Emanuel Strehler’s work on calcium pumps and calcium signaling

Emanuel E Strehler

Emanuel E Strehler, Biochemistry and Molecular Biology, Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN 55905, United States

Author contributions: Strehler EE solely contributed to this manuscript.

Supported by The National Institutes of Health (NS51769) and the Mayo Foundation for Education and Research

Correspondence to: Emanuel E Strehler, PhD, Professor of Biochemistry and Molecular Biology, Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, 200 First Street S.W., Rochester, MN 55905, United States. strehrer.emanuel@mayo.edu

Telephone: +1-507-2849372 Fax: +1-507-2842384

Received: February 3, 2011 Revised: March 16, 2011 Accepted: March 23, 2011 Published online: April 26, 2011

Abstract

Cells are equipped with mechanisms to control tightly the influx, efflux and resting level of free calcium (Ca^{2+}). Inappropriate Ca^{2+} signaling and abnormal Ca^{2+} levels are involved in many clinical disorders including heart disease, Alzheimer’s disease and stroke. Ca^{2+} also plays a major role in cell growth, differentiation and motility; disturbances in these processes underlie cell transformation and the progression of cancer. Accordingly, research in the Strehler laboratory is focused on a better understanding of the molecular "toolkit" needed to ensure proper Ca^{2+} homeostasis in the cell, as well as on the mechanisms of localized Ca^{2+} signaling. A long-term focus has been on the plasma membrane calcium pumps (PMCA), which are linked to multiple disorders including hearing loss, neurodegeneration, and heart disease. Our work over the past 20 years or more has revealed a surprising complexity of PMCA isoforms with different functional characteristics, regulation, and cellular localization. Emerging evidence shows how specific PMCA contribute not only to setting basal intracellular Ca^{2+} levels, but also to local Ca^{2+} signaling and vectorial Ca^{2+} transport. A second major research area revolves around the calcium sensor protein calmodulin and an enigmatic calmodulin-like protein (CALML3) that is linked to epithelial differentiation. One of the cellular targets of CALML3 is the unconventional motor protein myosin-10, which raises new questions about the role of CALML3 and myosin-10 in cell adhesion and migration in normal cell differentiation and cancer.

© 2011 Baishideng. All rights reserved.

Key words: Calcium signaling; Calcium transport; Calmodulin; Membrane trafficking; Myosin-10; Calcium ATPase; Calcium pumps; Structure-function relationship

Peer reviewers: Elena Zocchi, MD, PhD, Full Professor of Biochemistry, Department of Experimental Medicine, University of Genova, Viale Benedetto XV 1, 16132 Genova, Italy; Enzo Martegani, PhD, Professor, Dipartimento di Biotecnologie e Bioscienze, Università di Milano Bicocca, Piazza della Scienza 2, 20126, Milano, Italy; Sheng-Tao Hou, Professor, Institute for Biological Sciences, National Research Council of Canada, 1200 Montreal Road, Bldg M-54, Ottawa, Ontario, K1A 0R6, Canada

Strehler EE. Emanuel Strehler’s work on calcium pumps and calcium signaling. World J Biol Chem 2011; 2(4): 67-72 Available from: URL: http://www.wjgnet.com/1949-8454/full/v2/i4/67.htm DOI: http://dx.doi.org/10.4331/wjbc.v2.i4.67
INTRODUCTION AND EDUCATIONAL EXPERIENCE

