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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 deletion 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; *plc1*Δ 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, *plc1*Δ 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 *plc1*Δ cells are partially suppressed by deletion of histone deacetylase *HDA1*. The histone hypoacetylation in *plc1*Δ 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 *plc1*Δ 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.

Phospholipase C (PLC)[3] hydrolyzes phosphatidylinositol 4,5-bisphosphate, yielding two prominent eukaryotic second messengers: 1,2-diacylglycerol and inositol 1,4,5-trisphosphate. In yeast cells, PLC (Plc1p encoded by the *PLC1* gene) and four inositol polyphosphate kinases (Ipk2p/Arg82p, Ipk1p, Kcs1p, and Vip1p) constitute a nuclear signaling pathway that is responsible for synthesis of inositol polyphosphates (InsPs) and inositol pyrophosphates (PP-InsPs) (1, 2). InsPs and PP-InsPs play an important role in regulation of DNA and RNA metabolism; they affect transcriptional control (3, 4), export of mRNA from the nucleus (5–8), homologous DNA recombination (9), telomere length (10, 11), and kinetochore function (12, 13). PP-InsPs also bind to the CDI·cyclin·CDK complex Pho81p·Pho80p·Pho85p and promote its inactivation (14, 15), and inhibit Akt signaling (16). Plc1p is required for the initial step of InsPs and PP-InsPs synthesis, and yeast cells with deletion of the *PLC1* gene are completely devoid of any InsPs and PP-InsPs (5).

Plc1p and InsPs also regulate recruitment and activity of chromatin remodeling complexes and thus in addition to transcription may affect other chromatin-based processes such as replication, repair, and recombination (17, 18). Eukaryotic DNA is packaged into nucleosomes that represent basic building units of chromatin. The nucleosome structure limits access to DNA and thus the position and modification state of nucleosomes affect many processes in DNA metabolism (19). Chromatin-modifying complexes are classified into two categories. The first category includes ATP-dependent nucleosome-remodeling complexes that noncovalently modify and reposition nucleosomes, such as yeast Swi/Snf and RSC complexes (20–22). The second category includes complexes that post-translationally modify histones by acetylation, methylation, phosphorylation, and ubiquitynation (23–26). Charge neutralization of the histone tails by acetylation of the lysine residues is believed to weaken histone-DNA interactions and alter interactions between neighboring nucleosomes (24, 27–29). In addition, bromodomain-containing proteins such as Swi2p bind acetyl-lysine motifs in the histone tails and facilitate transcription (30). The enzymes responsible for histone acetylation are the histone acetyltransferases (HATs), whereas histone deacetylases (HDACs) remove acetyl groups from histones.

The activity of HATs depends on the concentration of acetyl-CoA in the nucleocytosolic compartment. The acetyl-CoA is produced by intermediary metabolism from glucose; however, the connection between intermediary metabolism and histone acetylation has been appreciated only recently (31–35). In mammalian cells, ATP-citrate lysase (ACL) generates acetyl-CoA in the nucleocytosolic compartment from glucose-derived citrate, and glucose availability affects histone acetylation in an ACL-dependent manner (36). Yeast cells do not have ACL and cytosolic acetyl-CoA is generated from acetate by acetyl-CoA

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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 lysase.

synthetase, encoded by the *ACS1* and *ACS2* genes. Inactivation of *ACS2* impairs global histone acetylation and transcriptional regulation (37). The cellular level of acetyl-CoA is also regulated by acetyl-CoA carboxylase Acc1p that catalyzes carboxylation of acetyl-CoA to malonyl-CoA. Decreased activity of Acc1p results in globally increased histone acetylation (38). In addition, the decreasing concentration of glucose in the medium as cells enter the stationary phase is accompanied by decreased histone acetylation (39, 40), whereas addition of glucose induces histone acetylation by picNuA4 and SAGA HAT complexes (41). The nucleocytosolic acetyl-CoA thus links histone acetylation with the metabolic state of the cell and perhaps provides an additional fine-tuning of the transcriptional regulation (31–33, 41, 42).

The changes in transcriptional control in cells with altered synthesis of InsPs prompted us to further explore the role of InsPs in regulation of the chromatin structutre. We now show that InsPs are required for normal levels of histone acetylation; *plc1*Δ cells display global histone hypoacetylation. Consequently, *plc1*Δ mutation is synthetically lethal with mutations in the SAGA and NuA4 complexes, the major yeast HATs. Conversely, deletion of histone deacetylase *HDA1* partially suppressed the slow growth phenotype and improved overall fitness of *plc1*Δ cells. The histone hypoacetylation phenotype of *plc1*Δ cells is caused by altered synthesis of acetyl-CoA due to the failure to degrade repressor Mth1p. Increasing the cellular level of acetyl-CoA improves growth rate and suppresses the transcriptional defects of *plc1*Δ cells. Together, our data show that InsPs affect histone acetylation and transcriptional regulation by a mechanism that involves regulation of the acetyl-CoA homeostasis.

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 (acH3K14; 07-353, Upstate Biotechnology), anti-hyperacetylated histone H4 polyclonal antibody (acH4K5,8,12,16; 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 probed5′ with anti-Pgk1p monoclonal antibody 22C5 (A6457; Invitrogen).

