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PROTEASES IN HEALTH AND DISEASE

EDITED BY
ENRICO DI CERA



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**Molecular Biology and
Translational Science**
**Proteases in Health
and Disease**

edited by

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Preface

Proteases act as positive or negative effectors of numerous biological processes, either as nonspecific catalysts of protein degradation or highly selective agents controlling physiological events. Many biological pathways involving protease activity have been characterized, and a wealth of information is available. Five classes of proteolytic enzymes are recognized on the basis of their catalytic mechanism: aspartic, cysteine, metallo-, threonine, and serine peptidases. With the advent of whole genome sequencing, this classification system has expanded by the necessity to encompass the diverse catalytic repertoire found in nature. Much work remains to define the diversity of proteolytic events in biological systems and their spatial and temporal distribution in health and disease. This volume contains seven chapters contributed by leaders in their field and provides illustrative examples of the key role of proteases in biological processes, and how proteolytic function and regulation can be harnessed to define new strategies of therapeutic intervention. The reader will find these chapters both stimulating and informative.

In Chapter 1, Antalis, Bugge, and Wu discuss the exciting group of membrane-anchored serine proteases that have emerged as crucial contributors to processes related to development and maintenance of homeostasis.

In Chapter 2, Zogg and Brandstetter review the assembly of multiprotein complexes in blood coagulation, with emphasis on their structural organization, modes of action, and regulation, and outline therapeutic opportunities for the treatment of hemophilia and thrombosis.

In Chapter 3, Lancellotti and De Cristofaro detail the structural and functional properties of ADAMTS13, a disintegrin and metalloprotease involved in the pathogenesis of thrombotic microangiopathies via processing of matrix components.

In Chapter 4, Di Cera reviews the structure, function, and regulation of thrombin and how this knowledge has led to the rational engineering of thrombin variants with intriguing anticoagulant and antithrombotic properties, both *in vitro* and *in vivo*.

In Chapter 5, Olson and Gettins offer an extensive review of protein inhibitors of the serpin superfamily, detailing their structural and kinetic properties and how this knowledge may lead to engineering variants with desired specificity toward protease targets for therapeutic applications.

In Chapter 6, Yang, Hill, and Rosenberg discuss the multiple roles of metalloproteases in neurological disorders, especially their ability to open the blood-brain barrier and degrade the extracellular matrix around blood vessels, and outline the advantages of more specific inhibitors.

In Chapter 7, Troy, Akpan, and Jean deal with the regulation of caspases in the nervous system and discuss their function in the context of neurodegenerative diseases like Alzheimer's and cerebral ischemia.

I thank all the authors for their excellent contributions and their efforts to submit outstanding chapters by the stringent deadlines agreed with the publisher. Expert editorial assistance from Tracey Baird, Senior Administrative Assistant, is gratefully acknowledged and was key to the timely execution of this project.

September 1, 2010

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Membrane-Anchored Serine
Proteases in Health and
Disease

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Serine proteases of the trypsin-like family have long been recognized to be critical effectors of biological processes as diverse as digestion, blood coagulation, fibrinolysis, and immunity. In recent years, a subgroup of these enzymes has been identified that are anchored directly to plasma membranes, either by a carboxy-terminal transmembrane domain (Type I), an amino-terminal transmembrane domain with a cytoplasmic extension (Type II or TTSP), or through

a glycosylphosphatidylinositol (GPI) linkage. Recent biochemical, cellular, and *in vivo* analyses have now established that membrane-anchored serine proteases are key pericellular contributors to processes vital for development and the maintenance of homeostasis. This chapter reviews our current knowledge of the biological and physiological functions of these proteases, their molecular substrates, and their contributions to disease.

I. Introduction

Proteolytic enzymes comprise over 2% of the known proteome, and their participation in many essential biological processes is well established. The serine proteases constitute one of the largest families of proteolytic enzymes and are well recognized for their pivotal roles in physiological processes as diverse as development, digestion, coagulation, inflammation, and immunity. These enzymes share a common catalytic mechanism for selective cleavage of specific substrates and are frequently involved in consecutive proteolytic reactions or protease cascades, where one protease precursor or zymogen is the substrate for an active protease. This shared mechanism confers the advantage that a single signal may be specifically and irreversibly amplified every time a downstream zymogen is activated, providing the capacity for unleashing a burst of proteolytic potential.

