

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

Jin Kyu Lee and Michael Pierce

Complex Carbohydrate Research Center and Department of Biochemistry and Molecular Biology  
University of Georgia, Athens, GA 30602

## Introduction

In 1988, Herman Stillmark discovered a hemagglutinin (that he originally called ricin) in extracts of castor beans. Although lectins were first described in plants in relation to their cell agglutinating properties[1, 2], molecules that bind carbohydrate residues have now been discovered in viruses, microorganisms, plants, and animals [1, 3, 4]. It has been shown that lectins serve to mediate diverse carbohydrate recognition events in plants and animal tissues of both embryonic and adult origins.

Animal lectins have been found associated with the cell surface, the cytoplasm, and the nucleus. At the cell surface, lectins can act as receptors involved in selective intercellular adhesions and cell migration[5, 6], as well as in the recognition of circulating glycoproteins[7]. Lectins have also been shown to function as receptors for the extracellular matrix proteins, elastin and laminin[8, 9] for glycosaminoglycans that mediate the binding of the proteoglycans to the sugars of other matrix glycoproteins[10], and mediate the first steps in immune cell adhesion to endothelia[11]. Proteins with carbohydrate residue affinities also function in the innate immune system of vertebrates[12].

Although the number of animal lectins discovered continues to increase, these lectins can be classified into distinct families based on protein sequence homologies[13, 14]. A recent classification indicates that most of fall into one of five major groups:  $\text{Ca}^{2+}$ -dependent lectin (C-type lectins), Galectins (S-type lectins), Mannose 6-phosphate receptors (P-type lectins), siglec and other immunoglobulin-like sugar binding lectins (I-type lectins) and lectins related in sequence to the leguminous plant lectins (L-type lectins)[15]. In this chapter, we review a relatively new family of animal lectins, the “X-lectins”, that have no C-type lectin domain (CRD) in their primary sequences, but have been shown to display binding activity to carbohydrate residues only in the presence of  $\text{Ca}^{2+}$ . These lectins appear to function in innate immunity, but members have been shown to have specialized functions, as well.

The X-lectin family was first studied as the *Xenopus laevis* oocyte cortical granule lectin (XL35) in oocyte and embryos. The cDNA encoding XL35 was isolated from a *Xenopus* oocyte cDNA library and its predicted amino acid sequence does not display the C-type lectin motif, although it does require calcium for binding [16]. The lack of sequence similarity of the XL35 lectin with other known calcium-dependent lectin families suggested that XL35 represents the first member of a new family of lectins. XL35 was shown to consist of 313 amino acids with three potential N-linked oligosaccharide sites and includes predicted signal sequence.

Several nucleic acid sequences that predict proteins homologous to XL35 have since been reported in frog, human, mouse, lamprey, trout, and ascidian. These predicted proteins also show 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 of all of these members have not been studied in detail, they appear to have common characteristics. Several independent studies on this new family of lectins strongly suggest that some members are expressed and stored in specialized vesicles that may be released upon infection by pathogens. In addition, some family members have been shown to bind to oligosaccharides of bacterial pathogens. Therefore, this family of lectins likely participates in pathogen surveillance as part of the innate immune system in humans, murine, and frogs. We proposed the name “X-lectin” family for these homologs, since these lectins are all clearly homologs of XL35[17].

**I. *Xenopus laevis* oocyte cortical granule lectin (XL-35) was the first identified lectin among the X-lectin family.**

