Yeast Phospholipase C Is Required for Normal Acetyl-CoA Homeostasis and Global Histone Acetylation*

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**Background:** Glucose metabolism provides acetyl-CoA for histone acetylation.

**Results:** Inositol polyphosphates (InsPs), produced by the phospholipase C-dependent pathway, are required for degradation of the transcriptional repressor Mth1p, expression of glucose transporters, and normal acetyl-CoA homeostasis.

**Conclusion:** Defect in InsP synthesis results in global histone hypoacetylation and altered transcriptional regulation.

**Significance:** InsPs affect synthesis of glucose-derived acetyl-CoA and global histone acetylation.

Phospholipase C (Plc1p) is required for the initial step of inositol polyphosphate (InsP) synthesis, and yeast cells with deletions of the PLC1 gene are completely devoid of any InsPs and display aberrations in transcriptional regulation. Here we show that Plc1p is required for a normal level of histone acetylation; plc1Δ cells that do not synthesize any InsPs display decreased acetylation of bulk histones and global hypoacetylation of chromatin histones. In accordance with the role of Plc1p in supporting histone acetylation, plc1Δ mutation is synthetically lethal with mutations in several subunits of SAGA and NuA4 histone acetyltransferase (HAT) complexes. Conversely, the growth rate, sensitivity to multiple stresses, and the transcriptional defects of plc1Δ cells are partially suppressed by deletion of histone deacetylase HDA1. The histone hypoacetylation in plc1Δ cells is due to the defect in degradation of repressor Mth1p, and consequently lower expression of HXT genes and reduced conversion of glucose to acetyl-CoA, a substrate for HATs. The histone acetylation and transcriptional defects can be partially suppressed and the overall fitness improved in plc1Δ cells by increasing the cellular concentration of acetyl-CoA. Together, our data indicate that Plc1p and InsPs are required for normal acetyl-CoA homeostasis, which, in turn, regulates global histone acetylation.

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3 The abbreviations used are: PLC, phospholipase C; InsPs, inositol polyphosphates; PP-InsPs, inositol pyrophosphates; HAT, histone acetyltransferase; HDAC, histone deacetylase complex; ACL, ATP-citrate lyase.
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**TABLE 1**

Yeast strains used in this study

| Strain      | Genotype                  | Source/Ref.     |
|-------------|---------------------------|-----------------|
| W303-1a     | MATa ade2-1 his3-11,15 leu2-3,112 trpl-1 ura3-1 sod1-d2 can1–100 | R. Rothstein    |
| W303-1x     | MATa ade2-1 his3-11,15 leu2-3,112 trpl-1 ura3-1 sod1-d2 can1–100 | R. Rothstein    |
| W303        | MATa/MATa ade2-1/ade2-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trpl-1/trpl-1 lur3-1/lur3-1 can1–100/ can1–100 | R. Rothstein    |
| HLI-1       | W303-1a plc1::LIR3         | 12              |
| HLI-3       | W303-1a plc1::LIR3         | 103             |
| WPA023      | W303-1a plc1::TRP1         | 103             |
| WPL046      | W303-1a plc1::TRP1         | 103             |
| A0004       | W303-1a ipk1::kanMX       | 5               |
| A0003       | W303-1a ipk2::HIS3         | 3               |
| LSY507      | W303-1a kc1::HIS3          | 11              |
| DYS5116     | W303-1a gcn5::CEN4         | 104             |
| FY1292      | MATa gcn5::HIS3 leu2Δ1 lys2–173 trp1Δ36 vas3–52 | 105             |
| ND818       | W303-1a gcn5::HIS3         | This study      |
| ND703       | W303-1a gcn5::TRP1         | 45              |
| DY6707      | W303-1a gcn5::LEU2         | 45              |
| YTT2256     | W303-1a gcn5::HIS3         | 106             |
| YTT3122     | W303-1a gcn5::LEU2         | 107             |
| YTT2392     | W303-1a gcn5::HIS3         | 107             |
| DYS5068     | W303-1a hda1::LIR3         | 104             |
| LG167       | W303-1a hda1::LIR3         | This study      |
| LG159       | W303-1a plc1::TRP1 hda1::LIR3 | This study   |
| CYW1128     | W303-1a grr1::LEU2         | 84              |
| CYW1310     | W303-1a MTH1–6x-myc::KanMX2 | 84              |
| LG442       | W303-1a plc1::LIR3         | This study      |
| LG510       | W303-1a MTH1–6x-myc::KanMX2| This study      |
| AUY009      | MATa tetO–ACC1 ura3–52 trpl–63 leu2Δ1::HIS3 | 82              |
| LG362       | W303-1a tetO–ACC1         | 38              |
| LG364       | W303-1a tetO–ACC1 plc1::LIR3 | 38              |
| YMY6266     | MATa his3Δ leu2a::LIR3 | 108             |
| mth1::kanMX2| W303-1a mth1::kanMX2       | This study      |
| LG471       | W303-1a mth1::kanMX2       | This study      |
| LG474       | W303-1a plc1::LIR3 mth1::kanMX2 | This study   |

