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Review

Human clade B serpins (ov-serpins) belong to a cohort of evolutionarily dispersed *intracellular* proteinase inhibitor clades that protect cells from promiscuous proteolysis

G. A. Silverman[a,*], J. C. Whisstock[b], D. J. Askew[a], S. C. Pak[a], C. J. Luke[a], S. Cataltepe[a], J. A. Irving[b] and P. I. Bird[b]

[a] Division of Newborn Medicine, Children's Hospital, Dept. of Pediatrics, Harvard Medical School, 300 Longwood Ave, Enders 970, Boston, Massachusetts 02115 (USA), Fax: +1 617 731 8962,
e-mail: gary.silverman@tch.harvard.edu
[b] Department of Biochemistry and Molecular Biology, Monash University 3800, Melbourne, Victoria (Australia)

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Abstract. Serpins are unique among the various types of active site proteinase inhibitors because they covalently trap their targets by undergoing an irreversible conformational rearrangement. Members of the serpin superfamily are present in the three major domains of life (*Bacteria*, *Archaea* and *Eukarya*) as well as several eukaryotic viruses. The human genome encodes for at least 35 members that segregate evolutionarily into nine (A-I) distinct clades. Most of the human serpins are secreted and circulate in the bloodstream where they reside at critical checkpoints intersecting self-perpetuating proteolytic cascades such as those of the clotting, thrombolytic and complement systems. Unlike these circulating serpins, the clade B serpins (ov-serpins) lack signal peptides and reside primarily within cells. Most of the human clade B serpins inhibit serine and/or papain-like cysteine proteinases and protect cells from exogenous and endogenous proteinase-mediated injury. Moreover, as sequencing projects expand to the genomes of other species, it has become apparent that *intracellular* serpins belonging to distinct phylogenic clades are also present in the three major domains of life. As some of these serpins also guard cells against the deleterious effects of promiscuous proteolytic activity, we propose that this cytoprotective function, along with similarities in structure are common features of a cohort of *intracellular* serpin clades from a wide variety of species.

Key words. Serpin; ov-serpin; clade B serpin; intracellular serpin; cysteine proteinase; serine proteinase; proteinase inhibitor; cell death; apoptosis; innate immunity; tumor invasion; evolution.

Introduction

Peptide bond hydrolysis is essential to both the life and death of an organism. The importance of this activity is underscored by the enormous expanse of genomic landscape committed to the expression of different classes of proteinases. Due to the catastrophic effects of excessive proteolysis, it is not surprising that a complex array of inhibitory molecules have evolved in parallel. Of these regulatory molecules, the serpins have evolved one of the most sophisticated means of blocking proteinase activity. The serpins are a family of proteins characterized by a unique tertiary structure and, unlike standard mechanism inhibitors, employ a suicide-substrate-like mechanism to neutralize their target proteinases (for recent reviews see [1, 2]). Although a few serpins have acquired novel functions such as hormone transport and blood pressure regulation, most serve as serine and/or cysteine proteinase inhibitors. Moreover, it has become apparent that serpins participate alone or in concert with other family members to regulate complex proteolytic cascades associated with

* Corresponding author.

coagulation, fibrinolysis, complement activation, certain cell death pathways, kinninogen activation, dorsal-ventral patterning, prophenoloxidase activation and *Toll* receptor signaling [1, 2].

In 1993, Remold-O'Donnell identified a subset of five serpins based on seven criteria: (i) >39% amino acid sequence identity, (ii) the absence of N- or C-terminal extensions relative to the prototypical serpin, α1-antitrypsin (SERPINA1), (iii) a *Ser* rather than an *Asn* residue at the penultimate position, (iv) a variable residue rather than a *Val* at position 388 (α1-antitrypsin numbering), (v) a variably sized loop between helices C and D (CD-loop), (vi) similar gene structures and most significantly (vii) the absence of a cleavable N-terminal signal peptide [3]. Since the archetypal member of this group was chicken ovalbumin, it was originally designated the ovalbumin (ov)-serpin family, but is now known as the clade B family under the revised nomenclature guidelines. [2]. The clade B serpins contain mostly inhibitory types, but at least one has significant noninhibitory activity (table 1). Of the inhibitory serpins, several neutralize serine and/or cysteine proteinases. The ability of these serpins to target

Table 1. Human clade B serpins.

Gene (GenBank)	No. amino acids	M_r	pI	Disease association or function	Cellular distribution		Potential proteinase targets
					Cytoplasmic	Nuclear	
SERPINB1 (P30740)	379	42741	5.90	inhibits or enhances apoptosis depending on inducer	+	+	neutrophil elastase, pancreatic elastase, chymotrypsin, catG, PR3, PSA, chymase
SERPINB2 (P05120)	415	46596	5.46	inhibits apoptosis, motility, inflammation, angiogenesis	+	+	u-PA, t-PA, acrosin, plasmin
SERPINB3 (P29508)	390	44564	6.35	inhibits apoptosis	+	?	catK, L, S, V
SERPINB4 (P48594)	390	44853	5.86	inhibits apoptosis	+	?	chymase, catG
SERPINB5 (P36952)	375	42138	5.72	inhibits cell motility, angiogenesis inhibitor, enhances apoptosis, class II tumor suppressor	+	+	–
SERPINB6 (P35237)	376	42589	5.18	protects cells from granule proteinases, upregulated in prostate cancer and complexes with kallikrein 2 in cancer cells	+	+	thrombin, trypsin, factor Xa, u-PA, chymotrypsin, cathepsin G, kallikrein 2, neuropsin
SERPINB7 (NP_003775)	380	42904	6.34	IgA, diabetic nephropathy	+	?	? plasmin
SERPINB8 (P50452)	374	42785	5.43		+	+	furin, trypsin, factor Xa, subtilisin A, thrombin, chymotrypsin,
SERPINB9 (P50453)	376	42403	5.61	antiinflammatory, atherosclerosis,	+	+	granzyme B, neutrophil elastase, subtilisin A, caspase-1, -4, -8
SERPINB10 (P48595)	397	45402	5.80	inhibits TNF-induced apoptosis	+	+	thrombin, trypsin
SERPINB11 (NP_536723)	392	44098	8.42				?
SERPINB12 (NP_536722)	405	46276	5.36				trypsin, plasmin
SERPINB13 (NP_036529)	391	44276	5.48	downregulated by UVB irradiation and in SCC of oropharynx, upregulated in psoriasis, protects against UV-induced apoptosis	+	–	catK and L

different mechanistic classes of proteinases may relate to their unique subcellular localization. Although clade B serpins can be detected in the extracellular space, their predominant location appears to be within the nucleocytoplasm. Collectively, these findings suggest that clade B serpins serve a protective role within the cell.

To date, serpins fitting the strict Remold-O'Donnell criteria have been found only in vertebrates. However, as genome sequencing projects progress, it has become apparent that *intracellular* serpins belonging to separate clades are present in the three major domains of life. These findings suggest that the clade B serpins, along with this larger cohort of *intracellular* serpins, may have evolved to guard against the deleterious effects of promiscuous proteolytic activity. Although this review focuses primarily on the human clade B serpins, we propose that this cytoprotective function, along with similarities in structure, is a common feature of a cohort of *intracellular* serpin clades from a wide variety of species.

Evolution

The origin of the serpin family is unclear. As recently as 2000, it appeared that serpins were restricted to higher eukaryotes (metazoans) and eukaryotic viruses [4]. The apparent absence of serpins in fungi and prokaryotes, and their scattered appearance in plants, suggested that serpins evolved in metazoans and appeared in plants by lateral gene transfer. However, the accelerating pace of genome research and increasing size of sequence database have revealed the presence, albeit sporadic, of serpins in some lower eukaryotes (fungi) and prokaryotes, including archaebacteria [5]. These findings suggest that the ancestral serpin gene arose in a primordial cell and was lost as prokaryotic and eukaryotic species adapted to distinct environments, or it evolved after the prokaryotic/eukaryotic separation and appeared in the other domains by lateral gene transfer.

Phylogenic analyses of serpin protein sequences suggest that the superfamily can be divided into at least 17 clades, with vertebrate serpins falling into 9 clades (A–I) [4]. Analysis of intron number and position of the vertebrate serpin genes identifies six or seven groups (fig. 1A) [6, 7]. Discrepancies between protein and gene analyses arise because the protein comparisons place three serpins (antithrombin III [SERPINC1], heparin cofactor II [SERPIND1], and C1 inhibitor [SERPING1]) in their own separate clades (C, D and G respectively). However, gene comparison reveals that heparin cofactor II (SERPIND1) has a similar gene structure to clade A serpins, and clades

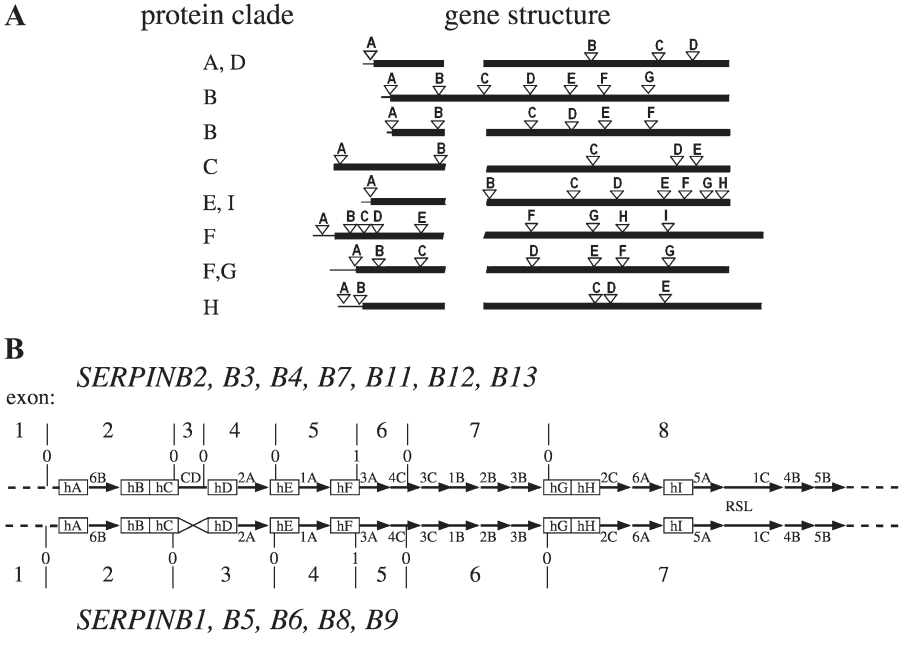

Figure 1. (*A*) Human serpin gene structures. Serpins can be grouped by gene structure. This correlates well with protein phylogeny since serpins in different protein clades have dissimilar gene structures, and serpins within a clade share similar gene structures. Shown are representatives of each clade (A–I) aligned using mRNA sequence (and hence amino acid sequence) similarity. Thin lines indicate untranslated regions and thick lines indicate open reading frames. Arrowheads and capital letters denote positions and designations of introns. Figure is not to scale. Derived from [6, 7]. (*B*) Maps of the eight-exon (top) and seven-exon (bottom) containing clade B serpin cDNAs relative to their structural elements. The rectangles and arrows represent helices and β strands, respectively. The dashed lines depict 5′ and 3′ untranslated sequences. Vertical lines indicate the positions of splice sites with intron phasing noted above or below these sites, respectively. Numbering for eight- and seven-exon classes are depicted above and below the lines, respectively. The CD-loop (eight-exon class only) and RSLs are indicated by thick lines. The CD-loops of SERPINB2, -3, -4, -7, -10, -13, -12 and -13 contain 33, 12, 12, 8, 18, 13, 22 and 22 residues, respectively. This figure is modified from [9].

E (PN-1 [SERPINE2], PAI-1 [SERPINE1]) and I (neuroserpin [SERPINI1], pancpin [SERPINI2]) are most probably linked. Similarly, Clades F (α2-AP [SERPIN F2], PEDF [SERPINF1]) and G (C1 inhibitor [SERPING1]) also can be combined.

The use of both amino acid sequence and gene structure comparisons increases the reliability of tracing the evolution of eukaryotic serpins. Gene structure is useful for studying evolution of serpin families, while protein trees facilitate the analyses of relationships within families and may give insight into function. In tracing prokaryotic serpin evolution, protein comparison combined with analysis of DNA GC content or evidence of transposition is likely to increase reliability. Finally, the increasing number of serpin structures available will enhance our ability to accurately build structure-based alignments and improve the accuracy of phylogenic analysis.

As described below, the signature clade B serpin gene structure comprises eight exons and seven introns, with the first encoding the 5′ untranslated region, and the third encoding the variable CD-loop (fig. 1B). This gene structure is not recognizable in the genomes of *Caenorhabditis elegans* or *Drosophila melanogaster,* suggesting that the clade B serpin family arose no earlier than the Deuterostomes (fig. 2). Since genes with a similar structure are present in both birds and mammals, clade B serpins must have originated at least 300 million years ago [8].

