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Review

Subtilisin-like proteases in nematodes

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Abstract

Cleavage by subtilisin-like proteases (subtilases) is an essential step in post-translational processing of proteins found in organisms ranging from yeast to mammals. Our knowledge of the diversity of this protease family in nematodes is aided by the rapid increase in sequence information, especially from the *Brugia malayi* genome project. Genetic studies of the subtilases in *Caenorhabitis elegans* give valuable insight into the biological function of these proteases in other nematode species. In this review, we focus on the subtilases in filarial nematodes as well as other parasitic and free-living nematodes in comparison to what is known in *C. elegans*. Topics to be addressed include expansion and diversity of the subtilase gene family during evolution, enhanced complexity created by alternative RNA splicing, molecular and biochemical characterization of the different subtilases and the challenges of designing subtilase-specific inhibitors for parasitic nematodes.

Keywords: Subtilisin; Proprotein convertase; Nematode; Protease

Contents

1. The subtilisin-like protease family

Proteolytic cleavage or activation of proteins by subtilisin-like proteases (subtilases) plays a major role in nematode biology. This family of serine proteases is involved in multiple processes such as the construction and maintenance of the cuticle, neural signaling and nematode development. Our knowledge of the number and location of subtilases in nematodes is

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Table 1
Expansion of the Kexin subfamily of subtilases during evolution

	S.cerevisiae	Nematodes	Mammals
Kexin subfamily	kexin	furin PC1 PC2	furin PC1 PC2
		blisterase *aex-5*	PC4 PC5 PACE 4 PC7
Pyrolysin subfamily	ND	TPP-II SKI-1	TPP-II SKI-1
Proteinase K subfamily	PRB1 YSP3 YCR045C	ND	NARC-1

Proteases conserved between nematodes and mammals are boxed and highlighted in red. ND = not detected.

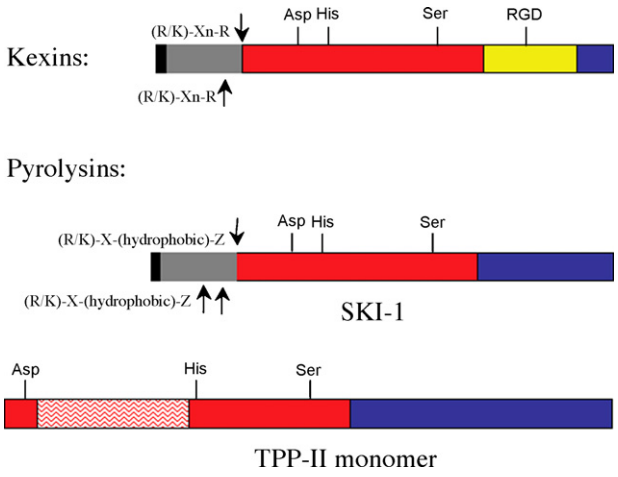

Fig. 1. Nematode subtilase domain architecture. The Kexins are composed of a signal peptide (black), pro (grey), catalytic (red), p (yellow) and carboxy (blue) domains. Arrows denote concensus cleavage motifs involved in protease activation. Cleavage occurs on the carboxy end of motifs described by (R/K)-Xn-R[1] where X is any aa and $n = 0$, 2, 4 or 6 [4]. The catalytic domain contains the Asp, His and Ser residues of the catalytic triad and exhibits the highest identity with the bacterial subtilisins. P domains stabilize the folding of the catalytic domain as well as regulate the pH and calcium dependence of the Kexins [67]. Most p domains express an RGD motif that may be involved with intracellular sorting as well as cell surface binding via integrins [68]. Carboxy domains are the least conserved among Kexins and contain signals responsible for trafficking and localization of these proteases in cells. The domain architecture of SKI-1 is similar to the Kexins except it has no p domain [52]. Activation of the SKI-1 zymogen occurs at multiple motifs described by (R/K)-X-hydrophobic-(L/T) (where X is any aa and Z is preferentially Leu or Thr) [6]. TPP-II monomers consist of a catalytic domain with an insert of approximately 200 aa (wavey red box) between the Asp and His catalytic residues and a carboxy domain both of which may play a role in stabilizing TPP-II complexes [8].

more advanced then our knowledge of their potential substrates. The essential role of these proteases and their diverse activities make them attractive drug targets. In this review, we will summarize the characteristics of the nematode subtilases in comparison to what is known about this interesting gene family in yeast and humans.

Subtilases are distinguished from other serine proteases by the order (Asp, His, Ser) of the catalytic residues along the linear protein sequence and by a unique protein scaffold. They are divided into six subfamilies (Subtilisin, Thermitase, Proteinase K, Lantibiotic, Kexin and Pyrolysin) based on sequence homology within the catalytic domain [1].

Two of these subfamilies, Kexin and Pyrolysin, are present in nematodes (Table 1). Kexin subfamily members are also known as subtilisin-like proprotein convertases (SPCs). Kexin, the first SPC to be identified, is encoded by the *Saccharomyces cerevisiae kex2* gene and is responsible for processing alpha mating factor and killer toxin [2]. Genbank searches with kexin identified furin, the first mammalian SPC, originally thought to be a growth factor receptor [3]. Kexins are only found in eukaryotes but are distantly related to subtilases in the bacteria *Anabaena variabilis* and *Aeromonas salmonicida* [1]. Members of the Kexin subfamily found in nematodes are furin, blisterase, proprotein convertase 1 (PC1), proprotein convertase 2 (PC2), and *aex-5* (Table 1).

Proteases of the Kexin subfamily are composed of multiple domains. They are synthesized as zymogens and are activated by removal of their prodomains (Fig. 1 and [4]). A general model has emerged for activation of most Kexins [5]. The first step is cleavage at the basic aa cluster at the pro/catalytic domain boundary (Fig. 1). After cleavage, the prodomain remains associated with the protease, inhibiting its activity until the complex is translocated from the endoplasmic reticulum (ER) to the trans Golgi network (TGN). Once in the Golgi apparatus, a change in the calcium and pH conditions triggers cleavage at the second basic aa cluster located upstream from the primary cleavage site and the prodomain fragments dissociate from the mature enzyme.

The other nematode subtilases, Subtilisin/Kexin Isoenzyme 1 (SKI-1) and Tripeptidyl Peptidase II (TPP-II) belong to the Pyrolysin subfamily (Table 1 and [1,6]). The Pyrolysin subfamily is a diverse group of subtilases characterized by large insertions and long C-terminal extensions [1]. Pyrolysin is a degradative endoprotease from the hyperthermophilic archaeon, *Pyrococcus furiosus* [7]. Pyrolysin-like subtilases are also found in gram negative and positive bacteria and eukaryotes [1]. Mature pyrolysin is composed of a catalytic and carboxy domain. The catalytic domain encodes an insert of ~150 aa between the Asp and His residues of the catalytic triad [7]. TPP-II monomers, like pyrolysin, have a large insertion between the Asp and His residues of the catalytic triad as well as a long carboxy tail (Fig. 1 and [8]). Mature SKI-1, like pyrolysin, is an endopeptidase composed of a catalytic

[1] The substrate cleavage site is designated as – P3-P2-P1/P1′-P2′-P3′- with the scissile bond between P1 and P1′ [66].