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The X-lectins: A new family with homology to the *Xenopus laevis* oocyte lectin XL-35

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The *Xenopus laevis* oocyte cortical granule lectin (XL35) has been studied in fertilization and embryonic development. Several nucleic acid sequences that predict proteins homologous to XL35 have since been reported in frog, human, mouse, lamprey, trout, ascidian worm. These proteins also showed high degrees of amino acid sequence homology to a common fibrinogen-like motif that may involve carbohydrate binding. Although their biological functions and carbohydrate binding specificities have not been studied in detail, this new family of lectins has common characteristics. Several independent studies on this new family of lectins strongly suggest that the lectins are expressed and stored in specialized vesicles that may be released upon the infection by pathogens. In addition, some family members have been shown to bind to oligosaccharides from bacterial pathogens. Therefore, this family of lectins likely participates in pathogen surveillance as part of the innate immune system. We propose the name X-lectin family for these homologs of XL35.
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Introduction

The *Xenopus laevis* oocyte cortical granule lectin XL-35 is produced and stored in the cortical granules of the oocyte, where it represents about 70% of the protein found in these secretory vesicles. At fertilization, the contents of the granules are released from the oocyte, and the multimeric lectin binds to its oligosaccharide ligands found in the surrounding egg jelly [1,2]. These ligands are expressed on ~500 kDa mucin-like glycoproteins cross-linked by disulfide bonds, each containing hundreds of O-linked saccharides. XL35 has a remarkable ability to bind a wide variety of both monovalently and polyvalently presented D-galactopyranosides, and binding is calcium-dependent [3]. The result of this binding reaction is an aggregation of lectins and large molecular weight glycoprotein ligands that participate in the formation of the fertilization membrane and serve as a block to polyspermy [1,4,5]. The cDNA encoding XL35 from *Xenopus* oocyte cDNA library was isolated and its predicted amino acid sequence does not display the C-type lectin motif, although it does require calcium for binding [6]. The lectin has an oligomeric structure with an apparent molecular weight of 500 kDa under non-reducing conditions [2,7]. Reducing SDS-PAGE reveals a monomer of about 43–45 kDa with size heterogeneity due to N-linked oligosaccharides, suggesting the native structure of the lectin is a 12-mer [7,8]. The lack of sequence similarity of the XL35 lectin with other known lectin families suggested that XL35 represents the first member of a new family of lectins.

Since the molecular cloning of XL35, mammalian homologs of XL35 have been identified. Two human XL-35 homologs, termed HL-1 and HL-2 [9], have been characterized. The overall amino acid identity between HL-1 and XL35 was 60% (similarity, 74%) with a 56% amino acid identity (similarity, 74%) with XL35. In addition, the cDNA of a murine homolog of XL35, termed intelectin, was isolated using a large-scale *in situ* hybridization screening method [10] and appeared to be expressed in intestinal Paneth cells. Recently, two groups reported the isolation of a cDNA from human placenta and human fetal intestine that shows identical sequence to that of HL-1 [11,12]. In addition to human and mouse, several cDNA sequences showing high amino acid sequence identity with XL35 have been reported from *Lathenteron japonicum* (lamprey), *Oncorhynchus mykiss* (trout), *Halocynthia roretzi* (ascidian worm), *Silurana tropicalis* (western clawed frog), as well as other, related sequences in *Xenopus* [13–15]. These findings demonstrate that a family of lectin-like proteins, homologs of XL35, is present in broad range of species. Although the biological functions for each of these putative lectins require further study, this review

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will summarize what is known about each and discuss the possible functions of these proteins in pathogen surveillance.

Xenopus laevis oocyte cortical granule lectin (XL35)

The cortical granule lectin (CGL) was initially purified from *Xenopus laevis* oocytes by melibiose affinity chromatography. The CGL agglutinated trypsinized rabbit erythrocytes in the presence of Ca^{2+}, and this reaction was strongly inhibited by α-galactosides such as melibiose, suggesting that the lectins bind to the abundant glycolipids on these erythrocytes terminated by α-galactose residues [7]. Structural characterization of the CGL, also known as XL35, was performed, and the hydrophobicity of the XL35 C-terminus and the lack of oligomerization after limited acid digestion of XL35 suggest that the region responsible for forming oligomers lies in the C-terminus selection of the polypeptide [16]. Interestingly, mass spectrometric studies demonstrated that the majority of the purified XL35 polypeptide from the oocyte does not show cleavage of the secretion signal sequence, although removal of the signal peptide was detected on a small fraction, with less than 10% of XL35 showing removal of the signal peptide [16]. This result suggests a unique biosynthetic pathway for XL35 in the oocyte which allows glycosylation and packaging into the cortical granules without cleavage of the signal sequence.

