New horizons for lipoprotein receptors: communication by β-propellers

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Abstract  The lipoprotein receptor (LR) family constitutes a large group of structurally closely related receptors with broad ligand-binding specificity. Traditionally, ligand binding to LRs has been anticipated to involve merely the complement type repeat (CR)-domains omnipresent in the family. Recently, this dogma has transformed with the observation that β-propellers of some LRs actively engage in complex formation too. Based on an in-depth decomposition of current structures and sequences, we suggest that exploitation of the β-propellers as binding targets depends on receptor subgroups. In particular, we highlight the shutter mechanism of β-propellers as a general recognition motif for NxxI-containing ligands, and we present indications that the generalized β-propeller-induced ligand release mechanism is not applicable for the larger LRs. For the giant LR members, we present evidence that their β-propellers may also actively engage in ligand binding. We therefore advocate for an increased focus on solving the structure-function relationship of this group of important biological receptors.—Andersen, O. M., R. Dagil, and B. B. Kragelund. New horizons for lipoprotein receptors: communication by β-propellers. J. Lipid Res. 2013. 54: 2763–2774.

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The family of proteins containing Tyr-Trp-Thr-Asp (YWTD)-repeat β-propellers constitutes a group of structurally related glycoproteins with extracellular domains that varies in size but has a broad ligand-binding profile. Most of the members belong to the lipoprotein receptor (LR) family engaged in classical endocytosis pathways, but it includes also nidogen proteins with no known roles in lipoprotein metabolism (1). Overall, the proteins subdivide into three groups according to their size and domain clustering with the SMALL receptors [low-density lipoprotein receptor (LDLR), very low density lipoprotein receptor (VLDLR), apoER2, nidogen-1, nidogen-2, and sorLA/LR11], the LARGE receptors [low-density lipoprotein receptor-like protein 4, 5, and 6 (LRP4, LRP5, and LRP6)] and the GIANT receptors [low-density lipoprotein receptor-like protein 1, protein 1B, and protein 2 (LRP1, LRP1B, and LRP2 (also known as megalin))] (Fig. 1A). Unrelated to size, the LRs are all modularly constructed from a limited subset of three different domains: the complement type repeat (CR)-domains, the epidermal growth factor (EGF)-like domains, and the β-propellers containing YWTD-repeats (YWTD β-propeller). The related members contain additional structural entities, such as fibronectin type III (FNIII) domains, vacuolar protein sorting 10 (VPS10) homology domains, and G-domains (2, 3). Despite this apparent structural simplicity, only few structures have been solved, and the foundation for an explication of their structure-function relations is far from complete.

Very early, around the 1980s (4), it was established that ligand binding to LRs involved the small (~40 residues) CR-domains (also termed receptor class A repeats), which are omnipresent in the family, Fig. 1B. These domains are clustered modularly in disulphide-bonded and Ca2+-stabilized regions, and for more than two decades, they have been seen as the sole ligand interaction regions in LRs. In contrast, the β-propellers and the EGF-like domains have merely been seen as spacer regions ensuring a proper distance between clusters of CR-domain binding sites aiding optimized adaptation in ligand binding (5). In 1998, an analysis of the YWTD-repeat sequences of LRs suggested that each region folds into a β-propeller containing six blades (1), and in 2001, this was confirmed by the crystal structure of this region of LDLR (6). Shortly after, the structure of nidogen-1 followed (7), showing that

Abbreviations:  CR, complement type repeat; CUB, complement C1r/C1s, Uegf, Bmp1; DFPase, diisopropyl fluorophosphatase; Dkk, Dickkopf; EGF, epidermal growth factor; FNIII, fibronectin type III; HRV2, human rhinovirus serotype 2; LDLR, low density lipoprotein receptor; LR, lipoprotein receptor; LRP, low density lipoprotein receptor-like protein; MuSK, muscle-specific kinase; PAI-1, plasminogen activator inhibitor-1; PCSK9, proprotein convertase subtilisin/kexin type 9; RAP, receptor-associated protein; uPA, urokinase plaminogen activator; VPS10, vacuolar protein sorting 10; Wnt, Wingless and INT-1.