TABLE 1
Yeast strains used in this study

Strain	Genotype	Source/Ref.
W303-1a	*MATa ade2-1 his3-11,15 leu2–3,112 trp1-1 ura3-1 ssd1-d2 can1–100*	R. Rothstein
W303-1α	*MATα ade2-1 his3-11,15 leu2–3,112 trp1-1 ura3-1 ssd1-d2 can1–100*	R. Rothstein
W303	*MATa/MATα ade2-1/ade2-1 his3-11,15/his3–11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1ura3-1/ura3-1* can1–100/ can1–100	R. Rothstein
HL1-1	W303-1α *plc1::URA3*	12
HL1-3	W303-1a *plc1::URA3*	103
WPA023	W303-1a *plc1::TRP1*	103
WPL046	W303-1α *plc1::TRP1*	103
A0004	W303-1α *ipk1::kanMX*	5
A0003	W303-1α *ipk2::HIS3*	3
LSY507	W303-1α *kcs1::HIS3*	11
DY5116	W303-1a *gcn5::TRP1*	104
FY1292	*MATa gcn5::HIS3 leu2Δ1 lys2–173 trp1Δ63 ura3–52*	105
ND818	W303-1a *gcn5::HIS3*	This study
ND703	W303-1a *spt3::TRP1*	45
ND713	W303-1a *spt7::LEU2*	45
DY6707	W303-1a *spt20::HIS3*	106
YTT2256	W303-1a *yng2*::NatMX	107
YTT3122	W303-1a *htz1*::NatMX	107
YTT2329	W303-1a *eaf1*::NatMX	107
DY5068	W303-1α *hda1::URA3*	104
LG167	W303-1a *hda1::URA3*	This study
LG159	W303-1a *plc1::TRP1 hda1::URA3*	This study
CWY1128	W303-1a *grr1::LEU2 RGT1–3HA::KanMX2*	84
CWY1310	W303-1a *MTH1–6x-myc::KanMX2*	84
LG442	W303-1a *plc1::URA3 MTH1–6x-myc::KanMX2*	This study
LG510	W303-1a *ipk2::HIS3 MTH1–6x-myc::KanMX2*	This study
AUY009	*MATa tetO$_7$-ACC1 ura3–52 trp1–63 leu2Δ1::tTA-LEU2*	82
LG362	W303-1a *tetO$_7$-ACC1*	38
LG364	W303-1a *tetO$_7$-ACC1 plc1::URA3*	This study
YM6266	*MATα his3Δ leu2Δ lys2Δ ura3Δ mth1::kanMX2*	108
LG471	W303-1a *mth1::kanMX2*	This study
LG474	W303-1a *plc1::URA3 mth1::kanMX2*	This study

Real-time RT-PCR Analysis—Total RNA was isolated from cultures grown in YPD medium to optical density $A_{600\ nm} = 1.0$ by the hot phenol method, treated with RNase-free DNase (Qiagen), and purified with an RNeasy 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′-TATGTGTAAAGCCGGTTTTGC-3′ and 5′-GACAATACCGTGTTCAATTGGG-3′), *RPS22B* (5′-AGCTGATGCTTTGAATGCCA-3′ and 5′-TTCGCCAATGTAACCATGCT-3′), *RPL18B* (5′-CCACCTGTTTCAGTCTCCAGAAT-3′ and 5′-TTGGGAATTCGAAGATCCTG-3′), *HXT1* (5′-CAACTTAAGTGAAAGTCAAGTGCAAC-3′ and 5′-ATGAAACCACCGAAAGCAAC-3′), *HXT3* (5′-GCCTTCGAATAGCTCTCAGGTA-3′ and 5′-CACAGTGACATATGCACCTTTACC-3′), *HXT4* (5′-TGCCTATCAAGAGGATACAGCAG-3′ and 5′-GTCATCTCTTTCAGCTTTGTTGG-3′), *YCR095C* (5′-GAGGTCAAGAACCATCCAAGTTT-3′ and 5′-CAGAAGAGCTTTTTACCGGAAC-3′), *GIT1* (5′-GGAAGACAAAGATATCACATCGG-3′ and 5′-AGGTTTCAGTACGGGTTGCA-3′), *YCR100C* (5′-TGTCATCTACGGACATCTGGAT-3′ and 5′-CCTTCCGATAGAATCTTCACGA-3′), *YCR106W* (5′-CTC-