Quill *et al*. purified the ligand of CGL from oocyte fractions by gel filtration, anion-exchange, and affinity chromatography [17]. The purified ligand was a single, heavily glycosylated, high-molecular weight protein (Mr > 250,000). The CGL ligand was rich in the potentially glycosylated β-hydroxy amino acids, Ser, Thr, and Gly, which are typical of glycoproteins containing O-linked glycans such as mucins. Treatment of the CGL ligand with N-glycanase did not affect the binding of CGL while ligand function was lost under hydrolysis conditions that cleave O-linked glycans (alkaline β-elimination). This demonstrated that CGL/XL35 was recognizing O-linked glycans on the glycoprotein ligand. Digestion of the ligand with several exoglycosidases showed that terminal α-galactoside residues are an essential carbohydrate moiety recognized by CGL. The structure of several neutral oligosaccharides released from glycoproteins of *Xenopus laevis* jelly coat by β-elimination has been reported [18]. Many of these oligosaccharide structures were found to contain a terminal α-galactose residue:

Galα1-4(Fucα1-2)Galβ1-3GalNac-, Galα1-4(Fuc1-2)Galβ1-3(GlcNAcβ1-6)GalNac-,
Galα1-4(Fucα1-2)Galβ1-3(Fucα1-2)Gal-, and
Galα1-4(Fuc1-2)Galβ1-3(Fucα1-2)Galβ1-3(GlcNAcβ1-6)GalNAc-.

The interaction of XL35 with the jelly coat protein (JCP) was investigated using a range of chemically synthesized mono-, di-, and trisaccharide derivatives that are analogs of JCP-derived oligosaccharides. Independent analytical techniques, including enzyme-linked lectin assays (ELLA) and surface plasmon resonance (SPR), were exploited to probe the interactions of compounds with XL35. The results revealed that XL35 has remarkably broad specificity for galactose-terminating saccharides, and the affinity of XL35 for different galactose-terminated saccharides is only slightly affected by secondary features, such as anomeric configuration of the terminal sugar or the identity and linkage pattern of branching sugars. Broad specificity was also observed when the saccharides terminating with galactose were presented in a polyvalent fashion.

XL35 was shown to consist of 313 amino acids with three potential N-linked oligosaccharide sites. Although this lectin, termed XL35, requires calcium ions for oligosaccharide binding, its sequence does not contain the sequence motif defined for C-type lectins [6]. To examine the expression patterns of lectin mRNA at fertilization and during embryo development, Northern analysis was performed on total RNA purified from Stage VI oocytes and from embryos at various stages of development. These results showed that relatively high levels of XL35 mRNA was present in Stage VI oocytes and persisted through gastrulation, after which it declined. Compared to the levels of expression in gastrulae, low levels of XL35 mRNA was present in hatching tadpoles. Since it is highly unlikely that maternal mRNA persisted until tadpole stages, together with the observation of an increase of RNA levels at gastrulation, it appeared that XL35 mRNA is newly transcribed at the mid-blastula transition along with many other zygotic RNAs. The fact that these RNA are transcribed zygotically, as well as maternally, would strongly support the hypothesis that XL35 displays other functions in addition to its role in fertilization [4,8].

Nomura *et al*. showed that monoclonal antibodies against human blood group-B-type trisaccharides (B-substance, Galα1-4(Fucα1-2)Galβ1-3-) completely block the Ca^{2+}-dependent cell-cell adhesion system in *Xenopus laevis* embryonic (blastula stage) cells [19]. Synthetic B-substance glycopeptides also disrupt the Ca^{2+}-dependent cell-cell adhesion. These authors purified membrane glycoproteins that reacted with B-substance saccharides and showed they are glycosylphosphatidyl inositol (GPI)-anchored proteins. Amino acid sequence analysis of the purified protein showed that these proteins are homologs of XL35 [19]. These results indicate that the GPI-anchored XL35 homologs that recognize the B-substance trisaccharide are directly involved in Ca^{2+}-dependent cell-cell adhesion of *Xenopus* embryonic blastula cells.

Recently, Ishino *et al*. have been identified two amino acid sequences deduced from proteins purified and cloned from *Xenopus laevis* serum, termed the 35 kDa serum lectin (accession no: AB061238) and lectin type 2 (accession no: AB061239). These lectins showed a high degree of amino acid sequence homology with XL35 (Figure 1). The overall amino acid identity between the 35 kDa serum protein and XL35 was 59% (similarity, 81%), while there was 59% amino acid identity (similarity, 84%) between lectin type 2 and XL35. The open reading frame

