Abstract  Translationally controlled tumor protein (TCTP) has both extra- and intracellular functions. Our group recently reported that TCTP interacts with Na,K-ATPase and suppresses its activity. Our studies led to the identification of sorting nexin 6 (SNX6) which binds with TCTP as a potential negative regulator of TCTP. SNX6 does not interact directly with any cytoplasmic domains of Na,K-ATPase. However, when overexpressed, it restores the Na,K-ATPase activity suppressed by TCTP. This was confirmed by measurements of purified plasma membrane Na,K-ATPase activity after incubation with recombinant TCTP and SNX6. SNX6 alone has no effect on Na,K-ATPase activity, but activates Na,K-ATPase via inhibition of TCTP. Inhibition of endogenous TCTP by the overexpression of SNX6 or knockdown of TCTP expression by siTCTP increased Na,K-ATPase activity above the basal level. The interaction between SNX6 and TCTP thus appears to regulate Na,K-ATPase activity.

Keywords: Na,K-ATPase; Translationally controlled tumor protein; Sorting nexin 6

1. Introduction

Translationally controlled tumor protein (TCTP), also variously known as IgE-dependent histamine-releasing factor (HRF), p23/p21, and fortlin, is distributed in all normal cell types. It exhibits a high degree of homology among various species, suggesting that TCTP may play an essential role in cellular processes [1]. Many studies demonstrated that TCTP is a multifunctional protein [2]. TCTP was reported to be involved, extracellularly in human allergic response as HRF [3], intracellularly as a microtubule-stabilizing protein [4], as an antiapoptotic protein [5], and as a guanine nucleotide dissociation inhibitor in protein synthesis [6]. Recently, TCTP has also been reported as a suppressor of Na,K-ATPase by our group [7].

Na,K-ATPase, sometimes called Na⁺ pump, is a multimembrane-spanning enzyme, and is essential for establishing and maintaining high K⁺ and low Na⁺ in the cytoplasm, required for various cellular activities such as regulation of cell volume, maintenance of membrane potential, and nutrient transport. This ionic homeostasis is critical for cell growth, differentiation, and cell survival [8,9]. Na,K-ATPase is composed of a catalytic α and a regulatory β subunit [10]. TCTP interacts with the third large cytoplasmic domain (CD3) of α subunit believed to be involved in the regulation of Na,K-ATPase activity by protein kinases and hormones [7,11].

In the present study, we looked for TCTP-interacting proteins that might regulate the activity of Na,K-ATPase. We found that sorting nexin 6 (SNX6) inhibits the Na,K-ATPase-suppressing activity of TCTP. SNX6, also named the tumor necrosis factor receptor-associated factor 4 (TRAF4)-associated factor 2 (TFAF2) [12], was originally isolated as an interacting partner for Smad1 [13]. Later, this molecule was found to bind with a variety of other receptors. These include: the receptor serine/threonine kinase TGF-βR, receptor tyrosine kinases of EGFR, PDGFR, IR, and the long form of the leptin receptors [14] such as other members of sorting nexin family believed to function in intracellular trafficking of target proteins [15]. SNX6 is widely expressed in multiple tissues [14]. It is located in the cytoplasm and cell membrane [16], and the nucleus after binding to Pim-1 [12]. However, little is known of the cellular function of SNX6 other than that it inhibits TGF-β signaling.

2. Materials and methods

2.1. Yeast two-hybrid analysis

Yeast two-hybrid screening was performed as described previously [17], using rat TCTP as bait and a mouse thymocyte cDNA library. Protein–protein interaction results in activation of LexAop-Lex+2 and LexAop-LacZ reporter genes. Clones encoding TCTP-interacting proteins were identified by DNA sequencing. One of these clones corresponded to SNX6. The cytoplasmic domains (CDs) of rat Na,K-ATPase α1, x2 and β subunits were cloned into the LexA fusion plasmid pEG202 as previously described [7]. Yeast cells were transformed with both resulting in LexA fusion constructs and a SNX6 clone. The binding activity was evaluated by β-galactosidase activity as reported previously [18], using the formula: β-galactosidase unit = A₄₂₀ × 1000/(min) × volume of extract (ml) × protein (µg/ml).

