## BIOGRAPHICAL SKETCH

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NAME: Iris Lindberg

eRA COMMONS USER NAME (credential, e.g., agency login): ILINDB

POSITION TITLE: Professor, Anatomy and Neurobiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing,* ***include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)***

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | Completion Date | FIELD OF STUDY |
|---|---|---|---|
| Univ. of California, Berkeley, CA | A.B. | 06/1976 | Biochemistry |
| Univ. of Wisconsin, Madison, WI | Ph.D. | 12/1980 | Pharmacology |
| National Institute of Mental Health | Postdoc | 09/1984 | Neuropharmacology |

### A. Personal Statement

My research focuses on the molecules within the secretory pathway required for the successful production of bioactive peptide hormones and neuropeptides from precursor proteins: for example the synthesis of enkephalins from proenkephalin and beta endorphin and alpha MSH from POMC. This process requires chaperones; abundant secretory proteins such as granins; and processing enzymes such as prohormone convertases PC1/3 and PC2. We demonstrated that only PC2 has the requisite specificity to liberate the pentapeptide enkephalins from proenkephalin, while PC1/3 produces the larger, mu receptor-active proenkephalin-derived peptides such as Peptide F. We have defined the cellular regulation of convertase activity; established enzyme crystal structures; and identified activators and inhibitors through various pharmacological collaborations. The widespread involvement of proprotein convertases in the physiology of nearly every tissue means that our work is linked to a variety of pathological processes, including obesity and diabetes. Our work has resulted in the publication of 139 peer-reviewed papers and 17 reviews/book chapters which have collectively been cited over 5400 times to date (Google Scholar): (http://www.ncbi.nlm.nih.gov/sites/myncbi/collections/bibliography/40560230/)

The current proposal to investigate the cell biology of human PC1/3 mutants with regard to POMC processing in obesity is a logical progression of our early work detailing the biosynthetic pathway of this convertase (given in section **C1** below). Though this work we acquired a number of valuable tools relevant to the study of PC1/3, including monoclonal and polyclonal antisera; human and mouse cDNA vectors; and recombinant proteins. Our collaboration with Dr. M. Martin (UCLA) during the past four years has introduced us to the fascinating physiology of human *PCSK1* mutations, and led to a number of collaborative projects with this group (described in **C5** below). Our very recent studies showing that mouse *pcsk1* mutations affect both the half-life and targeting of wild-type PC1/3 protein form the basis of the work described here. Our efforts in this area were recognized by an editorial highlighting our work in the July 2014 Endocrinology issue and an invitation to submit a review in the well-regarded book series shown below.

a. Blanco, E.H., Ramos-Molina, B. and **Lindberg, I.** (2015) Revisiting PC1/3 mutants: dominant-negative effect of endoplasmic reticulum-retained mutants. Endocrinology, *in press.*

b. Ramos-Molina, B., Martin, M.G., and **Lindberg, I.** (2015) "*PCSK1* variants and human obesity", in Genetics of Monogenic and Syndromic Obesity, in the series *Progress in Molecular Biology and Translational Science*P. Michael Conn, Ed; Academic Press. *in press.*

### B. Positions and Honors

9-75- 12-80 Graduate research assistant in Pharmacology, University of Wisconsin- Madison
1-81 - 6-81 Individual NIH F32 postdoctoral trainee, NIMH (Dr. Erminio Costa)
6-81 - 9-84 Pharmacology Research Associate Traineeship, NIMH
9-84 - 6-89 Assistant Professor of Biochemistry and Molecular Biology at Louisiana Health Sciences Ctr.
7-89 - 6-94 Associate Professor of Biochemistry and Molecular Biology; member, Neuroscience Center
7-94- 8/07 Professor of Biochemistry and Molecular Biology; Neuroscience Center member
8/07- present Professor, Department of Anatomy and Neurobiology, University of Maryland Medical School; Secondary appointment, Department of Biochemistry; Greenebaum Cancer Center Member

