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Membrane Properties that Shape the Evolution of Membrane Enzymes

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Abstract

Spectacular recent progress in structural biology has led to determination of the structures of many integral membrane enzymes that catalyze reactions in which at least one substrate also is membrane bound. A pattern of results seems to be emerging in which the active site chemistry of these enzymes is usually found to be analogous to what is observed for water soluble enzymes catalyzing the same reaction types. However, in light of the chemical, structural, and physical complexity of cellular membranes plus the presence of transmembrane gradients and potentials, it seems likely that these enzymes are subject to membrane-specific regulatory mechanisms that are only now beginning to be uncovered. We review the membrane-specific environmental traits that shape the evolution of membrane-embedded biocatalysts.

Introduction

In the early 1950s Frank Westheimer, William Jencks, and others built on the inspired work of Maud Menten, Leonor Michaelis and other early pioneers of biocatalysis to usher in the classical era of mechanistic enzymology in which the tools of physical organic chemistry were applied to elucidate the chemical and structural basis for enzyme rate acceleration. A high water mark of this era was the mid-1970's introduction of “perfect enzyme theory” by Albery and Knowles, which offered a rigorous conceptual and quantitative reckoning of the energetic hurdles that confront the evolution of enzymes [1,2]. They defined the nature of the energy landscapes in enzyme reaction pathways that, if attained through the process of natural selection, leads to “catalytic perfection”. For a “perfect” enzyme the rate-limiting step of the overall enzyme reaction at physiological substrate concentrations is diffusion of the substrate(s) to the active site. Once this condition is satisfied there is no selective pressure to further evolve the reaction chemistry, substrate affinity, or product release. Understandably, classical enzymology was devoted mainly to the study of water soluble enzymes that catalyze reactions involving soluble substrates. This Opinion is devoted to integral membrane enzymes of biosynthesis, metabolism, and proteolysis that catalyze

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reactions for which at least one of the substrates is also membrane-associated. The focus is on integral membrane enzymes that function autonomously (rather than as part of multi-protein complexes) and that are not coupled to transport. We also glance at a couple of unusual enzymes where a water soluble catalytic domain is tethered to an integral membrane regulatory domain. We respectfully apologize to our colleagues whose voluminous work on enzymes of the respiratory chain, photosynthesis, and various types of ATPases is not treated herein because of limitations in scope and space.

What unique environmental restraints confront the evolution of membrane enzymes? We offer here a very brief survey, which complements another recent review on membrane biocatalysis. This topic seems timely in light of the remarkable progress in membrane protein structure biology over the past decade, such that there are now more than a mere handful of membrane enzyme structures available (see Stephen White's well-maintained compilation: http://blanco.biomol.uci.edu/mpstruc/). Indeed, the structural biology of membrane enzymes seems, in a great many cases, to have by-passed our knowledge of their mechanisms [3]. In writing this Opinion we acknowledge with admiration that the road from water soluble to integral membrane enzymes is bridged by an impressive body of work on the phospholipases and other soluble enzymes that bind to the membrane surface to execute interfacial catalysis (c.f. [4-9]). An especially important development from these studies is the recognition that enzyme reactions on or in membranes generally conform to the Michaelis-Menten model provided that the concentrations of the enzyme and substrate are treated using membrane mole fraction or related surface concentration units rather than bulk molarity [10].

Membrane enzymes function within a fabulously heterogeneous medium

Integral membrane enzymes can only be removed from the bilayer when it is dissolved. A given biological membrane will contain many dozens, if not hundreds, of chemically distinct lipid molecules [11,12]. This heterogeneity is amplified for the case of eukaryotic membrane enzymes that traffic through more than one organelle, each with a distinct lipid composition. Biological membranes are also asymmetric: the lipid composition on the outer leaflet of a bilayer does not match that on the inner leaflet [11,12]. As is the case for all membrane proteins, membrane enzymes must to some degree be structurally and functionally *tolerant* of such lipid compositional heterogeneity (see review[13]). At the same time specific protein-lipid interactions may commonly be exploited by evolution as the basis for regulating enzymes. Long-running studies [14-18] of protein-lipid interactions ranging from fleeting solvent-like contacts to stoichiometric complex formation are currently being transformed by mass spectrometry-based approaches for detecting and quantitating specific lipid-protein interactions [19,20].

