[Frontiers in Bioscience 10, 2018-2026, September 1, 2005]

REGIONS CONFERRING ISOFORM-SPECIFIC FUNCTION IN THE CATALYTIC SUBUNIT OF THE NA,K-PUMP

Thomas A. Pressley [1], Marie-Josee Duran [1], and Sandrine V. Pierre [2]

[1] Department of Physiology, Texas Tech University Health Sciences Center, Lubbock, TX 79430 and [2] Department of Pharmacology and Therapeutics, Medical College of Ohio, Toledo, OH 43614

TABLE OF CONTENTS

1. ABSTRACT

The Na,K-pump (i.e., Na,K-ATPase) is critical for maintaining the ionic gradients across the plasma membranes of animal cells. Its component subunits are expressed in multiple forms, but the physiological relevance of this subunit diversity remains unknown. The primary contributor to overall catalysis, the alpha subunit, exists in four isoforms. There are observed kinetic differences among these isoforms, but their subtlety makes them an unlikely basis for physiological significance. Instead, recent work suggests that the major functional distinction among the isoforms is their interaction with regulatory proteins. Moreover, the isoform-specific effects of modulatory agents such as protein kinase C seem to originate within two regions of structural divergence: the amino terminus and eleven residues near the center of the alpha subunit, the isoform-specific region.

2. INTRODUCTION

A fundamental problem in modern biology is the unexpected heterogeneity found in most proteins. There are few enzymes that do not exist as two or more isozymes or structural proteins that do not exist in multiple forms. It is difficult to imagine that such diversity would be maintained across wide phylogenetic differences in the absence of a strong selective advantage, and indeed, in some cases, the potential physiological importance of multiple isozymes is obvious. For example, the differences in glucose affinity displayed by the hexokinase isozymes have profound effects on sugar entry into downstream catabolic pathways, creating a distinct tissue specificity in sugar metabolism (1). Unfortunately, such straightforward examples are in short supply, and the physiological advantage of diversity in most enzyme systems remains unclear. A case in point is the Na,K-pump, the plasma membrane-spanning protein complex that generates the electrochemical gradients for Na^+ and K^+ in animal cells.

3. HETEROGENEITY IN THE NA,K-PUMP

The pump extrudes Na^+ from the cell and absorbs K^+ at the expense of ATP hydrolysis. Indeed, Na^+,K^+-stimulated hydrolysis of ATP (Na,K-ATPase) is often used as a measure of pump function. This exchange of cations is central to ionic homeostasis in most cells, contributing to the electrochemical gradients across the plasma membrane. Nevertheless, it is not obvious why these actions cannot be accomplished by a single form of the pump, as originally

assumed by transport physiologists of the mid-twentieth century. Instead, we have an embarrassment of riches, with a growing list of alternative forms among the pump's constituent subunits (2).

A 110,000-dalton alpha subunit is the primary contributor to overall catalysis. It contains the binding sites for the substrates required by the enzyme and exists as a phosphorylated intermediate during the catalytic cycle (3). Heterogeneity within the alpha subunit was first detected in the late 1970's as differences in electrophoretic mobility (4), and this diversity has been confirmed repeatedly by molecular cloning. Four isoforms of the alpha subunit have been identified in mammals (5-7), and they are clearly the products of different genes. Their expression is tissue specific, with a nearly ubiquitous alpha1 isoform and three others, alpha2, alpha3, and alpha4, with increasingly restrictive expression patterns. The alpha2 isoform is expressed principally in nervous and adipose tissue, heart, and skeletal muscle. The alpha3 isoform is found primarily in nervous tissue, although it has been detected in a number of unexpected places, including the pineal gland, multinucleated macrophages, and corneal endothelium (8-10). All the results to date suggest that alpha4 expression is restricted to male reproductive tissues (11, 12). Although there may yet be additional catalytic isoforms, the sequencing efforts of the Human Genome Project have revealed no alpha-like isoforms beyond those already identified.

Molecular cloning has also identified heterogeneity within the 55,000-dalton beta subunit, a glycosylated protein that co-purifies with alpha and is required for normal pump function. In addition to the beta1 isoform originally isolated from kidney, beta2 and beta3 isoforms have been identified in various tissues (13, 14). From observations in heterologous expression systems, it is apparent that the different beta isoforms are capable of assembly with each alpha isoform (15, 16), suggesting that the number of potentially different pump complexes may be greater still. Association of alpha with a beta isoform is thought to play a role in stabilizing the pump complex in the membrane (17). Moreover, variation in the beta subunit can have subtle effects on the kinetic parameters of the enzyme (18). Indeed, it now seems clear that association with beta is just one example of protein-protein interactions that can influence pump function. Although beta also contributes to pump structure, others seem to play strictly a regulatory role, such as the FYXD family of proteins (19).

This impressive diversity of structure and function goes beyond the Na,K-pump to include other mediators of active transport. Indeed, it is clear that the P-type ATPases (so called because their catalytic cycle includes a phosphorylated intermediate) are a superfamily of alpha-like genes that include H,K-pumps, Ca-pumps, H-pumps, and various heavy metal pumps (20). As will be discussed, a high-resolution structural model proposed for the Ca-pump of sarcoplasmic reticulum has provided significant insight into the probable structure of the Na,K-pump.

