Regulation of SREBP Processing and Membrane Lipid Production by Phospholipids in *Drosophila*

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Animal cells exert exquisite control over the physical and chemical properties of their membranes, but the mechanisms are obscure. We show that phosphatidylethanolamine, the major phospholipid in *Drosophila*, controls the release of sterol regulatory element–binding protein (SREBP) from *Drosophila* cell membranes, exerting feedback control on the synthesis of fatty acids and phospholipids. The finding that SREBP processing is controlled by different lipids in mammals and flies (sterols and phosphatidylethanolamine, respectively) suggests that an essential function of SREBP is to monitor cell membrane composition and to adjust lipid synthesis accordingly.

The lipid composition of membranes in animal cells is maintained within strict limits, primarily by feedback regulation of lipid biosynthesis. The mechanism for this homeostasis is beginning to be understood. Recent insights have emerged from the study of membrane-bound transcription factors called SREBPs that activate genes encoding enzymes of lipid biosynthesis in insect cells (*1*) as well as in mammalian cells (*2*, *3*). The activities of SREBPs are inhibited in a feedback fashion by membrane lipids, but these regulatory lipids differ in mammalian and *Drosophila* cells. In mammalian cells, SREBP activity is inhibited by sterols and polyunsaturated fatty acids (*2*, *4*, *5*). In *Drosophila* cells, SREBP activity is blocked when palmitate but not sterols or other fatty acids is added to the culture medium (*1*). Although the regulatory agents differ, the mechanism is conserved. In *Drosophila* and mammalian cells, control is attained through regulated proteolytic release of the active fragments of SREBPs from cell membranes.

Similarities and differences in SREBP processing in *Drosophila* and mammalian cells. The SREBPs are synthesized as intrinsic proteins of the endoplasmic reticulum (ER) membrane. To activate transcription, SREBPs must be transported to the Golgi complex, where they are cleaved by two proteases that liberate the basic helix-loop-helix-leucine-zipper domains so they can enter the nucleus (*3*). This transport requires an escort protein, SCAP (SREBP cleavage–activating protein) (*6*). In mammalian cells, SCAP serves as a sterol sensor; it loses the ability to move to the Golgi complex when sterol concentrations are high. *Drosophila* cells express genes that encode a single SREBP (dSREBP) and orthologs of mammalian SCAP and the two SREBP proteases (*1*). Experiments with RNA interference (RNAi) indicate that SCAP is required for dSREBP processing in *Drosophila* cells (*1*), as it is in animal cells (*2*, *6*).

A major difference between mammalian and *Drosophila* cells relates to the genes activated by SREBPs. In mammalian cells, SREBPs activate genes that encode enzymes of cholesterol and unsaturated fatty acid biosynthesis (*2*, *5*, *7*). *Drosophila* cells, like those of other insects, do not produce sterols (*8*). The major SREBP targets in *Drosophila* S2 cells are enzymes required for saturated fatty acid biosynthesis (*1*).

Palmitate as the regulatory lipid in *Drosophila*: direct or indirect? An important question is whether palmitate regulates SREBP processing in *Drosophila* cells or whether it must be incorporated into another product, such as a phospholipid, in order to act. The inhibitory effect of palmitate [16 carbons, 0 double bonds (16:0)] on dSREBP processing in *Drosophila* cells is highly specific (*1*). Other saturated fatty acids such as stearate (18:0) were much less effective, as was the monounsaturated fatty acid oleate (18:1). Polyunsaturated fatty acids had no activity. This finding suggested that palmitate might act by incorporation into another lipid through the action of a highly specific enzyme. We used enzyme inhibitors and RNAi to block incorporation of palmitate into various end products in *Drosophila* S2 cells. The results indicate that palmitate must be converted to phosphatidylethanolamine (PE) to inhibit SREBP cleavage and that this conversion occurs through the sphingolipid pathway.

Metabolic conversion of palmitate to PE. PE synthesis in eukaryotic cells has been well characterized (*9*–*13*) (Fig. 1). Activated palmitate (palmitoyl-CoA) can be converted to PE by condensing with serine through the action of serine palmitoyltransferase (SPT), which forms an intermediate that is converted to sphinganine. Addition of another fatty acid and introduction of a double bond converts sphinganine to ceramide, which is converted to sphingosine through loss of the additional fatty acid. Phosphorylation by either of two sphingosine kinases (SK1 or SK2) produces sphingosine-1–phosphate, which is broken down by sphingosine-1–phosphate lyase (SPL) to produce the key intermediate phosphoethanolamine plus *trans*-2-hexadecenal (*11*).

The net result of this pathway is to convert palmitate plus serine plus phosphate [from adenoside triphosphate (ATP)] into *trans*-2-hexadecenal plus phosphoethanolamine. The *trans*-2-hexadecenal can be converted back to palmitate via hexadecanal by reducing the double bond and oxidizing the carbonyl with fatty aldehyde dehydrogenase (FALDH) (*14*, *15*). Phosphoethanolamine is attached to cytidine 5′-diphosphate (CDP) by phosphoethanolamine cytidylyltransferase (PECT). CDP-ethanolamine donates phosphoethanolamine to diacylglycerol to produce PE (*13*). When ethanolamine is available, it can be converted directly to phosphoethanolamine by ethanolamine kinase (EK), bypassing the sphingolipid intermediates. PE can also be created from phosphatidylserine through decarboxylation (via phosphatidylserine decarboxylase) or by base exchange when free ethanolamine is available (via phosphatidylserine synthase) (*13*).

