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Identification of a Vesicular Nucleotide Transporter

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YM and HO designed research, analyzed data and wrote the paper. KS, NE, NJ, TM, MO performed research. AY contributed immunoelectronmicroscopy.

The authors declare no conflict of interest.

Key words: chromaffin granule, synaptic vesicle, ATP, storage and exocytosis, purinergic signaling.

ATP is a major chemical transmitter in purinergic signal transmission. Before secretion, ATP is stored in secretory vesicles found in purinergic cells. Although the presence of active transport mechanism(s) for ATP has been postulated for a long time, the protein(s) responsible for its vesicular accumulation remains unknown. The transporter encoded by the human and mouse SLC17A9 gene, a novel member of an anion transporter family, was predominantly expressed in the brain and adrenal gland. The mouse and bovine counterparts were associated with adrenal chromaffin granules. Proteoliposomes containing purified transporter actively took up ATP, ADP and GTP using membrane potential as the driving force. The uptake properties of the reconstituted transporter were similar to that of the ATP uptake by synaptic vesicles and chromaffin granules. Suppression of endogenous SLC17A9 expression in PC12 cells decreased exocytosis of ATP. These findings strongly suggest that SLC17A9 protein is a vesicular nucleotide transporter and should lead to the elucidation of the molecular mechanism of ATP secretion in purinergic signal transmission.

Vesicular storage and subsequent exocytosis of neurotransmitters is essential for chemical transmission in neurons and endocrine cells. Thus far four distinct classes of transporters are known to participate in the uptake of neurotransmitters into neuronal synaptic vesicles and secretory granules in endocrine cells. These are vesicular monoamine transporters (VMAT), vesicular acetylcholine transporters (VAChT), vesicular inhibitory amino acid transporters (VIAAT) and vesicular glutamate transporters (VGLUT) (1-7). These vesicular transporters mediate the active accumulation of the respective neurotransmitters through an electrochemical gradient of protons across the membrane generated by vacuolar proton-ATPase. ATP is stored in secretory vesicles and subsequently exocytosed, which leads to the various purinergic responses such as central control of autonomic functions, pain and mechanosensory transduction, neural-glia interactions, control of vessel tone and angiogenesis, and platelet aggregation through purinoceptors, thus establishing its role as a chemical transmitter (8-12). Since the concentration of nucleotides in the vesicles was maintained at around 0.1 to 1 M, an active transport mechanism(s) to accumulate nucleotides has been postulated (8-18). Although evidence increasingly supports the presence of a vesicular ATP transporter(s) in secretory vesicles such as synaptic vesicles and adrenal chromaffin granules (13-19), the protein(s) responsible for the ATP accumulation has not yet been identified.

Here, we report the expression and function of human and mouse SLC17A9, a novel isoform of SLC17 phosphate transporter family. We present evidence that the SLC17A9 protein acts as a vesicular nucleotide transporter and that it plays an essential role in vesicular storage of ATP in the ATP-secreting cells.

Results and discussion

Gene Organization and Expression of SLC17A9. SLC17 is a type I phosphate transporter family consisting of eight genes that are classified into three distinct subfamilies:

a) SLC17A1-4 (NPT1, NPT3, NPT4 and Na⁺/PO₄²⁻-cotransporter homologue), Na⁺ and inorganic phosphate co-transporters, b) SLC17A5 (sialin), a lysosomal H⁺/sialic acid co-transporter and c) SLC17A6-8, vesicular glutamate transporters (VGLUT) (5). We found that a fourth subfamily tentatively designated SLC17A9 was identified in a genome data bank screening (Fig. 1A). SLC17A9 was located on chromosome 20 and contained 14 exons and 13 introns (supporting information (SI) Fig. 7). The SLC17A9 protein was 430 amino acid residues long with 12 putative transmembrane helices with around 23 - 29% identity and 41 - 48% similarity to that of other SLC17 members (Fig. 1B and SI Fig. 8). Orthologues of other mammalian species have been identified. Although amino acid sequences of the amino terminal region in mammals exhibited species-specific variation, the remaining regions showed the relatively well-conserved features (83% identity) (SI Fig. 9).

We cloned cDNA encoded SLC17A9 (SI Fig. 8). Northern blot analysis indicated that the SLC17A9 and mouse counterpart (mSLC17A9) were widely expressed in various organs, but predominantly in the adrenal gland, brain and thyroid gland (Fig. 2A). In mouse adrenal gland, the mSLC17A9 protein was specifically expressed in the medulla and was associated with chromaffin granules (Fig. 2B,C). The presence of a SLC17A9 counterpart in bovine adrenal chromaffin granule membrane has been confirmed (Fig. 2D).

SLC17A9 protein is an ATP transporter. We hypothesized that SLC17A9 is responsible for the storage of nucleotides in chromaffin granules, in which high concentrations of ATP and other nucleotides (~0.2 M) are accumulated in a membrane potential ($\Delta\psi$)-dependent manner (8, 13, 16). To test this working hypothesis, the SLC17A9 protein was expressed in High Five cells, solubilized from the membranes and purified by Ni-NTA column chromatography (Fig. 3A). The purified fraction showed two major protein bands (68 and 63kDa) on SDS polyacrylamide gel, which corresponded to SLC17A9 protein because of their immunological reactivity to anti-SLC17A9 antibodies

and ability to bind ATP upon UV light illumination (Fig. 3A). The observation of two SLC17A9 protein bands upon polyacrylamide gel electrophoresis could have been due to conformational differences or modification.

We then reconstituted SLC17A9 protein into liposomes, and investigated whether or not the proteoliposomes took up [α - 32 P]ATP. When an internal inside positive $\Delta\psi$ was established such as a K⁺-diffusion potential through the addition of valinomycin, the proteoliposomes took up [α - 32 P]ATP in a time-dependent manner (Fig. 3B, C and SI Table 1). ATP uptake was not observed in liposomes that did not contain SLC17A9 protein even when $\Delta\psi$ was already established (Fig. 3C). In contrast, artificially imposing pH gradient (Δ pH) did not evoke the ATP uptake (SI Table I). The valinomycin-evoked ATP uptake exhibited dose-dependence with K_m and V_{max} values of 0.8 mM and 138 nmol/min/mg protein, respectively (Fig. 3C). These results demonstrated that the SLC17A9 protein was an active transporter of ATP and used $\Delta\psi$ (positive inside) but not Δ pH (acidic inside) as the driving force.

Characterization of Nucleotide Transport. We further characterized the ATP uptake by SLC17A9 protein. Since ATP is a trivalent anion at the physiological pH and behaves as a monovalent anion upon chelation with divalent cations such as Mg²⁺, we asked which forms were recognized as transport substrates. We found that Mg²⁺ and Ca²⁺ did not affect the ATP uptake (Fig. 4A), suggesting that this transporter recognized ATP as a substrate irrespective of its charge status. Cl⁻ dependence on the ATP uptake is of interest because VGLUT, another subfamily of SLC17, absolutely requires Cl⁻ for transport activity (20), and Cl⁻ stimulates ATP uptake in chromaffin granule membrane vesicles (16). As expected, the presence of Cl⁻ was an absolute requirement for ATP transport activity in the SLC17A9 protein (Fig. 4B). The transport activity reached steady state at ~4 mM Cl⁻, while the magnitude of $\Delta\psi$ was unchanged. Br⁻ also activated ATP transport, while

sulfate was not effective (SI Fig. 10). Various anions such as iodine and thiocyanate were rather inhibitory. The anion dependence of the SLC17A9 protein was similar to that of VGLUT (21, 22), suggesting the presence of a similar anion binding site and regulatory mechanism in this transporter family. *Cis*-inhibition studies suggested that this transporter preferentially recognized ATP, GTP and ADP as transport substrates. AMP-PNP and γ S-ATP, non-hydrolysable ATP analogues, and diadenosine triphosphate (AP₃A), one of the unique physiological ATP derivatives that accumulates in chromaffin granules and synaptic vesicles (9, 18), strongly inhibited the uptake. In contrast, adenosine and adenine were less effective inhibitors (Fig. 4C). The transporter-mediated uptake of GTP and ADP was confirmed by the direct measurement of ADP and GTP uptake (Fig. 4D). The ATP uptake was greatly inhibited by 4,4'-diisothiocyanatostilbene-2, 2'-disulfonate (DIDS), an inhibitor of VGLUT (23). The concentration required for 50% inhibition (ID₅₀) was 1.5 μ M (Fig. 4E). DIDS also inhibited the uptake of GTP and ADP (Fig. 4D). Evans blue, a specific inhibitor of VGLUT (23), also potently inhibited the ATP uptake with an ID₅₀ of 40 nM (Fig. 4F). Atractyloside, an inhibitor of the mitochondrial ATP/ADP exchanger, inhibited the ATP uptake of the SLC17A9 protein only in the presence of Mg²⁺ as reported for ATP uptake in chromaffin granules (16) (Fig. 4G). Thus, the SLC17A9 protein is a Cl⁻-dependent nucleotide transporter, whose properties are very similar, if not identical, to those observed for ATP uptake in chromaffin granules (8, 13, 15-17).

Involvement of SLC17A9 protein in vesicular storage and exocytosis of ATP. Finally, we investigated whether SLC17A9 is responsible for vesicular storage and subsequent exocytosis of ATP in purinergic cells. PC12 cells are known to store and secrete ATP through secretory granule-mediated exocytosis (24). We found that mSLC17A9 protein was associated with secretory granules (Fig. 5A) and that RNA interference suppressed about 53% of SLC17A9 expression and about 50% of KCl-stimulated exocytosis of ATP

from PC12 cells, while the control treated with scrambled siRNA did not show any inhibitory effect (Fig. 5B and C). These results supported the participation of SLC17A9 protein in vesicular storage and exocytosis of ATP.

