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Mechanisms of serpin dysfunction in disease

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The serpin superfamily encompasses hundreds of proteins, spread across all kingdoms of life, linked by a common tertiary fold. This review focuses on five diseases caused by serpin dysfunction: variants of antithrombin III lose their ability to interact with heparin; the α_1-antitrypsin Pittsburgh mutation causes a change in target proteinase; the α_1-antitrypsin Z mutation and neuroserpin, polymerisation of which lead to cellular cytotoxicity; and a loss of maspin expression resulting in cancer.

The serpins (whose name derives from 'serine protease inhibitor') comprise a superfamily of proteins that share a conserved tertiary structure and have evolved primarily to control the activity of serine and papain-like cysteine proteinases. To date, over 800 serpin sequences have been identified in the genomes of species from almost all phyla including viruses, bacteria, metazoans and plants (Refs 1, 2). Phylogenetic analyses have divided eukaryotic serpins into 16 clades, together with several orphan sequences (Ref. 2). Serpins function as proteinase inhibitors in a wide range of physiological processes such as complement activation, blood coagulation and apoptosis (Refs 3, 4, 5). However, an increasing number of serpins have been identified that have evolved roles outside of proteinase inhibition in blood pressure regulation, chromatin condensation, tumour progression, protein folding and hormone transport (Refs 6, 7, 8, 9, 10, 11). Here, we summarise characteristics of the serpin superfamily before focusing on five specific serpin diseases: loss of cofactor binding, altered serpin specificity, polymerisation and loss of function in cancer.

The serpin superfamily

Serpin clades

The increasing number of serpin sequences deposited in databases has necessitated the construction of a systematic method of classification. Irving and colleagues put forward a system dividing the serpins into 16 clades based on phylogenetic analysis (Ref. 2). Plant and insect serpins occupy a single clade each, with viral serpins split into two clades. Animal serpins form the 12 remaining clades, with a single clade for nematodes, blood fluke and horseshoe crab. A phylogeny of the nine serpin clades present in higher animals shows that they cluster on the basis of function rather than species.

Serpin structure

Several serpin crystallographic structures (reviewed in Ref. 12) have shown that all serpins, both inhibitory and non-inhibitory, contain the same tertiary fold of eight or nine α-helices (designated A–I) and three β-sheets

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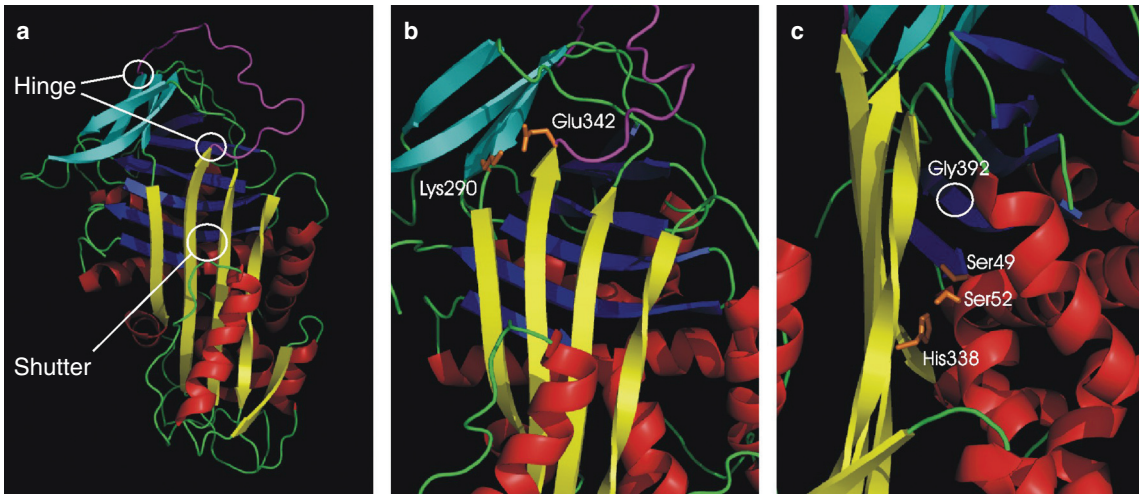
(termed A–C) (Fig. 1a). Whereas the B and C β-sheets are antiparallel, the five-stranded A sheet is a mixture of parallel and antiparallel strands. Extending from the top of the molecule between the A and C β-sheets lies an exposed loop termed the reactive centre loop (RCL).

The first serpin structure to be solved was that of α_1-antitrypsin (A1AT) cleaved within the RCL. Surprisingly, it was found that the cleaved residues were separated by 67Å, with the N-terminal portion of the RCL inserted between the strands of the A β-sheet to create a six-stranded antiparallel sheet (Ref. 13). Later analyses of crystals of uncleaved serpins have shown that the native state of the RCL is the exposed conformation (Fig. 1a), and that its insertion into the A sheet is part of the inhibitory mechanism (Refs 14, 15, 16). The necessary flexibility of the RCL is mediated by two highly conserved motifs (the proximal and distal hinges), whereas insertion into the A β-sheet is facilitated by the breach and shutter regions that assist in the opening of the A β-sheet and movement of the F helix, both of which are necessary for RCL insertion (Ref. 17).