Use of enzyme inhibitors and RNAi to block palmitate conversion to PE. To study conversion of palmitate into phospholipids, we incubated *Drosophila* S2 cells in a chemically defined medium (IPL-41) supplemented with delipidated, dialyzed fetal calf serum (FCS). This medium is devoid of fatty acids and contains all 20 amino acids, including 1.9 mM serine. The medium contains 140 μM choline but no ethanolamine. To this defined medium, we added palmitate, ceramide, or sphingosine for 4 hours before harvest (Fig. 2A). We subjected cell extracts to SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and blotted them with an antibody to dSREBP. In the absence of any additions, we detected the membrane-bound precursor and the cleaved nuclear forms of dSREBP, which migrated with apparent molecular masses of ~125 and 72 kD, respectively (lanes 1, 3, and 5). Addition of palmitate, ceramide, or sphingosine selectively reduced the nuclear form, which indicates

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inhibition of proteolytic processing (lanes 2, 4, and 6). To show the requirement for converting palmitate to a sphingolipid, we used ISP-1 (*16*), a specific inhibitor of SPT, which catalyzes the first step in this conversion (*11*) (Fig. 1). In the absence of ISP-1, palmitate and ceramide both inhibited dSREBP processing (Fig. 2B). In the presence of ISP-1, palmitate no longer inhibited dSREBP processing, but ceramide remained effective (Fig. 2B, bottom). To confirm the specificity of ISP-1, we reproduced the block by treating the cells with double-stranded RNAs (dsRNAs) directed at the mRNAs for the two subunits of SPT. Such treatment abolishes the corresponding endogenous mRNAs selectively through RNAi (*17*). As a control, we treated the cells with dsRNA directed against an irrelevant messenger RNA (mRNA) (rat CYP7A1) (Fig. 2C). RNAi against either of the two subunits of SPT blocked the ability of palmitate to inhibit dSREBP cleavage (lanes 6 and 8), whereas the control CYP7A1 dsRNA had no effect (lane 4).

To determine whether inhibition of dSREBP cleavage by palmitate or sphingosine requires converting sphingolipids to PE, we eliminated some of the necessary enzymes by RNAi (Fig. 3). Elimination of SPL blocked inhibition by palmitate, ceramide, and sphingosine (Fig. 3A), which indicates that the inhibitory effect of all three of these compounds requires conversion to either phosphoethanolamine or *trans*-2-hexadecenal (see Fig. 1). Elimination of PECT also blocked the actions of palmitate, ceramide, and sphingosine (Fig. 3B), which indicates that the required metabolite is phosphoethanolamine and that the phosphoethanolamine must be converted to CDP-ethanolamine, the final precursor of PE. To test the effect of the other product of the SPL reaction, *trans*-2-hexadecenal, we added hexadecanal to cells (Fig. 3C). Hexadecanal is produced in cells from *trans*-2-hexadecenal by reducing the double bond (*14*). Although hexadecanal inhibited dSREBP processing, RNAi directed against FALDH abolished its effect, indicating that hexadecanal acts by being converted to palmitate, which initiates the whole sequence of reactions. FALDH RNAi did not block inhibition by palmitate, and it had only a partial effect on the action of ceramide.

The preceding data indicate that palmitate inhibits dSREBP processing at least in part by supplying phosphoethanolamine through the sphingolipid pathway. Palmitate may also supply the fatty acid component necessary for PE biosynthesis (Fig. 1). To sort out these separate effects, we incubated cells with palmitate alone, ethanolamine alone, or the two together (Fig. 4A). As before, palmitate had a major effect in reducing nuclear dSREBP (lane 3). Ethanolamine alone had no effect (lane 2). Palmitate plus ethanolamine was similar to palmitate alone (lane 4). As observed here, the palmitate effect was abolished when the sphingolipid pathway was blocked by RNAi directed against SPT-I (lane 7) or SPL (lane 15). It was also abolished by RNAi against SK1 plus SK2 (lane 11). Addition of ethanolamine restored com-