**Experimental Procedures**

**Strains and Media**—All yeast strains are listed in Table 1. All the strains used in this study are isogenic to W303. Standard genetic techniques were used to manipulate yeast strains and introduce mutations from non-W303 strains into the W303 background (43). Cells were grown in rich medium (YPD; 1% yeast extract, 2% Bacto-peptone, 2% glucose) or under selection in synthetic complete medium containing 2% glucose and, when appropriate, lacking specific nutrients to select for a plasmid or strain with a particular genotype. Meiosis was induced in diploid cells by incubation in 1% potassium acetate.

**Western Blotting**—Denatured proteins were separated on 15% denaturing polyacrylamide gels and Western blotting with anti-histone H3 polyclonal antibody (ab1791; Abcam), anti-histone H4 polyclonal antibody (2592; Cell Signaling), anti-acetyl histone H3 (Lys-14) polyclonal antibody (ab97K14; 07-353, Upstate Biotechnology), anti-hyperacetylated histone H4 polyclonal antibody (abH4K5,8,12,16; 06-946; Upstate Biotechnology), anti-Htz1p polyclonal antibody (ab4626; Abcam), anti-acetyl-Htz1p (Lys-14) polyclonal antibody (07-719; Upstate), and anti-myc (A-14) polyclonal antibody (sc-789; Santa Cruz Biotechnology) was carried out as described previously (44). To confirm equivalent amounts of loaded proteins, the membranes were also probed with anti-Pgk1p monoclonal antibody 22C5 (A6457; Invitrogen).

**Real-time RT-PCR Analysis**—Total RNA was isolated from cultures grown in YPD medium to optical density A600 nm = 1.0 by the hot phenol method, treated with RNase-free DNase (Qiagen), and purified with an RNaseasy Mini Kit (Qiagen). Reverse transcription and real-time PCR amplification were performed with the iScript kit (Bio-Rad) using 100 ng of RNA and the following primers: ACT1 (5′-TATGTGTAAGCCG-3′ and 5′-GACATACCGTTGTCAAATTGGG-3′), RPS22B (5′-AGCTGATGTGGTGAATA-3′ and 5′-TTCCGCAATATGACACCATGCT-3′), RPL18B (5′-CCACCTGTTCGAGTCTCAGAAT-3′ and 5′-TGGGAATTGCAA-GATCTGT-3′), HXT1 (5′-CAVCTGTAATCCTAATGCTA-3′ and 5′-ATGAAACACCCGAGAAGAC-3′), HXT3 (5′-GCTCTTCGAAATGCTCAGTGA-3′ and 5′-CACATCGTGCATATGACCTTACC-3′), HXT4 (5′-TGGATCTCAAGAGGATAACGAG-3′ and 5′-GTCATCTCAGTTGTGG-3′), YCR095C (5′-AGGTTGTCAGA-CATCCAAAGTTT-3′ and 5′-CGAGAGCTTTTTC-ACCAGA-3′), GIT1 (5′-GGAAGAACAAAGATATA-3′ and 5′-AGGTTCCATGTCCTGGTGA-3′), YCR100C (5′-TGTCATCTACAGGACATCTG-3′ and 5′-CCTTCGGAATAGATCCTCAGA-3′), YCR106W (5′-CTC-
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GGCATGCAAGAAAAATTC-3' and 5'-TGAATTCGACGACGGTCTTTCGAC-3', HHT1/2 (5'-GAATTTCGGTCTTTCGAC-3' and 5'-ATCTGGGAGGTCTTTCGAC-3', HHF1/2 (5'-CAAGGCGTCAACGGATAATTCTA-3' and 5'-ACCAGAATCTGCTTGACCA-3'), HTA1/2 (5'-CCGTTGCCAAGGTTGATGAC-3' and 5'-TGGAGGCCAAGAACTTCTGAC-3', HTB1/2 (5'-CAAAG-3' and 5'-CGTTAAGGAATCACCCT-3' and 5'-GCCAATTACGTAATTCA-3'), HTZ1 (5'-CATGGAGGTCAAGAACC-3' and 5'-GTAGCGTGCTTTTCAAGGT-3'), Primer pairs for the canonical histones were designed so that they measure expression of both genes for that particular histone (HTA1 and HTA2, HTB1 and HTB2, HHT1 and HHT2, HHF1 and HHF2). Gene expression was normalized to ACT1 expression, which is not altered in plc1Δ, ipk2Δ, ipk1Δ, and kcs1Δ cells (44).

