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FURIN AT THE CUTTING EDGE: FROM PROTEIN TRAFFIC TO EMBRYOGENESIS AND DISEASE

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Abstract

Furin catalyses a simple biochemical reaction – the proteolytic maturation of proprotein substrates in the secretory pathway. But the simplicity of this reaction belies furin's broad and important roles in homeostasis, as well as in diseases ranging from Alzheimer's disease and cancer to anthrax and Ebola fever. This review summarizes various features of furin – its structural and enzymatic properties, intracellular localization, trafficking, substrates, and roles *in vivo*.

Furin is a cellular ENDOPROTEASE that was identified in 1990 (BOX 1); it proteolytically activates large numbers of PROPROTEIN substrates in secretory pathway compartments. As well as activating pathogenic agents (BOX 2), furin has an essential role in embryogenesis, and catalyses the maturation of a strikingly diverse collection of proprotein substrates. These range from growth factors and receptors to extracellular-matrix proteins and even other protease systems that control disease. Until recently, furin was thought to be an unglamorous housekeeping protein; however, furin's crucial role in so many different cellular events — and in diseases ranging from from anthrax and bird flu (BOX 2) to cancer, dementia and Ebola fever — has caused researchers to re-evaluate it. In this review, I summarize the various features of furin: its structural and enzymatic properties, autoactivation, intracellular localization and trafficking; its substrates; and its roles *in vivo*, including the requirement for furin in determining the PATHOGENICITY of many viruses and bacteria.

ENDOPROTEASE

An enzyme that hydrolyses peptide and protein substrates at specific internal peptide bonds. By contrast, exoproteases remove either the carboxy-terminal (carboxypeptidase) or amino-terminal (aminopeptidase) residues.

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OMIM: <http://www.ncbi.nlm.nih.gov/Omim/> Alzheimer's disease | familial British dementia | familial Danish dementia | rheumatoid arthritis

Swiss-Prot: <http://www.expasy.ch/> ADAM10 | ADAM17 | ADAMTS-4 | APP | APRIL | ARF1 | ATR | BAFF | BMP-4 | CI-MPR | CPD | EDAR | EF | Furin | gelsolin | GGA3 | HA | IGF1R | KIF13A | Lefty-2 | LF | MMP2 | MT1-MMP | β -NGF | Nodal | PA | PACE4 | PACS-1 | PC5/6A | PC5/6B | PC7 | RAB6 | β -secretase | SMAD2 | SNX-15 | Sortilin | TGF- β 1 | TNF- α

PROPROTEIN

A precursor protein that is proteolytically cleaved, typically at sites formed by doublets or clusters of basic amino acids, to produce the mature and active protein or, in the case of prohormones, the mature peptide hormones.

PATHOGENICITY

A measure of the ability of a pathogen to invade a host and cause disease. The degree of the pathogenicity is measured as its virulence.

The biochemical properties of furin

Domain structure

Furin is a ubiquitously expressed 794-amino-acid TYPE-I TRANSMEMBRANE PROTEIN that is found in all vertebrates and many invertebrates^{1,2}. Its large luminal/extracellular region has an overall homology with the same regions of other members of the PROPROTEIN CONVERTASE (PC) family (FIG. 1; TABLE 1), which belongs to the SUBTILISIN SUPERFAMILY of serine endoproteases. The greatest sequence similarity resides in the subtilisin-like catalytic domain; the aspartate (Asp), histidine (His) and serine (Ser) residues that form the CATALYTIC TRIAD are rigorously conserved, and the catalytic domains of the other PCs are 54–70% identical in sequence to furin. In addition to the signal peptide, which directs translocation of the pro-enzyme into the endoplasmic reticulum (ER), furin and the other PCs contain prodomains that are flanked by the signal peptidase cleavage site on the amino-terminal side and by a conserved set of basic amino acids that comprise the autoproteolytic cleavage site on the carboxy-terminal side. This essential prodomain has a crucial role in the folding, activation and transport of PCs, and in the regulation of PC activity. Furin and the other PCs also share a conserved P domain, which is essential for enzyme activity and the modulation of pH and calcium requirements³; this P domain is absent from the related bacterial enzymes. The furin cytoplasmic domain controls the localization and sorting of furin in the *trans*-Golgi network (TGN)/endosomal system, and furin is an important model for understanding the regulation of protein trafficking in mammalian cells.

pH and ion requirements

Furin has a broad pH optimum; it has more than 50% of its enzymatic activity between pH 5 and 8, depending on the substrate being cleaved. Like other members of the subtilisin superfamily, furin is strictly calcium dependent, requiring approximately 1 mM calcium for full activity. Modelling studies based on the alignment of furin with the solved structure of BACTERIAL THERMITASE — a well-characterized subtilisin that shares 29% sequence identity with the furin catalytic domain — indicate that furin has two calcium-binding pockets: one with medium affinity and one with high affinity⁴. Furin also binds weakly to potassium, and 20 mM potassium increases furin activity by enhancing the rate of deacylation, which is important in furin's catalytic cycle⁵.

Box 1 The discovery of furin

In 1990, the identification of furin as the first *bona fide* mammalian proprotein convertase (PC) ended a nearly quarter-century-long search for the mammalian enzymes that catalyse the proteolytic maturation of prohormones and proproteins (for a review see REFS 150,151). The quest for the PCs began with seminal studies by Donald Steiner in 1967 (REFS 152, 153), who showed for the first time that peptide hormones are post-translationally excised from larger prohormones by cleavage of the precursor at doublets, or clusters, of basic amino

acids (for example, $-\text{Lys}-\text{Arg}^{\downarrow}-$ and $-\text{Arg}-\text{Arg}^{\downarrow}-$, where \downarrow identifies the cleavage site). These studies were as revolutionary as those by Krebs and Fischer, which showed that protein phosphorylation is a universal modification in signal transduction. Concurrent studies by Michel Chrétien and Choh Hoh Li on the structural relationships between β -melanocyte stimulating hormone (β -MSH), γ -lipotropin (γ -LPH) and β -lipotropin (β -LPH)¹⁵⁴ — a subset of peptides derived from a complex pituitary prohormone, proopiomelanocortin (POMC) — provided the first clues to the greater generality of proprotein processing.

Together, these studies set the foundations of subsequent research in protein processing over the next 20 years, which showed that virtually all peptide hormones, numerous bioactive proteins (for example, growth factors, receptors and cell-adhesion molecules), and many bacterial toxins and viral envelope glycoproteins, follow this fundamental maturation scheme to generate the mature and biologically active molecule (for a review, see REF.¹⁵¹). The enzymes that catalyse these vital reactions, however, were not identified until 1984, when the yeast endoprotease, kexin or Kex2, was isolated¹⁵⁵.

Kex2 excises α -MATING PHEROMONE and killer toxin from their precursors in late Golgi compartments. Because Kex2 could also correctly process mammalian proproteins, one or more of the mammalian PCs were anticipated to share structural features with the yeast enzyme¹⁵⁶. A database search identified a previously reported protein encoded by the *FUR* ('fes/fps upstream region') locus¹⁵⁷, an open reading frame adjacent to the *fes/fps* proto-oncogene, as the first mammalian Kex2 homologue¹⁵⁸. The product of this gene — furin — was soon shown to correctly process precursors for neurotrophic factors, serum proteins and pathogen molecules^{6,159,160}. PCR strategies were then used to identify the remaining six members of the PC family (FIG. 1; TABLE 1). Together, the PCs catalyse the proteolytic maturation of an enormous collection of bioactive peptides and proteins that regulate virtually every process controlling homeostasis and disease.

TYPE-I TRANSMEMBRANE PROTEIN

Proteins that contain a single membrane-spanning domain, with the carboxyl terminus oriented towards the cytoplasm and the amino terminus oriented towards the lumen of membrane compartments or extracellularly.

PROPROTEIN CONVERTASE

Members of the family of calcium-dependent, subtilisin-like serine endoproteases that are structurally related to Kex2 and furin and that cleave proprotein substrates at the carboxy-terminal side of doublets or clusters of basic amino acids.

α -MATING PHEROMONE

A peptide pheromone that is secreted by yeast α -cells to stimulate mating with yeast α -cells. The α -mating pheromone is synthesized as a proprotein substrate that is cleaved by Kex2. Yeast α -cells lacking Kex2 are sterile.

Consensus cleavage site

The consensus site that furin cleaves, which is positioned after the carboxy-terminal arginine (Arg) residue in the sequence $-\text{Arg}-\text{X}-\text{Lys}/\text{Arg}-\text{Arg}^{\downarrow}-$ (where Lys is lysine, X is any amino

acid and ↓ identifies the cleavage site), was determined biochemically using two *bona fide in vivo* furin substrates — anthrax toxin protective antigen (PA) and avian influenza virus haemagglutinin (HA)^{6,7}. Because anthrax toxin PA and influenza virus HA are cleaved at the cell surface and in the TGN/biosynthetic pathway, respectively, these studies provided the first indication that furin is active in several cellular compartments and is a key enzyme in the activation of diverse pathogens (FIG. 2). Arg residues at the p1 AND p4 positions in this cleavage site are essential, whereas the P2 basic amino acid (Lys/Arg) is not, but it can greatly enhance processing efficiency. Therefore, –Arg–X–X–Arg↓– represents the minimal furin cleavage site, although favourable residues at P2 and P6 can compensate for less favourable ones at position P4 (REF. 8). Accordingly, in exceptional cases, –Lys/Arg–X–X–X–Lys/Arg–Arg↓– can be cleaved by furin.