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that many of the LRs are engaged in cellular signaling pathways via their intracellular domains (12, 13). This leaves the concept of LRs as merely endocytosis receptors obsolete. Examples, such as wingless and INT-1 (Wnt) signaling by LRP5/6 (14–17) in which deregulation (i.e., in forms of inherited mutations) leads to osteoporosis, LRP2 interaction with sonic hedgehog protein and bone morphogenic protein 4 in the forebrain development (18–21), and the association of LRP1B as tumor suppressor in numerous cancers (22–24), add value to the notion that the function of these receptors is not easily compatible with a simple interaction of ligands with a fast endocytic receptor. The new insight emerging from structures of complexes between ligands and $\alpha$-propellers now partly explains the function of LRs with no known ligand-binding activity in their CR-domains (such as LRP5/6). Still, apoE does indeed bind to LRP5, and although it has not been shown, this most likely happens through the CR-domains (25, 26).

The new structures also pose several important questions that warrant further investigations into the structural biology of this unique receptor family. Here, we utilize simple sequence analyses to address $\alpha$-propeller diversity.

By these recent findings, the diversity of ligand repertoire of LRs is now potentially broadened, and at the same time, they also raise the question of the role of the $\beta$-propellers in the giant members LRP1, LRP1B, and LRP2 is completely unknown.

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within the LR family and observe that β-propellers, in a similar manner to the CR-domain, exploit conserved binding motifs in ligand binding. Based on an in-depth decomposition of current structures and sequences, we suggest that exploitation of the β-propellers as binding targets is dependent on receptor subgrouping.

**EXPERIMENTAL PROCEDURES**

Fasta sequences of all human LR receptors were extracted from the Uniprot database (27) (LDLR, P01130, VLDLR, P98155, ApoER2, Q14114, LRP4, O75096, LRP5, O75197, LRP6, O75581, LRP1, P07054, LRP1B, Q9N8Z2, LRP2, P98164, Nidogen-1, P14543, Nidogen-2, Q14112, SorLA/LR11, Q92673). The β-propeller domains were identified using the Simple Modular Architecture Research Tool (SMART) server (28, 29). All sequence alignments were performed using the T-coffee multiple alignment server (30). The 3D-homology models of β-propeller domains were built with Swiss model workspace (31) using a high-resolution crystal structure of LRP6 as template model.

**RESULTS AND DISCUSSION**

**YWTD β-propeller superfamily**

The β-propeller fold is found in a number of different proteins and many even have enzymatic activity. They are formed by 4–10 blade-shaped β-sheets arranged in a compact dough-nut-like form. For the LR family, 6 blades of 4 antiparallel β-strands each form around a central axis with a diameter spanning around 40 Å, and they comprise in their sequence up to six YWTD- (or YWTD-like) repeats (Fig. 1C). The evolution of these receptors has included exon shuffling of the full β-propellers with flanking EGF-like domains (1) (Fig. 1D).

Besides LRs, the 6-bladed, YWTD-domain structural superfamily also includes nidogens, which are important for the assembly of basement membranes by interaction with laminin (32, 33) and SorLA/LR11, which is a sorting receptor for the amyloid precursor protein genetically associated with Alzheimer’s disease (34). So far it has not been established how (if at all) these receptors are evolutionarily linked; however, it is important to notice that shuffling of individual YWTD-repeats cannot occur (1).

Based on the CR-domains, of which nidogen has none, the LRs were previously divided into four subclasses depending on the total number of CR-domains (35). An alternative approach for classification is to base subgrouping on the total number of β-propellers. This results in three subclasses of the human YWTD β-propeller superfamily, suggesting a nomenclature designating LRs according to the number of β-propellers, which is used throughout this article: LR1β (LR with one β-propeller domain; LDLR, VLDLR, apoER2, SorLA/LR11, nidogen-1, and nidogen-2), LR4β (LR with four β-propeller domains; LRP4, LRP5, and LRP6) and LR8β (LR with eight β-propeller domains; LRP1, LRP1B, and LRP2) (Fig. 1).

By the identification of external ligands binding also to EGF-like domains (25, 36), it is now apparent that all three prototypes of domains of the LRs are involved in external communication. Although no general conclusions can be drawn regarding ligand selection by EGF-like domains, the structures of LDLR EGF-A module with an extrinsic ligand, PCSK9, show specific salt bridges and hydrogen bonds within a small interface similar but not identical to complexes of CR-domains (25, 36). Before focusing on the details of the sequences of the β-propellers as well as on their structures and complexes, we revisit the CR-domains and how they exploit a commonly found minimal motif in ligand binding.