4. ISOFORM-SPECIFIC DIFFERENCES

4.1. Function

Given that these isoforms of the Na,K-pump exist, what is the evidence for their physiological relevance? Their presence might be easily justified if they displayed dramatic kinetic differences, and numerous laboratories have spent the last few years analyzing their enzymatic properties. In rodents, the most obvious isoform-specific difference is a thousand-fold variation in affinity for cardiac glycosides between alpha1 and the three others (21, 22). As early as the mid-1970s, for example, it was noted that preparations of mouse brain displayed two apparent affinities for glycosides (23). Site-directed mutagenesis and heterologous expression have localized the structural basis of this difference to distinct regions within the isoforms, most notably the first extracellular domain (24, 25). Unfortunately, the difference in affinity for glycosides seen in rodents is far less dramatic in most mammalian species, including humans (26). Careful work in cultured cells and heterologous expression systems suggests that there are also small differences in the affinity for Na^+, K^+, and ATP. The alpha3 isoform, for instance, displays a three-fold higher affinity for extracellular K^+ and a four-fold lower affinity for intracellular Na^+ relative to alpha1 and alpha2 (27). A more striking difference among the isoforms involves the rate of K^+ deocclusion (28, 29). An ion is said to be occluded when it is bound to the Na,K-pump but not accessible from either side of the membrane. The release of occluded K^+ (i.e., deocclusion) is the rate-limiting step in the overall catalytic cycle. The alpha2 isoform displays a faster rate of release, suggesting a fundamental difference in the stability of the occluded, $E_2(K^+)$ conformation. However, an important caveat to this kinetic difference in deocclusion is the relatively nonphysiological condition needed for its detection. As interesting as these variations in kinetics may be to enzymologists, their subtlety would seem to argue against a large contribution to overall physiological function.

Nevertheless, that pump isoforms confer some selective physiological advantage is suggested by their widespread presence. The expression of multiple forms is conserved across a wide range of species, including both invertebrates and vertebrates. Since the late 1970's, for example, there has been evidence for heterogeneity in the alpha subunit of brine shrimp, an inference that has since been confirmed by molecular cloning (30, 31). By comparison with the four alpha isoforms of mammals, teleosts display a far more complex pattern of diversity, with at least eight versions of the catalytic subunit and five beta isoforms (32). Disruption of alpha isoform expression in mutant zebrafish and transgenic mice has confirmed that this diversity is necessary for normal growth and function (33-35). The variations in tissue expression discussed above provide additional evidence for the physiological importance of heterogeneity within the catalytic isoforms. Moreover, the alpha isoforms respond differently to ontogeny, differentiation, and changes in endocrine status (36-38). For example, the alpha2 isoform of muscle responds to insulin by translocation from an intracellular pool to the plasmalemma (39).

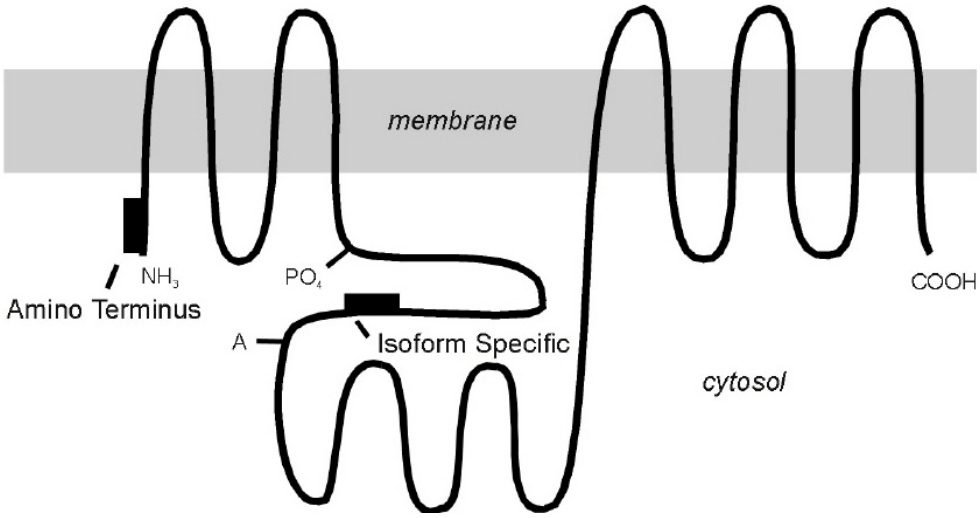

Figure 1. Structure of the alpha subunit showing the locations of isoform-specific regions (*dark boxes*). Although this topology has been somewhat controversial, the consensus in the field proposes ten transmembrane domains. The site of catalytic phosphorylation (PO_4) and the site of ATP binding (A) are depicted.