As described below, the various permutations of the consensus furin cleavage site have important implications for the proprotein substrate, both in terms of the compartmental specificity and the sequential ordering of the furin cleavage events. A model of the furin catalytic domain, based on the structures of the bacterial subtilisins BPN' and thermitase, predicts that negatively-charged residues in the S1, S2 and S4 subsites of the binding pocket might interact with the basic amino acids in the substrate^{4,9}. The lack of a three-dimensional furin structure, however, precludes any certainty about the substrate and cofactor binding sites.

Inhibitors

Furin's cleavage site requirements have been used to produce potent peptide- and protein-based inhibitors that block furin activity *in vitro* and *in vivo*¹⁰⁻¹². Perhaps the two most widely used furin inhibitors are the stoichiometric peptidyl inhibitor decanoyl–Arg–Val–Lys–Arg–CH₂Cl (where Val is valine) and α₁-antitrypsin Portland (α₁-PDX), a bioengineered variant of α₁-ANTITRYPSIN. Decanoyl–Arg–Val–Lys–Arg–CH₂Cl inhibits all PCs with a low nanomolar *K_i* (REF. 13), although the alkylating properties of the reactive group limit the usefulness of this reagent. Nonetheless, in cell-culture studies, decanoyl–Arg–Val–Lys–Arg–CH₂Cl blocks the processing of several furin substrates. The α₁-PDX inhibitor was generated by mutating the reactive-site loop of α₁-antitrypsin to contain the minimal consensus sequence for furin cleavage (–Arg–Ile–Pro–Arg–)¹⁴ (where Ile is isoleucine and Pro is proline), and it is highly selective for furin *in vitro* (*K_i* = 600 pM), although at higher concentrations it will also inhibit other PCs¹³. In biochemical, cellular and animal studies, α₁-PDX has been used to block furin activity and to prevent the production of pathogenic viruses, bacterial toxin activation, and cancer metastasis¹⁰ (see below).

Multistep furin autoactivation

The 83-amino-acid furin prodomain acts as an intramolecular chaperone propeptide that guides the folding and activation of the endoprotease¹⁵. The folding of furin's catalytic centre into its correct conformation is driven by a multistep, compartment-specific pair of cleavages in the prodomain, which yield the active enzyme (FIG. 3). To accomplish this task, furin exploits its own cleavage-site rules and cuts the prodomain twice. The first, and most rapid, cut (*t*_{1/2} = 10 mins) takes place in the neutral pH environment of the ER after Arg107 in the consensus furin cleavage site (–Arg–Thr–Lys–Arg₁₀₇ ↓– (where Thr is threonine)), which is located at the border of the catalytic domain. The second, slower, cut (*t*_{1/2} < 2 hrs) is made after Arg75 of the pH-sensitive furin propeptide cleavage site in the prodomain (–Arg₇₀–Gly–Val–Thr–Lys–Arg₇₅↓– (where Gly is glycine)) during trafficking of the propeptide–furin complex within the mildly acidic TGN/endosomal system. Furin's internal-propeptide-cleavage-site pH sensitivity is again shown by the mildly acidic conditions that are needed for the cleavage of proalbumin and *Pseudomonas* exotoxin A at similar furin sites¹⁰ (FIG. 2; also see below).

Box 2 Furin and bioterrorism

The bioterrorism plot following the World Trade Center tragedy on 11 September 2001 attempted to inflict countless deaths by disseminating *Bacillus anthracis* spores through the mail system. Twenty-two people were diagnosed with anthrax that was contracted from contact with contaminated mail — five died within days of exposure^{161,162}. However, the affected sorting facilities processed 85 million pieces of mail after the contaminated letters were sent, reinforcing just how close we came to disaster. And anthrax is not alone in its capacity to ignite disaster. It is eerily reminiscent of the influenza pandemic that could have erupted in Hong Kong in 1997, when a renegade pathogenic avian influenza virus — able to jump directly from birds to humans — killed six of the 18 people who were clinically diagnosed as having ‘bird flu’ in a week. If it had not been for the attenuated infectivity of this H5N1 influenza virus, the death toll from the outbreak could have been far worse. As well as illustrating our vulnerability to deadly microbes, there is another link between these two close calls, and that link is furin, a host-cell endoprotease that activates the toxic agents of these pathogens (see text for more details).

SUBTILISIN SUPERFAMILY

The serine proteases are composed of the subtilisin and chymotrypsin (including trypsin, thrombin and elastase) superfamilies. Surprisingly, the two superfamilies are evolutionarily distinct, yet the atoms that form the catalytic centre are in nearly identical positions — a remarkable example of convergent evolution.

CATALYTIC TRIAD

In serine endoproteases, the catalytic triad comprises the three spatially-optimized active-site residues — serine, histidine and aspartate — that conspire to bring about peptide-bond hydrolysis.

BACTERIAL THERMITASE

A thermostable bacterial subtilisin with a solved crystal structure.

P1 AND P4

A broadly used nomenclature that identifies the amino-acid residues that flank the cleavage site (scissile bond) in protein and peptide substrates. The amino acid that is amino-terminal to the scissile bond is designated P1. P2 is the amino acid that is amino-terminal to P1. So, P4 is the amino acid that is four residues to the amino-terminal side of the scissile bond. The amino acids that are carboxy-terminal to the scissile bond are labelled P1', P2', and so on.

α 1-ANTITRYPSIN

The circulating protein inhibitor of neutrophil elastase. It inhibits serine proteases by acting as a slow tight-binding inhibitor or suicide substrate. Inhibitors that use this type of mechanism are called serpins. The reactive site, which lures the protease, can be engineered to target specific proteases.

K_i

The inhibitor constant that describes the dissociation of an enzyme (E)–inhibitor (I) complex, $K_i = [E][I]/[EI]$.

JUXTACRINE VERSUS PARACRINE

Juxtacrine is communication between adjacent cells, whereas paracrine is communication between cells that are further apart.

The furin activation pathway shows evolutionary conservation with the activation pathways of bacterial subtilisin and α -lytic proteases that use their propeptide excision sites to guide the folding of the catalytic centre, which results in a structural reorganization of the folded proenzyme¹⁶⁻¹⁹. Furin's 'measure once, cut twice' method also seems to be a biochemical template that is used by members of the transforming growth factor- β (TGF- β) family to control JUXTACRINE VERSUS PARACRINE signalling, and by paramyxoviruses to produce fusion-competent envelope glycoproteins.

Furin localization and trafficking

The release of furin's propeptide fragments unmasks its endoprotease activity and enables it to cleave substrates *in TRANS*. Furin localizes to the TGN — a late Golgi structure that is responsible for sorting secretory pathway proteins to their final destinations, including the cell surface, endosomes, lysosomes and secretory granules^{20,21}. From the TGN, furin follows a highly regulated trafficking itinerary through several TGN/endosomal compartments and the cell surface^{10,22} (FIG. 4). This itinerary explains, in part, the ability of furin to process a diverse collection of proprotein substrates *in vivo*. Moreover, analysis of furin trafficking has uncovered novel roles for protein phosphorylation, the actin cytoskeleton and sorting adaptors in the regulation of protein traffic that controls cellular homeostasis and disease.

Localization to the trans-Golgi network

As with many itinerant type-I membrane proteins, both the TGN localization of furin and its dynamic cycling are controlled by sequences in its 56-amino-acid cytoplasmic domain (FIG. 5). Localization of furin to the TGN requires a bipartite motif that is composed of the casein kinase 2 (CK2)-phosphorylated acidic cluster (EECPpSDpSEEDE, where p highlights the serine residues that are phosphorylated) and a membrane-proximal segment containing two hydrophobic motifs (YKGL and LI)²³⁻²⁷ (FIG. 5). The bipartite motif controls two stages of a local cycling loop — the membrane-proximal segment is necessary for the efficient budding of furin from the TGN to endosomes, whereas the phosphorylated acidic cluster directs the efficient retrieval of endosomal furin to the TGN²⁸.

The steady-state localization of furin to the TGN has led to the supposition that this endoprotease cleaves proprotein substrates in this compartment, and several studies support this model^{29,30}. However, recent studies indicate that the processing compartments might be formed by the fusion of endocytic furin-containing compartments with export vesicles that contain substrate proteins^{31,32}. What, then, might be the role of the TGN in furin localization? Perhaps, in addition to housing the processing of some substrates, this compartment might also serve as a strategically located reservoir of furin molecules that are not active in proprotein processing. Such a mechanism would help to explain the control of furin processing *in vivo*.

Basolateral sorting

In polarized cells, furin targeting to the basolateral surface is similarly controlled by a bipartite signal that is composed of the carboxy-terminal part of the acidic cluster — EEDE — and the FI motif³³ (FIG. 5). The FI motif seems to bind to the sorting adaptor protein (AP)-4, and AP-4 is required for the basolateral sorting of furin³⁴ (FIG. 4). This role of the ubiquitously expressed AP-4 explains the lack of a function for the epithelial-specific basolateral sorting adaptor AP-1B in furin trafficking^{35,36}.