Studies by two groups have demonstrated that *Xenopus laevis* oocytes and embryos contain soluble, calcium-dependent lectins that form an oligomeric structure with an apparent molecular weight of 500 kDa under non-reducing conditions [18, 19]. 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 [18, 20]. 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[21]. Interestingly, mass spectrometric studies demonstrated that the majority of the purified XL35 polypeptide from the oocyte does not show cleavage of its secretion signal sequence, although removal of the signal peptide was detected on a small fraction, less than 10% [21]. This result suggests a unique biosynthetic pathway for XL35 in the oocyte that allows glycosylation and packaging into the cortical granules without cleavage of the signal sequence. XL-35 is contained within the cortical granules of the oocytes, as well as in several other intracellular locations, and it is released from the cortical granules at fertilization[22]. Several lines of evidence suggest that the released multimeric lectins bind to oligosaccharide targets on glycoproteins in the egg jelly coat where they participate in the formation of the fertilization envelope that blocks sperm entry[23]. These ligands are expressed on ~500 kDa mucin-like glycoproteins cross-linked by disulfide bonds, each containing hundreds of O-linked saccharides[24]. XL35 has a remarkable ability to bind a wide variety of both monovalently and polyvalently presented D-galactopyranosides, and binding is calcium dependent [25]. Hydrolysis of the purified XL-35 ligand with a series of exoglycosidases showed that a terminal  $\alpha$ -galactose was the ligand structure required for recognition by XL-35[24]. The ligand was rich in the potentially glycosylated  $\beta$ -hydroxy amino acids, Ser, Thr, and Gly, which are typical of glycoproteins containing O-linked glycans such as mucins. The structure of several neutral oligosaccharides released from glycoproteins of *Xenopus laevis* jelly coat by  $\beta$ -elimination has been reported [26]. Many of these

oligosaccharide structures were found to contain a terminal  $\alpha$ -galactose residue: Gal $\alpha$ 1-4(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3GalNAc-, Gal $\alpha$ 1-4(Fuc1-2)Gal $\beta$ 1-3(GlcNAc $\beta$ 1-6)GalNAc-, Gal $\alpha$ 1-4(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3(Fuc $\alpha$ 1-2)Gal- and Gal $\alpha$ 1-4(Fuc1-2)Gal $\beta$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3(GlcNAc $\beta$ 1-6)GalNAc-.

Recent results from a glycan array screening analysis with fluorescent labeled XL-35 showed the very clear carbohydrate binding specificity of XL-35 (Figure 1) (<http://www.functionalglycomics.org/glycomics/publicdata/selectedScreens.jsp>). The glycan array production and assay was performed by Core H of the Consortium for Functional Glycomics (CFG) (<http://www.functionalglycomics.org/fg/index.shtml>). The array consists of 285 glycans in replicates of 6. The results show that in the presence of calcium, specificity was restricted to glycans with a terminal alpha linked galactose. XL35 prefers to be attached to a GalNAc residue, more than any other residue assayed (Figure 1). These results are consistent with earlier studies and graphically demonstrate the specificity of XL35.

### **Homologs of XL35 in frog embryos**

To examine the expression patterns of XL35 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 were present in Stage VI oocytes and persisted through gastrulation, after which it declined[16]. 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, strongly supports the hypothesis that XL35 displays other functions in addition to its role in fertilization [20, 27].

Nomura et al. showed that monoclonal antibodies against human blood group-B-type trisaccharides (B-substance, Gal $\alpha$ 1-4(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3-) completely block the Ca<sup>2+</sup>-dependent cell-cell adhesion system in *Xenopus laevis* embryonic (blastula stage) cells [28]. Synthetic B-substance glycopeptides also disrupt this Ca<sup>2+</sup>-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 [28]. These results indicate that the GPI-anchored XL35 homologs that recognize the B-substance trisaccharide are directly involved in Ca<sup>2+</sup>-dependent cell-cell adhesion of *Xenopus* embryonic blastula cells.

The oocyte cortical granule lectin from *Silurana tropicalis* (western clawed frog) has also been reported in Genbank (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 protein is clearly a homolog of XL35 (Figure 2) and it will be interesting to determine if its binding specificity and functions are similar.

Recently, Ishino et al. have identified two amino acid sequences 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 2). 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 (Figure 2). The open reading frame 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 co-workers had reported that serum from estrogen-induced *Xenopus laevis* contained a 69 kDa protein that was weakly reactive against antibody against the cortical granule lectin. The serum protein also bound to immobilized melibiose in a  $\text{Ca}^{2+}$  dependent manner, and a peptide mapping analysis suggested some similarity with the cortical granule lectin (XL35) [29]. It will be interesting to determine the relationship of the 35 kDa serum lectin and the lectin type 2 may be related to this 69 kDa protein.

