By virtue of the strength of hydrophobic interactions and the malleability of phospholipid acyl chains, membrane lipids become one of the important glues of life. The liquid-crystalline organization of the bilayer component of biological membranes enables the membranes to be effective, cohesive, and yet flexible, barriers separating two fluid compartments. Integral membrane proteins are imbedded into the bilayer, where they catalyze the selective transfer of information and material between the two compartments. Hydrophobic interactions between the proteins’ bilayer-spanning domains and the bilayer lipids cause the lipids to pack tightly around the proteins, thereby maintaining the barrier properties. Because the bilayer lipids are organized as a liquid-crystalline sheet, the flexible lipids will accommodate protein conformational changes that involve the protein/bilayer boundary. Individual lipid molecules also may be imbedded at protein–protein interfaces where they not only may “plug” potential leaks but also stabilize supramolecular assemblies of bilayer-spanning proteins.

Lipid bilayers, however, are not just thin sheets of liquid hydrocarbon, stabilized by the lipids’ polar head groups, which serve as “solvents” for the bilayer-spanning proteins. Some polar head groups serve as ligands that bind to specific protein domains, and some head groups carry a net negative charge that in a less specific manner attract positively charged protein domains to the membrane/solution interface, where adsorption/binding may be stabilized by hydrophobic interactions. The polar head groups thus serve to direct and organize protein targeting to different plasma and organellar membrane/solution interfaces. This means that even though membrane lipids possess no intrinsic catalytic activity, they become key participants in the regulation of membrane turnover and cell metabolism, a regulation that becomes exquisite due to the regulated turnover of membrane lipids.

Lipid bilayers also are material bodies with well-defined elastic moduli, such that bilayer perturbations caused by membrane protein conformational changes involving the protein/bilayer boundary will incur an energetic cost. The hydrophobic cohesion between bilayer-spanning proteins and the host bilayer therefore couples the energetics of membrane protein conformational changes to the associated bilayer perturbation energy, which provides for additional mechanisms by which membrane lipids regulate biological function.

Recently, the importance of membrane lipids for many different physiological processes was highlighted at the 58th Annual Meeting of the Society of General Physiologists, which took place in Woods Hole, MA, September 8–12, 2004. Donald W. Hilgemann from University of Texas Southwestern Medical Center, Scott D. Emr from University of California, San Diego School of Medicine, and Pietro De Camilli from Yale University School of Medicine organized the symposium on Lipid Signaling in Physiology, which highlighted the recent progress that has taken place in understanding the physiological importance of membrane lipids for cell signaling, membrane turnover, and membrane protein function. With 164 participants and 104 invited and poster presentations covering a broad range of topics related to the sundry roles of lipids in cell physiology, the meeting was lively and informative.

Among the 200+ different phospholipid species that can be identified in the average cell membrane, phosphatidylinositol (PI) and the related polyphosphoinositides (PIPs) stand out because numerous physiological functions depend on the regulated turnover of phosphoinositides, which serve as messengers in membrane and protein trafficking events and as regulators of protein function. The importance of phospholipid turnover was recognized more than 50 yr ago by Lowell and Mabel Hokin (J. Biol. Chem. 203:967–977, 1953), who showed that cholinergic stimulation of pancreatic secretion is associated with phosphorylation of membrane phospholipids. Subsequently it became apparent that only the phosphoinositides, which constitute just 5–10% of the membrane phospholipids, are involved in this stimulated turnover. Moreover, though only a small fraction of the membrane lipids are phosphoinositides, regulated phosphoinositol turnover occurs in wide variety of physiological process, such as secretion (including synaptic neurotransmitter release), vesicle budding/fusion and trafficking, and the regulated protein targeting underlying cell proliferation and growth. In all these different functions, the phosphoinositides serve as both structural and signaling molecules.
The basic scheme underlying signaling by phosphoinositides is simple: active messengers are produced and removed by kinases and phosphatases (Fig. 1). Different phosphoinositides are localized at only a select set of bilayer/solution interfaces, where they interact with (bind to) different enzymes and cytoskeletal proteins. The phosphoinositides thus serve as localization signals/targets at specific membrane compartments. The mix of phosphoinositides at a given interface depends on the mix of phosphoinositide kinases and phosphatases, whose localization activities themselves are subject to regulation, and active messengers may be produced or removed by both phosphorylation and dephosphorylation. Thus, phosphoinositide turnover forms the basis for the spatially and temporally regulated production of transient messengers that control a multitude of downstream events. This control is exquisite because the inositol ring can be phosphorylated at multiple locations, such that there are three different PI-P species as well as three different PI-P₂ species and PI-P₃ (Fig. 2).

