and that high cholesterol levels may dissolve rafts (*7*). Zacharias *et al.* found that clustering of prenylated test proteins was insensitive to cholesterol depletion, probably indicating that these proteins clustered in a type of lipid microdomain that did not depend on cholesterol. The ability to extract prenylated test proteins with detergent (and the irregular structure of the prenyl tail) suggested that the lipid microdomains in which they congregated contained highly disordered lipids. Interestingly, replacing the saturated fatty acids bound to a signaling kinase with polyunsaturated fatty acids reduced the association of the kinase with lipid rafts, perhaps resulting in modulation of a signaling pathway (*8*). By using fluorescent test proteins that do not dimerize, the authors were able to avoid the pitfalls of cross-linking, which induces movement of proteins into less fluid membrane microdomains (*2*).

The new work still leaves many questions unanswered. What are the physical characteristics of lipid rafts in the inner leaflet, and which lipids do they contain? Why are lipid rafts in the plasma membrane outer leaflet observed under some conditions but not others (*7*, *9*)? How do proteins recognize appropriate lipid rafts, and are the increased thickness and decreased fluidity of these rafts involved? Do raft transmembrane adaptor proteins that contain palmitoyl groups, such as LAT and PAG (*1*), couple lipid microdomains in the outer leaflet with those in the inner leaflet? How is the actin cytoskeleton involved in raft migration and coalescence? Answers should arrive with the next wave of exciting experiments, especially those performed on cells of the immune system. Biophysicists—who have revolutionized the field by applying techniques such as single-particle tracking, single–fluorescent molecule microscopy, and atomic force microscopy—will be able to thoroughly characterize the different types of lipid rafts. Finally, it should not be forgotten that the notion of lipid rafts was developed by cell biologists studying intracellular lipid transport, a research area that will no doubt provide many more surprises (*10*, *11*).

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PERSPECTIVES: CELL BIOLOGY

Fats, Flies, and Palmitate

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The membranes of cells are composed of a bewildering array of lipids. Yet despite their complexity, biological membranes maintain stable compositions that are characteristic for different organisms, tissues, and intracellular organelles. Evidently, homeostatic mechanisms exist that maintain the concentrations of different membrane lipids at particular levels. The best understood mechanism is a feedback system in mammals in which cholesterol and fatty acids negatively regulate their own synthesis. Reporting on page 879 of this issue, Dobrosotskaya, Seegmiller, and colleagues (*1*) present surprising findings demonstrating that the fruit fly *Drosophila melanogaster* has an identical system, but one that is regulated by phosphatidylethanolamine rather than by cholesterol or fatty acids.

More commonly known as fats, lipids are biological compounds that are soluble in organic solvents but only sparingly so in water. Lipids found in membranes have both water-soluble and water-insoluble moieties. It is this common amphipathic feature that enables the formation of the lipid bilayer, the foundation of most membranes in nature. Two abundant types of membrane lipids, phosphoglycerides and sphingolipids, are rooted in the membrane bilayer by virtue of their hydrophobic tails, which are derived from fatty acids (long hydrocarbon chains with terminal carboxylates). These molecules also carry one of several different polar head groups that can interact with water. Cells generate a staggering diversity of lipids by combining fatty acid tails (which vary in the number of carbon atoms and in the positions of double bonds) with one of several different polar head groups. Another important class of lipids is sterols, which contain a series of fused rings. One such sterol, cholesterol, is a major component of the membranes of many animal cells.

In mammals, the transcription of several crucial genes required for lipid synthesis is activated by a small family of transcription factors called SREBPs (sterol response element binding proteins). A remarkable feature of SREBPs is that their entry into the nucleus depends on their release from the membrane by proteolysis (see the figure). During their synthesis, SREBPs are inserted into the membrane of the endoplasmic reticulum, a membranous network in the cytoplasm of the cell. In the endoplasmic reticulum, SREBPs form a complex with a membrane-embedded protein called SCAP, which escorts the SREBPs to another cellular compartment called the Golgi apparatus. Here, the SREBPs are sequentially cleaved by two Golgi-specific proteases (see the figure), releasing a soluble fragment from the amino terminus. This fragment is a transcription factor, which, as a result of cleavage, is free to migrate to the nucleus, where it activates the expression of genes involved in the synthesis of cholesterol and fatty acids. Homeostasis is achieved by a negative feedback loop in which cholesterol and fatty acids block the proteolytic release of SREBPs from Golgi membranes. Interestingly, one of the SREBPs (SREBP-1c) is subject to an additional regulatory step that takes place at the promoter for SREBP-1c itself. Fatty acids, the end products of the SREBP-1c pathway, inhibit the action of a transcription factor called LXR, which is required for optimal expression of the *SREBP-1c* gene (*2*, *3*).

