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Serine-Dependent Sphingolipid Synthesis Is a Metabolic Liability of Aneuploid Cells

Sunyoung Hwang
University of Massachusetts

H. Tobias Gustafsson
University of Massachusetts


Ciara O'Sullivan
University of Massachusetts

Gianna Bisceglia
University of Massachusetts

Xinhe Huang
University of Kentucky, xinhe.huang@uky.edu

See next page for additional authors

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Authors

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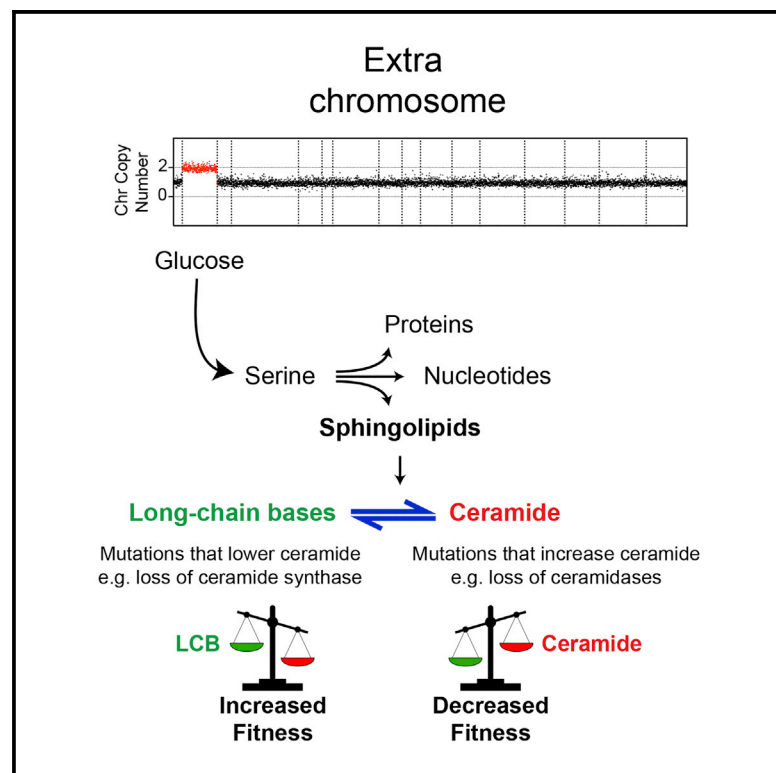
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Serine-Dependent Sphingolipid Synthesis Is a Metabolic Liability of Aneuploid Cells

Graphical Abstract



Authors

Sunyoung Hwang, H. Tobias Gustafsson, Ciara O'Sullivan, ..., Paola Cavaliere, Noah Dephoure, Eduardo M. Torres

Correspondence

eduardo.torres@umassmed.edu

In Brief

Hwang et al. demonstrate that aneuploid yeast cells rely on the synthesis of the amino acid serine for their viability. Serine is used for the synthesis of sphingolipids that control the fitness of aneuploid cells. Aneuploid cells are vulnerable to combined inhibition of serine and sphingolipid biosynthesis.

Highlights

- Aneuploid cells rely on increased serine synthesis to proliferate
- Increased serine synthesis leads to the accumulation of sphingolipids
- Mutations that lower ceramide levels increase the fitness of aneuploid cells
- Combined inhibition of serine and sphingolipid synthesis is lethal to aneuploid cells

Data and Software Availability

GSE93762



Serine-Dependent Sphingolipid Synthesis Is a Metabolic Liability of Aneuploid Cells

Sunyoung Hwang,^{1,7} H. Tobias Gustafsson,^{1,7} Ciara O'Sullivan,^{1,7} Gianna Bisceglia,¹ Xinhe Huang,^{2,5} Christian Klose,^{3,6} Andrej Schevchenko,³ Robert C. Dickson,² Paola Cavaliere,⁴ Noah Dephoure,⁴ and Eduardo M. Torres^{1,8,*}

¹Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, Worcester, MA 01605, USA

²Department of Molecular and Cellular Biochemistry and the Lucille Markey Cancer Center, University of Kentucky College of Medicine, Lexington, KY 40506, USA

³Max Planck Institute of Molecular Cell Biology and Genetics, Dresden 01307, Germany

⁴Department of Biochemistry, Weill Cornell Medical College, New York, NY 10021, USA

⁵Present address: School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, China

⁶Present address: Liptype GmbH, Dresden 01307, Germany

⁷These authors contributed equally

⁸Lead Contact

*Correspondence: eduardo.torres@umassmed.edu

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SUMMARY

Aneuploidy disrupts cellular homeostasis. However, the molecular mechanisms underlying the physiological responses and adaptation to aneuploidy are not well understood. Deciphering these mechanisms is important because aneuploidy is associated with diseases, including intellectual disability and cancer. Although tumors and mammalian aneuploid cells, including several cancer cell lines, show altered levels of sphingolipids, the role of sphingolipids in aneuploidy remains unknown. Here, we show that ceramides and long-chain bases, sphingolipid molecules that slow proliferation and promote survival, are increased by aneuploidy. Sphingolipid levels are tightly linked to serine synthesis, and inhibiting either serine or sphingolipid synthesis can specifically impair the fitness of aneuploid cells. Remarkably, the fitness of aneuploid cells improves or deteriorates upon genetically decreasing or increasing ceramides, respectively. Combined targeting of serine and sphingolipid synthesis could be exploited to specifically target cancer cells, the vast majority of which are aneuploid.

INTRODUCTION

Chromosome missegregation events leading to aneuploidy cause severe developmental defects in organisms (Torres et al., 2008). In the absence of other genomic alterations, losing chromosomes is usually lethal to cells whereas gaining chromosomes disrupts cellular homeostasis and hampers proliferation. At both the cellular and organismal levels, the deleterious effects of gaining chromosomes correlate with an increased number of encoding genes (Torres, 2015). However, the effects of aneuploidy on cell physiology can depend on the microenvironment as aneuploidy can confer a proliferative advantage under stress

conditions or resistance to a particular drug (Pavelka et al., 2010; Selmecki et al., 2006; Yona et al., 2012). Aneuploidy is a common characteristic of cancer cells, and gaining or losing chromosomes provides a mechanism by which cells gain copies of oncogenes or lose tumor suppressor genes, thereby driving tumorigenesis (Davoli et al., 2013). Importantly, the mechanisms by which cancer cells overcome the deleterious consequences associated with aneuploidy are not known.

To investigate how aneuploidy affects cellular physiology in eukaryotes, we generated and characterized a series of aneuploid yeast strains, each carrying an extra copy of a given chromosome (referred to as disomes; Torres et al., 2007). A direct consequence of acquiring an extra chromosome is the increased expression of the duplicated genes (Torres et al., 2016). On average, duplicated transcripts are translated, leading to proportional increases in protein abundance, with the notable exception of subunits of macromolecular complexes whose stability depends on complex assembly (Dephoure et al., 2014; McShane et al., 2016). The general increase in protein synthesis can cause several phenotypes shared by all aneuploid cells independent of the identity of the extra chromosome (Oromendia et al., 2012; Torres et al., 2007). Such phenotypes include decreased proliferation rates, increased glucose utilization, and signs of proteotoxic stress, all of which are also observed in aneuploid human cells (Santaguida and Amon, 2015; Stingle et al., 2012).

We previously identified aneuploidy-tolerating spontaneous mutations that improve the fitness of aneuploid cells (Torres et al., 2010). Among these, loss of function mutation in the deubiquitinating enzyme Ubp6 was shown to improve the fitness of 4 out of 12 aneuploid strains. Global proteome quantification revealed that loss of *UBP6* leads to the attenuation of the levels of overexpressed proteins, likely through a general increase in proteasome activity (Bashore et al., 2015; Dephoure et al., 2014; Hanna et al., 2006). This provides at least one mechanism by which altering a cellular process—increasing protein turnover—improves the fitness of aneuploid cells independent of karyotype.