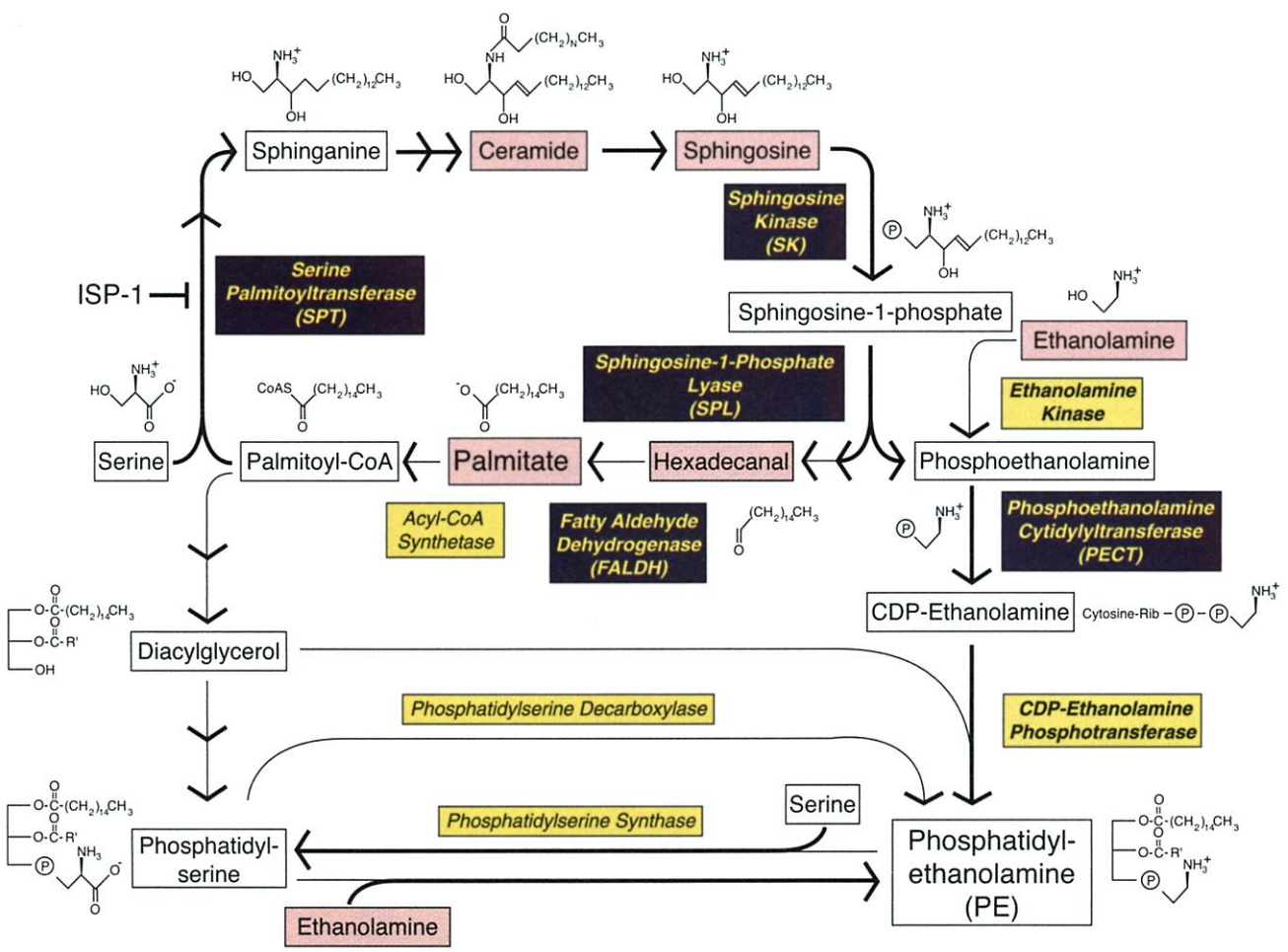

Fig. 1. Pathways of PE synthesis in eukaryotic cells. Scheme is based on data from mammalian and yeast systems (*9*–*15*). Intermediates not shown are indicated by multiple arrowheads. Pink boxes, compounds that inhibit dSREBP cleavage when added to *Drosophila* S2 cells; white boxes, compounds not tested because of poor entry into cells; black boxes, enzymes required for inhibition as revealed by RNAi experiments; yellow boxes, enzymes that were not tested. ISP-1 is an inhibitor of SPT (*16*).

plete inhibition by palmitate in the presence of these RNAi treatments (lanes 8, 12, and 16). These data strongly suggest that the sphingolipid pathway is required only to produce phosphoethanolamine and that the alternative supply of phosphoethanolamine through the direct pathway obviates the need for sphingolipid intermediates in the palmitate-mediated inhibition of dSREBP processing.

Inasmuch as ethanolamine is never sufficient to inhibit processing in the absence of palmitate, the data in Fig. 4A indicate that palmitate is required for one or more other reactions in addition to production of phosphoethanolamine. One of these reactions may be the glycerol 3–phosphate acyltransferase (GPAT)–catalyzed addition of fatty acids to glycerol 3-phosphate to form diacylglycerol (*13*), which is a precursor of all phospholipids, including PE (Fig. 1). If this is true, then other fatty acids should inhibit dSREBP processing in the presence of ethanolamine, because the fatty acid substrate specificity of GPAT is broader than that of SPT (*13*). We tested this idea by treating S2 cells with various fatty acids in the absence and presence of exogenous ethanolamine (Fig. 4B). In the absence of ethanolamine, only palmitate completely blocked processing of dSREBP (lane 2). Palmitoleate (16:1) had no effect (lane 3), whereas oleate (18:1) caused partial inhibition (lane 4). In the presence of ethanolamine, palmitoleate inhibited partially (lane 7), and oleate inhibited strongly (lane 8). These data suggest that the palmitate specificity stems from its requirement for phosphoethanolamine synthesis through the sphingolipid pathway. Once this requirement is satisfied, oleate can replace palmitate for the nonspecific function.