Budding from the trans-Golgi network

Little is known about the cytoplasmic machinery that directs TGN budding of furin. Earlier studies showed that furin localizes to clathrin-coated regions of the TGN, which indicates a role for the sorting adaptor protein AP-1. Indeed, both the LI and YKGL motifs bind to the μ 1 chain of AP-1 (REF. 37) (FIGS 4, 5). The recent identification of the Golgi-localized, γ -ear-containing, ADP-ribosylation-factor-binding (GGA) proteins, which control budding of the mannose-6-phosphate receptor from the TGN, raises the possibility of a second pathway through which furin could exit the TGN. Consistent with such a possibility, the furin LI motif is contained within a GGA3 consensus binding sequence (DXXLI)³⁸⁻⁴⁰ (FIG. 5).

CIS VERSUS TRANS CLEAVAGE

cis cleavage refers to the autoproteolytic intramolecular cleavage of an endoprotease propeptide by the catalytic triad that is part of the same molecule. *trans* cleavage refers to the processing of other molecules.

ANTEROGRADE AND RETROGRADE

Anterograde refers to trafficking from the endoplasmic reticulum (ER) towards the plasma membrane, whereas retrograde refers to trafficking from the plasma membrane towards the ER.

Retrieval from endosomes

Unlike the budding of furin from the TGN, the retrieval of furin from endosomes to the TGN is much better understood. The CK2-phosphorylated furin acidic cluster binds to the sorting protein PACS-1 (phosphofurin acidic cluster sorting protein-1) — a sorting connector that links furin to the AP-1 clathrin adaptor and transports furin from endosomes to the TGN^{28,41} (FIG. 4). The fact that binding of AP-1 to PACS-1 is crucial for endosome-to-TGN sorting concurs with the discovery that mutation of the AP-1 binding site in PACS-1 or genetic deletion of AP-1 both cause a similar mislocalization of furin to endosomal compartments^{36,41,42}. PACS-1 is not exclusively dedicated to furin; it controls the endosome-to-TGN sorting of several membrane proteins that contain acidic cluster-sorting motifs. These include cellular proteins, such as the cation-independent mannose-6-phosphate receptor (CI-MPR), the furin homologue PC5/6B (FIG. 1), carboxypeptidase D (CPD), Sortilin, and several pathogen proteins, including HIV-1 Nef (for ‘negative factor’) and several herpes virus envelope glycoproteins, such as varicella zoster virus gE and human cytomegalovirus gB^{28,43-45} (L. Wan and G.T., unpublished observations). PACS-1 binding to HIV-1 Nef is required for immunoevasion through the downregulation of cell-surface major histocompatibility class-I (MHC-I) molecules, and PACS-1 binding to herpes virus envelope glycoproteins is involved in the production of infectious virus^{41,44} (C. M. Crump and G.T., unpublished observations). How AP-1 might contribute both to ANTEROGRADE AND RETROGRADE transport between the TGN and the endosomes is not known, but reports showing that AP-1 links the CI-MPR to an anterograde

kinesin — KIF13A— for delivery to the cell surface from the TGN indicate that sorting proteins might combine to provide transport directionality⁴⁶.

Endocytosis

Many sorting motifs that localize furin to the TGN also direct its endocytic sorting itinerary. Endocytosis of furin is directed principally by the YKGL motif, which binds to the μ 2 subunit of the AP-2 adaptor³⁷. Recruitment of cell-surface furin molecules into endocytic compartments is regulated by tethering through its VY motif to filamin, a subcortical actin-binding protein that is involved in cell locomotion and signalling^{47,48} (FIGS 4-5; G. Liu and G.T., unpublished observations). In addition, sorting nexin-15 (SNX-15) affects furin internalization (FIG. 4), and overexpression of this sorting protein impedes the internalization of furin-containing chimaeras⁴⁹.

In early endosomes, furin can either be recycled to the cell surface or trafficked to the TGN. Recycling to the cell surface requires CK2 phosphorylation of the furin acidic cluster and PACS-1, whereas transport to the TGN requires dephosphorylation of the furin acidic cluster by specific isoforms of protein phosphatase 2A (PP2A)⁵⁰, which apparently occurs before transit through a late endosome intermediate⁵¹ or through sorting/recycling endosomes (FIG. 4).

So, PACS-1 and CK2 seem to place furin in one of two local cycling loops — one at the TGN and one between the plasma membrane and early endosomes. Sorting between these two loops requires dephosphorylation by PP2A. The sorting of CPD is similarly controlled by the phosphorylation state of its acidic cluster. Moreover, PP2A binds directly to the CPD cytoplasmic domain and this binding is essential for the control of CPD transport between the TGN and the cell surface⁵². The highly coordinated sorting itineraries of furin and CPD correlate with their sequential roles in the processing of proprotein substrates *in vivo*.

The regulated secretory pathway

The study of furin trafficking in endocrine and neuroendocrine cells has challenged the long-held view regarding the separation of the REGULATED AND CONSTITUTIVE PATHWAYS. In these cell types, furin buds into nascent immature secretory granules (ISGs), together with hormones and other molecules that are destined for dense core mature secretory granules (MSGs) (FIG. 4). ISGs are short-lived AP-1/clathrin-coated compartments that undergo homotypic fusions and extensive membrane remodelling during micro-tubule-based transport to the cell periphery⁵³⁻⁵⁵. At the cell periphery, furin is removed from ISGs, apparently during the brefeldin A (BFA)-sensitive, ADP ribosylation factor-1 (ARF1)-dependent remodelling of the ISG membrane^{56,57}, and is returned to the TGN^{55,58}. BFA blockage of ISG remodelling is likely to be due to inhibition of the ARF1-mediated recruitment of AP-1/clathrin and the subsequent membrane budding. Removal of furin from the ISGs requires a CK2-phosphorylated acidic cluster and PACS-1 (REFS^{41,58}). Similar results have been reported for the retrieval of CPD and the vesicular monoamine transporter-2, which highlights important roles for CK2 and PACS-1 in granule maturation⁵⁹⁻⁶¹. Several furin substrates are sorted to the regulated pathway⁶²⁻⁶⁵, which indicates that furin and CPD have crucial roles in proprotein processing in ISGs.

REGULATED VERSUS CONSTITUTIVE PATHWAYS

The regulated pathway in endocrine and neuroendocrine cells constitutes the anterograde pathway, leading from the *trans*-Golgi network (TGN) to the peptide-hormone-containing, mature secretory granules that can be stimulated to exocytose following an influx of extracellular calcium. Secretion by the constitutive pathway is not stimulated by calcium.

NEUROTROPHINS

A class of molecules that control various aspects of neuronal survival and plasticity, and that include nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4/5. The neurotrophins are synthesized as proproteins that require cleavage at consensus furin sites.

Furin in development, homeostasis and disease

The insights gained by the analysis of furin trafficking are equalled by those obtained from recent studies that indicate that furin has broad and important roles in embryogenesis, homeostasis and disease. Paradoxically, although furin is an enzyme that is essential for embryogenesis, its activity can also lead to fatal diseases in adults. Owing to space limitations, I will limit this discussion primarily to work reported during the past two years.

To cleave or not to cleave — neuronal innervation and dementia

The 16-kDa β -nerve growth factor (β -NGF) is the prototypic target-derived NEUROTROPHIN, and biochemical studies show that furin is the principal endoprotease that cleaves pro- β -NGF^{10, 22} (FIG. 6a). Surprisingly, the furin-catalysed processing of pro- β -NGF controls whether the neurotrophin activates cell-survival or cell-death pathways within innervating neurons⁶⁶. Processed β -NGF mediates cell survival through high-affinity binding to the Trk proto-oncogene receptor tyrosine kinases, which mediate the trophic effects of β -NGF and other neurotrophins. Conversely, secreted, unprocessed pro- β -NGF mediates apoptosis by high-affinity binding to the 75-kDa neurotrophin receptor (p75^{NTR}). This receptor is a member of the tumour necrosis factor (TNF) receptor/FAS family that antagonizes the trophic signalling mediated by β -NGF and Trk receptors. Regulation of furin activity might therefore have a central role in determining which neurons form synaptic complexes and which neurons die (FIG. 6a).

This control of furin activity might well extend to additional developmental programmes. For example, furin cleavage of the transmembrane receptor Notch is required for the release of the Notch intracellular domain by γ -secretase proteolysis. This intracellular domain then binds to the transcriptional regulator CSL (for 'C promoter binding factor/Suppressor of Hairless/Lag-1'), which activates genes required for cell-cell communication during development⁶⁷. By contrast, uncleaved Notch mediates a distinct signalling pathway that inhibits cell differentiation⁶⁸. How furin activity is controlled to regulate the processing of these substrates is unknown.