SLC17A9 protein as a vesicular nucleotide transporter. Based on this information, we concluded that SLC17A9 and its orthologues encode the vesicular nucleotide transporter (VNUT). SLC17A9 protein may accumulate ATP and other nucleotides in the secretory vesicles such as adrenal chromaffin granules and synaptic vesicles. SLC17A9 protein is a long-sought nucleotide transporter and seems to be a missing link in purinergic chemical transmission (Fig. 6). Our immunohistochemical studies with anti-mSLC17A9 antibodies indicated that mSLC17A9 protein is expressed in a population of astrocytes, suggesting involvement of this transporter in storage and subsequent exocytosis of ATP from these cells (manuscript in preparation) (25-27). Thus, identification of SLC17A9 protein as a vesicular nucleotide transporter may reveal the molecular mechanisms how ATP is secreted from purinergic cells, and provide a novel molecular target for the pathophysiology of purinergic signaling and its therapeutic potential (9-11). Phylogenetic analysis indicates that the orthologues of SLC17A9 are widely distributed among various invertebrates as well as vertebrates (SI Fig. 11). Thus, our findings suggest the occurrence of common molecular mechanisms of the purinergic chemical transmission in animals.

Materials and Methods

Expression and purification of SLC17A9 protein. Recombinant baculovirus containing human SLC17A9 cDNA were constructed using the Bac-to-Bac baculovirus expression systems (Invitrogen) according to the manufacture's protocol. SLC17A9 cDNA was amplified by PCR using the primers (5'-

CACCATGACCCTGACAAGCAGGCGCCAGGA -3' and 5'-
CTAGAGGTCCTCATGGGTAGAGCTC -3'). Sf9 or High Five cells were used for expression of SLC17A9 protein. Insect cells were infected by recombinant baculoviruses at a multiplicity of infection (m. o. i.) of 2 and cultured a further 72 h for Sf9 cells and 48 h for High Five cells. The cell ($1\sim 2 \times 10^8$ cells) were suspended in a buffer containing 20 mM Tris-HCl, pH 8.0, 0.1 M potassium acetate, 10% glycerol, 0.5 mM dithiothreitol, 10 μ g/ml pepstatin A and 10 μ g/ml leupeptin and disrupted by sonication with a TOMY UD200 tip sonifier. Cell lysates were centrifuged at 700 x g for 10 min to remove debris and the resultant supernatant was centrifuged at 160,000 x g for 1 h. The pellet (membrane fraction) was suspended in buffer containing 20 mM MOPS-Tris, pH 7.0, 10% glycerol, 10 μ g/ml pepstatin A and 10 μ g/ml leupeptin at approximately 6 mg protein/ml, and was solubilized with 2% octylglucoside. After centrifugation at 260,000 x g for 30 min, the supernatant was taken and applied to 1 ml of Ni-NTA Superflow resin (Qiagen). After incubation for 4 h at 4°C, the resin was washed with 10 ml of 20 mM MOPS-Tris pH 7.0, 5 mM imidazole, 20% glycerol and 1% octylglucoside. SLC17A9 protein was eluted from the resin with 3 ml of the same buffer containing 60 mM imidazole and could be stored at -80°C without loss of activity for at least a few months.

Reconstitution. Reconstitution of purified SLC17A9 protein into liposomes was carried out by the freeze-thaw method as described (20). In brief, 10 μ g SLC17A9 protein was mixed with asolectin liposomes (0.5 mg lipid), frozen at -80°C and left at this temperature for at least 5 min. The mixture was thawed quickly by holding the samples tube in the hands and diluted 60-fold with reconstitution buffer containing 20 mM MOPS-Tris, pH 7.0, 0.5 mM dithiothreitol, 0.15 M sodium acetate and 2 mM magnesium acetate. Reconstituted proteoliposomes were sedimented by centrifugation at 200,000 x g for 1 h

at 4°C, suspended in 0.2 ml of 20 mM MOPS-Tris, pH 7.0 containing 0.15 M sodium acetate and 2 mM magnesium acetate, and used within a day of preparation. Asolectin liposomes were prepared as follows: soybean lecithin (20 mg; Sigma Type IIS) was suspended in 2 ml of 20 mM MOPS-NaOH, pH 7.0 containing 0.5 mM dithiothreitol. The mixture was sonicated in a bath-type sonicator until clear, divided into small aliquots and stored at -80°C until use.

Nucleotides transport. Reconstituted proteoliposomes (0.5 µg protein per assay) were suspended in 500 µl of 20 mM MOPS-Tris, pH 7.0, 5 mM magnesium acetate, 4 mM KCl and 0.15 M potassium acetate and incubated for 3 min at 27°C.

Valinomycin was added to give a final concentration of 2 µM and the mixture incubated for a further 3 min. The assay was initiated by addition of 0.1 mM [α -³²P] ATP (3.7 GBq/mmol) and 130 µl aliquots were taken at the times indicated and centrifuged through a Sephadex G-50 (fine) spin column at 760 x g for 2 min (20). Radioactivity and protein concentration of the eluate were measured. In the case of GTP or ADP transport, [α -³²P] GTP (3.7 GBq/mmol) or [2, 8-³ H] ADP (0.37 GBq/mmol) were used for the substrates instead of ATP.

Cell culture and RNA interference (RNAi)

PC12 cells were cultured as described (24). HiPerFect transfection reagent (Qiagen) was used for transfection of 25 nM AllStars negative control siRNA or rat SLC17A9 siRNA: UAUUCGAGAGAAUGUCACG. KCl-stimulated ATP secretion was assayed as described 3 days later (24).

Data analysis. All numerical values are shown as the mean \pm SEM. n = 3-6. Statistical significance was determined by the Student's t-test. * <0.1, ** < 0.01, *** < 0.001.

Northern blot analysis, cDNA cloning, immunohistochemistry and other detailed methods are provided in SI Supplementary materials and methods.

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Figure Legends

Figure 1. Phylogenetic tree of SLC17 family (A) and putative secondary structure of SLC17A9 protein (B).

Figure 2. Expression of SLC17A9 and its association with chromaffin granules. (A) Northern blotting showing expression of SLC17A9 in human and mouse brain and adrenal gland. Expression of G3PDH is also shown as a control. (B) Immunohistochemical localization of SLC17A9 protein in mouse adrenal gland. Inset shows control staining with preabsorbed antibodies. C, cortex; M, medulla. Bar = 10 μ m. (C) Immunoelectronmicroscopy showing that SLC17A9 proteins (gold particles) are associated with chromaffin granules. The inset shows background labeling with control serum. Mit, mitochondrion. Bar = 100 nm. (D) Western blot indicates the presence of a SLC17A9 counterpart in the membrane of bovine adrenal chromaffin granule with a relative mobility of 61 kDa. The preabsorbed antibodies did not bind to the protein. The positions of marker proteins are indicated on the left.

Figure 3. Purification and reconstitution of SLC17A9 protein. (A) Purification. (*left*) Purified fraction (7 μ g protein) was analyzed in an 11% polyacrylamide gel electrophoresis in the presence of SDS and visualized by Coomassie Brilliant Blue staining. (*center*) A duplicate gel was analyzed by Western blotting with anti-SLC17A9 antibodies. (*right*) Labeling of SLC17A9 protein upon UV light illumination with [α -³²P]ATP. (B) Formation of $\Delta\psi$ (positive inside) was measured by oxonol-V fluorescence quenching. Proteoliposomes (0.5 μ g protein) or liposomes (20 μ g lipid) containing trapped Na⁺ was suspended in buffer containing 0.15 M K-acetate plus 4 mM KCl and 1 mM oxonol-V and fluorescence quenching was measured. Final concentration of valinomycin and CCCP

were added at 1 μM . (C) Time course of [α - ^{32}P]ATP uptake. Na^+ -trapped proteoliposomes or liposomes were suspended as above in the presence or absence of valinomycin. Upon the addition of [α - ^{32}P]ATP, samples were taken at the indicated times and radioactivity taken up by the proteoliposomes was counted. Inset. Dose dependence of ATP uptake.

Figure 4. Characterization of nucleotides transport by SLC17A9 protein. (A) The effect of divalent cations on the [α - ^{32}P]ATP uptake in the absence or presence of 2 mM Mg^{2+} or Ca^{2+} or 0.5 mM EGTA. (B) Dependence of [α - ^{32}P]ATP uptake on $[\text{Cl}^-]$. The uptake was measured after 2 min. Part of the potassium acetate in the reaction mixture was replaced with the indicated concentration of KCl. The magnitude of $\Delta\psi$ (oxonol V-fluorescence quenching) was little affected under the assay conditions employed. (C) [α - ^{32}P]ATP uptake in the presence of various nucleotides at 1 mM. (D) Uptake of radiolabeled GTP or ADP transport in the presence or absence of valinomycin. When indicated, DIDS at 2 μM was included. (E-G) The effect of DIDS, Evans blue and atractyloside on [α - ^{32}P]ATP uptake in the presence or absence of Mg-acetate. Atractyloside was included at 200 μM .

