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AQUAPORINOLOGY

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Abstract

The water channels milestones include: the vague idea of "hydrophilic pores" or "water-filled channels" in the red blood cell (RBC), the proposal that water channels (WCh) are accommodated in proteins, experiments associating WCh with the major RBC membrane protein called Band 3 (the anion exchanger), and the crucial experiment performed in 1985 by Benga group in Cluj-Napoca, Romania, proving the presence and location of a minor protein of the RBC membrane involved in water transport. In the landmark papers of 1986, Benga introduced the concept of the WCh being a protein specialized in water transport, i.e. a water channel protein (WCP). The first WCP discovered by our group was re-discovered in 1992 by Agre group. In the same year two other WCPs were discovered. The name aquaporins was proposed in 1993. In subsequent years hundreds of WCPS have been discovered in organisms from all kingdoms of life. WCPs are a family of membrane proteins, belonging to the Membrane Intrinsic Proteins superfamily. WCPs family include three subfamilies: 1) aquaporins (AQPs) which are mainly water selective channels; 2) aquaglyceroporins are permeable to water and to other small uncharged molecules; 3) S-aquaporins (subcellular or superaquaporins). Benga called aquaglyceroporins and S-aquaporins the "relatives of aquaporins". Twelve WCPs were identified in the human body, having a great importance in a lot of physiological phenomena, as well as in pathological conditions, from well defined "water channelopathies" to a wide range of diseases. Benga propose the name of aquaporinology for the domain of biomedical and natural sciences dedicated to the integrated approach of WCPs (aquaporins and relatives), which is also a chapter of Cellular and Molecular Biology.

Key words: aquaporinology, aquaporins, aquaglyceroporins, water channel proteins, new scientific domain.

INTRODUCTION

The milestones in the discovery of water channels were presented in detail previously (1-5). First, a vague idea of

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"hydrophilic pores" (6) or "water-filled channels" (7) in the red blood cell (RBC) membrane was formulated, however it was not clear which component of the membrane (lipid, protein or both) constitutes the pore. Second, Macey and Farmer (8) proposed that proteins in the RBC membrane accommodate water channels, however there was no idea in which protein could be located. Third, there were experiments aimed at associating water channels with specific membrane proteins using radioactive-sulfhydryl labeling methods, without correlating binding with inhibition of water transport. The conclusion was that water channels are located in the major protein in the RBC called band 3 (according to the position on the electrophoretogram of RBC membrane proteins, with a molecular weight of about 95 kDa, protein already known to be the anion exchanger) (9, 10). Moreover, it was even proposed that band 3 protein is a common pore for water, cations, anions and nonelectrolytes (11). After a decade of systematic research on water transport in red blood cells (RBCs) the group of Benga in Cluj-Napoca, Romania, clearly demonstrated for the first time, by experiments performed in 1985, that the water channel is located in "a minor protein binding PCMBS", migrating in the region of 35-60 kDa on the electrophoretogram (12,13). In fact we identified (hence discovered) the first water channel protein (WCP) in the RBC membrane; in addition, by these landmark papers Benga also introduced the concept of the water channel being in fact a protein specialized in water transport, i.e. a water channel protein (WCP). In subsequent reviews Benga emphasized the novelty of our findings (14-16).

In 1988 the group of Agre in Baltimore, USA, purified by chance the same protein and called it CHIP28 (*CH*annel forming Integral membrane Protein of 28 kDa), suggesting that it may play a role in the linkage of the membrane skeleton to the lipid bilayer (17); the group identified the water transport property of this protein in 1992 (18). As emphasized by Vandenberg and Kuchel (19) in fact our group in Romania discovered the glycosylated component of CHIP28 protein several years before the group of Agre.

In 1993 two other WCPs were discovered and cloned: WCH-CD (Water *CH*annel of the kidney Collecting Ducts) from the rat kidney (20) and γ-TIP (γ-Tonoplast Intrinsic Protein) from the vacuolar membrane (tonoplast) of *Arabidopsis thaliana* (21). Thus it became obvious that a family of WCPs exists and the name "aquaporins" was proposed for this class of membrane proteins, from the Latin words: aqua=water and porus=passage (22). The WCP first discovered by my group in 1985 and re-discovered by the group of Agre in 1988-1992 was called aquaporin 1 (AQP1).

The utmost importance of this discovery was recognized by the Nobel Foundation and The Royal Swedish Academy of Sciences, as half of the 2003 Nobel Prize in Chemistry was awarded to Agre "for the discovery of water channels" (water channels are synonymous with water channel proteins). As a graduate in both medicine and chemistry, I was pleased when

the Nobel Committee for chemistry selected water channels as the topic to be recognized in 2003. However, I was very disappointed that my group's priority in the discovery was overlooked.

Benga formulated a Petition for the recognition of his priority in the discovery of the first WCP (see *http://www.gheorghebenga.ro/wordpress*).