The meeting began with two feature lectures by L.C. Cantley (Harvard Medical School) and S.D. Emr (UCSD School of Medicine). Emr provided an overview of the phosphoinositides and the PI kinases and PI-P phosphatases that control the interconversion among the eight different phosphoinositides (Fig. 2). In yeast there are six PI kinases and seven PI-P phosphatases; in humans there are ~20 kinases and 25–30 phosphatases. These enzymes, especially the kinases, tend to have restricted localization targeting such that the synthesis (and localization) of a given phosphoinositide is compartment specific, meaning that the phosphoinositides become spatial landmarks within the cell to which proteins bind by virtue of their phosphoinositide-specific recognition domains, e.g.: phox homology (PX) domains and Fab1p/YOTB/Vac1p/EEA1 homology (FYVE) domains, which tend to bind selectively to PI(3)P; and pleckstrin homology (PH) domains, which with varying specificity bind to PI(3,4)P₂, PI(3,5)P₂, PI(4,5)P₂, or PI(3,4,5)P₃ (and in some cases to PI(3) and PI(4)). The different membrane-restricted poly-phosphoinositides serve to program/control vesicular trafficking decisions, which in turn regulate a multitude of cell signaling events. Further, protein binding to the cognate phosphoinositide may serve not only to anchor the protein to the target membrane but also to regulate protein function through allosteric mechanisms. The PI phosphatases serve to eliminate inappropriate poly-phosphoinositide synthesis and to terminate the signal, which in its own right may create the messenger for a different trafficking event.

Because different phosphoinositide kinases are localized to specific target sites, phosphoinositide turnover regulates exocytosis/secretion, endocytosis/membrane retrieval, and intracellular membrane trafficking.

**Figure 1.** The phosphoinositide cycle. Starting with phosphatidylinositol (PI), the inositol ring can be phosphorylated by PI-kinases at three different positions (3, 4, and 5) to yield monophosphorylated PI (PI-P), which in turn can be phosphorylated by PI-P kinases at one of the two remaining positions to yield the di-phosphorylated PI (PI-P₂). The di- or monophosphorylated PIs are degraded by phosphoinositide phosphatases. Because both PI-P and PI-P₂ function as signaling/targeting molecules, the effector lipids can be removed either by dephosphorylation or by further phosphorylation.

**Figure 2.** The phosphoinositides and related signaling molecules and their metabolic interrelationships. The major phosphorylation and dephosphorylation reactions are indicated by red and blue arrows, respectively. Similarly, kinases and phosphatases are denoted in red and blue. Lipases and their associated reactions are in green. DAG, diacylglycerols; DAGK, diacylglycerol kinase; MTMR, myotubularin-related proteins; PIKfyve, PI(3)P 5-kinase; PI(4)P, PI(5)P, PIKfyve, PI(3)P 5-kinase; PI(4)P, PI(5)P, PI(4)P 5-kinase; PLA₂, cytosolic phospholipase A₂; PLD, phospholipase D; PUFA, polyunsaturated fatty acid.
The phosphoinositides regulate these different functions because they serve as (transient) targets for numerous proteins involved in membrane turnover (vesicle budding and retrieval). Emr described the so-called ESCRT complexes of proteins (for endosomal sorting complex required for transport), which are involved in the down-regulation/recycling of growth factor and G protein–coupled receptors and for the budding of viruses at the plasma membrane (vesicle budding into endosomes is equivalent to budding out of cells).