Chromatin Immunoprecipitation and Quantitative Real-time PCR Analysis—In vivo chromatin cross-linking and immunoprecipitation were performed as described previously (13, 44–46) with the following antibodies: anti-myc polyclonal antibody A-14 (Santa Cruz Biotechnology), anti-histone H3 polyclonal antibody (ab1791; Abcam), anti-acetyl histone H3 (Lys-14) polyclonal antibody (acH3K14; 07-353, Upstate Biotechnology), and anti-hyperacetylated histone H4 polyclonal antibody (acH4K5.8,12,16; 06-946; Upstate Biotechnology). Total input DNA and coimmunoprecipitated DNA was then analyzed by real-time PCR with the Bio-Rad MyIQ single-color real-time PCR detection system (Bio-Rad). Each immunoprecipitation was performed at least three times using different chromatin samples, and the occupancy was calculated using the POL1 coding sequence as a negative control and corrected for the efficiency of the primers. The results were calculated as fold-increase in occupancy of the particular protein at the particular locus in comparison with the POL1 locus. Primers used for real-time PCR analysis are as follows: PGK1 (5'-CATTG-GACGTTAAGAAGATC-3' and 5'-TGAAGGACGCAAGAAATTC-3'), ACT1 (5'-CTCTTGTATCTTCTTCCCTTCTC-3' and 5'-ATGTTGCAAGGCCTCGTTTAC-3'), ADH1 (5'-AATCCACCCGTTAAGTGGTACT-3' and 5'-AAGCGGTTCAACTGTTGGAC-3'), YPIK (5'-TTGTGCTGTTGCTGCTGGAG-3' and 5'-CAATGTTTCCAAAACGGTCTC-3'), RPL18B (5'-CTGGTTACCCCGGCCAATCTTCTGAGCTTATTTAC-3' and 5'-CTTTTGTTGAAAGTTTACAGGTTTTG-3'), RPS22B (5'-GGCCATGTTTGGAGGAGG-3' and 5'-ATCCAGTAAAGACGGAGGAG-3'), PHOS (5'-CCATTTGGGATAGGTTAACATC-3' and 5'-AAAGATGAAGGCCATACAACTC-3'), MDN1 promoter region (5'-GATCCGCTTCTGTTATGTTTC-3' and 5'-GGACATTTCACTACAGGACG-3'), MDN1 coding region-promoter region (5'-ATGCCCTGTTTGGATCATACG-3' and 5'-ATGGAACACGCTACTCTTGG-3'), MDN1 coding region-3' end (5'-TCTGATGTATTTGGCAAAG-3' and 5'-TGCCCTGTTATACTGCTGG-3'), MPA1 promoter region (5'-TGTTGGTATCGCTTATGCT-3' and 5'-TTAG ATGTGAACGATAATGATAG-3'), MPA1 coding region-promoter region (5'-GACTGTAGTTGCTGCTGTG-3' and 5'-TGACTTCTGCTGCTGCTTG-3'), MPA1 coding region-3' end (5'-TGA-CTGGTCGCTTCTGCTGGAT-3' and 5'-CCGTTTCATCAATCGATCCAAGG-3'), YCR905C (5'-GAGTCAAGACGACGGTGTTTAC-3' and 5'-CAGAAGAGCTTTTTTACGAA-3'), GIT1 (5'-GGAAGAAATAGATCATCCTGG-3' and 5'-AGTTTGTACGGTTTGGCAG-3'), YCR100C (5'-TCTCTCATGACGATCTGGA-3' and 5'-CCGATTGGATTTTG-3'), and POL1 (5'-CTCTGGACAAAGGGCAAATG-3' and 5'-TAAACACCTGTACCTCG-3').

Acetyl-CoA and Pyruvate Assay—Cells were grown in YPD medium to an optical density of A500 nm = 1.0. Sodium azide was added to a final concentration of 10 mM and 3 x 10⁶ cells were harvested by centrifugation and lysed in 200 µl of 10% perchloric acid with pre-chilled glass beads. The lysate was neutralized with 10 mM KOH to pH 7.5. Acetyl-CoA was assayed with a ELISA kit (Cusabio-Antibodies-online GmbH), and pyruvate was assayed with a pyruvate colorimetric assay kit (Abcam).

RESULTS

plc1Δ Cells Display Hypoacetylation of Histones H3 and H4—We have found previously that Plc1p and InsPs are required for recruitment of the HAT complex SAGA to osmoinducible promoters (45). The recruitment was not associated with increased histone acetylation in the corresponding promoters (45), most likely because of the simultaneous recruitment of the Rpd3p HDAC complex (47). However, the role of Plc1p and InsPs in SAGA recruitment prompted us to test whether InsPs affect targeted and/or global histone acetylation. To assess whether plc1Δ mutation affects acetylation of histones H3 and H4, we performed a Western blot analysis of cell lysates prepared from wild-type and plc1Δ cells and found a significant decrease in the levels of both acH3 and acH4 (Fig. 1A). To identify the specific inositol polyphosphate that is required for normal histone acetylation, we also analyzed lysates from ipk2Δ, ipk1Δ, and kcs1Δ strains. Ipk2p converts Plc1p-generated InsP₄ into InsP₅ and InsP₆ (3, 5). Ipk1p converts InsP₅ into InsP₆ and Kcs1p produces inositol pyrophosphates PP-InsP₄ and PP-InsP₅ (10, 11, 48). The results show that, similar to plc1Δ cells, the ipk2Δ strain also displays decreased acetylation of histones H3 and H4 (Fig. 1A). Because ipk1Δ and kcs1Δ strains display a wild-type level of histone acetylation, synthesis of InsP₅ and InsP₆ thus appears to be required for normal histone acetylation.

