Knowing When to Let Go: Endosomal Release of LDL from the LDL-Receptor

In this issue of Molecular Cell, Blacklow and colleagues (Beglova et al., 2004) use NMR structural analyses and the combination of deletion mapping, site-directed mutagenesis and LDL release assays to examine the molecular basis for low pH-induced release of LDL from the LDL receptor.

A substantial portion of the underlying mechanisms for receptor-mediated endocytosis has been disclosed through studies of low-density lipoprotein receptors (LDL-Rs) (Brown and Goldstein, 1986). LDL-Rs play a critical role in the maintenance of cholesterol homeostasis by removing cholesterol-rich LDL particles from the circulation. After binding LDL on the cell surface at neutral pH, the LDL-R associates with clathrin-coated pits, and the receptor-ligand complex is internalized into endosomes. The acidic environment of endosomes (pH < 6) promotes dissociation of LDL-Rs from their bound LDL, which are subsequently degraded in lysosomes. Meanwhile, LDL-Rs efficiently recycle to the cell surface to undergo additional rounds of internalization and ligand release. Nature has provided something resembling sat-
than 1000 different mutations have been recognized. The authors' attention, and it is interesting to note that revealed in the LDL-R crystal structure (Figure 1). Forma-
tor for intramolecular closure upon exposure to acidic
the acidic endosomal environment, the LDL-R adopts upon the corresponding region in the crystal structure
et al., 2004). Strikingly, the NMR structure of the LA7-
receptor doubles back on itself, assuming a closed con-
edly found the structure of this linker to be somewhat
of the LDL-R extracellular domain at endosomal pH was the four-residue linker between LA7 and EGF-A. Through
sor domain appears essential for ligand release (Russell
levels, and consequently their total plasma cholesterol
ating mutagenesis of the LDL-R. Humans with LDL-R
mutations come to attention because their plasma LDL
levels, are elevated and they suffer heart attacks. More than 1000 different mutations have been recognized.
Their effects on receptor function were divided into five
classes (Hobbs et al., 1990). Class 5 mutations produced
receptors that bound ligands but failed to release them
in endosomes at acid pH. As a result, these receptors
could not return to the surface and were instead de-
graded. In this issue of Molecular Cell, Beglova et al.
(2004) provide insight into the molecular basis for the
class 5 mutations. Their study is built on the recent
determination of the structure of the LDL-R extracellular
domain at acid pH (Rudenko et al., 2002).

The LDL-R is a type-1 transmembrane protein of 839
amino acids. Its extracellular domain can be divided into
a LDL binding domain, consisting of seven cysteine-
rich LDL-R type-A (LA) modules, an epidermal growth
factor (EGF) precursor homology domain consisting of
two EGF-like repeats, and a β-propeller that harbors the
consensus sequence Tyr-Trp-Thr-Asp (YWTD) (Springer
et al., 1989; Davis et al., 1987). In late 2002 the structure
of the LDL-R extracellular domain at neutral pH was
revealed (Rudenko et al., 2002). In this structure, the
receptor doubles back on itself, assuming a closed con-
formation such that the ligand binding domain arches
over the EGF-precursor domain (Figure 1). This observa-
tion led to an intriguing proposal that upon exposure to
the acidic endosomal environment, the LDL-R adopts
the closed conformation, and as a result, the β-propeller
displaces bound LDL by acting as an alternative sub-
strate for the ligand binding region.

The Blacklow group embarked upon testing this intra-
molecular ligand model by focusing on two interfaces
revealed in the LDL-R crystal structure (Figure 1). Forma-
tion of the first interface, which is created by contacts
between the central LA4 and LA5 ligand binding repeats
and the β-propeller domain, underlies the proposition of
β-propeller-mediated displacement of LDL. Combining
deletion mapping and mutagenesis experiments with
LDL release assays, it was directly demonstrated that
the β-propeller domain plays an essential role in promot-
ing the release of LDL. Three histidine residues, which
are found at the LA4/LA5-β-propeller interface, caught
the authors' attention, and it is interesting to note that
mutations in two of the three histidines have been found
in FH patients (Sun et al., 1994; Hopkins et al., 1999).
LDL-Rs harboring mutations in these histidine residues
retained their ability to bind LDL, yet their low-pH release
activity was blunted. This observation raises the intrigu-
ing possibility that these histidine residues act coopera-
tively as pH-sensitive switches for interactions between
the LA4/LA5 and the β-propeller that excludes LDL bind-
ing at endosomal pH (Beglova et al., 2004). Interestingly,
the histidine residues carry a partial positive charge at
pH 5.3 and stabilize nearby negatively charged residues
as salt bridges. Thus, it is reasonable to speculate that
throughout a range of pH, the formation of salt bridges
mediating interface I could change as a function of pKa
values for the histidine residues and their ligands.

The second interface of interest is located between
LA7 and EGF-A (Figure 1), the first EGF-like repeat of the
EGF precursor domain. Upon its identity in the receptor
crystal structure, it was proposed that the linker between
LA7 and EGF-A (which encompasses the second inter-
face) acts as a hinge that allows the receptor to close
upon exposure to low pH. Flexibility of the hinge was
thought to be provided by a glycine residue that lies in
the four-residue linker between LA7 and EGF-A. Through
NMR spectroscopy, Blacklow and colleagues unexpect-
edly found the structure of this linker to be somewhat
constrained throughout a range of pH values (Beglova
et al., 2004). Strikingly, the NMR structure of the LA7-
EGF-A domain pair at neutral pH was superimposable
upon the corresponding region in the crystal structure
determined at endosomal pH. This fixed, pH-invariant
orientation of LA7 and EGF-A argues against models
predicting the region acts as a hinge that allows the
receptor to adopt the closed, low pH conformation. In
fact, the reorganization of this region primes the recep-
tor for intramolecular closure upon exposure to acidic
pH. Coordination of calcium at the N-terminal end of
the EGF-A region permits formation of a hydrophobic
interface between LA7 and EGF-A, thereby rendering
rigidity upon the region. Importantly, disrupting this ri-

Figure 1. Model for Low pH-Induced Release of LDL from the LDL-Receptor

The model put forth by Beglova et al. (2004) assumes the LDL-receptor (LDL-R) forms an extended structure (open conformation) at neutral pH, exposing LDL-R repeats for LDL binding. Exposure to the acidic environment of endosomes triggers the LDL-R to adopt the closed conformation observed in the crystal structure of the receptor solved at pH 5.3 (Rudenko et al., 2002). At endosomal pH the
β-propeller, rather than LDL, associates with the LDL binding repeats, resulting in displacement of LDL.

“Open Conformation”

“Closed Conformation”
ing residues both uncouples the second interface and abolishes low pH-induced release of LDL.

The studies of the Blacklow group (Beglova et al., 2004), together with the data of Rudenko et al. (2002), have provided an important model for receptor recycling, but huge questions remain unresolved. (1) How does the LDL-R bind proteins as diverse as apolipoprotein B (550 kDa) and apoE (32 kDa)? Does this binding involve different conformations of the extended chain of the ligand binding repeats? (2) How does the “finely tuned balance of interdomain rigidity and flexibility” allow other members of the LDL-R family to bind an almost inexhaustible list of protein ligands? Some of the LDL-R family members have extracellular domains that are five times as large as that of the LDL-R and contain as many as 31 binding repeats dispersed with 20 EGF repeats and YWTD propellers. How do these giants snare their ligands and release them in acidic endosomes? Further excitement clearly will ensue as the structural secrets of the LDL-R family are revealed.

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Selected Reading