Among the other aneuploidy-tolerating mutations in yeast were three independent spontaneous mutations in a gene that



regulates sphingolipid synthesis (*SVF1*), implicating these molecules in the physiological responses to aneuploidy (Brace et al., 2007; Torres et al., 2010). Aneuploid primary mouse embryonic fibroblasts (MEFs), cancer cell lines, and tumors show elevated levels of sphingolipids, yet a functional link between changes in sphingolipid levels and aneuploidy is not known (Erez-Roman et al., 2010; Guillermet-Guibert et al., 2009; Morad and Cabot, 2013; Tang et al., 2017). Sphingolipids are synthesized from serine and palmitoyl-coenzyme A (CoA) (Figure 1A). Both long-chain bases (LCBs) and ceramides increase rapidly upon stress and function as signaling molecules (Dickson et al., 1997; Jenkins et al., 1997). Whereas ceramides serve to slow proliferation by delaying entry into the cell cycle (Nickels and Broach, 1996), LCBs activate transcriptional responses and signaling pathways associated with cell wall integrity and survival (Coward et al., 2003; Dickson and Lester, 2002). Here, we find that the levels of both LCBs and ceramides are elevated in aneuploidy and that increased serine synthesis accounts for the accumulation of sphingolipids in aneuploid cells. Remarkably, the proliferation of several aneuploid strains improves significantly upon genetically decreasing the levels of ceramides and increasing those of LCBs. Transcriptome and proteome analyses of disomes harboring a mutation that improves their fitness indicate that sphingolipids regulate membrane protein composition, RNA biosynthesis, and metabolic pathways associated with energy production and biosynthesis.

RESULTS

Aneuploid Cells Rely on Increased *De Novo* Synthesis of Sphingolipids to Proliferate

To determine how regulation of sphingolipids affects the viability of aneuploid cells, we treated cells with myriocin, a specific inhibitor of serine palmitoyltransferase (SPT), which catalyzes the first and rate-limiting step of sphingolipid synthesis (Figure 1A). We found that 10 out of 13 disomes are hypersensitive to myriocin compared to wild-type cells (IV, V, VIII, IX, X, XI, XII, XIV, XV, and XVI; Figures 1B and 1C). Four other aneuploid strains harboring extra copies of either 2 or 3 chromosomes were also sensitive to myriocin treatment (Figure 1B). Furthermore, we found that 33 of 35 aneuploid yeast strains derived from random meiosis and that harbor complex karyotypes are sensitive to myriocin (Figure S1A; Pavelka et al., 2010). These results indicate that, despite the phenotypic variability and non-genetic individuality of aneuploid cells (Beach et al., 2017; Pavelka et al., 2010), the majority of aneuploid strains are sensitive to inhibition of sphingolipid synthesis, independent of karyotype identity.

Similar sensitivities were not observed in strains carrying yeast artificial chromosomes (YACs) that contain mammalian DNA with no known protein-coding genes (Figure 1B), indicating that enhanced myriocin sensitivity results from increased gene expression in aneuploid cells rather than increased DNA content. To determine whether sensitivity to inhibition of sphingolipid synthesis is a reflection of a more general need for lipids, we treated cells with cerulenin, an inhibitor of fatty acid synthase. We found that cerulenin does not specifically affect the viability of aneuploid cells, suggesting that lipid synthesis, in general, is not affected by aneuploidy (Figures 1D and S1B). Notably, three

hypomorphic alleles of essential cell cycle genes that exhibit reduced proliferation rates at the permissive temperature were not sensitive to myriocin (Figure 1E), indicating that impaired proliferation itself does not confer myriocin sensitivity. These results indicate that aneuploid cells rely on increased sphingolipid production for their viability.

Aneuploid Cells Have Elevated Levels of Long-Chain Bases and Ceramides

To test whether aneuploidy alters the cellular composition of LCBs or ceramides, we performed quantitative mass spectrometry (MS) on lipid extracts from 13 disomes as well as wild-type cells (Table S1; Bielawski et al., 2009). We found that the levels of LCBs or ceramides are increased in the aneuploid strains compared to wild-type cells (Figures 1F, 1G, and S1C). Interestingly, disomes with modest cell cycle delays, including disomes I, II, V, IX, X, and XIII (Torres et al., 2007), exhibited the smallest increases in ceramide levels (Figure 1G). Indeed, increases in ceramide levels correlated with decreased proliferation rates of the disomes (Figure S1D; Pearson $r = 0.65$; p value = 0.008). These results support the hypothesis that accumulation of ceramides plays a role in slowing the cell cycle in response to aneuploidy.

To investigate whether other lipids also increase in aneuploid cells, we comprehensively examined the cellular lipid composition using a MS-based global lipidomic technique in several strains (6 disomes analyzed: V, XI, XII, XIV, XV, XVI, and wild-type cells; Ejsing et al., 2009). These measurements confirmed a consistent and significant increase in ceramide levels in the disomes compared to wild-type cells. This is in contrast to most other lipids, the levels of which were only mildly and inconsistently altered (Figures S1E and S1F; Table S1). Lastly, we used a third approach based on quantitative high-performance liquid chromatography (qHPLC) to specifically quantify LCBs and their phosphorylated forms LCB-*Ps* (Lester and Dickson, 2001). We validated that the levels of LCBs and LCB-*Ps* are elevated in several disomes relative to wild-type cells (5 disomes analyzed: V, XI, XII, XV, XVI, and wild-type cells; Figures S1G–S1I; Table S2). Altogether, these results demonstrate, via three independent methodologies, that the levels of both LCBs and ceramides are increased in aneuploid cells and indicate that altered sphingolipid metabolism is a general feature of aneuploid cells.

Increased Serine Synthesis Accounts for Higher Levels of Sphingolipids in Aneuploid Cells

We next sought to understand the mechanisms leading to increased sphingolipid levels in aneuploid cells. Dozens of genes are involved in the regulation of sphingolipid biosynthesis (Table S3; Cherry et al., 2012). With the exception of the genes present in the duplicated chromosomes, mRNA and protein expression of this network of genes do not show significant differences between disomes and wild-type cells, indicating that transcriptional regulation does not play a significant role in the accumulation of sphingolipids in aneuploid cells (Figures S2A and S2B; Dephoure et al., 2014). We next examined whether aneuploidy increases SPT enzymatic activity. We found that, with the exception of disome IV, which contains two copies of the catalytic

