Down-Regulation of the Myoinositol Transporter SMIT by JAK2

Zohreh Hosseinzadehab Shefalee K. Bhavsarb Florian Langa

*Department of Physiology I, University of Tübingen, Tübingen, a,b contributed equally and thus share first authorship

Key Words
Inositol transport • Osmolyte • Cell volume • Energy depletion

Abstract

Background/Aims: Janus-activated kinase-2 JAK2 is activated by energy depletion and hyperosmotic shock and modifies the activity of several Na⁺ coupled transporters. The Na⁺ coupled osmolyte transporter SMIT (myoinositol transporter) is upregulated by osmotic shock and downregulated by energy depletion. The present study thus explored whether JAK2 contributes to the regulation of SMIT activity. Methods: To this end, cRNA encoding SMIT was injected into Xenopus oocytes with or without additional injection of cRNA encoding wild type JAK2, constitutively active V617F JAK2 or inactive K882E JAK2. Inositol-induced current (I_{SMIT}) was determined by dual electrode voltage clamp and taken as measure for electrogenic inositol transport. Results: No appreciable I_{SMIT} was observed in water injected oocytes. In SMIT expressing oocytes I_{SMIT} was significantly decreased by additional coexpression of JAK2 or V617F JAK2, but not by coexpression of K882E JAK2. According to kinetic analysis coexpression of JAK2 decreased maximal I_{SMIT}, without significantly modifying the concentration required for halfmaximal I_{SMIT} (K_M). In oocytes expressing both, SMIT and JAK2, I_{SMIT} was gradually increased by JAK2 inhibitor AG490 (40 µM). Disruption of carrier insertion with brefeldin A (5 µM) was followed by a decline of I_{SMIT} to a similar extent in Xenopus oocytes expressing SMIT with JAK2 and in Xenopus oocytes expressing SMIT alone, suggesting that JAK2 did not affect carrier stability in the cell membrane. Conclusion: JAK2 contributes to the regulation of the inositol transporter SMIT.
Introduction

The janus-activated kinase-2 JAK2 participates in the signaling of several hormones and cytokines [1-3] including leptin [4], growth hormone [5, 6], erythropoietin [3], thrombopoietin [3] and granulocyte colony-stimulating factor [3]. Excessive JAK2 activity fosters the development of malignancy and JAK2 inhibitors may be effective in the treatment of myeloproliferative disorders [7-12]. The gain of function mutation \( V617F \)JAK2 presumably contributes to the pathogenesis of myeloproliferative disease [13-16].

JAK2 is activated by oxidative stress & ischemia [17] and by hypertonicity [18, 19] and has most recently been shown to up-regulate the betaine/GABA transporter BGT1 (SLC6A12) [20], an osmolyte transporter participating in the regulation of cell volume [21-23]. Another \( \text{Na}^+ \) coupled osmolyte transporter is the myoinositol transporter SMIT, which accumulates myoinositol in osmotically shrunken cells [21, 24]. SMIT is genomically up-regulated by osmotic cell shrinkage, neuronal excitation and radiation [21-23, 25-39], an effect involving the transcription factor NFAT5 [40]. Little is known about posttranslational regulation of SMIT activity.

The present study explored whether JAK2 regulates the activity of SMIT. To this end, SMIT was expressed in \textit{Xenopus} oocytes with or without wild type JAK2, constitutively active \( V617F \)JAK2 or inactive \( K882E \)JAK2 and inositol transport estimated from inositol induced current.

Materials and Methods

**Constructs**

Constructs were used encoding wild type SMIT [41], wild-type human JAK2 (Imagenes, Berlin, Germany), inactive \( K882E \)JAK2 mutant [42] and overactive \( V617F \)JAK2 mutant [13] generated by site-directed mutagenesis (QuickChange II XL Site-Directed Mutagenesis Kit; Stratagene, Heidelberg, Germany) according to the manufacturer's instructions [43]. The constructs were used for generation of cRNA as described previously [44].