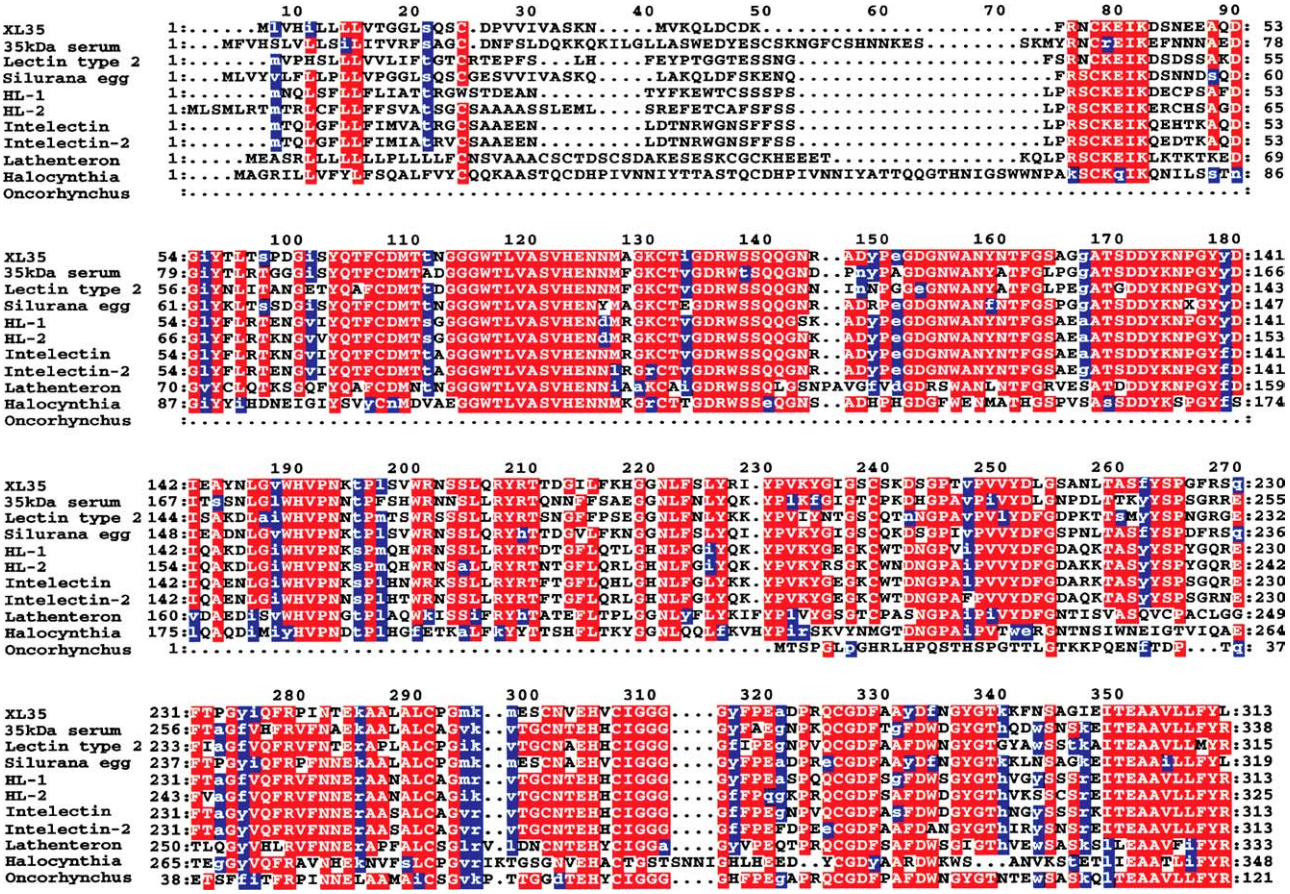

Figure 1. Comparison of amino acid sequences of X-lectin family. Amino acid sequences of all putative homologues from various eukaryotes are shown. Sequence information was obtained from the published paper or GeneBank data base. XL35, *Xenopus laevis* oocyte cortical granule lectin (U86699) [6]: 35 kDa serum, *Xenopus laevis* 35 kDa serum lectin (AB061238): Lectin type 2, *Xenopus laevis* lectin type 2 (AB061239): Silurana egg, *Silurana tropicalis* egg cortical granule lectin (AY079196): HL-1, human HL-1 (AY065972) [9,11,12]: HL-2, human HL-2 (AY065973) [9]: Intelectin, mouse Intelectin (AB016496) [10]: Intelectin-2, mouse Intelectin-2 (AY217760): Lathenteron, *Lathenteron japonicum* lamprey serum lectin (AB055981): Halocynthia, *Halocynthia roretzi* ascidian galactose lectin [15]: Oncorhynchus, *Oncorhynchus mykiss* (rainbow trout) (AF281350) [14]: The identical amino acids are shown in gray background characters, and similar amino acids are shown in black background.

for the 35 kDa serum lectin was predicted to encode 338 amino acids, while lectin type 2 was 315 amino acids. The predicted N-terminal region of these two lectins is composed of hydrophobic amino acids, suggesting the presence of a signal peptide sequence that causes proteins to enter the secretory pathway, similar to XL35. Earlier, Barondes and coworkers had reported that serum from estrogen-induced *Xenopus laevis* contained a 69 kDa protein that was weakly reactive against the anti cortical granule lectin antibody . The serum protein also bound to immobilized melibiose in a Ca^{2+} dependent manner, and a peptide mapping analysis suggested some similarity with the cortical granule lectin (XL35) [20]. It will be interesting to study how the 35 kDa serum lectin or lectin type 2 may be related to this 69 kDa protein.