Lipids and other small molecules in the membrane sometimes play a direct coenzyme role in membrane enzyme chemistry

Membrane-associated small molecules can serve as coenyzmes for membrane enzymes. A key step in chromophore regeneration in the rhodopsin photocycle of vision is the conversion of all-*trans* 11-retinol back into 11-*cis*-retinol [21]. Lecithin retinol acyl

transferase (LRAT) uses the stored energy of the ester linkage in phosphatidylcholine as the source of the energy that drives this otherwise energetically uphill trans-to-cis double bond isomerization reaction [21,22]. Another example is provided by the lipophilic ubiquinone coenzyme Q, which is used to shuttle electrons across the membrane in various reactions and pathways. These include the reaction in which electrons originating from disulfide bond formation in the periplasm of Gram negative bacteria are transferred from the periplasmic DsbA protein to membrane-embedded DsbB and thence to the freely membrane-diffusible coenzyme Q [23-25]. A similar reaction is catalyzed in the endoplasmic reticulum (ER) membrane by vitamin K epoxide reductase as part of the vitamin K cycle, which is essential for blood coagulation [26-28]. A final example is the exotic isoprenoid lipid dolichol phosphate, with its long polyprenyl tail. This lipid serves as the membrane-anchored covalent scaffold on which oligosaccharides destined for attachment to N-linked glycoproteins are synthesized. The initial reactions to add sugars to the scaffolded glycoside occur on the cytosolic face of the ER membrane. The dolichol/oligosaccharide conjugate is then actively flip-flopped to the luminal face of the membrane, followed by additional reactions to complete biosynthesis of the complex glycoside. This is followed by transferal of the fully elaborated glycoside from the dolichol phosphate head group to the asparagine side chains of nascent N-glycoproteins [29]. The cycle is completed when dolichol phosphate flip-flops back across the membrane for reuse as a scaffold. Undecaprenol phosphate serves an analogous function in related pan-membrane biosynthetic pathways in microbes, such as peptidoglycan biosynthesis [29,30].

Specific lipids are also thought to sometimes play allosteric cofactor roles in regulating membrane enzyme activity (c.f. [31,32]).

Diffusion of membrane enzymes and substrates is quasi-two dimensional, but there are caveats

In an ideal fluid mosaic membrane, proteins bob up and down in the membrane plane and execute rapid axial rotation around their long axes (review in [33]). They typically also undergo lateral 2-D Brownian diffusion in the membrane plane [34,35]. Bulk membrane phases usually approximate the fluid liquid-disordered phase of ideal bilayers, although the effective viscosity of the membrane is higher than in aqueous solution [34,36]. In terms of dictating the rate at which two solutes will bump into each other, the drag of increased viscosity is offset by the reduced dimensionality of the bilayer [34,37,38] such that there is no reason, *a priori*, to suppose that the rate at which a small molecule substrate in the membrane reaches a membrane enzyme is very different than for a corresponding substrate/enzyme pair in solution.

It is now appreciated that while the fluid mosaic model may apply to a significant fraction of the total area of any real biological membrane, this model is not uniformly applicable across the whole membrane [36,39,40], particularly for the plasma membrane of multicellular organisms. Some enzymes may be associated reversibly or irreversibly with the membrane cytoskeleton and are thereby fixed in the membrane. Even for free molecules, diffusion of both membrane enzymes and substrates may be transiently impeded by barriers or fences in

the membrane imposed by the cytoskeleton, connections to the extracellular matrix, tight junctions, large membrane protein complexes, or other fixed molecular assemblies [36,39-41]. Moreover, as noted by Donald Engelman, “membranes are more mosaic than fluid” [42]: as for the cytosol they too represent a protein-crowded milieu. Finally, while the term and actual manifestation of “lipid rafts” in cellular membranes remain controversial [43-46], there seems no doubt that the plasma membranes of many cells contain transient nanodomains composed of certain lipids (particularly cholesterol and sphingolipids and proteins (usually palmitoylated) that do not diffuse or mix freely in the 2-D plane of the membrane. These transient bilayer domains are generally thought to exhibit properties that resemble the liquid-ordered phase, which has been well-characterized in synthetic lipid vesicles [47-49]. If resident in membrane nanodomains, membrane enzymes and substrates are expected to diffuse more slowly than in the bulk membrane phase, exhibit dampened conformational motions, and will interact with a different cohort of lipids and membrane proteins than in the surrounding (fluid phase-like) bulk membrane.

There are reports of various membrane enzymes, including both β-secretase and y-secretase of Alzheimer's disease notoriety, that seem to have an affinity for inclusion in membrane nanodomains [50,51]. In this regard it is notable that the amyloidogenic β-secretase competes for initial cleavage of the full length amyloid precursor protein (APP) with yet another membrane protease, α-secretase, which initiates non-amyloidogenic processing of APP. α-Secretase has been reported to disfavor membrane nanodomains and has been proposed to cleave APP in bulk (disordered) phase membranes [51,52]. The product of the β-secretase reaction, the transmembrane C99 fragment of the APP, was shown to preferentially partition into disordered phase rather than into the liquid-ordered phase of model vesicles [53], but possibly may exhibit more complex preferences *in vivo*. Combined, these observations suggest that the balance between amyloidogenic and non-amyloidogenic processing of APP may be based, in part, on the differentially-regulated distribution between different membrane domains of full length APP, its C99 fragment, and the three secretases.