2.2. Epitope-tagged constructs

Full-length human SNX6 cDNA was obtained from Dr. Jong-Hoon Park (Sookmyung Women University, Seoul, Korea). Green fluorescence protein (GFP)-tagged SNX6 was generated by PCR.
amplification of the coding region of human SNX6 cDNA. The amplified fragments were digested with appropriate restriction enzymes and cloned into corresponding sites of mammalian expression vector pEGFP-N1 (Clontech). His-tagged SNX6 was constructed in bacterial expression vector pRSET (Invitrogen) for recombinant SNX6 protein in E. coli. HA-tagged TCTP, GFP-tagged TCTP, and His-tagged TCTP constructs were made from our previous study [19].

2.3. Co-immunoprecipitation

HeLa cells were transiently transfected with HA-TCTP construct using LipofectAMINE PLUS™ (Invitrogen) or WelFect-EX™ PLUS (WelGENE). After 24–48 h, the cells were lysed with an ice-cold lysis buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.025% sodium deoxycholate, 1 mM NaF, 1 mM Na3VO4, and Complete™ protease inhibitor cocktail tablet (Roche Applied Science). After centrifugation, the supernatants were mixed with mouse 12CA5 anti-HA monoclonal antibody and incubated with protein G-agarose. The bound immune complexes were pelleted and washed with ice-cold phosphate-buffered saline (PBS, 136.9 mM NaCl, 2.7 mM KCl, 8 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.4). The precipitated proteins were separated on 12% SDS–polyacrylamide gel (SDS–PAGE). The blots obtained were detected with mouse 12CA5 anti-HA monoclonal antibody, mouse anti-Na,K-ATPase α1 monoclonal antibody and rabbit anti-SNX6 polyclonal antibody (Zymed Laboratories Inc.). Same experiments were done with untransfected HeLa cells. In this case, after centrifugation of cell lysates, the supernatants were mixed with mouse anti-Na,K-ATPase α1 monoclonal antibody (Upstate Biotechnology) and blots were detected with mouse anti-Na,K-ATPase α1 monoclonal antibody, rabbit anti-SNX6 polyclonal antibody (Zymed Laboratories Inc.), and affinity purified rabbit anti-TCTP polyclonal antisera from recombinant His-tagged TCTP in our lab.

2.4. Knockdown of TCTP expression by small interfering RNA (siRNA)

siTCTP, construct for intracellular suppression of TCTP siRNA was made in pSUPER vector (OligoEngine). The functional siRNA corresponding to 5′-AGGGTACCGAAGCACAGTA-3′ of human TCTP gene was synthesized inside mammalian cells transfected with siTCTP construct [20]. The level of suppression of TCTP gene was measured from the amount of TCTP protein.

2.5. 86Rb+ uptake assay

HeLa cells were plated in 24-well plate at 5 × 104 cells/well and were transfected with GFP, GFP-TCTP, GFP-SNX6, or siTCTP constructs as mentioned above. After 24–48 h, the cells were incubated for 3 h at 37 °C in serum-free DMEM. The serum-starved cells were washed with 5 mM HEPES buffer (KRP, 140 mM NaCl, 5 mM KCl, 10 mM Na2HPO4, 1 mM MgSO4, and 2.5 mM glucose, 0.1% BSA, pH 7.4) and incubated with or without 0.1 mM ouabain (a specific inhibitor of Na,K-ATPase) and/or 0.1 mM furosemide (an inhibitor of Na+,K+,2Cl– and 2.5 mM glucose, 0.1% BSA, pH 7.4) and incubated for 3 h at 37 °C. 86Rb+ was used as a tracer to measure the uptake of K+. After incubation with 86RbCl (1 μCi/ml) for 15 min at 37 °C, cellular 86Rb+ uptake was terminated by washing with ice-chilled KRP and the cells were lysed with lysis buffer (50 mM NaOH, 1% Triton X-100) for 5 min. The radioactivity of the cell lysate from one well was counted. The value obtained was adjusted for the protein concentration.

2.6. Western blot analysis

Cells were prepared as described for the 86Rb+ uptake assay. The precipitated proteins of total cell lysates were separated on 12% SDS–PAGE, blotted, and incubated with anti-Na,K-ATPase α1 monoclonal antibody, rabbit anti-GFP polyclonal antibody (Clontech), rabbit anti-TCTP polyclonal antisemur.