**Voltage clamp in Xenopus oocytes**

\textit{Xenopus} oocytes were prepared as described [45] Where not indicated otherwise, 25 ng SMIT cRNA were injected on the first day and 10 ng of wild type JAK2 cRNA on the second day or at the same day after preparation of the oocytes [46]. The oocytes were maintained at 17\(^\circ\)C in a solution containing (in mM): 96 NaCl, 4 KCl, 1.8 MgCl\(_2\), 0.1 CaCl\(_2\), 5 HEPES, pH 7.4, gentamycin (50 mg/l), tetracycline (50 mg/l), ciprofloxacin (1.6 mg/l), refobacin (100 mg/l), and theophiline (90 mg/l). Where indicated, the JAK2 inhibitor AG490 (40 \( \mu \)M), brefeldin A (5 \( \mu \)M) or actinomycin D (10 \( \mu \)M) were added to the respective solutions. The voltage clamp experiments were performed at room temperature 7-10 days after injection [47]. Two-electrode voltage-clamp recordings were performed at a holding potential of -60 mV. The data were filtered at 10 Hz and recorded with a Digidata A/D-D/A converter and Clampex 9.2 software for data acquisition and analysis (Axon Instruments) [48]. The control superfusate contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl\(_2\), 1 mM MgCl\(_2\), and 5 mM HEPES, pH 7.4. Inositol was added to the solutions at a concentration of 1 mM, unless otherwise stated. The flow rate of the superfusion was approx. 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s.

**Statistical analysis**

Data are provided as means \( \pm \) SEM, \( n \) represents the number of oocytes investigated. All experiments were repeated with at least 2-3 batches of oocytes; in all repetitions qualitatively similar data were obtained. Data were tested for significance using ANOVA or t-test, as appropriate. Results with \( p < 0.05 \) were considered statistically significant.
To explore whether Janus kinase-2 (JAK2) modifies the function of the myoinositol transporter (SMIT), cRNA encoding SMIT was injected into *Xenopus* oocytes with or without additional injection of cRNA encoding JAK2. Electrogenic transport of inositol was apparent from inositol (1 mM) induced inward current (I_{SMIT}). No appreciable current was observed following exposure of non-injected or water-injected *Xenopus* oocytes to 1 mM inositol. Thus, *Xenopus* oocytes do not express appreciable endogenous electrogenic inositol transport (Fig. 1A,B). A sizable I_{SMIT} was, however, observed in *Xenopus* oocytes injected with cRNA encoding SMIT alone (b), SMIT with wild type JAK2 (c), SMIT with constitutively active V617F JAK2 (d), or SMIT with the inactive mutant K882E JAK2 (e). The additional injection of cRNA encoding wild type JAK2 was followed by a significant decrease of I_{SMIT} in SMIT expressing *Xenopus* oocytes (Fig. 1A,B). The effect of wild type JAK2 coexpression tended to be lower than that of active V617F JAK2 but not of inactive mutant K882E JAK2 (Fig. 1A,B). Accordingly, I_{SMIT} was significantly lower in *Xenopus* oocytes expressing SMIT together with V617F JAK2 than in *Xenopus* oocytes expressing SMIT alone. In contrast, I_{SMIT} was similar in *Xenopus* oocytes expressing SMIT together with K882E JAK2 and in *Xenopus* oocytes expressing SMIT alone. The effect of V617F JAK2 coexpression tended to be lower than that of wild type JAK2 coexpression.

Kinetic analysis was performed to determine whether JAK2 influenced the maximal transport rate or the affinity of the carrier. As illustrated in Fig. 2, inositol-induced currents...
in SMIT-expressing *Xenopus* oocytes increased following increase of inositol concentration. The currents allowed calculating a maximal current, which approached 6.30 ± 0.03 nA (n = 9) in *Xenopus* oocytes expressing SMIT alone, and 3.84 ± 0.02 (n = 9) in *Xenopus* oocytes expressing SMIT together with JAK2. Accordingly, the maximal transport rate was significantly lower in *Xenopus* oocytes expressing SMIT together with JAK2 than in *Xenopus* oocytes expressing SMIT alone. The inositol concentration required for halfmaximal current (K_M) approached 0.06 ± 0.01 µM (n = 9) in *Xenopus* oocytes expressing SMIT alone, and 0.12
± 0.04 µM (n = 9) in Xenopus oocytes expressing SMIT together with JAK2. The $K_M$ was not significantly different between Xenopus oocytes expressing SMIT together with JAK2 and Xenopus oocytes expressing SMIT alone (Fig. 2).

