Minireview

Proteolytic Processing in the Secretory Pathway*

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Many cellular processes, including embryogenesis (1–4), gene expression (5), cell cycle (6), programmed cell death (7), intracellular protein targeting (8) and endocrine/neural functions (9–13) are regulated by limited proteolysis of precursor proteins (14, 15). These functions are carried out by proteolytic enzyme families that are strategically localized within cells or on cell surfaces (3, 5–7, 9, 10). This review focuses on the serine proteases that process protein precursors (proproteins) traversing the secretory pathway (for recent reviews, see Refs. 9, 11–13, 16–19, 62). The early development of this field is reviewed in Ref. 14.

The Subtilisin-like Proprotein Convertases

The secretory pathway processing enzymes are calcium-dependent serine endoproteases related to subtilisin and the yeast processing protease Kex2p, or kexin (9, 10, 16), and hence have been called subtilisin-like proprotein convertases (SPCs) or more simply PCs. Seven members of this family in mammals have now been identified and characterized (Fig. 1). Although a three-dimensional structure is not yet available, their catalytic modules have been modeled on the basis of the x-ray structure of subtilisin (20, 21). Like subtilisin, these proteases become active by autocatalytic cleavage of an N-terminal propeptide, which is required for folding of the proenzymes (12, 13, 22). A downstream domain of about 150 amino acids, called the P- or Homo B-domain (Fig. 1) (9, 10), is also required for folding and activity. This domain plays a regulatory role, influencing both the calcium dependence and pH optima (23). The variable C-terminal regions of the PCs (Fig. 1) are less conserved and play a role in their subcellular routing (12, 13, 18).

The classical motif for processing by the PCs is KR or RR (9, 13). However, upstream basic residues at the P4 and/or P6 position also contribute to substrate recognition (16, 21, 25). Furin preferentially recognizes the motif RX/RKR but also is known to cleave RXXXR sites in some precursors (11, 26). Endoproteolytic cleavage is followed by exoproteolytic removal of the exposed C-terminal basic residues (14) by CPE in neuroendocrine and some other tissues, as well as by other recently discovered carboxypeptidases such as CPD (27, 28), CPZ (29, 30), and/or CPM (31).

Autoactivation Mechanism—The autoactivation of furin (11, 32) serves as a model for the other PCs, with the exception of PC2 (discussed below). Intramolecular cleavage of the prodomain allows furin to exit the ER (33). However, the prodomain remains attached noncovalently until the cleaved inactive proenzyme reaches the TGN where the more acidic (pH 6.5) and calcium-enriched environment facilitates dissociation of the prodomain (32). A sec-

ond cleavage within the prodomain then precludes further inhibitory interactions, resulting in full activation (32). A similar mechanism of activation has been demonstrated for PC1/PC3 (9), PC4 (13), PC5 (34), and PC7 (35, 36). Pro-PACE4 autoactivation is slow but probably also occurs prior to exit from the ER (37).

The Neuroendocrine Protein 7B2 Is Essential for the Activation of Pro-PC2—PC2 is unique in that it requires the acidic conditions of a late post-Golgi compartment for its autoactivation (38). In the ER pro-PC2 interacts with 7B2, a 27-kDa neuroendocrine secretory protein that is coexpressed with PC2 in many neuroendocrine tissues (39, 40). In the absence of 7B2, autocleavage of the PC2 prodomain will occur but give rise only to inactive enzyme (41). Biosynthetic studies show that 7B2 binds to pro-PC2 after it has folded and then facilitates its intracellular transport and activation (42). During its transport 7B2 undergoes cleavage at a polybasic site toward the C terminus, most likely by furin or related TGN proteases (43), resulting in the release of an N-terminal 21-kDa form and an inhibitory C-terminal fragment (44, 45). A KK site in this fragment is required for its specific inhibitory action on PC2 (42, 44) but also is slowly cleaved by PC2 (46). Whether the Cterminal peptide normally retards PC2 activity is unclear (47).

The 21-kDa N-terminal domain of 7B2 is capable of both generating and stabilizing active PC2 (48). It contains a polypeptide helix-like segment that interacts with pro-PC2 via structural determinants that appear to reside mainly within the catalytic domain (49); site-directed mutagenesis studies indicate that mutation of Tyr98 of pro-PC2 to Asp (as in PC1/PC3) blocks its binding to PC2 and subsequent activation (50). Interestingly, mutation of the unusual oxyanion residue, Asp-309, to Asn in pro-PC2 also inhibits binding to pro-7B2 (51).