2.7. In vitro Na,K-ATPase assay

The purified plasma membrane Na,K-ATPase fraction was obtained from twenty 100-mm dishes of HeLa cells using the procedures described in a previous report [21]. 0.04 mg/ml of plasma membrane Na,K-ATPase fraction positioned at 30% sucrose was preincubated with various concentrations of ouabain (10−9–10−3 M), recombinant TCTP (0.5–2 μg/ml), recombinant SNX6 (0.5–2 μg/ml), or a combination of recombinant TCTP and SNX6 at the appropriate concentrations in assay buffer (18 mM histidine, 18 mM imidazole, 80 mM NaCl, 15 mM KCl, 3 mM MgCl2, and 0.1 mM EGTA, pH 7.4). The assay was then started with the addition of ATP to a final concentration of 5 mM. After the incubation for 60 min at 37 °C, the release of inorganic phosphate from ATP was quantified using the colorimetric method described previously [22]. Recombinant TCTP and SNX6 were produced by the bacterial expression pRSET vector system (Invitrogen) which contains the sequence for a His6 tag at the N-terminus of the protein.

2.8. Data analysis

Data are presented as the means ± S.E. Statistical analysis utilized one-way analysis of variance (ANOVA) followed by post hoc Duncan test.

3. Results

3.1. TCTP interacts with both SNX6 and Na,K-ATPase

To better understand the functional role of TCTP in the regulation of Na,K-ATPase activity, we searched for TCTP-binding proteins using the yeast two-hybrid system. Using this approach, we were able to isolate several cDNA clones. One of these clones corresponded to the gene for SNX6 which encodes 406 amino acids (Fig. 1A). Fig. 1B, which shows the results of β-galactosidase activity assay, suggests that the interaction between TCTP and SNX6 occurred inside the yeast cell, and between TCTP and the third cytoplasmic domain of Na,K-ATPase α1 subunit (α1CD3). We confirmed that the interactions of TCTP with both SNX6 and Na,K-ATPase occur in vivo by co-immunoprecipitation. After checking that HeLa cells transfected with HA-TCTP construct expressed 23 kDa HA-TCTP, the immunoprecipitated complex prepared with anti-HA antibody was blotted with anti-Na,K-ATPase α subunit and anti-SNX6 antibody. HA-TCTP co-precipitated both 100 kDa Na,K-ATPase α subunit and 50 kDa SNX6 (Fig. 1C). We also performed immunoprecipitation with untransfected HeLa cells to see if endogenous TCTP and SNX6 are complexed with Na,K-ATPase. Immunoprecipitation with anti-Na,K-ATPase antibody detected 50 kDa SNX6 subunit (Fig. 1D), suggesting that TCTP associates with SNX6 as well as Na,K-ATPase, and their complex exists in HeLa cells.

3.2. SNX6 does not interact with Na,K-ATPase

To identify whether SNX6 itself interacts with the cytoplasmic domains of Na,K-ATPase like TCTP, individual Ura"-His" Trp+ transformants were streaked onto Glc Ura His" Trp" Leu" and Gal Ura His" Trp" Leu" plates and assayed for β-galactosidase activity. No transformant of pEG202/cytolactosin domains of Na,K-ATPase subunits and pJG4–5/SNX6 activated the transcription of the respective reporter genes, suggesting that the cytoplasmic domains of Na,K-ATPase α1, α2 and β subunits do not interact with SNX6 (Table 1). Although the cytoplasmic domain of Na,K-ATPase β subunit showed a slight increase in β-gal signal, it did not activate Leu2 reporter. One cannot determine whether the N-terminal portion of Na,K-ATPase α subunit interacts with SNX6 because the yeast cells transfected with pEG202/s1NT or pEG202/s2NT always activated reporter genes.
3.3. Overexpression of SNX6 restores the Na⁺,K⁺-ATPase activity suppressed by overexpression of TCTP without change of Na⁺,K⁺-ATPase protein level

To explore whether SNX6 is involved in the regulation of Na⁺,K⁺-ATPase activity through interaction with TCTP, we overexpressed SNX6 in HeLa cells and measured Na⁺,K⁺-ATPase activity using 