Phosphoinositides are not just involved in membrane turnover. PI3Ks serve as signaling components in receptor tyrosine kinase signaling cascades, and PI(3,4,5)P$_3$ contributes to the recruitment and activation of numerous targets, including the serine/threonine protein kinase Akt (also known as protein kinase B). Because Akt is involved in the control of cell proliferation and growth, defective phosphoinositide turnover, which compromises the production and removal of PI(3,4,5)P$_3$, may cause tumorigenesis due to defective Akt regulation. L.C. Cantley summarized recent work on the regulation of Akt (Fig. 4). PI(3,4,5)P$_3$ not only targets Akt to the plasma membrane, it also serves as an allosteric activator of Akt. The activated Akt phosphorylates several downstream targets, including tuberin, a GTPase activating protein (GAP) for the Ras-like small G protein Rheb. Akt-phosphorylated tuberin is inactive, leading to increased Rheb activity, and Rheb is an activator of mammalian target of rapamycin (mTOR), which controls protein synthesis and cell growth by activating a 70-kD ribosomal S6 kinase (S6K1) and inhibiting the elongation initiation factor 4E binding protein 1 (4E-BP1). The PIK3→Akt→tuberin→mTOR cascade thus is emerging as a key contributor to tumorigenesis through the PI(3,4,5)P$_3$→Akt pathway. Given the key role of PI(3,4,5)P$_3$, the above scheme also explains why the PI(3,4,5)P$_3$ phosphatase PTEN is a tumor suppressor.

What are the mechanisms underlying protein binding to phosphoinositides and other lipids? This question was addressed in several presentations. J.H. Hurley (National Institute of Diabetes and Digestive and Kidney Diseases) presented a new x-ray structure for β2-chimaerin, a GAP for the small G protein Rac, which provided structural insights into β2-chimaerin activation by DAG (and phorbol esters). The activators bind to a single C1 domain in the protein, similar to the C1 domains in PKC, which leads to a massive exposure of hydrophobic residues that stabilizes the membrane binding. S. McLaughlin (State University of New York at Stony Brook) showed that the juxtamembraneous region of the epidermal growth factor receptor (EGFR) binds to phospholipid bilayers through a combination of electrostatic and hydrophobic interactions, where the latter are stabilized by aromatic residues pointing in toward the bilayer’s hydrophobic core. He further emphasized that the limited amounts of the different phosphoinositides in a cell means that protein binding to a given phosphoinositide not only targets the protein(s) to selected membranes, it also “hides” the phosphoinositide such that it becomes unavailable to bind other proteins, thereby introducing yet another regulatory element into phosphoinositide signaling.

M. Overduin (University of Birmingham, UK) presented NMR results on PI(3)P binding to FYVE and PX domains, which are relatively selective for PI(3)P. Using micelle-incorporated PI(3)P, it could be shown that the lipid–protein interactions reflected a combination of chemically specific interactions, and electrostatic and hydrophobic interactions. Taken together, the results from these presentations led to a picture in which the binding of phosphoinositides to phosphoinositide-recognizing domains is governed by a combination of specific chemical interactions and less specific physical interactions.

T. Meyer (Stanford University School of Medicine) presented results on phosphoinositide turnover in che-
motaxis using fluorescently labeled phosphoinositide-specific binding domains to track different phosphoinositide species. Using variants of GFP, it becomes possible to monitor several phosphoinositides simultaneously in living cells using evanescent wave microscopy. Using the PH domain from Akt, which binds to PI(3,4,5)P$_3$, it was possible to relate the formation/localization of P(3,4,5)P$_3$ to cell motility. When human dendritic cells are immersed in a gradient of chemoattractants, there is a wave front of PI(3,4,5)P$_3$ (and actin) at the leading edge as the cells move up the gradient. Similar wave fronts (and cell movements) occur in very shallow gradients, which lead to the conclusion that cells self-polarize by forming lamellipod extensions that are correlated over a few µm. In this picture, cell migration becomes a consequence of local, stochastic lamellipod extensions that end up being correlated through sensory inputs that activate PI(3)Ks and thereby increase the formation of PI(3,4,5)P$_3$. The local increase in PI(3,4,5)P$_3$, in turn, recruits actin and other cytoskeletal proteins to membrane, which leads to the formation and local extension of lamellipods.

