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Purification and Partial Characterization of the M_r 30,000 Integral Membrane Protein Associated with the Erythrocyte Rh(D) Antigen*

(Received for publication, April 29, 1987)

Peter Agre‡, Ali M. Saboori, Andrew Asimos, and Barbara L. Smith

From the Departments of Medicine and Cell Biology/Anatomy, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

surface (8, 9). A protein of M_r approximately 28,000–33,000 was recently identified which can be surface ^{125}I-labeled on Rh(D) bearing erythrocytes and specifically precipitated with Rh(D) immune globulin (10–12). This Rh-associated protein contains no carbohydrate (13) and is linked to the membrane skeleton (14, 15). Purification and additional characterization of this Rh-associated protein was considered a direct approach to better understanding of this important and complex blood group system.

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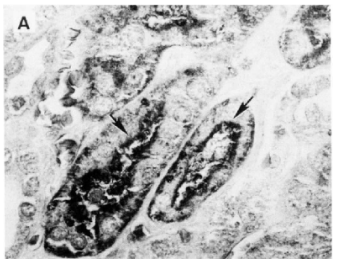
Identification, Purification, and Partial Characterization of a Novel M_r 28,000 Integral Membrane Protein from Erythrocytes and Renal Tubules*

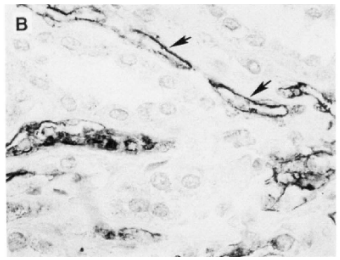
(Received for publication, April 19, 1988)

Bradley M. Denker, Barbara L. Smith, Francis P. Kuhajda, and Peter Agre‡

From the Departments of Medicine and Cell Biology/Anatomy, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

characterized. This paper describes the isolation and partial characterization of an abundant erythrocyte integral membrane protein of M_r 28,000 ("28kDa") which is linked entirely to the membrane skeleton. While the physiologic importance of 28kDa remains uncertain, a role in linkage of the membrane skeleton to bilayer is a possibility.





The physiologic role of the 28kDa polypeptide is uncertain. The existence of a polypeptide nearly identical to 28kDa in the apical brush borders of renal proximal-convoluted tubules is provocative. There is yet no evidence that 28kDa is itself a membrane transporter, but such a role is consistent with its physical behavior. The 28kDa could conceivably be a noncatalytic subunit of one or more transporters by interacting side-to-side between the leaflets of the lipid bilayer with catalytic subunits which would be restricted to a favorable orientation within the cell membrane. Renal proximal-convoluted tubule

Murer and Gmaj, 1986). Cytoskeletal elements related to those of the erythrocyte have recently been colocalized to the basolateral surfaces of polarized kidney epithelia apparently in association with specific transport polypeptides. Analogs to ankyrin and spectrin were colocalized with the anion transporter in basolateral membranes of rat kidney distal tubules (Drenckhahn *et al.*, 1985). Fodrin colocalized with Na,K-

eral membranes. The abundance of 28kDa in apical brush borders suggests that it may be a structural component of transport. Moreover, a skeleton-linked integral membrane protein like 28kDa may provide the mechanism whereby the state of cell volume is sensed from the degree of membrane skeleton distension and transduced back to the appropriate membrane transporters or channels which respond by restricting the entry of additional water or electrolyte. The relative ease with which 28kDa can be isolated and the existence of potent antibodies to 28kDa may make the evaluation of such potential roles feasible.

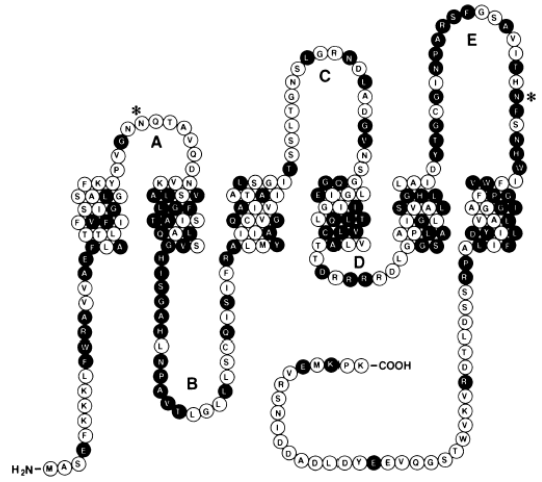
Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 11110–11114, December 1991
Cell Biology