Clade B serpin genomic organization

Clade B serpin genes have nearly identical genomic structures consisting of seven or eight exons [7, 9] (fig. 1B). In the seven-exon-containing genes, a C-terminal portion of helix C, the turn between helices C and D, and helix D are encoded on exon 3. In the eight-exon-containing genes, an additional coding sequence (CD-loop), interrupted by an intron, is located between the gene segments encoding helices C and D. The CD-loop may be involved in protein-protein interactions or may harbor subcellular localization signals (see below). The size of the CD-loops varies, with SERPINB2, -B3, -B4, -B7, -B10, -B13, -B12 and -B13 containing 33, 12, 12, 8, 18, 13, 22 and 22 residues, respectively (fig. 3).

All members of the eight-exon class show conserved splice-site phasing: 0, 0, 0, 0, 1, 0, 0 and 0 (fig. 1 B). The splice-site phasing of the seven-exon class is nearly identical: 0, 0, –, 0, 1, 0, 0 and 0. The exception is the fusion of the exons designated 3 and 4 in the seven-exon-containing class. In both classes, the translational start site resides in exon 2 and the reactive site loop (RSL) in the final exon (fig. 3). The presence of only eight-exon-containing genes in chickens suggests that the seven-exon-containing genes in mammals arose subsequently by intron loss [7]. The human clade B serpins map to one of two clusters located at 18q21.3 (*SERPINB2, -B3, -B4, -B5, -B7, -B8, -B10, -B11, -B12* and *-B13*) [9, 10] and 6p25 (*SERPINB1, -B6* and *-B9*) [11, 12] (fig 4).

Human clade B serpin expression patterns

The tight clustering of the human clade B serpin genes to two chromosomal locations raises the possibility that their expression is governed by some form of coordinate regulation. However, qualitative assessment of the tissue expression patterns of the human clade B serpins suggests that the control of their transcriptional activity is more complex than originally appreciated (table 2). For example, SERPINB1 and -B6 were detected in 23 and 28 of the 31 tissues examined, respectively. In contrast, SERPINB10 and -B13 were confined to 5 or fewer tissues. The remaining clade B serpins were detected in the range of 10–18 different tissues. When individual tissues were examined for clade B serpin expression, the results also were variable. Of the 31 tissues examined, 28 different clade B serpin expression patterns were detected. Only the eye and larynx, pancreas and placenta and muscle and liver showed expression patterns that were similar to the other, respectively. On average, each tissue expressed 6 serpin genes (range 1–10). Although these expression profiles will change as more sensitive detection systems are employed and as larger sample sizes are assayed under different conditions, these data underscore two general observations. First, the clade B serpins are expressed in a wide variety of human tissues. Second, the anti-proteinase defense system of most tissues contains different combinations of clade B serpins.

Structural studies on clade B serpins

Serpins have been the subject of intense structural study for the past 2 decades. The first serpin structure to be determined was that of cleaved α1-antitrypsin (SERPINA1) [13]. The structure revealed the nature of the serpin fold and also highlighted the puzzle that has subsequently proved defining for the field: the region responsible for interacting with the target protease, the RSL, lies buried and inaccessible, forming the 4th strand of the A β sheet (fig. 5A). Based upon these data, Loebermann and colleagues postulated that serpins must exist in an alternative conformation whereby the RSL must be available to interact with a target proteinase [13]. This prediction was confirmed through determination of the X-ray crystal structures of intact ovalbumin (a noninhibitory avian serpin that forms the major component of egg white, fig. 5B) [14] as well as that of plakalbumin (an RSL cleaved form of ovalbumin, fig. 5C) [15]. The structure of ovalbumin revealed that in the native state, the RSL is held at the top of the molecule as an exposed helix. The helical nature of the RSL most probably reflects the nonin-

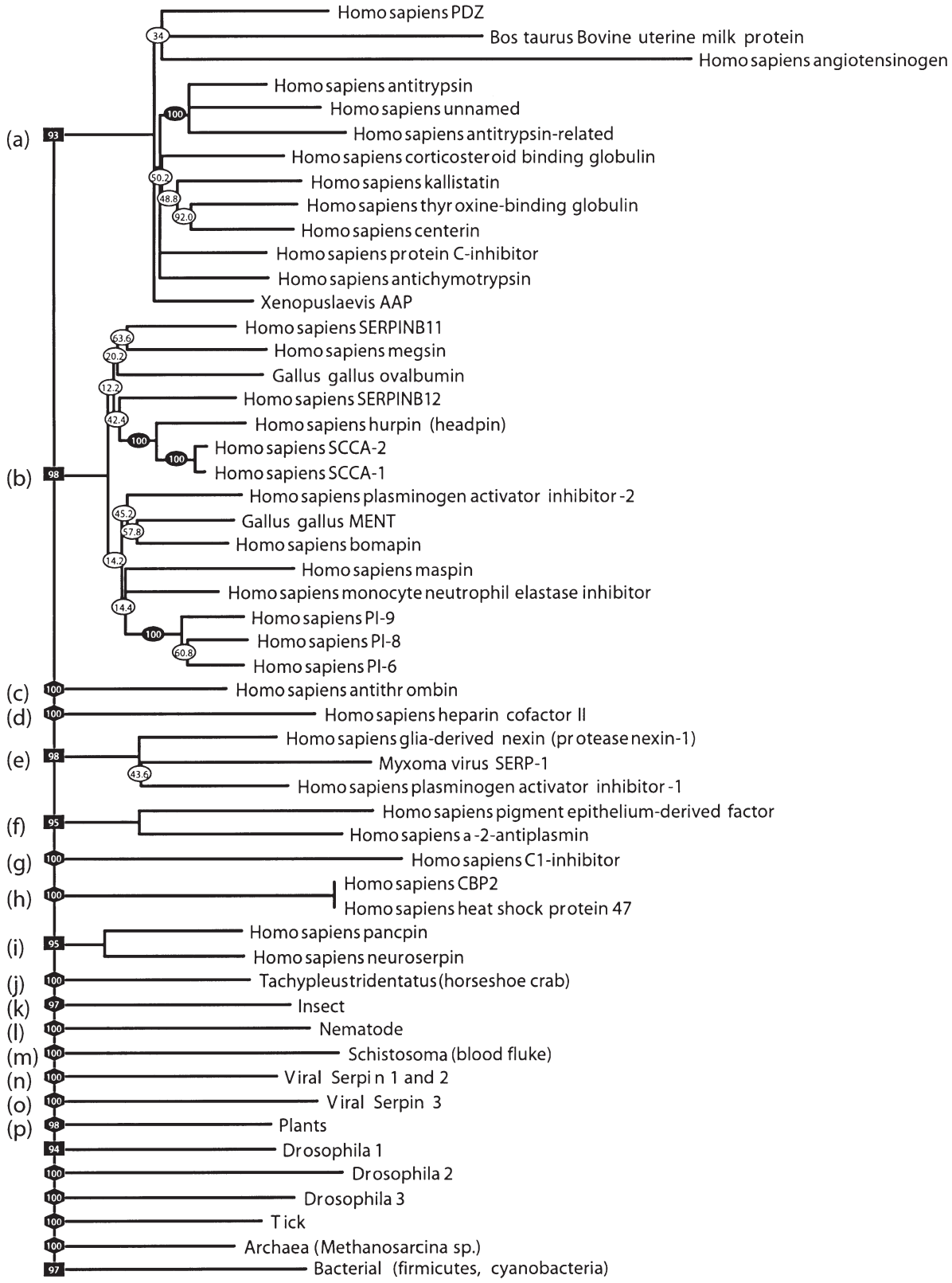

Figure 2. Multifurcating phylogenic tree showing the relationship between 35 human serpins and other members of the serpin superfamily. The tree was constructed as described by Irving and colleagues [4]. Conventional bootstrap values derived from maximum parsimony trees are highlighted by ovals. Hexagons indicate clades identified using the strict consensus method, and rectangles highlight clades identified using the comparison method. Each major clade (a–p) is consistent with the nomenclature described by Silverman and colleagues [2]. Figure from [164] (http://www. ehgonline.net/) and reproduced with permission from the Nature Publishing Group © 2003 Macmillan Publishers Ltd.

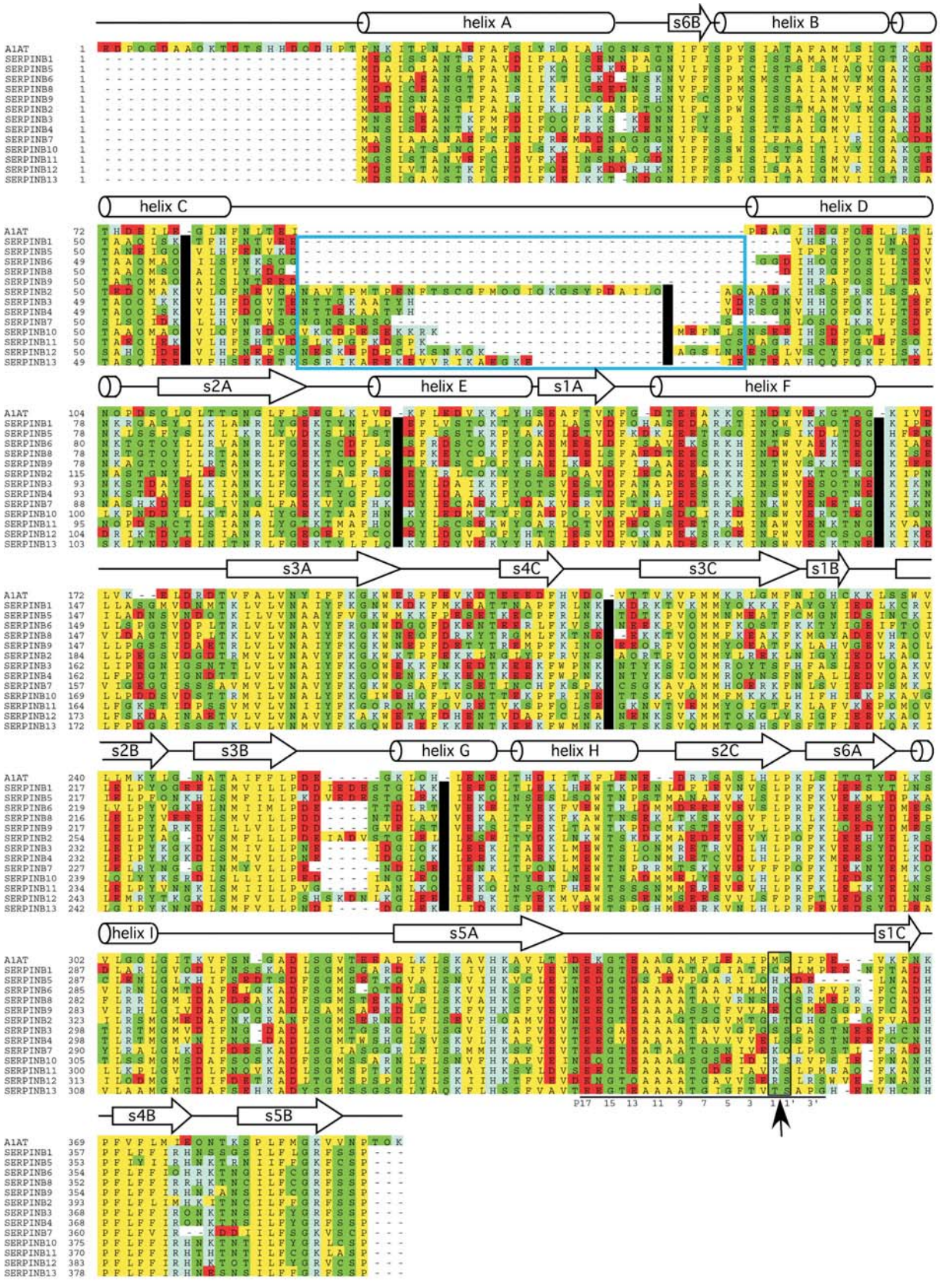

Figure 3. Amino acid sequence alignment of the 13 human clade B serpins. Amino acid sequences were aligned using ClustalW 1.8. The SeqVu 1.01 program (J. Gardner, Garvan Institute of Medical Research, Australia) was used to display the alignment. Colors indicate polar (green), nonpolar/hydrophobic (yellow), acidic (red) and basic (blue) residues. The CD-loop is boxed. The RSL is underlined and numbered from P17 to P3′. The canonical scissile bond is marked by an arrow. Thick vertical bars indicate positions of corresponding splice sites. Note the absence of a splice site and CD-loop for SERPINB1, -5, -6, -8 and -9. Structural motifs are indicated above the alignment, with cylinders and arrows indicating α helices and β strands, respectively. Amino acid numbering is relative to that of the prototypical serpin, α1-antritrypsin (A1AT, SERPINA1, top line). Figure modified (to include SERPINB11) from [9] with permission from the American Society for Biochemistry and Molecular Biology.