Treatment of Xenopus oocytes expressing both, SMIT and JAK2, with the JAK2 inhibitor AG490 (40 µM) was followed by an increase of $I_{SMIT}$ (Fig. 3). The effect of the inhibitor on $I_{SMIT}$ reached statistical significance within 8 and 24 hours of preincubation with AG490.

The decrease of $I_{SMIT}$ in SMIT expressing Xenopus oocytes following coexpression of JAK2 could have been due to accelerated clearance of carrier protein from the cell membrane. In order to estimate the stability of $I_{SMIT}$ the SMIT-expressing Xenopus oocytes were treated with 5 µM brefeldin A, a substance blocking the insertion of new carrier protein into the cell membrane. As shown in Fig. 4A, in the presence of brefeldin A $I_{SMIT}$ declined within 8 and 24 hours to a similar extent in Xenopus oocytes expressing SMIT together with JAK2 and in Xenopus oocytes expressing SMIT alone. The observation suggests that JAK2 decreases $I_{SMIT}$ by a mechanism other than accelerating carrier clearance from the cell membrane.

Inhibition of transcription by incubation (24-48 hours) with actinomycin D (10 µM) did not significantly modify $I_{SMIT}$, which remained significantly lower in Xenopus oocytes expressing SMIT together with JAK2 than in Xenopus oocytes expressing SMIT alone (Fig. 4B). Thus, the effect of JAK2 did not involve altered transcription.

Discussion

The present study discloses that JAK2 contributes to the regulation of the myoinositol transporter SMIT. Coexpression of the Janus Kinase 2 (JAK2) significantly decreased the electrogenic transport of inositol in SMIT expressing Xenopus oocytes. Down-regulation of SMIT was similarly observed following coexpression of the gain of function mutant V617F JAK2 but not following coexpression of inactive K882E JAK2. Thus, JAK2 is effective as kinase.

Coexpression of the kinase significantly decreased the maximal current. Thus, JAK2 is at least partially effective by decreasing the maximal transport rate of the carrier. JAK2 expression did not significantly affect $K_M$. However, the large scatter of calculated $K_M$ values precludes safe conclusions on alterations of substrate affinity.

JAK2 has previously been shown to foster cellular glucose uptake by influencing facilitative glucose carriers [49, 50] and Na$^+$ coupled glucose transport [51]. Moreover, JAK2 participates in the regulation of Na$^+$ coupled neutral amino acid transporter B0AT (SLC6A19) [52], Na$^+$ coupled glutamate transport [48] and Na$^+$ and Cl$^-$ coupled transport of betaine and GABA [20].

Inhibition of carrier insertion into the cell membrane by brefeldin A leads to a decline of SMIT activity, which is similar in Xenopus oocytes expressing SMIT together with JAK2 and Xenopus oocytes expressing SMIT alone. Thus, the clearance of carrier from the cell membrane appears not to be sensitive to JAK2 expression. Alternatively, JAK2 may be effective by fostering carrier insertion into the cell membrane or by activating the carrier within the cell membrane.

Exposure of Xenopus oocytes expressing SMIT together with JAK2 to the JAK2 inhibitor AG490 was followed by a gradual increase of SMIT induced current indicating that the inhibitor counteracts the suppression of the carrier by the kinase. The effect appears to be slow, which may point to indirect regulation of the carrier. The observations in the presence of actinomycin D suggest that the observed effect is not due to an effect of JAK2 on gene transcription.

The presently observed down-regulation of SMIT by JAK2 may be considered surprising in view of the stimulating effect of hypertonicity on JAK2 [18, 19]. Osmotic cell shrinkage stimulates the expression of osmolyte transporters [53-55] including SMIT [21, 24-30, 35-40]. Along those lines, JAK2 increases the activity of the osmolyte transporter BGT1 [20] and Na$^+$/H$^+$ exchanger [56], which similarly serve to increase cell volume following osmotic cell shrinkage [57]. However, JAK2 is similarly activated by energy depletion [17] and the
kinase may inhibit SMIT to counteract cell swelling, a major threat of energy depleted cells [57].

In theory, the observed downregulation of SMIT by JAK2 may be relevant for the effects of JAK2 activating hormones and cytokines [1-3], such as leptin [4], growth hormone [5, 6], erythropoietin [3], thrombopoietin [3] and granulocyte colony-stimulating factor [3]. To the best of our knowledge, however, an effect of those hormones on SMIT expression and function has never been explored.

