INTRODUCTION / INTRODUCTION

Report on the Na^+/H^+ Exchanger Satellite Meeting at the 53rd Annual Meeting and Conference of the Canadian Society of Biochemistry, Molecular and Cellular Biology

R.T. Alexander and L. Fliegel

Abstract: The Satellite Meeting on Na^+/H^+ Exchangers, held on 17 April 2010, covered a range of new developments in this field. The symposium was chaired by Dr. Larry Fliegel, University of Alberta, and the speakers were Dr. John Orlowski of McGill University, Dr. Jan Rainey of Dalhousie University, Dr. Etana Padan of The Hebrew University of Jerusalem, Dr. Masa Numata of The University of British Columbia, Dr. Pavel Dibrov from the University of Manitoba, Dr. Todd Alexander of the University of Alberta, and Grant Kemp of the University of Alberta. Talks ranged from organellar pH homeostasis to structure and function of Na^+/H^+ exchanger proteins. Highlights of the symposium included elucidation of the structure of transmembrane regions of the NHE1 isoform and development of a new model of the NHE1 protein based on the *E. coli* Na^+/H^+ exchanger. The symposium brought together scientists from different corners of the world. The discussions that followed were lively and many scientists received constructive comments from their peers.

Résumé : Le symposium satellite sur l'échangeur Na^+/H^+, tenu le 17 avril 2010, a couvert un éventail de nouvelles découvertes dans ce domaine. Le symposium était présidé par le Dr. Larry Fliegel, University of Alberta, et les conférenciers étaient le Dr. John Orlowski de l'Université McGill, le Dr. Jan Rainey, Dalhousie University, le Dr. Etana Padan, Hebrew University of Jerusalem, le Dr. Masa Numata, University of British Columbia, le Dr. Pavel Dibrov, University of Manitoba, le Dr. Todd Alexander, University of Alberta et Grant Kemp, University of Alberta. Les présentations allaient de l'homéostasie du pH des organelles à la structure et fonction des échangeurs Na^+/H^+. Les points saillants du symposium comprenaient notamment l'élucidation de la structure de la région transmembranaire de l'isoforme NHE1 et le développement d'un nouveau modèle de NHE1 basé sur l'échangeur Na^+/H^+ de *E. coli.* Le symposium a réuni des scientifiques des quatre coins du monde, et les discussions qui ont suivi ont été animées, plusieurs scientifiques ayant reçu des commentaires constructifs de leurs pairs.

[Traduit par la Rédaction]

The Satellite Meeting on Na^+/H^+ Exchangers was held on 17 April 2010 in conjunction with the 53rd Annual Meeting of the CSBMCB: Membrane Proteins in Health and Disease. The session was chaired and organized by Dr. Larry Fliegel of the Department of Biochemistry of the University of Alberta. The first speaker was Dr. John Orlowski of McGill University. His talk was titled "Organellar pH Homeostasis and Neurological Disease". Dr. Orlowski gave a dynamic talk, first introducing the various subclasses of the Na^+/H^+ exchangers and then providing background on the organellar isoforms NHE6, NHE7, and NHE9. He described how these isoforms can move K^+ and how they regulate organellar pH in concert with the vacuolar H^+-ATPase. He described how organellar pH can be measured with fluorescent probes, which is important because NHE6 and NHE7 are in recycling vesicles. The linkage of NHE6 mutation to several human diseases, including mental retardation and epileptic seizures, was described. Dr. Orlowski also detailed studies of NHE6 localization and function and how varying NHE6 levels affect endosomes and their traffic. Overall, this was a fast-paced and enjoyable talk packed with information well beyond what would normally be present in a 25 minute lecture.

The second speaker was Dr. Jan Rainey of Dalhousie University. His talk was titled "Correlating Function to Structure and Dynamics in NHE1 Using the 'Divide and Conquer' Approach". Dr. Rainey described how nuclear magnetic resonance (NMR) spectroscopy can be used to examine the structure of membrane proteins. He reviewed how the structure of fragments of membrane proteins deduced by NMR spectroscopy has been shown to correlate very well with the structure of those regions in full-length crystallized membrane proteins. He then reviewed the structure of trans-

Published on the NRC Research Press Web site at bcb.nrc.ca on .