4.2. Regulation

This last observation may offer our first real clue to the physiological significance of pump isoforms, at least with respect to the alpha subunit. It may be that isoforms allow for selective regulation of the Na,K-pump, in some cases achieving that regulation in a specific manner by altering explicitly the distribution between cellular compartments. The arguments supporting this regulatory role for the isoforms are drawn from two key lines of evidence: ***1)*** Shifts between intracellular compartments and the plasmalemma are a major mechanism for regulation of the Na,K-pump by second messenger systems. Data accumulated by several laboratories indicate that kinase-mediated phosphorylation of the alpha1 isoform serves as a signal to regulate the cycling of pump complex to and from the plasma membrane. For instance, activation of protein kinase C (PKC) produces clear shifts in the distribution of the pump that are mediated by modulation of clathrin-dependent endocytosis (40-42). ***2)*** The alpha isoforms vary in their response to regulatory phosphorylation by protein kinases. Isoform-specific changes in activity have been observed in response to protein kinase A and G in baculovirus-infected insect cells (43). That such differential responses depend on shifts to and from the plasmalemma is suggested by the work of Teixeira et al. (44), who found that treatment of neostriatal neurons with dopamine decreases the amount of alpha2 in the membrane, without altering alpha1 abundance. It therefore seems likely that the differences seen in response to second messengers are achieved by the specific targeting of alpha isoforms for translocation between an intracellular compartment and the plasmalemma.

5. STRUCTURAL BASIS OF REGULATORY DIFFERENCES

The differences seen in regulation of the isoforms imply that signaling and translocation proteins are capable of distinguishing between them. It could almost go without saying that the structural basis for this recognition must reside within specific regions of diversity, yet the primary structures of the alpha isoforms are nearly identical. Nevertheless, there are nonconserved residues among the four isoforms. More importantly, these divergent amino acids are mainly clustered into discrete domains, rather than appearing randomly along the length of the molecule (45). It is most likely that the differences displayed by the isoforms originate within these regions of divergent structure. Two such regions have been identified as playing a critical role in regulation: the amino terminus and an eleven-residue sequence near the center of the molecule, the isoform-specific region (Figure 1).

5.1. The Amino Terminus

A major region of sequence divergence among the alpha isoforms is the amino terminus (Figure 2). One isoform-specific difference involves co- or post-translational processing. The first five amino acids of alpha1 and alpha2 predicted from the complementary DNAs are cleaved enzymatically during or after translation (the shaded residues in the figure). In contrast to the first two isoforms, there is no evidence that alpha3 or alpha4 undergo such proteolysis. The development of various amino terminal mutants for use in heterologous expression systems has allowed the identification of structures required for this post-translational processing (29). More importantly, however, these experiments have shown that isoform-specific processing is not required for function, and its physiological importance remains unclear. We must look elsewhere for some functional consequence of the heterogeneity found in this region.

Despite the divergence seen in the four alpha isoforms, their amino termini share a highly charged, lysine-rich region slightly downstream. One of the lysines within this highly charged region is a target for tryptic

Figure 2. Aligned amino acid sequences of alpha isoform amino termini. All were derived from corresponding complementary DNA. The first five residues of alpha1 and alpha2 (*shaded*) are removed co- or post-translationally. The target serines phosphorylated by protein kinase C are denoted with *horizontal lines*. A leucine-lysine pair (*LK*) on the right-hand side is conserved among all four isoforms.

digestion whose accessibility is much greater when the alpha subunit is in the E_1 conformation. This has prompted speculation that the amino terminus may function as an ion-selective gate (46) or somehow influence the interaction between cations and the enzyme (47). Arguing against a critical role for the amino terminus, however, are mutants containing deletions of the region that are fully capable of supporting active transport when expressed in heterologous systems (48-50). Nevertheless, the amino terminus clearly influences the rate of K^+ deocclusion, one of the few kinetic properties that differ dramatically among the isoforms. For example, removal of 37-40 residues from the amino terminus of alpha1 results in an increased rate of deocclusion similar to that of the alpha2 isoform (28, 29).

More relevant to potential regulation by second messengers are serines 11 and 18 of alpha1 (numbering derived from the mature protein of rat), which are targets for PKC-mediated phosphorylation and subsequent changes in plasma membrane abundance (40, 51). Despite their obvious effects on pump function, the specific roles of these residues remain problematic. The former is conserved among mammalian alpha1 isoforms, but it is only weakly phosphorylated. The latter is missing in many species. Neither residue is contained within a PKC consensus sequence, and neither residue is conserved in alpha2 or alpha3. Nevertheless, there are other serines and threonines in the amino termini of these isoforms that may serve as phosphorylation sites. The differences among species and isoforms suggest that additional regions of the alpha subunit must contribute to the regulatory response in an isoform-specific manner (as discussed below). Consistent with its role in kinase-mediated regulation, the amino terminus is thought to be exposed to the cytoplasm, as deduced from comparisons with the high-resolution structure proposed for the sarcoplasmic reticulum Ca-ATPase (Figure 3). This places it on the exterior of the A or "actuator" domain, which is thought to link the energy of ATP hydrolysis to the movement of cations. Such localization may allow the amino terminus to both influence the rate of deocclusion and remain accessible for phosphorylation by PKC isozymes.

