LDL Receptor-Related Protein 1: Unique Tissue-Specific Functions Revealed by Selective Gene Knockout Studies

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I. INTRODUCTION

Protease activity in the blood is carefully regulated by a variety of proteinase inhibitors that are usually found circulating at high concentrations in the plasma. Early work led to the idea that once a proteinase forms a complex with its inhibitor, it is cleared from the circulation via a receptor system. Proof of this concept was demonstrated by showing that the proteinase inhibitor \( \alpha_2 \)-macroglobulin (\( \alpha_2 \)M) was rapidly cleared by a liver receptor after forming a complex with a protease (73). These and other studies provided evidence for the existence of a receptor responsible for removal of \( \alpha_2 \)M-proteinase complexes, and using affinity chromatography approaches, two groups isolated the receptor responsible for the clearance of these complexes (3, 169).

While this work was ongoing, a large hepatic receptor with remarkable resemblance to the LDL receptor was identified (92). This receptor, originally termed the LDL receptor-related protein (LRP), but now termed LRP1 or occasionally CD91, was shown to bind to apolipoprotein E (12), raising the possibility that LRP1 might function in lipoprotein metabolism as a chylomicron remnant receptor. Sequencing studies soon revealed that the \( \alpha_2 \)M receptor was identical to LRP1 (124, 264), revealing a large endocytic receptor that is widely expressed in several tissues. LRP1 is a member of the LDL receptor family that plays diverse roles in various biological processes including lipoprotein metabolism, degradation of proteases, activation of lysosomal enzymes, and cellular entry of bacterial toxins and viruses. Deletion of the LRP1 gene leads to lethality in mice, revealing a critical, but as of yet, undefined role in development. Tissue-specific gene deletion studies reveal an important contribution of LRP1 in the vasculature, central nervous system, macrophages, and adipocytes. Three important properties of LRP1 dictate its diverse role in physiology: 1) its ability to recognize more than 30 distinct ligands, 2) its ability to bind a large number of cytoplasmic adaptor proteins via determinants located on its cytoplasmic domain in a phosphorylation-specific manner, and 3) its ability to associate with and modulate the activity of other transmembrane receptors such as integrins and receptor tyrosine kinases.

II. STRUCTURAL ORGANIZATION OF LRP1

LRP1 is a member of the LDL receptor family, which contains several structurally homologous receptors that are composed of modular structures. This receptor family includes seven family members that are closely related and include the LDL receptor, very-low-density lipoprotein (VLDL) receptor, apoE receptor 2, multiple epidermal growth factor-like domains 7 (MEGF7), glycoprotein 330 (gp330/megalin/LRP2), LRP1, and LRP1B (Fig. 1). In addition, the family also includes additional members that are more distantly related, such as LRP5, LRP6, and SorLA/LRP11. Like other members of the LDL receptor family, the modular structures within LRP1 include cysteine-rich complement-type repeats, EGF repeats, \( \beta \)-propeller domains, a transmembrane domain, and a cytoplasmic domain.

A. Cysteine-Rich Complement-Type Repeats

All members of this receptor family contain clusters of two or more cysteine-rich complement-type repeats...
Table 1. Ligands known to bind to LRP1

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<td>Pregnancy zone protein-protease complexes</td>
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<td>Pseudomonas exotoxin A</td>
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<td>αβ peptide (monomer)</td>
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(α1M, α2M-macroglobulin; uPA, urokinase plasminogen activator; tPA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor 1; TSP, thrombospondin; MMP, matrix metalloproteinase; RAP, receptor associated protein.)

(CR) that are also commonly referred to as ligand-binding repeats, since most of the ligands bind to these repeats. The first insight into the folding properties of CR came from the NMR spectroscopy study of Daly et al. (50). This group solved the structure of the first repeat of the LDL receptor (CR1) revealing that this module consists of a β-hairpin structure followed by a series of β-turns. Subsequently, a crystal structure of CR5 from the LDL receptor was solved (65), revealing that the module forms a cage surrounding a calcium ion that stabilizes the structure. Since these early studies, a number of additional structures of CR from various members of the LDL receptor family have been reported and include CR3 (60), CR7 (248), CR5-CR6 (104), and CR8 (97) from LRP1 (Fig. 2A), and CR2 (49), CR1-CR2 (125), and CR6 (188) from the LDL receptor. In LRP1, the CR are localized into regions as clusters and are termed clusters I–IV, each containing variable numbers of CR. Binding experiments indicate that most LRP1 ligands bind to clusters II and IV (183, 292).

B. EGF and β-Propeller (YWTD) Domains

In addition to the CR, all LDL receptor family members contain one or more regions that are homologous to the EGF precursor which consists of two cysteine-rich EGF repeats, a YWTD repeat predicted from modeling to be folded as a β-propeller domain (254), followed by a third EGF-like repeat. Crystallization of the β-propeller domain along with the COOH-terminal EGF repeat from the LDL receptor (105) confirmed that the YWTD repeat forms a six-bladed β-propeller that packs tightly against the COOH-terminal EGF module (Fig. 2B). The function of this region was discovered when investigators deleted it from the LDL (51) and VLDL receptor (162) and found that the mutant receptors failed to release their ligands in the low pH environment of the endosomal compartments. The structural basis for the involvement of the β-propeller domains in ligand uncoupling was finally understood when a crystal structure of the LDL receptor extracellular domain at pH 5.3 was solved (236). In this structure, shown in Figure 2C, CR2 through CR7 fold back over the two EGF repeats and the β-propeller domain. At this low pH, CR4 and CR5 were found to associate with the β-propeller domain via their calcium-binding loop. This observation led to the proposal that the β-propeller domain functions as an alternate ligand for CR4 and CR5, which bind in a calcium-dependent manner promoting ligand release.

C. Transmembrane and Cytoplasmic Domains

Each member of the LDL receptor family contains a single-pass transmembrane domain and a cytoplasmic domain of varying length. In the case of LRP1, the cytoplasmic domain consists of 100 amino acid residues and includes two dileucine (LL) motifs and two NPxY motifs. The terminal NPxY motif is tyrosine phosphorylated by the PDGFR-β (23, 142), by connective tissue growth factor (CTGF, also known as CCN2) (302), and by v-Src (6). The LRP1 cytoplasmic domain interacts with numerous adaptor molecules (Table 2) (80, 274) including Shc, disabled, and Fe65, which are involved in directing cellular traffic or in cell signaling events. Additionally, LRP1 has been shown to undergo regulated intramembrane proteolysis (154), and in vitro, its intracellular domain (LRP1-ICD) has been implicated in transcriptional modulation (113).
III. CELL TRAFFICKING OF LRP1 AND THE ROLE OF THE RECEPTOR ASSOCIATED PROTEIN

Because LRP1 recognizes such a wide variety of different ligands, mechanisms exist to prevent newly translated LRP1 from prematurely associating with ligands in the endoplasmic reticulum (ER), which leads to aggregation and degradation instead of proper targeting to the plasma membrane. A chaperone, termed the receptor associated protein (RAP), binds tightly to LRP1 and other members of the LDL receptor family at neutral pH values and antagonizes ligand binding while these receptors are in the ER enabling them to be successfully delivered to the plasma membrane.

A. Discovery of RAP

RAP was discovered as a protein that copurified with LRP1 by ligand-affinity chromatography (3, 264). While it was discovered as a protein that copurified with LRP1 by ligand-affinity chromatography (3, 264). While it was

FIG. 1. Modular domain organization of LDL receptor family members. In LDL receptor-related protein (LRP1), the four clusters of complement-type repeats are numbered I–IV.

FIG. 2. Structure of modules from LDL receptor family members. A: X-ray structure of CR7 from LRP1 (248) showing the basic folding of these modules with the structural calcium residue. B: X-ray structure of epidermal growth factor (EGF) and β-propeller (YWTD) domain from the LDL receptor (105) showing the six-bladed β-propeller domain. C: X-ray structure of the LDL receptor ectodomain solved at pH 5.2 (236) showing the interaction of CR4 and CR5 with the β-propeller domain at this reduced pH.
was originally thought to represent the COOH-terminal region of the rat Heyman nephritis antigen (LRP2/megalin) (209), subsequent work revealed that RAP is a distinct ER-resident protein (263) that binds tightly to multiple sites on LRP1 and prevents ligands from binding to this receptor (91, 289).

B. Structure of RAP

Attempts to crystallize the entire RAP molecule have not been successful, and our knowledge of the structure is derived from solved structures of individual domains. A three-domain structure of RAP was originally proposed by Bu et al. (30) based on the prediction of an internal triplication in the primary structure of RAP. Experimental evidence supporting this proposal was later obtained by Ellgaard et al. (62) and Lazic et al. (129) who prepared recombinant fragments representing domains 1, 2, and 3 and showed that the functional integrity of these domains is preserved when isolated. The structures of domain 1 (D1) (187, 298), domain 2 (D2) (130), and domain 3 (D3) (131) of RAP were solved using NMR spectroscopy (Fig. 3A). RAP D3 was also solved by X-ray crystallography (68) as a complex with CR4 and CR5 of the LDL receptor. These studies suggest that each RAP domain can be represented by a three-helix bundle connected by flexible loops. In D1, three distinct α-helices are present and consist of residues 23–35 (α1), 39–65 (α2), and 72–88 (α3). D2 is also comprised of three α-helices consisting of the residues 117–127 (α4), 132–161 (α5), and 184–210 (α6) (the α-helices are numbered in the context of the full-length RAP). The NH2-terminal peptide segment of D2 comprising residues 101–116 is flexible and disordered. The linker between α4 and α5 is well defined, whereas the linker between α5 and α6 is 23 amino acids long and is disordered and susceptible to protease digestion (219). The D3 structure reveals that this RAP domain is also composed of a three-helix bundle containing a short helix followed by two longer helices. These helices consist of residues 222–230 (α7), 238–274 (α8), and 281–315 (α9). D1, D2, and D3 show a remarkably similar topology. Each domain is stabilized by hydrophobic interactions within the core of each structure, with the relative arrangement of the three helices in each domain mainly determined by a number of hydrophobic contacts. It is noteworthy that the linkers connecting the two long helices in D1 and D3 are short and relatively well structured.

To determine if the structures of individual domains of RAP are representative of those in full-length RAP, the chemical shifts of the backbone amide groups of the individual domain constructs were compared with those

| Table 2. Adaptor proteins known to bind to the cytoplasmic domain of LRP1 |
|-----------------|----------------------------------|
| Displed-1 (Dab1)| Src activation, neuronal migration |
| Shc             | Signal transduction by protein-tyrosine kinases |
| PKCa           | Proliferation, apoptosis, differentiation and motility |
| FE65           | Actin, APP processing |
| PSD95          | Coupling to NMDA receptors |
| SEMCAP-1       | Axon guidance |
| JIP1, JIP2     | MAPK pathway |
| Talin-like protein | Coupling to actin cytoskeleton |
| CAPON          | NO synthase |
| PIP4,5 kinase like protein | Inositol signaling |
| ICAP1          | Integrin-mediated signaling |
| Cbl            | E3 ligase, receptor tyrosine kinase |
|                | downregulation |

...was originally thought to represent the COOH-terminal region of the rat Heyman nephritis antigen (LRP2/megalin) (209), subsequent work revealed that RAP is a distinct ER-resident protein (263) that binds tightly to multiple sites on LRP1 and prevents ligands from binding to this receptor (91, 289).