GCGATGCCAACAAAATTC-3′ and 5′-TGAATCCATCAGAGTCGTTTGC-3′), *HHT1/2* (5′-GAAGCCTCACAGATATAAGCCAG-3′ and 5′-ATCTTGAGCGATTTCTCTGACC-3′), *HHF1/2* (5′-CCAAGCGTCACAGAAAGATTCTA-3′ and 5′-ACCAGAAATACGCTTGACACCA-3′), *HTA1/2* (5′-CGGTGGTAAAGGTGGTAAAGC-3′ and 5′-TGGAGCACCAGAACCAATTC-3′), *HTB1/2* (5′-CAAAGTTTTGAAGCAAACTCACCC-3′ and 5′-GCCAATTTAGAAGCTTCAGTAGC-3′), *HTZ1* (5′-CATGGAGGTAAAGGTAAATCCG-3′ and 5′-GTAGCGTGCCTTTTCAGGTAAC-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*, and *HHF1* and *HHF2*). Gene expression was normalized to *ACT1* expression, which is not altered in *plc1*Δ, *ipk2*Δ, *ipk11*Δ, and *kcs1*Δ 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′-CATTGGACGGTAAGAAGATCAC-3′ and 5′-TGAGAAGCCAAGACAACGTATC-3′), *ACT1* (5′-CTCTTGTATTCTTCCTTCCCCTTTC-3′ and 5′-ATGGTGCAAGCGCTAGAACATAC-3′), *ADH1* (5′-AATCCCACGGTAAGTTGGAATAC-3′ and 5′-AAGCGTGCAAGTCAGTGTGAC-3′), *PYK1* (5′-TTGTTGCTGGTTCTGACTTGAG-3′ and 5′-CAATGTTCAAACCAGCCTTTCTC-3′), *RPL18B* (5′-CGTTACCCGACCTCGTTATTTTAC-3′ and 5′-CCTTTGGTGAAACAGGTAGTTTTG-3′), *RPS22B* (5′-GCCCATGTGTTGGAGGGAAGG-3′ and 5′-ATCAGCTAAAACGGAAGAGCGAG-3′), *PHO5* (5′-CCATTTGGGATAAGGGTAAACATC-3′ and 5′-AGAGATGAAGCCATACTAACCTCG-3′), *MDN1* promoter region (5′-GTACCTCTGAGTTAATTTGACATCG-3′ and 5′-GGACATTCTCAGCAGATTATAACGG-3′), *MDN1* coding region-middle (5′-ATTGGCCGTTGGTACTTGTTG-3′ and 5′-ATGGAAAACACGTCTACTCTGGG-3′), *MDN1* coding region-3′ end (5′-TCTGATGGTATTTGCGAAGACC-3′ and 5′-TGGCTCATGTCTAAGATGGATTC-3′), *PMA1* promoter region (5′-TGGTGGGTACCGCTTATGCT-3′ and 5′-TTAG ATGTTAGACGATAATGATAGGACA-3′), *PMA1* coding region-middle (5′-GACTTGATGTTGACTGCTTGTTTG-3′ and 5′-TGTACTTGGTCAAAGCGTCCTT-3′), *PMA1* coding region-3′ end (5′-TTACTGTCGTCCGTGTCTGGAT-3′ and 5′-CCGTTCATCAATCTGTCAAAGG-3′), *YCR095C* (5′-GAGGTCAAGAACCATCCAAGTTT-3′ and 5′-CAGAAGAGCTTTTTACCGGAAC-3′), *GIT1* (5′-GGAAGACAAAGATATCACATCGG-3′ and 5′-AGGTTTCAGTACGGGTTGCA-3′), *YCR100C* (5′-TGTCATCTACGGACATCTGGAT-3′ and 5′-CCTTCCGATAGAATCTTCACGA-3′), *YCR106W* (5′-CTCGCGATGCCAACAAAATTC-3′ and 5′-TGAATCCATCAGAGTCGTTTGC-3′), and *POL1* (5′-TCCTGACAAAGAAGGCAATAGAAG-3′ and 5′-TAAAACACCCTGATCCACCTCTG-3′), *HMR* (5′-GCAGGTACTCCTGGTTTTTGTT-3′ and 5′-TCGCCTACCTTCTTGAACAAGAT-3′), *YEF3* (5′-TAACGCCATGCAAGCTGTTG-3′ and 5′-GGAAATCAAAGTTTCGGAAGC-3′), *ERG11* (5′-CAGCAGGCTTGAATAGAAACAGA-3′ and 5′-GCCAAGAAATGACTTAAACCAATG-3′).

Acetyl-CoA and Pyruvate Assay—Cells were grown in YPD medium to an optical density of $A_{600\,nm} = 1.0$. Sodium azide was added to a final concentration of 10 mM and 3×10^8 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 M 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

*plc1*Δ *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 *plc1*Δ mutation affects acetylation of histones H3 and H4, we performed a Western blot analysis of cell lysates prepared from wild-type and *plc1*Δ cells and found a significant decrease in the levels of both acH3 and acH4 (Fig. 1*A*). To identify the specific inositol polyphosphate that is required for normal histone acetylation, we also analyzed lysates from *ipk2*Δ, *ipk1*Δ, and *kcs1*Δ strains. Ipk2p converts Plc1p-generated $InsP_3$ into $InsP_4$ and $InsP_5$ (3, 5). Ipk1p converts $InsP_5$ into $InsP_6$, and Kcs1p produces inositol pyrophosphates PP-$InsP_4$ and PP-$InsP_5$ (10, 11, 48). The results show that, similar to *plc1*Δ cells, the *ipk2*Δ strain also displays decreased acetylation of histones H3 and H4 (Fig. 1*A*). Because *ipk1*Δ and *kcs1*Δ strains display a wild-type level of histone acetylation, synthesis of $InsP_4$ and $InsP_5$ 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 *plc1*Δ and *ipk2*Δ cells. To determine, whether the lower abundance of histones H3 and H4 in *plc1*Δ cells is due to decreased transcription, we determined the mRNA level for histones H2A, H2B, H3, and H4 as well as the histone H2A variant *HTZ1* (Fig. 1*C*). In agreement with the protein levels, the mRNA levels for individual core histones in *plc1*Δ cells were reduced to 55–85% of the wild-type levels. However, the expression of *HTZ1* in *plc1*Δ cells was increased to ~130% of the wild-type level. To eliminate the possibility