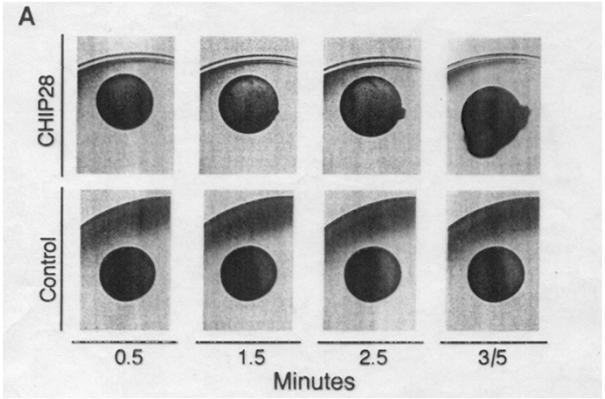
Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: Member of an ancient channel family

GREGORY M. PRESTON AND PETER AGRE
Departments of Medicine and Cell Biology/Anatomy, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205

Communicated by Pedro Cuatrecasas, September 20, 1991 (received for review August 21, 1991)

Analysis of the deduced amino acid sequence suggests that CHIP28 protein contains six bilayer-spanning domains, two exofacial potential N-glycosylation sites, and intracellular N and C termini. Search of the DNA sequence data base revealed a strong homology with the major intrinsic protein of bovine lens, which is the prototype of an ancient but recently recognized family of membrane channels. These proteins are believed to form channels permeable to water and possibly other small molecules. CHIP28 shares homology with all known members of this channel family, and it is speculated that CHIP28 has a similar function.