We also noted that the level of non-acetylated histones H3 and H4 is somewhat reduced in ipk2Δ and ipk1Δ cells. To determine whether the lower abundance of histones H3 and H4 in plc1Δ cells is due to decreased transcription, we determined the mRNA level for histones H2A, H2B, and H3 and H4 as well as the histone H2A variant Htz1 (Fig. 1C). In agreement with the protein levels, the mRNA levels for individual core histones in plc1Δ cells were reduced to 55–85% of the wild-type levels. However, the expression of Htz1 in plc1Δ cells was increased to ~130% of the wild-type level. To eliminate the possibility of
that the lower level of acetylated histones H3 and H4 in plc1Δ cells is due to decreased expression of the histones, we determined the acetylation level of Htz1p. Htz1p is acetylated at Lys-14 by NuA4 and SAGA complexes (49, 50). The level of acetylated histone H4 (acH4K5,8,12,16) at the promoter regions of PGK1, ACT1, ADH1, PYK1, and PHOS5. We used anti-H3 antibody that recognizes the C-terminal region of H3 that is not post-translationally modified. The ChIP signal obtained with this antibody thus represents total H3 occupancy and can be used to calculate the histone acetylation levels per nucleosome content (51–54).

Histone H3 was 1.1 to 2 times less acetylated and histone H4 was 1.2 to 1.7 times less acetylated in the promoter regions of plc1Δ cells than in the wild-type cells (Fig. 2, A, C, E, G, and I). To account for differences in nucleosome density at the different promoters, we corrected the acH3 and acH4 occupancies for histone H3 content, and generated values that represent acetylation per nucleosome. The acetylation of histones H3 and H4 per nucleosome in the promoter regions was 2.2 to 5.8 and 1.9 to 4.6 times lower in plc1Δ cells than in the wild-type cells, respectively (Fig. 2, B, D, F, H, and J).

To test whether the decreased acetylation of histones found in the promoter regions of plc1Δ cells is also found in the coding regions, we evaluated the occupancy of acetylated histones in the long coding regions of MDN1 and PMA1. Because the DNA fragments obtained through sonication in the ChIP protocol are randomly generated, the long coding regions of MDN1 (15 kb) and PMA1 (3 kb) allowed us to design 3 sets of primers (promoter, middle of the coding region, and 3’ end of the cod-
ing region) far from each other to avoid overlap of the DNA fragments. We found that the total levels of acH3 and acH4 in \( \text{plc1}/\text{H9004} \) cells were decreased 1.5 to 5.1 and 1.5 to 3.8 times, respectively, in the \( \text{MDN1} \) coding region (Fig. 3, A and C), and decreased 1.3 to 4.0 and 1.2 to 2.5 times in the \( \text{PMA1} \) coding region (Fig. 3, E and G), when compared with the wild-type cells. When we corrected the acetylation levels of histones H3 and H4 per nucleosome content, the \( \text{plc1}/\text{H9004} \) cells showed a decrease of 1.6 to 6.9 and 1.7 to 5.1 times, respectively, in the \( \text{MDN1} \) coding region (Fig. 3, B and D), and a decrease of 1.4 to 4.6 and 1.2 to 3.2 times, respectively, in the \( \text{PMA1} \) coding region (Fig. 3, F and H), when compared with the wild-type cells. The decreased acetylation of both histones H3 and H4 at all tested loci suggests that the lack of InsPs in \( \text{plc1}\Delta \) cells results in decreased acetylation of chromatin histones in a global, untargeted manner, and is in agreement with the decreased acetylation of bulk histones, shown by Western blot analysis (Fig. 1A).

**FIGURE 3.** \( \text{plc1}\Delta \) cells display decreased histone acetylation at \( \text{MDN1} \) and \( \text{PMA1} \) promoters and coding regions. Wild-type and \( \text{plc1}\Delta \) cells were grown at 30 °C in YPD medium to an \( \text{OD}_{600} = 0.8 \). ChIP experiments were performed with antibodies against total histone H3 (H3), histone H3 acetylated at lysine 14 (acH3), and hyperacetylated histone H4 (acH4). Occupancies of H3, acH3, and acH4 were determined in the promoter region, middle of the coding region, and 3’ end of the coding region of \( \text{MDN1} \) and \( \text{PMA1} \) for wild-type (A and E) and \( \text{plc1}\Delta \) (C and G) cells. Acetylation per nucleosome was calculated as the ratios of acH3 to total H3 and acH4 to total H3 for wild-type (B and F) and \( \text{plc1}\Delta \) (D and H) cells. The experiments were repeated three times and results are shown as mean ± S.D.