Intriguingly, some model membrane studies have also suggested that some membrane proteins appear to preferentially localize to the interface between nanodomains and the surrounding bulk membrane [54,55]. One can speculate that this could be a mechanism sometimes used in nature to generate locally high concentrations of an otherwise surface-dilute enzyme within a membrane.

Membrane enzymes are usually vectorially-oriented

As posited in the original fluid mosaic model, the vast majority of membrane proteins are inserted into the membrane with a near-100% orientational preference [56,57]. The active site of a membrane enzyme with a water soluble substrate will always face either the extracellular/luminal milieu or the cytosol, but almost never both. Lipids and other small molecules often are also preferentially distributed in the inner or outer leaflet, but usually much less exclusively than for membrane protein orientation [11,12]. Depending on the topology of a specific membrane enzyme a good its substrate may have to flip-flop across the bilayer to reach the active site, perhaps as facilitated by a transporter. This scenario appears to apply to microbial diacylglycerol kinase, as will be summarized later in this

Opinion. The orientation of a membrane enzyme may also be critical in determining how various transmembrane gradients shape catalysis in cells, as described below.

Membrane enzymes are subject to the influence of transmembrane potentials and gradients

Both the plasma membrane (especially) and the membranes of intracellular organelles delineate transmembrane potentials and gradients, both fixed and variable, that shape the evolution and function of membrane enzymes.

First, most biological membranes serve as boundaries between aqueous phases that have different redox potentials. The cytosol is usually a reducing environment, whereas the lumen of sealed organelles, the periplasm, and the extracellular milieu are usually oxidizing environments. Membrane proteins therefore differ from most soluble proteins in that individual proteins often contain both disulfide bonds and free cysteine residues. Membranes represent an unusually well-suited environment for redox chemistry, in part because membrane enzymes bridge this gradient in redox potential. Membranes are also often subject to gradients in osmolarity and/or pH, gradients that membrane enzymes must, at the very least, adapt to. Finally, some membranes are subject to varying transmembrane electrical potentials that can alter protein structure and function, as is most extensively characterized for voltage-gated ion channels.

The impact of these various transmembrane gradients on membrane enzyme structure, function, and regulation have received scant attention outside of the respiratory chain, active transport, and related bioenergetic systems. In part, this is because it is difficult to establish and manipulate these gradients in the model membrane milieu in which membrane enzymes are often characterized. However, the notion that there may be interesting discoveries waiting to be made in this area is supported by studies of the voltage sensing lipid phosphatase [58]. While the phosphatase domain of this enzyme is water soluble, it is tethered to a transmembrane domain that closely resembles the tetraspan voltage sensor domain present in voltage-gated ion channels [59-61] (Figure 1A). Membrane depolarization activates the phosphatase activity of this enzyme, presumably via electroconformational coupling between the voltage sensor domain and the catalytic domain [60,61]. One wonders how many other enzymes may be regulated by variations in membrane gradients and potentials.

Membranes have varying lateral pressure, curvature, and thickness

These properties of biological membranes can vary from membrane to membrane or, for curvature and thickness, may vary even within a single membrane. These properties may also be time-dependent and can impact membrane protein structure, folding, and stability [62-68].

It has been shown that the activity of a membrane-associated form of glycerol-3-phosphate dehydrogenase [69] is modulated by membrane lateral pressure. V_{max} for this enzyme decreases as the membrane lateral pressure is increased [70]. The activity of leader peptidase

has also been shown to vary with changes in membrane lateral pressure [71]. The question of how varying membrane curvature alters integral membrane function has been little-explored. However, given that numerous peripheral membrane enzymes have activities that depend on this membrane trait [72], it will be surprising if many integral enzymes do not as well.

The prokaryotic (integral membrane) diacylglycerol kinase exhibits a catalytic preference for membranes of a particular thickness [73]. Varying membrane thickness appears to regulate the catalytic activity of the microbial DesK histidine kinase, a temperature sensor [74,75] (Figure 1B). DesK has five transmembrane helices, to which is tethered its soluble cytosolic kinase domain. It functions as homodimer. Varying temperature alters the membrane thickness, thereby perturbing the structure of the DesK transmembrane domain in a manner that is transduced to the kinase domain, to regulate its catalytic activity [76,77]. It has been shown that the transmembrane (TM) domain can be whittled down to a single composite TM segment that is able to sense and transduce temperature-dependent changes in membrane thickness to the kinase domain to regulate its catalytic activity [78-80]. Varying membrane thickness is known to shift the distribution of cleavage sites in the amyloid precursor protein by the gamma secretase proteolytic complex, resulting in alteration of the production ratio between the more toxic long forms of the amyloid-beta polypeptide (42-43mers) and the shorter (38-40mer) forms [81-83]. This may be related to the unusual way the substrate for this reaction, the APP C99 domain, adapts topologically to changes in membrane thickness [84]. Major hydrophobic mismatch between the transmembrane domain of a protein and the thickness of the surrounding membrane can be expected to sometimes have an adverse impact on catalytic function (c.f. [85]).