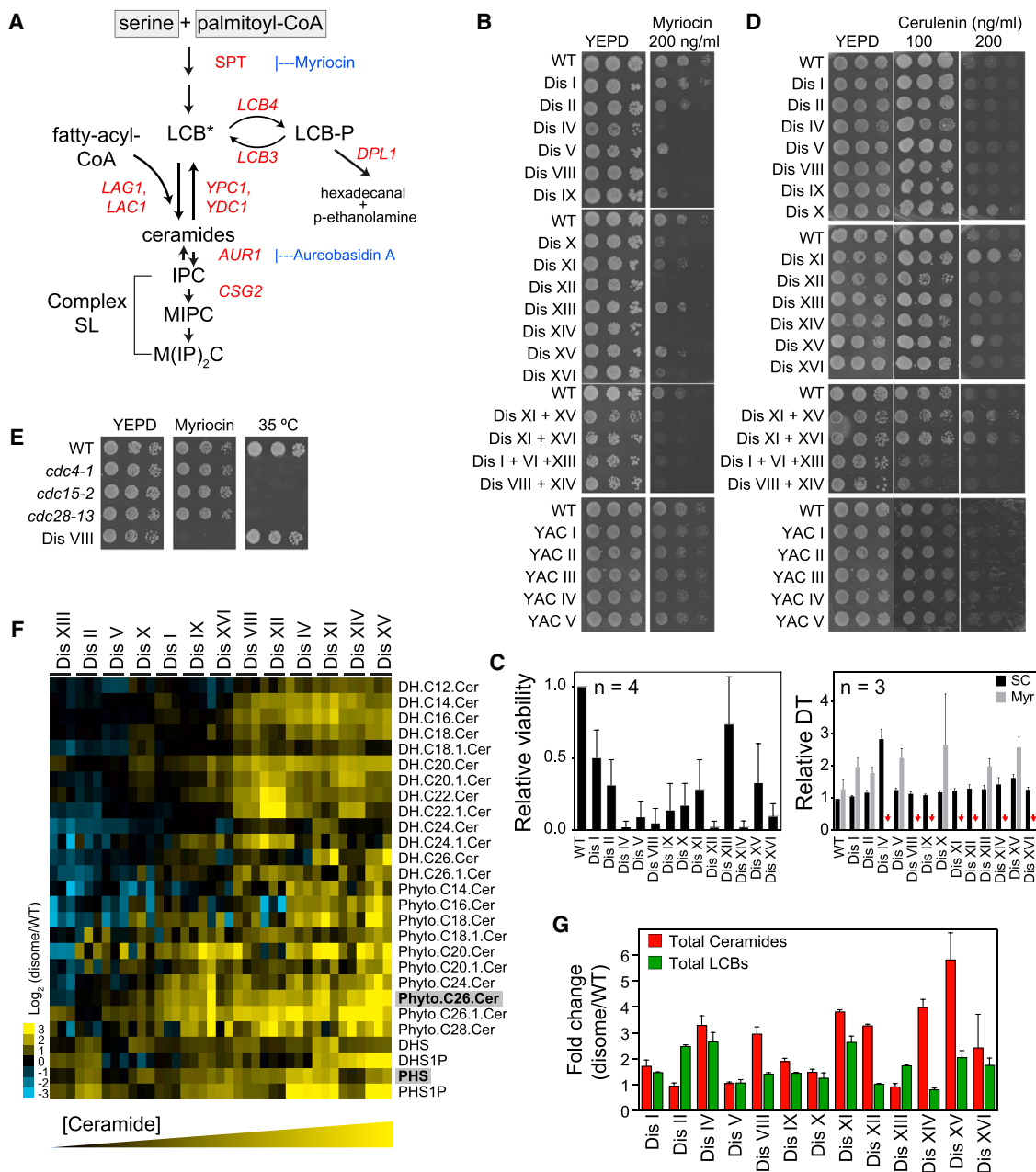


Figure 1. Aneuploidy Increases Sphingolipid Biosynthesis

(A) Biochemical pathway of *de novo* synthesis of sphingolipids in yeast. Genes used in this study are shown in red. LCB, long-chain bases, asterisk (*) indicates that LCBs need to be phosphorylated/dephosphorylated to be converted to ceramide; IPC, inositol-phosphorylceramide; MIPC, mannosyl-IPC; M(IP)₂C, mannosyl-diinositol-phosphorylceramide; SPT, serine palmitoyltransferase.

(B) Proliferative capability of wild-type cells (WT), disomes, and strains harboring YAC in the presence of myriocin.

(C) Quantification of the viability of cells treated with 200 ng/mL myriocin in the left panel. Right panel shows the doubling times of disomes in synthetic complete media alone (SC) (black bars) and with 200 ng/mL myriocin (Myr) (gray bars) relative to WT. Red arrows point to strains that did not grow in culture.

(D) Proliferative capability of WT, disomes, and strains harboring YAC in the presence of cerulenin.

(E) Proliferative capability of WT, cell cycle mutants, and disome VIII in the presence of myriocin (200 ng/mL) and at a restrictive temperature for the cell cycle mutants.

(F) Liquid chromatography (LC)/LC-MS analysis of LCBs and ceramides in the disomes compared to WT. Columns represent experiments (3 biological replicates shown). Rows represent lipid species. Cer, ceramide; DH, dihydro; DHS, dihydrosphingosine; PHS, phytosphingosine. Most abundant LCB and ceramide are in gray boxes.

(G) Fold change of total LCBs and ceramides in the disomes relative to WT.

Error bars represent ± SD. See [Supplemental Information](#) for details on the strains used in all figures. See also [Figure S1](#) and [Tables S1](#) and [S2](#).

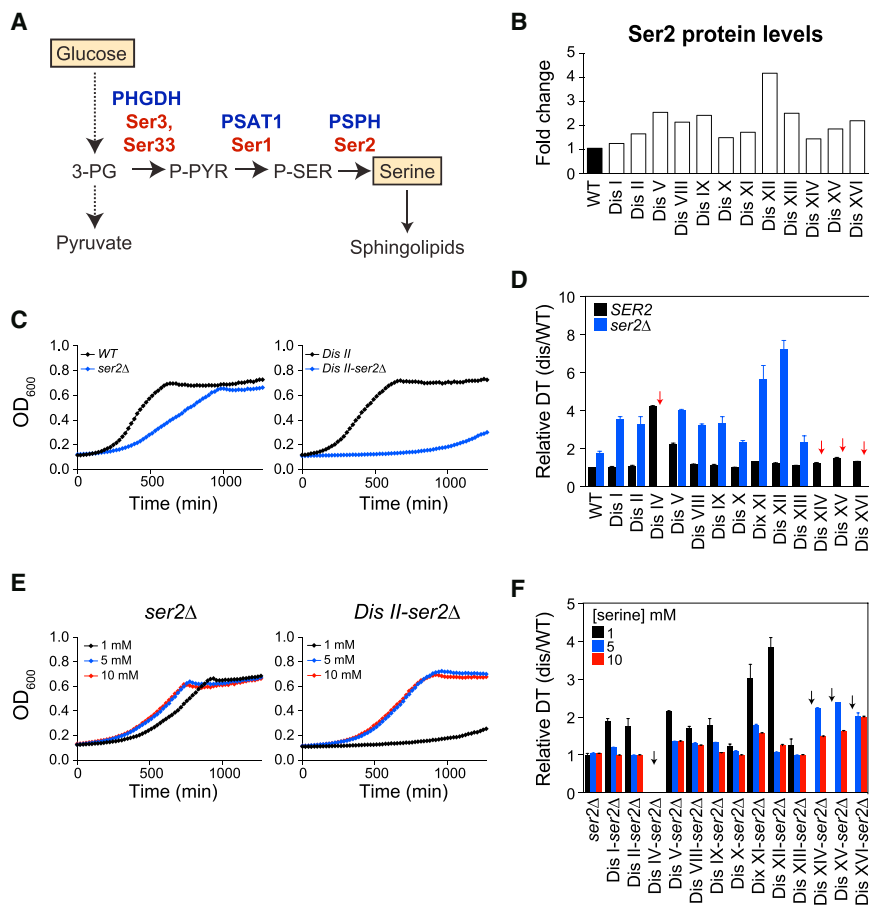


Figure 2. Aneuploid Cells Require Increased Serine Biosynthesis for Their Survival

(A) Biochemical pathway of *de novo* serine synthesis from glucose. Human genes (blue) and yeast genes (red) are shown. 3-PG, 3-phosphoglycerate; P-PYR, phosphohydroxypyruvate; P-SER, phosphoserine.

(B) Ser2 protein levels in the disomes relative to WT (Dephoure et al., 2014).

(C) Growth curves of cells with wild-type *SER2* (black line) or harboring *ser2Δ* (blue line). WT and disome II are shown.

(D) Doubling times of disomes (black bars) and disomes harboring *ser2Δ* (blue bars) relative to WT. Red arrows point to strains that did not grow in culture. Error bars represent \pm SD; $n = 3$.

