INTRODUCTION

# THE EVER EXPANDING SAGA OF THE PROPROTEIN CONVERTASES: FROM BENCH TO BEDSIDE

NABIL G. SEIDAH

*Laboratory of Biochemical Neuroendocrinology, Institut de Recherches Cliniques de Montreal, 110 pine Ave West, Montreal, Quebec, Canada H2W 1R7*

The number of protein/peptide products that result from a given genome depends on multiple factors that generate both diversity and specificity. Prominent among these are processes that regulate post-translational modifications of the primary product of mRNA translation, the precursor protein. The primary events governing the modification of the amino acid chain of secretory proteins include the N-glycosylation and signal peptide cleavage by signal peptide peptidase. This is then followed by trimming of the glycosylation chain and remodeling up until it reaches its final form in the Trans Golgi Network (TGN). Since the early/mid 1960s it was realized that most secretory proteins undergo at least one peptide bond cleavage along their trafficking pathway, e.g., by signal peptide peptidase in the endoplasmic reticulum (ER) and/or by one or more proteinase in the Golgi apparatus to release the final form of the protein and/or its processing products. Proteolysis is essentially an irreversible process, because no known enzyme can repair broken peptide bonds under normal physiological conditions. The primary event of peptide bond cleavage induces conformational changes in the resulting product, thereby generating productive biological activity. The repertoire of the secretory protein precursors that undergo limited proteolysis is large and varied. It includes many proteins that are translocated across membranes such as polypeptide endocrine and neural hormones, growth factors and their receptors, membrane bound transcription factors, adhesion molecules, extracellular matrix proteins, proteases and other types of enzymes, as well as a number of surface glycoproteins of opportunistic pathogenic viruses and bacteria.

While it is predicted that the mammalian genome codes for 460 human and 525 mouse functional proteases [1], only a handful of these are implicated in the intracellular limited proteolysis of precursor proteins.



*A-Majid Khatib (ed.), Regulation of Carcinogenesis, Angiogenesis and Metastasis by the Proprotein Convertases*, 1–5.

Prominent amongst the proprotein processing enzymes are the members of the family of subltilisin/kexin-like proprotein convertases (PCs). It took more than 15 years to identify these serine proteinases that can be subdivided into three subfamilies: [**A**] The basic amino acid specific kexin-like PCs include seven members: PC1/3, PC2, Furin, PC4, PC5/6, PACE4 and PC7 [2]; [**B**] The pyrolysin-like subtilisin-kexin isoform SKI-1/S1P, also known as site 1 protease S1P [3]; and [**C**] The proteinase K-like neural apoptosis regulated convertase NARC-1/PCSK9 [4]. The last two convertases cleave at non-basic residues and process precursors that are distinct form those of the basic amino acid-specific convertases [3–6].

The discovery of these convertases from 1989–2003, elicited a wide interest in the scientific community as it was realized that these enzymes play key roles in various homeostatic as well as pathogenic events [2, 5–10]. The most evident role came from studies of the tumorigenic potential of these convertases, where it was shown that overexpression of one or more of the basic amino acid specific PCs leads to increased cell proliferation and enhanced metastasis, while their inhibition reverses this effect [11–14]. However, this is not universally the case, as a decreased expression of the Cys-rich domain containing PC5 [15, 16] and PACE4 [17] has been observed in various cancers including breast and ovarian cancers, as well as the increased metastatic potential of the human colon carcinoma HT-29 cells overexpressing $\alpha$1-PDX, a potent inhibitor of the constitutively secreted convertases [18].

On another front, the implication of the PCs in viral infections became apparent from the processing sites of the surface glycoproteins of infectious viruses and of bacterial toxins [19]. In fact, data on various infectious viruses and bacterial toxins showed that cleavage of surface/spike glycoprotein precursors of these pathogens by one or more member of the PC-family, including the basic amino acid- specific Furin, PC7, PACE4 and/or PC5 (2) and the pyrolysin-like SKI-1/S1P (20) is a required step for the acquisition of fusiogenic potential and thus for their infectious and/or cell-cell spreading capacity [19, 21].

Recently, some of the convertases such as PC5/6, SKI-1/S1P and NARC-1/PCSK9, were implicated in cardiovascular complications. Examples include the vital role of SKI-1/S1P in the regulation of the synthesis of cholesterol and fatty acids via the cleavage within the Golgi of the two master switches of sterol, and fatty acid metabolism, the sterol regulatory element binding proteins [SREBP-1 and SREBP-2] [22, 23]. The convertase PC5/6 has also been implicated in vascular remodeling and the development of atherosclerosis [24, 25], as well as in the phenomenon known as restenosis that occurs following balloon angioplasty or stint implantation [26]. In addition, PC5/6, which is highly expressed in endothelial cells [27, 28] has been implicated in the activation of endothelial lipase, and hence could positively regulate the level of high density lipoproteins (HDL) [29].

Finally, the last member of the family NARC-1/PCSK9 has clearly been associated with the development of dyslipidemias, as specific mutations in its coding sequence are directly responsible for the development of a dominant form of either familial hyper-cholesterolemia [5] or hypo-cholesterolemia [30]. This is

the first case of a dominant disease associated with mutations in one of the PCs. It seems that these mutations [6] result in either a gain/enhancement of an existing function, for those causing hyper-cholesterolemia [5], or in a loss of function in hypo-cholesterolemia patients [30]. The mechanism behind these pathologies is essentially related to one of the major roles of NARC-1/PCSK9 which is to enhance the degradation of the low density lipoprotein receptor (LDLR) [31] through a mechanism requiring entry into low pH endocytotic vesicles [32]. This exciting development opens the way to the development of anti-cholesterogenic drugs that could supplement the widely prescribed HMG-CoA reductase inhibitors, known as "statins" that themselves upregulate the expression of NARC-1/PCSK9 [33]. Indeed, supplementation of statins to the diet of mice lacking the expression of *PCSK9*, resulted in a marked additional decrease in the level of circulating total cholesterol [34].

The present monogram deals with multiple aspects of the proprotein convertases, from their discovery, to their analysis and to the projected pharmacological and clinical applications that may result from the inhibition of these enzymes. Thus, this is one example of "bench to bedside" directly applicable to the convertases. It is hoped that the use of modern day multiplexing technologies including various RNA and protein/peptide arrays should result in the development of specific convertase inhibitors that should find applications to control a wide variety of pathologies, including cancer and associated metastasis as well as dyslipidemias such as atherosclerosis and hypercholeste-rolemia. The importance of the PCs in the self renewal and maintenance of cancer stem cells [35] is a future area that begs extensive investigation, as it may opens the door towards stem cell-specific targeting of convertase inhibition. It took more than 30 years to unravel some of the mysteries of the proprotein convertases. It is hoped that the next decade will consolidate and expand the genetic, cellular and molecular knowledge of the PCs, including their 3D structures [36], in order to rationally design potent drugs that regulate their levels and/or activities *in vivo*.

## REFERENCES

[1] Puente XS, Sanchez LM, Overall CM, Lopez-Otin C (2003) Human and mouse proteases: A comparative genomic approach. Nat Rev Genet **4**:544–558

[2] Seidah NG, Chretien M (1999) Proprotein and prohormone convertases: A family of subtilases generating diverse bioactive polypeptides. Brain Res **848**: 45–62

[3] Seidah NG, Prat A (2002) Precursor convertases in the secretory pathway, cytosol and extracellular milieu. Essays Biochem **38**:79–94

[4] Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, Basak A, Prat A, Chretien M (2003) The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): Liver regeneration and neuronal differentiation. Proc Natl Acad Sci USA **100**:928–933

[5] Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, Derre A, Villeger L, Farnier M, Beucler I, Bruckert E, Chambaz J, Chanu B, Lecerf JM, Luc G, Moulin P, Weissenbach J, Prat A, Krempf M, Junien C, Seidah NG, Boileau C (2003) Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet **34**:154–156

[6] Attie AD, Seidah NG (2005) Dual regulation of the LDL receptor—some clarity and new questions. Cell Metab **1**:290–292

[7] Seidah NG, Mowla SJ, Hamelin J, Mamarbachi AM, Benjannet S, Toure BB, Basak A, Munzer JS, Marcinkiewicz J, Zhong M, Barale JC, Lazure C, Murphy RA, Chretien M, Marcinkiewicz M (1999) Mammalian subtilisin/kexin isozyme SKI-1: A widely expressed proprotein convertase with a unique cleavage specificity and cellular localization. Proc Natl Acad Sci USA **96**:1321–1326

[8] Fugere M, Day R (2005) Cutting back on pro-protein convertases: The latest approaches to pharmacological inhibition. Trends Pharmacol Sci **26**:294–301

[9] Khatib AM, Siegfried G, Chretien M, Metrakos P, Seidah NG (2002) Proprotein convertases in tumor progression and malignancy: Novel targets in cancer therapy. Am J Pathol **160**:1921–1935

[10] López de Cicco R, Bassi DE, Zucker S, Seidah NG, Klein-Szanto AJ (2005) Human carcinoma cell growth and invasiveness is impaired by the propeptide of the ubiquitous proprotein convertase furin. Cancer Res **65**:4162–4171

[11] Bassi DE, Mahloogi H, Klein-Szanto AJ (2000) The proprotein convertases furin and PACE4 play a significant role in tumor progression. Mol Carcinog **28**:63–69

[12] Cheng M, Xu N, Iwasiow B, Seidah N, Chretien M, Shiu RP (2001) Elevated expression of proprotein convertases alters breast cancer cell growth in response to estrogen and tamoxifen. J Mol Endocrinol **26**:95–105

[13] Khatib AM, Siegfried G, Prat A, Luis J, Chretien M, Metrakos P, Seidah NG (2001) Inhibition of proprotein convertases is associated with loss of growth and tumorigenicity of HT-29 human colon carcinoma cells: Importance of insulin-like growth factor-1 (IGF-1) receptor processing in IGF-1-mediated functions. J Biol Chem **276**:30686–30693

[14] Siegfried G, Basak A, Cromlish JA, Benjannet S, Marcinkiewicz J, Chretien M, Seidah NG, Khatib AM (2003) The secretory proprotein convertases furin, PC5, and PC7 activate VEGF-C to induce tumorigenesis. J Clin Invest **111**:1723–1732

[15] Nour N, Mayer G, Mort JS, Salvas A, Mbikay M, Morrison CJ, Overall CM, Seidah NG (2005) The Cysteine-rich domain of the secreted proprotein convertases PC5A and PACE4 functions as a cell surface anchor and interacts with tissue inhibitors of metalloproteinases. Mol Biol Cell **16**:5215–5226

[16] Cheng M, Watson PH, Paterson JA, Seidah N, Chretien M, Shiu RP (1997) Pro-protein convertase gene expression in human breast cancer. Int J Cancer **71**:966–971

