MINI-REVIEW

The role of a group III AQP, AQP11 in intracellular organelle homeostasis

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Abstract : AQP11 is a member of a new aquaporin subfamily which includes many aquaporin homologs with low amino acid identities, around 20% of previously identified AQPs. Although these AQPs have unusual NPA sequences, these AQPs have a completely conserved and functionally indispensable cysteine residue downstream of the second NPA box, suggesting that they belong to a specific AQP subfamily, which we propose to name the group III AQPs. On the other hand, the NPA boxes are highly conserved in previous AQP subfamilies : the group I AQPs, original water-selective aquaporin family and the group II AQPs, aquaglyceroporin family. Currently the roles of the group III AQPs are only known with AQP11 as the disruption of intracellularly located AQP11 in mice produced huge vacuoles in the proximal tubule leading to fatal polycystic kidneys at one month old. This review focused on the classification of AQPs based on primary structures to obtain insights into the function and the role of AQPs. With the accumulation of new AQP-like sequences through genome projects, this classification will be useful to predict their functions as each group may have specific characteristics in its function, distribution and regulation. J. Med. Invest. 56 Suppl. : 312-317, December, 2009

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NEW CLASSIFICATION OF AQPS

Aquaporins (AQPs) have evolved mainly to facilitate the permeation of small molecules at the plasma membrane (1). The AQP family represents a group of proteins whose primary sequences are similar to AQP1, a prototype of AQP. With the progress of genome projects, more and more AQP-like sequences have been identified on the basis of amino acid sequence similarities. Most of them, however, have not yet been functionally characterized and may not transport water. AQPs have six transmembrane domains with N- and C- termini in the cytoplasm. The pore is made of two highly conserved short hydrophobic stretches of amino acid residues named NPA boxes, which are the signature sequences for AQPs.

The AQP family was previously divided into two groups from their primary sequences especially around NPA boxes, which generally corresponds to their functions : water-selective and glycerol-permeable (2-4). The latter are also called aquaglyceroporins as they permeate both water and glycerol (3). In this review, they will be called the group I AQPs and the group II AQPs, respectively, because the name aquaglyceroporin is sometimes used to indicate a glycerol-permeating group I AQP (5), which is confusing. While the arginine (R) in the loop including the second NPA box forms a narrow route for solute passage (ar/R), the corresponding aspartic acid (D) in the group II AQPs expands the pore to accept larger molecules such as glycerol (6). Therefore, this D will be a signature sequence for the group II AQPs, while its absence suggests that

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the protein belongs to the group I AQPs.

Interestingly, genome projects have revealed many AQP-like sequences with low homologies (less than 20%) to the class I and II AQPs especially around the NPA boxes (7-9), which may not belong to the AQP family with different loop sequences including the NPA box. Such low homologies will indicate that they do not transport water and that they belong to an AQP supergene family. Accordingly, we named them 'superaquaporins' indicating their properties of supergenes (7). However, we now realized that 'super' suggests functional superiority as is the case with superantigen and one of them in fact has been shown to function as a water channel (10), suggesting that they indeed are members of the AQP family. Therefore, we now believe that 'the group III AQP' is a more appropriate naming than 'superaquaporin' and will be used in this review. Although the sequences of the loops including the NPA box of the group III AQPs are highly variable, a cysteine residue downstream of the second NPA box is completely conserved (Fig. 1). This cysteine is expected to be located at the extracellular loop between 5th and 6th transmembrane domains near the pore and will be important for channel function. In fact, its mutation in AQP11 produced a similar phenotype with AQP11 null mice (11). Therefore, this cysteine will establish the authenticity and independency of the group III AQPs and should be the signature sequence for this group. Accordingly, plant SIPs, short intrinsic basic proteins, previously included in superaquaporin subfamily (7-9) are now excluded from the group III AQPs because this signature cysteine residue is missing in all SIPs. They will belong to the group I AQPs as D is absent in the second NPA box (Fig. 1). In fact, overall sequences of SIPs are closer to the group I than the group III AQPs.

Although more and more AQP-like sequences with variations of 'NPA' at the NPA box have been identified particularly in unicellular micro-organisms, they all belong to the group I or II AQPs because the sequences around the NPA box are similar to the group I and II AQPs and the signature cysteine residue is absent. For example, an AQP of *Dictyostelium discoideum* has a highly deviated 'APN' in the second 'NPA' box (D.disD in Fig. 1), but the signature-like cysteine residue after the second NPA is deviated and the sequence around the first NPA box is similar to those of the group I or II AQPs. Accordingly, this AQP should not belong to the group III AQPs but belong to the group I AQPs as the signature residue (D) of the group II AQPs is absent following the second NPA box.