Hypoacetylation of Histones in \( \text{plc1}\Delta \) Cells Results in Spread of the SIR Complex and Lower Expression of HMR- and Telomere-proximal Genes—Yeast heterochromatin occupies ribosomal DNA, the silent mating-type loci \( \text{HMR} \) and \( \text{HML} \), and chromatin domains adjacent to telomere ends (55). Silencing at the silent mating loci and telomeres is mediated by Rap1p and the SIR complex that includes Sir1p, Sir2p, Sir3p, and Sir4p. The assembly of heterochromatin involves Sir2p-medi-
ated deacetylation of histone H4 K16, and binding of Sir3p and Sir4p to deacetylated histone tails. The formation of the boundary regions that prevent the spread of heterochromatin into adjoining euchromatin requires acetylation of histone H4 K16 by the HAT SAS (56, 57). This acetylation then allows the incorporation of the histone variant Htz1p (58, 59), acetylation of which is also required for the efficient anti-silencing function of the boundary regions (49). In addition, the anti-silencing function of the boundary regions also requires Gcn5p- and Elp3p-mediated histone H3 acetylation (60). Because the balance between histone acetylation and histone deacetylation demarcates heterochromatin, we wanted to evaluate whether histone hypoacetylation of plc1Δ cells results in the spread of heterochromatin. We analyzed histone acetylation, occupancy of the SIR complex, and expression of genes YCR095C, GIT1, and YCR100C, flanking the HMR silent cassette, and YCR106W, localized close to the telomere of chromosome III (Fig. 4A). As expected, in comparison to the HMR- and telomere-proximal genes, the histone acetylation levels in the HMR locus were significantly lower in both wild-type and plc1Δ cells (Fig. 4B). In plc1Δ cells, the acetylation levels of histones H3 and H4 in the HMR- and telomere-proximal genes were 1.7 to 3.5 and 1.2 to 3.2 times lower than in the wild-type cells, respectively (Fig. 4B). Correcting the acetylation values for nucleosome content also showed a decrease in plc1Δ cells of 1.3 to 2.8 and 1.2 to 2.5 times for histones H3 and H4, respectively, when compared with the wild-type cells (Fig. 4C). The decrease in the histone acetylation level in plc1Δ cells resulted in significantly increased occupancy of Sir3p in HMR- and telomere-proximal genes of chromosome III in comparison with the wild-type cells (Fig. 4D), causing a defective boundary function as reflected by the decreased expression of these genes (Fig. 4E). Interestingly, Sir3p occupancy at the HMR locus was slightly decreased in plc1Δ cells in comparison to the wild-type cells. Our results suggest that histone hypoacetylation in plc1Δ cells affects the spread of Sir3p from heterochromatin regions, and are consistent with a previous study (49) that showed that two main HATs, SAGA and NuA4, are required for proper acetylation of nucleosomes in the HMR and telomere-proximal genes and that mutation in NuA4 severely affects the boundary function.

**Mutation in Histone Deacetylase HDA1 Improves Growth Rate and Fitness of plc1Δ Cells**—The dynamic balance between histone acetylation and deacetylation, mediated by the activities of HATs and HDACs, is well regulated and required for proper execution of the transcriptional program. The NuA4 and SAGA complexes are the major HAT activities that are counteracted by HDAC activities of the HDA and Rpd3 complexes (61). The NuA4, SAGA, HDA, and Rpd3 complexes provide the bulk control of the dynamic balance of global histone acetylation and deacetylation, with the HDA complex providing the major counterbalancing effect on the HAT activities, indicating that the HDA complex removes the largest amount of acetyl groups (61, 62). Because plc1Δ cells display decreased acetylation of histones H3 and H4 (Figs. 2–4) and number of aberrant phenotypes, including slow growth and temperature sensitivity (63, 64), we tested whether inactivating the HDA complex would improve the growth rate and fitness of plc1Δ cells. Hda1p is the catalytic subunit of the HDA complex that deacetylates H3 and H2B (65, 66). Deletion of HDA1 was shown to reverse the hypoacetylation of H3K9,14 caused by gcn5Δ mutation (61). Introducing the hda1Δ mutation in plc1Δ cells resulted in improved growth rate, as well as increased benomyl resistance, temperature resistance, osmotic resistance, and the improved ability to utilize carbon sources other than glucose (Fig. 5A). These results also suggest that histone hypoacetylation in plc1Δ cells is global and leads to a decreased growth rate and overall fitness of plc1Δ cells. The fact that the rpd3Δ mutation does not suppress the slow growth phenotype of plc1Δ cells (data not shown) can be explained by the role of Rpd3C(S) in regulating transcriptional elongation (67–69). If Plc1p is required for normal acetylation of histones, then one would expect that the plc1Δ mutation would display synthetic genetic interactions with the two major HATs, SAGA and NuA4. In
agreement with this prediction, we found that the plc1Δ mutation is synthetically lethal with deletions of several subunits of the SAGA and NuA4 complexes (Table 2).

