Oxidant Effects on Epithelial Na,K-ATPase Gene Expression and Promoter Function

Christine H. Wendt,1 Renuka Sharma,1 Robert Bair,1 Howard Towle,2 and David H. Ingbar1

1Departments of Medicine and 2Biochemistry, University of Minnesota School of Medicine, Minneapolis, Minnesota

The lung epithelium resorbs alveolar fluid through combined action of sodium channels and the sodium pump, Na,K-ATPase. The lung often is exposed to hyperoxia in disease states and hyperoxia generates a mixture of reactive oxygen species. In vivo and in vitro exposure of rat lung and alveolar type II cells, respectively, increases expression of both the α-1 and β-1 subunits of the sodium pump. In contrast to the primary type II cells, several type II cell lines did not increase sodium pump gene expression with hyperoxia, but the renal tubular epithelial MDCK cell line did. Using promoter-reporter constructs transfected into MDCK cells, hyperoxia did not markedly increase transcription of the α-1 subunit but doubled transcription of the β-1 subunit gene. Using 5'-deletion constructs, the region required for the β-1 increase was localized to a 40-base pair region from -460 to -64. The hyperoxic responsiveness of this region was confirmed using constructs with one or two copies of this region placed in minimal promoter-luciferase reporters. This 5' promoter region contains a consensus binding sequence for SP-1, a basal transcription factor but not for binding of other known transcription factors. Thus, hyperoxia induces Na,K-ATPase β-1 promoter transcription, likely acting through a novel mechanism.- Environ Health Perspect 106(Suppl 5):1213-1217 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-5/1213-1217wendt/abstract.html

Key words: sodium pump, lung injury, alveolar epithelium, ion transport, oxidants, MDCK cells

---

Introduction

The sodium pump, Na,K-ATPase, moves sodium out of cells and potassium into cells against concentration gradients, using a significant fraction of cellular ATP content (1,2). The maintained sodium gradient promotes entry of glucose, amino acids, and other vital molecules into cells. Thus Na,K-ATPase is essential for the survival of all cells. It also is important in electrical activity and other cell functions. Although the enzyme is ubiquitous, the levels of Na,K-ATPase are much higher in cells specialized for vectorial transport of solutes and/or fluid, such as epithelial cells.

Structurally, the sodium pump is an integral membrane protein composed of α and β subunits in a heterotetramer. The α subunit passes through the plasma membrane 7 to 9 times and contains the catalytic site, ATP binding site, and ouabain binding site. The β subunit is smaller, has a single transmembrane domain, and is glycosylated. There are three isoforms of each subunit, with α-1 and β-1 predominating in most epithelia (3,4). The αβ stoichiometry is 1:1 in the active enzyme, but different cell types have varied ratios of alpha and beta subunit mRNA and protein. The heterotetramer is assembled in the endoplasmic reticulum and then is transported to the plasma membrane where it becomes active (5).

The processing and regulation of Na,K-ATPase have been recently reviewed (1-3). Many organs are exposed to oxidant stress, particularly the lung and the kidney. The lung is exposed to oxidative stress in a variety of physiologic and pathologic conditions and with high levels of supplemental oxygen. In disease, this occurs in oxidant gas inhalation, lung inflammation, fibrotic lung diseases, and acute lung injury. Oxidants can lead to endothelial and epithelial injury, increasing permeability and resulting in alveolar edema. How does the lung compensate for this injury and maintain effective gas exchange? One mechanism is to augment the molecular systems that resorb alveolar solute and fluid. In type II alveolar epithelial cells, sodium and fluid resorption is accomplished primarily through combined action of the apical amiloride-sensitive sodium channel and the basolateral Na,K-ATPase (6,7). The kidney is another organ specialized for epithelial transport that commonly undergoes oxidant stress during ischemia–reperfusion injury (8). What are the effects of oxidant stress on these transport proteins?

