ECTO-NOX Proteins

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Growth, Cancer, and Aging

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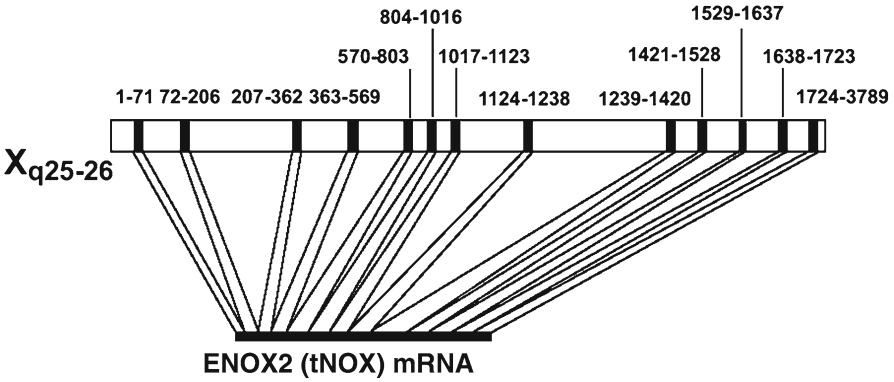
Preface

One does not discover new lands without consenting to lose sight of the shore for a very long time.

André Gide



The discovery of the ENOX proteins was inexorably interwoven with the concept of a plasma membrane electron transport first indicated from the findings of Cathy Frantz, a masters student at Purdue University. Cathy found microsomal electron transport activities associated with highly purified plasma membranes from rat livers. The first report of these findings in 1973 at the American Society of Cell Biology meetings was greeted with indifferent disbelief. Clear exceptions were Prof. Fred Crane of Purdue University, a member of Cathy's examining committee and Fred's close friend and colleague, Prof. Hans Löw of the Karolinska Institute in Sweden. The Morrés continued to pursue these observations in their efforts to solidify the flow-differentiation model of Golgi apparatus functioning whereas Fred and Hans set upon a quest to discover the elusive plasma membrane electron transport system. That quest culminated with two co-edited volumes, one on animals in 1990

and one on plants in 1991 entitled *Oxidoreduction at the Plasma Membrane: Relation to Growth and Transport* published by CRC Press (Crane et al. 1990b, 1991). The existence of growth-related NADH oxidases of the plant and animal plasma membrane were first reported in 1986 from plants (Morré et al. 1986a) but it was not until 1990 (Morré and Crane 1990; Fig. 4.8; cover) that their role as the terminal oxidases of the plasma membrane electron transport chain was correctly formulated.

We thank our good friends and colleagues Frederick L. Crane and Hans Löw for championing the concept that the plasma membranes might contain redox active proteins with important functions in growth and disease even in the face of general non-acceptance of that notion by the overall scientific community and a universal lack of enthusiasm by extramural funding agencies for studies of plasma membrane electron transport no matter how well conceived.

It is a singular but sometimes lonely privilege to write a book on a potentially important new family of proteins virtually single handedly identified, cloned, characterized and clinically implemented with minimal independent outside confirmation at the time. While masquerading as intractable proteins, they have and continue to offer remarkable opportunities for research, commercial development and outside confirmation (Chap. 11; Table 11.1). The latter underscores the complexity of these proteins and the many unusual difficulties especially in their assay. We recall a visit from Dr. Warren McKellar of Eli Lilly to our laboratory in the very early days of anti-cancer sulfonylurea research to carry out a spectrophotometric assay on his own to validate the activity. He was pleased to see a rate only to remark a few moments later that it had stopped momentarily only to start up again a few minutes later. Several more years were required before the true meaning of that observation and its subsequent reproduction with other systems finally led to the still controversial conclusion that ENOX activities were oscillatory.