Most of the well-characterized members of the S1 family of serine proteases are either secreted enzymes or exocytosed from secretory vesicles into the extracellular environment. Trypsin and chymotrypsin, the main intestinal digestive enzymes, are prototype members of the S1 family. Over the past 10 years, a structurally distinct group of S1 serine proteases, termed broadly as the membrane-anchored serine proteases, has emerged that are synthesized with amino- or carboxy-terminal extensions that serve to anchor their serine protease catalytic domains directly at the plasma membrane^{1,2} (Fig. 1).

The largest group of membrane-anchored serine proteases is the Type II transmembrane serine proteases or TTSPs.¹ These proteases are synthesized with an amino-terminal signal anchor that is not removed during synthesis, but serves as a transmembrane domain that positions the protease in the plasma membrane with a cytoplasmic amino-terminal domain of variable length (20–160 amino acids) and the catalytic serine protease domain at the carboxy-terminus.¹ These serine proteases are synthesized as single-chain precursors or zymogens; activation produces a two-chain form with the chains linked by a disulfide bridge, so that the active enzyme remains membrane bound. Nineteen human TTSPs have been identified and may be categorized into four subfamilies: Hepsin/TMPRSS, Matriptase, HAT/DESC, and Corin^{3,4} (Fig. 1).

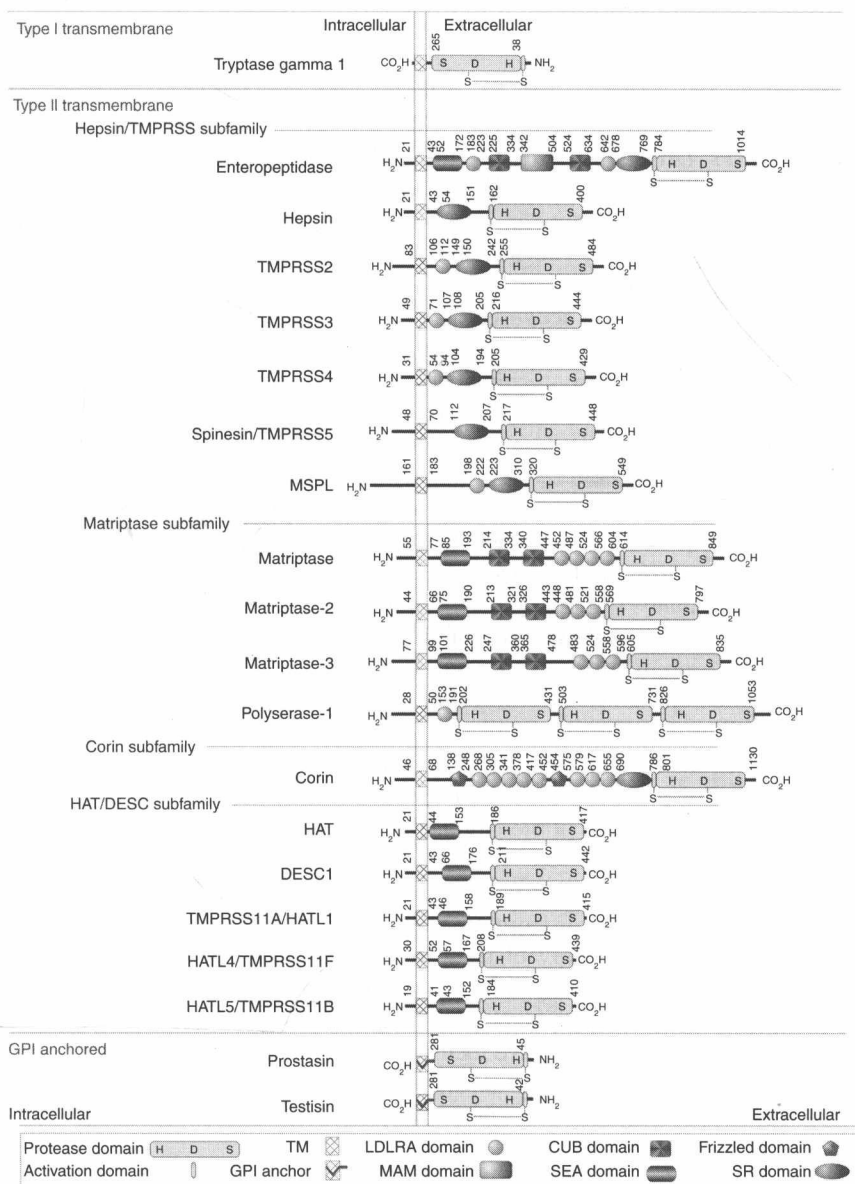


FIG. 1. Domain structures of the human membrane-anchored serine proteases. Structures are grouped according to similarity in domain structure to each other. Consensus domains are as indicated at the bottom of the figure. The location of each protein domain (amino acid numbering) is indicated above the domain. Amino and carboxy termini are as indicated. Protease domain: serine protease domain; activation domain: pro-domain; TM: transmembrane domain; GPI anchor: glycosylphosphatidylinositol linkage domain; LDLRA: LDL receptor class A domain; MAM: meprin, A5 antigen, and receptor protein phosphatase μ domain; CUB: Cls/Clr, urchin embryonic growth factor and bone morphogenetic protein-1; SEA: sea urchin sperm protein, enteropeptidase, agrin domain; Fz: frizzled domain; SR: Group A scavenger receptor domain. Modified from Ref. 2.

Mammalian orthologs, as well as different isoforms between humans and rodents, exist for many if not all of the TTSPs.⁴⁻⁶ There also exist two nonmammalian TTSPs, *Drosophila* stubble-stubblod (st-sb)⁷ and corin.⁸

In contrast to the TTSPs, additional membrane-anchored serine proteases of the S1 family each possess an amino-terminal signal peptide and enter the secretory pathway. These enzymes are also synthesized as zymogens, with an amino-terminal extension that acts as a pro-peptide, requiring proteolytic cleavage to generate the active enzyme. The Type I transmembrane serine protease, tryptase $\gamma 1$, is the only human membrane-anchored serine protease synthesized with a carboxy-terminal hydrophobic extension that serves as a transmembrane domain.^{9,10} The carboxy-terminal extensions of prostasin and testisin are modified posttranscriptionally with a glycosylphosphatidylinositol (GPI) linkage that anchors these proteases in the plasma membrane.¹¹⁻¹⁵