\[ ^{86}\text{Rb}^+ \] uptake assay. Because it has been reported that 60% of \( ^{86}\text{Rb}^+ \) uptake in HeLa cells is accounted for by Na⁺,K⁺-ATPase and another 30% by Na⁺,K⁺,2Cl⁻ cotransporter, for a total of 90% [23], we determined the individual transporter activity using 0.1 mM ouabain (a specific inhibitor of Na⁺,K⁺-ATPase) and 0.1 mM furosemide (an inhibitor of Na⁺,K⁺,2Cl⁻ cotransporter). Fig. 2A shows that Na⁺,K⁺-ATPase activity of GFP-TCTP-transfected cells was 14.5 ± 5.4% lower than that of mock. This confirms the previous report that overexpression of TCTP inhibits Na⁺,K⁺-ATPase activity [7]. However, Na⁺,K⁺-ATPase activity of cells co-transfected with both GFP-TCTP and GFP-SNX6 recovered to the mock level and Na⁺,K⁺,2Cl⁻ cotransporter and the residual transporter activities were similar. These results suggest that the overexpression of SNX6 restores the Na⁺,K⁺-ATPase activity decreased by overexpression of TCTP. To clarify whether the increase in Na⁺,K⁺-ATPase activity from overexpression of SNX6 is caused by the change of Na⁺,K⁺-ATPase expression level, Western blot of cells transfected as in the \( ^{86}\text{Rb}^+ \) uptake assay was performed using anti-Na⁺,K⁺-ATPase α subunit antibody. Fig. 2B demonstrates that the expression level of α subunit was the same in all transfected cells. This suggests that recovery of Na⁺,K⁺-ATPase activity in TCTP and SNX6-co-transfected cells is not due to the increase of Na⁺,K⁺-ATPase protein level. Although the expression level of SNX6 as shown in Fig. 2B appeared to be much lower than that of TCTP, SNX6 was able to restore the Na⁺,K⁺-ATPase activity suppressed by overexpression of TCTP. This might be due to different cellular localization of the protein after transfection. Indeed, subcellular fractionation analysis showed that 51–28% of cellular SNX6 is localized in plasma membrane (PM), whereas only 10–7% of cellular TCTP is localized in plasma membrane (unpublished data). Thus, SNX6 localized in PM seems to reverse the inhibition of Na⁺,K⁺-ATPase by TCTP in PM.

3.4. Recombinant SNX6 prevents recombinant TCTP from suppressing purified plasma membrane Na⁺,K⁺-ATPase activity in vitro

Using HeLa cell plasma membrane fraction enriched with ouabain-sensitive Na⁺,K⁺-ATPase by removal of extraneous protein, we investigated the effect of SNX6 and TCTP on purified membrane-bound Na⁺,K⁺-ATPase in vitro. Ouabain effectively decreased the activity of the purified plasma membrane Na⁺,K⁺-ATPase used in this assay (IC₅₀ = 2.85 × 10⁻⁷ M) (Fig. 3A). Addition of recombinant TCTP to the purified Na⁺,K⁺-ATPase resulted in a significant decrease in its activity in a dose-dependent manner (IC₅₀ = 1.84 μg/ml) and finally complete inhibition at 10 μg/ml above (data not shown) as previously reported [7]. However, this inhibition was prevented by recombinant SNX6 (Fig. 3B). Moreover, although the recombinant SNX6 did not affect Na⁺,K⁺-ATPase activity significantly, the purified Na⁺,K⁺-ATPase which existed firstly with recombinant SNX6 was not inhibited by the addition of SNX6.
activity was calculated by 86Rb+ uptake assay. As expected, overexpressed in HeLa cells and the individual transporter will be activated. To test this hypothesis, SNX6 alone was SNX6 and TCTP is increased simultaneously, Na,K-ATPase activation domain. Protein–protein interaction results in activation of as a positive control (25).

Although siTCTP caused incomplete knockdown of TCTP, cells, endogeneous TCTP expression was reduced by 70–80%. Increased expression of Na,K-ATPase. In siTCTP-transfected cells, endogeneous TCTP expression was reduced by 70–80%, this incomplete knockdown had the same effect on the activation of Na,K-ATPase as overexpression of SNX6, implying the breakout of Na,K-ATPase activity. As indicated before, this may be, because, based on the subcellular fractionation analysis of cellular TCTP (unpublished data), only 10–7% of the remaining TCTP is probably localized in plasma membrane. Thus the quantity of the remaining TCTP is probably insignificant.