Furin's role in the α -, β - and γ -secretase-mediated processing of the β -amyloid precursor protein (APP) helps to determine whether APP-derived peptides enhance NGF signalling to innervating neurons or cause the massive neurodegeneration that is associated with Alzheimer's disease^{69,70}. The extracellular domain of APP is cleaved by α -secretase to produce soluble APPs that enhance the anti-apoptotic and neuroprotective activities of NGF^{71,72}. By contrast, cleavage of APP by a combination of the β - and γ -secretases releases the amyloidogenic β APP₁₋₄₀, β APP₁₋₄₂ and related peptides, which form amyloid plaques and are responsible for the neurodegeneration that is suffered by individuals with Alzheimer's disease⁷³.

AMYLOID DEMENTIA

Neurological abnormality caused by deposits of proteinaceous matter.

ENAMEL KNOT

Signalling centre in the dental epithelium that controls tooth morphogenesis.

Recent studies point to an essential role for furin in the activation of both α - and β -secretase. Two members of the ADAMs (for ‘a disintegrin and metalloproteinase-like’) family of zinc metalloproteinases — ADAM10 and ADAM17 — have been implicated as the α -secretase^{74,75}. Characteristic of this protein family, both ADAM10 and ADAM17 contain propeptides that are linked to their catalytic domains by a consensus furin motif, and cleavage at this site is required for their activation^{76,77}. Interestingly, PC7 might activate the α -secretase under basal conditions, whereas furin might activate it following protein kinase C activation, which increases the α -secretase-catalysed release of soluble APP⁷⁷. The β -secretase, also called β -site APP-cleaving enzyme (BACE), is a type-I membrane protein that localizes to the TGN/endosomal system and requires proteolytic removal of its proregion by furin at a minimal furin site ($-\text{Arg}-\text{Leu}-\text{Pro}-\text{Arg}-\downarrow$)⁷⁸⁻⁸⁰. The similarities in BACE and furin trafficking further support the idea that furin is the BACE-activating enzyme^{81,82}.

Alzheimer's is only one type of AMYLOID DEMENTIA in which furin has a crucial role. Two separate mutations in the *BRI* gene that encodes a widely expressed type-II membrane protein cause either familial British dementia (FBD) or familial Danish dementia (FDD). In healthy individuals, cleavage of this protein by furin, or possibly PC7, at an atypical furin site, which contains a lysine at P6 ($-\text{Lys}-\text{Gly}-\text{Ile}-\text{Gln}-\text{Lys}-\text{Arg}-\downarrow$ (where Gln is glutamine)), releases a 23-residue carboxy-terminal peptide with an unidentified function^{83,84}. However, the nucleotide transversion or decamer duplication in FBD and FDD, respectively, causes aberrant 34-residue amyloidogenic peptides to be produced on furin cleavage⁸³⁻⁸⁵.

Recent studies also show a role for furin in both Finnish- and Danish-type familial amyloidoses. Both diseases are caused by mutations that disrupt the binding of calcium to plasma gelsolin, which is a circulating scavenger of extracellular actin^{31,86,87}. The disrupted calcium binding causes aberrant cleavage by furin at the carboxy-terminal side of a cryptic $-\text{Arg}-\text{Val}-\text{Val}-\text{Arg}-\downarrow$ site, which is normally buried in the core of the wild-type molecule. The furin-mediated cleavage initiates the release of a 70-amino-acid amyloidogenic peptide⁸⁷.

Furin, the TNFs, and the TGF- β s — short- versus long-range signalling in development and disease

The proteolytic release of TNF- α from the plasma membrane by ADAM17 has long been regarded as a key mechanism that mediates juxtacrine- versus paracrine-signalling of this cytokine family⁸⁸. Recently, however, furin has been shown to control the signalling range of another TNF family member — ectodysplasin-A (Eda-1; FIG. 6b). Eda-1 is a type II plasma membrane protein that controls the formation of several epithelial tissues, including hair, teeth and eccrine sweat glands. The earliest expression of Eda-1 and its receptor — EDAR — is in the partially overlapping regions of the thickened dental epithelium⁸⁹. During proliferation of the epithelium into the underlying mesenchyme to form the tooth bud, EDAR expression accompanies the leading edge of the epithelial layer and is ultimately confined to the ENAMEL KNOT, whereas Eda-1 remains several cell distances away in the outer epithelium. A furin-mediated switch from juxtacrine to paracrine signalling might accompany the spatial uncoupling of the receptor and ligand (FIG. 6b). Mutations in the furin cleavage site of Eda-1 account for ~20% of all known mutations in X-linked hydrohidrotic ectodermal dysplasia, and block the ability of Eda-1 to signal in a paracrine fashion^{90,91}.

The importance of furin for the signalling of two other TNF family members — B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) — indicates a broad role for furin in controlling TNF function⁹²⁻⁹⁴.

Furin's role in activating members of the TNF family is surpassed by its role in controlling TGF- β -family signalling. Inactivation of the furin gene in mice creates an embryonic lethal phenotype, with death occurring at an early embryonic stage⁹⁵. Furin is required both in the extra-embryonic tissues and in the cardiogenic mesoderm to promote yolk sac vasculogenesis and ventral closure, heart-looping and axial rotation. The failure to maintain asymmetry in the embryo is likely to arise from a block in the furin-catalysed production of the TGF- β family members Nodal and Lefty-2 (REF. ⁹⁶). Consistent with this model, furin cleaves several TGF- β members, including TGF- β 1 and bone morphogenetic protein-4 (BMP-4)^{97,98}. Moreover, disrupting pro-BMP-4 maturation in four-cell *Xenopus laevis* embryos results in a dorsalized phenotype that mimics the phenotype that is observed when the BMP-4 signalling pathway is disrupted⁹⁸.

MORPHOGENS

Secreted signalling molecules that govern developmental patterns and axis formation by producing a concentration gradient emanating from the cells in which they are synthesized.

GLIOBLASTOMAS

Aggressive malignant brain tumours that are derived from astrocytes and that account for ~30% of all primary brain tumours.

Furin's autoactivation method also seems to be used by BMP-4 to control its signalling strength and range during embryogenesis⁹⁹. Furin first cleaves pro-BMP-4 at the consensus furin site that joins the pro- and BMP-4 domains (–Arg–Ser–Lys–Arg[↓]–), followed by a second cleavage at a minimal consensus furin site within the propeptide (–Arg–Ile–Ser–Arg[↓]–). The context of the two sites ensures the ordered processing of pro-BMP-4 and the correct activity of this MORPHOGEN. The presence of consensus and minimal furin sites in other BMP-4-related signalling molecules^{10,22,99} indicates that the ‘measure once, cut twice’ method is used to control signalling gradients in many organisms. Moreover, this method might extend to viral pathogenesis, in which generation of the correctly folded, respiratory-syncytial-virus (RSV) fusion protein requires sequential cleavage at two furin sites to produce infectious progeny^{100,101}.

Although furin-catalysed TGF- β activation is essential for embryogenesis, this pathway causes disease in adults. Furin and TGF- β cooperate in a novel positive feedback loop that exacerbates rheumatoid arthritis (FIG. 6c). TGF- β can bind to its own receptor to stimulate furin gene transcription by a SMAD2 and mitogen-activated protein kinase (MAPK) convergent pathway¹⁰²⁻¹⁰⁴. In synoviocytes, which are fibroblast- and macrophage-like cells that line the synovium of joints, the amplified levels of furin and TGF- β combine to increase the levels of ADAMTS-4 (a disintegrin and metalloprotease with thrombospondin motifs-4). ADAMTS-4 (previously identified as aggrecanase-1) is a member of a new family of ADAMs proteases, and it degrades the cartilage protein aggrecan and causes rheumatoid arthritis^{105, 106} (FIG. 6c).

Furin and tumour metastasis

Furin is upregulated in several cancers, including non-small-cell lung carcinomas, squamous-cell carcinomas of the head and neck, and GLIOBLASTOMAS¹⁰⁷. Moreover, the increased levels of

furin in tumours correlate both with the increased aggressiveness of head, neck and lung cancers and with an increase in the levels of one of its substrates — membrane type 1-matrix metalloproteinase (MT1-MMP)^{108,109}. MT1-MMP activates extracellular pro-MMP2 (pro-gelatinase) to induce rapid tumour growth and NEOVASCULARIZATION¹¹⁰ (FIG. 6d). Activation of MMPs classically uses a cysteine-switch mechanism, in which the catalytic-site zinc atom that is bound to a cysteine residue in the pro-region of the latent pro-enzyme switches to binding a water molecule in the active protease. However, activation of MT1-MMP and related family members seems decidedly more complex and requires furin-mediated cleavage of their pro-region^{111,112}. Furin inhibitors, including α_1 -PDX, block the activation of MT1-MMP in head, neck and oral squamous-cell carcinomas, which leads to a block in both MMP2 activation and tumour metastasis in transplanted mice^{109,113}. The fact that the MT1-MMP/MMP2 axis is essential for ALVEOLIZATION of the embryonic lung¹¹⁴ provides another example of a furin-activated cascade that is essential in embryogenesis but that is detrimental in adults.

A second furin substrate, insulin-like growth factor-1 (IGF1), is upregulated in colon, breast, prostate and lung cancers. Its receptor, IGF1R, which is also a furin substrate, is upregulated on the surface of the tumour cells¹¹⁵. IGF1 and IGF1R processing are catalysed by furin or PC5/6A, and inhibition of this processing by α_1 -PDX reduces the incidence, size and vascularization of tumour development in transplanted mice¹¹⁶.