The RCL is the most variable sequence between serpins, defining their inhibitory specificity and biological functions by acting as a pseudo-substrate for the cognate proteinases. The nomenclature of Schechter and Berger designates those residues N-terminal to a proteinase cleavage site as P residues (P17–P1), and those C-terminal as P′ residues (P1′–P10′) (Ref. 18). Cleavage of the RCL occurs at the P1–P1′ peptide bond and the P1 residue is the major determinant of serpin specificity. Some serpins have been shown to inhibit multiple proteinases through the use of alternative P1 residues within the RCL. For instance, proteinase inhibitor 8 (PI8) is capable of inhibiting trypsin-like proteinases by utilising Arg339 (the predicted P1), or chymotrypsin-like proteinases by utilising Ser341 (the predicted P2′ residue) (Ref. 19). Likewise, proteinase



Structure of native α_1-antitrypsin (A1AT)

Figure 1. Structure of native α_1-antitrypsin (A1AT). (a) Secondary structural elements and functional motifs of native A1AT [Research Collaboratory for Structural Bioinformatics Protein Databank (http://www.rcsb.org/pdb/) identifier 1ATU]. Helices are red, loops are green, β-sheet A is yellow, β-sheet B is blue, β-sheet C is cyan and the reactive centre loop is magenta. (b) The Z mutant of A1AT involves mutation of glutamic acid (Glu)342 to lysine, disrupting a salt bridge formed naturally with Lys290. Side chains of Glu342 and Lys290 are shown in orange. (c) The four residues mutated in a novel form of inherited encephalopathy known as FENIB (familial encephalopathy with neuroserpin inclusion bodies) cause polymerisation of neuroserpin and are indicated in orange on the A1AT structure. Gly392 is circled as the side chain cannot be visualised.

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inhibitor 6 (PI6) inhibits trypsin through Arg341 and chymotrypsin through Met340, which are the predicted P1 and P2 residues, respectively (Refs 20, 21).

Serpin inhibitory mechanism

The native, active serpin fold is metastable, placing the molecule under considerable strain. During inhibition of a target proteinase, conformational change within the serpin releases the strain, a process termed the 'stressed-to-relaxed' transition. Initial interactions between the serpin RCL and proteinase active site result in the formation of a reversible Michaelis–Menten complex (Fig. 2) that is rapidly converted to a covalently attached serpin–proteinase complex. During this process, the serpin RCL is cleaved at the P1 position, releasing the P1′ residue, and an ester bond is formed between the proteinase catalytic serine and serpin P1 residues. At this point, the RCL inserts into the A sheet, dragging the proteinase to the opposite pole of the serpin, and the serpin–proteinase complex remains trapped as an acyl–enzyme intermediate (Refs 22, 23, 24). The recent solution of the structure of a serpin–proteinase complex provided an explanation for the interruption of the cleavage event at this point.

