FEBS 30184 FEBS Letters 579 (2005) 6827–6833

Identification of the novel evolutionary conserved *obstructor* multigene family in invertebrates

Matthias Behr, Michael Hoch*

Institut für Molekulare Physiologie und Entwicklungsbiologie der Universität Bonn, Rheinische Friedrich-Wilhelms-Universitat Bonn, Abt. für Molekulare Entwicklungsbiologie, Poppelsdorfer Schloss, 53115 Bonn, Germany

Received 14 October 2005; revised 4 November 2005; accepted 9 November 2005

Available online 28 November 2005

Edited by Takashi Gojobori

Abstract Insects have evolved chitin-containing structures such as the cuticle or peritrophic membranes that serve to protect their bodies against the hostile environment. The specific mechanisms by which these structures are produced, are mostly unknown. We have identified a novel multigene family, the *obstructor* family, which encodes ten putatively secreted chitin-binding proteins that are characterized by a stereotype arrangement of a N-terminal signaling peptide and 3 chitin-binding-domains. Gene expression studies in *Drosophila melanogaster* embryos demonstrate that *obstructor* family members are expressed in cuticle forming tissues. Using computational and phylogenetic analysis, we show that *obstructor* genes represent an evolutionary conserved multigene family in invertebrates.

Keywords: Obstructor; Cuticle; Chitin secretion; Epithelial barrier

1. Introduction

Multicellular organisms have to protect their body against mechanical disruption, digestion or inflammatory processes mediated by their hostile environment. In insects, septate junctions and cuticular structures are the key components for protective barriers [1–4]. Cuticles are established by sclerotization of chitin polymers and extracellular matrix proteins at the apical surface of epidermal cells [5]. They are composed of lipids, sclerotized proteins and the polysaccharide chitin which functions as scaffold material [4]. Chitin is also an integral part of insect peritrophic matrices providing a permeability barrier at the proventriculus organ and the midgut epithelium [4].

Insect growth and development are strictly dependent on the capability to remodel chitinous structures to allow molting and regeneration of the cuticular structures. However, the regulation of the chitin metabolism and secretion are still largely elusive [5–7]. Using computational and phylogenetic analysis, and gene expression studies we have identified and characterized a novel multigene family encoding ten putatively secreted chitin-binding proteins evolutionary conserved in invertebrates, but not in vertebrates.

*Corresponding author. Fax: +49 228 734480.
E-mail address: m.hoch@uni-bonn.de (M. Hoch).

2. Materials and methods

2.1. Computer-based analysis

Blast searches [8] were performed with the flybase (http://flybase.net/blast/) and the NCBI blast (http://www.ncbi.nlm.nih.gov/), domain prediction with SMART (http://smart.embl-heidelberg.de/) [9], NCBI Conserved Domain search (http://www.ncbi.nlm.nih.gov/), and the CBS Prediction server (http://www.cbs.dtu.dk/services/). 3D-JIGSAW was used for protein 3D modeling (http://www.bmm.icnet.uk/servers/3djigsaw/) [10]. The phylogenetic tree was carried out with MegAlign (Lasergene, DNASTAR). Parameters are indicated in supplementary data.

2.2. Gene expression

Micro-array data were searched via BDGP [11] (http://www.fruitfly.org/). RNA-antisense in situ hybridizations were done as described by Bauer et al. [12]. For signal amplification, we used the Elite ABC kit (Vector Laboratories) and the TSA-Cyanine-3 system (NEN). cDNA clones obtained from the *Drosophila* gene collection (http://www.fruitfly.org/EST/index.shtml) were used for RNA antisense in situ expression and sequence analysis.

3. Results and discussion

3.1. The obstructor multigene family

In order to understand more about the mechanisms underlying cuticle formation, cuticle rearrangement and its function in epithelial barrier formation, we screened the BDGP expression pattern database for novel genes that are strictly expressed in ectodermal derived cuticle forming organs such as the epidermis, the trachea and the fore- and hindgut. We identified the gene locus CG17052 that is expressed in chitin-secreting tissues (see below) and we could show that mutant embryos for CG17052 display a barrier brake down phenotype (M. Behr and M. Hoch, unpublished data); we thus named the locus *obstructor* (*obst*). Blasting the *obst* Open Reading Frame (ORF) against flybase and NCBI DNA and protein databases, we discovered four additional genes with significant expectation value (*E*-value) and similarities in size, length and putative protein features.

obst and the four related genes show more than 50% identity in their putative ORF DNA sequences (693–1014 bp; Fig. 1A). Thus, according to their obvious relationship we termed *obst* and the four related as *obst-A* to *obst-E* (Fig. 1A); *obst-C* is synonymous to the previously described *gasp* gene [13]. *obst* genes most likely possess one transcript with the exception of *obst-E* encoding for two different transcripts E1 and E2 due to alternative splicing (Fig. 1A). Translations of *obstructor* ORFs result in putative proteins with 219–353 amino acids

