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

Graphical Abstract

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

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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.

Data and Software Availability

GSE93762
Serine-Dependent Sphingolipid Synthesis Is a Metabolic Liability of Aneuploid Cells

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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; Selmeci 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 (Öromendia 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; Stingele 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 mole-
cules 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; Jen-
kings 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 (Cowart 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 geneti-
cally 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 in-
hibitor 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 myrio-
cin 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 individu-
ality 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 re-
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 spectrom-
etry (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). Interest-
ingly, 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 composi-
tion 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 inco-
sistently 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 be-
tween disomes and wild-type cells, indicating that transcrip-
tional regulation does not play a significant role in the accumula-
tion 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 excep-
tion 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.
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 disomes 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 14C-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...
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 deoxothymidine 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 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 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
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 CSS2, a protein that regulates the synthesis of complex sphingolipids (Beeler et al., 1997). We found that 9 out of 12 disomes harboring css2Δ 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 (Cowart et al., 2010; Saba et al., 1997). We found that loss of DPL1 is detrimental 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 ceramidas 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 μM C2-ceramide. We found that the proliferation of the majority of the disomes is impaired compared to wild-type cells in the presence of C2-ceramide (IV, V, VIII, X, XI, XII, XIII, XIV, XV, and XVI; Figure 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.
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 log2 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...
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 overexpressed 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 ceramide 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 myristicin 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
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 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 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 sphingolipid levels is experimentally challenging, because sphingolipids 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; Skrypek 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.

**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-lcb3∆ 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.
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 strains 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 μL 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 water.

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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