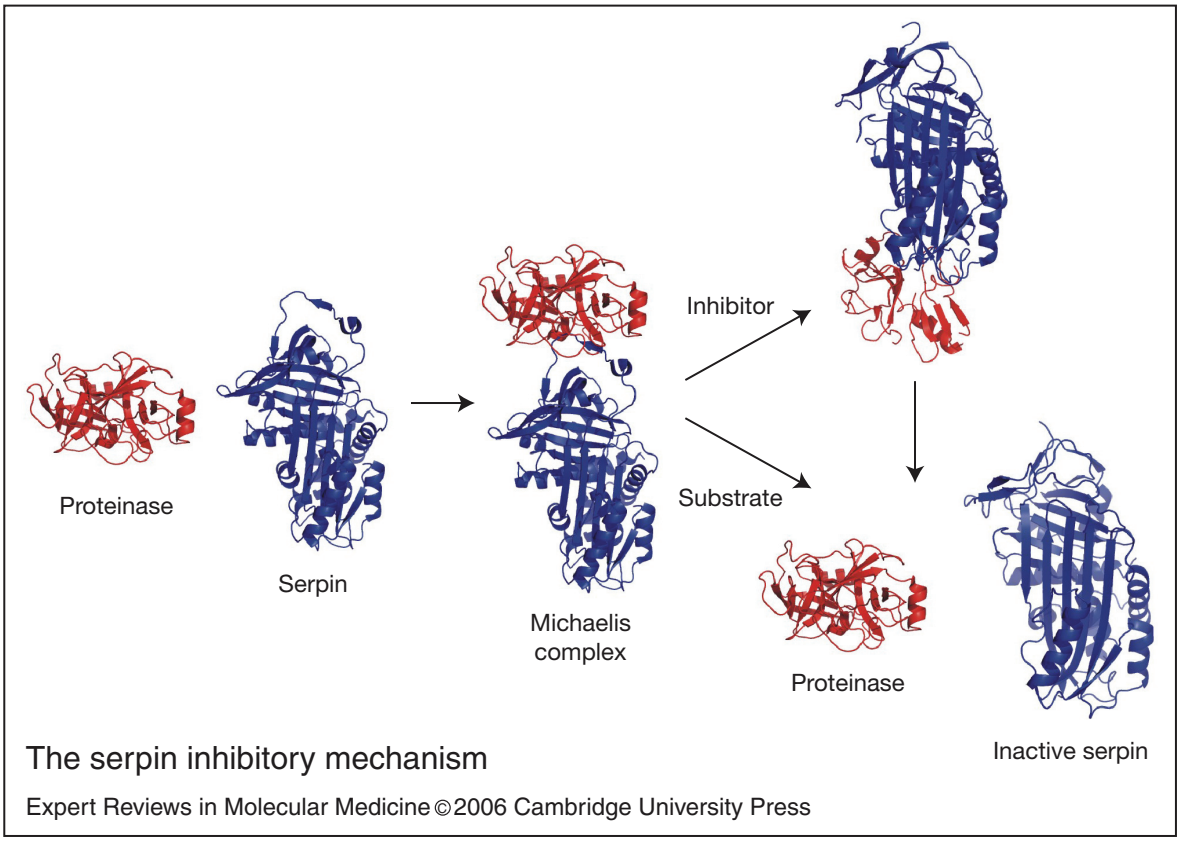

Figure 2. The serpin inhibitory mechanism. The exposed serpin reactive centre loop (RCL) acts as a pseudo-substrate for the target proteinase and initial interactions result in the formation of a reversible, non-covalent Michaelis complex. The RCL is then cleaved at the P1 position and inserts into the A β-sheet. During an inhibitory interaction, the serpin conformational change traps the proteinase in a covalent inhibitory complex in which both molecules are inactivated, although over time this complex can decay to release the active proteinase and the cleaved, inactivated serpin. Alternatively, the serpin may act as a classical proteinase substrate. In this instance, no covalent complex is formed and the serpin is cleaved and inactivated while the proteinase remains unaffected. Research Collaboratory for Structural Bioinformatics Protein Databank (http://www.rcsb.org/pdb/) identifiers: 1ATU (native α_1-antitrypsin); 1DP0 (native trypsin); 1OPH (Michaelis complex between α_1-antitrypsin Pittsburgh and trypsin S195A); 2ACH (cleaved α_1-antitrypsin); 1EZX (inhibitory complex between α_1-antitrypsin and trypsin).

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Loss in secondary structure and a dramatic rearrangement of the proteinase active site is observed. Distortion around the proteinase active site moves the loop containing the catalytic serine 6Å away from the catalytic histidine residue and disrupts the oxyanion hole (Ref. 25). These effects both contribute to the inhibition of deacylation. However, active proteinase may be released from this inhibitory complex as a result of complex breakdown over very long periods of time (Ref. 26).

If the serpin interacts with a non-target enzyme, its RCL is cleaved and released by the proteinase before translocation can occur. This 'substrate pathway' results in the inactivation of the serpin and release of active proteinase. Whether or not a serpin is likely to be a physiological inhibitor of a given proteinase is dependent on the association constant (K_{ass}) and the stoichiometry of inhibition (SI) of the interaction. These kinetic parameters measure the rate at which inhibition occurs and the number of serpin molecules required to inhibit a single proteinase molecule, respectively. A physiological interaction will have a K_{ass} of 10^5 to 10^7 $M^{-1}s^{-1}$ and SI approaching 1 (Ref. 27).

Non-functional serpin states

The metastable nature of the serpin fold is necessary to store the energy required for RCL translocation and deformation of the proteinase in the final inhibitory complex. However, this flexibility also allows the serpin to adopt structural conformations that, although more energetically favourable, are not able to perform the primary inhibitory role. These non-functional serpin states include the latent, delta and polymerised forms (Fig. 3) (Refs 28, 29, 30). Latency occurs as a result of RCL insertion into the A β-sheet, without its cleavage by a proteinase. As such, the RCL becomes inaccessible to proteinases and the serpin is inactivated. The delta conformation has been observed in mutant α_1-antichymotrypsin and is similar to the latent conformation except that the RCL is only partially inserted into the β-sheet. The remainder of strand 4A is formed by the residues of a loop between the F helix and strand 3A.

Serpin polymers are formed by the RCL of one molecule interacting with the A β-sheet of a second, thus forming a continuous chain of inactivated molecules. Under certain conditions, some serpins can also polymerise through RCL interactions with the C β-sheet (Refs 31, 32, 33). The polymer conformation of several serpins has been associated with many diseases resulting from a lack of serpin function or the accumulation of misfolded protein.

Serpin diseases

Serpin diseases arise as a result of mutations affecting the inhibitory mechanism and/or protein stability (Table 1). Here, we focus on five specific serpin diseases: loss of cofactor binding [the interaction between antithrombin III (ATIII) and heparin], altered serpin specificity (the A1AT Pittsburgh mutation), polymerisation (the A1AT Z mutant and neuroserpin pathology) and loss of function in cancer (maspin).

