Vol. 217, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 5, 1995 Pages 150-153

THE YEAST *YBR235W* GENE ENCODES A HOMOLOG OF THE MAMMALIAN ELECTRONEUTRAL Na^+-(K^+)-Cl^- COTRANSPORTER FAMILY

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Received October 17, 1995

Summary : The *YBR235w* gene of the yeast *Saccharomyces cerevisiae* was found during sequencing of chromosome II. Here, we show that the 1120 aa protein (Ybr235p) encoded by this gene shares strong sequence similarity with the highly related electroneutral Na^+-Cl^- and Na^+-K^+-Cl^- cotransporters of animal cells. We hypothesize that this yeast protein also mediates active uptake of Cl^- into the cell.

Materials and Methods

To establish a preliminary classification of membrane transport proteins in *S. cerevisiae* (1), release 4.1 of the YPD database containing 4305 proteins (all Genbank *S. cerevisiae* sequences through July 7) (2) was screened for proteins having at least 4 predicted transmembrane domains (3; H_{19} method). Of the 301 proteins thus retrieved, those of known function uninvolved in transport were discarded. The remaining ones (most of which were discovered by genome sequencing) were compared with each other and used in protein similarity searches perfomred by means of the BLAST algorithm (4).

Results and Discussion

Active transport of Cl^- ions into many epithelial and non-epithelial animal cells is ensured by electrically silent Na^+-Cl^- symporters, the driving force for Cl^- entry being provided by the energetically favourable uptake of Na^+ down its electrochemical gradient (maintained by the [Na^+:K^+] ATPase) (5-6). The Na^+-Cl^- cotransporters can be grouped according to their sensitivities to inhibitors and their requirements for K^+. Thus, some mediate Na^+-Cl^-

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0006-291X/95 $12.00

cotransport tightly coupled to that of K^+ with a stoechiometry of $1Na^+$-$1K^+$-$2Cl^-$. These Na^+-K^+-Cl^- cotransporters are sensitive to bumetanide and have been found in many different cell types. For instance, in the thick ascending limb of the loop of Henle in the kidney, epithelial cells reabsorbe NaCl in excess water through an apical Na^+-K^+-$2Cl^-$ cotransporter. Epithelial salt secretion involves a basolateral Na^+-K^+-$2Cl^-$ cotransporter that is considered to provide the main entry pathway for Cl^- and Na^+. Other electroneutral Na^+-Cl^- cotransporters are K^+-independent, insensitive to bumetanide but highly sensitive to the thiazides. These Na^+-Cl^- cotransporters have been identified in renal and non-renal epithelia. The genes encoding thiazide-sensitive Na^+-Cl^- and bumetanide-sensitive Na^+-K^+-Cl^- cotransporters have been recently isolated (5-6). The deduced amino acid sequences defined a new family of highly similar proteins presumably made of 12 membrane-spanning regions flanked by large cytoplasmic N- and C-termini. It was speculated that other cation-Cl^- cotransporters, such as bumetanide-sensitive Na^+-Cl^- cotransporters or the K^+-Cl^- cotransporters detected in erythrocytes and epithelia, could also be protein members of this family (5).

In the course of a systematic computer-aided analysis of putative transport proteins in the yeast *Saccharomyces cerevisiae* (1), our attention was drawn by the *YBR235w* gene of chromosome II (7). This gene encodes a 1120 amino acid protein with 12 predicted transmembrane domains and sharing sequence homology with the Na^+-(K^+)-Cl^- cotransporter family described above. Figure 1 shows the sequence alignment of the yeast *YBR235w* product with the secretory Na^+-K^+-Cl^- cotransporter from shark rectal gland epithelia (sCCC1) (8), the mammalian absorptive Na^+-K^+-Cl^- cotransporter of basolateral kidney cells (rCCC2) (9-10) and the thiazide-sensitive Na^+-Cl^- cotransporter from the urinary bladder of the teleost *P. americanus* (tCCC3) (11). The highest degree of amino-acid identity is in the predicted transmembrane regions. The yeast Ybr235 protein and the vertebrate Na^+-(K^+)-Cl^- cotransporters share 30-32 % identical residues within this region. The predicted intracellular loop connecting predicted transmembrane segments 2 and 3 was previously noted as being extremely well conserved among members of the Na-K-Cl family; this region is as well conserved in yeast Ybr235p (Fig. 1). The C-terminal hydrophilic extremity of Ybr235p is much smaller compared with those of vertebrate Na^+-(K^+)-Cl^- cotransporters.