**A minimal ligand-binding motif of CR-domains**

It has been an enigma to understand not only what governs ligand-binding specificity and high affinity but also whether there are conserved mechanisms within the LR family that involve large domain movements or even dimerization of importance for ligand binding. The LR family is known to have a very broad ligand-binding specificity with the largest LR receptor, LRP2, reported to bind more than 50 different ligands (Ref. 37 and references herein). Solely based on our knowledge of complex formation between various ligands and highly similar CR-domains, a general minimal motif was defined (38). Correspondingly, the built-in multivalency in ligand binding has largely been seen as a result of the different charge distributions of the CR-domains combined with linker adaptability.

In all known CR-domain structures in complex with a ligand, the minimal motif is utilized. An aromatic tryptophan and a charged aspartic acid residue near the calcium-binding site from the CR-domain make up the first part of the motif and are involved in and crucial for association with the ligand. From the ligand, a lysine residue interacts with the aspartic acid residue, and the receptor tryptophan is sandwiched between the lysine and a hydrophobic residue of the ligand; these two residues form the second part of the motif (Fig. 2, top). The aromatic residue makes a π-stacking interaction with the two residues from the ligand (38). This motif has proved its ubiquitous utilization, and mutation analyses of key residues of the motif have revealed energetic contribution to binding from all four residues (38–41). It thus appears that most (if not all) CR-domains are exploiting this common motif in ligand binding, applying additional residues surrounding the fingerprint residues for ligand selection (Fig. 3A).

To date, the structure of eight ligand:CR-domain complexes have been solved, all adhering to this concept, namely, the β-propeller domain of LDLR with its own CR-domains (8); reelin with apoER2 (42, 43); human rhinovirus serotype 2 (HRV2) with VLDLR (44); receptor-associated protein (RAP) with LDLR and LRP1 (38, 45); β2-glycoprotein with LDLR (46); apoE with LRP1 (47); and gentamicin with LRP2 (48) (Table 1). In the latter complex, gentamicin adopts the characteristics of the ligand using several positively charged amino groups, which act as the Lys side-chain mimic, and instead of an aliphatic π-interaction, the cyclic 2-deoxystreptamine group stacks against the Trp residue of the minimal motif.

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Besides direct insight from structures of CR-domain:ligand complexes, several biochemical studies support the conservation of ligand binding by the described minimal motif and fingerprint residues. The binding of α2-macroglobulin to LRP1 was shown to critically depend on the acidic residues of the fourth CR-domain (49) and key lysine residues in the receptor-binding domain of the proteinase inhibitor (50). Similar mutagenesis studies also demonstrated that the complex between urokinase plaminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) binds to its cognate receptors by positively charged ligands (51) exploiting the Trp/Asp-fingerprint residues of, for example, LRP1 (52, 53) and VLDLR (40). The minimal motif is apparently exploited broadly among the different ligands, and several ligands are indeed shared among the LR family, including RAP and apoE, suggesting that these common ligands may only need to associate with a string of correct fingerprint-containing CR-domains (54–56). Still, each receptor also has its own separate ligands, strongly indicating that besides the minimal motif, a certain degree of specificity must be linked to the surrounding patches of the CR-domains or even span across several CR-domains. However, such additional specificity progenies have so far not been clearly identified and are only starting to emerge from close inspection of structures of receptor:ligand complexes.

Some β-propellers bind the minimal motif at low pH, but is this a general model for ligand release?

CR-domains have been suggested to be displaceable during endocytic cycling of the LDLR complex into low-pH compartments (57), and an identical release mechanism has been hypothesized for other ligands as they apparently associate with highly similar CR-domains. However, such mechanism remains questionable for the giant receptors as they could be speculated to suffer from large entropic penalties should similar mechanisms exist.