0014-5793/$30.00
doi:10.1016/j.febslet.2005.11.021

A gene - ID	genomic localization	cDNA	ORF (bp)	ORF id (%)	E-val DNA-Prot
obst-A - CG17052	19C1	LD43683	714		
obst-B CG4778	31A1	GH02976	1014	57	8e-63
obst-C (gasp) - CG10287	83D4	LD05259	777	55	3e-35
obst-D - CG17058	19C1	LD20973	693	52	1e-15
obst-E1/E2 - CG11142A/B	25F4	GH01453	729/750	48/51	1e-35

B protein - ID	length aa	kDa	protein id/sim%	CBD2_a id/sim %	CBD2_b id/sim %	CBD2_c id/sim %
Obst-A	237	27				
Obst-B	337	38	30/36	51/61	43/51	52/59
Obst-C	258	28	32/38	44/53	35/44	44/59
Obst-D	230	26	28/34	36/39	38/43	25/37
Obst-E1/E2	249/242	27/26	29/37	35/42	31/34	34/50

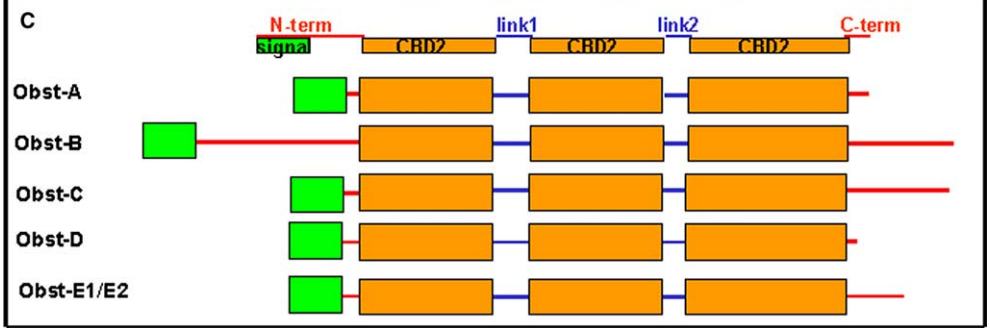

Fig. 1. The obstructor multigene family subgroup 1. (A,B) *obst-A* and the four homologous candidates *obst-B*, *-C*, *-D* and *-E* are summarized in both tables; identities (id) and similarities (sim). (C) Protein domain analysis among Obst-A to E: one N-terminal signal peptide, three regularly spaced CBD2s, two linker regions and individual C-terminal stretches. The domain nomenclature is indicated in the upper drawing.

(aa) and 26–38 kDa in size (Fig. 1B). Protein sequence alignments revealed 28–32% identities and 34–38% similarities among Obst-A to E (Fig. 1B). In addition, Obst proteins evidently share specific and regular domain arrangements. For all Obsts, we discovered a putative signal peptide (Fig. 1C). The signal peptide is followed by three specific chitin-binding-domains type2 (CBD2; Fig. 1C), which show identities and similarities of 25–52% and 34–61%, respectively, between Obst-A and E (Fig. 1B). Furthermore, the presence of linker regions that are similar in length among Obst-A to E (Fig. 1C), results in a stereotype spacing of the three CBD2 domains which is characteristic for these genes. In a SMART database search to identify more genes encoding proteins with the characteristic obst-like domain arrangement, we discovered five more *Drosophila* genes, containing again a small ORF (660–1062 bp) and 38–41% identity to the *obst-A* DNA sequence (Fig. 2A). We classified the genes as *obst-F* to *J*. Two putative transcripts exist for *obst-H* and a single transcript for the others. The putative proteins are small in length (219–326 aa) and predicted size (24–39 kDa; Fig. 2B). Alignments of the protein sequences to Obst-A and among each other (not shown) reveal 19–24% identity and 24–29% similarity (Fig. 2B). Domain analyses show an obvious relation between Obst-F to J and Obst A. All contain a putative N-terminal signal peptide (Fig. 2C) and three CBD2s; the spacing of the CBD2s is more variable in case of Obst-F to J due to variable linker regions, as compared to Obst-A to E. In summary, the specific gene and putative protein relationship between all *obst* family members indicates that we identified a novel multigene family, that can be subdivided into a subgroup 1 (*obst-A* to *E*) and into a subgroup 2 (*obst-F* to *J*).

3.2. Obstructor protein domain arrangements

The signal peptide sequences at the N-terminus of Obst proteins are 16–21 aa in length (Fig. 3A). They contain a consensus cleavage site (not shown), indicating that these proteins are secreted. The most prominent protein motif of the Obst proteins is, however, the CBD2 domain which occurs in a triple arrangement. This domain (SMART accession: SM00494) is mainly found in animal and baculovirus proteins. CBD2s, also characterized as the so-called Peritrophin-A domain [14], are in average 65 aa in length and have been identified as a common structural motif for chitin-binding proteins in invertebrates [15,16] that are required for different classes of proteins involved in degradation [17] or cross-linking of chitin fibrils [18,19]. Consistent with this the Obst CBD2s vary between 52 and 69 aa residues, although for Obst-J they are little smaller in length (45–55 aa). As a common consensus sequence, Obst CBD2s contain the typical motif of 6 cysteines probably forming intradomain disulfide bonds, as it might be the case for other chitin binding proteins [7] and has been described for the well-studied CBD2 of Tachycitin, an immunoregulatory peptide [16]. In addition, they exhibit a core region between cysteine 1 and 5 that is more conserved compared to other domain regions and possess four regular aromatic residues (Fig. 3B).