Dr. Ernst Emanuel Strehler (Figure 1) is a Professor of Biochemistry and Molecular Biology at the Mayo Clinic College of Medicine in Rochester, Minnesota, USA. Since 2005, he has also been an Adjunct Professor in the Department of Pharmacology, College of Graduate Studies and Research, of the University of Saskatchewan in Saskatoon, Canada. Dr. Strehler received his MS degree in Biochemistry, Cell and Molecular Biology in 1977 and his PhD in Cell Biology in 1981, both from the Swiss Federal Institute of Technology (ETHZ) in Zurich, Switzerland. He did his MS thesis under the mentorship of Professor Claus W Heizmann and his PhD thesis (on M-line muscle proteins) under Professor Hans M Eppenberger. Both his MS and his PhD theses were recognized with a prize and the Silver Medal of the ETHZ. After a short stint with Professor Lars-Eric Thornell at the University of Umeå in Sweden (1981), Dr. Strehler pursued postdoctoral training in recombinant DNA technology and molecular biology with Professor Bernardo Nadal-Ginard at the Children’s Hospital and Harvard Medical School in Boston, USA, from 1982 to 1986. He was supported by a short-term EMBO postdoctoral fellowship (1981) and a stipend for young advanced scientists from the Swiss National Science Foundation (1982-1984). In 1986, Dr. Strehler returned to the ETHZ as an Assistant Professor in the Laboratory of Biochemistry directed by Professor Ernesto Carafoli. There, he first applied his expertise in molecular biology to the identification and characterization of the plasma membrane calcium pump (PMCA) family. In 1990, he was awarded the prestigious ETHZ Latsis prize of the International Latsis Foundation for work on the molecular biology of the PMCAs, as well as a START (Swiss Talents in Research and Teaching) Career Development Award from the Swiss NSF. He moved to his current position at the Mayo Clinic College of Medicine in 1992. Dr. Strehler served as Molecular Neuroscience Program Director in the Mayo Graduate School from 2000 to 2007. He has been an invited speaker at national and international meetings, a peer reviewer for numerous scientific journals and grant proposals, and has served on the Editorial Boards of the Journal of Biological Chemistry, Current Chemical Biology, and Biochemistry Research International. He has held grants from multiple foundations and federal organizations (including the Swiss NSF and the US NIH) and has published over 120 peer-reviewed journal articles and reviews.

ACADEMIC STRATEGIES AND GOALS

Dr. Strehler’s research is focused on the biochemistry, molecular cell biology, and structure/function analysis of the PMCA family as it contributes to intracellular calcium signaling in health and disease. Using a wide range of techniques, including protein and Ca$^{2+}$ transport biochemistry, recombinant DNA technology, and cell biology methods such as fluorescence microscopy and immunocytochemistry, his research group has been studying the human PMCA gene family and its multiple encoded isoforms and alternative splice variants. Helped by productive and long-standing collaborations, his group has contributed to a better understanding of the mechanism of regulation of different PMCAs, their tissue expression and cellular targeting, their interactions with scaffolding and signaling proteins, and their different contribution to handling cellular Ca$^{2+}$ spikes. The ultimate goal of these studies is to comprehend how the diverse PMCAs are integrated in the complex Ca$^{2+}$ signaling networks that control physiological and pathophysiological cell function. Dr. Strehler’s research has also uncovered a surprising complexity of the human calmodulin gene family in which multiple genes code for an identical protein, and has led to the characterization of a calmodulin-like protein with a putative role in cell differentiation and migration. The overarching goal of Dr. Strehler’s research is to understand the molecular underpinnings and regulation of intracellular Ca$^{2+}$ signaling as it relates to normal cell physiology and disease.

ACADEMIC ACHIEVEMENTS

The following sections provide brief vignettes of discoveries and research accomplishments of Dr. Strehler’s laboratory in the field of the calcium pumps and calcium sensor proteins.

Characterization of the human PMCA gene family and discovery of alternative splicing as a mechanism to generate isoform complexity

PMCAs are generally low-abundance membrane proteins, therefore, they are difficult to isolate and purify. The first cloning of a PMCA therefore required immuno-isolation of clones from λgt11 bacteriophage human cDNA expression libraries and low stringency hybridization cloning using degenerate oligonucleotides. After the breakthrough cloning of the first human PMCA cDNA[1], additional PMCA isoforms were rapidly isolated and sequenced. Dr. Strehler was involved in the cloning of all four human PMCAs, and his team also reported the first human PMCA gene structure[2]. The molecular cloning work revealed an (at the time) unexpected complexity of alternative RNA splicing in the PMCA genes[3]. Work over several years has shown that all human PMCA genes are subject to alternative splicing, which affects two major sites of functional importance: one in the first cytosolic loop of the pump (site A) and one in the C-terminal tail (site C)[4]. In total, over 30 different human PMCA variants can be generated from the four genes and through combinatorial alternative splicing. A schematic overview of the PMCA and its major splice options is shown in Figure 2. This body of work has provided a comprehensive description of the human PMCA family, and has opened the door to detailed molecular, biochemical, and functional studies on this important calcium extrusion system.
PMCA splice variants are functionally different - implications for Ca\(^{2+}\) handling