The low-pH crystal structure of LDLR, with intramolecular interactions between CR4 and CR5 (LA4 and LA5) and its single β-propeller (8), shows exploitation of the minimal motif with the ligand being intrinsic and mimicked by the β-propeller. In the binding interface, two histidines in the β-propeller replace the positively charged lysine and thereby introduce a pH-regulated intrinsic ligand-release mechanism, which is operational at the low-pH characteristic for endosomes. The resolution of the structure of LDLR at low pH (PDB 1N7D) is 3.7 Å. This introduces some uncertainty in regard to the position of the side chains; however, a number of conclusions have been made based on this structure. In the binding site, one histidine acts as a base (His586; β-prop) to form a salt bridge with the acidic residue (Asp149; CR-domain), and here a lysine (Lys560; β-prop) stacks with the aromatic tryptophan from the CR-domain. Also, a second histidine (His562; β-prop) contributes to binding by forming a hydrogen bond to the same aspartic acid side-chain group from the CR-domain, increasing the affinity at low pH. Since all members of the LR family have β-propellers, the concept of intrinsic ligand release at low pH prompted by protonation of the β-propeller histidines has almost become a dogma, although vaguely documented for receptors other than LDLR.

Therefore, we asked how general this release mechanism may be and addressed the question by analyzing the complete set of 42 β-propellers of the humanYWTD β-propeller superfamily. In particular, we looked for the conservation of the two key histidines (His562 and His586) and the lysine (Lys560) residue of the LDLR β-propeller important for intrinsic CR-domain binding (Fig. 5B). From multiple sequence alignments (T-coffee) and 3D-model building (Swiss model), the comparison of the resulting structures showed a conserved fold with much shorter loop regions between the YWTD-repeats compared with other six-bladed β-propellers [e.g., brain tumor protein (PDB 1Q7F) (58); DFPase (PDB 1E1A) (59); and paraoxonase 1.

Fig. 2. Binding motifs of the LR family - minimal and SBiN motifs. A schematic and structure example is shown for the two different types of binding motifs so far identified in the LR family. The known structures are listed in Table 1. (Top) Minimal motif: the W/D-minimal motif from CR-domains is shown by the interaction between HRV2 and VLDLR CR3 (PDB 1V9U). (Bottom) SBiN motif: the interaction between Shutter-Binding NxI residues from the β-propeller of nidogen are shown interacting with the NxI motif in the complex with laminin (PDB 1NPE).
Extrinsic ligand recognition by LR/H9252-propeller domains together with a histidine residue corresponding to position His586. Unexpectedly, none of the remaining 38 LR-propellers had the combination of all three important residues, although many of these LR-propellers contain a single histidine, analogue to His586 of LDLR. In contrast, we found that many LR-propellers had an acidic residue at the corresponding positions of Lys560 or of His562 (Fig. 3B). In a matchup with the acidic minimal motif of the CR-domain, this would lead to repulsion, arguing against the formation of an pH-dependent, intramolecular CR-domain:LR-propeller complex. These observations therefore (PDB 1VO4 (60)]. Recent studies of LDLR have shown that although mutation of either histidines to tyrosine impacted the acid-related ligand release, the substitution of His562 to tyrosine showed an effect over a much broader pH range, suggesting this residue plays a key function in pH-regulated ligand release (61, 62). Surprisingly, from our alignment we observed that in addition to LDLR only the LR-propellers of VLDLR, apoER2, and LRP4-1 conferred to a potential release mechanism by having both a lysine (no. “i”), and a histidine (no. “i + 2”) in their sequences at the corresponding positions of Lys560 and His562, together with a histidine residue corresponding to position His586. Unexpectedly, none of the remaining 38 β-propellers had the combination of all three important residues, although many of these β-propellers contain a single histidine, analogue to His586 of LDLR. In contrast, we found that many β-propellers had an acidic residue at the corresponding positions of either Lys560 or of His562 (Fig. 3B). In a matchup with the acidic minimal motif of the CR-domain, this would lead to repulsion, arguing against the formation of an pH-dependent, intramolecular CR-domain:β-propeller complex. These observations therefore
strongly suggest that protonation of β-propeller histidines as a mechanism for ligand release in the large and giant LR family members is not likely to mimic the mechanism derived for the small LRs. However, as both LRP1 and LRP2 are notoriously famous for their function as highly efficient endocytosis receptors, this leaves the question open of how ligands dissociate from the large and giant receptors upon internalization.

Can other general ligand binding motifs be defined in β-propeller interactions?