The oocyte cortical granule lectin from *Silurana tropicalis* (western clawed frog) has also been reported in Genebank (accession no: AY079196). The amino acid identity between the cortical granule lectin from *Silurana tropicalis* and XL35 was 85% (similarity, 95%). The total number of amino acids in the open reading frame was 320 compared to 313 in XL35. This lectin is clearly a homolog of XL35, and it will be interesting to determine if its binding specificity and functions are similar to those of XL35.

Human homologs of XL35

XL35 cDNA and amino acid sequences were used to search protein and DNA sequence databases, and a single entry was identified from non-*Xenopus* sources, a human-expressed sequence tag (EST) data bank (GenBank, Accession number Z36760) [6,9]. Using the human EST sequence, we isolated two different cDNAs (HL-1 and HL-2) from a human small intestine λgt 10 cDNA library [9]. These two cDNA sequences showed 85% identity to one another at the deduced amino acid level. The

overall amino acid identity between HL-1 and XL35 was 60% (similarity, 74%) with a 56% amino acid identity (similarity, 74%) between HL-2 and XL35 (Figure 1). The open reading frame for HL-1 was the same size as that of XL35, 313 amino acids, while HL-2 was predicted to have 325 amino acids. Both HL-1 and HL-2 have signal peptide sequences and are encoded at chromosome 1q21.3 and 1q22–23.5, respectively, which has been designated as an "endothelial cell adhesion locus" because the genes for a number of cell adhesion molecules are found at this location, including the selectins. BLAST analysis against a human genomic DNA database showed the distance between the two genes encoding HL-1 and HL-2 is only 7,000 base pairs.

Northern blot analysis showed selective expression of HL-1, in heart, small intestine, colon, thymus, with lower levels in ovary, testis, and spleen. Other tissues showed detectable level of expression: skeletal muscle, placenta, and spleen. HL-2, however, was expressed only in small intestine [9]. Using a rabbit polyclonal antiserum raised to XL35, Immunohistochemical analysis showed that, in the human tissues that highly express their mRNA, these proteins are present in the endothelial cells lining small blood vessels. Using peptide-specific antibody, HL-2 was localized by immunostaining to the Paneth cells, specialized secretory cells whose main function is in pathogen surveillance (Lee *et al*., manuscript in preparation). The Paneth cells in the small intestine of most mammals produce α-defensins and other antimicrobial proteins including lysozyme and secretory phospholipase A2 [21]. These findings strongly implicate the involvement of the human homologs of XL35 in host defense against pathogens in the small intestine, thus making them a part of the innate immune system.

Tsuji *et al*. purified and cloned a protein from human placenta and named this protein, human Intelectin (hIntL) [12]. Surprisingly, the deduced amino acid sequence of hIntL was the same as that of HL-1. The protein was expressed as a secreted form in the rabbit kidney cell line RK-13. The hIntL/HL-1 absorbed to galactose-Sepharose and was completely eluted with 10 mM EDTA. About 50% of the absorbed HL-1 was eluted by buffers containing 100 mM galactose, 100 mM *N*-acetylgalactosamine, or 100 mM fructose. The same concentrations of mannose, glucose, *N*-acetylmannosamine, Nacetylglucosamine, sorbose, D-fucose, L-fucose, L-rhamnose and 2-deoxy-D-glucose did not elute hIntL from galactose-Sepharose. The protein was also effectively eluted by D-pentose, D-Xylose, D-ribose, and 2-deoxy-D-ribose. Interestingly, however, hIntL was not eluted from galactose-Sepharose by melibiose or lactose. These results suggest that the carbohydrate binding specificities of hIntL (HL-1) and XL35 are distinct. The hIntL was also shown to bind to the bacterial arabinogalactan from the cell wall of *Nocardia ruba* containing D-galactofuranosyl residues. The binding was completely inhibited by EDTA, D-ribose, D-galactose, and D-arabinose, but not D-glucose. Pentoses (D-ribose, D-xylose, D-lyxose, and D-arabinose); D-galactose inhibited the binding of HL-1 to arabinogalactan more effectively than D-mannose or D-glucose.

Another group cloned the entire coding region of the lactoferrin receptor cDNA by PCR based on amino acid sequences of the purified native lactoferrin receptor from fetal intestine [11]. Surprisingly, the amino acid sequence of this lactoferrin receptor showed a 100% match with that of HL-1. The apparent molecular mass was 136 kDa under non-reducing conditions and 34 kDa under reducing conditions suggesting a tetramer under native conditions. Phosphoinositol phospholipase C treatment indicated that the lactoferrin receptor is GPI anchored. The C-terminal region of the GPI anchored proteins should consist of a predominantly hydrophobic region of 8 to 20 amino acids, which directs the addition of preformed GPI anchor. There is a potential cleavage site in the sequence of lactoferrin receptor/HL-1 at residue 298 that could then be attached to the GPI anchor. This is a very interesting observation, in light of the finding of *Xenopus* homologs of XL-35 that were GPI-linked and involved in cell adhesion, as mentioned above [19]. In addition, lactoferrin is known to have a variety of antimicrobial activites [22,24]. It is possible, therefore, that HL-1, in its role as the lactoferrin receptor can modulate the antimicrobial effects of lactoferrin [11]. Since lactoferrin also functions in the mammalian embryo [25], it is also possible that HL-1 expressed in the mammalian oocyte and blastula, is the lactoferrin receptor, and mediates the function of lactoferrin during fertilization and early embryogenesis. Lactoferrin bound to HL-1 in a Ca^{2+}-dependent manner during affinity chromatography. This binding may involve the glycans on lactoferrin, since it is a glycoprotein, although the role of oligosaccharides on lactoferrin binding to HL-1 has not been directly examined.