Hyperoxia is a model for oxidant injury, since it generates a complex mixture of reactive oxygen species and mimics the pathologic changes seen in acute lung injury. The impact of hyperoxia has been studied in the lung much more than in the kidney. With acute hyperoxia in the lung (>95%), permeability increases and edema occurs prior to type I alveolar epithelial cell death, inflammation, and type II cell proliferation. The severity of injury is a function of the antioxidant defenses and is partially determined by age and the length of hyperoxic exposure. If hyperoxia continues, animals die of seizures and lung injury, but if they are returned to room air, repair restores normal architecture. In contrast, chronic, subacute hyperoxia (85% oxygen for 7–10 days) is not lethal and causes type II cell proliferation. In the chronic hyperoxic model, alveolar fluid resorption usually increases with increased sodium channel and sodium pump mRNA, protein, and activity in the type II cells (9,10). In acute hyperoxia, the results are more complex, with the impact varying depending on the severity of injury and with heterogeneous responses in different animals of the same strain. Mild injury has little impact on fluid resorption (11,12). Moderate injury causes heterogeneous responses, with decreases of active alveolar

---

This paper is based on a presentation at the Second International Meeting on Oxygen/Nitrogen Radicals and Cellular Injury held 7–10 September 1997 in Durham, North Carolina. Manuscript received at EHP 23 March 1998; accepted 10 July 1998.

The authors thank G. Gick, SUNY Brooklyn, New York, NY; K. Kawakami, Jichi Medical School, Minamikawachi-machi, Japan; and E. Benz, The Johns Hopkins University, Baltimore, MD, for their generous gifts of sodium pump constructs and critical advice. These studies were partially supported by National Institutes of Health grants SCOR P50 HL50152 (DI) and K08 HL03114 (CW); an American Lung Association Career Investigator Award (DI); and American Heart Association Research Grants-in-Aid (DI and CW).

Address correspondence to D.H. Ingbar, Pulmonary, Allergy and Critical Care Medicine, University of Minnesota, Box 278, 420 Delaware St. S.E., Minneapolis, MN 55455. Telephone: (612) 624-8423. Fax: (612) 624-2174. E-mail: ingbar001@maroon.tc.umn.edu

Abbreviations used: ARE, antioxidant response element; ATCC, American Tissue Culture Collection; NF-κB, nuclear factor kappa B; MDCK, Madin Darby canine kidney.
sodium resorption in mild to moderate injury but normal to increased resorption in the moderately severe injury (13). Severe injury decreases sodium and fluid resorption along with Na,K-ATPase activity (14). The biochemical effects also are complex, with alveolar type II cell Na,K-ATPase mRNA, protein, and activity changing in a nonparallel fashion (15). This complexity has been reviewed in detail and will not be considered further here (1,16). Consistent across all these models is the increase in sodium pump mRNA levels. The goal of this study was to assess the mechanisms of the hypoxic increase in gene expression in lung and renal epithelial cells (17).

Experimental Studies
First we sought to determine whether the increase in steady-state mRNA levels in alveolar type II (ATII) cells was due to decreased degradation, as occurs for catalase with hyperoxia, or to increased gene transcription. Primary cultures of rat alveolar type II cells were isolated using elastase digestion and panning in standard fashion. After overnight recovery in 5% CO₂/95% air in Dulbecco's modified Eagle's medium with 10% fetal bovine serum, cells exposed to hyperoxia were transferred to a Billups plexiglass container. The container was filled with 95% O₂ and 5% CO₂ gas mixture at 1 atmosphere pressure, and the humidified container was sealed (18). All studies of hypoxic cells were performed at 24 hr and prior to significant cell death. Nuclei were isolated from normoxic and hypoxic alveolar type II cells and nuclear run-on transcription assays were performed using standard techniques. Transcription of both the α-1 and β-1 Na,K-ATPase subunits increased with hyperoxia approximately 2.5- and 5-fold, respectively (Table 1).

Analyzing transcriptional regulation in primary cultures of alveolar type II cells is very difficult because the primary ATII cells are isolated in relatively small numbers, do not proliferate, and also undergo phenotypic change to resemble type I cells in culture. Therefore an epithelial cell line that also increased steady-state Na,K-ATPase mRNA in response to hyperoxia was sought. Three type II cell lines were examined: A549 human adenocarcinoma cells from the American Tissue Culture Collection (ATCC); L2 cells from ATCC; and MP48 transformed rat type II cells (gift of G. Hunninghake, University of Iowa), but for each of these the Na,K-ATPase mRNA levels were stable with hyperoxia, unlike the primary alveolar type II cell cultures. A proximal renal tubular epithelial cell line, MDCK cells, increased steady-state levels of Na,K-ATPase α-1 and β-1 mRNA approximately 3- and 5-fold after 24 hr of hyperoxia (Figure 1). Like the primary alveolar type II cells, these cells form tight junctions and vectorially transport sodium in culture with basolateral Na,K-ATPase. We speculate that the MDCK and primary alveolar type II cells may have similar Na,K-ATPase responses to hyperoxia because they are capable of solute transport unlike the alveolar type II cell lines mentioned above, which do not form tight junctions.