[17] Fu Y, Campbell EJ, Shepherd TG, Nachtigal MW (2003) Epigenetic regulation of proprotein convertase PACE4 gene expression in human ovarian cancer cells. Mol Cancer Res **1**:569–576

[18] Nejjari M, Berthet V, Rigot V, Laforest S, Jacquier MF, Seidah NG, Remy L, Bruyneel E, Scoazec JY, Marvaldi J, Luis J (2004) Inhibition of proprotein convertases enhances cell migration and metastases development of human colon carcinoma HT-29 cells in a rat model. Am J Pathol **164**:1925–1933

[19] Thomas G (2002) Furin at the cutting edge: From protein traffic to embryogenesis and disease. Nat Rev Mol Cell Biol **3**:753–766

[20] Lenz O, ter Meulen J, Klenk HD, Seidah NG, Garten W (2001) The Lassa virus glycoprotein precursor GP-C is proteolytically processed by subtilase SKI-1/S1P. Proc Natl Acad Sci USA **98**:12701–12705

[21] Bergeron E, Vincent MJ, Wickham L, Hamelin J, Basak A, Nichol ST, Chretien M, Seidah NG (2005) Implication of proprotein convertases in the processing and spread of severe acute respiratory syndrome coronavirus. Biochem Biophys Res Commun **326**:554–563

[22] Brown MS, Ye J, Rawson RB, Goldstein JL (2000) Regulated intramembrane proteolysis: A control mechanism conserved from bacteria to humans. Cell **100**:391–398

[23] Pullikotil P, Vincent M, Nichol ST, Seidah NG (2004) Development of protein-based inhibitors of the proprotein of convertase SKI-1/S1P: Processing of SREBP-2, ATF6, and a viral glycoprotein. J Biol Chem **279**:17338–17347

[24] Stawowy P, Kallisch H, Kilimnik A, Margeta C, Seidah G, Chretien M, Fleck E, Graf K (2004) Proprotein convertases regulate insulin-like growth factor 1-induced membrane-type 1 matrix metalloproteinase in VSMCs via endoproteolytic activation of the insulin-like growth factor-1 receptor. Biochem Biophys Res Commun **321**:531–538

[25] Stawowy P, Marcinkiewicz J, Graf K, Seidah N, Chretien M, Fleck E, Marcinkiewicz M (2001) Selective expression of the proprotein convertases furin, pc5, and pc7 in proliferating vascular smooth muscle cells of the rat aorta in vitro. J Histochem Cytochem **49**:323–332

[26] Veinot JP, Prichett-Pejic W, Picard P, Parks W, Schwartz R, Seidah NG, Chretien M (2004) Implications of proprotein Convertase 5 (PC5) in the arterial restenotic process in a porcine model. Cardiovasc Pathol **13**:241–250

[27] Beaubien G, Schafer MK, Weihe E, Dong W, Chretien M, Seidah NG, Day R (1995) The distinct gene expression of the pro-hormone convertases in the rat heart suggests potential substrates. Cell Tissue Res **279**:539–549

[28] Essalmani R, Marcinkiewicz E, Chamberland A, Mbikay M, Chretien M, Seidah NG, Prat A (2006) Genetic deletion of PC5/6 leads to early embryonic lethality. Mol Cell Biol **26**:354–61

[29] Jin W, Fuki IV, Seidah NG, Benjannet S, Glick JM, Rader DJ (2005) Proprotein covertases are responsible for proteolysis and inactivation of endothelial lipase. J Biol Chem **280**:36551–36559

[30] Cohen J, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH (2005) Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nat Genet **37**:161–165

[31] Maxwell KN, Breslow JL (2004) Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype. Proc Natl Acad Sci USA **101**:7100–7105

[32] Benjannet S, Rhainds D, Essalmani R, Mayne J, Wickham L, Jin W, Asselin MC, Hamelin J, Varret M, Allard D, Trillard M, Abifadel M, Tebon A, Attie AD, Rader DJ, Boileau C, Brissette L, Chretien M, Prat A, Seidah NG (2004) NARC-1/PCSK9 and Its Natural Mutants: Zymogen cleavage and effects on the low density lipoprotein (LDL) receptor and LDL cholesterol. J Biol Chem **279**:48865–48875

[33] Dubuc G, Chamberland A, Wassef H, Davignon J, Seidah NG, Bernier L, Prat A (2004) Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. Arterioscler Thromb Vasc Biol **24**:1454–1459

[34] Rashid S, Curtis DE, Garuti R, Anderson NN, Bashmakov Y, Ho YK, Hammer RE, Moon YA, Horton JD (2005) Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9. Proc Natl Acad Sci USA **102**:5374–5379

[35] Wang JC, Dick JE (2005) Cancer stem cells: Lessons from leukemia. Trends Cell Biol **15**:494–501

[36] Henrich S, Cameron A, Bourenkov GP, Kiefersauer R, Huber R, Lindberg I, Bode W, Than ME (2003) The crystal structure of the proprotein processing proteinase furin explains its stringent specificity. Nat Struct Biol **10**:520–526

CHAPTER 1

# DISCOVERY OF THE PROPROTEIN CONVERTASES AND THEIR INHIBITORS

ABDEL-MAJID KHATIB[1], NATHALIE SCAMUFFA[1], FABIEN CALVO[1], MICHEL CHRÉTIEN[2] AND NABIL G. SEIDAH[3]

[1] *INSERM U 716/AVENIR, Institut de Génétique Moléculaire, Paris, France*
[2] *Regional Protein Chemistry Centre, Diseases of Ageing Unit, Ottawa Health Research Institute, Loeb Building, 725 Parkdale Ave., Ottawa, Ontario, Canada*
[3] *Laboratory of Biochemical Neuroendocrinology, Institut de Recherches Cliniques de Montréal, 110 pine Ave West, Montreal, Quebec, Canada*

**Abstract:** The members of the convertase family play a central role in the processing of various protein precursors ranging from hormones and growth factors to viral envelope proteins and bacterial toxins. The proteolysis of these precursors that occurs at basic residues is mediated by the proprotein convertases (PCs), namely: PC1, PC2, Furin, PACE4, PC4, PC5 and PC7. The proteolysis at non-basic residues is performed by subtilisin/kexin-like isozyme-1 (S1P/SKI-1) and the newly identified neural apoptosis-regulated convertase-1 (NARC-1/PCSK9). These proteases have key roles in many physiological processes and various pathologies including cancer, obesity, diabetes, neurodegenerative diseases and autosomal dominant hypercholesterolermia. Here we summarize the discovery of the proprotein convertases and their inhibitors, discuss their properties, roles, resemblance and differences

**Keywords:** Proprotein convertases, SKI-1/S1P, NARC-1/PCSK9, Prosegments, α1-PDX, 7B2, ProSAAS

## 1. PROPROTEIN CONVERTASES (PCs)

To date, seven mammalian members of subtilisin-related PCs that process substrates at basic residues have been identified. These include Furin/*PACE*, PC1/*PC3*, PC2, PC4, PACE4, PC5/*PC6*, and PC7/*LPC/PC8/SPC7* (Figure 1).

This somewhat confusing nomenclature arose from the simultaneous discovery of some of these enzymes by different groups. PCs are multi-domain serine proteinases



*A-Majid Khatib (ed.), Regulation of Carcinogenesis, Angiogenesis and Metastasis by the Proprotein Convertases*, 7–26.

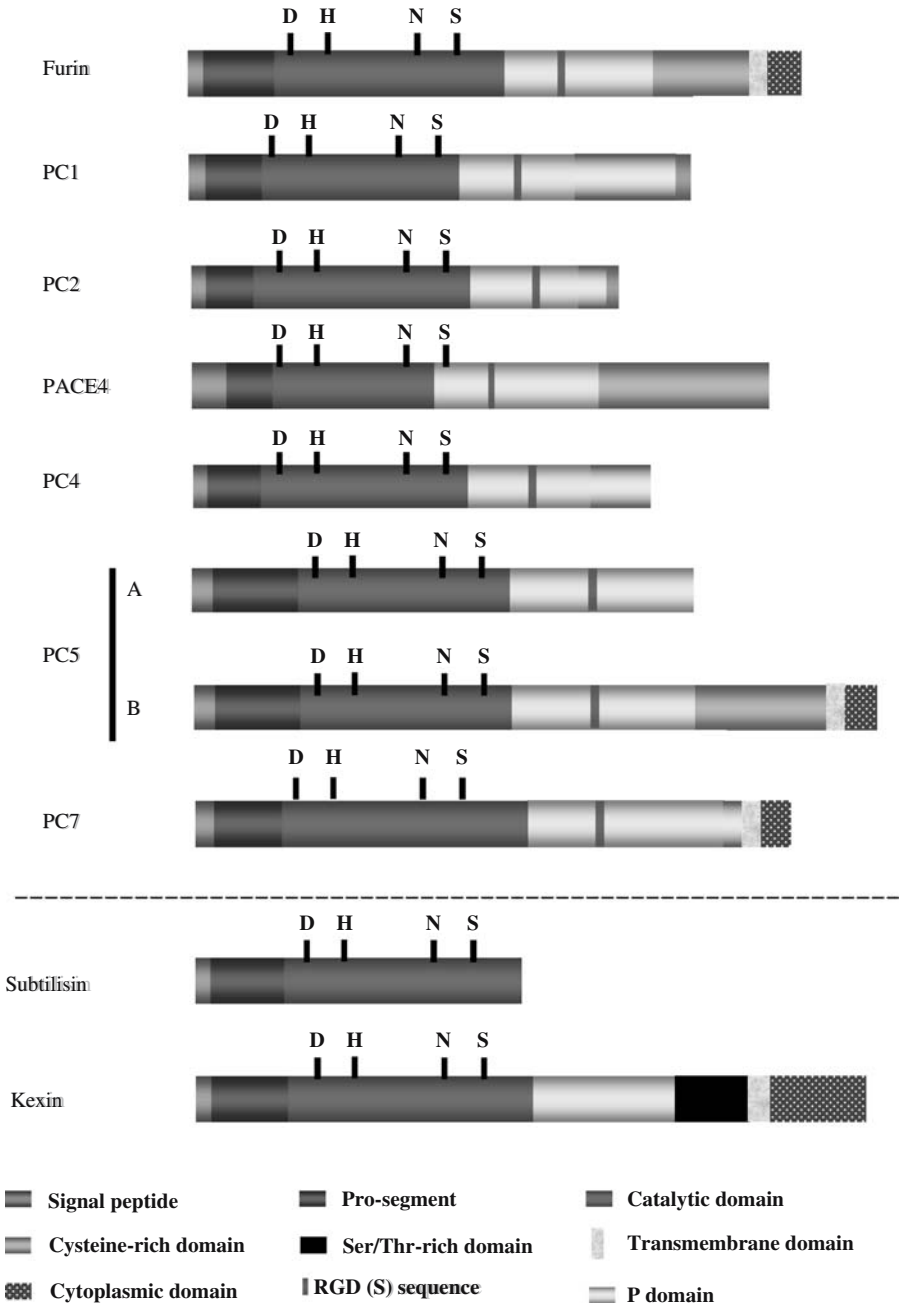


*Figure 1.* Schematic representation of the prohormone convertases PC1, PC2, Furin, PACE4, PC4, PC5 (A and B isoforms) and PC7. These PCs are multi-domain serine proteinases consisting of a signal peptide followed by prosegment, catalytic, middle, and cytoplasmic domains. Homology is highest in the catalytic domains and lowest in the carboxyl-terminal domains. The schematic representation for Kexin and subtilisin are given for comparison