The barrier properties of lipid bilayers result from cohesion among adjacent acyl chains, which are quite mobile in the liquid-crystalline bilayers of physiological interest. That is, the stability of lipid bilayers is due to hydrophobic interactions, as opposed to more specific steric interactions. Given the importance of hydrophobic interactions also for bilayer–protein interactions, it becomes important to consider membrane function not only in terms of the membrane properties that arise from specific “chemical” interactions (in which structural details are important) but also in terms of the membrane properties that arise from physical interactions (in which structural details can be ignored), and to know where chemistry ends and mesoscale physics begins. E. Evans (University of British Columbia, Canada and Boston University) showed how important lipid bilayer properties can be understood “simply” in terms of mesoscopic physics of elastic bodies. This is possible because the length constant for the decay of local bilayer perturbations is comparable to the bilayer thickness (or ~3 nm). Over length scales from >10 nm (to at least 10 µm), lipid bilayers behave as fairly uniform bodies with well-defined elastic (expansion/compression, $K_a$ and bending, $K_c$) moduli. These moduli can be determined experimentally by measuring how the bilayer area increases when a lateral tension is applied. At very low tensions (<0.5 mN/m), the apparent resistance to area expansion is small and the apparent area expansion varies as a logarithmic function of tension because the expansion is achieved primarily by dampening the amplitude of thermal bending fluctuations in the bilayer. In this region of the area–tension relation, the bilayer bending modulus can be determined to be $K_c = 22 k_BT$ for phospholipids with mono-unsaturated acyl chains, where $k_B$ is Boltzmann’s constant and $T$ is the temperature in kelvin. At higher tensions, the resistance to area expansion increases and approaches a direct stretch in which the area expansion varies as a linear function of tension. In this region of the area–tension relation, the area expansion/compression modulus can be determined to be $K_a = 240$ mN/m (60 $k_BT$/nm$^2$). Both $K_a$ and $K_c$ are functions of the intermolecular interactions between the lipid acyl chains, which also determine the magnitude of the hydrocarbon/water surface tension, $\gamma_{hc/w} \approx 40$ mN/m (which effectively is an invariant for all lipid species). These different lipid bilayer characteristics therefore should be interrelated. According to “simple” mesoscopic theories of elastic behavior, $K_a = 6\gamma_{hc/w}$ and $K_c = (d_h^2/24) \cdot K_a$, where $d_h$ is the average thickness of the bilayer’s hydrophobic core when no tension is applied (~3 nm). The predicted relations between $K_a$, $K_c$, and $\gamma_{hc/w}$ are in remarkable agreement with the experimental observations, for phospholipids with mono-unsaturated acyl chains.

More complex behaviors arise in the case of phospholipids with polyunsaturated acyl chains, where $K_c$ is twofold less than would be predicted from the mesoscopic theory using the measured $K_c$ (which is similar for phospholipids with mono- and polyunsaturated acyl chains). This means that the length constant for the decay of bilayer perturbations, which scales as $\sqrt{K_c/K_a}$, is less in bilayers composed of poly-unsaturated phospholipids. Similarly, cholesterol increases both $K_a$ and $K_c$ much more than would be expected from the $K_a\gamma_{hc/w}$ relation. In either case, it becomes necessary to incorporate more specific chemical features into the description of the bilayer. It is in this context relevant that sphingomyelin:cholesterol bilayers are much stiffer than sphingomyelin:dioleoylphosphatidylcholine:cholesterol bilayers, again underscoring that though many bilayer properties can be understood simply in terms of the intermolecular interactions among the acyl chains (as expressed in $\gamma_{hc/w}$), other properties can be understood only by invoking more chemically specific interactions.