Both PC2 and 7B2 are highly conserved in evolution; homologues of 7B2 have recently been described in the molluscan Lymnaea stagnalis (52) and in Caenorhabditis elegans (53). Recently, mice with a disruption of the 7B2 gene have been produced and, as expected, they lack active PC2 but have other defects (54). The nature of the 7B2-induced structural alterations that facilitate pro-PC2 autoactivation are of great interest as a topic for further study.

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‡ The abbreviations used are: SPC, subtilisin-like proprotein convertase; PC, proprotein convertase; CPE, carboxypeptidase E; CPD, carboxypeptidase D; CPZ, carboxypeptidase Z; CPM, carboxypeptidase M; ER, endoplasmic reticulum; TGN, trans-Golgi network; TM, transmembrane; MSH, melanocyte-stimulating hormone; POMC, proopiomelanocortin; ACTH, adrenocorticotropic hormone; TGF, transforming growth factor.
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Two Major Functional Branches

The mammalian SPCs function in either the regulated or constitutive branches of the secretory pathway. The convertases PC2 and PC1/PC3 (Fig. 1) are the major forms expressed in the neuroendocrine system and brain, where they act on prohormone and neuuropeptide precursors within dense core vesicles of the regulated secretory pathway (9, 13). PC4, which is expressed only in the testis (55), and an isoform of PC6 that lacks a TM domain, PC6A, also belong to this group (34). The differential expression of PC2 and PC1/PC3 in various endocrine cells and neurons gives rise to varied mixtures of peptide products with divergent or opposing activities, sometimes derived from the same precursor (Fig. 2). Both the transcription and translation of PC2 and PC1/PC3 mRNA are regulated in neuroendocrine cells by glucose, second messengers, and other factors (9, 56–58), usually in parallel with regulatory changes in prohormone expression.

The other major convertase family branch includes furin, PACE4, PC6B, and the more recently discovered PC7 (59–61) (Fig. 1). These convertases are expressed in many tissues, including the neuroendocrine system, liver, gut, and brain, where their active forms are localized in the TGN and small secretory vesicles of the constitutive pathway (11, 13, 62, 63). Because of alternative splicing, some of these exist in multiple forms, e.g., PACE4 (64) and PC5/PC6 (34). All of these convertases more closely resemble the yeast homologue, kexin, which also is localized in the TGN by a TM/cytosolic tail and functions analogously (65). The convertase genes share intron/exon structure (9, 13), implying their origin in ancestrally conserved repeats. PACE4 contains two dileucine motifs that might be involved in its retrieval from the TGN (78). Binding of PACS-1 (81) to the phosphorylated cytosolic domain promotes the return of furin to the TGN from the plasma membrane

Effects of Mutations and/or Disruptions in Convertase Genes

A Furin Null Mutation Results in Embryonic Lethality—Null embryos appear normal until day 8.5 but then fail to undergo axial rotation. Consequent disruption of the development of many systems, but especially of the heart and vascular systems, results in embryonic death between days 10.5 and 11.5 (4). Disruption of the gene encoding PC4 leads to severe impairment of fertility in homozygous males (88). The fertility of the PC4 null spermatozoa is reduced, and fertilized ova fail to develop. The results suggest that PC4 is required for the production of fertile and developmentally competent spermatozoa.

Multiple Effects of a PC2 Null Mutation—Mice lacking active PC2 because of deletion of exon 3 survive and reproduce but with reduced fertility (69, 70, 14). The males are sterile due to abnormal growth rate (89). Homozygous null mice exhibit a complex polyendocrine phenotype, whereas heterozygotes are normal. Pancreatic proinsulin stores are elevated to 35–40% of total insulin-like material (normal levels are below 5%), and these are the source of elevated circulating proinsulin. In biosynthetic labeling experiments half-times for the conversion of both mouse proinsulins I and II are prolonged approximately 3-fold (25). Larger than normal amounts of des-31,32-proinsulin, an intermediate cleaved at the B chain-C-peptide junction (the preferential site of action of PC1/PC3), are also generated (see Fig. 2).
Despite the defective processing of proinsulin the PC2 nulls have no tendency to develop diabetes. Instead, their blood glucose level is lower than normal, and the rise in response to glucose is reduced (89). Rouillé et al. (90, 91) have demonstrated that PC2 acts alone to generate the characteristic alpha cell pattern of processing of the multifunctional proglucagon molecule, resulting in the selective release of only glucagon (see Fig. 2). Accordingly, mature glucagon is not detectable in the plasma, although large amounts of proglucagon and some partially processed larger forms are present in the alpha cells and the circulation (89). The chronic hypoglycemia confirms the major role of glucagon in physiology as a tonic antagonist of insulin.

