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Sequence analysis of the non-recurring C-terminal domains shows that insect lipoprotein receptors constitute a distinct group of LDL receptor family members

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Abstract

Lipoprotein-mediated delivery of lipids in mammals involves endocytic receptors of the low density lipoprotein (LDL) receptor (LDLR) family. In contrast, in insects, the lipoprotein, lipophorin (Lp), functions as a reusable lipid shuttle in lipid delivery, and these animals, therefore, were not supposed to use endocytic receptors. However, recent data indicate additional endocytic uptake of Lp, mediated by a Lp receptor (LpR) of the LDLR family. The two N-terminal domains of LDLR family members are involved in ligand binding and dissociation, respectively, and are composed of a mosaic of multiple repeats. The three C-terminal domains, viz., the optional O-linked glycosylation domain, the transmembrane domain, and the intracellular domain, are of a non-repetitive sequence. The present classification of newly discovered LDLR family members, including the LpRs, bears no relevance to physiological function. Therefore, as a novel approach, the C-terminal domains of LDLR family members across the entire animal kingdom were used to perform a sequence comparison analysis in combination with a phylogenetic tree analysis. The LpRs appeared to segregate into a specific group distinct from the groups encompassing the other family members, and each of the three C-terminal domains of the insect receptors is composed of unique set of sequence motifs. Based on conservation of sequence motifs and organization of these motifs in the domains, LpR resembles most the groups of the LDLRs, very low density lipoprotein (VLDL) receptors, and vitellogenin receptors. However, in sequence aspects in which LpR deviates from these three receptor groups, it most notably resembles LDLR-related protein-2, or megalin. These features might explain the functional differences disclosed between insect and mammalian lipoprotein receptors.

Keywords: Lipid transport; Lipoprotein; Lipophorin; Lipophorin receptor; LDL receptor family; Protein domains; Sequence motif; Sequence comparison; Phylogeny; Glycosylation; Transmembrane; Intracellular

1. Introduction

Lipoprotein-mediated transport of lipid in the circulation is a general mechanism in animals, where lipid is being used for storage or metabolic purposes. The lipoprotein in insects, lipophorin (Lp) is proposed to function as a reusable shuttle, in which Lp selectively unloads lipids at the cell surface, subsequently followed by a new round of lipid loading. Consequently, in this process, Lp is not taken up into the cells by endocytosis (see Van der Horst, 1990; Ryan and Van der Horst, 2000; Van der Horst et al., 2002; Rodenburg and Van der Horst, 2005). On the other hand, lipid delivery in mammals finally results in the endocytic uptake of remnant lipoprotein by cells, which is mediated by members of the low density lipoprotein (LDL) receptor (LDLR) family. After endocytic uptake, the complex of lipoprotein and receptor dissociates and the lipoprotein undergoes lysosomal degradation, whereas the receptor is

Abbreviations: apoER2, apolipoprotein E receptor 2; cA/GDPK, cAMP/cGMP-dependent protein kinase; CK2, casein kinase-2; EGF, epidermal growth factor; ICD, intracellular domain; LDL, low density lipoprotein; LDLR, LDL receptor; Lp, lipophorin; LpR, lipophorin receptor; LRP, LDLR-related protein; OLGD, O-linked glycosylation domain; PKC, protein kinase C; TMD, transmembrane domain; VLDLR; very low density lipoprotein receptor; VGR, vitellogenin receptor

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recycled back to the cell surface for another round of lipoprotein uptake (see Goldstein et al., 1985; Krieger and Herz, 1994; Herz and Bock, 2002; Jeon and Blacklow, 2005). In view of the reusable shuttle functioning of Lp, there would be no need for insects to express LDLR family members. However, recently it has become apparent that in insects also endocytic uptake of Lp is occurring, which is mediated by Lp receptors (LpRs) (Dantuma et al., 1999; Lee et al., 2003; Seo et al., 2003; Van Hoof et al., 2003, 2005a) (see Van der Horst et al., 2001, 2002; Van der Horst and Ryan, 2004; Rodenburg and Van der Horst, 2005). Remarkably, sequence comparison indicates that these LpRs belong to the LDLR family (Dantuma et al., 1999; Lee et al., 2003; Seo et al., 2003; Van Hoof et al., 2003, 2005a, b). In addition, LpR of *Locusta migratoria*, the only LpR for which the endocytic process to date has been characterized in details at the cellular level, appears to be able to recycle its ligand, a function not reported for any other LDLR family member (Van Hoof et al., 2002, 2003, 2005a, b; see Van der Horst et al., 2002; Van der Horst and Ryan, 2004; Rodenburg and Van der Horst, 2005).