(E) Growth curves of cells harboring *ser2Δ* in medium containing 1 mM serine (black line), 5 mM serine (blue line), and 10 mM serine (red line).

(F) Doubling times of disomes harboring *ser2Δ* in medium containing 1 mM (black), 5 mM (blue), or 10 mM (red) serine. Error bars represent \pm SD; $n = 3$. Black arrows point to strains that did not grow in culture.

See also Figure S2 and Table S3.

subunit of the SPT complex (*LCB2*), SPT activity is not significantly altered in the disomes compared to wild-type cells (Figures S2C and S2D). This indicates that signaling pathways known to regulate SPT activity do not play a critical role in the cellular responses to aneuploidy (Roelants et al., 2011).

Because substrate availability is rate limiting for the *de novo* synthesis of sphingolipids (Alvarez-Vasquez et al., 2005; Cowart and Hannun, 2007), we next examined the serine requirements for the proliferation of the disomes. We recently identified Ser2, the phosphoserine phosphatase that catalyzes the final and rate-limiting step in the synthesis of serine from glucose, to be upregulated in the disomes, indicating that serine biosynthesis from glucose may be critical for aneuploid cells (Figures 2A, 2B, S2A, and S2B; Dephoure et al., 2014). Strikingly, deletion of *SER2* significantly hampered the proliferation of all disomes tested while only causing a modest effect on the proliferation of wild-type cells (Figures 2C and 2D). Disomes harboring the largest chromosomes were most affected by *ser2Δ*, and four of these (IV, XIV, XV, and XVI) did not grow in liquid medium. Standard synthetic medium contains 1 mM serine, a condition under which the disomes proliferate very poorly. Supplementation of 5 or 10 mM serine significantly improved the proliferation of the disomes while minimally affecting that of wild-type cells (Figures 2E and 2F). Addition of other amino acids did not result in similar effects (Figure S2E). These data indicate that the dis-

omes exhibit a higher metabolic demand for serine and that the lack of serine alone and not the accumulation of metabolic intermediates upon *SER2* loss of function is deleterious to aneuploid cells. Similar results were obtained when the phosphoserine aminotransferase *SER1* was

deleted in the disomes (Figure S2F), thus corroborating our findings. Increased levels of sphingolipids and higher dependence on serine synthesis suggest that, compared to euploid cells, aneuploid cells utilize more serine for the production of sphingolipids. Indeed, we found that despite their impaired proliferation, most disomes (10 out of 12: I, II, VIII, X, XI, XII, XIII, XIV, XV, and XVI) have increased serine utilization compared to wild-type cells (Figures 3A and 3B). To determine whether changes in serine metabolic flux leads to increased sphingolipid synthesis, we quantified ^{14}C -serine incorporation into ceramides in wild-type cells and disomes. Using this approach, we detected increased serine incorporation into ceramides in 10 out of 12 disomes (I, V, VIII, IX, X, XI, XII, XIV, XV, and XVI; Figure 3C). The differences in serine intake account for the increased ceramide synthesis, because the fold change in serine intake strongly predicts the fold changes in ceramide levels (Pearson $r = 0.7$; Figure 3D). To validate whether impaired serine synthesis affects the synthesis of sphingolipids, we used quantitative MS to measure the levels of LCBs in four strains harboring the *ser2Δ* (wild-type and 3 representative disomes: V, XI, and XIV; Figure S3A) and confirmed that indeed LCBs are reduced when serine synthesis is impaired. These results show that aneuploid cells rely on increased serine synthesis for their survival and indicate that increased serine synthesis due to the upregulation of the

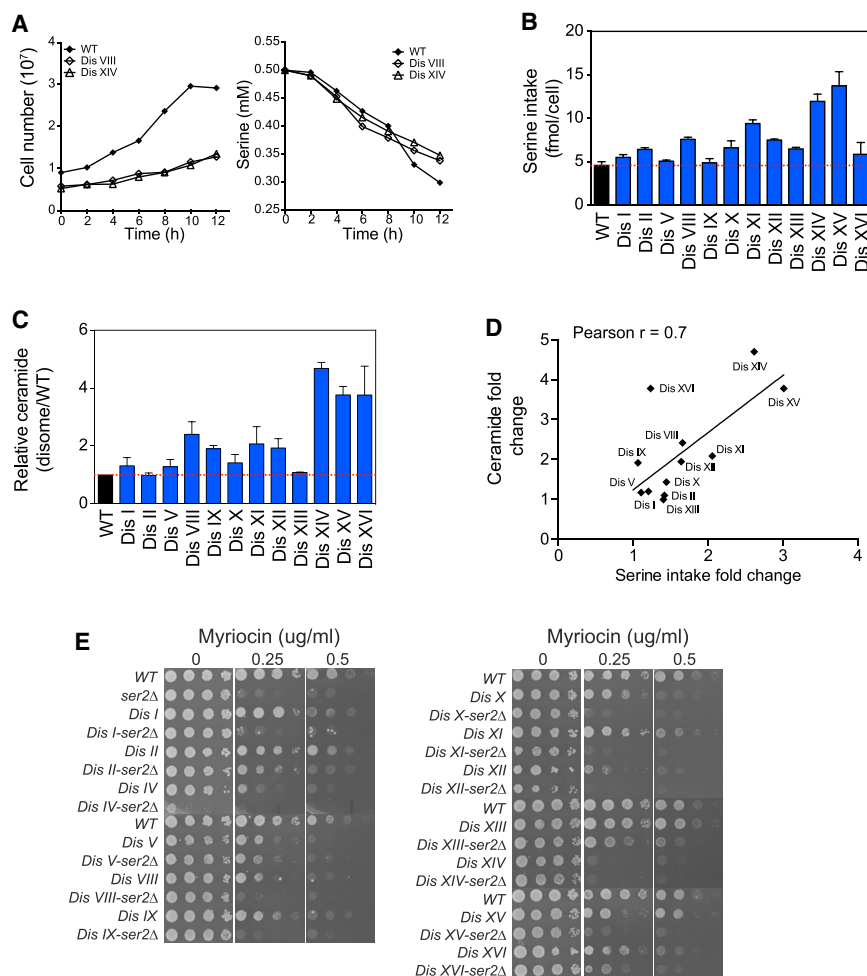


Figure 3. Aneuploid Cells Use Serine for the Synthesis of Sphingolipids

(A) Growth curves of WT, disome VIII, and disome XIV are shown in the left panel. Amount of serine in the medium as a function of time is shown (right panel).

(B) Serine utilization per cell during exponential growth of WT and disomes. Error bars represent \pm SD; $n = 3$ biological replicates.

(C) Relative amount of serine incorporated into ceramides in the disomes relative to WT. Error bars represent \pm SD; $n = 3$ biological replicates.

(D) Linear correlation between the fold change in serine intake and fold change in ceramide synthesis in the disomes.

(E) Proliferative capability of WT, *ser2Δ*, disomes, and the disomes harboring *ser2Δ* in the presence of myriocin.

See also Figure S3.

synthesis did enhance sensitivity to DHFR inhibitors in the disomes (Figure S3E). Our results indicate that serine flux into sphingolipid synthesis plays an important role in the cellular responses to aneuploidy and that inhibiting serine biosynthesis can sensitize aneuploid cells to drugs that target nucleotide or sphingolipid synthesis.

Reducing Ceramide Synthesis Improves the Proliferation of Aneuploid Cells

To investigate the physiological consequences of the altered levels of sphingolipids in aneuploid cells, we performed

phosphoserine phosphatase Ser2 is a major path through which aneuploid cells accumulate sphingolipids.

