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REVIEW

Roles of sphingolipids in *Drosophila* development and disease

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Abstract

The last 10 years have seen a rebirth of interest in lipid biology in the fields of *Drosophila* development and neurobiology, and sphingolipids have emerged as controlling many processes that have not previously been studied from the viewpoint of lipid biochemistry. Mutations in sphingolipid regulatory enzymes have been pinpointed as affecting cell survival and growth in tissues ranging from muscle to retina. Specification of cell types are also influenced by sphingolipid regulatory pathways, as genetic interactions of glycosphingolipid biosynthetic enzymes with many well-known signaling receptors such as Notch and epidermal growth factor receptor reveal. Furthermore, studies in flies are now uncovering unexpected roles of sphingolipids in controlling lipid storage and response to nutrient availability. The sophisticated genetics of *Drosophila* is particularly well suited to uncover the roles of sphingolipid regulatory enzymes in development and metabolism, especially in light of conserved pathways that are present in both flies and mammals. The challenges that remain in the field of sphingolipid biology in *Drosophila* are to combine traditional developmental genetics with more analytical biochemical and biophysical methods, to quantify and localize the responses of these lipids to genetic and metabolic perturbations.

Keywords: development, *Drosophila*, genetics, metabolism, nervous system, sphingolipids.

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Time was, when the worst nightmare of every student of *Drosophila* development was to find out that a laboriously cloned gene turned out to encode, in the words of one PhD advisor, 'probably *just* a lipase!' How things have changed over the course of the last 20 years, as the role of lipases and everything else having to do with lipid metabolism has moved from the realm of so-called housekeeping genes to center stage in one of the most exciting and current aspects of developmental and cell biology. Part of the reason for this decades-long lapse in general appreciation for lipids and their role in developmental events was surely because of the fact that fewer tools existed then (and now) to be able to visualize particular lipids in the developing animal, and to study their effects on morphological processes. With the low-hanging fruit of obvious embryonic phenotypes having been plucked the earliest, these difficult-to-analyze mutations in lipid-modulatory genes largely began to emerge only later.

This review focuses on work over the last decade that discovered novel roles of the sphingolipids, and how they feature in the development and health of the fly. With respect to sphingolipid biology, effects on normal embryonic and pupal development are only part of the news. Lipid regulation of such phenomena as energy household, lifespan, and degenerative processes that afflict adult animals were only recently made accessible by new screening and quantitative methods to detect these phenotypes. Now that these approaches have become routine, regulatory functions of sphingolipids in metabolic homeostasis are revealing themselves. Also coming of age is the part that lipids, in

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Abbreviations used: CIP, ceramide-1-phosphate; Dacer, *Drosophila* alkaline ceramidase; EGF-R, epidermal growth factor receptor; ERK, extracellular signal-related kinase; GalNAc, *N*-acetylgalactosamine; GalNAc-T, *N*-acetylgalactosaminyl transferase; GlcNAc, *N*-acetylglucosamine; GlcT-1, glucosylceramide transferase-1; JNK, c-jun N-terminal kinase; MAPK, mitogen-activated protein kinase; nCDase, neutral ceramidase; NPC, Niemann–Pick type C disease; PE, phosphoethanolamine; PI3K, phosphinositide-3-kinase; PLC, phospholipase C; S1P, sphingosine-1-phosphate; SBD, sphingolipid-binding domain peptide; SMase, sphingomyelinase; SMS, sphingomyelin synthase; spt, serine-palmitoyl transferase; SREBP, serum response element binding protein; TAG, triacylglycerol; TGFα, transforming growth factor α.

particular sphingolipids and sterols, may have in the development of progressive neurodegenerative disorders. We will discuss both the peculiarities of *Drosophila* sphingolipids and the commonalities with mammals that make the fly a good model in which to study nervous system development, degeneration, and metabolic disorders where sphingolipids and sphingolipid-regulated mechanisms figure prominently.

Fly-specific structures and species

Studying the functions of sphingolipid metabolic enzymes in *Drosophila* has an obvious genetic advantage in that flies usually have many fewer gene candidates for a particular enzymatic step, making analysis of mutant phenotypes much more clear-cut (Acharya and Acharya 2005). In addition, over-expression or knockout of a given gene in *Drosophila*, even in a tissue-specific way and in any desired combination, is experimentally straightforward – a matter of weeks or months of work, not years. On top of this, since *Drosophila* larvae are very reliable and consistent eating machines, nutrient conditions, or drugs that might alter sphingolipid metabolism are very easy to administer to flies in the larval stage, simply by adding agents to the food (Adachi-Yamada *et al.* 1999; Mair *et al.* 2005; Ye *et al.* 2007; Vargas *et al.* 2010).

Many of the major sphingolipid biosynthetic and processing enzymes have known orthologs in *Drosophila*, including all the ones required for the production of sphingosine and ceramide, as well as those involved in generating metabolites of and breaking down these sphingolipids, e.g. ceramide kinase and different ceramidases (CDase; Adachi-Yamada *et al.* 1999; Acharya *et al.* 2003; Gorski *et al.* 2003; Fyrst *et al.* 2004; Herr *et al.* 2004; Rohrbough *et al.* 2004; Acharya and Acharya 2005; Bauer *et al.* 2009). Sphingosine-metabolizing enzymes, such as the sphingosine kinases, which generate the important signaling molecule sphingosine-1-phosphate (S1P), and S1P-lyase which breaks it down, have interesting developmental defects when absent, and these are discussed further below. A comprehensive review on sphingolipid-metabolizing enzymes in the fly, and information on the suspected or known orthologs is available (Acharya and Acharya 2005). There are important differences in lipid structure, however, the length of the sphingoid base product of serine-palmitoyl transferase (spt) in *Drosophila* being much shorter, generally containing only 12 carbons derived from laurate instead of the 16 carbons from palmitate as in mammals. This gives a sphingoid base alkyl chain of C14 instead of C18, whereas the fatty acyl chain, which is attached by an amide linkage to the sphingoid base, is quite long, with C20 being the major species (Fyrst *et al.* 2004; Acharya and Acharya 2005; Fig. 1). Shorter chain length may have the advantage of not solidifying at the preferred fly body temperature of ~ 25°C (Holthuis *et al.* 2001).

In spite of the differences in structure of fly sphingolipids, and the lower abundance of sterols in fly membranes, sphingolipid- and sterol-rich microdomains have been documented by several authors in *Drosophila* cells and tissues (Rietveld *et al.* 1999; Karpen *et al.* 2001; Zhai *et al.* 2004; Hebbar *et al.* 2008), and the raft-associated adaptor proteins reggie/flotillins are expressed in flies (Stuermer *et al.* 2001; Hoehne *et al.* 2005). So-called 'detergent resistant membranes' can be isolated from fly cell membranes, and the lipid

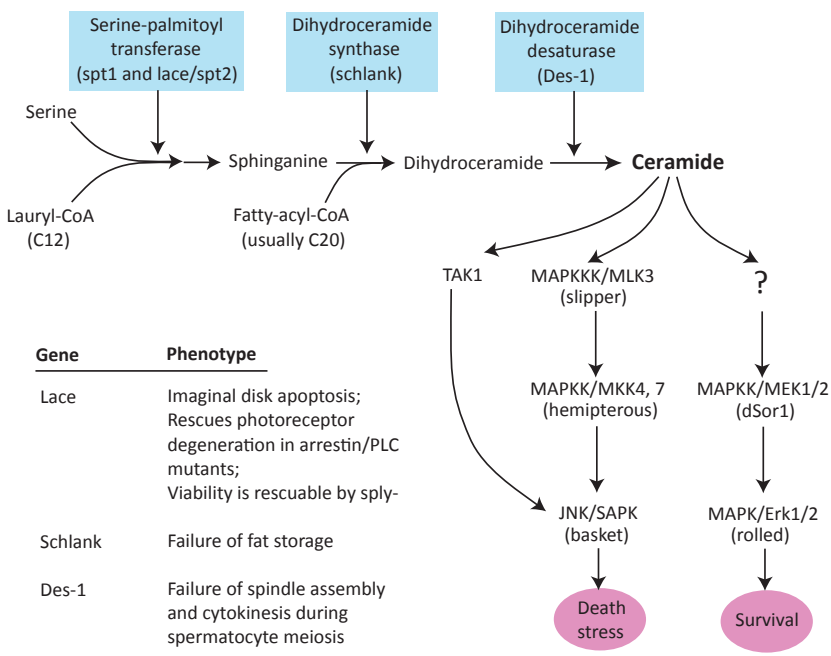


Fig. 1 Ceramide biosynthesis in *Drosophila*. In each figure, enzyme names and the corresponding fly homologs (in parentheses) are boxed in blue; physiological effects of the given pathways are circled in purple. Mutant phenotypes of the given genes and genetic interactions are listed in the tables at the bottom of the figures.

content of these membranes roughly corresponds to what would be expected if as in mammals, these domains contain sphingolipids and sterol. Moreover, detergent resistant membranes from fly membranes co-segregate with markers thought to associate with ordered sphingolipid-containing domains (Zhai *et al.* 2004; Hebbar *et al.* 2008). Sterol depletion by methyl-β-cyclodextrin results in ∼ 50% reduction in sterol levels in cultured *Drosophila* cells, and also changes the distribution of markers, such as flotillin and sphingolipid-binding domain peptide (SBD; Hebbar *et al.* 2008; Steinert *et al.* 2008). Sterol was also required for translocation of the InaD-containing phototransduction machinery in fly photoreceptors (Sanxaridis *et al.* 2007), and certain neurotransmitter receptors and ion channels likewise appear to be activated by raft domains (Eroglu *et al.* 2003; Gasque *et al.* 2005). Depletion of sterols or prevention of sphingolipid biosynthesis using the fungal antibiotic Fumonisin B1 prevents recognition of fly neuronal cells by the SBD peptide, suggesting that Fumonisin B1 may be able to inhibit production of sphingolipids in *Drosophila* in a functionally analogous way to its inhibition of dihydroceramide synthase in mammalian cells (Merrill *et al.* 1996; Hebbar *et al.* 2008).

Sphingolipid metabolizing enzymes in fly

Even though flies appear to make negligible amounts of sphingomyelin, there are several orthologs of sphingomyelin synthases (SMSs) in the fly genome (Huitema *et al.* 2004; Tafesse *et al.* 2006), that are candidate enzymes for the synthesis of the much more abundant species, phosphoethanolamine ceramide (PE-ceramide). Sphingomyelin synthase candidate orthologs were identified in *Drosophila* by virtue of their domain similarity to yeast AUR1 which synthesizes the yeast equivalent of sphingomyelin, inositol-phosphoryl-ceramide (Dickson 1998), and the presence of conserved domains found in lipid phosphate phosphatases (Huitema *et al.* 2004). A recent study determined that one of these synthases, SMSr (sphingomyelin synthase 1-related) can synthesize PE-ceramide, but does not do this *in vivo*. In fact, it functions in mammalian and fly cells as a sensor and regulator of ceramide homeostasis, which prevents accumulation of ceramide in the endoplasmic reticulum. In the absence of this protein, ceramide builds up and leads to fragmentation of Golgi stacks (Vacaru *et al.* 2009). The more likely candidate for the actual PE-ceramide generating enzyme is evidently one of the other three SMS homologous genes.