| Convertases | Amino acid number | Autocatalytic site | Accession number |
|---|---|---|---|
| **Furin** | **794** | **$^{101}$A-K-R-R-T-K-R-D** | **NP_002560** |
| **PC1** | **751** | **$^{105}$K-E-R-S-K-R-S-V** | **P21662** |
| **PC2** | **638** | **$^{103}$G-F-D-R-K-K-R-G** | **P16519** |
| **PACE4** | **969** | **$^{141}$Q-E-V-K-R-R-V-K** | **P29122** |
| **PC4** | **654** | **$^{105}$R-R-R-V-K-R-S-L** | **A54306** |
| **PC5** | **1870** | **$^{109}$V-K-K-R-T-K-R-D** | **Q04592** |
| **PC7** | **785** | **$^{134}$R-L-L-R-R-A-K-R** | **NP_004707** |
| **SKI-1** | **1052** | **$^{131}$K-V-F-R-S-L-K-Y** | **NP_003782** |
| **NARC-1** | **692** | **$^{145}$E-D-S-S-V-F-A-Q** | **NM_174936** |

*Figure 2.* Amino acid sequences of the autocatalytic sites of the PCs. Like their substrates, the pro-segments of the PCs are removed at sites cleaved by the PCs. Indicated are the number of amino acid and accession number for every PC

consisting of a signal peptide followed by prosegment, catalytic, middle, and cytoplasmic domains (Figure 1). Homology is highest in the catalytic domains and lowest in the carboxyl-terminal domains.

These enzymes cleave precursor proteins at basic residues within the general motif $(\boldsymbol{K/R})$-$(\boldsymbol{X})_{\boldsymbol{n}}$-$(\boldsymbol{K/R})\downarrow$, where n = 0, 2, 4 or 6 and X any amino acid except Cys [1–4]. Usually most of the PCs cleave their substrates at pairs of basic amino acids, but several of them, with monobasic sites are also cleaved [1–4]. Some PCs, such as PC1, PC2 and PC5A, are sorted and activated in the regulated secretory pathway and thus process protein precursors whose secretion is regulated. In contrast, the transmembrane proteins Furin, PACE4, PC5B and PC7 (Figure 1), cycle between the cell surface and the *trans* Golgi Network (TGN) and are involved in the processing of precursor proteins in the constitutive secretory pathway [1–4]. Like their substrates, the pro-segments of the PCs are also removed at a cleavage site containing a basic–amino acid PC motif (Figure 2), befitting their autoactivation [1–4].

## 1.1 Furin

Furin was the first convertase identified. Its discovery was made just after the availability of the Kex2 cDNA sequence. Kex2 is a cellular processing endoprotease that is required for cleavage at dibasic sites within the killer toxin and the mating pheromone, α–factor precursors [5, 6]. In 1989, in an effort to find other

| Convertases/ Inhibitors | Human | | Mouse | |
|---|---|---|---|---|
| | Chromosomes | Cytogenetic | Chromosomes | Cytogenetic |
| **Furin** | **15** | **15q26.1** | **7** | **7 D1-E2** |
| **PC1** | **5** | **5q15-q21** | **13** | **13C2** |
| **PC2** | **20** | **20p11.2** | **2** | **2G1** |
| **PACE4** | **15** | **15q26** | **7** | **7B5** |
| **PC4** | **19** | **19p13.3** | **10** | **10C1** |
| **PC5** | **9** | **9q21.3** | **19** | **19B** |
| **PC7** | **11** | **11q23.3** | **9** | **9A5.2** |
| **SKI-1** | **16** | **16q24** | **8** | **8E1** |
| **NARC-1** | **1** | **1p32.3** | **4** | **4C7** |
| **7B2** | **15** | **15q13-q14** | **2** | **2E5** |
| **Prosaas** | **X** | **Xp11.23** | **X** | **XA1.1** |

*Figure 3.* Chromosome localisation of mouse and human PCs and the inhibitors Prosaas and 7B2. Note the approximate position of PACE4 gene to fur gene on the human chromosome 15 and mouse chromosome 7 suggesting their probable common ancestry by gene duplication

related Kex2 enzymes, Fuller et al., identified the first mammalian homologue of Kex2 [7], *fur* gene. This gene is located on the human chromosome 15 and on mouse chromosome 7 (Figure 3). The *Furin* gene (*PCSK3*) was unexpectedly discovered by Roebroek et al., a few years earlier due to its proximity to the c-fes/fps protooncogene (***fur*** being: *c-**fes/fps*** **u**pstream **r**egion) [8]. At that time the product of the *fu*r gene was believed to be a growth factor receptor because of the presence of a cysteine-rich domain and a putative trans-membrane domain in its sequence (Figure 1, [9]). Subsequently, the cloning of full-length Furin cDNA revealed that Furin was structurally analogous to Kex2, although the Ser/Thr-rich domain in Kex2 was replaced by a cysteine-rich domain (Figure 1, [10]). Furin is a membrane protein, initially produced as a 104 kDa pro-furin precursor which is rapidly converted into a 98 kDa form by an autocatalytic process (Figure 2, [11, 12].) This autocatalytic cleavage of the pro-Furin occurs in the endoplasmic reticulum (ER) and is a perquisite for the exit of the mature Furin molecule out of the ER to reach the cell surface [13, 14]. Unlike most other convertases, Furin has a widespread distribution being present in all tissues and cells examined so far.

## 1.2 PC1 and PC2

In an effort to find additional Furin-like enzymes, the polymerase chain reaction was used successfully to detect and amplify conserved sequences within the catalytic domain of Furin and Kex2. In 1990, Seidah et al., identified, in mouse pituitary, the cDNA of two additional PC-related enzymes that were called PC1 and PC2 [15]. At approximately the same time, Smeekens and Steiner identified in human insulinoma a cDNA coding for PC2 [16]. The human and mouse *PC1* genes (*PCSK1*) are localized on chromosomes 5 and 13, respectively, whereas the *PC2* gene (*PCSK2*) is localized on human chromosome 20 and on mouse chromosome 2 (Figure 3). The corresponding protein of the full-length cDNA of PC1 is a 751-residue protein and the cDNA of PC2 encodes a 638-residue protein. Contrary to Kex2 and Furin, both PC1 and PC2 lack a transmembrane domain (Figure 1) [15, 16]. In 1991, using similar approaches, Smeekens et al., identified a PC-related enzyme highly expressed in the mouse AtT20 anterior pituitary cell line that unfortunately was called PC3 [17], since it turned out to be identical to PC1 [18, 19, 20]. Studies in various laboratories revealed that PC1 and PC2 process peptide hormones and neuropeptide precursors within the dense core vesicles of the regulated secretory pathway of the brain and the neuroendocrine system [21, 22]. Although PC1 and PC2 are structurally very similar, each convertase has definite substrate site preferences. Among the major substrates of these enzymes are proopiomelanocortin (POMC), proinsulin and proglucagon [23, 24]. Regulation of the activity of PC1 occurs by both its N-and C-terminal domains. Following its N-terminal autocatalytic cleavage within the endoplasmic reticulum, the 84 kDa form of PC1 is transported to the *trans* Golgi Network (TGN) and secretory granules to undergo two other autocatalytic cleavages, one within the inhibitory prosegment and the other at its carboxy-terminal domain to generate the fully active 66-kDa form [25], the major form found in islets of Langerhans and in secretory granules of AtT20 cells [25]. Although PC2 is also autocatalytically processed prior to activation like PC1 (Figure 2), the removal of its prosegment is less efficient and PC2 slowly exits from the ER as a zymogen (proPC2) and is processed to PC2 only in immature secretory granules. This difference in the time course of activation of PC1 and PC2 was reportedly linked to pH and calcium levels [26]. The cleavage of proPC1 to the 84 kDa PC1 occurs at a neutral pH and is calcium-independent, whereas PC2 is activated much more slowly in the immature secretory granules at pHs 5–6 in a calcium-dependent fashion [26]. As a consequence of the different temporal activation of PC1 and PC2 in cells expressing both enzymes, PC1 will cleave precursors before PC2, leading to an ordered cleavage mechanism that may explain the first cleavage of POMC into β-LPH and then into β-endorphin, peptide products that require the consecutive action of PC1 and PC2, respectively [27, 28].

## 1.3 PACE4

With a polymerase chain reaction methodology similar to the one used for the identification of Furin, PC1 and PC2, Kiefer et al., identified the convertase PACE4 using specific primers for the paired basic amino acid residue processing motifs of

the available PCs [29]. PACE4 contains distinct features that are not present in the previously identified three convertases. These include an extended signal peptide region and large carboxyl-terminal cysteine-rich region (Figure 1) [29, 30]. PACE4 is expressed in most tissues, with highest levels occurring in the liver [29]. It processes a variety of substrates [30]. Like other PCs, the maturation of proPACE4 occurs via an intramolecular autocatalytic cleavage of its propeptide (Figure 2). This is the rate-limiting step for the secretion of the mature PACE4 [31, 32]. Furthermore, the secretion and the maturation of PACE4 are also controlled by the carboxy terminal sequence of PACE4 [31, 32]. Deletion of the last 25 residues of PACE4 has been shown to induce a marked acceleration in both the maturation and secretion of mature PACE4 [31]. Another property of PACE4 is its ability to bind heparan sulfate proteoglycans in the extracellular matrix (ECM) [33]. The PACE4 heparin-binding region was localized in the cationic region of amino acids between residues 743 and 760. This suggests a spatial role for PACE4 in the regulation of the biological activities of its substrates [33]. Very recently, we have shown that the C-terminal Cys-rich domain of PACE4 anchors the secreted enzyme to the plasma membrane *via* a complex with one or more member of the tissue inhibitor of metalloproteases (TIMPs) through binding of the complex to cell surface heparan sulfate proteoglycans [34]. Localization of the *PACE4* gene (*PCSK6*) revealed its closeness to the *fur* gene on the human chromosome 15 and mouse chromosome 7 (Figure 3), suggesting a probable common ancestry by gene duplication [29].

Despite a likely common origin, the regulation of Furin and PACE4 expression appears quite different. While both are up-regulated by phorbol 12-myristate 13-acetate (PMA) and tumor necrosis factor (TGF), PACE4 is also upregulated by platelet derived growth factor-BB (PDGF-BB), indicating a unique role for PACE4 in platelet production [35, 36]. Recent studies revealed that the expression of PACE4 is down-regulated by the basic helix-loop-helix transcription factors hASH-1 and MASH-1, suggesting co-regulation of PACE4 and its substrates by these transcription factors [37].

