Membrane-Anchored Serine Proteases: Host Cell Factors in Proteolytic Activation of Viral Glycoproteins

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Abstract

Over one third of all known proteolytic enzymes are serine proteases. Among these, the trypsin-like serine proteases comprise one of the best characterized subfamilies due to their essential roles in blood coagulation, food digestion, fibrinolysis, or immunity. Trypsin-like serine proteases possess primary substrate specificity for basic amino acids. Most of the well-characterized trypsin-like proteases such as trypsin, plasmin, or urokinase are soluble proteases that are secreted into the extracellular environment. At the turn of the millennium, a number of novel trypsin-like serine proteases have been identified that are anchored in the cell membrane, either by a transmembrane domain at the N- or C-terminus or via a glycosylphosphatidylinositol (GPI) linkage. Meanwhile more than 20 membrane-anchored serine proteases (MASPs) have been identified in human and mouse, and some of them have emerged as key regulators of mammalian development and homeostasis. Thus, the MASP corin and TMPRSS6/matriptase-2 have been demonstrated to be the activators of the atrial natriuretic peptide (ANP) and key regulator of hepcidin expression, respectively. Furthermore, MASPs have been recognized as host cell factors activating respiratory viruses including influenza virus as well as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) coronaviruses. In particular, transmembrane protease serine S1 member 2 (TMPRSS2) has been shown to be essential for proteolytic activation and consequently spread and pathogenesis of a number of influenza A viruses in mice and as a factor associated with severe influenza virus infection in humans.

This review gives an overview on the physiological functions of the fascinating and rapidly evolving group of MASPs and a summary of the current knowledge on their role in proteolytic activation of viral fusion proteins.

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8.1 Introduction

The designation trypsin-like serine proteases has originally been used for a large group of soluble proteolytic enzymes, which are involved in digestion, blood coagulation, fibrinolysis, and immunity. Analyses of vertebrate genomes at the turn of the millennium have identified a novel subfamily of trypsin-like serine proteases that are anchored in the cell membrane, either by a carboxy-terminal transmembrane domain (type I), an amino-terminal transmembrane domain (type II), or via a glycosylphosphatidylinositol (GPI) linkage at the carboxy terminus. Over the past two decades, these membrane-anchored serine proteases (MASPs) have emerged as key regulators of mammalian development and homeostasis in processes such as epithelial tight junction formation, skin development, epithelial sodium channel activation, cellular iron homeostasis, blood pressure, inner ear development, placental morphogenesis, neural tube closure, and male fertility. Moreover, dysregulated expression and/or activity of a number of MASPs is observed in many cancer tissues. Within the past decade, MASPs expressed in the human airways have furthermore been identified as host cell factors that may support proteolytic activation and spread of respiratory viruses including influenza virus, human metapneumovirus, severe acute respiratory syndrome (SARS) , and Middle East respiratory syndrome (MERS) coronavirus (CoV).

The largest group of the MASPs is the family of type II transmembrane serine proteases (TTSPs) (Hooper et al. 2001). To date, the TTSP family comprises 18 known members in humans and 20 in mice. All members of the TTSP family share a common domain structure possessing a short N-terminal cytoplasmic domain, a transmembrane domain, a C-terminal serine protease domain, and a variable stem region that may contain 1–11 protein domains of 6 different types (Fig. 8.1). The most prominent member of the TTSP, enteropeptidase, has been identified over a century ago by Pavlov and coworkers due to its essential role in food digestion; however, only the cloning of the enteropeptidase cDNA in 1994 revealed the presence of a membrane anchor and explained its localization to the plasma membrane of duodenal cells. The modular structure of the enteropeptidase protein was found to be similar to that of a previously cloned protease, hepsin (Leytus et al. 1988).

The first of the GPI-anchored serine proteases identified was prostasin (Yu et al. 1994; Chen et al. 2001a, b). A second human GPI-anchored serine protease, testisin, was identified in 1998/1999 (Inoue et al. 1998, Hooper et al. 1999). Tryptase gamma 1 is the only type I transmembrane serine protease identified to date. Prostasin, testisin, and tryptase gamma 1 are composed of a single protease domain linked to a GPI anchor or a transmembrane domain at the C-terminus (Fig. 8.1).

MASPs belong to the chymotrypsin (S1)-like serine protease family (reviewed in Perona and Craik 1995 and Hooper et al. 2001). They are synthesized as inactive single-chain zymogens that are activated by cleavage following an arginine or lysine residue within a highly conserved activation motif preceding the catalytic domain. After activation, the catalytic domain remains linked to the membrane-anchored domains by a disulfide bond but can also be released as a soluble protease (Hooper et al. 2001). All MASPs have a highly conserved S1 serine protease domain that

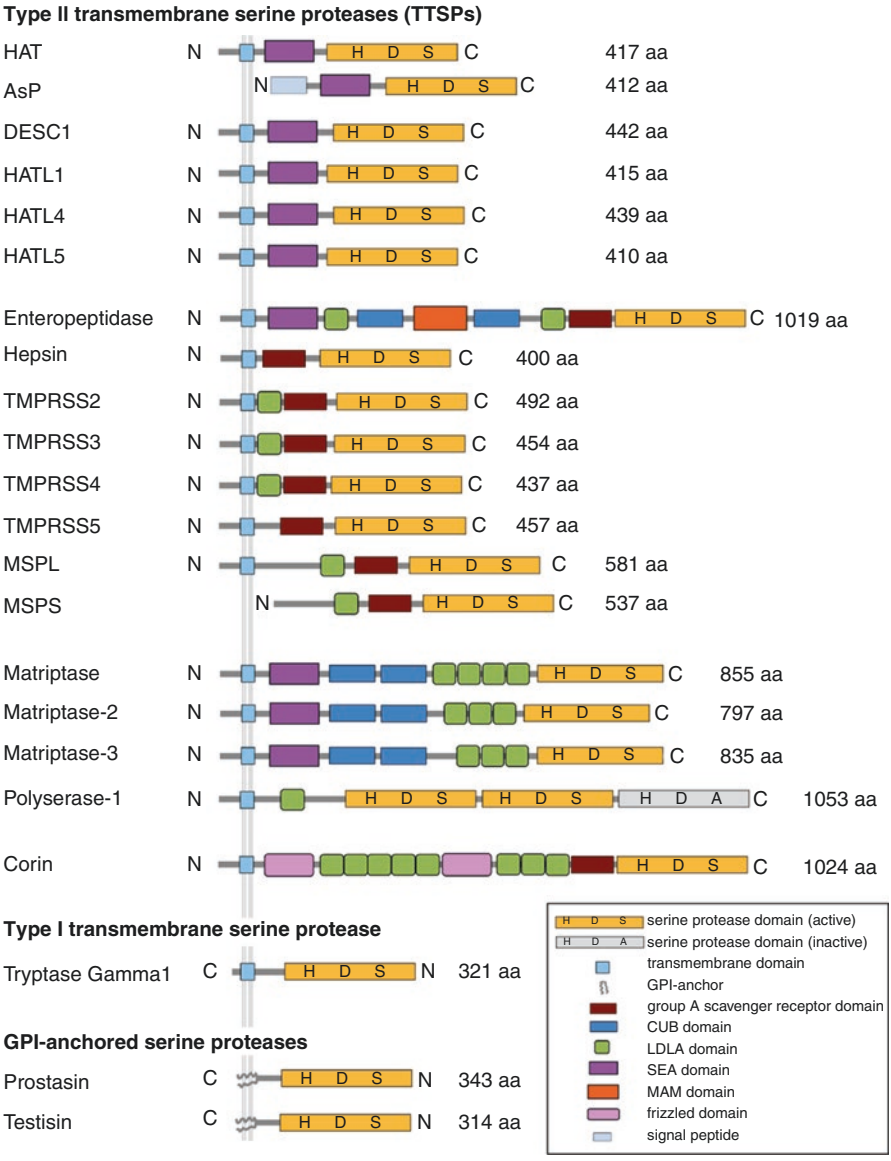

Fig. 8.1 Domain structures of membrane-anchored serine proteases. *HAT* human airway trypsin-like protease; *HATL* HAT-like protease; *AsP* adrenal secretory serine protease; *DESC1*, differentially expressed in squamous cell carcinoma; *TMPRSS*, transmembrane serine protease S1; *MSPL/MSPS* mosaic serine protease large/short-form; *GPI* glycosylphosphatidylinositol; *CUB* Cls/Clr, urchin embryonic growth factor and bone morphogenetic protein-1; *SEA* sea urchin sperm protein, enterokinase, and agrin; *LDLA* low-density lipoprotein receptor A; *MAM* meprin, A5 antigen, and receptor protein phosphatase μ. Amino and carboxy termini are indicated by N and C, respectively. *aa* amino acids

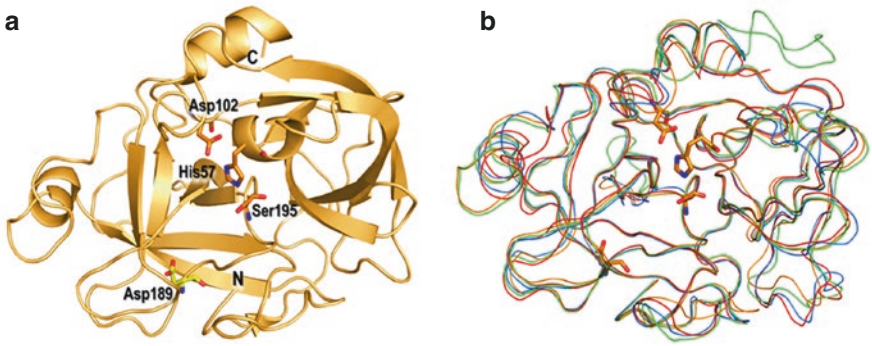

Fig. 8.2 Crystal structure of the catalytic domain of human DESC1. (**a**) DESC1 (PDB: 2OQ5, Kyrieleis et al. 2007) is shown in cartoon style. The residues of the catalytic triad (carbons in orange, nitrogen in blue, and oxygen in red) and of Asp189 (carbons in yellow) are shown as sticks. N-terminus and C-terminus are labeled. (**b**) DESC1 (ribbon style, orange) superimposed with the catalytic domains of human matriptase (blue, PDB: 1EAX, Friedrich et al. 2002), human enteropeptidase (red, PDB: 1EKB, Lu et al. 1999), and human hepsin (green, PDB: 1P57, Somoza et al. 2003). The residues of the active site of DESC1 and Asp189 are shown as sticks (carbons in orange, nitrogen in blue, and oxygen in red)

contains the histidine, aspartate, and serine residues (catalytic triad) necessary for the catalytic activity (Fig. 8.2). In addition, all MASPs show a strong preference for cleavage of substrates after a basic residue (arginine or lysine) due to a negatively charged aspartate located at the bottom of the S1 pocket that is highly conserved in enzymes with trypsin-like activity (reviewed in Perona and Craik 1995 and Hooper et al. 2001). Accordingly, autocatalytic activation of several TTSPs has been observed in vitro, suggesting that some of the TTSPs could function as initiators of proteolytic cascades (Hooper et al. 2001).

This review aims to give an overview on the physiological functions of this fascinating and rapidly evolving group of enzymes and a summary of the current knowledge on their role in proteolytic activation of viral fusion proteins.

8.2 Type II Transmembrane Serine Proteases (TTSPs)

Based on the arrangements of their extracellular protein domain, the phylogenetic analysis of the serine protease domain, and the chromosomal arrangement of the cognate genes, human TTSPs are divided into four subfamilies: the HAT/DESC (human airway trypsin-like protease/differentially expressed in squamous cell carcinoma) family, the hepsin/TMPRSS (transmembrane protease/serine S1) family, the matriptase family, and the corin family (Szabo et al. 2003) (Fig. 8.1). TTSP genes are found in all vertebrate genomes. There also exist two nonmammalian TTSPs in *Drosophila*, stubble-stubbloid (st-sb), and corin, indicating that the TTSP family may have originated from two ancestral genes, one giving rise to the HAT/DESC family and one to the corin family (Appel et al. 1993; Bugge et al. 2009).

8.2.1 HAT/DESC Family

The HAT/DESC family contains five human members: HAT, DESC1, HAT-like 1 protease (HATL1), HATL4, and HATL5. In addition, HATL2 and HATL3 have been described in rodents (Table 8.1). The stem region of all HAT/DESC members is composed of a single SEA (sea urchin sperm protein, enterokinase, and agrin) domain. The genes encoding the HAT/DESC family members are all located in tandem on human chromosome 4 and mouse chromosome 5, respectively (Hobson et al. 2004). To date, the functional significance of the HAT/DESC1 family is largely unknown with HAT being the best studied exemption.

8.2.1.1 HAT: A Trypsin-Like Protease Associated with Airway Diseases

Human airway trypsin-like protease (HAT) was originally isolated from sputum of patients with chronic airway diseases as a soluble active protease with an apparent molecular mass of 27 kDa (Yoshinaga et al. 1998). Subsequent cloning of HAT cDNA from human trachea cDNA revealed that it encodes for a protease of 417 amino acids with a predicted molecular mass of 47 kDa that contains a transmembrane region near the N-terminus (Yamaoka et al. 1998). The HAT zymogen undergoes autocatalytic activation in vitro (Kato et al. 2012). HAT has been shown to be expressed as an active protease on the cell surface of HAT-expressing Madin-Darby canine kidney (MDCK) cells (Böttcher-Friebertshäuser et al. 2010). Surface biotinylation analysis showed that both the zymogen and the mature form of HAT are present on the cell surface, indicating that autoactivation might take place at the cell surface.