Prosomatostatin also is not processed normally to somatostatin 14 in the islet delta cells (89) or in the brain. Because PC2 is the predominant convertase in all the non-beta islet cells (92), it is likely that the biosynthesis of all of the other islet hormones is adversely affected. Lack of PC2 in the beta cells may also impair IAPP/familin production (93). The metabolic consequences of these defects are not known.

The PC2 null islets are enlarged and show marked hypertrophy and hyperplasia of the alpha, delta, and gamma cells in the periphery of the islets, whereas the central beta cell mass appears to be diminished significantly (89). Hyperplasia in the alpha and delta cell populations presumably represents an attempt to compensate for the lack of their normal processed hormonal products. Preliminary results indicate that long term administration of glucagon to PC2 null mice results in normalization of blood sugar levels and reversal of alpha cell hyperplasia.3 These results demonstrate that the lack of glucagon in the PC2 null animals accounts for at least two of the major phenotypes and dramatically illustrates the existence of dynamic feedback mechanisms that regulate the growth and relative size of these islet cellular compartments.

The PC2-deficient mice also have multiple defects in neuroendocrine function. Recent studies have shown marked reductions in IAPP/amylin production (93). The metabolic consequences of these defects like that of the PC2 null mice. However, they develop a fulminating form of Cushing’s disease because of excessive secretion of ACTH from the pituitary intermediate lobe. ACTH accumulates in this lobe because of the lack of PC2 to convert it to α-MSH (see Fig. 2), and PC2 may play a role in regulating its secretion (54).

PC1/PC3 Deficiency in Man—Although a mouse model of PC1/PC3 deficiency is not yet available, an adult subject with severe obesity and hyperinsulinemia (97) has been found to be a compound heterozygote for inactivating gene mutations (98). Multiple endocrine deficiencies include elevated proinsulin and ACTH precursors (POMC and intermediates) in the plasma (97); PC1/PC3 is the major convertase that cleaves ACTH from POMC in the anterior pituitary corticotrophs (16) (see Fig. 2). Increased amounts of des-64,65-proinsulin intermediates accompany elevated intact proinsulin, as would be expected from a lack of PC1/PC3-mediated cleavage at the B chain-C-peptide junction to generate des-31,32-proinsulin (Fig. 2), an intermediate that is more readily cleaved by PC2 than is intact proinsulin (99). The absence of detectable insulin in the blood suggests that PC1/PC3 plays a major role in proinsulin processing.

The early and marked obesity probably is the result of defective processing of neuropeptides involved in hypothalamic regulation of food intake (100). Hypogonadotropic hypogonadism in this subject (97) suggests that PC1/PC3 may also be involved in processing gonadotropin-releasing hormone.

CPE Deficiency Syndrome—CPE<sup>−/−</sup> mice are obese, hyperglycemic, and hyper/pro-insulinemic because of an inactivating point mutation in the CPE gene (Ser-202 → Pro) (101, 102). Other processing abnormalities such as CPD (27) and CP2 (29) partially offset this defect in brain and some tissues. Pancreatic extracts contain 40–50% proinsulin and arginine-extended forms of insulin. The rapid buildup of such C-terminally extended intermediates may inhibit the SPC endopeptidases in various neuroendocrine tissues (96, 103, 104).

A recent proposal that CPE is a sorting receptor for the regulated secretory pathway (105, 106) is not supported by studies directly measuring the efficiency of proinsulin sorting in islets of CPE<sup>−/−</sup> mice. Intermediate forms of proinsulin and POMC are found in abundant secretory granules in islet and pituitary cells, respectively, of the CPE<sup>−/−</sup> mice, consistent with normal sorting.

Perspective

Although a large body of data strongly supports the notion that the SPC family of enzymes plays a central role in the processing of most precursor proteins in the secretory pathway, another possibility that unidentified enzymes may participate in some cleavages at single basic residues and other unusual cleavage sites. For example, a recently discovered ER-localized protease with a subtilisin-like catalytic domain is distantly related to the SPCs and cleaves at sites having the sequence RSVL↓ (5, 111). This enzyme cleaves the precursor of the sterol regulatory element-binding protein and may well represent the first member of a novel subfamily of ER-active processing enzymes (5), i.e., similar cleavage sites have been noted in prolactin and several other precursor proteins (see Ref. 13). Efforts to identify other types of processing proteases, such as thiol, aspartic, or metallopeptases (reviewed in Refs. 108 and 109), have yet to lead to definitive genetic evidence to support their participation in neuroendocrine precursor processing. Much remains to be done to elucidate the structural features of the SPCs that lead to their great selectivity, sorting behavior, pH sensitivity, and calcium activation and to define more fully their normal substrates and the regulation of their tissue-specific expression.

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