**Awards:** Pharmacology Research Associate Traineeship, 1981-1984; Research Career Development Award, 1988-1993; Research Scientist Development Award, 1993-1998; 1998-2003
**Scientific Societies**: ASBMB, 1985 – present; Society for Neuroscience, 1982- present; WCBR, 1985-present; Endocrine Society, 2001- present
**Study section service**: *ad hoc* reviewer on NLS-1, 1994, 1995, 1996; NIDA-B, 10/97; DK Program Project Review 12/98; ACS, 6/99; MCDN5, 12/02; Endocrinology, 1989, 1990, 1995; 10/00; 2/02; 6/02; 2/03;10/03; 6/04; 10/04; 6/05; 2/06; 2/07; Eureka SEP 3/2011; SBIR, 3/2013; MCE 6/2015
*Standing member*, Endocrinology (renamed MCE) 1996- 2000 and 2006- 2009
**Reviewer, international grants:** Finnish National Academy of Sciences; Canada Research Chairs; Wellcome Trust; Belgian Foundation for Scientific Research; Canadian Institutes of Health Research; Danish Research Council
**Editorial Board,** J. Biol. Chem. 2000 – 2005; 2012- 2017
**Chair**, Gordon Conference on Proprotein Processing, Trafficking, and Secretion (formerly Hormonal and Neural Peptide Synthesis) 2004; GRC Proprotein Processing Advisory Committee 2002, 2006, 2008, 2010, 2014, 2016)

**Patents:**
1. Patent # 6,548,736 on the 7B2 null mouse as a model for pituitary Cushing's was granted to C.H. Westphal, **I. Lindberg**, and P. Leder.
2. Patent # 7,033,991 on polyarginine furin inhibitors in inhibiting bacterial disease and cancer was granted on April 25, 2006 to **I. Lindberg**, A. Cameron, J. Appel, and R.A. Houghten.

**C. Contributions to Science**

**C1. Proprotein convertase biosynthesis and structure.** The novel family of enzymes known as the proprotein convertases contains two members, PC1/3 and PC2, which are almost exclusively expressed in endocrine and neural tissues and function in the proteolytic maturation of peptide precursors such as proenkephalin and POMC. Our contribution to this field (largely supported by NIDA) was to biochemically characterize PC1/3 and PC2 and to describe the biosynthetic pathway of PC1/3 in both endocrine and constitutive cell lines. We demonstrated that the active enzyme is first produced as a stable 87 kDa precursor which gives rise to a much more active but unstable C-terminally truncated form. We showed using antisense methodology that only PC2 is able to generate opioid-active enkephalins from proenkephalin. We used the dihydrofolate reductase-coupled method of overexpression to massively overexpress and purify this protein, as well as the related convertase furin, in CHO cells. Our soluble furin preparation yielded the first structure of a proprotein convertase. The furin structure, still one of only two – the yeast protein was crystallized almost simultaneously – has guided structural analyses and inhibitor development of all convertases during the past dozen years. We later contributed to the structural biochemistry of proPC2 by establishing that proPC2 activation is a pH-dependent process that occurs spontaneously within secretory granules. The following four original research papers have been cited 632 times at this time, supporting their importance to the field.

a. Vindrola, O., and **Lindberg, I.** (1992) Biosynthesis of the prohormone convertase mPC1 in AtT-20 cells. Mol. Endocrinol. 6, 1088-1094.

b. Zhou, Y., and **Lindberg, I.** (1993) Purification and characterization of the prohormone convertase PC1 (PC3) J. Biol. Chem. 268, 5615- 5623.

c. Lamango, N., Zhu, X., and **Lindberg, I.** (1996) Purification and enzymatic characterization of recombinant PC2: stimulation of activity by 21 kDa 7B2. Arch. Biochem. Biophys. 330, 238-250.

d. Henrich, S., Cameron, A., Bourenkov, G.P., Kiefersauer, R., Huber, R., **Lindberg, I.,** Bode, W., and Than, M.E. (2003) The crystal structure of the proprotein processing proteinase furin explains its stringent specificity. Nature Structural Biology 10, 520-526.