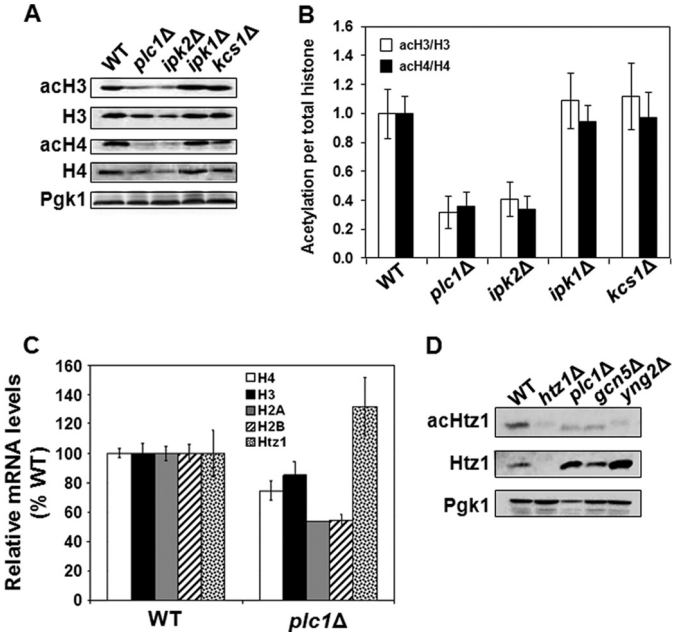

FIGURE 1. **Histones H3 and H4 are hypoacetylated in *plc1*Δ and *ipk2*Δ cells.** *A, plc1*Δ and *ipk2*Δ cells display lower levels of acH3 and acH4. Samples from the indicated strains were analyzed by Western blotting with antibodies against total histone H3 and H4, histone H3 acetylated at lysine 14 (*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. *B,* quantitative analysis of the Western blots was performed by densitometric analysis of the band intensities using UN-SCAN-IT software (Silk Scientific) and the ratios of acH3 to total histone H3 and acH4 to total histone H4 were plotted. The ratios represent mean ± S.D. from three independent experiments. *C,* expression of histones H4, H3, H2A, and H2B is reduced and expression of the H2A variant Htz1 is elevated in *plc1*Δ cells. WT and *plc1*Δ strains were grown in YPD medium at 30 °C to an A_{600} of 1.0 and the total RNA was isolated and assayed for H4, H3, H2A, H2B, and *HTZ1* 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. *D,* acetylation of Htz1p is reduced in *plc1*Δ cells. Samples from the indicated strains were analyzed by Western blotting with antibodies against Htz1p, acetylated Htz1p (acHtz1), and Pgk1. The experiment was performed three times, and representative results are shown.

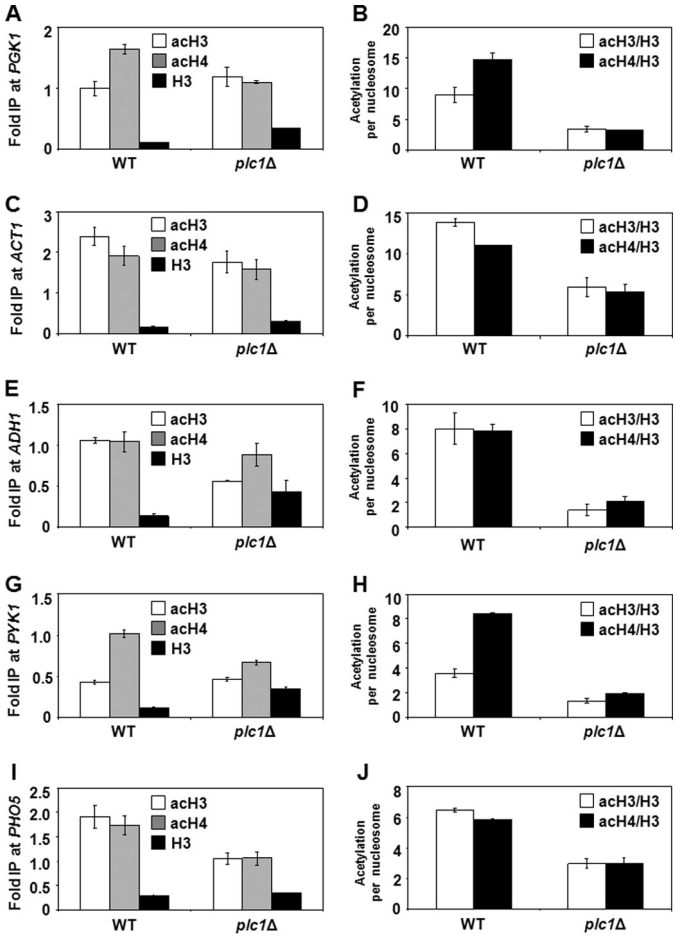

FIGURE 2. ***plc1*Δ cells display decreased untargeted acetylation of chromatin histones.** Wild-type and *plc1*Δ cells were grown at 30 °C in YPD medium to an A_{600} = 0.8. ChIP experiments were performed with antibodies against total histone H3 (*H3*), histone H3 acetylated at lysine 14 (*acH3*), and hyperacetylated histone H4 (*acH4*). Occupancies of H3, acH3, and acH4 were determined in the promoter region of *PGK1* (*A*), *ACT1* (*C*), *ADH1* (*E*), *PYK1* (*G*), and *PHO5* (*I*). Acetylation per nucleosome was calculated as ratios of AcH3 to total H3 and acH4 to total H3 (*B*, *D*, *F*, *H*, and *J*). The experiments were repeated three times and results are shown as mean ± S.D.

that the lower level of acetylated histones H3 and H4 in *plc1*Δ 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 Htz1p is increased in *plc1*Δ cells and is thus in agreement with the increased mRNA level (Fig. 1, *C* and *D*). However, the level of acK14 Htz1p in *plc1*Δ cells is lower than in the wild-type cells (Fig. 1*D*). The results thus suggest that the decreased acetylation of histones H3 and H4 in *plc1*Δ cells is not due to the lower expression of both histones.