5.2. The Isoform-Specific Region

A second region of sequence divergence among the alpha isoforms is found within the major cytoplasmic loop (Figure 4). It is an 11-amino acid sequence beginning with Lys-489 of mature rat alpha1 that protrudes into the cytoplasm from the N or "nucleotide-binding" domain, as deduced from comparisons with the structure of the Ca-ATPase (Figure 5). Its distinctive conservation among the alpha isoforms of different species suggests a critical isoform-specific function. The region from alpha3, for example, is conserved among many different phyla. Immunological detection of the region in multiple species using site-directed antibodies provided some of the first evidence that the isoform-specific region from alpha3 may be more ubiquitous than expected (52). Indeed, with the continued sequencing of genomes, it has become one of the hallmarks of alpha3 orthologs in non-mammalian species, and the identical sequence is found in nearly all the vertebrates that have been examined, including trout, *Xenopus*, and chicken (45, 53, 54). In contrast, the analogous region of alpha2 seems to be less constrained, with identical sequences appearing only in mammals. Least conserved is the region from alpha1, which varies a great deal even among closely-related species. It is not yet clear how conserved the region from alpha4 will prove to be.

Arguing that these differences in conservation point towards an important role for the region, what might that role be? Our recent work with the heterologous expression of chimeric molecules in which this region from rat has been exchanged among the isoforms clearly shows a specific influence on PKC-mediated changes in active transport (55). By analogy with the translocation of pump complex seen with dopamine, we have proposed that this region serves as a dynamic retention signal that controls internalization of the pump. The isoform-specific region in alpha1 contains a dileucine motif of the structure, $n(p)_{2\text{-}4}LL$, where n is a negatively charged residue and p is a polar residue (56). This motif may serve as a target for interaction with adaptor proteins such as AP-2, although this remains to be tested explicitly. On the other hand, Done et al. (57) have reported that changing the second leucine did not influence the PKC response, but work with other proteins has shown a more relaxed requirement for the second leucine (58). Notwithstanding these concerns, it may be that the variability within this site among the alpha isoforms contributes to their differential response to PKC and subsequent internalization.

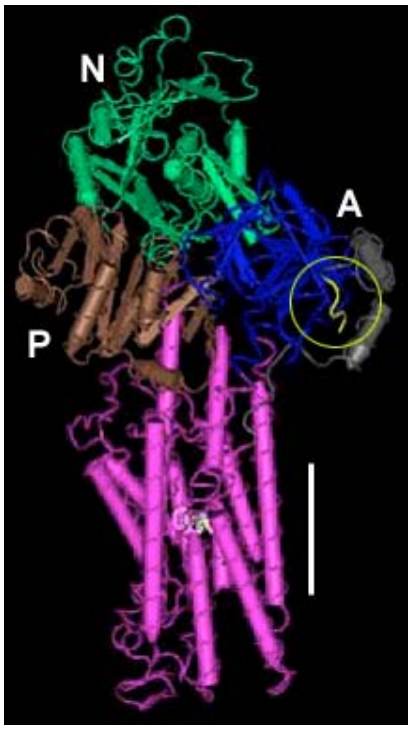

Figure 3. Likely position of the amino terminus in the structure of the alpha subunit. Proposed by comparison with the high-resolution model of the Ca-ATPase (70). The *yellow circle* highlights the amino terminus. *A*, *N*, and *P* denote the actuator, nucleotide-binding, and phosphorylation domains, respectively. A *white bar* indicates the membrane-spanning domains.

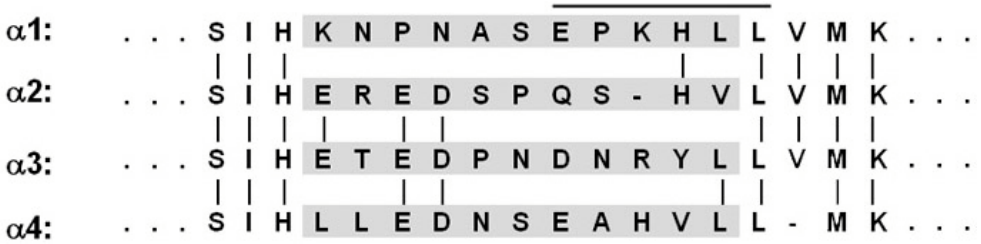

Figure 4. Aligned amino acid sequences of alpha isoform-specific region. All were derived from corresponding complementary DNA. The divergent sequences are *shaded*. A *thin line* denotes the di-leucine motif of alpha1.

6. PROTEIN-PROTEIN INTERACTIONS

The complexity of Na,K-pump regulation is increased further still by the heterogeneity seen within the proteins with which it interacts. For example, PKC exists in multiple forms with diverse expression patterns. These various forms fall into three groups: the conventional (i.e., Ca^{++}-dependent), novel, and atypical isozymes (59, 60). Recent data suggests that the consequences of alpha1 phosphorylation are dependent on the particular isozyme of PKC. For example, the increase in plasmalemma Na,K-ATPase abundance seen with phorbol esters in cultured kidney cells is mediated by one of the conventional beta isozymes of PKC, yet the dopamine-induced decrease is mediated by the atypical PKC-zeta (40, 61). It is perhaps not surprising that zeta seems to be relevant in the latter condition, since it does not respond to phorbol esters (62). With respect to rat alpha1, the specific response appears to be controlled, at least in part, by which serines are targeted. It is far from clear, however, which PKC isozymes are needed for regulation of the other alpha isoforms, yet it seems likely that a cell-specific response to PKC is achieved by interaction of a unique matrix of PKC isozymes with the alpha isoforms.