Figure 5. The impact of rat SLC17A9 siRNA on the endogenous expression of SLC17A9 in PC12 cells was compared to cells treated with scrambled siRNA (control). (A) Double labeling immunohistochemistry indicated that SLC17A9 protein was co-localized at least in part with synaptotagmin, a marker of secretory granule, but not with synaptophysin, a marker of synaptic-like microvesicles in cultured PC12 cells. (B, C) Both SLC17A9 mRNA levels (B), as determined by real-time PCR, and the KCl-dependent secretion of ATP after 30 min (C) were assessed. $n=9$, 3 independent experiments.

Figure 6. Schematic presentation of purinergic chemical transmission. VNUT is present in secretory vesicles and is responsible for vesicular storage of nucleotides. The accumulated

nucleotides, in particular ATP, are exocytosed upon stimulation, bind to purinoceptors at the surface of target cells which then triggers intracellular signal transmission.

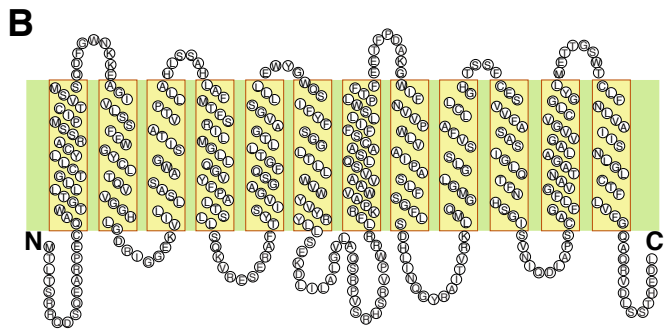
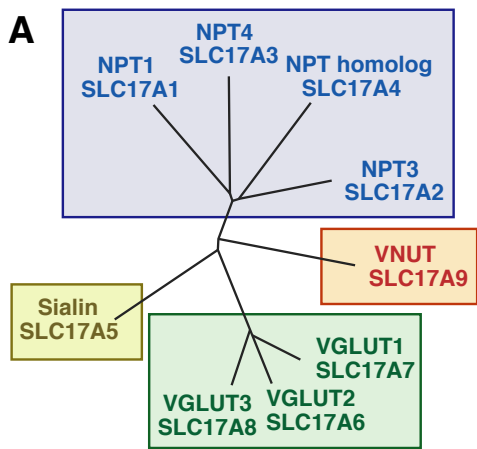


Figure 1

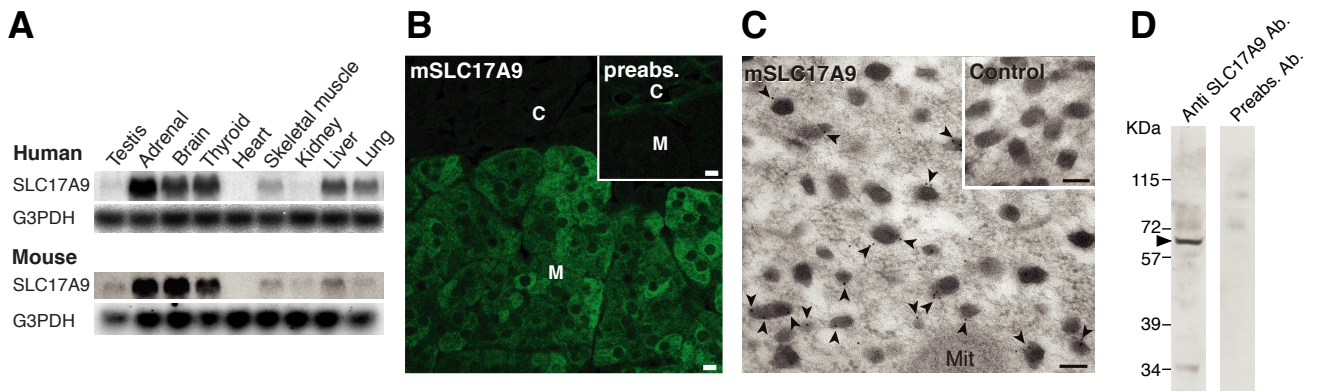


Figure 2

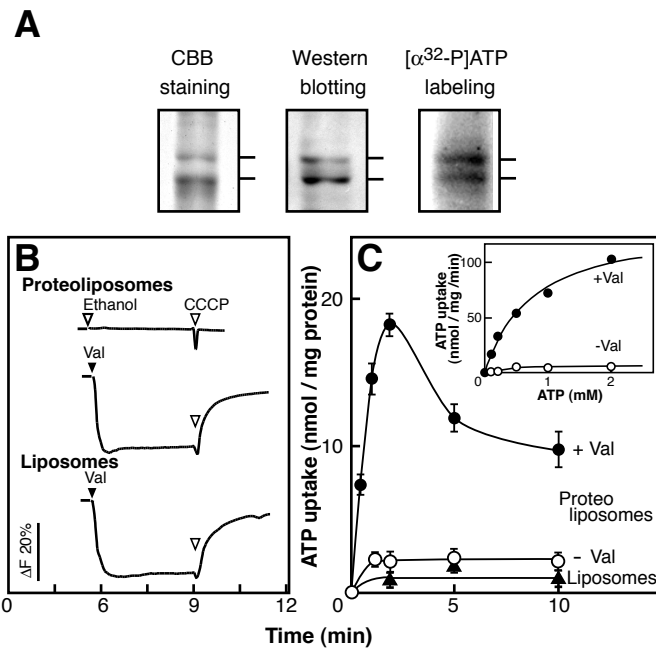


Figure 3

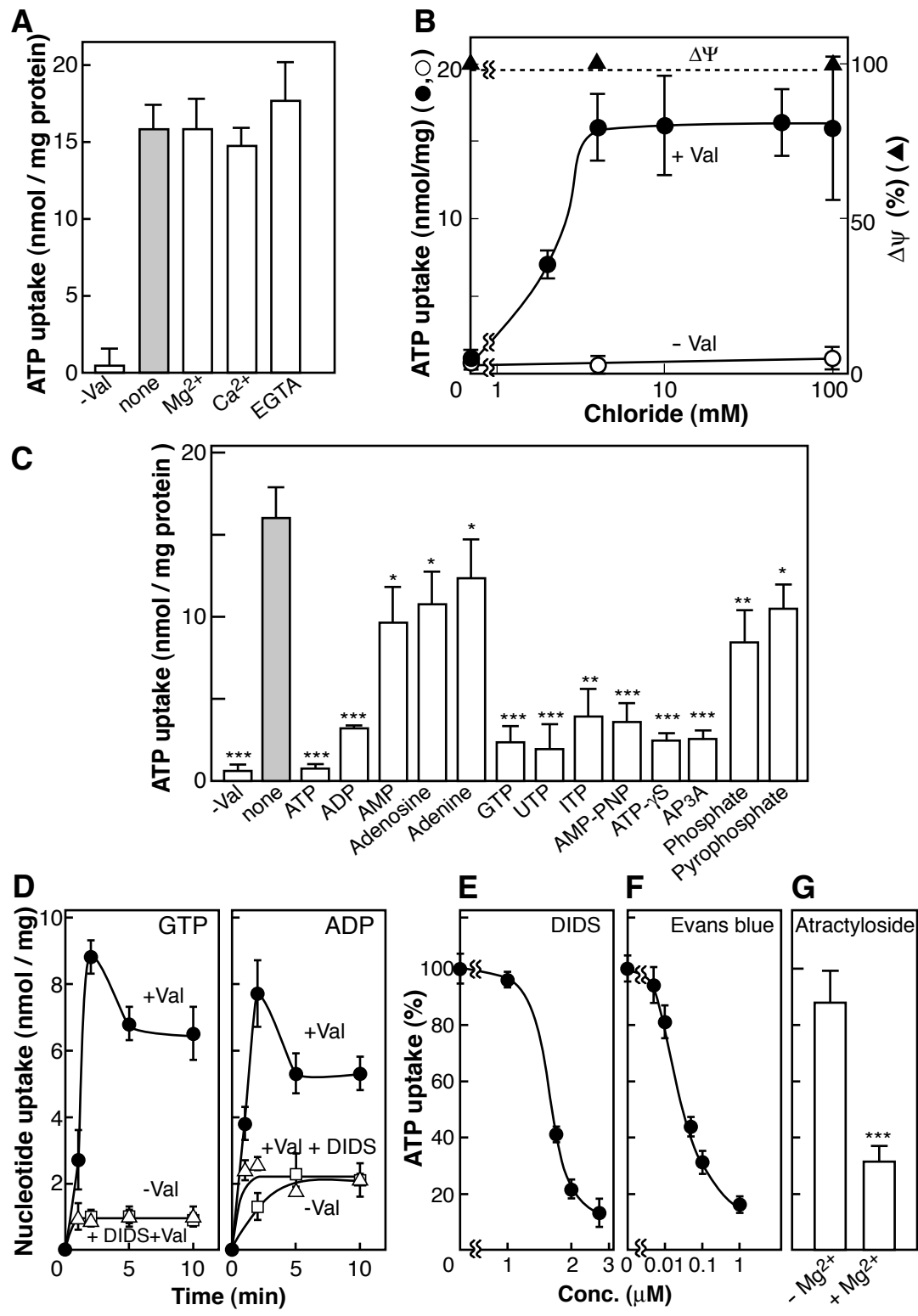


Figure 4

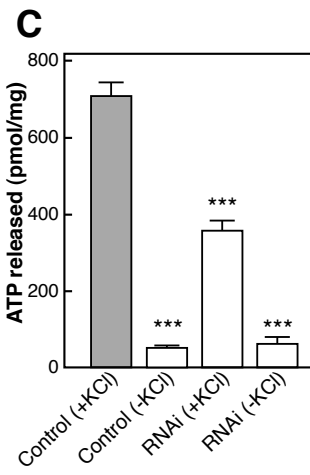
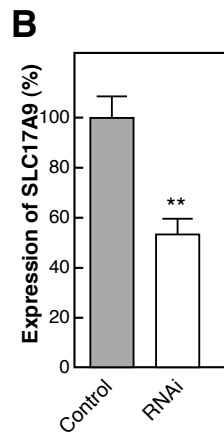
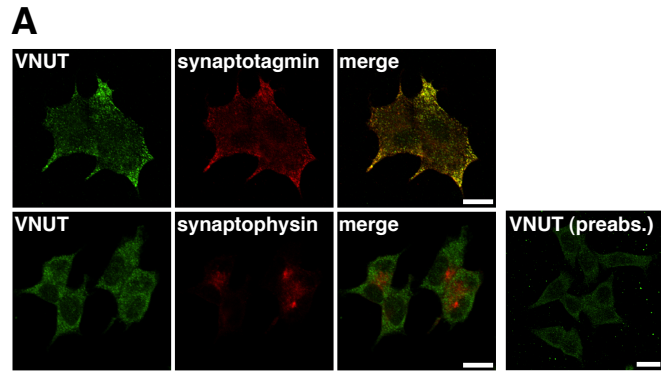