Mouse homologs of XL35

Using a large-scale *in situ* hybridization screening method, Komiya *et al*. isolated a cDNA termed intelectin whose amino acid sequence revealed a 61% homology with XL35 [10]. The mRNA of intelectin appeared to be expressed in small intestine Paneth cells. Northern blot analysis revealed the mRNA corresponding to the cDNA was 1.2 kb in length, and expression was specific to small intestine. Intelectin is likely an ortholog of HL-2 because both are expressed only in small intestine Paneth cells. It is not clear, however, if the mouse expresses a homolog of HL-1. Interestingly, an intelectin sequence has also been identified in a 10-day-old mouse pancreas cDNA library [26] (accession number: AK065973). A very low level of HL-2 cDNA has been detected in human pancreas using quantitative RT-PCR, although a Northern blot showed no expression signal in this tissue (Kaneko *et al*., manuscript in preparation). These results also support that intelectin is a mouse ortholog of HL-2.

To analyze the location of intelectin in a mouse endothelial cell line, SVEC (SV40 transformed mouse lymph node endothelial cell) cells were studied by immunofluorescence and confocal microscopy using the rabbit polyclonal antiserum to XL35. The cells were stained with or without permeabilization by saponin, to distinguish cell surface and intracellular

Table 1. Summary of the X-lectins (homologs of XL35) from various sources

Organism	*Name*	*Tissue expressed*	*No. of amino acids*	*Accession No.*	*Proposed biological functions (Binding Specificity)*	*References*
Xenopus laevis	XL35	oocyte (cortical granule)	313	U86699	Transformation of fertilized envelop, Embryogenesis (Galactose)	Lee *et al.* (1997)
	35 kDa serum lectin	serum	338	AB061238	?	Ishino *et al.* (2002)
	lectin type 2	serum	315	AB061239		Ishino *et al.* (2002)
Silurana tropicalis	egg cortical granule lectin	egg	320	AY079196	?	Lindsay *et al.* (2002)
Mouse	Intelectin	small intestine (Paneth cell)	313	AB016496	?	Komiya *et al.* (1999)
		Pancreas		AK007447		Carninci *et al.* (2000)
	Intelectin-2	Intestinal epithelium	313	AY217760		Knight *et al.* (2003)
Human	HL-1	heart, colon, small intestine, etc.	313	AY065972	Recognizes bacterial arabinogalactan of Norcardia	Lee *et al.* (2001)
					(Affinity for D-galactose and D-galactofuranosyl)	Suzuki *et al.* (2001)
						Tsuji *et al.* (2001)
	HL-2	Small intestine (paneath cell)	325	AY065973	Bind to Cryptococcus neoformans and Candida albicans	Lee *et al.* (2001)
Lathenteron japonicum	lamprey serum lectin	serum	333	AB055981	?	Yoshimura *et al.* (2001)
Ciona intestinalis		serum	659	AK113061	?	Satou *et al.* (2002)
Oncorhynchus mykiss		liver	121	AF281350	Induced by the injection of Vibrio angyillarum	Bayne *et al.* (2001)
Halocynthia roretzi	asidian galactose lectin	serum	327	AY217760	Enhances phagocytosis by Halocynthia roretzi hemocytoes (galctose)	Abe *et al.* (1999)

*Reference 1, 2, 3, 4 and 5 have been taken from GenBank.