Heterogeneity is also the hallmark of another group of proteins that associate with the Na,K-ATPase, the members of the FXYD family, so called because they share a signature motif of four residues. These are small, single-span membrane proteins that have been shown to associate with a number of channels and transporters. Early work in purification and affinity-labeling of the Na,K-pump from mammalian kidney suggested an association with a small hydrophobic protein, the gamma subunit (63, 64). Its existence was confirmed by molecular cloning, and comparisons with other sequences quickly revealed the

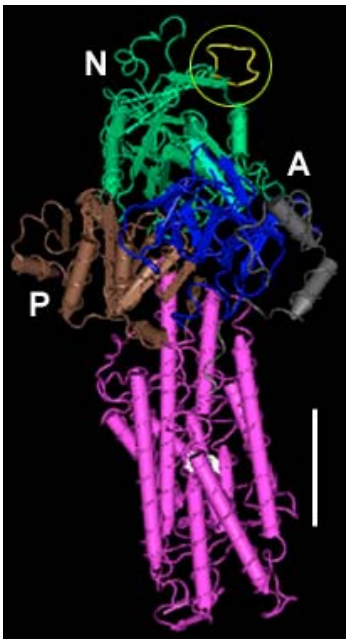

Figure 5. Likely position of the isoform specific region in the structure of the alpha subunit. Proposed by comparison with the high-resolution model of the Ca-ATPase (70). The *yellow circle* highlights the divergent region. The remaining notations are as in Figure 3.

existence of a family of proteins capable of modulating membrane transport (65). Work from a number of laboratories has shown that interactions of the various FXYD proteins and their splice variants with the pump can influence its kinetics, in particular the affinity for Na^+ (66-69) Although it is not yet clear if the various FXYD proteins have differential effects on the Na,K-pump isoforms, the many tissues that express multiple FXYD proteins suggest the presence of yet another mechanism for influencing Na,K-pump function.

7. CONCLUSIONS AND PERSPECTIVES

The majority of studies focusing on translocation of the pump in response to PKC has taken advantage of heterologous expression of exogenous subunits in cultured cells that express only one isoform of alpha. A limitation of this strategy, however, may be that the expression of an introduced isoform in the wrong context (i.e., cells that usually have only one form) may not reflect its regulation under more physiological conditions. Although we have learned much about the structural constraints on isoform-specific regulation from these more straightforward experiments, it remains to be seen whether the proposed mechanisms for modulating the pump are important in cells that express more than one isoform. Nervous tissue is probably the first that comes to mind, since neurons and glia seem to express a broad panel of alpha and beta isoforms. The combination of multiple alpha isoforms and variability among the kinase isozymes may provide a versatility that could not be achieved by the single Na,K-pump proposed by physiologists of the 1940's and '50's. Future studies exploring this question may take us full circle, with a return to the broader question of heterogeneity and its impact on cellular physiology and homeostasis.

8. ACKNOWLEDGMENTS

This work was supported by a grant from the National Center for Research Resources, RR-19799.

9. REFERENCES

1. Cornish-Bowden A. & M.L. Cardenas: Hexokinase and 'glucokinase' in liver metabolism. *Trends Biochem Sci* 16, 281-282 (1991)

2. Blanco G. & R.W. Mercer: Isozymes of the Na-K-ATPase: heterogeneity in structure, diversity in function. *Am J Physiol* 275, F633-F650 (1998)

3. Lingrel J.B. & T. Kuntzweiler: Na^+,K^+-ATPase. *J Biol Chem* 269, 19659-19662 (1994)

4. Sweadner K.J.: Two molecular forms of (Na^++K^+)-stimulated ATPase in brain. Separation, and difference in affinity for strophanthidin. *J Biol Chem* 254, 6060-6067 (1979)

5. Shull G.E., J. Greeb & J.B. Lingrel: Molecular cloning of three distinct forms of the Na^+,K^+-ATPase alpha-subunit from rat brain. *Biochemistry* 25, 8125-8132 (1986)

6. Herrera V.L.M., J.R. Emanuel, N. Ruiz-Opazo, R. Levenson & B. Nadal-Ginard: Three differentially expressed Na,K-ATPase alpha subunit isoforms: structural and functional implications. *J Cell Biol* 105, 1855-1865 (1987)

7. Shamraj O.I. & J.B. Lingrel: A putative fourth Na^+K^+-ATPase gene is expressed in testis. *Proc Natl Acad Sci USA* 91, 12952-12956 (1994)

8. Shyjan A.W., V. Cena, D.C. Klein & R. Levenson: Differential expression and enzymatic properties of the Na^+,K^+-ATPase alpha3 isoenzyme in rat pineal glands. *Proc Natl Acad Sci USA* 87, 1178-1182 (1990)

9. Vignery, A., F. Wang, H.-Y. Qian, E.J. Benz, Jr. & M. Gilmore-Hebert: Detection of the Na^+-K^+-ATPase $alpha_3$-isoform in multinucleated macrophages. *Am J Physiol* 260, F704-F709 (1991)

10. Huang B, G. Blanco, R.W. Mercer, T. Fleming & J.S. Pepose: Human corneal endothelial cell expression of Na^+,K^+-adenosine triphosphatase isoforms. *Arch Ophthalmol* 121, 840-845 (2003)