For subgroup 1, the regular spacing of the three CBD2s is determined by two linkers that are similar in size, with 10 aa

A gene - ID	genomic localization	cDNA	ORF (bp)	ORF identity(%)
obst-F - CG7306	77A2	LP10853	981	41
obst-G - CG9781	68E3	-	840	41
obst-H - CG7973 (short)	71E1	-	810	38
obst-I - CG32304	62D5	-	660	41
obst-J - CG7348	76F2	RE16222	1062	39

B protein – ID	length aa	kDa	protein id/sim %	CBD2_1 id/sim %	CBD2_2 id/sim %	CBD2_3 id/sim %
Obst-F - CG7306	326	36	19/24	30/35	25/31	35/44
Obst-G - CG9781	279	31	24/29	38/40	25/28	34/43
Obst-H (short) - CG7973	269	31	19/26	27/35	35/43	27/35
Obst-I - CG32304	219	24	18/24	33/41	27/33	31/42
Obst-J - CG7348	353	39	20/24	31/35	33/38	27/45

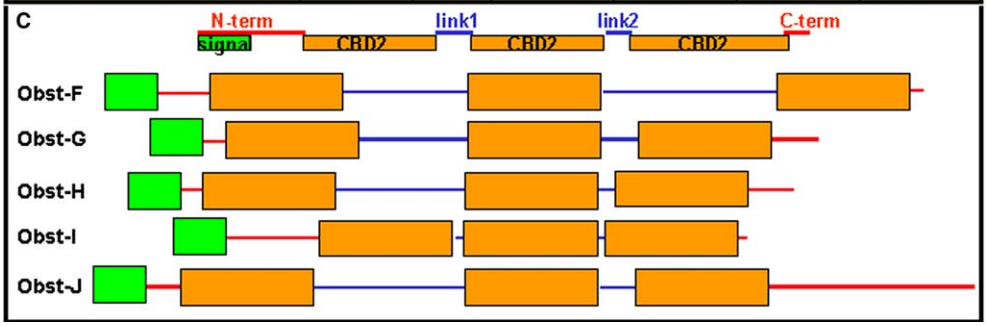

Fig. 2. The obstructor multigene family subgroup 2. (A,B) Both tables summarize the *obst-A* related proteins *obst-F*, *-G*, *-H*, *-I*, *-J*. Identities (id), similarities (sim). (C) Obst-F to -J reveal characteristic Obst domain arrangements containing an N-terminal signal peptide that is followed by three irregular spaced CBD2s. The domain nomenclature is indicated in the upper cartoon.

for linker 1 and 6–8 aa for linker 2. Also, residues at the transition sites between CBD2s and linkers are conserved for subgroup 1 members (Fig. 3C and D). In contrast, subgroup 2 proteins possess variable linkers with no specific sequence similarities (Fig. 3C and D). The most variable region among the Obst family members comprises the C-termini with no significant similarities (Fig. 3E).

The structure of the chitin-binding-protein Tachycitin has been well characterized by nuclear magnetic resonance spectroscopy [16]. Suetake et al. showed that a short part of the Tachycitin CBD2 (Cys 20 to Gly 60) is conserved among invertebrates and plants for certain residues of Cys, Pro and Gly that are significant for structural constructions. In addition conservation of specific polar and hydrophobic residues have been discovered. We compared the corresponding region of the second CBD2s of all 10 Obst proteins (Fig. 3F) and could identify all Cys and in most cases the Pro and Gly residues. Furthermore, Obst CBD2s show polar and hydrophobic residues similar to Tachycitin or other typical chitin-binding proteins as the Chitinase (Ag-Chit; aa 486–524) [20] and the Adult Peritrophin-1 (Ag-Aper1; aa 19–79) [21] of *Anopheles gambiae*, the *Trichoplusia ni* intestinal Mucin (aa 419–476) [22] and the *Lucilia cuprina* Peritrophin-44 (Per-44; aa 29–85) [23]. To improve putative Obst chitin-binding function, we compared the predicted structure of all Obst CBD2s (Fig. 4 left and right panel) with Tachycitin and the other chitin-binding proteins (Fig. 4, middle panel). The Tachycitin structure includes a backbone of 3 β-sheets and a conserved antiparallel β-sheet within the CBD2 (Fig. 4, frame). Resembling structures are found for the other chitin-binding proteins. Similar, most of the Obst CBD2s display a Tachycitin like folding in particular for the antiparallel β-sheets. Thus chitin binding of Obst proteins is highly probable according to the sequence and structure similarities of the Obst CBD2 domains.