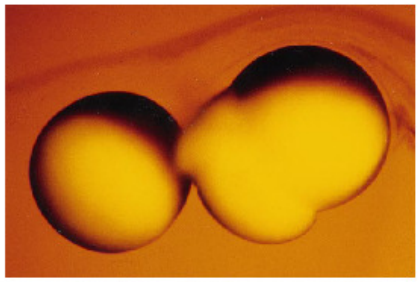
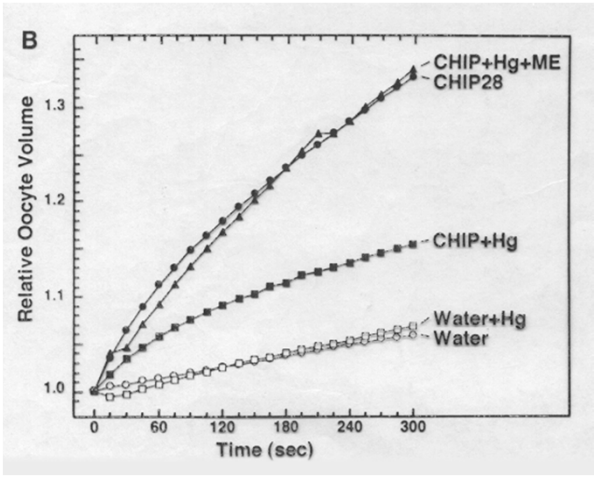
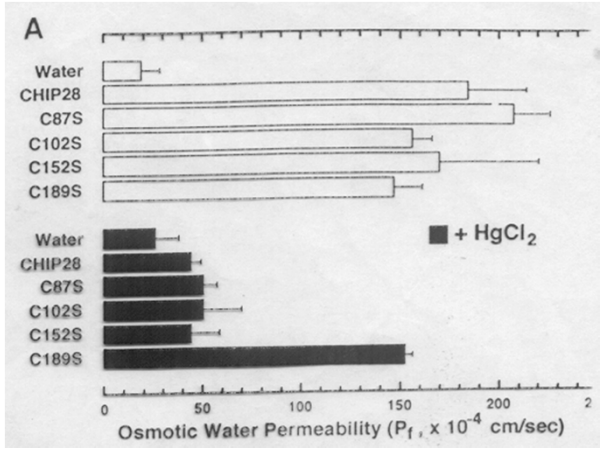
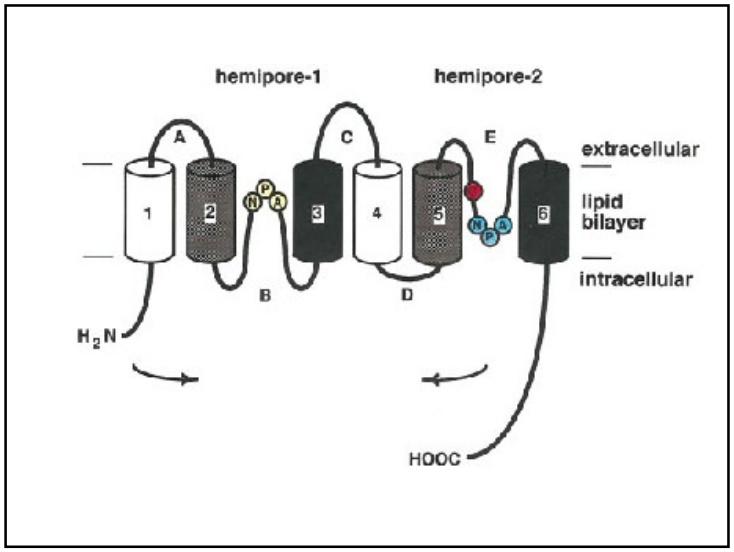
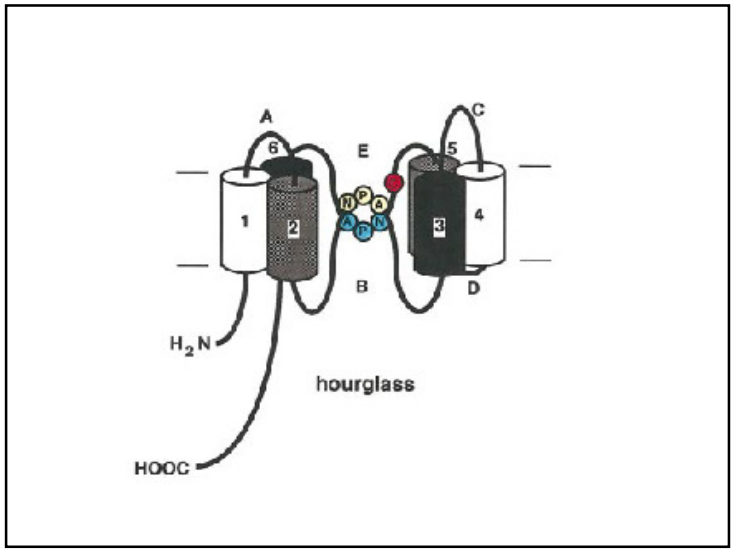


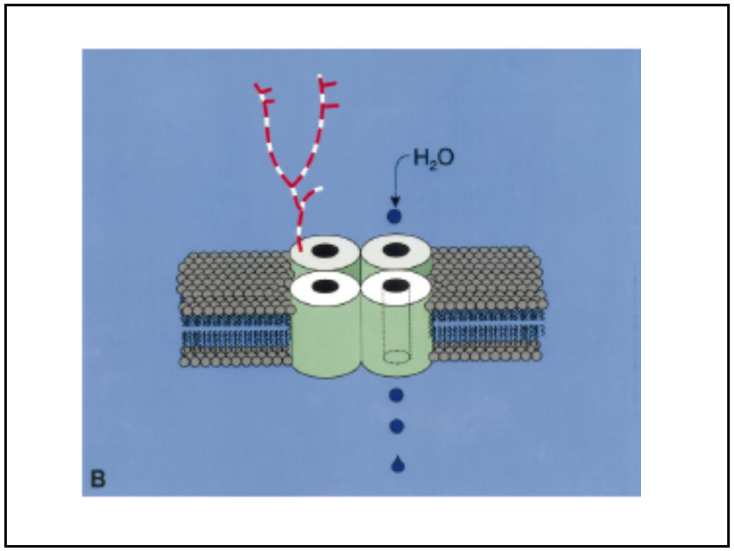
FIG. 1. **Water permeability of AQP1 expressed in *Xenopus* oocytes.** Control oocytes exhibit negligible water permeability in hypo-osmotic buffer (*left*) whereas oocytes expressing AQP1 rapidly swell and explode (*right*).