11. Blanco G., G. Sanchez, R.J. Melton, W.G. Tourtellotte & R.W. Mercer: The alpha4 isoform of the Na,K-ATPase is expressed in the germ cells of the testes. *J Histochem Cytochem* 48, 1023-1032 (2000)

12. Duran M.J., M. Chosseler & T.A. Pressley:. Regulation of Na,K-pump-mediated transport by prolactin in cultured human prostate epithelial cells. *Cell Mol Biol* 50, 809-814 (2004)

13. Martin-Vasallo P., W. Dackowski, J.R. Emanuel & R. Levenson: Identification of a putative isoform of the Na,K-ATPase beta subunit. Primary structure and tissue-specific expression. *J Biol Chem* 264, 4613-4618 (1989)

14. Malik N., V.A. Canfield, M.-C. Beckers, P. Gros & R. Levenson: Identification of the mammalian Na,K-ATPase beta3 subunit. *J Biol Chem* 271, 22754-22758 (1996)

15. Jaunin P., J.-D. Horisberger, K. Richter, P.J. Good, B.C. Rossier & K. Geering: Processing, intracellular transport, and functional expression of endogenous and exogenous alpha-beta3 Na,K-ATPase complexes in *Xenopus* oocytes. *J Biol Chem* 267, 577-585 (1992)

16. Blanco G., G. Sanchez & R.W. Mercer: Comparisons of the enzymatic properties of the Na,K-ATPase alpha3beta1 and alpha3beta2 isozymes. *Biochemistry* 34, 9897-9903 (1995)

17. Geering K., I. Theulaz, F. Verrey, M.T. Hauptle & B.C. Rossier: A role for the beta-subunit in the expression of functional Na^+-K^+-ATPase in *Xenopus* oocytes. *Am J Physiol* 257, C851-C858 (1989)

18. Eakle K.A., R.M. Lyu & R.A. Farley: The influence of beta subunit structure on the interaction of Na^+/K^+-ATPase complexes with Na^+. A chimeric beta subunit reduces the Na^+ dependence of phosphoenzyme formation from ATP. *J Biol Chem* 270, 13937-13947 (1995)

19. Cornelius F. & Y.A. Mahmmoud: Functional modulation of the sodium pump: the regulatory proteins "Fixit." *News Physiol Sci* 18, 119-124 (2003)

20. Lutsenko S. & J.H. Kaplan: Organization of P-type ATPases: significance of structural diversity. *Biochemistry* 34, 15607-15613 (1995)

21. Sweadner K.J.: Enzymatic properties of separated isozymes of the Na,K-ATPase. Substrate affinities, kinetic cooperativity, and ion transport stoichiometry. *J Biol Chem* 260, 11508-11513 (1985)

22. McGill D.L. & G. Guidotti: Insulin stimulates both the alpha1 and the alpha2 isoforms of the rat adipocyte (Na^+,K^+) ATPase: two mechanisms of stimulation. *J Biol Chem* 266, 15824-15831 (1991)

23. Marks M.J. & N.W Seeds:. A heterogeneous ouabain-ATPase interaction in mouse brain. *Life Sci* 23, 2735-2744 (1978)

24. Jewell E.A. & J.B. Lingrel: Comparison of the substrate dependence properties of the rat Na,K-ATPase alpha1, alpha2, and alpha3 isoforms expressed in HeLa cells. *J Biol Chem* 266, 16925-16930 (1991)

25. Crambert, G., D. Schaer, S. Roy & K. Geering: New molecular determinants controlling the accessibility of ouabain to its binding site in human Na,K-ATPase alpha isoforms. *Mol Pharmacol* 65, 335-341 (2004)

26. Wang J., J.B. Velotta, A.A. McDonough & R.A. Farley: All human Na^+-K^+-ATPase alpha-subunit isoforms have a similar affinity for cardiac glycosides. *Am J Physiol* 281, C1336-C1343 (2001)

27. Munzer J.S., S.E. Daly, E. A. Jewell-Motz, J.B. Lingrel & R. Blostein: Tissue- and isoform-specific kinetic behavior of the Na,K-ATPase. *J Biol Chem* 269, 16668-16676 (1994)

28. Daly S.E., L.K. Lane & R. Blostein: Structure/function analysis of the amino-terminal region of the alpha1 and alpha2 subunits of Na,K-ATPase. *J Biol Chem* 271, 23683-23689 (1996)

29. Petrosian S.A., D.L. Carr, G. Guerrero & T.A. Pressley: Mutagenesis disrupts posttranslational processing of the Na,K-ATPase catalytic subunit. *Arch Biochem Biophys* 357, 249-258 (1998)

30. Peterson G.L., R.D. Ewing, S.R. Hootman & F.P. Conte: Large scale partial purification and molecular and kinetic properties of the (Na + K)-activated adenosine triphosphatase from *Artemia salina* nauplii. *J Biol Chem* 253, 4762-4770 (1978)

31. Macias M.T., I. Palmero & L. Sastre: Cloning of a cDNA encoding an *Artemia franciscana* Na/K ATPase alpha-subunit. *Gene* 105, 197-204 (1991)