![Fig. 3. A: NMR structure of RAP domains 1 (D1) (298), D2 (130), and D3 (131) showing the three helical bundle organization of each domain. The helices are numbered as α1–α9. B: since the three domains of RAP are independent and do not interact, but are connected by long flexible loops, the protein is expected to adopt a variety of conformations in solution, one of which is shown.](http://physrev.physiology.org/.../10.1152/physrev.00164.2007)
of intact RAP using a combination of 2D-\([^{15}\text{N},^{1}\text{H}]\)-TROSY and 3D-TROSY-HNCA spectra (130). The chemical shift differences between the individual domains and those in full-length RAP are \(<0.05\) ppm, except for the linker regions where domain constructs break off, suggesting that the structures of the individual domains are preserved and that there is no detectable chemical shift perturbation due to the presence of other domains. Thus the individual domain structures are faithful representations of their structure in the full-length protein.

An idea of the overall structure of RAP was obtained by employing experimental small-angle neutron scattering (SANS) data and a novel simulated annealing protocol to characterize the overall structure of RAP (130). Since RAP consists of three independent domains joined by two flexible linkers, the protein is expected to have an ensemble of conformers in solution, one of which is shown in Figure 3B. RAP adopts a unique structural architecture consisting of three independent three-helix bundles that are connected by long and flexible linkers. The flexible linkers and the quasi-repetitive structural architecture may allow RAP to adopt various possible conformations when interacting with the LDL receptors, which are also made of repetitive substructure units.

C. Chaperone Function of RAP

To understand the in vivo function of RAP, gene targeting was utilized to generate mice in which the RAP gene was deleted (291). The studies revealed that in RAP-deficient mice the amount of mature, processed LRP1 is substantially deleted (291). The studies revealed that in RAP-deficient mice the amount of mature, processed LRP1 is substantially deleted (291). The studies revealed that in RAP-deficient mice the amount of mature, processed LRP1 is substantially deleted (291).

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D. A Histidine Switch in RAP Modulates LRP1 Binding

A major function of RAP is to escort LRP1 from the ER to the Golgi apparatus, where RAP then dissociates from LRP1 as a result of a lowered pH encountered later in the secretory pathway (30, 293). This function of RAP has been ascribed to the RAP D3 domain (194). When the surface charge distribution of RAP D3 at pH 7.2 (corresponding to the ER) was compared with the distribution at a more acidic pH, i.e., pH \(<6.5\), corresponding to the Golgi, the positively charged regions on the surface of D3 increased dramatically under the acidic conditions, mainly due to protonation of solvent-exposed histidine side chains (131). These results suggested that histidine residues, especially highly conserved histidines, may function as a switch in response to the environmental change encountered when the RAP/LRP1 complex shuttles from the ER to the Golgi, leading to dissociation of RAP from LRP1. To test the involvement of histidine residues in modulating the pH-dependent binding of RAP to LRP1, mutant molecules were prepared in which all the conserved histidine residues in D2 and D3 were replaced with alanine residues. Binding studies revealed that mutation of the histidines in RAP D3 significantly reduced the pH sensitivity of D3 binding to LRP1 and failed to promote the secretion of soluble fragments of LRP1 from cells (131). Thus structure-based mutagenesis studies confirm that the protonation of histidine residues as a consequence of the pH changes modulate the binding/release of RAP from LRP1.

IV. MODEL FOR LIGAND RECOGNITION BY LRP1

One of the major structural questions that remains to be solved is how LRP1 is capable of recognizing such a wide variety of structurally distinct ligands. Site-directed mutagenesis studies point to an important role of basic residues present on the ligand that contribute to LRP1 recognition, while the first solved structure of a receptor fragment/RAP complex gives some insight into the mode by which these residues may interact with CR present within LDL receptor family members.

A. Role of Basic Residues in the Recognition of Ligands by LRP1

ApoE is a ligand recognized by most LDL receptor family members. Earlier studies highlighted the importance of lysines found between residues 140-160 (126) and Arg-172 (172) within the apoE molecule that contributed to its interaction with the LDL receptor. In the case of lipoprotein lipase, its interaction with LRP1 has been localized to the COOH-terminal domain (191, 290) and involves two regions within this domain that include residues 380-384 and residues 404-430 (186). Mutation of Lys-407 to alanine resulted in a 10-fold reduction in the affinity of the COOH-terminal domain of lipoprotein lipase for LRP1 (290).
A number of serpin enzyme complexes have been identified that interact with LRP1, including complexes consisting of proteases with plasminogen activator inhibitor 1 (PAI-1). Mutagenesis studies have identified basic residues in PAI-1 that appear important for its interaction with LRP1 (229). Thus conversion of Lys-82 and Arg-120 to alanine reduced the ability of LRP1 to recognize complexes of PAI-1 complexed to urokinase plasminogen activator (uPA). Likewise, mutation of Arg-78 and Lys-124 to alanine also resulted in loss of binding of the complex to LRP1. Stefansson et al. (259) found that a PAI-1 molecule with Arg-76 mutated to glutamic acid resulted in a loss of binding to LRP1.

Critical lysine residues were located in another ligand for LRP1, α2M. This molecule only binds to LRP1 following a conformational change induced by complex formation with proteases. Site-directed mutagenesis implicated two lysine residues, Lys-1370 and Lys-1374, in binding to LRP1, and mutation of these two residues significantly reduced the affinity of the α2M receptor-binding domain for LRP1 (185).

The D3 domain of RAP binds with high affinity to LRP1, and to gain insight into amino acids that are required for the binding of RAP to LRP1, Migliorini et al. (160) performed random mutagenesis of D3 of RAP, which identified two critical lysine residues, Lys-256 and Lys-270, within the α8 helix of the third domain of RAP (Fig. 4A) that are necessary for binding of D3 to LRP1. Mutation of either lysine residue significantly reduced the affinity RAP D3 for LRP1.

Not all LRP1 binding sites are composed of lysine residues, and, in the case of another serpin, protease nexin 1 (PN-1), a region has been identified corresponding to Pro-47 to Ile-58 of PN-1 that appears responsible for interacting with LRP1 (118). Thus a synthetic peptide representing this region (PHDNIVISPHGI) was shown to competitively inhibit the LRP1-dependent endocytosis of thrombin:PN1 complexes. An antibody prepared against this synthetic peptide inhibited degradation of the PN1:thrombin complex by 70%, but it had no effect on binding of the complex to cell surfaces (117). Furthermore, point mutations within the corresponding region of PN-1 (His-48A and Asp-49A) reduced the catabolism rate of mutated PN-1 to 15% of wild type (117).

B. Structure of RAP D3 Receptor Complex

Recently, Fisher et al. (68) solved the X-ray structure of a complex between a two-module region of the CR3 and CR4 of the LDL receptor and the third domain of RAP. In the complex, RAP D3 contains two docking sites for the LDL receptor CR involving Lys-256 and Lys-270 (Fig. 4A). A relatively small interface between D3 and CR3-CR4 of the LDL receptor is dominated by electrostatic interactions between the two basic residues (Lys-256 and Lys-270) and the surface-exposed aspartate residues that participate in calcium coordination on CR3 and CR4 (Fig. 4, B and C). Each CR consists of four residues that provide a “docking” site for a lysine side chain protruding for helix α8 of the RAP D3 domain. Carboxylate oxygen atoms from three aspartates (Asp-147, Asp-149, and Asp-151 in CR4 and Asp-108, Asp-110 and Asp-112 in CR3) surround the ε-amino lysine group to form a salt bridge. In addition, two aromatic residues, (Phe-105 in CR3 and Trp-144 in CR4) pack up against the aliphatic portion of the lysine side chain. Importantly, all four residues participate in coordination of the calcium ion, and their position is therefore fixed in the structure.

C. Summary

As highlighted by Fisher et al. (68), the aspartic acid residues that form the acidic pocket responsible for dock-
ing the basic lysine residues in RAP are highly conserved among the CR of LDL receptor family members and thus are representative of most CR repeats in these receptors. This suggests that lysine docking may represent a general mechanism for ligand recognition by LRP1 and other LDL receptor family members (68). If true, high-affinity binding would require avidity effects resulting from the interaction of multiple lysine residues with multiple CR on the receptors. Interestingly, optimal high-affinity (K_D = 1.6 nM) binding of RAP to CR in cluster II of LRP1 requires three CR (CR5-CR7) (279); no binding of RAP to two repeats was detected in these experiments. Titration calorimetry experiments reveal that the binding of RAP or RAP D3 to two repeats, CR7-CR8 of LRP, is relatively weak (K_D = 1 μM). These studies suggest that optimal binding of RAP D3 to LRP1 may require contact with at least three CR. Other ligands, such as activated forms of α2M, also appear to optimally require three CR for their binding as well (59).

V. HEPATIC FUNCTION OF LRP1 IN THE CLEARANCE OF PLASMA PROTEINS

LRP1 is abundantly expressed in the liver in hepatocytes and resident macrophages (Kupffer cells). Here, LRP1 recognizes a variety of distinct molecules in the circulation, including proteinase-inhibitor complexes, activated coagulation factors, and chylomicron remnants and mediates their endocytosis and intracellular degradation.

A. α2-Macroglobulin

α2M is a highly conserved proteinase inhibitor capable of inhibiting target proteinases from all four major classes. The target proteinase cleaves α2M at a “bait” region, which triggers a conformational change in the molecule that entraps the proteinase in a cagelike structure and exposes a receptor binding site that is recognized by LRP1 on hepatocytes (251). Abundant evidence exists indicating that LRP1 is the key hepatic receptor responsible for clearing α2M-proteinase complexes. First, LRP1 was purified from tissue extracts by ligand affinity chromatography using the activated form of α2M coupled to Sepharose (3, 169). Second, cells genetically deficient in LRP1 lack the ability to mediate the internalization and degradation of 125I-labeled α2M-proteinase complexes (69, 214). Third, RAP was demonstrated to inhibit the clearance of 125I-labeled α2M-proteinase complexes from the circulation when coinjected with α2M (122) or when overexpressed in the liver (294). Finally, decreased hepatic levels of LRP1 using RAP-deficient mice resulted in delayed clearance of 125I-labeled α2M-proteinase complexes from the circulation (291). Together, these data provide compelling evidence that LRP1 participates in vivo in the clearance of α2M-proteinase complexes.

B. Serpin-Enzyme Complexes

The serine proteinase inhibitors (serpins) are a large family of proteins, some of which are found circulating in the plasma where they function as inhibitors of serine proteinases (247). These proteinase inhibitors form a complex with target proteinases that is initiated when the protease cleaves an exposed loop present in the inhibitor which in turn triggers a conformation change in the serpin. This results in the formation of a covalent complex with the target proteinases. Serpin-enzyme complexes (SECs) are unstable and will slowly break down releasing the active enzyme. Fortunately, SECs are recognized by a receptor system that is responsible for mediating their endocytosis and subsequent degradation. The existence of a hepatic receptor-based clearance mechanism was first suggested from the early work of Ohlsson et al. (196) who investigated the clearance of trypsin-inhibitor complexes from the circulation. Further studies revealed that the SEC receptor is specific for the serpin only after it has complexed with an enzyme and does not effectively recognize the cleaved or native serpin (73, 153).

Attempts to identify the receptor system responsible for the clearance of SECs led to the description of an SEC receptor that recognizes a pentapeptide sequence located at the COOH-terminal fragment of α1-antitrypsin (107). This peptide appeared to bind to a cell-surface receptor and was reported to prevent the internalization and degradation of a number of SECs by HepG2 cell lines. However, mutation of this region in heparin cofactor II failed to diminish the binding, internalization, or degradation of thrombin:heparin cofactor II complexes by Hep G2 cells (147), revealing that other regions on the serpin are involved in receptor recognition.