A dihydroceramide desaturase was identified in flies, and named Des-1 because of its degenerative spermatocyte phenotype (Basu and Li 1998). In spermatocytes, Des-1 has a role in spindle assembly and meiosis, and seems to associate strongly with mitochondria and with the contractile ring during division, suggesting that the production of ceramide might be important somehow in anchoring cytoskeletal elements. This is reminiscent of the more recent observations by Bieberich's group that ceramides are present in and required for the formation of cytoskeleton-dependent structures like cilia and tight junctions in epithelia (Wang *et al.* 2009a,b).

Other sphingolipid biosynthetic and processing enzymes have been identified in the fly, including ceramide transfer protein, which as in mammals is required to move ceramide from the site of its synthesis in the endoplasmic reticulum to the Golgi, where it is further processed to glycosphingolipids and, in the case of flies, PE ceramide instead of phosphocholine-ceramide (sphingomyelin). Ceramide transfer protein mutations in the fly, as might be expected given the membrane-gelling properties of ceramide, result in phenotypes related to altered fluidity of membranes, including heightened sensitivity to oxidative stress and premature aging (Rao *et al.* 2007; Goñi and Alonso 2009). This outcome has interesting implications for the use of flies to study the role of ceramide in age-related diseases, such as neurodegeneration and type II diabetes.

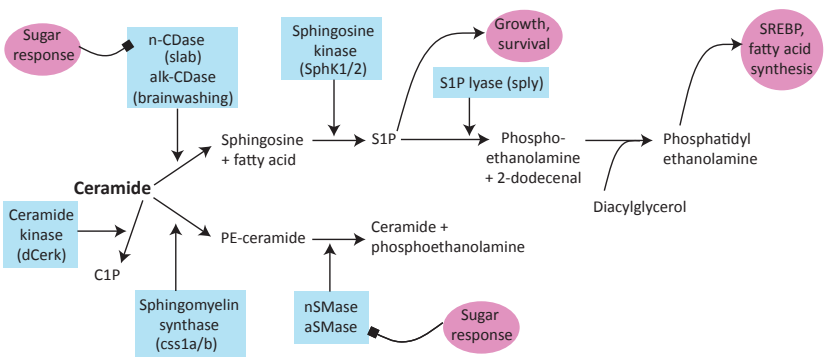
Life, death, and sphingolipids

One of the first sphingolipid metabolic enzymes to be described in *Drosophila*, and its loss of function phenotype studied was serine palmitoyl transferase (spt), the enzyme that is responsible for the rate-limiting step in de novo sphingolipid biosynthesis. A mutant in the spt2 subunit, known as *lace*, was identified because it suppressed hyperactivation in the growth-mediating mitogen-activated protein kinase (MAPK)/extracellular signal-related kinase (ERK) pathway, achieved by over-expressing the fly MAPK and MAPKK orthologs *rolled* and *dSor1* (Adachi-Yamada *et al.* 1999; Fig. 1). Spt2/lace mutants showed abnormal development and ectopic apoptosis in imaginal discs triggered by c-jun N-terminal kinase (JNK) activity (*basket* in flies), and these effects were rescued by increased signaling through the opposing MAPKK survival pathway. These interactions imply that ceramide or its metabolites might suppress a suppressor of the MAPK/ERK survival pathway such that removal of *spt* would reduce *dSor1* and *rolled* hyperactivation. A candidate for this might be the transforming growth factor β (TGFβ)-activated kinase 1 homolog TAK1, which is activated by ceramide and regulates both the JNK pathway in flies (Takatsu *et al.* 2000) and the MAPK/ERK pathway (Sharma and Shi 1999; Zhang and Dong 2007). Alternatively, a sphingolipid metabolite might activate the presumptive ras/raf initiator of this pathway (Raabe 2000), such that removing *spt* would turn down signaling. Interestingly, the lethality of *lace* was rescued by feeding sphingosine during larval growth, but not by feeding sphingomyelin or ceramide, suggesting that sphingosine or an immediate metabolite may activate the MAP kinase kinase kinase → MAPK/ERK

kinase (dSor1) → Erk (rolled) survival pathway. A second MAPKKK upstream of death-mediating JNK, the mixed-lineage kinase (dMLK) was found to be controlled by ceramide in *Drosophila* S2 cells (Sathyanarayana *et al.* 2002). Thus, the one product of sphingolipid metabolism, sphingosine, or more likely its metabolite S1P, mediates survival and prevents apoptosis, whereas the other, ceramide, induces death (Spiegel and Milstien 2002; Fyrst and Saba 2010). Presumably, since the activities of sphingosine and S1P have extremely different consequences for cell survival (Hannun and Obeid 2002), and the concentrations of these molecules differs greatly in the cell, the conversion by sphingosine kinases must be highly regulated. Likewise the single CDase step that converts ceramide to sphingosine must be tightly controlled. Indeed, other ceramide conversion pathways also rescue cell survival, e.g. to glycosylated ceramides by the glucosylceramide synthase GlcT-1, and the *N*-acetylgalactosamine-transferase (GalNAc-T) α4GT1. GlcT-1 over-expression prevents induction of apoptosis by reaper and grim, and RNAi knockouts in GlcT-1 result in widespread embryonic cell death (Kohyama-Koganeya *et al.* 2004). Likewise, α4GT1 was shown to be an inhibitor of apoptosis in the eye (Protzer *et al.* 2009).

Another case in which the balance of sphingolipid metabolites regulates cell growth versus death during development was revealed by the loss of S1P-lyase in *sply* mutants. Sply, unlike most other sphingolipid metabolic enzymes, irreversibly removes sphingolipids from circulation by converting S1P to 2-dodecenal and PE, so mutants accumulate huge excesses in both S1P and unphosphorylated sphingoid base metabolites (Herr *et al.* 2003; Fyrst and Saba 2010; Fig. 2). *sply* animals showed severely abnormal flight muscles and excessive apoptosis in reproductive organs, which could be suppressed by mutation in pro-apoptotic genes *reaper* and *hid* (Herr *et al.* 2003; Phan *et al.* 2007). This suggested that although there is an overabundance of sphingosine in mutants, the death knell in *sply* may be dealt ultimately by ceramide, since *reaper* expression can lead to the production of ceramide (Bose *et al.* 1998). Reduction in *lace* (spt2) strongly reduced *sply* sphingosine/S1P accumulation, as well as its muscle phenotype. Conversely, *sply* mutations also rescued viability in the *lace* mutants, supporting the idea that *lace* apoptosis and lethality may be primarily brought about by a loss of sphingosine or S1P, whereas *sply* defects presumably arise from excess sphingosine or S1P. Mutations in sphingosine kinases, of which there are two in *Drosophila*, *Sphk1* and *Sphk2*, should clear up the ambiguity in the effects of these two metabolites, since they should only accumulate sphingosine, but lack S1P. *Sphk2* mutants in fact impair flight and reduce fecundity, but do not resemble *sply* mutants in which the ovaries and testes degenerate. Rather, *Sphk2* mutants retain eggs (Herr *et al.* 2004). Thus, it would appear that accumulation of sphingosine alone cannot explain the defects in *sply*.

With regard to sorting out the roles of the phosphorylated and non-phosphorylated sphingoid base metabolites, it will also be interesting to find out whether the lipid phosphate phosphatases *wunen* and *wunen2* (Zhang *et al.* 1997; Starz-Gaiano *et al.* 2001), among whose targets may be S1P and/or ceramide-1-phosphate (C1P), exhibit similar growth or apoptotic defects to the *sply* mutants described earlier, which result in excesses of both sphingosine and S1P (Herr *et al.*



Gene	Phenotype
dCerk	Phototransduction defect; Fail to localize PLC-β; Effect on PIP2 distribution in photoreceptor membrane
nCDase	Failure of synaptic vesicle fusion and synaptic transmission; Overexpression rescues retinal degeneration in arrestin and PLC mutants; Down-regulated upon sugar feeding

Gene	Phenotype
alkCDase	Increase in ceramide levels; Increased resistance to oxidative stress; Increased lifespan; Defective mushroom and ellipsoid body.
SphK1/2	Flight impairment; Reduced fecundity
sply	Increased sphingosine, S1P levels; Increased Δ4,6-sphingadienes; Flight muscle degeneration; Apoptotic ovaries and testes; Rescuable by mutation in lace

Fig. 2 Biosynthetic pathways of ceramide metabolites sphingosine-1-phosphate (S1P) and phosphoethanolamine (PE)-ceramide, and sphingolipid catabolism via S1P lyase. SREBP, serum response element binding protein activation.

2003). So far, these genes, which are expressed in the nervous system, are known to affect migration of embryonic germ cells by controlling the release of a repellent; notably, the expression of these genes can not only repel the germ cells, but also kill them (Hanyu-Nakamura *et al.* 2004; Sano *et al.* 2005). It is very likely that the *wunen* genes have other functions in the nervous system, since they were also identified in a screen for genes that when over-expressed in the nervous system lead to altered patterns of neuromuscular connectivity (Kraut *et al.* 2001). This finding, along with a subsequent report that S1P affects axon guidance in the visual system of Xenopus (Strochlic *et al.* 2008), may point to S1P as being at least one target of the wunens.

Recently, the same group as cited earlier (Fyrst *et al.* 2009) detected a novel species of sphingolipid that was present during *Drosophila* development, namely sphingadienes having two double bonds (designated $\Delta^{4,6}$) instead of the usual one ($\Delta^{4)}$ in the sphingoid base. These were also strongly over-expressed in *sply* mutants, and similarly to the previously characterized Δ^4 monounsaturated sphingoid bases in *sply*, were brought back toward normal levels by introduction of a *lace* (spt2) mutation (Fyrst *et al.* 2008). Interestingly, sphingadienes were shown to be powerful anti-growth agents and suppressors of tumor cell proliferation via their suppression of Akt kinase; conversely, they were inducers of apoptosis accompanied by caspase activation and poly ADP-ribose polymerase cleavage. At the same time, sphingadienes localized with autophagosomal markers, and induced autophagy, which when dysregulated can also lead to cell death (Fyrst *et al.* 2009; Pattingre *et al.* 2009b). Thus, it was postulated that the overabundance of $\Delta^{4,6}$sphingadienes and corresponding $\Delta^{4,6}$ceramides were responsible for the muscle degeneration in the *sply* mutants; indeed these compounds were able to decrease cell proliferation when added to cultured wing disc cells, and were extremely enriched (28×) in mutants in exactly the muscles that were most susceptible (Fyrst and Saba 2008).