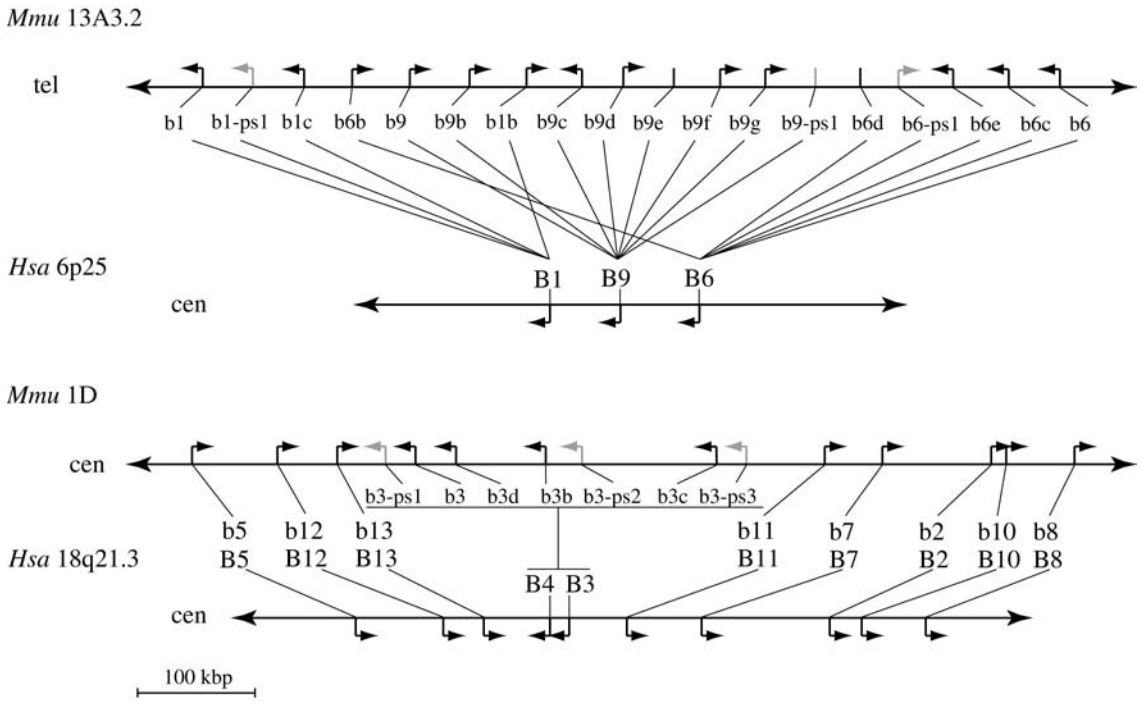

Figure 4. Comparison between human (*Hsa*) and mouse (*Mmu*) clade B serpin clusters. Synteny between the clade B serpins at *Mmu* 13A3.2 and *Hsa* 6p25 (upper panel and [145]) and *Mmu* 1D and *Hsa* 18q21 (bottom panel and [9]). Transcriptional orientation depicted by arrows. Pseudogenes (ps) are in grey. Data and portion of figure for *Mmu* 1D from D. J. Askew et al., unpublished.

hibitory nature of this serpin, since an α helix is nonideal for docking with a target proteinase. Indeed, subsequent structural studies on native inhibitory serpins have revealed the RSL of inhibitory serpins to be highly flexible, extended or β-strand-like. However, until the X-ray crystal structure of α1-antichymotrypsin (SERPINA3) was determined in 1994, ovalbumin represented the only native 'template' for the entire family [16].

Biochemical studies reveal that the native state of inhibitory serpins is thermally labile in comparison with that of the cleaved conformation. Thus the native fold of serpins is metastable, and the conformational change characteristic of inhibitory serpins is termed the 'stressed' (S) to 'relaxed' (R) transition [17]. In contrast to most serpins, ovalbumin is noninhibitory, and biochemical studies reveal that it is unable to undergo the S to R transition [18]. Consistent with these data, the structure of cleaved ovalbumin (plakalbumin) reveals the RSL of this serpin remains at the top of the molecule (fig. 5C) and is not able to insert into the A sheet. These data highlight the central role of the serpin conformational change in the inhibitory mechanism. Recent structural studies reveal that after docking and cleaving the RSL, the covalently attached proteinase is translocated 70 Å to the bottom of the serpin, where it resides in a distorted, inactive form [19].

In addition to the fundamental insight into the nature of the native state, structural studies on ovalbumin have proven invaluable in understanding the mechanism of the S to R transition. The RSL hinge region of most inhibitory serpins contains the conserved consensus motif $G_{P15}TEAAAT_{P8}$ (the standard P and P′ nomenclature is used to number amino acid positions N- and C-terminal outward from the scissile bond [P1-P1′], respectively [20]) that functions to facilitate rapid RSL insertion. This hinge region is mutated in ovalbumin and other noninhibitory serpins. An elegant structural study by Yamasaki and colleagues revealed that a single point mutation restoring the consensus residue at P14 (P14 R → T) is sufficient to allow RSL insertion in cleaved ovalbumin [21]. However, the mutant is still unable to function as a protease inhibitor, presumably because the rate of RSL insertion is not rapid enough. It is clear, however, that despite the extreme divergence in function, the ability to undergo the characteristic serpin conformational change remains programmed within the ovalbumin fold. Indeed, a recent study by Sugimoto and colleagues, reveals that ovalbumin exists in a labile conformation in newly deposited eggs and converts to a more stable form as the embryo within the egg develops [22].

Interestingly, while it is possible to unlock the ability to undergo conformational change in ovalbumin via a single site-directed mutant, the opposite effect in an inhibitory serpin cannot be achieved so easily. For example, a P14 T → R mutation in α1-antichymotrypsin (SERPINA3), whilst abolishing inhibitory activity, does not remove the ability to undergo a conformational change [23].

Table 2. Normal tissue expression patterns for human clade B serpins*.

SERPINB	Neuro-muscular			Resp.		Endocrine						Heme-lymphoid				GI tract						GU system					CV		Misc.		
	brain	eye	muscle	lung	larynx	adrenal	breast	pancreas	parathyroid	prostate	thyroid	blood	bone marrow	lymph node & tonsil	spleen	colon	esophagus	liver	stomach	tongue	bladder	cervix	kidney	testis	uterus	ovary	heart	blood vessel	bone	skin	placenta
1	+	+		+	+		+	+	+	+	+	+	+	+		+			+			+	+	+	+	+		+	+	+	+
2	+			+			+	+		+		+				+			+						+	+	+	+		+	+
3	+			+			+	+		+	+		+	+		+	+			+		+		+	+	+		+		+	+
4	+			+				+		+	+		+	+		+	+			+		+		+	+					+	+
5				+				+		+						+			+	+	+				+	+	+			+	+
6	+	+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
7	+						+	+					+				+						+			+		+		+	+	+
8	+		+	+			+	+		+				+		+		+	+				+			+	+		+	+	+
9	+		+	+			+	+					+	+		+		+	+				+		+	+	+	+	+			+
10													+																			
11				+						+				+																		+
12	+		+	+				+					+	+	+			+					+	+	+	+	+				+	+
13	+						+									+						+										

* Expression detected by dbEST, SAGE, RT-PCR, Northern blotting and/or immunohistochemistry. Data on SERPINB11 provided by S. Cataltepe and G. A. Silverman [unpublished].
Table modified from [9] with permission from the American Society for Biochemistry and Molecular Biology.

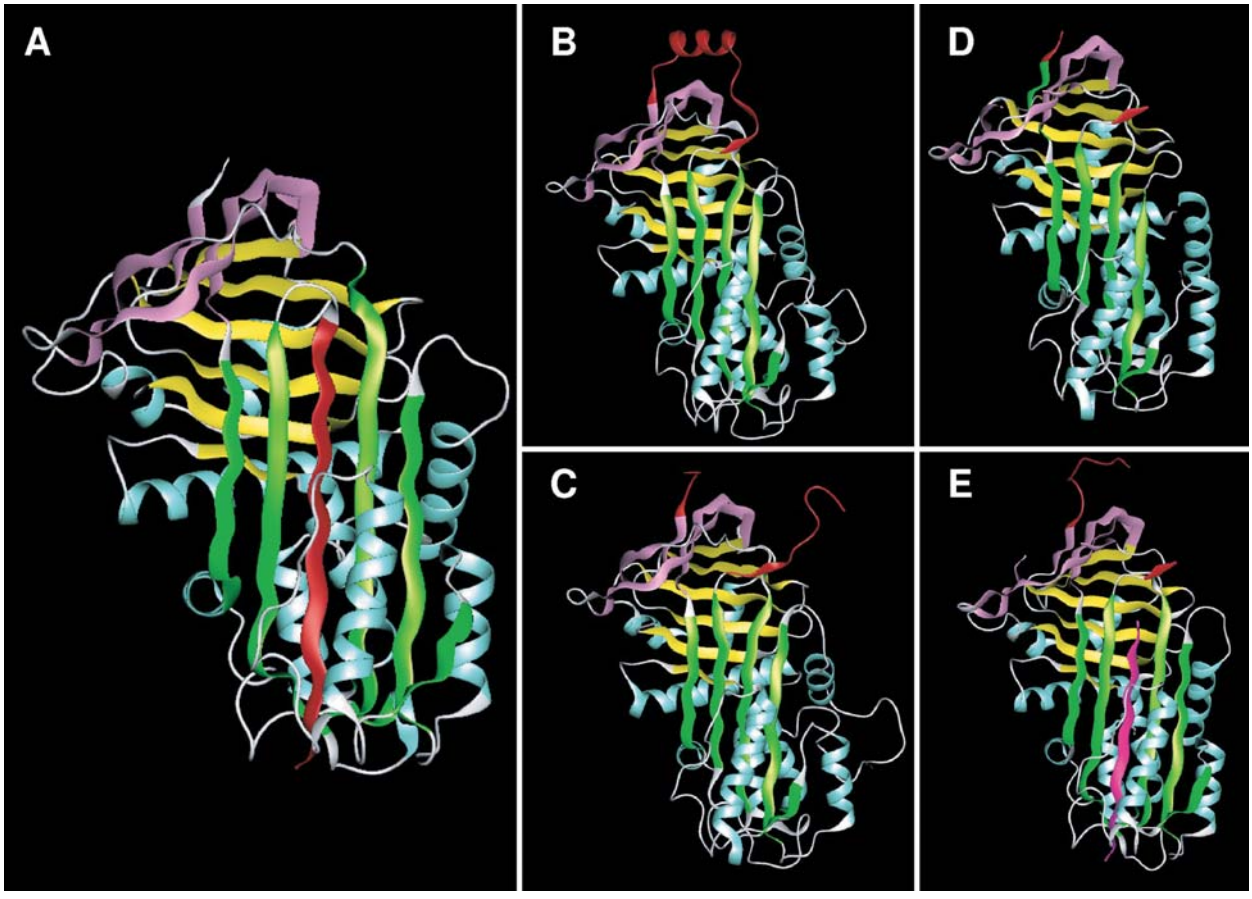

Figure 5. X-ray crystal structures of intracellular serpins. In all parts the A sheet is in green, the B sheet in yellow, the C sheet in pink and the RSL in red. Helices are shown in cyan. (*A*) Cleaved horse leukocyte elastase inhibitor (PDB: 1HLE), (*B*) intact ovalbumin (PDB: 1OVA), (*C*) cleaved ovalbumin (plakalbumin, PDB: 1JTI), (*D*) SERPINB2 (PAI2, PDB: 1BY7) and (*E*) SERPINB2 in complex with an RSL peptide (purple) (PDB: 1JRR).

To date, only two other clade B serpins have been the subject of structural studies, horse leukocyte elastase inhibitor (HLEI), which is ~80% identical to human SERPINB1, and SERPINB2 (PAI2). The high-resolution structure of HLEI (fig. 5A) represents the only cleaved inhibitory-type clade B serpin [24]. The structure of this molecule closely resembles that of cleaved α1-antitrypsin (SERPINA1) and α1-antichymotrypsin (SERPINA3).

The X-ray crystal structure of the native conformation of a SERPINB2 (PAI2) variant lacking the CD-loop has been determined (fig. 5D) [25]. In addition, the structure of this variant bound to a peptide mimicking the RSL has also been solved (fig. 5E) [26]. The RSL is partially disordered in both structures, indicating a high degree of flexibility in this region and consistent with the requirement for the RSL to act as an attractive 'bait' for target proteases. While the absence of the CD-loop does not affect the inhibitory capacity of the molecule, this region performs an important role in modulating function, most probably through interaction with specific cofactors or protein-binding partners [27]. No structural information yet exists for the CD-loop, as this part of the molecule probably adopts a flexible unstructured conformation that may prove resistant to structural studies in the absence of stabilizing cofactors.

Structural comparisons of native, inhibitory serpins with their cleaved and peptide-bound counterparts reveal a cluster of conserved residues underneath the A β sheet that move to accommodate and form hydrogen bonds to residues in the inserting RSL [4, 25, 28]. In particular, three residues within the shutter region (Ser^{56}, Asn^{186} and His^{334} (α1-antitrypsin [SERPINA1] numbering) interact with one another in the native state and function to receive the sidechain of the P8 position from the RSL upon transition to the cleaved form. A similar pattern of conserved residues exists at the top of the A sheet within the breach and functions to receive the RSL as it first inserts [28].