The next goal was to investigate further the mechanism of the increased Na,K-ATPase mRNA in the MDCK cells to determine whether hyperoxia increased the mRNA half-lives, transcription or rates; the former occurs with catalase mRNA. The mRNA half-life in MDCK cells was measured using actinomycin D to prevent new RNA synthesis; the remaining Na,K-ATPase mRNA was quantitated using Northern blot analysis and densitometry (19). The results for the α-1 and β-1 subunit mRNAs are shown in Figure 2A, B. Although hyperoxia increased the initial steady-state level of mRNA, for this analysis the time of actinomycin D addition (t=0) was considered 100% for each group. Other investigators have observed an initial hyperoxic increase in gene expression in lung and renal epithelial cells (17).

Table 1. Effect of hyperoxia on nuclear run on transcription of Na,K-ATPase α-1 and β-1 genes in alveolar type II and MDCK cells.

| Subunit | Alveolar type II cells* | MDCK cells** |
|---------|-------------------------|--------------|
| α-1     | 2.5                     | 34±1.2       |
| β-1     | 4.8                     | 52±1.1       |

Values shown are mean±SEM for the ratio of hyperoxic/normoxic transcription rates after normalization for actin transcription rate under each condition and are derived from a minimum of 2* or 3** experiments.

Figure 1. Effects of hyperoxia on MDCK cell steady-state Na,K-ATPase mRNA levels. The effects of 24 and 48 hr of hyperoxia on the relative steady-state levels of Na,K-ATPase (A) α-1 and (B) β-1 mRNA are demonstrated. For both subunits there were statistically significant (*p<0.05; **p<0.02) increases at 24 hr and the increase persisted at 48 hr for the β-1 subunit. n=4 separate experiments. Data adapted from Wendt et al. (17).

Figure 2. Half-lives of Na,K-ATPase mRNA in normoxic and hyperoxic MDCK cells. The effects of normoxia or 24 hr of hyperoxia on the subsequent survival of sodium pump (A) α-1 and (B) β-1 mRNA. The densitometric values were analyzed as a log-linear plot with least-squares fit for each of 3 separate experiments and the half-lives for normoxia and hyperoxia were determined. There was no significant change with hyperoxia for either subunit. Data adapted from Wendt et al. (17).
superinduction of Na,K-ATPase mRNA in actinomycin D studies, and we observed an initial stable level followed by a decrease. Because the curves did not fit classic first-order decay, half-lives were calculated with and without inclusion of this stabilization period. In each subunit, hyperoxia did not significantly affect the mRNA stability, independent of the method used for mRNA half-life calculation. Nor-moxic and hyperoxic half-lives were approximately 6 hr for both subunits. Not unexpectedly, there was some increased variation experiment to experiment at longer times of actinomycin D exposure such as 8 hr. As in the primary alveolar type II cells, nuclear run-on transcription experiments with normoxic and hyperoxic MDCK cells demonstrated increased transcription of both subunits with hyperoxia (Table 1). Thus, the MDCK cell increase in Na,K-ATPase mRNA was primarily transcriptional, as also occurred in alveolar type II cells.

Having demonstrated transcription increases using the global nuclear run-on assay, the next goal was to determine specific components needed for transcriptional upregulation of Na,K-ATPase by hyperoxia. To assess this, Na,K-ATPase promoters linked to luciferase reporter constructs were generously provided by Kawakami and Gick (20,21). These promoter–reporter constructs were transfected into 50% confluent MDCK cells by lipofection (GIBCO-BRL) and after 48 hr of either normoxia or hyperoxia, the reporter gene activity was assayed by chemiluminescence of luciferase. Controls for the efficiency of lipofection included use of a constitutive reporter expression construct for cotransfection (pEFBOS-Cat) (20) in some experiments. Reporter activity also was normalized to milligrams of protein. There was no difference in the normalizations to pEFBOS-Cat or protein in five separate experiments using multiple 5'-deletion constructs in each experiment; hence, some experiments were normalized only to the milligrams of protein. The amount of cellular protein also served as a control for the number of cells present, as cell number was affected by hyperoxia. For the α1 subunit of Na,K-ATPase, the full-length promoter and eight 5'-deletion constructs (20) were studied in normoxia and hyperoxia; no significant induction by hyperoxia was observed for any of the specific constructs (data not shown). In contrast, for the β1 Na,K-ATPase, there was a 1.9-fold reproducible induction of the full-length (817 nucleotides of 5' promoter upstream from the transcription initiation site) β1-817 construct. This construct contained 100 nucleotides of the first intron linked to luciferase, and the induction by hyperoxia was present with normalization to either pEFBOS-Cat or milligrams of protein. Although the magnitude of the increase was less than the steady-state mRNA increase we observed, this effect was consistent and of a magnitude similar to those changes in Na,K-ATPase gene expression and transcription that occur with ischemic and hormonal stimuli (22-24).