Ceramidase, sphingomyelinase, and nutrient metabolism

In mammals, sphingolipid balance has a central role in controlling nutrient utilization and growth (Unger 2003; Holland *et al.* 2007). In particular, the breakdown of sphingomyelin at the plasma membrane by neutral and acid sphingomyelinases (Bartke and Hannun 2009), which produces ceramide, has powerful effects on the storage versus export of cholesterol (Slotte and Bierman 1988; Ridgway 2000), on glucose uptake (Summers *et al.* 1998; Al-Makdissy *et al.* 2003; Liu *et al.* 2004), and on the storage of fats in the form of triacylglycerols (TAG; Deevska *et al.* 2009). Sphingolipids are also activators of serum response element binding protein (SREBP) signaling, which controls biosynthesis of fats and cholesterol in mammals (Scheek *et al.* 1997; Horton *et al.* 2002; Worgall 2008). Feeding rodents with saturated fat, as found in lard, or with pure palmitate supplemented in the food leads to drastic changes in fat metabolism and insulin resistance, owing to conversion of the fatty acid to sphingolipid (Holland *et al.* 2007). The key metabolite doing the damage is thought to be ceramide, through its repressive effects on protein kinase B/Akt and insulin downstream signaling, since these effects can be mitigated by blocking spt activity with myriocin.

Starting in the 1970s, experiments were done with feeding different high-fat diets, including palmitate, soy, and beef to flies (Driver and Cosopodiotis 1979; Ye *et al.* 2007). The high-palmitate diet led to shortened lifespan, suggesting possible involvement with insulin-like growth factor/phosphinositide-3-kinase (PI3K) signaling that is known to control longevity in flies (Britton *et al.* 2002). Since palmitate in flies also feeds into the sphingolipid biosynthetic pathway, whose immediate effects would be to increase levels of ceramide, one might expect the PI3K signaling to be suppressed, as in the Summers model (Dobrosotskaya *et al.* 2002; Holland and Summers 2008) where ceramide activates protein phosphatase 2A thereby dephosphorylating and down-regulating protein kinase B/Akt (Dobrowsky and Hannun 1992). It is clearly established that suppression of insulin-like growth factor/PI3K signaling extends lifespan (Broughton and Partridge 2009), but it is possible that very high doses of saturated fat and/or ceramide, as an acute stress and apoptotic signal, might also introduce other toxic or senescence-promoting signals (like sphingosine) that tip the balance toward decrepitude rather than long life (Hannun and Obeid 2008). High saturated fat diets may also be inflammatory and result in oxidative stress in flies, since fat-induced shortening of lifespan in flies was suppressible by the anti-oxidant Acai fruit (Sun *et al.* 2010). Acai not only increased transcription of stress-response detoxification genes, but in a fat-fed background, it also strongly lowered the levels of phosphoenolpyruvate carboxykinase, the first committed step in gluconeogenesis.

In flies, it is not known whether a sphingolipid-dependent pathway similar to that identified in rodents interferes with insulin signaling and could lead to a diabetic phenotype. However, it is intriguing that changes in sphingolipid metabolic genes were part of the transcriptional response to sugar feeding (but not starvation) of larvae in a study done by the Pankratz lab (Zinke *et al.* 2002). This microarray study identified an acid Sphingomyelinase (aSMase; CG15533) and a neutral CDase (nCDase/*slab*, CG1471) both as being down-regulated after sugar feeding, whereas fatty acid synthesis was up-regulated. This result would imply that the products of SMase and CDase, namely ceramide and sphingosine, may have some role in down-regulating fatty acid synthesis, and should be avoided if the desired

metabolic response is increased glucose and fatty acid storage.

Along the same lines, several exciting new studies clearly point to ceramide as playing a regulatory role in fat storage in flies. A fly homolog of the longevity assurance genes, a family of six mammalian dihydroceramide synthases responsible for the synthesis of the various chain-length ceramides (Pewzner-Jung *et al.* 2006), was identified by virtue of its abnormally thin larval phenotype in mutants. This gene, *schlank* (meaning slender in German), seems to be the functional homolog of the lass genes in the fly, since only one other homolog was found, and it does not rescue the *schlank* fat storage defect (Bauer *et al.* 2009). *schlank* mutants fail to bulk up during larval development, because of their suppression of fat storage in the form of triacylglycerides (TAGs), of which they have strongly reduced levels. This matched the up-regulation in *schlank*- animals of the TAG lipase encoded by *brummer* (Grönke *et al.* 2005), which is turned on by starvation conditions, and the TAG lipase3 gene, an indicator of the starvation response (Zinke *et al.* 1999; Fuss *et al.* 2006). Thus, one of the normal functions of ceramide synthesis by *schlank* is to participate in the accumulation of fat stores, and in keeping with this, overexpression of *schlank* gives somewhat higher levels of di- and triglycerides, fatty acids, and increased transcription of both SREBP and fatty acid synthase (Bauer *et al.* 2009). These results are perhaps consistent with the findings of Zinke *et al.*, where the response to sugar feeding would be to decrease nCDase levels, if ceramides are required to convert sugars into fats via fatty acid synthase and SREBP. Interestingly, *sply* mutants in mice, similarly to *schlank* in flies, have reduced adiposity, although they show increased lipid storage in the serum and liver, suggesting that the causative metabolite that leads to such a pathology could be excess sphingosine or sphinganine (see Figs 1 and 2) rather than strictly loss of ceramide (Bektas *et al.* 2010). However, a fat storage defect in *sply* flies was not noted.

Ceramide: death molecule or savior?

Interesting insight into the effects of ceramide on developmental maturation as well as oxidative stress and lifespan was provided by a recent paper that analyzed *Drosophila* alkaline CDase (Dacer) mutants (Yang *et al.* 2010). This CDase was previously identified as *brainwashing*, which affects the mushroom and ellipsoid bodies in the brain (Boquet *et al.* 2000). First, the current authors showed that Dacer does indeed have alkaline CDase activity, and that ceramide levels increase in mutants. Dacer mutants also significantly increased the activity of an enzyme that is required for the production of juvenile hormone, an epoxide that is required for larval growth and prevention of metamorphosis (Postlethwait 1974). Larval developmental time in *Dacer* mutants was accordingly lengthened by about 50% compared with wild type. In contrast to *dCert* mutants, which fail to transport ceramide to the plasma membrane (discussed earlier; Rao *et al.* 2007), loss of Dacer led to an increase in stress resistance. Interestingly, and similarly to yeast that are deficient in alkaline CDase, it also lengthened lifespan substantially (Powers *et al.* 2006; Yang *et al.* 2010). The interpretation of this study is complex, since excess ceramide is generally associated with increased apoptosis and deleterious effects, including shortened lifespan (see above; Kohyama-Koganeya *et al.* 2004; Hannun and Obeid 2008), whereas in this case an increase appears to be beneficial. Perhaps ceramide, as a stress-response coordinator and activator in the MAP kinase kinase kinase → JNK pathway (Ruvolo 2003; Fig. 1), is acting to mobilize defenses against acute stress (Hannun and Obeid 2002).

Although the relationships between sphingolipids and nutrient storage, oxidative stress, and lifespan in flies are as yet far from clear, the ability to manipulate at will the genetic background in these animals should allow one to determine definitively which sphingolipid metabolites are responsible for modulating these pathways.

Ceramide metabolites and neuronal function

A study carried out by Acharya *et al.* (2003) suggested that eliminating ceramide could be protective under certain circumstances, in this case in the background of arrestin and phospholipase C β (PLC; *norpA*) mutations that lead to photoreceptor degeneration in the fly eye (Acharya *et al.* 2003). Lowered ceramide levels were achieved by targeted over-expression of the nCDase in the eye, as well as by introducing the *lace* (spt2) mutation, which decreases de novo synthesis of ceramide (Adachi-Yamada *et al.* 1999). In both cases, the measured decrease in ceramide levels clearly rescued the degenerative phenotype, which is brought about by hyperstable arrestin–rhodopsin complexes and an accumulation of defective rhabdomeric membranes (Alloway *et al.* 2000; Kiselev *et al.* 2000). Both of these genetic backgrounds seemed to exert their effect through a dynamin-dependent process, suggesting that active endocytosis is particularly important in maintaining the health of neurons, especially photoreceptors. A later study using mutants in the same nCDase (Acharya *et al.* 2008), which themselves showed photoreceptor apoptosis, came out with the surprising result that the effect of CDase on photoreceptor neurons is non-cell autonomous, and is able to act from as far away as the fat body.

Possibly related to the findings above on the effects of CDase on membrane dynamics and turnover, the group of Broadie *et al.* found that it is also important for synaptic function of the neuromuscular junction in *Drosophila* (Rohrbough *et al.* 2004). *Slab* (*slugabed*) mutants in the same nCDase gene that was studied by Acharya *et al.*, although they did not show increased apoptosis in the

nervous system, had impaired pre-synaptic neuronal transmission and faulty synaptic vesicle fusion, as well as a shortage of fusion-ready vesicles.

The Broadie laboratory's findings with *slab* corroborate the reported role of sphingosine, the product of CDase, in exocytic vesicle fusion events, as suggested initially by genetic studies in yeast, where mutants in the synaptobrevin ortholog *snc* are rescuable by over-expressing the dihydrosphingosine phosphate lyase DPL1 (Grote *et al.* 2000), or by combining with certain mutations in ELO genes that are responsible for the elongation of very long chain fatty acids from which ceramides are synthesized (David *et al.* 1998). Darios *et al.* (2009) recently demonstrated how this works using an *in vitro* system that identified sphingosine as the key lipid: sphingosine allows the release of the v-SNARE (vesicular-soluble *n*-ethylmaleimide sensitive factor attachment protein receptor) synaptobrevin from association with phospholipids in the synaptic vesicular membrane such that SNARE fusion complexes can be formed with syntaxin/SNAP-25 (synaptosomal-associated protein 25) (reviewed in Verhage and van Meer 2009). Ceramides probably affect synaptic growth and morphology as well, by dint of their activation of autophagy (Pattingre *et al.* 2009a). Autophagy was reported to promote expansion of the larval neuromuscular junction, and this was mediated once again through MAPK pathway members *wallenda* (a fly MAPKKK) and *basket* (JNK; Collins *et al.* 2006; Shen and Ganetzky 2009).