Although ~50 serpin structures are deposited in the Protein Data Bank (pdb), the clade B serpins are severely underrepresented [29]. One likely explanation for this discrepancy is the limited availability of material, since many intracellular clade B serpins cannot be purified eas-

ily from their source (one exception, of course, being ovalbumin from egg white). In addition, many clade B serpins have proven difficult to produce in recombinant form. However, the available clade B serpin structures have already provided a wealth of information in regard to the serpin scaffold. Future studies examining the structural basis for interaction between clade B serpins and non-proteinase binding partners and cofactors should provide further insight into biological function.

Human clade B serpin family members (table 1)

SERPINB1 (MNEI)

In 1985, Remold-O'Donnell described a monocyte-derived protein that was a fast-acting inhibitor of neutrophil and pancreatic elastase [30]. Subsequent isolation of the protein and complementary DNA (cDNA) from horse and humans, respectively, confirmed that it was a member of the serpin superfamily [31, 32]. Like several other serpins, SERPINB1 uses two different residues to neutralize different types of proteinases (table 3) [33]. *Phe*^{343} functions as the P1 residue in the inhibition of chymase, chymotrypsin and cathepsin (cat) G, whereas *Cys*^{344} (the canonical P1 position relative to SERPINA1) assumes that function for the inhibition of neutrophil elastase, pancreatic elastase, proteinase 3 and prostate-specific antigen. SERPINB1 is unique, as it is the only human clade B serpin that effectively neutralizes all of the major proteinases of the azurophilic granule.

The biologic function of SERPINB1 has been examined in a rat lung injury model. Recombinant SERPINB1 was capable of blocking both the hemorrhagic and epithelial permeability changes associated with instillation of neutrophil elastase [34]. Other functions ascribed to SERPINB1 include a mitogenic effect on human tumor infiltrating lymphocytes [35] and a cation-independent, endonuclease activity [36]. A27-kDa C-terminal fragment from SERPINB1 (L-DNAse II) is associated with this DNAse activity. L-DNAse II is derived from SERPINB1 by proteolytic processing or incubation at an acidic pH [37]. BHK cells, overexpressing the porcine orthologue of SERPINB1, showed increased apoptosis, DNA degradation and L-DNAse II formation after cytosolic acidification by blocking a Na^+/H^+ antiporter with hexamethylene amiloride [38]. In contrast, these cells were protected from etoposide-induced apoptosis in comparison to mock-transfected cell lines. Thus, SERPINB1 may enhance or delay apoptosis depending on the mode of induction.

SERPINB2 (plasminogen activator inhibitor type 2, PAI2)

SERPINB2 is an inhibitor of two-chain urokinase-type plasminogen activator (u-PA) and tissue-type plasmino-

Table 3. Cleavage sites in the reactive site loops of human clade B serpins with overlapping reactive centers.

Serpin	Reactive site loop																						Ref.
	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	1′	2′	3′	4′	5′	
SERPINB1 (MNEI)	E	E	G	T	E	A	A	A	A	T	A	G	I	A	T	F[a]	C[e]	M	L	M	P	E	[33]
SERPINB4 (SCCA2)	E	E	G	V	E	A	A	A	A	T	A	V	V	V	V[f]	E	L[g]	S	S	P	S	T	[78]
SERPINB6 (PI6)	E	E	G	T	E	A	A	A	A	T	A	A	I	M	M	M[a]	R[c]	C	A	R	F	V	[110]
SERPINB8 (PI8)	E	E	G	T	E	A	A	A	A	T	A	V	V	R	N	S	R[i]	C	S[a]	R	M	E	[124]
SERPINB9 (PI9)	E	E	G	T	E	A	A	A	A	S	S	C	F	V	V	A	E[h]	C	C[e]	M	E	S	[124, 165]
Serpinb3a (mouse)	E	E	G	T	E	A	A	A	A	T	G	V	E	V[g]	S	L	T[f]	S	A	Q	I	A	[166]

Cleavage sites (vertical bar) for [a] chymotrypsin, [b] plasmin, [c] trypsin-like, [d] factor XII, [e] human neutrophil elastase, [f] catS, [g] catG, [h] granzyme B and [i] thrombin. Numbering of the reactive site loop is based on the canonical sequence of α1-antitrypsin (SERPINA1). Reprinted in modified form with permission from [166]. Copyright (2002) American Chemical Society.

gen activator (t-PA), and to a lesser degree, acrosin and plasmin (reviewed in [39, 40]). SERPINB2 also inhibits receptor bound u-PA [41, 42]. Although an inhibitor of u-PA was partially purified in the 1960s, an amino acid sequence was not obtained until 1986 [43]. SERPINB2 appears as an intracellular form with a molecular mass of ~47 kDa and an extracellular form with a mass of ~60–70 kDa. The difference in molecular mass between these forms is due to *N*- and *O*-linked glycosylation [44]. SERPINB2 was detected initially in cells of the monocytic lineage and the placenta. Constitutive or inducible SERPINB2 expression is found also in skin, hair follicles, endothelium, mesothelium and fibroblasts (table 2). In the skin, SERPINB2 is expressed primarily in the suprabasal layers and is integrated into the cornified envelope [45]. SERPINB2 is cross-linked covalently into this envelope via a transglutamination reaction involving three *Glu* residues in the CD-loop [46]. The function of this serpin in the cornified envelope is unclear but may serve as a structural component, as most of this protein is in the inactive, cleaved form. The presence of active SERPINB2 in the granular and spinous layers suggests that this serpin is involved in epidermal cell differentiation or serves as a regulator of the u-PA system that is activated upon wound healing. However, mice lacking *Serpinb2* show normal development of the epidermis and its appendages, as well as a normal wound-healing response to a punch biopsy [47]. Further analysis of these mice using different stressors should help reveal the biologic function of SERPINB2 in the epidermis.

Under certain circumstances, SERPINB2 demonstrates a cytoprotective role. SERPINB2 protects HT-1080 fibrosarcoma cells from tumor necrosis factor-α (TNFα) induced apoptosis [48]. SERPINB2 also protects HeLa cells from TNFα-induced apoptosis, but not that induced by ultraviolet (UV) or ionizing radiation [49]. This protection is dependent on the P1 *Arg* residue in the RSL and the CD-loop (see below) [27, 49]. Since SERPINB2 does not inhibit caspases directly, these findings suggest that SERPINB2 protects against apoptosis by neutralizing a serine-like proteinase that acts proximally or distally to the caspase activation sequence or by regulating a caspase-independent death pathway. SERPINB2 also protects HeLa cells from the cytopathic effects of alphavirus infection [50]. In this case, protection appeared to be due to the induction of antiviral genes such as interferons α and β.

Due to the importance of cell surface plasminogen activation and subsequent extracellular remodeling in facilitating tumor invasion and metastases, numerous studies have attempted to determine whether concomitant SERPINB2 expression might serve as a positive prognostic indicator for certain types of cancer (reviewed in [40]). In breast carcinomas, SERPINB2 expression is correlated with increased relapse-free survival only in those tumors that also expressed u-PA [51]. Low levels of SERPINB2 are associated with tumor invasiveness in non-small cell lung carcinomas [52]. Similarly, decreased SERPINB2 expression is associated with the progression of keratinocytes to squamous cell carcinomas [53] and with metastatic pancreatic carcinomas [54]. A human metastatic melanoma cell line, engineered to overexpress SERPINB2, became encapsulated upon transplantation into *scid*/*scid* mice [55]. Relative to nontransfected tumor cells, the SERPINB2-expressing cells yielded significantly fewer numbers of metastases.

Paradoxically, in other studies, increased SERPINB2 expression is associated with a poorer prognosis and decreased survival [56]. In part, this may be due to the ability of SERPINB2 to inhibit some apoptosis pathways. Indeed, transgenic mice overexpressing SERPINB2 in basal keratinocytes show normal skin development but enhanced papilloma formation using the classical DMBA/TPA (initiator/promoter) carcinogenesis model [57]. After cessation of treatment, papillomas in the control mice regressed by massive apoptosis, whereas tumors in the SERPINB2-overexpressing mice showed markedly diminished numbers of apoptotic cells, and many progressed to frank carcinomas.

The CD-loop of SERPINB2 contains 33 amino acids and is the longest of its type within the human clade B serpins. In addition to serving as a site for transglutamination, this loop is necessary for inhibition of apoptosis [27] and the binding of the cytosolic proteins annexin I, II, IV and V [58]. The significance of annexin binding is unknown. The CD-loop also regulates SERPINB2 polymerization [59]. Although most wild-type serpins can be induced to polymerize under experimental conditions, SERPINB2 polymerizes spontaneously under physiologic conditions. Polymerization is facilitated by the formation of an intramolecular disulfide bond between Cys^{79} of the CD-loop and Cys^{161} at the bottom of helix F. Under reducing conditions, the disulfide bond is broken, β-sheet A closes and the serpin is resistant to polymerization. The redox sensitivity of this process may explain why cytosolic SERPINB2 is monomeric and that in the secretory pathway is polymeric.

SERPINB3 (squamous cell carcinoma, antigen 1, SCCA1)

For over 20 years, the squamous cell carcinoma antigen (SCCA) has served as a anonymous, diagnostic serum marker for advanced squamous cell carcinomas (SCCs) of the uterine cervix, lung, esophagus, and head and neck (HNSCCs) (reviewed in [60]). For certain tumors, SCCA also serves as a prognostic indicator. Elevated pretreatment SCCA levels portend a bad outcome in stage IB and IIA cervical carcinomas [61, 62]. Moreover, a persistent increase in SCCA after radiation therapy is the most important negative predictor of disease-free survival in

HNSCC [63, 64]. Interestingly, radiation therapy induces a rapid increase in the synthesis and release of SCCA by SCC cells [65]. These findings suggest that the expression of SCCA is in some way associated with the enhanced survival of SCC cells. In addition to patients with certain tumors, serum SCCA levels are elevated in individuals with inflammatory conditions of the lung (e.g. sarcoidosis, pneumonia) and skin (e.g. eczema, psoriasis) (reviewed in [60]).

Initial biochemical analysis of SCCA revealed the presence of acidic and neutral isoforms [60]. However, it was not until cloning of the cDNA and genomic DNA sequence that it was realized that the SCCA isoforms were encoded by two nearly identical genes, *SCCA1* (*SERPINB3*) and *SCCA2* (*SERPINB4*), of the serpin superfamily [66, 67]. Although SERPINB3 and -B4 are nearly identical (92%) in their amino acid sequences, critical differences in their RSLs suggested that they inhibited distinct types of proteinases. Indeed, SERPINB3 paradoxically inhibited the lysosomal cysteine proteinases catK, L, S and V, whereas SERPINB4 inhibited the chymotrypsin-like serine proteinases catG and mast cell chymase [68, 69]. SERPINB3 was the first serpin shown to inhibit papain-like cysteine proteinases and this inhibition was mechanistically similar to the RSL-dependent mechanism used by other serpins to inhibit serine proteinases [70].

In normal tissues, SERPINB3 and -B4 are coexpressed in the suprabasal layers of stratified squamous epithelia, Hassall's corpuscles of the thymus and the pseudo-stratified columnar epithelium of the conducting airways (table 2) [71].

Since several serpins (e.g. crmA [72], SERPINB2 [49], SERPINB9 [73, 74]) inhibit cell death pathways, several investigators hypothesized that SERPINB3 and SERPINB4 might provide SCCs with a survival advantage by regulating an intracellular cell death cascade. This notion is supported by transduction studies reported by Suminami and colleagues [75]. PCI-51 is a human HNSCC cell line that does not express SERPINB3. PCI-51 cells, overexpressing SERPINB3, show increased resistance to apoptosis induced by SN-38, TNFα or natural killer (NK) cells as compared with mock-transduced controls. A mouse SCC line, KLN-205, transduced to overexpress SERPINB3 and injected into nude mice, developed tumors of larger volume in comparison with control cells [75]. Conversely, the SERPINB3-expressing SKG IIIa cervical squamous cell carcinoma cell, transduced to express an antisense transcript, showed increased apoptosis in vitro [75] and decreased tumor volume, in vivo [76]. Using high doses of γ-irradiation (5–20 Gy) as the inducer of cell death, Murakami and colleagues showed that transfected human kidney cells expressing either SERPINB3 or SERPINB4 showed a significant increase in survival as compared with mock transfected controls [77]. Transfected cells showed decreased amounts of caspase-9 and -3 activation, but no changes in caspase-2 or -8 activity. Transfected cells also appeared to show a decrease in activation of the mitogen-activated protein kinase (MAPK) pathway. Further studies are needed to determine whether SERPINB3 and SERPINB4 attenuate these stress response pathways by direct inhibition of a proteinase cascade or by some accessory function unrelated to RSL binding.

