Minireview

Furin/PACE/SPC1: a convertase involved in exocytic and endocytic processing of precursor proteins

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Abstract One of the most exciting breakthroughs of the 90's in the fields of biochemistry, cell biology and neuroendocrinology is the identification of a novel family of proteolytic enzymes called mammalian subtilisin-like convertases. This family is comprised so far of seven distinct endoproteases responsible for the proteolytic excision of biologically active polypeptides from inactive precursor proteins. Six years after the initial observation of a structural conservation between a characterized yeast enzyme (kexin) and a human gene product (furin), it is now well accepted that one of these convertases, furin, has the enzymatic capabilities to efficiently and correctly process a great variety of precursors. Furin's ability to cleave precursors within both the exocytic and endocytic pathways will require sustained efforts in order to delineate all of its physiological roles.

Key words: Furin; PACE; SPC1; Precursor processing; Convertase; Exocytic pathway; Endocytic pathway

1. Introduction

Biologically active polypeptides act as intercellular messengers of information (neuropeptides, hormonal peptides), direct many cellular activities (growth factors, enzymes, receptors) and are involved in the pathogenicity associated with certain viruses (viral glycoproteins) or bacteria (toxins). Since many of these polypeptides are initially synthesized as large, inactive precursors or pro-proteins, a common cellular mechanism must function to release the bioactive segment of the protein. This mechanism is the proteolytic cleavage of the precursor by cellular endoproteases which usually takes place at specific pairs of basic amino acids like Arg–Arg or Lys–Arg [1]. This processing event is found in bacteria, fungi, yeast, invertebrates and mammals and occurs both within the exocytic (secreted and membrane proteins) as well as the endocytic pathway (uptake, processing and degradation of proteins). At the end of 1989, it was observed that the yeast enzyme, kexin, which had been characterized as a Ca^{2+}-dependent serine protease able to cleave yeast pro-α factor and pro-killer toxin, had a high amino acid sequence similarity at its catalytic site with a human gene product, furin (also called SPC1 or PACE for *p*aired basic *a*mino acid *c*leaving *e*nzyme) [2]. The corresponding gene, *FUR*, was the upstream region of the fes/fps proto-oncogene and had been partially sequenced three years before [3].

This was the beginning of an extensive search for other proteases of this family. Presently, there are seven distinct convertases that we can tentatively assign to four subgroups based on their tissue localization; the furin and PC7 subgroup has a ubiquitous distribution, the PACE4 and PC5 subgroup has a distribution which overlaps that of the previous enzymes but not in all cases, the PC1 (also called PC3) and PC2 subgroup is restricted to endocrine and neuroendocrine cells while PC4 is limited to round spermatids [4]. Although these enzymes may diverge in their tissue distribution and in their cellular localization, one common thread resides in their specificity to process protein precursors at some single basic residues and/or selective pairs of basic amino acids permitting release of the active peptide. Here, we will focus on one of these convertases, furin.

2. Tissue and sub-cellular localization of furin

Furin (EC 3.4.21.85) is the first and so far the best characterized enzyme of the mammalian subtilisin-like family of convertases. Northern blot analysis has demonstrated that the major 4.4 kb furin transcript is ubiquitously distributed [4]. In human, this mRNA gives rise to a type I membrane-bound protein localized mainly in the trans-Golgi network (TGN). Recent reports have demonstrated that furin can translocate between the cell surface and the TGN [5,6]: this translocation event is highly reminiscent of the retrieval pathway observed in the case of the TGN38 protein [7]. Translocation to the cell surface and from early endosomes back to the TGN may depend on two routing signals found in the cytoplasmic domain of the enzyme [8]. The first of these, the CPSDSEEDEG sequence (residues 771–780), contains a casein kinase II phosphorylation motif (S/T-D/E-D/E-D/E) which may be subjected to different phosphorylation patterns at the Ser^{773} and Ser^{775} residues [9,10]. The result of these phosphorylation/dephosphorylation events would be the routing of furin towards specific organelles, either the TGN or the cell surface via endosomes or granules. The other targeting signal is the YKGL sequence (residues 757–760), a determinant for routing furin to the endosomes [8]. In light of these observations, it is interesting to note that proteins containing the YXXØ motif, where Ø is a bulky hydrophobic side chain, have recently shown to bind medium chains of clathrin-associated protein complexes [11]. The physiological role of such translocation events is unknown but cell surface furin may mediate processing of bacterial toxin precursors as well as viral fusion protein precursors (see Table 1), both of which are detrimental to the host cell. It had been originally suggested that the *FUR* gene product could act as a receptor molecule due to the fact that it contains a cysteine-rich region,