Substantial evidence now indicates that LRP1 and other members of the LDL receptor family (LRP2/gp330/megalin and the VLDL receptor) function as prominent receptors in mediating the clearance of SECs (2, 89, 111, 122, 192, 258). Given that LRP1 is the only one of these receptors that is abundant in the liver, this receptor is likely important in the hepatic removal of SECs from the plasma. LRP1 binds numerous SECs and, as expected for the SEC clearance receptor, does not recognize the native or cleaved serpin (122). The role of LRP1 in mediating the cellular uptake of SECs has been demonstrated by using cell lines genetically deficient in LRP1 and by in vivo clearance studies showing that RAP blocks removal of 125I-labeled SECs from the circulation (122).
C. Factor VIII

Factor VIII (fVIII) is a key plasma protein and a member of the coagulation cascade that is deficient in the well-characterized bleeding disorder hemophilia A. fVIII, which normally circulates in a complex with its carrier protein von Willebrand factor, is an inactive cofactor (66). Upon injury within the vasculature, this cofactor is activated to fVIIIa by limited proteolysis. This results in its dissociation from von Willebrand factor and subsequent assembly on the membrane surface with an enzymatically active form of factor IX (fIXa) to form a macromolecular Xase complex. This complex effectively activates factor X, the next proenzyme in the coagulation cascade. The fact that deficiencies in both fVIIIa and fIXa lead to bleeding disorders attests to the significant role that the macromolecular Xase complex plays in the blood coagulation cascade. While the functional and structural properties of fVIII are well described, only recently have the mechanisms by which this protein is metabolized become a key focus of investigation. Key hepatic receptors contributing to the clearance of fVIII are both members of the LDL receptor family, LRP1 and the LDL receptor.

Saenko et al. (238) and Lenting et al. (135) were the first to describe the potential of LRP1 to mediate the catabolism of fVIII. Both studies reported that LRP1 binds to fVIII with $K_D$ values between 60 and 116 nM (135, 238). Furthermore, both studies found that cells expressing LRP1, but not cells genetically deficient in LRP1, were able to mediate the uptake of fVIII in an LRP1-dependent manner. The in vivo significance of these observations was demonstrated by showing that RAP blocked the in vivo clearance of $^{125}$I-labeled fVIII (238) from the circulation. Importantly, von Willebrand factor was shown to inhibit the LRP1-mediated clearance of fVIII (135).

Genetic studies confirmed an important role for LRP1 in the metabolism of fVIII (25). This was demonstrated using an a cre/loxP-mediated recombination strategy to develop mice with LRP1 specifically deleted in the liver. This mutation resulted in an increase in the plasma levels of fVIII, from 1.9 U/ml in control mice to 3.4 U/ml in LRP1-deficient mice. Furthermore, the clearance of fVIII was delayed in the hepatic LRP1-deficient mice. Together, these studies reveal that LRP1 functions in vivo and modulates fVIII plasma levels. More recent work has suggested that the LDL receptor, in addition to LRP1, also contributes to the clearance of fVIII from the plasma (26). Using hepatic LRP1 and LDL receptor double-deficient mice, Bovenschen et al. (26) demonstrated that mice with combined deficiency displayed a much greater increase of fVIII levels (~4 to 5-fold) than mice lacking LRP1 alone. In clearance studies, the mean residence time of fVIII was also dramatically prolonged (~5-fold) in mice with combined receptor deficiency (26). These findings, together with the fact that both LRP1 and the LDL receptor are predominantly expressed in the liver, reveal that LRP1 and the LDL receptor cooperate in regulating fVIII levels and clearance in vivo.

A puzzling question raised by these studies is how LRP1 and the LDL receptor function to effectively remove fVIII from the plasma. These receptors’ affinity for fVIII is relatively weak ($K_D$ values from 60 to 116 nM) but is well above the levels of fVIII circulating in the plasma. It could be that other cofactor molecules, such as cell surface heparan sulfate proteoglycans (HSPG), facilitate the uptake of fVIII by LRP1 and the LDL receptor. Additionally, it is now known that activation of fVIII generates a molecule with a significantly higher affinity for LRP1 (27). Thus the removal of fVIII from the circulation may require prior activation and dissociation from its carrier protein, von Willebrand factor.

Interestingly, a familial study of factors influencing plasma fVIII levels revealed an association of fVIII levels with polymorphisms within the LRP1 gene (171). Specifically, the N allele of the LRP1/D2080N polymorphism was associated with slightly decreased plasma levels of fVIII ($90.4 \pm 8.7$ vs. $102.2 \pm 3.5$ IU/dl, $P = 0.02$) (171).

D. Chylomicron Remnants

Dietary lipids, cholesterol, and fat-soluble vitamins are incorporated into large lipoproteins in the intestine known as chylomicrons (45). These triglyceride-rich lipoproteins are absorbed into the lymphatics and transferred to the general circulation via the thoracic duct (45). The enzyme lipoprotein lipase, expressed on endothelial cells especially in muscle and adipose tissue, selectively removes and hydrolyzes triglycerides, transferring free fatty acids to the tissue (260). The residual lipoprotein particles, called chylomicron remnants, are enriched in cholesteryl esters and contain apoE and apolipoprotein B48. After using exogenous fats, the liver can release excess lipids in the form of VLDL into the blood (11). VLDL is another substrate for lipoprotein lipase, and VLDL remnants can be taken up by the liver, in an apoE-mediated process or hydrolyzed to LDLs (11).

Remnant lipoproteins are rapidly cleared from the plasma by the liver. This process requires apoE, which mediates binding of the lipoprotein particle to members of the LDL receptor family (LRP1 and LDL receptor) and to HSPG, which have been shown to play independent and cooperative roles in remnant lipoprotein clearance (148). The findings that the absence of normal LDL receptor activity leads to accumulation of LDL, but not remnant lipoproteins (116, 235), led to the search for additional receptors that might be involved in remnant lipoprotein uptake, and the early discovery that LRP1 recognizes apoE (12) led to the notion that LRP1 might function as a remnant receptor. Evidence that LRP1 plays an in vivo
role in remnant removal was provided by Willnow et al. (291). They bred mice genetically deficient in RAP, which have reduced hepatic LRP1 levels, to mice lacking the LDL receptor and demonstrated that the progeny mice have high levels of remnantlike lipoproteins in their circulation. Additionally, infection of mice lacking LDL receptors with an adenovirus that expressed RAP resulted in the accumulation of remnant lipoproteins in the plasma, supporting a role for a hepatic RAP-sensitive receptor, most likely LRP1, in the clearance of these particles (294). Rohlmann et al. (230) confirmed a role for LRP1 in remnant metabolism by using a viral Cre-mediated recombination technique to reduce LRP1 expression in the livers of mice on an LDLR-deficient background. Inactivation of LRP1 in the livers of these mice led to accumulation of cholesterol-rich remnant lipoproteins in their circulation, confirming a contribution of LRP1 in this process.

In addition to the two receptors, LRP1 and the LDL receptor, HSPGs are also known to participate in the removal of chylomicron remnants. Interestingly, inactivation of the biosynthetic gene GlcNAc N-deacetylase/N-sulfotransferase 1 (Ndst1) in hepatocytes, which results in a reduction of the sulfation of liver heparan sulfate, was found to have a dramatic effect on the accumulation of triglyceride-rich lipoprotein particles (145), revealing that hepatic HSPG directly contribute to the clearance of triglyceride-rich lipoproteins.

The current concept of remnant lipoprotein uptake recently was reviewed (148) and is summarized in Figure 5. The first step in this process involves sequestering of the remnant lipoprotein particles in the space of Disse via association with HSPG. Here the binding of remnant lipoproteins is primarily mediated by apoE. The purpose of this step is to assemble the participants in remnant clearance. In the second step, lipases (lipoprotein lipase and hepatic lipase) continue their lipolytic processing of the particles that began before their entry into the space of Disse, preparing them for the third step in the process: uptake into hepatocytes. It has been proposed that the LDL receptor, HSPG, and the HSPG/LRP1 complex all serve as receptors or coreceptors mediating lipoprotein uptake. Interestingly, Wilsie and Orlando (295) discovered that LRP1 immunoprecipitates with HSPGs, although they reported that the LRP1/HSPG complex is unable to bind VLDL particles, which suggests a distinctly different model than the synergistic model proposed in Figure 5.

E. Summary

LRP1 in the liver plays an important role in facilitating the plasma removal of a number of molecules, including enzymes and cofactors involved in blood coagulation and fibrinolysis, enzyme-inhibitor complexes, and certain lipoprotein particles. Its function in the liver is important for normal homeostasis of these pathways. Deletion of

![Diagram of remnant lipoprotein uptake](http://physrev.physiology.org/)

**FIG. 5.** Proposed model for the involvement of LRP1 in remnant metabolism in the liver. Remnant lipoprotein particles entering the space of Disse in the liver are first thought to be sequestered by association with heparan sulfate proteoglycans (HSPG). Here they are remodeled by the action of lipoprotein lipase (LPL) and hepatic lipase (HL). Internalization by the hepatocytes is mediated directly by HSPG, the LDL receptor, or HSPG/LRP1 complexes. [Model adapted from MacArthur et al. (145) and Mahley and Huang (148).]
hepatic LRP leads to increased plasma levels of certain molecules and accelerates the development of atherosclerosis (see below).

VI. ROLE OF LRP1 IN PROTECTING THE VASCULATURE

Atherosclerosis is a leading cause of death and disability in industrialized nations, including the United States. It is the underlying medical problem in most patients with coronary artery disease, stroke, abdominal angina, and peripheral vascular disease. Elevated levels of serum lipoproteins, especially modified lipoproteins and triglyceride-rich lipoproteins, play a critical role in the development of this disease. Lipoproteins accumulate in the intima of large vessels where monocytes are then recruited in a step critical for initiating and sustaining lesion formation. There, monocytes differentiate into macrophages, scavenge subendothelial lipoproteins, transform into foam cells, and accelerate plaque formation and lesion progression (143, 233). Substantial evidence now exists from gene knockout studies in vascular smooth muscle cells (24), hepatocytes (64), and macrophages (95, 203), that LRP1 functions to protect the vessel wall from injury.

A. Role of Vascular Smooth Muscle Cell LRP1 in Modulating PDGF Receptor Function

Steps leading to the development of atherosclerosis are complex and are thought to result from an excessive response of the vascular endothelium and smooth muscle cells (SMC) in the artery wall to insult (232). SMC respond to the insult by undergoing proliferation and migration, mediated in part by PDGF released from endothelial cells. The mammalian family of PDGFs is comprised of four molecules (PDGF-A, PDGF-B, PDGF-C, and PDGF-D), which differentially associate with two receptor tyrosine kinases: PDGFR-α and PDGFR-β. PDGF is a potent mitogen for fibroblasts and smooth muscle cells, and studies in mice in which either the PDGF-B or PDGFR-β gene has been deleted exhibit an almost complete lack of pericytes, mesenchymal-like cells which can differentiate into smooth muscle cells, fibroblasts, or macrophages, in certain vascular beds (16, 140).

Involvement of PDGF in the development of atherosclerosis has been demonstrated by employing balloonicatheterization injury of rat carotid arteries as a model. Balloon catheterization results in damage to the endothelial cells and an increase in the level of activated PDGF receptors in the vessel wall (1, 204). Furthermore, the intimal thickening that follows this treatment is inhibited by administration of neutralizing PDGF antibodies (67). In addition, infusion of PDGF-B into rats after carotid injury (103), or expression of recombinant PDGF-B in porcine arteries (181), caused a significant increase in vessel wall thickening. Within atherosclerotic lesions, PDGF stimulates smooth muscle cells to migrate from the media of the vessel to the intimal layer and to proliferate and produce matrix molecules at this site (215).

