

The remarkable precision achieved by Kiss *et al.* (2) is thus within reach for glycolysis and may eventually come to the world of circadian clocks with the use of suspensions of cyanobacteria or suprachiasmatic nucleus neurons in tissue culture.

## References and Notes

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## PERSPECTIVES: STRUCTURAL BIOLOGY

# LDL Receptor's $\beta$ -Propeller Displaces LDL

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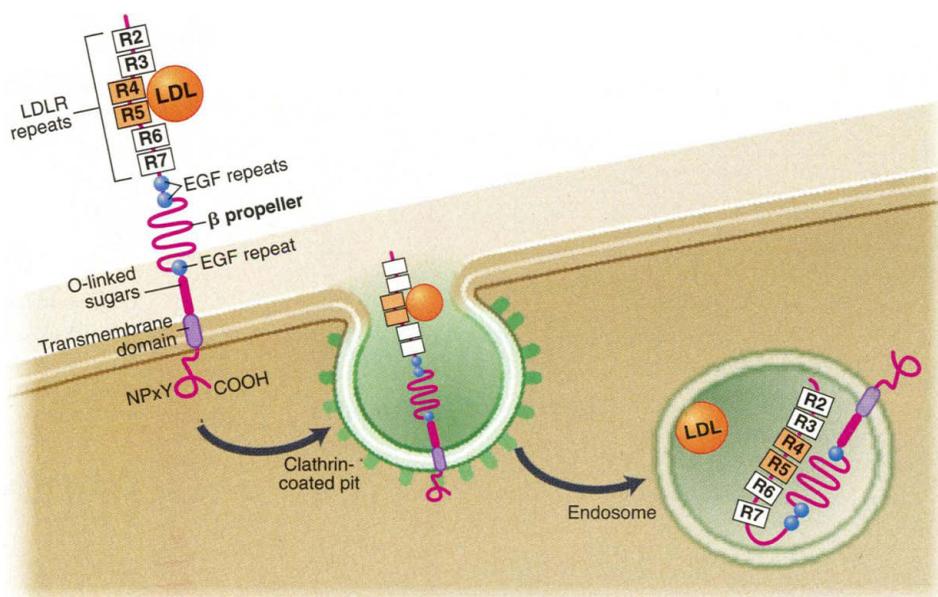
Much of what is known about receptor-mediated endocytosis comes from studies of the low density lipoprotein receptor (LDLR) pathway (1). LDLR binds cholesterol-carrying LDL, associates with clathrin-coated pits, and is internalized into acidic endosomes where it separates from its ligand. The ligand is degraded in lysosomes, while the receptor returns to the cell surface. Mutations in the LDLR gene can lead to elevated plasma cholesterol levels, resulting in coronary heart disease and atherosclerosis (1). Seminal observations by Rudenko *et al.* on page 2353 in this issue (2) shed light on a mystery of LDLR recycling—how the LDLR releases its lipoprotein ligand in the endosome.

The LDLR has several domains (see the figure). The ligand-binding domain contains seven imperfect repeats, each with three disulfide bonds and a coordinated  $\text{Ca}^{2+}$  ion. Extracellularly, it binds two ligands: apolipoprotein (apo) B100 (the only protein in LDL) and apoE (a protein in other lipoproteins). The second domain (411 amino acids in length) is analogous to the membrane-bound precursor of the epidermal growth factor (EGF). It consists of two EGF repeats, followed by a  $\beta$ -propeller region that contains the consensus sequence Tyr-Trp-Thr-Asp, and another EGF repeat (see the figure). LDLR with the EGF precursor domain deleted still binds apoE, but not LDL. However, apoE is not released in the endosome, and the ligand-receptor complex is degraded in the lysosome. Thus, the EGF precursor domain is critical for ligand release and recycling of the receptor, but until now the mechanism remained a mystery (3).