PMCA isoforms and splice variants are expressed in tissue- and developmental stage-specific patterns, which suggests functional specialization among different pumps. Much work has therefore been dedicated to determination of the regulatory and kinetic differences between PMCA isoforms. The PMCAs are regulated (activated) by Ca\(^{2+}\)-calmodulin, which binds to a specific region in the C tail of the pump\(^{[6-8]}\). The discovery that alternative splicing at site C affects the calmodulin-binding region of the pump immediately suggests a mechanism for differential regulation of the different splice variants. Work by different groups, including that of Dr. Strehler, has shown that different C-terminal splice variants indeed differ in calmodulin affinity and, of particular interest, in their activation and inactivation kinetics\(^{[9-11]}\). This work is highly significant because it demonstrates that different PMCA isoforms and splice variants handle Ca\(^{2+}\) signals differently. Tissues and cells with a need for fast and efficient expulsion of Ca\(^{2+}\) therefore express “fast” PMCAs, whereas cells and tissues with more modest (slow-tonic) changes in [Ca\(^{2+}\)] are well served by “slow” PMCAs such as PMCA4b\(^{[12]}\).

Elucidation of the activated complex of calmodulin with the calmodulin-binding domain of the PMCA

Detailed kinetic work has shown that calmodulin binds to the slow PMCA4b in a multistep process, and has tentatively identified important anchor residues in the PMCA that tightly interact with Ca\(^{2+}\)-calmodulin\(^{[13,14]}\). In recent collaborative work, the Strehler group has determined the NMR structure of the full-length calmodulin binding domain of PMCA4b in complex with holo-calmodulin\(^{[15]}\). This structure represents a novel (18-1 motif) calmodulin-target peptide complex, and confirms the identity of anchor residues proposed in earlier kinetic studies of the interaction of calmodulin with the PMCA. This work is of further significance because it suggests the possibility of obtaining more extensive structural information on the regulatory C tail of this difficult-to-study membrane protein.

Discovery of rapidly regulated alternative splicing in the PMCA

The discovery of a large family of PMCA isoforms and splice variants immediately raises the question about the specific roles of these isoforms. In 1996, Dr. Strehler’s laboratory showed for the first time that a specific alternative splice shift in PMCA2 occurs as a consequence of a transient increase in intracellular Ca\(^{2+}\)\(^{[15]}\). The isoform switch was independent of new protein synthesis and was observed within 30 min or less of the Ca\(^{2+}\) stimulus. This work has shown that rapid changes in alternative RNA splicing may be induced by a change in [Ca\(^{2+}\)], which has given rise to new hypotheses concerning the molecular mechanisms that are responsible for second-messenger-induced reprogramming of the splicing machinery. The results also suggested a dynamic role for specific PMCA isoforms in Ca\(^{2+}\) regulation rather than the static function originally assigned to these pumps.
and clustering as well as signaling proteins. The identification of these novel protein interactions is a major finding because it reveals an additional role for alternative splicing in the PMCA family, and suggests that isoform-specific interactions with PDZ proteins are responsible for the incorporation of the Ca\(^{2+}\) pumps into multiprotein complexes involved in local Ca\(^{2+}\) signaling. This work lends strong support to the emerging hypothesis that the PMCAs are dynamic components of local Ca\(^{2+}\) control at the plasma membrane\(^{[19]}\).

**Alternative splicing controls the membrane targeting of PMCs in polarized cells**

When searching for a role of alternative splicing at site A (Figure 2), Dr. Strehler and his co-workers discovered that the size of the splice insert in the first cytosolic loop of PMCA2 affects the apical or basolateral targeting of the pump. The large w-insert is responsible for directing the PMCA2 to the apical stereocilia in cochlear hair cells or the apical membrane in polarized kidney epithelial cells\(^{[20]}\). Recent work has shown that interaction with PDZ proteins, such as the apical scaffolding protein NHERF2\(^{[21]}\), further enhances the recruitment of PMCA2w/b to the apical membrane and leads to reduced lateral mobility and decreased endocytosis of the pump\(^{[20]}\). These studies suggest that an interplay of effects of altered loop structure and specific protein-protein interactions of the C tail determines the outcome of the membrane targeting and final localization of different PMCAs in the cell. Ongoing work in the Strehler laboratory aims to understand the molecular mechanism of the specific membrane targeting of the PMCAs, and the functional consequences of the selective deployment of PMCA isoforms for local Ca\(^{2+}\) signaling.