Recent in vivo and in vitro studies reveal that LRP1 is a physiological modulator of the PDGF signaling pathway. A tissue-specific deletion of the LRP1 gene in vascular SMC (smcLRP1−/−) on a background of LDL receptor deficiency led to SMC proliferation, aneurysm formation, and increased susceptibility to cholesterol-induced atherosclerosis (24). The smcLRP1−/−/LDL receptor −/− mice demonstrated significantly more atherosclerotic lesions and abnormal activation of PDGFR-β compared with the smLRP1−/− mice. These effects could be inhibited by treatment of the mice with Gleevec, a known inhibitor of PDGF signaling. These studies indicate that LRP1 plays a role in protecting the integrity of the vascular wall and preventing atherosclerosis by suppressing PDGF activation. At this time, the mechanism by which LRP1 modulates PDGF function remains incompletely understood, but several possibilities exist. First, it was discovered that LRP1 directly binds PDGF-B (142) and thus LRP1 may function to reduce PDGF-B levels, thereby reducing PDGF-β activation. However, in vitro binding experiments reveal that the affinity of PDGF-B for LRP1 is somewhat weaker than its affinity for PDGF-β, and thus a simple clearance mechanism is probably not the entire story (142).

A second possibility is that LRP1 may direct the trafficking of the PDGFR-β following its activation. Newton et al. (184) found that activated forms of the PDGFR-β coimmunoprecipitate with LRP1, and in cells, PDGFR-β associates with LRP1 within endosomes following addition of PDGF and mediates the tyrosine phosphorylation of the LRP1 cytoplasmic domain (23, 142). Takayama et al. (270) also found that LRP1 binds to Cbl, a ubiquitin-protein ligase that associates with the PDGFR and other receptor tyrosine kinases (167, 168), mediating their monoubiquitination which seems necessary for lysosomal-mediated degradation of the PDGFR complex. The potential of Cbl to associate with LRP1 provides a plausible mechanism that LRP1 might modulate PDGFR-β recycling/degradation. At this time, it is not known whether LRP1 phosphorylation is required for Cbl association. Using pulse-chase experiments, Takayama et al. (270) found that the steady-state turnover rate of PDGFR-β was accelerated in LRP1-deficient fibroblasts. While these effects are not consistent with the in vivo experiments indicating that LRP1 downregulates PDGF signaling, the results do indicate that LRP1 can modulate PDGFR-β levels.

Finally, it is possible that LRP1 may modulate other signaling pathways that, in turn, could modulate PDGF
levels. In this regard, it is interesting to note that LRP1 has been identified as a receptor for TGF-β (see below), which itself is known to induce expression of the PDGFR (86, 101).

B. LRP1 as a TGF-β Receptor

Transforming growth factor-β (TGF-β) regulates multiple biological processes, in a context-dependent and cell-specific manner, including proliferation, extracellular matrix biosynthesis, angiogenesis, immune response, apoptosis, and differentiation (32). The biological activities of TGF-β are mediated by cellular receptors, and a variety of cell surface receptors have been identified by cross-linking 125I-labeled TGF-β to cells. One of these receptors, termed the TGF-βR-V, was recently identified as LRP1 (96). LRP1 appears to be required for mediating the growth inhibitory response of TGF-β, in conjunction with Smad2/3 signaling through TGF-βRI and II (96, 275). In vivo, LRP1 appears to regulate TGF-β signaling pathways as well, as smooth muscle cell deletion of LRP1 also results in a Marfan-like syndrome with nuclear accumulation of phosphorylated Smad 2/3, disruption of elastic layers, and increased expression of thrombospondin 1 and PDGFR-β in the vessel wall (22).

C. Hepatic LRP1 Protects Against Development of Atherosclerosis

To investigate the role of hepatic LRP1 in atherogenesis independent of its role in the removal of apoE-rich remnant lipoproteins, Espirito Santo et al. (64) crossed mice that are susceptible to inducible inactivation of hepatic LRP1 with mice deficient in both the LDL receptor and apoE (MX1Cre12/15-LO mx1creLDLR1/12/15-LO apoE12/15-LO). On an LDLR−/− APOE−/− background, hepatic LRP1 deficiency resulted in decreased plasma cholesterol and triglycerides. Interestingly, these mice showed a two-fold higher atherosclerotic lesion area compared with control mice, revealing that hepatic LRP1 plays a protective role in the development of atherogenesis that is independent of plasma cholesterol levels. The mechanism by which LRP1 exerts its protective effect is not clear, but may be due to its ability to reduce plasma levels of proatherogenic lipids such as coagulation factor VIIII, whose levels are increased in the hepatic LRP1 knockout mouse.

D. Macrophage LRP1 Protects Against the Development of Atherosclerosis

A crucial role of macrophages in the development of atherosclerosis has been demonstrated by studies in which mice with a defective macrophage colony-stimulating factor (M-CSF) gene were bred into an apoE-deficient background (249). M-CSF is a hematopoietic growth factor that stimulates survival, proliferation, differentiation, and multiple functions of cells derived from the mononuclear phagocytic lineage (70). The double-mutant mice had significantly smaller lesions in the aortic root region than their apoE-deficient control littermates, revealing that monocyte-derived macrophages play a key role in atherosclerosis (225).

To investigate the in vivo role of LRP1 in macrophages and its contribution to the development of atherosclerosis, two groups (95, 203) prepared mice with targeted deletion of LRP1 in macrophages. The first study (95) generated LRP1-deficient macrophages on a combined apoE/low density lipoprotein receptor deficient background and found a 1.8-fold increase in atherosclerotic lesion area in the aortic root of 18-wk-old LRP-deficient mice. There were no changes in the lipoprotein profiles in these mice, and the mechanism by which macrophage LRP1 is protective to the vessel wall remains unclear. The second study (203) generated the targeted deletion of LRP1 in macrophages and then performed a bone marrow transplantation into sublethally irradiated female LDL receptor−/− recipient mice. This resulted in a 40% increase in atherosclerosis as determined by measuring lesion area in the proximal aorta. The increased lesion area was not caused by altered serum lipoprotein levels but was speculated to result from a putative role for LRP1 in regulating inflammatory responses. In vitro studies using macrophages isolated from LRP1-deficient mice revealed increased production of tumor necrosis factor (TNF)-α by these macrophages upon LPS treatment, although this was not confirmed in vivo.

In summary, the two separate studies performed in different mouse models (apoE/low density lipoprotein receptor-deficient mice versus LDLR-deficient mice) confirmed an atheroprotective effect of macrophage LRP1. The mechanism by which macrophage LRP1 protects against the development and progression of atherosclerosis remains to be elucidated.

E. Potential Role of LRP1 in Facilitating LDL Oxidation

Formation and uptake of oxidized LDL are thought to be critical to foam cell formation and the progression of atherosclerosis (143). Oxidative modification of LDL can occur by a variety of processes (261) including the action of lipoxigenases (LOs). One of these enzymes, 12/15-LO, which is capable of oxidizing esterified unsaturated fatty acids in LDL particles (35, 252), is implicated in the development of atherosclerosis. Thus disruption of the 12/15-LO gene in apoE-deficient mice (47) or in LDLR−/− mice (77) was found to retard the initiation and progression of atherosclerosis.
To determine the mechanism by which 12/15-LO oxidizes extracellular LDL, resident peritoneal macrophages from LDLR−/− mice were utilized. It was confirmed that the LDL receptor is not required for cell-mediated LDL oxidation (271). However, incubation of thioglycollate-elicited peritoneal macrophages with anti-LRP1 antibodies inhibited LDL oxidation by 56% (300), implicating LRP1 in this process. These studies were confirmed by using 12/15-LO-transfected J774A.1 cells and showing that anti-LRP1 antibodies, RAP, and antisense oligonucleotides to knock down LRP1 reduced cell-mediated oxidation of LDL (300). Together, this work suggests that LRP1 mediates oxidation of LDL by 12/15-LO in macrophages. In further studies investigating the potential mechanism, LRP1 was found to promote the translocation of 12/15-LO from the cytosol to the plasma membrane (308), which is thought to be important for its activity.

F. Summary

Tissue-selective gene deletion studies in vascular smooth muscle cells, hepatocytes, and macrophages have all revealed a protective role for LRP1 in the development of atherosclerosis. The mechanism by which LRP1 expressed in these distinct cell types protects the vasculature is not fully understood. In the case of smooth muscle cells, LRP1 suppresses PDGF signaling pathways. In hepatocytes and macrophages, however, the pathway by which LRP1 alters the progression of atherosclerosis is not known. Finally, if LRP1 is definitively shown to play a role in the production or uptake of oxidized LDL by macrophages, this would add yet another facet to LRP1’s roles in macrophage function, especially in inflammatory states.

VII. ROLE OF LRP1 IN ADIPOCYTES

LRP1 is abundantly expressed in adipocytes (55), and some insight into its function in this tissue was derived from generating mice with an adipocyte-specific inactivation of the LRP1 gene (94). The adipocyte LRP1 knockout mice (adLRP1−/−) displayed delayed postprandial lipid clearance, smaller fat stores, and lipid-depleted brown adipocytes which resulted in reduced body weight. This work highlights the importance of adipocyte LRP1 in postprandial triglyceride metabolism, where LRP1 in collaboration with lipoprotein lipase mediates both the lipolytic and endocytic processes responsible for triglyceride catabolism (37, 38, 157, 189). In addition to the delay in postprandial triglyceride clearance, the adLRP1−/− mice also had an overall decrease in fat mass and were resistant to diet-induced obesity. While the molecular mechanism by which deletion of the LRP gene in adipocytes leads to resistance of diet-induced obesity is not known, it was speculated that this may be due to a lack of LRP1-mediated lipid delivery to white adipocytes which in turn results in increased muscular activity in order for the mice to maintain their core body temperature. Although many questions remain, the study reveals a prominent role of adipocyte LRP1 in modulating energy metabolism and sensitivity to diet-induced obesity.

VIII. MODULATION OF BLOOD-BRAIN BARRIER FUNCTION BY LRP1

A. The Neurovascular Unit

The endothelial lining of vessels functions as a permeability barrier. In the brain, endothelial cells are one component of a functional unit that forms a barrier, termed the blood-brain barrier (BBB), which protects the brain from the entrance of potentially harmful substances present in the blood and maintains the homeostatic environment of the central nervous system (CNS) (234). This functional unit, often called the neurovascular unit, is composed of endothelial cells with extensive tight junctional associations, astrocytes, neurons, and a contractile apparatus of either SMCs or pericytes. A number of grafting and cell culture experiments have suggested that the barrier property of CNS endothelial cells also requires the cooperation of astrocytes (28, 102, 262), which appear to secrete factors that initiate signaling pathways necessary for BBB development (133). Although BBB permeability is carefully regulated, in pathological situations such as stroke, dysregulation of the BBB leads to vascular leakage resulting in severe edema (4, 74).