The solution came from the structure of the extracellular domain of the human LDLR crystallized at pH 5.3. In this structure, the  $\beta$ -propeller region of the EGF precursor domain interacts with the main ligand-binding repeats of the LDLR (R4 and R5) (see the figure). Rudenko *et al.* (2) propose that in the endosomes, the  $\beta$ -propeller region displaces the bound lipoprotein ligand by acting as an alternate substrate for the ligand-binding domain. This compelling model is supported by other key evidence: mutations in the ligand-binding and EGF precursor regions that abolish function, phylogenetic evidence of conserved amino acids, and biochemical evidence that the ligand-binding repeats associate with the EGF

precursor at pH 6 but not at pH 8. Clusters of histidines in the  $\beta$ -propeller region likely act as pH-sensitive switches for the domain interactions. The histidines carry no net charge at pH 7.3, but are partially charged at pH 5.3 and participate in the formation of salt bridges in the crystal structure.

The interaction of the  $\beta$ -propeller region with repeats 4 and 5 appears to have much in common with the interactions between lipoprotein ligands and the ligand-binding repeats and clarifies a controversy about lipoprotein-receptor interactions. The interaction of the two domains of the LDLR, as shown by the crystal structure, is based on six hydrophobic bonds and seven salt bridges between R4/R5 and the  $\beta$ -propeller region. Previous studies indicated that ionic or salt bridges are also critical for LDLR-ligand interactions (4, 5), with conserved acidic amino acids in the ligand-binding repeats forming ionic interactions with positively charged amino acids in the receptor binding site of the lipoprotein ligands. However, this ionic interaction model has



**Catch and release.** A model for how LDLR releases LDL. A crystal structure of the extracellular domain of LDLR at pH 5.3 (2) shows that ligand-binding repeats R4 and R5 interact with the  $\beta$ -propeller region of the EGF precursor domain. This interaction may displace LDL from the receptor in acidic endosomes.

been questioned because many of the conserved acidic amino acids in the ligand-binding repeats coordinate  $\text{Ca}^{2+}$  and are completely or partially buried in a  $\text{Ca}^{2+}$  cage. Hence they are presumably unavailable to bind to apoB100 or apoE. Instead, a hydrophobic concave face on the opposite side of the  $\text{Ca}^{2+}$  cage was proposed to interact with the lipoproteins (6).

The crystal structure reported by Rudenko *et al.* (2) resolves this conundrum by illustrating that some conserved acidic amino acids that coordinate  $\text{Ca}^{2+}$  also participate in the formation of salt bridges with basic residues of the  $\beta$  propeller. Although the negative charge potential is somewhat attenuated, the three disulfide bonds and the  $\text{Ca}^{2+}$  coordination lock the negatively charged side chains of R4 and R5 in place for optimal interaction with the basic residues of the  $\beta$ -propeller region (2).

Rudenko *et al.* (2) point out that the ligand-binding repeats are not in contact with each other and can accommodate different-sized ligands. ApoE (relative molecular mass 33,000) and apoB100 (rel-

ative molecular mass 550,000) differ dramatically in size and have no common structural features or amino acid sequence similarity with the exception of a short sequence that serves as the receptor binding site and main proteoglycan binding site in both (5, 7, 8). Extensive studies on apoE show that basic residues in this region are critical for receptor binding (4, 5, 9), and a three-dimensional structure of the 22-kD LDLR binding domain of apoE shows that the receptor binding site is a positively charged, amphipathic helix (10).

The larger apoB100 is less well understood, but an analogous sequence is the likely site for receptor binding. Mutation of basic amino acids in this site to neutral amino acids abolishes receptor binding (8). Because only one small site common to both apoE and apoB100 appears critical for receptor binding, it is likely these proteins have critical interactions with only one or two ligand-binding repeats of the LDLR. This is analogous to the  $\beta$ -propeller, which only interacts with two ligand-binding repeats (R4, R5).

The model of acidic-triggered ligand release by binding to an alternate tethered site will probably be the paradigm for other members of the LDLR family. Will other receptors engaged in receptor-mediated endocytosis outside of the LDLR family have a similar mechanism? Finally, although this study provides insights into the binding of the LDLR with its ligands, a definitive answer will only come from the cocrystallization of a receptor-binding active fragment of apoE with the seven ligand-binding repeats of the LDLR.