HAT is encoded by the TMPRSS11D gene, located on human chromosome 4q13.2. The TMPRSS11D gene is the human ortholog of long splice variants of the airway trypsin-like protease from mouse (MAT1) and rat (RAT1) (Hansen et al. 2004). An alternatively spliced isoform of MAT1 and RAT1 has been identified in rat (RAT2) and mouse (MAT2), respectively. It is also known as adrenal secretory serine protease (AsP) and contains an N-terminal signal peptide instead of a transmembrane domain and a SEA domain (Fig. 8.1). There have been no reports of a short isoform of HAT in humans.

Within human tissues, HAT expression is prominent in the trachea and bronchi and was also detected in the gastrointestinal tract, the skin, and the brain (Sales et al. 2011; Bertram et al. 2012). In the airway epithelium, HAT has been shown to be expressed at the apical membrane of ciliated cells, but not in goblet cells, submucosal glands, and mast cells (Takahashi et al. 2001). A number of studies suggest a potential role for HAT in the pathophysiology of bronchial asthma and chronic bronchitis (Chokki et al. 2004; Matsushima et al. 2006; Yasuoka et al. 1997; Yoshinaga et al. 1998). Among other functions HAT has been shown to cleave fibrinogen, to modulate structure and functions of the urokinase-type plasminogen activator receptor (uPAR, CD87), and to activate the protease-activated receptor 2 (PAR-2). Protease-activated receptors (PARs) are a family of seven transmembrane domain G-protein-coupled receptors that are activated by serine proteases through

Table 8.1 Tissue distribution and physiological functions of MASPs in human and mouse

Protease	Other names	Chromosomal localization human/ mouse	Tissue distribution	Physiological functions	Physiological substrate(s)	Phenotype of mutant mice
HAT	TMPRSS11D	4q13.2/5E1	air, bra, bro, es, ey, pro, sk, si, spc, st, tes, to, tr, ub	Unknown	Unknown	Deficiency: no effect on development, postnatal growth, or long-term health
DESC1	TMPRSS11E	4q13.3/5E1	air, epd, es, pro, sk, sg, tes, to, tr	Unknown	Unknown	n.d.
HAT-like 1 (human)	TMPRSS11A, DESC3, ECRG1	4q13.2/E51	bl, eye, es, sk, st, to, tr	Unknown	Unknown	Deficiency: no effect on development, postnatal growth, or long-term health
HAT-like 2 (mouse)	DESC4	-/5E1	air, bl, bra, co, ey, he, li, lu, sg, tg, to	Unknown	Unknown	n.d.
HAT-like 3 (mouse)	TMPRSS11C, neurobin	-/5E1	bra, spc	Unknown	Unknown	n.d.
HAT-like 4	TMPRSS11F	4q13.2/5E1	es, pl, sk, tes, to, tr	Unknown	Unknown	Deficiency: viable and fertile, increased body fluid loss
HAT-like 5	TMPRSS11B	4q13.3/E51	es, eye, tes, to, tr	Unknown	Unknown	n.d.
Enteropeptidase	PRSS7	21q21/16C3.1	psi	Converting trypsinogen to trypsin in the intestine	Trypsinogen	n.d.
Hepsin	TMPRSS1	19q11-q13.2/7A3	hyp, ie, ki, li, lu, pa, pro, thm, thr	Cochlear development and hearing (mice)	Unknown	Deficiency: viable and fertile, deafness, abnormal cochlear development, thyroid hormone deficiency

TMPRSS2	Epitheliasin	21q22.3/16C2	air, bro, co, he, ki, li, lu, pa, pro, sg, si, st, tr	Unknown	Unknown	Deficiency: no effect on development, postnatal growth, or long-term health, no apparent phenotype
TMPRSS3	TADG-12	21q22.3/17A3.3	co, coc, he, ie, ki, li, lu, ova, pa, pl, pro, si, spl, tes, thm	Hearing, survival of cochlear hair cells and spiral ganglion neurons	Unknown	Expression of truncated nonfunctional TMPRSS3 mutant: deafness
TMPRSS4	CAP2	11q23.3/9A5.2	bro, bl, co, es, ki, lu, si, st	Unknown	Unknown	Deficiency: no effect on development, postnatal growth, or long-term health, no apparent phenotype
Spinesin	TMPRSS5	11q23.3/9A5.2	bra, ie, spc	Hearing	Unknown	n.d.
TMPRSS12		12q13.13/15F1	chicken: lu, phx, spl, tr	n.d.	Unknown	n.d.
TMPRSS13	MSPL	11q23.2/9A5.2	lu, pa, pl, pro	Unknown	Unknown	Deficiency: abnormal skin development
Matriptase	MT-SP1, ST14, CAP3, PRSS14, epithin	11q25/9A4	Variety of epithelial tissues	Epidermal and hair follicle development, epithelial barrier functions (tight junction formation) Inhibition of matriptase by HAI-1 is essential for formation of the placental labyrinth in mice	Prostasin	Deficiency: early postnatal mortality, impaired epithelial barrier function leading to severe skin dehydration Hypomorphic (100-fold reduced epidermal matriptase expression): viable and fertile, impaired epidermal prostasin zymogen activation and profilaggrin processing, impaired tight junction formation, ichthyosis, hypotrichosis

(continued)

Table 8.1 (continued)

Protease	Other names	Chromosomal localization human/ mouse	Tissue distribution	Physiological functions	Physiological substrate(s)	Phenotype of mutant mice
Matriptase-2	TMPRSS6	22q13.1/15E2	bra, ki, li, lu, spl, tes, ut	Key regulator in iron homeostasis, suppression of hepcidin expression	Hemojuvelin	Deficiency or lack of catalytic activity increases hepcidin levels leading to iron-refractory iron-deficiency anemia (IRIDA)
Matriptase-3	TMPRSS7	3q13.2/16B5	bra, epd, ey, lu, ova, pro, sg, sk, tes, tr, ut	Unknown	Unknown	n.d.
Polyserase-1	TMPRSS9	19p13.3/10C1	bra, he, ki, li, pl, skm	Unknown	Unknown	n.d.
Corin	TMPRSS10	4p13-p12/5C3.2	he, hf, ie, ki, tes, ut	Pro-ANP convertase, processing of peptides critical for regulation of blood pressure, role in specifying the hair shaft pigmentation	Pro-atrial natriuretic peptide (ANP)	Deficiency: fertile, develop normally, increased body weight, salt-sensitive hypertension, reduced sodium excretion, lack of pro-ANP processing, abnormal hair pigmentation
Tryptase gamma 1	PRSS31	16p13.3/17A3.3	mc	Unknown	Unknown	Deficiency: viable, no effect on development

Prostasin	PRSS8, CAP1	16p11.2/7F3	Variety of epithelial tissues	Epidermal and hair follicle development and epithelial barrier functions, regulation of ENaC-mediated alveolar sodium and water transport (mice)	Matriptase, ENaC (gamma subunit)	Deficiency: early postnatal mortality due to severe skin dehydration, impaired profilaggrin processing, impaired tight junction formation Expression of catalytically inactive prostasin: normal prenatal and postnatal survival, no profound defects in epidermal development and barrier function, abnormal hair growth, and delayed skin wound healing
Testisin	PRSS21, ESP-1	16p13.3/17A3.3-B	eos, sperm, tes	Directs epididymal sperm maturation and fertilizing ability	Unknown	Deficiency: subfertility, aberrant sperm morphology

HAT human airway trypsin-like protease, *DESC* differentially expressed in squamous cell carcinoma, *TMPRSS* transmembrane protease serine S1, *ECRG* esophageal carcinoma-related gene, *PRSS* protease serine S1, *CAP* channel-activating protease, *TAGD* tumor-associated differentially expressed gene, *MSPL* mosaic serine protease large-form, *ST* suppression of tumorigenicity, *ESP* eosinophil serine protease. Tissue distribution (human and mouse) experimentally confirmed at mRNA and/or protein level (for references, see text and Bugge et al. 2009). *air* upper airways; *bl* bladder; *bra* brain; *bro* bronchioles; *co* colon; *coc* cochlea; *epd* epididymis; *eos* eosinophils; *es* esophagus; *ey* eye; *he* heart; *hf* hair follicle; *hyp* hypophysis; *ie* inner ear; *ki* kidney; *li* liver; *lu* lung; *mc* mast cells; *ova* ovary; *pa* pancreas; *phx* pharynx; *pl* placenta; *pro* prostate; *psi* proximal small intestine; *sg* salivary gland; *si* small intestine; *sk* skin; *skm* skeletal muscle; *spc* spinal cord; *spl* spleen; *st* stomach; *tes* testis; *tg* tear gland; *thm* thymus; *thr* thyroid; *to* tongue; *tr* trachea; *ub* urinary bladder; *ut* uterus; *n.d.* not determined

specific N-terminal proteolytic cleavage and the unmasking of a tethered ligand. Activated PAR-2 plays a pivotal role in cell adhesion and early inflammatory processes and has been reported to mediate allergic airway inflammation of the mouse airways in vivo (Schmidlin et al. 2002). HAT has been described to increase mucin gene expression and to stimulate bronchial fibroblast proliferation in airway epithelial cells through PAR-2-mediated signaling pathways (Yoshinaga et al. 1998; Matsushima et al. 2006).

HAT expression has been shown to be deregulated in skin diseases such as psoriasis vulgaris. Higher expression of HAT was found in psoriatic epidermal lesions (Iwakiri et al. 2004). It has been reported that HAT might promote PAR-2-mediated interleukin 8 (IL-8) production to accumulate inflammatory cells in the epidermal layer of psoriasis vulgaris.

The physiological function of HAT in the airways and in the skin, however, remains unknown. Knockout of HAT/TMPRSS11D expression in mice does not affect development, postnatal growth, or long-term health (Sales et al. 2011), indicating that HAT/TMPRSS11D activity is dispensable or can be compensated by other proteases. A recent study found that HAT expression is lost during the dedifferentiation of epithelial cells in high-grade tumors, a hallmark of squamous cell carcinogenesis (Duhaime et al. 2016). Therefore, HAT might act as an activator and initiator of a proteolytic cascade during terminal differentiation of squamous epithelia.

HAT and TMPRSS2 (see later chapter) were the first human proteases identified to cleave and activate the influenza A virus surface glycoprotein hemagglutinin (HA) with a monobasic cleavage site and to support multicycle replication and spread of the virus in cell culture (Böttcher et al. 2006). The role of HAT and other MASPs in activation of viral glycoproteins will be described in more detail at the end of this review and is summarized in Table 8.2.

Table 8.2 Proteolytic activation of viral glycoproteins by MASPs

Protease	Influenza virus hemagglutinin (HA)	Coronavirus spike protein S	Other viruses	References
HAT/ TMPRSS11D	Avian and mammalian IAV (R/K↓) (subtypes H1, H2, H3, H9, H11, H12) IBV	SARS-CoV MERS-CoV	n.d.	Böttcher et al. (2006), Chaipan et al. (2009), Bertram et al. (2011), Böttcher-Friebertshäuser et al. (2012) and Galloway et al. (2013)
DESC1	IAV (subtypes H1, H2, H3, H17)	SARS-CoV MERS-CoV	n.d.	Zmora et al. (2014) and Hoffmann et al. (2016)
HATL1	n.d.	SARS-CoV	n.d.	Kam et al. (2009)
HATL5	No	No	n.d.	Bertram et al. (2010)
HATL4	No	No	n.d.	Bertram et al. (2010)
Hepsin	No	n.d.	Fusion protein F of subtype B avian metapneumovirus (RKKR↓)	Bertram et al. (2010) and Yun et al. (2016)

Table 8.2 (continued)

Protease	Influenza virus hemagglutinin (HA)	Coronavirus spike protein S	Other viruses	References
TMPRSS2	Avian and mammalian IAV (R/K↓) (subtypes H1–H7, H9–H11, H11–H18) IBV Essential for proteolytic activation of H7N9 and H1N1 IAV in mice	SARS-CoV MERS-CoV Human CoV-229E Porcine epidemic diarrhea virus	Fusion protein F of paramyxoviruses: human metapneumovirus; human parainfluenza viruses 1, 3, 4a, and 4b; and Sendai virus	Böttcher et al. (2006), Shirogane et al. (2008), Matsuyama et al. (2010), Glowacka et al. (2011), Shirato et al. (2013), Shulla et al. (2011), Böttcher-Friebertshäuser et al. (2012), Abe et al. (2013), Bertram et al. (2013), Ferrara et al. (2013), Galloway et al. (2013), Gierer et al. (2013), Hatesuer et al. (2013), Tarnow et al. (2014) Sakai et al. (2014), Hoffmann et al. (2016) and Fan et al. (2017)
TMPRSS3	No	No	n.d.	Bertram et al. (2010) and Zmora et al. (2014)
TMPRSS4	IAV (H1, H3)	No: SARS-CoV	n.d.	Chaipan et al. (2009) and Glowacka et al. (2011)
TMPRSS12	n.d.	n.d.	Fusion protein F of subtype B avian metapneumovirus (RKKR↓), no activation of F with RQSR↓ motif	Yun et al. (2016)
MSPL/ TMPRSS13	Highly pathogenic avian IAV of subtype H5N2 (KKKR↓) IAV (subtypes H1, H2, H3, H17 monobasic motifs)	SARS-CoV MERS-CoV Porcine epidemic diarrhea virus	n.d.	Okumura et al. (2010), Zmora et al. (2014), Hoffmann et al. (2016) and Fan et al. (2017)
Matriptase	IAV (subtype H9 with RSS/ RR↓ motif but not VSSR↓ motif, some H1N1 strains (IQSR↓))	n.d.	n.d.	Hamilton et al. (2012), Baron et al. (2013) and Beaulieu et al. (2013)
Matriptase-2	No	n.d.	n.d.	Bertram et al. (2010)