PE as a feedback regulator of SREBP processing in *Drosophila* cells. To determine whether the immediate precursor of PE, CDP-ethanolamine, regulates SREBP processing, we examined RNAi directed against CDP-ethanolamine phosphotransferase (Fig. 1). Inhibition of this enzyme should block regulation by palmitate or ceramide if the ultimate regulator of SREBP processing is PE but not if it is CDP-ethanolamine. A search of the *Drosophila* genome sequence revealed three enzymes with high identity to both CDP-ethanolamine phosphotransferase and CDP-choline phosphotransferase. Each of these enzymes might have the capacity to synthesize PE as well as phosphatidylcholine (PC) (*13*). The multiplicity of these enzymes and their overlapping substrate specificities made it impractical to eliminate this activity by RNAi, because this would also stop synthesis of PC. This issue might be clarified if we could add PE to cells directly, but this was not possible because PE does not cross intact cell membranes. Although we were unable to rule out CDP-ethanolamine as the regulatory agent, the data favor the hypothesis that PE is the regulator for three reasons: (i) SCAP seems designed to monitor membrane composition, and PE is the major constituent of *Drosophila* cell membranes (~55% of total phospholipids) (*18*); (ii) feedback inhibition of biosynthetic pathways is usually mediated by the end product (in this case PE) and not by an intermediate such as CDP-ethanolamine; and (iii) if CDP-ethanolamine were the sole regulator, exogenous ethanolamine should have inhibited dSREBP processing.

Although the above data suggest that PE

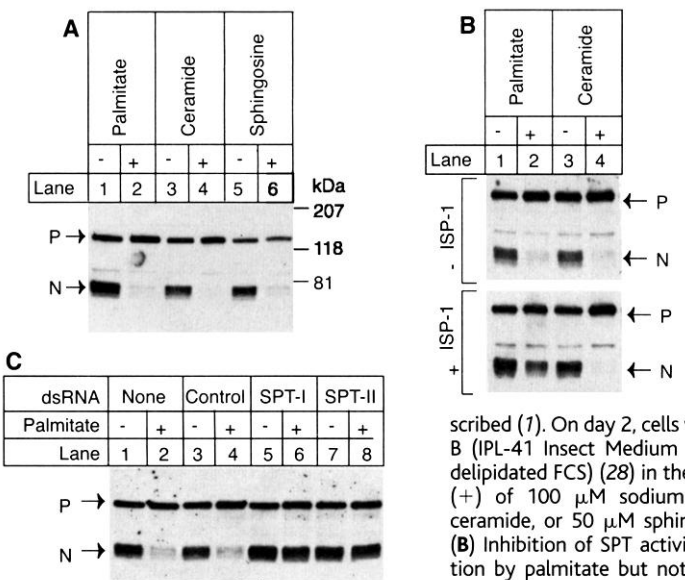

Fig. 2. Requirements for inhibition of dSREBP cleavage in *Drosophila* S2 cells. (**A**) Cleavage of dSREBP is inhibited by palmitate, ceramide, or sphingosine. On day 0, monolayers of S2 cells were set up for experiments at 10^6 cells per 60-mm dish in 3 ml of medium A (Schneider's *Drosophila* medium supplemented with 10% FCS) (*28*) and incubated at 23°C as described (*1*). On day 2, cells were switched to medium B (IPL-41 Insect Medium supplemented with 10% delipidated FCS) (*28*) in the absence (−) or presence (+) of 100 μM sodium palmitate, 50 μM C6-ceramide, or 50 μM sphingosine (*28*) as indicated. (**B**) Inhibition of SPT activity by ISP-1 blocks inhibition by palmitate but not by ceramide. On day 0, cells were set up as in (A). On day 2, cells were switched to medium B containing 100 μM sodium palmitate or 50 μM C6-ceramide in the absence or presence of 10 μM ISP-1 (*28*) as indicated. On day 2, 4 hours after addition of the various lipid regulators, cells were harvested, and aliquots of total cell protein [(A), 25 μg; (B), 15 μg] were subjected to SDS-PAGE and immunoblot analysis with monoclonal antibody to dSREBP (IgG-3B2) at 2 μg/ml as described (*1*). Filters were exposed to film for ~20 s. P and N denote precursor and cleaved nuclear forms of dSREBP, respectively. (**C**) RNAi-mediated reduction in either subunit of SPT (SPT-I or SPT-II) blocks ability of palmitate to inhibit cleavage of dSREBP. On day 0, S2 cells were suspended in medium C (Ultimate Insect Serum-Free Medium without serum and antibiotics) (*28*) containing the indicated dsRNA (50 μg/ml). One milliliter of cell suspension was plated at a density of 10^6 cells per 37-mm well as described (*1*). Six hours later, 2 ml of medium C containing 15% heat-inactivated FCS was added directly to each well. On days 1, 2, and 3, cells were resuspended, re-treated with dsRNA (50 μg/ml), and replated (*28*). On day 4, cells were switched to medium B in the absence (−) or presence (+) of 100 μM sodium palmitate. After incubation for 4 hours, cells were harvested, and aliquots of total cell protein (50 μg) were analyzed by immunoblotting as described. Filters were exposed to film for ~30 s. dsRNA for rat CYP7A1 was used as a control (*1*). SPT-I and SPT-II denote dsRNAs corresponding to the messages for subunits I and II of *Drosophila* SPT.