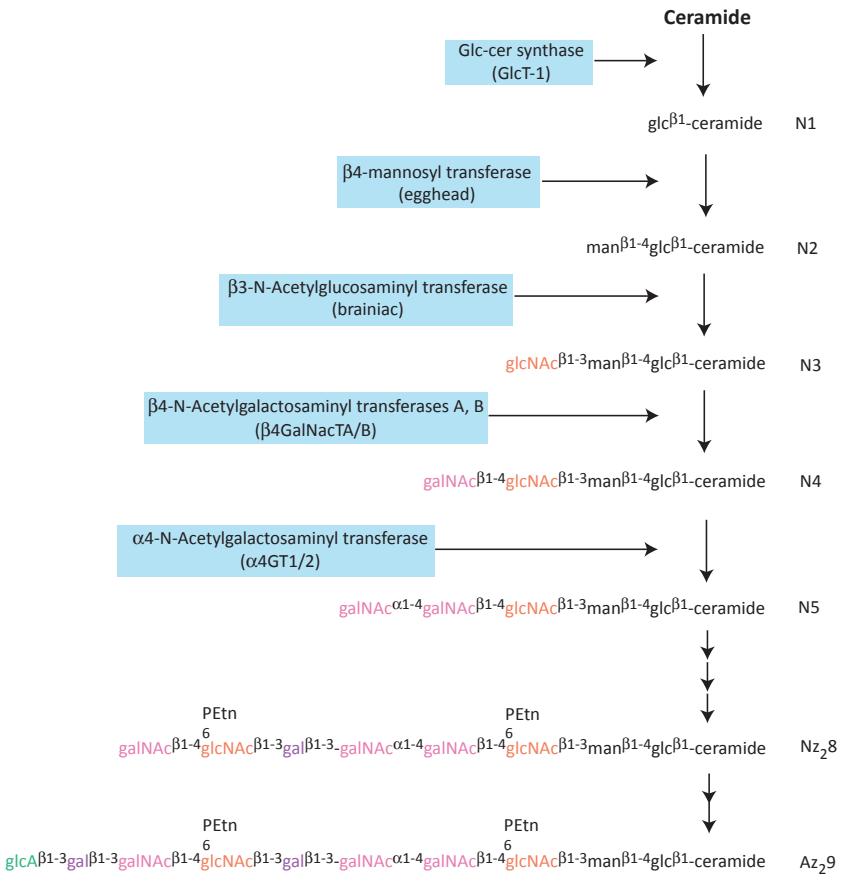
Ceramide metabolites have further functions in photoreceptors beyond maintaining membrane turnover and preventing degeneration. Dasgupta *et al.* (2009) showed that the product of ceramide kinase, C1P, is involved in phototransduction by PLC activity, and alters the balance and distribution of phosphatidylinositol-4,5-bisphosphate at the membrane. Ceramide kinase (*dcerk*) mutants, which lack C1P but have more ceramide, fail to localize PLC to membranes. PLC thus degrades, resulting in an excess of phosphatidylinositol-4,5-bisphosphate (PIP2) (Fig. 2). This work stands out in that it is one of the few that combines genetic methods with biophysical tests – in this case, fluorescence image correlation spectroscopy on supported lipid bilayers – to try to relate the behaviors of sphingolipids in membranes with their physiological functions in a biological process like phototransduction. This is an approach that will surely reap insight into the interplay between the interesting membrane properties of sphingolipids and their effects on signaling and developmental events.

Glycosphingolipids in development and the nervous system

Arthropod glycosphingolipids have been known for many years (Wiegandt 1992), but their composition specifically in *Drosophila* was only determined recently by Seppo *et al.* (2000) and Wandall *et al.* (2003, 2005) using TLC. Fly glycosphingolipids are quite different from their mammalian counterparts in that the core disaccharide consists of $Man^{\beta 1-4}Glc^{\beta 1}$-Cer, aka mactosyl-ceramide, instead of $Gal^{\beta 1-4}Glc^{\beta 1}$-Cer in mammals (Seppo and Tiemeyer 2000; Fig. 3). There are 12 major species of glycosphingolipid, none of which are sialylated as in mammals, but many containing one or two PE-substituted-GlcNAc(Seppo *et al.* 2000). Glycosphingolipids can be very long in flies, the longest species having 10 sugar residues (GlcNAc, GalNAc, galactose, or a terminal glucuronic acid) in a linear chain.

The first evidence that glycosphingolipids have major effects on well-known developmental pathways in flies came from the identification of two mutants, called *egghead* and *brainiac*, so named because they had embryonic neuronal hypertrophy phenotypes similar to *Notch* mutants (Goode *et al.* 1992). In addition to Notch-like phenotypes, *egghead* and *brainiac* led to identical defects in polarity and adhesion of oocyte follicular epithelial cells, which was proposed to arise from an interaction with epidermal growth factor receptor (EGF-R), the receptor mediating a back-and-forth signaling between the developing oocyte and the surrounding follicle cells (Goode *et al.* 1996a,b; Nilson and Schupbach 1999). *Egghead* and *brainiac* turned out to encode the glycosyl transferases responsible for sequential addition of the second and third sugar residues, mannose and GlcNAc, that form the core of all *Drosophila* glycosphingolipids, and whose products in insect cells were determined by TLC, mass spectrometry, and NMR *in vitro* (Müller *et al.* 2002; Schwientek *et al.* 2002; Wandall *et al.* 2003) and *in vivo* (Wandall *et al.* 2005). Interestingly, the blockage of the core mactosyl-ceramide and the resultant phenotype in *egghead* animals could be fully rescued by introducing a transgene for the human galactosyltransferase enzyme, which makes lactosyl ceramide (Wandall *et al.* 2005). Apparently, flies can function perfectly well with the mammalian lactosyl core instead of their own mannose-containing core.

Enzymes that catalyze further steps in more complex glycosphingolipids have also been identified, among them the β4-*N*-GalNAc transferases A and B, which in the case of transferase A (*GalNAc-T A*) mutants alone, had neuromuscular innervation and coordination defects (Haines and Irvine 2005; Haines and Stewart 2007). Unlike the *egghead* and *brainiac* deficiencies in core glycolipids, flies seem to be able to do without these GalNAc-ylated complex glycolipids for most essential functions, as the β*4GalNAc-TA, B* double mutants are viable and fertile (Haines and Irvine 2005; Stolz *et al.* 2008). The group of Cohen, however, not only confirmed the results of Haines *et al.* that transferase A displays un-coordination and abnormal climbing behavior, but that the other transferase mutant β*4GalNAc-TB* showed ventralized ovarian follicles similar to *egghead*, *brainiac*, and EGF-R knockouts (Chen *et al.* 2007), and moreover that both of these phenotypes interacted genetically with *egghead* and *brainiac*. Further, it was shown that the transferase acts



Gene	Phenotype
GlcT-1	Apoptosis in embryo; overexpression suppresses apoptosis induced by reaper and grim
Egghead/ brainiac	Notch-like neuronal hypertrophy; Defective EGF-R signaling in oocyte (ventralized follicle cells)
β4GalNacTA/B	(A) Defective neuromuscular junction innervation and coordination; (B) Similar to egghead/brainiac defective oocyte
α4GT1/2	Overexpression suppresses Mindbomb Notch-like phenotype and inhibits apoptosis in eye disk (no phenotype alone)

Fig. 3 Glycosphingolipid biosynthesis in *Drosophila*. Notation with *N* = number of sugar residues in the neutral core, and *z* = number of phosphoethanolamine (PEtn) substituted *N*-acetylglucosamine (GlcNAc) residues, is according to Seppo *et al.* (2000). Shorter variants with fewer sugars (colored) also exist, but are not shown.

in the oocyte, which produces TGFα-like ligand *gurken* and secretes it to the follicle cells, leading to the speculation that N4 or higher order glycosphingolipids might be involved in the secretion or distribution of the TGFα-like signal, or interact with its EGF-R receptor (Chen *et al.* 2007). In fact, a follow-up study by the same group demonstrated that glycosphingolipid is required for the distribution of the *gurken* ligand gradient, but not for its secretion or for EGF-R activity *per se* (Pizette *et al.* 2009).

An α1,4GalNAc-T in flies has also been identified (Protzer *et al.* 2009), in which a specific (but again, not lethal) interaction was discovered with Notch signaling, in a recent study by the Schweisguth group (Hamel *et al.* 2010). Here, Gal4-mediated over-expression of the α1,4GalNAc-T gene was found to suppress the Notch-like phenotype of loss of function in *mindbomb* (*Mib1*), an E3 ubiquitin ligase that functions in Notch endocytosis and activation. The Notch ligand Serrate was also found to bind to glycosphingolipids, suggesting that glycosphingolipids act at multiple steps in the Notch signaling cascade. Although the disruption of α1,4GalNAc-T by itself has no detectable phenotype, the interaction of its glycosphingolipid products with Notch possibly contribute to the more severe phenotypes of *egghead* and *brainiac*, which disrupt an earlier step in glycosphingolipid biosynthesis (Hamel *et al.* 2010).

On the subject of signaling receptors interacting with sphingolipid-rich domains, it is interesting to note that a study of olfactory receptor neurons in the moth Manduca was able to detect what appeared to be cholesterol-dependent membrane domains containing both glycosphingolipid, recognized by fluorescent lectins, and various signaling receptors including EGF-R and the cell-adhesion molecules FasII and neuroglian (Gibson *et al.* 2009). In humans and mice, the EGF-receptor (Puri *et al.* 2005; Lajoie *et al.* 2007) and insulin receptors are thought to be able to translocate into sphingolipid-rich domains where they are regulated by particular gangliosides, in both cases GM3 (Rebbaa *et al.* 1996; Yamashita *et al.* 2003; Kabayama *et al.* 2007). Taking a cue from mammalian cells, where glycosphingolipids are known in many cases to interact directly with cell surface receptors and regulate their lateral organization and signaling capability (Proia 2003; Lopez and Schnaar 2009), the above studies of fly glycosphingolipid biosynthesis mutants hint that the same is very likely to be true in *Drosophila*. As the previous examples of different glycosphingolipid deficiencies show, flies provide an excellent system in which to detect genetic interactions that will reveal the players involved.

Sphingolipids and cholesterol

The usefulness of *Drosophila* as a model for metabolic processes and diseases that involve cholesterol has been questioned because flies regulate sterols – the single most abundant structural component of mammalian membranes – completely differently from mammals. Flies are sterol auxotrophs, meaning that they do not synthesize their own sterols, but rather absorb them (primarily ergosterol) from their main food source, yeast. Fly membranes also contain considerably less sterol proportionately than mammalian membranes, being only $\sim$ 18% versus $\sim$ 30% (Rietveld *et al.* 1999; http://www.Cyberlipid.org). Unexpectedly, *Drosophila* cells in culture, like Schneider S2 cells, are able to survive and divide after sterol depletion (Gupta *et al.* 2009), casting serious doubt on any critical function of sterol in their basic physiology. By extension, sphingolipid biosynthesis and storage, which is known to be closely coupled with cholesterol (Guan *et al.* 2009), would be expected to be regulated differently from mammals, although very little is known about this at present.