Figure 5

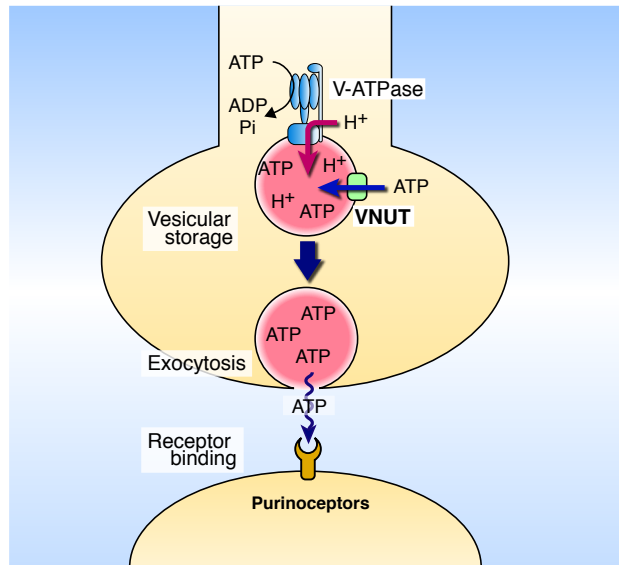


Figure 6

Supplementary information

Supplementary materials and methods

cDNAs. cDNA of SLC17A9 was cloned by PCR from Mammalian Gene Collection clone 4563089. For the construction of cDNA, we used pENTR Directional TOPO cloning Kits (Invitrogen). The primers used were based on database sequences (Genbank No. CAC28600): sense primer, CACCATGACCCTGACAAGCAGGCGCCAGGA; antisense primer, CTAGAGGTCCTCATGGGTAGAGCTC. The sequence was confirmed by comparison with the human genome sequence and any differences corrected. cDNAs of mouse SLC17A9 was cloned as described above. cDNA of mouse SLC17A9 was amplified by RT-PCR using RNA derived from mouse adrenal gland. The following primers were used; sense primer, 5'- CACCATGCCATCCCAGCGCTCTA-3', antisense primer, 5'- TTCACTGACAGGGTTGTTATCC -3'.

Northern blot analysis. Human and mouse multiple-tissue Northern blots (MTN) were purchased from Clontech. For Northern blot analysis, PCR fragments encoding the N-terminal 425 bps (223-647) of human SLC17A9 and C-terminal 758 bps (891 – 1648) of mouse SLC17A9 labeled with ³²P-dCTP were used as probes. Hybridization was performed at 68 °C for 1 h in Express Hyb hybridization solution (Clontech) and washed under high-stringency conditions at 50 °C.

Antibodies. Site-specific polyclonal antibodies against human and mouse SLC17A9 were prepared by repeatedly injecting GST-fusion polypeptides encoding either M1- I40 or L8 - R97, respectively. The antiserum (100 µl) was preabsorbed by incubating it with the antigen (1 mg) at 4 °C overnight. Monoclonal antibodies against synaptotagmin were kindly supplied by Dr. M. Takahashi (28). Anti-His-tag monoclonal antibody was from

Novagen. Alexa Fluor 488-labeled goat anti-rabbit IgG was obtained from Invitrogen (Molecular Probes) and colloidal gold conjugated goat anti-rabbit IgG from British Biocell International.

Nucleotide binding. Binding of nucleotides to the purified transporter was assayed by UV light illumination according to the published procedures (29). In brief, the reaction was performed in 50 μ l reaction mixture containing 20 mM MOPS-Tris, pH 7.0, 50 mM potassium acetate, 2 mM magnesium acetate, 4 mM KCl, 7 μ g protein and 5 μ M concentration of [α - 32 P] ATP (15 TBq/mmol). The reaction mixture was placed in flat bottom plastic test tubes, on ice, and at a 5-cm distance from an unfiltered UV lamp (UVP Inc., 15W). After a 10 min illumination, the reaction was terminated by the addition of 10 μ l of concentrated dissociation buffer to give final concentration of 2% SDS and β -mercaptoethanol. Samples (50 μ l) were electrophoresed in the presence of SDS and were exposed to an IP image plate.

Immunohistochemistry

Indirect immunofluorescence microscopy was performed as previously described (30). Adrenal glands were obtained from adult C57BL/6 mice perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The first antibody treatment was performed with 1:500 diluted antibodies in phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) for 1 h at room temperature. The specimens were observed under an Olympus FV300 confocal laser microscope.

Immunoelectronmicroscopy

The LR White embedding immunogold method used (31). Adult C57BL/6 mice were anesthetized with ether and then perfused intracardially with saline, followed by 0.1%

glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Adrenal gland was washed with PBS, dehydrated in ethanol series, and then embedded in LR White. Ultrathin sections (80 nm) on nickel grids were incubated with PBS containing 2% goat serum and 2% BSA for 10min, and then treated with either rabbit antiserum against mouse SLC17A9 diluted 1:20 or normal rabbit serum diluted 1:20 for 1hr at room temperature. The sections were washed and treated with secondary antibodies conjugated with 10nm colloidal gold diluted 1:50. After washing with sodium cacodylate buffer (pH 7.4), the sections were postfixed in 2.5% glutaraldehyde in the cacodylate buffer, stained sequentially uranyl acetate for 10 min and lead citrate for 1 min. Specimens were observed under a Hitachi H-7100S electron microscope.

Measurement of $\Delta\psi$ and ΔpH by fluorescence quenching.

$\Delta\psi$ (positive inside) was assayed by measuring the fluorescence quenching of oxonol V as described previously (20).

Preparation of chromaffin granule membrane vesicles from bovine adrenal glands

Chromaffin granules were isolated from bovine adrenal glands by differential centrifugation and successive sucrose density gradient centrifugation as described previously (32). After isolation, the granules were immediately disrupted under low osmotic conditions, and the resulting membrane vesicles suspension was stocked at -80 °C.

Other procedures. Polyacrylamide gel electrophoresis in the presence of SDS and Western blotting were performed as described (20). Protein concentration was assayed using bovine serum albumin as a standard (33).

Supplementary Figures

Figure 7. Chromosomal localization and gene organization of SLC17A9. The exon and intron organization of SLC17A9 is also shown. The open and shaded boxes show noncoded and coded exons, respectively.

Figure 8. Amino acid sequences comparison of members of SLC17 family. Identical residues are indicated by asterisks. Predicted transmembrane regions are shaded.

Figure 9. The amino acid sequences of SLC17A9 orthologues are aligned. Identical residues are indicated by asterisks. Predicted transmembrane regions are shaded. The positions of polypeptide used for preparation of antibody were boxed in red.