The recent findings of extrinsic ligand binding to the β-propellers of the large receptors prompted us to perform an analogous analysis as was done for the CR-domains looking for any general ligand-binding motifs utilized by the β-propellers. First, a comparison of all the known 3-D structures of β-propellers from the LRs and nidogens (Table 1) showed an overall similar fold, with root mean square deviations (RMSD) ranging from 1.0 Å to 1.4 Å. In support of our hypothesis, their unique short-loop regions compared with other families of β-propellers suggest a distinct ligand-binding mechanism for the YWTD β-propeller superfamily.

The first paradigmatic structure of a complex involving a YWTD β-propeller and an extrinsic ligand was that of nidogen with laminin (7). Inspection of the interface between the β-propeller of nidogen and the LE4-domain of laminin identified two hydrophobic residues of the β-propeller, a tryptophan (Trp1138) and a phenylalanine (Phe1124), positioned in the central cavity formed by the blades. The large tryptophan side chain acts as a shutter orients the side chain of Phe1124 into the central cavity, thereby improving the overall hydrophobicity of the interface (hence, the FW pair is termed shutter residues) (7). Laminin, on the other hand, utilizes a so-called Nxi-motif (x is any residue) distinctly different from the motif used for ligand binding to the CR-domains of LRs. Hence, the complex between β-propeller and ligand uses the shutter residues to recognize and bind the Nxi-motif. Accordingly, the overall binding mechanism is termed the Shutter Binding Nxi (SBiN) motif (Fig. 2, bottom). Recently, a peptide phage display library screening identified an antibody binding to LRP6β1-β2, enabling the researchers to determine the crystal structure of an LRP6:antibody complex (9). Both in this complex and in the one involving nidogen and laminin, binding occurs directly above the central cavity of the β-propeller with contacts to residues from several blades. Besides Trp1138 (β-prop) and Phe1124 (β-prop), additional residues take part in ligand binding and, in particular, Asn1082 (β-prop) forms bifurcated interactions with the asparagine side chain of the Nxi motif. This interaction is supported by another tryptophan [Trp1053 (β-prop)] and an arginine [Arg1038 (β-prop)], and the isoleucine residue of the Nxi-motif in the ligand interacts with Trp1138 (β-prop). Analysis of the interface in the also recently determined complex between LRP4 and agrin (63) revealed a very similar way of coordinating the Nxi motif-bearing ligand making substantial interactions with the shutter residues (FW) and conserved residues following an almost identical pattern.

Subsequently, we analyzed the sequences of all the 42 β-propellers of the human YWTD family for the presence of the SBiN motif, which includes the shuttle residues Phe1124 and Trp1138, as well as the essential asparagine Asn1082 and the supporting Arg1038-Trp1053 pair. From the alignment of sequences, we observed that many, but far from all, β-propellers have the hallmark FW pair (Fig. 3C). Apparently, as described above for both the nidogen-laminin, the LRP6:antibody, and the LRP4:agrin complexes, the residues of the β-propellers engaging in extrinsic ligand interactions seem not to be sequentially linked in a simple motif but nevertheless are identified in an extended stretch of approximately 100 residues (Fig. 3C). Likewise, many, but not all, have the important asparagine (homologue to Asn1082) positioned to interact specifically with the Asn of the Nxi motif in the
potential ligands. Concerning the supporting Arg-Trp residues, these are also only conserved in a subset of β-propellers, but importantly, those propellers with both the Asn and the FW-shutter residues all had the pair of supporting Arg-Trp residues, showing a strong evolutionary selection for the entire (extended) shutter motif, called the SBiN motif, suggesting an important functional role (Fig. 3C).

From the sequence analysis (Fig. 3C), it appears that all but the small LR members (LDLR, VLDLR, and apoER2) have at least one β-propeller with a SBiN motif primed to bind Nxl motif-bearing ligands. Notably, all the small family members were devoid of the tryptophan in the shutter motif. In particular, the N- and C-terminal β-propellers of the giant receptors, as well as β4 of all large and giant receptors, could not present this motif, suggesting that either they recognize ligands distinct from the Nxl motif or they have a function not related to ligand binding (e.g., spacer region or overall conformation). In some instances, as for LRP5 and LRP6, all but one of the β-propellers (β4) harbored the shutter residues. Thus, it is quite likely that these β-propellers all recognize extrinsic ligands with an Nxl motif. In support of this hypothesis, the recently solved structures of LRP5 and LRP6 with Dickkopf (Dkk1) showed that Dkk1 indeed binds to its receptors using an Nxl motif in its N-terminal part (9). For the C-terminal part of Dkk1 the binding to β-propeller III involves similar residues, except the residues corresponding to Asn(1082), as if binding an Nxl motif, although no Nxl motif was seen in this part of Dkk1 (10, 11). Furthermore, it has been elegantly demonstrated by Holdsworth et al. that sclerostin also binds its cognate receptors LRP5 and LRP6 via its Nxl motif (64). Moreover, as LRP2 contains three β-propellers with an SBiN motif, it is interesting that an analysis of the sequences of known LRP2 ligands reveals that cubilin, vitamin D-binding protein, transcobalmin-vitamin B12, serum albumin, and apoB all contain one or two copies of exposed Nxl motifs, although LRP2 binding using this motif has not yet been shown.