3.3. The Obstructor family is conserved among invertebrates

To investigate whether the Obst family is evolutionary conserved, we searched via SMART, NCBI and BDGP databases for Obst homologues in *Drosophila pseudoobscura* and the more distant *A. gambiae* and *Apis mellifera* insect species. With the exception of Obst-I, we could identify homologues for all Obst proteins in *D. pseudoobscura* (Fig. 5). In contrast, *A. gambiae* and *A. mellifera* orthologous were discovered only for subgroup 1 members (Fig. 5). All putative proteins are similar to their corresponding Obst homologues (40–97% identity) and contain a typical Obst domain arrangement (not shown).

To unravel phylogenetic relationships among the Obst family, a phylogenetic tree was constructed based on protein sequences (Fig. 5A). We found that Obst family members group together with their corresponding partners, suggesting a high conservation during insect evolution. The phylogenetic tree reveals two main branches: subgroup 1 and subgroup 2. We conclude from these data that the Obst family is conserved among insects.

Interestingly, Obst-related proteins exist in the nematodes *Caenorhabditis elegans* (*C. elegans*; NCBI database W03F11.1; 235 aa) and *Caenorhabditis briggsae* (*C. briggsae*; CAE60308; 236 aa) with remote sequence identities (20%)

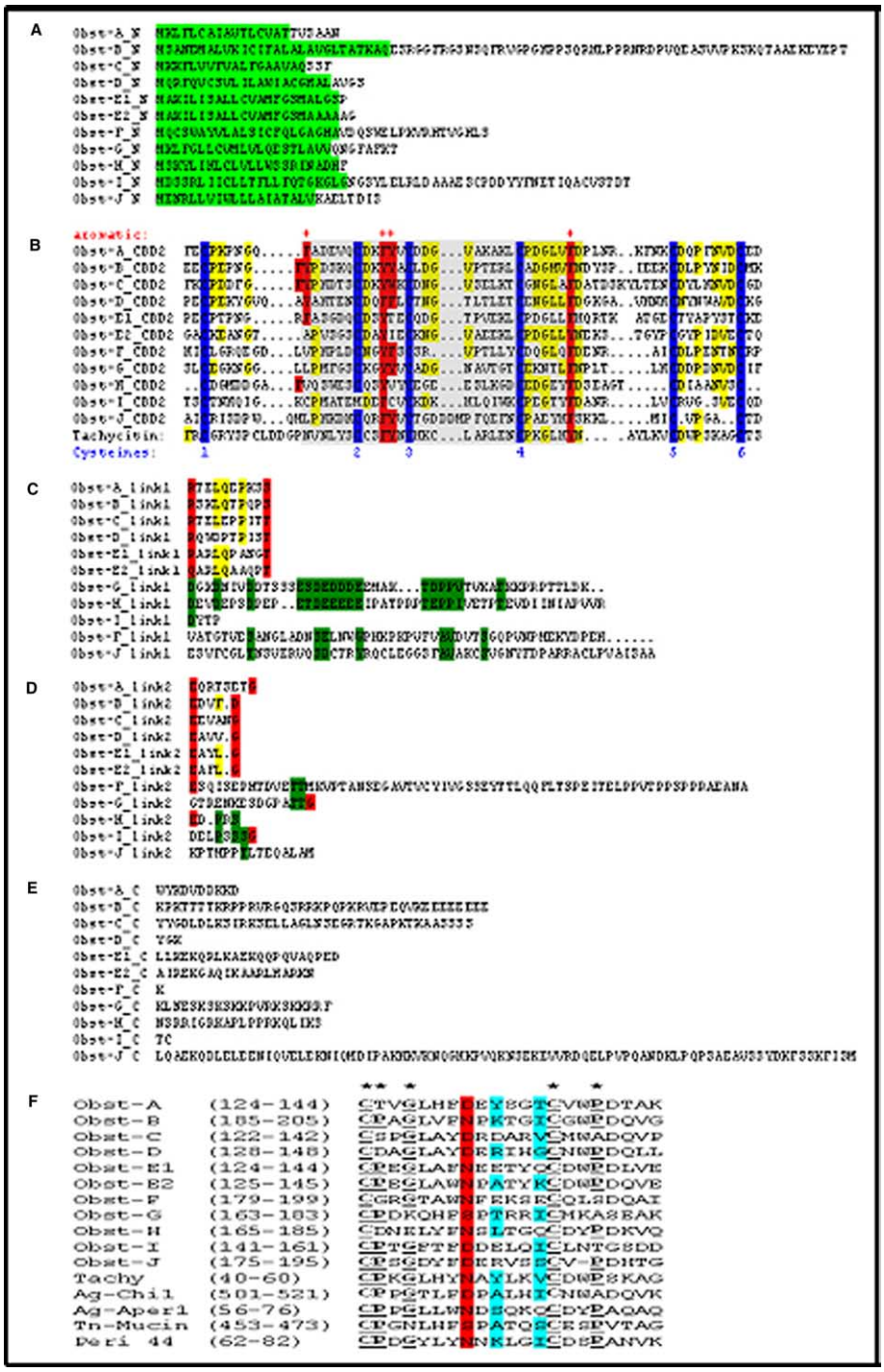

Fig. 3. Obstructor sequence and domain comparison. (A) Obst proteins encode at their N-terminus a signal peptide (marked in green). (B) As an example the first Obst CBD2s is compared among the 10 Obst proteins and Tachycitin (6 cysteines are indicated in blue and a core region in gray and aromatic residues in red). Homologous aa among Obst proteins are pointed out in yellow. (C,D) Obst linker regions between their CBD2s: Subgroup 1 transition sites are marked in red; homologous aa residues are marked in yellow. Obst subgroup 2 homologous aa residues are marked in green. (E) C-terminal part of Obst proteins. (F) Parts of second CBD2s of Obst and known chitin-binding proteins are compared. Conserved aa are bold & underlined (see asterisks on top), specific polar aa are in red and hydrophobic aa are in turquoise indicated.

and similarities (26%). Both putative proteins are *yet* uncharacterized. The nematode Obst proteins reveal subgroup 1-related domain composition (Fig. 5B). In contrast, we did not find Obst-related proteins in vertebrates, neither by sequence searches nor by domain composition analysis. Thus, Obst proteins are conserved in invertebrate species but very likely not in vertebrates.