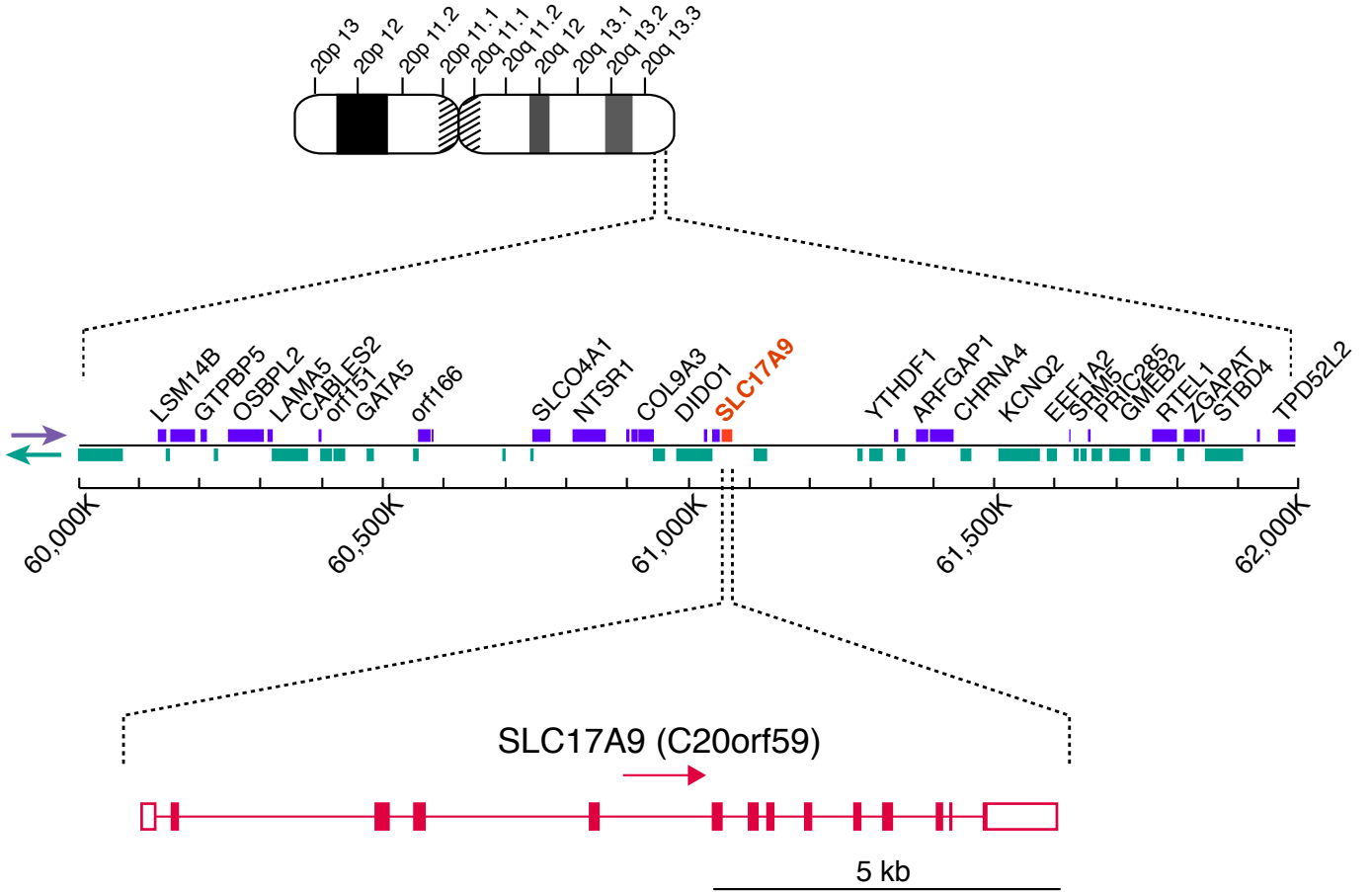
Figure 10. The effect of various anions on $\Delta\psi$ -mediated uptake of [α - ^{32}P] ATP by SLC17A9 protein. A. The uptake was performed under the standard assay conditions except that KCl was replaced with various potassium salts as indicated. B. The effects of anion species on Cl^- -dependent [α - ^{32}P]ATP uptake at 2 min in the presence of 4 mM KCl plus the indicated potassium salts (4 mM) was measured.

Figure 11. VNUT orthologues are widely distributed in the animal kingdom. A phylogenetic tree of selected genes encoding VNUT-type transporters from the animal kingdom is shown. VNUT from mammals are shown in beige, birds in coral, amphibians in dark pink, fish in blue and insects in green.

Supplementary References

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Human Chromosome 20



SLC17A1_NPT1 -----MQMDNRLPPKKVPGFCSFRYGLSF 24
 SLC17A2_NPT3 -----MDGKPAATRKGPDFCSLRYGLAL 22
 SLC17A3_NPT4 -----MATKTELSPTARESNAQDMQVDETLIPRKVPSLCSARYGIAL 43
 SLC17A4_NPThomolog -----MSTGPDVKATVGDISSDGNLNVAQECCSRKGFCSVRHGLAL 41
 SLC17A5_Sialin -----MRSPVRDLARNDGEESTDRTPLLPGAPRAEAAPVCCSARYNLAI 44
 SLC17A6_VGLUT2 -----MESVKQRILAPGKGLKFNAGKSLGQIYRVLEKKQDTGETIELTEDGKPLEVPERKAPLDCDCTCFGLPRRYIIAI 75
 SLC17A7_VGLUT1 -----MEFRQEFERKLAGRALGKLRLEKQEGEAETLELSADGRPVTTQTRDPPVVDCTCFGLPRRYIIAI 67
 SLC17A8_VGLUT3 MFPKAFDFTFKEKILKPGKGEVKNVAVGDSLGILQRKIDGTTEEDDNIELNEEGRPVQTSRPSPLCDCHCCGLPKRYIIAI 80
 SLC17A9_VNUT -----MTLTSRRQDSQEAREPCEQAWTG 22

* * * * *
 SLC17A1_NPT1 LVHCCNVIITAQRACLNLTVMVNVNSTDPHGLPNTSTKLLDN-----IKNPMYNWSPDIQGIILSSTSYGV 91
 SLC17A2_NPT3 IMHFSNFTMITQRVSLSAIIAMVNTTQQGLSNASTEGPVADAFNNSISISKEFDTKASVYQWSPETQGIIFSSINYGI 102
 SLC17A3_NPT4 VLHFCNFTTIAQNVIMNITMVMVNSTSPQSQNDSSVLPVDSFGGLSKAPKSLPAKAPVYDWSPOIQGIIFGAVGYGG 123
 SLC17A4_NPThomolog ILQLCNFSIYTOQMNLISAIIPAMVNNSTAPPSPQNASTERPSTDSQGYWNETLKEFKAMAPAYDWSPEIQGIILSSLNYS 121
 SLC17A5_Sialin LAFFGFFIVYALRVNLSVALVDMVDSNTTLEDNRTSKACPEHSAPIKVHNN-----QTGKKYQWDAETQWILGSPFFGY 119
 SLC17A6_VGLUT2 MSGLGFICISFGIRCNLGVAVDMVNNSTIHRGGKVI-----KEKAKFNWDPETVGMIHGSFFWGY 135
 SLC17A7_VGLUT1 MSGLGFICISFGIRCNLGVAVDMVNNSTTHRGHVV-----VQKAQFSWDPETVGLIHGSFFWGY 127
 SLC17A8_VGLUT3 MSGLGFICISFGIRCNLGVAVDMVNNSTVYVDGKPE-----IQTAQFNWDPETVGLIHGSFFWGY 140
 SLC17A9_VNUT TLLLTGTCLLYCARSSMPICTVSMS-----QDFGWNKKEAGIIVLSFFWGY 67

* * * * *
 SLC17A1_NPT1 IIVQVPVGYFSGIYSTKMKIGFALCLSSVLSLLIPPAAGIG---VAVVVVCRAVQGAQGVIVATAQFEIYVKWAPPLER 168
 SLC17A2_NPT3 ILTLIPSGYLAGIFGAKKMLGAGLLISLLTLFTPLAADFG---VILVIMVRTVQMAQGMWGTQFTIWKWAPPLERS 179
 SLC17A3_NPT4 ILTMAPSGYLAGRVGTRVVGISLFAFSFTLCLIPLATDFG---IVLLIVTRIVQGLSQSSILGGQFAIWEKWGPQERS 200
 SLC17A4_NPThomolog FLAPIPSGYVAGIFGAKYVVGAGLFISSEFTLFIPLAANAG---VALLIVLRIVQGIQVVMVLTGQYSIWKWAPPLERS 198
 SLC17A5_Sialin IITQIPGGYVASKIGKMLLGFILGTAVLTFTPIAADLG---VGPLIVLRALEGLGEGVTFPAMHAMSSWAPPLERS 196
 SLC17A6_VGLUT2 IITQIPGGYIASRLAANRVFGAALLTSTLNMLIPSAARVH---YGCVIFVRILQGLVEGVTYPACHGIVSKWAPPLERS 212
 SLC17A7_VGLUT1 IVTQIPGGYICQKFAANRVFGFAIVATSTLNMLIPSAARVH---YGCVIFVRILQGLVEGVTYPACHGIVSKWAPPLERS 204
 SLC17A8_VGLUT3 IMTQIPGGYISNKFAANRVFGAAIFLTSTLNMLIPSAARVH---YGCVMCVRILOGLVEGVTYPACHGMWSKWAPPLERS 217
 SLC17A9_VNUT CLTQVVGHGLGDRIGGEKVIILLSASAWGSITAVTPLLHLSSAHLAFMFTFSRILMGLLQGVYPPALTSLLSQKVRRESERA 147

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 SLC17A1_NPT1 RLTSMTSGFLLGPFVILLVTGVICESLQWPMVVFYIFGACGCAVCLLWVLFVYDDPKDHPICISISEKEYITSSLVQOV-- 246
 SLC17A2_NPT3 KLTTIAGSGSAFGSFIILCVGGLISQALSWPFIYIFGSTGCVCLLWFTVIYDDPMHHPICISVREKEHILSSLAQQP-- 257
 SLC17A3_NPT4 RLCSIALSGMLLGCFTAILIGGFISETLWGFVYFIFGCVGCCLLWVVIYDDPVSYPWISTEKEYIIVSLKQOV-- 278
 SLC17A4_NPThomolog QLTIIAGSGMLGSFIVLLAGLLCQTIGWPYVYFIFGICGACCLWPLIYDDPVNHFFISAGEKRIIVCSLAQQD-- 276
 SLC17A5_Sialin KLLSISYAGAQLGTVILSLPLSGIICYMNTWYVYFYFCTIGIFWFLWLWLVSDTPQKHKRISHYEKEYILSSLRNQL-- 274
 SLC17A6_VGLUT2 RLATTSFCGSYAGAVIAMPLAGILVQYTGWSSVFYVYGSFGMVWYMFLLVSVESPAKHPTITDEERRYIEESIGESANL 292
 SLC17A7_VGLUT1 RLATTAFCGSYAGAVVAMPLAGVLVQYIGWSSVFYIYGMFGI IYWMFWLLQAYECPAAHPTISNEEKTYIETSIGEGANV 284
 SLC17A8_VGLUT3 RLATTSFCGSYAGAVVAMPLAGVLVQYIGWSSVFYIYGMFGI IYWMFWLLQAYECPAAHPTISNEEKTYIETSIGEGANV 297
 SLC17A9_VNUT FTYSIVGAGSQFGLTLLTGAVGSLLEWYWGQSIFYFSGGLTLLWVWVYRYLLSEKDLILALGVLAQSRPVS----- 219