In conclusion, the present paper reveals that JAK2 is a powerful regulator of the Na⁺, Cl⁻ coupled myoinositol transporter SMIT. JAK2 down-regulates the transporter and thus limits the cellular uptake of Na⁺, Cl⁻ and myoinositol.

Acknowledgements

The authors acknowledge the meticulous preparation of the manuscript by Lejla Subasic and technical support by Elfriede Faber. This study was supported by the Deutsche Forschungsgemeinschaft.

References

1. Lopez AF, Hercus TR, Ekert P, Little DR, Guthridge M, Thomas D, Ramshaw HS, Stomski F, Perugini M, D'Andrea R, Grimbaldeston M, Parker MW: Molecular basis of cytokine receptor activation. IUBMB Life 2010;62:509-518.
2. Noon-Song EN, Ahmed CM, Dasic B, Canton J, Johnson HM: Controlling nuclear JAKs and STATs for specific gene activation by IFNgamma. Biochem Biophys Res Commun 2011;410:648-653.
3. Spivak JL: Narrative review: Thrombocytosis, polycythemia vera, and JAK2 mutations: The phenotypic mimicry of chronic myeloproliferation. Ann Intern Med 2010;152:300-306.
4. Morris DL, Rui L: Recent advances in understanding leptin signaling and leptin resistance. Am J Physiol Endocrinol Metab 2010;297:E1247-E1259.
5. Brooks AJ, Waters MJ: The growth hormone receptor: mechanism of activation and clinical implications. Nat Rev Endocrinol 2010;6:515-525.
6. Yang N, Jiang J, Deng L, Waters MJ, Wang X, Frank S: Growth hormone receptor targeting to lipid rafts requires extracellular subdomain 2. Biochem Biophys Res Commun 2010;391:414-418.
7. Baskin R, Majumder A, Sayeski PP: The recent medicinal chemistry development of Jak2 tyrosine kinase small molecule inhibitors. Curr Med Chem 2010;17:4551-4558.
8. Ko K, Valdez F, Garcia R, Tirado CA: JAK2 Translocations in hematological malignancies: Review of the literature. J Assoc Genet Technol 2010;36:107-109.
9. Oh ST, Gotlib J: JAK2 V617F and beyond: role of genetics and aberrant signaling in the pathogenesis of myeloproliferative neoplasms. Expert Rev Hematol 2010;3:323-337.
10. Santos FP, Verstovsek S: JAK2 inhibitors: What's the true therapeutic potential? Blood Rev 2011;25:53-63.
11. Tefferi A: Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: JAK2, MPL, TET2, ASXL1, GBL, IDH and J2F1. Leukemia 2010;24:1128-1138.
12. Mahfouz RA, Hoteit R, Salem Z, Bazarbachi A, Mugharbel A, Farhat F, Ziyadeh A, Ibrahim A, Taher A: JAK2 V617F Gene Mutation in the Laboratory Work-Up of Myeloproliferative Disorders: Experience of a Major Referral Center in Lebanon. Genet Test Mol Biomarkers 2011;15:263-265.
13. Shen X, Wei W, Xu H, Zhang M, Qin X, Shi W, Jiang Z, Chen Y, Chen F: JAK2V617F/STAT5 signaling pathway promotes cell proliferation through activation of Pituitary Tumor Transforming Gene 1 expression. Biochem Biophys Res Commun 2010;398:707-712.
14. Venkitachalam S, Chueh FY, Yu CL: Nuclear localization of lymphocyte-specific protein tyrosine kinase (Lck) and its role in regulating LIM domain only 2 (Lmo2) gene. Biochem Biophys Res Commun 2012;417:1058-1062.
16 Yao X, Balamurugan P, Arvey A, Leslie C, Zhang L: Heme controls the regulation of protein tyrosine kinases Jak2 and Src. Biochem Biophys Res Commun 2010;403:30-35.

17 Kurdi M, Booz GW: JAK redux: a second look at the regulation and role of JAKs in the heart. Am J Physiol Heart Circ Physiol 2009;297:H1545-H1556.

18 Garnovskaya MN, Mukhin YV, Vasova TM, Raymond JR: Hypertonicity activates Na⁺/H⁺ exchange through Janus kinase 2 and calmodulin. J Biol Chem 2003;278:16908-16915.