In conclusion, it appears that WYTD β-propellers with a conserved SBiN motif conferring to the shutter mechanism may bind Nxl-bearing ligands. To date, four complexes confirm this general SBiN-binding mechanism, as complexes for nidogen:lamamin, LRP6:Dkk1, LRP6:antibody, and LRP4:agrin all comply with this binding pattern. However, as several of the β-propellers do not contain the SBiN motif residues, there are likely additional ways for proteins without Nxl motifs to interact with LR β-propellers. Whether these are linked by other motifs, such as hydroxyl-bearing or hydrophobic side-chains matching, remains to be explored.

**Intramolecular interactions: a prerequisite for modular packing**

Besides the identification of extrinsic ligand binding to the β-propellers, it has from the recent structural studies of the β-propellers become evident that they form a tight interaction with adjacent EGF-like domains (6). Indeed, no structures of β-propellers have been determined in isolation, suggesting that the presence of the EGF-like domain is essential for a stable and structured β-propeller (6). Recently, the structures of tandem β-propellers from LRP6 (βII- and βII-IV) have been solved (10), showing very close contact between neighboring β-propellers of the LR4β family (10). This is in strong contrast to the CR-domains where numerous studies have shown that these domains behave like beads on a string (i.e., necklace), providing maximal flexibility to interact with the broad variety of structurally unrelated ligands (Fig. 2, top). In terms of the β-propeller fold, it is now obvious that the YWTD regions introduce much more structural restraint in terms of the overall folding of the entire receptor. It was a surprise when Rudenko and coworkers described the folding back of the CR-cluster onto the β-propeller at acidic pH, but besides this single study, it is still largely unknown how the receptor’s overall conformations look and behave. Although it is anticipated that the LR1β subfamily folds similarly to the LDLR, it was not known how the conformation of LR4β and LR8β members would manifest. Some light onto this has now been shed due to the findings that at least two adjacent propellers function as a single rigid body with strong restraints on their relative positions (10, 11, 65), and electron microscopy data suggest that the LR4β subgroups adopt elongated conformations in which the two rigid bodies are not intimately linked and where all four propellers can be either elongated after each other or exist with a bend between the two units of dimer propellers (65).