B. LRP1 Expression in the Brain

In the adult human brain, LRP1 immunoreactivity is abundant on neuronal cell bodies and proximal processes (31, 224, 273, 296). In situ hybridization assays revealed that within the cerebellum, LRP1 expression is observed in neurons diffusely scattered throughout the granular cell layer and is more intensely noted in the large Purkinje cells, but is not found in the molecular cell layer. In the dentate gyrus region of the hippocampus, LRP1 is expressed in neurons of both the granule and pyramidal cell layers (31). LRP1 immunostaining has been identified in astrocytic foot processes (213, 296) and discontinuously along capillary membranes (273). Electron microscopy confirmed that along the capillaries, LRP1 is expressed in the pericytes but not the endothelial cells. This early study was confirmed by immunohistochemical analysis of mouse brain sections (139), where prominent neuronal staining of LRP1 was detected, but no endothelial cell staining was observed. In contrast to these reports, Shi-
bata et al. (245) reported expression of LRP1 in brain microvessels by immunocytochemical approaches. Curiously, this study did not observe the prominent neuronal staining of LRP1 that has been reported by others. The investigation also reported that LRP1 expression was decreased in the brains of older animals. The reason for the differences between this study and prior work is not apparent at this time. In cells, LRP1 message is extremely low in human umbilical cord vein endothelial cells (243), while LRP1 antigen has been detected at low levels in human cerebral microvascular endothelial cells by immunoblot analysis (283). Interestingly, LRP1 is abundantly expressed in bovine aortic endothelial cells (201). Thus, to summarize all of the data, LRP1 is abundantly expressed in vascular smooth muscle cells, pericytes, astrocytes, and neurons, but is not as abundant in the endothelium.

C. Role of LRP1 in Maintaining the Integrity of the blood-brain Barrier

A contribution of LRP1 to maintaining BBB function was discovered by Yepes et al. (305), who found that tissue-type plasminogen activator (tPA) regulates permeability at the BBB via a process that appears to be dependent upon LRP1. This was discovered when tPA injected into the cerebrospinal fluid led to increased vascular permeability even in the absence of ischemia (305). Furthermore, blockade of LRP1 by coinjection of RAP abolished this effect (305). This study identifies an important role for LRP1 in controlling the permeability of the BBB in response to tPA. Interestingly, treatment with either RAP or anti-LRP1 IgG results in a faster recovery of motor activity and protection of the integrity of the neurovascular unit following middle cerebral artery occlusion (213). These findings are of significant clinical importance, as thrombolytic therapy for ischemic stroke with tPA may be accompanied by significant intracerebral bleeding. The mechanism by which tPA and LRP1 modulate BBB integrity is not yet known but may involve the potential of tPA and LRP1 to modulate or initiate signaling pathways. Interestingly, ischemic insult appears to induce shedding of LRP1’s ectodomain from perivascular astrocytes into the basement membrane (213), which appears to be associated with a detachment of astrocytic end-foot processes and the formation of areas of perivascular edema. The shedding of LRP1’s ectodomain is significantly decreased in tPA−/− mice. Furthermore, shedding of LRP1’s ectodomain occurs in cultured astrocytes under conditions of oxygen and glucose deprivation, is increased when tPA is added, and is inhibited by RAP.

IX. ROLE OF LRP1 IN NEURONS

A. Alzheimer’s Disease and the Amyloid Hypothesis

Alzheimer’s disease is the most common age-related neurodegenerative disorder. Pathological findings include neuronal loss, neurofibrillary tangle formation, and the extracellular deposition of insoluble protein fibrils called plaques (244). Neurofibrillary tangles are bundles of protein filaments found in the cytoplasm of neurons, while plaques are composed of a small, hydrophobic peptide termed β-amyloid (Aβ), which is derived from a ubiquitous type I transmembrane protein, β-amyloid precursor protein (APP) (272). Aβ generation is thought to be central to the development of the disease (244).

Generation of Aβ from APP occurs in both secretory and endocytic compartments by regulated intramembrane proteolysis (RIP) (29), a sequential, two-step cleavage of transmembrane proteins with the second cleavage occurring within the transmembrane domain. In the case of APP, RIP is initiated by the β-site APP-cleaving enzyme BACE (280), an aspartyl proteinase that cleaves APP’s ectodomain and liberates the NH2 terminus of Aβ. Aβ generation is completed by intramembrane cleavage of APP, which requires presenilin-1 (PS-1), an unusual aspartic proteinase with eight transmembrane domains (138). This cleavage can occur at slightly different positions, resulting in two principal forms of Aβ: Aβ40 and Aβ42, peptides with 40 and 42 amino acid residues, respectively. Once formed, the Aβ is released outside the cell. While Aβ40 constitutes ~90% of the total Aβ generated, the slightly longer Aβ42 has a higher tendency to form fibrils. Since all known genetic risk factors for AD impact Aβ metabolism, it is believed that the accumulation of Aβ fibrils into amyloid plaques plays a key role in the onset and/or progression of the disease.

B. Interaction of LRP1 With APP and the Effect on Aβ Production

Kounnas et al. (123) were the first to demonstrate that LRP1 can bind and mediate the cellular catabolism of the longer forms of APP (APP751, APP770) which contain Kunitz-type protease inhibitor (KPI) domains. Knauer et al. (119) subsequently found that transmembrane isoforms of APP containing KPI domains form complexes with a proteinase ligand, EGF-binding protein, and are internalized by a RAP-sensitive receptor, most likely LRP1. Together, these studies suggested a common LRP1-mediated internalization pathway for both soluble and transmembrane forms of APP containing KPI domains. Following these findings, Ulery et al. (276) tested the hypothesis that LRP1 can alter the trafficking of APP,
thereby modulating the production of the Aβ peptide. This study found that restoring LRP1 function in LRP1-deficient CHO cells increased the amyloidogenic pathway of APP processing, reducing the amount of soluble forms of APP generated by α-secretase cleavage (sAPPα) detected in the media, and increasing the production of the Aβ peptide.

Subsequent work (210) confirmed this initial study and further found that, not only does LRP1 affect Aβ production and the amount of sAPP released from the cell, but it also affects APP internalization, turnover of full-length APP, and the stability of APP COOH-terminal fragments. These LRP1-dependent changes occurred in all APP isoforms. With the use of deletion constructs, the critical region in LRP1 that modulates APP processing was mapped to the LRP1 cytoplasmic domain at the second NPYX motif and appears to be dependent on Fe65 (211), an adaptor protein that binds to the cytoplasmic domains of LRP1 and APP, linking them together. Together, all of these studies suggest that LRP1 functionally modulates APP steps critical for Aβ production and APP processing. Exactly how association of LRP1 with APP leads to enhanced Aβ production is not clear at present. One possibility is that the association of APP with LRP1 leads to increased trafficking of APP through the endosomal compartments where BACE and PS1 are known to reside, leading to enhanced proteolysis of APP (Fig. 6).

Konoshita et al. (114) confirmed that LRP1 interacts with APP in cells via both ectodomain and cytoplasmic domain interactions using fluorescence resonance energy transfer (FRET) measurements. The study identified interactions that were sensitive to RAP and assumed to be mediated by ectodomain interactions, as well as interactions that were insensitive to RAP and therefore assumed to represent cytoplasmic domain interactions. By using COOH-terminally tagged LRP1 and APP, the potential of the COOH termini of both APP695 and APP770 to interact with the COOH terminus of LRP1 was confirmed. These interactions were not sensitive to RAP treatment. FRET studies also confirmed a close proximity between the amino Fe65 phosphotyrosine binding (PTB) domain and LRP1 cytoplasmic domain and between the carboxyl Fe65 PTB domain and the APP cytoplasmic domain. These findings demonstrate that LRP1 and APP interact in cells.

To determine if LRP1 influences Aβ production in vivo, Zerbinatti et al. (306) generated a transgenic mouse overexpressing a functional LRP1 mini-receptor in neurons and crossed this mouse with the PDAPP mice, a well-known mouse model of amyloid deposition in which mice express a mutated version of human APP under the control of the PDGF promoter (152). Overexpression of a functional LRP1 minireceptor in the brain of PDAPP mice results in age-dependent increase of soluble brain Aβ, with no changes in Aβ plaque burden. Importantly, soluble brain Aβ was found to be primarily in the form of monomers/dimers and to be highly correlated with deficits in spatial learning and memory. These results provide in vivo evidence that LRP1 may contribute to memory...
deficits typical of Alzheimer’s disease by modulating the pool of small soluble forms of Aβ.

C. Regulation of LRP1 Expression by APP

Cao and Sudhof (34) were the first to discover that the intracellular tail of APP, which is released following γ-secretase cleavage, formed a multimeric complex with the nuclear adaptor protein Fe65 and the histone acetyltransferase Tip60. This complex was found to stimulate transcription via heterologous Gal4- or LexA-DNA binding domains and led these investigators to propose that the APP cytoplasmic tail may function to regulate gene expression. Interestingly, Liu et al. (141) discovered that LRP1 expression is increased in mouse embryonic fibroblasts from APP knockout mice. They further showed that expression of the APP intracellular domain together with Fe65 and Tip60 interacts with the LRP1 promoter and suppresses its transcription. These studies uncovered an unexpected role for APP in suppressing LRP1 expression. Given that LRP1 recognizes numerous molecules, this observation may have a drastic impact on our understanding of neuronal physiology.

D. Role of LRP1 in Clearance of Aβ From the Brain

While the Aβ peptide is produced as a normal consequence of APP metabolism (85), Aβ fibrils do not accumulate in large quantities in healthy individuals, indicating the existence of clearance mechanisms. To date, three known pathways have been characterized that reduce the levels of Aβ: extracellular proteolysis, transport across the BBB, and receptor-mediated endocytosis. A number of proteinases are known to cleave the Aβ peptide (for review, see Ref. 150) and include insulin-degrading enzyme (216) and a neutral endopeptidase similar or identical to nephrilysin (98). The significance of proteolytic pathways has been demonstrated by infusion of neutral endopeptidase inhibitors in the rat brain resulting in abnormal deposition of endogenous Aβ (98).

Aβ transport across the BBB is less well understood, and the relative importance of this pathway to the overall removal of the Aβ peptide in vivo has not yet been demonstrated. However, injection of 125TI-AB40 into the brain resulted in a rapid removal mainly by transport across the BBB (52). This process was significantly reduced by RAP, antibodies against LRP1, and αvβ3 integrins implicating LRP1 in the removal of the Aβ peptide. These studies will have to be confirmed with tissue-selective LRP1 knockout studies to establish the contribution of LRP1 to this process in vivo.

The final mechanism that results in reduction of Aβ levels involves direct uptake by endocytic receptors. The class A and class B scavenger receptors can bind to and internalize fibrillar forms of Aβ (205). On the other hand, Aβ can form complexes with LRP1 ligands such as apoE (301), lactoferrin (217), and activated αvM (109, 217), which can then be internalized via LRP1. More recent work (52) reveals that Aβ40 directly binds to LRP1 clusters II and IV with relatively high affinity, while Aβ42 binds with slightly weaker affinity. Interestingly, it appears that the affinity of Aβ for LRP1 decreases with increasing β-sheet content, suggesting that LRP1 binds with higher affinity to monomeric forms of Aβ and, therefore, has the potential to mediate the cellular uptake of Aβ.

Studies (110) have reported that a C766T polymorphism in exon 3 of LRP1 is under-represented in AD and associated with later age of disease onset; however, this is controversial and has been reproduced (120) and refuted by additional studies (36, 134, 240). Interestingly, Kang et al. (109) also suggested that lower levels of LRP1 in AD correlated with the CC genotype within the LRP1 exon 3 polymorphism locus and reported a reduction in the levels of LRP1 in the brains of patients with Alzheimer’s disease. Based on the suspected ability of LRP1 to mediate the transport of the Aβ peptide out of the brain, the study proposed that decreased levels of LRP1 may reduce Aβ clearance, thereby contributing to increased Aβ levels and enhanced disease. In contrast, Causevic et al. (36) found no correlation between LRP1 levels and Alzheimer’s disease. Thus any connection between levels of LRP1 and Alzheimer’s disease requires further clarification.