## 1.4 PC4

Like other PCs, identification of PC4 was based on PCR strategies and was simultaneously identified from mouse testis by Nakayama and our group [38, 39]. It is a 654-residue protein, which possesses the same subtilisin-like catalytic domain found in Furin, PC1, PC2, and Kex2 (Figure 1). Distribution analysis in various cell lines and tissues revealed that PC4 appears to be exclusive to testis and ovarian cells [38–41]. Northern blot analysis indicates that PC4 mRNA is detectable only in the testis after the $20^{th}$ day of postnatal development and was primarily expressed in round spermatids, suggesting that PC4 is involved in the maturation of precursor proteins found in testicular germ cells. Subsequently, the importance of PC4 in these processes was shown by PC4 gene expression during spermatogenesis [38–40]. Although PC4 is able to efficiently process various protein precursors in the testis, a specific substrate for PC4 expressed only in this organ remains unknown.

The *PC4* gene (*PCSK4*) is located on chromosome 19 and 10 in human and mouse, respectively (Figure 3).

### 1.5 PC5 (Isoforms PC5A and PC5B)

The 915 amino acid isoform PC5A was identified and cloned by our group using RT-PCR and oligonucleotide sequences derived from conserved sequences of PC1, PC2, Furin, and PC4, in both mouse and rat tissues [41]. The same year, the group of Nakagawa et al., cloned this convertase and named it PC6 [42]. The *PC5* gene (*PCSK5*) is localized on human chromosome 9 and mouse chromosome 19 (Figure 3). The human *PCSK5* gene encodes two isoforms: the 915 amino acid PC5A and a C-terminally extended 1870-residue protein (PC5B) with multiple Cys-rich domains. Both isoforms contain a subtilisin-like catalytic domain and PC5A exhibits a high similarity to PACE4, especially at the COOH-terminal Cys-rich region (Figure 1) [42]. Northern blot analysis revealed that PC5 mRNA, as with Furin and PACE4 mRNA, was expressed in various tissues and cell lines [42–45]. Its highest expression is in adrenal cortex and small intestine suggesting possible roles in stress response and in processing protein substrates of gastrointestinal peptides [42–45]. Like PACE4, the expression of PC5 is upregulated by PDGF-BB and during cell proliferation [44]. Many substrates have been reported to be efficiently processed by PC5; including growth factors such PDGF-A [45], PDGF-B [46] and VEGF-C [47], receptors such as IGFI-1 receptor (1) and various integrins [48]. While these substrates were also shown to be processed by other PCs, certain precursor proteins are processed effectively mostly by PC5, such as neural adhesion molecule L1 [49] and Lefty protein [50]. Similar to other PCs, the activity and secretion of PC5 is also regulated by its prosegment. The pro-region of PC5 was shown to prevent IGF-1 receptor (1) and VEGF-C processing by PC5, both *in vitro* and *in vivo* [47, 51] suggesting an inhibitory role of the PC5 propeptide.

### 1.6 PC7

This convertase was identified in 1996 by our group [52], Bruzzaniti et al. [53] and Meerabux et al. [54]. Meerabux identified PC7 through its involvement in a chromosome translocation that occurred in a particular lymphoma [54]. This translocation is the result of a fusion between an intron in the 3′-untranslated region of PC7 with a sequence close to the switch region S gamma 4 of the IGH locus. The product of the *PC7* gene (*PCSK7*) encodes a 785 residue protein with a large homology to all members of the PC family (Figure 1). Using PCR and degenerate primers to conserved amino acid residues in the catalytic region of the PCs, Bruzzanti et al., predicted the product of the gene they identified (called PC8) to be 785 residues [53]. The catalytic region of this protein is more than 50% identical in primary sequence to the other PCs. Using similar technologies, we also isolated a cDNA coding for a gene from the rat anterior pituitary that we named PC7. We found the open reading frame codes for a prepro-PC with a 36-amino

acid signal peptide, a 104-amino acid prosegment, and a 747-amino acid type I membrane-bound glycoprotein, representing the mature form of PC7 [52]. Distinct from Furin (*PCSK3*) and PACE4 (*PCSK6*) genes, both mapping to chromosome 15, PCSK7 maps to chromosome 11 (Figure 3). Phylogenetic analysis suggested that PC7 is the most ancestral member of the seven basic amino acid-specific proprotein convertases [52]. Northern blot analyses demonstrated significant expression of PC7 mRNA in the colon and lymphoid-associated tissues. *In situ* hybridization and histochemistry analysis in various tissues revealed that PC7 co-localizes with Furin, suggesting widespread proteolytic functions of PC7 and its participation with Furin in the activation of several substrates [52–57].

## 2. PROPROTEIN CONVERTASES THAT PROCESS SUBSTRATES AT NON-BASIC RESIDUES

### 2.1 Subtilisin/Kexin-like Isozyme-1 (SK-1)

In 1999, using reverse transcriptase-PCR and degenerate oligonucleotides, derived from the active-site residues of subtilisin/kexin-like serine proteinases, we identified in human, rat, and mouse, a type I membrane-bound proteinase, which we called subtilisin/kexin-isozyme-1 (SKI-1) [58]. It was so named because of the homology of its catalytic domain to the bacterial subtilisin BPN (Figure 4). In contrast to the basic amino acid-specific PCs, this convertase appears to prefer processing precursors at residues within the general motif $\boldsymbol{RX}(\boldsymbol{V},\boldsymbol{I},\boldsymbol{L})(\boldsymbol{K},\boldsymbol{F},\boldsymbol{L})\downarrow$, with the preferred critical basic Arg/Lys and aliphatic (Leu/Ile/Val) residues occupying positions P4 and P2, respectively [58].

Data bank searches revealed that Sakai et al., also identified a few month earlier a similar hamster enzyme from CHO cells, which they named Site-1 protease (S1P). They determined that this enzyme was involved in the control of lipid metabolism by mediating the cleavage of Sterol Regulatory Element-Binding Proteins (SREBPs) in its luminal loop [59]. Previously, SREBPs were described to play a key role in the fundamental feedback mechanism of cellular lipid homeostasis.

The transcriptional activation of genes containing sterol responsive elements (SRE) is known to be regulated by sterols through modulation of the proteolytic maturation of SREBPs [59]. The two known SREBPs (SREBP1 and SREBP2) are inserted into the membrane of the endoplasmic reticulum envelope in a wide variety of tissues. In sterol-deficient cells, proteolytic cleavage of SREBPs by SKI-1 and S2-P protease releases their N-terminal mature form from the membrane into the cytosol enabling them to enter the nucleus (Figure 5), where they bind to the SREs and activate genes involved in the biosynthesis of cholesterol, triglycerides, and fatty acids [59]. In the presence of sterols, the proteolytic process is inhibited and the transcription of the genes is reduced [59] (Figure 5).

The gene of SKI-1/S1P (*PCSK8*) is located on human chromosome 16 and mouse chromosome 8 (Figure 3), and is expressed in most tissues and cells. To date, several viral glycoproteins in addition to SREBPs, as well as the brain-derived

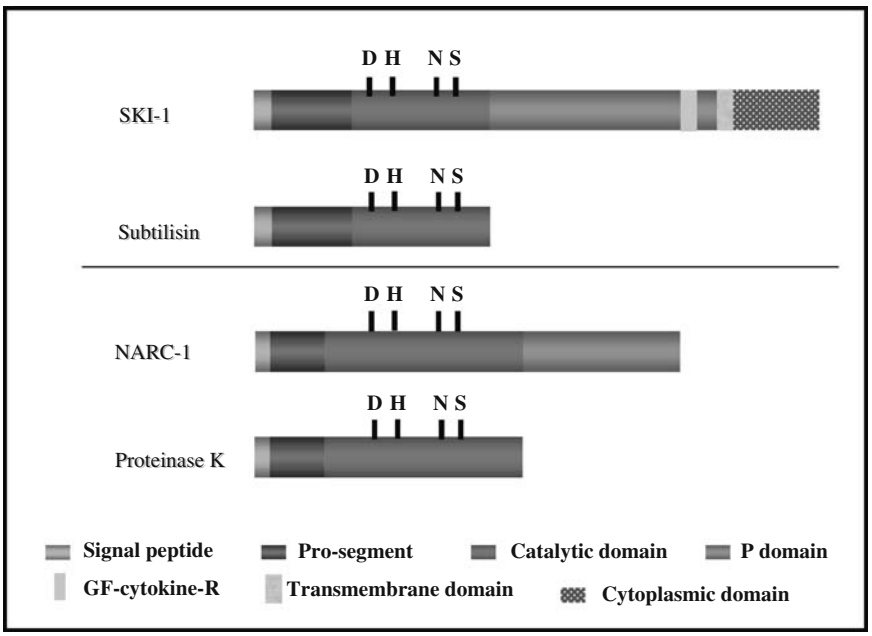


*Figure 4.* Schematic representation of the prohormone convertases SK-1 and NARC-1. The convertase subtilisin/kexin-isozyme-1 (SKI-1) possesses a catalytic domain with high homology to bacterial subtilisin BPN, whereas the neural apoptosis-regulated convertase-1 (NARC-1) belongs to the proteinase K-like subtilases

neurotrophic factor, ATF-6 and endocrine polypeptide somatostatin were found to be SKI-1 substrates [59–64]. New substrates include CREB-containing precursors, such as CREB-4 were also reported to be cleaved by SKI-1/S1P [65]. As with the PCs, the precursor protein of SKI-1 is also autocatalyticaly cleaved (Figure 2) and can be further processed into two membrane-bound forms of SKI-1 (120 and 106 kDa), differing by the nature of their N-glycosylation. Some of these SKI-1 forms are shed into the medium as a 98-kDa form.