4. Discussion

Because Na,K-ATPase is closely involved in physiological and pathological phenomena, it is important to understand the mechanisms that regulate its activity. We propose here that Na,K-ATPase activity may be regulated by a complex mechanism involving the interaction of TCTP with Na,K-ATPase and SNX6. Our study suggests the existence of a tertiary complex of Na,K-ATPase, TCTP, and SNX6 that SNX6 acts as the negative regulator of TCTP which was previously identified as a suppressor of Na,K-ATPase [7]. Furthermore, our data demonstrate that the inhibition of Na,K-ATPase by endogenous TCTP is a natural phenomenon existing for the maintenance of cellular Na,K-ATPase activity at normal resting state and that Na,K-ATPase can be activated by inhibition of endogenous TCTP via the interaction with SNX6. Although siTCTP construct caused an incomplete knockdown of the expression of endogenous TCTP (70–80%), this incomplete knockdown had the same effect on the activation of Na,K-ATPase as overexpression of SNX6, implying the breakout of normal resting Na,K-ATPase activity. It was recently reported that knockdown of TCTP promotes apoptosis [24,25]. It appears that the ultimate role of endogenous TCTP is to carry out critical functions for cell survival, although this needs to be confirmed by studying the effect of knocking out the TCTP gene.

It has been reported that TCTP and SNX6 are ubiquitously expressed in multiple tissues and localized throughout the cell.

Table 1

<table>
<thead>
<tr>
<th>Na,K-ATPase subunit</th>
<th>Construct</th>
<th>Selective media plate</th>
<th>β-Galactosidase activity (Unit)</th>
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</thead>
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<tr>
<td></td>
<td>pEG202 (DNA-binding)</td>
<td>pJG4-5 (transcription activation)</td>
<td>Gln U H T L</td>
</tr>
<tr>
<td>Control</td>
<td>Negative</td>
<td>– –</td>
<td>1</td>
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<tr>
<td></td>
<td>Positive</td>
<td>– +++</td>
<td>0</td>
</tr>
<tr>
<td>x1</td>
<td>NT (Met1-Cys93)</td>
<td>SNX6</td>
<td>+</td>
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<tr>
<td></td>
<td>CD2 (Glu151-Ile292)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>CD3 (Lys354-Val759)</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>CD4 (Glu825-Arg848)</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>CT (Ile935-Tyr1023)</td>
<td>–</td>
<td>–</td>
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<tr>
<td>x2</td>
<td>NT (Met1-Cys91)</td>
<td>SNX6</td>
<td>+</td>
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<tr>
<td></td>
<td>CD2 (Glu149-Ile290)</td>
<td>–</td>
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<td></td>
<td>CD3 (Lys352-Val756)</td>
<td>–</td>
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<tr>
<td></td>
<td>CD4 (Glu822-Arg845)</td>
<td>–</td>
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<tr>
<td></td>
<td>CT (Ile932-Tyr1020)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>β</td>
<td>CD (Ala1-Lys33)</td>
<td>SNX6</td>
<td>–</td>
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The CDs of the Na,K-ATPase x1, x2 and β subunits were fused to the LexA DNA-binding domain. SNX6 was fused to the B42 transcription activation domain. Protein–protein interaction results in activation of LexAop-Leu2 and LexAop-Leu2-Z reporter genes. The yeast cell transformed with empty vector pEG202 and pJG4-5 was used as a negative control, and the yeast cell transformed with pEG202/1CD3 and pJG4-5-S267(cofilin) as a positive control (25). β-Galactosidase activity values are the average of four independent determinations converted to percentage of positive control. Gal, galactose; Glc, glucose; U, uracil, histidine, tryptophan, leucine; NT, N-terminal portion; CD, cytoplasmic domain; CT, C-terminal portion.

recombinant TCTP (Fig. 3C). These results show that SNX6 specifically inhibits the ability of TCTP to suppress Na,K-ATPase activity, which ultimately results in indirect activation of Na,K-ATPase.