(continued)

Table 8.2 (continued)

Protease	Influenza virus hemagglutinin (HA)	Coronavirus spike protein S	Other viruses	References
Matriptase-3	No	No: SARS-CoV	n.d.	Chaipan et al. (2009) and Glowacka et al. (2011)
Polyserase-1	No	No	n.d.	Zmora et al. (2014)
Corin	No	No	n.d.	Bertram et al. (2010)
Prostasin	No	No	n.d.	Bertram et al. (2010), Böttcher-Friebertshäuser et al. (2010) and Zmora et al. (2014)
Testisin	IAV (subtype H1, H3, H9)	n.d.	n.d.	E. Böttcher-Friebertshäuser, A. Arendt, unpublished data

IAV influenza A virus; *IBV* influenza B virus; *HA* hemagglutinin; *S* spike protein; *F* fusion protein; *SARS* severe acute respiratory syndrome; *MERS* Middle East respiratory syndrome; *CoV* coronavirus; *n.d.* not determined. Amino acids are indicated as single-letter code. The cleavage site is indicated by an arrow

8.2.1.2 DESC1 and TMPRSS11A/HATL1: Tumor Suppressors in Esophagus Cancer

DESC1 (differentially expressed in squamous cell carcinoma, also designated as transmembrane protease serine S1 family member 11E (TMPRSS11E)) is expressed in the human head, neck, skin, prostate, and testis. DESC1 was first identified as a gene downregulated in squamous cell carcinoma of the head and neck (Lang and Schuller 2001). Moreover, induction of normal keratinocyte differentiation by calcium challenge was accompanied by an increase in DESC1 expression in vitro (Sedghizadeh et al. 2006). A recent study found that DESC1 sensitizes cells to apoptosis by downregulating the epidermal growth factor receptor (EGFR)/AKT pathway in esophageal squamous cell carcinoma (Ng et al. 2016). Protease activity was required for this function, suggesting that DESC1 cleaves EGFR, which subsequently leads to the downregulation of the AKT pathway. Moreover, DESC1 has been demonstrated to reduce tumor growth kinetics in an orthotopic nude mouse model for study of esophageal squamous cell carcinoma. Taken together, there is accumulating evidence suggesting the association of DESC1 downregulation with cancer development. However, its tumor suppressive role remains to be characterized in more detail in future studies. The crystal structure of the catalytic domain of DESC1 has been solved (Kyrieleis et al. 2007) and is shown in Fig. 8.2.

TMPRSS11A, also designated as HAT-like 1 protease (HATL1) and esophageal carcinoma-related gene 1 (ECRG1), has been identified as a gene downregulated in esophagus cancers (Li et al. 2006). TMPRSS11A-specific mRNA is present in the eye, testis, glandular stomach, tongue, trachea, bladder, forestomach, and skin of mice (Sales et al. 2011). Knockout of TMPRSS11A expression

does not affect development, postnatal growth, or long-term health in mice (Sales et al. 2011). TMPRSS11A/ECRG1 is one among many tumor suppressor genes that may play a role in the initiation and development of esophageal squamous cell carcinoma (Netzel-Arnett et al. 2003). Overexpression of TMPRSS11A/ECRG1 has been shown to inhibit cell growth and to induce G1/S cell cycle arrest through upregulation of p15INK4b expression in esophageal cells in vitro (Zhao et al. 2004).

8.2.1.3 TMPRSS11F/HATL4 and TMPRSS11B/HATL5

There is little known about TMPRSS11F/HATL4 and TMPRSS11B/HATL5. TMPRSS11B/HATL5 expression has been detected in the cervix, esophagus, and oral cavity. It was found to be significantly decreased in squamous cell carcinomas of these tissues as compared to normal and tumor adjacent samples (Miller et al. 2014), and thus it might have a similar physiological function like DESC1 and TMPRSS11A. TMPRSS11F/HATL4-specific mRNA was found to be present in the skin, esophagus, trachea, tongue, eye, bladder, testis, uterus, and stomach (Sales et al. 2011). Recently, TMPRSS11F-knockout mice were shown to be viable and fertile (Zhang et al. 2017). Compared with wild-type controls, TMPRSS11F-deficient newborn mice had greater body fluid loss and higher mortality in a trans-epidermal body fluid loss test, indicating that TMPRSS11F/HATL4 is involved in epidermal barrier function to prevent body fluid loss.

8.2.2 Hepsin/TMPRSS Family

The hepsin/TMPRSS family comprises eight members: hepsin/TMPRSS1, TMPRSS2, TMPRSS3, TMPRSS4, spinesin/TMPRSS5, TMPRSS12, TMPRSS13/MSPL (mosaic serine protease large-form), and enteropeptidase. All members of this family have a group A scavenger receptor domain in their stem region linked to the serine protease domain, preceded by a single LDLA (low-density lipoprotein receptor A) domain in TMPRSS2, TMPRSS3, TMPRSS4, and MSPL. Enteropeptidase is unique for this family, having multiple protein domains between the transmembrane domain and the catalytic domain, including a SEA domain, two CUB (C1s/C1r, urchin epidermal growth factor and bone morphogenetic protein 1) domains, two LDLA domains, a MAM (meprin, A5 protein, tyrosine phosphatase μ) domain, and a group A scavenger receptor domain (Fig. 8.1).

8.2.2.1 Enteropeptidase: Activation of Pancreatic Hydrolases by Converting Trypsinogen to Trypsin

As already mentioned enteropeptidase, originally named enterokinase, has been discovered in 1899 in the laboratory of Ivan Pavlov as an activity of extracts of small intestinal mucosa that was able to activate hydrolytic enzymes in pancreatic fluid. In 1939, Moses Kunitz demonstrated that purified porcine enteropeptidase converts crystalline trypsinogen to trypsin (Kunitz 1939). In the 1970s, purification of enteropeptidase from porcine, bovine, and human intestine revealed that it

consists of a heavy chain (82–140 kDa) and a light chain (35–62 kDa) connected by a disulfide bond. Edman degradation of the amino-terminal sequence of the light chain of bovine enteropeptidase revealed its homology to other trypsin-like proteases. However, only cloning of the enteropeptidase cDNA in 1994 revealed the presence of a membrane anchor near the amino-terminus and indicated that the active two-chain form is derived from a single-chain precursor (Kitamoto et al. 1994).

Enteropeptidase activity is almost exclusively localized to the duodenum. The enzyme is localized in the brush border of enterocytes and some goblet cells of the human duodenal mucosa and, at lower levels, in the mucosa of the proximal jejunum (Hermon-Taylor et al. 1977; Yuan et al. 1998). Enteropeptidase specifically cleaves trypsinogen at the activation site DDDDK↓I that is highly conserved among vertebrates (Zheng et al. 2009). Trypsin, in turn, activates a number of pancreatic zymogens such as chymotrypsinogen, proelastase, procarboxypeptidases, and prolipases in the lumen of the gut. Such unique sequence specificity is thought to provide an important mechanism to ensure that trypsinogen is activated only by enteropeptidase, but not by other proteases in pancreas, avoiding damage of the pancreas due to excess protease activities.

The cDNA sequences of enteropeptidase have been determined for several vertebrate species including cattle, swine, mouse, Japanese rice fish (medaka), and man (Zheng et al. 2009). The amino acid sequences of the human and bovine protease are 82% identical. In humans, enteropeptidase is encoded by the PRSS7 gene localized on chromosome 21q21 (Kitamoto et al. 1994, 1995). Enteropeptidase is synthesized as a zymogen of 1019 amino acids, which requires activation by another protease at K784 within the activation site sequence ITPK↓IVGG. Trypsin and the protease duodenase that is secreted by Brunner's glands of the proximal segment of duodenum have been shown to activate enteropeptidase (Zamolodchikova et al. 1997, 2000). However, duodenase is synthesized as a zymogen, too, and requires activation by another protease. Moreover, the measured rate of activation of purified bovine recombinant proenteropeptidase by duodenase was about 70-fold lower than that by trypsin (Zamolodchikova et al. 2000). Thus, the role for duodenase as primary activator of proenteropeptidase remains to be established.

The molecular basis of human enteropeptidase localization to the apical membrane is not yet completely understood. The transmembrane domain anchors enteropeptidase in the brush border of duodenal enterocytes. In addition, mucin-like repeats in the SEA domain and N-linked glycosylation of the catalytic domain have been found necessary for apical delivery in MDCK cells (Zheng and Sadler 2002; Zheng et al. 2009). In addition, enteropeptidase is present as a soluble form in the small intestinal lumen. Shedding may be due to the action of biliary or pancreatic proteases and possibly to local effects of gastrointestinal hormones (Götze et al. 1972). However, it remains unknown whether shedding of enteropeptidase plays a role in regulating its activity in the gut.

The enteropeptidase serine protease domain contains a basic tetrapeptide segment consisting of R/K96-R-R-K99, which is not conserved in other serine proteases (Matsushima et al. 1994; Kitamoto et al. 1994; Yuan et al. 1998). Computer modeling suggested that this basic segment is located on the protein surface where

it might bind the acidic P2–P5 residues of trypsinogen activation sites. The crystal structure of the bovine enteropeptidase catalytic domain in complex with the inhibitor V-$(D)_4$-K-chloromethane confirmed this extended substrate binding exosite (Lu et al. 1999). The K99 residue was identified as major determinant for recognition of the P2 and P4 aspartate residues. It is conserved among enteropeptidases of many species. Substitution of the lysine by alanine prevented enteropeptidase from cleaving trypsinogen (Lu et al. 1999). However, two-chain enteropeptidase cleaves trypsinogen about 500-fold more rapidly than does the isolated light chain (Lu et al. 1997), indicating that the heavy chain is necessary for optimal cleavage of trypsinogen.

The physiological importance of enteropeptidase is indicated by severe intestinal malabsorption in congenital deficiency of this enzyme. A number of cases of primary enterokinase deficiency have been reported since it was first described in 1969 (Hadorn et al. 1969). Patients suffer from severe intestinal malabsorption with diarrhea, vomiting, and growth failure in early infancy. Nonsense or frameshift mutations in the *PRSS7* gene have been shown in patients (Holzinger et al. 2002). Congenital enteropeptidase deficiency can be treated successfully by administration of pancreatic extract in early infancy (Zheng et al. 2009).

Because of its unique substrate specificity and protein stability, enteropeptidase is also of biotechnological interest. Introduction of the DDDDK↓I enterokinase recognition sequence is widely used as a tool to specifically cleave and activate recombinant proproteins or fusion proteins. More recently, enteropeptidase from the Japanese rice fish medaka has been cloned and characterized (Ogiwara and Takahashi 2007). The E173A mutant of medaka enteropeptidase showed an even stricter specificity for the DDDDK sequence compared to bovine enteropeptidase and may therefore provide the most appropriate protease to cleave recombinant proteins containing the DDDDK motif.

8.2.2.2 TMPRSS2: Prostate Cancer Progression and Proteolytic Activation of Influenza A Viruses

TMPRSS2 cDNA was originally cloned by exon trapping when the transcription map of human chromosome 21 was developed (Paoloni-Giacobino et al. 1997). The human gene is mapped to 21q22.3 and encodes for a protein of 492 amino acids. Murine TMPRSS2 is also designated as epitheliasin and is encoded on chromosome 16. The modular structure of TMPRSS2 is illustrated in Fig. 8.1.

TMPRSS2 is widely expressed in epithelial cells of the respiratory, gastrointestinal, and urogenital tract with high expression levels in the prostate and colon (Bugge et al. 2009; Bertram et al. 2012). Immunohistochemical studies revealed that TMPRSS2 is also expressed in cardiac myocytes (Bertram et al. 2012). TMPRSS2 is associated with prostate cancer. The protease has been shown to be overexpressed in prostate cancer tissue, and the TMPRSS2 level has been shown to be correlated with prostate cancer progression (Lucas et al. 2008, 2014; Chen et al. 2010). Moreover, fusion of the androgen-regulated TMPRSS2 promoter to E26 transformation-specific (ETS) transcription factor genes, particularly the ETS-regulated gene (ERG), resulting in overexpression of ERG is seen in nearly 50% of

patients and is used as a prognostic marker of prostate carcinogenesis (Tomlins et al. 2005; Gasi Tandefelt et al. 2014). The TMPRSS2 gene and the ERG gene are located approximately 3 Mb apart in the same relative orientation on chromosome 21. However, the role of these gene fusions in the development and progression of prostate cancer is not understood in detail. The TMPRSS2 gene is furthermore flanked by the Mx1 (myxovirus resistance 1) gene, encoding a GTPase that is part of the antiviral response induced by type I and type III interferons (Paoloni-Giacobino et al. 1997; Verhelst et al. 2013).

TMPRSS2 promoted prostate cancer cell invasion and metastasis to distant organs in a mouse model of prostate carcinogenesis (Lucas et al. 2014). TMPRSS2 has been shown to activate PAR-2 and hepatocyte growth factor (HGF)/c-Met signaling pathways and to downregulate E-cadherin expression in prostate cancer cells (Wilson et al. 2005; Lucas et al. 2014; Leshem et al. 2011). More recently, TMPRSS2 has been described to promote prostate cancer tumor growth and metastasis, via activation of the TTSP matriptase and degradation of extracellular matrix (ECM) laminin β1 and nidogen-1 in vitro and in a xenograft mouse model of prostate cancer (Ko et al. 2015). TMPRSS2 protein level was shown to correlate with increased levels of active matriptase as well as increased metastases (Ko et al. 2015).