Several transcription factors are sensitive to reduction–oxidation and can activate gene expression when oxidized, such as nuclear factor kappa B (NF-κB), the Fos/Jun/AP-1 system, and the antioxidant response element that is activated by hydrogen peroxide or cyclic aromatic hydrocarbons. Two 5'-deletion constructs of the sodium pump β-1 promoter (β1-102 and β1-41) were kindly provided by G. Gick. Both these constructs lack the consensus sequences for known reduction–oxidation–sensitive transcription factors (Figure 3). When these two constructs were analyzed for hyperoxic induction, the β1-102 construct had less basal normoxic transcription than the full-length β1-817 construct and had an equal degree of inducibility by hyperoxia (1.9-fold) (Table 2). In contrast, the β1-41 construct was reduced and not induced by hyperoxia. A concern in these experiments was the very low basal transcription with the β1-41 promoter, raising the possibility that the lack of inducibility was an artifact of the severe truncation of the promoter, rather than due to specific elements between nucleotides -41 and -102 that were missing. To assess this possibility, the -44/-82 region was cloned and placed in front of a minimal mouse mammary tumor virus promoter linked to luciferase. When this construct was transfected into MDCK cells, hyperoxia led to a 2-fold increase in transcription. When a dimer of the region from -44 to -82 was inserted in front of this minimal promoter, hyperoxia induced transcription 5-fold. This confirmed that the -44/-82 region is both necessary and sufficient for 2-fold hyperoxic induction of the β-1 subunit transcription in MDCK cells.

At present, the transcription factor(s) involved in the hyperoxic induction in this region remains uncertain. The basal transcription factor SP1 has two consensus sequences for binding in this region and binds there by electrophoretic mobility shift assay (Figure 4). A homologous transcription factor, SP-3, binds to these sequences also. However, it is not clear that these transcription factors are affected by oxidation and whether they are involved in the hyperoxic induction. Electrophoretic mobility shift assays, site-specific mutagenesis, and DNase I hypersensitivity

---

**Table 2. Effect of hyperoxia on Na,K-ATPase β-1 subunit promoter activity in MDCK cells.**

| Construct | Normoxia | Hyperoxia | Hyperoxia/normoxia |
|-----------|----------|-----------|-------------------|
| β1-102    | 100      | 190 ± 21.2| 1.9 ± 0.2*        |
| β1-41     | 2.7 ± 1.9| 14 ± 0.7  | 0.5 ± 0.1         |

*p<0.01 versus β1-41; **p<0.02 versus β1-41.

---

**Figure 3. Diagram of the Na,K-ATPase rat β-1 gene promoter with marking of the consensus sequences for binding of reduction–oxidation-sensitive transcription factors.** The full-length β1-817 nucleotide promoter sequence is shown, including 1 consensus sequence for binding of NF-κB and 2 antioxidant response element (ARE) sequences. The arrows indicate the lengths of the two 5'-deletion promoter–reporter constructs used (provided by G. Gick). Data adapted from Wendt et al. (17).

**Figure 4. Nucleotide sequence of the -82/-44 5' sequence of the rat β-1 gene promoter with indication of partial SP-1 consensus binding sites.** The canonical consensus sequence for SP-1 binding is GCCGGG and the two putative binding sites with partial consensus in this region are underlined.
studies in vivo and in vitro are being used to analyze further the critical components of hyperoxic induction.

Discussion and Summary

These studies with MDCK cells indicate that hyperoxia causes at least a 2-fold induction in transcription of Na,K-ATPase and localizes the portion of the β-1 subunit promoter required for this response. Although the 2-fold degree of induction in the promoter–reporter experiments is not impressive compared with the 10- to 100-fold induction of other genes by stress such as heat shock proteins or heme oxygenase, the sodium pump is a critical housekeeping gene that is expressed at high basal levels in these cells. In addition, other physiologic stresses, such as decreased intracellular potassium or high levels of mineralocorticoids or glucocorticoids, lead to similar degrees of sodium pump transcriptional activation. Thus the 2-fold increase in gene transcription and the increased steady-state levels of mRNA with hyperoxia likely are of physiologic significance in maintaining the Na,K-ATPase activity in the face of oxidative stress.