The importance of understanding how the chemical identities of the bilayer lipids regulate function was also highlighted by P. De Camilli (Yale University School of Medicine). Key bilayer properties, such as the propensity to form nonbilayer structures (which are essential in fusion intermediates) or lipid microdomains, as well as protein–bilayer interactions vary as a function of the specific bilayer lipid composition. Moreover, just as bilayers serve to regulate protein function, proteins modify bilayer properties. Phosphoinositide turnover due to the action of kinases, phosphatases, and lipases (Figs. 1 and 2); other proteins, such as amphiphysin, catalyze...
the formation of tubular structures through the interaction of BAR domains with highly curved negatively charged lipid interfaces. PI(4,5)P2 is a key player in both exocytosis and endocytosis at the plasma membrane, whereas PI(3,4,5)P3 is involved only in endocytosis. The complexity of phosphoinositide signaling is highlighted by the fact that PI(4,5)P2 is involved in both vesicle fusion and retrieval, which implies that turnover per se becomes important in determining the direction of the membrane flow, and suggests a key role for polyphosphoinositide phosphatase synaptojanin.

N. Brose (Max-Planck-Institute for Experimental Medicine, Göttingen, Germany) described a different type of lipid-binding protein involved in synaptic function and vesicle fusion, Munc13 (the mammalian homologue of Caenorhabditis elegans unc13). Munc13 is involved in vesicle priming through activation of the tsgnre synaptin. Munc13 has a C1 domain (similar to β2-chimaerin) and translocates to the plasma membrane because it binds to DAG. It is the only relevant DAG receptor in synaptic function, where it regulates short-term plasticity.

Phosphoinositides are important not only in normal cell function, but also are implicated in infectious processes and the cytoidal action of bacterial toxins. G. van der Goot (University of Geneva, Switzerland) described how the anthrax toxin manages to co-opt the cell’s membrane recycling machinery to precisely choreograph its uptake into the cell. S. Grinstein (Hospital for Sick Children, Toronto, Canada) showed how the Salmonella protein SigD (a PI(4,5)P2 phosphatase) causes membrane ruffling in mammalian cells, which is a prerequisite for the bacterial invasion of the cells.

To understand further the spatial and temporal regulation of membrane turnover, it becomes necessary to understand which of the numerous phosphoinositide kinases and phosphatases are involved, and how they are regulated. H. Yin (University of Texas Southwestern Medical Center) pointed out that clustering of PI kinases and PI-P kinases allows for precisely tuned spatial and temporal control. She then summarized work on dissecting out the relative importance of the PI(4)P 5-kinase isoforms (α, β, and γ) using small interfering RNAs (RNAi) that selectively reduce the expression of each isoform in a dose-dependent manner. Though all three isoforms are found at the plasma membrane, they are involved in rather diverse function: α is important in membrane ruffling; β in the organization of the actin cytoskeleton and receptor-mediated endocytosis; and γ in the focal adhesions and synaptic vesicle dynamics. The γ isoform also is the major player in the generation of the PI(4,5)P2 that is the substrate for the generation of (3,4,5)P3.

Phosphoinositides are key players in the coupling of the cytoskeleton to the plasma and organelar membranes. M.P. Sheetz (Columbia University) showed how the strength of the plasma membrane tether force, which is measured by pulling membrane tethers out from the membrane, is reduced by “occlusion” by proteins that bind to phosphoinositides, such as PH domains, D.R. Klopfenstein (University of California San Francisco) showed how the C. elegans kinesin motor UNC-104 binds to PI(4,5)P2 through a PH domain. This interaction is important for synaptic vesicle transport, and the rate of transport is increased when the PI(4,5)P2 is clustered, suggesting that PI(4,5)P2 stimulates the rate of transport in a cooperative manner. P. Devreotes (Johns Hopkins University) showed how the downstream response to chemoattractants in Dictyostelium discoideum involves a highly polarized accumulation of PI(3,4,5)P3, with subsequent actin polarization at the “upstream” edge of the cell, and the concomitant removal of PI(3,4,5)P3 by the phosphatase PTEN at the “downstream” edge.