Impaired Serine Synthesis Enhances Sensitivity to Inhibition of Sphingolipid Synthesis

Our results raise the possibility that impaired *de novo* serine biosynthesis may render cells vulnerable to drugs that target sphingolipid synthesis. Indeed, we found that loss of either *SER2* or *SER1* enhanced sensitivity of all strains to myriocin (Figures 3E and S3B). Serine also serves as a precursor molecule for the synthesis of nucleotides. Therefore, it is possible that aneuploid cells show synthetic interaction with *ser2Δ* or *ser1Δ* because these cells have increased serine demand for nucleotide biosynthesis. To assess whether nucleotide biosynthesis is rate limiting for the proliferation of the disomes, we treated cells with drugs that inhibit dihydrofolate reductase (DHFR) activity, thereby blocking deoxythymidine monophosphate (dTMP) synthesis from serine (methotrexate and aminopterin; Figure S3C). Surprisingly, we found that, whereas most disomes are sensitive to the DNA-damaging agent 4-nitroquinoline 1-oxide (4NQ), the majority of disomes do not display enhanced sensitivity to DHFR inhibitors (Figure S3D). Nonetheless, impairing serine

epistasis analysis between genes that regulate ceramide synthesis and aneuploidy. If increased ceramide levels serve to slow cell cycle progression, deleting a gene that lowers ceramide synthesis may enhance the proliferation of aneuploid cells. To test this hypothesis, we deleted the ceramide synthase *LAG1* in 12 disomes and compared the doubling times with and without the *LAG1* deletion (*lag1Δ*). Remarkably, we found that loss of *LAG1* enhanced the proliferation of 9 disomes at 30°C (Figure S4A). Consistently, at 37°C, where the difference in proliferation between disomes and wild-type cells is more apparent, all disomes showed improved proliferation upon loss of *LAG1* (Figures 4A and S4B).

SVF1, the gene mutated in evolved isolates of disome XIV cells, acts in concert with the LCB kinase *LCB4* and the LCB-P phosphatase *LCB3* to control the phosphorylation of LCBs (Brace et al., 2007). The phosphorylation-dephosphorylation cycle of LCBs is required for their proper compartmentalization and acylation by ceramide synthases (Funato et al., 2003; Qie et al., 1997). Consequently, loss of *LCB3*, *LCB4*, or *SVF1* lowers ceramides and may suppress cell cycle defects in aneuploid cells (Figures S4D and S4E; Mandala et al., 1998). Remarkably, we found that deletion of any one of these

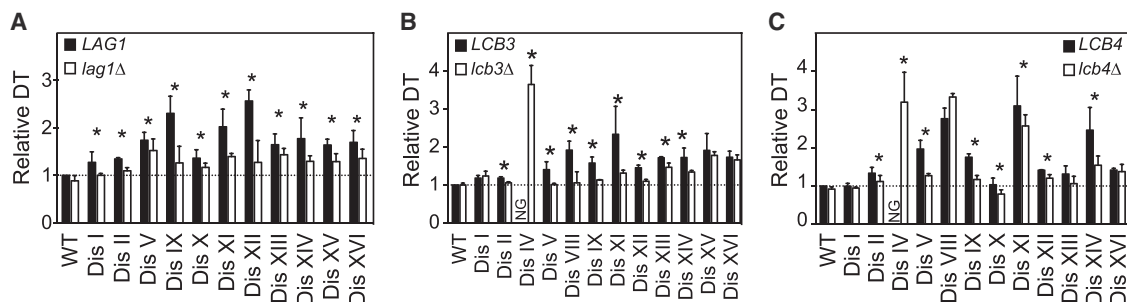


Figure 4. Reduced Ceramide Synthesis Improves the Fitness of Aneuploid Cells

(A) Doubling times of disomes (black bars) and disomes harboring *lag1Δ* (open bars) relative to WT at 37°C.

(B) Doubling times of disomes (black bars) and disomes harboring *lcb3Δ* (open bars) relative to WT at 37°C.

(C) Doubling times of disomes (black bars) and disomes harboring *lcb4Δ* (open bars) relative to WT at 37°C.

The average of 3 biological replicates is shown. Error bars represent \pm SD. * represents p value < 0.05; paired Student's t test. See also Figure S4.

three genes suppresses the proliferation defects of several aneuploid strains. Indeed, in addition to disome XVI, the proliferation of 5 other disomes improved upon deleting *SVF1* at 30°C (Figure S4C). Moreover, deletion of *LCB3* significantly improved the proliferation of 7 disomes at 30°C (Figure S4D) and that of 9 disomes at 37°C (Figure 4B). Furthermore, whereas *LCB4* loss of function did not affect the fitness of wild-type cells, it significantly improved the fitness of 5 disomes at 30°C (Figure S4E) and that of 8 disomes at 37°C (Figure 4C). These results indicate that distinct gene mutations leading to lowered ceramide synthesis have beneficial effects on the proliferation of the aneuploid strains.

Increased Ceramide Levels Can Be Exploited to Target Aneuploid Cells

Because ceramides are elevated in the disomes and ceramides specifically arrest cells in G1 (Figure S5A), we next examined whether further increases in ceramide levels could be exploited to kill aneuploid cells. To this end, we performed three different genetic approaches that can result in elevated ceramide levels. First, we analyzed the consequences of deleting *CSG2*, a protein that regulates the synthesis of complex sphingolipids (Beeler et al., 1997). We found that 9 out of 12 disomes harboring *csg2Δ* show impaired fitness and that their proliferation is significantly affected at 37°C (Figure 5A). Second, we examined the effects of inhibiting the hydrolysis of sphingolipids by deleting the LCB lyase *DPL1* (Coward et al., 2010; Saba et al., 1997). We found that loss of *DPL1* is deleterious to the majority of the disomes (9 out of 12 disomes; Figure S5B). Lastly, we examined the effects of deleting the ceramidase *YPC1* and found that this deletion reduces the fitness of four disomes (I, VIII, XII, and XVI; Figure S5C). We hypothesize that expression of the other yeast ceramidase *YDC1* may compensate for the loss of *YPC1*. We could not test the effects of deleting both ceramidases due to lethality to yeast.

To validate these findings, we compared the viability of cells grown in rich medium alone or in the presence of 20 μ M C_2 -ceramide. We found that the proliferation of the majority of the disomes is impaired compared to wild-type cells in the presence of C_2 -ceramide (IV, V, VIII, X, XI, XII, XIII, XIV, XV, and XVI; Fig-

ure S5D). We next examined the effects of increasing ceramide levels in the disomes by inhibiting their conversion to complex sphingolipids by treating cells with aureobasidin A, a specific inhibitor of *AUR1*, the inositol phosphorylceramide synthase (Nagiec et al., 1997). We found that most disomes are hypersensitive to this drug compared to wild-type cells (IV, V, VIII, IX, XI, XII, XIV, XV, and XVI; Figure S5E). These results demonstrate that the majority of aneuploid strains are sensitive to drugs that elevate ceramide levels, with a few exceptions. Given the complexity and number of genes involved in the regulation of ceramide synthesis, expression of one or more genes on the duplicated chromosomes may confer resistance to these drugs or help cells tolerate increases in ceramide levels.

In sum, our studies reveal that loss of function of either *LAG1*, *LCB3*, *LCB4*, or *SVF1*, which leads to increased LCB and lowered ceramide levels, improves the proliferation of the majority of aneuploid strains; in contrast, increasing ceramide levels either genetically or pharmacologically decreases their fitness (Figure 5B). The concomitant increase in the production of LCBs and ceramides in response to aneuploidy supports the hypothesis that ceramides slow down the proliferation of aneuploid cells whereas LCBs promote their survival.