NEOVASCULARIZATION

De novo stimulation of new blood supplies to a growing tumour.

ALVEOLIZATION

Encompasses the latter stages of lung development, which begin with bronchial and respiratory-tree development, and culminate in the formation of terminal saccules and alveoli to facilitate efficient gas exchange.

Furin is not the only PC that is associated with a poor prognosis for many cancers. The furin homologue, PACE4 (FIG. 1; TABLE 1), is upregulated in breast tumours¹¹⁷, and expression of this PC increases the invasiveness of mouse squamous-cell carcinomas by converting them to more aggressive, poorly differentiated, spindle-cell carcinomas¹¹⁸. Together, these studies indicate that inhibiting PCs might be a novel approach to combating various aggressive cancers.

Anthrax, AIDS, Ebola> — what next?

Early studies showing furin's role in both anthrax toxin activation and avian influenza virus HA maturation merely provided a glimpse into the devastating role of furin in the activation of various bacterial and viral pathogens. The analysis of bacterial toxin activation has further illuminated distinct roles for furin-catalysed proprotein processing at the cell surface or early endosomes, providing a single perspective for unravelling the regulation of protein trafficking in mammalian cells. Cell-surface furin activates the anthrax toxin^{6,119} — a now infamous weapon of bioterrorism¹²⁰ (BOX 2) — as well as the aerolysin toxin, which is a causative agent in many food-borne illnesses¹²¹, and *Clostridium septicum* α -toxin, which causes gas gangrene¹²² (FIG. 2). Cleavage of each toxin by furin is an obligatory step in making the toxin able to form pores in cell membranes.

The anthrax toxin comprises three proteins: PA, protective antigen, so-called for its ability to elude immune protection against anthrax; and two toxic proteins — lethal factor (LF) or oedema factor (EF)¹²³. LF is a metalloproteinase that cleaves MAPK kinases, whereas EF is a calmodulin-dependent adenylate cyclase¹²³. The 83-kDa PA molecule that is secreted from

the bacterium binds to the anthrax toxin receptor (ATR)¹²⁴, and is then cleaved by cell-surface furin to generate a cell-associated 63-kDa PA and a free 20-kDa PA (FIG. 7). The cell-associated PA molecule heptamerizes, binds to either of the two toxic factors, and is then internalized into early endosomes¹²⁵. In the early endosomal acid pH environment, the PA heptamer forms a membrane channel that shuttles the toxic factors into the host-cell cytoplasm, which results in oedema, systemic shock and death (FIG. 7). In the absence of furin, the toxin fails to assemble and is not lethal¹²⁶. Moreover, mutation of the furin cleavage site results in a dominant-negative protein that binds to ATR but fails to oligomerize¹²⁷. Both proaerolysin and *Clostridium septicum* α -toxin bind to glycosylphosphatidylinositol-anchored molecules, and, similar to PA, furin cleavage of both molecules is required for them to form ion-permeable heptameric pores in the host-cell plasma membrane, which leads to cell toxicity^{128,129}.

Early endosomal furin activates other bacterial toxins, including *Pseudomonas* exotoxin A (PEA), shiga toxin (ST), shiga-like toxin-1 (ST-1) and diphtheria (DT) toxins¹⁰ (FIG. 2). Unlike the cell-surface-activated toxins, these toxins are all A/B-type toxins that contain an active domain (A) and a binding domain (B) that are joined by a furin cleavage site¹²⁹. Following receptor binding, each toxin is endocytosed into early endosomes, where it is cleaved by furin. Cleavage of ST, ST-1 and PEA by furin requires the acidic pH that is characteristic of early endosomal compartments, whereas cleavage of DT does not¹⁰. As in the cancer models discussed, inhibition of furin activity by the extracellular delivery of α_1 -PDX protects cells from PEA and other bacterial toxins¹³ (F. Jean and G.T., unpublished observations). The sensitivity of cells to PEA is increased in the absence of filamin (FIG. 4), which normally tethers furin to the cell surface, indicating that filamin might control the formation of endosomal furin-processing compartments⁴⁷. The crystal structure of PEA shows that exposure of the molecule to an acidic pH unmasks the furin cleavage site, which at least partially explains the requirement for acidic pH-dependent furin processing¹³⁰.

Surprisingly, furin cleavage enables PEA, ST/ST-1 and DT to translocate to the cytosol through three distinct trafficking pathways. Following cleavage, the DT B domain forms a channel in the early endosomal membrane that shuttles the A fragment into the host-cell cytosol¹³¹. By contrast, cytosol delivery of both PEA and ST/ST-1 requires retrograde trafficking to the ER, where the toxins are apparently translocated to the cytosol through the SEC61 CHANNEL^{132,133}. The retrograde trafficking of PEA requires the KDEL RECEPTOR, which binds to the processed PEA and retrieves the toxin to the ER^{132,133}, whereas cleaved ST/ST-1 traffics to the ER through a pathway that is both KDEL-receptor- and COPI-independent, which indicates that vesicle coats other than COPI direct their retrieval to the ER. However, retrieval of ST/ST-1 is dependent on RAB6, a small GTPase that controls intra-Golgi transport and the cycling of Golgi-resident glycosyltransferases through the ER¹³²⁻¹³⁴. As it has also been suggested that furin might be sorted to the ER to metabolize misfolded insulin receptors, it will be important to determine whether furin is also sorted through the ST/ST-1 pathway¹³⁵.

SEC61 CHANNEL

The main protein that forms the pore in the endoplasmic reticulum (ER) membrane that facilitates the translocation of nascently synthesized proteins into the secretory pathway. The SEC61 channel might also be a conduit for the reverse translocation (dislocation) of proteins from the ER into the cytoplasm.

KDEL RECEPTOR

Golgi-localized membrane protein that binds to carboxy-terminal KDEL (–Lys–Asp–Glu–Leu–) motifs, which are present on many resident endoplasmic reticulum (ER) proteins that escape to the Golgi, and that retrieves them to the ER.

COPI

(Coatomer protein complex I). A specific type of coat on vesicles that traffic principally between Golgi cisternae and from the Golgi to the endoplasmic reticulum. Also reported on early endosomes.

VIRULENCE

The extent or degree to which a pathogen can cause disease.

The broad role of furin in activating bacterial toxins is exceeded by its role in activating numerous pathogenic viruses. Many pathogenic viruses, including avian influenza virus, HIV-1, measles virus and RSV, express envelope glycoproteins that must be cleaved at consensus furin sites to form the mature and fusogenic envelope glycoprotein^{10,22}. For example, processing of HIV-1 gp160 unveils the amino-terminal gp41 fusogenic peptide that is contained within the trimeric gp120/gp41 envelope complex. Whether furin or one of the other PCs (for example, PACE4, PC5/6B or PC7) is the *in vivo* gp160 convertase is unknown¹³⁶. Lovo cells, which lack furin, process HIV-1 gp160 (REF. 137). Nonetheless, furin inhibitors block processing of HIV-1 gp160 and, in turn, the production of infectious HIV-1, as well as blocking other viruses that require processing of their envelope glycoproteins at consensus furin sites¹⁰. Furin cleaves HIV-1 gp160 at the carboxy-terminal side of the consensus sequence –Arg–Glu–Lys–Arg–↓. The P3 glutamate in this cleavage site reduces the efficiency of furin processing, and the conservation of this residue in several HIV isolates has raised doubts about furin's involvement in gp160 processing¹³⁶. Indeed, mutation of this cleavage site to make a site containing all basic amino acids (–Arg–Arg–Lys–Arg–↓) enhances processing by furin¹³⁸. Surprisingly, however, recombinant HIV containing this all-basic site is attenuated, which indicates a selective advantage for the inefficiently cleaved –Arg–Glu–Lys–Arg–↓ site in HIV-1.

HIV-1 seems to maintain a selective growth advantage by using a suboptimal furin site in its envelope glycoprotein, whereas the analysis of viral tropism — that is, the molecular determinants that enable a virus to spread throughout the body — shows that the VIRULENCE of many deadly viruses (including avian influenza virus, Newcastle Diseases virus and, potentially, Ebola virus) is directly correlated with the ability of these viruses to incorporate a consensus furin cleavage site within their envelope proteins¹³⁹⁻¹⁴². For example, the pathogenicity of avian influenza viruses has long been recognized to correlate directly with the cleavability of its fusion protein precursor HA₀, which is cut by furin to generate the fusion-competent HA₁–HA₂ complex. Similar to HIV-1 gp160, cleavage of HA₀ exposes the fusogenic peptide located at the amino terminus of HA₂, which can fuse with target-cell membranes.

