AQUAPORINOLOGY (THE STUDY OF WATER CHANNEL PROTEINS - AQUAPORINS AND RELATIVES) AS A NEW DOMAIN OF NATURAL SCIENCES

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Abstract. A new domain of natural sciences, for which I propose the scientific term "aquaporinology", began with the discovery in 1985 by BENGA group in Cluj-Napoca, Romania, of the first water channel protein from the human red blood cell membrane. This protein was re-discovered in 1992 by AGRE group in Baltimore, USA, and was called aquaporin 1 (AQP1) in 1993. In subsequent years, hundreds of water channel proteins have been discovered in unicellular organisms (archaea, bacteria, yeasts, and protozoa) and multicellular organisms (plants, animals, and humans). In addition to aquaporins, other classes of water channel proteins (that I called "relatives of aquaporins") have been discovered: aquaglyceroporins, S-aquaporins, etc. The study of water channel proteins became a new domain of natural sciences and a very hot field of research with a lot of theoretical and practical issues.

Keywords: Aquaporinology, aquaporins, water channel proteins, natural sciences, new scientific domain.

Rezumat. Aquaporinologia (studiul proteinelor canal pentru apă - aquaporine şi rudele lor) ca un domeniu nou al ştiinţelor naturii. Un domeniu nou al ştiinţelor naturii, pentru care propun termenul ştiinţific de „aquaporinologie", a început cu descoperirea în 1985 de către grupul BENGA la Cluj-Napoca, România, a primei proteine canal pentru apă din membrana celulei roşii umane. Această proteină a fost re-descoperită în 1992 de către grupul AGRE la Baltimore, USA, fiind numită în 1993 aquaporina 1 (AQP1). In anii următori s-au descoperit sute de proteine canal pentru apă în organisme unicelulare (archea, bacterii, drojdii, protozoare) şi organisme multicelulare (plante, animale, oameni). Pe lângă aquaporine s-au descoperit alte clase de proteine canal pentru apă (pe care le-am numit "rude ale aquaporinelor": aquagliceroporinele, S-aquaporinele). Studiul proteinelor canal pentru apă a devenit un domeniu nou al ştiinţelor naturii şi un foarte fierbinte domeniu de cercetare cu o mulţime de aspecte teoretice şi practice.

Cuvinte cheie: aquaporinologie, aquaporine, proteine canal pentru apă, ştiinţele naturii, nou domeniu ştiinţific.

The discovery of the water channel proteins (WCPs) is considered by many scientists as being of utmost importance. As formulated by WOLBURG et al., (2011): "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., 1986a, b; DENKER et al., 1988; PRESTON et al., 1992)." The first WCP, called today aquaporin 1 (AQP1), was discovered in the red blood cell (RBC) membrane by BENGA group in 1985 in Cluj-Napoca, Romania, reported in publications in 1986 (BENGA et al., 1986a,b) and reviewed in subsequent years (BENGA et al., 1988; 1989a, b). I have previously reviewed the discovery of the first WCP and presented in detail the landmarks leading to this discovery (BENGA 2003; 2004; 2006a-c, 2009; 2011; 2012a, b).

This protein was purified by chance in 1988 by AGRE group in Baltimore, USA, and called CHIP28 (*CH*annel forming *I*ntegral membrane *P*rotein of *28* kDa) (DENKER et al., 1988). The AGRE group found the water transport property of this protein only in 1992 (PRESTON et al., 1992). In the same year, other WCPs were discovered and cloned: WCH-CD (*W*ater *CH*annel of the kidney *C*ollecting *D*ucts) from the rat kidney (FUSHIMI et al., 1993) and γ-TIP (γ-*T*onoplast *I*ntrinsic *P*rotein) from the vacuolar membrane (tonoplast) of *Arabidopsis thaliana* (MAUREL et al., 1993). Thus, it became obvious that the 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 (AGRE et al., 1993). The WCP first discovered by my group in 1985 and re-discovered by AGRE group in 1992 was called aquaporin 1 (AQP1).

I claim now that this discovery was in fact a crucial event in science, opening a new domain of natural sciences, dedicated to all aspects of WCPs, domain for which I suggest here the term of "aquaporinology". As I have been working in the field for more than 25 years, I could see that this domain of science became a very hot area of research embracing many branches of natural sciences, as 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). These proteins are now studied from the molecular and cellular level (structure-function relationships, expression in various cells, regulation) to the level of whole organisms and of populations. New very important aspects are uncovered every day, the diversity of hundreds of WCPs is revealed, with increasing practical implications, including the physiological and medical implications. Thousands of publications appeared on these topics, including many reviews (see references in BENGA 2009). I am still actively working in the field as it can be seen from the articles of my group (reviewed by BENGA 2013).

The scientific importance of the discovery of WCPs was also recognized by the Nobel Foundation, as half of the 2003 Nobel Prize in Chemistry "for the discovery of water channels" was awarded to Peter AGRE (USA), a physician from Baltimore. Water channels are synonymous with water channel proteins. As a graduate in both medicine and chemistry field, I was pleased that The Nobel Committee for Chemistry selected the water channels as the area of interest in 2003. However, I was very disappointed that my landmark discovery of the first WCP in the human RBC

membrane was overlooked by The Nobel Committee and The Royal Swedish Academy of Sciences. My claim is now supported by thousands of scientists from the whole world.

A **water channel protein** (WCP) can be defined as a transmembrane protein that has a specific three-dimensional structure with a pore that provides a pathway for water permeation across biological membranes (BENGA 2009). The three-dimensional structure of AQP1 is represented in figure 1.

