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Review

Amphibian aquaporins and adaptation to terrestrial environments: A review ☆

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Abstract

In many anurans, the pelvic patch of the ventral skin and the urinary bladder are important osmoregulatory organs. Since the discovery of water channel protein, aquaporin (AQP), in mammalian erythrocytes, 17 distinct full sequences of AQP mRNAs have been identified in anurans. Phylogenetic tree of AQP proteins from amphibians and mammals suggested that anuran AQPs can be divided into six types: *i.e.* types 1, 2, 3, and 5, and anuran-specific types a1 and a2. Among them, two types of anuran AQPs (types 1 and a2) are localized in the skin and urinary bladder by immunohistochemistry. Tree frog type-a2 AQPs, AQP-h2 and AQP-h3, are vasotocin-regulated water channels predominant in the osmoregulatory organs. Both the AQP-h2 and AQP-h3 are expressed at the granular cells underneath the keratinized layer in the pelvic patch, whereas only AQP-h2 is detected at the granular cells in the urinary bladder. In response to vasotocin, both the molecules seem to be translocated from the cytoplasmic pool to the apical plasma membrane of the granular cells. On the other hand, type-1 AQPs, *Rana* FA-CHIP and *Hyla* AQP-h1, are detected at the endothelial cells of blood capillaries in frog osmoregulatory organs. These findings suggest that AQP-h2 and AQP-h3 are key players for transepithelial water movement, and that FA-CHIP and AQP-h1 might be important for the transport of absorbed water into the blood flow. Comparative investigation of type-a2 AQPs in anurans further revealed that AQP-h2 and -h3-like molecules might exist at the urinary bladder and the pelvic skin, respectively, in various anurans from aquatic species to arboreal dwellers. AQP-h2-like protein is also detected in the pelvic skin of terrestrial and arboreal species. It is possible that this molecule might have occurred in the pelvic skin as anurans penetrated into drier environments.

Keywords: Aquaporin; Water channel; Tree frog; Pelvic patch skin; Urinary bladder; Vasotocin; Immunohistochemistry; Anurans

Contents

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1. Introduction

Amphibians represent the first vertebrates that emerged from aquatic habitats to terrestrial environments. To adapt to dryer environments, many adult anurans have evolved specialized osmoregulatory organs: the ventral pelvic patch, or seat patch, to absorb water from the external environments and urinary bladder that stores water and reabsorbs it in times of need (Bentley and Yorio, 1979; Hillyard, 1999; Bentley, 2002). Hydromineral transport across the tight epithelium has been studied intensively, using these organs as model systems (Macknight et al., 1980; Jorgensen, 1997). Electrophysiological and pharmacological studies revealed numerous key molecules such as epithelial sodium channel (ENaC) (Bentley, 1968; Garty and Palmer, 1997), H^+-ATPase (Harvey, 1992), cystic fibrosis transmembrane conductance regulator (CFTR)/chloride channel (Willumusen et al., 2002; Jensen et al., 2003), and Na^+/K^+-ATPase (Koefoed-Johnsen and Ussing, 1958). The CFTR in toad skin was further identified by cDNA cloning (Amstrup et al., 2001). Considering the functions of these players and tight junction, several models are proposed to explain molecular mechanisms for ion transport across the amphibian skin (Larsen, 1991; Jensen et al., 2003) and urinary bladder (Macknight et al., 1980).

Coupled with ion transport, water moves across the tight epithelium through two pathways, transcellular and paracellular. Although the paracellular water transport through the tight junction area occurs in amphibian epithelia (Guo et al., 2003; Orce et al., 2004), the main route is the transcellular pathway. Classically, the transcellular water transport was believed to be passive diffusion through the lipid bilayer of cell membranes. However, biophysical, physiological, and electron microscopic studies predicted the existence of water movement mediated by membrane channel proteins, called aggregates (Chevalier et al., 1974; Brown et al., 1983; Yasui, 2004). During the early 1990s such water channel proteins were discovered, and are now called aquaporins (AQP) (Agre et al., 1993). As for amphibians, approximately 20 AQP cDNAs have been registered in DNA data bank, and it is getting more important to characterize the function and localization of AQPs for the understanding of water pathways in the amphibian body. In this review, we focus on amphibian AQPs and discuss molecular mechanisms of the transepithelial water permeability in the pelvic patch and urinary bladder, in terms of AQPs. Furthermore, AQPs present in the osmoregulatory organs are compared among several anurans to consider the role of AQPs in the terrestrial adaptation of anurans. The kidney is also involved in amphibian osmoregulation (Bentley, 2002; Uchiyama and Konno, 2006), but this topic is not dealt with here.