The physiological role of TMPRSS2 is unknown so far. TMPRSS2-deficient mice lack a discernible phenotype (Kim et al. 2006). The protease has been shown to cleave the epithelial sodium channel (ENaC) in *Xenopus* oocytes in vitro and suggested to be involved in regulation of the airway surface liquid (ASL) volume by proteolytic cleavage of ENaC (Donaldson et al. 2002). Interestingly, by using TMPRSS2-deficient mice, three recent studies identified TMPRSS2 as a host cell factor essential for activation and spread of H1N1 and H7N9 influenza A viruses in mice (see below). Intriguingly, knockout of TMPRSS2 expression protected mice from an otherwise lethal infection due to inhibition of activation of progeny virus and, thus, spread along the respiratory tract (Hatesuer et al. 2013; Tarnow et al. 2014; Sakai et al. 2014).

TTSPs are believed to be situated at the cell surface, and shedding of TMPRSS2 has been described from prostate and prostate cancer cells and from human nasal epithelial cells exposed to ozone in vitro (Afar et al. 2001; Kesic et al. 2012). Studies on the subcellular localization of influenza A virus HA cleavage by TMPRSS2 upon co-expression in MDCK cells, however, indicate that TMPRSS2 cleaves HA in the trans-Golgi network (TGN) or during its transport to the plasma membrane, where virus assembly and budding take place (Böttcher et al. 2009; Böttcher-Friebertshäuser et al. 2010, 2013) (see Sect. 8.5). In contrast, TMPRSS2 present on the cell surface and soluble TMPRSS2 shed from MDCK cells showed poor if any enzymatic activity and were not able to cleave HA (Böttcher-Friebertshäuser et al. 2010). The reason for the lack of TMPRSS2 activity on the cell surface and in cell supernatants is unknown and might be related to the expression of protease inhibitors or missing cofactors. However, intracellular activation of HA by TMPRSS2 revealed that TTSPs may not only act as proteolytic enzymes on the cell surface but can process their substrates also (or already) in intracellular compartments.

8.2.2.3 Dysregulation of TMPRSS3, TMPRSS5/Spinesin, and Hepsin Is Associated with Deafness

TMPRSS3 was first identified as a novel multi-domain serine protease overexpressed in ovarian cancers and therefore originally named tumor-associated differentially expressed gene 12 (TADG-12) (Underwood et al. 2000). Independently, mutations in the TMPRSS3 gene were associated with congenital and childhood onset autosomal recessive deafness (Scott et al. 2001). This was the first description of a protease involved in hearing loss. The TMPRSS3 gene maps on chromosome 21 at q22 and encodes for a protein of 454 amino acids with an overall domain structure similar to TMPRSS2 and TMPRSS4. TMPRSS3 is synthesized as a zymogen and undergoes autoactivation (Guipponi et al. 2002). RT-PCR analysis and RNA in situ hybridization experiments revealed expression of TMPRSS3 in the thymus, stomach, testis, ovary, kidney, and eye and in a variety of inner ear tissues, including inner hair cells, stria vascularis, spiral ganglion neurons, modiolus, and organ of Corti (Scott et al. 2001; Guipponi et al. 2002, 2008).

A number of different mutations in the TMPRSS3 gene have been identified in patients with non-syndromic autosomal recessive deafness (DFNB8/10) (Scott et al. 2001; Ben-Yosef et al. 2001; Masmoudi et al. 2001, Guipponi et al. 2008). Mutations occur in all functional domains and have been shown to disrupt the proteolytic activity of TMPRSS3, indicating that TMPRSS3 protease activity is critical during inner ear development (Guipponi et al. 2002; Wattenhofer et al. 2005). It remains to be elucidated how missense mutations in the LDLA and SRCR domains affect the proteolytic activity of TMPRSS3. Studies in a mouse model carrying a protein-truncating nonsense mutation in TMPRSS3, Y260X (X = stop codon), revealed that TMPRSS3 is essential for mouse cochlear hair cell survival at the onset of hearing (Fasquelle et al. 2011). Mice expressing TMPRSS3-Y260X are completely deaf due to rapid and massive degeneration of hair cells. Moreover, loss of spiral ganglion neurons was observed in TMPRSS3-Y260X mice at the age of 4 months (Fasquelle et al. 2011). Proteomic analyses revealed that TMPRSS3 deficiency leads to a decrease in the expression of Kcnma1 potassium channels in inner hair cells (Molina et al. 2013). However, it remains to be investigated in more detail how TMPRSS3 regulates the abundance of functional Kcnma1 channels expression in hair cells. A recent study by Li et al. demonstrated that knockdown of TMPRSS3 inhibited cell viability of spiral ganglion neurons in vitro (Li et al. 2014). Moreover, they observed that microRNA miR-204 suppressed spiral ganglion neuron survival in vitro by targeting TMPRSS3 (Li et al. 2014). TMPRSS3 mRNA was found to have a putative miR-204 binding site within its 3′-UTR that is highly conserved among the vertebrates.

In addition to its role in hearing, TMPRSS3 was found to be overexpressed in pancreatic and ovarian cancer and to promote proliferation, invasion, and migration of ovarian cancer cells via activation of the ERK1/2 pathway in vitro (Wallrapp et al. 2000, Zhang et al. 2016).

Hepsin was identified in cDNA clones obtained from human liver and was the first serine protease characterized to contain a transmembrane domain

(Leytus et al. 1988). Hepsin is abundant in the liver. Other tissues such as kidney, pancreas, lung, stomach, prostate, and thyroid express low levels of hepsin mRNA (Tsuji et al. 1991). The human hepsin gene has been localized to chromosome 19q11-13.2. Hepsin consists of an N-terminal cytoplasmic domain, a transmembrane domain, and an extracellular portion composed of a SRCR domain and a C-terminal protease domain. The crystal structure of a soluble human hepsin that included the SRCR and protease domains has been solved (Somoza et al. 2003).

A number of potential hepsin substrates have been identified in vitro including blood clotting factors VII, IX, and XII, prourokinase, promatriptase, proprostasin, macrophage-stimulating protein (MSP), and laminin-332 (reviewed in Antalis et al. 2011), suggesting that hepsin may play a role in blood coagulation and embryonic development. The evidence for these functions, however, remains inconclusive since hepsin-deficient mice are viable and fertile, and do not exhibit obvious defects in growth and blood coagulation (Wu et al. 1998; Brunati et al. 2015). Unexpectedly, a study by Guipponi et al. found that hepsin-deficient mice exhibited profound hearing loss (Guipponi et al. 2007). Hepsin knockout mice have abnormal cochlea and reduced myelin protein expression in the auditory nerve (Guipponi et al. 2007). Furthermore, low levels of plasma thyroxine, a thyroid secreted hormone important for cochlear development, have been found in hepsin-deficient mice (Hanifa et al. 2010). The molecular mechanisms by which hepsin regulates normal hearing are not understood, and so far it is unknown if hearing loss in hepsin-knockout mice is a result of thyroid hormone deficiency.

Further studies of knockout mouse models of hepsin demonstrated that the protease activates pro-hepatocyte growth factor (pro-HGF) in the liver and is responsible for cleavage and urinary secretion of uromodulin (Tamm-Horsfall protein) (Hsu et al. 2012; Brunati et al. 2015). It was proposed that HGF/c-Met signaling may regulate expression of connexins, gap junction proteins, in hepatocytes in mice. Loss of hepsin was found to increase expression of connexins, resulting in an expansion of hepatocyte size and a concomitant narrowing of sinusoids. Interestingly, systemic delivery of tumor cells by tail-vein injection showed preferential colonization of tumor cells in the liver of hepsin-deficient mice compared to wild-type mice. These data suggest that loss of hepsin enhances the colonization of liver by tumor cells, probably through increased retention of tumor cells because of narrower sinusoids related to enlarged hepatocytes.

Hepsin has been identified as one of the most upregulated genes in prostate cancer. Hepsin increases early in prostate cancer initiation, and its high levels are maintained throughout progression and metastasis and are indicative of poor outcome (Dhanasekaran et al. 2001; Stephan et al. 2004). Overexpression of hepsin has also been shown in many other cancers including breast, ovarian, and endometrial cancer (Murray et al. 2016).

Although a number of studies demonstrated that hepsin is involved in prostate cancer progression, little is known about the basis of its functions. Overexpression of hepsin in a mouse model of non-metastasizing prostate cancer caused disorganization and disruption of basement membrane and promoted primary prostate cancer progression and metastasis to the liver, lung, and bone (Klezovitch et al. 2004).

Overexpression of hepsin also promoted ovarian tumor growth in a mouse model, and proteolytic activity of hepsin was shown to be necessary for promoting tumor progression (Miao et al. 2008). Hepsin and HGF are present in desmosomes. Desmosome dissociation is known to be one of the first steps during HGF-induced epithelial-mesenchymal transition, indicating that increased levels of hepsin and its substrate HGF may play a role in ovarian cancer progression through their interaction with desmosomes (Miao et al. 2008). At present, the mechanism of this interaction and the functional importance of hepsin localization in the desmosomes warrant further investigation.

TMPRSS5/spinesin was cloned from a human spinal cord cDNA library (Yamaguchi et al. 2002). The human TMPRSS5 gene is located on chromosome 11q23 and encodes a simple TTSP of 457 amino acids consisting of a short cytoplasmic domain, a transmembrane domain, a stem region containing a SRCR domain, and a C-terminal serine protease domain (Yamaguchi et al. 2002). Northern blot analyses and immunohistochemical staining revealed that TMPRSS5 is predominantly expressed in the brain and the spinal cord. Guipponi et al. demonstrated that TMPRSS5-specific mRNA is furthermore present in inner ear tissues and the testis of rats (Guipponi et al. 2008). A mutation screen in a cohort of ca. 360 sporadic deafness cases revealed three mutations in the TMPRSS5 gene (A317L, F369L, and Y438X). These TMPRSS5 mutants showed reduced (F369L) or no (A317S, Y438X) proteolytic activity in yeast-based protease assays, suggesting that impaired TMPRSS5 activity might cause hearing impairment. However, TMPRSS5/spinesin has not been characterized in more detail, and it remains to be investigated how it affects hearing and which role it may play in the central nervous system.

8.2.2.4 TMPRSS4: An Emerging Potential Therapeutic Target in Cancer

TMPRSS4 (also known as channel-activating protease 2 (CAP2)) was originally identified as a gene expressed in most pancreatic tumors but not in the healthy pancreas (Wallrapp et al. 2000). Meanwhile, TMPRSS4 has been shown to be highly expressed also in many other cancers including thyroid, colon, gastric, and lung cancers; association with poor prognosis has been consistently described (reviewed in Kim and Lee 2014; Tanabe and List 2017).

The TMPRSS4 gene is located on chromosome 11 at q23.3 and encodes a protein of ~437 amino acids. The domain structure of TMPRSS4 is similar to TMPRSS2 and TMPRSS3. TMPRSS4 undergoes autoactivation in vitro (Antalis et al. 2011). The catalytic domain of TMPRSS4 can be released as an active form in cell culture (Min et al. 2014a). TMPRSS4 mRNA was detected in the bladder, esophagus, stomach, small intestine, colon, kidney, larynx, trachea, bronchi, and lung (Wallrapp et al. 2000; Jung et al. 2008; Böttcher-Friebertshäuser unpublished data). In murine lung TMPRSS4 is expressed in type II pneumocytes (Kühn et al. 2016).

Overexpression of TMPRSS4 has been shown to promote invasion and metastasis of human tumor cells by facilitating epithelial-mesenchymal transition (EMT) (Kim et al. 2010; Jung et al. 2008; Cheng et al. 2009). Knockdown of TMPRSS4

expression in lung and colon cancer cells by using siRNA reduced cell proliferation and invasion (Jung et al. 2008). A number of mechanisms, by which TMPRSS4 may modulate tumor cell proliferation and invasion, have been described in vitro. Overexpression of TMPRSS4 in colon cancer cells promoted EMT through the upregulation of integrin α5, thereby enhancing motility and invasiveness (Kim et al. 2010). In addition, TMPRSS4 was shown to induce invasion through upregulation of both expression and activity of urokinase plasminogen activator (uPA) via activation of the transcription factors AP-1, Sp1, and Sp3 and via processing of pro-uPA precursor into its active form in vitro (Min et al. 2014a, b). The serine protease uPA converts inactive plasminogen to active plasmin, which in turn can degrade most extracellular proteins and activate MMPs. Moreover, uPA and its receptor, uPAR (CD87), interact with integrin coreceptors to activate intracellular signaling pathways for cell migration, invasion, proliferation, and survival (reviewed in Hildenbrand et al. 2008). It has been shown that TMPRSS4 can interact with uPAR (Min et al. 2014b). Furthermore, TMPRSS4 has been shown to induce downregulation of E-cadherin, a well-known hallmark of EMT, via activation of transcriptional repressors Sip1/Zeb2 (Jung et al. 2008). Increased TMPRSS4 expression in cancer could be partially due to epigenetic dysregulation. The TMPRSS4 promoter has been shown to be hypomethylated in hepatocellular carcinoma and non-small cell lung cancer (Stefanska et al. 2011; Villalba et al. 2016). Hypomethylation of TMPRSS4 promoter was associated with worse prognosis in non-small cell lung cancer patients. Taken together, TMPRSS4 may be an important upstream regulator of the EMT and the invasiveness of cancer cells and a useful biomarker for the prognosis of certain types of cancers and could be employed for diagnostics and therapeutics.