32. Rajarao S.J., V.A. Canfield, M.A. Mohideen, Y.L. Yan, J.H. Postlethwait, K.C. Cheng & R. Levenson: The repertoire of Na,K-ATPase alpha and beta subunit genes expressed in the zebrafish, *Danio rerio*. *Genome Res* 11, 1211-1220 (2001)

33. Shu X., K. Cheng, N. Patel, F. Chen, E. Joseph, H.-J. Tsai & J.-N. Chen: Na,K-ATPase is essential for embryonic heart development in the zebrafish. *Development* 130, 6165-6173 (2003)

34. James P.F., I.L. Grupp, G. Grupp, A.L. Woo, G.R. Askew, M.L. Croyle, R.A. Walsh & J.B. Lingrel: Identification of a specific role for the Na,K-ATPase alpha2 isoform as a regulator of calcium in the heart. *Mol Cell* 3, 555-563 (1999)

35. Moseley A.E., S.P. Lieske, R.K. Wetzel, P.F. James, S. He, D.A. Shelly, R.J. Paul, G.P. Boivin, D.P. Witte, J.M. Ramirez, K.J. Sweadner & J.B. Lingrel: The Na,K-ATPase alpha2 isoform is expressed in neurons, and its absence disrupts neuronal activity in newborn mice. *J Biol Chem* 278, 5317-5324 (2003)

36. Sweadner K.J. & S.K. Farshi: Rat cardiac ventricle has two Na^+, K^+-ATPases with different affinities for

ouabain: Developmental changes in immunologically different catalytic subunits. *Proc Natl Acad Sci USA* 84, 8404-8407 (1986)

37. Gick G.G., J. Melikian & F. Ismail-Beigi: Thyroidal enhancement of rat myocardial Na,K-ATPase: Preferential expression of alpha2 activity and mRNA abundance. *J Membr Biol* 115, 273-282 (1990)

38. Hensley C.B., K.K. Azuma, M.-J. Tang & A.A. McDonough: Thyroid hormone induction of rat myocardial Na^+-K^+-ATPase: $alpha_1$-, $alpha_2$-, and $beta_1$-mRNA and -protein levels at steady state. *Am J Physiol* 262, C484-C492 (1992)

39. Hundal H.S., A. Marette, Y. Mitsumoto, T. Ramlal, R. Blostein & A. Klip: Insulin induces translocation of the alpha2 and beta1 subunits of the Na^+/K^+-ATPase from intracellular compartments to the plasma membrane in mammalian skeletal muscle. *J Biol Chem* 267, 5040-5043 (1992)

40. Efendiev R., A.M. Bertorello, T.A. Pressley, M. Rousselot, E. Feraille & C.H. Pedemonte: Simultaneous phosphorylation of Ser11 and Ser18 in the alpha-subunit promotes the recruitment of Na^+,K^+-ATPase molecules to the plasma membrane. *Biochemistry* 39, 9884-9892 (2000)

41. Khundmiri S.J. & E. Lederer: PTH and DA regulate Na-K ATPase through divergent pathways. *Am J Physiol* 282, F512–F522 (2002)

42. Bertorello A.M., Y. Komarova, K. Smith, I.B. Leibiger, R. Efendiev, C.H. Pedemonte, G. Borisy & J.I. Sznajder: Analysis of Na^+,K^+-ATPase motion and incorporation into the plasma membrane in response to G protein-coupled receptor signals in living cells. *Mol Biol Cell* 14, 1149-1157 (2003)

43. Blanco G., G. Sanchez & R.W. Mercer: Differential regulation of Na,K-ATPase isozymes by protein kinases and arachidonic acid. *Arch Biochem Biophys* 15, 139-150 (1998)

44. Teixeira V.L., A.I. Katz, C.H. Pedemonte & A.M. Bertorello: Isoform-specific regulation of Na^+,K^+-ATPase endocytosis and recruitment to the plasma membrane. *Ann NY Acad Sci* 986, 587-594 (2003)

45. Takeyasu K., V. Lemas & D.M. Fambrough: Stability of Na^+-K^+-ATPase alpha-subunit isoforms in evolution. *Am J Physiol* 259, C619-C630 (1990)

46. Jorgensen P.L. & J.H. Collins: Tryptic and chymotryptic cleavage sites in sequence of alpha-subunit of (Na^+ + K^+)-ATPase from outer medulla of mammalian kidney. *Biochim Biophys Acta* 860, 570-576 (1986)

47. Wierzbicki W. & R. Blostein: The amino-terminal segment of the catalytic subunit of kidney Na,K-ATPase regulates the potassium deocclusion pathway of the reaction cycle. *Proc Natl Acad Sci USA* 90, 70-74 (1993)

48. Burgener-Kairuz, P., J.D. Horisberger, K. Geering & B.C. Rossier: Functional expression of N-terminal truncated alpha-subunits of Na,K-ATPase in *Xenopus laevis* oocytes. *FEBS Lett* 290, 83-86 (1991)

49. Vasilets, L.A., H.S. Omay, T. Ohta, S. Noguchi, M. Kawamura & W. Schwarz: Stimulation of the Na^+/K^+ pump by external [K^+] is regulated by voltage-dependent gating. *J Biol Chem* 266, 16285-16288 (1991)