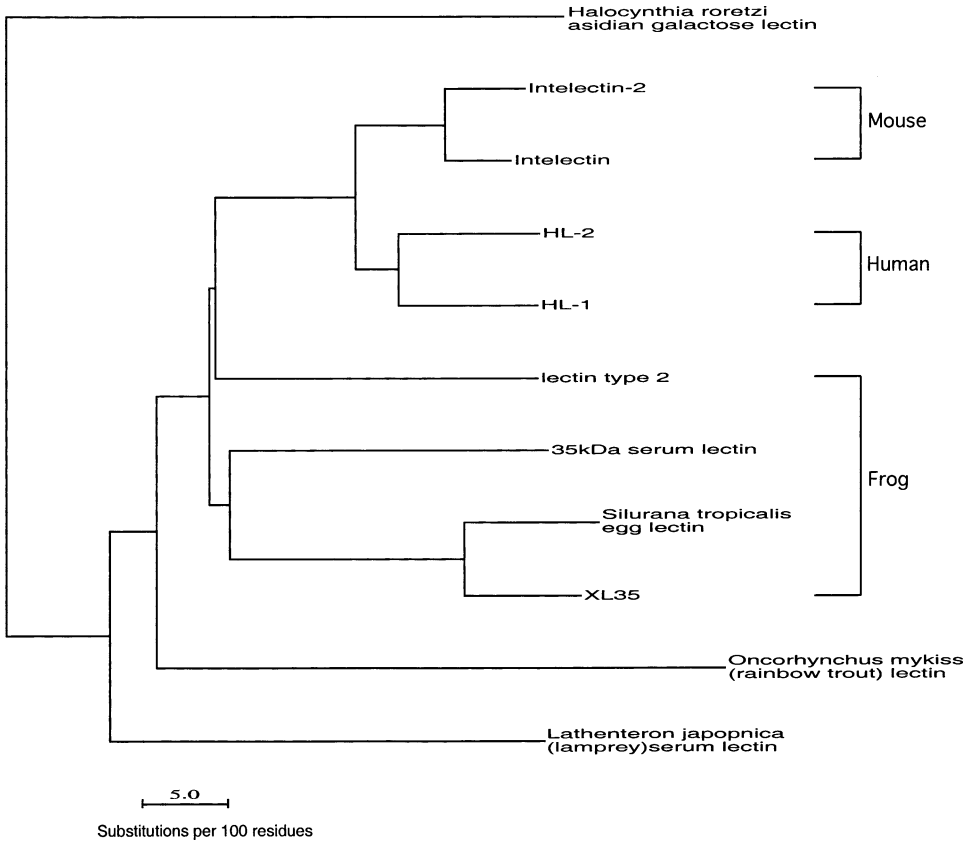

Figure 2. Dendogram of the X-lectin family. Sequences were compared by multiple sequence alignment using the CLUSTAL W algorithm [33]. The output from this alignment was used to construct a dendogram with the distance/growtree program. N-terminal regions including signal sequences were not used for construction of this dendogram.

Figure 3. Comparison of fibrinogen-like motif between homologues of XL35 and Ficolin/Opsonin/p35 lectin family. Identical amino acids are shown in gray background characters and similar amino acids are shown in black. Only the fibrinigen-like motif regions were compared: XL35, amino acids 66–134; HL-1, amino acids 66-134; HL-2, amino acids 77–165; Intelectin, amino acids 66–134; Fibrinogen-beta chain, amino acids 267–134; Ficolin-1, amino acids 142–200; Hakata antigen, amino acids 117–175; p35, amino acids 129–187.

locations, and bound antibody visualized by FITC-conjugated goat anti-rabbit IgG. There was no detectable binding of the anti-XL-35 antiserum to the surface of the non-permeabilized SVEC cells. The cells permeabilized by saponin, however, showed strong fluorescence intracellularly highlighted by the presence of distinct labeled vesicles, indicating that an XL35-related protein was present in the cells (Lee *et al.*, manuscript in preparation). These immunofluorescence results suggest that an XL35-related protein such as intelectin is expressed in the secretory pathway of SVEC, stored in a type of secretory vesicle. Further experiments will determine if specific stimuli can cause release of the vesicular XL35-related protein, as would occur in an *in vivo* defense response.

Recently, Knight *et al.* registered a mouse cDNA sequence of a homolog of intelectin in GeneBank (accession number: AY217760). A cDNA was isolated from intestinal epithelium and named Intelectin 2 (Figure 1). The amino acid identity between Intelectin (Intelectin 1) and Intelectin 2 was 91% (similarity, 98%). The total number of amino acids in open reading frame was 313, the same as Intelectin 1. A functional study

of Intelectin 2 has not been reported as yet. It is not clear if the sequencedifferences between these two Intelectins result from strain differences and whether their functions are identical.

XL35 homologs in other organisms

Besides human, mouse, and frog, sequence homologs of XL35 have been reported in several other eukaryotes (Table 1). Yokosawa *et al*. investigated the defense mechanism in the ascidian *Halocynthia reretzi*, which occupies a phylogenic position between the vertebrate and invertebrates [27]. They isolated several candidate defense molecules from plasma and hemocytes. Among them, a galactose-specific lectin was purified from plasma and demonstrated to stimulate the production of superoxide anions by mammalian polymorphonuclear leukocytes [28]. The complete amino acid sequence of the galactose-specific lectin from the plasma of the ascidian *Halocynthia roretzi* was determined by sequential Edman degradation, analysis of peptide fragments derived by proteolytic fragmentation, and chemical cleavage of the reduced S-pyridylethylated lectin. The amino acid sequence was verified by cDNAs isolated from a *H. roretzi* hepatopancreas cDNA library. The protein consisted of a total of 348 amino acids, including a putative signal sequence [15]. The putative amino acid sequence showed ∼40% identity (∼70% similarity) to XL35 (Figure 1). The authors reported that this lectin functions as a phagocytosis-stimulating molecule [15]. Although its effect appeared to be weak, they suggested that the lectin in the plasma may bind to and agglutinate invading foreign materials via galactose residiues which would enhance the phagocytosis by the hemocytes.