Although many of the critical lipid metabolic enzymes are there, including hydroxymethylglutaryl (HMG)-CoA reductase, which synthesizes mevalonate, the precursor to cholesterol biosynthesis, the SREBP pathway that regulates cholesterol and lipid biosynthesis in mammals is not controlled by feedback through cholesterol concentration in the membrane (Dobrosotskaya *et al.* 2002). Moreover, and somewhat surprisingly, SREBP does not control hydroxymethylglutaryl (HMG)-CoA reductase, but rather the synthesis of saturated fatty acids. Although most SREBP pathway components are present in the fly, its activity is instead regulated by the glycerol lipid phosphatidylethanolamine (PE), the major constituent of cell membranes in *Drosophila* (Jones *et al.* 1992; Rawson 2003). Sphingolipids also play a regulatory role in SREBP cleavage by controlling the availability of PE, since the ethanolamine group on this lipid can be donated indirectly by S1P (Herr *et al.* 2003). Notably, palmitate, but not sterols or other fatty acids, block cleavage of SREBP when added to *Drosophila* cultured cells, and this blockage is dependent on cycling through sphingolipids. Another possible source of PE would be cleavage of PE-ceramide by a SMase (Fig. 2).

Niemann–Pick models in the fly

Fly models of diseases that arise from aberrant cholesterol storage have come on the stage recently, with the analysis of mutants in the various orthologs of the genes responsible for the neurodegenerative disease Niemann–Pick type C, NPC-1 and NPC-2. These are important in considering the potential validity of fly models of sphingolipid storage diseases and neurodegeneration, because of the established interdependence of sterol and sphingolipid trafficking, degradation, and metabolism (Puri *et al.* 1999, 2003; Ridgway 2000) even in eukaryotes much less advanced than *Drosophila* (Guan *et al.* 2009). In the first of these NPC models, one of two fly homologs of NPC-1, dnpc1a, was mutated and this led to accumulated sterol throughout the body and developmental arrest in first instar larvae. Dnpc1a mutants could be fully rescued by feeding with the sterol precursors of the ecdysone molting hormone, suggesting that the main problem was that sterol was being sequestered in aberrant compartments and unable to be released for ecdysone production (Huang *et al.* 2005; Fluegel *et al.* 2006). Of the eight NPC-2 genes in *Drosophila*, *Dnpc2a* was the most conserved with human NPC-2, and mutants looked similar to *Dnpc1a* in their aberrant punctate accumulation of sterol in some tissues, including brain (Huang *et al.* 2007).

Since NPC in humans is severely neurodegenerative, presumably owing to a backup of cholesterol in degradative compartments of neurons, and resultant malfunctioning of endolysosomal trafficking (Vance *et al.* 2005), it was hoped that NPC models in *Drosophila* would show parallels to the disease, even though flies are sterol auxotrophs. In fact, alteration of cholesterol metabolism and storage was documented in another neurodegenerative mutant called *löchrig* (German for full of holes, referring to the brain degeneration in mutants), which corresponds to the gamma subunit of AMP-kinase (Tschäpe *et al.* 2002). It is interesting that a degenerative phenotype was accompanied by these changes in fat metabolism, but the degeneration was not shown to be actually caused by the increase in free cholesterol. The first clear indication that sterols in the fly may indeed play a part

in the metabolic health of neurons, instead of being just a precursor to ecdysone hormone and a comparatively minor structural component of membranes, was that certain mutant combinations of Dnpc2 and mutants in Dnpc1a led to not only sterol accumulation but also to other defects, such as lipofuscin-like membrane whorls and axonal accumulations of synaptotagmin, as well as shortened lifespan and neurodegeneration, which was rescuable in a cell-autonomous way (Huang *et al.* 2007; Phillips *et al.* 2008).

It was not established in these NPC models whether sphingolipid metabolism and trafficking were also affected, but this is certainly not far-fetched, given the situation in yeast and humans (Puri *et al.* 1999; Simons and Gruenberg 2000; Guan *et al.* 2009). A convincing case has been made that the defect in NPC-1 disease in humans is traceable to a primary accumulation of sphingosine in acidic compartments, which depletes lysosomal calcium and as a result ultimately leads to storage of other sphingolipids and cholesterol in endolysosomal compartments (Lloyd-Evans *et al.* 2008).

The observation that *Drosophila* appear to recapitulate neurodegenerative features of NPC disease, in spite of having a very different mode of sterol metabolism from humans, makes one optimistic that flies could also present a viable model for the numerous sphingolipid storage diseases that share a common cell biology and pathology with NPC. That sphingolipid misregulation can indeed lead to neurodegenerative defects in *Drosophila* was suggested by studies of the gene *spinster*, whose zebrafish ortholog Spns2 was deduced to be a S1P transporter, based on its phenocopying the defect of the S1P-receptor mutation in heart development (Kupperman *et al.* 2000; Kawahara *et al.* 2009). Although a S1P-receptor has not been identified in flies, *spinster* mutants accumulate multilamellar lipofuscin granules in the nervous system, and undergo some neuronal degeneration preceded by a defect in programmed cell death (Nakano *et al.* 2001; Dermaut *et al.* 2005). Intriguingly, loss of the Spinster protein (also called Benchwarmer), which is a late endolysosomal 12-pass transmembrane protein very distantly related to sugar transporters, also leads to enlargement of late endolysosomal compartments at synaptic termini and in oocytes in *Drosophila* (Sweeney and Davis 2002; Dermaut *et al.* 2005), where purportedly carbohydrate accumulation recognizable by the periodic acid Schiff stain occurs (Dermaut *et al.* 2005). Given the link to sphingolipid transporters, and the lysosomal accumulation of lipofuscin-like material in *spinster*, the question that springs to mind is whether the carbohydrate accumulation seen in these animals may actually arise from non-degraded glycolipids. If this is the case, *spinster* mutants would serve as an excellent model for lysosomal sphingolipid storage diseases, such as the gangliosidoses (Kolter and Sandhoff 2005).

Of potential relevance to these models, recent studies using sphingolipid tracers in *Drosophila* cultured neurons showed that increasing or decreasing the levels of sterols in the cells altered the trafficking behavior of a fluorescently tagged glycosphingolipid (boron-dipyrromethene [BODIPY]-lactosyl-ceramide; Hortsch *et al.* 2010) and a raft- and sphingolipid-interacting peptide, the SBD (Hebbar *et al.* 2008; Steinert *et al.* 2008), in ways that would suggest that cellular sterol content controls a toggle switch between biosynthetic versus degradative pathways, as is the case in mammalian cells (Puri *et al.* 2001; Choudhury *et al.* 2004; Zhang *et al.* 2009). These studies provide support for the validity of fly models of lipid storage diseases that arise from sphingolipid and/or sterol accumulation, such as NPC, the gangliosidoses, and the lipofuscinoses, since it appears that the trafficking and storage mechanisms of sphingolipids are similar. More such cell biological analyses in primary cells from mutants and drug-fed or nutrient-controlled animals should enable a quite precise analysis of changes in sphingolipid trafficking and accumulation under different conditions that might affect lipid metabolism. This type of study would be facilitated by the development and validation of more sphingolipid-recognizing labels in fly cells as well as the application of high resolution imaging methods.

Conclusion

In *Drosophila* as much as in other systems, one of the major obstacles to analyzing the interplay between sphingolipids and the response to nutrients, or the response of sphingolipids to pathological conditions like diabetes and neurodegeneration, is the intrinsically self-correcting property of metabolism. Feedback loops and compensatory mechanisms, often coming from multiple enzymes whose activity can result in the same product (Merrill and Sandhoff 2010), may obscure fluctuations in levels of sphingolipids such that at steady state, they cannot be detected. Since the analysis of phenotypes and metabolites is generally done after extensive developmental time and averaged over many samples, it is difficult to determine the immediate responses to certain conditions or treatments. This probably results in acute changes in the representation of different sphingolipid species being masked by compensatory responses in the animal's physiology. In flies, this problem could be solved by analyzing lipids in genetic backgrounds that may reduce compensatory pathways (see, e.g., Breslow *et al.* 2010), and in animals that over-express sphingolipid metabolic enzymes in a spatially and temporally controlled manner.

In spite of differences from humans in both the variety of sphingolipid species present in the fly, and its means of regulating the sterol content of its membranes, it is obvious that sphingolipids are just as critical to developmental and metabolic processes in the fly as they are in mammals. With the growing popularity of *Drosophila* as a model for metabolic diseases, such as obesity and diabetes (Baker and Thummel 2007; Al-Anzi *et al.* 2009), more studies have

appeared that model these pathologies in the fly as a surprisingly good parallel to their human counterparts. Given the similarities, sphingolipids will also surely play a role in fly models of other diseases with a metabolic component, such as neurodegeneration. Importantly, novel visualization tools, as well as traditional and high accuracy mass spectrometry-based lipid analytic studies are showing that the nuts and bolts of sphingolipid cell biology and physiology are conserved. This by itself is a major advance, since with this knowledge one can now begin to combine very sophisticated fly genetics with analytical and pharmacological methods that are standard in lipid biochemistry, but not yet commonplace among Drosophilists.

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References

Acharya U. and Acharya J. K. (2005) Enzymes of sphingolipid metabolism in *Drosophila melanogaster*. *Cell. Mol. Life Sci.* **62**, 128–142.

Acharya U., Patel S., Koundakjian E., Nagashima K., Han X. and Acharya J. K. (2003) Modulating sphingolipid biosynthetic pathway rescues photoreceptor degeneration. *Science* **299**, 1740–1743.

Acharya J. K., Dasgupta U. and Rawat S. S. *et al.* (2008) Cell-nonautonomous function of ceramidase in photoreceptor homeostasis. *Neuron* **57**, 69–79.

Adachi-Yamada T., Gotoh T., Sugimura I., Tateno M., Nishida Y., Onuki T. and Date H. (1999) De novo synthesis of sphingolipids is required for cell survival by down-regulating c-Jun N-terminal kinase in *Drosophila* imaginal discs. *Mol. Cell. Biol.* **19**, 7276–7286.

Al-Anzi B., Sapin V., Waters C., Zinn K., Wyman R. J. and Benzer S. (2009) Obesity-blocking neurons in *Drosophila*. *Neuron* **63**, 329–341.

Alloway P. G., Howard L. and Dolph P. J. (2000) The formation of stable rhodopsin-arrestin complexes induces apoptosis and photoreceptor cell degeneration. *Neuron* **28**, 129–138.

Al-Makdissy N., Younsi M., Pierre S., Ziegler O. and Donner M. (2003) Sphingomyelin/cholesterol ratio: an important determinant of glucose transport mediated by GLUT-1 in 3T3–L1 preadipocytes. *Cell. Signal.* **15**, 1019–1030.

Baker K. D. and Thummel C. S. (2007) Diabetic larvae and obese flies-emerging studies of metabolism in *Drosophila*. *Cell Metab.* **6**, 257–266.

Bartke N. and Hannun Y. A. (2009) Bioactive sphingolipids: metabolism and function. *J. Lipid Res.* **50**(Suppl.), S91–S96.