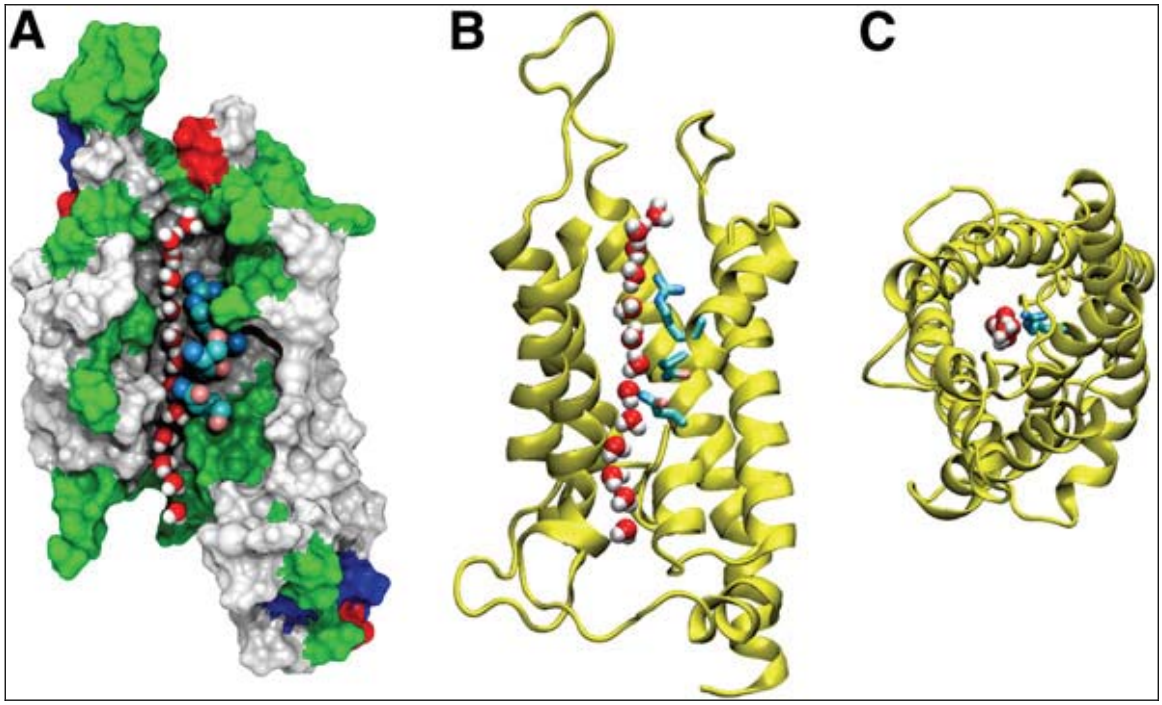

Figure 1. The structure of an AQP monomer (a,b: side views; c: topview).
(From WANG & TARKHORJID 2007).

The monomer is shown in surface representation in a and in yellow cartoon representation in b,c. In a, charged residues are drawn in red (negative) and blue (positive), polar residues in green, and nonpolar hydrophobic residues in white. Water molecules form a single file inside the channel, with a unique bipolar configuration that disfavours proton transfer through the file (24). The interior of the channel is mostly hydrophobic. Key hydrogen-binding residues that line the pore, namely an Arg at the *selectivity filter* and 2 asparagines from the conserved *asparagineproline-alanine (NPA) motifs*, are explicitly shown. Reproduced from Wang and Tajkhorshid, 2007, with permission of the copyright holder.

WCPs, as a family of membrane proteins, belong to the Membrane Intrinsic Proteins (MIPs) superfamily with more than 1000 members (reviewed by ZARDOYA 2005). In addition to WCPs, MIPs also include proteins with no identified channel activity. WCPs are characterized by a homotetrameric structure, with each monomer having a pore formed by two highly conserved regions in the amino acid sequence, called NPA boxes (or motifs) with three amino acid residues (asparagine-proline-alanine, NPA) and several surrounding amino acids. The NPA boxes have been called the "signature" sequence of WCPs (reviewed by BENGA 2012c).

WCPs family of proteins include three subfamilies. 1) ***Aquaporins*** (abbreviated as AQPs) are mainly water selective or specific water channels; they were also named by various authors as "orthodox", "ordinary", "conventional", "classical", "pure", "normal", or "sensu strictu" aquaporins; 2) ***Aquaglyceroporins*** are permeable to water, but also to other small uncharged molecules, in particular glycerol; 3) The third subfamily of WCPs have little conserved amino acid sequences around the NPA boxes, unclassifiable to the first two subfamilies. They received various names: "superaquaporins", "aquaporins with unusual (or deviated) NPA boxes", "subcellular aquaporins", or "sip-like aquaporins". I recommended (BENGA, 2012a) to use always for this subfamily the name ***S-aquaporins***. I called aquaglyceroporins and S-aquaporins the "relatives of aquaporins".

Some confusion regarding the nomenclature of WCP exists in the scientific literature. Some authors say: "Aquaporins are divided into aquaporins, aquaglyceroporins and superaquaporins". It is like saying: "Men are divided into men, women and children" (with a note that "children" are "supermen"!). As discussed previously (BENGA 2012d) since it appears more appropriately to say: "People are divided into men, women and children", I believe 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 ("sensu strictu", "orthodox", "ordinary", "conventional", "classical", "pure" or "normal") is avoided, as well as that of naming the WCPs with deviated NPA boxes as "superaquaporins" or "unorthodox" (why not "non-Catholic"?).

In order to debate all aspects of WCPs I 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 is already scheduled to take place in Cluj-Napoca (May 20-24, 2015).***

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