E. Tissue-Selective Deletion of LRP1 in Neurons

While LRP1 is abundantly expressed in neurons, its exact function here is unknown. To investigate the role of LRP1 in neurons, tissue selective deletion of LRP1 was accomplished (155). Mice lacking LRP1 in differentiated neurons develop severe behavioral and motor abnormalities, including hyperactivity, tremor, and dystonia. In these mice, no histological abnormalities were noted, indicating that gross developmental processes were not impaired. The hypothesis that LRP1 may participate in neurotransmitter-dependent postsynaptic responses resulted from the finding that LRP1 is in close proximity to the N-methyl-D-aspartate (NMDA) receptor in dendritic synapses in neurons and coprecipitates with NMDA receptor subunits and the postsynaptic density protein PSD-95 from neuronal cell lysates. If true, LRP1, like other ApoE receptors, may modulate synaptic transmission in the brain.

F. Summary

LRP1 is abundantly expressed in neurons where its function is yet to be established. Studies have raised the
possibility that LRP1 may function in synaptic transmission in the brain, perhaps in cooperation with tPA. Additional work is required to determine if this is the case. In the brain, LRP1 can also associate with APP and modulate the trafficking of this molecule, resulting in increased production of the Aβ peptide. On the other hand, LRP1 may also participate in the removal of the Aβ peptide by directly binding to it and mediating its cellular uptake and degradation. Thus LRP1 appears positioned to modulate the levels of this peptide and, in so doing, possibly to regulate the progression of AD.

X. ROLE OF LRP1 IN CELL MIGRATION AND INTEGRIN FUNCTION

The interaction of cells with the extracellular matrix is important for cellular physiology, as these interactions regulate cell survival, proliferation, migration, and differentiation. The bidirectional communication between the extracellular matrix (ECM) and the actin cytoskeleton is regulated by integrins, a large family of cell surface receptors that regulate cell adhesion and migration. Cell migration is important in many physiological and pathological processes including wound healing, bone remodeling, development, angiogenesis, and invasion of cancer cells. During the process of cell migration, cells form and remodel their focal adhesions, both through reorganization of the cytoskeleton and through modulation of integrin signaling (72, 170, 228). Inside-out and outside-in signaling events activate integrins, which lead to conformational changes in the integrin dimer and increased affinity for its ECM ligands. Activated integrins are recruited to the leading edge of the migrating cell (115) where they also recruit proteases to enhance degradation of the ECM (174). While not yet fully understood, LRP1 is able to modulate integrin action by directly associating with integrins or by cooperation with other molecules, such as thrombospondin.

A. Calreticulin/Thrombospondin-1 and the Role of LRP1 in Focal Adhesion Disassembly

The adhesive remodeling that is requisite for cell migration requires disassembly or restructuring of the integrin-linked focal adhesion scaffold. Focal adhesion disassembly can be triggered by a number of proteolytic and stimulatory signals. Members of a class of ECM proteins termed “matricellular” proteins can all perform such a trigger function. This class of proteins includes SPARC, tenascin-C, and thrombospondins-1 and -2, all of which function in focal adhesion disassembly. Thrombospondin-1 (TSP1), a large 420 kDa, homotrimeric, extracellular matrix protein (21), is released from platelet α-granules following platelet aggregation (128). TSP1 is also expressed by most cell types in culture, including epithelial cells, fibroblasts, endothelial cells, smooth muscle cells, and immune cells (99, 100, 173, 222, 278). Expression of TSP1 is highly regulated: it is induced by growth factors, serum, hypoxia, and oxidative stress (61, 149, 208). Consequently, TSP1 expression is increased where there is tissue remodeling, which occurs in response to injury and fibrosis, during wound healing, and in development (193, 223, 226, 227, 278, 287). Structurally, TSP1 is comprised of different domains that interact with a variety of cellular receptors. The NH2-terminal domain (NTD) of TSP1 binds LRP1 (164), calreticulin (CRT), HSPGs, and integrins (156, 158, 257). This TSP domain can be cleaved from the remaining COOH-terminal portion of TSP1 by a wide array of serine proteases and has functions distinct from those of the intact TSP1 molecule (58, 127, 132, 218).

TSP1 in its soluble form has antiadhesive properties and causes reorganization of actin stress fibers and focal adhesion disassembly (83, 177). Focal adhesions are signaling scaffolds composed of both structural and signaling proteins that link the extracellular environment to the cytoskeleton (41, 46). Signaling through focal adhesions regulates cell shape, motility, survival, and differentiation (241, 242, 297). The stimulation of focal adhesion disassembly by TSP1 is thought to enable cell migration, potentially by triggering changes in cytoskeletal organization that are optimal for cell motility. Both intact TSP1 and the NTD can stimulate focal adhesion disassembly (176). This activity is localized to a 19-amino acid sequence in the NTD of TSP1 (amino acids 17-35) that binds to cell-surface CRT (78, 176). A peptide mimetic of this sequence (hep I) has been used to probe TSP1 actions specific to this sequence (176).

CRT isolated from bovine aortic endothelial cells was identified as a TSP1 (hep I) binding protein, and it was shown that expression of CRT on the cell surface is necessary for TSP1 to signal focal adhesion disassembly and cell motility in endothelial cells and fibroblasts (79, 198). The TSP1 binding sequence in CRT has been localized to an 18-amino acid sequence, amino acids 19-36, in the NTD of CRT (79). CRT, also identified as a C1q receptor, is best known as an ER chaperone protein that serves as an important regulator of both intracellular Ca2+ stores and antigen presentation (165, 250, 281). However, CRT is also localized on the cell surface of many cell types, where its expression is upregulated by cellular stress (78, 81, 106, 212, 286, 299). CRT binds to integrins, LRP1, and collagens (201, 207, 231). Mice lacking the CRT gene die during embryogenesis due to defects in myocardial development (159). Initial studies, which showed that cell-surface CRT mediated TSP1 signaling, were perplexing since CRT neither contains a transmembrane domain nor is GPI anchored. This suggested that CRT may form a complex with a binding partner to signal in response to TSP1 binding.
Studies from Orr et al. (201) identified LRP1 as the coreceptor which mediates TSP1 signaling of focal adhesion disassembly and stimulation of cell motility through binding to CRT (Fig. 7A). This work revealed that an antibody to LRP1 or RAP blocks focal adhesion disassembly by TSP1 and hep I (201). Furthermore, mouse embryonic fibroblasts (MEFs) deficient in LRP1 are unable to undergo focal adhesion disassembly in response to either TSP1 or hep I, although they retain the ability to undergo focal adhesion disassembly in response to tenascin A-D, a matricellular protein that induces focal adhesion disassembly through annexin II (44, 178, 198, 201). Basal CRT-LRP1 interactions are not sufficient to trigger this signaling. Binding of TSP1 (hep I) is necessary to stimulate both increased association of CRT with LRP1 and downstream signaling events in endothelial cell membranes (198, 201). The binding site(s) between CRT and LRP1 have not yet been identified. It is likely that the extracellular domain of LRP1 is important for CRT signaling, since RAP can inhibit cellular responses to TSP1 (201). Furthermore, cells expressing LRP1 mini-receptor constructs that lack most of the extracellular domain of LRP1 fail to respond to TSP1/hep I (Van Duyn, Murphy-Ullrich, and Strickland, unpublished results). Focal adhesion disassembly by hep I and TSP1 also requires the surface expression of Thy-1, a GPI-linked protein; the role of Thy-1 is unclear, and there is no evidence that Thy-1 interacts directly with either CRT or LRP1 (5).

Stimulation of the CRT-LRP1 co-complex by the hep I sequence of TSP1 induces the transient association of the G\textsubscript{q/11}-H\textsubscript{9251}i-2 protein subunit with LRP1. This PTX-sensitive event triggers phosphorylation of FAK and Src, activation of ERK and phosphatidylinositol 3-kinase (PI3K), and culminates in RhoA inactivation. Cells lacking either CRT or LRP1 fail to activate FAK, P3K, or ERK in response to TSP1 (5, 198–201). PTX-sensitive G proteins also are involved in focal adhesion disassembly by fibroblast-derived motility factor and uPA (53, 267). Although heterotrimeric G protein signaling is typically associated with seven-transmembrane spanning receptors, LRP1 has been linked to PTX-sensitive G proteins in other systems. For example, apolipoprotein E4 induces apoptosis of neuronal cells in a process thought to involve LRP1 and PTX-sensitive G proteins (88). In addition, lactoferrin signaling through LRP1 in macrophages induces a PTX-sensitive increase in inositol 1,4,5-trisphosphate (IP\textsubscript{3}) and intracellular calcium (166).

TSP1 signaling through the CRT-LRP1 co-complex is distinct from LRP1’s role in the endocytosis of TSP1 (Fig. 7B). Thus LRP1 can bind, internalize, and degrade TSP1 through LRP1 binding to the NTD of TSP1 (163, 164). The sequence of TSP1 recognized by LRP1, and necessary for its endocytosis, is contained within amino acids 1–90 of the NTD (282). However, endocytosis of TSP1 by LRP1 does not involve the NTD sequence mimicked by hep I, since the hep I peptide does not bind LRP1 (201). Importantly, TSP1 also acts as a bridging molecule to facilitate clearance of other proteins by LRP1. TSP1 interacts with both matrix metalloproteinase (MMP)2 and MMP9 (10). LRP1 endocytoses pro-MMP2 that is bound to TSP1, and clearance of pro-MMP2 can be blocked by an anti-TSP1 antibody (304). Interestingly, endocytosis of the pro-MMP2 is impaired in LRP1-deficient cells (266).
and tPA. uPA and tPA activate plasminogen to plasmin, trypsin metalloproteinases and the serine proteinases uPA and tPA. uPA and tPA activate plasminogen to plasmin, which can digest the ECM and activate MMPs (161, 285). Because LRP1 is involved in the processing of multiple enzymes which regulate matrix turnover and cell adhesion, it is not surprising that LRP1 was found to modulate cell migration. This was first demonstrated by Okada et al. (197) who used a Transwell filter migration assay with fibronectin-coated filters and found that anti-LRP antibodies or RAP inhibited cell migration. A similar inhibitory effect of RAP on smooth muscle cell migration and invasion was observed by Wijnberg et al. (288).

The exact mechanism by which LRP1 influences cell migration is not known, but it appears that multiple and distinct mechanisms exist. First, accumulating data suggest that certain LRP1 ligands can stimulate cell migration through engagement of LRP1 signaling. Okada et al. (197) observed that addition of either uPA or tPA stimulated vascular smooth muscle cell migration. Other mesenchymal cell types also appear to be stimulated by ligand binding to LRP1. MEFs were stimulated to migrate on a vitronectin and fibronectin matrix by TSP1 signaling through the CRT-LRP1 receptor complex (198). The migration was blocked with Rap, indicating that ligand binding (CRT) to LRP1 is required for this process. Furthermore, LRP1- and CRT-null MEFs failed to migrate in response to TSP1 and hep I, confirming the involvement of LRP1 and CRT in this process (198). Degryse et al. (54) similarly reported that PAI-1-stimulated rat smooth muscle cell migration was blocked by an anti-LRP1 antibody or Rap. Interestingly, LRP1-deficient cells also exhibited defective migration in response to serum and time-lapse microscopy of these cells suggests that LRP1-deficient cells have impaired lamellipodia formation (198).