*plc1*Δ *Cells Display Globally Decreased Untargeted Acetylation of Chromatin Histones*—To test whether mutation in *PLC1* decreases histone acetylation globally or only at specific loci, we evaluated by chromatin immunoprecipitation (ChIP) the occupancy of histone H3 acetylated at lysine 14 (acH3K14) as well as hyperacetylated histone H4 (acH4K5,8,12,16) at the promoter regions of *PGK1*, *ACT1*, *ADH1, PYK1*, and *PHO5*. 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 *plc1*Δ 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 *plc1*Δ 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 *plc1*Δ 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-

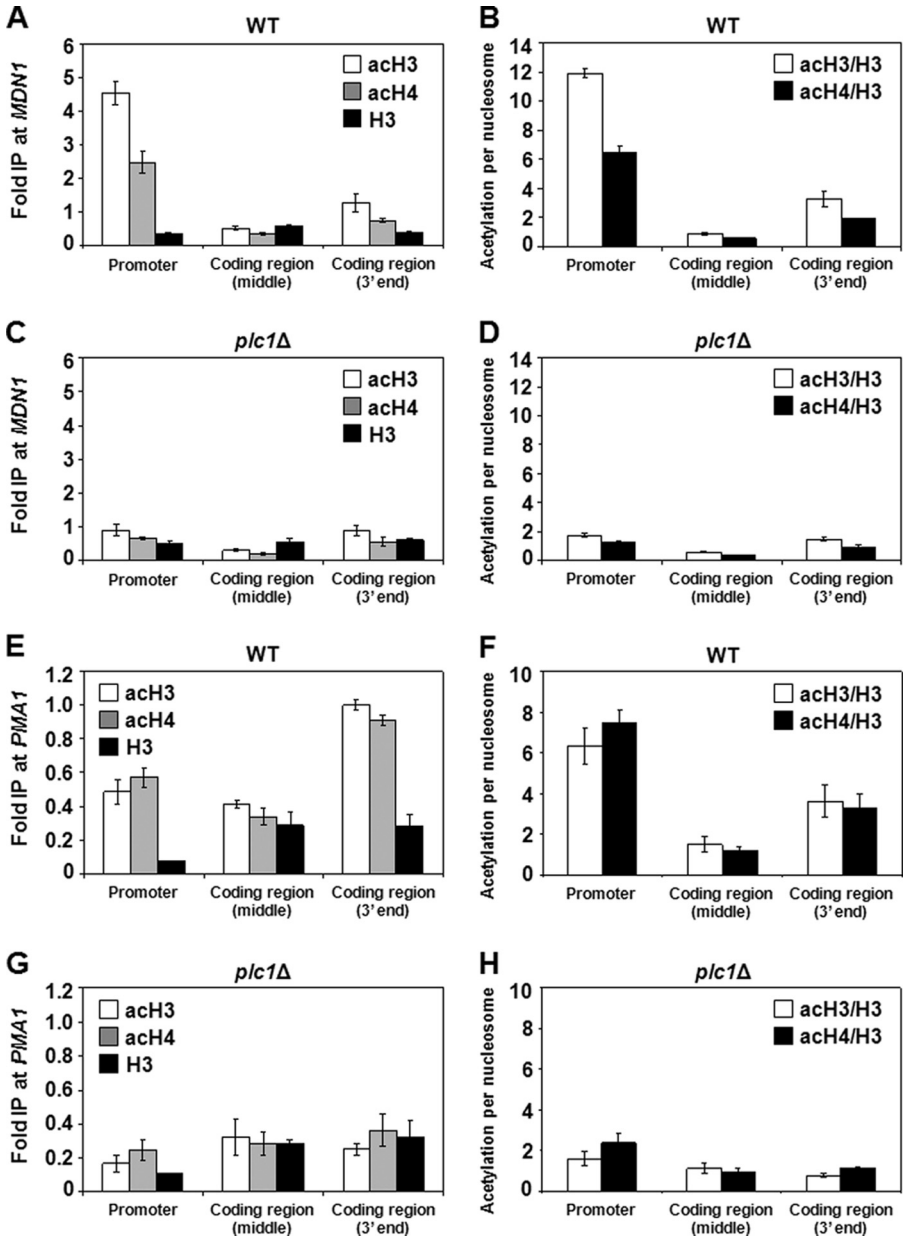

FIGURE 3. ***plc1*Δ cells display decreased histone acetylation at *MDN1* and *PMA1* promoters and coding regions.** Wild-type and *plc1*Δ cells were grown at 30 °C in YPD medium to an A_{600} = 0.8. ChIP experiments were performed with antibodies against total histone H3 (*H3*), histone H3 acetylated 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 *MDN1* and *PMA1* for wild-type (*A* and *E*) and *plc1*Δ (*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 *plc1*Δ (*D* and *H*) cells. The experiments were repeated three times and results are shown as mean ± S.D.

ing region) far from each other to avoid overlap of the DNA fragments. We found that the total levels of acH3 and acH4 in *plc1*Δ cells were decreased 1.5 to 5.1 and 1.5 to 3.8 times, respectively, in the *MDN1* coding region (Fig. 3, *A* and *C*), and decreased 1.3 to 4.0 and 1.2 to 2.5 times in the *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 *plc1*Δ cells showed a decrease of 1.6 to 6.9 and 1.7 to 5.1 times, respectively, in the *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 *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 *plc1*Δ 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. 1*A*).