The membrane-anchored serine proteases are proving to be key components of the cell machinery for activation of precursor molecules in the pericellular microenvironment, with several playing vital roles during development and the maintenance of homeostasis. There is also growing evidence for their participation in the pathogenesis of inflammatory and neoplastic diseases. Endogenous protein substrates targeted by membrane-anchored serine proteases include peptide hormones, growth and differentiation factors, receptors, enzymes, adhesion molecules, and viral coat proteins.¹⁶ A number of insights into our understanding of the unique physiological functions of the membrane-anchored serine proteases and their involvement in human pathology have come from a combination of biochemical analyses, animal models, and human patient studies. However, our current understanding of the impact of the membrane-anchored serine proteases on many biological, physiological, and pathological processes is far from complete. This chapter provides a historical perspective on the discovery of these enzymes, current knowledge of their activities and their regulation, and the functional consequences of the activities of these proteases in mammalian physiology and disease. For the interested reader, several other reviews have focused on different aspects of their nomenclature, classifications into subgroups, gene structure and chromosomal localization, tissue- and cell-specific distribution, and biochemical properties.^{1-4,16,17}

II. Structural Features

All of the membrane-anchored serine proteases have membrane-anchoring domains and structurally conserved serine protease catalytic domains. The TTSPs have additional extracellular stem regions that separate the catalytic domains from their transmembrane domains. The extracellular regions of the membrane-anchored serine proteases are believed to be essential to the biological and physiological functions ascribed to these enzymes.

A. Catalytic Domains

The zymogen forms of the membrane-anchored serine proteases are activated by proteolytic cleavage following an arginine or lysine amino acid present in a highly conserved activation motif separating the pro- and catalytic domains. The catalytic mechanism of the membrane serine proteases involves a catalytic triad of three amino acids, serine (nucleophile), aspartate (electrophile), and histidine (base), present in highly conserved sequence motifs. While the geometric orientations of these amino acid residues are similar, the protein folds are variable, which contribute to their selective substrate specificities. The catalytic reaction follows a two-step mechanism for hydrolysis of substrates in which a covalently linked enzyme-peptide intermediate is formed, with the loss of a peptide fragment.¹⁸ This acylation step is followed by a deacylation step which occurs by a nucleophilic attack on the intermediate by water, resulting in hydrolysis of the peptide.