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#### PERSPECTIVES: CIRCADIAN RHYTHMS

## Carbon Monoxide and Clocks

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The gases nitric oxide (NO) and carbon monoxide (CO) are increasingly appreciated as major neurotransmitters (1). One of the principal means by which NO and CO transmit signals between neurons is through binding to a heme moiety at the active site of soluble guanylyl cyclase. This enzyme then becomes activated, leading to an increase in the intracellular second messenger molecule cGMP (guanosine 3',5'-monophosphate). NO and CO have been implicated in long-term neural alterations such as learning and memory, and thus it has been presumed that these gases could influence events in the nucleus such as transcription. Independent lines of research have shown that the proteins Clock and NPAS2 are transcription factors that regulate circadian rhythms. On page 2385 of this issue, Dioum *et al.* (2) unite the fields of circadian rhythms and neurotransmission by showing that NPAS2 is a hemoprotein whose DNA binding activity is selectively regulated by CO.

NPAS2 (neuronal PAS domain protein 2) was first identified as a member of the basic

helix-loop-helix (bHLH) family of transcription factors (3). When Takahashi and colleagues (4, 5) identified Clock as a crucial regulator of circadian rhythms, they noted its close sequence similarity to NPAS2. Clock and NPAS2 regulate the activating portion of the circadian transcriptional feedback cycle by forming a heterodimeric complex with another bHLH transcription factor, BMAL1. NPAS2-BMAL1 and Clock-BMAL1 heterodimers direct the transcription of period (Per) and cryptochrome (Cry) proteins, which are the negative regulatory components of the circadian clock. Per and Cry inactivate the Clock-BMAL1 and NPAS-BMAL1 heterodimers, thus completing the transcriptional loop.

One of the salient features of circadian clocks is their entrainment by environmental stimuli such as light, temperature, activity, and food intake. The molecular mechanisms that enable environmental stimuli to abruptly alter circadian rhythms remain obscure. However, modulation of Clock and NPAS2 activity according to the redox state of the cell may provide a clue. The reduced cofactors NADH and NADPH (nicotinamide adenine dinucleotide and its phosphate) greatly enhance binding of Clock-BMAL1 and NPAS2-BMAL1 heterodimers to DNA, whereas the oxidized forms of the same molecules, NAD

and NADP, inhibit the DNA binding of these dimers (6). Alterations in DNA binding activity occur abruptly with modest changes in the ratio of oxidized to reduced cofactors. This implicates the cofactors as molecular switches that direct NPAS2-BMAL1 and Clock-BMAL1 dimers to bind to DNA in response to changes in cellular redox state.

In their current study, Dioum *et al.* (2) have discovered a new way in which NPAS2 responds to environmental stimuli. PAS domains—modules of 130 amino acids previously characterized in organisms ranging from bacteria to mammals—respond to variations in stimuli including oxygen, voltage, light, and redox potential. The PAS domains of some bacterial proteins operate as oxygen sensors via a heme prosthetic group. During purification of NPAS2, Dioum and colleagues discovered that both PAS domains of the NPAS2 monomer contain a heme molecule. Heme was not required for the NPAS2-BMAL1 dimer to bind to DNA, nor did it affect the regulation by redox cofactors. The authors noted that the absorption spectrum of heme-bound NPAS2 resembles that of gas-sensing proteins from bacteria, including the CO-sensor protein CooA from *Rhodospirillum rubrum*. Therefore, they examined the effects of CO, NO, and  $\text{O}_2$  on the DNA binding activity of NPAS2. CO bound to heme-containing NPAS2 with a dissociation constant of  $\sim 1 \mu\text{M}$  but failed to bind to NPAS2 lacking heme. In addition, CO inhibited the DNA binding capacity of NPAS2-BMAL1 heterodimers with a similar molar potency. By contrast, NO did not bind to NPAS2 at physi-

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