*Hypoacetylation of Histones in plc1*Δ *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 *HMR* and *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 *plc1*Δ 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. 4*A*). 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 *plc1*Δ cells (Fig. 4*B*). In *plc1*Δ 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. 4*B*). Correcting the acetylation values for nucleosome content also showed a decrease in *plc1*Δ 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. 4*C*). The decrease in the histone acetylation level in *plc1*Δ 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. 4*D*), causing a defective boundary function as reflected by the decreased expression of these genes (Fig. 4*E*). Interestingly, Sir3p occupancy at the HMR locus was slightly decreased in *plc1*Δ cells in comparison to the wild-type cells. Our results suggest that histone hypoacetylation in *plc1*Δ 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.

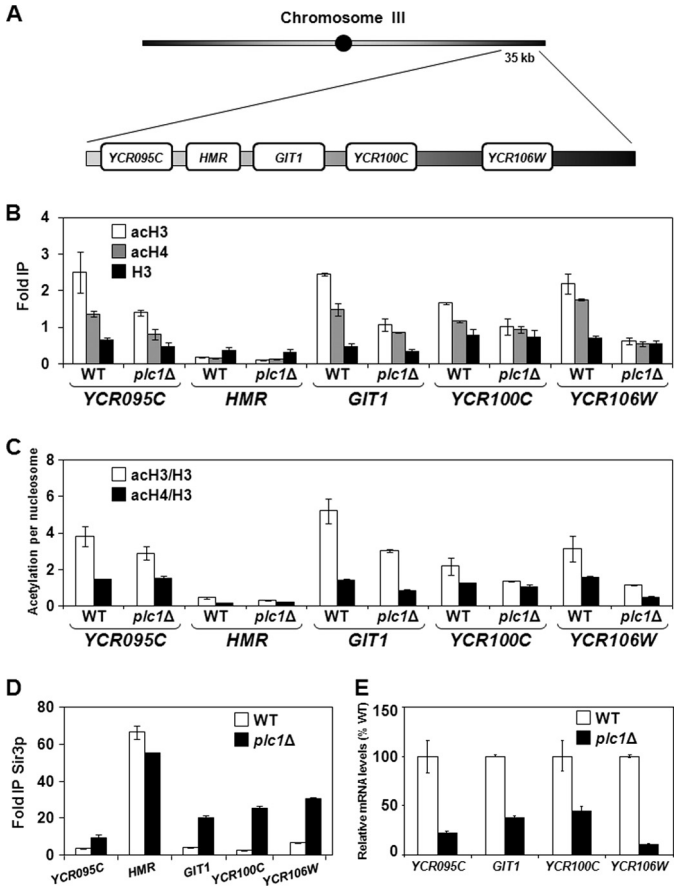

FIGURE 4. **Histone hypoacetylation in *plc1*Δ cells leads to spread of Sir3p and lower expression of HMR- and telomere-proximal genes.** *A,* diagram of the right arm of chromosome III containing the *HMR* locus and the flanking genes. *B* and *D*, wild-type and *plc1*Δ cells were grown at 30 °C in YPD medium to an A_{600} = 0.8 and ChIP experiments were performed with antibodies against total histone H3 (*H3*), histone H3 acetylated at lysine 14 (*acH3*), hyperacetylated histone H4 (*acH4*), and anti-myc (*Sir3p*). Occupancies of H3, acH3, acH4, and Sir3p were determined in the promoter regions of *YCR095C*, *GIT1*, *YCR100C*, *YCR106W*, and at the *HMR* locus. *C,* acetylation per nucleosome was calculated as ratios of AcH3 to total H3 and acH4 to total H3. The experiments were repeated three times and the results are shown as mean ± S.D. *E*, expression of *YCR095C*, *GIT1*, *YCR100C*, and *YCR106W* is reduced in *plc1*Δ cells. WT and *plc1*Δ strains were grown in YPD medium at 30 °C to an A_{600} = 1.0 and the total RNA was isolated and assayed for *YCR095C*, *GIT1*, *YCR100C*, and *YCR106W* 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.

*Mutation in Histone Deacetylase HDA1 Improves Growth Rate and Fitness of plc1*Δ *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 *plc1*Δ 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 *plc1*Δ 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 *gcn5*Δ mutation (61). Introducing the *hda1*Δ mutation in *plc1*Δ 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. 5*A*). These results also suggest that histone hypoacetylation in *plc1*Δ cells is global and leads to a decreased growth rate and overall fitness of *plc1*Δ cells. The fact that the *rpd3*Δ mutation does not suppress the slow growth phenotype of *plc1*Δ 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 *plc1*Δ mutation would display synthetic genetic interactions with the two major HATs, SAGA and NuA4. In