The lack of consensus sequence for known reduction–oxidation-sensitive transcription factors in the required portion of the β-1 promoter suggests that a novel transcription factor may be involved or that there is a complex interaction of multiple transcription factors. Sorting out this challenging question likely will require multiple simultaneous approaches. At present, relatively little has been defined about transcriptional regulation of the β-1 subunit. In contrast, many cis regulatory elements for the α-1 subunit have been defined (25–29).

The physiologic significance of Na,K-ATPase upregulation in renal tubular epithelial cells by hyperoxia is not proven at present. However, it is likely an important homeostatic response to oxidative stress in ischemia–reperfusion injury. Once the critical elements in the MDCK cell transcriptional response are further defined, it will be possible to return to alveolar type II cells to determine whether their mechanism of hyperoxic induction is similar or different. It also may be interesting to assess why the ATII cell lines do not exhibit hyperoxic induction. Is a critical transcription factor missing, are repressor elements active, or are the antioxidant defenses of the cells different? The underlying premise of this investigation is that there are homeostatic defenses that maintain sodium pump activity during oxidant stress in the kidney with ischemia–reperfusion injury and in the lung with incipient pulmonary edema. The importance of the active resorption of alveolar fluid is supported by studies in humans with pulmonary edema (30) and animal models in the developing and adult lung (31).

Finally, it is likely that other factors not yet defined also contribute to the increased steady-state mRNA levels, especially since the promoter-reporter responses for α-1 and β-1 do not match the degree of increase in steady-state levels. Thus there may be distal 5' enhancer elements that were not included in the promoter-reporter constructs, as occurs with heme oxygenase. Alternatively, intronic or 3' sequence can regulate transcription in some genes.

In summary, Na,K-ATPase induction during hyperoxia is observed in transporting epithelia of the alveolus and renal tubule. In both it seems to be at least partially through increased gene transcription. In MDCK cells, there is a 40-nucleotide region of the promoter that is necessary and sufficient for the 2-fold hyperoxic induction of transcription. However, the transcription factors involved in stimulating this response are unknown. The transcription factors may be novel or the hyperoxia response may involve phosphorylation of a bound factor or altered assembly of a complex of known transcription factors. Understanding oxidant regulation of gene expression is of great importance for many genes and homeostatic mechanisms in the lung, kidney, and other organs.

REFERENCES AND NOTES

1. Horisberger J-D. The Na,K-ATPase: Structure-Function Relationship. Austin, TX:R.G. Landes, 1994.
2. Ingbah DH, Wendt CH, Crandall EC. Na,K-ATPase and the clearance of pulmonary edema fluid. In: Pulmonary Edema (Matthay MA, Ingbah DH, eds). New York: Marcel Dekker, 1998:477–499.
3. Lingel JB, Orlovski J, Shull MM, Price EM. Molecular genetics of Na,K-ATPase. Progr Nucleic Acid Res 38:37–89 (1990).
4. Jewell EA, Shamraj OI, Lingel JB. Isomers of the subunit of Na,K-ATPase and their significance. Acta Physiol Scand 146:161–169 (1992).
5. Geering K. Subunit assembly and posttranslational processing of Na-pumps. Acta Physiol Scand 146:177–181 (1992).
6. Matthay MA, Folkesson HG, Verkman AS. Salt and water transport across alveolar and distal airway epithelia in the adult lung. Am J Physiol 270:L487–503 (1996).
7. Matalon S. Mechanisms and regulation of ion transport in adult mammalian alveolar type II pneumocytes. Am J Physiol 261:C727–738 (1991).
8. Winko-Sarsar V, Friedlander M, Capeiffe-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, Jungers P, Descamps-Latsha B. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 49:1304–1313 (1996).
9. Olivera WG, Ridge KM, Wood LDH, Snajder JI. Active sodium transport and alveolar epithelial Na,K-ATPase increase during subacute hyperoxia in rats. Am J Physiol 266:L577–584, 1994.
10. Yue G, Russell WJ, Benos DJ, Jackson RM, Olman MA, Matalon S. Increased expression and activity of sodium channels in alveolar type II cells of hyperoxic rats. Proc Natl Acad Sci USA 92:8418–8422 (1995).
11. Garat C, Meignan M, Matthay MA, Luo DF, Jay C. Alveolar epithelial fluid clearance mechanisms are intact after moderate hyperoxic lung injury in rats. Chest 111:1381–1388 (1997).
12. Nici I, Dowin R, Jamieson JD, Ingbah DH. Upregulation of rat type II pneumocyte Na,K-ATPase during hyperoxic lung injury. Am J Physiol 261:L307–314 (1991).
13. Carter EP, Wangensteen OD, O’Grady SM, Ingbah DH. Effects of hyperoxia on type II cell Na,K-ATPase function and expression. Am J Physiol (Lung) 272:L542–L551 (1997).
14. Olivera WG, Ridge KM, Snajder JI. Lung liquid clearance and Na,K-ATPase during acute hyperoxia and recovery in rats. Am J Respir Crit Care Med 152:1229–1234 (1995).
15. Carter EP, Ingbah DH, Dunitz JM, Wangensteen OD. Hyperoxic effects on alveolar sodium resorption and lung Na,K-ATPase. Am J Physiol (Lung) 273:L1191–L1202 (1997).
16. Ingbah DH, Wendt CH. The sodium pump and oxidant stress: if only it were so simple. J Lab Clin Med 130:119–122 (1997).
17. Wendt CH, Towe H, Sharma R, Duvick S, Kawakami K, Gick G, Ingbah DH. Regulation of Na,K-ATPase gene expression in hyperxia. Am J Physiol (Cell) 274:C356–C364 (1998).
18. Carter EP, Duvick SE, Wendt CH, Dunitz J, Nici L, Wangensteen OD, Ingbah DH. Hyperoxia increases active alveolar Na resorption in vivo and type II cell Na,K-ATPase in vitro. Chest 105:755–785 (1994).
HYPEROXIC INDUCTION OF SODIUM PUMP GENE TRANSCRIPTION