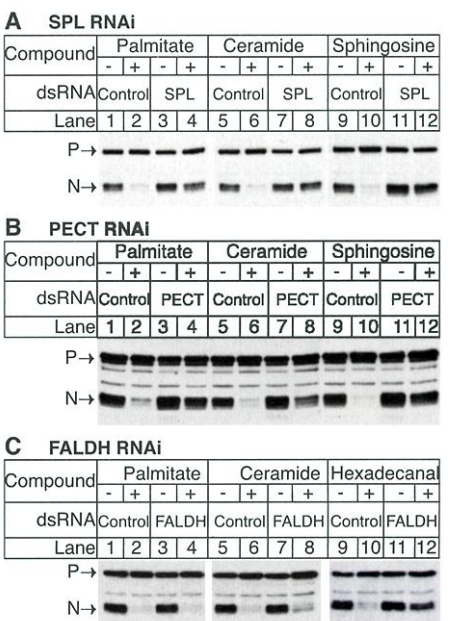

Fig. 3. Palmitate-mediated inhibition of dSREBP cleavage requires multiple enzymes in the PE biosynthetic pathway. S2 cells were treated three (**B**) or four (**A** and **C**) times with the indicated dsRNA (50 μg/ml) as described in Fig. 2C. On day 3 (B) or day 4 (A and C), cells were switched to medium B in the absence (−) or presence (+) of 100 μM sodium palmitate, 50 μM C6-ceramide, 50 μM sphingosine, or 100 μM hexadecanal (*28*) as indicated. After incubation for 4 hours, cells were harvested, and aliquots of protein (50 μg) were analyzed by immunoblotting as described in Fig. 2. Filters were exposed to film for ~40 s. P and N denote precursor and cleaved nuclear forms of dSREBP, respectively.

is the regulator, the possibility exists that a relative of PE, such as PE plasmalogen (*19*), is the active molecule. Against this hypothesis, neither palmitoyl alcohol nor stearoyl alcohol, biosynthetic precursors of PE plasmalogen (*20*), inhibited dSREBP processing in either the absence or presence of exogenous ethanolamine (*21*).

RNAi treatments directed against key enzymes in phosphatidylserine (PS synthase) and PC (phosphocholine cytidylyltransferase) synthesis had no effect on palmitate-mediated inhibition of dSREBP processing (*21*). These findings are consistent with a specific role of PE, as opposed to other phospholipids.

We used quantitative real-time polymerase chain reaction (PCR) assays to measure target mRNAs that are potentially down-regulated in response to PE (Fig. 5). We did this by comparing the effects obtained when nuclear dSREBP was eliminated in a regulatory fashion by the combination of palmitate plus ethanolamine (vertical axis) and when it was eliminated by RNAi directed against dSREBP (horizontal axis). The results are expressed relative to the expression level of each mRNA in control cells incubated without palmitate and ethanolamine or the dSREBP RNAi. We observed a high correlation (r = 0.76) when dSREBP was reduced by the two methods, which suggests that, together, palmitate plus ethanolamine acts solely by inhibiting nuclear dSREBP. We observed the most profound reductions (<30% of control) in enzymes of fatty acid biosynthesis [fatty acid synthase, acetyl-CoA synthase, and acetyl-CoA carboxylase (numbers 1–3)]. We showed previously that this reduction leads to a marked reduction in the overall rate of fatty acid synthesis (*1*). We also observed inhibition of enzymes involved in the synthesis of phospholipids [phosphocholine cytidylyltransferase, fatty acyl-CoA synthetase, and PECT (numbers 4–6)]. Elimination of dSREBP had no effect on mRNAs encoding enzymes of polyisoprenoid biosynthesis (numbers 8–10) or SCAP (number 11).

PE synthesis stimulated by palmitate and reduced by dSREBP RNAi. If the conclusions drawn from the above data are correct, then addition of palmitate should enhance the synthesis of PE in *Drosophila* cells, and this effect should be abolished when the sphingolipid pathway is blocked. To test this hypothesis, we measured the effect of palmitate on incorporation of [^{32}P]orthophosphate into ^{32}P-labeled phospholipids in S2 cells (Fig. 6, A–C). Palmitate (16:0) enhanced the synthesis of PE, but the closely related palmitoleate (16:1) and oleate (18:1) did not (Fig. 6A). The effect of palmitate was abolished in the presence of 10 μM ISP-1, an inhibitor of serine palmitoyltransferase, which indicates that palmitate was acting through the sphingolipid pathway (Fig. 6B). The effect of palmitate was specific for PE; there was no stimulation of the synthesis of phosphatidylglycerol (PG)