SERPINB4 (squamous cell carcinoma antigen 2, SCCA2)

Although SERPINB4 inhibits the chymotrypsin-like serine proteinases, catG and human mast cell chymase, it can inhibit the cysteine proteinase, catS, albeit at a rate 50-fold less than that of SERPINB3 [78]. The inhibition of catS by a mutant SERPINB4 containing the RSL of SERPINB3 is comparable to that of wild-type SERPINB3. This finding suggested that there were no motifs outside the RSL, and only eight residues within, that were directing catS-specific inhibition. SERPINB4 molecules containing different RSL mutations showed that no single amino acid substitution could convert SERPINB4 into a more potent cysteine proteinase inhibitor. Rather, different combinations of mutations led to incremental increases in catS inhibitory activity with residues in four positions (P1, P3′, P4′ and P11′) accounting for 80% of the difference in activity between SERPINB3 and SERPINB4. These studies also showed the importance of a *Pro* residue in the P3′ position for efficient inhibition of catS by both wild-type SERPINB3 and mutated SERPINB4. Molecular modeling studies suggested that this residue might facilitate positioning of the RSL within the active site of the cysteine proteinase.

As described above with SERPINB3, SERPINB4 may protect cells from injury. In additional support of this hypothesis, HeLa cells transfected with the SERPINB4 cDNA showed increased resistance to TNFα-induced apoptosis [79]. Moreover, this protective effect was dependent upon residues in the reactive center, suggesting that this serpin inhibits a catG-like proteinase involved in induction of the cell death pathway.

SERPINB5 (maspin, PI5)

Using differential display, Zou and colleagues identified a novel member of the serpin family that was expressed in normal breast epithelial and myoepithelial cells but was downregulated or absent in most mammary carcinoma cell lines and histological samples from advanced carcinomas [80]. Breast carcinoma cell lines engineered to over-express SERPINB5 showed decreased invasiveness and motility in vitro, which correlated with their diminished capacity to form tumors or metastases in nude

mice in vivo. Cell motility and invasiveness could be inhibited by the addition of exogenous recombinant serpin where the protein localized to the cell surface [81]. In addition to its effects on cell motility and invasiveness, the restoration of SERPINB5 expression in breast carcinoma cells show enhanced susceptibility to apoptosis [82, 83]. Based on these observations and the knowledge that the gene was not deleted or mutated in breast cancers, the authors concluded that SERPINB5 functions as a class II tumor suppressor gene in mammary gland epithelia. The downregulation of SERPINB5 expression was associated with cytosine methylation, chromatin deacetylation and chromatin condensation of the maspin promoter [84]. In addition to epigenetic mechanisms, the SERPINB5 promoter is a p53 target and may be downregulated in tumors with p53 mutations [85].

The ability of SERPINB5 to inhibit tumor invasiveness suggests that the loss of SERPINB5 expression would be associated with a poor outcome in patients with cancer. Indeed, the loss of SERPINB5 expression was found to be a poor prognostic indicator in several series of patients with breast [86–88], prostate [89] and colon [90] carcinomas. However, SERPINB5 expression was upregulated in a proportion of pancreatic [91], ovarian [92] and lung (non-small cell) [93] carcinomas. This paradoxical relationship between SERPINB5 and different tumors may be explained by the pleiotropic activity of SERPINB5 in different tissues, its association with other interacting proteins or the nuclear to cytoplasmic serpin ratio [94]. In one study, preferential localization of SERPINB5 to the nucleus or the cytoplasm was associated with a good or poor prognosis, respectively [95].

Although SERPINB5 requires an intact RSL to inhibit tumor cell motility [96], this serpin is unlikely to inhibit a proteinase by the canonical suicide-substrate-like mechanism. The hinge region of the RSL of SERPINB5 contains *Gly*, *Ser*, *Glu* and *Pro* residues instead of the consensus *Thr*, *Ala*, *Ala* and *Thr* residues at the P14, P12, P10 and P8 positions, respectively. The presence of these residues in the RSL of SERPINB5 is likely to impair loop insertion into β-sheet A and permit proteinase dissociation before the formation of stable acyl-enzyme complex [97]. Consistent with this hypothesis, recombinant SERPINB5 is unable to undergo the stressed to relaxed conformation characteristic of inhibitory-type serpins [98]. Preliminary studies suggested that SERPINB5 inhibited t-PA [99]. However, subsequent studies could not confirm this observation [100, 101]. We conclude that SERPINB5, like ovalbumin, is a noninhibitory type member of the serpin family.

SERPINB5 may participate in other biologic functions. Zhang and colleagues showed that SERPINB5 has antiangiogenic activity, as recombinant protein blocked endothelial cell migration induced by vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (FGF), inhibited corneal neovascularization and decreased the density of microvessels in a xenograft tumor transplant model [102]. SERPINB5 plays a role in normal development, as homozygous null mutations (by targeted deletion) result in embryonic lethality [103]. Unlike other clade B serpin knockouts studied to date, heterozygous loss of *Serpinb5* also results in an abnormal phenotype. *Serpinb5* haploinsufficiency results in abnormal ovulation and is manifest by decreased fertility and smaller litter sizes. Targeted overexpression of SERPINB5 to the mammary gland inhibits the development of lobular-alveolar structures during pregnancy [101]. SERPINB5 expression may play a role in limiting cytotrophoblast invasion after implantation [104].

SERPINB5 is expressed also in the thymus, testis and epithelium of the lung, small intestine, skin, prostate and mammary gland (table 2) [84, 105]. The function of SERPINB5 in these tissues under normal conditions is unknown.

SERPINB6 (placental thrombin inhibitor, cytoplasmic antiproteinase, CAP, PI6)

In 1993, Coughlin and colleagues purified a protein from placental extracts that bound to and inhibited thrombin [106]. A similar protein was isolated from the cytosolic extracts of a monkey kidney epithelial cell line and shown to inhibit thrombin, as well as trypsin, u-PA and factor Xa [107]. Amino acid sequencing and subsequent molecular cloning yielded a cDNA that encoded for a ~42-kDa serpin with several oxidation-sensitive residues surrounding the reactive center (*Met-Met-Met-Arg-Cys*^{342} residues at the putative P4-P3-P2-P1-P1′ positions, respectively) [108, 109]. This hypothesis was confirmed, as preincubating the serpin with iodoacetamide eliminated its ability to bind thrombin [109].

In addition to inhibiting trypsin-like proteinases, SERPINB6 neutralizes chymotrypsin [110], catG [111] and the kallikrein family member neuropsin [112]. Of these proteinases, it most efficiently inhibits catG. The inhibition of chymotrypsin utilizes *Met*^{340} (the canonical P2 position) as the reactive center, in contrast to the *Arg*^{341} utilized by thrombin and catG. This is another example of a clade B serpin using a different reactive center to expand its inhibitory profile (table 3).

A tissue survey revealed that SERPINB6 is widely distributed including heart, lung, liver muscle, kidney and pancreas (table 2). SERPINB6 is also found in hematopoietic cells, including megakaryocyte cell lines, platelets, monocytes, neutrophils and myelomonocytic cell lines (e.g. THP-1, U947 and PMA-treated HL60 cells) [111]. In some cell extracts, SERPINB6 was found in a complex with catG. While complex formation occurred after cell lysis and was secondary to the admixing of cytosolic contents with granule proteinases, this phenome-

non supports the notion that cytosolic serpins can guard the cytoplasm from adventitious proteinase activity.
Like many other clade B serpins, such as SERPINB1, -B5, -B7, -B9 and -B12, SERPINB6 is expressed in the suprabasal layers of the epidermis [113]. SERPINB6 expression increases 24-fold during the differentiation of primary keratinocytes, and parallels the expression of neuropsin [F. L. Scott and P. I. Bird, unpublished]. The role of this serpin in the epidermis is unknown, but it may regulate ectopic neuropsin. Alternatively, like SERPINB2, it could be associated with the cornified envelope or may be needed to neutralize endogenous proteinases as the keratinocyte undergoes a form of apoptosis in which a modified cell corpse is retained. SERPINB6 also is detected in a complex with kallikrein 2 in extracts from prostate cancer cells and, to a lesser extent, normal prostate tissue [114]. This complex appears to occur in the extracellular space after cell lysis and is not found in the seminal plasma [115]. This complex may serve as a marker for the tissue damage associated with prostate cancer.
SERPINB6 has a nucleo-cytoplasmic subcellular distribution and is incapable of being secreted (see below).

SERPINB7 **(megsin)**
The cDNA for this gene was cloned from a cell line synthesizing a factor that induced the maturation of megakaryocytes from cultures of interleukin (IL)-3-stimulated murine bone marrow cells [116]. The activity of this megakaryocyte maturation factor (MMF) was confirmed using a recombinant protein. Miyata and colleagues performed random DNA sequencing of cDNA clones derived from a human mesangial cell library [117]. They isolated an abundant transcript that by database searching appeared to be unique to this cell type. However, the cDNA sequence of this clone, megsin (SERPINB7), was identical to MMF. SERPINB7 RNA and protein are upregulated in glomerular diseases associated with mesangial cell proliferation and/or mesangial matrix deposition such as immunoglobulin (Ig)A and diabetic nephropathy (reviewed in [118]). SERPINB7 may be a causal factor in these disorders, as transgenic mice overexpressing the human protein showed increased mesangial cell proliferation and extracellular matrix deposition [119].
By amino acid sequence analysis, SERPINB7 appears to have a functional RSL with a *Lys* residue at the putative P1 position. Limited analysis using recombinant proteins suggested that SERPINB7 inhibits plasmin, but not t-PA or thrombin [119]. However, this inhibitory activity conflicts with an earlier study [116].

SERPINB8 **(cytoplasmic antiproteinase 2, CAP2, PI8)**
SERPINB8 and SERPINB9 were isolated by screening a human λgt11 placental cDNA library under conditions of low hybridization stringency using SERPINB6 cDNA as a probe [120]. By Northern blotting, SERPINB8 has a broad expression pattern with the highest levels in skeletal muscle, liver, lung and placenta (table 2). By immunohistochemistry, SERPINB8 is detected in the nuclei of stratified squamous epithelia, monocytes and neuroendocrine cells of the pancreas pituitary gland and gastrointestinal tract [121]. The biologic function of SERPINB8 is unknown, but its expression is up-regulated in response to the proinflammatory cytokine TNFα [122].
The inhibitory profile of SERPINB8 is derived from its use of at least two active centers (table 3). Using Arg^{339} at the canonical P1 position, SERPINB8 neutralizes thrombin, trypsin and factor Xa [123]. In contrast, Ser^{341}, located at the canonical P2′ position, is used to inhibit chymotrypsin [124]. SERPINB8 also inhibits subtilisin A and furin, but the RSL cleavage sites have not been mapped [123, 125]. Most likely, furin cleaves after Arg^{339} rather than Arg^{342}. Of all of these SERPINB8-proteinase interactions, the binding to furin-like proteinases may be the most physiologic, as demonstrated by a low stoichiometry of inhibition and relatively high second-order rate constant.

SERPINB9 **(cytoplasmic antiproteinase 3, CAP3, PI9)**
SERPINB9 was originally isolated from placental cDNA libraries using SERPINB6 probes [120], and from bone marrow messenger RNA (mRNA) by degenerate reverse transcriptase-polymerase chain reaction (RT-PCR) [73]. SERPINB9 is the only human serpin with an acidic residue (*Glu*) at the canonical P1 position. This is significant, because caspases and granzyme B prefer an acidic residue (*Asp*) at this position and group I, II and III caspases prefer aromatic, aspartic and aliphatic nonaromatic residues at the P4 positions, respectively. SERPINB9 is an efficient granzyme B inhibitor [73] and plays an important biological function in cytotoxic lymphocytes and antigen-presenting cells by serving as an antidote to misdirected granzyme B [74, 126]. Cytotoxic T cells mediate cell death (apoptosis) by Fas receptor ligation or by cytotoxic granule exocytosis. Cytotoxic granules contain perforin and granzyme B, which are taken up by the target cell and released into the cytoplasm. In turn, granzyme B can activate caspases, and apoptotic death ensues. SERPINB9 appears to protect cytotoxic lymphocytes from self-inflicted injury by neutralizing cytosolic granzyme B that may have leaked from cytotoxic granules or by the reuptake of granule contents extruded into the extracellular space. Strong evidence for this hypothesis is derived from experiments showing that transfected cells expressing SERPINB9 show a dose-dependent resistance to granzyme B and perforin mediated injury [74]. Also, SERPINB9 that is associated with the cytoplasmic face of cytotoxic lymphocyte granules [127] and cytolytic

lymphocytes overexpressing SERPINB9 have enhanced killing potency [127]. Interestingly, SERPINB9 does not, but a SERPINB9 *Glu*P1*Asp* mutant does, protect cells from Fas-mediated cell death. This supports the notion that wild-type SERPINB9 is not a physiologic inhibitor of initiator or executioner caspases, and that it does not interfere with death receptor-mediated downsizing of dendritic or cytotoxic lymphocyte populations at the end of the immune response.