How do lipids inhibit the proteolytic processing of SREBPs? This is best understood for cholesterol, which regulates the export of the SCAP-SREBP complexes from the endoplasmic reticulum. When cholesterol levels are low, SCAP escorts SREBPs to the Golgi, where processing takes place. When cholesterol levels are high, SCAP retains SREBP in the endoplasmic reticulum, processing is prevented, and cholesterol synthesis is curtailed (*4*).

Highlighting the central importance of the SREBP pathway for lipid homeostasis in animals, a similar cast of protein characters exists in *Drosophila*. These include a SREBP (flies only have one), a SCAP, and orthologs of the two proteases that release SREBP from the membrane. The SREBP pathway in flies directs the expression of genes involved in fatty acid synthesis as well as genes involved in the production of at least one membrane lipid, phos-

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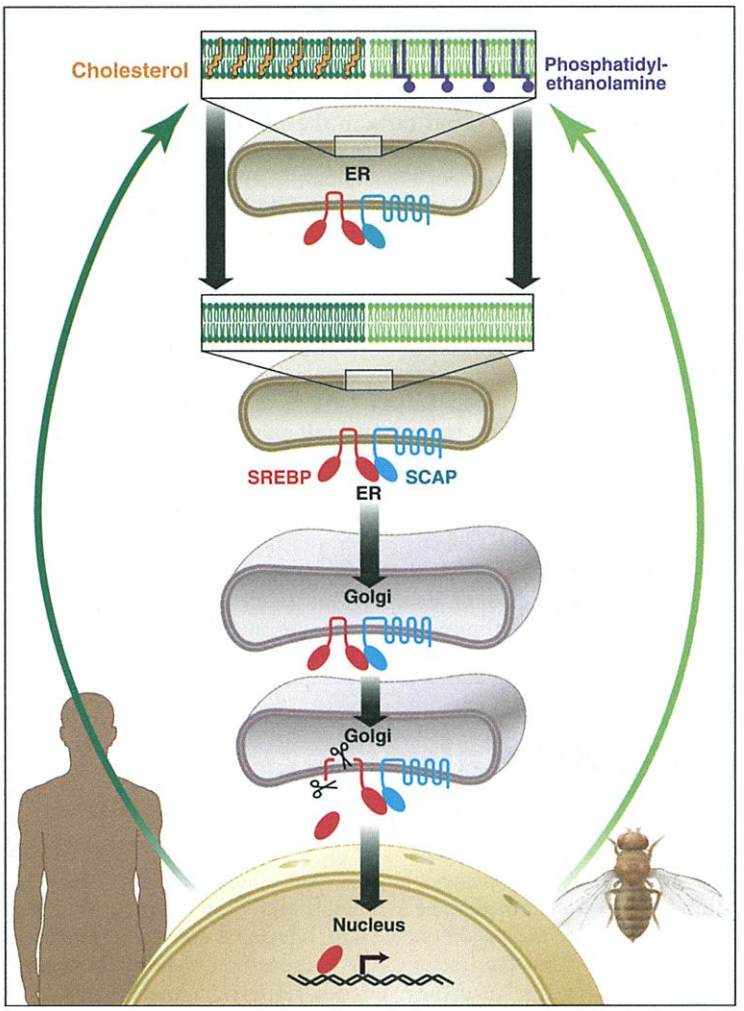
phatidylcholine. How is this pathway controlled? Similar to SREBPs in mammals, *Drosophila* SREBP is regulated by its proteolytic release from the membrane, which depends on SCAP. But insects are incapable of producing sterols, and proteolytic processing of SREBP in *Drosophila* cells does not respond to sterols. Instead, as recently reported by Seegmiller, Dobrosotskaya, and colleagues (*5*), processing is inhibited by a common saturated fatty acid called palmitate. This finding raised the intriguing question of whether palmitate itself inhibits SREBP cleavage or whether the regulatory compound derives from palmitate through a specific metabolic pathway.