3.4. Expression patterns of obstructor family members in Drosophila melanogaster

In *Drosophila melanogaster*, chitin is strongly secreted during late embryogenesis [24,6]. Time course micro-array-charts from the BDGP database show a weak expression level for *obst* subgroup 1 genes during early and mid embryogenesis (Fig. 6A, E and H). In parallel to cuticle formation, the expres-

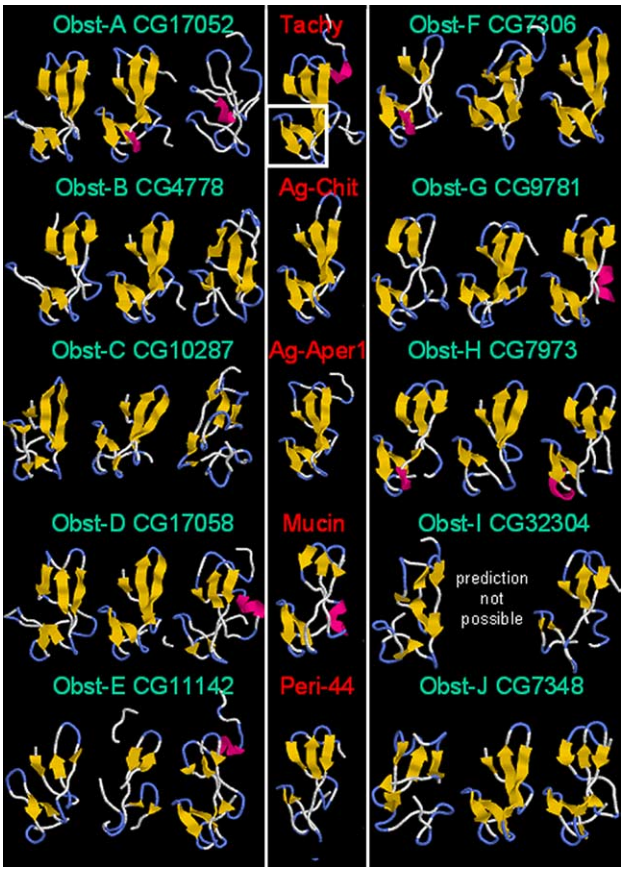

Fig. 4. CBD2 structure determination. Tachycitin and the CBD2s of other chitin-binding proteins are shown in the middle panel and CBD2s (1–3, from left to right) of subgroup 1 and subgroup 2 Obst proteins in the left and right panel, respectively.

sion of subgroup 1 genes increases dramatically during late embryogenesis with the exception of *obst-E*. Enhanced embryonic chitin secretion takes place in ectodermally derived organs [24,25]. In particular within the tracheal system, first chitin fibrils are formed when tubes start to expand with the beginning of embryonic stage (st) 14 [26]. Consistent with this, our *obst-A* RNA antisense in situ hybridizations (Fig. 6) demonstrate strong expression within the tracheal system starting at late st 13 until st 17. Further expression is detectable at st 14 in the epidermis and at st 15, we find weaker expression in the fore- (not shown) and hindgut (Fig. 6B–D). Similarly, (Fig. 6F and G) *obst-B* is active within the tracheal system and epidermis starting at st 14. We detect low expression levels in the hindgut and in the midgut at late embryogenesis. *obst-C* (*gasp*) RNA can be observed within the tracheal system, as shown by Barry et al. [13]. *obst-D* and *obst-E* are predominantly active within the midgut, starting at st 14, and within the epidermis (Fig. 6I and L). During later stages also the tracheal system and the fore- and hindgut express *obst-D* and *-E* (Fig. 6J and M). Thus, subgroup 1 genes are activated slightly prior to chitin secretion specifically in tubular epithelial organs, consistent with a functional role during this process. In contrast, the expression of subgroup 2 genes is not elevated; micro-array-chart analysis shows continuous, low level expression during embryogenesis (Fig. 6N, not shown). *obst-J* RNA antisense hybridizations reveal restricted gene activity only at late embryogenesis in the recurrent layer of the proventriculus (Fig. 6O), which is known to secrete low level of chitin within the peritrophic matrix [7]. Further expression is found in the midgut at the end of embryogenesis (Fig. 6P).