Membrane proteins can distort the membrane

There is a wealth of data that proteins can directly alter membrane curvature and thickness in a regulated manner (c.f. [65,68,86-88]). Among other possible mechanisms, membrane enzymes such as phosphatidylserine decarboxylase likely help to control membrane curvature by altering the ratio between lipids with large head groups such as phosphatidylserine (favoring convex leaflet curvature) and those with smaller head groups, such as phosphatidylethanolamine (c.f. [89]). It has also been proposed that certain membrane enzymes, such as the Gram negative outer membrane phospholipase A, thin the membrane in the vicinity of their transmembrane domains [90], most likely as a mechanism to help promote access of water to active sites in which it participates in the reaction chemistry.

The barriers to water penetration into the interior of the bilayer are anomalously low

Even though water is a highly polar molecule it has a surprisingly high membrane permeability, indicating that the energy barrier for transient excursions deep into the membrane surface is lower than one might guess [11]. This is thought to be because of the very small size of water molecules. Water at or near the membrane surface is thought to have significantly different properties than solvent water [91].

Water-soluble enzymes have active site structures and related dynamic properties that enable them to control water access, excluding from the active site excess water that might otherwise interfere with reaction pathways, while at the same time allowing in water molecules needed to promote substrate binding, reaction chemistry, and product release. It clear that the evolution of membrane enzymes has navigated the same water management issues. Many membrane proteins exhibit bound water molecules in their transmembrane domains [92-94]. Indeed, the introduction of bound water molecules into TM domains appears to be one mechanism used to evolve thermostability in some membrane proteins [95]. At least one membrane enzyme contains a small sealed cavity filled with multiple water molecules that seem to be tapped for use in the reaction cycle (see next section). Some enzymes exhibit aqueous cavities leading to an active site located below the membrane surface (c.f. [96,97]), while still others have aqueous caverns within the transmembrane domain. The Ste24p and ZMPSTE24 CAAX proteases (not to be confused with the architecturally-distinct CAAX protease Rce1) exhibits a barrel-like architecture with transmembrane helices providing the staves [97-101] (Figure 2). Within this barrel is a water-filled cavity large enough for hundreds of water molecules. The active site is located just under an interfacial lid to this barrel, with substrate entry and product exit through fenestrations located near the upper end of the barrel at or just under the water-bilayer interface. The original thinking regarding the substrate specificity of this protease is now in flux [102], but its unusual structure may be related to the facts that (i) it cleaves its substrates near their C-termini but at different numbers of residues from the end from substrate to substrate, (ii) some substrates include attached isoprenoid chains near the cleavage site, and (iii) some substrates are successively cleaved without release of the protein substrate after the first cleavage event, with the two reactions releasing C-terminal peptides of different lengths and different states of posttranslational modification.

We conclude by examining four case studies for membrane enzymes.

Rhomboid protease

Rhomboid is one of several membrane proteases currently under intense study [103,104] and has become a major system for studies of membrane enzyme catalysis, as well as folding and stability [105,106]. Rhomboid proteases are ubiquitous in all domains of life [107,108]. The best characterized forms of rhomboid, such as *E. coli* GlpG (276 residues) cleave specific single domain membrane protein substrates inside the membrane but near the surface to release the water soluble ectodomain of the substrate. This enzyme usually has 6 transmembrane helices and does not structurally resemble any water soluble protease [109,110], even though its reaction chemistry is similar to that of a classical serine protease [108,111]. Some rhomboids appear to be constitutively active, which implies they are regulated only by substrate availability in the contiguous membrane domain. Rhomboid may thin the adjacent lipid bilayer, facilitating access of water to the active site. Conversely, its activity may in some cases be regulated by hydrophobic mismatch [112-115]. There is also a small cavity in the GlpG rhomboid containing ordered water molecules adjacent to the scissle site. At least one of these ordered waters likely is used during the reaction cycle [116]. Both these internal waters and water contiguous with the aqueous phase are almost

certainly subject to controlled access to and exclusion from the active site during the rhomboid reaction.

