**Lipocalin receptors**  
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**Introduction**

The research interests of our group are concerned with the biological role and mechanism of action of lipocalin receptors as a platform for understanding their structure/function relationships and the interaction networks through which they fulfill their biological role. The lipocalins are a large family of small proteins that occur throughout all eukaryotic life forms, and bind small ligands with a range of specificities (retinoids, pheromones, odours). Lipocalins and their ligands play critical roles in crucially important biological processes such as vision, reproduction, olfaction, stress responses, development, inflammation, infection control and so on. Almost 20 years ago, we produced evidence that at least some of these functions were mediated through membrane-bound receptors. That for retinol binding protein was characterised and shown to mediate the uptake of vitamin A. Since then, further data have confirmed the presence of these receptors for a number of lipocalins in a variety of biological systems. The first of these receptors to be cloned - that for tear lipocalin - was shown to be a member of a quite new family of proteins (Figure 1A). Most recently, the one for the retinol-binding-protein (RBP) has also been described and shown to possess all the features described in our earlier reports (Figure 1B). It too belongs to yet another new family, the only previously known property being its induced expression by retinoic acid.

![Figure 1. Putative Lipocalin Receptor Topology. Panel A for Lcn 1, and Panel B. for RBP](image)

**The RBP receptor-system**

The RBP : RBP receptor system has been studied only in this laboratory. Recent work has demonstrated that as well as being the receptor for RBP on the outside of the cell, it also appears to be the receptor for cellular RBP at the cytosolic surface.

Using surface plasmon resonance, and observing the binding phenomenon of the two purified lipocalins with preparations of solubilised HEK membranes, we obtained binding of both soluble partners to a protein entity in the membrane preparations, but only when RBP was conjugated with retinol and CRBP was in its free form. These results supported our hypothesis on the mechanism of action of the system *in vivo* (Figure 2). Further, domain swapping experiments both confirmed the site on RBP responsible for its interaction with the receptor and demonstrated that occupancy of the binding site on a heterologous lipocalin could signal to the RBP domain thereby generating a high affinity conformation for receptor interaction.

Current work utilising 2-hybrid systems for integral membrane proteins has identified receptors, ion channels and soluble proteins which can interact with RBP and CRBP (used as
baits). In the light of the recent dramatic paper that RBP may be heavily involved in causing insulin resistance (and hence its development into type 2 diabetes), this work promises to reveal new receptors and pathways important in homeostatic regulation and as drug targets.

Figure 2. The mechanism of RBP/CRBP-receptor binding

**Lipocalin-1 interacting membrane protein (LIMR)**
The cloning of the first validated receptor for a lipocalin was achieved via the demonstration of an interaction between tear lipocalin (Lcn1) and a phage bearing what turned out to be the N-terminal putative extracellular domain of the intact receptor. Subsequent work confirmed this region as the receptor site for lipocalin binding. We are expressing this receptor, and that for RBP, in transient and stable *Drosophila* S2 cultures to determine the oligomeric structure, topography and key functional sites in both proteins, using a combination of biochemical, cell biological and biophysical techniques. The study will also begin to reveal the interacting networks in which these receptors participate.

**Publications**


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