Mice deficient in TMPRSS4 are viable, fertile, and do not show any obvious abnormalities (Keppner et al. 2015; Kühn et al. 2016). However, so far no cancer studies have been performed in TMPRSS4-deficient mice. Like TMPRSS2, TMPRSS4 has been shown to be able to activate the epithelial sodium channel (ENaC) when co-expressed in *Xenopus* oocytes (Vuagniaux et al. 2002). TMPRSS4 cleaves the gamma subunit of ENaC at a site distinct from the site that is processed by the GPI-anchored protease prostasin (cf. prostasin chapter). However, a recent study demonstrated that regulation of the ENaC-mediated sodium balance is not affected in TMPRSS4-deficient mice (Keppner et al. 2015), indicating that TMPRSS4 is not crucial for processing of ENaC in vivo.

8.2.2.5 TMPRSS13/MSPL: Preferential Recognition of Paired Basic Residues at the Cleavage Site

TMPRSS13 (transmembrane protease, serine 13, also known as mosaic serine protease large-form (MSPL)) was isolated in 2001 from a human lung cDNA library. The human TMPRSS13 is located on chromosome 11q23.2. Alternatively spliced forms from this gene have been identified, one encoding a type II transmembrane protease (MSPL) and a second form encoding a protease without a transmembrane domain (mosaic serine protease short-form (MSPS) that comprises 581 and 537 amino acids, respectively (Kim et al. 2001). RT-PCR analysis revealed that TMPRSS13-specific

mRNA is present in various human tissues including lung, liver, kidney, spleen, pancreas, small intestine, prostate, and placenta (Kido et al. 2008).

The biological functions of TMPRSS13 are not well understood. TMPRSS13-deficient mice display abnormal skin development, leading to a compromised barrier function (Madsen et al. 2014). Interestingly, tight junction formation and profilaggrin processing were not affected in TMPRSS13-deficient mice. Thus, TMPRSS13 supports stratum corneum formation and epidermal barrier formation by a mechanism that is independent of both profilaggrin processing and tight junction formation (see below).

TMPRSS13 shows unique substrate specificity among TTSPs, with preferential recognition of paired basic residues (R/K at P1 and P2 positions) (Kido et al. 2008). TMPRSS13 also efficiently cleaved peptide substrates with K at position P4 that were not cleaved by furin and has been shown to activate the HA of highly pathogenic avian influenza viruses of subtype H5N2 possessing this motif at the cleavage site (Okumura et al. 2010). TMPRSS13-specific mRNA was detected in the blood vessels, lungs, trachea, colon, small intestine, and kidney of chickens.

8.2.3 Matriptase Family

The matriptase family has four members: matriptase, matriptase-2, matriptase-3, and polyserase-1. The matriptases have a SEA domain, two CUB domains, and three or four LDLA domains in their stem region. Polyserase-1 is unique among serine proteases, having one enzymatically inactive and two active serine protease domains (Fig. 8.1).

8.2.3.1 Matriptase: Crucial Roles in Epidermal Differentiation and Tight Junction Formation

Matriptase (also known as membrane-type serine protease 1 (MT-SP1), epithin, suppressor of tumorigenicity 14 (ST14), channel-activating protease 3 (CAP3)) was originally identified in 1993 as a new gelatinolytic activity in conditioned medium from cultured breast cancer cells (Shi et al. 1993). Matriptase is encoded by the *ST14* gene located on human chromosome 11q24–25 and encodes a polypeptide of 855 amino acids with a molecular weight of 95 kDa (Lin et al. 1999). Orthologs of matriptase are present in all vertebrate genomes examined to date (review Miller and List 2013).

Matriptase shows the most ubiquitous expression pattern of TTSPs being expressed in epithelial cells of most embryonic and adult tissues (Miller and List 2013). Matriptase has been shown to be required for postnatal survival in mice and has essential physiological functions in terminal differentiation of the oral and intestinal epithelium and the epidermis (List et al. 2002, 2003; Buzza et al. 2010). Mice deficient in matriptase die within 48 h after birth due to a severe dehydration resulting from an impaired epidermal barrier function. Matriptase is also critical for hair follicle growth and thymic development (List et al. 2002, 2003). Interestingly, it was observed that matriptase-deficient mice and mice deficient in expression of the GPI-anchored serine protease prostasin display identical epidermal phenotypes

and that prostasin zymogen activation by matriptase is a key function in epidermal development (List et al. 2002, 2003; Netzel-Arnett et al. 2006; Leyvraz et al. 2005; also see prostasin chapter). These observations led to the hypothesis that matriptase may be part of a matriptase-prostasin proteolytic cascade in the epidermis (Netzel-Arnett et al. 2006). Moreover, this cascade has been shown to regulate different steps in terminal epidermal differentiation: tight junction formation, profilaggrin processing, epidermal lipid synthesis, and induction of desquamation through promoting expression or activation of kallikrein-related peptidases (KLKs) (for review see Szabo and Bugge 2011). It remains to be analyzed in more detail which target substrates of the matriptase-prostasin proteolytic cascade are involved in the different steps of epidermal differentiation. A number of studies during the last years, however, revealed that the matriptase-prostasin cascade is much more complex. While matriptase activates prostasin zymogen in the epidermis, prostasin is crucial for matriptase zymogen activation in the intestine and the placenta (Buzza et al. 2013; Szabo et al. 2016; cf. prostasin chapter).

Activity of matriptase during development is controlled by the transmembrane inhibitors hepatocyte growth factor activator inhibitor 1 (HAI-1) and HAI-2. Loss of HAI-1 or HAI-2 in mice results in embryonic lethality (Szabo et al. 2009; Tanaka et al. 2005). HAI-1 is essential for placental differentiation and overall embryonic and postnatal survival in mice (Nagaike et al. 2008; Szabo et al. 2007; Tanaka et al. 2005). Loss of HAI-2 is associated with defects in neural tube closure in mice (Szabo et al. 2009, 2012). Remarkably, all developmental defects in HAI-1- or HAI-2-deficient mice are rescued in whole or in part by reducing or eliminating the expression of matriptase (Szabo et al. 2009, 2012). These observations indicate that matriptase is not essential for placental development in mice but that its activity in the placenta needs to be regulated.

Activation of the single-chain matriptase zymogen is very complex requiring two sequential proteolytic processing events and the transient interaction with its cognate inhibitors HAI-1 and HAI-2 (Oberst et al. 2003, 2005; Nonboe et al. 2017). The first cleavage occurs after G149 in the SEA domain, releasing the enzyme from its transmembrane anchor. However, matriptase remains membrane-bound due to interactions with the cleaved domain and/or with HAI-1 and HAI-2. The second cleavage, which appears to be autocatalytic, occurs after R614 within the highly conserved R↓VVGG activation motif, converting the single-chain form into the active two-chain form. Co-expression of HAI-1 and HAI-2 has been shown to be necessary for matriptase expression, stability, and intracellular trafficking in vitro (Oberst et al. 2005; Larsen et al. 2013; Nonboe et al. 2017). Matriptase can be shed from the cell surface. Shed matriptase was identified originally in complex with an inhibitor HAI-I in human milk, and additional shed forms have been reported in conditioned media of cultured epithelial cell lines (reviewed in List et al. 2006a).

Apart from its physiological roles, matriptase has been extensively studied in the context of tumor progression. Expression of matriptase is upregulated in a variety of epithelial cancers including breast, prostate, ovarian, cervical, gastric, colon, renal cell, esophageal, and oral squamous cell carcinoma (reviewed in Tanabe and List 2017). List et al. demonstrated that matriptase causes strong proliferation of keratinocytes and formation of squamous cell carcinomas when only modestly

overexpressed in the epidermis of transgenic mice (List et al. 2006b). Matriptase may promote tumor growth, invasion, and metastasis by converting pro-HGF and pro-uPA into its active forms, by degrading ECM components, or by activating PAR-2. Activation of matriptase by androgen-induced TMPRSS2 has been shown to promote prostate cancer tumor growth and metastasis in vitro and in a xenograft mouse model of prostate cancer (Ko et al. 2015).

Matriptase has furthermore been linked to other pathological processes. Impaired matriptase proteolytic activity due to mutations in the *ST14* gene was linked to a rare form of skin disease named autosomal recessive ichthyosis syndrome with hypotrichosis (ARIH) (condition of sparse hair) (List et al. 2007; reviewed in Antalis et al. 2011). Hypomorphic mice with 100-fold reduced expression levels of matriptase mRNA have been shown to phenocopy the key features of ARIH (List et al. 2007). Moreover, expression of matriptase is elevated in osteoarthritis, and the protease was identified as initiator of cartilage matrix degradation in osteoarthritis (Milner et al. 2010). In contrast, matriptase expression is significantly downregulated in intestinal tissues of patients with Crohn disease and ulcerative colitis (Netzel-Arnett et al. 2012).

8.2.3.2 TMPRSS6/Matriptase-2: Maintenance of Systemic Iron Homeostasis

TMPRSS6/matriptase-2 is composed of a transmembrane domain, followed by a SEA domain, two CUB domains, three LDLR, and a C-terminal trypsin-like serine protease domain (Wang et al. 2014). Matriptase-2 shares high structural and enzymatic similarities with matriptase, which contains four LDLR repeats instead of three. The matriptase-2 zymogen (90 kDa) undergoes autocatalytic cleavage at R567 within the R↓IVGG activation motif and remains membrane-anchored through a disulfide bond linking the pro- and catalytic domains (Stirnberg et al. 2010). Soluble forms of matriptase-2 were detected in the conditioned medium of transfected cells. Interestingly, shedding of matriptase-2 has been found to be due to cleavage at R404 and/or R437 within the second CUB domain and seems to be required for converting matriptase-2 into the active form via cleavage at R567. Activation of matriptase-2 was prevented in cells expressing matriptase-2 mutant R404E/R437E, which cannot be shed (Stirnberg et al. 2010).

TMPRSS6/matriptase-2 is expressed predominantly in the liver. TMPRSS6/matriptase-2-specific mRNA has been also detected to a lower extent in the kidney, spleen, lung, brain, mammary gland, testis, and uterus (reviewed in Ramsay et al. 2009; Wang et al. 2014).

TMPRSS6/matriptase-2 plays a key role in the maintenance of systemic iron homeostasis. Systemic iron homeostasis is maintained by regulating the iron absorption in the duodenum, by recycling of iron from senescent erythrocytes in macrophages, and by mobilizing stored iron in the liver. Increases in iron levels stimulate the production of the hepatic hormone hepcidin, which blocks iron export into the circulation by binding to and targeting the iron exporter ferroportin on the plasma membrane of duodenal enterocytes, macrophages, and hepatocytes for degradation. Hepcidin production is suppressed in the case of iron deficiency (reviewed in Ganz

and Nemeth 2012 and Wang et al. 2014). Therefore, hepcidin is a key regulator of intestinal iron absorption, plasma iron concentrations, and tissue iron distribution. Lack of hepcidin causes juvenile hemochromatosis, a particularly severe form of iron overload disorder. In contrast, inappropriately high levels of hepcidin cause chronic inhibition of iron absorption and consequent anemia (Ganz and Nemeth 2012; Wang et al. 2014).

Mutations in the TMPRSS6 gene were found to cause increased hepcidin expression, which leads to iron-refractory iron-deficiency anemia (IRIDA) (Finberg et al. 2008). Similar phenotypes were also observed in mouse models deficient in TMPRSS6/matriptase-2 expression or with expression of a truncated protease form that lacks the catalytic domain (Du et al. 2008). Thus, results in mice and humans indicated that TMPRSS6/matriptase-2 is required to sense iron deficiency in mammals. Silvestri et al. demonstrated that TMPRSS6/matriptase-2 negatively regulates expression of hepcidin by cleaving the GPI-anchored protein hemojuvelin from the plasma membrane (Silvestri et al. 2008). Hemojuvelin is a coreceptor in the bone morphogenetic protein (BMP)/SMAD signaling pathway that upregulates hepcidin in response to increased iron (Babitt et al. 2006; Finberg et al. 2010). Cleavage of hemojuvelin by TMPRSS6/matriptase-2 downregulates BMP/SMAD signaling and inhibits hepcidin expression.

Studies have shown that TMPRSS6/matriptase-2 expression can be modulated by iron status. The underlying mechanism, however, is not fully understood. Beliveau et al. found that TMPRSS6/matriptase-2 is constitutively internalized from the plasma membrane in cell culture due to motifs within its cytoplasmic tail (Béliveau et al. 2011). Internalized TMPRSS6/matriptase-2 was detected in LAMP-2-labeled vesicles, suggesting that the protease transits to lysosomes, where it is degraded. However, it is still not clear whether this mechanism of protein degradation regulates TMPRSS6/matriptase-2 expression depending on the iron status.

8.2.3.3 Matriptase-3

Matriptase-3 was identified by bioinformatic analysis in 2005 (Szabo et al. 2005). The TMPRSS7 gene encoding matriptase-3 is located on human chromosome 3q13.2 and encodes a N-glycosylated TTSP of ca. 90 kDa expressed on the cell surface in vitro. Orthologs of the matriptase-3 gene are present in all vertebrates analyzed to date, including chimpanzee, dog, rodents, chicken, and fish (Szabo et al. 2005). In human tissues, matriptase-3 mRNA has been detected in the testis, ovary, brain, salivary gland, lung, and trachea (Bugge et al. 2009). The generation of matriptase-3-deficient mice has not been reported so far, and the physiological substrates and function of matriptase-3 are unknown.