Bayne *et al*. analyzed differentially expressed genes in the livers of *Oncorhynchus mykiss* (rainbow trout) in the course of an acute phase response, using suppression subtractive hybridization of cDNAs from the livers of un-stimulated trout and of trout given a potent inflammatory stimulus (injection with a bacterial emulsion made by killing a suspension of *Vibrio anguillarum*). They isolated a putative homolog of XL35 (accession no. AF281350) along with 25 other genes thought to be potentially immune-related [14]. One of the isolated cDNAs showed a partial open reading frame of 121 amino acids that showed 46% identity (∼73% similarity) to the C-terminal region of XL35 (Figure 1). These findings strongly suggest that adult fish also express at least one type of XL35 homolog in response to infection by a pathogenic microorganism.

Yoshimura *et al*. reported the cDNA sequence of a putative homolog of XL35 from lamprey, *Lethenteron japonicum* (accession number: AB055981), termed the Lampetra japonica serum lectin. This cDNA encoded 333 amino acids and showed 47% identity (∼75% similarity) with XL35 (Figure 1). No functional studies on this lectin have been reported as yet.

Since these lectins are all clearly homologs of XL35, the first member of the family to be cloned, we have termed the family the X-lectins. Figure 2 depicts the evolutionary relationship between the deduced amino acid sequences of X-lectins from different species.

Conclusions

Lectins showing high homology to the amino acid sequence of XL35 have been reported from several eukaryotes since the original cloning of XL35. In addition to its function in formation of the fertilization envelope which blocks sperm entry [1,4,5], XL35 likely functions as well in cell-cell or cell-matrix adhesion events in the embryo [4,6,8]. Although several homologs of XL35 have been discovered in lower eukaryotes as well as human or mouse, studies on their biological functions and carbohydrate-binding specificities have been very restricted (Table 1). None of these proteins has the C-type lectin domain (CRD) [29], even though some have been shown to display binding activity to carbohydrate residues only in the presence of Ca^{2+}. Instead of the CRD domain, the X-lectins have a fibrinogen-like motif that is in the region of sequence that shows the highest degree of homology based on amino acid sequence alignment (Figures 1 and 3). Members of the Ficolin/Opsonin/p35 lectin family also contain significant homology to this fibrinogen-like motif, although members of this family do not share any other similarity with the X-lectins [30,31]. The Ficolin/Opsonin/p35 family of proteins is found in serum where it is thought to bind to oligosaccharide structures on the surfaces of microorganisms, leading to the killing of bound microbes through complement activation and phagocytosis, although their binding specificities are not well understood [32]. It has been hypothesized that their carbohydrate-binding activity depends on the fibrinogen-like domain. All X-lectins contain this fibrinogen-like motif (Figure 3); therefore, this region may function in some way in carbohydrate recognition, as well.

Although the biological function of each member of the X-lectin family has not been elucidated, their combined characterizations suggest that pathogenic infection can cause induction of their transcription and release from specialized vesicles. This family of lectins, therefore, is no doubt involved in the surveillance of the pathogens in the innate immune reaction, and their activities may involve opsonization, immobilization, or agglutination of the pathogens. Definition of biological functions and carbohydrate- binding specificities will facilitate development of novel antimicrobial therapeutics based on this new lectin family. Importantly, as the X-lectins may recognize invariant saccharide components of pathogens, these molecules may be involved in the rapid recognition and control of microbial pathogens at the "front lines" of the innate immune response.

References

1 Wyrick RE, Nishihara T, Hedrick JL, Agglutination of jelly coat and cortical granule components and the block to polyspermy in the amphibian *Xenopus laevis*, *Proc Natl Acad Sci USA* **71**(5), 2067–71 (1974).

2 Nishihara T *et al*., Isolation and characterization of a lectin from the cortical granules of Xenopus laevis eggs, *Biochemistry* **25**, 6013–20 (1986).

3 Arranz-Plaza E *et al*., High-avidity, low-affinity multivalent interactions and the block to ploryspermy in *Xenopus Laevis*, *J Am Chem Soc* (2002).

4 Roberson MM, Barondes SH, *Xenopus laevis* lectin is localized at several sites in Xenopus oocytes, eggs, and embryos, *J Cell Biol* **97**, 1875–81 (1983).

5 Monk BC, Hedrick JL, The cortical reaction in *Xenopus laevis* eggs: Cortical granule lectin release as determined by radioimmunoassay, *Zool Sci* **3**, 459–66 (1986).

6 Lee JK *et al*., Cloning and expression of a *Xenopus laevis* oocyte lectin and characterization of its mRNA levels during early development, *Glycobiol* **7**, 367–72 (1997).

7 Roberson MM, Barondes SH, Lectin from embryos and oocytes of *Xenopus laevis*: Purification and properties, *J Biol Chem* **257**, 7520–6 (1982).

