absolutely conserved and interact differently with the ring analogs found in synthetic ligands. Smith et al. rightly note that this raises the possibility for design of even more specific ligands for the RhlR and LasR transcription factors [14]. Some of the library compounds that failed to show agonist activity are actually antagonists of RhlR and LasR. One compound in particular, *N*-(2-oxocyclohexyl)-3-oxododecanamide, is a strong antagonist of the quorum-sensing system that significantly reduces production of several virulence factors and prevents biofilm formation by *P. aeruginosa* strains.

The ease of synthesis, chemical stability, and strong agonist and antagonist activity of these autoinducer analogs make them productive leads for future research. The observation of differences in how regulatory proteins bind these homoserine lactone analogs will certainly allow further exploration through focused libraries. Extensive screening of analog libraries will probably yield other types of antagonists as well. The report that long chain *N*-acyl-HSLs can display immunomodulatory effects may also open new avenues of investigation for these compounds [15]. After a long history of bacterial communication, it appears that humans are starting to join the conversation.

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Membrane Proteins: Adapting to Life at the Interface

A recent publication by Cravatt and colleagues which describes the structure of an integral membrane protein (FAAH) highlights that the structural differences between membrane proteins and soluble proteins are not as disparate as is sometimes believed.

Soluble proteins and membrane proteins are sometimes thought of as two completely different classes of biomolecules that inhabit two completely different worlds. Their solubility, the nature of their molecular surfaces, the pathways by which they fold, and the forces that stabilize them are widely considered so different that it is hard to even find a common basis for comparison. But are the distinctions really so clear cut? A new structural study of an integral membrane protein, fatty acid amide hydrolase (FAAH; [1]), has evolutionary implications that highlight the shades of gray between the black and white extremes of soluble versus integral membrane proteins.

Amphitropic membrane proteins have a dual life as soluble and membrane proteins. Under some circumstances, they can be just as soluble as ordinary soluble proteins, yet they are also capable of binding to membranes with high affinity and, in many cases, inserting themselves deeply into the hydrocarbon core of one of the two leaflets of a phospholipid bilayer. The translocation of amphitropic membrane proteins between membrane and soluble phases is often used as a regulatory mechanism in processes such as signaling, cytoskeletal regulation, and membrane trafficking.

The phosphoinositide lipids, which play central roles in all of the above-mentioned cell processes, are turned over by amphitropic enzymes that catalyze their synthesis, hydrolysis, phosphorylation, and dephosphorylation [2]. Phosphoinositide signaling is rich in examples of soluble enzyme folds that have been cannibalized for membrane activity. The kinases that produce polyphosphoinositides have the same catalytic fold as the protein kinase superfamily, but their folds have been drastically modified for action at the interface [3, 4]. Phosphatidylinositol phosphate kinase (PIPK) has a flattened face that interacts electrostatically with phospholipid bilayers through extensive basic patches [5]. Moreover,

this kinase is a dimer with both active sites on the same flattened face, allowing simultaneous access of both sites to the membrane-bound substrate. PI 3-kinase [4] does not have such a flat face, but rather contains a membrane binding C2 domain fused to the catalytic domain that targets to the bilayer surface. The phosphoinositide phosphatase PTEN is an adaptation of the protein tyrosine phosphatase fold [6]. The active site of PTEN is flatter and more open than that of the PTPases, allowing access to the membrane-bound phosphoinositide headgroup, while a C2 domain is fused to the catalytic domain and makes extensive interdomain contacts.

The phosphoinositide phosphatase and kinase catalytic domains do not penetrate deeply into the membrane, since they act on chemical bonds that are distal to the membrane surface, but phospholipases are a different story. Phosphoinositide-specific phospholipase C-δ (PLC-δ) is a membrane-interacting adaptation of the ubiquitous TIM barrel fold of many soluble enzymes [7, 8]. The TIM barrel active site is surrounded by a rim of hydrophobic side chains, which facilitates membrane penetration by the enzyme. Cytosolic phospholipase A2 (cPLA$_2$), a distant structural cousin of soluble α/β hydrolases, has an even more hydrophobic active site [9]. As the name implies, however, the enzyme can be found in the cytosol. Its membrane binding and substrate access requires the removal of a flexible lid over the active site. The conformational change that opens the lid allows cPLA$_2$ to convert to a membrane binding form.

Like many amphitropic membrane proteins, monotopic integral membrane proteins insert into just one leaflet of the bilayer. Monotopic membrane proteins typically insert only a small fraction of their total surface area into the membrane, and in this respect they resemble amphitropic and soluble proteins. They are stabilized by large hydrophobic cores, as are soluble proteins. In other respects, they more closely resemble their bilayer-crossing cousins, the polytopic integral membrane proteins. In the cell, they have no stable existence away from the membrane. They must be solubilized in detergent for their purification and biochemical and structural analysis. The archetypal structural study for this class of proteins was done by Garavito and colleagues in their landmark work on prostaglandin synthase [10].

Now Cravatt and coworkers have solved the structure of another monotopic integral membrane protein, FAAH [1], and added to our knowledge of the structures of this class of membrane proteins. FAAH is a member of the amidase signature (AS) family of serine hydrolases. One other structure is known for an enzyme of the AS family, that of the soluble enzyme malonamidase (MAE2) from the bacterium *Bradyrhizobium japonicum*. The comparison between the soluble and membrane-bound structures is the heart of the study. FAAH contains a hydrophobic helix α18 not present in its soluble homologs. This helix inserts into the bilayer with its axis running roughly parallel to the bilayer surface; orthogonal to the orientation most often seen in integral membrane proteins. Furthermore, although both FAAH and MAE2 are dimers, the dimer interface is modified such that the actives sites of FAAH are on the same face, even though the active sites of MAE2 are not. The adaptations have many analogies to those seen for the phosphoinositide signaling enzymes, but the consequences are more drastic in that FAAH is an integral rather than an amphitropic membrane protein.

The amphitropic membrane proteins and monotopic integral membrane proteins fill the gray zone in the continuum between the soluble and membrane worlds. How and why have these intermediate classes of membrane proteins evolved? While the membrane interface comprises a minute fraction of the three-dimensional volume of a cell, it plays a profound role in cell physiology that is completely out of proportion to its small volume. Reactions occurring at the interface are the source of many of the most important mediators of cell signaling, including the endogenous ligands for the cannabinoid receptor that are downregulated by FAAH. Regulation of the interface is critical for diverse processes from endocytosis and secretion to motility and cell structure. Thus, a wide range of regulatory enzymes and other proteins have adapted themselves for an odd existence at the interface. The sometimes unexpected structural adaptations of soluble enzymes for life at the interface have been compared to a well-known adaptation at the level of whole-organism structure, the morphology of the flounder: perhaps bizarre and graceless, but uniquely adapted to its life at the interface between ocean and sea floor.

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