Avirulent avian influenza viruses, which lack a consensus furin site in HA₀, cause a localized infection in the intestinal tract. However, mutation of the HA₀ cleavage site to a consensus furin site enables the virus to be activated by the ubiquitously expressed furin, invariably enabling the virus to spread systemically throughout the bird, including infection of the central nervous system¹³⁹. This ability relates to the deadly flu outbreak in Hong Kong in 1997 (BOX 2). Analysis of the H5N1 influenza virus, which killed at least six people, showed that just two

mutations were required to generate the lethal virus — a mutation in a subunit of the viral RNA polymerase PB2, together with the generation of a tandem furin site in the cleavage junction between HA₁ and HA₂ (–Arg–Glu–Arg–Arg–Arg–Lys–Lys–Arg–↓)¹⁴³. Exactly how the tandem furin site and the PB2 mutation contribute to the increased virulence of influenza H5N1 is unknown. Fortunately, the attenuated INFECTIVITY of this virus, which is also poorly understood, impeded its spread through the population. Nonetheless, the propensity for the rapid mutation and reassortment rate in avian influenza and its documented ability to jump directly from birds to humans underscore our vulnerability to this pathogen¹⁴⁴.

The importance of furin for pathogen virulence extends to other viruses, including Ebola virus. For example, the highly pathogenic Ebola Zaire and Ivory Coast strains — which cause a massive and sudden (fulminant) haemorrhagic fever that is characterized by massive internal and external bleeding and that kills 90% of the people who contract it — contain a consensus furin site in their envelope glycoprotein (GP)¹⁴⁵. But, by contrast, GP of the relatively milder Ebola Reston strain lacks a consensus furin site. This isolate is not pathogenic to humans¹⁴¹. Surprisingly, however, despite the apparent underlying structural similarities between HIV-1 gp160, influenza virus HA and Ebola virus GP, furin-catalysed cleavage of GP is not required for membrane fusion in cell-culture models¹⁴⁶. The lack of a requirement for GP processing for membrane fusion is consistent with the presence of an internal fusion sequence in Ebola GP and related flaviviruses¹⁴⁷. What, then, might account for the furin-dependent tropism of Ebola virus? One clue lies in the severe cytotoxicity and marked increase in vascular permeability of GP from the highly pathogenic Ebola Zaire, but not from the apathogenic Reston isolate^{148,149}. Interestingly, the crucial region of GP required for this toxicity is adjacent to the furin cleavage site, indicating that proteolysis might unveil the cytotoxic domain.

INFECTIVITY

The ability of a pathogen to invade a host and replicate, irrespective of its ability to cause disease.

Conclusions and perspectives

In summary, the proteolytic reaction catalysed by furin, which once seemed so ordinary, now clearly has enormous ramifications in biological research. Furin's 'measure once, cut twice' method of autoactivation has yielded new understanding of the interplay between protein folding and the distinct microenvironments of early and late secretory pathway compartments. Moreover, this activation method seems to extend beyond the PCs — it is used to control the formation of morphogen gradients during embryogenesis, as well as the correct folding of some viral envelope glycoproteins. The intracellular trafficking of furin is decidedly complex and has provided new insights into the regulation of protein traffic, including new roles for protein phosphorylation, the actin cytoskeleton and sorting adaptors. Surprisingly, the analysis of furin trafficking has also provided new insights into diverse processes ranging from secretory-granule formation to HIV immunoevasion.

Ongoing studies of furin should further clarify its activity *in vivo*, as well as its relationships with other PCs. Analyses of neuronal innervation, cell fate and juxtacrine- versus paracrine-signalling indicate that furin activity might be dynamically controlled, yet we do not know how. One possibility is that cellular furin inhibitors might be temporally expressed, or that the complex and diverse machinery that directs the highly regulated furin trafficking itinerary might control whether furin interacts with its various substrates. Furthermore, our models of furin processing must also account for the roles of the other broadly expressed PC family

members that traffic similarly to furin — namely PC7, PC5/6B and PACE4. The extent of their roles in the furin pathway remains unclear.

Not only will the analysis of furin trafficking continue to lead to a better understanding of the basic processes that are involved both in controlling membrane traffic and in integrating these dynamic sorting steps with signal-transduction cascades, cell fate, other PCs and protease systems, but it will also clarify furin's role in a broad spectrum of human diseases. The finding that deadly bacterial and viral pathogens usurp the furin pathway to exert their virulence strongly argues for a strategy that targets furin for therapeutic intervention, even though such a strategy would have to consider that the very prevalence of furin might also create toxicity in the drugs that target it. Nevertheless, furin holds great promise as a key to solving both theoretical and practical questions in cell biology.

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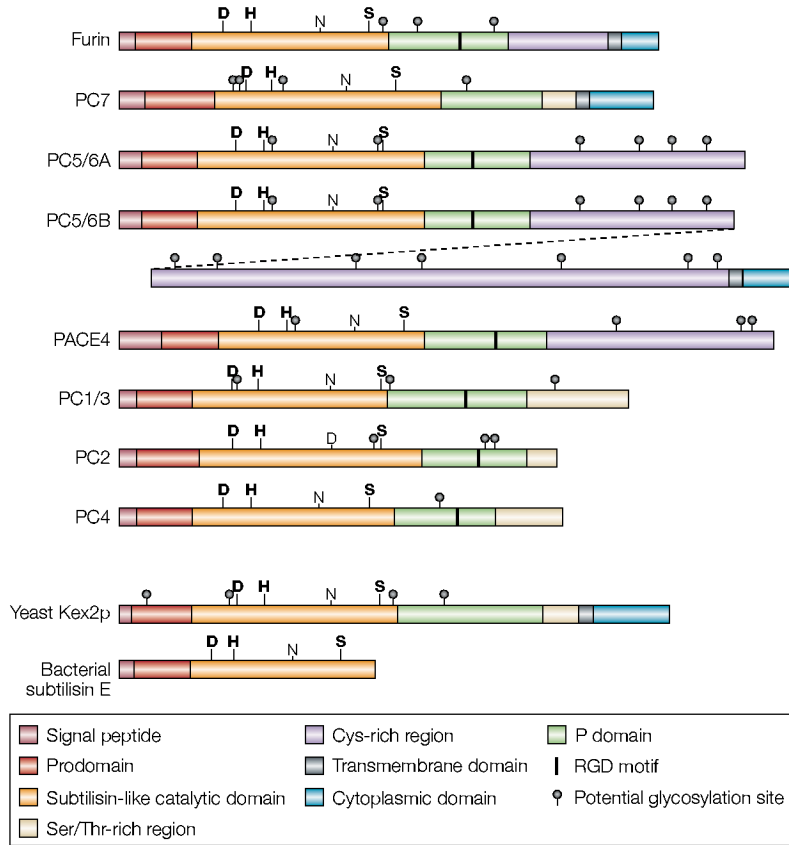


Figure 1. Schematic diagram of the proprotein convertase (PC) family. Shown are schematics for furin and the six other PCs. Schematics of yeast Kex2 and the evolutionarily related bacterial subtilisin (which lacks the conserved P domain) are also shown. PC5/6 is expressed as either the A or B isoform. These isoforms are generated by alternative splicing, and the diagonal dashed line links the two halves of PC5/6B. The PC5/6B isoform contains all of PC5/6A except for a small part of its carboxyl terminus that is positioned after the splice site. The bold labels D, H and S highlight the active-site residues, whereas the non-bold labels N and D highlight the oxyanion-hole residues.

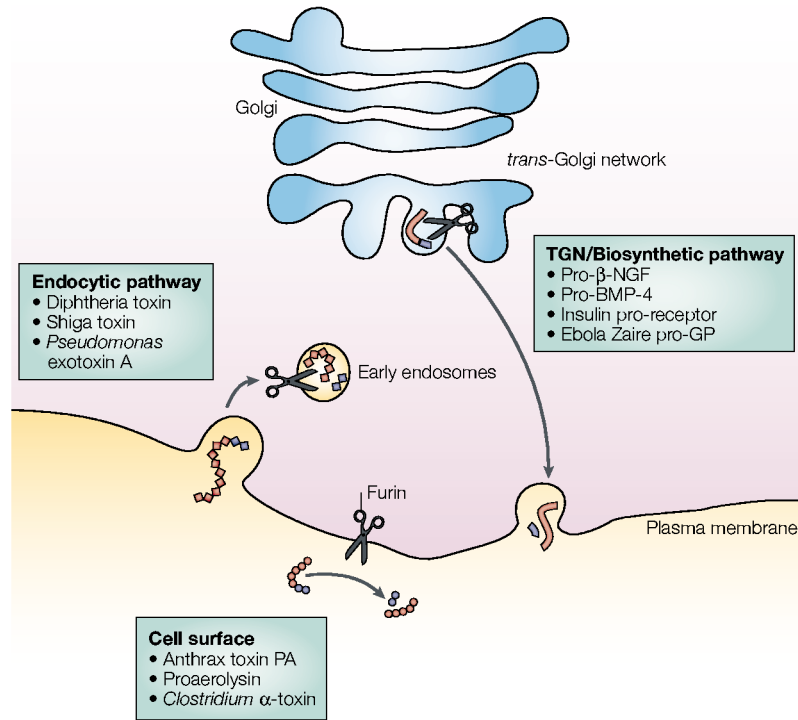


Figure 2.

