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# Do clustered β-propeller domains within the N-terminus of LRP1 play a functional role?

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**Abstract**

The six β-propellers located within the N-terminus of low density lipoprotein receptor-related protein 1 (LRP1) are arranged in two clusters that contain two and four β-propellers, respectively. Working with LRP1 deletion mutants, we found that randomly removing large segments of amino acid sequences did not affect the intracellular trafficking of LRP1 as long as the clustered β-propeller domains were retained. However, deletion mutants with crippled β-propeller clusters invariably exhibited retarded exit from the endoplasmic reticulum (ER). To determine potential functions of the clustered β-propellers, we generated a series of deletion mutants in which the β-propellers were systematically removed from the C-terminal end of the second cluster. The resulting minireceptors, designated LRPβ1–6, β1–5, β1–4, β1–3, and β1–2 containing decreasing numbers of the β-propellers, were stably expressed in LRP1-null CHO cells. Binding/degradation assays with receptor-associated protein or $\alpha_2$-macroglobulin showed that removing one or more β-propellers had little effect on binding or degradation of these ligands. However, minireceptors containing odd number of β-propellers (i.e., LRPβ1–3 and β1–5) showed prolonged retention within the ER and remained endoglycosidase H-sensitive, whereas minireceptors containing even number of β-propellers (i.e., LRPβ1–2, β1–4 and β1–6) exited ER at variable rates. Cell surface biotinylation experiments showed that LRPβ1–3 was absent from the cell surface. Prolonged retention of LRPβ1–3 within the ER was accompanied by increased association with molecular chaperone Grp78/Bip. These results suggest that the clustered β-propellers may play a role in folding and intracellular trafficking of LRP1.
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*Keywords:* LRP minireceptor; β-propeller; CHO LRP1-null cell; Trafficking

## 1. Introduction

The low density lipoprotein receptor-related protein 1 (LRP1) is a large member (600 kDa) of the low density lipoprotein receptor (LDLR) family recognizing at least 30 different ligands [1]. Because of its broad ligand-binding spectrum, LRP1 has been considered to play a role in diverse biological processes, ranging from lipid metabolism, homeostasis of proteinases and proteinase inhibitors, cellular entry of viruses and toxins, activation of lysosomal enzymes, cellular signal transduction, to neurotransmission [1]. Synthesized as a single polypeptide chain in the endoplasmic reticulum (ER), LRP1 undergoes proteolytic

*Abbreviations:* LRP1, low density lipoprotein receptor-related protein 1; LDLR, low density lipoprotein receptor; ER, endoplasmic reticulum; EGF, epidermal growth factor; RAP, receptor-associated protein; CHO, Chinese hamster ovary; endo H, endoglycosidase H; PNGaseF, peptide: *N*-glycosidase F; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle medium; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; $\alpha_2$M, $\alpha_2$-macroglobulin; HBSS, Hanks balanced salt solution; BSA, bovine serum albumin; TCA, trichloroacetic acid

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cleavage by the endopeptidase furin in the *trans*-Golgi compartment. The resulting 515-kDa extracellular α-chain and 85-kDa transmembrane β-chain remain associated non-covalently as they traverse to the cell surface. The extracellular region of LRP1 has a highly modular domain organization and is comprised of three structural repeats, namely the class A ligand-binding (complement type) repeats, the class B epidermal growth factor (EGF)-type repeats, and repeats that form a six-bladed β-propeller structure with a Tyr–Trp–Thr–Asp (YWTD) consensus motif within the core of each blade. The ligand-binding repeats are arranged in four clusters (I through IV, beginning from the N-terminus); each cluster contains a different number of the ligand-binding repeats. To date, binding of most of the known LRP1 ligands, including $\alpha_2$-macroglobulin and the 39-kDa receptor-associated protein (RAP), has been mapped to cluster II of the ligand-binding repeats [2]. Several lines of evidence indicate that RAP, by