Phosphoinositides are important also for the function of bilayer-spanning proteins (ion channels and transporters). D.W. Hilgemann (University of Texas Southwestern Medical Center), who some 10 yr ago discovered that integral membrane proteins may be regulated by phosphoinositides, presented results suggesting that this regulation involves not only the direct control of protein function by adjacent phosphoinositides, but also membrane turnover. Using a sophisticated capillary perfusion system that allows for the perfusion of whole-cell voltage-clamped cells, it is possible to show that exogenous P(4,5)P2 causes an inhibition of Na+/Ca2+-exchanger activity in parallel with membrane retrieval as measured by changes in membrane capacitance. This regulation of transport activity is under the control of not only PI(4,5)P2, but also DAG and Ca2+. Altogether, the results show that the tapestry of phosphoinositide-dependent activities displays a richness that will keep many investigators busy for years to come.

R.C. Hardie (University of Cambridge, UK) showed how phototransduction in Drosophila is dependent on phosphoinositide turnover. The photoreceptor cells’ response to light is mediated by TRP (transient receptor potential) channels that are activated downstream of rhodopsin, G protein activation, and PLC activation. In contrast to some TRP channels, however, I(3,4,5)P3 does not appear to be involved in photoreceptor TRP channel activation. To understand better the relative roles of different phosphoinositide metabolites, the photoreceptor cells were transfected with a PI(4,5)P2 sensitive inward rectifier potassium channel (Kir2.1), and the PI(4,5)P2 levels were monitored by recording the Kir2.1 current. It thus was possible to show that TRP channel activation depends on the coordinated interplay of PI(4,5)P2 depletion, which occurs at a rate of ~150% per second, and DAG production (Fig. 2). It re-
mains unclear whether DAG itself is activating the TRP channels, or if further downstream metabolites, such as PUFAs, are the key activators. The process is under the control of Ca^{2+}, which can enter the photoreceptors through the highly Ca^{2+}-permeable TRP channels and inhibit the PLC (at [Ca^{2+}] > 100 μM), and thus limit the PI(4,5)P2 depletion.

The role of phosphoinositides in the regulation of channel function was described also by B. Hille (University of Washington), who summarized results on the regulation of M currents (potassium currents activated by muscarinic stimulation) using GFP-labeled PI(4,5)P2 and DAG probes. M currents were completely inhibited by PI(4,5)P2 depletion due to PLC activation. DAG does not seem to be a key player in the regulation; but the response does depend on cytoplasmic Ca^{2+} transients; if the intracellular [Ca^{2+}] transients that occur in response to muscarinic stimulation are quenched by Ca^{2+} buffering, the PLC activation is reduced. The role of PI(4,5)P2 in the regulation of G protein–regulated inward rectifier potassium channels (GIRKs) was described by D.E. Logothetis, who pointed out that PI(4,5)P2 can regulate ion channels by mechanisms that do not depend on any of the canonical lipid-binding domains, even though the channels may be able to distinguish among the different phosphoinositides. As is the case for M currents, GIRK channel activity is critically dependent on the PI(4,5)P2 levels in the plasma membrane, and the channels desensitize due to PLC activation and PI(4,5)P2 depletion.

The regulation of membrane protein function by the membrane lipids tends to be couched in terms of interactions between the lipid polar head groups and binding site on the protein, i.e., as a variant of protein regulation by soluble second messengers. It long has been known that the membrane bilayer can regulate membrane function by a different mechanism, based on the hydrophobic coupling between the bilayer-spanning domains of integral membrane proteins to the bilayer hydrophobic core. Protein conformational changes that involve the protein/bilayer boundary will perturb the surrounding bilayer, and the energetic cost of this bilayer perturbation will contribute to the free energy difference of protein conformational change. In the simplest case, the bilayer deformation can be represented as a local bilayer thinning (or thickening) due to a mismatch between the length of the protein’s hydrophobic domain and the average thickness of the unperturbed bilayer, which incurs an energetic cost because lipid bilayers are elastic bodies with well-defined elastic properties. O.S. Andersen (Weill Medical College of Cornell University) summarized results showing that lipid bilayers are sufficiently stiff that the energetic cost associated with such bilayer deformations is large enough to modulate the equilibrium distribution between different protein conformations and thus protein function.