Furin-processing compartments of the *trans*-Golgi network (TGN)/endosomal system. At steady-state, furin (represented by scissors) is localized principally to the TGN, where it cycles between this sorting compartment, the cell surface and the early endosomes. In the TGN/biosynthetic pathway, furin cleaves many substrates including pro- β -nerve growth factor (pro- β -NGF), pro-bone morphogenetic protein-4 (pro-BMP-4), the insulin pro-receptor and Ebola Zaire pro-glycoprotein (pro-GP). At the cell surface, furin cleaves substrates such as anthrax protective antigen (PA), proaerolysin and *Clostridium septicum* α -toxin. In mildly acidic early endosomes (endocytic pathway), furin cleaves substrates including diphtheria toxins, shiga toxin and shiga-like toxin-1, and *Pseudomonas* exotoxin A. See text for more details.

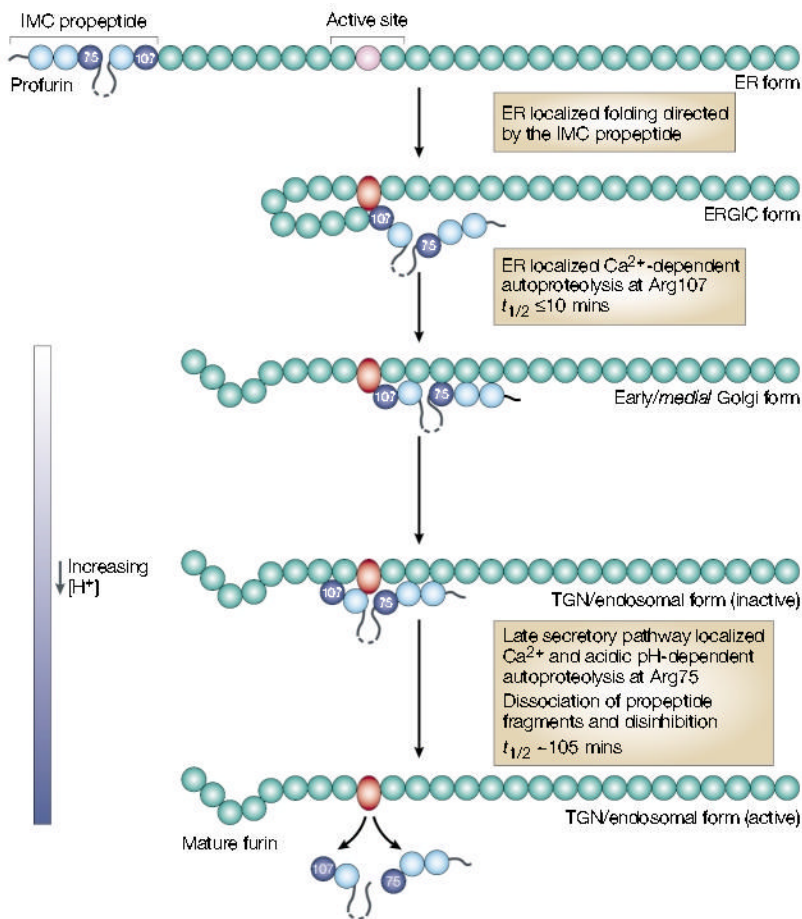


Figure 3.

The furin autoactivation pathway. Following translocation and signal sequence removal, the furin prodomain acts as an intramolecular chaperone (IMC) to facilitate folding of the unstructured, inactive catalytic domain (pink circle) into the active conformation (red oval). After the initial endoplasmic reticulum (ER) folding events, furin undergoes autoproteolytic intramolecular excision of the propeptide at Arg107. The propeptide, however, remains associated with the mature domain and functions as a potent autoinhibitor in *trans* during transport to the late secretory pathway. Propeptide excision can be blocked by inactivating furin and results in the accumulation of an apparent folding intermediate in the ER–Golgi intermediate compartment (ERGIC)/*cis*-Golgi network. These data indicate that both the ER and ERGIC compartments participate in the initial steps of furin activation. Following propeptide excision, the inactive propeptide complex transits to late secretory compartments — *trans*-Golgi network (TGN)/endosomes — where the relatively acidic pH promotes autoproteolytic, intramolecular cleavage of the propeptide at a second, internal site (Arg75). The Arg75 cleavage is followed by the rapid dissociation of the propeptide fragments and disinhibition of furin. Adapted with permission from REF. 15. © (2002) American Society for Biochemistry and Molecular Biology.

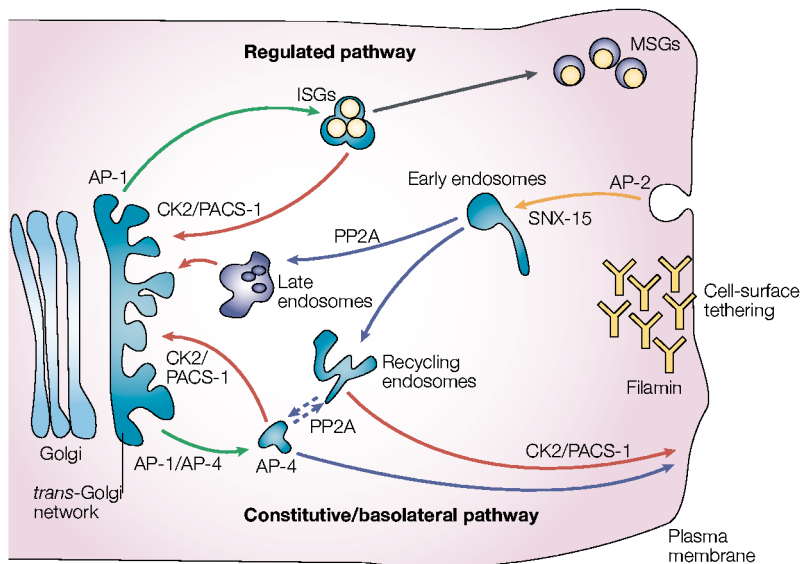


Figure 4. **Model of furin trafficking.** Budding of furin from the *trans*-Golgi network (TGN) is mediated by the binding of the tyrosine-based or di-leucine-like hydrophobic sorting motifs to adaptor protein (AP)-1, which targets furin to endosomes, or binding to AP-4, which targets furin to the basolateral surface from either the TGN or possibly endosomes. In endocrine and neuroendocrine cells, AP-1 directs furin budding from the TGN into immature secretory granules (ISGs). Phosphofurin acidic cluster sorting protein-1 (PACS-1) connects the casein kinase 2 (CK2)-phosphorylated furin acidic cluster to AP-1/clathrin to retrieve furin to the TGN either from ISGs, prior to their progression to mature secretory granules (MSGs), or from endosomes. Furin molecules arriving at the cell surface can be tethered by the cytoskeletal protein filamin, which is also called actin-binding protein (ABP)-280. The dynamin/clathrin-dependent internalization of cell-surface furin is mediated principally by the tyrosine-based motif, which binds to AP-2. Once inside early endosomes, furin molecules that are dephosphorylated by specific protein phosphatase 2A (PP2A) isoforms are delivered to the TGN, apparently through a late endosomal compartment. By contrast, CK2-phosphorylated furin is recycled back to the plasma membrane in a PACS-1-dependent step. Hence, the TGN- and peripheral-cycling loops are essentially mirror images of each other. On the basis of the similar itinerary of carboxypeptidase D (CPD), movement of furin from a post-TGN endosomal compartment to the cell surface might also require PP2A (dashed lines). Sorting nexin-15 (SNX-15), a PX-domain containing protein that binds phosphoinositides, modulates furin sorting through endosomes.

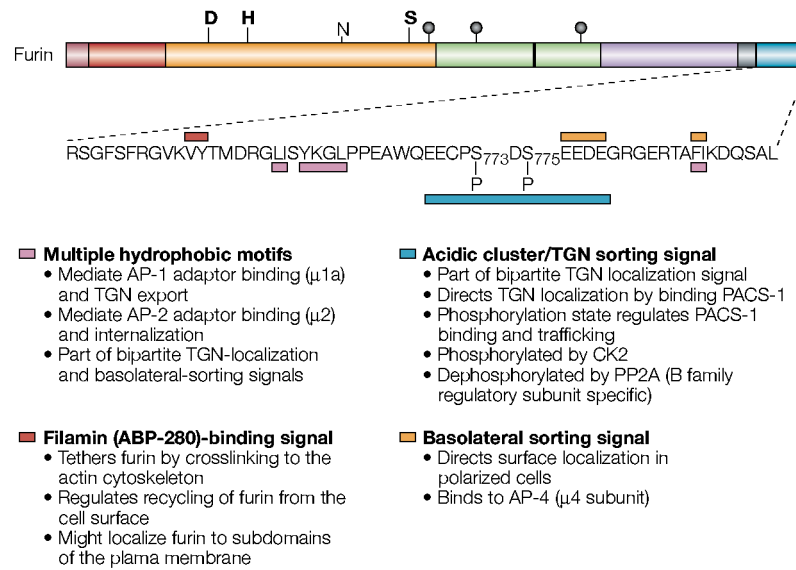


Figure 5.
The sorting motifs of the furin cytoplasmic domain. Shown is the sequence of the human furin cytoplasmic domain. The various intracellular sorting motifs are indicated. See text for more details. ABP, actin-binding protein; AP, adaptor protein; CK2, casein kinase 2; PACS-1, phosphofurin acidic cluster sorting protein-1; PP2A, protein phosphatase 2A; TGN, *trans*-Golgi network. For a key to furin domain organization, see FIG. 1.