Basu J. and Li Z. (1998) The Des-1 protein, required for central spindle assembly and cytokinesis, is associated with mitochondria along the meiotic spindle apparatus and with the contractile ring during male meiosis in *Drosophila melanogaster*. *Mol. Gen. Genet.* **259**, 664–673.

Bauer R., Voelzmann A., Breiden B., Schepers U., Farwanah H., Hahn I., Eckardt F., Sandhoff K. and Hoch M. (2009) Schlank, a member of the ceramide synthase family controls growth and body fat in *Drosophila*. *EMBO J.* **28**, 3706–3716.

Bektas M., Allende M. L., Lee B. G., Chen W., Amar M. J., Remaley A. T., Saba J. D. and Proia R. L. (2010) Sphingosine 1-phosphate lyase deficiency disrupts lipid homeostasis in liver. *J. Biol. Chem.* **285**, 10880–10889.

Boquet I., Hitier R., Dumas M., Chaminade M. and Preat T. (2000) Central brain postembryonic development in *Drosophila*: implication of genes expressed at the interhemispheric junction. *J. Neurobiol.* **42**, 33–48.

Bose R., Chen P., Loconti A., Grullich C., Abrams J. M. and Kolesnick R. N. (1998) Ceramide generation by the Reaper protein is not blocked by the caspase inhibitor, p35. *J. Biol. Chem.* **273**, 28852–28859.

Breslow D. K., Collins S. R., Bodenmiller B., Aebersold R., Simons K., Shevchenko A., Ejsing C. S. and Weissman J. S. (2010) Orm family proteins mediate sphingolipid homeostasis. *Nature* **463**, 1048–1053.

Britton J. S., Lockwood W. K., Li L., Cohen S. M. and Edgar B. A. (2002) *Drosophila*'s insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. *Dev. Cell* **2**, 239–249.

Broughton S. and Partridge L. (2009) Insulin/IGF-like signalling, the central nervous system and aging. *Biochem. J.* **418**, 1–12.

Chen Y. W., Pedersen J. W., Wandall H. H., Levery S. B., Pizette S., Clausen H. and Cohen S. M. (2007) Glycosphingolipids with extended sugar chain have specialized functions in development and behavior of *Drosophila*. *Dev. Biol.* **306**, 736–749.

Choudhury A., Sharma D. K., Marks D. L. and Pagano R. E. (2004) Elevated endosomal cholesterol levels in Niemann-Pick cells inhibit rab4 and perturb membrane recycling. *Mol. Biol. Cell* **15**, 4500–4511.

Collins C. A., Wairkar Y. P., Johnson S. L. and DiAntonio A. (2006) Highwire restrains synaptic growth by attenuating a MAP kinase signal. *Neuron* **51**, 57–69.

Darios F., Wasser C. and Shakirzyanova A. *et al.* (2009) Sphingosine facilitates SNARE complex assembly and activates synaptic vesicle exocytosis. *Neuron* **62**, 683–694.

Dasgupta U., Bamba T. and Chiantia S. *et al.* (2009) Ceramide kinase regulates phospholipase C and phosphatidylinositol 4, 5, bisphosphate in phototransduction. *Proc. Natl Acad. Sci. USA* **106**, 20063–20068.

David D., Sundarababu S. and Gerst J. E. (1998) Involvement of long chain fatty acid elongation in the trafficking of secretory vesicles in yeast. *J. Cell Biol.* **143**, 1167–1182.

Deevska G. M., Rozenova K. A. and Giltiay N. V. *et al.* (2009) Acid Sphingomyelinase Deficiency Prevents Diet-induced Hepatic Triacylglycerol Accumulation and Hyperglycemia in Mice. *J. Biol. Chem.* **284**, 8359–8368.

Dermaut B., Norga K. K., Kania A., Verstreken P., Pan H., Zhou Y., Callaerts P. and Bellen H. J. (2005) Aberrant lysosomal carbohydrate storage accompanies endocytic defects and neurodegeneration in *Drosophila* benchwarmer. *J. Cell Biol.* **170**, 127–139.

Dickson R. C. (1998) Sphingolipid functions in Saccharomyces cerevisiae: comparison to mammals. *Annu. Rev. Biochem.* **67**, 27–48.

Dobrosotskaya I. Y., Seegmiller A. C., Brown M. S., Goldstein J. L. and Rawson R. B. (2002) Regulation of SREBP processing and membrane lipid production by phospholipids in *Drosophila*. *Science* **296**, 879–883.

Dobrowsky R. T. and Hannun Y. A. (1992) Ceramide stimulates a cytosolic protein phosphatase. *J. Biol. Chem.* **267**, 5048–5051.

Driver C. J. and Cosopodiotis G. (1979) The effect of dietary fat on longevity of *Drosophila melanogaster*. *Exp. Gerontol.* **14**, 95–100.

Eroglu C., Brugger B., Wieland F. and Sinning I. (2003) Glutamate-binding affinity of *Drosophila* metabotropic glutamate receptor is

modulated by association with lipid rafts. *Proc. Natl Acad. Sci. USA* **100**, 10219–10224.

Fluegel M. L., Parker T. J. and Pallanck L. J. (2006) Mutations of a *Drosophila* NPC1 gene confer sterol and ecdysone metabolic defects. *Genetics* **172**, 185–196.

Fuss B., Becker T., Zinke I. and Hoch M. (2006) The cytohesin Steppke is essential for insulin signalling in *Drosophila*. *Nature* **444**, 945–948.

Fyrst H. and Saba J. D. (2008) Sphingosine-1-phosphate lyase in development and disease: sphingolipid metabolism takes flight. *Biochim. Biophys. Acta* **1781**, 448–458.

Fyrst H. and Saba J. D. (2010) An update on sphingosine-1-phosphate and other sphingolipid mediators. *Nat. Chem. Biol.* **6**, 489–497.

Fyrst H., Herr D. R., Harris G. L. and Saba J. D. (2004) Characterization of free endogenous C14 and C16 sphingoid bases from *Drosophila melanogaster*. *J. Lipid Res.* **45**, 54–62.

Fyrst H., Zhang X., Herr D. R., Byun H. S., Bittman R., Phan V. H., Harris G. L. and Saba J. D. (2008) Identification and characterization by electrospray mass spectrometry of endogenous *Drosophila* sphingadienes. *J. Lipid Res.* **49**, 597–606.

Fyrst H., Oskouian B., Bandhuvula P., Gong Y., Byun H. S., Bittman R., Lee A. R. and Saba J. D. (2009) Natural sphingadienes inhibit Akt-dependent signaling and prevent intestinal tumorigenesis. *Cancer Res.* **69**, 9457–9464.

Gasque G., Labarca P. and Darszon A. (2005) Cholesterol-depleting compounds modulate K+-currents in *Drosophila* Kenyon cells. *FEBS Lett.* **579**, 5129–5134.

Gibson N. J., Tolbert L. P. and Oland L. A. (2009) Roles of specific membrane lipid domains in EGF receptor activation and cell adhesion molecule stabilization in a developing olfactory system. *PLoS ONE* **4**, e7222.

Goñi F. M. and Alonso A. (2009) Effects of ceramide and other simple sphingolipids on membrane lateral structure. *Biochim. Biophys. Acta (BBA) – Biomembr.* **1788**, 169–177.

Goode S., Wright D. and Mahowald A. P. (1992) The neurogenic locus brainiac cooperates with the *Drosophila* EGF receptor to establish the ovarian follicle and to determine its dorsal-ventral polarity. *Development* **116**, 177–192.

Goode S., Melnick M., Chou T. B. and Perrimon N. (1996a) The neurogenic genes egghead and brainiac define a novel signaling pathway essential for epithelial morphogenesis during *Drosophila* oogenesis. *Development* **122**, 3863–3879.

Goode S., Morgan M., Liang Y. P. and Mahowald A. P. (1996b) Brainiac encodes a novel, putative secreted protein that cooperates with Grk TGF alpha in the genesis of the follicular epithelium. *Dev. Biol.* **178**, 35–50.

Gorski S. M., Chittaranjan S. and Pleasance E. D. *et al.* (2003) A SAGE approach to discovery of genes involved in autophagic cell death. *Curr. Biol.* **13**, 358–363.

Grönke S., Mildner A., Fellert S., Tennagels N., Petry S., Müller G., Jäckle H. and Kühnlein R. P. (2005) Brummer lipase is an evolutionary conserved fat storage regulator in *Drosophila*. *Cell Metab.* **1**, 323–330.

Grote E., Vlacich G., Pypaert M. and Novick P. J. (2000) A snc1 endocytosis mutant: phenotypic analysis and suppression by overproduction of dihydrosphingosine phosphate lyase. *Mol. Biol. Cell* **11**, 4051–4065.

Guan X. L., Souza C. M. and Pichler H. *et al.* (2009) Functional interactions between sphingolipids and sterols in biological membranes regulating cell physiology. *Mol. Biol. Cell* **20**, 2083–2095.

Gupta G. D., M. G S., Kumari S., Lakshminarayan R., Dey G. and Mayor S. (2009) Analysis of endocytic pathways in *Drosophila* cells reveals a conserved role for GBF1 in internalization via GEECs. *PLoS ONE* **4**, e6768.

Haines N. and Irvine K. D. (2005) Functional analysis of *Drosophila* beta1, 4-N-acetlygalactosaminyltransferases. *Glycobiology* **15**, 335–346.

Haines N. and Stewart B. A. (2007) Functional roles for beta1, 4-N-acetlygalactosaminyltransferase-A in *Drosophila* larval neurons and muscles. *Genetics* **175**, 671–679.

Hamel S., Fantini J. and Schweisguth F. (2010) Notch ligand activity is modulated by glycosphingolipid membrane composition in *Drosophila melanogaster*. *J. Cell Biol.* **188**, 581–594.

Hannun Y. A. and Obeid L. M. (2002) The Ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. *J. Biol. Chem.* **277**, 25847–25850.

Hannun Y. A. and Obeid L. M. (2008) Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat. Rev. Mol. Cell Biol.* **9**, 139–150.

Hanyu-Nakamura K., Kobayashi S. and Nakamura A. (2004) Germ cell-autonomous Wunen2 is required for germline development in *Drosophila* embryos. *Development* **131**, 4545–4553.

Hebbar S., Lee E., Manna M., Steinert S., Kumar G. S., Wenk M., Wohland T. and Kraut R. (2008) A fluorescent sphingolipid binding domain peptide probe interacts with sphingolipids and cholesterol-dependent raft domains. *J. Lipid Res.* **49**, 1077–1089.

Herr D. R., Fyrst H., Phan V., Heinecke K., Georges R., Harris G. L. and Saba J. D. (2003) Sply regulation of sphingolipid signaling molecules is essential for *Drosophila* development. *Development* **130**, 2443–2453.

