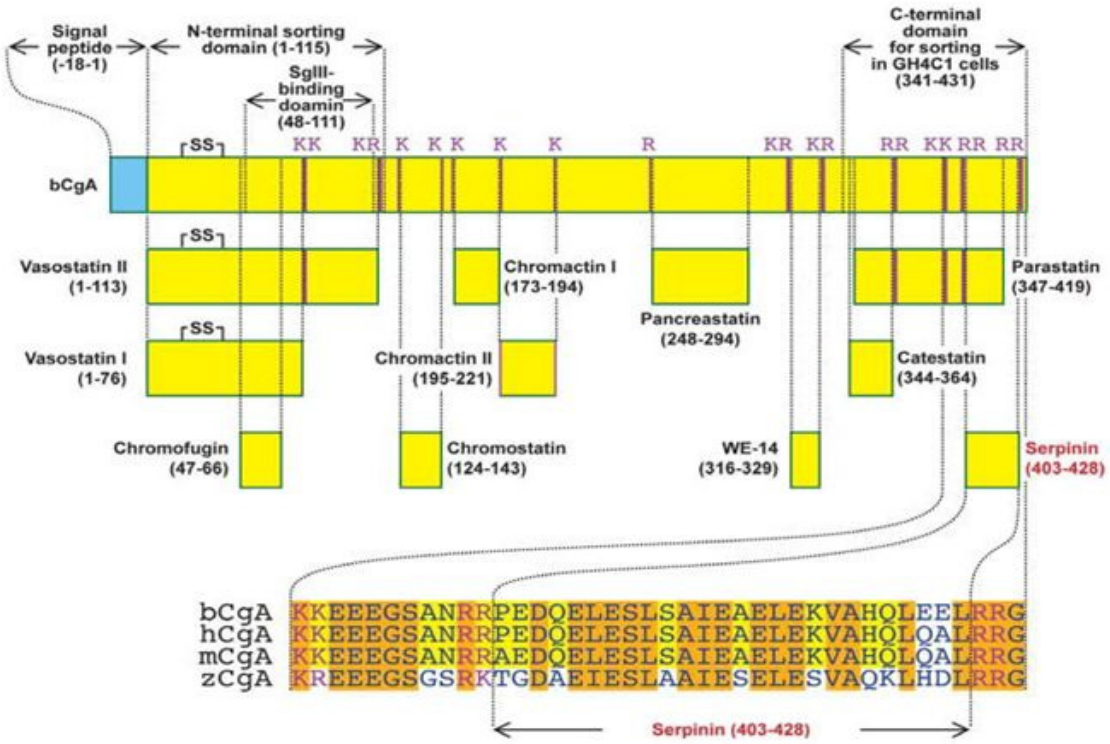
Serpinin

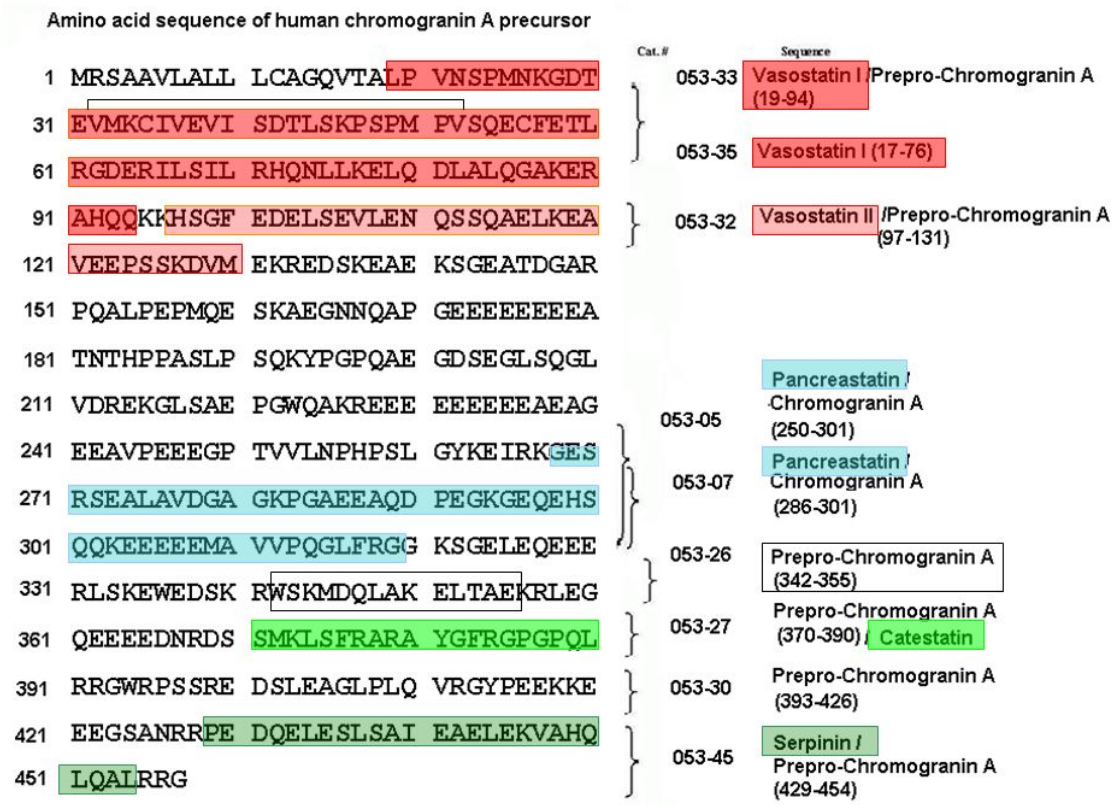
Chromogranin A-derived, secreted peptide up-regulates Nexin-1 and granule biogenesis



Serpinin: A Novel Chromogranin A-Derived, Secreted Peptide Up-Regulates Protease Nexin-1 Expression and Granule Biogenesis in Endocrine Cells.

Previously we demonstrated that chromogranin A (CgA) promoted secretory granule biogenesis in endocrine cells by stabilizing and preventing granule protein degradation in the Golgi, through up-regulation of expression of the protease inhibitor, protease nexin-1 (PN-1). However, the mechanism by which CgA signals the increase of PN-1 expression is unknown. Here we identified a 2.9-kDa CgA-C-terminus peptide, which we named serpinin, in conditioned media from AtT-20 cells, a corticotroph cell line, which up-regulated PN-1 mRNA expression. Serpinin was secreted from AtT-20 cells upon high potassium stimulation and increased PN-1 mRNA transcription in these cells, in an actinomycin D-inhibitable manner. CgA itself and other CgA-derived peptides, when added to AtT-20 cell media, had no effect on PN-1 expression. Treatment of AtT-20 cells with 10 nm serpinin elevated cAMP levels and PN-1 mRNA expression, and this effect was inhibited by a protein kinase A inhibitor, 6-22 amide. Serpinin and a cAMP analog, 8-bromo-cAMP, promoted the translocation of the transcription factor Sp1 into the nucleus, which is known to drive PN-1 expression. Additionally, an Sp1 inhibitor, mithramycin A inhibited the serpinin-induced PN-1 mRNA up-regulation. Furthermore, a luciferase reporter assay demonstrated serpinin-induced up-regulation of PN-1 promoter activity in an Sp1-dependent manner. When added to CgB-transfected 6T3 cells, a mutant AtT20 cell line, serpinin induced granule biogenesis as evidenced by the presence of CgB puncta accumulation in the processes and tips. Our findings taken together show that serpinin, a novel CgA-derived peptide, is secreted upon stimulation of corticotrophs and plays an important autocrine role in up-regulating PN-1-dependent granule biogenesis via a cAMP-protein kinase A-Sp1 pathway to replenish released granules.