Here we propose a simple method for classifying AQPs into three groups based on the primary sequences without using overall homology analyses. First, identify the second NPA box and search for an aspartic acid residue (D) at two residues downstream of the second NPA box. If there is a D at the right site, the protein will be a group II AQP. If not, then search for the cysteine residue (C) at the ninth residue downstream of the second NPA box. If there is C at the right site and the loops including the NPA box are less conserved, the protein will be a group III AQP. If there is no C at the right site and the loops including the NPA box are well conserved, the protein will be a group I AQP (Table 1).

FUNCTIONAL ROLES OF THE GROUP III AQP

As the primary structure of each AQP group is distinct, each may have a specific function and a physiological role. Current knowledge on the function of each group is summarized in Table 2. There are several overlaps and unknowns making it difficult to determine the characteristics of each group. In the near future, the functional bases for this classification will be clarified by the accumulation of more data.

The absence of water channel activity in *Xenopus oocytes* expressing AQP11 was reported (12), although we found poor expressions of AQP11 at the plasma membrane (13). In contrast, a recent reconstruction vesicle study clearly showed that AQP11 is indeed a water channel which transports water as efficient as AQP1 (10). Whether AQP11 also functions as a water channel inside the cell is not clear and requires further studies. Moreover, the permeability of glycerol and other substrates is unknown and no functional data other than AQP11 has yet been reported in this group.

AQP11 is widely expressed in many cells including proximal tubular cells, hepatocytes, intestinal epithelial cells, neurons, and spermatids (12-14). However, AQP11 disruption only affected the kidney leading to uremic death from polycystic kidneys with unknown mechanisms (13). Interestingly, before developing the cysts, the proximal tubules accumulated huge intracellular vacuoles, which have not been observed in other cystic kidney diseases. The vacuoles were also observed in hepatocytes

	First NPA boxes	Second NPA boxes
AQPZ	-VGHISGGHFNPAVTIGLWAG-	-SIPVTNTSVNPARSTAVAIFQG-
GlpF	-TAGVSGAHLNPAVTIALWLF-	-MGPLTGFAMNPAR**D**FGPKVFAW-
Entero	-LFVFGGVCINPAMALAQAIL-	-LGGTTGFAMNQAR**D**LGPRIAYQ-
Meth	-FGRISGCHINPAVTIALFAT-	-IGNLTGASLNPARTFGPYLGDW-
Cripto	-FFRVSGGLFNPAVSLGMVLA-	-GVPYSGGALNPVRSLGPAVVTH-
Tryp1	-FGYISGGHFNPAVTMAVFLV-	-VGRISGGAFNPAAATGLQLALC-
Tryp2	-FGYISGAHFNPAITFATFIN-	-VGGFTGGAFNPAVATGTQLVGC-
Leish	-FGYISSSHFNPAVSIAVFLV-	-AGRISGGAFNPAAASGLQVAMC-
P.viv	-AAKLSGAHLNLAVTVGFATI-	-FGGNTGFALNPSR**D**LGARLLSL-
D.disA	-VSGVSGCNLNPAVTLANLLS-	-GFNFSGGALNPVRVLGPSIISG-
D.disB	-ISGISGCQLNPAVTVGCVTT-	-LNLFTGGSLNPARSFGPAVFSD-
D.disC	-FADVSGAHFNPAVTFATCVT-	-GGSVSGGAFNPARVFGTALVGN-
D.disD	-CAPVSGGHLNPSITLATFFA-	-LSIIASGGIAPNYIFGFNIARC-
D.disE	-CAPVSGGHLNPSITIATFFS-	-FSIIASSGISPNYIFGFNMARC-
TIP1.1	-GANISGGHVNPAVTFGAFIG-	-GGAFSGASMNPAVAFGPAVVSW-
PIP2.6	-TAGISGGHINPAVTFGLFLA-	-TIPITGTGINPARSFGAAVIYN-
NIP1.2	-LGHISGAHFNPAVTIAFASC-	-AGPVSGASMNPGRSLGPAMVYS-
SIP1.1	-TVIFGSASFNPTGSAAFYVA-	-GSKYTGPAMNPAIAFGWAYMYS-
AQP1	-VGHISGAHLNPAVTLGLLLS-	-AIDYTGCGINPARSFGSAVLTR-
AQP3	-AGQVSGAHLNPAVTFAMCFL-	-MGFNSGYAVNPAR**D**FGPRLFTA-
AQP8	-LGNISGGHFNPAVSLAVTVI-	-GGSISGACMNPARAFGPAVMAG-
CeAQP9	-IEFQRDAVAHPCPLVTNCYR-	-GINYTGMYANPIVAWACTFN**C**L-
CeAQP10	-NIFNRGAMTNCAPIFEQFVF-	-LYVVGVPGLNPIVATARLYG**C**R-
CeAQP11	-ALCNRTAFCSPLAPIEQYLF-	-VTFVGDQALDPLVASTLFFG**C**R-
Dros	-GRVWGDASACPYTHMEDVVE-	-AFNFSGGYFNPVLATALKWG**C**R-
Urch1	-LTFDGDSTANTCMIWQSMLK-	-GLEWTGMMFNPALAAGITLN**C**G-
Urch2	-NEELSNAGDAPLGQAVQVQP-	-GLEYTGAPMNPILGFASGWG**C**K-
ZF1	-GFSFRGAICNPTGALELLSR-	-GGRLTGAVFNPALAFSIQFP**C**P-
ZF2	-TAVMQDVSGNPAVTLLRLLQ-	-ANNYTSGYVNPALAYAVTLT**C**P-
Xeno	-GFTFNKASGNSAVSLQDFLL-	-AGSYTGAFFNPTLAAALTFQ**C**S-
Chic11	-GLTLPGSTCNPCGTLQPLWG-	-GGNLTGAIFNPALAFSLHPH**C**F-
Chic12	-AACANGAASNPTVSLQEFLL-	-AAPATGAFFNPALATASTFL**C**A-
AQP11	-GLTLVGTSSNPCGVMMQMML-	-GGSLTGAVFNPALALSLHFM**C**F-
AQP12	-GVTLDGASANPTVSLQEFLM-	-AGPFTSAFFNPALAASVTFA**C**S-