2. Aquaporin family

Aquaporins have been discovered in a vast and varied array of organisms, ranging from bacteria to animals and plants. These AQPs are divided into two subfamilies: *i.e.* orthodox aquaporins conducting only water and aquaglyceroporins transporting solutes and water. Thus far, 13 isoforms of AQPs (AQP0-12) have been identified in mammals (Borgnia et al., 1999; Takata et al., 2004; Itoh et al., 2005; Gorelick et al., 2006). AQP0 and AQP12 are unique to the lens and acinar cells of the pancreas, respectively, whereas the other isoforms are detected in various cells and tissues (Takata et al., 2004). Multiple isoforms of AQPs are expressed in many organs, but each of them shows specific cellular and subcellular localization, thereby serving important physiological functions. As for the mammalian kidney, 8 AQPs are located at specific segments of the nephron and other components: *i.e.* AQP1, AQP7, AQP8, and AQP11 at the proximal tubule (Maunsbach et al., 1997; Nejsum et al., 2000; Elkjaer et al., 2001; Morishita et al., 2005), AQP1 at the descending thin limb of Henle's loop (Maunsbach et al., 1997), AQP2, AQP3, AQP4, AQP6, and AQP8 at the collecting duct (Ishibashi et al., 1994; Frigeri et al., 1995; Nielsen et al., 1995a; Ohshiro et al., 2001; Elkjaer et al., 2001), AQP3 at the renal pelvis (Matsuzaki et al., 1999), and AQP1 at the vasa recta (Sabolic et al., 1992). Among these AQPs, AQP2 is the vasopressin-sensitive water channel that facilitates water reabsorption in response to antidiuretic hormone, vasopressin, by translocating from intracellular vesicles to the apical membrane of collecting duct principal cells (Nielsen et al., 1995a; Noda and Sasaki, 2005; Valenti et al., 2005). AQP2 mutations and disruption of AQP2 gene cause nephrogenic diabetes insipidus, a disease characterized by a massive loss of water through the kidney (Deen et al., 1994; Yang et al., 2001). Other renal AQPs are also shown to play critical roles in water reabsorption and urine concentration in the kidney (Verkman, 2006).

3. Amphibian aquaporins

The full sequences of 17 AQP cDNAs have been elucidated in anurans, but only expressed sequence tags (ESTs) are registered in urodeles. A phylogenetic analysis suggested that anuran AQPs can be assigned to six clusters: types 1, 2, 3, and 5, and two anuran-specific types, designated as a1 and a2 (The letter "a" represents *a*nuran) (Fig. 1). The cluster of type-a1 AQPs is composed of AQPxlo from *Xenopus laevis* oocytes (Virkki et al., 2002) and another *X. laevis* AQP (accession number: BC090201). The cluster of type-a2 AQPs contains AQP-h2 (Hasegawa et al., 2003) and AQP-h3 from the frog, *Hyla japonica* (Tanii et al., 2002), and AQP-t2 (AF020621) and AQP-t3 from the toad, *Bufo marinus* (AF020622). As for anuran osmoregulatory organs, little information is available for the types 2, 3, 5, and a1. Therefore, this section summarizes published data on the type-1 AQPs and type-a2 AQPs, especially highlighting the structure and function of *Hyla* AQPs.

3.1. Type-1 AQPs

The AQP1 cluster includes mouse AQP1 (Moon et al., 1995), AQP-h1 from *H. japonica* (Hasegawa et al., 2003), FA-CHIP from *Rana esculenta* (Abrami et al., 1994), and AQP-t1 from *B. marinus* (Ma et al., 1996) (Fig. 1). *Hyla* AQP-h1 is composed of 271 amino acid residues, and its predicted structure is shown in Fig. 2A. Hydropathy analysis has