Mutations of the ATIII heparin-binding domain

The blood coagulation pathway involves ordered activation of a serine proteinase cascade from inactive zymogen precursors, leading to the activation of thrombin and clot formation. ATIII has been shown to inhibit many of these proteinases including activated factors IX, X, XI, XII and thrombin, although its in vivo role is probably limited to inhibition of thrombin and activated factor Xa (reviewed by Ref. 34). However, the structure of native ATIII shows that two N-terminal residues of the RCL are partially inserted into the A β-sheet (Refs 15, 35, 36). Furthermore, the P1 residue forms interactions with the body of the serpin, making it relatively unavailable to interact with target proteinases. This results in relatively poor interaction kinetics with its target proteinases. At the site of vessel injury, ATIII binds to glycosaminoglycans, such as heparin, initiating a conformational change that expels the RCL (Fig. 4) and increases the rate of ATIII–proteinase interactions by several orders of magnitude (Refs 37, 38). For example, heparin raises the K_{ass} for the ATIII–factor Xa interaction over 1000 times from 1.9×10^5 $M^{-1}s^{-1}$ to 2.4×10^8 $M^{-1}s^{-1}$ (Ref. 39).

The ATIII heparin-binding domain consists of a patch of positive amino acids (Lys11, Arg13, Arg24, Arg46, Arg47, Lys114, Lys125, Arg129) clustered around the A helix, D helix and the N-terminus of ATIII (Refs 40, 41, 42, 43, 44, 45, 46, 47, 48). Mutation of many of these residues has been identified in heterozygote individuals as

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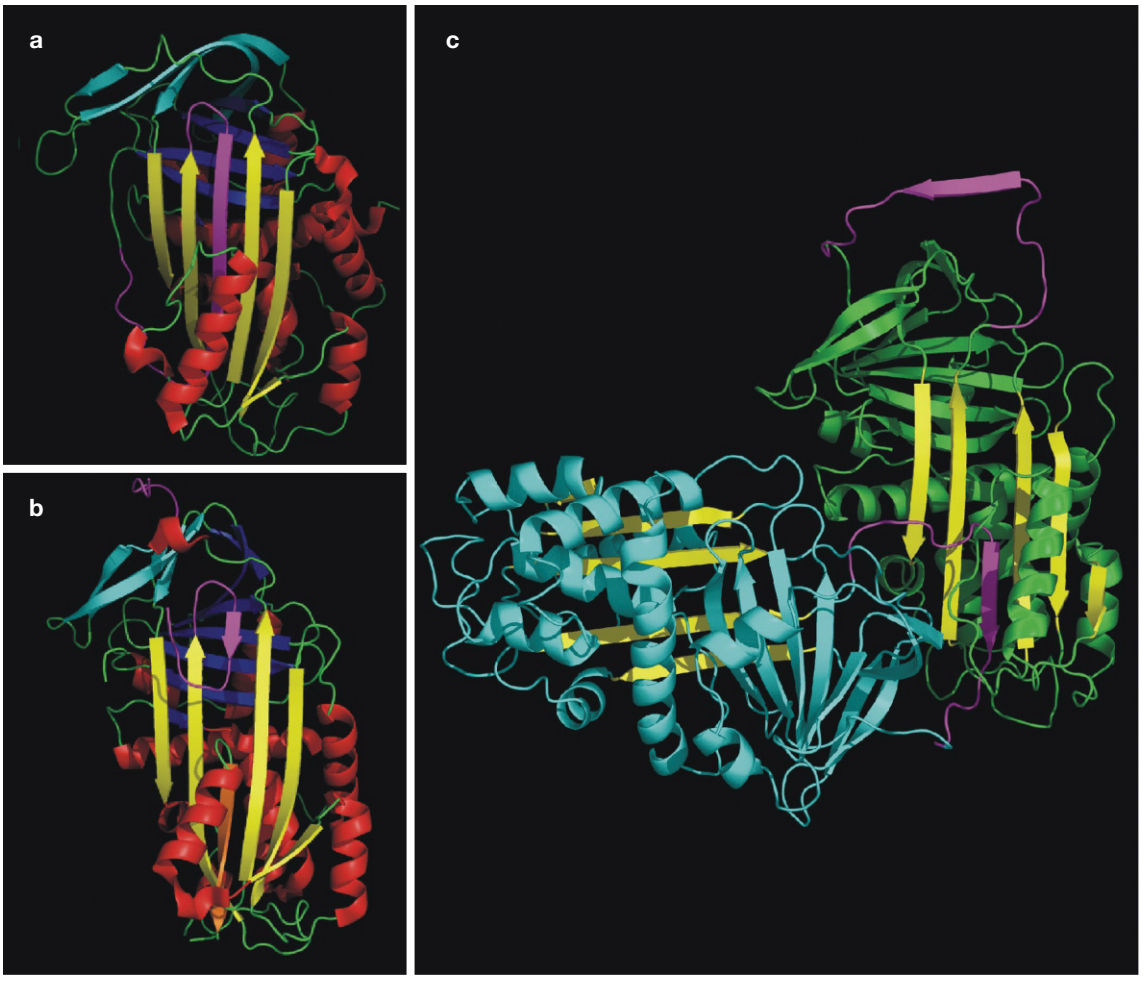