Recent studies have provided an intriguing body of data supporting a model in which rhomboid appears to have a non-conventional mode of substrate recognition. Potential substrates with a single N-terminal-out topology form a complex with rhomboid, but do not appear to initially engage the active site [117]. If the segment of the bound transmembrane helix located at or near the potential cleavage site transiently unravels (an event that is likely facilitated by water shepherded by rhomboid into the binding site via membrane thinning), the now vulnerable segment is engaged by the actual active site and proteolysis proceeds [117,118]. Transmembrane helices that are completely stable during the lifetime of their complex with rhomboid dissociate from the enzyme unscathed. This “interrogation” of substrate TM helix stability may be the critical determinant of rhomboid substrate specificity. While the lifetime of the interrogation complexes has yet to be measured, the turnover number for cleavage is very slow—an average of 1 cleavage event every 2.5 minutes [119]. Interestingly, good substrates and bad substrates appear to have similar Km, leading one to wonder if non-substrate single span membrane proteins bind to the “interrogation” site with a Kd that equals Km for actual substrates [119]. The substrate K_m has been proposed to be orders of magnitude higher than physiological substrate concentrations [119]. Combined, these results suggest that rhomboid binds N-terminal-out single span membrane proteins indiscriminately (with similar Kd values) and only then makes a judgement as to whether or not the bound protein is an actual substrate, a decision based on interrogating helix stability. Rhomboid is therefore unusual as an enzyme in that substrates are distinguished from abundant non-substrates only after complex formation.

It is interesting to note that the seemingly unrelated aspartyl protease gamma-secretase also seems to exhibit an extremely low k_{cat} and substrate K_m values that likely are orders of magnitude higher than physiological concentrations [120]. Gamma-secretase functions as a heterotetrameric complex of membrane proteins and has a very broad substrate specificity toward N-terminal-out single span membrane proteins with stubby ectoplasmic domains [121-123]. Like rhomboid, there is evidence that the site of initial engagement between gamma-secretase and its substrates may be adjacent to, but not actually at the active site [124-126]. It also is interesting that addition of hydrophobic small molecules known to modulate the location of the preferred final cleavage site of the APP C99 domain by gamma secretase (“gamma secretase modulators”—GSMs [127,128]) also dramatically expands the substrate specificity of human and *Drosophila* rhomboids [129]. While the mechanisms underlying these phenomena are not understood, one wonders if some of the unusual mechanistic features of the rhomboids are shared by gamma secretase, despite their vastly different structural properties.

Phosphatidylglycerophosphate phosphatase B (PgpB)

This *E. coli* form of this enzyme is a member of the type II phosphatidic acid phosphatase (PAP2) family [130,131]. Its common name is a misnomer in that its main function is as a pyrophosphatase that cleaves undecaprenyl pyrophosphate to release the monophosphate form of this carrier lipid to activate its reuse as a membrane-anchored biosynthetic scaffold

[132]. PgpB is an integral membrane enzyme with 6 helical transmembrane segments and a V-shaped cleft in its transmembrane domain that leads to an interfacial active site bounded by a small periplasmic domain [133]. What is remarkable is that elements of the PgpB core structure, particularly three transmembrane segments (that include much of the active site), are similar to a trio of helices (and encompassing much of the active site) within the much larger water soluble PAP2 phosphatases, to which PgpB shares weak sequence homology [133] (Figure 3). Nature evidently re-engineered an existing water soluble enzyme into an integral membrane to serve as undecapryl pyrophosphatase rather than evolve a new membrane enzyme for this purpose. This strategy appears to sharply contrast to the approach taken by nature for enzymes such as rhomboid, gamma-secretase, microbial diacylglycerol kinase (below) and the CAAX proteases, which do not appear to have water soluble relatives.

Monotopic membrane enzymes

Monotopic membrane enzymes have water-exposed catalytic domains that are tightly associated with the membrane via an amphipathic platform that sits deeply in one leaflet of the bilayer. The structures of these platforms vary in details from enzyme to enzyme [134]. These enzymes include prostaglandin H synthase [135,136] (often referred to as cyclooxygenase), fatty acid amide hydrolase [137], and certain cytochrome P450s [138]. The evolutionary strategy underlying the structure-function relationships of these enzymes is to maintain well-evolved water soluble catalytic domains as water-exposed enzymes. However, they have been adapted for catalysis on lipophilic substrate by evolution of a channel leading from the membrane surface up into the enzyme active site located well above the membrane plane in the aqueous phase. These membrane platforms appear not only to anchor the catalytic domain with the correct orientation but in some cases also serves as a septum to seal off water access to the hydrophobic substrate channel leading from the membrane to the active site located well above the membrane surface [134].

Diacylglycerol kinase (DAGK)

Microbial diacylglycerol kinase is the smallest of all kinases—ca. 120 residues—and functions as a homotrimer with three transmembrane domains per subunit and three actives sites—1 at each subunit-subunit interface. It has no homology or structural similarity to the eukaryotic DAGKs, which are water soluble or peripheral membrane enzymes, or to any other kinases [139,140]. Nevertheless its active site chemistry has been shown to be analogous to that observed for water soluble kinases [141]. The convergent evolution of reaction chemistry for water soluble enzymes and for evolutionarily unrelated membrane enzymes seems to be a recurrent theme. The structure of the active form DAGK was determined based on lipidic cubic phase crystallization by the elegant and patient work Li and Caffrey [139,141], superseding the previously published NMR structure of what now is thought to be a heat-inactivated domain-swapped homotrimeric form of the enzyme in DPC micelles [142]. While in some ways DAGK is tolerant of amino acid mutations[143,144], it also has a significant propensity to misfold (c.f. [145] and review in [140]). DAGK has been shown to function after reconstitution into nearly every model membrane system currently available on earth (review in [13,140]). However, it does generally prefer to have at least

some lipid present to attain maximal catalytic activity [146-148], with the exception that it is nearly fully active in certain long chain detergents [149]. Activation of DAGK by non-substrate lipid was originally described as reflecting a co-factor role [147,148], but except for its DAG substrate binding site DAGK does not seem to have a discrete high affinity lipid site [139,141].