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a motif found in the tumor necrosis factor receptor, insulin receptor and epidermal growth receptor molecules [12]. The recent observations of a furin-like protein in the cellular membranes of tomato leaves which would interact with systemin [13], a powerful inducer of proteinase inhibitor synthesis, could lend weight to this hypothesis.

Once furin has reached the cell membrane it may also be further processed either by an autocatalytic process or via another protease. This possibility is brought about by evidence demonstrating the presence of a shortened form of the enzyme in the media of cells overexpressing native furin [14] although the likelihood exists that overexpression systems may artefactually be responsible for the production of this secreted product. More experiments are needed to measure levels of furin outside the cell which may lead to the elucidation of the possible physiological role of this enzyme in cleavage of extracellular substrates.

3. Biochemical and enzymatic features of furin

The human *FUR* gene, mapped to chromosome 15q25-q26 [3,4], was cloned in 1990 from a cDNA library constructed from a human liver cell line, HepG2 cells [15,16]. This led to the identification of *Rattus norvegicus* [17], *Mus musculus* [18], *Drosophila melanogaster* [19,20], *Caenorhabditis elegans* [21], *Aplysia californica* [22], *Xenopus laevis* [23], *Lymnaea stagnalis* [24], *Bos taurus* [6], *Cricetulus griseus* [25] enzymes corresponding to or closely related to furin.

Upon examination of the human furin cDNA sequence, we can predict a translation product of 794 amino acids. As depicted in Fig. 1, the general organization of the various domains of this Ca^{2+}-dependent protease is as follows: (1) a signal peptide enabling access to the secretory pathway; (2) a pro-region which may act as a pseudo-chaperone and when removed from the zymogen leads to enzyme activation; (3) a catalytic domain whose structure is closely related to subtilisin-like enzymes; (4) a homo or P-domain whose partial or total integrity might be necessary for proper enzymatic activity and which contains a 'receptor like' cysteine-rich region; (5) a hydrophobic transmembrane spanning domain anchoring the enzyme to membranes and (6) a cytoplasmic tail, possibly involved in translocation of the protease from one organelle to the other. Additionnally, various motifs exist within the protease which may help in delineating some of its properties as previously mentioned (see Fig. 1).

Initial efforts to express furin relied on a recombinant *vaccinia* virus expression system [26]. Extracts of BSC-40 cells infected with the recombinant virus contain two furin translation products (90 and 96 kDa) corresponding to the active and zymogen form of the enzyme, respectively. It was later determined that the 96 kDa form underwent an intramolecular autocatalytic activation [27] by removal of the pro-region in the endoplasmic reticulum [6]. To fully examine furin's enzymatic properties, a secreted form of furin (termed soluble furin), having its trans-membrane domain removed, was constructed which facilitated its purification and characterization [27,28].