## **II. Mouse homologs of XL35: Intelctin-1 and Intelectin-2**

The first report on a mouse homolog of XL-35 was the isolation of its cDNA using a large-scale *in situ* hybridization screening method. They named the protein encoded by this cDNA “Intelectin”, since it was shown to be expressed in small intestine. The term, intelectin, is now sometimes used for homologs of XL-35. The intelectin amino acid sequence revealed a 61 % homology with XL 35 [30] (Figure 2). Northern blot analysis revealed the mRNA corresponding to the cDNA was 1.2 kb in length, and expression was specific to small intestine. The mRNA of Intelectin appeared to be expressed in small intestine Paneth cells *in situ* hybridization studies. Interestingly, an Intelectin sequence has been also identified in a 10 day old mouse pancreas cDNA library [31] (accession no: AK065973), no further characterization of the protein encoded by this cDNA can be found.

Another mouse homolog, intelectin-2, has been detected by the proteomic analysis of mouse jejunal epithelium and its response to infection with the intestinal nematode, *Trichinella spiralis*[32]. Intelectin-2 was cloned from BALB/c mRNA extracted on day 14 of infection of *T. spiralis*, and was found to have 91% amino acid identity with mouse intelectin (now termed Intelectin-1) (Figure 2).

Intelectin-2 transcripts were up-regulated early (day 3) during infection with *T. spiralis* in BALB/c mice and levels remained high through to day 14 (time of parasite rejection), but returned to undetectable levels at day 56, following resolution of infection[33]. Immunohistochemistry of jejunal sections followed a similar pattern, with intense labeling of goblet and Paneth cells at day 14. However, intelectin-2 transcripts and protein were absent when C57BL/10 mice were infected with *T. spiralis*. Genomic PCR and Southern blotting confirmed that the intelectin-2 gene is absent from the C57BL/10 genome. In uninfected BLAB/c mice, mRNA for intelectin-1 was expressed at high levels throughout the gut, but was not detected in significant amount in other tissues. Nematode infection did not cause any apparent change in Intelectin-1 expression in the gut, however. These observations suggest that intelectin-2 may serve a protective role in the innate immune response to parasite infection and may be more similar to HL-2 function (see below).

Mouse Intelectin-1 is also expressed in the airway epithelial cells of IL-13-over-expressing transgenic mice. IL-13 over-expression in a mouse model is necessary and sufficient for the development for experimental asthma. These mice develop airway hyperreactivity and mucus overproduction. Intelectin was highly expressed (20 times in microarray and 146 times in PCR analysis) in airway epithelial cells from mice with asthma compared to controls[34]. Intelctin-1 in the airway might alter the response of subjects with asthma to infection or colonization with bacterial or fungal pathogens.

### **III. XL35 homologs in fish have important functions in their innate immune response.**

In addition to human, mouse, and frog, sequence homologs of XL35 have been reported in several other eukaryotes, especially in fish (Table 1) (Figure 2), in which innate immune responses are the dominant defense system against pathogens. The cDNA encoding grass carp Intelectin was isolated from

a kidney cDNA library, and termed gcIntL[35]. The deduced amino acid sequence of gcIntL consists of 318 amino acids, and was about 55% identical and 74% similar to a human homolog, HL-1 (see below). Using real-time quantitative RT-PCR analysis, gcIntL transcripts were significantly induced in head kidney, trunk kidney, spleen, and intestine from LPS-stimulated fish. RT-PCR and Western blotting analysis demonstrated that gcIntL mRNA and protein were detected in brain, gill, intestine, head kidney, trunk kidney, spleen, and heart. Furthermore, gcIntL protein could also be detected in gill, intestine, trunk kidney, head kidney, spleen, heart, and brain.