Fig. 1 Sequence alignments of aquaporins at the first and the second NPA boxes

The sequences above the line are conventional aquaporins (group I and II AQPs) ; the sequences below the line are group III AQPs. Highly conserved NPAs (asparagine-proline-alanine) are underlined. The aspartic acid (D in bold print) in the second NPA box will be a signature residue for group II AQPs. The second NPA box has a conserved cysteine (C in bold print) in group III AQPs : NPA (L/V/A/I)AXXXXXXC. This cysteine will be a signature residue for group III AQPs.

AQPZ, GlpF : *Escherichia coli* (NP_415396, NP_418362), Entero : *Enterococcus faecalis* V583 (Gene ID : 1200713), Meth : *Methanoculleus marisnigri* JR1(Gene ID : 4846532), Cripto : *Cryptococcus neoformans* var. neoformans JEC21(Gene ID : 4935143), Tryp1/2 : *Trypanosoma cruzi* (XP_815990, AF31269.1), Leish : *Leishmania major* ; CAJ08765.1), D.disA, B, C, D, E : *Dictyostelium discoideum* (Gene ID : 3398231, 3392160, 3392764, 3395408, 3387173, 3391439), TIP1.1 (tonoplast intrinsic protein), PIP2.6 (plasma membrane intrinsic protein), NIP1.2 (NOD26-like intrinsic proteins), SIP1.1 (Short basic intrinsic protein) : *Arabidopsos thaliana* (P25818, Q9ZV07, Q8LFP7, Q9M8W5), AQP1/3/8 : *Mus musculus* (NP_031498, NP_057898, NP_031500), CeAQPs : *C. elegans* (NP_001021552.1, NP_496105.1, NP_499821.2), Dros : *Drosophila melanogaster* (AAF58409.2), Urch1/2 sea urchin : *Strongylocentrotus purpuratus* (XP_780933.1, XP_787329.1), ZF1/2 : zebrafish : *Danio rerio* (AAH95775.1, AAH95564.1) ; Xeno : *Xenopus laevis* (AAH82904.1), Chic11/12 : *Gallus gallus* ; (XP_424343.1, NP_001030011.1), AQP11/12 : *Mus musculus* (NP_780314, NP_808255).