Loss of *LCB3* Remodels Membrane Protein Composition of Aneuploid Cells

To gain mechanistic insight into how sphingolipids improve the fitness of aneuploid cells, we analyzed the transcriptome profiles of 10 disomes harboring *lcb3Δ* compared to wild-type cells (Figure 6A; Table S4). We focused on the effects of *LCB3* loss for two reasons: (1) competition assays revealed that whereas the fitness of wild-type cells is not affected by *lcb3Δ*, that of 9 disomes improved significantly at 30°C (Figure S6A) and (2) MS measurements revealed that loss of *LCB3* restores ceramide levels close to those of wild-type while significantly increasing LCB levels (Figures S6B and S6C). This allows us to determine gene expression changes mainly driven by increases in LCBs. In addition, we included in our analysis the transcriptional profiles of 13 disomes without any deletions (*LCB3*, *UBP6*) and 13 disomes harboring *ubp6Δ* (Dephoure et al., 2014). We refer to the latter 26 strains as control strains.

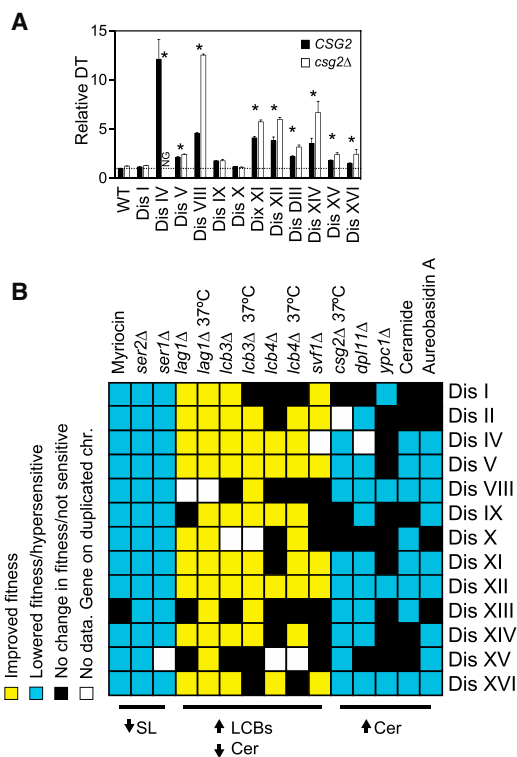


Figure 5. Effects of Shifting the Balance between Ceramide and Long-Chain Bases in Aneuploid Strains

(A) Doubling times of disomes (black bars) and disomes harboring *csg2Δ* (open bars) relative to WT at 37°C. The average of 3 biological replicates is shown. Error bars represent \pm SD. * represents p value < 0.05 ; paired Student's t test.

(B) Summary of the effects of several gene deletions and drugs that target the synthesis of sphingolipids on the fitness of thirteen disomes.

See also Figure S5.

We found that 62% of the transcriptome of the disomes with *lcb3Δ* show significant changes compared to wild-type cells (3,107 of 5,016 genes). Sorting by intensities, we identified groups of genes with similar profiles and categorized them into several clusters (Figure 6B; see Supplemental Experimental Procedures). Cluster 1 includes 655 genes that are upregulated in the control strains but are significantly attenuated upon loss of *LCB3*. Cluster 2 includes 624 genes whose expression is not altered in the control strains but are downregulated by the *lcb3Δ*. Gene Ontology enrichment (GO) analysis revealed that genes encoding membrane proteins are significantly enriched in clusters 1 and 2 ($n = 496$; p value = $8E-26$; Table S4). Among these are several transmembrane transporters ($n = 145$; p value = $5E-08$; Table S4). Notable downregulated genes include ATP-binding cassette (ABC) transporters and transporters of metabolites, such as amino acids, uracil, copper, and phosphate. LCBs, whose levels increase upon loss of *LCB3*, are known to downregulate nutrient intake by mechanisms that are not fully understood (Chung et al., 2001; Skrzypek et al., 1998). Our results indicate that transcriptional regulation of transporters is one mechanism by which LCBs regulate nutrient intake.

Loss of *LCB3* Upregulates Genes Involved in Transcription and Metabolic Processes

Cluster 3 includes 533 genes that do not significantly change in the disomes but are upregulated by *lcb3Δ*. Cluster 4 includes 657 genes that are downregulated in the disomes, and their levels increase upon loss of *LCB3*. GO analysis revealed a significant enrichment for genes encoding proteins that localize to the nucleus in these clusters ($n = 558$; p value = $3E-17$), including nucleolar proteins ($n = 96$; p value = $3E-06$) and proteins that regulate RNA biosynthesis ($n = 361$; p value = $7E-11$; Figure 6B; Table S4). In addition, within the most upregulated genes, we found a significant enrichment for those that regulate metabolic processes, including mitochondrial genes (p value = $1E-06$). Our results indicate that accumulation of LCBs upon loss of *LCB3* activates signaling pathways that drive the described transcriptional changes, raising the hypothesis that changes in sphingolipids improve the fitness of aneuploid cells by altering membrane physiology, promoting RNA biosynthesis, and affecting metabolic functions. Notably, genes whose levels increase or decrease in all 36 aneuploid strains are associated with a transcriptional response termed the environmental stress response and are not affected by the loss of *LCB3* in the disomes (Gasch et al., 2000).

Consequences of *LCB3* Loss of Function on Cellular Protein Composition of Aneuploid Cells

To gain further insight into how sphingolipids improve fitness of aneuploid cells, we used tandem mass tag (TMT)-based quantitative mass spectrometry to determine the proteome profiles of 10 disomes harboring *lcb3Δ* (72% of verified open reading frames [ORFs]; $n = 3,682$; Table S5). As previously shown for the control strains, we found that, on average, increases in gene copy number lead to proportional changes in protein levels in 10 disomes analyzed, all harboring *lcb3Δ* (Figure 7A). In addition, we found that protein levels of duplicated genes that code for subunits of macromolecular complexes are attenuated to the same extent as in the control strains (Figure S7A).

To reveal the most relevant changes in protein levels elicited by the loss of *LCB3*, we sorted by the averaged \log_2 ratios and identified a subset of proteins that specifically change in the disomes upon loss of *LCB3* (Figure 7B). We found that 655 and 290 proteins are upregulated and downregulated, respectively, in the disomes harboring *lcb3Δ*. Consistent with the transcriptome analysis, we found that the subset of downregulated proteins is enriched for membrane proteins whereas those that are upregulated are significantly enriched for metabolic processes (Figure 7B). GO term analysis of the upregulated proteins revealed more specific cellular processes than the transcriptome analysis because as much as 58% (383 proteins) of the upregulation in protein levels takes place independent of transcription. Remarkably, several metabolic pathways associated with cellular anabolism and energy production were induced by the loss of *LCB3* (Figure 7B). Among the most enriched proteins were enzymes involved in gluconeogenesis, the tricarboxylic acid cycle (TCA) and glyoxylate cycles, trehalose and glycogen biosynthesis and breakdown, and ATP synthesis. In addition, among the most upregulated proteins was Hsp12, a membrane protein involved in maintaining membrane integrity and required for

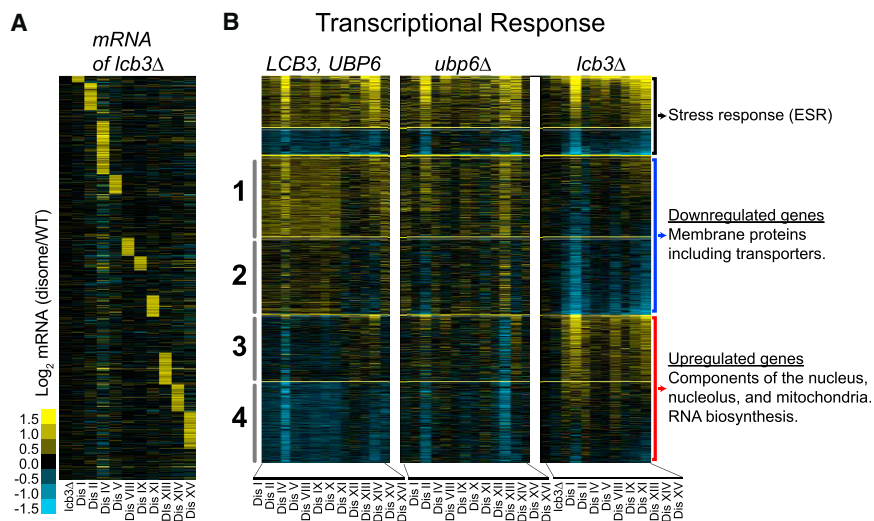


Figure 6. Loss of *LCB3* Remodels Membrane Protein Composition and Promotes RNA Biosynthesis

(A) Gene expression analysis of disomes harboring *lcb3Δ* grown in batch culture, ordered by chromosome position. Experiments (columns) are ordered by the number of the chromosomes present in two copies. Data were normalized to account for the extra chromosome present in disomic strains. Upregulated genes are shown in yellow and downregulated ones in blue.