Though the intricacies of phosphoinositide signaling can seem daunting, compared with cholesterol the phosphoinositides are easy. This point was made by M.S. Brown (University of Texas Southwestern Medical Center) in his feature lecture on the regulation of cholesterol synthesis. Cholesterol is a key component of mammalian cell membranes, as well as a precursor in the biosynthesis of hormones and bile acids. Cholesterol esters also are key participants in the development of atherosclerotic plaques. There are two sources of cellular cholesterol: uptake through the low-density lipoprotein (LDL) receptors, and de novo synthesis. Both of these pathways are tightly regulated by cell cholesterol, a regulation that normally maintains the cellular cholesterol levels within narrow limits. In fact, cell cholesterol normally is confined to the membranes; only when the regulation of cholesterol homeostasis breaks down does it accumulate in the lysosomes, as a first step in the atherogenic process. A key question thus becomes: how do cells regulate the amount of cholesterol in their membranes? K. Bloch and F. Lynen provided a partial answer by elucidating the metabolic pathways involved in cholesterol and fatty acid synthesis; but the key question is not so much how cholesterol is synthesized, but how much cholesterol is synthesized. The regulation of cholesterol synthesis is implemented through regulated gene activation, in which transcription factors are generated by the controlled proteolysis of sterol binding element binding proteins (SREBPs) in the ER. The synthesis of many different genes is regulated by SREBPs, not only cholesterol but also saturated fatty acids and stearoyl-coenzyme A desaturases. The specificity of the activation arises from the presence of the SREBP cleavage-activating protein (SCAP), which possesses a sterol-sensing domain and thereby monitors the membrane cholesterol level. When the cell cholesterol levels are low, SACP binds to SREBP, which causes the SACP–SREBP complex to move from the ER to the Golgi apparatus, where SREBP is cleaved by the membrane-attached serine protease S1P (Fig. 5).

The cleavage product is cleaved a second time, by the membrane-spanning Zn^{2+} protease S2P, which releases a basic helix-loop-helix transcription factor that then moves to the nucleus to activate gene transcription. A long-standing puzzle has been: what is the signal for SCAP dissociation from SREBP? When cell cholesterol levels rise, SCAP undergoes a conformational change that presumably causes the dissociation of the complex. Similar conformational changes can be induced by a variety of cationic amphiphiles, which could indicate that SCAP senses some change in bilayer elasticity. Cholesterol does indeed change bilayer elasticity, but
changes in bilayer elasticity are unlikely to be the primary cause of SCAP dissociation from SREBP. This is because the ER cholesterol levels are so low (<10%, or so) that the changing cholesterol levels are likely to have only modest effects on bilayer elasticity. What then? Brown presented results culminating a long search for specific cholesterol binding to SCAP, which show that cholesterol indeed binds to SCAP. This result provides a key insight into the long-standing puzzle of how cells regulate their cholesterol levels.

How do membrane-spanning proteins become bilayer incorporated, and can charged residues be incorporated into the bilayer core? G. von Heijne (Stockholm University, Sweden) summarized results on a novel method that allowed for the construction of the elusive hydrophobicity scale for amino residues that are buried in the bilayer core. The trick is to fix the side chain in question at a defined position in the bilayer, which can be done by incorporating the side chain into bilayer-spanning α-helices that cotranslationally become incorporated into the bilayer by moving from the ER translocon into the adjacent bilayer. If the helix is too polar, it will not incorporate into the bilayer, and the distribution between bilayer-incorporated and -unincorporated helices can be distinguished by inserting a glycosylation site that only will be glycosylated if the helix is bilayer incorporated. It thus is possible to construct a hydrophobicity scale for residues that are anchored at specific depths in the bilayer, and to show that even (potentially) charged residues can be buried in the bilayer. It remains unclear, however, whether this hydrophobicity scale reflects a true equilibrium situation because translation and translocation are energy-dependent processes.