**Identification of human calmodulin-like protein (CALML3) as a tumor-sensitive protein involved in epithelial differentiation**

The serendipitous discovery of an intronless gene for a human calmodulin-like protein\(^{[22]}\) gained interest when it became clear that calmodulin-like protein (CLP) (human genome nomenclature CALML3) is a tumor-sensitive protein specifically expressed in normal epithelial cells but downregulated in tumorigenesis\(^{[23]}\). Dr. Strehler’s group has characterized the biochemical properties of CLP\(^{[24,25]}\) and provided the first description of the gene and its regulation\(^{[26]}\), and has shown that downregulation of the protein is an early event in breast cancer development\(^{[27]}\). However, the causal role of CLP in epithelial differentiation and malignant transformation remains to be determined.

**Discovery of myosin-10 as a specific target of CLP/CALML3: role in filopodial motility and wound healing**

One of the most pressing questions raised by the discovery of CLP/CALML3 is that of its potential targets. Although it is 85% identical to human calmodulin, the distinct properties of CLP suggest that it has specific targets or targets that only partially overlap with those of calmodulin. Research in Dr. Strehler’s group has identified the unconventional myosin-10 (Myo10) as a specific target of CLP\(^{[28]}\). This work also has shown that CLP functions as a light chain for Myo10 by binding to one or several of the three IQ domains in the neck region of the myosin (Figure 3). CLP competes effectively with calmodulin for Myo10 binding\(^{[16]}\), and upregulation of CLP results in in-
increased Myo10 expression and function. The discovery of Myo10 as a specific target of CLP is highly significant and suggests multiple lines of further research such as investigations of the Ca\(^{2+}\) regulation of Myo10 and the role of the loss of CLP in epithelial differentiation, adhesion, and cancer.

CONCLUSION

Cellular processes ranging from fertilization to programmed cell death are regulated by precisely timed changes in the intracellular free Ca\(^{2+}\) concentration. Cells therefore must be equipped with mechanisms to control tightly the influx, efflux and resting level of Ca\(^{2+}\). Dr. Strehler's research has made major contributions to the understanding of the biochemical, structural, regulatory, and functional properties of the human PMCa. These pumps represent the primary high-affinity Ca\(^{2+}\) efflux system of all eukaryotic cells and are now recognized as integral and dynamic players in cellular Ca\(^{2+}\) signaling. Dr. Strehler's research has also shed light on the organization and function of the human calmodulin gene family and has shown that calmodulin-like isoforms may assume unique roles in the transmission of the Ca\(^{2+}\) signal to specific cellular targets, and thereby have an impact on crucial processes such as cell differentiation, motility, and adhesion.

ACKNOWLEDGMENTS

I am grateful to all the past and present members of my laboratory for their enthusiastic contributions to our studies, including many stimulating discussions during after-hours. I would also like to thank my many collaborators over the years and on different continents; their expertise and dedication to joint projects were (and are) essential for the success of these studies.