SERPINB9 is detected in the lung [120], vascular smooth muscle cells [128], endothelial cells of arteries and veins, mesothelial cells [129], cytotrophoblast of the placenta, dendritic cells, T lymphocytes, B cells of the marginal zone, Sertoli cells and spermatagonia of the testis, granulosa cells of the ovary and the lens of the eye [127, 130] (table 2).

SERPINB9 expression can be induced by inflammatory stimuli in endothelial cells, hepatocytes, dendritic cells and cytotoxic lymphocytes [126, 127, 129, 131]. Interestingly, SERPINB9 can be induced in HepG2 cells by incubation with IL-1β, lipopolysaccharide (LPS) or TPA [132]. Activation occurs via transcription factors NFκB (comprising the Rel family heterodimers p50 and p65) and AP-1 (comprising heterodimers containing either c-Jun and c-Fos or JunD and c-Fos), which bind to their respective DNA motifs within the SERPINB9 promoter. Collectively, these studies suggest that SERPINB9 expression is upregulated to protect hepatocytes from inflammation and to initiate a negative feedback loop by limiting the conversion of IL-1β [132]. What is most intriguing about this hypothesis is that this regulatory pathway is reminiscent of the feedback loop induced in *Drosophila* by activation of the *Toll* pathway [133]. Stimulation of the *Toll* pathway by fungal or Gram-positive bacterial infection leads to the generation of antimicrobial peptides and activation of the melanization cascade. *Toll* activation also upregulates the expression of Serpin-27A, which neutralizes a critical enzyme in the melanization cascade, prophenoloxidase-activating enzyme [134]. Thus, signaling via NFκB-related pathways in both humans and flies leads to upregulation of a serpin that, in turn, temporizes the inflammatory response.

Since the canonical P4-P1′ residues of SERPINB9 (*Val-Val-Ala-Glu-Cys*) are similar to those of the viral serpin and caspase inhibitor, CrmA (*Leu-Val-Ala-Asp-Cys*), it has been suggested that SERPINB9 could be a physiologic regulator of caspase activity. Relative to serpin-proteinase interactions in general and CrmA-caspase (e.g. caspase-1) interactions in particular, SERPINB9 appears to be a very weak ($k$ass $< 10^3$ M^{-1} s^{-1}) inhibitor of caspases-1, -4 and -8 [135], compared with its potent ability to inhibit granzyme B [73]. Interestingly, a *Glu*P1*Asp* SERPINB9 mutant is a less effective granzyme B inhibitor and, as expected, a more efficient caspase inhibitor [74]. Together, these findings suggest that SERPINB9 selectively inhibits granzyme B-induced injury but has a minimal effect on that induced by initiator (group III) or executioner (group II) caspases. Nevertheless, there is evidence to suggest that SERPINB9 plays a role in limiting the IL-1β (generated by caspase-1)-induced inflammation associated with atherosclerotic plaques [128]. Normal arterial endothelial cells contain abundant amounts of SERPINB9 and minimal amounts of IL-1β, whereas atheromas show the inverse relationship. Conceivably, a decrease in SERPINB9 activity allows caspase-1 to generate more IL-1β. In turn, IL-1β increases inflammation and arterial wall damage.

Finally, consistent with the hypothesis that SERPINB9 may interfere with the activation of certain apoptotic pathways triggered by cytotoxic T or NK cells, increased SERPINB9 expression correlates with a poor prognosis in anaplastic large cell lymphomas [136]. Thus, increased SERINB9 expression might help tumor cells evade cytolytic T or NK cell surveillance mechanisms [137].

SERPINB10 (bomapin, PI10)

Riewald and Schleff used PCR to isolate a unique serpin sequence from a human bone marrow cDNA library [138]. In vitro translated SERPINB10 formed SDS-stable complexes with thrombin and trypsin. Although a detailed kinetic analysis has yet to be published, this inhibitory profile is consistent with the *Arg* residue located at the canonical P1 position. Of all the clade B serpins, SERPINB10 appears to have the most restricted expression pattern (table 2). It is expressed almost exclusively in cells of the monocytic lineage [139]. In addition, it is unique among the human serpins in possessing a nuclear localization signal, which is present in the CD-loop.

The biologic function of SERPINB10 in monocytes is unknown. However, transfection of HeLa cells with a SERPINB10 expression plasmid protects these cells from TNFα/cylohexamide-induced apoptotic death [140]. Moreover, this protection was correlated with the appearance of high molecular weight SERPINB10 complexes in the cytoplasm. Although no proteinase was isolated from the complex per se, the authors suggest that the protective role of SERPINB10 was due to the neutralization of a cytosolic enzyme.

SERPINB11

Using high-throughput genomic sequence, a novel member of the human clade B serpin family, *SERPINB11,* was identified [S. Cataltepe et al., unpublished]. This gene maps to the serpin cluster at 18q21.3. Several cDNA clones were isolated and sequenced. Unlike most clade B serpins, the *SERPINB11* sequence contains several single-nucleotide polymorphisms (SNPs) that alter the coding sequence. Although all of the sequence variants contain a

functional RSL with *Lys-Ser* at the putative P1-P1′ positions, target proteinases have yet to be identified. However, only a few of the variants have been analyzed, and some SNPs may have a profound effect on serpin activity, as several alter well-conserved residues in the serpin backbone. Preliminary analysis using the highly conserved mouse orthologue suggests that SERPINB11 will neutralize some trypsin-like proteinases. SERPINB11 (and its mouse orthologue) also has an unusually high isoelectric point (pI = 8.42), which is reminiscent of the chicken clade B serpin, Ment (pI = 9.38). Since the other human clade B serpins possess a pI in the range of 5.18–6.35, this positively charged serpin may associate with distinct subcellular structures such as the nucleus or the lysosome, or with acidic components of the extracellular matrix.

SERPINB12

SERPINB12 was isolated also by screening high-throughput genomic sequence data [9]. The gene mapped to the clade B serpin cluster at 18q21 and resided between *SERPINB5* and *SERPINB13*. The presence of SERPINB12 in silico was confirmed by cDNA cloning and RT-PCR. Expression studies showed that SERPINB12, like SERPINB6, was present in many tissues, including brain, bone marrow, lymph node, heart, lung, liver, pancreas, testis, ovary and intestines (table 2). Based on the presence of *Arg* and *Ser* at the reactive center (P1-P1′) of the RSL, SERPINB12 appeared to be an inhibitor of trypsin-like serine proteinases. This hypothesis was confirmed, as recombinant SERPINB12 inhibited human trypsin and plasmin, but not thrombin, coagulation factor Xa or uPA.

SERPINB13 (headpin, hurpin, PI13)

The cDNA for SERPINB13 was isolated independently by two different groups [141, 142]. Abts and colleagues identified a transcript that was downregulated in response to UVB irradiation (hurpin), whereas Spring and colleagues isolated a cDNA (headpin) that was underexpressed in several squamous cell carcinomas of the oral cavity. In contrast, SERPINB13, which is present in the mucosa, normal keratinocytes and skin, is upregulated in psoriasis.

Genomic analysis revealed the presence of several alternative donor splice sites at the 3′ end of exon 3. One splice variant (confirmed by cDNA cloning) would add an additional nine amino acids to the CD-loop [142]. The functional significance of the alternative splicing is not known but may alter subcellular distribution or the repertoire of proteins capable of interacting with SERPINB13. Biochemical analysis of recombinant SERPINB13 shows that it can neutralize the cysteine proteinases catK and L [143, 144]. RSL cleavage occurs between the *Thr* and *Ser* residues at the canonical P1and P1′ positions, with *Val* at the P2 position most likely directing proteinase binding and cleavage.

Although the biological function of SERPINB13 is unknown, experimental evidence suggests that cells overexpressing this serpin show increased resistance to UV-induced apoptosis [144]. This protective mechanism may involve the neutralization of lysosomal cysteine proteinases that leak from the damaged organelles.

Mouse-human clade B serpin relationships

Human clade B serpins are grouped into clusters of 3 and 10 genes that map to 6p25 and 18q21.3, respectively [7, 9]. There is remarkable conservation in gene order between the mouse and human clade B serpin clusters (fig. 4). However, the mouse clade B serpin clusters on both chromosomes 1 and 13 show an expanded gene repertoire relative to their human counterparts. On human chromosome 6, *SERPINB1*, *-B6* and *-B9* span a distance of ~350 kbp. In contrast, the mouse serpin cluster on chromosome 13 spans ~1000 kbp and contains three, five and seven paralogues of *Serpinb1*, *-b6* and *-b9*, respectively [145]. In addition, the locus harbors at least 3 pseudogenes and some gene fragments. On human chromosome 18, the 10 serpin genes span a distance of ~500 kbp [9]. The syntenic locus on mouse chromosome 1 spans ~ 800 kbp. However, unlike the serpin locus on mouse chromosome 13, only the region corresponding to *SERPINB3* and *-B4* is amplified [D. J. Askew and G. A. Silverman, unpublished]. This region contains four paralogs of a *SERPINB3-B4*-like gene plus 3 pseudogenes. The human and mouse genes in this region appeared to evolve from a single common ancestor followed by local duplication events within each species. The designation of Serpinb3a is somewhat misleading, as this protein demonstrates the same inhibitory profile and tissue distribution pattern as both SERPINB3 and -B4. Thus, of the 4 mouse genes in this locus, Serpinb3a is likely to be the direct descendant of the ancestral SERPINB3-B4-like gene. The other paralogues in this locus, Serpinb3b, -b3c and -b3d, all contain different RSLs and probably have evolved functions distinct from that of Serpinb3.

The amplification of serpin genes in the mouse genome is not restricted to the clade B serpin clusters. For example, the clade A serpin genes on mouse chromosome 12 are amplified greatly in comparison to the syntenic locus on human chromosome 14 [146]. The expansion of serpin gene numbers in the mouse is associated with a concomitant diversification of RSL amino acid sequences. This finding suggests that mice have expanded their inhibitory repertoire to regulate a broader array of endogenous and/or exogenous (e. g. bacterial) proteinases.

Mice with targeted deletions of *Serpinb2* [47], *Serpinb3a* [D. J. Askew and G. A. Silverman, unpublished], *Serpinb5*

or *Serpinb6* [P. I. Bird et al., unpublished] have been generated. Animals with homozygous deletions of *Serpinb2, -b3a* and *-b6* show normal development and longevity. However, an abnormality may be revealed upon exposure to different types of stressors. Animals with homozygous loss of *Serpinb5* die in development (see above) [103].

Clade B serpin cellular and subcellular distribution

Studies examining the subcellular distribution of the clade B serpins show a pattern distinct from that of serpins from the other human clades. All of the human clade B serpins can be detected in the cytosol, and some appear to be associated with cytoplasmic organelles such as cytotoxic or azurophilic granules (P. I. Bird et al., unpublished and [127]). Bird and colleagues used subcellular fractionation techniques and immunofluorescence microscopy to show that SERPINB1, -B2, -B6, -B8 and -B9 are present in both the cytoplasm and the nucleus [147]. In the case of SERPINB9 and depending on the cell type, 25–41% of the protein is detected in the nucleus. Nuclear transport occurs in the absence of a classical nuclear localization signals (NLS), but appears to be an active process involving cytoplasmic factors but not ATP. SERPINB9 does not appear to bind avidly to an intranuclear site. Nuclear export of SERPINB9 is sensitive to leptomycin B, thereby implicating Crm1p (exportin) in this process.

Unlike the serpins described above, SERPINB10 uses an NLS within the CD-loop to access the nucleus [148]. This motif, *Lys-Lys-Arg-Lys*[77], is very similar to the monopartite *Pro-Lys-Lys-Lys-Arg-Lys-Val* NLS of SV40 T antigen. The biologic function of nuclear clade B serpins is unknown but may involve protecting the nucleus from exogenous proteolytic activity. Indeed, there are several examples of proteinases that localize to the nucleus (e.g. granzyme B). Another possible role for nuclear serpins is to facilitate chromatin condensation. Ment, a chicken clade B serpin with similarity to SERPINB10, also contains an NLS (*Arg-Arg-Arg-Arg*) in the CD-loop as well as an AT hook motif and a nuclear lamin-like chromatin-binding region [149]. Ment is the major non-histone chromatin protein, is bound stably to compact nucleosomes and requires both its chromatin-binding CD-loop and RSL to induce proper chromatin condensation. Ment is also a potent papain-like cysteine proteinase inhibitor [150].

Extracellular clade B serpins

There are numerous reports describing the presence of different clade B serpins in extracellular fluids such as blood, saliva, breast milk, urine and bronchiolar secretions. In many cases, concentrations of clade B serpins in these fluids are substantial and thereby likely to play an important physiologic role. Thus, the absence of a prototypical N-terminal signal sequence does not preclude an extracellular existence for some of the clade B serpin family members. For example, ovalbumin is secreted from the chicken oviduct [151, 152]. Secretion occurs via the endoplasmic reticulum (ER)-Golgi pathway but appears to require an unusual internal hydrophobic secretion signal located between residues 22 and 41 [151]. Of the 13 human clade B serpins, there is evidence for a dual intracellular and extracellular existence for SERPINB1-B5 [105, 153, 154]. However, with the exceptions of SERPINB2 and -B5, there is little compelling experimental evidence to suggest that the release of clade B serpins from cells is anything more than a passive process associated with loss of cell surface membrane integrity or cell lysis. The human clade B serpin SERPINB2 is found primarily within monocytes and macrophages [39]. However, upon stimulation with PMA, LPS or TNFα, relatively small amounts of SERPINB2 are secreted [39]. Depending on the cell type, this facultative secretion is dependent or independent of the ER-Golgi pathway. In the former case, SERPINB2 contains both complex-type *N*-linked and *O*-linked oligosaccharides [44]. In the latter case, SERPINB2 contains no detectable *N*-linked oligosaccharides, and the process is not blocked by brefeldin A [155].