Figure 3. Structure of inactive serpin states. (a) Latent plasminogen activator inhibitor 1. The reactive centre loop (RCL, magenta) is fully inserted into the body of the molecule and is therefore inaccessible to proteinases. Thus, the inhibitory activity of the serpin is lost. Helices are red, loops are green, β-sheet A is yellow, β-sheet B is blue, β-sheet C is cyan and the RCL is magenta. (b) α_1-Antichymotrypsin in the delta conformation. The RCL is only partially inserted, whereas the rest of strand 4A is formed by a loop (orange) between the F helix and strand 3A. Colours are as for part a. (c) Model of two subunits in a serpin polymer based on the structure of a cleaved α_1-antitrypsin polymer. The monomers are coloured green and cyan, with both A β-sheets in yellow and both RCLs in magenta. Research Collaboratory for Structural Bioinformatics Protein Databank (http://www.rcsb.org/pdb/) identifiers: 1C5G (latent plasminogen activator inhibitor 1), 1QMN (delta α_1-antichymotrypsin), 1D5S (cleaved α_1-antitrypsin polymer).

causing an increased risk of venous thromboses; however, a more severe defect is observed in homozygous individuals. For example, several case studies have shown that homozygous mutation of Arg47 to Cys leads to a loss of heparin binding. This results in recurrent venous and arterial thromboses due to uncontrolled activation of the coagulation cascade (Refs 41, 49, 50, 51, 52, 53, 54). Pathogenic ATIII mutations have been identified in residues Pro41, Leu99, Ser116 and Gln118, which do not contact the heparin molecule but are associated with the tight packing of side chains within the binding domain (Refs 55, 56, 57, 58).

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Table 1. Correlation between serpin mutations and human disease

Cladistic name[a]	Other name	Function	Correlation(s) with disease[b]	Gain/loss-of-function mutation and molecular basis of disease[c]
A1	α_1-Antitrypsin	Extracellular inhibition of elastase	Thrombosis	GOF Pittsburgh mutation (M358R): inhibition of thrombin due to altered P1 (Ref. 61)
			Emphysema	LOF Z mutation (E342K): polymerisation and retention in hepatocytes due to hinge mutation (Ref. 66)
A2	α_1-Antitrypsin-like	Unknown	ND	
A3	α_1-Antichymotrypsin	Extracellular inhibition of cathepsin G and chymase	Vascular disease	Isehara 1 (M389V): unknown mechanism (Ref. 130)
A4	Kallistatin	Extracellular inhibition of tissue kallikrein	Pancreatitis	LOF (Ref. 131)
A5	Protein C inhibitor	Extracellular inhibition of anticoagulant enzymes	ND	
A6	Corticosteroid-binding globulin	Non-inhibitory: hormone transport	Low serum cortisol	LOF Leuven mutation (L93H): enhanced substrate dissociation rate (Ref. 132)
A7	Thyroxine-binding globulin	Non-inhibitory: hormone transport	Low serum thyroxine	LOF TBG-A (A191T): decreased affinity for thyroxine (Ref. 133)

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Table 1. Correlation between serpin mutations and human disease *(continued)*

Cladistic name[a]	Other name	Function	Correlation(s) with disease[b]	Gain/loss-of-function mutation and molecular basis of disease[c]
A8	Angiotensinogen	Blood pressure regulation	Hypertension	GOF L10F: increased cleavage by ACE (Ref. 134)
			Hypotension	LOF Y248C: decreased secretion due to abnormal glycosylation (Ref. 135)
A9	Centerin	Unknown	ND	
A10	Protein Z-dependent Protease inhibitor	Extracellular inhibition of factor IXa, Xa, XIa	Venous thrombosis	LOF (Ref. 136)
A11	–	Unknown	ND	
A12	Vaspin	Unknown	Diabetes and obesity	(Ref. 137)
B1	Monocyte/neutrophil elastase inhibitor	Intracellular inhibition of elastase	ND	
B2	Plasminogen activator inhibitor 2	Intracellular inhibition of unknown proteinase(s); extracellular inhibition of uPA, tPA	Cancer marker	
B3	Squamous cell carcinoma antigen 1	Intracellular inhibition of unknown cysteine proteinase(s)	Cancer marker	
B4	Squamous cell carcinoma antigen 2	Intracellular inhibition of unknown serine proteinase(s)	Cancer marker	
B5	Maspin	Non-inhibitory: tumour suppressor	Cancer progression	LOF (Ref. 9)
B6	Proteinase inhibitor 6	Intracellular inhibition of cathepsin G	ND	
B7	Megsin	Intracellular inhibition of unknown proteinase(s)	IgA nephropathy	GOF (Ref. 138)

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Table 1. Correlation between serpin mutations and human disease *(continued)*

Cladistic name[a]	Other name	Function	Correlation(s) with disease[b]	Gain/loss-of-function mutation and molecular basis of disease[c]
B8	Proteinase inhibitor 8	Intracellular inhibitor	ND	
B9	Proteinase inhibitor 9	Intracellular inhibition of granzyme B	ND	
B10	Bomapin	Intracellular inhibition of unknown proteinase(s)	ND	
B11	Epipin	Intracellular inhibition of unknown proteinase(s)	ND	
B12	Yukopin	Intracellular inhibition of unknown proteinase(s)	ND	
B13	Hurpin	Intracellular inhibition of cathepsin L	ND	
C1	Antithrombin III	Extracellular inhibitor of activated clotting factors	Venous thrombosis	LOF: decreased affinity for heparin (Ref. 139)
D1	Heparin cofactor II	Extracellular inhibition of thrombin	Venous thrombosis	LOF Oslo mutation (R189H): decreased affinity for dermatan sulphate (Ref. 140)
E1	Plasminogen activator inhibitor 1	Extracellular inhibitor of uPA and tPA	Bleeding disorders	LOF (Ref. 141)
			Myocardial infarction	GOF (Ref. 142)
E2	Glial-derived nexin	Extracellular inhibition of plasmin, uPA, tPA, thrombin	ND	
F1	Pigment epithelium-derived factor	Non-inhibitory: anti-angiogenic factor	Age-related macular disease	LOF (Ref. 143)