8.2.3.4 Polyserase-1

Polyserase-1 (polyserine protease 1, also named TMPRSS9) is a unique TTSP with three tandem serine protease domains, of which two display catalytic activity (Cal et al. 2003). Polyserase-1 was originally cloned from human liver cDNA. The protease is widely expressed in mouse and human tissues. In addition, a shorter splice

variant, termed serase-1B, which contains only the first of the three serine protease domains of polyserase-1, has been described in mice and humans, with its highest expression detected in liver, small intestine, pancreas, testes, and peripheral blood CD14+ and CD8+ cells (Okumura et al. 2006). The putative functional advantages derived from the complex structural organization of polyserase-1 and its functional significance remain unknown in both normal and pathological conditions.

8.2.4 Corin Family

8.2.4.1 Corin: The Pro-Atrial Natriuretic Peptide (pro-ANP) Activating Enzyme

Corin is the only member of the corin family and has a complex stem region (Fig. 8.1). At the turn of the millennium, corin was identified as a serine protease in the heart and as the physiological activator of atrial natriuretic peptide (ANP), also called ANF (atrial natriuretic factor) (Yan et al. 1999, 2000). ANP is an important hormone that regulates blood pressure and cardiac function by promoting natriuresis, diuresis, and vasodilation (Li et al. 2017, review). In cardiomyocytes, ANP is synthesized as a precursor protein pro-ANP that is stored in intracellular granules and converted to active ANP by corin upon secretion in response to high blood volume or pressure.

The human corin gene (*TMPRSS10*) located on chromosome 4p12–13 consists of 22 exons and spans ca. 200 kb, making it one of the largest protease genes in the human genome. Human corin consists of 1042 amino acids and includes an N-terminal cytoplasmic tail, a transmembrane domain, and an extracellular region that contains two frizzled domains, eight LDLA repeats, a SRCR domain, and a C-terminal serine protease domain (Li et al. 2017). Corin is the only serine protease containing frizzled-like domains. Corin zymogen is activated by cleavage at a conserved site between Arg801 and Ile802. In 2015, Chen et al. identified the proprotein convertase paired basic amino acid-cleaving enzyme 4 (PACE4), also designated as proprotein convertase subtilisin/kexin 6 (PCSK6), as the long-sought physiological activator of corin (Chen et al. 2015). Co-expression of PACE4 and corin enhanced corin activation in HEK293 cells. Moreover, knockout of PACE4 expression in mice led to impaired corin activation, decreased pro-ANP processing, and development of salt-sensitive hypertension.

Corin is extensively N-glycosylated (Liao et al. 2007; Gladysheva et al. 2008; Wang et al. 2015). Human corin has a predicted molecular mass of 116 kDa; however, native and recombinant corin appears as a protein of 200 kDa. Human and mouse corin have 19 and 16 predicted N-glycosylation sites in its extracellular domains, respectively. It has been shown that N-glycosylation at Asn697 in the SRCR domain and Asn1022 in the protease domain are required for corin cell surface expression and zymogen activation.

Corin is primarily expressed in cardiomyocytes. Furthermore, corin has been detected in the kidney, blood, and urine. The protease is shed from the cell surface

of transient corin expressing HEK293 cells and from the surface of cardiomyocytes as three distinct soluble fragments of 180, 160, and 100 kDa, respectively, which represent activated two-chain forms linked by a disulfide bond containing the 40 kDa catalytic domain (Jiang et al. 2011). The metalloproteinase ADAM10 was shown to cleave corin in its juxtamembrane region to release the 180-kDa fragment, corresponding to the nearly entire extracellular region. In contrast, the 160- and 100-kDa fragments were generated by corin autocleavage at Arg164 in frizzled domain 1 and Arg427 in LDLA domain 5, respectively. Further studies revealed that the 180-kDa fragment exhibited the biological activity in processing pro-ANP, whereas the two other fragments had little activity (Li et al. 2017).

The presence of soluble corin in human blood indicates that shed corin can enter the circulation. Remarkably, corin remains active in the presence of human plasma, indicating that circulating protease inhibitors do not block corin activity. To date, no physiological corin inhibitors have been identified.

The importance of corin in regulating blood pressure has been shown in corin-deficient mice, which exhibited reduced sodium excretion and salt-sensitive hypertension due to impaired pro-ANP processing (Chan et al. 2005). Reduced corin expression has been detected in animal models of cardiomyopathies. Furthermore, decreased levels of circulating corin have been reported in patients with hypertension, pre-eclampsia, and cardiovascular diseases including acute myocardial infarction, heart failure, and stroke. Mutations in the corin gene that result in defects in intracellular trafficking of the protease, cell surface expression, and zymogen activation have been found in these patients (reviewed in Li et al. 2017). Moreover, a PCSK6 mutation with impaired corin activation has been identified in a hypertensive patient (Chen et al. 2015). Latest studies show that plasma corin concentrations provide a valuable prognostic marker for risk stratification of patients with acute myocardial infarction (AMI) and low levels of circulating corin are related with poor clinical outcomes (Zhou et al. 2016).

In addition to ANP, the mammalian natriuretic peptide family comprises two additional members: BNP (B-type or brain natriuretic peptide) and CNP (C-type natriuretic peptide). ANP and BNP are primarily expressed in the heart, while CNP is of endothelial origin and present in various tissues. pro-CNP is converted to active CNP by furin (Wu et al. 2003). It has been shown that both corin and furin cleave pro-BNP in vitro (Semenov et al. 2010). However, pro-BNP processing was not abolished in corin-deficient mice, indicating that corin is not essential for pro-BNP cleavage in vivo (Chen et al. 2015).

Corin expression has also been detected in noncardiac tissues including kidney and in human urine (Fang et al. 2013). In rat models of kidney disease, reduced renal corin expression was associated with sodium retention (Polzin et al. 2010). Latest studies show reduced urinary and renal corin levels in patients with chronic kidney disease (Fang et al. 2013). Further studies are necessary to fully understand the role of corin in regulating renal function and sodium homeostasis.

Curiously, corin-deficient mice appear to have a lighter coat color (dirty blond), and this phenotype depends on the agouti gene (Enshell-Seijffers et al. 2008).

In summary, corin is a key enzyme in the natriuretic peptide system, and corin defects may contribute to major diseases such as hypertension, heart failure, pre-eclampsia, and kidney disease.

8.3 Type I Transmembrane Serine Proteases

8.3.1 Tryptase Gamma 1

Tryptase gamma 1 (also known as transmembrane tryptase (TMT) and protease serine member S31 (PRSS31)) was identified in 1999 as a tryptase present in human and mouse mast cells that differs from all other known tryptases, which are soluble proteases, by containing a C-terminal transmembrane domain (type I transmembrane serine protease) (Wong et al. 1999). The physiological role(s) of tryptase gamma 1 are unknown. PRSS31-deficient mice exhibit no obvious developmental abnormalities but show markedly reduced experimental chronic obstructive pulmonary disease (COPD) and colitis compared to wild-type littermates, indicating a role of tryptase gamma 1 in mast cell-dependent inflammatory diseases (Hansbro et al. 2014).

8.4 GPI-Anchored Serine Proteases

8.4.1 Prostasin: Proteolytic and Non-Proteolytic Functions in Epithelial Development and Tissue Homeostasis

Prostasin was purified and characterized as an active soluble protease from human seminal fluid in 1994 (Yu et al. 1994). The protease is expressed in a variety of epithelial tissues with high expression in the prostate, bronchus, lung, and kidney in mouse and human. Prostasin is also known as channel-activating protease (CAP)-1 and was the first membrane serine protease found to activate the epithelial sodium channel (ENaC) (Vallet et al. 1997).

The *PRSS8* gene encoding prostasin is conserved in all vertebrate species examined. In humans, the *PRSS8* gene is located on chromosome 16p11.2 and encodes a protein of 343 amino acids. Prostasin is GPI-anchored in the cell surface and associates with lipid rafts (Chen et al. 2001b; Verghese et al. 2006). In polarized cells, prostasin is present on the apical membrane. Using substrate libraries, prostasin was shown to have a preference for polybasic substrates with R/K in P4, H/K/R in P3, basic or large hydrophobic amino acids in P2, and R/K in P1 (Shipway et al. 2004). No activity was seen with substrates containing isoleucine in P1′, providing an explanation for prostasin being not capable of undergoing autoactivation (Shipway et al. 2004). Prostasin zymogen conversion in the epidermis requires matriptase (Netzel-Arnett et al. 2006; cf. matriptase chapter). The crystal structure of the catalytic domain of prostasin has been solved (Rickert et al. 2008). Prostasin has been shown to be an essential regulator of the ENaC and thereby regulates the

homeostasis of extracellular fluid volume, blood pressure, and intestinal sodium and water absorption (Frateschi et al. 2012; Planès et al. 2010). Prostasin cleaves the ENaC gamma subunit at K186 within the cleavage motif RKRK↓ distal to a furin cleavage site at R144 (RKRR↓) (Bruns et al. 2007). Dual cleavage of the gamma subunit releases a 43-amino acid inhibitory peptide and causes full activation of the channel, resulting in an increased cellular uptake of Na^+. Studies show that prostasin is highly expressed in cystic fibrosis airways and is a strong basal activator of ENaC in cystic fibrosis airway epithelial cells (Donaldson et al. 2002; Tong et al. 2004). Increased levels of soluble prostasin are also found in urine of hypertensive patients (Narikiyo et al. 2002). Prostasin may be released from the cell surface by an endogenous GPI-specific phospholipase D1 or via cleavage in its C-terminal hydrophobic domain (Yu et al. 1994; Verghese et al. 2006). Soluble prostasin purified from human seminal fluid terminates at R323 (Yu et al. 1994). On the other hand, prostasin expression is reduced in a number of cancers including prostate, breast, and colorectal cancers, and prostasin has been shown to inhibit prostate and breast cancer cell invasion in vitro (Chen et al. 2001a; Chen and Chai 2002; Bao et al. 2016).

Prostasin-deficient mice display impaired epidermal barrier function, abnormal hair follicle maturation, impaired profilaggrin processing, defects in tight junction formation, and fatal dehydration (Leyvraz et al. 2005; Netzel-Arnett et al. 2006; Szabo et al. 2016). Moreover, constitutive knockout of prostasin leads to embryonic lethality due to placental insufficiency (Hummler et al. 2013). The identical phenotypes of matriptase- and prostasin-deficient mice suggested that both proteases are components of one proteolytic cascade (reviewed in Netzel-Arnett et al. 2006; cf. matriptase chapter). This hypothesis, however, proved incompatible with studies demonstrating that prostasin acts upstream of matriptase in intestinal epithelial cells and in the placenta through matriptase zymogen activation (Buzza et al. 2013; Szabo et al. 2016). Thus, the prostasin-matriptase cascade turned out to be more complex than previously thought.

Paradoxically, a number of studies revealed that prostasin requires neither zymogen conversion nor catalytic activity to execute its essential functions in the epidermal development (Peters et al. 2014; Friis et al. 2016). Already in 2006/2007, studies by Andreasen et al. and Bruns et al. reported that catalytically inactive prostasin mutant S238A was able to activate ENaC in *Xenopus* oocytes in vitro, indicating that the catalytic activity of prostasin appears to be dispensable for cleavage of the ENaC gamma subunit (Andreasen et al. 2006; Bruns et al. 2007). But cell surface expression of prostasin via the GPI anchor was essential for ENaC activation. More recent studies in mouse models expressing catalytically inactive prostasin mutant S238A or zymogen-locked mutant R44Q revealed that prostasin supports both epidermal development and long-term survival in a non-catalytic manner (Peters et al. 2014; Friis et al. 2016). In contrast, prostasin proteolytic activity was found to be crucial for matriptase zymogen activation in the placenta (Szabo et al. 2016). In addition, mice expressing catalytically inactive or zymogen-locked prostasin displayed impaired hair follicle development and delayed skin wound healing,

indicating that some of the physiological functions of prostasin require its proteolytic activity. The V170D (low enzymatic activity) and the G54-P57 deletion mutations of prostasin have been identified in mouse frizzy (fr) and rat hairless (frCR) animals, respectively, and have been proposed to be responsible for their skin phenotypes (Spacek et al. 2010; Szabo et al. 2012).

Taken together, prostasin is unique among trypsin-like proteases in that it has essential functions as an enzymatically active protease as well as an enzymatically inactive zymogen. The specific mechanism(s) by which catalytically inactive prostasin supports epidermal development remain to be established. Matriptase and prostasin form a reciprocal zymogen activation complex *in vitro* that results in the formation of both active matriptase and active prostasin (Friis et al. 2013).

8.4.2 Testisin: Roles in Sperm Maturation and Motility

Testisin (also referred to as PRSS21, testicular protease 5 (TESP5), or eosinophil serine protease 1 (ESP-1)) is aberrantly expressed in male germ cells and sperm and is also expressed in microvascular endothelial cells and in eosinophils (Hooper et al. 1999; Inoue et al. 1998; Aimes et al. 2003). The protease was originally cloned from human eosinophils and from HeLa cells (Inoue et al. 1998; Hooper et al. 1998). Testisin expression is lost in testicular germ cell tumors (Hooper et al. 1999) and is found overexpressed in ovarian tumors (Shigemasa et al. 2000). The testisin gene, *PRSS21*, is located on human chromosome 16p13.3. Several isoforms of human testisin have been identified that are generated by alternative pre-mRNA splicing (Hooper et al. 2000; Inoue et al. 1998).

Testisin is synthesized as a 43-kDa precursor in the testis, and the zymogen is converted into the 42- and 41-kDa active enzymes during sperm transport in the epididymis (Honda et al. 2002). Testisin is anchored to the membrane via a GPI moiety at its carboxy terminus and is included into lipid rafts on the sperm membrane (Honda et al. 2002). Unlike other membrane-anchored serine proteases, testisin has not been found to be naturally shed from the plasma membrane, but the protease can be release from cells in vitro using exogenous bacterial phosphatidylinositol-specific phospholipase C (Honda et al. 2002).