SERPINB5 also resides in the cytosol but can associate with secretory granules and the cell membrane [105]. Using an in vitro transcription, translation and translocation assay, Pemberton and colleagues showed that SERPINB5 partitioned to microsomal membranes and appears capable of sorting into the classical secretory pathway.

In contrast to SERPINB2 and -B5, SERPINB6 resides exclusively within the cell [156]. SERPINB6 is not detected in the culture media of a variety of cell types treated with inducers of the protein kinase A (dibutyl cAMP) or C (PMA) signal transduction pathways or TNFα [156]. Fusion of a signal peptide (influenza HA) to the N-terminus of SERPINB6 resulted in its translocation across the ER where it was retained in a nonfunctional form. This finding suggests that conditions within the ER prevent correct folding of SERPINB6 so that it cannot transit the remainder of the secretory pathway. Experiments with SERPINB1, -B8 and -B9 yielded results similar to those for SERPINB6 [F. Scott et al. unpublished].

Previous reports suggest that SCC cells actively secrete SERPINB3 and -B4 into the circulation or culture medium [154, 157, 158]. However, using pulse-chase analysis, under conditions that increase SERPINB3 and -B4 synthesis (incubation with PMA or TNFα), relatively scant amounts of SERPINB3 and -B4 appeared in the culture medium. These pulse-chase studies were performed using a squamous cell tumor line that was considered to be a high 'secretor' of SERPINB3 and -B4

[154]. The increased amount of LDH in the medium suggested that cell lysis rather than an active secretory process contributed to the scant amount of SERPINB3 and -B4 in the culture supernatant. Relative to the few clade B serpins that have been studied to date, the cellular fate of SERPINB3 and -B4 appeared more like that of SERPINB6 [156] rather than that of SERPINB2 [44] or -B5 [105]. In contrast to SERPINB6, when a signal peptide was fused to the N-terminus of SERPINB3, this serpin readily appeared in the culture supernatant. Moreover, these molecules retained their biologic activity as proteinase inhibitors. Thus, if the SERPINB3 and -B4 can traverse the ER membrane, then secretion along the classical pathway is feasible. However, in vitro experiments using microsomal membranes suggest that the translocation of wild-type SERPINB3 or -B4 across the ER membrane is a formidable task and is unlikely to occur in vivo. Mutagenesis studies show that two embedded (noncleaved) N-terminal hydrophobic motifs, H1 (residues 4–16) and H2 (residues 28–46), play an important role in directing clade B serpin secretion [105, 151, 153, 159]. Examination of the amino acid sequences of SERPINB3, -B4 and -B6 revealed the presence of a charged *Glu* residue within the H1 domain. SERPINB3 and -B4 also harbor a *Lys* residue in this domain. However, substitution of one or both of these residues for those present in SERPINB2 failed to increase the secretion of SERPINB3. This finding suggested that the charged residues in the H1 domains of SERPINB3 and -B4 were not in themselves an impediment to secretion via the classical or alternative pathways. Conversely, these data indicated that the hydrophobicity of the H1 domain was insufficient to augment clade B serpin secretion and that other residues or motifs within the serpin backbone may participate in the secretory process.

These data also indicated that SERPINB3 and -B4 in the circulation of cancer patients may be a simple function of the mass of SERPINB3 and -B4 synthesized and released by necrotic tumor cells. This notion is consistent with the diagnostic and prognostic data collected for serum SERPINB3 and -B4 levels in SCC patients as well as an intracellular site for clade B serpin function. However, interactions between serpins and potential target proteinases depend on the relative concentrations of the reactants, binding constants and local environmental factors. The latter factors are particularly important, as serpin-proteinase binding is influenced by redox state, pH, solute concentrations and the presence of accessory molecules such as heparans, vitronectin, defensins and other proteinases [160, 161]. Thus, a precise knowledge of an intra- and/or extracellular location is a prerequisite to understanding the actual range of target proteinases and biologic functions of serpin molecules. The detection of SERPINB3 and -B4 in the cytosol suggests that under normal conditions, these serpins (like SERPINB6) regulate intracellular proteolytic cascades rather than those in the circulation or extravascular space. However, under pathologic conditions in which they are actively or passively released into the circulation, a systemic role for SERPINB3, -B4 and other clade B serpins cannot be precluded [162].

Intracellular serpins in other species

Based on information currently available in the genomic and expressed sequence tag (EST) databases, clade B members, per se, have been detected only in vertebrates. Moreover, they are the only serpin clade within this taxon with a predominately intracellular distribution. Since it is generally accepted that the primordial serpin was intracellular [4] and that all species harboring serpin family members also contain a subset of intracellular serpins, it can be asked whether there is a deeper evolutionary relationship between the clade B serpins and these other intracellular serpin groups. The answer to this question hinges on our ability to identify and trace a sequence pattern or structural motif common to all intracellular serpins. At present such a trait has not been identified, and it is not possible to detect an insect/nematode/mammalian/plant/jellyfish/bacterial/archaeal intracellular serpin group that excludes the extracellular serpins. The reason for this is that relationships at this depth in the serpin tree are not clear. Furthermore, since there is presently no example of a conserved proteinase regulated by intracellular serpins in widely separated species, the possibility of convergent evolution in generating intracellular serpin families cannot be evaluated. Thus, it is not yet possible to determine whether the intracellular serpins have arisen once or multiple times during evolution.

Despite the current lack of a clear evolutionary distinction between the intracellular and extracellular serpins, all of the intracellular serpin groups share several subtle structural and functional features in common with clade B serpins: (i) the absence of typical (cleavable) N-terminal signal peptides (i.e. they are leader-less), (ii) the lack of N- or C-terminal extensions comparable to those of the canonical clade A serpin, α1-antitrypsin (SERPINA1), (iii) a cytosolic or nucleocytosolic subcellular distribution (for those that have been examined), (iv) inhibition of serine and/or cysteine proteinases (each group also contains as a few noninhibitory types), (v) a conserved *Ala* residue at the canonical P10 position of the inhibitory type RSLs and (vi) a significant likelihood of containing one or more *Cys* residues in the RSL and/or the contiguous β strand, 1C.

The ability of these serpins to inhibit both serine and/or cysteine proteinases may reflect the need of cells to guard efficiently their intracellular space against a broader array of misdirected proteinases or to possess strategic regulators at key nodes of interaction between cysteine and

serine proteinase cascades. The significance of the conserved P10 *Ala* is unknown, but may be necessary to facilitate RSL movement and inhibitory function within the intracellular space. The presence of *Cys* residues in the RSL-β s1C in the intracellular serpins is intriguing. In humans, only serpins in the B clade contain *Cys* residues in this region (table 4). A more global analysis reveals that ~65% of 70 putative intracellular serpins, but only ~11% of 92 putative extracellular serpins contain at least one *Cys* residue in the RSL- s1C region (table 4). This association of these *Cys* residues with serpins lacking signal peptides is significant (χ^2 = 50.37, P ≤ 0.001) and does not take into account the recent findings that even the relatively few 'extracellular' serpins with RSL-containing *Cys* residues may actually reside intracellularly. For example, the gene for mouse Serpina3g (Spi2-1, serpin 2A) contains *Cys-Cys* residues at the reactive center (P1-P1′) and encodes for a N-terminal signal peptide, as do all clade A serpins. However, by immunohistochemistry this protein demonstrates a nucleocytoplasmic distribution [163]. This subcellular distribution appears to be a consequence of an alternative 5′splicing event that eliminates that portion of the transcript encoding for the N-terminal signal peptide. It has yet to be determined

Table 4. Presence of *Cys* residues in RSLs of intracellular (Signal Peptide, SP –) and extracellular (SP +) serpins.