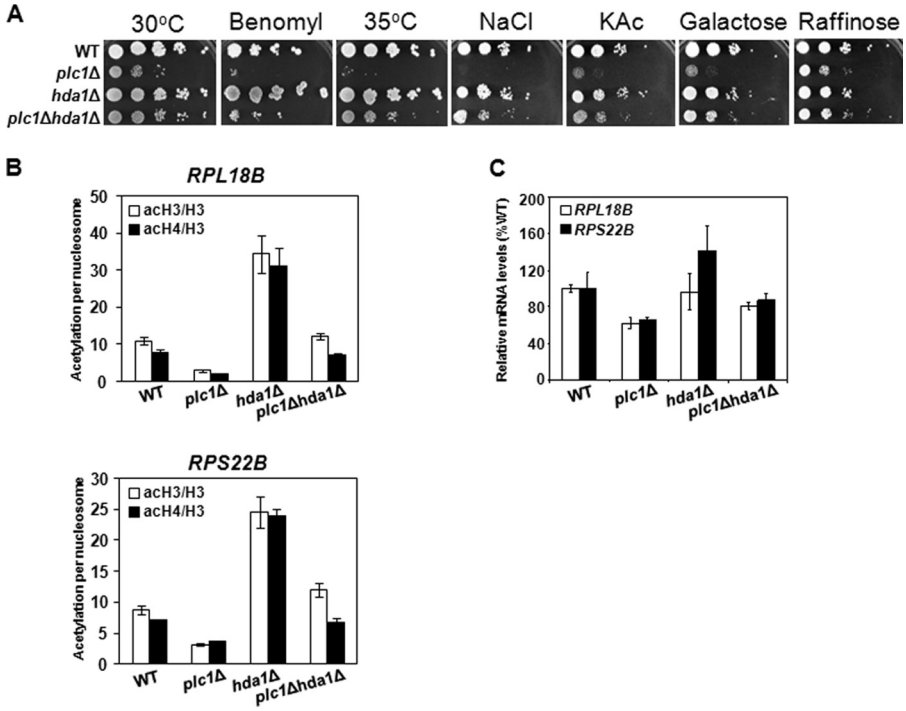

FIGURE 5. **Mutation in histone deacetylase *HDA1* improves growth rate and suppresses defects in expression of ribosomal protein genes in *plc1*Δ cells.** *A*, slow growth of *plc1*Δ cells on YPD medium and different carbon sources, as well as sensitivity to benomyl, high temperature, and osmolarity are partially suppressed by *hda1*Δ mutation. Cells were grown to log phase at 30 °C and 10-fold serial dilutions were spotted onto YPD plates at 30 or 35 °C, with YPD plates containing benomyl (10 μg/ml) or NaCl (0.4 M), or YP plates containing 2% potassium acetate (KAc), 2% galactose, or 2% raffinose, and grown for 2 days at 30 °C. Typical results from three independent experiments are shown. *B,* histone hypoacetylation in the promoter regions of *RPL18B* and *RPS22B* of *plc1*Δ cells is suppressed by *hda1*Δ mutation. Occupancies of H3, acH3, and acH4 were determined in the promoter region of *RPL18B* and *RPS22B* for wild-type, *plc1*Δ, *hda1*Δ, and *plc1*Δ*hda1*Δ cells. Acetylation per nucleosome was calculated as ratios of acH3 to total H3 and acH4 to total H3 and shown as mean ± S.D. for three independent experiments. *C,* low expression of ribosomal protein genes in *plc1*Δ cells is suppressed by *hda1*Δ mutation. Wild-type, *plc1*Δ, *hda1*Δ, and *plc1*Δ*hda1*Δ cells were grown in YPD medium at 30 °C to an A_{600} = 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.

agreement with this prediction, we found that the *plc1*Δ 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 *plc1*Δ cells by the *hda1*Δ mutation suggests that many loci are hypoacetylated in *plc1*Δ 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 *plc1*Δ cells (44). Consistently with the notion that *plc1*Δ cells display global histone hypoacetylation, the acetylation per nucleosome in the promoters of two ribosomal protein genes, *RPL18B* and *RPS22B*, was decreased in *plc1*Δ cells in comparison with wild-type cells, and the decreased acetylation in *plc1*Δ cells was suppressed by the *hda1*Δ mutation (Fig. 5*B*). As expected, the expression of *RPL18B* and *RPS22B* genes was reduced in *plc1*Δ cells to about 60% of the wild-type level and the defect was again partially suppressed by the *hda1*Δ mutation (Fig. 5*C*).

*plc1*Δ *Cells Display Reduced Level of Acetyl-CoA*—Nucleocytosolic acetyl-CoA is the common substrate for all HATs, and

TABLE 2
Genetic interactions between *plc1*Δ and HAT mutations

Heterozygous diploids were prepared by standard genetic crosses and the synthetic lethality was deduced from the failure to recover any double mutants after dissecting at least 50 tetrads.

HAT complex	Mutations synthetically lethal with *plc1*Δ
SAGA	*gcn5*Δ, *spt7*Δ, *spt3*Δ, *spt20*Δ
NuA4	*yng2*Δ, *eaf1*Δ

defect in the acetyl-CoA synthesis results in global untargeted histone hypoacetylation (37). To test the possibility that the histone hypoacetylation phenotype of *plc1*Δ cells is due to a defect in acetyl-CoA synthesis, we determined the cellular level of acetyl-CoA in wild-type and *plc1*Δ cells. The acetyl-CoA level in *plc1*Δ cells was only 12% of the wild-type level (Fig. 6*A*). In budding yeast, the majority of glycolytically produced pyruvate is converted to acetaldehyde and subsequently into ethanol; only a small fraction of acetaldehyde is converted into acetate (77–79). This acetate is subsequently converted to acetyl-CoA by nucleocytosolic acetyl-CoA synthetase 2 (Acs2p) (37). Because glucose represses tricarboxylic cycle and respiration in *Saccharomyces cerevisiae*, only a very small fraction of glycolytically produced pyruvate is translocated into mitochondria and converted to acetyl-CoA by the pyruvate dehydrogenase complex (77–79). The mitochondrial pool of acetyl-CoA in glucose-grown cells is very small and because it is