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 SLC17A1_NPT1 ---SSRQSLPIKAILKSLPVWVAISIGSFTFFWSHNIMTYTPMFINSMHLVNIKENGFLSSLPYFAWICGNLAGQLSDF 324
 SLC17A2_NPT3 ---SSPGRVPIKAMVTCPLWALIFLGFPSHFWLCTIILTYLPTIYIITLLHVNIRDSSVLSLPLFAAACTILGGQLADF 335
 SLC17A3_NPT4 ---GSSQPLPIKAMLRSLPIWISICLGCFSHQWLVSVMVYIPTYISSVYHVNIRDNGLLSALPFIWAVVIGMVGYLADF 356
 SLC17A4_NPThomolog ---CSPGWSLPIRAMIKSLPLWAILVSYFCEYWFYTIMAYTPTYISSVLQANLRDGSILSALPFVVGICICIILGGLADF 354
 SLC17A5_Sialin ---SSQK-SVPWVPILKSLPLWAVVAHFSYNWFTYLLTLLPTYMKEILRFNVQENGLFSSLPVWGLMILSGQAADN 351
 SLC17A6_VGLUT2 LGAMEKFKTPWRKFFTSMPVYAIIVANFCRSWTFYLLLSIQPAYFEEVFGFEISKVGMLSAVPHLVMTIIVPIGGQIADF 372
 SLC17A7_VGLUT1 MNPLTKFSTPWRKFFTSMPVYAIIVANFCRSWTFYLLLSIQPAYFEEVFGFEISKVGLVSALPHLVMTIIVPIGGQIADF 364
 SLC17A8_VGLUT3 VS-LSKFSTPWRKFFTSMPVYAIIVANFCRSWTFYLLLSIQPAYFEEVFGFEISKVGLLSAVPHLVMTIIVPIGGQIADF 376
 SLC17A9_VNUT ---RHSRVPWRRILFRKPAVWAAVVSQLSAACSFIFILLSWLPTFFFEETFPDAKG--WIFNVVPLVVAIPASLFGSGLSDH 293

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 SLC17A1_NPT1 FLTRNILSVIAVRKLFTAAGFLPAIFGVCLPVLSSTFYSIVIFLILAGATGSFCLGGVFINGLDIAPRYFGFIKACSTL 404
 SLC17A2_NPT3 LLSRNLLRLITVRKLFSSLGLLPSICAVALPFVASSYVITILLILIPGTSNLCDSGFIINTLDIAPRYASFLMGISRG 415
 SLC17A3_NPT4 LLTKKFR-LITVRKIATILGSLPSSALIVSLPLNSGYITATALLTSCGLSTLCQSGIYINLVDIAPRYSSFLMGASRG 435
 SLC17A4_NPThomolog LLRSKILRLITVRKLFTAIGVLFPVSVILVSLPWRSSHMTMPLFVLSAISSPCESGALVNFIDIAPRYTGFLKGLLQV 434
 SLC17A5_Sialin LRAKWNFTLVCRRIFSLIGMIPAVLVAAGFIGCDYSLAVAFLTISTLGGFCSSGFIINHLDIAPRYAGILGITIT 431
 SLC17A6_VGLUT2 LRSKQILSTTTVRKIMNCGGFGMEATLLLVVGYSHTRGVAISFLVLAVGVFSGFAISGFNVNHLDIAPRYASILMGISNG 451
 SLC17A7_VGLUT1 LRSRRIMSTTNVRKIMNCGGFGMEATLLLVVGYSHSKGVAISFLVLAVGVFSGFAISGFNVNHLDIAPRYASILMGISNG 443
 SLC17A8_VGLUT3 LRSRQILTTTAVRKIMNCGGFGMEATLLLVVGYSHHTKGVAISFLVLAVGVFSGFAISGFNVNHLDIAPRYASILMGISNG 455
 SLC17A9_VNUT LIN-QGYRAITVRKLMQGMGLGLSSVFALCLGHT-SSFCEVVFASASIGLQTFNHSIGISVNIQDLAPSAGFLFGVANT 371

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 SLC17A1_NPT1 TGMIGGLIASTLTGLILKQDPESAWFKTIFILMAAINVTGLIFYLIVATAEIQDWAKEKQHTRL----- 467
 SLC17A2_NPT3 FGLIAGIISSTATGFLISQDFESGWRNVFFLSAAVNMFLVYLTFGQAELODQWAKERTLTRL----- 478
 SLC17A3_NPT4 FSSIAPVIVPTVSGFLLSQDPEFGWRNVFFLFAVNLGLLFLYLI FGEADVQEWAKERKTRL----- 498
 SLC17A4_NPThomolog FAHIAGAI SPTAAGFFISQDSEFGWRNVFFLSAAVNSGLVYLYIFGRADVQDWAKEQTFTHL----- 497
 SLC17A5_Sialin FATIPGMVGPVIAKSLTPDNTVGEWQTVFYIAAAINVFAGAIFFTLFAKGEVQNWALNDHHGHRH----- 495
 SLC17A6_VGLUT2 VGTLSGMVCPPIVIGAMTKNKSFRGEWQYVFLIAALVHYGGVIFYGVFASGEKQPWAEPEEMSEKCGFVGHDLQAGSDDSE 531
 SLC17A7_VGLUT1 VGTLSGMVCPPIVIGAMTKHKTREEWQYVFLIASLVHYGGVIFYGVFASGEKQPWAEPEEMSEKCGFVGHDLQAGSDDSE 523
 SLC17A8_VGLUT3 VGTLSGMVCPPIVIGAMTRHKTREEWQNVFLIAALVHYSGVIFYGVFASGEKQWADPENLSEKCGI IDQDELAEIEIEN 535
 SLC17A9_VNUT AGALAGVVGVLGGYLKMTTG--SWTCLFNLVAIISNLGLCTFLVFGQAQRVDSLSTHEDL----- 430

SLC17A1_NPT1 ----- 467
 SLC17A2_NPT3 ----- 478
 SLC17A3_NPT4 ----- 498
 SLC17A4_NPThomolog ----- 497
 SLC17A5_Sialin ----- 495
 SLC17A6_VGLUT2 TQNYINYGTTKSYGATTQANGWPSGWEKKEEFVQGEVDQSHSYKDRVDYS--- 582
 SLC17A7_VGLUT1 MEDEAEPGAPPAPPSPYSYATHSTFPQPRPPPPVVDY----- 560
 SLC17A8_VGLUT3 HESFASPKKMSYGATSNCEVQKKEWKQQRGATLDEBELTSYQNEERNFSTIS 589
 SLC17A9_VNUT ----- 430

Human ----- MTLTSRRQDSQEARPECOAWTGTLLGLTCLLYCARSSMPI CTVSMSQDFGW 51
Bovine ----- MQPPDETRRDAEDTQWSRPECOAWTGTLLGLTCLLYCARVSMVPVCAASMSQDFGW 57
Mouse ----- MPSQRSSLMQPIPEETRKTPSAAAEDTRWSRPECOAWTGTLLGLTCLLYCARVMPVCTVAMSQDFGW 68
Chicken ----- MAAGGGRNAPVCGGERGSRAPQHDGMWKDGGGEQYWSRPECRAWTAVLLGLTCLLYCARVTVPI CAVALSAHFGW 75
Xenopus VPGNFMASTSLGGKIAGYQQLLGGDVQILERLRSSRDPFWSRWESRVWATLLVGTCLLYCARANMPI CAVAMSEDFGW 82
Zebrafish -MAVLQKHGKNSCPDLSSIKENPADNIGAAGSQKKTWESSQNWTRPVARIWTVVLLGLTCLLYCARVAMPI CAVSMAERFSW 81
Sea urchin ----- -MAMKHTKAKVLARYNSERRLWTVLLFVMTSVLFSARTI VPLVAVNLSKEFGW 53
Drosophila ----- -MDEKLKYSLLRGLVDTQSIWTRHEKRVWFI TLI TGTCLMYSTR TTMPLLVPAVASAQKW 60
C elegans ----- -MLKRPEYEPPLGKIWTRAESRMWTITMFSGTCLVYASRASLPI SAAAVAKEFAW 55
Sea anemone ----- -MEEEDI VLVAKELLSEKESAESKECSSHWPKDRS QWLIITLMASTASYVTR IIMPLCEPTLALDMGW 68