Yokosawa et al. investigated the defense mechanism in the ascidian *Halocynthia roretzi*, which occupies a phylogenic position between the vertebrate and invertebrates [36] (Figure 2 and Figure 3). 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 [37]. 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 [38]. The putative amino acid sequence showed ~40% identity (~70% similarity) to XL35 (Figure 2). The authors reported that this lectin functions as a phagocytosis-stimulating molecule [38]. 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 residues, which would enhance 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 (after intraperitoneal injection with a *Vibrio* bacterin). They isolated a cDNA of putative homolog of XL35 along with 25 other genes thought to be potentially immune-related [39, 40]. 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 2). 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 no. AB055981), termed the Lampetra japonica serum lectin. This cDNA encoded 333 amino acids and showed 47% identity (~75% similarity) with XL35 (Figure 2). No functional studies on this lectin have been reported as yet.

Because invertebrates lack an adaptive immune system, they had to evolve effective intrinsic defense strategies against a variety of microbial pathogens. The innate immune system of invertebrates includes a hemolymph coagulation system, which participates both in defense against microbes and in hemostasis. In invertebrates, it has been shown that lectins present in the hemolymph play key roles in biological defense against invading foreign materials as nonspecific defense molecules[38]. Although X-lectin homolog function have just begun to be studied in fish, lamprey, and ascidian defense mechanism, they likely play an important role in innate immune responses.

#### **IV. Human homologs of XL35 have been reported with various biological functions and names: HL-1, HL-2, Intelectin, Lactoferrin receptor, and Omentin.**

Since the molecular cloning of XL35, several mammalian homologs of XL35 have been identified. Two human XL-35 homologs, termed HL-1 and HL-2 [41] were identified and cloned rapidly

after XL35 cloning using the human expressed sequence tag (EST) data bank (GenBank, Accession number Z36760) [16, 41]. 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 2). 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 are predicted to have signal peptide sequences and are encoded at chromosome 1q21.3 and 1q22-23.5, respectively, the same locus that encodes the selectins[42]. 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, implying a gene duplication event was likely involved.

Northern blot analysis showed selective expression of HL-1, in heart, small intestine, colon, and thymus, with lower levels in ovary, testis, and spleen. Other tissues showed detectable levels of expression: skeletal muscle, placenta, and spleen. Colon, liver and thymus expressed HL-1 in their vascular endothelial cells using immunohistochemistry on tissue sections[41]. Human aortic endothelial cells express HL-1 but the expression was apparently unaltered in cultured cells by the addition of an endothelial cell activating agent[41]. HL-2, however, was expressed only in small intestine [41] by Northern and PCR analysis. Using a peptide-specific antibody, HL-2 was localized by immunostaining to the Paneth cells, specialized secretory cells whose main function is in pathogen surveillance (unpublished data). The Paneth cells in the small intestine of most mammals produce  $\alpha$ -defensins and other antimicrobial proteins including lysozyme, secretory phospholipase A2, HIP/PAP and RegIII $\gamma$  [12, 43]. Microbial colonization of germ-free mice triggers epithelial expression of RegIII $\gamma$ , a secreted C-type lectin. RegIII $\gamma$  binds intestinal bacteria but lacks the complement recruitment domains present in other microbe-binding mammalian C-type lectins. Recently, Cash et al. showed that RegIIIg is by itself an