In summary, we have identified a novel, evolutionary conserved multigene family, the obstructor family, which encodes putatively secreted chitin-binding proteins. Obst proteins are characterized by a stereotype arrangement of a N-terminal

protein	gene bank	*D.pseudo.*	*A. gambiae*	*A. mellifera*
Obst-A	NP_608378	EAL31995.1 (GA14300)	XP_309184	no hit
Obst-B	NP_609339	EAL33377.1 (GA18242)	XP_318884	XP_392261.1
Obst-C	NP_649611	EAL28077.1 (GA10221)	XP_319536	XP_397215.1
Obst-D	NP_728324	EAL31994.1 (GA14302)	XP_560209	XP_393551.1
Obst-E1 Obst-E2	NP_608957 NP_723116	EAL34295 (GA10970)	XP_310082	XP_397120.1
Obst-F	NP_649186	EAL30804 (GA20250)	no hit	no hit
Obst-G	NP_648529	EAL30787 (GA22032)	no hit	no hit
Obst-H	NP_730052	EAL30648 (GA20733)	no hit	no hit
Obst-I	NP_728731	no hit	no hit	no hit
Obst-J	NP_649179	EAL30797 (GA20283)	no hit	no hit

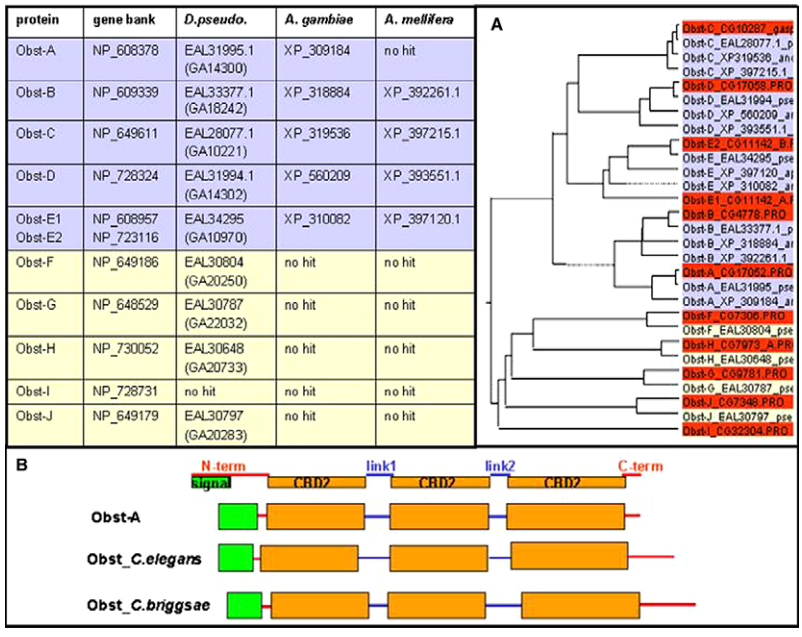

Fig. 5. The Obstructor protein family is conserved among invertebrates. The table shows Obst subgroups homologous proteins in *D. pseudoobscura*, *A. gambiae* and *A. mellifera*. (A) A phylogenetic tree of Obst proteins. *D. melanogaster* proteins are boxed in red; Obst subgroup 1 proteins are shadowed in blue and subgroup 2 proteins in yellow. (B) Obst-related proteins exist in nematodes *C. elegans* and *C. briggsae*. The domain nomenclature is indicated in the upper cartoon.