The suppression of the slow growth phenotype of plc1Δ cells by the hda1Δ mutation suggests that many loci are hypoacetylated in plc1Δ cells and the corresponding genes have altered expression. The ribosomal protein genes are among the most highly transcribed genes in the yeast genome, and their expression correlates with growth rate (70, 71). Following the general trend that promoters of highly transcribed genes are generally associated with increased histone acetylation (72–74), the acetylation of histones in ribosomal protein gene promoters by NuA4 is known to regulate their transcription (75, 76). Our previous results showed that many ribosomal protein genes have decreased expression in plc1Δ cells (44). Consistently with the notion that plc1Δ cells display global histone hypoacetylation, the acetylation per nucleosome in the promoters of two ribosomal protein genes, RPL18B and RPS22B, was decreased in plc1Δ cells in comparison with wild-type cells, and the decreased acetylation in plc1Δ cells was suppressed by the hda1Δ mutation (Fig. 5B). As expected, the expression of RPL18B and RPS22B genes was reduced in plc1Δ cells to about 60% of the wild-type level and the defect was again partially suppressed by the hda1Δ mutation (Fig. 5C).

plc1Δ Cells Display Reduced Level of Acetyl-CoA—Nucleocytosolic acetyl-CoA is the common substrate for all HATs, and
biochemically isolated cannot be used for histone acetylation (37). Only the nucleocytosolic level of acetyl-CoA in plc1Δ cells is partially suppressed by reduced ACC1 expression. The indicated strains were grown in YPD medium containing 0.05 μg/ml of doxycycline to an A600 = 0.8. The cells were harvested by centrifugation, lysed with glass beads in perchloric acid, and acetyl-CoA was determined in neutralized lysates. The experiment was repeated three times, and the results are shown as mean ± S.D. 100% wild-type levels of acetyl-CoA equals to 1.6 nmol/10^7 cells.

A, low intracellular level of acetyl-CoA in plc1Δ cells is partially suppressed by reduced ACC1 expression. The indicated strains were grown in YPD medium containing 0.05 μg/ml of doxycycline to an A600 = 0.8. Samples were analyzed by Western blotting with antibodies against histone H3 acetylated at lysine 14 (acH3), hyperacetylated histone H4 (acH4), and total histone H3. Even loading of protein samples was confirmed with anti-Pgk1p antibody. The experiment was performed three times, and representative results are shown.

C, temperature sensitivity of plc1Δ cells is partially suppressed by reduced ACC1 expression. 10-Fold serial dilutions of the indicated strains were spotted onto YPD plates without doxycycline and YPD plates containing 0.1 μg/ml of doxycycline and grown for 2 days at 30 and 35 °C. Typical results from three independent experiments are shown. D, histone hypoacetylation in the promoter regions of RPL18B and RPS22B and the acetylation per nucleosome was calculated as ratios of acH3 to total H3 and acH4 to total H3. The experiment was repeated three times, and the results are shown as mean ± S.D. E, low expression of ribosomal protein genes in plc1Δ cells is suppressed by the tetO-ACC1 allele. Wild-type, plc1Δ, tetO-ACC1, and plc1Δ tetO-ACC1 cells were grown in YPD medium containing 0.05 μg/ml of doxycycline at 30 °C to an A600 = 1.0 and the total RNA was isolated and assayed for RPL18B and RPS22B transcripts by real-time RT-PCR. The results were normalized to ACT1 RNA and expressed relative to the value for the WT strain. The experiment was repeated three times, and the results are shown as mean ± S.D.