REFERENCES

1. Verma AK, Filoteo AG, Stanford DR, Wieben ED, Penniston JT, Strehler EE, Fischer R, Heim R, Vogel G, Mathews S. Complete primary structure of a human plasma membrane Ca\(^{2+}\) pump. J Biol Chem 1988; 263: 14152-14159
2. Hilfiker H, Strehler-Page MA, Stauffer TP, Carafoli E, Strehler EE. Structure of the gene encoding the human plasma membrane calcium pump isoform 1. J Biol Chem 1993; 268: 19717-19725
3. Strehler EE, Strehler-Page MA, Vogel G, Carafoli E. mRNAs for plasma membrane calcium pump isoforms differing in their regulatory domain are generated by alternative splicing that involves two internal donor sites in a single exon. Proc Natl Acad Sci USA 1989; 86: 6908-6912
4. Strehler EE, Zacharias DA. Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. Physiol Rev 2001; 81: 21-50
5. Zacharias DA, Strehler EE. Change in plasma membrane Ca\(^{2+}\)-ATPase splice-variant expression in response to a rise in intracellular Ca\(^{2+}\). Curr Biol 1996; 6: 1642-1652
6. Penniston JT, Filoteo AG, McDonough CS, Carafoli E. Purification, reconstitution, and regulation of plasma membrane Ca\(^{2+}\)-pumps. Methods Enzymol 1988; 157: 340-351
7. Penniston JT, Enyedi A. Modulation of the plasma membrane Ca\(^{2+}\) pump. J Membr Biol 1998; 165: 105-109
8. Strehler EE. Recent advances in the molecular characterization of plasma membrane Ca\(^{2+}\) pumps. J Membr Biol 1991; 120: 1-15
9. Caride AJ, Elwess NL, Verma AK, Filoteo AG, Enyedi A, Bajzer Z, Penniston JT. The rate of activation by calmodulin of isoform 4 of the plasma membrane Ca\(^{2+}\) pump is slow and is changed by alternative splicing. J Biol Chem 1999; 274: 32277-32285
10. Caride AJ, Filoteo AG, Penheiter AR, Pászty K, Enyedi A, Penniston JT. Delayed activation of the plasma membrane calcium pump by a sudden increase in Ca\(^{2+}\): fast pumps reside in fast cells. Cell Calcium 2001; 30: 49-57
11. Caride AJ, Filoteo AG, Penniston JT, Strehler EE. The plasma membrane Ca\(^{2+}\) pump isoform 4a differs from isoform 4b in the mechanism of calmodulin binding and activation kinetics: implications for Ca\(^{2+}\) signaling. J Biol Chem 2007; 282: 25640-25648
12. Strehler EE, Filoteo AG, Penniston JT, Caride AJ. Plasma membrane Ca\(^{2+}\) pumps: structural diversity as the basis for functional versatility. Biochem Soc Trans 2007; 35: 919-922
13. Penheiter AR, Bajzer Z, Filoteo AG, Thorgate R, Török K, Caride AJ. A model for the activation of plasma membrane calcium pump isoform 4b by calmodulin. Biochemistry 2003; 42: 12115-12124
14. Penheiter AR, Filoteo AG, Penniston JT, Caride AJ. Kinetic analysis of the calmodulin-binding region of the plasma membrane calcium pump isoform 4b. Biochemistry 2005; 44: 2009-2020
15. Juranic N, Atanasesova E, Filoteo AG, Macura S, Prendergast FG, Penniston JT, Strehler EE. Calmodulin wraps around its binding domain in the plasma membrane Ca\(^{2+}\) pump anchored by a novel 18-1 motif. J Biol Chem 2010; 285: 4015-4024
16. Kim E, DeMarco SJ, Martatla SM, Chishti AH, Sheng M, Strehler EE. Plasma membrane Ca\(^{2+}\) ATPase isoform 4b binds to membrane-associated guanylate kinase (MAGUK) proteins via their PDZ (PSD-95/Dlg/ZO-1) domains. J Biol Chem 1998; 273: 1591-1595
17. DeMarco SJ, Strehler EE. Plasma membrane Ca\(^{2+}\)-atpase isoforms 2b and 4b interact promiscuously and selectively with members of the membrane-associated guanylate kinase family of PDZ (PSD95/Dlg/ZO-1) domain-containing proteins. J Biol Chem 2001; 276: 21594-21600
18. Strehler EE, Caride AJ, Filoteo AG, Xiong Y, Penniston JT, Enyedi A. Plasma membrane Ca\(^{2+}\) ATPases as dynamic regulators of cellular calcium handling. Ann N Y Acad Sci 2007; 1099: 226-236
19. Chicka MC, Strehler EE. Alternative splicing of the first intracellular loop of plasma membrane Ca\(^{2+}\)-ATPase isoform 2 alters its membrane targeting. J Biol Chem 2003; 278: 18464-18470
20. Grati M, Aggarwal N, Strehler EE, Wenthold RJ. Molecular determinants for differential membrane trafficking of PMCA1 and PMCA2 in mammalian hair cells. J Cell Sci 2006; 119: 2995-3007
21. DeMarco SJ, Chicka MC, Strehler EE. Plasma membrane Ca\(^{2+}\) ATPase isoform 2b interacts preferentially with Na+/H+ exchanger regulatory factor 2 in apical plasma membranes. J Biol Chem 2002; 277: 10506-10511
22. Padanyi R, Xiong Y, Antibody G, Löv K, Pászty K, Strehler EE, Enyedi A. Apical scaffolding protein NHERF2 modulates the localization of alternatively spliced plasma membrane Ca\(^{2+}\) pump 2b variants in polarized epithelial cells. J Biol Chem 2010; 285: 3104-3116
23. Fischer R, Koller M, Flura M, Mathews S, Strehler-Page MA, Krebs J, Penniston JT, Carafoli E, Strehler EE. Multiple divergent mRNAs code for a single human calmodulin. J Biol Chem 1988; 263: 17055-17062
Strehler EE. Strehler’s profile