antimicrobial protein that binds to bacterial targets via interactions with peptidoglycan carbohydrate [12]. These findings strongly implicated 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) [44] because of its homology to the mouse intelectin. Surprisingly, the deduced amino acid sequence of hIntL was the same as that of HL-1. The protein was reported to be expressed at very low levels as a secreted form from the rabbit kidney cell line RK-13. The hIntL/HL-1 was reported to be absorbed to galactose-Sepharose and was completely eluted with EDTA. About 50% of the absorbed HL-1 was eluted by buffers containing 100 mM galactose, N-acetylgalactosamine, or fructose, however. The same concentrations of mannose, glucose, N-acetylmannosamine, N-acetylglucosamine, sorbose, D-fucose, L-fucose, L-rhamnose and 2-deoxy-D-glucose did not appear to elute hIntL from galactose-Sepharose. The protein was also reported to be 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 demonstrated that the carbohydrate binding specificities of hIntL (HL-1) and XL35 are distinct, but suggested that the specificity was not clear. The hIntL also appeared to bind to the bacterial arabinogalactan from the cell wall of *Nocardia rubra* containing D-galactofuranosyl residues. This 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) and D-galactose inhibited the binding of HL-1 to arabinogalactan more effectively than D-mannose or D-glucose. In conclusion, hIntL appears to be a lectin that can at least partially recognize galactofuranose and likely plays a role in the recognition of bacteria-specific component in the host.

Another report suggested a possible, additional function for HL-1. The entire coding region of a lactoferrin receptor cDNA was reported to be cloned by PCR, based on amino acid sequences of a protein

from fetal intestine that bound lactoferrin [45]. Surprisingly, the amino acid sequence of this lactoferrin receptor was reported to be a 100% match with that of HL-1. The apparent molecular mass of recombinantly expressed protein in a baculovirus-insect cell system 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 8-20 amino acids, which directs the addition of preformed GPI anchor. There is a putative 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 [28]. In addition, lactoferrin is known to have a variety of antimicrobial activities [46-48]. It is possible, therefore, that HL-1, in its role as the lactoferrin receptor can modulate the antimicrobial effects of lactoferrin [45]. Since lactoferrin also functions in the mammalian embryo [49], it is also possible that HL-1 expressed in the mammalian oocyte and blastula, functions as a lactoferrin receptor, and mediates the function of lactoferrin during fertilization and early embryogenesis. Lactoferrin bound to this lactoferrin receptor 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 lactoferrin receptor (HL-1) was not examined. A recent publication suggested that lactoferrin receptors are present in both the secretory granules of lysozyme-positive Paneth cells in the bottom of the intestinal crypts, as well as in goblet cells along the crypt-villus axis[50]. But quantitatively, the major site of lactoferrin receptor localization was the enterocyte brush border in small intestine. This membrane is organized in stable glycolipid-based lipid raft microdomains and has long been known to harbor receptor for lactoferrin. Enterocytes synthesize a number of brush border GPI-anchored proteins, showing this cell type harbors

the enzymes required for anchorage of GPI in the endoplasmic reticulum. A major part of lactoferrin receptors in enterocytes was released by phosphatidylinositol-specific phospholipase C, indicating a membrane insertion by a GPI anchor. Interestingly, lactoferrin receptor (HL-1) was suggested to be a major component of microvillar lipid rafts and superrafts. This strategic localization suggests that the lactoferrin receptor (HL-1) serves as an organizer and stabilizer of the brush border membrane, preventing loss of digestive enzymes to the gut lumen and protecting the glycolipid microdomains from pathogens[50].

Another interesting study of HL-1 (Intelectin-1) has been done on malignant pleural mesothelioma (MPM), which is a fatal neoplasm with no acceptable curative approaches[51]. Serial analysis of gene expression (SAGE) was used to compare the gene expression pattern of a surgically resected MPM to the autologous normal mesothelium. Intelectin gene overexpression (>139-fold) was found in the tumor. Online SAGE datasets revealed Intelectin to be consistently present in mesothelioma(s), ovarian cancer, and colon cancer. Intelectin mRNA expression was found by RT-PCR in resected MPM tumors, and Intelectin protein expression was confirmed by immunohistochemistry in MPM tumors. These observations suggest that Intelectin over-expression in mesothelioma could have potential screening, and therapeutic implications