Furin's enzymatic activity and specificity has been evaluated in vitro and in vivo. In vitro assays relying on the use of either the shed form of furin [14] and/or the soluble furin [28] measure the cleavage of various substrates such as the protective antigen (PA) precursors or fluorogenic tetrapeptides or using recently developped internally quenched fluorogenic peptidyl substrates [28–30]. In vivo, by co-expressing various substrate precursors along with furin, more than 25 precursors have now been cleaved by furin (Table 1) at what has been described as the minimal furin recognition site, R-X-X-R, though it has also been shown that other members of the convertase family could cleave this motif efficiently [31]. In this vein, it has been observed that characterization of furin enzymatic activity must take into account the level of produced enzyme which, in overexpression systems, leads to an elevated, non-physiologically relevent protease to substrate ratio and thus broadens furin's specificity [32]. Thus, complementation of in vivo studies with in vitro enzymatic digestions using purified enzyme and defined substrates are necessary to compensate for such discrepancies. Overall however, a definition of the processing site used by furin permits the inclusion of other determinants that this convertase accepts for eventual cleavage. Arginine residues in the P8, P6, P4, P2 and P1 position relative to the scissile bond (R-X-R-X-R-X-X-R↓) will in certain cases either be critical (P1 and P4) or contribute (P2, P6 and P8) to cleavage while the + 1 (P_1') position should not be an amino acid containing a hydrophobic aliphatic side chain [33]. For example, the use of prorenin as a model substrate in vivo coupled to in vitro incubations demonstrated a sequence specificity of RXRX-KR > XXRXKR ≥ RXRXXR > RXXXKR > XXRXXR for furin while XXXXKR and RXXXXR sites were not cleaved [33].

However, in addition to the inherent limitations of the in vivo expression system as already noted, the delineation of furin enzymatic specificity in vivo is complicated by the fact that, in

Table 1

PROTEIN PRECURSORS CLEAVED BY FURIN

Viral glycoproteins and proteins	cleavage site	
Human cytomegalovirus glycoprotein B	LTHNR**TKR**↓	*ST*[b]
Measles virus glycoprotein F_0	ASSR**RHKR** ↓	*FA*
Newcastle disease virus glycoprotein F_0	SGGR**RQRR** ↓	*FI*
Human immunodeficiency virus glycoprotein 160 (gp160)	RVVQ**REKR** ↓	*AV*
Fowl plague (influenza A) virus hemagglutinin	PSKK**REKR** ↓	*GL*
Sindbis virus gpE2	GSSG**RSKR** ↓	*SV*
Mouse mammary tumor virus-7 superantigen	GIEN**RKRR** ↓	*ST*
Human parainfluenza virus type 3 glycoprotein F_0	NTDP**RTKR** ↓	*FF*
Toxins		
Shigella dysenteria type 1 shiga toxin subunit A	HHSA**RVAR** ↓	*MA*
Corynebacterium diphtheriae diphtheria toxin	CAGN**RVRR** ↓	*SV*
Bacillus anthracis protective antigen (PA)	SSNS**RKKR** ↓	*ST*
Pseudomonas aeruginosa exotoxin A	FTRH**RQPR** ↓	*GW*
Receptors		
Human insulin pro-receptor	PRPS**RKRR** ↓	*SL*
Hepatocyte growth factor/scatter factor receptor (HGF/SF)	LTEK**RKKR** ↓	*ST*
Plasma proteins		
Human proalbumin	SRGVFF**RR** ↓	*DA*
Human complement pro-C3	QPAA**RRRR** ↓	*SV*
Human pro-von Willebrand factor	PLSH**RSKR** ↓	*SL*
Human pro-factor IX	KILN**RPKR** ↓	*YN*
Human vitamin K-dependant pro-factor X	QTLE**RRRR** ↓	*SV*
Hormones and growth factors		
Human pro-parathyroid related peptide	GLSR**RLKR** ↓	*AV*
Human pro-parathyroid hormone	DGKSV**KKR** ↓	*SV*
Mouse pro-β-nerve growth factor	NRTH**RSKR** ↓	*SS*
Human pro-transforming growth factor type β	LQSS**RHRR** ↓	*AL*
Human pro-endothelin-1 (pET-1)	WRLR**RSKR** ↓	*CS*
Others		
Human pro-furin	QVAK**RTKR** ↓	*DV*
Human stromelysin-3	SARN**RQKR** ↓	*FV*

[a] Residues in boldface are those that constitute a furin recognition motif.
[b] Residues in italic are serine residues and hydrophobic residues found in many precursors at P1' and P2' positions respectively.