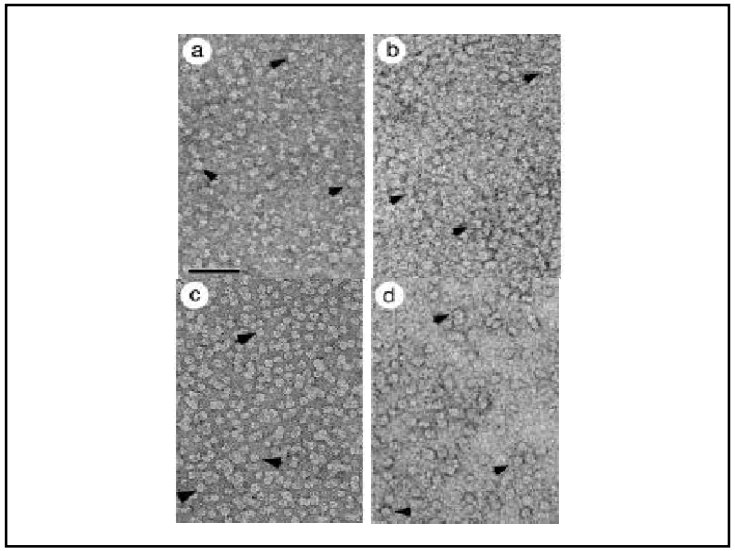


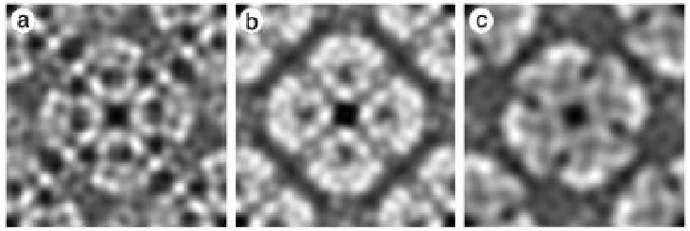



hemipore-1
hemipore-2
A
C
E
1
2
3
4
5
6
extracellular
lipid
bilayer
intracellular
B
D
H_2N
HOOC