**C2. Identification of potent convertase inhibitors.** The only convertase inhibitor available for over a decade was a highly cytotoxic chloromethyl ketone-containing compound. We discovered that polyarginine-rich peptides represent potent furin inhibitors that can be used *in vitro*; in cell lines; and in animals to combat disease – for example anthrax and *Pseudomonas* toxicity. Our stable polyarginine inhibitor, hexa-D-arginine amide (D6R), is now commercially available and is widely used by others as a convertase inhibitor (*e.g*. Virol. J. 11:165, 2014). Our recent work shows that popular cationic protein transduction agents, such as Chariot and the TAT-derived peptide, also represent potent furin inhibitors (Ramos-Molina *et al*., *in press.*) We have also collaborated with various institutes, companies, and research groups to identify novel furin and prohormone convertase inhibitors; these collaborations have collectively resulted in 23 papers to date, and include small molecule inhibitors of PC1/3 and PC2 in addition to furin. Perhaps the most successful collaboration in this regard was our 1998 collaboration with the Torrey Pines Institute for Molecular Studies, in which we screened a hexapeptide combinatorial library for inhibitors of our recombinant PC1/3. Two years later, the exact sequence of the best inhibitory hexapeptide we identified, LLRVKR, was identified within the natural PC1/3 inhibitor, proSAAS. The four inhibitor papers listed below have been cited 368 times to date, with the polyarginine papers gaining steady interest over the years with increasing use of polyarginines as protein transduction tags. We have patented polyarginine convertase inhibitors as potential anti-bacterial and anti-cancer therapeutics.

a. Apletalina, E., Appel, J., Lamango, N.S., Houghten, R., and **Lindberg, I.** (1998) Identification of potent inhibitors of prohormone convertases 1 and 2 using a peptide combinatorial library. J. Biol. Chem. 273, 26589-26595.

b. Cameron, A., Appel, J., Houghten, R.A. and **Lindberg, I.** (2000) Polyarginines are potent furin inhibitors. J. Biol. Chem. 75, 36741-36749.

c. Sarac, M.S., Peinado, J.R., Leppla, S.H., and **Lindberg, I**. (2004) Protection against anthrax toxemia by hexa-D-arginine *in vitro* and *in vivo*. Infection and Immunity 72, 602-605.

d. Kacprzak, M., Peinado, J.R., Than, M., Appel, J., Henrich, S., Bode, W., Houghten, R.A., and **Lindberg, I.** (2004) Inhibition of furin by polyarginine-containing peptides: nanomolar inhibition by nona-D-arginine. J. Biol. Chem. 279, 36788-9463

**C3. Production of active PC2 requires the 7B2 protein.** Surprisingly, CHO cell overexpression of the convertase proPC2 resulted only in catalytically inert protein; our enzymatic studies to characterize this enzyme therefore first focused on immunopurified enzyme. In collaboration with the Martens group, we found that the neural and endocrine chaperone protein 7B2 represents a nanomolar inhibitor of active PC2, with all of its inhibitory activity contained within the C-terminal 31-residue peptide. We discovered that co-expression of proPC2 together with the remaining 21 kDa domain of 7B2 is both necessary and sufficient to produce enzymatically active PC2. Structure-function studies of 7B2 revealed that it contains a 36-residue internal peptide with specific biochemical features which maintain proPC2 in an activatable state. We then showed that in the absence of 7B2, proPC2 becomes severely aggregated; it is that aggregation which renders proPC2 incapable of maturation to an enzymatically competent species. The work is significant because it demonstrates that 7B2 levels control the activity of this important enzyme; in agreement, we and others later showed that circulating glucagon levels vary in mice expressing different amounts of 7B2.

In 1998 we were contacted by Dr. Philip Leder (Harvard) who had created a 7B2 knockout mouse that inexplicably died between 5-8 weeks of age. Upon receipt of the mice, I noticed an unusual dorsal fat pad reminiscent of the "buffalo hump" present in patients with Cushing's disease. This observation led us to diagnose multiple endocrinological problems, all of which originate from overproduction of intermediate lobe ACTH (due to the inability of PC2 to cleave - and thereby inactivate- ACTH); and the lack of glucagon, a PC2-specific product. The high circulating levels of ACTH in this null mouse result in adrenal cortex hypertrophy, generating corticosterone levels 50-fold above normal. Given mouse's total inability to generate glucagon this severe endocrine dysbalance eventually culminates in a lethal hypoglycemic crisis. Our group showed that adrenalectomy can rescue the 7B2 null; that intrapituitary adenoviral administration of 7B2 extends life span and partially reverses endocrinological deficiencies; and that the lethal phenotype of the 7B2 knockout is highly strain-specific. The 7B2 knockout mouse was jointly patented by the Leder group and our group as a partial

model for Cushing's disease, and the animals archived as a public resource at Jackson Laboratories. The following four papers have collectively been cited over 550 times.

