999), we found that AQP1 deletion in mice reduces the growth and vascularity of implanted tumors (Saadoun et al., 2005a). Cultured aortic endothelial cells from *Aqp1*-null mice migrated slower than cells from wild-type mice in response to a chemotactic stimulus, and cell migration was increased when various cells not normally expressing AQPs were transfected with AQPs. Further evidence, including AQP polarization to the leading edge of migrating cells, increased lamellipodial dynamics in AQP-expressing cells and AQP-dependent cell migration in different cells types and with different AQPs, suggested a mechanism for AQP-facilitated migration (Papadopoulos et al., 2008; Loitto et al., 2009) (poster panel 6).

On the basis of these results, we have proposed that actin depolymerization and ion influx increase cytoplasmic osmolality at the leading edge of a migrating cell, driving water influx through the plasma membrane (Saadoun et al., 2005a). This idea of water flow into and out of migrating cells is supported by data showing that migration can be modulated by changes in extracellular osmolality and transcellular osmotic gradients (Saadoun et al., 2005b). Our model postulates that water influx causes expansion of the adjacent plasma membrane by increased hydrostatic pressure, which is followed by actin repolymerization to stabilize the cell membrane protrusion. In support of this idea is the observation that regional hydrostatic pressure changes within cells do not equilibrate throughout the cytoplasm, on a distance scale of 10 μm and a time scale of 10 seconds, and could thus contribute to the formation of localized cell membrane protrusions (Charras et al., 2005). However, this mechanism remains unproven and other mechanisms, such as AQP-dependent changes in cell volume during migration and interaction of AQPs with other proteins, are potential alternatives.

Regardless of the exact biophysical mechanism, AQP-facilitated cell migration appears to be a general phenomenon relevant not only in angiogenesis but also in tumor spread, wound healing and immune cell chemotaxis. AQP expression in tumor cells increases their ability to extravasate across blood vessels and to invade locally (Hu and Verkman, 2006), which might account for the high level of AQP expression in many tumor types and the correlation between AQP expression and tumor grade in some tumors, such as glioblastomas (Verkman et al., 2008a). Expression of AQP4 in brain astrocytes increases their migration towards a chemotaxic stimulus and increases glial scarring (Saadoun et al., 2005b; Auguste et al., 2007), and expression of AQP3 in skin and cornea facilitates wound healing (Levin et al., 2006; Hara-Chikuma and Verkman, 2008b).

Although the evidence remains preliminary, AQPs might also be involved in intracellular vesicular transport processes, such as pancreatic granule exocytosis and astrocyte cytokine secretion (Cho et al., 2002; Li et al., 2011), perhaps by a mechanism involving rapid AQP-dependent changes in cell volume, which might accompany vesicle fusion.

Aquaglyceroporin AQP3 in skin hydration

The functional significance of glycerol transport by the aquaglyceroporins, such as AQP3 in skin and AQP7 in adipocytes was, for many years, unclear. However, more recent studies have indicated roles for these channels in skin hydration and fat metabolism. AQP3-facilitated glycerol transport in skin is an important determinant of epidermal and stratum corneum hydration (Ma et al., 2002) (poster panel 7). Mice lacking AQP3, which is normally expressed in the basal layer of proliferating keratinocytes in the epidermis, manifest reduced stratum corneum hydration and skin elasticity. This is caused by reduced epidermal cell glycerol permeability, resulting in reduced glycerol content in the stratum corneum and epidermis, where it acts as a 'humectant', or water-retaining osmolyte (Hara et al., 2002). Topical or systemic administration of glycerol normalized stratum corneum glycerol content and corrected the skin-hydration defect in AQP3 deficiency (Hara and Verkman, 2003). These findings provided a rationale for the common use of glycerol in cosmetics and various skin medical formulations and generated interest in the involvement of AQP3 in skin diseases (reviewed by Hara-Chikuma and Verkman, 2008c).

AQP3 in cell proliferation

An unanticipated role of AQP3 in cell proliferation was observed in several AQP3-expressing tissues, including skin, colon and cornea. AQP3-deficient mice manifest impaired cutaneous and corneal wound healing (Hara-Chikuma and Verkman, 2008b; Levin and Verkman, 2006) and colonic epithelial cell regeneration (Thiagarajah et al., 2007). In addition, a remarkable tumor phenotype was found in *Aqp3*-null mice, which showed complete resistance to the formation of skin tumors in response to a tumor initiator-promoter protocol that produces multiple tumors in wild-type mice (Hara-Chikuma and Verkman, 2008a). Biochemical studies in epidermal cells of these AQP3-deficient mice showed impaired cellular glycerol metabolism and biosynthesis, with reduced ATP content and impaired MAPK signaling (poster panel 8). Thus, we proposed that AQP3-facilitated glycerol transport is a key determinant of cell proliferation through a mechanism involving reduced epidermal glycerol concentration in AQP3 deficiency, impaired lipid biosynthesis, reduced glycerol metabolism and ATP, impaired MAPK signaling (particularly p38 kinase) and, finally, reduced cell proliferation. Further investigation of glycerol metabolism in AQP3-expressing cells is needed to verify this proposed mechanism. In any case, the possibility of AQP3 inhibition being used to prevent or treat certain tumors is intriguing because AQP3 inhibition is predicted to reduce both migration and proliferation in tumor cells.