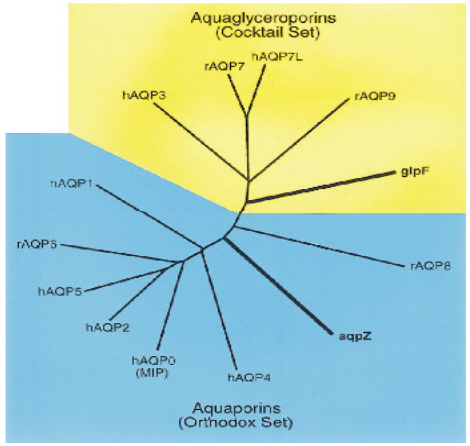

A
C
E
6
5
1
2
3
4
B
D
H_2N
hourglass
HOOC

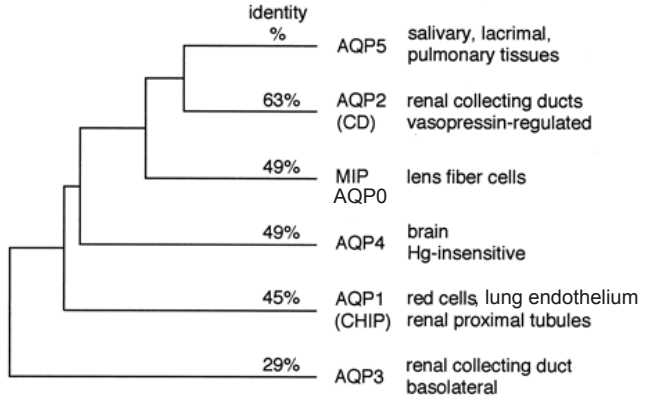

H_2O
B

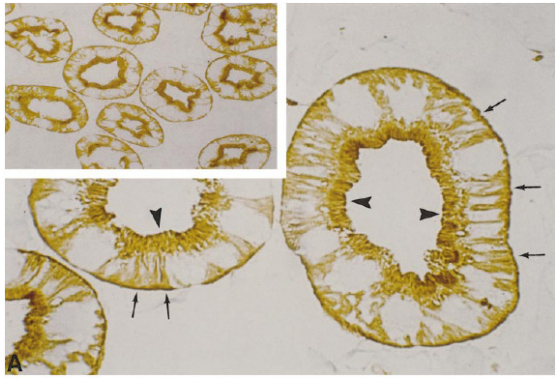

a
b
c
d



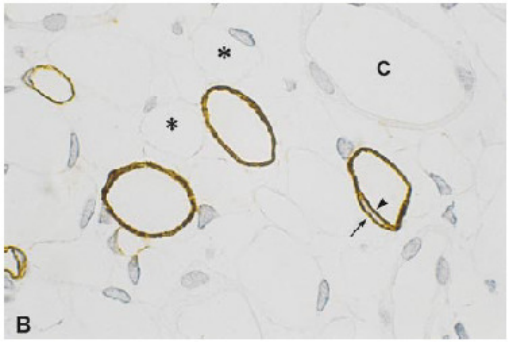
AQP1 (globuli rossi) | AQP0 (cristallino) | AqpZ (E. Coli)



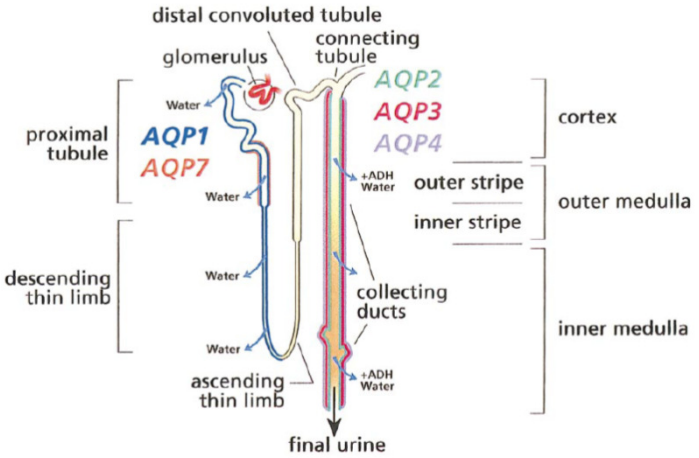


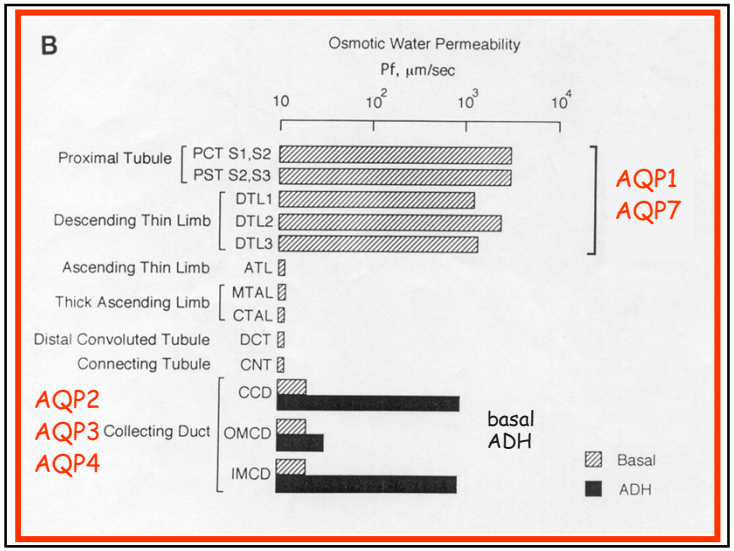


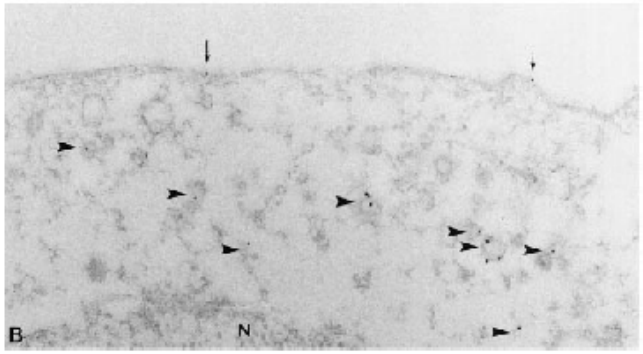
Sezione sottile di corticale renale di ratto, anticorpi anti-AQP1, colorazione con perossidasi