### 2.2 Neural Apoptosis-regulated Convertase 1 (NARC-1/PCSK9)

Through a search of patent databases, using as a bait a small sequence of the conserved catalytic domain of SKI-1/S1P, we identified a protein belonging to proteinase K-like subtilases (Figure 4) called neural apoptosis-regulated convertase 1 (NARC-1) or PCSK9. NARC-1/PCSK9 was previously identified by two pharmaceutical companies [66], based on the cloning of up-regulated cDNAs after the induction of apoptosis by serum deprivation in the primary cerebellar neurons and by means of global cloning of secretory proteins [66]. Like other convertases, NARC-1/PCSK9 is also synthesized as a zymogen that undergoes autocatalytic intramolecular processing in the ER (Figure 2). This cleavage occurs within the

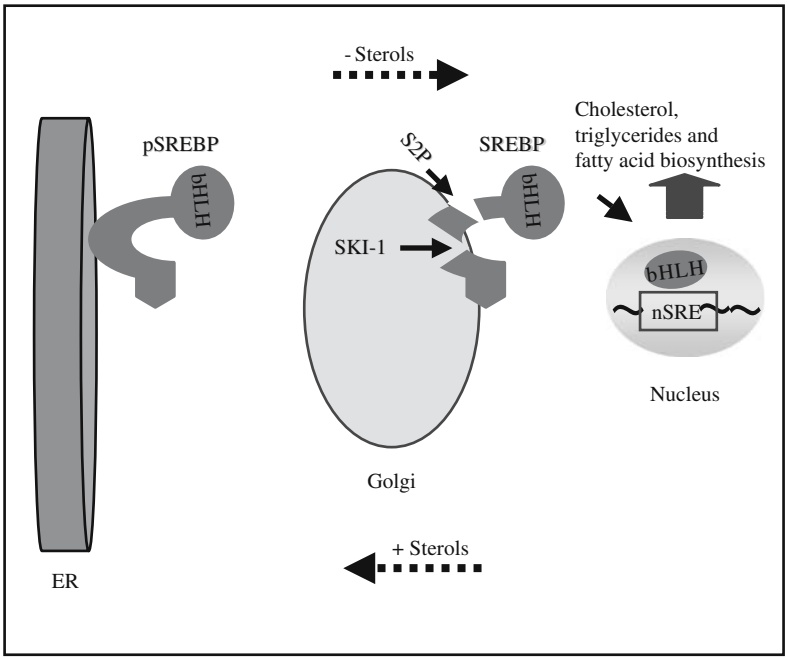


*Figure 5.* Role of SKI-1/S1P in the processing of SREBP. The sterol regulatory element binding protein precursors (SREBPs) are inserted into the membrane of the endoplasmic reticulum (ER) envelope in various tissues and the amino-terminal transcription-factor domain (bHLH-zip) is located in the cytoplasmic compartment. Under insufficient amount of sterols, the SREBP precursor protein travels to the Golgi apparatus where SKI-1/S1P cleaves at site-1 in the luminal loop and produce the substrate for the Site-2 protease (S2P), which cleaves at site-2. This second cleavage releases the transcription-factor domain from the membrane that enters the nucleus and induces the increased transcription of target genes. In the presence of sterols, the proteolytic process is inhibited and the transcription of the genes is reduced. bHLH-zip: basic helix-loop-helix leucine-zipper

motif ***SSVFAQ SIP*** [67]. Northern blots and *in situ* hybridization analyses revealed that in the adult NARC-1/PCSK9 mRNA expression is restricted to the liver, kidney and small intestine. Unlike PC7 and SKI-1, but similar to Furin, PC5 and PACE4, the mRNA of NARC-1/PCSK9 was up-regulated during liver regeneration following partial hepatectomy [68]. Overexpression of NARC-1/PCSK9 in primary culture of embryonic telencephalon cells at day 13.5 induced differentiation of neuronal progenitors, suggesting a role for NARC-1/PCSK9 in enhancing the differentiation/proliferation of cortical neurons [66]. Recently, we have shown that point mutations in human PCSK9 are associated with the development of severe hypercholesterolemia phenotypes [69], likely through a grain of function [70]. Conversely, other mutations resulting in early termination of the coding region (non-sense mutations) resulted in a loss of function and hence familial hypocholesterolemia [71]. Thus, mutations in PCSK9 results in a dominant form of either hypo-or hyper-cholesterolemia, suggesting that inhibitors of these enzymes may

lead to novel pharmaceutical drugs to further lower circulating cholesterol levels as a supplement to the conventional HMG-CoA reductase inhibitors known as "statins".

## 3. PROPROTEIN CONVERTASE INHIBITORS

To date, the propeptides or prosegments of the PCs constitute the only naturally occurring intracellular PC inhibitor found in the mammalian constitutive secretory pathway [1–4] and, in the case of PC1, its C-terminal domain [72]. Aside from the prosegment inhibitors, the activities of the regulated secretory pathway convertases PC1 and PC2 are also regulated by their selective and specific inhibitors/binding partners, known as proSAAS [73, 74] and 7B2 [75] respectively.

### 3.1 Naturally Occurring PC2 Inhibitor 7B2

In 1982, during the purification of the POMC N-terminal glyco-segment from pig anterior pituitaries, we discovered the protein 7B2 [75]. Subsequently, the homologues of this peptide were cloned in tissues and organs of other species, including human, and showed high homology between mammals [75–78]. Studies on the tissue distribution and secretion of 7B2 revealed its predominance in endocrine and neural tissues, including the brain and adrenal medulla, as well as the pituitary, thyroid and pancreas [75].

The gene for 7B2 is located on human chromosome 15 and mouse chromosome 2 (Figure 3). It is produced as an intracellular precursor of 25–29 kDa. This 7B2 precursor is converted into a secreted form of 18–21 kDa by PC cleavage after the $RRRRR^{155}$ motif, followed by carboxypeptidase E (CPE) removal of the 5 basic residues. After processing, 7B2 proteins are packaged into dense-core vesicles and are secreted upon exocytotic stimulation [75]. Pulse–chase studies showed that proPC2 is bound to pro7B2 in the early compartments of the secretory pathway dissociates from it in later ones and serves as an intracellular proPC2 chaperone that prevents the premature activation of the zymogen during its transit in the regulated secretory pathway [75]. Attachment of pro7B2 to proPC2 in the ER generates an inactive complex that is transported to the TGN where pro7B2 is cleaved into an N-terminal protein and an inhibitory C-terminal 31 aa peptide (CT-7B2). ProPC2 is then autocatalytically cleaved after the prodomain as the complex is transported into the immature secretory granules [75]. In the acidic environment of these organelles, the prodomain and 7B2 dissociate from the enzyme, which then cleaves the PC2-specific inhibitory CT-7B2 resulting in fully active PC2.

### 3.2 Naturally Occurring PC1 Inhibitor ProSAAS

ProSAAS was identified by Fricker et al. during an analysis of peptides not properly processed in $Cpe^{fat}/Cpe^{fat}$ mice lacking carboxypeptidase E activity due to a point mutation in the carboxypeptidase E gene [79, 80]. These mice accumulate peptides

with C-terminal Lys and/or Arg extensions. Using an affinity column, peptides with C-terminal basic residues from $Cpe^{fat}/Cpe^{fat}$ tissues were isolated and analyzed. Five of these peptides were found to be encoded by proSAAS [81]. Subsequent overexpression of proSAAS in endocrine cells revealed its selective inhibitory effect on PC1 [81]. The proSAAS gene is located on the human and mouse chromosome X (Figure 3) and, similarly to 7B2, proSAAS is largely expressed in neuroendocrine cells and its inhibitory domains are located at the C terminus. In contrast to 7B2, which is required for the expression and secretion of active convertase PC2 [82–84], active PC1 can be expressed in cells lacking proSAAS [82–84]. Despite the absence of data on proSAAS null mice, taking together with its inhibitory role on PC1, and similarities to 7B2, proSAAS may be assumed to have other functions such as the control of the body mass blood glucose levels as recently revealed by analysis of transgenic mice expressing proSAAS [85].

### 3.3 Prosegments and Exogenous Inhibitors

Since the discovery of Furin, many attempts have been made to develop inhibitors to control the activity of the PCs. Initially, taking advantage of the fact that PCs are synthesized as inactive zymogens autocatalytically activated, Anderson et al., demonstrated that the prosegment of Furin, when used as a fusion protein to glutathione S-transferase, exhibits a potent *in vitro* inhibitory activity on Furin [86]. Previously, we found that purified prosegments and synthetic peptides derived from the prosegments of PC1, PC7 and Furin are potent inhibitors of their corresponding enzymes [87–91]. Using these inhibitors, we were able to intracellularly inhibit the processing of various PC substrates, including PDGF-A [45], PDGF-B [46] VEGF-C [47] and IGF-1 receptor (1.)

In addition to these naturally occurring inhibitors, many exogenous inhibitors were proposed to control the activity of the convertases. Of these molecules, the trypsin inhibitor and the third domain of turkey ovomucoid have been reported to be inhibitors for furin [92]. Subsequently, Garten et al. [93] have shown that acylated peptidyl chloromethane, containing a consensus furin cleavage sequence, decanoyl-Arg-Glu-Lys-Arg-$COCH_2Cl$, that inhibits Furin activity *in vitro* at low micromolar concentrations to block the cleavage of influenza-virus HA. While these inhibitors were useful for study of the processing of various proteins by Furin, they appear to be unstable and unable to completely block the processing of various PC substrates *in vivo* due to their inefficiencies and/or decreased capability in entering cells. In 1988, Bathurst et al., and Brennan et al., proposed the use of protein-based inhibitors to control the activity of PCs [94]. They demonstrated that the variant of α1-antitrypsin, called α1-anti-trypsin Pittsburgh (α1-PIT), which has a replacement of the reactive-site Met residue by Arg, inhibits, *in vitro*, the processing of proalbumin by Kex2p [94]. Subsequently, the group of G. Thomas developed another variant of $\alpha_1$-antitrypsin, called $\alpha_1$-anti-trypsin Portland ($\alpha_1$-PDX), in which the reactive-site Ala-Ile-Pro-Met has been replaced by Arg-Ile-Pro-Arg. This serpin inhibits Furin in the subnanomolar range, three times lower than that $\alpha_1$-PIT.

Kinetic analysis showed that a portion of bound $\alpha_1$-PDX operates as a suicide inhibitor [94–97]. Once bound to Furin's active site, $\alpha_1$-PDX can either undergo proteolysis by Furin or form a kinetically trapped SDS-stable complex with the enzyme. Furthermore, when expressed in cells, $\alpha_1$-PDX, was shown to be a potent inhibitor of Furin-mediated cleavage of HIV gp160 [97], and subsequently demonstrated to inhibit all PCs involved in processing within the constitutive secretory pathway [1, 97–101]. Inhibition of PCs by $\alpha_1$-PDX has been shown to reduce the production of the APP$\alpha$ [102] and block the activation of the pore-forming toxin proaerolysin [103], the maturation of infectious pathogens glycoproteins [97], the proteolytic activation of BMP4 [104] and the cleavage of IGF-1R [1, 105], PDGF-A [45], PDGF-B [46] and VEGF-C [47].

In an attempt to produce other PC inhibitors, researchers mutated the bait region of the general protease inhibitor $\alpha_2$-macroglobulin (RVGFYESDVM$^{690}$ into RVRSKRSLVM$^{690}$) [106]. This variant was reported to inhibit processing of several Furin substrates including HIV type 1 glycoprotein gp160, von Willebrand factor and TGF-$\beta$1 [106]. Other inhibitors were suggested, such as the ovalbumin-type serpin human proteinase inhibitor-8, which contains two instances of the minimal Furin recognition sequence (VVRNSRCSRM$^{343}$). Although this inhibitor was shown to inhibit Furin in a rapid and tight binding manner, it required the addition of a signal peptide before it could inhibit Furin *in vivo* [107]. Additionally, the hexa-D-arginine was reported to be a potent and relatively specific Furin inhibitor; however, it showed reduced ability to cross the cell membrane [108].