Table 1 The distribution of aquaporins

Organisms		group I	group II	group III
Bacteria				
	E. coli	1	1	
	H. influenzae	1	1	
	P. aeruginosa		1	
	S. typhimurium	1		
	M. marburgensis	1		
Fungi				
	S. cervisiae	2	2	
	A. nidulans	1	4	
	U. mydis	2	3	
	M. grisea	3	1	
Protozoan				
	L. major	4	1	
	T. cruzi	4		
	T. brucei		3	
	T. gondii	1		
	P. falciparum		1	
	D. discoideum	5		
Nematode				
	C. elegans	3	5	3
Plant				
	A. thaliana	35		
Insect				
	D. melenogaster	7		1
Vertebrate				
	human	7	4	2

group I : 'water-selective', group II : aquaglyceloprin, group III ; superaquaporin

Table 2 The functions of aquaporins

Functions	group I	group II	group III
Water transport	++	+	(++AQP11)
Glycerol transport	- (+NIP)	++	?
Ion channel	- (+bib, AQP6)	-	?
CO_2 transport	(+AQP1, PIP)	-	?
H_2O_2 transport	(+TIP, AQP8)	-	?
Arsenite transport	- (+NIP)	+	?
Cell adhesion	(+AQP0, 4)	?	?
Cell movement	(+AQP1, 4)	(-AQP3)	?
Cell proliferation	(-AQP1)	(+AQP3)	?
Organelle maintenance	(+bib)	?	(+AQP11)

group I : water-selective, group II : aquaglyceloprin, group III ; superaquaporin

close to portal veins and intestinal epithelial cells near the tip of the villi where water is massively absorbed (13).

How can a defective water transport inside the cell lead to the vacuole formation as AQP11 is expressed intracellularly (12-14) and functions as a water channel (10)? Currently, little is known about the movement of intracellular water although its importance in cell biology has been speculated. In fact primary cultured proximal tubular cells from AQP11 null mice had a defective endosomal pH regulation (13), which may parallel the Big Brain (bib) defect

in Drosophila accumulating cytoplasmic endosomes (15). The mechanism for the endosomal dysfunction remains to be clarified.

EMERGING PROPERTIES OF INTRACELLULAR AQPS

Although most AQPs are localized at the plasma membrane, some have been shown to be present inside the cell. In plants, most TIPs are present at intracellular vacuoles, tonoplasts, while SIPs and some NIPs have been shown to be localized at the endoplasmic reticulum (ER) (16-18). Therefore, intracellular AQPs are not unusual in plants. On the other hand, intracellular AQPs are relatively rare and not well characterized in animals. In insects, bib has been shown to be localized at endosomes to regulate Notch signalings by modulating endosome maturation, trafficking, and acidification (15). In mammals, AQP8 and AQP9 were reported to be localized at mitochondria (19, 20). AQP1 and AQP6 were also shown to be localized at exocrine vesicles and synaptic vesicles, respectively (21, 22). These results, however, require further confirmations since different results have also been reported (23). AQP10 was also shown to be localized at intracellular vesicles of enterochromaffin cells in human although its role in these cells is unclear (24). As mentioned above, AQP11 was reported to be localized inside the cell in the kidney, brain and testis (12-14). AQP12, another member of the group III AQPs was also intracellularly localized when expressed at a fibroblast cell line although its exact localization in the pancreas remains to be clarified (25).

What is the role of intracellular AQPs? Their physiological significance in water transport is debated as intracellular organelles are so small that surface-to-volume ratios may be large enough to facilitate water movement without water channels. In plants, very abundant AQPs (TIPs) are present at vacuoles (tonoplasts), where more than 10% of membrane proteins are TIPs (26). TIPs may enable tonoplasts to transport water freely inside the cell where one or several vacuoles occupy more than 30 to 90% of the cell volume. Alternatively, as TIPs also transport ammonia and H_2O_2, they may be needed for decomposition, a breakdown of dead organisms or storing substrates in the tonoplast (27). TIP null plants, however, revealed no abnormalities under optimal growth conditions (28). The result may indicate that TIP is not essential for plants or that some members of TIP family may redundantly transport water across the tonoplast. In animals, the role of intracellular AQPs is not clear as gene disruption studies showed no functionally significant water channels in mitochondria (23). Only AQP11 disruption in mice revealed endosomal pH dysregulation (13). Possible roles of AQPs in intracellular water movement (8) and in the mitochondrial function (29) were recently reviewed.

PERSPECTIVES

Faced with arrays of AQP functions, we need a guide for sorting out these functions. We have proposed a classification of AQPs into three groups based on primary structures, which may also reflect evolution of AQPs. As this classification primarily depends on the pore-forming sequences of the second NPA box, it may also reflect permeating substrates, thus channel functions of AQPs. With accumulation of more members through genome projects, further divisions will be necessary, especially in the group III AQPs as their homologies with each other are relatively low. This classification will be useful to speculate functions of AQP-like sequences as each group may have specific characteristics in its function, distribution and regulation.

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