a. Martens, G.M., Braks, A.M., Eib, D., Zhou, Y., and **Lindberg, I.** (1994) The neuroendocrine polypeptide 7B2 is a naturally occurring inhibitor of the prohormone convertase PC2. Proc. Nat. Acad. Sci. 91, 5784-5785.

b. Zhu, X., and **Lindberg, I.** (1995) 7B2 facilitates the maturation of proPC2 in neuroendocrine cells and is required for the expression of enzymatic activity. J. Cell Biol. 129, 1641-1650.

c. Muller, L., Zhu, X., and **Lindberg, I.** (1997) Mechanism of facilitation of PC2 maturation by 7B2: involvement in PC2 transport and activation, but not folding. J. Cell. Biol. 139, 625-638.

d. Westphal, C.H., Muller, L., Zhou, A., Zhu, X., , Bonner-Fraser, S., Schambelan, M., Steiner, D.F., **Lindberg, I.***, and Leder, P.* (1999) The neuroendocrine protein 7B2 is required for peptide hormone processing *in vivo* and provides a novel mechanism for pituitary Cushing's disease. Cell 96, 689-700. (*co-senior authors)

**C4. Anti-aggregant chaperones in the secretory pathway.** While the regulated secretory pathway is known to contain extremely high concentrations of proteins destined for export, no specialized anti-aggregant proteins had been identified within the lumen of this particular pathway. Based on our work demonstrating that 7B2 is a highly effective anti-aggregant for proPC2, we surmised that it might also represent a general secretory anti-aggregant. This hypothesis was borne out in experiments using Abeta and other fibrillating secretory peptides and proteins such as human islet amyloid polypeptide and alpha synuclein. We later found that proSAAS, the PC1/3 binding protein whose expression is restricted to neural, neuroendocrine, and endocrine tissues, is also an effective protein anti-aggregant. These results may explain the much wider brain distribution of proSAAS and 7B2 than of the two prohormone convertases. Our recent work has shown that 7B2 is an intrinsically disordered protein which itself forms oligomers; we believe that this oligomerization process plays an important role in its anti-aggregant activities. These results are significant in their identification of novel protein anti-aggregant chaperones that are specific to neural and endocrine tissues.

a. Dasgupta, I.*, Sanglas, L.*, Enghild, J. and **Lindberg I.** (2012) The neuroendocrine protein 7B2 is an intrinsically disordered protein. (*co-first authors) Biochemistry 51(38):7456-64. PMID:22947085

b. Helwig, M., Hoshino, A., Berridge, C., Lee, S.N., Lorenzen, N., Otzen, D., Eriksen, J., and **Lindberg, I**. (2013) The neuroendocrine protein 7B2 suppresses neurodegenerative disease-related protein aggregation. J. Biol. Chem. 288:1114–1124. PMID: 23172224

c. Peinado, J.R., Sami, F., Rajpurohit, N., and **Lindberg, I.** (2013) Blockade of islet amyloid polypeptide fibrillation and cytotoxicity by the secretory chaperones 7B2 and proSAAS. FEBS Lett., Nov 1;587(21):3406-11 PMID: 24042052

d. Hoshino, A., Helwig, M., Rezaei, S., Berridge, C., Eriksen, J.L., and **Lindberg, I**. (2013) A novel function for proSAAS as an amyloid anti-aggregant in Alzheimer's disease. J. Neurochem. 128(3):419-30 PMCID: PMC3946950

**C5. Mutations in PC1/3 result in deleterious dominant-negative interactions.** Mutations in secretory proteins which result in poorly folded proteins are responsible for a variety of genetic diseases, for example the proinsulin mutations which cause neonatal diabetes. Because many secretory proteins naturally form oligomers, folding mutations can also result in deleterious dominant-negative effects on wild-type proteins. We showed that PC1/3 also naturally oligomerizes, and demonstrated that a portion of the N222D mouse mutant PC1/3 protein undergoes endoplasmic reticulum (ER) retention and increased ER-associated degradation. Further, when mutant protein oligomerizes with wild-type PC1/3, both become degraded. These findings of dominant-negative interactions are significant in that they explain the obesity which occurs in N222D PC1/3 heterozygote mice -- but not in PC1/3 knockout heterozygotes.