## 4. SUMMARY AND CONCLUDING REMARKS

Since the discovery of Furin, the first mammalian convertase identified, cumulative knowledge has been acquired regarding the physiological and physiopathological role of these enzymes. The data obtained on the functional role of these enzymes by the use of null mice provided exceptional information, not only on the precursor proteins that are processed by one or more PCs, but also precious information on the importance of these enzymes in normal physiological situations. To date, based on the available PC-null mice, only the absence or dysfunction of Furin [109], PC5 [110] and SKI-1/S1P [111] are lethal at the embryonic stage. Mice with disrupted PC1or PC2 are viable despite their hormonal and/or neuro-endocrinal deficiency [112, 113]. PACE4 deficient animals show bone defects [114] and PC4 null mice are infertile or subfertile [115]. These varieties in the PC knockout phenotypes reveal the complexity and wide array of the protein precursors that are processed by these enzymes. Protein precursors may be processed by one specific convertase, a limited set or multiple convertases. The determination of the knockout phenotype observed in the PC-null mice seems to be more likely due to a defect in the processing of specific protein precursors by specific PCs.

While the PC null mice studies confirm the critical role of these enzymes in the activation of proteins involved in physiological processes, there is also growing evidence of their role in various pathological processes and diseases. Some PCs

have been reported to be involved in Alzheimer's disease, rheumatoid arthritis, cancer and other pathologies. In this chapter, we have described the progress made in establishing potent and specific inhibitors to control PC activity. Some of these inhibitors, particularly $\alpha_1$-PDX, were shown to dramatically reduce tumor growth and the malignant phenotype of various caner cells [1, 105]. $\alpha_1$-PDX was also shown to inhibit the processing of the HIV-1 GP 160 protein and other viral glycoproteins and, in turn, the production of infectious viruses. Recently, inhibition of Furin by the inhibitor Dec-RVKR-CH(2)Cl was revealed to prevent cartilage degradation induced by cytokines, suggesting the inhibition of PCs as a potential therapeutic intervention in arthritic diseases [116].

## ACKNOWLEDGEMENTS

This work was supported by the grant from the Fondation pour la Recherche Médicale and Avenir INSERM Award to AM K, Paris, France.

## REFERENCES

[1] Khatib AM, Siegfried G, Chretien M, Metrakos P, Seidah NG (2002) Proprotein convertases in tumor progression and malignancy: Novel targets in cancer therapy. Am J Pathol **160**:1921–1935
[2] Nakayama K (1997) Furin: A mammalian subtilisin/Kex2p-like endoprotease involved in processing of a wide variety of precursor proteins. Biochem J **327**:625–635
[3] Zhou A, Webb G, Zhu X, Steiner DF (1999) Proteolytic processing in the secretory pathway. J Biol Chem **274**:20745–20748
[4] Seidah NG, Chretien M, Day R (1994) The family of subtilisin/kexin like pro-protein and pro-hormone convertases: Divergent or shared functions. Biochimie **76**:197–209
[5] Julius D, Brake A, Blair L, Kunisawa R, Thorner J (1984) Isolation of the putative structural gene for the lysine-arginine-cleaving endopeptidase required for processing of yeast prepro-alpha-factor. Cell **37**:1075–1089
[6] Mizuno K, Nakamura T, Ohshima T, Tanaka S, Matsuo H (1988) Yeast KEX2 genes encodes an endopeptidase homologous to subtilisin-like serine proteases. Biochem Biophys Res Commun **156**:246–254
[7] Fuller RS, Brake AJ, Thorner J (1989) Intracellular targeting and structural conservation of a prohormone-processing endoprotease. Science **246**:482–486
[8] Roebroek AJ, Schalken JA, Bussemakers MJ, van Heerikhuizen H, Onnekink C, Debruyne FM, Bloemers HP, Van de Ven WJ (1986) Characterization of human c-fes/fps reveals a new transcription unit (fur) in the immediately upstream region of the proto-oncogene. Mol Biol Rep **11**:117–125
[9] Roebroek AJ, Schalken JA, Leunissen JA, Onnekink C, Bloemers HP, Van de Ven WJ (1986) Evolutionary conserved close linkage of the c-fes/fps proto-oncogene and genetic sequences encoding a receptor-like protein. EMBO J **59**:2197–2202
[10] van de Ven WJ, Voorberg J, Fontijn R, Pannekoek H, van den Ouweland AM, van Duijnhoven HL, Roebroek AJ, Siezen RJ (1990) Furin is a subtilisin-like proprotein processing enzyme in higher eukaryotes. Mol Biol Rep **14**:265–275
[11] Leduc R, Molloy SS, Thorne BA, Thomas G (1992) Activation of human furin precursor processing endoprotease occurs by an intramolecular autoproteolytic cleavage. J Biol Chem **267**:14304–14308
[12] Creemers JW, Siezen RJ, Roebroek AJ, Ayoubi TA, Huylebroeck D, Van de Ven WJ (1993) Modulation of furin-mediated proprotein processing activity by site-directed mutagenesis. J Biol Chem **268**:21826–21834

[13] Takahashi S, Nakagawa T, Kasai K, Banno T, Duguay SJ, Van de Ven WJ, Murakami K, Nakayama K (1995) A second mutant allele of furin in the processing-incompetent cell line, LoVo. Evidence for involvement of the homo B domain in autocatalytic activation. J Biol Chem **270**:26565–26569

[14] Molloy SS, Thomas L, VanSlyke JK, Stenberg PE, Thomas G (1994) Intracellular trafficking and activation of the furin proprotein convertase: Localization to the TGN and recycling from the cell surface. EMBO J **13**:18–33

[15] Seidah NG, Gaspar L, Mion P, Marcinkiewicz M, Mbikay M, Chretien M (1990) cDNA sequence of two distinct pituitary proteins homologous to Kex2 and furin gene products: Tissue-specific mRNAs encoding candidates for pro-hormone processing proteinases. DNA Cell Biol **9**:415–424

[16] Smeekens SP, Steiner DF (1990) Identification of a human insulinoma cDNA encoding a novel mammalian protein structurally related to the yeast dibasic processing protease Kex2. J Biol Chem **265**:2997–3000

[17] Smeekens SP, Avruch AS, LaMendola J, Chan SJ, Steiner DF (1991) Identification of a cDNA encoding a second putative prohormone convertase related to PC2 in AtT20 cells and islets of Langerhans. Proc Natl Acad Sci U S A **88**:340–344

[18] Seidah NG, Marcinkiewicz M, Benjannet S, Gaspar L, Beaubien G, Mattei MG, Lazure C, Mbikay M, Chretien M (1991) Cloning and primary sequence of a mouse candidate prohormone convertase PC1 homologous to PC2, Furin, and Kex2: Distinct chromosomal localization and messenger RNA distribution in brain and pituitary compared to PC2. Mol Endocrinol **5**:111–122

[19] Korner J, Chun J, Harter D, Axel R (1991) Isolation and functional expression of a mammalian prohormone processing enzyme, murine prohormone convertase 1. Proc Natl Acad Sci U S A **88**:6834–6838

[20] Steiner DF, Smeekens SP, Ohagi S, Chan SJ (1992) The new enzymology of precursor processing endoproteases. J Biol Chem **267**:23435–23438

[21] Seidah NG, Benjannet S, Hamelin J, Mamarbachi AM, Basak A, Marcinkiewicz J, Mbikay M, Chretien M, Marcinkiewicz M (1999) The subtilisin/kexin family of precursor convertases. Emphasis on PC1, PC2/7B2, POMC and the novel enzyme SKI-1. Ann N Y Acad Sci **885**:57–74

[22] Bell ME, Myers TR, Myers DA (1998) Expression of proopiomelanocortin and prohormone convertase-1 and -2 in the late gestation fetal sheep pituitary. Endocrinology **139**:5135–5143

[23] Steiner DF, Rouille Y, Gong Q, Martin S, Carroll R, Chan SJ (1996) The role of prohormone convertases in insulin biosynthesis: Evidence for inherited defects in their action in man and experimental animals. Diabetes Metab **22**:94–104

[24] Mineo I, Matsumura T, Shingu R, Namba M, Kuwajima M, Matsuzawa Y (1995) The role of prohormone convertases PC1 (PC3) and PC2 in the cell-specific processing of proglucagon. Biochem Biophys Res Commun **207**:646–651

[25] Vindrola O, Lindberg I (1992) Biosynthesis of the prohormone convertase mPC1 in AtT-20 cells. Mol Endocrinol **6**:1088–1094

[26] Shennan KI, Taylor NA, Jermany JL, Matthews G, Docherty K (1995) Differences in pH optima and calcium requirements for maturation of the prohormone convertases PC2 and PC3 indicates different intracellular locations for these events. J Biol Chem **270**:1402–1407

[27] Crine P, Gossard F, Seidah N, Blanchette L, Lis M, Chretien M (1979) Concomitant synthesis of beta-endorphin and alpha-melanotropin from two forms of pro-opiomelanocortin in the rat pars intermedia. Proc Natl Acad Sci U S A **76**:5085–5089

[28] Benjannet S, Rondeau N, Day R, Chretien M, Seidah NG (1991) PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues. Proc Natl Acad Sci U S A **88**:3564–3568

[29] Kiefer MC, Tucker JE, Joh R, Landsberg KE, Saltman D, Barr PJ (1991) Identification of a second human subtilisin-like protease gene in the fes/fps region of chromosome 15. DNA Cell Biol **10**:757–769

[30] Laprise MH, Grondin F, Cayer P, McDonald PP, Dubois CM (2002) Furin gene (fur) regulation in differentiating human megakaryoblastic Dami cells: Involvement of the proximal GATA recognition motif in the P1 promoter and impact on the maturation of furin substrates. Blood **100**:3578–3587

[31] Nagahama M, Taniguchi T, Hashimoto E, Imamaki A, Mori K, Tsuji A, Matsuda Y (1998) Biosynthetic processing and quaternary interactions of proprotein convertase SPC4 (PACE4). FEBS Lett **434**:155–159

[32] Taniguchi T, Kuroda R, Sakurai K, Nagahama M, Wada I, Tsuji A, Matsuda Y (2002) A critical role for the carboxy terminal region of the proprotein convertase, PACE4A, in the regulation of its autocatalytic activation coupled with secretion. Biochem Biophys Res Commun **290**:878–884

[33] Tsuji A, Sakurai K, Kiyokage E, Yamazaki T, Koide S, Toida K, Ishimura K, Matsuda Y (2003) Secretory proprotein convertases PACE4 and PC6A are heparin-binding proteins which are localized in the extracellular matrix. Potential role of PACE4 in the activation of proproteins in the extracellular matrix. Biochim Biophys Acta **1645**:95–104