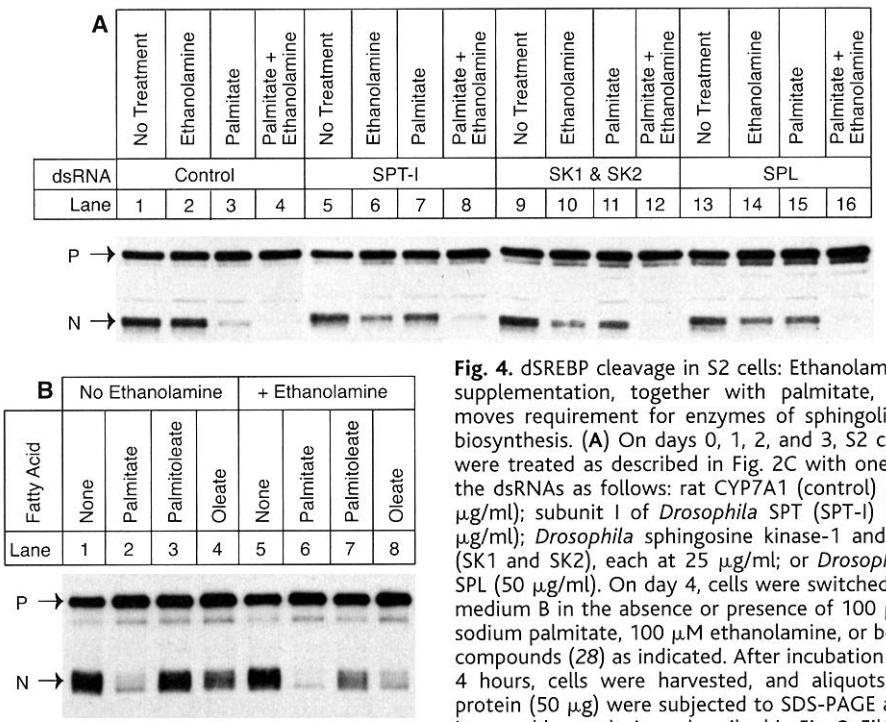

Fig. 4. dSREBP cleavage in S2 cells: Ethanolamine supplementation, together with palmitate, removes requirement for enzymes of sphingolipid biosynthesis. (**A**) On days 0, 1, 2, and 3, S2 cells were treated as described in Fig. 2C with one of the dsRNAs as follows: rat CYP7A1 (control) (50 μg/ml); subunit I of *Drosophila* SPT (SPT-I) (50 μg/ml); *Drosophila* sphingosine kinase-1 and -2 (SK1 and SK2), each at 25 μg/ml; or *Drosophila* SPL (50 μg/ml). On day 4, cells were switched to medium B in the absence or presence of 100 μM sodium palmitate, 100 μM ethanolamine, or both compounds (*28*) as indicated. After incubation for 4 hours, cells were harvested, and aliquots of protein (50 μg) were subjected to SDS-PAGE and immunoblot analysis as described in Fig. 2. Filters were exposed to film for ~40 s. (**B**) On day 0, S2 cells were set up as described in Fig. 2A. On day 2, cells were switched to medium B containing the indicated fatty acid (100 μM) in the absence or presence of 100 μM ethanolamine (*28*). Four hours later, the cells were harvested for immunoblotting as described in Fig. 2. Filters were exposed to film for ~5 s. P and N denote precursor and cleaved nuclear forms of dSREBP, respectively.