19 Gatsios P, Terstegen L, Schliess F, Haussinger D, Kerr IM, Heinrich PC, Graeve L: Activation of the Janus kinase/signal transducer and activator of transcription pathway by osmotic shock. J Biol Chem 1998;273:22962-22968.

20 Hosseinzadeh Z, Shojaiefard M, Bhavsar SK, Lang F: Up-regulation of the betaine/GABA transporter BGT1 by JAK2. Biochem Biophys Res Commun 2012;420:172-177.

21 Handler JS, Kwon HM: Regulation of renal cell organic osmolyte transport by tonicity. Am J Physiol 1993;265:C1449-C1455.

22 Kempson SA, Montrose MH: Osmotic regulation of renal betaine transport: transcription and beyond. Pflugers Arch 2004;449:227-234.

23 Takenaka M, Preston AS, Kwon HM, Handler JS: The toxicity-sensitive element that mediates increased transcription of the betaine transporter gene in response to hypertonic stress. J Biol Chem 1994;269:29379-29381.

24 Huang Z, Tunnacliffe A: Response of human cells to desiccation: comparison with hyperosmotic stress response. J Physiol 2004;558:181-191.

25 Bitoun M, Tappaz M: Gene expression of the transporters and biosynthetic enzymes of the osmolytes in astrocyte primary cultures exposed to hyperosmotic conditions. Glia 2000;32:165-176.

26 Bitoun M, Tappaz M: Gene expression of taurine transporter and taurine biosynthetic enzymes in brain of rats with acute or chronic hyperosmotic plasma. A comparative study with gene expression of myo-inositol transporter, betaine transporter and sorbitol biosynthetic enzyme. Brain Res Mol Brain Res 2000;77:10-18.

27 Burger-Kentischer A, Muller E, Neuhof F, Haussinger D, Beck F: Expression of aldose reductase, sorbitol dehydrogenase and Na⁺/myo-inositol and Na⁺/Cl⁻/betaine transporter mRNAs in individual cells of the kidney during changes in the diuretic state. Pflugers Arch 1999;437:248-254.

28 Denkert C, Warskulat U, Hensel F, Haussinger D: Osmolyte strategy in human monocytes and macrophages: involvement of p38MAPK in hyperosmotic induction of betaine and myo-inositol transporters. Arch Biochem Biophys 1998;354:172-180.

29 Ibsen L, Strange K: In situ localization and osmotic regulation of the Na⁺/myo-inositol cotransporter in rat brain. Am J Physiol 1996;271:F877-F885.

30 Matsuoka Y, Yamauchi A, Nakaniishi T, Sugiuira T, Kitamura H, Hori M, Nakamitsu Y, Ando A, Imai E, Hori M: Response to hypertonicity in mesothelial cells: role of Na⁺/myo-inositol co-transporter. Nephrol Dial Transplant 1999;14:1217-1223.

31 Neuhof W, Bartels H, Fraek ML, Beck FX: Relationship between intracellular ionic strength and expression of tonicity-responsive genes in rat papillary collecting duct cells. J Physiol 2002;543:147-153.

32 Nonaka M, Kohmura E, Yamashita T, Yamauchi A, Fujinaka T, Yoshimine T, Tohyama M, Hayakawa T: Kainic acid-induced seizure upregulates Na⁺/myo-inositol cotransporter mRNA in rat brain. Brain Res Mol Brain Res 1999;70:179-186.

33 Wiese TJ, Dunlap JA, Conner CE, Grzybowski J, Lowe WL, Yorek MA: Osmotic regulation of Na⁺/myo-inositol cotransporter mRNA level and activity in endothelial and neural cells. Am J Physiol 1996;270:C990-C997.

34 Wiese TJ, Dunlap JA, Conner CE, Grzybowski J, Lowe WL, Jr, Yorek MA: Osmotic regulation of Na⁺/myo-inositol cotransporter mRNA level and activity in endothelial and neural cells. Am J Physiol 1996;270:C990-C997.

35 Wiese TJ, Dunlap JA, Conner CE, Grzybowski J, Lowe WL, Jr, Yorek MA: Osmotic regulation of Na⁺/myo-inositol cotransporter mRNA level and activity in endothelial and neural cells. Am J Physiol 1996;270:C990-C997.
Yamauchi A, Miyai A, Shimada S, Minami Y, Tohyama M, Imai E, Kamada T, Ueda N: Localization and rapid regulation of Na+/myo-inositol cotransporter in rat kidney. J Clin Invest 1995;96:1195-1201.