[34] Nour N, Mayer G, Mort JS, Salvas A, Mbikay M, Morrison CJ, Overall CM, Seidah NG (2005) The cysteine-rich domain of the secreted proprotein convertases PC5A and PACE4 functions as a cell surface anchor and interacts with tissue inhibitors of metalloproteinases. Mol Biol Cell **16**:5215–5226

[35] Bando M, Matsuoka A, Tsuji A, Matsuda Y (2002) The proprotein convertase PACE4 is upregulated by PDGF-BB in megakaryocytes: Gene expression of PACE4 and furin is regulated differently in Dami cells. J Biochem (Tokyo) **132**:127–134

[36] Blanchette F, Day R, Dong W, Laprise MH, Dubois CM (1997) TGFbeta1 regulates gene expression of its own converting enzyme furin. J Clin Invest **99**:1974–1983

[37] Yoshida I, Koide S, Hasegawa SI, Nakagawara A, Tsuji A, Matsuda Y (2001) Proprotein convertase PACE4 is down-regulated by the basic helix-loop-helix transcription factor hASH-1 and MASH-1. Biochem J **360**:683–689

[38] Nakayama K, Kim WS, Torii S, Hosaka M, Nakagawa T, Ikemizu J, Baba T, Murakami K (1992) Identification of the fourth member of the mammalian endoprotease family homologous to the yeast Kex2 protease. Its testis-specific expression. J Biol Chem **267**:5897–5900

[39] Seidah NG, Day R, Hamelin J, Gaspar A, Collard MW, Chretien M (1992) Testicular expression of PC4 in the rat: Molecular diversity of a novel germ cell-specific Kex2/subtilisin-like proprotein convertase. Mol Endocrinol **6**:1559–1570

[40] Torii S, Yamagishi T, Murakami K, Nakayama K (1993) Localization of Kex2-like processing endoproteases, furin and PC4, within mouse testis by in situ hybridization. FEBS Lett **316**:12–16

[41] Li M, Mbikay M, Nakayama K, Miyata A, Arimura A (2000) Prohormone convertase PC4 processes the precursor of PACAP in the testis. Ann N Y Acad Sci **921**:333–339

[42] Lusson J, Vieau D, Hamelin J, Day R, Chretien M, Seidah NG (1993) cDNA structure of the mouse and rat subtilisin/kexin-like PC5: A candidate proprotein convertase expressed in endocrine and nonendocrine cells. Proc Natl Acad Sci U S A **90**:6691–6695

[43] Nakagawa T, Hosaka M, Torii S, Watanabe T, Murakami K, Nakayama K (1993) Identification and functional expression of a new member of the mammalian Kex2-like processing endoprotease family: Its striking structural similarity to PACE4. J Biochem (Tokyo) **113**:132–135

[44] Stawowy P, Blaschke F, Kilimnik A, Goetze S, Kallisch H, Chretien M, Marcinkiewicz M, Fleck E, Graf K (2002) Proprotein convertase PC5 regulation by PDGF-BB involves PI3-kinase/p70(s6)-kinase activation in vascular smooth muscle cells. Hypertension **39**:399–404

[45] Siegfried G, Khatib AM, Benjannet S, Chretien M, Seidah NG (2003) The proteolytic processing of pro-platelet-derived growth factor-A at RRKR(86) by members of the proprotein convertase family is functionally correlated to platelet-derived growth factor-A-induced functions and tumorigenicity. Cancer Res **63**:1458–1463

[46] Siegfried G, Basak A, Prichett-Pejic W, Scamuffa N, Ma L, Benjannet S, Veinot JP, Calvo F, Seidah N, Khatib AM (2005) Regulation of the stepwise proteolytic cleavage and secretion of PDGF-B by the proprotein convertases. Oncogene **24**:6925–6935

[47] Siegfried G, Basak A, Cromlish JA, Benjannet S, Marcinkiewicz J, Chretien M, Seidah NG, Khatib AM (2003) The secretory proprotein convertases furin, PC5, and PC7 activate VEGF-C to induce tumorigenesis. J Clin Invest **111**:1723–1732

[48] Lissitzky JC, Luis J, Munzer JS, Benjannet S, Parat F, Chretien M, Marvaldi J, Seidah NG (2000) Endoproteolytic processing of integrin pro-alpha subunits involves the redundant function of furin

and proprotein convertase (PC) 5A, but not paired basic amino acid converting enzyme (PACE) 4, PC5B or PC7. Biochem J **346**:133–138

[49] Kalus I, Schnegelsberg B, Seidah NG, Kleene R, Schachner M (2003) The proprotein convertase PC5A and a metalloprotease are involved in the proteolytic processing of the neural adhesion molecule L1. J Biol Chem **278**:10381–10388

[50] Ulloa L, Creemers JW, Roy S, Liu S, Mason J, Tabibzadeh S (2001) Lefty proteins exhibit unique processing and activate the MAPK pathway. J Biol Chem **276**:21387–21396

[51] Nour N, Basak A, Chretien M, Seidah NG (2003) Structure-function analysis of the prosegment of the proprotein convertase PC5A. J Biol Chem **278**:2886–2895

[52] Seidah NG, Hamelin J, Mamarbachi M, Dong W, Tardos H, Mbikay M, Chretien M, Day R (1996) cDNA structure, tissue distribution, and chromosomal localization of rat PC7, a novel mammalian proprotein convertase closest to yeast kexin-like proteinases. Proc Natl Acad Sci U S A **93**:3388–3393

[53] Bruzzaniti A, Goodge K, Jay P, Taviaux SA, Lam MH, Berta P, Martin TJ, Moseley JM, Gillespie MT (1996) PC8 [corrected], a new member of the convertase family. Biochem J **314**:727–731

[54] Meerabux J, Yaspo ML, Roebroek AJ, Van de Ven WJ, Lister TA, Young BD (1996) A new member of the proprotein convertase gene family (LPC) is located at a chromosome translocation breakpoint in lymphomas. Cancer Res **56**:448–451

[55] Taylor NA, Van De Ven WJ, Creemers JW (2003) Curbing activation: Proprotein convertases in homeostasis and pathology. FASEB J **17**:1215–1227

[56] Constam DB, Robertson EJ (1999) Regulation of bone morphogenetic protein activity by pro domains and proprotein convertases. J Cell Biol **144**:139–149

[57] Constam DB, Calfon M, Robertson EJ (1996) SPC4, SPC6, and the novel protease SPC7 are coexpressed with bone morphogenetic proteins at distinct sites during embryogenesis. J Cell Biol **134**:181–191

[58] Seidah NG, Mowla SJ, Hamelin J, Mamarbachi AM, Benjannet S, Toure BB, Basak A, Munzer JS, Marcinkiewicz J, Zhong M, Barale JC, Lazure C, Murphy RA, Chretien M, Marcinkiewicz M (1999) Mammalian subtilisin/kexin isozyme SKI-1: A widely expressed proprotein convertase with a unique cleavage specificity and cellular localization. Proc Natl Acad Sci U S A **96**:1321–1326

[59] Sakai J, Rawson RB, Espenshade PJ, Cheng D, Seegmiller AC, Goldstein JL, Brown MS (1998) Molecular identification of the sterol-regulated luminal protease that cleaves SREBPs and controls lipid composition of animal cells. Mol Cell **2**:505–514

[60] Brown MS, Goldstein JL, (1997) The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell **89**:331–340

[61] Lenz O, ter Meulen J, Klenk HD, Seidah NG, Garten W (2001) The Lassa virus glycoprotein precursor GP-C is proteolytically processed by subtilase SKI-1/S1P. Proc Natl Acad Sci U S A **98**:12701–12705

[62] Ye J, Rawson RB, Komuro R, Chen X, Dave UP, Prywes R, Brown MS, Goldstein JL (2000) ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell **6**:1355–1364

[63] Yang J, Goldstein JL, Hammer RE, Moon YA, Brown MS, Horton JD (2001) Decreased lipid synthesis in livers of mice with disrupted Site-1 protease gene. Proc Natl Acad Sci U S A **98**:13607–13612

[64] Mouchantaf R, Watt HL, Sulea T, Seidah NG, Alturaihi H, Patel YC, Kumar U (2004) Prosomatostatin is proteolytically processed at the amino terminal segment by subtilase SKI-1. Regul Pept **120**:133–140

[65] Stirling J, O'hare P (2006) CREB4, a Transmembrane bZip Transcription Factor and Potential New Substrate for Regulation and Cleavage by S1P. Mol Biol Cell **17**:413–426

[66] Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, Basak A, Prat A, Chretien M (2003) The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): Liver regeneration and neuronal differentiation. Proc Natl Acad Sci U S A **100**:928–933

[67] Naureckiene S, Ma L, Sreekumar K, Purandare U, Frederick Lo C, Huang Y, Chiang LW, Grenier JM, Ozenberger BA, Steven Jacobsen J, Kennedy JD, DiStefano PS, Wood A, Bingham B (2003)

Functional characterization of Narc 1, a novel proteinase related to proteinase K. Arch Biochem Biophys **420**:55–67

[68] Seidah NG (2001) Cellular limited proteolysis of precursor proteins and peptides. In: Dalbey RE, Sigman DS (eds), The Enzymes: Co- and Posttranslational Proteolysis of Proteins, San Diego, CA, Academic Press, pp 237–258

[69] Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, Derre A, Villeger L, Farnier M, Beucler I, Bruckert E, Chambaz J, Chanu B, Lecerf JM, Luc G, Moulin P, Weissenbach J, Prat A, Krempf M, Junien C, Seidah NG, Boileau C (2003) Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet **34**:154–156

[70] Attie AD, Seidah NG (2005) Dual regulation of the LDL receptor – some clarity and new questions. Cell Metab **1**:290–292

[71] Cohen J, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH (2005) Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nat Genet **37**:161–165

[72] Jutras I, Seidah NG, Reudelhuber TL, Brechler V (1997) Two activation states of the prohormone convertase PC1 in the secretory pathway. J Biol Chem **272**:15184–15188

[73] Fricker LD, McKinzie AA, Sun J, Curran E, Qian Y, Yan L, Patterson SD, Courchesne PL, Richards B, Levin N, Mzhavia N, Devi LA, and Douglass J (2000) Identification and characterization of proSAAS, a granin-like neuroendocrine peptide precursor that inhibits prohormone processing. J. Neurosci **20**:639–648

[74] Basak A, Koch P, Dupelle M, Fricker LD, Devi LA, Chretien M, and Seidah NG (2001) Inhibitory specificity and potency of proSAAS-derived peptides toward proprotein convertase 1. J. Biol. Chem **276**:32720–32728

[75] Mbikay M, Seidah NG, and Chretien M (2001) Neuroendocrine secretory protein 7B2: Structure, expression and functions. Biochem. J **357**:329–342