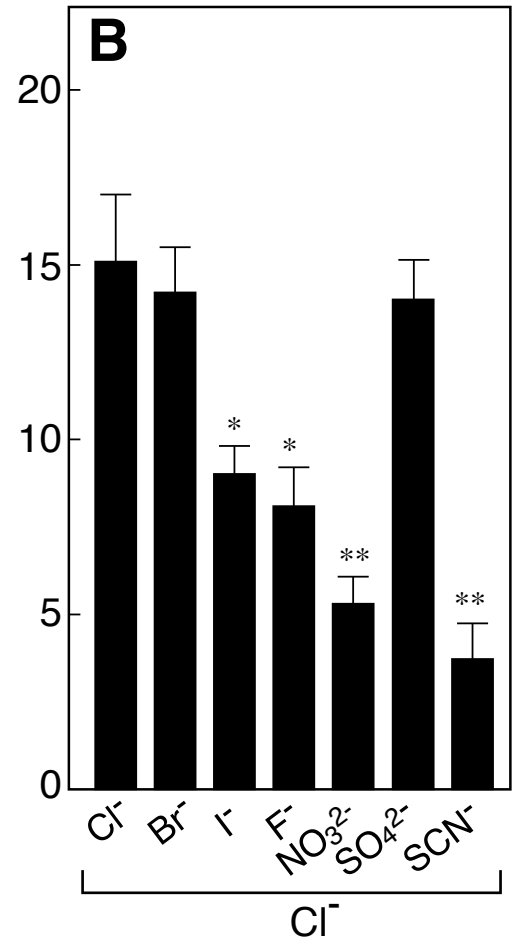
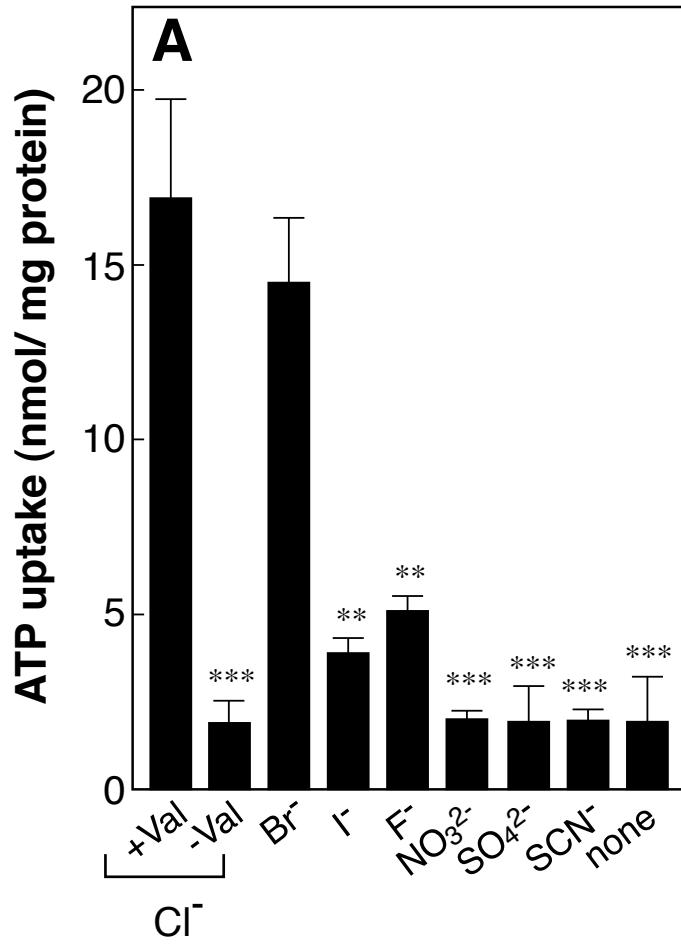
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Mouse NKKEAGIVLSSFFWGYCLTQVVGHLGDRIGGEKVILLSASAWGFI TVTTP-LLAHLG---SGHLAFLTFSRILTGLLQGVY 146
Chicken DKKQSGVLLSFFWGYCLTQVIGGHSIQIGGEKVILLSASAWGFI TFI TP-LLTQIT---SAHLVFMTCVSRFLMGLLQGVY 153
Xenopus NKRGIVLSSFFWGYCLTQVVGHLGDRIGGEKVI FLSALTWGLI TAMTP-LVAHVT---SVPLILVSVLRFMLGLLQGVH 160
Zebrafish SKRETMVLSGFFWGYCFTQVGGVSDRVGGEKVMLLSAAAWGAMTAFTP-ILAHFC---SQPIFMSLTFSRILMGLLQGVH 159
Sea urchin DKTDGLVLSGFFWGYPIAQIPGGMMSDRIGGDYVI I RAAP I WGVVTLVTP-LVPYLF TTKAGT IMSMTVLRFLMGLTQGVH 134
Drosophila SKTDSGTVLSGFFWGYTLTQVVGYPFSDRFGGQVILFAAIGWSLITFFLMPTI IWTAGSIKSYAIPV I VAI R I L N G A L Q G V H 142
C elegans NKTDSGTVLSGFFWGYALTQVFAGR IADKYGAEKILPYSSLAWTMLTFFT PHLFDFAFWTN- YPLVLLAVRILTGVCAQFH 136
Sea anemone NKREGLVLSGFFWGYLITQIPGGFSLDVYGAERLLLWAVTGCSSLTLVI PLVASQKVT---SPITLVLISRLALGVFOGIY 147

Human FPALTSLLSQKVRERAFYTSIVGAGSQFTLLTGAVGSLLEWYQWQSI FYFSGGLTLLVWVYRYLLSEK----- 203
Bovine FPALTSLLSQKVRERAFYTSIVGAGSQFTLVTGAVGSLLDWYQWQSVFYFSGGLTLLVWVYRYRCLL SERGPSSHLDG 217
Mouse FPALTSLLSQKVRERAFYTSIVGAGSQFTLVTGGVGSVLLDQCQWQSVFYFSGGLTLLWAYVYRYLLNEK----- 220
Chicken FPSLASLLSQKVRERAFYTSIVGAGSQFTLVIGAAGSLLLDWYQWQSVFYFSGGLTLLVYCTCKYLLVEK----- 227
Xenopus FPALASLFSQRVRETERAFYTSIVGAGSQFTLVMGAGSLLLEWYQWQSVFYFAGFLALLWGYCTSYLLKEK----- 234
Zebrafish YPSLASLCSQKVVESERGFLLMSTVSGSYLGLTLVIGVGSMLLDLYGWESVYFVSGLLSVLWAYCMWKYLLKGEGP----- 235
Sea urchin YPSLASLTSQKVTADKRAYVMSCVFAASLGLTLLTGAI GSI I MDHNSWHYVYFPGT I SIV I A T I Q S F S R K Q R R K ----- 210
Drosophila FPSMISLTSQKVNERSFPGLLTAGSALGTLTLMGSLLDYFQWQSVYFVIGLGMIAWALVLRMYAMAG----- 215
C elegans IPSLASIVSKHLAAADKGRVFGIVLAGSHWGTVLAGAIGSL I I E W I G W R A L F Q F V G I I S L I W C W V R W V L D R A K G P G G -- R S 216
Sea anemone YPSLYSLLAKSLPIERSSCSAFALAGGPVGLVIGGLGSLVLRARCWRWVFLSFGS I G L L W A V L W K K I F I D H N ----- 221

Human ----- DLILALGVLAQSRPVSRRHNVPRRLFRKPAVWAAVVSQLSAACSFF ILLSWLPTFFF EETFPDAKGI FNVVVP 276
Bovine FHVDLPPPSDLILALGILAQGLPVRHTKVPWRQLFRKPSVWAAI I S Q L S A A C S F F I L L S W L P T F F F K E T F P S K G W F N V V P 299
Mouse ----- DLVLA LGFLA QGLPVT K P S K V P W R Q L F R K A S V W A A I C S Q L C S A C S F F I L L S W L P T F F F K E T F P S K G W F N V V P 293
Chicken ----- DLVPI D Y L R R G I S I S K Q S P W K Q L F K K A P I W A V I A Q L C T A S T F C T L L S W L P T F F F K E T F P S K G W F N V V P 300
Xenopus ----- E R I V T V E D L K K C F A G S Q A N G Q W K L F R K A P V W A V I L A Q L C V A S T A F T I F S W M P T F F F K E R F P E S K G W F N V V P 307
Zebrafish ----- I I T L E S L G S A G T Q S K I P K R N W L R L F R Q P A V C A V I I T H L C T A S T F F T L L S W L P T F F F K D T F P D A K G I F N V V P 306
Sea urchin ----- T F F S F Q N G R A G T R K D D E K K S L P V L K L L T K K P F W A M A I S H L C V I F S Y F I I I N W M P T F F F K E V P E S K G W F N V V P 284
Drosophila ----- E R N R I I N I A T P S R L C A N K S P E K T S A V P W L R Y F R R L S F W A C V L T H A C E M N C F F L L S W L P T Y F H D G F P H A K G W F N V V P 293
C elegans SPLPDEEVLLDKKHDTIESHLAATS PCPSVPWGT LFRHPAFWAAVAQYTGGNYS I L F N W L P S Y F H E T F P T A K G F V N V V P 298
Sea anemone ----- S Q M N P T Y S P S H T K S E Q S S A R K N V P W G K I I S E P A I W A V V I V H F C H N C M Y F T L I S W M P T Y F H E N F P E S Q G W F N V V P 296