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Table 1. Correlation between serpin mutations and human disease *(continued)*

Cladistic name[a]	Other name	Function	Correlation(s) with disease[b]	Gain/loss-of-function mutation and molecular basis of disease[c]
F2	α_2-Antiplasmin	Extracellular inhibition of plasmin	Bleeding disorders	LOF Enschede mutation [Ala insertion in proximal hinge (P8-P12)]: converts serpin to a substrate, rather than inhibitor (Ref. 144)
G1	C1 inhibitor	Extracellular inhibition of complement proteinases	Hereditary angioedema	LOF A436T: proteinase will no longer interact with the serpin (Ref. 145)
			Systemic lupus erythematosus	GOF A443V: inhibition of trypsin due to P2 mutation (Ref. 146)
H1	Heat shock protein-47	Non-inhibitory: collagen chaperone	Fibrosis	(Ref. 147)
I1	Neuroserpin	Extracellular inhibition of tPA.	FENIB	Polymerisation due to perturbation of the shutter (Ref. 109)

Abbreviations: ACE, angiotensin-converting enzyme; FENIB, familial encephalopathy with neuroserpin inclusion bodies; GOF, gain-of-function; LOF, loss-of-function; TBG, thyroxine-binding globulin; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator.

[a]Cladistic nomenclature for serpins includes a SERPIN prefix (e.g. SERPINA1) that has been removed here.
[b]ND: no disease or disease correlation has been described.
[c]This is not a comprehensive list of serpin mutations in disease, but rather a brief description of some mutants for which molecular mechanisms have been defined.

A1AT Met358Arg (Pittsburgh mutation)

The importance of the P1 residue in determining the inhibitory spectrum of a serpin is best illustrated by the lethal A1AT Pittsburgh mutation. Wild-type A1AT is the major extracellular inhibitor of neutrophil elastase (Ref. 59), and the Pittsburgh mutation was first identified in 1978 in a boy with episodic uncontrolled bleeding (Ref. 60). The symptoms were initially thought to be caused by a variant of ATIII, which bears significant structural and biochemical homology to A1AT (Ref. 60).

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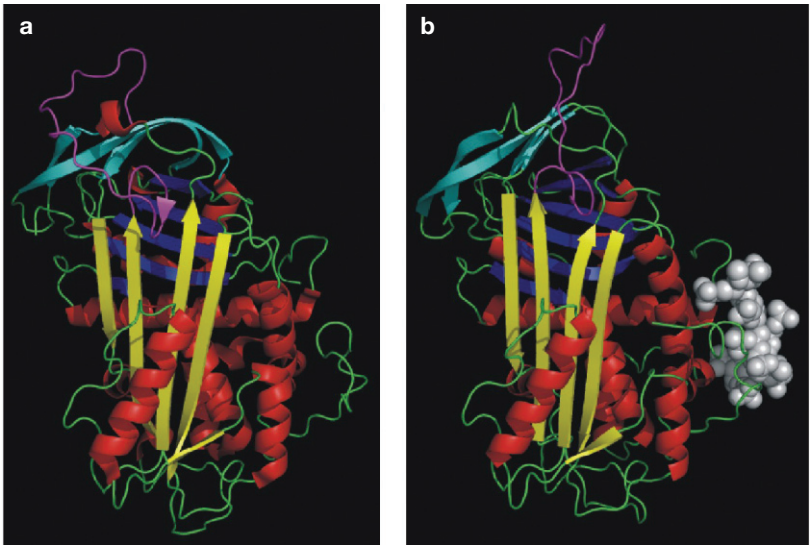

Activation of antithrombin III (ATIII) by heparin

Figure 4. Activation of antithrombin III (ATIII) by heparin. (a) In the native state of ATIII, the reactive centre loop (RCL, magenta) is partially inserted into the A β-sheet (blue). (b) Binding of heparin (grey spheres) induces a conformational change that expels the RCL, thereby enhancing the rate of interaction between ATIII and its target proteinases. Helices are red, loops are green, β-sheet A is yellow, β-sheet B is blue, β-sheet C is cyan and the RCL is magenta. Research Collaboratory for Structural Bioinformatics protein databank (http://www.rcsb.org/pdb/) identifiers: 1T1F (native ATIII), 1NQ9 (ATIII bound to heparin pentasaccharide).

However, further investigation indicated a mutation in the gene encoding A1AT resulting in a single amino acid substitution of the P1 residue (Met358) to arginine (Ref. 61). This resulted in a new inhibitory specificity for A1AT, creating a potent inhibitor of the arginine-specific pro-coagulation enzymes thrombin, kallikrein, factor XIa and factor XII_f (Refs 62, 63), thus leading to haemorrhagic disease.