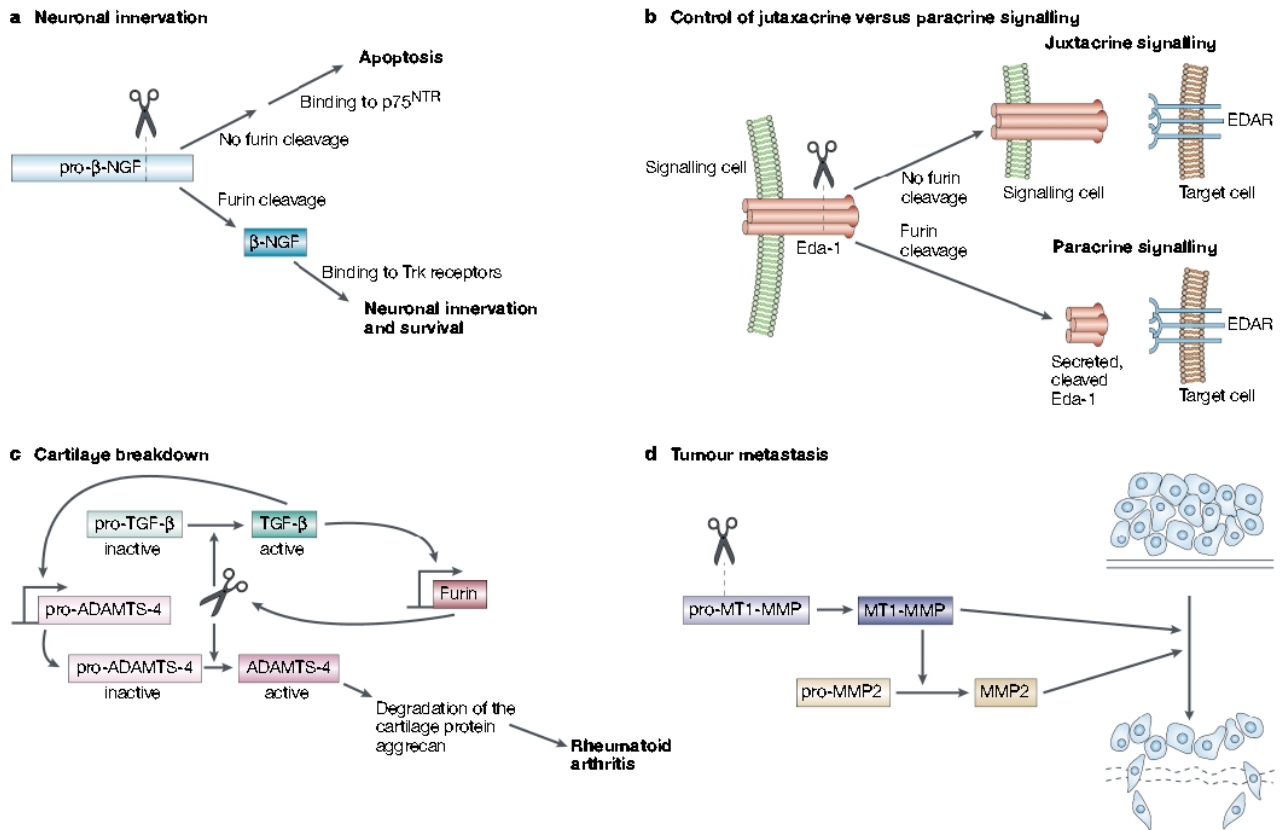


Figure 6.

Furin in development, homeostasis and disease. **a** | Furin-mediated cleavage of pro- β -nerve growth factor (NGF) produces the 13-kDa β -NGF neurotrophin that binds to Trk receptors to promote synaptic innervation. By contrast, inhibition or sequestering of furin results in the secretion of pro- β -NGF that binds to the 75-kDa neurotrophin receptor ($p75^{\text{NTR}}$) to promote cell-death pathways. **b** | Ectodysplasin-A (Eda-1) is a trimeric tumour necrosis factor family member that stimulates morphogenesis of ectodermal structures by activation of its receptor, EDAR, on target cells. Eda-1 can signal in a juxtacrine manner by binding to EDAR on adjacent cells. However, cleavage of membrane anchored Eda-1 by furin releases the ligand and enables it to signal through EDAR on distant cells in a paracrine manner. **c** | In synoviocytes, furin and transforming growth factor (TGF)- β participate in a positive feedback loop that results in elevated levels of ‘a disintegrin and metalloprotease with thrombospondin motifs-4’ (ADAMTS-4, or aggrecanase-1). Furin cleaves both pro-TGF- β and pro-ADAMTS-4 to yield the active growth factor and protease, respectively. The secreted mature form of TGF- β then binds to its receptor and, through a SMAD2 and mitogen-activated protein kinase (MAPK) convergent pathway, increases furin expression. The increased levels of furin lead to an increase in TGF- β , which creates a positive-feedback loop. In synoviocytes, TGF- β also stimulates the expression of pro-ADAMTS-4. So, because furin and TGF- β are in this positive loop, the levels of active ADAMTS-4 are greatly elevated, which leads to destruction of the cartilage protein aggrecan and hence to rheumatoid arthritis. **d** | Furin activates several membrane-type matrix metalloproteinases (MT-MMPs) that are involved in tumour formation and metastasis. Furin-activated MT-MMP1 activates MMP2 (gelatinase), which degrades the extracellular matrix, and MT-MMP1 also directly degrades extracellular matrix itself.

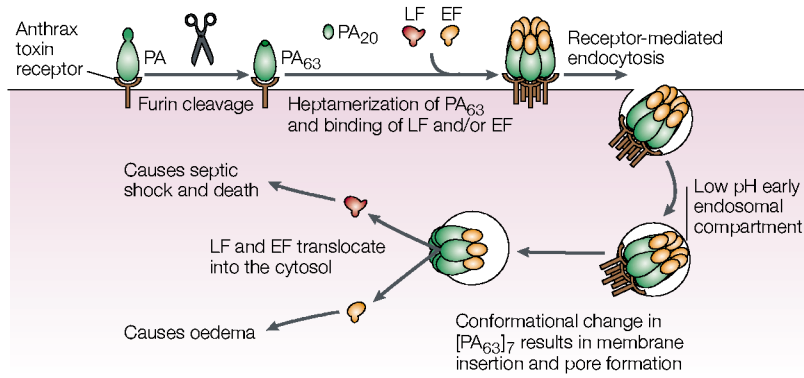


Figure 7.

Furin activation of the anthrax toxin. Cleavage of anthrax protective antigen (PA) by furin leads to internalization and activation of lethal factor (LF), which is a zinc metalloproteinase that cleaves mitogen-activated protein kinase (MAPK) kinases, and oedema factor (EF), which is a calmodulin-dependent adenylate cyclase. EF causes eschar formation, which are black masses of scab-like necrotized tissue, and massive soft-tissue oedema¹²³. In lung macrophages, phagocytosed LF leads to changes in the levels of tumour necrosis factor- α and interleukin-1 β , as well as apoptosis. The altered cytokine levels coupled with the paralysed innate immune system rapidly result in anthrax-induced systemic shock and death by a complex, unresolved process. See text for more details. Modified with permission from REF. 163. © (2002) Birkhäuser Publishing Ltd.

Table 1

The proprotein convertase family

Proprotein convertase	Size (amino acids)	Tissue distribution	Subcellular localization	Knockout phenotype
Furin	794	Broad	TGN/endosomal	Embryonic lethality (day 10.5), impaired axial rotation
PC7	785	Broad	TGN/endosomal	Viable; misexpression causes thymic defects
PC5/6A*	915	Broad	Secretory granules	ND
PC5/6B*	1877	Broad	TGN/endosomal	ND
PACE4	963	Broad	TGN/endosomal	Embryonic lethality (day 15.5), craniofacial and CNS defects
PC1/3	753	Neuroendocrine system	Secretory granules	Mouse: severe growth defects, defective GHRH and POMC processing, hyperproinsulinaemia ‡Humans: severe early obesity, adrenocortical insufficiency, hyperproinsulinaemia
PC2	638	Neuroendocrine system	Secretory granules	Hypoglycaemia, proinsulinaemia, glucagon deficiency, defects in opioid peptide processing
PC4	655	Testicular and ovarian germ cells	ND	Males: infertility Females: mild infertility

Included are the phenotypes of the knockout mice for furin⁹⁵, PACE4 (REF. 164), PC2 (REF. 165), PC4 (REF. 166), PC7 (REF. 167 and N. Seidah, personal communication) and PC1/3 (REF. 168).

* PC5/6 is expressed as either the A or B isoform.

‡ The phenotype of a patient lacking PC1/3 (REF. 169). See REFS 150,151 and 170 for more comprehensive reviews on the PC family. CNS, central nervous system; GHRH, growth hormone-releasing hormone; ND, not determined; PC, proprotein convertase; POMC, proopiomelanocortin; TGN, *trans*-Golgi network.