The high degree of similarity between Ybr235p and vertebrate Na^+-(K^+)-Cl^- cotransporters suggests that Ybr235p might also correspond with an active uptake system for Cl^- ion. This putative Cl^- transporter could function as a H^+-Cl^- symporter or a Na^+-Cl^- symporter. Although the vacuolar membrane of yeast cells has been shown to contain Cl^- transport systems contributing to the formation of a chemical H^+ gradient across the vacuolar membrane (13), there is as yet no reported experimental evidence of the existence of an active Cl^- uptake system in the plasma membrane of yeast cells. Imaginably, such a Cl^- cotransporter might play a role in regulating the water content of yeast cells, since the role of Na^+-K^+-Cl^-

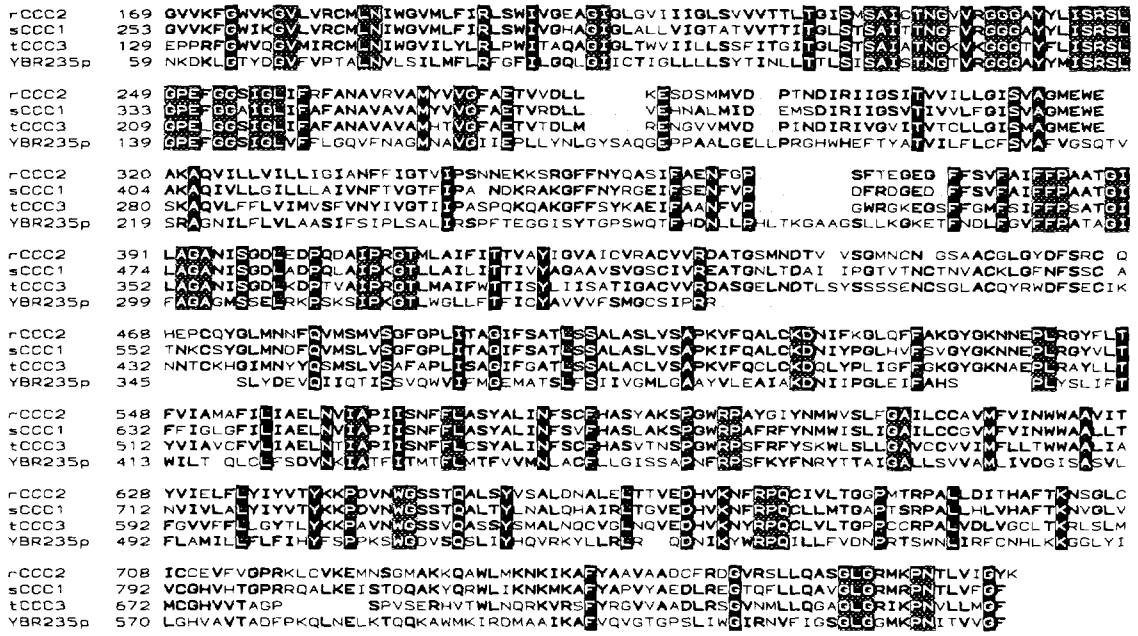

Figure 1. Alignment of the deduced amino-acid sequence of the *S. cerevisiae YBR235w* gene product (7) with that of the secretory Na^+-K^+-Cl^- cotransporter from shark rectal gland epithelia (sCCC1) (8), the mammalian absorptive Na^+-K^+-Cl^- cotransporter of basolateral kidney cells (rCCC2) (9-10) and the thiazide-sensitive Na^+-Cl^- cotransporter from the urinary bladder of the teleost *P. americanus* (tCCC3) (11). Sequence comparison was restricted to the regions covering the predicted membrane-spanning domains. Identical residues are marked in black boxes. Similar residues are marked in bold.

cotransporters in maintaining and regulating cell volume has indeed been well documented in epithelial and non-epithelial animal cells (5). Functional analysis of the *YBR235w* gene by means of reverse genetics and study of its expression in cells grown under various conditions should help in determining the function of this putative transporter.

Acknowledgments

We are grateful to R. Beauwens for reading the manuscript. This work was supported by the Fund for Medical Scientific Research (Belgium) (contract n° 3.4602.94). B. A. is Senior Research Assistant at the National Fund for Scientific Research (Belgium).

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