FIGURE 6. plc1Δ cells that display reduced levels of acetyl-CoA and histone hypoacetylation in plc1Δ cells can be suppressed by increasing the pool of cytosolic acetyl-CoA. A, low intracellular level of acetyl-CoA in plc1Δ cells is partially suppressed by reduced ACC1 expression. The indicated strains were grown in YPD medium containing 0.05 μg/ml of doxycycline to an A600 = 0.8. The cells were harvested by centrifugation, lysed with glass beads in perchloric acid, and acetyl-CoA was determined in neutralized lysates. The experiment was repeated three times, and the results are shown as mean ± S.D. 100% wild-type levels of acetyl-CoA equals to 1.6 nmol/10^7 cells. B, histone hypoacetylation in plc1Δ cells is partially suppressed by the tetO-ACC1 allele. Occupancies of H3, acH3, and acH4 were determined in the promoter regions of RPL18B and RPS22B and the acetylation per nucleosome was calculated as ratios of acH3 to total H3 and acH4 to total H3. The experiment was repeated three times, and the results are shown as mean ± S.D. E, low expression of ribosomal protein genes in plc1Δ cells is suppressed by the tetO-ACC1 allele. Wild-type, plc1Δ, tetO-ACC1, and plc1Δ tetO-ACC1 cells were grown in YPD medium containing 0.05 μg/ml of doxycycline at 30 °C to an A600 = 1.0 and the total RNA was isolated and assayed for RPL18B and RPS22B transcripts by real-time RT-PCR. The results were normalized to ACT1 RNA and expressed relative to the value for the WT strain. The experiment was repeated three times, and the results are shown as mean ± S.D.
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Plc1p Is Required for Expression of the HXT Genes—Availability of glucose modulates histone acetylation through glycolysis flux and synthesis of acetyl-CoA (41), which implies that defects in sensing, transport, or catabolism of glucose affect the acetyl-CoA level and global histone acetylation. To test whether plc1Δ cells are able to respond to glucose addition by up-regulating the transcription of glucose transporter genes, we analyzed expression of HXT1, HXT3, and HXT4 genes, which are regulated in response to different glucose concentrations (83, 84). HXT1 is induced in high but not low glucose, HXT3 is efficiently expressed in both high and low glucose, and HXT4 is induced in low but not high glucose (83, 85). Interestingly, all glucose transporters tested were expressed less in plc1Δ cells than in wild-type cells (Fig. 7, A–C). Because the ipk2Δ strain also displays hypoacetylation of histones H3 and H4 (Fig. 1A) and Ipk1p and Kcs1p are required for synthesis of Insp₆ and PP-InsP₄/PP-InsP₅, respectively, we also analyzed expression of HXT1, HXT3, and HXT4 genes in ipk2Δ, ipk1Δ, and kcs1Δ strains. The results show that similarly to plc1Δ cells, the ipk2Δ strain also displays decreased expression of the tested glucose transporters (Fig. 7, A–C). The expression pattern of HXT1, HXT3, and HXT4 genes in ipk1Δ and kcs1Δ strains was similar to the pattern in the wild-type cells. The results suggest that the absence of Insp₆ and Insp₅ in plc1Δ and ipk2Δ cells hinders expression of glucose transporters. As a negative control we used grr1Δ cells. The F-box protein Grr1p is a component of the Skp1/Cullin/F-box (SCF) E3 ubiquitin ligase complex. SCF-Grr1 is required for degradation of Mth1p, a negative regulator of expression of HXT genes (84, 86, 87). Cells lacking Grr1p are unable to degrade Mth1p in response to glucose and therefore cannot express HXT genes (84).

Plc1p Is Required for Mth1p Degradation and Normal Glucose Metabolism—In S. cerevisiae cells grown under aerobic conditions, glucose is metabolized mainly by glycolysis, generating pyruvate. If glucose sensing, transport, or catabolism is hindered in plc1Δ cells, one would expect a low intracellular level of pyruvate. Indeed, plc1Δ cells contain only 32% of the wild-type pyruvate level (Fig. 8A). This finding is in agreement with the lower level of acetyl-CoA found in plc1Δ cells (Fig. 6A). To elucidate the mechanism responsible for the altered glucose metabolism in plc1Δ cells, we evaluated the kinetics of Mth1p degradation in response to glucose. Transcription of HXT genes is regulated by the repressor Rgt1p and co-repressors Mth1p and Std1p (88). In the absence of glucose, Rgt1p represses transcription of all HXT transporters (89–93). This repression requires co-repressors Mth1p and Std1p (88). The glucose signal results in Grr1p-dependent degradation of Mth1p (84), which exposes Rgt1p to phosphorylation, probably by PKA (94, 95). This phosphorylation results in dissociation of Rgt1p from HXT promoters and alleviates the repressive activity of Rgt1p (Fig. 9). Although Mth1p was rapidly degraded upon addition of glucose in the wild-type cells, Mth1p was still detectable 20 min after addition glucose in plc1Δ and ipk2Δ cells (Fig. 8B). Thus, it seems that Plc1p and Ipk2p are required for proper Mth1p degradation that is needed for efficient expression of the HXT genes. To test whether the defect in Mth1p degradation is responsible for the decreased level of acetyl-CoA and histone hypoacetylation in plc1Δ cells, we introduced the mth1Δ mutation in plc1Δ cells. Indeed, mth1Δ mutation not only increased the cellular level of acetyl-CoA and histone acetylation (Fig. 8, C and D), but also improved the growth rate and partially suppressed temperature sensitivity of plc1Δ cells (Fig. 8E). The notion that proper degradation of the Mth1p repressor and transcription of the glucose transporters is required for histone acetylation is in agreement with the finding that grr1Δ cells also display histone hypoacetylation (Fig. 8F). Cumulatively, our results show that Plc1p and InspSs are important for Mth1p degradation, normal glucose metabolism, and acetyl-CoA synthesis, and highlight the connection between regulation of the intermediary metabolism and global histone acetylation.