To address this question, the authors have now used enzyme inhibitors and RNA interference (a method for eliminating specific messenger RNAs in cells) to systematically block the various steps involved in the conversion of palmitate to other lipid intermediates (*1*). The result of these analyses was surprising: Palmitate must be converted to the sphingolipid ceramide in order to block *Drosophila* SREBP processing. Next, ceramide must be converted to the two-carbon molecule phosphoethanolamine, which eventually serves as the polar head group of the membrane lipid phosphatidylethanolamine. Thus, in *Drosophila* phosphatidylethanolamine or a closely related molecule is responsible for blocking the SCAP-dependent formation of mature SREBP.

How do cholesterol and phosphatidylethanolamine control the action of SCAP? Mammalian SCAP is thought to respond to cholesterol through a stretch of five transmembrane segments called the sterol-sensing domain. Certain amino acid substitutions in the sterol-sensing domain—which is homologous to sequences in other proteins with known or suspected roles in sterol sensing—render SCAP insensitive to cholesterol. According to one model, cholesterol directly interacts with the sterol-sensing domain, thereby inducing a conformational change in SCAP that prevents its exit with SREBP from the endoplasmic reticulum. In another model, SCAP responds not to cholesterol directly but rather to a cholesterol-induced change in the physical properties of the membrane bilayer. The new findings of Dobrosotskaya *et al.* (*1*) tell us, however, that SCAP in flies can respond to a lipid that is entirely unrelated in structure to cholesterol. This may mean that the so-called sterol-sensing domains of SCAP in mammals and flies are each capable of recognizing different ligands. Alternatively, and as suggested by Dobrosotskaya *et al.*, cholesterol and phosphatidylethanolamine may both perturb the endoplasmic reticulum membrane in a similar way, which in turn is sensed by SCAP. Yet a third possibility is that cholesterol and phosphatidylethanolamine are each separately recognized by an as yet unknown protein that interacts with SCAP. In this case, the sterol-sensing domain might actually represent a recognition sequence for the unknown lipid-sensing proteins.



A lipid sensor in the ER. Feedback inhibition of the SREBP pathway in mammals and flies. Depletion of cholesterol (orange) or phosphatidylethanolamine (purple) from the membranes of the endoplasmic reticulum (ER) allows the protein complex composed of SCAP (blue) and SREBP (red) to move to the Golgi apparatus. Here, two proteases (scissors) cause the release of a transcriptionally active fragment of SREBP. This fragment is able to enter the nucleus, where it switches on the expression of genes involved in lipid synthesis.

One of the mysteries raised by the Dobrosotskaya *et al.* work is the question of why *Drosophila* SREBP stimulates the production of fatty acids, which are likely to be incorporated into phosphatidylcholine and many other membrane lipids. Yet, as we have seen, the SREBP pathway in flies responds to one lipid only, phosphatidylethanolamine. How then do insect cells prevent the excessive accumulation of fatty acid–containing lipids other than phosphatidylethanolamine? Additional regulatory pathways might exist that respond to lipids other than the one sensed by SCAP. But the general solution might be simpler than that. The composition of membranes depends largely on the activities of key enzymes in the metabolic pathways that control the distribution of fatty acids among the various lipids. Because the metabolic building blocks of membrane lipids are constantly exchanged, the use of a single surrogate, phosphatidylethanolamine, might suffice as a device for sensing and controlling the levels of the entire repertoire of fatty acid–derived membrane lipids. We note that SCAP-dependent processing of SREBPs in mammals is inhibited by fatty acids as well as by cholesterol. Perhaps the effect of fatty acids on mammalian cells is also mediated by a phosphatidylethanolamine-like membrane lipid.

The Dobrosotskaya *et al.* work is also noteworthy for its ingenious use of RNA interference in probing the intricate web of metabolic interconversions in the cell. This application highlights the extraordinary power of this relatively new molecular genetics tool in dissecting complex networks of interactions in higher cells.

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