(B) Gene expression of disomes, disome harboring *ubp6Δ*, and disomes harboring *lcb3Δ*. Columns represent experiments. Rows are genes organized by pattern of expression of disomes-*lcb3Δ* compared to the control strains (13 disomes and 13 disomes-*ubp6Δ*). Top two clusters are up- and downregulated in all 36 strains. Clusters 1 and 2 are downregulated by *lcb3Δ*. Clusters 3 and 4 are upregulated by *lcb3Δ*. See Table S4 for GO enrichment analysis details. See also Figure S6 and Table S4.

dietary-restriction-induced lifespan extension (Herbert et al., 2012). These results are consistent with our conclusions from the transcriptome analysis and indicate that LCBs improve the fitness of aneuploid cells through transcriptional and posttranscriptional mechanisms to remodel membrane protein composition and promote metabolic pathways associated with anabolism and ATP synthesis.

Importantly, loss of *LCB3* did not attenuate the levels of over-expressed proteins in the disomes, indicating that the mechanisms by which changes in sphingolipids improve the fitness of aneuploid cells are distinct from those induced by the loss of *UBP6* (Figure S7B). In support for this, we found that concomitant loss of *UBP6* and *LCB3* enhances the fitness of aneuploid cells to a greater extent than the loss of either alone, indicating a cooperation between both gene deletions (Figure S7C).

DISCUSSION

Aneuploidy Increases *De Novo* Synthesis of Sphingolipids

Here, we found that aneuploid cells accumulate higher levels of LCBs and ceramides and that this accumulation plays a key role in the cellular response to aneuploidy. Our studies support the hypothesis that LCBs activate survival pathways and ceramides function to slow down cell cycle progression in response to aneuploidy. Remarkably, we found that the changes in lipid composition elicited by aneuploidy are specific to sphingolipids rather than any other lipid class. Furthermore, we provide the important finding that changes in sphingolipid levels are driven by increased serine biosynthesis, which itself is driven by enhanced glucose utilization and upregulation of the Ser2 protein levels. In addition, we found that decreased serine synthesis significantly alters sphingolipid composition, indicating that sphingolipid levels are sensitive to the amount of intracellular serine available for their synthesis. These results indicate that altered serine metabolic demand can elicit dramatic physiological responses associated with sphingolipid biology. Serine

serves as a precursor molecule for nucleotides, amino acids, and other lipids, and its synthesis is increased in a large proportion of human tumors (Locasale et al., 2011; Possemato et al., 2011). In cancer, it is recognized that serine fuels metabolic pathways associated with the one-carbon cycle (Locasale, 2013). Here, we demonstrate that, as compared to euploids, aneuploid cells are dependent on increased serine utilization, which can lead to altered sphingolipid content and improved cellular fitness. As a result, serine and sphingolipid synthesis represent an important metabolic dependency of aneuploid cells.

Lowering Ceramides Improves the Fitness of Aneuploid Cells

Inhibition of the synthesis of both LCBs and ceramides by myriocin treatment is lethal to aneuploid cells. However, lowering ceramide synthesis while increasing LCB enhances their proliferation. These results suggest that aneuploid cells strictly rely on the survival functions of LCBs. If ceramide slows down the cell cycle, why would aneuploid cells accumulate higher levels of this molecule? Endogenous levels of LCBs are coupled to those of ceramides, as these molecules are generated from each other and are in a physiological balance. As it happens in response to extrinsic stresses, both the “good” (LCBs) and the “bad” (ceramide) lipids increase in response to aneuploidy. Thereby, higher ceramide levels may increase the selective pressure to acquire mutations to lower their synthesis. Lipidomics measurements indicate that the combined cellular amounts of LCBs and ceramides are two orders of magnitude lower than their products, the complex sphingolipids (0.1% versus 15% of total lipids; Figure S1). This suggests that a high metabolic flux exists through the pathway and that minor changes in enzyme activities or substrate concentrations could lead to significant changes in the levels of LCBs and ceramides. Four enzymatic activities control the interconversion of LCBs and ceramides, each encoded by at least 2 genes: LCB kinase (*LCB4* and *LCB5*); LCB-P phosphatase (*LCB3* and *YRS3*); ceramide synthase (*LAG1* and *LAC1*); and ceramidase (*YPC1* and *YDC1*). Given this genetic redundancy, we

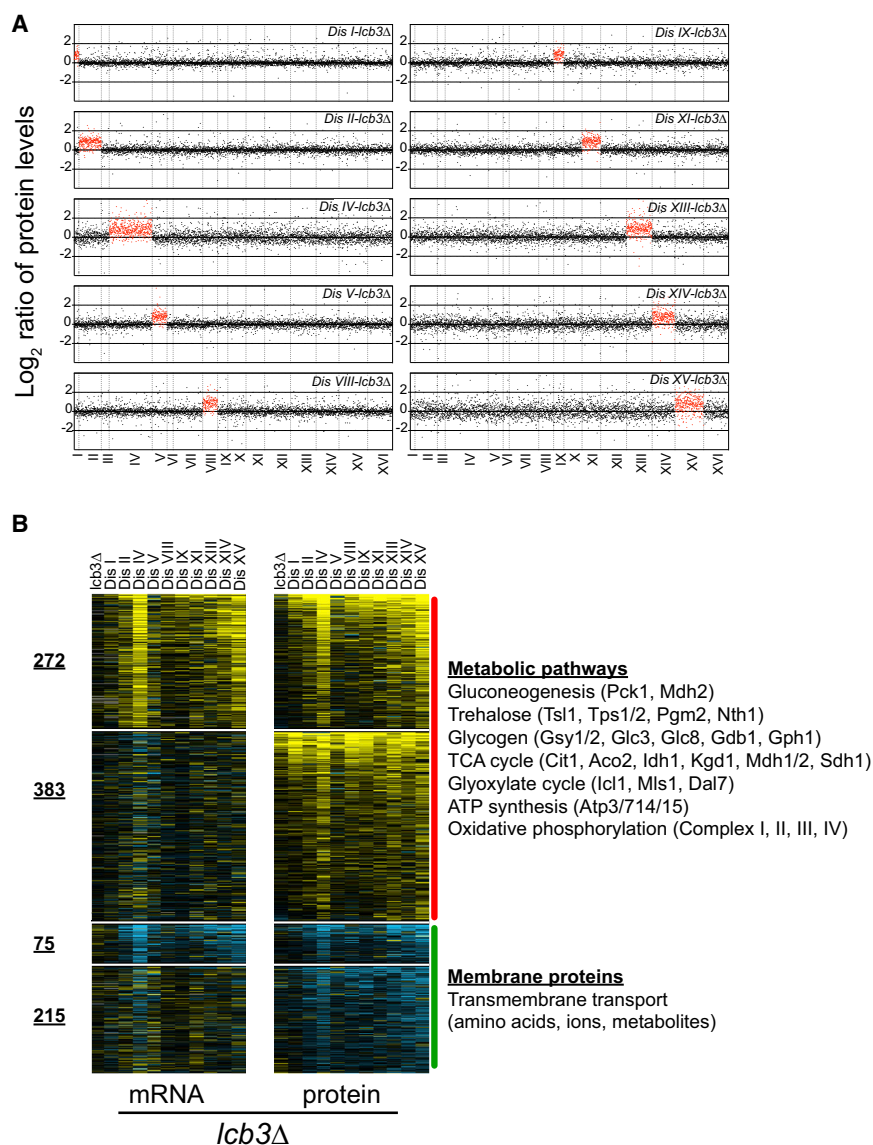


Figure 7. Loss of LCB3 Remodels Membrane Protein Composition and Promotes Anabolism and Mitochondrial Function