DISCUSSION

Our results show that Plc1p and InspSs are important for the normal level of histone acetylation. This notion is supported by several lines of evidence. First, bulk histones H3 and H4 in total cell lysates are hypoacetylated in plc1Δ cells. Second, ChIP experiments show that different chromosomal loci are
cells have low intracellular levels of pyruvate. Cells were grown in YPD medium. In wild-type and plc1Δ strains, pyruvate was assayed with colorimetric assay kit. The experiment was repeated three times, and the results are shown as mean ± S.D. B, samples from wild-type, plc1Δ, and ipkΔ cells expressing Mth1-myc were grown inYP medium containing 2% galactose to an A600 = 0.8. Glucose was subsequently added to 4% and samples were taken just before addition of glucose and at the times indicated after the addition of glucose. Cell extracts were analyzed by Western blotting with anti-myc antibodies. Even loading of protein samples was confirmed with anti-Pgk1p antibody. The experiment was repeated three times, and representative results are shown.

C, low intracellular level of acetyl-CoA in plc1Δ cells is partially suppressed by the mth1Δ mutation. The indicated strains were grown in YPD medium to an A600 = 0.8. The cells were harvested by centrifugation, lysed with glass beads in perchloric acid, and acetyl-CoA was determined in neutralized lysates. The experiment was repeated three times, and the results are shown as mean ± S.D. 100% wild-type levels of acetyl-CoA equals 1.6 nmol/10⁷ cells. D, hypoacetylation of bulk histones in plc1Δ cells is suppressed by mth1Δ mutation. Samples from the indicated strains were analyzed by Western blotting with antibodies against total histone H3, histone H3 acetylated at lysine 14 (acH3), and hyperacetylated histone H4 (acH4). Even loading of protein samples was confirmed with anti-phosphoglycerate kinase (Pgk1p) antibody. The experiment was performed three times, and representative results are shown.

Our results show that InsPs are important for histone acetylation. Deletions of genes encoding components of both the SAGA complex (gcn5Δ, spt20Δ, and spt7Δ) and NuA4 complex (yng2Δ and eaf1Δ) are synthetically lethal with plc1Δ mutation. Perhaps most importantly, the slow growth phenotype and overall fitness of plc1Δ cells is significantly improved by inactivation of the HDA complex by hda1Δ mutation.

Another mechanism that could account for the role of InsPs in glucose transport and acetyl-CoA homeostasis was suggested by the finding that Plc1p negatively regulates endocytosis of hexose transporters in Rsp5p-dependent manner (96). Rsp5p, a HECT-type ubiquitin ligase, is involved in ubiquitination of several transporters and permeases in the plasma membrane. The association of Rsp5p with the plasma membrane is likely mediated by its C2 domain, which has a strong affinity for phosphatidylinositol 4,5-bisphosphate. It is possible that the constitutive endocytosis of the hexose transporters in plc1Δ cells is caused by increased recruitment of Rsp5p to the plasma membrane and increased ubiquitination of the hexose transporters (96). However, the ipk2Δ mutation is not expected to affect the level of phosphatidylinositol 4,5-bisphosphate and
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the association of Rsp5p with the plasma membrane, but it results in reduced transcription of the HXT genes (Fig. 7) and histone hypoacetylation (Fig. 1). Therefore, we believe that the histone hypoacetylation phenotype of plc1Δ and ipk2Δ cells is due to a defect in Mth1p degradation by the ubiquitin/proteasome pathway (84).

How do Plc1p and InsPs affect the ubiquitin/proteasome pathway? There are several indications that Plc1p and InsPs may be involved in regulation of proteasome. First, 26 S proteasome-mediated destruction of C-type cyclin Ume3p/Srb11p/Ssn3p upon oxidative stress requires Plc1p (97). Second, genome-wide identification of protein complexes revealed that Plc1p interacts with Cfl130p (98), a component of the Ccr4-Not transcriptional regulatory complex. The Ccr4-Not complex associates with the proteasome (99). One of the subunits of the Ccr4-Not complex is Not4p, an ubiquitin E3 ligase that is required for the activity of the proteasome (100). Alternatively, InsPs may be involved in regulation of Grr1p, a component of the Skp1/Cullin/F-box (SCF) E3 ubiquitin ligase complex. The Arabidopsis homolog of Grr1p, TIR1, is also an F-box protein and a subunit of the SCF(TIR1) ubiquitin ligase complex. TIR1 is related to the yeast Grr1p (101) and contains inositol hexakisphosphate (InsP6) as a co-factor (102). Thus, it is possible that Grr1p also binds and is activated by one of the InsPs.

An important conclusion of this work is that Plc1p and InsPs are required for normal acetyl-CoA homeostasis and global histone acetylation. The histone hypoacetylation in plc1Δ cells is due to the defect in Mth1p degradation, and consequently reduced synthesis of glucose-derived acetyl-CoA.

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