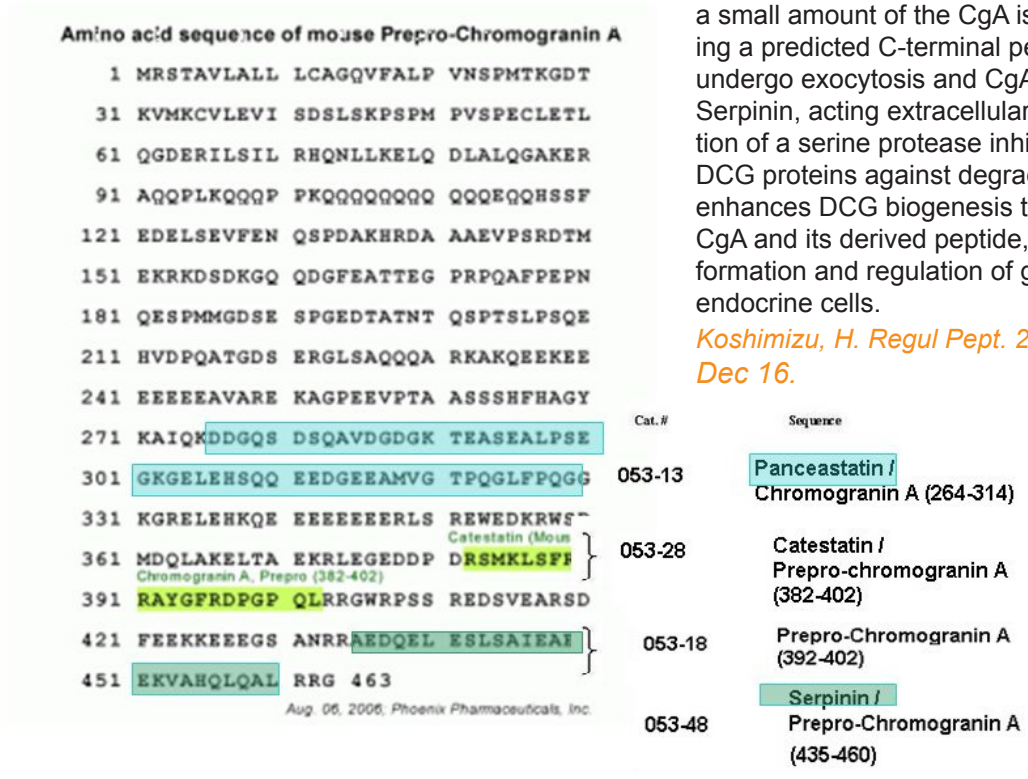
Koshimizu H, Mol Endocrinol. 2011 Mar 24. [Epub ahead of print]

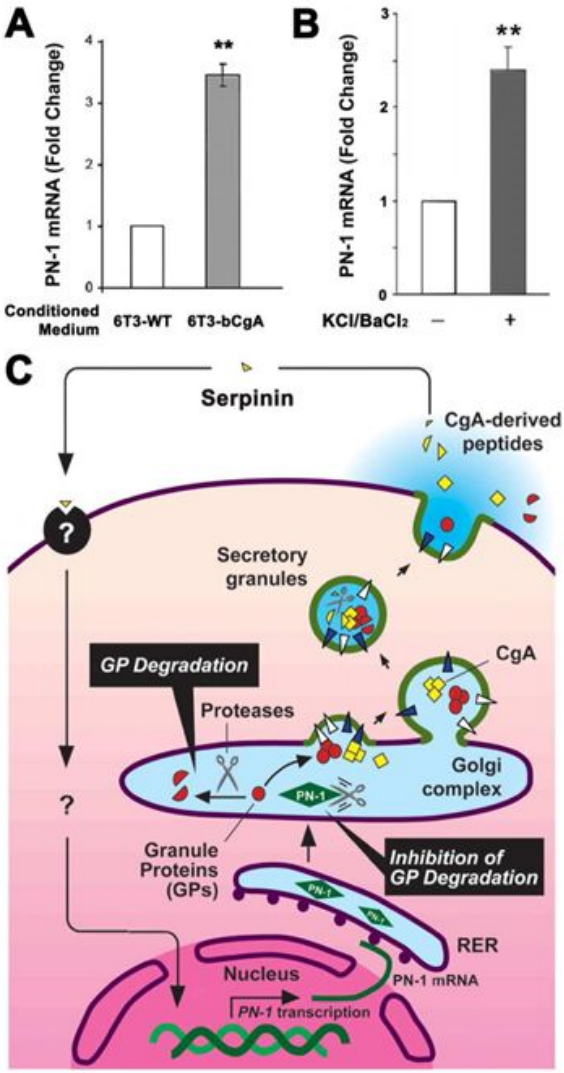


Chromogranin A: a new proposal for trafficking, processing and induction of granule biogenesis.