Some insights into the structural features that contribute to the unique catalytic and substrate specificities of the membrane-anchored serine proteases have been obtained through comparative analyses of amino acid sequences^{1,2,19} combined with tertiary structural analyses.²⁰⁻²⁶ Consistent with the family of S1 serine proteases, each catalytic domain possesses two adjacent, six-stranded β -barrel domains that are connected by three trans-domain segments. The catalytic triad amino acids are located along the junction between the two barrels, with the active site cleft running perpendicular to this junction.²⁷ The size, shape, and charge distribution within the formed binding pocket of the active enzyme are determinants of substrate specificity. These pockets are defined by differing substrate-binding subsites (e.g., S_4 - S_2') and loop regions that surround the active site cleft.²³

The specificity for cleavage of substrates with the positively charged amino acid residues, lysine or arginine, in the P_1 position (the position directly preceding the cleaved peptide bond) is conferred by the presence of a conserved aspartate residue at the bottom of the binding pocket of all of the activated enzymes.² The rate of cleavage is influenced by the amino acid residues surrounding the P_1 residue, numbered P_1 to P_n , counting outward from the amino-terminal side of the peptide bond that is cleaved during hydrolysis, and numbered P_1' through P_n' from the carboxy-terminal side.²⁸ In recent years, recombinant catalytic domains of several of the membrane-anchored serine proteases have been produced in various laboratories and peptide screening assays applied to quantitatively identify cleavage preferences for the P_1 and P_1' amino acids, and surrounding amino acid positions.²⁹⁻³⁵

Several of the biochemically purified TTSPs rapidly undergo autocatalytic activation *in vitro* (matriptase^{29,36} matriptase-2,³⁷ hepsin,³⁸ TMPRSS2,³⁹ TMPRSS3,⁴⁰ TMPRSS4,⁴¹ HAT-like 3/TMPRSS11C⁴²). Thus, mutation of

active site catalytic triad residues of several of these TTSPs prevents activation site cleavage.^{3,36,42,43} It has been speculated that autoactivation of these TTSPs could contribute to the initiation of proteolytic cascades at the cell membrane, although the signals that induce internal cleavage or autoactivation *in vivo* have not yet been defined.

B. Extracellular "Stem" Regions

While the catalytic domains of the Type I and GPI-anchored serine proteases lie directly proximal to the membrane anchoring domain, the TTSPs are characterized by the presence of up to 11 extracellular structural domains present between their catalytic domains and the transmembrane domain (Fig. 1). These "stem" regions appear to serve as regulatory and/or binding interaction domains,¹ and are believed to variously contribute to the cellular localization, activation, inhibition, and/or substrate specificity of these enzymes.⁴⁴ The most common stem region structural domain is the low-density lipoprotein (LDL) receptor class A domain (LDLRA): corin contains eight, matriptase contains four, enteropeptidase two, and TMPRSS2 and TMPRSS4 one each of these domains (Fig. 1). In addition to the LDLRA domains, there are Group A scavenger receptor (SR) domains, frizzled domains, Cls/Clr, urchin embryonic growth factor and bone morphogenic protein 1 (CUB) domains, sea urchin sperm protein, enterokinase, agrin (SEA) domains, and meprin, A5 antigen, and receptor protein phosphatase μ (MAM) domains. The specific contributions of each stem domain to TTSP proteolytic activities have not yet been well defined, although for several of the TTSPs, the stem domain is required for efficient cleavage of their physiological substrates.^{45,46}

Targeted release of the extracellular domain, or ectodomain shedding, occurs for several of the membrane-anchored serine proteases, enabling the release of the soluble catalytic domain into the extracellular space. Soluble shed protease forms have been reported for enteropeptidase, HAT, matriptase, matriptase-2, and prostasin.^{11,47-49}

C. Membrane-Anchoring Domains

Surface localization studies demonstrate that membrane-anchored serine proteases normally localize to the cell surface and are differentially distributed on apical or basolateral surfaces of polarized cells in patterns unique for each protease.^{3,4} Studies indicate that the TTSP transmembrane domains are dispensable for the catalytic activities of many of the TTSPs, including enteropeptidase, matriptase, hepsin, corin, and MSPL.⁴ It appears that the function of the transmembrane domain in TTSPs is not to enhance their catalytic activities but to target the TTSPs to plasma membranes and localize their activities at specific sites. Similarly, the GPI-anchored serine proteases are