The ENOX proteins were discovered as a result of a search for a growth-related protein at the cell surface that was the target for immobilized forms of the anticancer drug doxorubicin (Adriamycin®). In the late 1970s several groups demonstrated that doxorubicin exhibited an enhanced anti-cancer activity if it was first conjugated to an impermeant support and not permitted to enter the cell (Chap. 11). As doxorubicin is a redox-active quinone site inhibitor, a redox protein was sought that was at the cell surface and cancer-specific. The search led eventually to discovery of the ECTONOX (ENOX) family of external hydroquinone oxidases, also capable of oxidizing external NADH. One subset of the ENOX proteins, the tumor-specific tNOX or ENOX2 proteins, was inhibited by doxorubicin and other quinone site-targeted anticancer drugs, was cancer-specific, was absent from the surface of non-cancer cells and tissues and was the first ENOX protein to be cloned (Chueh 1997; Chueh et al. 2002b). Three additional family members including the constitutive human ENOX1 in 2008 (Jiang et al. 2008) followed with the most recent, an age-related ENOX protein, cloned in 2010 and a constitutive ENOX1 from plants in 2011. Being proteins of the external cell surface and lacking *trans*-membrane helices to anchor the protein in the membrane, ENOX proteins were found to be shed and to appear in soluble form in patient sera and urine where they serve as early diagnostic markers for cancer presence and

organ site to permit very early intervention strategies prior to advanced disease and metastatic spread (Chap. 12).

Findings of Chap. 8 suggest that the cancer- or tumor-related ENOX2 (tNOX) proteins are all splice variants from a single gene. More importantly, each major type of human cancer is characterized by characteristic transcript variants of unique molecular weight and isoelectric point (Chap. 12). Using a proteomics approach and a recombinant antibody specific for a common exon, it is possible not only to detect cancer but to diagnose it as well.

The purpose of this book is to document this unique family of cell surface proteins (the ECTO-NOX or ENOX protein family) involved in growth, biological time keeping, cancer, aging and viral infections and having properties of prions. The ENOX proteins are the exclusive discovery (subsequently confirmed by others) of the authors, Drs. D. James and Dorothy Morré, and their students and research associates at Purdue University. Roles in plasma membrane electron transport (Chap. 4), growth (Chap. 5), biological time keeping (Chap. 6), cancer (Chaps. 8, 11 and 12), prevention of viral infections (Chap. 7), crop production through control of plant growth (Chap. 10), and coronary artery disease and skin aging (Chap. 9), are among the many developing opportunities for new discovery and commercialization surrounding the ENOX proteins.

The book provides an ENOX-based mechanism for how cells become larger (increase in size) that is both unique and well documented with applications not only to cancer and cancer therapy but for production agriculture as well (Chap. 10) with increase of biomass for biofuel production as one exciting future prospect.

Finally, the concept of and the evidence for oscillations in the ratios of electron spin pairs defining *ortho* and *para* water as the basis for highly coordinated populations of coherent water that appear vital to water's biological and physical properties is completely new, of interest to the biological and physical sciences and now becoming widely accepted by the physical scientists involved with the study of the properties of water (Chap. 6).

Special mention is accorded to Michael Berridge, Frederick Crane, Iris Sun, Rita Barr and Hans Löw who have unwaveringly promoted plasma membrane redox and a functional role of ENOX proteins in the overall process, to the late Albert Overhauser for encouragement to seek an explanation of the oscillatory patterns of ENOX proteins at the atomic level (Chap. 6), to Michael Böttger for assistance with pivotal growth measurements (Chap. 5), to Ron Brightmore for his inspiring surveys of the literature and to Profs. Jacob Levitt and Hale Fletchall of the University of Missouri for planting the initial seeds of inquiry. Special thanks to Don Lee, Tom Shelton, Graham Kelly and Richard Greaves for recognizing the commercial potential of the ENOX protein family.

We express our appreciation to the many colleagues, postdoctorals, graduate students, undergraduate assistants, and technicians whose invaluable assistance made possible the experimental studies especially as graduate students, Andrew Brightman, P.-J. Chueh, Chinpal (James) Kim, Xiaoyu Tang and Ziying Jiang for ground breaking protein purification and molecular cloning efforts. Appreciation is extended as well to the even greater numbers who challenged and criticized the work to force us

to work even more diligently to distinguish among possible interpretations of the findings. We thank Peggy Runck for manuscript preparation and Aya Ryuzoji for preparation of the figures. We are especially indebted to the unwavering support of the Morré children, Connie, Jeffrey and Suzanne, and grandchildren, Christopher, Eric and Katherine Chalko, Matthew, Timothy and Nicholas Miner, Suzanna Morré and Aren and Mariah Rudder. May our ENOX proteins always oscillate in synchrony.

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Contents