Human WLVAIPASLFSGFLSDHLINQGYRAITVRKLMQGMGLGLSSVFALCLGHTSSFCESVVFASASIGLQTFNHSGISVNIQDLA 358
Bovine WLVAIPASLLSGLSDHLINQGYRTITVRKFMQVMGLGLSSVFALCLGHTSSFCNSVVFASASIGLQTFNHSGISVNIQDLA 381
Mouse WMLAIPASLFSGFISDRLLISQGYRITVRKFMQVMGLGLSSI FALCLGHTT SFLKAMI FASASIGFQTFNHSGISVNIQDLA 375
Chicken WLVAIPTSLFSGFLSDHLINQGYKTIITVRKFMQVIGSGVSSVFALCLGQTS SFC A I V F A S A S V G L Q T F N H S G I S V N I Q D L A 382
Xenopus WLFAIPAGILSGLSDHLITQGYQT V W V R K L M Q I V G M G F S S A F I F C L A H T T Y Y H Y A V A F A S A L A L Q T F N H S G I T V N I Q D L A 389
Zebrafish WFVAIPSSLFSGCLSDHLISQGFDTASVRKLMQFMSGVSSVFTLFLCGT T T F P A A V A F V S A T M G L T T F S H S G V S V N I Q D L A 388
Sea urchin WLAIPASIAAGGCLGDSLIAHKVPVGISRKMHTLTVMS-----SLSNSSTAPNVQDVA 339
Drosophila WLALPCTLFAKYLTRLLAREWHTTVRKVIQSCFAAQNLAFLVMSRTSDFHTALICMTI I I G G T G F H N N A V T V N P Q D L A 375
C elegans SLAIVVTSLVAPVMA SRALSEGKTYTRKLMEGASLLGIAFCLMLVPMTSSFWISLI IFTMAMAARGLHGGVSVNPHDFA 380
Sea anemone YIGNFVGLKGGGYVADKMIKMFSAVTRKFLLETGLTCVPALVLLSTSQAVHFVQALVCMMSMALCLCGASTSGSLMNIQDLS 378

Human PSCAGFLFGVANTAGALAGVGVCLGGYLMETGTS-WTCLFNLVAI I SNLGLCTFLVFGQAQRVLDLSTHEDL----- 430
Bovine PSCAGFLFGVANTAGALAGVGVCLGGYLIETTGS-WTSVFNLVAAISSLGLCTFLVFGKAQRVLDLSPAHEDL----- 453
Mouse PSCAGFLFGVANTAGALAGVGVCLSGYLIETTGS-WTCVFLHVAI I SNLGLGTFLVFGKAQRVLDLVP THEDL----- 447
Chicken PSCAGLLFGVNTGGALLGVVCLVYLAGYLMETGTS-WISVFNLVAVNSVGLCVFLVFGQAQRVLDLSDAYIDL----- 454
Xenopus PTCAGFLFGVANTGGALLGVVLYLSSYLIETTGS-WSSMFYLV I V N L M G L V V F L E F A K S E R V D T E T I H V ----- 459
Zebrafish PSCAGALFGVMNTCGAFTGVIMVYFSGYLIETTGS-WASVFLGITVNNLLGLTTFLSFAEARRVDIDLAKGRYHNIHI 465
Sea urchin PKHAGSVFGLVNAMGAVQGFVGTIYAGCILHSTGS-WSAVFNMSACVCLFWSVVVFTLFAVGTPIV----- 403
Drosophila PLHSGSVFGLMNTVGAI PGFLGVYLAGHILELTSQ-WPMVFSAAAAGINLVGWI I F I V F G S A E A I V ----- 439
C elegans PNHAGSVFVFNACGAI TGFVGVYIAGHILEATNNNSYV F V V T A A Q C V V G A M Y T L L G T G Q K I I ----- 445
Sea anemone PGFAGSISGVVFTISAI PGVLGVYATGYILHVTNS-WTTV F Q L T A V I C T A G C A V Y N V F A K G H K I L ----- 442



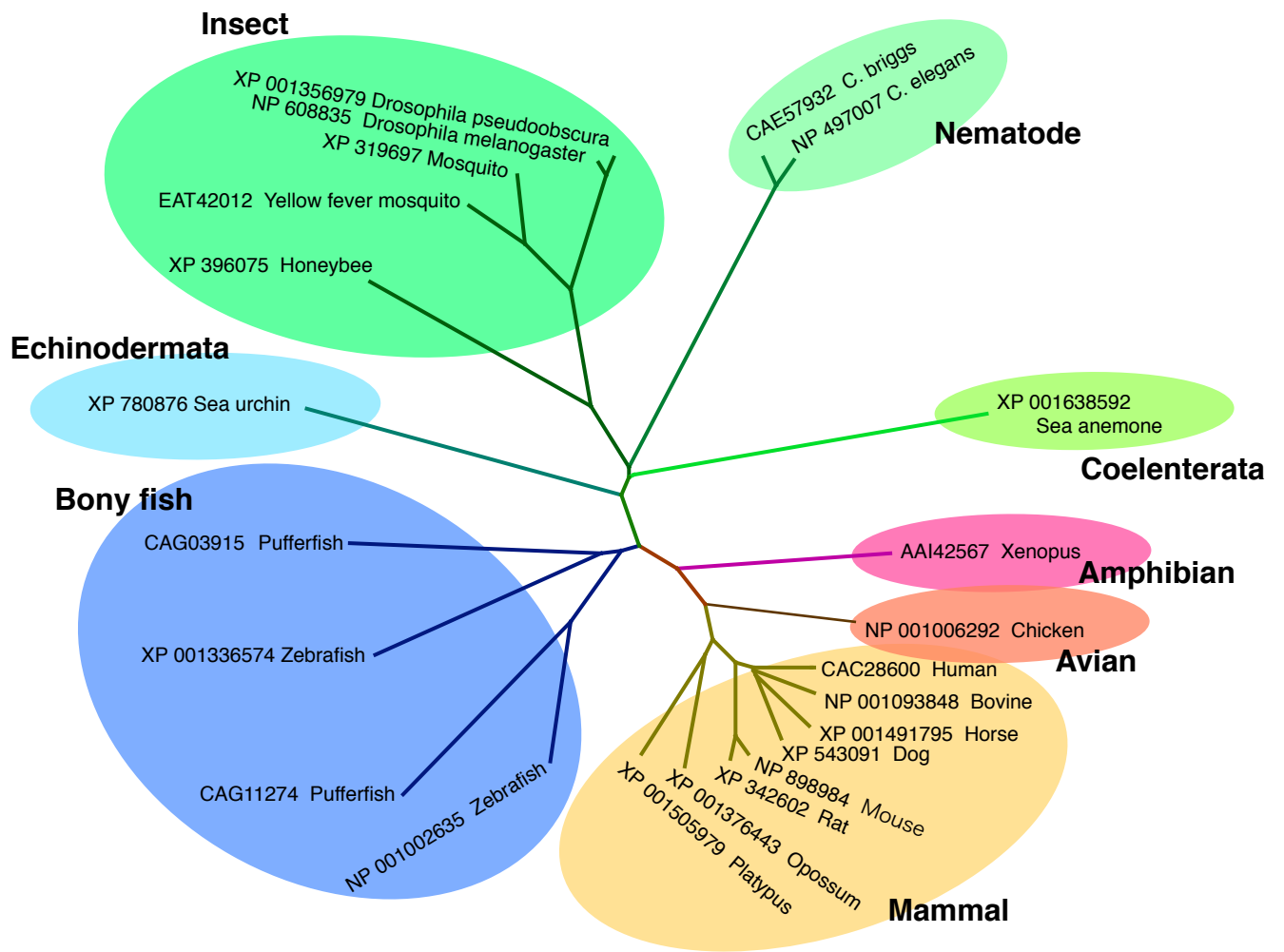


Table 1. Energetics of ATP transport.

Inside	Outside	Ionophores	Inside pH	Outside pH	ATP uptake (%)
Na ⁺	K ⁺	none			3.1 ± 1.7
		Val.	7.0	7.0	100.0
		Nig.			6.4 ± 2.1
Na ⁺	Na ⁺	none			7.6 ± 6.8
		Val.	7.0	7.0	6.5 ± 5.0
		Nig.			4.5 ± 4.1
K ⁺	K ⁺	none	7.0	7.0	5.9 ± 2.3
			5.5	7.0	2.6 ± 0.1
			5.5	7.5	2.4 ± 0.4
		Val.	7.0	7.0	5.9 ± 5.1
		Nig.			2.4 ± 3.0
K ⁺	Na ⁺	none			2.5 ± 0.6
		Val.	7.0	7.0	3.0 ± 2.1
		Nig.			3.3 ± 2.8

Na⁺-trapped proteoliposomes or K⁺-trapped proteoliposomes was prepared by dilution of purified transporter (10 µg protein, 150 µl) into the 3 ml of buffer consisting of 20 mM MOPS-tris, pH 7.0, 0.15 M Na-acetate (Na⁺-trapped proteoliposomes) or 0.15 M K-acetate (K⁺-trapped proteoliposomes), and centrifuged as described. The pellets were suspended in 200 µl of the same buffer. The proteoliposomes (0.5 µg protein) was added to the buffer containing 0.15 M K-acetate and 4 mM KCl or 0.15 M Na-acetate and 4 mM NaCl in the presence or absence of listed ionophores (1 µM each). After incubation for 5 min, ATP uptake was started as described. 100 % control corresponded to 16.0 ± 1.3 nmole/mg protein/2 min. In some experiments, proteoliposomes were prepared in the 20 mM MES, pH 5.5, 0.15 M K-acetate and 4 mM KCl. Then, proteoliposomes were added to the assay

solution, pH 7.0 or 7.5, 0.15 M K-acetate and 4 mM KCl. ATP uptake at 2 min was measured.