In contrast to ATIII, A1AT Pittsburgh does not interact with heparin and therefore effectively inhibits these enzymes in its absence (Ref. 61), acting as an uncontrolled anticoagulant. Compounding this, the concentration of A1AT is much higher than ATIII (Refs 61, 64), so that the basal thrombin inhibitory activity in the blood of the patient was higher than that achieved by fully heparinised ATIII in normal plasma (Ref. 61). In this manner, the single P1 residue mutation resulted in the patient's death by changing serpin inhibitory specificity and thereby bypassing the normal homeostatic controls.

A1AT Glu342Lys (Z mutation)

Wild-type A1AT is synthesised and secreted by the liver and is important in controlling extracellular elastase, thereby maintaining the integrity of many tissues, particularly the lungs. The most common cause of A1AT deficiency is the Z mutation, resulting in the mutant protein being retarded within the endoplasmic reticulum (ER) of hepatocytes (Refs 65, 66). The Z allele encodes a glutamic acid to lysine substitution at position 342, at the top of the A β-sheet (Fig. 1b). Although this results in the loss of a salt bridge formed with Lys290 (Ref. 13), this interaction alone is unlikely to be important in the folding or secretion of A1AT, as the reverse mutation (Lys290Glu) has little or no effect (Refs 67, 68, 69). It is likely that the loss of other interactions involving Glu342 results in a structural instability leading to a propensity to form polymers (Ref. 68).

A1AT Z polymerises through a 'loop–sheet' mechanism, whereby the RCL of one molecule

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inserts into the A β-sheet of a second, creating a continuous chain of inactivated molecules (Refs 70, 71). The accumulation of polymers within the secretory pathway leads to hepatocyte death and, ultimately, to liver cirrhosis. At the same time, the loss of circulating A1AT leads to uncontrolled elastase activity and hence excessive tissue destruction in the lungs, resulting in emphysema. This severe phenotype raises the question of why the Z allele remains relatively common in North European populations (Ref. 72). The observation that A1AT polymers are observed in the lungs (Ref. 73), as well as the liver, and act as pro-inflammatory chemoattractants (Refs 74, 75, 76) has lead to the theory that they might provide some protection from respiratory infection (Ref. 77).

Treatment for A1AT Z

Although several treatments for A1AT Z exist for either emphysema or liver cirrhosis, the only effective therapy targeting both pathologies is liver transplantation. Following transplantation, the cirrhotic tissue is removed and the hepatic phenotype of the donor leads to a normalisation of serum A1AT without the danger of disease recurrence in either the lungs or liver (Refs 78, 79, 80, 81). However, a lack of transplantable organs has necessitated the development of other therapies.

Augmentation therapy by intravenous infusion of A1AT purified from the pooled blood of healthy donors has been used to treat A1AT deficiency for approximately 20 years. This treatment has been shown to be effective at restoring functional A1AT to the circulation (Refs 82, 83, 84), with low risk to the patient (Refs 85, 86). However, the levels of A1AT are not stable over long periods of time (Ref. 87), and so constant treatment is required, leading to high cost.

Gene therapy has been investigated as a mechanism to induce long-term secretion of A1AT and thus alleviate tissue destruction of the lungs. Several promising animal studies have indicated that the approach can yield circulating levels of A1AT high enough to protect the lungs using various delivery techniques and targeting different tissues as the site of novel protein production (Refs 88, 89, 90, 91). Recently, a clinical trial has begun to assess the efficacy of gene therapy in humans to correct the serum concentration of A1AT in individuals carrying the Z mutation (Ref. 92).

Whereas augmentation and gene therapies target the effect of decreased serum A1AT in the lungs, RCL peptides have been assessed as a mechanism for inhibiting the polymerisation defect affecting the liver. The use of peptides mimicking the RCL has been shown to prevent the polymerisation of serpins by binding to the A β-sheet and thereby inhibiting access of a second RCL (Refs 93, 94, 95, 96). However, the side-effect of this binding is that the RCL cannot insert into the sheet during inhibition and therefore the serpin becomes a substrate rather than an inhibitor (Ref. 93). Recently, a variant of the A1AT RCL peptide has been produced that binds preferentially to the Z mutant (Ref. 97). Importantly, this peptide can dissociate from the serpin to release an active proteinase inhibitor (Ref. 98). However, the use of peptides as therapies remains problematic, primarily as the peptide must be delivered into the ER of hepatocytes to be effective.

Neuroserpin

Neuroserpin is another example of a serpin in which polymerisation of a mutant form leads to disease. First identified in 1989 (Ref. 99), neuroserpin exhibits a tissue distribution that is restricted mainly to the brain, although it has also been detected in the pancreas, heart, kidney and testis (Ref. 100). Neuroserpin is a functional proteinase inhibitor, with inhibitory complexes observed with tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA) and plasmin (Ref. 101), although its in vivo function is believed to centre on inhibition of tPA. The balance between neuroserpin and tPA has been shown to be important in several neuronal events such as synaptic plasticity (Refs 102, 103), behaviour (Ref. 104) and the control of ischaemia following stroke (Refs 105, 106).