Benga's claim has been supported by thousands of scientists, including George Emil Palade (Romanian born American scientist, by many renowned scientists, including Wolburg *et al.* (23) who stated: "The detection of water-specific membrane channels in red blood cells belongs to the fundamental discoveries in biology of the twentieth century (Benga *et al.* 1986 a,b; Denker *et al.*1988; Preston *et al.* 1992)."

WCPs, as a family of membrane proteins, belong to the Membrane Intrinsic Proteins (MIPs) superfamily with more than 1000 members (24). In addition to WCPs, MIPs also include proteins with no identified channel activity (Fig. 1, reprinted, with permission, from ref. 25).

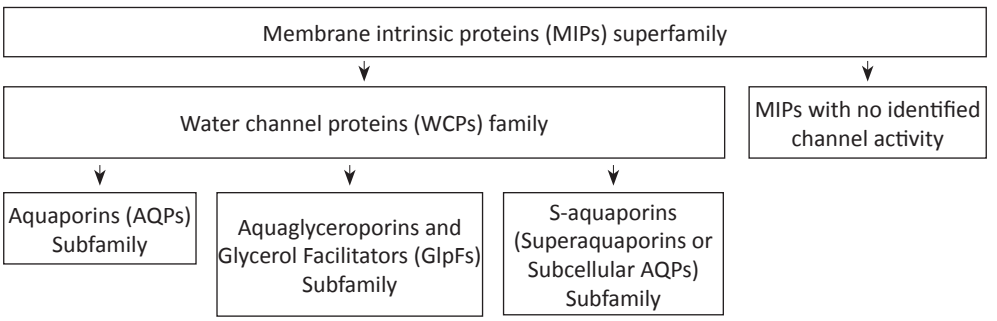

Figure 1. Proposed nomenclature and classification of water channel proteins and MIP proteins.

1974 Nobel Prize in Physiology and Medicine), who wrote: "… The idea of a petition has the merit of attracting the attention to the scientific community to the regrettable mistake of your omission from the group of laureates this year. …I signed the petition received from you. I wish you enough courage and strength to carry through this battle" (communicated by fax to The "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, on 4 December 2003).

The scientific importance of the discovery of WCPs and Benga group's contribution have also been recognized

WCPs family of proteins include three subfamilies: 1) aquaporins (abbreviated as AQPs) which are mainly water selective channels; they were also named by various authors as "classical", "sensu strictu" aquaporins", "orthodox", "ordinary", "conventional", "pure", "normal"; 2) aquaglyceroporins are permeable to water, but also to other small uncharged molecules, in particular glycerol; 3) the third subfamily of WCPs have amino acid sequences unclassifiable to the first two

subfamilies. Benga recommended (25) to use always for this subfamily the name S-aquaporins, although they received various names: "superaquaporins", "aquaporins with unusual (or deviated) NPA boxes", "subcellular aquaporins", "sip-like aquaporins", or "unorthodox". Benga called aquaglyceroporins and S-aquaporins the "relatives of aquaporins".

Regarding the nomenclature of WCP, Benga believes that some confusion existing in the scientific literature should be avoided. Some authors say: "Aquaporins are divided into aquaporins, aquaglyceroporins and superaquaporins". This is like saying: "Men are divided into men, women and children" (with a note that "children" are "supermen"!). As discussed previously (26), since it appears more appropriately to say: "People are divided into men, women and children", Benga believed that it is also more appropriate to say: "Water Channel Proteins (WCPs) are divided into aquaporins, aquaglyceroporins and S-aquaporins". In this way the requirement of adding another specification for aquaporins ("orthodox" etc.) or S-aquaporins ("superaquaporins" or "unorthodox", why not "non-catholic"?) is avoided.

So far, 13 WCPs have been discovered in humans (and other mammals). Out of these, seven are aquaporins (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8), four are aquaglyceroporins (AQP3, AQP7, AQP9, and AQP10), whereas AQP11 and AQP12 are S-aquaporins. WCPs play very important roles in a variety of cellular processes in all organs of the human body. On the other hand, the malfunction of WCPs is linked to a lot of diseases. Some of these are water channelopathies (the congenital cataract due to mutations in AQP0 gene, the nephrogenic diabetes insipidus due to mutations in AQP2 gene, or neuromyelitis optica due to autoantibodies against AQP4). WCPs are also implicated in a wide range of diseases including epilepsy (27), brain disorders, kidney diseases, cardiovascular diseases, gastrointestinal and hepatobiliary diseases, cancer, etc. (see Table 1 and articles in ref. 28).

Benga believes that in fact the discovery of WCPs was a crucial event in science, opening a new domain of biomedical and natural sciences, for which he suggested the term "aquaporinology" (29). There are several arguments to justify the proposal of this name for a new domain of science. Many scientific names are based on Latin and Greek words. In addition to Latin words "aqua" and "porus" (mentioned above), "logos" is a Greek word with several meanings; one of these refers to "subject of study or topic of discussion" (30).