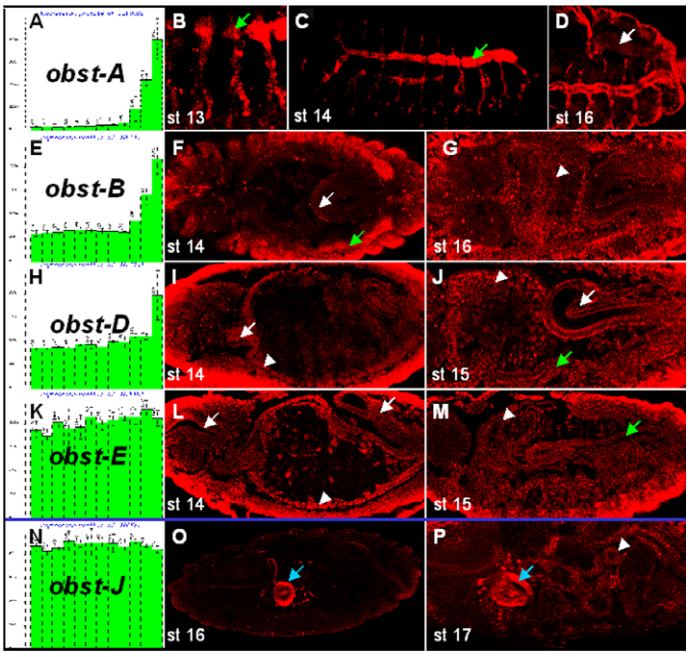

Fig. 6. *obstructor* genes are expressed in Chitin secreting organs. Shown are time course micro array gene chip data (BDGP; A, E, H, K, N st 1–14) and fluorescence RNA antisense hybridization in situ studies of Wild-type embryos for *obst-A* (B–D), *obst-B* (F,G), *obst-D* (I, J), *obst-E* (L,M) and *obst-J* (O,P). Arrows indicate fore (I,L) and hindgut (F,J,L), tracheal system (green) or cardia (blue); arrowheads points to midgut; dorsal view is shown in F, G, I, J; lateral view in B-D, L, M, O, P. anterior is left; dorsal is up.

signaling peptide and three CBD2s. SMART database analysis identifies 127 different CBD2 containing proteins in *D. melanogaster*, however, only few of those have been analyzed so far. Zhu and colleagues discovered recently by sequence homology searches chitinases possessing CBD2s [17]. Barry et al. described in 1999 the *gasp* gene (*obst-C*) [13]. *gasp* encodes a putative protein containing CBD2s that reveal sequence similarities to peritrophins of other insects and to the hypothetical uncharacterized *Drosophila* Peritrophin-A (Obst-D) sequence. However, the combination of a N-terminal signal peptide with three CBD2 domains is a specific feature of Obst proteins and identifies a novel class of proteins which we demonstrate, is highly conserved in invertebrates. Our gene expression studies in *Drosophila* embryos show that *obst* subgroup 1 genes are active during late embryogenesis in chitin secreting organs.

It has been determined that chitin synthesis takes place at stage 17 in the epidermis [25] whereas luminal chitin bundle assembly in the tracheae occurs at stage 14 [26]. The subgroup 1 obst proteins which are strongly expressed in the tracheal cells may be required during luminal chitin bundle assembly in the trachea. Interestingly, the expression pattern of *krotzkopf verkehrt* (*kkv; CG2666*) which encodes one of the two *Drosophila* chitin synthases [25,26] resembles the expression of *obst* subgroup 1 genes. *kkv* is essential for cuticle formation [25,27] and chitin localization in the *Drosophila* tracheal system [26]. In contrast to *kkv*, CG7464 the second *Drosophila chitin synthase* is expressed similar to the obst subgroup 2 genes, as revealed by micro-array-chart analysis. What function obst protein serve is not clear. It is likely, however, that the chitin-binding proteins may be required for chitin packaging during cuticle differentiation rather than assisting chitin synthesis per se. In this respect, it is of note that obst subgroup 1 and subgroup 2 family members differ in the spacing of the CBD2 domains which is more stereotype within subgroup 1 family members. The midgut chitinous peritrophic matrix is not as organized as the cuticle chitinous procuticle; this notion may explain why the spacing of CBD2 in subgroup 2 proteins is variable compared to subgroup 1 members.

Acknowledgements: We are grateful to Verena Arndt, Reinhard Bauer and Christian Wolf for critical reading of this manuscript and Dierk Niessing for CBD2 3D structure assistance. We thank the BDGP, CBS, EMBL and NCBI for open database access. M.B. was supported by a DFG Grant BE 3215 and M.H. by the SFB 645.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2005.11.021.

References

[1] Baumgartner, S., Littleton, J.T., Broadie, K., Bhat, M.A., Harbecke, R., Lengyel, J.A., Chiquet-Ehrismann, R., Prokop, A. and Bellen, H.J. (1996) A *Drosophila* neurexin is required for septate junction and blood–nerve barrier formation and function. Cell 87, 1059–1068.
[2] Behr, M., Riedel, D. and Schuh, R. (2003) The claudin-like megatrachea is essential in septate junctions for the epithelial barrier function in *Drosophila*. Dev. Cell 5, 611–620.
[3] Lamb, R.S., Ward, R.E., Schweizer, L. and Fehon, R.G. (1998) *Drosophila* coracle, a member of the protein 4.1 superfamily, has essential structural functions in the septate junctions and developmental functions in embryonic and adult epithelial cells. Mol. Biol. Cell 9, 3505–3519.
[4] Lehane, M.J. (1997) Peritrophic matrix structure and function. Annu. Rev. Entomol. 42, 525–550.
[5] Merzendorfer, H. and Zimoch, L. (2003) Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. J. Exp. Biol. 206, 4393–4412.