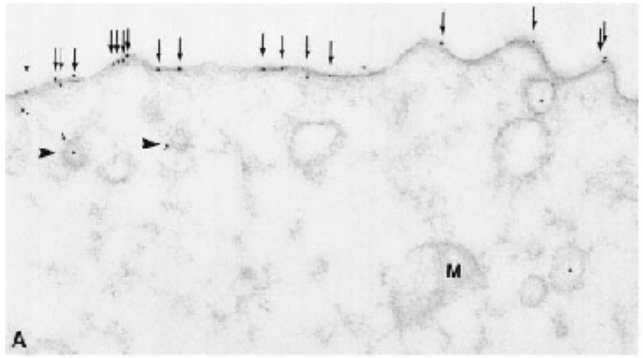
Sezione sottile di midollare interna di ratto, anticorpi anti-AQP1, colorazione con perossidasi



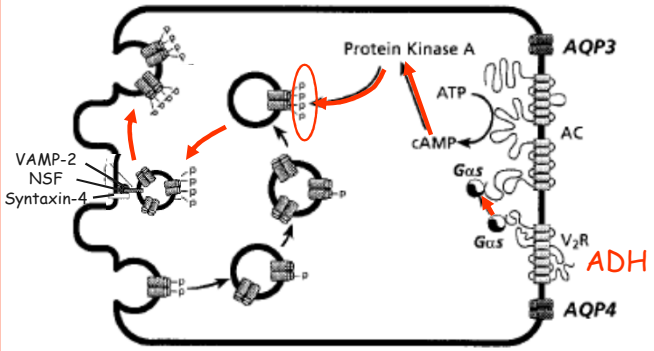


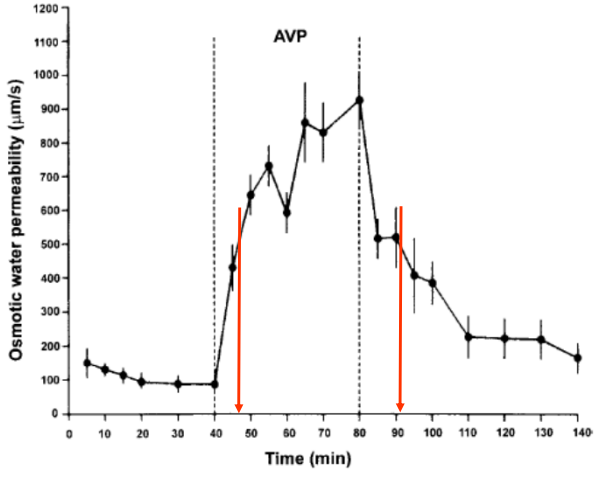


Sezione ultrasottile di dotto collettore di ratto in vitro. Microscopia elettronica dopo reazione con anticorpi anti-AQP2 coniugati con oro colloidale prima ……..



…. e dopo trattamento con ADH *in vitro*





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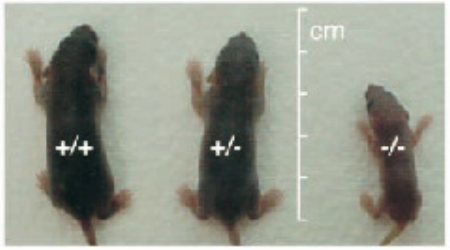
Neonatal Mortality in an Aquaporin-2 Knock-in Mouse Model of Recessive Nephrogenic Diabetes Insipidus*

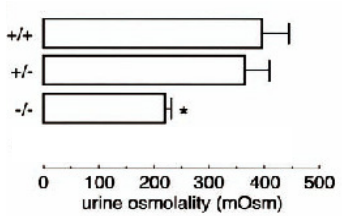
Received for publication, September 7, 2000, and in revised form, October 3, 2000
Published, JBC Papers in Press, October 16, 2000, DOI 10.1074/jbc.M008216200