While the DAGK active site is located at the plasma membrane/cytosol interface, under physiological conditions its substrate, diacylglycerol (DAG), is produced on the other (periplasmic) side of the membrane as a by-product of the membrane-derived oligosaccharide (MDO) cycle of Gram negative bacteria [150] (Figure 4). To reach the active site, DAG must first flip-flop across the membrane to reach the inner leaflet. Because its head group is comprised only of a hydroxyl moiety the spontaneous flip-flop rate of DAG is rather high, roughly 50 per second [151]. DAG flip-flop appears to establish the substrate diffusion rate limit for the DAGK reaction. Based on the physiological concentrations of DAG in E. coli membranes [152] and steady state kinetic analysis of DAGK [153] it has been determined that $k_{cat}/K_{m,DAG}$ DAGK turnover is similar to the DAG flip-flop/diffusion rate to the DAGK active site, indicating that DAGK satisfies the Albery-Knowles definition of being a “perfect” enzyme. However, as such it is still orders of magnitude slower than fully evolved water soluble kinases, which are rate-limited by the much more rapid diffusion of substrates in solution to the active site [153]. It seems that the evolutionary aspirations of DAGK have been limited by the low standard of catalytic perfection imposed by the membrane to its maximum possible reaction rate. As a consequence, except for its sister enzyme, Gram positive undecaprenol kinase, Nature never saw fit to duplicate its gene and then adapt it for paralogous reactions. In a world populated with large and powerful families of kinases, DAGK and undecaprenol kinase remain orphan siblings forever trapped in known metabolic pathways.

Conclusions

For many years membrane protein structural biology has been inspired by the notion that the studies of membrane protein structure lag many years behind the study of soluble proteins. But membrane protein structural biology is catching up. We opine that other areas of membrane protein biophysics, which include mechanistic studies of membrane enzymes, may now be critically underdeveloped. While a pattern seems already to have emerged that the active site chemistry of membrane enzymes usually involves variations on themes already established by studies of water soluble enzymes, the frontier most in need of exploration is how membrane enzymes are regulated by varying membrane composition, geometry, biochemical and physical properties, and by transmembrane gradients and potentials. This is especially the case under actual cellular conditions. Given the recent flood of structural information for membrane enzymes and a host of emerging chemical biological and biophysical approaches suitable for examining enzyme function in cells, the time seems ripe for such studies.

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Richard N. Armstrong, who had a long-standing interest in membrane enzymology and was also endearingly opinionated.

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● of special interest

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Highlights

- Recent progress in the structural biology of integral membrane enzymes has generated a renewal of interest in their mechanisms and modes of regulation.
- Membranes contain many distinct traits that shape the evolution of membrane enzymes, in ways that are completely different from water soluble enzymes.
- Results so far suggest that membrane enzyme reaction chemistries are largely conventional, but that the membrane-related mechanisms by which these enzymes are regulated are sometimes distinctive and complex.
- Our developing understanding of how membrane traits alter membrane enzyme function remains in its infancy.

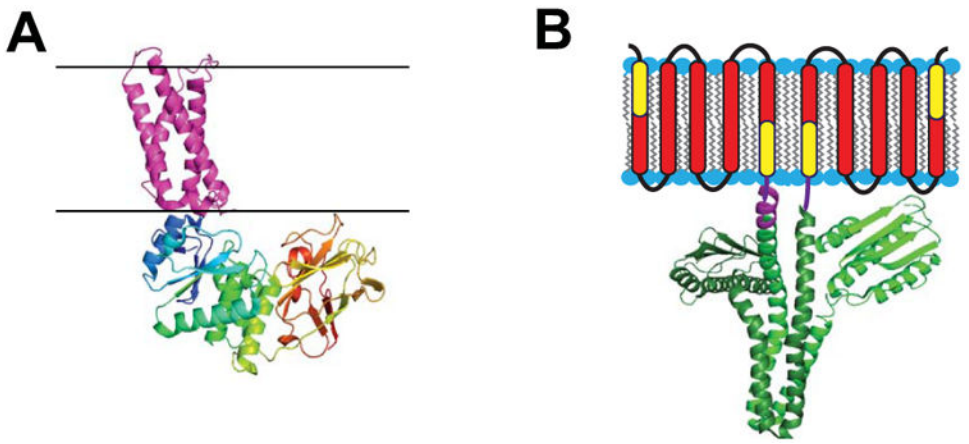

Figure 1.