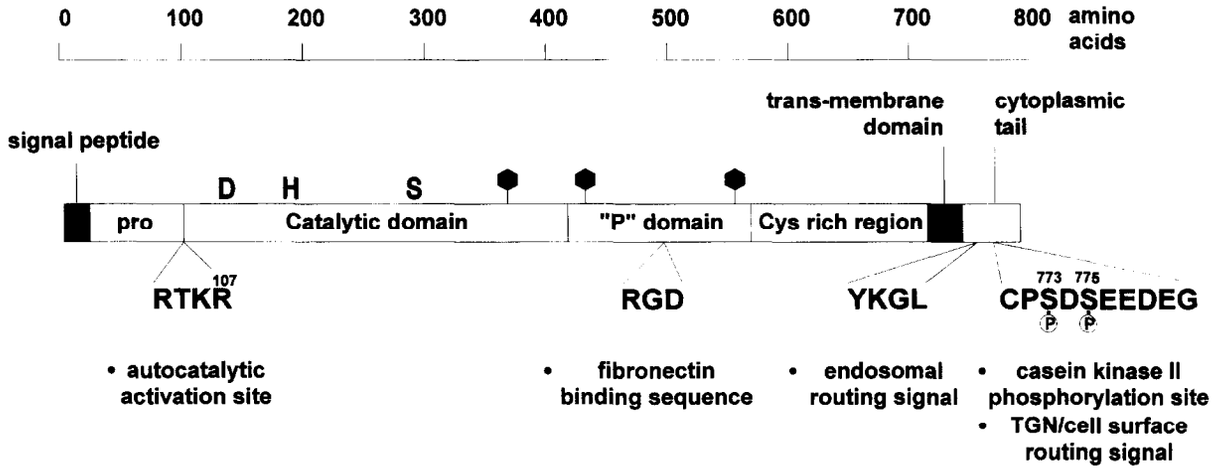

Fig. 1. Schematic representation of human furin convertase.

addition to furin and depending on the cell type used, some other convertases are present which might exhibit similar cleaving patterns and thus contribute to processing of a particular precursor; the choice of a suitable cell line then becomes crucial. Consequently, a few cellular models have been found and/or defined in an effort to eliminate endogenous participation of various enzymes. For instance *LoVo* cells, a human colon carcinoma cell line, contain a mutated *FUR* gene which renders the enzyme inactive [34]. However, these cells have elevated mRNA levels of PACE4, another convertase, and because of similar cleavage specificities observed between furin and PACE4 [35] caution must be taken in interpretation of data. Another cell type, RPE.40, have demonstrated processing deficiencies and thus have also been used to study furin-related cleavage of specific precursors [36].

4. Regulation of furin expression

Distinct promoters (P1, P1A and P1B), containing SP1 elements and a combination of regulated TATA and GC-rich regions, have been identified on the *FUR* gene [37] which may explain why multiple mRNA isoforms can be detected; these range from 4.0, 4.5, 6.8 and 8.4 kb [19,20] but usually a single furin transcript of 4.4 kb is detected in most tissues. Extensive studies performed in *Drosophila* indicate that splicing events seem to be occurring in this lower species with variations of mRNA levels and size at different larval stages and presence of furin in adult female nurse cells, in developing oocytes and brain cell soma [19,20]. This suggests a role for furin in development and behaviour. Again in *Drosophila* two homologous genes to human *FUR* have been identified; three proteins (dfurin1, dfurin1-CRR, dfurin1-X) are generated by the *Dfur1* gene while the *Dfur2* gene gives rise to dfurin-2 [38]. Interestingly, the biosynthetic fates of these molecules differ, some being efficiently converted from zymogen to active enzyme while others are detected as soluble enzymes.