Chromogranin A (CgA), a member of the granin family serves several important cell biological roles in (neuro)endocrine cells which are summarized in this review. CgA is a “prohormone” that is synthesized at the rough endoplasmic reticulum and transported into the cisternae of this organelle via its signal peptide. It is then trafficked to the Golgi complex and then to the trans-Golgi network (TGN) where CgA aggregates at low pH in the presence of calcium. The CgA aggregates provide the physical driving force to induce budding of the TGN membrane resulting in dense core granule (DCG) formation. Within the granule, a small amount of the CgA is processed to bioactive peptides, including a predicted C-terminal peptide, serpinin. Upon stimulation, DCGs undergo exocytosis and CgA and its derived peptides are released. Serpinin, acting extracellularly is able to signal the increase in transcription of a serine protease inhibitor, protease nexin-1 (PN-1) that protects DCG proteins against degradation in the Golgi complex, which then enhances DCG biogenesis to replenish those that were released. Thus CgA and its derived peptide, serpinin, plays a significant role in granule formation and regulation of granule biogenesis, respectively, in (neuro) endocrine cells.

Koshimizu, H. Regul Pept. 2010 Feb 25;160(1-3):153-9. Epub 2009 Dec 16.





A. CgA-dependent up-regulation of PN-1 mRNA expression in pituitary cell lines. Bar graphs show the effect of 20h treatment of 6T3-WT cells with conditioned medium from 6T3-WT cells, which lack CgA expression, or 6T3-bCgA cells, which express CgA, on PN-1 mRNA expression. Cells treated with 6T3-bCgA cell-conditioned medium showed a significant increase in PN-1 mRNA expression (3.30 ± 0.17 fold, ± SEM, $^{**}P < 0.01$, N = 3) relative to cells treated with 6T3-WT cell-conditioned medium (1.00 fold as control, N =3). **B.** AtT-20 cells were stimulated with 50 mM KCl/2mM BaCl2. The bar graph shows that the fold change in PN-1 mRNA of stimulated cells was 2.40 ± 0.24 (± SEM, N = 3, $^{*}P < 0.05$) relative to unstimulated cells (1.00 fold as control, N = 3). **C.** Model for serpinin-inducing PN-1-dependent granule biogenesis in (neuro)endocrine cells. CgA is proteolytically cleaved to form serpinin which is secreted in an activity-dependent manner. Secreted serpinin binds to a cognate receptor and up-regulates *PN-1* transcription. The increase in PN-1 protein stabilizes the secretory granule proteins at the Golgi apparatus to increase their levels which then promotes biogenesis of dense core granules.

From Koshimizu H. et al. Published in Regul Pept. 2010 Feb 25;160(1-3):153-9.

Catalog Number	Product Name	Standard Size
053-45	Serpinin / Prepro-Chromogranin A (429-454) (Human)	100ug
053-48	Serpinin/ Prepro-Chromagranin A (435-460) (Rat, Mouse)	100ug
B-053-45	Serpinin/ Prepro-Chromogranin A (429-454) (Human)- Biotin labled	100ug
T-053-47	Serpinin [Tyr0] / Prepro-Chromogranin A (429-454) [Tyr0] (Human)-I-125 Labled	10 µCi
T-053-49	Serpinin [Tyr0] / Prepro-Chromagranin A (435-460) [Tyr0] (Rat, Mouse)-I-125-Labeled	10 uCi

Phoenix Pharmaceuticals, Inc.
330 Beach Road, Burlingame CA, 94010, USA
Phone: **(650) 558-8898** email: **info@phoenixpeptide.com**
www.phoenixpeptide.com

Phoenix Europe GmbH
Viktoriastrasse 3-5, D-76133 Karlsruhe, Germany
phone: **+49-721-1611950** email: **germany@phoenixpeptide.com**
www.phoenixpeptide.com