Our ongoing collaboration with the clinical group of Dr. M. Martin (UCLA) has shown that *PCSK1* mutations are responsible for the loss of PC1/3 enzyme activity in a pediatric cohort who first exhibit severe malabsorptive diarrhea, and then go on to develop obesity. We demonstrated that the most severe human cases possess inactivating mutations in PC1/3 which result in ER retention. Ongoing work reveals that these human mutant PC1/3 proteins also interact in a dominant-negative fashion with wild-type enzyme and cause ER stress. These results extend an underlying cause of human obesity to cell biological disturbances, rather than to a simple lack of PC1/3 activity, and this phenomenon will be further explored in this application.

a. Hoshino, A., Kowalska, D., Jean, F., Lazure, C., and **Lindberg, I.** (2011) Modulation of PC1/3 activity by self-interaction and substrate binding. Endocrinology 152:1402-11 PMID:21303942

b. Pickett, L.A., Yourshaw, M., Chen, Z. Solorzano-Vargas, R.S., Nelson, S.F., Martín, M.G., and **Lindberg, I.** (2013) Functional consequences of a novel variant of *PCSK1*. PLoS One 8(1):e55065. PMCID: PMC3557230

c. Martín, M.G., **Lindberg, I**, Solorzano-Vargas, R.S., Wang, J., Avitzur, Y., Bandsma, R., Sokollik, C., Lawrence, S., Pickett, L.A., Chen, Z., Egritas, O., Dalgic, B., Albornoz, V., de Ridder, L., Hulst, J., Gok, F., Aydoğan, A., Al-Hussaini, A., Gok, D.E., Yourshaw, M., Wu, S.V., Cortina, G., Stanford, S., and Georgia, S. (2013) Congenital proprotein convertase 1/3 deficiency causes malabsorptive diarrhea and other endocrinopathies in a pediatric cohort. Gastroenterology 45:138-48 PMCID:PMC3719133

d. Prabhu, Y., Blanco, E.H., Liu, M, Peinado, J.R., Wheeler, M., Gekakis, N., Arvan, P. and **Lindberg, I.** (2014) Defective transport of the obesity mutant PC1/3 N222D contributes to loss of function. Endocrinology 155:2391-401 PMID: 24828610

## D. Research Support

### *Ongoing Research Support*

**The Secretory Chaperone 7B2 as an Endogenous Regulator of Amyloid Pathology** 9/2014 - 4/2016
R21 AG045741-01 I. Lindberg (PI)
NIH/NIA
This 2-year grant is to explore the idea that brain 7B2 levels modulate the pathologic aggregation of beta amyloid-derived peptides by crossing 7B2 null and transgenic mice with AD-model mice. **NO OVERLAP.**

### *Completed Research Support in Last Five Years*

**Opioid Peptide Synthesizing Enzymes** 4/1988- 2/2016
R01 DA05084-27 I. Lindberg (PI)
NIH/NIDA
This grant was to study the regulation of PC1/3 activity through oligomerization, and the role of proSAAS in mediating this process; to crystallize PC1/3; and to identify small molecule convertase inhibitors using combinatorial compound screening and molecular modeling. More recently we have examined the cell biology of mouse and human PC1/3 mutants. The 2010-2015 cycle of this grant (19% priority score, not currently funded) provided sole support for 29 papers and reviews, including 10 research papers from our laboratory.

**Control of Peptide Hormone Biosynthesis by PC2 and 7B2**
R01 DK49703-16 I. Lindberg (PI) 9/1996 - 3/2014
NIH/NIDDK
This grant was to investigate the role of the neuroendocrine protein 7B2 in PC2-mediated peptide hormone synthesis. **(see C3 above).**

**De-Orphanizing the Peptidome**
R01 DA27170-05 I. Lindberg and B. Roth (co-PIs) 7/2009 - 6/2014
NIH/NIDA
This grant attempted to identify novel ligand-receptor pairs through systematic screening of novel and known peptide products against known and orphan GPCRs**.**