**Biological implications of protein interactions of the LRP β-propellers**

Our analyses have shown that LRs communicate via their β-propellers and that these can serve as binding partners for extracellular proteins, for instance, by using the SBiN motif to recognize Nxl motifs of the target proteins, as seen for Dkk1 and agrin. Accordingly, not only are the CR-clusters and EGF-like domains of LRs functional in binding of extracellular ligands but also the β-propellers communicate. Intriguingly, as noted above for the LRP2 ligands, the Nxl motif is present in other cell surface receptors, not only in soluble extracellular ligands. It is interesting to note that cubilin has two Nxl motifs in its complement C1r/C1s, Uegf, Bmp1 (CUB) domain 18 conserved between the human and rat variants. Cubilin has been suggested to serve as a coreceptor with LRP2 (66, 67). However, CUB domains 17–22 did not in a LRP2-affinity chromatography study bind to LRP2 (68). Still, as LRP2-β7 contains a shutter motif, CUB18 could be a potential ligand hereof, perhaps binding in a stabilizing manner together with CUB domain 12–17 that binds LRP2 in a Ca2+ dependent manner and hence involves CR-domains (68). Accordingly, we suggest that giant receptors, such as LRP2, may associate with other receptor molecules, sometimes by interaction solely by their β-propellers and sometimes in combination with CR-domains or EGF-like domains, which in principle allows for an increase in affinity.
Fig. 4. Correlation between LR subgrouping and their main biological activities. (A) The LR core family members are depicted schematically with the β-propeller domains color coded according to conservation of the histidine-based pH release switch (in orange) or the SBiN motif (in green) (outlined in Fig. 3B, C, respectively). CR-domains containing the Trp/Asp residues of the minimal motif as described in Fig. 3A are shown in red. (B) All LR1β receptors are likely to contain the property of ligand displacement upon internalization of receptor-ligand complexes into acidic endosomal compartments. Ligands (Lig) bind to the CR-cluster at the cell surface at neutral pH, whereas the CR-domain associates with the β-propeller in the endosomal environment leading to displacement of the ligand. (C) All LR4β receptors have three to four SBiN-motif containing β-propellers, suggesting their main biological function relates to transduction of Nxl-induced signaling across the plasma membrane. The Nxl-motif is found either in coreceptors or in extracellular proteins binding directly the SBiN-propeller or being the link to coreceptors. (D) LR8β receptors contain neither β-propellers with the pH switch residues nor multiple SBiN-motif β-propellers in close contact. The main functions of these giant receptors are more difficult to hypothesize. One possible
Another interesting observation from comparison of the LR members is that only LR4β receptors contain clusters of β-propellers with SbiN motifs. In contrast, the SbiN-containing β-propellers of the giant receptors are spaced by clusters of CR-domains (Fig. 4A); therefore, it is highly possible that these receptors need to fold into a more compact structure that could bring the various SbiN motifs in close proximity, thus mimicking the high concentration of SbiN motifs found in the LR4β receptors (Fig. 4D).

It has been well established that LRP5/6 works in concert with several other coreceptors in the Wnt signaling pathways (e.g., the family of frizzled receptors and Kremen receptors) (69). The primary function of this coreceptor complex formation is anticipated to bring together various adaptor proteins at the cytoplasmic side of the plasma membrane. It could be speculated that in parallel to the suggested LRP2: cubilin complex, frizzled and Kremen also may bind directly to LRP5/6 extracellularly by exploitation of β-propellers. Frizzled-5 and frizzled-8 both contain an NxVR/NxVK motif in their extracellular region, indicating them as potential LRP5/6 β-propeller ligands. This is addi-
tionally indicated by data showing that Kremen, in concert with the Wnts, influences the endocytosis of LRP5/6 (70) (Fig. 4C). Several Wnt receptors are capable of binding to the β-propeller region on LRP5/6; however, binding occurs in different regions, depending on the specific Wnt variant. After analyzing the crystal structure of Wnt8 (71), it appears that its sole NxI motif is not accessible to LRP5/6 binding. Still, we cannot rule out that conformational changes may be induced in Wnt8 by possible binding to LRP5/6. Whether other Wnt variants bind to LRP5/6 through SbiN motifs remains unanswered.

Further demonstration of coreceptor function comes from LRP4 forming a complex with muscle-specific kinase (MuSK) for optimal transduction of the agrin signaling (agrin binds using an NxI motif to LRP4-βI) (63). As this interaction is likely not to be pH sensitive, it could in part be a very useful mechanism for receptors undergoing signaling and eventually establishing intracellular signaling endosomes/pathways, recently described for LRP6-signalsomes (72). Besides binding to other LRP-unrelated receptors, it has been reported that several LRs can form functional heterodimers under certain circumstances; for example, LRP binds to sorLA/LR11 (73), and apoER2 and VLDLR seems to work in dimer conformation with cadherin-related neuronal receptors in the reelin signaling pathways (74). However, it has not been addressed whether two LRs bind directly to each other, although this seems likely and may involve β-propeller:CR-domain interactions from different receptors. Collectively, these extensive and widespread observations point to LRs of having the potential to use their β-propeller domains to establish complexes with other receptors in coreceptor arrangements.

Interestingly, coreceptors are most commonly described for the LR4β and LR8β families, suggesting that the LR1β receptors are not steered for coreceptor involvement. This is in line with a primary function of LR1β receptors for endocytosis and acidic ligand release (Fig. 4B), as well as the lack of the shutter-associated FW-pair of residues in their β-propeller. These small receptors, however, are still able to elicit cell signaling, as they are clearly engaged in the reelin pathway (74, 75). Interestingly, LRP4 is the only other receptor also containing a potential pH-inducible switch β-propeller (Fig. 4A), suggesting this receptor is able to have biological functions depending on both its ability to displace bound ligands upon entry in the endosomal pathway and its interaction with NxI motif-containing ligands. Importantly, from the domain organization presented in Fig. 4A, the pH-release β-propellers are only seen in receptor molecules with a single ligand-binding CR-cluster preceding the β-propeller, suggesting that the molecular flexibility required for the intramolecular interactions to take place is only possible for this domain organization.