That (potentially) charged residues can be buried in the bilayer core becomes relevant when examining the structure of the bacterial potassium channel KvaP, which was discussed by R. MacKinnon (The Rockefeller University). MacKinnon pointed out that the overall hydrophobicity of the KvaP S4 segment, which has many positively charged residues distributed along its length, makes it feasible to bury the S4 segment in the bilayer hydrophobic core. Some aspects of the original KvaP structure may need to be revised, however, as the voltage sensor domain is very flexible. Indeed, the structure of the voltage sensor domain and its orientation relative to the channel “core” may differ in different KvaP structures, as deduced from a new structure, based on single particle electron microscopy. A common element in all the KvaP structures, however, is that the S4 segment is much more lipid exposed than would have been expected from a priori biophysical reasoning! This conformational plethora is unusual, even among membrane proteins; it suggests that the conformational constraints imposed by the bilayer are more important than often appreciated.

The meeting’s final presentation was a feature lecture by J.E. Dixon (University of California San Diego, School of Medicine), who discussed the so-called dual-specificity phosphatases that may function as both protein and phosphoinositide phosphatases. PTEN and myotubularin and the myotubularin-related phosphatases are among these dual-specificity phosphatases, but they turn out to be such poor protein phosphatases that they properly should be considered phosphoinositide phosphatases (Fig. 2). The structure of a novel MTMR, MTMR2, which is mutated in Charcot-Marie-Tooth syndrome 4B was presented. The structure dis-

Figure 5. Schematic model for the cholesterol-dependent proteolytic cleavage of SREBP. Cholesterol binding to SCAP breaks the association between the SCAP WD domain and SREBP Reg domain, thereby blocking the translocation of SREBP (as part of a SCAP–SREBP complex) to the Golgi apparatus. In the Golgi apparatus, SREBP is proteolytically processed by two membrane-associated/spanning proteases: the serine protease S1P and the Zn²⁺ protease S2P. S1P cuts the luminal linker between the two bilayer-spanning α-helices in SREBP. S2P releases the basis helix-loop-helix (bHLH) by cutting in the middle of the bilayer-spanning α-helix, which enables bHLH to translocate to the nucleus where it activates gene transcription.
played several unexpected features, including an unexpected PH domain, which could be involved in binding to membrane lipids (PH domains can bind to both lipids and proteins). Surprisingly, Charcot-Marie-Tooth syndrome 4B can be caused not only by mutations in the active phosphatase, but even by “dead” phosphatases with catalytically lethal mutations in the active site, suggesting that heterodimer formation between “live” and “dead” MTMR proteins may be important for targeting the proteins correctly.

Altogether, the meeting highlighted admirably the critical importance of lipids as structural and signaling components in many different physiological processes. Not so many years ago, a friend of mine, who shall remain unnamed, stated that “The study of lipid–protein interactions is the last realm of the intellectually bankrupt!” Today, one can more appropriately argue that to neglect the importance of lipid–protein interactions in cell signaling is intellectually suspect. In many cases, the lipid regulation of various proteins can be couched in terms of ligand activation of protein, terms that are familiar (and comfortable) to all of us. In other cases, the mechanistic basis for the regulation needs to be found in terms of mesoscale physics and bilayer elasticity. Could phosphoinositide regulation of membrane proteins, for example, depend not only on the head group but also on the poly-unsaturated acyl chain, which would decrease $K_c$ and thus the length constant describing the decay of bilayer perturbations? The challenge over the next years becomes to delineate the relative importance of the different energetic contributions to the regulation of protein function by the membrane lipids. We have an exciting time ahead of us.

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