Mammalian fertilization requires sperm to penetrate the cumulus matrix surrounding the oocyte to reach the zona pellucida (ZP), binding and invasion of the ZP, and finally fusion of the sperm and oocyte plasma membranes. The serine protease acrosin has been long believed to participate in limited proteolysis of ZP, thus enabling sperm to penetrate the egg coat. However, acrosin-deficient mice were fully fertile, although they displayed delayed sperm penetration of the ZP at the early stage of fertilization in vitro (Baba et al. 1994), indicating that additional serine protease(s) play important roles in the regulation of male fertility. Mice deficient in testisin expression are fertile, too, but display deformed spermatozoa with an increased tendency toward decapitation and reduced motility (Netzel-Arnett et al. 2009). Testisin was found to direct murine sperm cell maturation and

sperm-fertilizing ability during passage of spermatozoa through the epididymis to their site of temporary storage in the cauda (Netzel-Arnett et al. 2009). Combined knockout of acrosin and testisin in mice impairs fertility in vivo and causes complete loss of fertilization ability in vitro, which suggests that sperm trypsin-like activity is indispensable for in vitro fertilization but not particularly for fertilization in vivo in mice. Interestingly, these data suggest that the female reproductive tract partially compensates for the loss of the sperm function, presumably due to the presence of an acrosin-/testisin-like protease (Kawano et al. 2010; reviewed in Szabo and Bugge 2011).

Little is known regarding specific physiological substrates of testisin during epididymal sperm maturation and initiation of sperm motility as well as during testicular and ovarian tumor formation and progression. Recently, testisin was shown to be capable of activating PAR-2 in vitro (Driesbaugh et al. 2015). PAR-2 activation has been associated with the regulation of sperm motility following trypsin activation (Miyata et al. 2012). However, activation of PAR-2 by testisin in vivo and a possible role in sperm motility remain to be demonstrated.

8.5 MASPs in Viral Infections

The majority of viral fusion proteins is synthesized as precursors and requires processing by a host cell protease to trigger fusion of the viral lipid envelope and cellular membranes in order to release the virus genome into the host cell. For a large number of viruses, cleavage of the fusion protein occurs either at a single arginine (R↓) by trypsin-like proteases or at a multibasic motif of the consensus sequence R-X-R/K-R↓ by ubiquitous expressed subtilisin-like proteases such as furin and proprotein convertase 5/6 (PC5/6) (see Chap. 9).

The first report of proteolytic activation of viral fusion proteins was published in 1973 in a study using Sendai virus. It was demonstrated that the viral glycoprotein F is synthesized as an inactive precursor that is converted into its biological active form due to cleavage by a host cell protease and that F cleavage is a prerequisite for infectivity and multicycle virus replication (Homma and Ohuchi 1973; Scheid and Choppin 1974). In the following years, striking differences in glycoprotein activation have been observed with Newcastle disease virus and avian influenza viruses, which proved to be important determinants of the pathogenicity of these viruses (Nagai et al. 1976; Bosch et al. 1981). In 1992, the proprotein convertase furin was identified as protease that activates the hemagglutinin of the highly pathogenic avian influenza virus (HPAIV) strains at multibasic motifs (Stieneke-Gröber et al. 1992; reviewed by W. Garten in another chapter of this book) (Fig. 8.3a, b). Furin which is expressed in all tissues is responsible for the systemic infection typical for these viruses. However, less was known about the identity of the protease(s) that support HA cleavage at a single arginine residue.

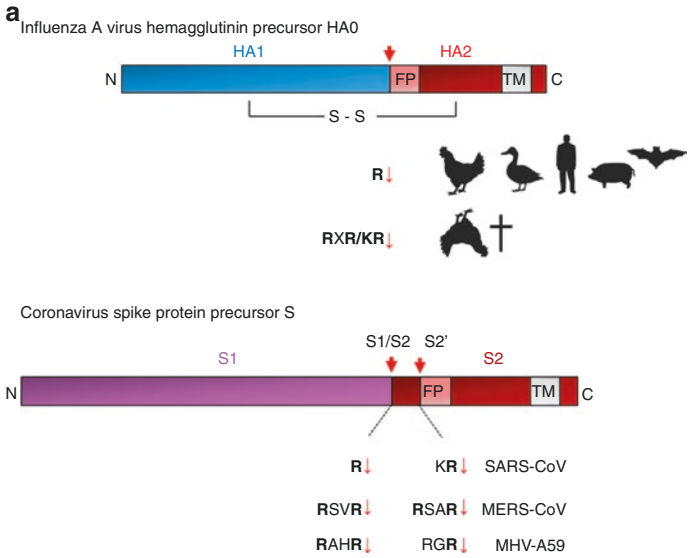

Fig. 8.3 Activation of the envelope glycoprotein hemagglutinin HA of influenza A virus and spike protein S of CoV by host cell proteases. (**a**) Schematic illustration of the precursor proteins HA0 and S of influenza A virus and coronaviruses (CoVs), respectively, and cleavage sites (red arrows). HA0 is cleaved into HA1 and HA2; both subunits remain linked by a disulfide bond. HA0 of highly pathogenic avian influenza A viruses is activated at a multibasic cleavage site, while HA0 of low pathogenic avian influenza A viruses and mammalian influenza A viruses is cleaved at a single basic residue. CoV S protein is cleaved at two distinct sites at monobasic motifs or the minimal furin consensus motif RXXR. The S1 and S2 domains are not held by disulfide bonds but remain associated non-covalently (Millet and Whittaker 2015). FP, fusion peptide. TM, transmembrane domain. Basic amino acids crucial for cleavage by relevant proteases are written in bold letters. (**b**) Compartmentalization of activation of HA and S by host cell proteases. The envelope proteins HA and S mediate virus entry into cells through receptor binding and fusion of the viral envelope with host cell membranes. Fusion delivers the viral genome into the host cell and initiates viral replication and generation of progeny virions. Newly synthesized virions are finally released via budding at the plasma membrane (influenza A virus) or at the ER-Golgi intermediate compartment (ERGIC) and subsequent exocytosis of new virions at the cell surface (CoV). HA and S require cleavage by host cell proteases to gain their fusion capacity. In the case of influenza A virus, newly synthesized HA is cleaved during its transport to the plasma membrane by furin (multibasic cleavage site) or TMPRSS2 (monobasic cleavage site) in the trans-Golgi network (TGN) or at the cell surface by HAT during assembly and budding of progeny virus. Thus, virus containing cleaved HA is released from infected cells. HA can also be cleaved by HAT on the cell surface prior to entry into a new cell. In contrast, TMPRSS2 present on the cell surface does not support HA cleavage. It remains unclear whether HAT is enzymatically active within the cell and thus can cleave HA already during its transit to the plasma membrane. Most CoVs, including SARS- and human 229E-CoVs, are released with non-cleaved S from the infected cells, indicating that S is not activated during its transit through the exocytic pathway. Therefore, the S protein of CoV is activated upon entry into the host cell. Entry can take place via fusion at or close to the plasma membrane or in early endosomes ("early entry") and may be pH-independent or via late fusion in late endosomes ("late entry") in a low pH-dependent manner. Early or late fusion seems to be dependent on the protease(s) that cleave S at the S2' site. Cleavage of S by furin and TTSPs is believed to support an early entry (e.g., MERS-CoV), whereas activation of S by cathepsins supports late entry via fusion in late endosomes (e.g., mouse hepatitis virus (MHV) A59) (Burkard et al. 2014; Millet and Whittaker 2015; Park et al. 2016). Newly synthesized S protein of some CoV, including MERS-CoV, has been reported to be cleaved by furin or TTSPs in the exocytic pathway, but the role of S cleavage in the secretory pathway for virus-cell and cell-cell fusion remains to be investigated in more detail. It also remains unknown whether furin and/or TTSPs such as TMPRSS2 are present as enzymatically active enzymes in the ERGIC and might cleave S in this compartment

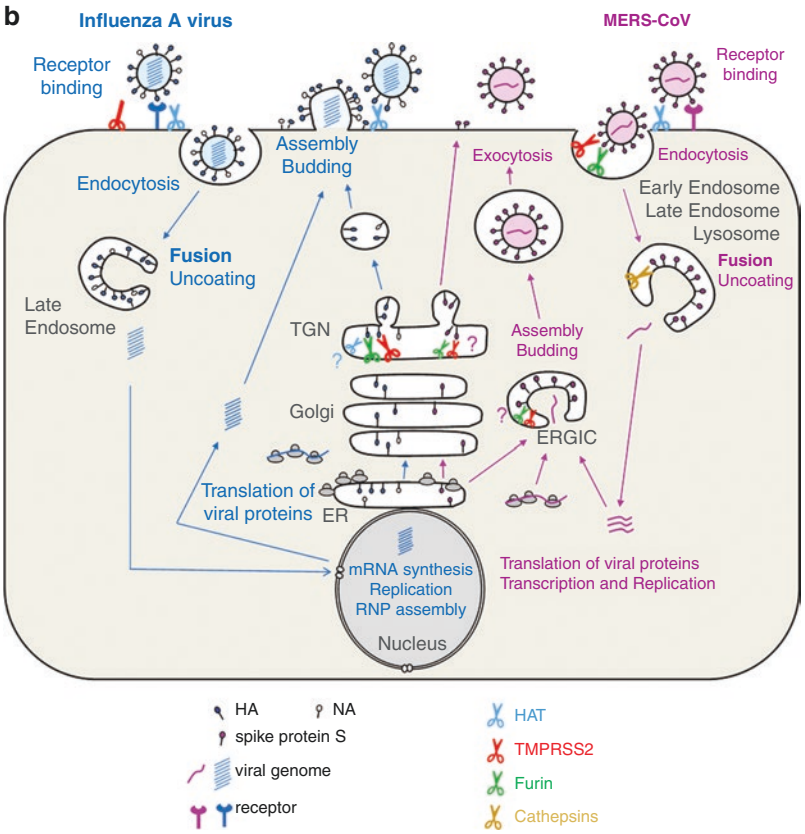

Fig. 8.3 (continued)

8.5.1 The Search for Virus-Activating Proteases in the Airway Epithelium

Human and mammalian influenza A and B viruses and low pathogenic avian influenza A viruses (LPAIV) are activated at monobasic HA cleavage site motifs by trypsin in vitro (Klenk et al. 1975; Lazarowitz and Choppin 1975). A number of soluble trypsin-like proteases isolated from rat and swine lung, such as tryptase Clara, mini-plasmin, or tryptase TC30, as well as a blood clotting factor Xa-homologous protease in embryonated chicken eggs have also been found to activate HA of these viruses as described in a chapter by H. Kido in this book. However, the genetic identity is still unknown for many of these enzymes, and it remains unclear whether they play a role in in vivo infection. Relevant HA-activating proteases in the human airways were unknown for a long time. Cleavage of HA by soluble proteases such as trypsin in cell culture takes place outside the cells during assembly and budding of new virions when HA is present on the plasma membrane or after progeny virus is released from the infected cell. Thus, it was believed for a long time that HA with a monobasic cleavage site is activated extracellularly and, therefore, differs from HA of HPAIV, which is cleaved in the TGN by furin.

In 2006, a number of proteases that possess trypsin-like activity were cloned from primary human tracheobronchial epithelial (HTBE) cells in search for human HA-activating proteases (Böttcher et al. 2006). Among a couple of candidates, the TTSPs HAT and TMPRSS2 were demonstrated to activate HA with monobasic cleavage site in vitro and thus were identified as the first human influenza virus-activating proteases in the respiratory tract (Böttcher et al. 2006) (Table 8.2).

Subsequently, HAT and TMPRSS2 were shown to activate also other respiratory viruses at monobasic cleavage site motifs in vitro, such as human coronaviruses (CoVs) including SARS-CoV and MERS-CoV, the human metapneumovirus, and human parainfluenza viruses (Shirogane et al. 2008; Glowacka et al. 2011; Matsuyama et al. 2010; Shulla et al. 2011; Shirato et al. 2013; Millet and Whittaker 2015; Abe et al. 2013). In addition, further TTSPs were tested for their ability to activate viral fusion proteins, and TMPRSS4, DESC1, TMPRSS11A, MSPL/TMPRSS13, and matriptase have been found to activate influenza virus HA as well as SARS-CoV and MERS-CoV spike protein S in cell cultures (Table 8.2). Other TTSPs, such as hepsin, TMPRSS3, matriptase-2, matriptase-3, or corin, did not support proteolytic activation of influenza viruses or CoV in cell culture, demonstrating that only certain trypsin-like proteases present in the airway epithelium support activation and spread of respiratory viruses. TMPRSS2-homologous proteases have been identified in swine, chicken, and mouse and were shown to be capable of activating HA at a single arginine, suggesting that homologous proteases are involved in HA cleavage in different host species (Bertram et al. 2012; Peitsch et al. 2014; Tarnow et al. 2014).