(A) The plots show the log₂ ratio of the relative protein abundance of disomes-*lcb3Δ* compared to WT. Protein levels are shown in the order of the chromosomal location of their encoding genes. Protein levels of duplicated chromosomes are shown in red.

(B) Transcript and protein abundances of genes that are specifically up- and downregulated in the disomes upon the loss of *LCB3*. See Table S5 for GO enrichment analysis details. See also Figure S7 and Table S5.

golipids regulate many aspects of cellular physiology. Because the *lcb3* deletion (1) improves the fitness of aneuploid cells without affecting wild-type cells and (2) increases LCB levels while restoring ceramide levels closer to those of wild-type levels, we were able to identify specific cellular processes affected by LCBs in aneuploid cells. Nearly 50% of the cellular transcriptome is affected in the disomes when *LCB3* is deleted. We found that nutrient transporters are transcriptionally downregulated by LCBs, indicating that transcription factors can act downstream of LCBs. Counter-intuitively, restriction of nutrient intake could be beneficial for aneuploid cells. In a manner similar to how dietary restriction promotes metabolic changes contributing to increased longevity and resistance to stress (Fontana et al., 2010), remodeling the cell's membrane protein composition in aneuploid cells may promote metabolic pathways associated with biosynthesis and energy production. These metabolic changes in turn are beneficial to aneuploid cells because gaining an extra chromosome increases the metabolic demands driven by enhanced transcription and protein synthesis, folding, and turnover. Consistently, loss of *LCB3* or *LAG1* (longevity assurance gene) induces resistance to stress and increases lifespan in yeast (D'mello et al., 1994; Skrzypek et al., 1999). Our results provide important insights into the molecular mechanisms by which several mutations in the sphingolipid pathway can promote longevity and enhance resistance to stress.

found that reducing ceramide levels while increasing those of LCBs (through deletion of one of two genes that hamper LCB kinase activities, LCB-P phosphatase or ceramide synthase) improves the fitness of aneuploid cells. Increasing ceramide levels by lowering ceramidase activity has the opposite effect. Interestingly, most of the deletions included in our studies have little effect on the proliferation of wild-type cells under non-stress conditions. This suggests that the genetic redundancy of this essential pathway that is conserved and expanded in humans—where at least 6 different genes code for ceramide synthases and 5 for ceramidases (Coant et al., 2017; Wegner et al., 2016)—allows flexibility of regulation during cellular responses to stress.

Long-Chain Bases Promote Anabolism and Energy Production in Aneuploid Cells

Identifying a specific cellular process affected by changes in sphingolipid levels is experimentally challenging, because sphin-

goid cells because gaining an extra chromosome increases the metabolic demands driven by enhanced transcription and protein synthesis, folding, and turnover. Consistently, loss of *LCB3* or *LAG1* (longevity assurance gene) induces resistance to stress and increases lifespan in yeast (D'mello et al., 1994; Skrzypek et al., 1999). Our results provide important insights into the molecular mechanisms by which several mutations in the sphingolipid pathway can promote longevity and enhance resistance to stress.

The mechanisms by which loss of *LCB3* improve the fitness of aneuploid cells are distinct from those elicited by the loss of *UBP6*. This highlights the complexity of the cellular responses to aneuploidy and indicates that parallel pathways can be acted upon to overcome the negative effects of aneuploidy. It is important to note that the fitness of the disomes harboring both deletions is lower than that of wild-type cells, indicating that other mutations could be acquired to further improve fitness.

Targeting Sphingolipids in Human Diseases

Aneuploid primary MEFs, cancer cell lines, and human tumors show elevated levels of sphingolipids. Despite numerous studies exploiting this correlation to target cancer cells, a general mechanism explaining how cells accumulate higher levels of sphingolipids is lacking. Here, we show that aneuploidy increases the biosynthesis of sphingolipids and that aneuploid cells are highly dependent on serine and sphingolipid synthesis. This metabolic codependency could therefore be exploited therapeutically to specifically target cancers, the vast majority of which are aneuploid. Furthermore, individuals with Trisomy 21 suffer from several immunological disorders, increased risk for leukemias, premature aging, and neurodegenerative disorders, such as Alzheimer's disease. Consequently, our results also provide a novel framework to study the mechanisms affecting sphingolipid metabolism in Down syndrome patients.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Conditions

All stains are derivatives of W303 (E187). Detailed methodologies, a list of strains, and media conditions are provided in [Supplemental Experimental Procedures](#).

Growth Rate Measurements

All cells were grown overnight to optical density 600 (OD₆₀₀) between 0.5 and 2 and then diluted to a starting OD₆₀₀ of 0.2 in 100 μ L of fresh medium in 96-well plates. Cell growth was monitored using a Tecan Infinite PRO microplate reader at indicated temperatures with continuous shaking. Relative doubling times (DTs) are simply the ratio of the DT of the strain divided by the DT of the WT measured in identical experimental conditions.

Mass Spectrometry of Sphingolipids

Lipidomics of wild-type cells and thirteen disomes were outsourced to the Lipidomics Shares Resource at the Medical University of South Carolina. Cells were treated with 5% trichloroacetic acid for 10 min on ice and washed with water 3 times before shipping in dry ice. Three independent cultures were analyzed for each strain, and relative levels of all lipids were normalized to total cell numbers, which were determined using a Beckman Coulter Counter. Protocols for lipid extraction and analysis are described in [Bielawski et al. \(2009\)](#).

Global Lipidomics of Yeast Cells

The lipid compositions of total cell extracts were determined by quantitative shotgun lipidomic analysis as previously described ([Ejsing et al., 2009](#); [Klose et al., 2012](#); [Supplemental Experimental Procedures](#)).

Gene Expression and Proteome Quantification

Cells were grown overnight at 30°C in selective medium. Batch cultures were diluted to OD₆₀₀ = 0.2 into YEPD medium the next day and harvested once they reached an OD₆₀₀ = 1.0. Gene expression was analyzed using Agilent microarrays as previously described in [Torres et al. \(2007\)](#). Proteome analysis was performed as described in [Dephoure et al. \(2014\)](#). See [Supplemental Experimental Procedures](#) for details.

DATA AND SOFTWARE AVAILABILITY

The accession number for the microarray data reported in this paper is GEO: GSE93762.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and five tables and can be found with this article online at <https://doi.org/10.1016/j.celrep.2017.11.103>.

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AUTHOR CONTRIBUTIONS

E.M.T. designed the study, and E.M.T., S.H., H.T.G., C.O., and G.B. performed all experiments. X.H. and R.C.D. contributed to the design of lipidomics experiments and performed qHPLC. C.K. and A.S. contributed to the design of lipidomics experiments and performed LC-MS. P.C. and N.D. contributed to the design and performed proteomics experiments. All authors contributed to data analysis and discussion. E.M.T. wrote the paper, and all authors contributed to editing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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