LDLR family members are composed of four or five characteristic domains (Fig. 1): (1) an N-terminal ligand binding domain, (2) an epidermal growth factor (EGF)-precursor homology domain, (3) an optional O-linked glycosylation domain (OLGD), (4) a single transmembrane spanning segment or transmembrane domain (TMD), and (5) a C-terminal intracellular domain (ICD). Of the two N-terminal domains, the ligand binding domain is composed of multiple cysteine-rich repeats that are involved in binding of different ligands, including lipoproteins. The EGF-precursor homology domain is composed of different repeats that function in ligand dissociation after endocytic uptake of the ligand. The three C-terminal domains are composed of non-recurring sequences. The OLGD, present in some but not all family members, contains several serine and threonine residues that potentially are O-linked glycosylated. The TMD spans the cell membrane once, to position the receptor properly during intracellular transport and at the cell surface. The ICD mediates the endocytic uptake of receptor in complex with lipoprotein via the NPXY sequence motif in the latter domain that acts as a clathrin-coated pit internalization signal (see Willnow, 1999; Herz and Bock, 2002; Schneider and Nimpf, 2003).

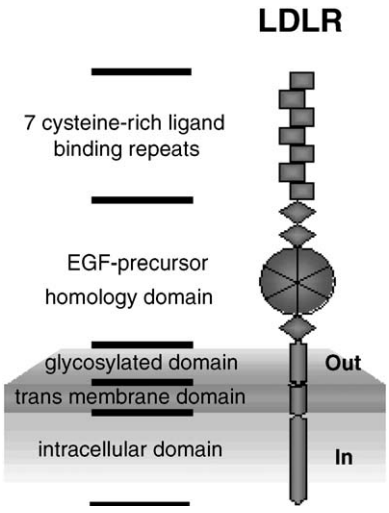

Fig. 1. Domain structure of the LDL receptor. The receptor is composed of the five domains that are the building blocks for the composition of all LDLR family members. The N-terminal part, or ectodomain of the receptor is exposed to the extracellular space (out), the transmembrane domain is integrated in the phospholipid bilayer, indicated as a grey three-dimensional plane, and the intracellular domain is exposed to the cytoplasm (in), represented by the light grey shaded area. The cysteine-rich repeats, represented by squares, make up the ligand binding domain. The EGF-type repeats, indicated as diamonds, and the six-bladed β-propeller domain (marked by the circle divided into six parts) make up the EGF precursor homology domain. The OLGD forms the connector domain of the ectodomain to the TMD.

The family of LDLR members is composed of a large variety of receptors that, most notably, contain a different number of cysteine-rich ligand binding repeats in the first domain, often in combination with multiple copies of the second (EGF-precursor homology) domain, thereby allowing the binding and dissociation of different ligands, both in number and specificity (Herz and Bock, 2002). In addition to their endocytic role, several family members have been shown to be involved in other processes: they effect signal transduction (LDLR-related protein (LRP); very low density lipoprotein receptor (VLDLR); and LRP8 [apolipoprotein E receptor-2 (apoER2)]), mediate polarized expression in cells (LDLR; LRP2, also known as megalin), perform transcytosis (LRP2), ligand recycling (LpR), activate transcription (LRP; LRP2), or interact with intracellular proteins for which a cellular event has not been identified yet (Herz and Strickland, 2001; Herz and Bock, 2002; May and Herz, 2003; Schneider and Nimpf, 2003; Strickland and Ranganathan, 2003; Van der Horst and Ryan, 2004; Rodenburg and Van der Horst, 2005). For LRP8 involvement of the OLGD in function has been characterized (May et al., 2002, 2003a, b), and the ICD for LDLR, VLDLR, LRP, LRP2, and LRP8 (Matter et al., 1992, 1994; Trommsdorff et al., 1999; Djordjevic et al., 2000; Gotthardt et al., 2000; Li et al., 2000; Oleinikov et al., 2000; Rader et al., 2000; Stockinger et al., 2000, 2002; Patrie et al., 2001; He et al., 2002; Melman et al., 2002; Mishra et al., 2002; Larsson et al., 2003; Nagai et al., 2003; Petersen et al., 2003; Takeda et al., 2003). Tissue specific splice variants in the OLGD have been identified for VLDLR, LRP8 (Martensen et al., 1997; Iijima et al., 1998; Nakamura et al., 1998; Clatworthy et al., 1999; Magrane et al., 1999; Sun and Soutar, 1999; Korschineck et al., 2001), and LpR from the insects *Aedes aegypti* (Seo et al., 2003) and *Galleria mellonella* (Lee et al., 2003), suggesting alternative functioning of these variants.

Currently, it appears that each LDLR family member performs its own specific set of functions, and that the OLGD and ICD of individual receptors are directly involved in the execution of many of these functions (see Willnow, 1999; Herz and Strickland, 2001; Herz and Bock, 2002; Andersen and Petersen, 2003; May et al., 2002,