A domain of science should have its own "object" of study, condition fully fulfilled by aquaporinology; its "object" of study, the WCPs (aquaporins and relatives), is quantitatively important, since hundreds of WCPs have been discovered in organisms from all kingdoms of life, including unicellular organisms (archaea, bacteria, yeasts, and protozoa) and multicellular ones (plants, animals, and humans) (reviewed in ref. 31). Hence, the new domain aquaporinology is implicated in many branches of biology: microbiology,

Table 1. The water channel proteins present in humans, some of their physiological implications and the well defined water channelopathies

Water channel protein	Permeability characteristics	Tissue distribution	Function	Water channelopathy
AQP0	Water (low)	Lens fiber cells	Maintaining the transparency and integrity of the lens	Congenital cataract (due to mutations in the AQP0 gene)
AQP1	Water (high)	Red blood cell Kidney proximal tubule Eye: ciliary epithelium Brain: choroid plexus Lung: alveolar epithelial cells	Multiple functions Concentration of urine Production of aqueous humor Production of cerebrospinal fluid Alveolar hydration state	
AQP2	Water (high)	Kidney: collecting ducts	Mediates antidiuretic hormone activity	Nephrogenic diabetes insipidus (due to mutations in AQP2 gene)
AQP3	Water (high), Glycerol (high), urea (moderate)	Kidney: collecting ducts Respiratory tract: bronchial epithelium, epithelial cells Skin Eye Colon	Reabsorption of water Tracheal and bronchial fluid secretion Hydration of skin	
AQP4	Water (high)	Kidney: collecting ducts Brain: ependymal cells Lung: bronchial epithelium	Reabsorption of water CSF fluid balance Bronchial fluid secretion	Neuromyelitis optica (due to autoantibodies against AQP4)
AQP5	Water (high)	Salivary glands Lacrimal gland Sweat gland Lung, cornea	Production of saliva Production of tears Production of sweat	
AQP6	Water (low), anions (NO^{3-} > Cl^-)	Kidney		
AQP7	Water (high), glycerol (high), urea (high), arsenite	Adipose tissue, kidney, testis		
AQP8	Water (high)	Testis, kidney, liver, pancreas, small intestine, colon		
AQP9	Water (high), glycerol (high), urea (high), arsenite	Liver, leukocytes, brain, testis		
AQP10	Water (low), glycerol (high), urea (high)	Small intestine		
AQP11	Water (high)	Testis, kidney proximal tubules, liver	Water movement across intracellular membranes	
AQP12	Water (high)	Pancreas	Water movement across intracellular membranes	

botanics, zoology, human biology.

Moreover, the study of WCPs is performed today from the molecular and cellular level (three-dimensional structure, structure-function relationships, regulation, expression in various cells, as described for AQP1 in ref. 5) to the level of whole organisms, of species (32) and of populations. Some of these aspects belong to structural chemistry, biochemistry and biophysics (see for example ref. 33).

In addition, as WCPs are involved in so many and important physiological and pathological processes Benga believes that aquaporinology is also a domain of biomedical sciences. What are biomedical sciences? There are many definitions available on the web. "Biomedical science is the application of the principles of the natural sciences to medicine" (*http://www/thefreedictionary.com/biomedical+science*). "Biomedical science is the study of human body in health and disease" (*http://www.ox.ac.uk/admissions/undergraduate_courses/courses/biomedical_sciences/biomedical_sciences.html*). "Biomedical science combines the fields of biology and medicine in order to focus on the health of both animals and humans" (*https://www.princetonreview.com/Majors.aspx?cip=260002*).

Among the great progresses of biology in the second half of the XXth century was "the creation of a new field of biology in which structure, biochemistry, function, and biogenesis of cells are considered together. This integrated approach known as Cellular and Molecular Biology proved to be remarkably productive." (G.E. Palade, personal communication).

Based on the above mentioned considerations aquaporinology embraces many disciplines of biomedical sciences and many branches of natural sciences: biology, (bio)chemistry, (bio) physics. Hence, Benga believes that aquaporinology can be defined as the domain of biomedical and natural sciences dedicated to the integrated approach of WCPs (aquaporins and relatives), as well as a chapter of Cellular and Molecular Biology.

In order to debate all aspects of WCPs, Benga organized in October 2011 The First World Congress on Water Channel Proteins (Aquaporins and Relatives) Celebrating the 25th Anniversary of the Discovery of the First Water Channel Protein (Later Called Aquaporin 1). The Second World Congress on Water Channel Proteins (Aquaporins and Relatives) Celebrating the 30th Anniversary of the Discovery of The First Water Channel Protein will take place in Cluj-Napoca (May 20-24, 2015).

Conflict of interest

The author declares that there is no conflict of interest.

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