[6] Moussian, B., Soding, J., Schwarz, H. and Nusslein-Volhard, C. (2005) Retroactive, a membrane-anchored extracellular protein related to vertebrate snake neurotoxin-like proteins, is required for cuticle organization in the larva of *Drosophila melanogaster*. Dev. Dyn. 233, 1056–1063.
[7] Tellam, R.L., Wijffels, G. and Willadsen, P. (1999) Peritrophic matrix proteins. Insect Biochem. Mol. Biol. 29, 87–101.
[8] Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.
[9] Letunic, I., Copley, R.R., Schmidt, S., Ciccarelli, F.D., Doerks, T., Schultz, J., Ponting, C.P. and Bork, P. (2004) SMART 4.0: towards genomic data integration. Nucleic Acids Res. 32, D142–D144.
[10] Bates, P.A., Kelley, L.A., MacCallum, R.M. and Sternberg, M.J. (2001) Enhancement of protein modeling by human intervention in applying the automatic programs 3D-JIGSAW and 3D-PSSM. Proteins Suppl. 5, 39–46.
[11] Tomancak, P., Beaton, A., Weiszmann, R., Kwan, E., Shu, S., Lewis, S.E., Richards, S., Ashburner, M., Hartenstein, V., Celniker, S.E. and Rubin, G.M. (2002). Systematic determination of patterns of gene expression during Drosophila embryogenesis. Genome Biol 3, RESEARCH0088.1–0088.14..
[12] Bauer, R., Lehmann, C., Fuss, B., Eckardt, F. and Hoch, M. (2002) The *Drosophila* gap junction channel gene innexin 2 controls foregut development in response to Wingless signalling. J. Cell. Sci. 115, 1859–1867.
[13] Barry, M., Triplett, A. and Christensen, A. (1999) A peritrophin-like protein expressed in the embryonic tracheae of *Drosophila melanogaster*. Insect Biochem. Mol. Biol. 29, 319–327.
[14] Gaines, P., Walmsley, S. and Wisnewski, N. (2003) Cloning and characterization of five cDNAs encoding peritrophin-A domains from the cat flea, *Ctenocephalides felis*. Insect Biochem. Mol. Biol. 33, 1061–1073.
[15] Shen, Z. and Jacobs-Lorena, M. (1999) Evolution of chitin-binding proteins in invertebrates. J. Mol. Evol. 48, 341–347.
[16] Suetake, T., Tsuda, S., Kawabata, S., Miura, K., Iwanaga, S., Hikichi, K., Nitta, K. and Kawano, K. (2000) Chitin-binding proteins in invertebrates and plants comprise a common chitin-binding structural motif. J. Biol. Chem. 275, 17929–17932.
[17] Zhu, Q., Deng, Y., Vanka, P., Brown, S.J., Muthukrishnan, S. and Kramer, K.J. (2004) Computational identification of novel chitinase-like proteins in the *Drosophila melanogaster* genome. Bioinformatics 20, 161–169.
[18] Wijffels, G., Eisemann, C., Riding, G., Pearson, R., Jones, A., Willadsen, P. and Tellam, R. (2001) A novel family of chitin-binding proteins from insect type 2 peritrophic matrix. cDNA sequences, chitin binding activity, and cellular localization. J. Biol. Chem. 276, 15527–15536.
[19] Wang, P., Li, G. and Granados, R.R. (2004) Identification of two new peritrophic membrane proteins from larval *Trichoplusia ni*: structural characteristics and their functions in the protease rich insect gut. Insect Biochem. Mol. Biol. 34, 215–227.
[20] Shen, Z. and Jacobs-Lorena, M. (1997) Characterization of a novel gut-specific chitinase gene from the human malaria vector *Anopheles gambiae*. J. Biol. Chem. 272, 28895–28900.
[21] Shen, Z. and Jacobs-Lorena, M. (1998) A type I peritrophic matrix protein from the malaria vector *Anopheles gambiae* binds to chitin. Cloning, expression, and characterization. J. Biol. Chem. 273, 17665–17670.
[22] Wang, P. and Granados, R.R. (1997) Molecular cloning and sequencing of a novel invertebrate intestinal mucin cDNA. J. Biol. Chem. 272, 16663–16669.
[23] Elvin, C., Vuocolo, T., Pearson, R., East, I., Riding, G., Eisemann, C. and Tellam, R. (1996) Characterization of a major peritrophic membrane protein, peritrophin-44, from the larvae of *Lucilia cuprina*. cDNA and deduced amino acid sequences. J. Biol. Chem. 271, 8925–8935.
[24] Campos-Ortega, J. and Hartenstein, V. (1997) The Embryonic Development of *Drosophila melanogaster*, Springer, Berlin.
[25] Moussian, B., Schwarz, H., Bartoszewski, S. and Nusslein-Volhard, C. (2005) Involvement of chitin in exoskeleton morphogenesis in *Drosophila melanogaster*. J. Morphol. 264, 117–130.
[26] Tonning, A., Hemphala, J., Tang, E., Nannmark, U., Samakovlis, C. and Uv, A. (2005) A transient luminal chitinous matrix is required to model epithelial tube diameter in the *Drosophila* trachea. Dev. Cell 9, 423–430.
[27] Ostrowski, S., Dierick, H.A. and Bejsovec, A. (2002) Genetic control of cuticle formation during embryonic development of *Drosophila melanogaster*. Genetics 161, 171–182.