Herr D. R., Fyrst H., Creason M. B., Phan V. H., Saba J. D. and Harris G. L. (2004) Characterization of the *Drosophila* sphingosine kinases and requirement for Sk2 in normal reproductive function. *J. Biol. Chem.* **279**, 12685–12694.

Hoehne M., de Couet H. G., Stuermer C. A. and Fischbach K. F. (2005) Loss- and gain-of-function analysis of the lipid raft proteins Reggie/Flotillin in *Drosophila*: they are posttranslationally regulated, and misexpression interferes with wing and eye development. *Mol. Cell. Neurosci.* **30**, 326–338.

Holland W. L. and Summers S. A. (2008) Sphingolipids, insulin resistance, and metabolic disease: new insights from in vivo manipulation of sphingolipid metabolism. *Endocr. Rev.* **29**, 381–402.

Holland W. L., Brozinick J. T. and Wang L. P. *et al.* (2007) Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab.* **5**, 167–179.

Holthuis J. C. M., Pomorski T., Raggers R. J., Sprong H. and Van Meer G. (2001) The organizing potential of sphingolipids in intracellular membrane transport. *Physiol. Rev.* **81**, 1689–1723.

Horton J. D., Goldstein J. L. and Brown M. S. (2002) SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* **109**, 1125–1131.

Hortsch R., Lee E., Erathodiyil N., Hebbar S., Steinert S., Lee J. Y., Chua D. S. and Kraut R. (2010) Glycolipid trafficking in *Drosophila* undergoes pathway switching in response to aberrant cholesterol levels. *Mol. Biol. Cell* **21**, 778–790.

Huang X., Suyama K., Buchanan J., Zhu A. J. and Scott M. P. (2005) A *Drosophila* model of the Niemann-Pick type C lysosome storage disease: dnpc1a is required for molting and sterol homeostasis. *Development* **132**, 5115–5124.

Huang X., Warren J. T., Buchanan J., Gilbert L. I. and Scott M. P. (2007) *Drosophila* Niemann-Pick type C-2 genes control sterol homeostasis and steroid biosynthesis: a model of human neurodegenerative disease. *Development* **134**, 3733–3742.

Huitema K., van den Dikkenberg J., Brouwers J. F. and Holthuis J. C. (2004) Identification of a family of animal sphingomyelin synthases. *EMBO J.* **23**, 33–44.

Jones H. E., Harwood J. L., Bowen I. D. and Griffiths G. (1992) Lipid composition of subcellular membranes from larvae and prepupae of *Drosophila melanogaster*. *Lipids* **27**, 984–987.

Kabayama K., Sato T., Saito K., Loberto N., Prinetti A., Sonnino S., Kinjo M., Igarashi Y. and Inokuchi J. (2007) Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. *Proc. Natl Acad. Sci. USA* **104**, 13678–13683.

Karpen H. E., Bukowski J. T., Hughes T., Gratton J. P., Sessa W. C. and Gailani M. R. (2001) The sonic hedgehog receptor patched associates with caveolin-1 in cholesterol-rich microdomains of the plasma membrane. *J. Biol. Chem.* **276**, 19503–19511.

Kawahara A., Nishi T., Hisano Y., Fukui H., Yamaguchi A. and Mochizuki N. (2009) The sphingolipid transporter Spns2 functions in migration of zebrafish myocardial precursors. *Science* **323**, 524–527.

Kiselev A., Socolich M., Vinos J., Hardy R. W., Zuker C. S. and Ranganathan R. (2000) A molecular pathway for light-dependent photoreceptor apoptosis in *Drosophila*. *Neuron* **28**, 139–152.

Kohyama-Koganeya A., Sasamura T., Oshima E., Suzuki E., Nishihara S., Ueda R. and Hirabayashi Y. (2004) *Drosophila* glucosylceramide synthase: a negative regulator of cell death mediated by proapoptotic factors. *J. Biol. Chem.* **279**, 35995–36002.

Kolter T. and Sandhoff K. (2005) Principles of lysosomal membrane digestion: stimulation of sphingolipid degradation by sphingolipid activator proteins and anionic lysosomal lipids. *Annu. Rev. Cell Dev. Biol.* **21**, 81–103.

Kraut R., Menon K. and Zinn K. (2001) A gain-of-function screen for genes controlling motor axon guidance and synaptogenesis in *Drosophila*. *Curr. Biol.* **11**, 417–430.

Kupperman E., An S., Osborne N., Waldron S. and Stainier D. Y. (2000) A sphingosine-1-phosphate receptor regulates cell migration during vertebrate heart development. *Nature* **406**, 192–195.

Lajoie P., Partridge E. A., Guay G., Goetz J. G., Pawling J., Lagana A., Joshi B., Dennis J. W. and Nabi I. R. (2007) Plasma membrane domain organization regulates EGFR signaling in tumor cells. *J. Cell Biol.* **179**, 341–356.

Liu P., Leffler B. J., Weeks L. K., Chen G., Bouchard C. M., Strawbridge A. B. and Elmendorf J. S. (2004) Sphingomyelinase activates GLUT4 translocation via a cholesterol-dependent mechanism. *Am. J. Physiol. Cell Physiol.* **286**, C317–C329.

Lloyd-Evans E., Morgan A. J. and He X. *et al.* (2008) Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium. *Nat. Med.* **14**, 1247–1255.

Lopez P. H. and Schnaar R. L. (2009) Gangliosides in cell recognition and membrane protein regulation. *Curr. Opin. Struct. Biol.* **19**, 549–557.

Mair W., Piper M. D. and Partridge L. (2005) Calories do not explain extension of life span by dietary restriction in *Drosophila*. *PLoS Biol.* **3**, e223.

Merrill A. H. and Sandhoff K. (2010) Sphingolipids: metabolism and cell signaling, in Biochemistry of Lipids, Lipoproteins, and Membranes (4th Edition; Vance J. E. and Vance D., eds), pp. 373–407, Elsevier, Amsterdam.

Merrill Jr., A. H., Liotta D. C. and Riley R. T. (1996) Fumonisins: fungal toxins that shed light on sphingolipid function. *Trends Cell Biol.* **6**, 218–223.

Müller R., Altmann F., Zhou D. and Hennet T. (2002) The *Drosophila melanogaster* brainiac protein is a glycolipid-specific beta 1, 3N-acetylglucosaminyltransferase. *J. Biol. Chem.* **277**, 32417–32420.

Nakano Y., Fujitani K. and Kurihara J. *et al.* (2001) Mutations in the novel membrane protein spinster interfere with programmed cell death and cause neural degeneration in *Drosophila melanogaster*. *Mol. Cell. Biol.* **21**, 3775–3788.

Nilson L. A. and Schupbach T. (1999) EGF receptor signaling in *Drosophila* oogenesis. *Curr. Top. Dev. Biol.* **44**, 203–243.

Pattingre S., Bauvy C., Carpentier S. p., Levade T., Levine B. and Codogno P. (2009a) Role of JNK1-dependent Bcl-2 phosphorylation in ceramide-induced macroautophagy. *J. Biol. Chem.* **284**, 2719–2728.

Pattingre S., Bauvy C., Levade T., Levine B. and Codogno P. (2009b) Ceramide-induced autophagy: to junk or to protect cells? *Autophagy* **5**, 558–560.

Pewzner-Jung Y., Ben-Dor S. and Futerman A. H. (2006) When do Lasses (longevity assurance genes) become CerS (ceramide synthases)? Insights into the regulation of ceramide synthesis. *J. Biol. Chem.* **281**, 25001–25005.

Phan V. H., Herr D. R., Panton D., Fyrst H., Saba J. D. and Harris G. L. (2007) Disruption of sphingolipid metabolism elicits apoptosis-associated reproductive defects in *Drosophila*. *Dev. Biol.* **309**, 329–341.

Phillips S. E., Woodruff, 3rd, E. A., Liang P., Patten M. and Broadie K. (2008) Neuronal loss of *Drosophila* NPC1a causes cholesterol aggregation and age-progressive neurodegeneration. *J. Neurosci.* **28**, 6569–6582.

Pizette S., Rabouille C., Cohen S. M. and Thérond P. (2009) Glycosphingolipids control the extracellular gradient of the *Drosophila* EGFR ligand Gurken. *Development* **136**, 551–561.

Postlethwait J. H. (1974) Juvenile hormone and the adult development of *Drosophila*. *Biol. Bull.* **147**, 119–135.

Powers 3rd R. W., Kaeberlein M., Caldwell S. D., Kennedy B. K. and Fields S. (2006) Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev.* **20**, 174–184.

Proia R. L. (2003) Glycosphingolipid functions: insights from engineered mouse models. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **358**, 879–883.

Protzer C. E., Preiss A. and Nagel A. C. (2009) *Drosophila* alpha-1, 4-glycosyltransferase (alpha-4GT1) inhibits reaper-mediated apoptosis in the eye. *Cell Tissue Res.* **336**, 149–158.

Puri V., Watanabe R., Dominguez M., Sun X., Wheatley C. L., Marks D. L. and Pagano R. E. (1999) Cholesterol modulates membrane traffic along the endocytic pathway in sphingolipid-storage diseases. *Nat. Cell Biol.* **1**, 386–388.

Puri V., Watanabe R., Singh R. D., Dominguez M., Brown J. C., Wheatley C. L., Marks D. L. and Pagano R. E. (2001) Clathrin-dependent and -independent internalization of plasma membrane sphingolipids initiates two Golgi targeting pathways. *J. Cell Biol.* **154**, 535–547.

Puri V., Jefferson J. R., Singh R. D., Wheatley C. L., Marks D. L. and Pagano R. E. (2003) Sphingolipid storage induces accumulation of intracellular cholesterol by stimulating SREBP-1 cleavage. *J. Biol. Chem.* **278**, 20961–20970.

Puri C., Tosoni D., Comai R., Rabellino A., Segat D., Caneva F., Luzzi P., Di Fiore P. P. and Tacchetti C. (2005) Relationships between EGFR signaling-competent and endocytosis-competent membrane microdomains. *Mol. Biol. Cell* **16**, 2704–2718.

Raabe T. (2000) The Sevenless signaling pathway: variations of a common theme. *Biochim. Biophys. Acta (BBA) – Mol. Cell Res.* **1496**, 151–163.

Rao R. P., Yuan C., Allegood J. C., Rawat S. S., Edwards M. B., Wang X., Merrill Jr., A. H., Acharya U. and Acharya J. K. (2007) Ceramide transfer protein function is essential for normal oxidative stress response and lifespan. *Proc. Natl Acad. Sci. USA* **104**, 11364–11369.

Rawson R. B. (2003) The SREBP pathway – insights from insigs and insects. *Nat. Rev. Mol. Cell Biol.* **4**, 631–640.

Rebbaa A., Hurh J., Yamamoto H., Kersey D. S. and Bremer E. G. (1996) Ganglioside GM3 inhibition of EGF receptor mediated signal transduction. *Glycobiology* **6**, 399–406.