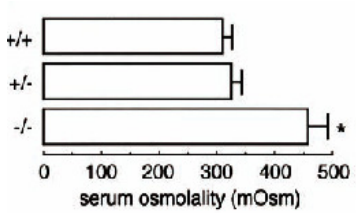
Baoxue Yang, Annemarie Gillespie, Elaine J. Carlson, Charles J. Epstein, and A. S. Verkman‡

From the Departments of Medicine, Physiology, and Pediatrics, Cardiovascular Research Institute, University of California, San Francisco, California 94143-0521

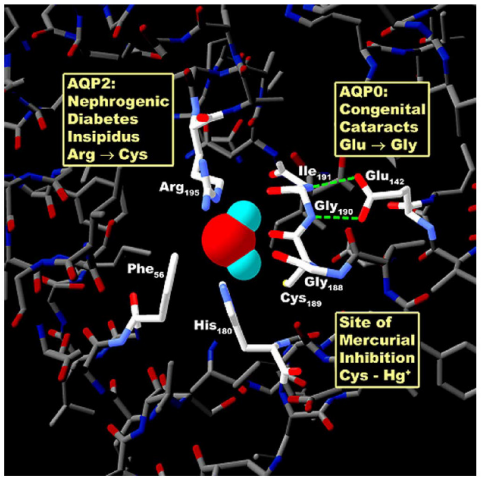


Hereditary non-X-linked nephrogenic diabetes insipidus (NDI) is caused by mutations in the aquaporin-2 (AQP2) water channel. In transfected cells, the human disease-causing mutant AQP2-T126M is retained at the endoplasmic reticulum (ER) where it is functional and targetable to the plasma membrane with chemical chaperones. A mouse knock-in model of NDI was generated by targeted gene replacement using a Cre-loxP strategy. Along with T126M, mutations H122S, N124S, and A125T were introduced to preserve the consensus sequence for *N*-linked glycosylation found in human AQP2. Breeding of heterozygous mice yielded the expected Mendelian distribution with 26 homozygous mutant offspring of 99 live births. The mutant mice appeared normal at 2–3 days after birth but failed to thrive and generally died by day 6 if not given supplemental fluid. Urine/serum analysis showed a urinary concentrating defect with serum hyperosmolality and low urine osmolality that was not increased by a V2 vasopressin agonist. Northern





P. Agre, D. Kozono/FEBS Letters 555 (2003) 72–78



NATURE REVIEWS | MOLECULAR CELL BIOLOGY

VOLUME 5 | SEPTEMBER 2004 | 689

FROM STRUCTURE TO DISEASE: THE EVOLVING TALE OF AQUAPORIN BIOLOGY

Landon S. King, David Kozono and Peter Agre

Abstract | Our understanding of the movement of water through cell membranes has been greatly advanced by the discovery of a family of water-specific, membrane-channel proteins — the aquaporins. These proteins are present in organisms at all levels of life, and their unique permeability characteristics and distribution in numerous tissues indicate diverse roles in the regulation of water homeostasis. The recognition of aquaporins has stimulated a reconsideration of membrane water permeability by investigators across a wide range of disciplines.

Aquaporins in the Kidney: From Molecules to Medicine

SØREN NIELSEN, JØRGEN FRØKLÆR, DAVID MARPLES, TAE-HWAN KWON, PETER AGRE, AND MARK A. KNEPPER

The Water and Salt Research Center, Institute of Anatomy, and Institute of Experimental Clinical Research, University of Aarhus, Aarhus, Denmark; University of Leeds, Leeds, United Kingdom; Dongguk University, Kyungju, Korea; Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore; and National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Nielsen, Søren, Jørgen Frøkiær, David Marples, Tae-Hwan Kwon, Peter Agre, and Mark A. Knepper. Aquaporins in the Kidney: From Molecules to Medicine. *Physiol Rev* 82: 205–244, 2002;10.1152/physrev.00024.2001.—

Physiol. Rev. (2002) 82, 205-244