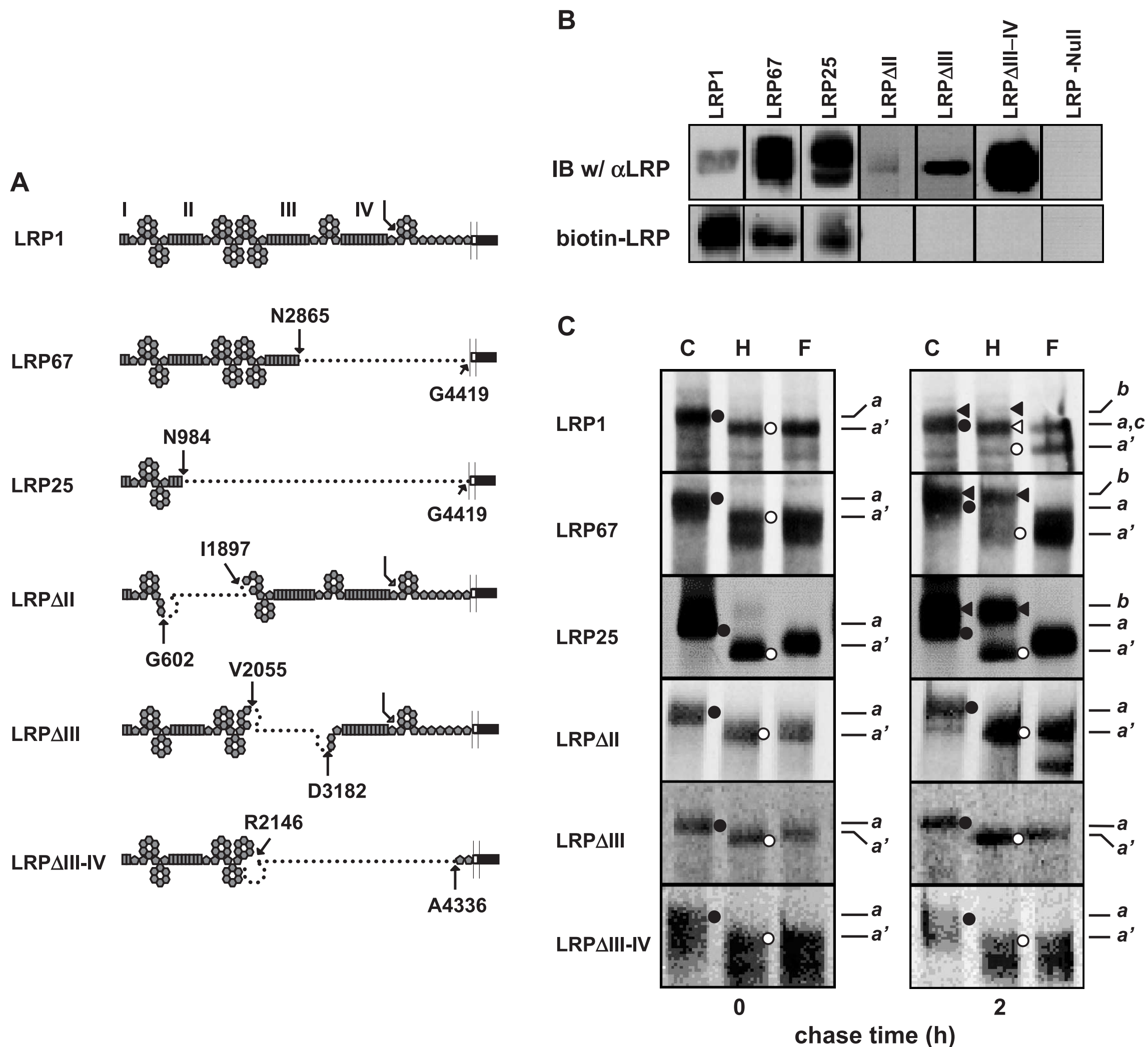


Fig. 1. Expression and analysis of intracellular trafficking of LRP minireceptors generated by random deletion mutagenesis. (A) Schematic representation of the full-length LRP1 and the LRP minireceptors. Vertical rectangles, class A repeats; pentagons, EGF type repeats; hexagons, YWTD repeats. Roman numerals denote clusters I through IV of the class A ligand-binding repeats. The bended arrow indicates position of the furin cleavage site within LRP1, LRPΔII, and ΔIII. The vertical double lines represent the transmembrane domain, and the closed rectangles represent the cytosolic domain. Numbers denote the boundaries encompassing the deleted LRP amino acid sequences (dotted lines). (B) Cells stably expressing the indicated LRP forms were subjected to cell surface biotinylation analysis by incubation with sulfo-NHS-biotin at 0 °C for 1 h. The biotinylated LRP proteins were recovered from cell lysate using a polyclonal antibody, resolved by SDS-PAGE (5% gel) under reducing conditions. Top: Immunoblots of cell-associated LRP minireceptors. Bottom: Cell surface biotinylated LRP minireceptors probed with horseradish peroxidase-conjugated streptavidin. (C) Cells (60-mm dish) expressing LRP1 or the respective minireceptors were labeled with [$^{35}$S]methionine/cysteine (100 μCi/dish) for 1 h and incubated with chase medium for 2 h. At 0 and 2 h of chase, the LRP proteins were recovered from cell lysates using a polyclonal antibody that precipitates both α- and β-chains. Aliquots of samples were incubated with endo H, PNGaseF, or buffer alone (as control). After incubation, the samples were resolved by SDS-PAGE (5% gel) and LRP proteins were visualized by fluorography. Simple glycosylated LRP proreceptors (*a* in lanes C (control), denoted by ●) are endo H-sensitive (*a*' in lanes H (endo H), denoted by ○), whereas complex glycosylated proreceptors (*b* in lanes C and H, denoted by ◄ ) are endo H-resistant. In the case of LRP1, the processed α-chain (*c*, denoted by ◁) was also endo H-resistant. PNGaseF treatment was used to verify the complex glycosylation status of LRP minireceptors (Lane F). The fluorograms are representatives of at least two independent experiments with identical results.

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