It has long been known that HAs with monobasic cleavage site differ in their sensitivity to host proteases. For instance, HAs of A/WSN/33 (H1N1), A/PR8/34 (H1N1), A/Asia/1/57 (H2N2), and A/duck/Ukraine/1/63 (H3N8) are activated by trypsin, plasmin, kallikrein, and uPA in vitro, whereas HA of A/chicken/Germany/49 (H10N7) is activated only by trypsin (Lazarowitz et al. 1973; Scheiblauer et al. 1992). More recent studies on cleavage of human influenza A virus HA by kallikrein (KLK) 5 and 12 showed that H1 is cleaved by both KLK5 and KLK12 in vitro, whereas H2 and H3 are only cleaved by KLK12 and KLK5 , respectively (Hamilton and Whittaker 2013). Furthermore, the sensitivity of 16 HA subtypes to cleavage by TMPRSS2, HAT, and pancreatic trypsin was demonstrated to vary significantly among the different subtypes (Galloway et al. 2013; see also Chap. 1). Interestingly, studies in TMPRSS2-deficient mouse models demonstrated that differences in the sensitivity of HA to cleavage by host proteases may affect influenza virus spread and pathogenesis (Hatesuer et al. 2013; Tarnow et al. 2014; Sakai et al. 2014) (see below). The mechanisms underlying protease specificity of HA with monobasic cleavage site, however, are still unknown and may be related to the structure and exposure of the cleavage site loop or steric hindrance by adjacent carbohydrate moieties (Kawaoka et al. 1984; Sakai et al. 2015).

8.5.2 Subcellular Localization and Time Point of Virus Activation by TTSPs

The identification of membrane-bound HA-activating proteases raised the question where in the host cell and at which step of the viral replication cycle the viral

glycoproteins are cleaved by TTSPs. The subcellular localization and enzymatic activity of HAT and TMPRSS2 in airway epithelial cells have not been investigated in more detail so far but have been studied in MDCK cells that proved to be a suitable model system (Böttcher et al. 2009). Interestingly, it was demonstrated that activation of influenza virus HA by HAT and TMPRSS2 differs in subcellular localization and can take place at different steps of the viral life cycle (Böttcher et al. 2009; Böttcher-Friebertshäuser et al. 2010, 2013; Fig. 8.3b). Incubation of HAT-expressing MDCK cells with fluorogenic peptides to measure the protease activity on the cell surface revealed that HAT is present as an enzymatically active protease on the cell surface. In addition, it was shown that HAT is capable of cleaving newly synthesized HA0, probably during assembly and budding of new virions on the plasma membrane, as well as HA0 of incoming influenza virus at the stage of entry during attachment to a new host cell. In contrast, only poor if any enzymatic activity of TMPRSS2 was measured on the surface of TMPRSS2-expressing MDCK cells, and the cells were not able to support activation of incoming influenza virions containing HA0, although TMPRSS2 was present as both zymogen and mature form on the surface of MDCK cells (Böttcher-Friebertshäuser et al. 2010). Cleavage of HA was found to take place intracellularly during its transport through the secretory pathway to the plasma membrane (Fig. 8.3b). Immunofluorescence studies of transient TMPRSS2 expression in mammalian cells revealed that the protease accumulates in the TGN, where it co-localizes with furin, suggesting that HA cleavage by TMPRSS2 and furin occurs in the same cellular compartment (Böttcher-Friebertshäuser et al. 2013). These data were in agreement with earlier studies by Zhirnov et al. that showed that cleavage of HA in human respiratory or intestinal epithelial cells occurs intracellularly and is performed by cell-associated proteases (Zhirnov et al. 2002; Zhirnov and Klenk 2003). These studies provided further evidence that cleavage of influenza virus HA can occur at different steps of the viral life cycle: during transport of HA along the secretory pathway to the cell surface, on the plasma membrane during assembly and budding, and late in infection upon entry into new cells (Boycott et al. 1994; Zhirnov et al. 2002; Böttcher-Friebertshäuser et al. 2010). However, shedding of TTSPs may be enhanced under stress, and soluble forms might contribute to virus activation under such conditions. For example, enhanced shedding of TMPRSS2 and HAT from human nasal epithelial cells and hence enhanced influenza virus replication have been observed upon exposure to ozone in vitro (Kesic et al. 2012).

Studies on proteolytic activation of the spike protein S of human CoV including SARS-CoV and MERS-CoV by HAT and TMPRSS2 revealed also that viruses may have different options to be activated by host cell proteases during the viral replication cycle (Fig. 8.3b). CoV S proteins possess two distinct cleavage sites, S1/S2 and S2', and can be cleaved by a number of proteases, including furin, cathepsin L, and trypsin, and trypsin-like proteases such as the TTSP HAT, TMPRSS2, DESC1, and MSPL (reviewed in Millet and Whittaker 2015, and Chap. 4 this book; see also Fig. 8.3). Cleavage of S by cathepsins occurs in late endosomes or lysosomes and is pH dependent, whereas activation by TTSPs may

support cathepsin- and low pH-independent CoV entry via fusion in early endosomes in vitro (Burkard et al. 2014; Millet and Whittaker 2015; Park et al. 2016). Both HAT and TMPRSS2 are believed to cleave S at or close to the cell surface (for review see Chap. 4). It remains to be investigated whether TMPRSS2 and/or HAT are present (and active) in endosomes. It further remains to be investigated why TMPRSS2 may cleave CoV S but not influenza virus HA at the cell surface. For some CoV, receptor binding has been shown to be required for proteolytic activation of S. Treatment of SARS-CoV particles with trypsin prior to binding to its receptor angiotensin I-converting enzyme 2 (ACE2) inactivates the virions in vitro, whereas SARS-CoV particles bound to its receptor are activated by trypsin for membrane fusion (cf. Chap. 4). Moreover, TMPRSS2 has been shown to cleave ACE2 and thereby to increase SARS-CoV S uptake into ACE2-expressing cells in vitro (Heurich et al. 2014). Thus, receptor binding of S or interactions of TMPRSS2 and ACE2 might trigger S cleavage by TMPRSS2 at the plasma membrane. In general, CoV are believed to be proteolytically activated upon entry into cells, but newly synthesized S may also be activated prior to virus release from the infected cell (Fig. 8.3b). The subcellular compartmentalization of S cleavage during its transit through the exocytic pathway and/or virus assembly and budding at the ER-Golgi intermediate compartment (ERGIC) (reviewed in Ujike and Taguchi 2015), however, are not understood in detail.

8.5.3 Identification of TMPRSS2 as Host Cell Factor Essential for Influenza A Virus Activation and Spread in Mice

In three recent independent studies, TMPRSS2 was identified as the solely HA-activating protease and as a host cell factor essential for spread and pneumotropism of human H1N1 and H7N9 influenza A viruses in mice (Hatesuer et al. 2013; Tarnow et al. 2014; Sakai et al. 2014). Intriguingly, knockout of TMPRSS2 expression prevented virus activation and consequently spread into the lungs and thereby protected the animals from influenza pathogenesis, whereas wild-type littermates succumbed to severe infection. These studies also revealed that other TTSPs that activate H1N1 virus in vitro, such as HAT/TMPRSS11D, TMPRSS4, DESC1, or MSPL, do not support HA activation in mice in vivo. For HAT/TMPRSS11D, this can be explained by the fact that the protease is expressed in the upper airways, trachea, and bronchi of mice, but is not present in the lungs and, thus, cannot support influenza virus pneumotropism (Sales et al. 2011; Tarnow et al. 2014). The same is probably true for DESC1. However, TMPRSS4 and MSPL/TMPRSS13 are present in lung tissue (Kühn et al. 2016; Kim et al. 2001), and it remains unclear why both proteases do not support activation of H1N1 and H7N9 influenza virus in the airways of TMPRSS2-deficient mice. Noteworthy, replication of human H3N2 influenza A virus and influenza B virus (IBV) was almost independent of TMPRSS2 expression in mice, indicating that

H3 and IBV HA can be activated by additional host proteases in contrast to H1 and H7 (Tarnow et al. 2014; Sakai et al. 2016). In a recent study, knockout of both TMPRSS4 and TMPRSS2 caused reduced body weight loss and mortality upon H3N2 influenza A virus infection in mice in comparison with wild-type animals, indicating that both proteases contribute to activation of H3N2 virus in mice (Kühn et al. 2016). Nonetheless, H3N2 influenza A virus was still proteolytically activated in TMPRSS2-TMPRSS4-knockout mice and caused severe disease with 30% mortality, indicating that another H3-cleaving protease(s) is present in murine airways. MSPL/TMPRSS13 might be a potential candidate.

Taken together, these studies demonstrated for the first time that expression of the appropriate HA cleaving protease along the respiratory tract is essential for influenza virus pneumotropism and pathogenicity in a mammalian host. In addition, TMPRSS2 emerged as a potential drug target for influenza treatment. It will be very interesting to analyze the role of TMPRSS2 and further TTSPs in activation and pathogenicity of other respiratory viruses including SARS-CoV and MERS-CoV and human parainfluenza viruses using suitable mouse models. Interestingly, a single nucleotide polymorphism in the TMPRSS2 gene that results in higher TMPRSS2 expression has been associated with increased susceptibility to H1N1 and H7N9 influenza virus and higher risk of severe infection, suggesting that TMPRSS2 may play a crucial role in influenza virus activation also in humans (Cheng et al. 2015). This view is supported by the observation that knockdown of TMPRSS2 expression in the human airway epithelial cell line Calu-3 strongly suppressed activation and multicycle replication of human H1N1 influenza A viruses (Böttcher-Friebertshäuser et al. 2011). Interestingly, knockdown of TMPRSS2 also strongly suppressed H3N2 virus replication in Calu-3 cells, suggesting that the differences in protease specificity of influenza virus HA observed in mice might be less pronounced in humans. However, further studies are needed to understand the role of TMPRSS2 in influenza virus activation in humans.

8.5.4 Activation of Viral Fusion Proteins with Di-/Multibasic Cleavage Site Motifs by TTSPs

Other TTSPs have been found to activate viral fusion proteins at di- or multibasic amino acid motif. MSPL/TMPRSS13 and the hepsin-related protease TMPRSS12 were able to activate avian H5N2 influenza virus and avian metapneumovirus, respectively, at multibasic cleavage site motifs (Okumura et al. 2010; Yun et al. 2016). This may be relevant particularly for unusual di- or multibasic cleavage site motifs that are not cleaved by furin. H9N2 viruses in Asia and the Middle East have acquired dibasic cleavage site motif R-S-S/R-R that are not activated by furin. A study demonstrated that H9 with R-S-S/R-R at the cleavage site can be activated by matriptase in addition to TMPRSS2 and HAT in vitro (Baron et al. 2013). Matriptase is widely expressed in multiple epithelial tissues and, therefore, may affect H9N2 virus spread, tissue tropism, and pathogenicity. Nephrotropism of H9N2 virus has

been described in chickens, and matriptase has been suggested to contribute to H9N2 virus replication in the kidney of chickens (Baron et al. 2013). Some H9N2 isolates have been reported to cause lethal infections in mice with virus replication in the lung and brain (Guo et al. 2000; Li et al. 2012).

Concluding Remarks and Key Research Questions

Described for the first time two decades ago, a number of MASPs have already been established as important regulators in mammalian physiology. However, for many MASPs the physiological functions and/or substrates are not fully characterized or still unknown at all. The TTSP TMPRSS2 was identified as influenza A virus-activating protease in human airway cells and in mice and as host cell factor essential for pneumotropism and pathogenesis of certain influenza A virus strains infecting humans in mice. The role of TMPRSS2 in activation of further respiratory viruses in vivo remains to be investigated, and its important role in influenza virus activation in humans needs to be demonstrated. As mentioned above, a number of TTSPs that have been shown to activate HA in vitro and to be present in the respiratory tract, including TMPRSS4 and TMPRSS13, were not able to compensate for the lack of TMPRSS2 expression in influenza virus activation in mice. Thus, if there is some redundancy among MASPs in processing of physiological substrates, and this is very likely, it is not the case for cleavage of HA. The underlying reasons are unclear so far. Detailed information on the substrate specificity of TMPRSS2 or TMPRSS4 is missing due to the lack of suitable systems for expression and purification of these proteases and available structural information of the protease domains. To date, the crystal structure of the protease domain has been solved for hepsin, DESC1, enteropeptidase, matriptase, and prostasin. Moreover, differences in (1) subcellular compartmentalization and activity, (2) expression levels, and/or (3) distribution in different airway cell types of TMPRSS2 in comparison with other TTSPs might account for the observed differences in activation of influenza A viruses. In particular, the compartmentalization of MASP activity should be investigated in more detail in future studies. MASPs are predicted to act as active enzymes on the cell surface, but studies on activation of influenza A virus HA by TMPRSS2 showed that cleavage takes place intracellularly, probably in the TGN. Thus, some MASPs may process their substrates already (or even exclusively) in intracellular compartments. Expression and enzymatic activity of MASPs in endosomes and lysosomes have not been studied so far but may play a role in processing of both physiological substrates and viral fusion proteins. Furthermore, the role of soluble MASP activity due to shedding of the catalytic domain is poorly understood.

Dysregulated MASP activities are associated with a number of pathophysiological processes, and specific inhibitors may provide promising pharmaceutical tools for the treatment of cancer, iron overload, or respiratory diseases. Potent inhibitors for some MASPs have already been developed (for review see Steinmetzer and Hardes, this book), but no inhibitor of host proteases has been

approved for the treatment of virus infections to date. The development of highly selective MASP inhibitors is hampered by the lack of available crystal structures of the catalytic domain as well as knowledge on the role of the different domains of the stem region in substrate specificity, protease conformation, and protein-protein interactions. It remains to be analyzed whether some TTSPs are upregulated during virus infection and thereby may support enhanced virus activation and/or organ tropism. Moreover, it will be interesting to analyze whether dysregulation of MASPs contributes to the susceptibility to virus infection.

The protection of TMPRSS2 knockout mice from influenza A virus pathogenesis strikingly demonstrated the crucial role of virus activation for viral spread in the host. However, we are just beginning to understand in more detail which roles MASPs may play in virus activation, spread, and organ tropism on one hand and whether the physiological functions of these enzymes can be suppressed or compensated during an acute virus infection in order to block virus multiplication by using protease inhibitors on the other hand.

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