In contrast to these reports citing LRP1-ligand induced stimulation of cell migration, Weaver et al. (284) found that LRP1-deficient MEFs grown in serum-containing media migrated faster than wild-type MEFs when subjected to an in vitro scratch assay, revealing that LRP1 expression delayed cell migration. In these experiments, MEFs were grown in serum-containing medium until 95% confluent on bacterial plates coated with either serum, vitronectin, fibronectin, Matrigel, or type I collagen. Cell layers were scratched, and cell migration was assessed under serum-free conditions in media supplemented with 20 ng/ml PDGF-BB. LRP1-deficient cells migrated faster in response to PDGF-BB than did wild-type MEFs when plated on serum-, vitronectin-, and fibronectin-coated plates. However, there was no difference in migration rates between LRP1-expressing and LRP1-deficient MEFs in cultures plated on either Matrigel or type I collagen, indicating the importance of the matrix environment. The increased migration noted in LRP1-deficient fibroblasts may relate to increased surface expression of the urokinase receptor (uPAR) noted in these cells (284).

The uPAR is a three-domain molecule attached to the plasma membrane by a GPI anchor (9, 19, 256) that binds to one of various adaptor proteins with the LRP cytoplasmic domain is modulated by its phosphorylation state and that serine and threonine phosphorylation reduces the association of LRP with adaptor molecules of the endocytic machinery. In contrast, serine and threonine phosphorylation was necessary for the interaction of LRP with Shc, an adaptor protein that participates in signaling events. Furthermore, serine and threonine phosphorylation increased the interaction of LRP with GULP with other adaptor proteins such as Dab-1 and CED-6/GULP. These results indicate that phosphorylation of LRP modulates the endocytic and signaling function of LRP by modifying its association with adaptor proteins. Grey et al. (84) found that lactoferrin, a mitogen for osteoblasts, requires LRP1 for signaling. However, blocking endocytosis by placing cells in a hypertonic solution, lowering the temperature to 4°C, or using a pharmacological inhibitor of endocytosis did not affect lactoferrin signaling through LRP1. This suggests that the signaling and endocytic functions of LRP1 are independent of each other (84). Gotthardt et al. (80) found that the adaptor protein DAB1, which regulates tyrosine kinase signaling and microtubule function in neurons, binds to the cytoplasmic tail of both LRP1 and the LDL receptor. In the presence of DAB1, LDL receptor degradation is reduced, suggesting that adaptor binding competes with the endocytic machinery. This suggests that engagement of the LDL receptor in signaling reactions precludes its ability to mediate endocytosis (80). Since TSP1, through binding to CRT, can engage LRP1 in G protein-mediated signaling and direct interactions of TSP1/2 with LRP1 stimulate endocytosis, it will be interesting to determine whether LRP1 signaling and scavenger activities are differentially regulated by TSPs.

B. Role of LRP1 in Cell Migration

Cell migration is regulated by proteases such as matrix metalloproteinases and the serine proteinases uPA and tPA. uPA and tPA activate plasminogen to plasmin, and TSP2 interactions with LRP1 are important for internalization of MMP2, and studies in TSP2 knockout animals suggest that a deficiency in MMP2 clearance in the absence of TSP2 results in defective connective tissue organization (303, 304). Recently, it also has been shown that TSP1 mediates the clearance of vascular endothelial growth factor (VEGF) through LRP1 (82). These results suggest that LRP1 plays an important role in tissue and vascular remodeling through endocytosis of factors involved in matrix degradation and angiogenesis.

Studies suggest that engagement of LRP1’s signaling function impedes its ability to act as a scavenger receptor. Thus Ranganathan et al. (221) discovered that the association of various adaptor proteins with the LRP cytoplasmic domain is modulated by its phosphorylation state and that serine and threonine phosphorylation reduces the association of LRP with adaptor molecules of the endocytic machinery. This suggests that the signaling and endocytic functions of LRP1 are independent of each other (84). Gotthardt et al. (198) observed that addition of either uPA or tPA stimulated vascular smooth muscle cell migration. Other mesenchymal cell types also appear to be stimulated by ligand binding to LRP1. MEFs were stimulated to migrate on a vitronectin and fibronectin matrix by TSP1 signaling through the CRT-LRP1 receptor complex (198). The migration was blocked with Rap, indicating that ligand binding (CRT) to LRP1 is required for this process. Furthermore, LRP1- and CRT-null MEFs failed to migrate in response to TSP1 and hep I, confirming the involvement of LRP1 and CRT in this process (198). Degryse et al. (54) similarly reported that PAI-1-stimulated rat smooth muscle cell migration was blocked by an anti-LRP1 antibody or Rap. Interestingly, LRP1-deficient cells also exhibited defective migration in response to serum, and time-lapse microscopy of these cells suggests that LRP1-deficient cells have impaired lamellipodia formation (198).

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tightly to uPA. uPAR plays an important role in a cell-based proteolytic system and is also known to stimulate signaling pathways (202). uPA activity is regulated by a serpin, PAI-1, and, upon complex formation with this inhibitor, a cryptic site is exposed that is recognized by LRPI (192). The consequence of this interaction is LRPI-dependent endocytosis of cell-associated uPAR complexed to uPA-PAI-1 (190), which leads to reduced steady-state levels of uPAR (284). Under high serum conditions, LRPI-deficient fibroblasts have elevated levels of Rac, the small GTPase that is critical for cell spreading and lamelipodia formation (144). Thus elevated pericellular proteolysis and/or persistence of Rac activating factors in the LRPI-deficient MEFs, due to the absence of LRPI scavenger activity, could account for the increased motility in these cells. This also suggests that LRPI-mediated clearance of pericellular proteases and other growth factors can attenuate cell motility (284).

Another mechanism by which LRPI can regulate cell migration is through the ability of certain LRPI ligands to block the effect of stimulatory ligands on cell migration. For example, apoE inhibits SMC migration induced by PDGF-B (288). This occurs when apoE binds to LRPI and initiates a signaling pathway that results in increased intracellular cAMP levels and protein kinase A activity, counteracting the stimulatory effects of PDGF (309).

LRPI also can directly modulate cell migration by mediating the internalization of integrins under certain conditions. Czekay et al. (48) found that, paradoxically, addition of exogenous PAI-1 and uPA to HT-1080 fibrosarcoma cells resulted in their detachment. This occurred through formation of a complex of PAI-1 with uPA bound to LRPI associated with integrins. Formation of the covalent PAI-1-uPA-uPAR complex led to internalization of the attached integrin by an LRPI-mediated process, which was detected by accumulation of α1,β3 and α1,β3-integrins in early endosomal fractions. This accumulation was completely blocked by anti-LRPI IgG or Rap, showing that LRPI is required for the uPA/PAI-1-induced internalization of integrins.

LRPI also has been reported to directly associate with β2-integrins on leukocytes (33, 253). In monocytes, immunofluorescence studies showed that LRPI colocalizes with β2-integrins (33, 253). In vitro binding experiments showed that recombinant cluster IV of LRPI binds directly to purified immobilized α3β1. The association of LRPI with β2-integrins is thought to regulate integrin recycling during cell migration and may depend on other LRPI ligands, such as tPA (33).

C. Role of LRPI in Integrin Processing

Unexpectedly, it was observed that loss of LRPI expression correlated with reduced cell-surface expression of β1-integrin (239). Further studies showed that LRPI plays a role in the posttranslational processing and delivery of mature integrins to the cell surface. Mature β1-integrin is glycosylated in the ER and Golgi, whereas the immature form is not fully glycosylated. Alterations in integrin glycosylation have variably been shown to alter three aspects of their maturation and function: transport through the Golgi, pairing with the α-subunit, and their ligand binding affinity (13). Although there was less mature β1-integrin on the cell surface of cells lacking LRPI, the total amount of β1-integrin protein in the cell was unchanged, and there was increased β1-integrin in the ER, suggesting that LRPI plays a role in β1-integrin trafficking from the ER to the cell surface. The effects of LRPI on β1-integrin maturation were TGF-β and ECM substrate independent, but dependent on culture confluency (239). Apparently, neither LRPI ligand binding nor endocytosis is involved in integrin maturation, since Rap did not affect maturation. It is unclear whether LRPI associates with chaperones or adaptor proteins in the ER during the transit of β1-integrins or whether LRPI affects integrin glycosylation directly. Direct interactions between LRPI and integrins do not appear to be involved, since the authors were unable to coimmunoprecipitate LRPI with β3-integrin. It was suggested that chaperones or adaptor proteins such as calreticulin, hsp90, Fe65, or ICAP-1 might act as a bridge between LRPI and β1-integrin.

D. LRPI and Tumor Invasion

Because of LRPI’s complex role in regulating pericellular proteases and cell migration, a role for LRPI in tumor cell invasion has been investigated. Various groups have investigated whether LRPI expression on tumor cells correlates with invasiveness. Kancha et al. (108) examined a panel of breast carcinoma cells with different degrees of invasiveness including nontumorigenic MCF10A breast cells, preneoplastic MCF10AT cells, noninvasive subclones of MCF10AT cells, and invasive subclones of MCF10AT cells. They evaluated LRPI expression by Northern blot analysis and through the use of a binding assay with radioactively labeled activated ωM to assess functional LRPI at the cell surface. These studies showed that MCF10ATs and their invasive subclones had decreased mRNA levels and expression of LRPI surface protein compared with the less invasive MCF10A and noninvasive subclones (108). In addition, invasion through Matrigel by a follicular thyroid carcinoma cell line was found to inversely correlate with LRPI expression, and inhibiting LRPI or increasing uPA levels increased invasiveness (246). These studies suggest that LRPI is associated with a less invasive phenotype, perhaps by mediating endocytosis of proteases, and that decreased LRPI levels correlate with increased invasive-
ness. Consistent with this idea is the observation that primary breast tumors did not exhibit levels of LRP1 detectable by immunohistochemical approaches, although LRP1 was found on stromal fibroblasts (40).

In contrast, other investigators provide evidence of a role for LRP1 in promoting breast cancer cell invasiveness (39, 136). Flow cytometric analyses of different breast cancer cell lines showed varying LRP1 surface expression levels. Cell lines MDA-MB-231, T47D, BT-20, and HS-578T, which are more highly invasive in vitro Matrigel invasion assays, had higher LRP1 expression levels compared with the less invasive cell lines HMEC, MCF-7, and MDA-MB-361, which exhibited lower LRP1 expression (136, 137). Furthermore, LRP1 was localized to the leading edge of breast cancer cells, suggesting that LRP1 might regulate cell-matrix interactions and/or cytoskeletal organization to enhance the protrusive activity needed for cell migration (39).

E. Summary

These results highlight the difficulty in determining the function of a protein as complex as LRP1. The inherent differences between tissues and established cell lines and the variability of in vitro culture conditions all potentially influence the results. The dual nature of LRP1, both scavenger and signaling receptor, and the variable functions of its numerous ligands adds to the complexity of deciphering the role of LRP1 in biological processes. However, evidence to date supports a role for LRP1 in regulating the pericellular microenvironment through clearance of adhesion and matrix-altering proteases. In this capacity, LRP1 would likely act to stabilize the matrix and cell adhesion, thus reducing migration and invasiveness. However, depending on the ligand, engagement of LRP1 as a signaling receptor can also directly trigger cellular de-adhesion and cytoskeletal reorganization to support increased cell motility. Clearly, the nature of the matrix and the extracellular milieu of LRP1 ligands will determine its function in a cell-, tissue-, and context-specific manner.