### **HL-1 is a novel adipocytokine and enhances glucose transport: Omentin**

Recently, a new adipocytokine expressed in human omental adipose tissue, termed Omentin, was reported and revealed to be 100% identical in amino acid sequence to that of HL-1[52, 53]. Omentin mRNA was predominantly expressed in visceral adipose tissue and was barely detectable in subcutaneous fat depots in human and rhesus monkeys. Whether there exists a unique type of cell in the omental

adipose depot or whether a unique local environment induces Omentin expression from a specific cell type is not clear, since an immunohistological study has not been performed. Omentin is among the first molecules known to exhibit dramatic difference in gene expression between the two major types of fat deposits. Furthermore, Omentin enhanced only insulin-mediated glucose transport and did not stimulate basal glucose transport[53]. Very recently, Batista et al. showed that plasma Omentin and mRNA expression levels are inversely related to obesity[54]. This result suggests that higher Omentin levels in serum may be seen as a positive factor that opposes the obese state.

These observations strongly suggest that Omentin is a novel adipokine that is expressed in omental adipose tissue in human and may regulate insulin action. It is certainly possible that Omentin (HL-1) may be present in endothelial cells in the vessels that vascularize the fat tissue, which would be consistent with the location identified in other tissues. Various adipocytokines (adipocyte-secreted hormones) have been described which profoundly affect insulin sensitivity and might potentially link obesity, insulin resistance and cardiovascular disease[55, 56]. Among these, adiponectin and visfatin appear as insulin-sensitising adipocytokines, whereas TNF- $\alpha$ , IL-6 and resistin induce insulin resistance. One of the adipocytokines, leptin, is a fat-derived key regulator of appetite and energy expenditure in mice [56]. Due to their profound effects on whole-body glucose and energy metabolism, Omentin is attracting interest as potential new therapeutics for diabetes mellitus and obesity related to pathogenesis. It will be interesting to define the Omentin receptor on adipocytes and analyze the possible carbohydrate binding specificity of Omentin to its receptor, since it is highly homologous to a known lectin, XL35.

## **Summary and Conclusions**

A new family of lectins showing significant homology to the amino acid sequence of XL35 has been reported from several eukaryotes since the original cloning of XL35 (Figure 3). These findings demonstrate that a family of lectins, homologs of XL35, is present in broad range of species. We have proposed the name “X-lectin” family for these homologs of XL35. In addition to its function in formation of the fertilization envelope which blocks sperm entry [22, 23, 27], XL35 likely functions as well in cell-cell or cell-matrix adhesion events in the embryo [16, 20, 27]. 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 not been done in detail (Table 1).

None of these proteins has the C-type lectin domain (CRD) [57], even though several have been shown to display binding activity to carbohydrate residues only in the presence of  $\text{Ca}^{2+}$ . Instead of the CRD domain, all X-lectins, including the fish homologs, 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 (Figure 2 and Figure 4). Fibrinogen is a principal protein of vertebrate blood clotting and is found universally in blood from fish and echinoderms to mammals [58]. Early work on the evolution of vertebrate fibrinogen suggested a common origin of the arthropod hemolymph coagulation and the vertebrate blood coagulation systems. A thorough analysis of the evolution of vertebrate fibrinogen suggested most of the proteins bearing fibrinogen-related domains are lectins [59]. 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 [60, 61]. The Ficolin/Opsonin/p35 family of proteins are found in serum where they are thought to bind to oligosaccharide structures on the surfaces of microorganisms, leading to the killing of bound microbes through complement activation and phagocytosis [62]. Collectins also bind to a wide range of sugar residues in a  $\text{Ca}^{2+}$ -dependent manner. In addition, Ficolins bind to sugar residues that are rich on microbial surfaces, for example, N-acetyl-D-

glucosamine, in a calcium dependent manner[63]. Therefore, the conserved fibrinogen-like motif in X-lectins may function in carbohydrate recognition, as well.