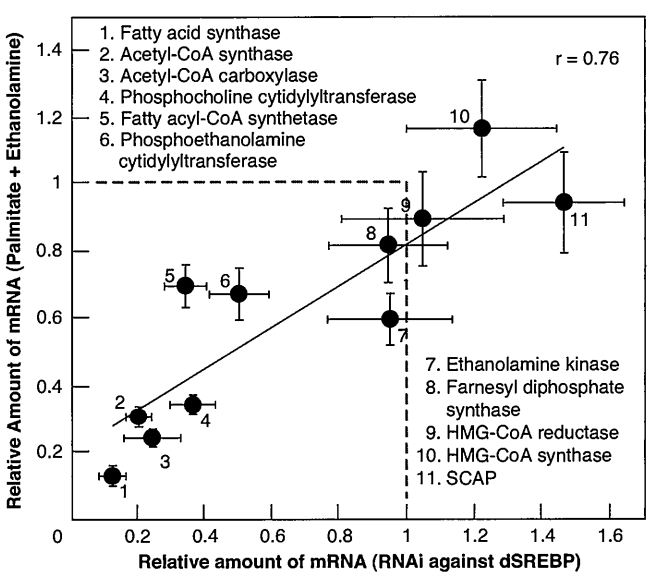

Fig. 5. Reductions in mRNAs encoding lipid biosynthetic enzymes in S2 cells treated with either palmitate plus ethanolamine (ordinate) or RNAi against dSREBP (abscissa). For the experiments with ethanolamine plus palmitate, cells were set up on day 0 in medium A, and 6 to 8 h later they were switched to serum-free medium B [1% (weight/volume) fatty acid–free bovine serum albumin instead of FCS] in the absence or presence of 100 μM sodium palmitate plus 100 μM ethanolamine. Cells were incubated for 20 hours, fed fresh medium again, and then harvested 4 hours later. For the RNAi experiments, cells were treated on day 0 once with dsRNA (50 μg/ml) directed against control mRNA (CYP7A1) or dSREBP mRNA as described in Fig. 2C and harvested 48 hours later. In both types of experiments, total RNA was isolated, and transcript abundance was determined by quantitative real-time PCR as described (*1*) with the use of primers shown in Supplementary table 1 (*28*). For each experiment, real-time PCRs were performed in triplicate. Isocitrate dehydrogenase mRNA was used as the invariant control. The amount of each mRNA is expressed relative to that in control cells, which is set at a value of 1. The average of two independent experiments is plotted for each treatment. Bars denote the range of values in the two experiments.

and/or ceramide phosphoethanolamine (CPE), PC, or phosphatidylinositol (PI) (Fig. 6C).

To demonstrate the role of dSREBP in phospholipid synthesis, we treated S2 cells with RNAi against dSREBP or the control CYP7A1 and then incubated the cells with [^{32}P]orthophosphate (Fig. 6D). RNAi against dSREBP reduced ^{32}P incorporation into PE by 60% compared with the control RNAi ($P < 0.001$) (Fig. 6D, left). Addition of ceramide enhanced PE synthesis in the control cells and restored PE synthesis to baseline levels in the dSREBP-deficient cells. RNAi against dSREBP also reduced PC synthesis by 45% (Fig. 6D, right), perhaps because of the reduction in the mRNA encoding phosphocholine cytidylyltransferase (Fig. 5). In contrast to PE synthesis, PC synthesis was not restored by ceramide.

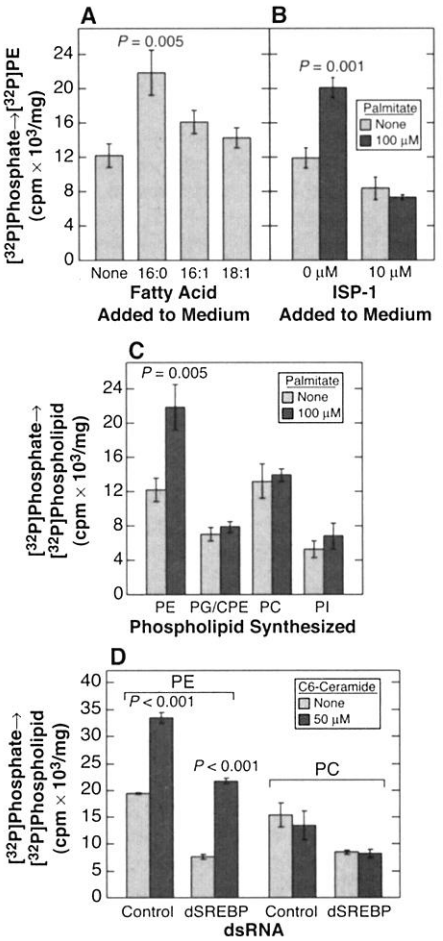

Fig. 6. Incorporation of [^{32}P]orthophosphate into phospholipids in S2 cells. (**A** through **C**) On day 0, S2 cells were set up at 10^6 cells per 37-mm well in medium A. On day 2, cells were switched to medium B containing the indicated fatty acid (100 μM) (A), 10 μM ISP-1 in the absence or presence of 100 μM sodium palmitate as indicated (B), or 100 μM sodium palmitate as indicated (C) (*28*). (**D**) On day 0, cells were set up and treated with the indicated dsRNA (50 μg/ml) as described in Fig. 2C. On day 2, cells were switched to medium B in the absence or presence of 50 μM C6-ceramide (*28*). (A through D) Each monolayer of cells received [^{32}P]orthophosphate at a final concentration of 10 μCi/ml. After incubation for 4 hours at room temperature, cells were harvested for measurement of phospholipid synthesis as described in Supplementary Material (*28*). Each value represents the mean ± SD of three incubations. Similar results were obtained in multiple independent experiments. *P* values were calculated according to Student's two-tailed *t* test.

Hypothesis: the SREBP pathway as a monitor of membrane lipids. Taken together, the data indicate that dSREBP controls membrane lipid production in *Drosophila* cells by regulating the synthesis of fatty acids and their incorporation into PE and PC. The activity of dSREBP is inhibited in a feedback fashion by the end product PE. The phosphoethanolamine component of PE can be derived from palmitate through the sphingolipid pathway, or it can be derived from external ethanolamine through EK. In contrast, in mammalian cells SREBP processing is controlled by cholesterol, and this regulates cholesterol synthesis (*22*).