50. Shanbaky N.M. & T.A. Pressley: Mammalian alpha1-subunit of Na^+-K^+-ATPase does not need its amino terminus to maintain cell viability. *Am J Physiol* 267, C590-C597 (1994)

51. Feschenko M.S., R.K. Wetzel & K.J. Sweadner: Phosphorylation of Na,K-ATPase by protein kinases. Sites, susceptibility, and consequences. *Ann NY Acad Sci* 834, 479-488 (1997)

52. Pressley T.A.: Phylogenetic conservation of isoform-specific regions within alpha-subunit of Na^+-K^+-ATPase. *Am J Physiol* 262, C743-C751 (1992)

53. Richards J.G., J.W. Semple, J.S. Bystriansky & P.M. Schulte: Na^+/K^+-ATPase alpha-isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. *J Exp Biol* 206, 4475-4486 (2003)

54. Klein S.L., R.L. Strausberg, L. Wagner, J. Pontius, S.W. Clifton & P. Richardson: Genetic and genomic tools for *Xenopus* research: The NIH *Xenopus* initiative. *Dev Dyn* 225, 384-391 (2002)

55. Pierre S.V., M.J. Duran, D.L. Carr & T.A. Pressley: Structure/function analysis of Na^+-K^+-ATPase central isoform-specific region: Involvement in PKC regulation. *Am J Physiol* 283, F1066 –F1074 (2002)

56. Kirchhausen T.: Adaptors for clathrin-mediated traffic. *Ann Rev Cell Dev Biol* 15, 705-732 (1999)

57. Done S.C., I.B. Leibiger, R. Efendiev, A.I. Katz, B. Leibeger, P.-O. Berggren, C.H. Pedemonte & A.M. Bertorello: Tyrosine 537 within the Na^+,K^+-ATPase alpha-subunit is essential for AP-2 binding and clathrin-dependent endocytosis. *J Biol Chem* 277, 17108-17111 (2002)

58. Dietrich J., J. Kastrup, B.L. Nielsen, N. Odum & C. Geisler: Regulation and function of the CD3gamma DxxxLL motif: a binding site for adaptor protein-1 and adaptor protein-2 in vitro. *J Cell Biol* 138, 271-281 (1997)

59. Newton A.C.: Protein kinase C: structure, function, and regulation. *J Biol Chem* 270, 28495-28498 (1995)

60. Battaini F.: Protein kinase C isoforms as therapeutic targets in nervous system disease states. *Pharmacol Res* 44, 353-361 (2001)

61. Efendiev R., A.M. Bertorello & C.H. Pedemonte: PKC-beta and PKC-zeta mediate opposing effects on proximal tubule Na^+,K^+-ATPase activity. *FEBS Lett* 456, 45-48 (1999)

62. Nishizuka Y.: Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J* 9, 484-496 (1995)

63. Forbush, B. III, J.H. Kaplan & J.F. Hoffman: Characterization of a new photoaffinity derivative of ouabain: labeling of the large polypeptide and of a proteolipid component of the Na, K-ATPase. *Biochemistry* 17, 3667-3676 (1978)

64. Collins, J.H. & J. Leszyk: The "gamma subunit" of Na,K-ATPase: a small, amphiphilic protein with a unique amino acid sequence. *Biochemistry* 26, 8665-8668 (1987)

65. Mercer R.W., D. Biemesderfer, D.P. Bliss Jr, J.H. Collins & B. Forbush III: Molecular cloning and immunological characterization of the gamma polypeptide, a small protein associated with the Na,K-ATPase. *J Cell Biol* 121, 579-586 (1993)

66. Arystarkhova E., C. Donnet, N.K. Asinovski & K.J. Sweadner: Differential regulation of renal Na,K-ATPase by splice variants of the gamma subunit. *J Biol Chem* 277, 10162-10172 (2002)

67. Crambert G., M. Fuzesi, H. Garty, S. Karlish & K. Geering : Phospholemman (FXYD1) associates with Na,K-ATPase and regulates its transport properties. *Proc Natl Acad Sci USA* 99, 11476-11481 (2002)

68. Zouzoulas A., A.G. Therien, R. Scanzano, C.M. Deber & R. Blostein: Modulation of Na,K-ATPase by the gamma subunit: studies with transfected cells and transmembrane mimetic peptides. *J Biol Chem* 278, 40437-40441 (2003)

69. Lindzen M., R. Aizman, Y. Lifshitz, I. Lubarski, S.J.D. Karlish & H. Garty: Structure-function relations of interactions between Na,K-ATPase, the gamma subunit, and corticosteroid hormone-induced factor. *J Biol Chem* 278, 18738-18743 (2003)

70. Toyoshima C., M. Nakasako, H. Nomura & H. Ogawa: Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Angstrom resolution. *Nature* 405, 647-655 (2000)

Key Words: Isozymes, Sequence comparisons, Structure-function relationships, Protein kinase C

Send correspondence to: Dr Thomas A. Pressley, Dept. of Physiology, Texas Tech University Health Sciences Center, 3601 4th Street, Lubbock, TX 79430, Tel: 806-743-4056, Fax: 806-743-1512, E-mail: Thomas.Pressley@ttuhsc.edu