8 Outenreath RL, Roberson MM, Barondes SH, Endogenous lectin secretion into the extracellular matrix of early embryos of *Xenopus laevis*, *Dev Biol* **125**(1), 187–94 (1988).

9 Lee JK *et al*., Human homologs of the Xenopus oocyte cortical granule lectin XL35, *Glycobiology* **11**(1), 65–73 (2001).

10 Komiya T, Tanigawa Y, Hirohashi S, Cloning of the novel gene intelectin, which is expressed in intestinal paneth cells in mice, *Biochem Biophys Res Commun* **251**(3), 759–62 (1998).

11 Suzuki YA, Shin K, Lonnerdal B, Molecular cloning and functional expression of a human intestinal lactoferrin receptor, *Biochemistry* **40**(51), 15771–9 (2001).

12 Tsuji S *et al*., Human intelectin is a novel soluble lectin that recognizes galactofuranose in carbohydrate chains of bacterial cell wall, *J Biol Chem* **276**(26), 23456–63 (2001).

13 Satou Y *et al*., A cDNA resource from the basal chordate Ciona intestinalis, *Genesis* **33**(4), 153–4 (2002).

14 Bayne CJ *et al*., Immune-relevant (including acute phase) genes identified in the livers of rainbow trout, Oncorhynchus mykiss, by means of suppression subtractive hybridization, *Dev Comp Immunol* **25**(3), 205–17 (2001).

15 Abe Y *et al*., A unique primary structure, cDNA cloning and function of a galactose-specific lectin from ascidian plasma, *Eur J Biochem* **261**(1), 33–9 (1999).

16 Tracy AS, *Biochemistry and Molecular Biology* (University of Georgia: Athens, 2001).

17 Quill TA, Hedrick JL, The fertilization layer mediated block to polyspermy in *Xenopus laevis*: Isolation of the cortical granule lectin ligand, *Arch Biochem Biophys* **333**, 326–32 (1996).

18 Strecker G *et al*., Primary structure of 12 neutral oligosaccharide-alditols released from the jelly coats of the anuran *Xenopus laevis* by reductive beta-elimination, *Glycobiology* **5**(1), 137–46 (1995).

19 Nomura KH *et al*., Involvement of blood-group-B-active trisaccharides in Ca^{2+}-dependent cell-cell adhesion in the Xenopus blastula, *Dev Genes Evol* **208**(1), 9–18 (1998).

20 Roberson M *et al*., Galactoside-binding serum lectin of *Xenopus laevis*. Estrogen-dependent hepatocyte synthesis and relationship to oocyte lectin, *JBC* **260**, 11027–32 (1985).

21 Ganz T, Defensins and host defense, *Science* **286**(5439), 420–1 (1999).

22 Teraguchi S *et al*., Glycans of bovine lactoferrin function as receptors for the type 1 fimbrial lectin of Escherichia coli, *Infect Immun* **64**(3), 1075–7 (1996).

23 Schaible UE *et al*., Correction of the iron overload defect in beta-2-microglobulin knockout mice by lactoferrin abolishes their increased susceptibility to tuberculosis, *J Exp Med* **196**(11), 1507–13 (2002).

24 Otto BR, Verweij-van Vught, MacLaren DM, Blood substitutes and infection, *Nature* **358**(6381), 23–4 (1992).

25 Ward PP *et al*., Restricted spatiotemporal expression of lactoferrin during murine embryonic development, *Endocrinology* **140**(4), 1852–60 (1999).

26 Carninci P *et al*., Normalization and subtraction of cap-trapper-selected cDNAs to prepare full-length cDNA libraries for rapid discovery of new genes, *Genome Res* **10**(10), 1617–30 (2000).

27 Yokosawa H *et al*., Galactose-specific lectin in the hemolymph of solitary ascidian, Halocynthia roretzi: Isolation and characterization, *Biochem Biophys Res Commun* **107**(2), 451–7 (1982).

28 Yokosawa H *et al*., Galactose-specific lectin in the hemolymph of solitary ascidian, Halocynthia roretzi. Molecular, binding and functional properties, *Biochim Biophys Acta* **870**(2), 242–7 (1986).

29 Drickamer K, Ca^{2+}-dependent carbohydrate-recognition domains in animal proteins, *Curr Opin Structural Biol* **3**, 393–400 (1993).

30 Kilpatrick DC, Fujita T, Matsushita M, P35, an opsonic lectin of the ficolin family, in human blood from neonates, normal adults, and recurrent miscarriage patients, *Immunol Lett* **67**, 109–12 (1997).

31 Ohashi T, Erickson HP, Two oligomeric forms of plasma ficolin have differential lectin activity, *J Biol Chem* **272**, 14220–6 (1997).

32 Matsushita M *et al*., A novel human serum lectin with collagen- and fibrinogen-like domains that functions as an opsonin, *J Biol Chem* **271**, 2448–54 (1996).

33 Thompson JD, Higgins DG, Gibson TJ, CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, *Nucleic Acids Res* **22**(22), 4673–80 (1994).