19. Chambers SK, Gilmore-Hebert M, Kacinski BM, Benz EJ. Changes in Na,K-ATPase gene expression during granulocytic differentiation of HL60 cells. Blood 80:1559–1564 (1992).

20. Yagawa Y, Kawakami K, Nagano K. Cloning and analysis of the 5' flanking region of rat Na+/K+-ATPase α1 subunit gene. Biochim Biophys Acta 1049:286–292 (1990).

21. Liu B, Gick G. Characterization of the 5'-flanking region of the rat Na+K-ATPase α1 subunit gene. Biochim Biophys Acta 1130:336–338 (1992).

22. McDonough AA, Tang M-J, Lescale-Matys L. Ionic regulation of the biosynthesis of Na,K-ATPase subunits. Semin Nephrol 10:400–409 (1990).

23. McDonough AA, TABrown, B Horowitz, Chin R, Schlotterbeck J, Bowen J, Schmitt CA. Thyroid hormone coordinates regulate Na-K-ATPase α1 and β subunit mRNA levels in kidney. Am J Physiol 254:C323–329 (1988).

24. Ewart HS, Klip A. Hormonal regulation of the Na-K-ATPase: mechanisms underlying rapid and sustained changes in pump activity. Am J Physiol 269:C295–311 (1995).

25. Kawakami K, Yanagisawa K, Watanabe Y, Tominaga S, Nagano K. Different factors bind to the regulatory region of the Na,K-ATPase α1-subunit gene during the cell cycle. FEBS Lett 335:251–254 (1993).

26. Kawakami K, Masuda K, Nagano K, Ohkuma Y, Roeder RG. Characterization of the core promoter of the Na/K-ATPase α1 subunit gene: elements required for transcription by RNA polymerase II and III in vitro. Eur J Biochem 237:440–446 (1996).

27. Kobayashi M, Kawakami K. ATF-1CREB heterodimer is involved in constitutive expression of the housekeeping Na,K-ATPase α1 subunit gene. Nucl Acids Res 23:2848–2855 (1995).

28. Nomoto M, Gonzales FJ, Mita T, Inoue N, Kawamura M. Analysis of cis-acting regions upstream of the rat Na/K-ATPase α1 subunit gene by in vitro footprinting. Biochim Biophys Acta 1264:35–39 (1995).

29. Yagawa YS, Kawakami K, Nagano K. Housekeeping Na,K-ATPase 1 subunit gene promoter is composed of multiple cis elements to which common and cell type-specific factors bind. Mol Cell Biol 12:4046–4055 (1992).

30. Matthay MA, Wiener-Kronish JP. Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. Am Rev Respir Dis 142:1250–1257 (1990).

31. O’Brodovich HM. The role of active Na transport by lung epithelium in the clearance of airspace fluid. N Horiz 3:240–247 (1995).