3.5. Overexpression of SNX6 activates Na,K-ATPase above the basal level like knockdown expression of endogenous TCTP

We hypothesized that if the interaction between endogenous SNX6 and TCTP is increased simultaneously, Na,K-ATPase will be activated. To test this hypothesis, SNX6 alone was overexpressed in HeLa cells and the individual transporter activity was calculated by 86Rb+ uptake assay. As expected, Na,K-ATPase activity of the GFP-SNX6-transfected cells was 18.7 ± 4.2% higher than that of mock (Fig. 4A). Overexpression of SNX6 can promote the interaction between SNX6 and endogenous TCTP, leading to upregulation of Na,K-ATPase above the basal level via inhibition of Na,K-ATPase-suppressive activity of endogenous TCTP. This suggests that inhibition of Na,K-ATPase by endogenous TCTP is a normal physiological event required for the maintenance of cellular Na,K-ATPase activity to basal level under normal environment. We made the siTCTP construct which markedly downregulates the level of endogenous TCTP, and examined whether the activation of Na,K-ATPase occurs in siTCTP-transfected cells. As shown in Fig. 4A, siTCTP caused the activation of Na,K-ATPase as did the overexpression of SNX6, suggesting Na,K-ATPase activity is usually inhibited to basal level by endogenous TCTP. Additionally, Na,K-ATPase activity of cells, co-transfected with both siTCTP and GFP-SNX6, was not different from that of SNX6 or siTCTP alone. Fig. 4B shows that Na,K-ATPase activity by overexpression of SNX6 and decreased expression of TCTP was not caused by increased expression of Na,K-ATPase. In siTCTP-transfected cells, endogeneous TCTP expression was reduced by 70–80%. Although siTCTP caused incomplete knockdown of TCTP, co-transfection with both siTCTP and GFP-SNX6 still activates Na,K-ATPase activity. As indicated before, this may be, because, based on the subcellular fractionation analysis of cellular TCTP (unpublished data), only 10–7% of the remaining TCTP is probably localized in plasma membrane. Thus the quantity of the remaining TCTP is probably insignificant.
Because the activation of Na,K-ATPase by SNX6 results from the latter’s role as an inhibitor of TCTP, any imbalance in the amounts of TCTP and SNX6 affects their interaction which in turn affect Na,K-ATPase activity. SNX9, a member of sorting nexins, was found to be translocated from cytosol to plasma membrane by insulin [26]. Inhibition of TCTP by SNX6 may contribute to upregulation of Na,K-ATPase by stimuli such as insulin. Studies are underway to test this possibility. Moreover, the interaction of TCTP with SNX6 may influence other functions of TCTP involved in growth-related processes, apoptosis and allergic response [2].

Currently, little is known of the cellular function of SNX6, other than that SNX6 plays the role of interfering with TGF-β signaling [14]. SNX6 is a member of sorting nexins, a family of cytoplasmic and membrane-associated proteins. A characteristic structural feature of the sorting nexins is the PH domain (PX domain), a phosphoinositide-binding module [27,28]. Because several sorting nexins with their PX domains localize mainly in PtdIns(3)P-rich endosomal and vacuolar, it has been suggested that they may function in the endocytosis of plasma membrane and/or the trafficking of...
proteins from one membrane compartment to another [29]. It has been suggested that for some sorting nexins, membrane association also requires their C-terminal coiled-coil regions (BAR domain) as well as PX domain [16]. Several sorting nexins including SNX6 consist of PX domain and a BAR domain [30]. They homo- and hetero-oligomerize and colocalize intracellularly through these domains [31]. Recently, the Bin/Amphipathin/Rvs (BAR) domain has been described as a dimerization and membrane binding module which can sense membrane curvature [32]. Moreover, this domain has the potential to function as a small G-protein binding module [32,33]. Thus, it is tempting to speculate that the sorting nexins that share PX and BAR domains may share common functions in the cell. However, the fact that individual sorting nexins have different specificities for interacting with the target protein [15] implies that they may serve to select the specific target protein to be conveyed within the particular transport vesicles, or regulate the specific cellular functions unrelated to their normal functions. Our demonstration that SNX6 is involved in cellular ionic homeostasis via inhibition of TCTP may be an example of such new functions.

Further studies are needed to understand the detailed mechanisms underlying the role of SNX6 as a TCTP-inhibitory protein. Elucidation of how Na,K-ATPase activation by SNX6 occurs may lead to more comprehensive approaches to TCTP regulation and the identification of therapeutic targets to diseases involving Na,K-ATPase, such as hypertension.

In conclusion, we identified SNX6 as a TCTP-binding protein and demonstrated that the interaction of TCTP with SNX6 interferes with suppression of Na,K-ATPase by TCTP. SNX6 therefore may be a key molecule serving as the negative regulator of TCTP in the regulation of Na,K-ATPase activity under physiological stimuli. Our demonstration of the indirect regulation of Na,K-ATPase activity by SNX6 suggests a novel molecular mechanism for the functional coupling of SNX6 and TCTP.

Acknowledgments: We thank Jaehoon Jung for advice on $^{86}$Rb$^+$ uptake and in vitro Na,K-ATPase activity assays. This work was supported by Korea Research Foundation Grant funded by Korea Government (MOEHRD, Basic Research Promotion Fund), Korean Health 21 R&D Project by Ministry of Health and Welfare (A050402), Seoul R&B Program, and the Korea Science and Engineering Foundation through CCSR at Ewha Womans University. T. Yoon was supported by the Brain Korea 21 Project Fellowship.

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