Enzymes for which water soluble catalytic domains are coupled to integral membrane sensor regulatory domains. A) Crystal structure of the voltage-sensor domain of the Ci-VSP phosphatase (4G80) [59] in purple juxtaposed to its cytosolic domain (3AWF) [154] with multicolored chains. Black lines indicate expected membrane boundaries. This figure is adapted from [58]. B) The DesK temperature sensor protein homodimer, with the transmembrane region represented in cartoon form and the cytosolic domain depicted as based on its crystal structure (3GIG) [155], which is that of a non-symmetric dimer. It has been shown that the indicated yellow components of the first and last DesK monomer transmembrane segments can be fused to create a temperature-sensing-functional single pass protein referred to as MS-DesK [78,80]. The linker between the transmembrane and cytosolic domain in each monomer is represented in purple (the crystallographically resolved segment of this linker is longer in the subunit on the left than in the subunit on the right). This figure is adapted from [76].

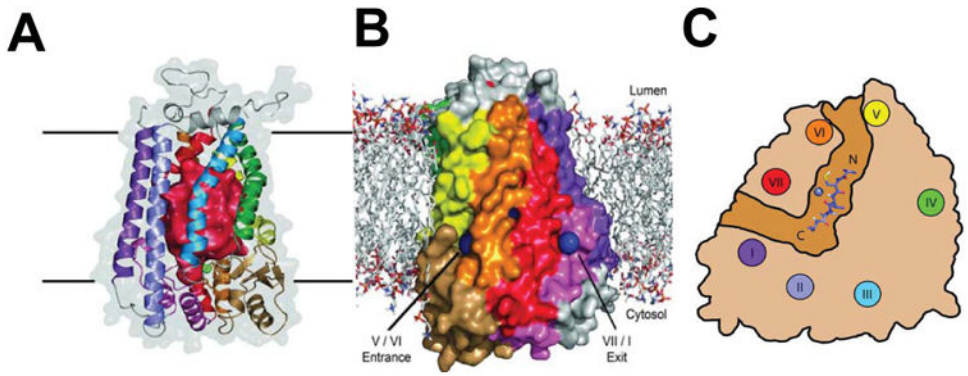

Figure 2. The Ste24p CAAX Protease

A) Structure of the Ste24p protease shown as a ribbon diagram. The red interior surface illustrates the surfaces of the large water-filled cavity. B) Surface representation of Ste24p in a lipid bilayer). Openings to the substrate binding groove/channel and active site are shown as blue spheres, with the indicated helices corresponding in location and color to those depicted in panel C. C) Cutaway representation of the Ste24p groove/channel viewed looking down on the membrane. The groove contains a 13 residue substrate-derived peptide docked in. These figures are reproduced from Pryor *et al.* [98] with permission.