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membrane (TM) segments IV, VII, and IX of the NHE1 isoform of the Na^+/H^+ exchanger. Of note, these regions are not continuous helices, but rather are interrupted by non-helical extended or bent regions. TM segment IV of NHE1 shows structural homology to TM segment IV of NhaA. TM segment IX shows a bent structure with amino acids Ser351 and Glu346, which are important functionally, on opposite faces of the peptide. This might indicate an alternating access region of the protein. Overall, the talk shed light on a new approach to study membrane proteins when production of the entire protein and its crystallization are problematic.

Dr. Etana Padan of The Hebrew University of Jerusalem talked about "NhaA Based Modeling of the Eukaryotic Exchanger NHE1 and NHA2". She outlined differences in the functional aspects of NhaA and NHE1, including differences in affinity and regulation by pH. She explained how a new model of NHE1 was developed based on a comparison with NhaA. This model shows critical differences in topology from a previous model of NHE1 based on cysteine scanning accessibility. Dr. Padan outlined some inconsistencies in the previous model, as some intracellularly located residues were accessible to reagents that cannot permeate the plasma membrane. A new model of NHA2 was presented based on comparison with NhaA. Structure-based mutagenesis studies were used to identify residues critical to the activity of the protein. This very sophisticated lecture showed new insights into how the topology of membrane proteins can now be predicted and used to plan structure-based mutagenesis.

Dr. Masa Numata of The University of British Columbia spoke about "Membrane Dynamics and Tumor Metastasis — Potential Role of NHE7". He explained the comparative effects of expression of NHE7 and NHE1 in causing metastasis. His studies examined the effects of their expression in a metastatic breast cancer cell line, MDA-MB-231. He described effects measured by use of cell overlay and cell infiltration assays. Cells with elevated NHE7 expression demonstrated greater saturation compared with controls or NHE1-containing cells. In cell invasion assays, increased expression of NHE7 enhanced colony formation. Preliminary experiments in nude mice suggest NHE7 expression may enhance tumor formation and metastasis.

After a lively discussion and break, Dr. Pavel Dibrov from the University of Manitoba gave a talk entitled "Peculiar Features of Na^+/H^+ Antiporters of NhaP Type in *Vibrio cholerae*". Dr. Dibrov reviewed the alternating access model of NhaA transport. He outlined the many isoforms of the Na^+/H^+ antiporter that exist in *Vibrio cholerae* and how some arose from gene duplication. He described work on vcNhaP2, which showed that chromosomal deletion of this isoform caused growth impairment at pH 6.0 in medium containing KCl. This isoform was also expressed in *E. coli* and its activity was characterized in vesicles using acridine orange. It exhibited transport in response to KCl addition and to a lesser degree in response to NaCl, but not in response to LiCl. The results were explained in terms of an interesting model describing how these ions bind to the putative cation-binding sides.

Dr. Todd Alexander of the University of Alberta gave a talk entitled "NHE3 is Necessary for Renal and Intestinal Calcium Absorption". Dr. Alexander described the proposed mechanism by which NHE3-mediated Na^+ flux might drive paracellular Ca^{2+} flux across renal and intestinal epithelia. To test this he employed NHE3 null mice. The knockout animals had normal serum calcium and parathyroid hormone levels but increased vitamin D levels. Functional studies of calcium flux across the small intestine revealed decreased flux in the knockout mice. Similarly, the knockout animals had increased urinary calcium excretion. These combined observations contributed to a decreased bone mineral density in the null animals. Dr. Alexander inferred from these findings that NHE3 plays a significant role in calcium homeostasis.

The final talk of the session was given by Grant Kemp, a graduate student in the Department of Biochemistry at the University of Alberta. Mr. Kemp described a yeast-based expression system that he developed to express milligram quantities of the NHE1 protein. The protein was used with electron microscopy and single particle reconstruction of negatively stained NHE1 to produce a molecular envelope for the [please italics for "in vitro"] wed that NHE1 is dimeric, each n d shape consistent with a general 12 membrane segment protein, similar to NhaA. Experiments were shown that demonstrated that the protein produced by yeast can be phosphorylated in vitro. Reconstitution of NHE1 demonstrated that it had a typical profile of inhibition with NHE1 inhibitors. Future experiments will further examine the structure of the protein.

Overall, the symposium gave a very state-of-the-art summary of Na^+/H^+ exchanger proteins spanning from the *E. coli* and *Vibrio cholerae* proteins to mammalian Na^+/H^+ exchanger isoforms. The discussions that followed were lively and many scientists received constructive comments from their peers.