Species	Serpin and accession number	SP	RSL-s1c
A. gambiae	SRPN3 (ATIII-like) [7723]	+?	GTEASAATEGTLVFTILDQPVKFIAN
	SRPN6 [0545]	+?	GTEGAAATSALVDRIGSQRQFNGN
	SRPN7 (B10, I1-like) [20725]	?	GTEAAAVTSVGTKFRVRNTQFRVD
	SRPN8 (B9-like) [14699]	-?	GTMTAAVTTGVFANKATPPRFLAN
	SRPN9 (B3-like) [16680]	-?	GSVAASATVAFSFRSSRPADPAMFHCN
	SRPN15 (B3-like) [15448]	-?	GSVAASATVAFSFRSSRPADPAMFHCN
	SRPN5 (OVA-like) [10507]	-?	GTEGGAVTITAMERSLPPVNFRVR
	spi21F-CAM CAD12781.1	-	GTEAAAATGMVMMTCAMIMYPMFTAD
	spi21F-FCM CAD12782.1	-	GTEAAAATAMIAVSFCMIISEPVKFTVD
	spi21F-RCM CAD12783.1	-	GTEAAAATGMIMMMRCMPMHPYFTVD
	spi21F-KRAL CAD12784.1	-	GTEAAAATAAVVRVKRALINRLKVRLD
B. mori	A1ATa P22922	+	GAEAAAAANAFTMTRSSKVYVRPPIVFNAN
	A1ATb P80034	+	GAEAAAAANAFAVVFMSAVVSQPLVFKAN
	ACT Q03383	+	GATAGAFTGLIAVPTSSLSRPPPSLLFKVD
	Serpin-2 AAF61252.1	-	GAEAAAATGMVMMMRCARPPSPSFHAD
B. taurus	SERPINC1 (ATIII) P41361	+	GSEAAASTVISIAGRSLNSDRVTFKAN
	SERPINE1 (PAI1) P13909	+	GTLASSSTALVVSARMAPEEIIMD
	SERPINB6 (B43) O02739	-	GTEAAAATGAVVMMRCLMVVPRFNAN
D. melano-gaster	Serpin 27A AAAF24518	+	GTEAYAATVVEIENKFGGSTAIEEFNVN
	CG6717	+	GEASAATGVLTRRKKSIDNLIQPPMEFIAD
	CG7219	+	GTEAAASSVTYLKKSGPDVLFRGD
	CG1859	+	GGNADDSFSFGDLFRRALPLVIN
	CG6680	+	GTTAGAVTEAALANKATPPKFLLN
	CG1342	+	ATMQARTPSVESTVLPVPEPEPELPGVERFEVN
	CG6687	+	GSTAAAATILLVSRSSRQPDPTKFNCN
	nec CG1857	+	GTEASAASYAKFVPLSLPPKPTEFVAN
	Spn43Aa CG12172	+	GCEAAGASYAAGVPMSLPLDPKTFVAD
	Spn43Ab CG1865	+	VTEAGVDQPLETGLLKGLFSRSKKFEAD
	Serpin-1 AJ251744.1	+	GAEAAGATSVAVTNRAGFSTFLMAD
	Serpin-2 AJ251745.1	+	GAEAAAATALLFVRLSVPMPSSQMVFNAD
	Serpin-3 AJ251746.1	+	GSEAAAATAVVFRYKSIRSPPMDFNVN
	Serpin-5 AJ251748.1	+	GSTAAAATVLFTYRSARPVEPAKFECN
	Serpin-4f CAD21899.1	-	GTEAAAATGMVMCYASMLTFEPQPVQFHVQ
	Serpin-4h CAD21901	-	GTEAAAATVWRVMAVAAFSRKHFIAN
	Serpin-4g CAD21900	-	GTEAAAATGMFMSLTSLPMPKPDPIRFNVD
	Serpin-4e CAD21897	-	GTEAAAATGMAVRRKRAIMSPEEPIEFFAD
	Serpin-6 AJ251749.1	-	GTEAAAATGMIMMTRMMTFPLQFQAD
	CG7722	-	GCEVAPEAEVQPEVLKKNPDRKFFKAD
	CG9460	-	GTTASGATFIKVSVESLTIGEEVFEFIAD
	CG9455	-	GSEAAAVSFMKIVPMMLNMNKKLFKAD
F. rubripes	SERPINE2 [134592]	+	GTKAAAATSMVLLKRSRAPVFKAD
	SERPINE2 [129619]	+	GTKASAATTAILIARSSPPWVAVD
	SERPING1 [126237]	+	GVEAVAVTSTTFSRTYNSFSAL
	SERPINI1 [151864]	+	GLEGAVGSGLVALTRTLVLYPQVMAD
	SERPINB1a [124335]	-	GTEAAAATAGMVAFCMLREEHFTAD
	SERPINB1b [131090]	-	GTEAAAATGVVFTLHCAVFPQNFYAD
	SERPINB2a [139667]	-	GTEASAATGAVFKFRCARRTETFVAD
	SERPINB2b [144408]	-	GTEAAAATGVVFTLLCAVFPQNFYAD
G. gallus	SERPINI1 Q90935	+	GSEAAAASGMIAISRMAVLYPQVIVD
	OVA P01012	-	GREVVGSAEAGVDAASVSEEFRAD
	Gene Y P01014	-	GTEATGSTGAIGNIKHSLELEEFRAD
H. sapiens	SERPINA1 sp|P01009|	+	GTEAAGAMFLEAIPMSIPPEVKFN
	SERPINA2 NP_006211	+	GTEATGAPHLEEKAWSKYQTVMFN
	SERPINA3 sp|P01011|	+	GTEASAATAVKITLLSALVETRTIVRFN
	SERPINA4 sp| P29622	+	GTEAAAATTFAIKFFSAQTNRHILRFN
	SERPINA5 sp| P05154	+	GTRAAAATGTIFTFRSARLNSQRLVFN
	SERPINA6 sp| P08185|	+	GVDTAGSTGVTLNLTSKPIILRFNQPF
	SERPINA7 NM_000354	+	GTEAAAVPEVELSDQPENTFL
	SERPINA10 NM_016186	+	GTEAVAGILSEITAYSMPPVIKVD
	SERPINB1 NP_109591	-	GTEAAAATAGIATFCMLMPEENFTAD
	SERPINB2 NP_002566.1	-	GTEAAAGTGGVMTGRTGHGGPQFVAD
	SERPINB3 NP_008850.1	-	GAEAAAATAVVGFGSSPTSTNEEFECN
	SERPINB4 NP_002965.1	-	GVEAAAATAVVVVELSSPSTNEEFECN
	SERPINB5 NP_002630.1	-	GGDSIEVPGARILQHKDELNAD
	SERPINB6 NP_004559.3	-	GTEAAAATAAIMMMRCARFVPRFCAD
	SERPINB7 NP_003775.1	-	GTEATAATGSNIVEKQLPQSTLFRA
	SERPINB8 NP_002631.1	-	GTEAAAATAVVRNSRCSRMEPRFCAD
	SERPINB9 NP_004146.1	-	GTEAAAASSCFVVAECCMESGPRFCAD
	SERPINB10 NP_005015.1	-	GTEAAAGSGSEIDIRIRVPSIEFNAN
	SERPINB11 NP_536723.1	-	GTEAAAATGDSIAVKSLPMRAQFKAN
	SERPINB12 NP_536722.1	-	GTQAAAATGAVVSERSLRSWVEFNAN
	SERPINB13 NP_036529.1	-	GTEAAAATGIGFTVTSAPGHENVHCN
	SERPINC1 sp|P01008|	+	GSEAAASTAVVIAGRSLNPNRVTFKAN
	SERPIND1 sp| P05546	+	GTQATTVTTVGFMPLSTQVRFTVD
	SERPINE1 sp| P05121|	+	GTVASSSTAVIVSARMAPEEIIMD
	SERPINE2 NP_006207	+	GTKASAATTAILIARSSPPWFIVD
	SERPINF1 sp|P36955|	+	GAGTTPSPGLQPAHLTFPLDYHLN
	SERPINF2 sp| P08697	+	GVEAAAATSIAMSRMSLSSFSVN
	SERPING1 sp| P05155	+	GVEAAAASAISVARTLLVFEVQQPFLFVLWDQQH
	SERPINH1 sp| P29043	+	GNPFDQDIYGREELRSPKLFYAD
	SERPINI1 sp| Q99574	+	GSEAAAVSGMIAISRMAVLYPQVIVD
	SERPINI2 gi|3724282|	+	GSEAATSTGIHIPVIMSLAQSQFIAN
Leporipox-virus	SERP1 P12393	+	GTTASSDTAITLIPRNALTAIVAN
	SERP2 Q9WPF7	-	GTTAASCTYGCVTDFGGTMDPVVLKVN
M. sexta	Serpin-1a AAC47342.1	+	GAEAAAAANAFFITRQARLDIRYFVAN
	Serpin-1b AAC47343.1	+	GAEAAAAANAFGIVPASLILYPEVHID
	Serpin-1z AAC47338.1	+	GAEAAAAANAFGIAYLSAVIRSPVFNAD
	Serpin-1k AAC47334.1	+	GAEAAAAANAFKITFYSFHFVPKVEIN
	Serpin-1j AAC47340.1	+	GAEAAAAANAFILTDRCCSDYDDNIEFDVN
	Serpin-1i AAC47337.1	+	GAEAAAAANEFGIVALSLEFSLNEIKFVVN
	Serpin-1h AAC47332.1	+	GAEAAAAANAFITYVESIDNFVPTIEFDVN
	Serpin-1g AAC47336.1	+	GAEAAAAANAFFIVGITSIQFEPPVIEFHVN
	Serpin-1f AAC47333.1	+	GAEAAAAANAFIAVVDSIDIFERTIEFHAD
	Serpin-1e AAC47335.1	+	GAEAAAAANVIRVVKKKFRVIPPVLKFHVD
	Serpin-1d AAC47341.1	+	GAEAAAAANVVRGIRPRPSVRPPTPKFEAD
	Serpin-1c AAC47339.1	+	GAEAAAAANAFFIIESYSSYEPVVPVFDID
	Serpin-2 AAB58491	-	GSGIDIRSISFMADAETRESAYFRAD
	Serpin-3a AAO21505	+	GSVAFSATQIGIQNKFGEDSDINYEVVAN
M. musculus	Serpina1a (SPI1-1) [21483]	+	GTEAAAVTVLQMVPMSMPPILRFD
	Serpina1b (SPI1-2) [58535]	+	GTEAAAATVFEAVPMSMPPILRFD
	Serpina1d (SPI1-4) [54291]	+	GTEAAAATVLQVATYSMPPIVRFD
	Serpina1e (SPI1-5) [21083]	+	GTEAAAATVLQGGFLSMPPILHFN
	Serpina2 ATR [21081]	+	GSKPSTNSCFKKLGSTDMGRMQLN
	Serpina3b [41449]	+	GTEGDAITIVGYNFMSAKL
	Serpina3c P29621	+	GTEGVAATGVNFRILSRRTSLWFN
	Serpina3d S15636	+	GTEADAATRFKIAPLSAKFDIVNVNFN
	Serpina3e S15635	+	GTEAAAATGVKVNLRCGKIYSMTIYFK
	Serpina3f S15629	+	GTEAAAATGYQNLCCQGVIYSMKIYFD
	Serpina3g [41250	+	GTEAAAATGMAGVGCHAVFDFLEIFFN
	Serpina3h S15633	+	GTEAAAATGVKLIICEKIYSMTIYFN
	Serpina3i S15635	+	GTEAAAATGVKVNLRCGKIYSMTIYFN
	Serpina3k [41449	+	GTEAAAATGVIGGIRKAVLPAVCFN
	Serpina3k P07759	+	GTEAAAATGVIGGIRKAILPAVHFN
	Serpina3m [41449	+	GTEAAAATGFIPGFRSRRLQTMTVQFN
	Serpina3n [21091	+	GTEAAAATGVKFVPMSAKLYPLTVYFN
	Serpina5 [41550	+	GTTAAAITGAIFTFRSARPSSLKIEFT
	Serpina6 [41595	+	GNVLPAATNGPPVHLPSESFTLKYN
	Serpina7 [31271	+	GTKEGASPEVGSLDQQEVPPL
	Serpina10 [41603	+	GTEAVSGTLSEIIAYSMPPAIKVN
	Serpina12 [41567	+	GMEGAAGSGAQTLPMETPRHMKLD
	Serpinb1 [44734	-	GTEAAAATGGIATFCMLLPEEEFTVD
	Serpinb1b [51029	-	GTEAAAATGGIIQVLCEKMPTPQEVFTVD
	Serpinb1c [21401	-	GTETDAAMPGTVVGCCLMPMEFTVD
	Serpinb2 [38771	-	GTVAAGGTGAVMTGRTGHGGPQFVAD
	Serpinb3 [44594	-	GTEAAAATGVEVSLTSAQIAEDFCCD
	Serpinb3b [26328	-	GTEADPASGEEVILRLAQVADFRCD
	Serpinb3c [23098	-	GTEAAAATGVEVSVRSAQIAEDFCCDHPF
	Serpinb3d [38810	-	GTEAAATTGIKSHNLSLQITEDFYCD
	Serpinb5 [46560	-	GGESIEVPGSRILQHKDEFNAD
	Serpinb6 [17044	-	GTEAAAATAGMMTVRCMRFTPRFCAD
	Serpinb6b AAB57819.2	-	GTEAAAATAANIGFRCMVPYFCAN
	Serpinb6c AAL65910.1	-	GSEATAATTIVLKGSSRSTPSFCVN
	Serpinb6e [17044	-	GTEAAVATEIVTVGSPLTQRCLLAD
	Serpinb6d [47889	-	GTKVAAATDIVMMGASPTTHTFCAD
	Serpinb7 [38779	-	GTEATAATENNIVEKQLPESTVFRAD
	Serpinb8 [26315	-	GTEAAAATAVIRNARCSRTEPRFCAD
	Serpinb9 Spi6 [6391	-	GTEAAAASAIIEFCCASSVPTFCAD
	Serpinb9b U96705	-	GTEAAAASSAEGIIPLCLGGGPSWFCAD
	Serpinb9c O08803	-	GTEATAATADDTVCSAETHDGQTFCAD
	Serpinb9d U96704	-	GTEAAAATAGKTIGCCLGSYPQTFCAD
	Serpinb9e U96709	-	GTEAVAASAGKIILFCDGPDPEVFCAD
	Serpinb9f U96708	-	GTEAAAASAVEFIFLCSGPDPETFCAD
	Serpinb9g AF425083	-	GTEAAAASAVKFIFLCSGPDPETFCAD
	Serpinb10 XP_136873	-	GTEAAAGTGSEISVRIKAPSIELNVD
	Serpinb11 [26327	-	GTEAAAATGESISVKRLPVTVQFTANC
	Serpinb12 [26323	-	GTQAAAASGVVAAEKALPSWVEFNAN
	Serpinb13 [48775	-	GVEATAGTGVGLKVSSAASCELVHCN
	Serpinc1 [26715	+	GSEAAASTSVVITGRSLNPNRVTFKAN
	Serpind1 [22766	+	GTQAAAVTTVGFMPLSTQVRFTVD
	Serpine1 [37411	+	GTVASSSTAFVISARMAPTEMVID
	Serpine2 [26249	+	GTKASAATTAILIARSSPPWFIVD
	Serpinf1 [00769	+	GAGSSPSPGLQPVRLTFPLDYHLN
	Serpinf2 [38224	+	GVEAAAATSVAMNRMSLSSFTVN
	Serping1 [23224	+	GVEAAAASAISFGRSLPIFEVQ
	Serpini1 [27834	+	GSEAAAASGMIAISRMAVLYPQVIVDH
	Serpini2 34139	+	GSEAAASTGINIPAIMSLTQTQFLAN
Orthopox-virus	SPI-3 AAG44097	+	GTVAEASTIMVATARSSPEQLEFN
	SPI-1 P42927	-	YTEASAITGVFMTNFSMVYRTKVYIN
	SPI-2/crmA P07385	-	YTEAAAATCALVADCASTVTNEFCAD
R. appendicu-latus	RAS-3 AAK61377.1	+	GTIATAVTGLGFVPLSAHYNPPPPIEFTVD
	RAS-4 AAK61378.1	+	GAAAATRQSSSKPRSSGGAIRFVVD
	RAS-2 AAK61376.1	-	GTEAAAATAITMMTYCARFDGTRFVVD
	RAS-1 AAK61375.1	-	GTEAAAATAVMMAACCLSSTPPQTYKFTVD

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whether other clade A-like serpins with *Cys* residues in the RSL follow a similar fate. The presence of *Cys* residues in the RSL- s1C of intracellular serpins may be required to facilitate or stabilize the interactions with the types of proteinases encountered within the cell. Moreover, the relative reducing environment of the cytosol may facilitate this process by deterring the formation of intramolecular disulfide linkages capable of interfering with inhibitory functions. Alternatively, these *Cys* residues may help maintain proteinase-inhibitor balance in the extracellular space by limiting the half-life of serpins released from necrotic cells. In unique circumstances, however (e.g. the Crenarchaeote thermophile, *Pyrobaculum aerophilum*), a *Cys* residue on the P′ side of the RSL could be employed to stabilize the serpin fold without interfering with serpin inhibitory function [5].

Although the precise biologic function of the majority of these serpins is unknown, they are likely to play critical roles in cellular metabolism by safeguarding the intracellular environment from endogenous or exogenous proteinase-mediated injury. Thus, *intracellular* serpins, which encompass the vertebrate clade B serpins and leader-less serpins from other taxa, have functionally converged to fulfill the common need of organisms to carefully regulate intracellular proteolysis.

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