Given the similarities in the pathway for SCAP-dependent proteolytic processing of SREBPs in *Drosophila* and mammalian cells, it is surprising that this pathway would be controlled by different end products. How can two different lipids such as cholesterol and PE regulate the same biological process? One clue emerges from a comparison of their physical properties. PE is a hexagonal (H_{II})-phase lipid (*23*, *24*). Unlike PC and PS, which form flat bilayers spontaneously on hydration, PE forms extended monolayer tubes in which the polar headgroups face inward. The exposed hydrophobic tails interact with those of other tubes to form stacked arrays that appear hexagonal in cross section. These structures have not been observed in vivo because the bilayer is stabilized by the presence of bilayer-forming lipids (*23*, *24*). However, some have speculated that the presence of H_{II}-phase lipids, such as PE in cell membranes, alters membrane structure and properties (*25*). This may be true in *Drosophila*, where PE is the predominant phospholipid in membranes (~55% of total for PE versus ~20% for PC) (*18*). In mammalian cells, on the other hand, the major membrane phospholipid is PC (~50% of total for PC versus ~20% for PE) (*23*). Although cholesterol does not form H_{II} structures in isolation, it is remarkable for its ability to induce H_{II}-phase formation in bilayer systems, especially those containing PE (*24*). These observations suggest that PE and cholesterol may perturb membranes through their tendency to form hexagonal structures. Inhibition of SREBP processing may result from such perturbations in the local environment surrounding SCAP.

References and Notes

1. A. C. Seegmiller, I. Dobrosotskaya, J. L. Goldstein, Y. K. Ho, M. S. Brown, R. B. Rawson, *Dev. Cell* **2**, 229 (2002).
2. M. S. Brown, J. L. Goldstein, *Cell* **89**, 331 (1997).
3. J. L. Goldstein, R. B. Rawson, M. S. Brown, *Arch. Biochem. Biophys.* **397**, 139 (2002).
4. X. Wang, R. Sato, M. S. Brown, X. Hua, J. L. Goldstein, *Cell* **77**, 53 (1994).
5. T. F. Osborne, *J. Biol. Chem.* **275**, 32379 (2000).
6. A. Nohturfft, D. Yabe, J. L. Goldstein, M. S. Brown, P. J. Espenshade, *Cell* **102**, 315 (2000).
7. P. A. Edwards, D. Tabor, H. R. Kast, A. Venkateswaran, *Biochim. Biophys. Acta* **1529**, 103 (2000).
8. A. J. Clark, K. Bloch, *J. Biol. Chem.* **234**, 2578 (1959).
9. A. H. Merrill, Jr. *et al.*, *Toxicol. Appl. Pharmacol.* **142**, 208 (1997).
10. R. C. Dickson, R. L. Lester, *Biochim. Biophys. Acta* **1438**, 305 (1999).
11. Y. A. Hannun, C. Luberto, K. M. Argraves, *Biochemistry* **40**, 4893 (2001).
12. S. Mathias, L. A. Pena, R. N. Kolesnick, *Biochem. J.* **335**, 465 (1998).
13. C. Kent, *Annu. Rev. Biochem.* **64**, 315 (1995).
14. P. P. van Veldhoven , G. P. Mannaerts, *Adv. Lipid Res.* **26**, 69 (1993).
15. W. B. Rizzo, *Mol. Gen. Metabol.* **65**, 63 (1998).
16. Y. Miyake, Y. Kozutsumi, S. Nakamura, T. Fujita, T. Kawasaki, *Biochem. Biophys. Res. Commun.* **211**, 396 (1995).
17. J. C. Clemens *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6499 (2000).
18. H. E. Jones, J. L. Harwood, I. D. Bowen, G. Griffiths, *Lipids* **27**, 984 (1992).
19. F. Snyder, in *Biochemistry of Lipids and Membranes*, D. E. Vance, J. E. Vance, Eds. (Benjamin/Cummings, Menlo Park, CA, 1985), chap. 9.
20. T. A. Rosenberger, J. Oki, A. D. Purdon, S. I. Rapoport, E. J. Murphy, *J. Lipid Res.* **43**, 59 (2002).
21. I. Y. Dobrosotskaya, A. C. Seegmiller, M. S. Brown, J. L. Goldstein, R. B. Rawson, data not shown.
22. Oxysterols, such as 25-hydroxycholesterol, are considerably more potent than cholesterol in inhibiting SREBP activity when added to tissue culture medium in ethanol dispersions (*4*). Oxysterols appear to achieve this regulation by causing cellular cholesterol to move from the plasma membranes to the ER (*26*, *27*), thereby allowing the cholesterol to block movement of the SCAP/SREBP complex (*6*).
23. P. Yeagle, *The Membranes of Cells* (Academic Press, San Diego, CA, ed. 2, 1993), pp. 1–349.
24. P. R. Cullis, M. J. Hope, in *Biochemistry of Lipids and Membranes*, D. E. Vance, J. E. Vance, Eds. (Benjamin/Cummings, Menlo Park, CA, 1985), chap. 2.
25. M. K. Storey *et al.*, *J. Biol. Chem.* **276**, 48539 (2001).
26. M. S. Brown, S. E. Dana, J. L. Goldstein, *J. Biol. Chem.* **250**, 4025 (1975).
27. Y. Lange, J. Ye, M. Rigney, T. L. Steck, *J. Lipid Res.* **40**, 2264 (1999).
28. Supplementary material is available on *Science* Online at www.sciencemag.org/cgi/content/full/296/5569/879/DC1.

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