Intestinal Paneth cells are the major producers of multiple peptides and proteins with antimicrobial activity in the intestine[64]. The most abundant and diverse of these are the defensins. They are highly microbicidal in vitro and probably important in vivo, yet their physiologic functions and mechanisms remain incompletely understood. Relative defensin deficiency may be a risk factor for Crohn's disease and infectious diarrhea. Antimicrobial lectins, particularly the hepatocarcinoma-intestine-pancreas/pancreatic-associated protein, RegIII $\gamma$  can lyse bacteria or interfere with their attachment to epithelial cells[12]. As discussed, several groups have shown that X-lectins members are expressed in mammalian Paneth cells. This location strongly suggests that mammal X-lectins function as a type of antimicrobial or microbicidal proteins secreted in Paneth cells in response to pathogens. Notably, the interactions of XL35 itself, after release from the cortical granules in complex and cross-linked with its high molecular weight glycoprotein ligand, participates in the formation of the fertilization membrane. This structure functions in the block of polyspermy, but may function as well as a barrier to microbial infection of the embryo.

It is likely that pathogenic infection can cause the induction of their transcription and release of members of the XL35 family from specialized vesicles. Some members are involved in the surveillance of the pathogens in the innate immune reaction, and their activities may involve opsonization, immobilization, or agglutination of the pathogens. The reg family is an example among proteins that have those biological functions. The *reg* gene family encodes a diverse group of secreted proteins that contain the conserved sequence motif found in C-type lectin domains. They bind to bacteria, but lack the complement recruitment domains present in other microbe-binding mammalian C-type lectins. Definition of the mechanisms of microbial cytotoxicity and carbohydrate binding specificities will facilitate development of possible novel antimicrobial therapeutics based on this new lectin family. Importantly, as

the X-lectins may recognize invariant saccharide(s) 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.

Very recently, HL-1 has been shown to function as a new member of the adipocytokine family in humans. Since there is now a link between several adipocytokines and the immune response[65], it is intriguing to speculate that through a gene duplication event, a cytotoxic lectin involved in the innate immune system (HL-2) gave rise to a homolog (HL-1) which is expressed in a subset of endothelial cells, including omental endothelia, that evolved to function as an adipocytokine. It will be very interesting to determine if HL-1 has retained a true carbohydrate binding specificity for its receptor on adipocytes, or whether its ligand is completely peptide in nature, or a hybrid of peptide and glycan, such is observed with P-selectin, which binds portions of both the sialylated Lewis X glycan and sulfated tyrosine residues on its ligand, PSGL-1.

### **Table 1. Summary of the X-lectin family from various sources.**

### **Legends to figures**

### **Figure 1. Glycan binding array analysis of XL-35 shows it binds to alpha linked Galactose.**

The fluorescent labeled XL-35 has been assayed for its binding specificities using a glycan array consisting of 285 different glycans structures. Its binding specificity was restricted to glycans with a terminal alpha-linked galactose. The glycan array was produced and assayed by Consortium for

Functional Glycomics (CFG). The full data is available at <http://www.functionalglycomics.org/fg/index.shtml>.

**Figure 2. Amino acid sequences comparisons 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: 35kDa 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): HL-2, human HL-2 (AY065973): Intelectin, mouse Intelectin (AB016496): Intelectin-2, mouse Intelectin-2 (AY217760): Lathenteron, *Lathenteron japonicum* lamprey serum lectin (AB055981): Halocynthia, *Halocynthia roretzi* ascidian galactose lectin: Oncorhynchus, *Oncorhynchus mykiss* (rainbow trout) (AF281350): The identical amino acids are shown in red background characters, and similar amino acids are shown in blue background.

**Figure 3. Dendogram of the X-lectin family. Sequence were compared by multiple sequence alignment using the CLUSTAL W algorithm [66].**

**Figure 4. Comparison of fibrinogen-like motif between homologues of XL-35 and Ficolin/Opsonin/p35 lectin family.**

The conserved fibrinogen-like motif in X-lectins may function in carbohydrate recognition. Only fibrinogen-like motif regions were compared: XL-35, amino acids 66-134; HL-1, 66-134; HL-2, 77-165; mouse inteletin-1, 66-134; Fibrinogen-beta chain, 267-134; ficolin-1, 142-200; Hakata antigen, 117-175; p35, 129-187. Identical amino acids are shown in red background characters and similar amino acids are shown blue.

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