Four separate pathological mutations (Fig. 1c) have been identified in neuroserpin that can lead to its polymerisation: Ser49Pro, Ser52Arg, His338Arg and Gly392Glu (Refs 107, 108, 109). Although these mutations are widely distributed in the primary sequence, they all map to the shutter region, which is involved in regulating the opening and closing of the A β-sheet (Ref. 110). Polymerisation of neuroserpin has been shown to result in a novel form of

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inherited encephalopathy known as FENIB (familial encephalopathy with neuroserpin inclusion bodies), which presents as a progressive neurological degeneration including myoclonic seizures and dementia (Ref. 107). Post-mortem analysis of brain tissues indicates the presence of Collins bodies (intracellular aggregates of neuroserpin polymers) within the neurons. Interestingly, the severity of symptoms and early disease onset correlate strongly with predictions of increasingly severe perturbation of the shutter region by the various point mutations (Ref. 109).

Mutant neuroserpin is an inefficient proteinase inhibitor (Ref. 31) and readily polymerises in the ER both in vitro (Refs 31, 111) and in vivo (Ref. 112). However, it is difficult to ascertain whether FENIB is caused by lack of extracellular neuroserpin and uncontrolled proteolysis (as in A1AT Z emphysema), or by death as a result of ER stress (as in A1AT liver cirrhosis).

Maspin

The disease states described above are the result of mutations in proteins for which the normal homeostatic roles have been defined. These mutations result either in loss of specificity or in predictable destabilisation of the highly conserved and flexible tertiary structure leading to loss of function. By contrast, equivalent mutations have yet to be described for any of the 13 human clade B serpins. Nevertheless, several clade B serpins are strongly associated with disease. For example, maspin is a clade B serpin first identified in 1994 among transcripts downregulated in breast cancer tumourigenesis (Ref. 9). Since then, maspin has been shown to be downregulated or lost in a variety of cancers, suggesting it is a tumour suppressor. Loss of maspin expression is most often a result of abnormal methylation of its promoter, rather than a result of mutation or chromosomal rearrangement (Refs 113, 114, 115, 116).

Expression of maspin in transgenic animal models of breast cancer has been shown to inhibit the metastatic potential, but not the incidence, of cancers (Ref. 117). This appears to be a result of extracellular effects that decrease the motility of cancer cells as well as their ability to invade through the extracellular matrix (Ref. 118). In addition to this, maspin is an inhibitor of angiogenesis, and can inhibit growth of the primary tumour mass (Ref. 119). Maspin can also act intracellularly to increase the susceptibility of cells to apoptosis, through a poorly described mechanism that is possibly dependent on members of the Bcl-2 family (Refs 120, 121, 122). As well as decreasing invasive potential and increasing susceptibility to apoptosis, transfection with maspin results in large-scale changes in the proteome. However, any mechanism by which maspin might mediate transcriptional effects remains unexplored (Ref. 123).

The maspin RCL is crucial for its various biological effects (Refs 124, 125, 126). However, understanding the underlying mechanism is complicated by the fact that maspin does not appear to be an inhibitory-type serpin. Analysis of the primary sequence shows that maspin does not have an inhibitory proximal hinge motif, and maspin fails to undergo the stressed-to-relaxed conformational change that characterises serpin–proteinase interactions (Refs 127, 128). Furthermore, although several extracellular functions have been described, maspin is a member of the clade B serpins that, by definition, lack classical secretory signals (Ref. 129), and no mechanism for maspin secretion has yet been proposed. Future studies of the dysregulation of maspin in cancer might shed light on these problems and yield some insight into its normal role in homeostasis.

Conclusions

The presence of serpins in organisms from every kingdom of life underscores their importance to the well being of the host. In humans, serpins play crucial roles in the maintenance of homeostasis, controlling such processes as blood clotting and cellular survival. As such, loss of serpin function often leads to disease and death. The unique inhibitory mechanism utilised by serpins is based on a metastable fold and specific recognition of the cognate proteinase by the RCL. This means that they are prone to mutations leading to loss of cofactor control (ATIII mutants in the heparin-binding domain), gain of function (A1AT Pittsburgh mutant), loss of function (emphysema caused by the A1AT Z mutant or cancer resulting from loss of maspin), and toxic polymerisation (A1AT Z liver cirrhosis or FENIB). By increasing our understanding of the molecular mechanisms underpinning serpin

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function in homeostasis, we might in the future be able to devise novel therapeutics to treat their altered functions in disease.

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Further reading, resources and contacts

Support for patients with α_1-antitrypsin deficiency, including the latest news on research and treatment, can be found at:

http://www.alpha1.org

Movies showing serpin structural rearrangements upon proteinase cleavage or heparin binding can be found on a website set up by Dr J.A. Huntington's laboratory (Thrombosis Research Unit, University of Cambridge, Cambridge Institute for Medical Research, Cambridge, UK):

http://huntingtonlab.cimr.cam.ac.uk/movies.html

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Features associated with this article

Figures

Figure 1. Structure of native α_1-antitrypsin (A1AT).
Figure 2. The serpin inhibitory mechanism.
Figure 3. Structure of inactive serpin states.
Figure 4. Activation of antithrombin III (ATIII) by heparin.

Table

Table 1. Correlation between serpin mutations and human disease.

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