[76] Hsi KL, Seidah NG, De Serres G, Chretien M (1982) Isolation and NH2-terminal sequence of a novel porcine anterior pituitary polypeptide. Homology to proinsulin, secretin and Rous sarcoma virus transforming protein TVFV60. FEBS Lett. **147**:261–266

[77] Seidah NG, Hsi KL, De Serres G, Rochemont J, Hamelin J, Antakly T, Cantin M, Chretien M (1983) Isolation and NH2-terminal sequence of a highly conserved human and porcine pituitary protein belonging to a new superfamily. Immunocytochemical localization in pars distalis and pars nervosa of the pituitary and in the supraoptic nucleus of the hypothalamus. Arch Biochem Biophys **225**:525–534

[78] Marcinkiewicz M, Benjannet S, Falgueyret JP, Seidah NG, Schurch W, Verdy M, Cantin M, Chretien M (1988) Identification and localization of 7B2 protein in human, porcine, and rat thyroid gland and in human medullary carcinoma. Endocrinology **123**:866–873

[79] Fricker LD, McKinzie AA, Sun J, Curran E, Qian Y, Yan L, Patterson SD, Courchesne PL, Richards B, Levin N, Mzhavia N, Devi LA, Douglass J (2000) Identification and characterization of proSAAS, a granin-like neuroendocrine peptide precursor that inhibits prohormone processing. J Neurosci **20**:639–648

[80] Naggert JK, Fricker LD, Varlamov O, Nishina PM, Rouille Y, Steiner DF, Carroll RJ, Paigen BJ, Leiter EH (1995) Hyperproinsulinaemia in obese fat/fat mice associated with a carboxypeptidase E mutation which reduces enzyme activity. Nat Genet **10**:135–142

[81] Qian Y, Devi LA, Mzhavia N, Munzer S, Seidah NG, Fricker LD (2000) The C-terminal region of proSAAS is a potent inhibitor of prohormone convertase 1. J Biol Chem **275**:23596–23601

[82] Zhu, X, 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

[83] Benjannet S, Rondeau N, Day R, Chretien M, Seidah N (1991) PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues. Proc. Natl. Acad. Sci. U. S. A **88**:3564–3568

[84] Boudreault A, Gauthier D, Rondeau N, Savaria D, Seidah N, Chretien M, Lazure C (1998) Molecular characterization, enzymatic analysis, and purification of murine proprotein convertase-1/3 (PC1/PC3) secreted from recombinant baculovirus-infected insect cells. Prot. Exp. Purif **14**:353–366

[85] Wei S, Feng Y, Che FY, Pan H, Mzhavia N, Devi LA, McKinzie AA, Levin N, Richards WG, Fricker LD (2004) Obesity and diabetes in transgenic mice expressing proSAAS. J Endocrinol **180**:357–368

[86] Anderson ED, VanSlyke JK, Thulin CD, Jean F, Thomas G (1997) Activation of the furin endoprotease is a multiple-step process: Requirements for acidification and internal propeptide cleavage. EMBO J **16**:1508–1518

[87] Zhong M, Munzer JS, Basak A, Benjannet S, Mowla SJ, Decroly E, Chrétien M, Seidah NG (1999) The prosegments of furin and PC7 as potent inhibitors of proprotein convertases: In vitro and ex vivo assessment of their specificity and selectivity. J. Biol. Chem **274**:33913–33920

[88] Bhattacharjya S, Xu P, Zhong M, Chretien M, Seidah NG, Ni F (2000) Inhibitory activity and structural characterization of a C-terminal peptide fragment derived from the prosegment of the proprotein convertase PC7. Biochemistry **39**:2868–2877

[89] Boudreault A, Gauthier D, Lazure C (1998) Proprotein convertase PC1/3-related peptides are potent slow tight-binding inhibitors of murine PC1/3 and Hfurin. J Biol Chem **273**:31574–31580

[90] Lazure C, Gauthier D, Jean F, Boudreault A, Seidah NG, Bennett HP, Hendy GN (1998) In vitro cleavage of internally quenched fluorogenic human proparathyroid hormone and proparathyroid-related peptide substrates by furin. Generation of a potent inhibitor. J Biol Chem **273**:8572–8580

[91] Sawada Y, Inoue M, Kanda T, Sakamaki T, Tanaka S, Minamino N, Nagai R, Takeuchi T (1997) Co-elevation of brain natriuretic peptide and proprotein-processing endoprotease furin after myocardial infarction in rats. FEBS Lett. **400**:177–182

[92] Lu W, Zhang W, Molloy SS, Thomas G, Ryan K, Chiang Y, Anderson S, Laskowski Jr M (1993) Arg15-Lys17-Arg18 turkey ovomucoid third domain inhibits human furin. J Biol Chem **268**:14583–14585

[93] Garten W, Hallenberger S, Ortmann D, Schafer W, Vey M, Angliker H, Shaw E, Klenk HD (1994) Processing of viral glycoproteins by the subtilisin-like endoprotease furin and its inhibition by specific peptidylchloroalkylketones. Biochimie **76**:217–225

[94] Bathurst IC, Brennan SO, Carrell RW, Cousens LS, Brake AJ, Barr PJ (1987) Yeast KEX2 protease has the properties of a human proalbumin converting enzyme. Science **235**:348–350

[95] Brennan SO, Peach RJ (1988) Calcium-dependent KEX2-like protease found in hepatic secretory vesicles converts proalbumin to albumin. FEBS Lett. **229**:167–170

[96] Anderson ED, Thomas L, Hayflick JS, Thomas G (1993) Inhibition of HIV-1 gp160-dependent membrane fusion by a furin-directed α 1-antitrypsin variant. J Biol Chem **268**:24887–24891

[97] Jean F, Stella K, Thomas L, Liu G, Xiang Y, Reason AJ, Thomas G (1998) α1-Antitrypsin Portland, a bioengineered serpin highly selective for furin: Application as an antipathogenic agent. Proc Natl Acad Sci USA **23**:7293–7298

[98] Dufour EK, Denault JB, Hopkins PC, Leduc R (1998) Serpin-like properties of alpha1-antitrypsin Portland towards furin convertase. FEBS Lett. **426**:41–46

[99] Vollenweider F, Benjannet S, Decroly E, Savaria D, Lazure C, Thomas G, Chrétien M and Seidah NG (1996) Comparative cellular processing of the human immunodeficiency virus (HIV-1) envelope glycoprotein gp160 by the mammalian subtilisin/kexin-like convertases. Biochem J **314**:521–532

[100] Benjannet S, Savaria D, Laslop A, Munzer JS, Chretien M, Marcinkiewicz M, Seidah NG (1997) Alpha1-antitrypsin Portland inhibits processing of precursors mediated by proprotein convertases primarily within the constitutive secretory pathway **272**:26210–26218

[101] Munzer JS, Basak A, Zhong M, Mamarbachi M, Hamelin J, Savaria D, Lazure C, Benjannet S, Chrétien M and Seidah NG (1997) In Vitro characterization of the novel proprotein convertase PC7. J Biol Chem **272**:19672–19681

[102] Lopez-Perez E, Seidah NG, Checler F (1999) Proprotein convertase activity contributes to the processing of the Alzheimer's β-amyloid precursor protein in human cells: Evidence for a role of the prohormone convertase PC7 in the constitutive α-secretase pathway. J Neurochem **73**:2056–2062

[103] Abrami L, Fivaz M, Decroly E, Seidah NG, Jean F, Thomas G, Leppla SH, Buckley JT, van der Goot FG, (1998) The pore-forming toxin proaerolysin is activated by furin. J Biol Chem **273**:32656–32661

[104] Cui Y, Jean F, Thomas G, Christian JL (1998) BMP-4 is proteolytically activated by furin and/or PC6 during vertebrate embryonic development. EMBO J **17**:4735–4743

[105] Khatib AM, Siegfried G, Prat A, Luis J, Chretien M, Metrakos P, Seidah NG (2001) Inhibition of proprotein convertases is associated with loss of growth and tumorigenicity of HT-29 human colon carcinoma cells: Importance of insulin-like growth factor-1 (IGF-1) receptor processing in IGF-1-mediated functions. J Biol Chem **276**:30686–30693

[106] Van Rompaey L, Ayoubi T, Van De Ven W, Marynen P (1997) Inhibition of intracellular proteolytic processing of soluble proproteins by an engineered alpha 2-macroglobulin containing a furin recognition sequence in the bait region. Biochem J **326**:507–514

[107] Dahlen JR, Jean F, Thomas G, Foster C, Kisiel W (1998) Inhibition of Soluble Recombinant Furin by Human Proteinase Inhibitor 8. J Biol Chem **273**:1851–1854

[108] Cameron A, Appel J, Houghten RA, Lindberg I (2000) Polyarginines Are Potent Furin Inhibitors. J Biol Chem **275**:36741–36749

[109] Roebroek AJ, Umans L, Pauli IG, Robertson EJ, van Leuven F, Van de Ven WJ, Constam DB (1998) Failure of ventral closure and axial rotation in embryos lacking the proprotein convertase furin. Development **125**:4863–4876

[110] Essalmani R, Hamelin J, Marcinkiewwicz E, Chamberland A, Mbikay M, Chretien C, Seidah N.G, Prat A (2006) Genetic deletion of PC/6 leads to early embryonic lethalithy. Mol.Cell Biol **26**:354–361

[111] Yang J, Goldstein JL, Hammer RE, Moon YA, Brown MS, Horton JD (2001) Decreased lipid synthesis in livers of mice with disrupted Site-1 protease gene. Proc Natl Acad Sci U S A **98**:13607–13612

[112] Furuta M, Yano H, Zhou A, Rouille Y, Holst JJ, Carroll R, Ravazzola M, Orci L, Furuta H, Steiner DF (1997) Defective prohormone processing and altered pancreatic islet morphology in mice lacking active SPC2. Proc Natl Acad Sci U S A **94**:6646–6651

[113] Zhu X, Zhou A, Dey A, Norrbom C, Carroll R, Zhang C, Laurent V, Lindberg I, Ugleholdt R, Holst JJ, Steiner DF (2002) Disruption of PC1/3 expression in mice causes dwarfism and multiple neuroendocrine peptide processing defects. Proc Natl Acad Sci U S A **99**:10293–10298

[114] Constam DB, Robertson EJ (2000) SPC4/PACE4 regulates a TGFbeta signaling network during axis formation. Genes Dev. **14**:1146–1155

[115] Mbikay M, Tadros H, Ishida N, Lerner CP, De Lamirande E, Chen A, El-Alfy M, Clermont Y, Seidah NG, Chretien M, Gagnon C, Simpson EM (2002) Disruption of PC1/3 expression in mice causes dwarfism and multiple neuroendocrine peptide processing defects. Proc Natl Acad Sci U S A **99**:10293–10298

[116] Milner JM, Rowan AD, Elliott SF, Cawston TE (2003) Inhibition of furin-like enzymes blocks interleukin-1alpha/oncostatin M-stimulated cartilage degradation. Arthritis Rheum **48**:1057–1066