That the LR family is found in many other organisms shows an early evolutionary function of these proteins. Interestingly, insects and other egg-laying species contain a branch of LRs with three β-propeller domains (LR3β) (76). For example, the vitellogenin receptor yolkless from Drosophila melanogaster (77) contains three β-propellers, which neither contain SbiN motifs nor have the pH release switch, and their functions thus deserve more attention. However, this article has focused solely on the human sequences of LRs, as sequence variations within species model involves simultaneous binding of both NxI and classical ligands (Lig) to different sites in the extracellular receptor regions (shown to the right). Another model is the folding of the giant receptors into more compact structures involving minimal and SbiN-motif interactions that may bring SbiN β-propellers into close proximity, forming optimal binding sites for NxI ligands (shown to the left).

### TABLE 2. Open questions

- Is the evolution of mammalian LRs and their functionality rooted in the clustering of the β-propellers?
- What is the mechanism for ligand release for the large and giant receptors upon internalization?
- What is the role of CR-domains not containing the Trp/Asp fingerprint residues?
- Do β-propellers from the giant receptors recognize extrinsic ligands with an NxI motif?
- Is there a distinct difference in the function of single-positioned β-propellers compared with twins and quadruplets?
- Are twin β-propellers important for an elongated form of the receptors?
- What is the function of the β-propellers positioned after cluster II in LRP2, which has an extreme broad ligand portfolio but lack SbiN motifs as well as histidines for pH-induced ligand release?
both with regards to receptors and targets may impact the conclusions drawn.

Concluding remarks and future perspectives

Our current understanding of ligand specificity, binding, and release in the LR family, in particular relating to the β-propellers, is limited. Recent research on these modules of the LR family is only now becoming appreciated, showing that it is the tip of an iceberg regarding the multitude of actions to be expected from these receptors. Despite the fact that the giant receptors were described 20 to 30 years ago [LRP1 in 1988 (78); LRP1B in 2000 (79), and LRP2 in 1994 (80)], it is still unclear how the giant receptors are structurally organized. It seems unlikely that we are to see schematic representations of elongated LRs in the near future, although elucidation of the overall structure of the giants is a highly needed task. In this respect, integrative structural, biophysical, and molecular analyses are essential for understanding LRs, and future research is expected to provide significant findings addressing many open questions (Table 2). Three important types of experiments can be suggested to directly address some of the more important and urgent questions applicable to all LR members. First, a domain swapping (or motif swapping) approach between LR members and the assessment of ligand binding and release of hybrid receptors is a strategy that has not been addressed for the giant LRs, although successfully applied to LRP1β receptors (81). Importantly, EGF-like domains linked to the β-propeller in question must be transferred along with it. Also, measuring ligand binding and ligand dissociation for deletion mutants and point-mutation variants will reveal mechanistic and biochemical insight. The use of fluorescence microscopy to analyze ligand interactions with LRs at the cellular level provides a powerful tool to dissect the role of the β-propellers. Second, pull-down assays and interactome profiling using β-propellers instead of receptor fragments focusing on the CR-domains (82) is clearly an important avenue of investigation that will provide broad insight into ligand-binding specificities of various β-propellers. Third, we find it of extreme importance that the structural platform is extended for the LR family, from single domains to entire molecules and complexes. This will provide the most detailed information of interaction determinants, allowing for more general conclusions in terms of biological functions and mechanisms to be drawn.

Based on the theoretical analyses presented here and with emphasis on deriving experimental evidence in the future, the answering of the open questions will be of obvious interest to basic science. The outcome should also strongly benefit research in diseases linked to LRs, such as hypercholesterolemia, Alzheimer’s disease, and cancer, and in drug development and transport, relieving severe side effects, such as nephro- and ototoxicity. By our current analyses, we hope to encourage the continued structural and molecular elucidation of the modus operandi of the LRs.

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