Ridgway N. D. (2000) Interactions between metabolism and intracellular distribution of cholesterol and sphingomyelin. *Biochim. Biophys. Acta* **1484**, 129–141.

Rietveld A., Neutz S., Simons K. and Eaton S. (1999) Association of sterol- and glycosylphosphatidylinositol-linked proteins with *Drosophila* raft lipid microdomains. *J. Biol. Chem.* **274**, 12049–12054.

Rohrbough J., Rushton E., Palanker L., Woodruff E., Matthies H. J., Acharya U., Acharya J. K. and Broadie K. (2004) Ceramidase regulates synaptic vesicle exocytosis and trafficking. *J. Neurosci.* **24**, 7789–7803.

Ruvolo P. P. (2003) Intracellular signal transduction pathways activated by ceramide and its metabolites. *Pharmacol. Res. Pharmacol. Ceramide Sphingolipids* **47**, 383–392.

Sano H., Renault A. D. and Lehmann R. (2005) Control of lateral migration and germ cell elimination by the *Drosophila melanogaster* lipid phosphate phosphatases Wunen and Wunen 2. *J. Cell Biol.* **171**, 675–683.

Sanxaridis P. D., Cronin M. A., Rawat S. S., Waro G., Acharya U. and Tsunoda S. (2007) Light-induced recruitment of INAD-signaling complexes to detergent-resistant lipid rafts in *Drosophila* photoreceptors. *Mol. Cell. Neurosci.* **36**, 36–46.

Sathyanarayana P., Barthwal M. K., Kundu C. N., Lane M. E., Bergmann A., Tzivion G. and Rana A. (2002) Activation of the *Drosophila* MLK by Ceramide Reveals TNF-α and Ceramide as Agonists of Mammalian MLK3. *Mol. Cell.* **10**, 1527–1533.

Scheek S., Brown M. S. and Goldstein J. L. (1997) Sphingomyelin depletion in cultured cells blocks proteolysis of sterol regulatory element binding proteins at site 1. *Proc. Natl Acad. Sci. USA* **94**, 11179–11183.

Schwientek T., Keck B. and Levery S. B. *et al.* (2002) The *Drosophila* gene brainiac encodes a glycosyltransferase putatively involved in glycosphingolipid synthesis. *J. Biol. Chem.* **277**, 32421–32429.

Seppo A. and Tiemeyer M. (2000) Function and structure of *Drosophila* glycans. *Glycobiology* **10**, 751–760.

Seppo A., Moreland M., Schweingruber H. and Tiemeyer M. (2000) Zwitterionic and acidic glycosphingolipids of the *Drosophila melanogaster* embryo. *Eur. J. Biochem.* **267**, 3549–3558.

Sharma K. a. S. and Shi Y. (1999) The yins and yangs of ceramide. *Cell Res.,* **9**(1), 1–10.

Shen W. and Ganetzky B. (2009) Autophagy promotes synapse development in *Drosophila*. *J. Cell Biol.* **187**, 71–79.

Simons K. and Gruenberg J. (2000) Jamming the endosomal system: lipid rafts and lysosomal storage diseases. *Trends Cell Biol.* **10**, 459–462.

Slotte J. P. and Bierman E. L. (1988) Depletion of plasma-membrane sphingomyelin rapidly alters the distribution of cholesterol between plasma membranes and intracellular cholesterol pools in cultured fibroblasts. *Biochem. J.* **250**, 653–658.

Spiegel S. and Milstien S. (2002) Sphingosine 1-phosphate, a key cell signaling molecule. *J. Biol. Chem.* **277**, 25851–25854.

Starz-Gaiano M., Cho N. K., Forbes A. and Lehmann R. (2001) Spatially restricted activity of a *Drosophila* lipid phosphatase guides migrating germ cells. *Development* **128**, 983–991.

Steinert S., Lee E. and Tresset G. *et al.* (2008) A fluorescent glycolipid-binding peptide probe traces cholesterol dependent microdomain-derived trafficking pathways. *PLoS ONE* **3**, e2933.

Stolz A., Haines N., Pich A., Irvine K. D., Hokke C. H., Deelder A. M., Gerardy-Schahn R., Wuhrer M. and Bakker H. (2008) Distinct contributions of beta 4GalNAcTA and beta 4GalNAcTB to *Drosophila* glycosphingolipid biosynthesis. *Glycoconj. J.* **25**, 167–175.

Strochlic L., Dwivedy A., van Horck F. P. G., Falk J. and Holt C. E. (2008) A role for S1P signalling in axon guidance in the Xenopus visual system. *Development* **135**, 333–342.

Stuermer C. A., Lang D. M., Kirsch F., Wiechers M., Deininger S. O. and Plattner H. (2001) Glycosylphosphatidyl inositol-anchored proteins and fyn kinase assemble in noncaveolar plasma membrane microdomains defined by reggie-1 and -2. *Mol. Biol. Cell* **12**, 3031–3045.

Summers S. A., Garza L. A., Zhou H. and Birnbaum M. J. (1998) Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. *Mol. Cell. Biol.* **18**, 5457–5464.

Sun X., Seeberger J., Alberico T., Wang C., Wheeler C. T., Schauss A. G. and Zou S (2010) Acai palm fruit (Euterpe oleracea Mart.) pulp improves survival of flies on a high fat diet. *Exp. Gerontol.* **45**, 243–251.

Sweeney S. T. and Davis G. W. (2002) Unrestricted synaptic growth in spinster-a late endosomal protein implicated in TGF-beta-mediated synaptic growth regulation. *Neuron* **36**, 403–416.

Tafesse F. G., Ternes P. and Holthuis J. C. (2006) The multigenic sphingomyelin synthase family. *J. Biol. Chem.* **281**, 29421–29425.

Takatsu Y., Nakamura M., Stapleton M., Danos M. C., Matsumoto K., O'Connor M. B., Shibuya H. and Ueno N. (2000) TAK1 Participates in c-Jun N-Terminal Kinase Signaling during *Drosophila* Development. *Mol. Cell. Biol.* **20**, 3015–3026.

Tschäpe J. A., Hammerschmied C., Mühlig-Versen M., Athenstaedt K., Daum G. and Kretzschmar D. (2002) The neurodegeneration mutant löchrig interferes with cholesterol homeostasis and Appl processing. *EMBO J.* **21**, 6367–6376.

Unger R. H. (2003) Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. *Endocrinology* **144**, 5159–5165.

Vacaru A. M., Tafesse F. G., Ternes P., Kondylis V., Hermansson M., Brouwers J. F. H. M., Somerharju P., Rabouille C. and Holthuis J. C. M. (2009) Sphingomyelin synthase-related protein SMSr controls ceramide homeostasis in the ER. *J. Cell Biol.* **185**, 1013–1027.

Vance J. E., Hayashi H. and Karten B. (2005) Cholesterol homeostasis in neurons and glial cells. *Semin. Cell Dev. Biol.* **16**, 193–212.

Vargas M. A., Luo N., Yamaguchi A. and Kapahi P. (2010) A role for S6 kinase and serotonin in postmating dietary switch and balance of nutrients in *D. melanogaster*. *Curr. Biol.* **20**(11), 1006–1011.

Verhage M. and van Meer G. (2009) Synaptobrevin, sphingolipids, and secretion: lube 'n' go at the synapse. *Neuron* **62**, 603–605.

Wandall H. H., Pedersen J. W., Park C., Levery S. B., Pizette S., Cohen S. M., Schwientek T. and Clausen H. (2003) *Drosophila* egghead encodes a β1, 4-mannosyltransferase predicted to form the immediate precursor glycosphingolipid substrate for brainiac. *J. Biol. Chem.* **278**, 1411–1414.

Wandall H. H., Pizette S., Pedersen J. W., Eichert H., Levery S. B., Mandel U., Cohen S. M. and Clausen H. (2005) Egghead and Brainiac Are Essential for Glycosphingolipid Biosynthesis in Vivo. *J. Biol. Chem.* **280**, 4858–4863.

Wang G., Krishnamurthy K. and Bieberich E. (2009a) Regulation of primary cilia formation by ceramide. *J. Lipid Res.,* **50**(10), 2103–2110.

Wang G., Krishnamurthy K., Umapathy N. S., Verin A. D. and Bieberich E. (2009b) The carboxyl-terminal domain of atypical protein kinase Czeta binds to ceramide and regulates junction formation in epithelial cells. *J. Biol. Chem.* **284**, 14469–14475.

Wiegandt H. (1992) Insect glycolipids. *Biochim. Biophys. Acta (BBA) – Lipids Lipid Metab.* **1123**, 117–126.

Worgall T. S. (2008) Regulation of lipid metabolism by sphingolipids. *Subcell. Biochem.* **49**, 371–385.

Yamashita T., Hashiramoto A. and Haluzik M. *et al.* (2003) Enhanced insulin sensitivity in mice lacking ganglioside GM3. *Proc. Natl Acad. Sci. USA* **100**, 3445–3449.

Yang Q., Gong Z. J. and Zhou Y. *et al.* (2010) Role of *Drosophila* alkaline ceramidase (Dacer) in *Drosophila* development and longevity. *Cell. Mol. Life Sci.* **67**, 1477–1490.

Ye J., Cui X. and Loraine A. *et al.* (2007) Methods for nutrigenomics and longevity studies in *Drosophila*: effects of diets high in sucrose, palmitic acid, soy, or beef. *Methods Mol. Biol.* **371**, 111–141.

Zhai L., Chaturvedi D. and Cumberledge S. (2004) *Drosophila* wnt-1 undergoes a hydrophobic modification and is targeted to lipid rafts, a process that requires porcupine. *J. Biol. Chem.* **279**, 33220–33227.

Zhang Y. and Dong C. (2007) Regulatory mechanisms of mitogen-activated kinase signaling. *Cell. Mol. Life Sci.* **64**, 2771–2789.

Zhang N., Zhang J., Purcell K. J., Cheng Y. and Howard K. (1997) The *Drosophila* protein Wunen repels migrating germ cells. *Nature* **385**, 64–67.

Zhang D., Manna M., Wohland T. and Kraut R. (2009) Alternate raft pathways cooperate to mediate slow diffusion and efficient uptake of a sphingolipid tracer to degradative and recycling compartments. *J. Cell Sci.* **122**, 3715–3728.

Zinke I., Kirchner C., Chao L. C., Tetzlaff M. T. and Pankratz M. J. (1999) Suppression of food intake and growth by amino acids in *Drosophila*: the role of pumpless, a fat body expressed gene with homology to vertebrate glycine cleavage system. *Development* **126**, 5275–5284.

Zinke I., Schutz C. S., Katzenberger J. D., Bauer M. and Pankratz M. J. (2002) Nutrient control of gene expression in *Drosophila*: microarray analysis of starvation and sugar-dependent response. *EMBO J.* **21**, 6162–6173.