XI. FUNCTION OF LR P1 IN INFLAMMATION AND PHAGOCYTOSIS

The removal of apoptotic cells, necrotic debris, or infectious agents by the process of phagocytosis is essential for maintenance of homeostasis, organogenesis, resolution of inflammation, and prevention of autoimmune responses (146). Phagocytosis requires receptor-mediated recognition of such targets followed by their delivery into phagosomes. In the case of apoptotic cells, recognition and phagocytosis are complex events modulated by several known, and probably many as of yet unidentified, interactions between phagocyte receptors and ligands on the surface of apoptotic cells (reviewed in Ref. 265). Evidence is beginning to accumulate to suggest an important role for LRP1 in this process.

A. Potential of LR P1 to Mediate Phagocytosis

Several lines of evidence suggest a role for LRP1 in the process of phagocytosis. Pathways involved in apoptotic cell recognition and phagocytic removal are highly conserved across species from fly to human. Transmembrane receptors responsible for recognizing apoptotic cells and initiating downstream signaling events to mediate actin rearrangement and phagocytosis have been identified in Caenorhabditis elegans (112, 307) and Drosophila (151, 237). Work in C. elegans revealed that two pathways regulate this process. In one of these pathways, a transmembrane receptor (CED-1) recognizes an unidentified ligand on apoptotic cells and recruits the adaptor protein CED-6, which binds to an NPYX motif on the CED-1 cytoplasmic tail (112). This recruitment activates CED-10, a Rac GTPase that initiates actin reorganization necessary for phagocytosis. LRP1 has been suggested to be a possible functional mammalian homolog of CED-1 (112) largely based on its status as a single-pass transmembrane receptor, its NPYX-containing intracellular domain (ICD), and the ability of its cytoplasmic tail to interact with phosphorylated forms of the adaptor protein GULP, the mammalian homolog of CED-6 (221, 266).

A critical role for the Drosophila CED-1 homolog Draper has also been identified. Draper is expressed in two types of phagocytes: glia and hemocytes/macrophages (71). Freeman et al. (71) demonstrated that deletion of the draper locus in embryos resulted in an increased number of apoptotic neurons in the CNS, suggesting the involvement of this molecule in glial phagocytosis of apoptotic neurons. Manaka et al. (151) confirmed the role of Draper in glia and, importantly, found that Draper is also involved in the phagocytosis of apoptotic cells by Drosophila hemocytes/macrophages. The importance of CED-1 and Draper in C. elegans and Drosophila, respectively, has been clearly established, and while it has not been shown as definitively, evidence is beginning to accumulate suggesting that LR P1 may play a similar role in mammalian cells, as discussed below.

By employing a chimeric receptor, the hypothesis that the LR P1 cytoplasmic domain contains the necessary structural information to mediate phagocytosis was confirmed (206). RAW 264.7 cells (a macrophage cell line) were transfected with a chimeric receptor consisting of the extracellular domain of CD2, a T-cell surface protein capable of binding nonopsonized sheep red blood cells (SRBC), fused with the transmembrane domain and cytoplasmic tail of LR P1. As a control, a construct containing
the LDL receptor transmembrane and intracellular domains was also generated. Cells transfected with the hybrid receptor containing the LDL receptor ICD were able to bind, but could not internalize, SRBC. In contrast, the phagocytes transfected with the chimeric receptor containing the LRP1 transmembrane and ICD were capable of internalizing SRBC, indicating that sufficient information is present within the LRP1 cytoplasmic domain to mediate phagocytosis. This work highlighted the potential of LRP1 to participate in phagocytosis.

B. Putative Role of LRP1 in Calreticulin-Mediated Phagocytosis of Apoptotic Cells

Members of the defense collagen family, including C1q and the collectins [lung surfactant proteins A and D (SP-A and SP-D), mannose binding lectin (MBL) and ficolin], assist in the recognition of particles and enhance the phagocytic activity of professional phagocytes (reviewed in Ref. 20). These molecules are characterized by their structural similarity and contain a globular head and conserved collagen-like tails connected via a hinge region. C1q, a component of the initiator of the classical pathway of complement activation, was shown to bind to apoptotic cells via its globular head region (121, 182). In 2001, Ogden et al. (195) provided evidence that LRP1 on phagocytes, in a complex with cell-surface CRT, mediates enhanced ingestion of apoptotic cells opsonized with C1q or MBL, by human monocyte-derived macrophages (vitro, suggesting that LRP1, complexed with CRT, on phagocytes is a crucial player in the recognition events required for triggering the enhancement of phagocytosis (195). Interestingly, Ogden et al. (195) reported that it is the collagen-like tails of C1q which actually interact with CRT/LRP1 on the phagocyte, linking apoptotic cells to phagocytes, and mediating enhanced ingestion.

Vandivier et al. (277) extended these observations and demonstrated that other defense collagens, in this case lung surfactant proteins A and D, enhance ingestion of apoptotic Jurkat T cells by murine alveolar macrophages in an LRP1 and CRT-dependent fashion. Again, anti-LRP1 and anti-CRT antibodies were the primary means used to implicate these two proteins in enhancement of phagocytosis.

While the aforementioned study by Ogden et al. proposed that LRP1 and CRT form a complex on the phagocyte to bind apoptotic cells opsonized with C1q or MBL, in 2005, Gardai et al. (75) proposed an alternative model in which CRT on the surface of apoptotic cells binds as a ligand to LRP1 on the phagocyte and triggers enhanced ingestion, independent of defense collagens. They reported reduced uptake of apoptotic fibroblasts, neutrophils, and Jurkat cells by fibroblasts in the presence of anti-CRT antibodies. Furthermore, they showed a defect in clearance of apoptotic, CRT-deficient, MEFs from the peritoneum of normal mice compared with apoptotic MEFs which expressed CRT normally. These results led them to suggest that CRT is a surface ligand found on apoptotic cells that is critical for their removal. Additionally, anti-LRP1 antibodies and treatment with RAP resulted in a decrease in LRP1-mediated phagocytosis of apoptotic neutrophils by the J774 macrophage cell line.

In addition to triggering engulfment, receptors responsible for mediating phagocytosis also act to define the consequences of phagocytosis as either proinflammatory or anti-inflammatory, depending on the combination of the target bound and the receptor involved (265). Gardai et al. (76) suggested that certain defense collagens may serve as surveillance molecules that are capable of signaling either pro- or anti-inflammatory responses, depending on conditions in vivo. They proposed a model whereby, under normal conditions, the globular head regions of the lung collectins SP-A and SP-D are free to bind signal inhibitory regulatory protein α (SIRP-α), activating the tyrosine phosphatase SHP-1 with downstream blockade of signaling through src-family kinases and p38 mitogen-activated protein (MAP) kinase leading to suppression of proinflammatory mediator production. Alternatively, in the setting of an infected or damaged lung, SP-A or SP-D bind foreign organisms or cell debris with their globular head regions while their tails interact with CRT/ LRP1 and enhance phagocytosis and, via the p38 MAP kinase pathway, signal upregulation of NFκB-mediated transcription of proinflammatory mediators. While a direct role for LRP1 was not demonstrated in this work (76), the concept that LRP1 may be involved in regulating both phagocytosis and the overall inflammatory responses to environmental challenges in mammalian organisms is of great interest and will surely be further investigated in the future.

C. Summary

While the molecular mechanism governing defense collagen-mediated recognition of apoptotic cells and the
role of LRP1/CRT-dependent or -independent of these molecules remain to be elucidated, evidence suggesting an important role for LRP1 in the process of phagocytosis is accumulating. To this point, indirect means of evaluating LRP1’s role in these processes have been employed, i.e., blocking antibodies and ligand-binding inhibitors. Use of the newly generated tissue-specific LRP1-deficient mice should enable direct testing of the role of LRP1 in this exciting area.

XII. ROLE OF LRP1 IN REGULATING IMMUNE RESPONSES

The receptor-mediated uptake of foreign molecules by endocytic receptors present on dendritic cells is an effective means of presenting antigens to MHC class II molecules (269). Although additional work is required, an emerging role for LRP1 in this process is suggested by studies showing that LRP1-mediated uptake of molecules covalently bound to α2M significantly enhances antigen presentation. Likewise, it is thought that LRP1 facilitates antigen presentation of peptides associated with various heat shock proteins by mediating their endocytosis.

A. Receptor-Mediated Antigen Delivery Via α2-Macroglobulin

In addition to its ability to inhibit proteases, α2M can form covalent complexes with diverse proteases during a transient protease-activated state (42, 43). The resulting complexes are then internalized after binding to LRP1. To determine if α2M enhances antigen delivery and presentation, Chu and Pizzo (43) used T hybridoma clones that respond only to hen egg lysozyme in a MHC-restricted manner. Macrophages that were incubated with lysozyme-α2M-elastase complexes required 200–250 times less antigen than those incubated with free lysozyme to achieve effective presentation to T cells. Furthermore, adding equimolar amounts of α2M-elastase complexes effectively blocked the presentation of lysozyme-α2M-elastase complexes but had no effect on free lysozyme presentation. These results indicate that LRP1-competent forms of α2M can enhance antigen processing by delivering antigens into macrophages through this LRP1-mediated process.

B. Role of LRP1 in Mediating the Endocytosis of Heat Shock Proteins

Heat shock proteins are conserved peptide binding molecules that control the folding of proteins by preventing their aggregation (87). In addition, heat shock proteins appear to be very effective in interacting with antigen presenting cells (APCs) and facilitating the delivery of peptides to the MHC complex (255). Since heat shock proteins are released from cells as a result of necrotic death (8), their ability to deliver peptides to the MHC complex provides a potential pathway through which antigens unique to cancer cells are cross-presented by the APCs to naïve T cells within the lymph node (7).

To identify the cellular receptor(s) responsible for the endocytosis of heat shock proteins, Binder et al. (17) performed affinity chromatography experiments with immobilized gp96 and identified an 80-kDa polypeptide that was eluted from the affinity column. Protein sequencing of this fraction identified four peptides that originated from the NH2-terminal portion of the LRP1 515-kDa heavy chain, and thus represent a proteolytic fragment of this chain. Curiously, an antibody prepared against the material eluted from the affinity column failed to recognize the 515-kDa LRP1 heavy chain from macrophage extracts, as one would expect if the 80-kDa polypeptide was a proteolytic cleavage product of the LRP1 515-kDa subunit. Based on this work and additional studies, it has been suggested that LRP1 functions as a receptor for gp96, hsp90, and hsp70 (7, 17) and plays an essential role in presentation of peptides to MHC complexes (18). However, the contribution of LRP1 to this process appears controversial at this time, as Berwin et al. (14) found that excess forms of activated α2M or RAP failed to compete for the binding and uptake of gp96 in macrophages, revealing that receptors distinct from LRP1 are involved in gp96 internalization. Indeed, subsequent work identified scavenger receptor A as the primary receptor for mediating gp96 internalization in macrophages (15). Thus the exact role that LRP1 plays in mediating the internalization of various heat shock proteins remains to be firmly established.

XIII. CONCLUSIONS

The complexity of LRP1’s role in biology arises from its ability to interact with a variety of ligands, each of which uniquely contributes to different aspects of physiology. Additionally, the cytoplasmic domain of LRP1 has the potential to engage a variety of adaptor molecules involved in endocytosis, phagocytosis, and cell signaling. Together, these important properties place LRP1 in a unique position to impact normal and abnormal mammalian physiology in a variety of ways. Indeed, studies employing tissue-selective deletion of LRP1 in murine neurons, vascular smooth muscle cells, hepatocytes, adipocytes, and macrophages have revealed additional unique and distinct functions for LRP1. We eagerly anticipate development of additional tissue-selective LRP1 knockout mice and look forward to the continued clarification of both normal and pathological processes that future investigations of LRP1 promise to bring.
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