Aquaglyceroporin AQP7 in fat metabolism

A quite unexpected role of an aquaporin in obesity has been discovered. AQP7 is expressed in the plasma membrane of adipocytes. *Aqp7*-null mice manifest a remarkable progressive increase in fat mass and adipocyte hypertrophy, accumulating glycerol and triglycerides in their adipocytes, as they age (Hara-Chikuma et al., 2005; Hibuse et al., 2005). Biochemical studies suggested that this adipocyte hypertrophy is the consequence of reduced plasma membrane glycerol permeability, resulting in cellular glycerol and triacylglycerol accumulation, and glycerol kinase upregulation (poster panel 9). These results focus attention on adipocyte glycerol permeability as a novel regulator of adipocyte size and whole-body fat mass, suggesting that the modulation of adipocyte AQP7 expression and/or function can alter fat mass. AQP9 has been suggested as an important route for hepatic glycerol uptake (Carbrey et al., 2003), and AQP7 and AQP9 as key metabolic regulators in diabetes and obesity (Maeda et al., 2009). However, additional work is needed to validate these ideas.

'Aquaporinopathies' – human aquaporin diseases

In humans, loss-of-function mutations in AQPs exist but are rare. As mentioned above, mutations in *AQP2* produce non-X-linked NDI by a recessive mechanism involving defective AQP2 protein folding and retention in the endoplasmic reticulum (ER), as well as by a dominant mechanism, which results from interactions between wild-type and mutant AQP2 in the ER and/or Golgi that prevent plasma membrane targeting of wild-type AQP2 (Bichet, 2006). The incidence of NDI caused by AQP2 mutations is fewer than one in 20 million births.

For other AQPs, only a handful of subjects have been identified with loss-of-function mutations. A few subjects that lack functional AQP1, which were identified by blood-group screening, are phenotypically normal but manifest defective urinary concentrating function when deprived of water (King et al., 2001). Because of the rarity of AQP1-deficient individuals, as well as a few subjects that apparently lack functional AQP3 or AQP7 (Roudier et al., 2002), and because of wide phenotype variations in humans, little useful information is available about the roles of these AQPs in humans.

Mutations in AQP0 (MIP) in lens fiber cause congenital cataracts by a mechanism that is speculated to involve defective cell–cell adhesion rather than impaired water transport (Berry et al., 2000; Engel et al., 2008; Chepelinsky, 2009). Neither disease-causing mutations of other AQPs in humans nor strong associations between AQP polymorphisms and disease have so far been described.

The neuroinflammatory demyelinating disease neuromyelitis optica (NMO) is an interesting aquaporinopathy that has recently received considerable attention. It is a relatively rare variant of multiple sclerosis that primarily affects the optic nerve and the spinal cord, causing blindness, paralysis and death, and it has a poor prognosis, even with aggressive immunosuppressive therapy. A defining feature of NMO is the presence of serum antibodies directed against extracellular epitopes on AQP4 (autoantibodies) (Lennon et al., 2005). NMO autoantibodies (NMO-IgG) are thought to mediate their pathogenic effect by binding to AQP4 on astrocytes, which activates complement- and cell-mediated cytotoxicity, elaborates inflammatory mediators and stimulates leukocyte infiltration (Wingerchuk et al., 2007) (poster panel 10). It is not known what triggers the production of NMO-IgGs, nor how they enter the CNS or why lesions are largely absent in brain and in peripheral AQP4-expressing tissues. Active research is being carried out to establish useful animal models of NMO (Bennett et al., 2009; Saadoun et al., 2010) and to develop new therapeutic approaches, such as small-molecule inhibitors or monoclonal antibodies ('aquaporumabs') that block the binding of NMO-IgG to AQP4.

Perspective

The AQP field has matured since the molecular identification of the first AQP two decades ago. By now we have a substantial base of knowledge on AQP structure, cellular expression and biological functions. However, substantial gaps in knowledge, continued surprises and unrealized opportunities to translate basic AQP research to the clinic remain.

Further work is needed to establish cellular mechanisms for the many interesting phenotypes produced by AQP deficiency, such as impaired cell migration, cell proliferation, neuroexcitation and fat metabolism. Interesting new phenotypes will continue to be discovered and will challenge existing paradigms about water transport physiology, such as the impaired pain nociception in AQP1 deficiency (Zhang and Verkman, 2010a) and the reduced autoimmune neuroinflammation in AQP4 deficiency (Li et al., 2011).

Phenotype studies in knockout mice suggest that AQP-based therapy for human diseases might be useful. AQP inhibitors, or 'AQP-aquaretics', are predicted to reduce urine concentration, producing a water greater than salt diuresis for therapy of diuretic-refractory edematous states, such as severe congestive heart failure. AQP4 inhibitors are predicted to reduce brain swelling in cytotoxic edema, potentially offering neuroprotection following brain injury and stroke, and reducing mortality in meningitis and various encephalitides. Inhibitors of AQPs in tumor cells and microvessels are predicted to reduce tumor spread and angiogenesis, offering adjunctive tumor chemotherapy. Inhibition of AQP4-facilitated glial cell migration is predicted to inhibit glial scar formation following brain and spinal cord injury, promoting axonal regeneration and improving long-term neurological outcome. Topical inhibitors of AQP3 in the skin might reduce skin cancer, and increasing adipocyte glycerol permeability, perhaps by enhancement of AQP7 expression, might provide a novel therapy for obesity. There is early stage work investigating the possibility of AQP5 gene replacement for salivary gland dysfunction (Baum et al., 2009). Validation of the utility of aquaporin-based therapeutics will require the development of AQP-specific modulators, which is the subject of ongoing drug discovery efforts in numerous laboratories and pharmaceutical companies. Thus, many opportunities remain to translate AQP research from the bench into clinical practice.

Individual poster panels are available as JPEG files at http://jcs.biologists.org/cgi/content/full/124/13/2107/DC1

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