Yamauchi A, Sugita T, Ito T, Miyai A, Horio M, Imai E, Kamada T: Na+/myo-inositol transport is regulated by basolateral tonicity in Madin-Darby canine kidney cells. J Clin Invest 1996;97:263-267.

Zhang Z, Ferraris JD, Brooks HL, Brisc I, Burg MB: Expression of osmotic stress-related genes in tissues of normal and hyposmotic rats. Am J Physiol Renal Physiol 2003;285:F688-F693.

Arroyo JA, Garcia-Jones P, Graham A, Teng CC, Battaglia FC, Galan HL: Placental TonEBP/NFAT5 Osmolyte Regulation in an Ovine Model of Intrauterine Growth Restriction. Biol Reprod 2012;86:94.

Matskevitch J, Wagner CA, Risler T, Kwon HM, Handler JS, Waldegger S, Busch AE, Lang F: Effect of extracellular pH on the myo-inositol transporter SMIT expressed in Xenopus oocytes. Pflugers Arch 1998;436:854-857.

Mohamed MR, Alesutan I, Foller M, Sopjani M, Bress A, Mair M, Salama RH, Bakr MS, Mohamed MA, Blin N, Lang F, Pfister M: Functional analysis of a novel I71N mutation in the GJB2 gene among Southern Egyptians causing autosomal recessive hearing loss. Cell Physiol Biochem 2010;26:959-966.

Eckey K, Strutz-Seeborn N, Katz G, Fuhrmann G, Henrion U, Pott L, Linke WA, Arad M, Lang F, Seebohm G: Modulation of human ephB4 a gogo related channels by CASQ2 contributes to etiology of catecholaminergic polymorphic ventricular tachycardia (CPVT). Cell Physiol Biochem 2010;26:503-512.

Bohmer C, Sopjani M, Klaus E, Lindner R, Laufer J, Jeyaraj S, Lang F, Palmada M: The serum and glucocorticoid inducible kinases SGK1-3 stimulate the neutral amino acid transporter SLC6A19. Cell Physiol Biochem 2010;25:723-732.

Dermaku-Sopjani M, Sopjani M, Saxena A, Shojaiefard M, Bogatikov E, Alesutan I, Eichenmuller M, Lang F: Downregulation of NaPi-IIa and NaPi-IIb Na-coupled phosphate transporters by coexpression of Klotho. Cell Physiol Biochem 2011;28:251-258.

Hosseinzadeh Z, Bhavsar SK, Alesutan I, Saxena A, Dermaku-Sopjani M, Lang F: Regulation of the glutamate transporters by Jak2. Biochem Biophys Res Commun 2011;408:208-213.

Hosseinzadeh Z, Bhavsar SK, Shojaiefard M, Saxena A, Merches K, Sopjani M, Alesutan I, Lang F: Stimulation of the glucose carrier SGLT1 by AK2. Biochem Biophys Res Commun 2011;408:208-213.

Bhavsar SK, Hosseinzadeh Z, Merches K, Gu S, Broer S, Lang F: Stimulation of the amino acid transporter SLC6A19 by Jak2. Biochem Biophys Res Commun 2011;414:456-461.

Handler JS, Kwon HM: Regulation of the myo-inositol and betaine cotransporters by tonicity. Kidney Int 1996;49:1682-1683.

Hofmann EK, Pedersen SF: Sensors and signal transduction pathways in vertebrate cell volume regulation. Contrib Nephrol 2006;152:54-104.

Pasantes-Morales H, Cruz-Rangel S: Brain volume regulation: osmolytes and aquaporin perspectives. Neuroscience 2010;168:871-884.

Coaxum SD, Garnovskaya MN, Gooz M, Baldys A, Raymond JR: Epidermal growth factor activates Na+/H+ exchanger in podocytes through a mechanism that involves Janus kinase and calmodulin. Biochem Biophys Acta 2009;1793:1174-1181.

Lang F, Busch GL, Ritter M, Volk H, Waldegger S, Gubins E, Haussinger D: Functional significance of cell volume regulatory mechanisms. Physiol Rev 1998;78:247-306.