Although very little information in higher organisms is presently available on the control of furin expression, the recent observations that the *FUR* gene contains three distinct promoters, as above mentioned, may provide clues as to the onset of expression or as to different levels of expression detected in various cell types and tissues. Thus, a delicate balance of furin activity is probably required by all cells since overexpression of this enzyme could be toxic to cells or could lead to pathological aberrations. In support of this, it has been observed that treatment of synovial cells and NIH-3T3 cells with a cytokine, TGF-β, resulted in a significant increase in furin transcript levels [39]. Since furin is the prime candidate as the proTGF-β cleaving enzyme [40], the cytokine may auto-regulate its own convertase and hence its conversion rate.

Regulation or modulation of enzymatic activity can also be examined at the post-translational level. Initial efforts to impede convertase activity relied on the use of active-site-directed compounds such as the chloroalkylketones eventhough other types of inhibitors have also been prepared [41]. These highly reactive compounds, although very useful in biochemical and cellular studies suffer from a lack of specificity when, as in the case of the convertases, closely related active sites are to be targeted. Careful tailoring of the peptidyl sequence may, however, improve the specificity of these reagents. An example of in vivo convertase inhibition using chloromethylketones was the inhibition of furin-derived processing of HIV-1 gp160 to the gp120 protein [42]. It was shown that interference of cleavage of the glycoprotein by decanoyl-Arg-Glu-Lys-Arg-chloromethylketone, not the best of the tetrapeptidyl sequences recognized by furin, led to the diminished formation of infectious particles. In a similar type of experiment, in vivo processing of proendothelin-1 was markedly reduced by use of a similar compound, decanoyl-Arg-Val-Lys-Arg-chloromethylketone [43]. Failure to completely abolish processing of these precursors may be due to (1) the efficacy of penetration of the peptide within cells or (2) the presence of other convertases not inhibited by the use of a specific compound and being able to correctly process the precursor, albeit less efficiently or both.

Protein-based serine protease inhibitors have also been evaluated for their capacity to abolish furin activity. Initially, a turkey ovomucoid third domain variant was shown to be inhibitory towards furin [44]. Later, an engineered variant of the endogenous elastase inhibitor, the serpin α_1-antitrypsin (AT), was designed and tested [45]. This protein, called α_1-antitrypsin Portland (AT-PDX) where the reactive site of the wild type protein (Ala^{355}-Ile-Pro-Met^{358}) is replaced with a furin recognition site (Arg^{355}-Ile-Pro-Arg^{358}), has a $K_{0.5}$ of 30 ng/ml (550 pM) which compares well to inhibition constants of compounds

used in therapeutic intervention for inhibition of other types of enzymes. This serpin has been recently described in a measles virus model to inhibit furin-related processing of fusion glycoprotein which resulted in loss of syncitia formation [46] and thus may offer novel approaches to antiviral therapy.

5. Future perspectives

Because of the ubiquity of furin, the broad distribution and shared similarity in specificity to other mammalian convertases along with the possible role of furin in processing essential cellular factors, inhibition of furin in physio-pathological states associated with elevated levels of biologically active factors, is somewhat of an equivocal issue. However, a potential target of furin inhibitors may be the extracellular membrane form of the enzyme. Though no physiological role has been attributed to extracellular furin, its involvement and consequently its inhibition to convert various bacterial pro-toxins may be addressed in vivo in infected animal models. Another interesting aspect is the putative furin interacting or binding proteins of the kinase or phosphatase class; attempts to identify these proteins are in progress. Also, further characterization of the various domains of the enzyme, notably the pro-region, the cys-rich region and the cytoplasmic tail could be revealing. What is most certain is the evergrowing interest in this field of research which will undoubtedly lead to multifaceted discoveries.

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