24 Berchtold MW, Egli R, Rhynner JA, Hameister H, Strehler EE. Localization of the human bona fide calmodulin genes CALM1, CALM2, and CALM3 to chromosomes 14q24-q31, 2p21.1-p21.3, and 19q13.2-q13.3. Genomics 1993; 16: 461-465

25 Toutenhoofd SL, Foletti D, Wicki R, Rhynner JA, Garcia F, Tolon R, Strehler EE. Characterization of the human CALM2 calmodulin gene and comparison of the transcriptional activity of CALM1, CALM2 and CALM3. Cell Calcium 1998; 23: 323-338

26 Koller M, Baumer A, Strehler EE. Characterization of two novel human retropseudogenes related to the calmodulin-encoding gene, CaMII. Gene 1991; 97: 245-251

27 Koller M, Strehler EE. Characterization of an intronless human calmodulin-like pseudogene. FEBS Lett 1988; 239: 121-128

28 Toutenhoofd SL, Strehler EE. The calmodulin multigene family as a unique case of genetic redundancy: multiple levels of regulation to provide spatial and temporal control of calmodulin pools? Cell Calcium 2000; 28: 83-96

29 Yaswen P, Smoll A, Peehl DM, Trask DK, Sager R, Stampfer MR. Down-regulation of a calmodulin-related gene during transformation of human mammary epithelial cells. Proc Natl Acad Sci USA 1990; 87: 7360-7364

30 Rhynner JA, Koller M, Durussel-Gerber I, Cox JA, Strehler EE. Characterization of the human calmodulin-like protein expressed in Escherichia coli. Biochemistry 1992; 31: 12826-12832

31 Durussel I, Rhynner JA, Strehler EE, Cox JA. Cation binding and conformation of human calmodulin-like protein. Biochemistry 1993; 32: 6089-6094

32 Koller M, Strehler EE. Functional analysis of the promoters of the human CaMIII calmodulin gene and of the intronless gene coding for a calmodulin-like protein. Biochim Biophys Acta 1993; 1163: 1-9

33 Rogers MS, Foley MA, Crotty TB, Hartmann LC, Ingle JN, Roche PC, Strehler EE. Loss of immunoreactivity for human calmodulin-like protein is an early event in breast cancer development. Neoplasia 1999; 1: 220-225

34 Rogers MS, Kobayashi T, Pittelkow MR, Strehler EE. Human calmodulin-like protein is an epithelial-specific protein regulated during keratinocyte differentiation. Exp Cell Res 2001; 267: 216-224

35 Rogers MS, Strehler EE. The tumor-sensitive calmodulin-like protein is a specific light chain of human unconventional myosin X. J Biol Chem 2001; 276: 12182-12189

36 Caride AJ, Bennett RD, Strehler EE. Kinetic analysis reveals differences in the binding mechanism of calmodulin and calmodulin-like protein to the IQ motifs of myosin-10. Biochemistry 2010; 49: 8105-8116

37 Bennett RD, Mauer AS, Strehler EE. Calmodulin-like protein increases filopodia-dependent cell motility via up-regulation of myosin-10. J Biol Chem 2007; 282: 3205-3212

38 Bennett RD, Mauer AS, Pittelkow MR, Strehler EE. Calmodulin-like protein upregulates myosin-10 in human keratinocytes and is regulated during epidermal wound healing in vivo. J Invest Dermatol 2009; 129: 765-769

S- Editor Cheng JX  L- Editor Kerr C  E- Editor Zheng XM