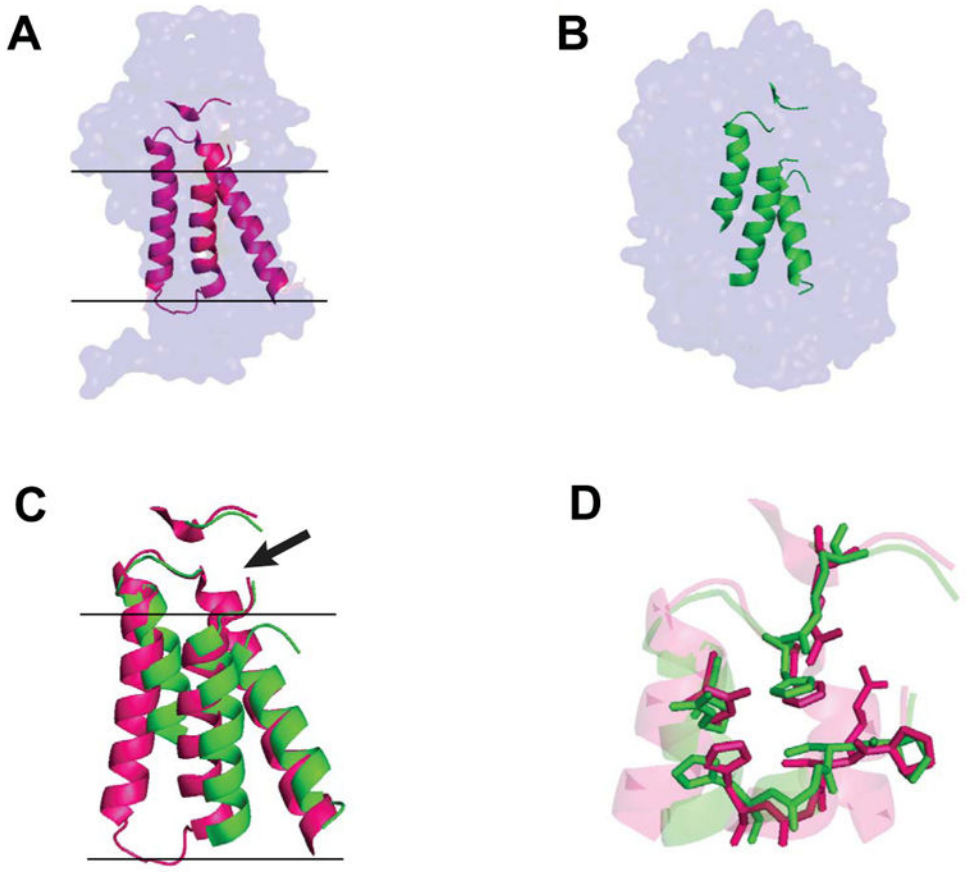

Figure 3. A key structural element of soluble PAP2 phosphatase has been adapted and inserted into the membrane for the PgpB phosphatase

A) Surface representation of the ecPgpB phosphatase crystal structure with key helices highlighted in pink (4PX7) [133]. Black lines indicate estimated membrane boundaries. B) Surface representation of the crystal structure of the water soluble ciCPO PAP2 phosphatase, with key helices highlighted in green. (1VNC) [156]. C) Aligned core helices of membrane-integral ecPgpB (pink) and souble ciCPO (green). Non-core portions of the protein are removed for clarity. The location of the active site is indicated by the arrow. D) Zoom view of the active sites from panel C, with side chains of key catalytic residues now being shown. This figure is adapted from [133].

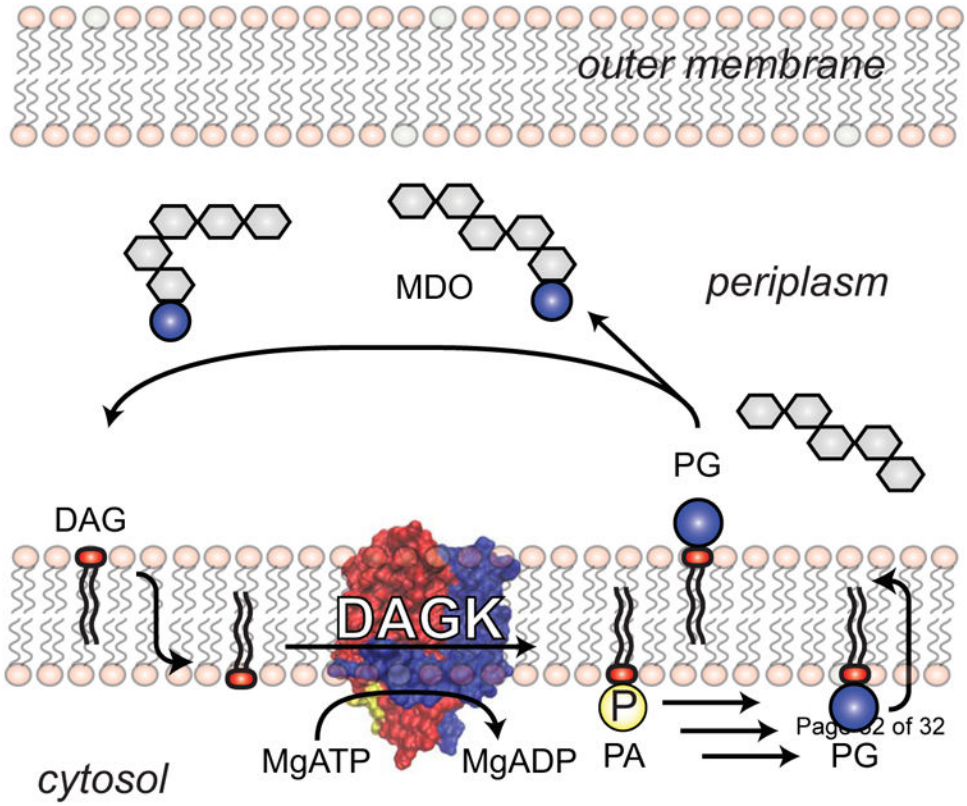

Figure 4.

DAGK in the membrane-derived oligosaccharide (MDO) cycle of Gram negative bacteria. Glycerol-1,3-diphosphate is transferred in the periplasm from the head group of phosphatidylglycerol (PG) to decorate the nascent MDOs, with DAG being produced as a byproduct. DAG then flip-flops from the periplasmic to the cytosolic leaflet of the plasma membrane where it is phosphorylated by DAGK to generate phosphatidic acid, which can then be recycled back into PG. The rate of diffusion of DAG to the DAGK active site is thought to be limited by the rate of spontaneous DAG flip-flop to the cytosolic leaflet. DAGK has evolved so as to be able to convert DAG into PA at a rate that roughly matches this lipid translocation event [153]. This figure was adapted from [140].