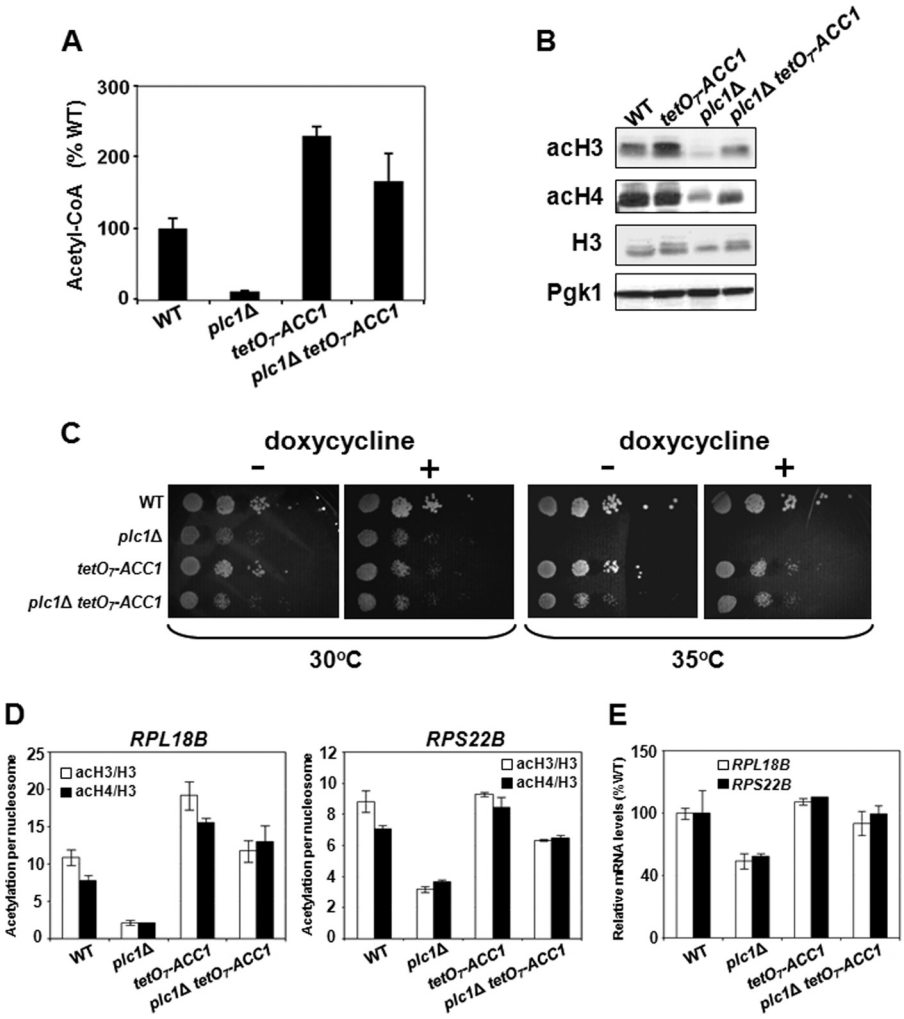

FIGURE 6. ***plc1*Δ cells that display reduced levels of acetyl-CoA and histone hypoacetylation in *plc1*Δ cells can be suppressed by increasing the pool of cytosolic acetyl-CoA.** *A,* low intracellular level of acetyl-CoA in *plc1*Δ cells is partially suppressed by reduced *ACC1* expression. The indicated strains were grown in YPD medium containing 0.05 μg/ml of doxycycline to an A_{600} = 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 *plc1*Δ cells is partially suppressed by the *tetO*$_7$*-ACC1* allele. The indicated strains were grown in YPD medium containing 0.05 μg/ml of doxycycline to an A_{600} = 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 *plc1*Δ 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* of *plc1*Δ cells is partially suppressed by the *tetO*$_7$*-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 *plc1*Δ cells is suppressed by the *tetO*$_7$*-ACC1* allele. Wild-type, *plc1*Δ, *tetO*$_7$*-ACC1*, and *plc1*Δ *tetO*$_7$*-ACC1* cells were grown in YPD medium containing 0.05 μg/ml of doxycycline at 30 °C to an A_{600} = 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.

biochemically isolated cannot be used for histone acetylation (37). Only the nucleocytosolic acetyl-CoA is available to HATs and its level regulates global histone acetylation (37); however, this acetyl-CoA can be also used for *de novo* synthesis of fatty acids (80). The first and rate-limiting reaction in *de novo* synthesis of fatty acids is carboxylation of acetyl-CoA to malonyl-CoA, catalyzed by acetyl-CoA carboxylase (Acc1p) (80). We recently showed that histone acetylation and synthesis of fatty acids compete for the same acetyl-CoA pool and that reduced expression of *ACC1* results in globally increased histone acetylation (38). To test the possibility that decreased expression of *ACC1* in *plc1*Δ cells would increase acetyl-CoA and histone acetylation levels, we suppressed expression of the *ACC1* gene using the regulatable *tetO*$_7$ promoter fused to the *ACC1* coding region (*tetO*$_7$*-ACC1*) (38, 81, 82). Indeed, reduced *ACC1* expression rendered by the *tetO*$_7$*-ACC1* allele increased the acetyl-CoA level (Fig. 6*A*), increased acetylation of bulk histones H3 and H4 (Fig. 6*B*), and suppressed the temperature sensitivity of *plc1*Δ cells (Fig. 6*C*). Suppression of the histone hypoacetylation phenotype of *plc1*Δ cells by reduced *ACC1* expression was also detectable by ChIP analysis at *RPL18B* and *RPS22B* promoters (Fig. 6*D*) and resulted in increased expression of these genes in *plc1*Δ*tetO*$_7$*-ACC1* cells than in the *plc1*Δ cells (Fig. 6*E*).

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 *plc1*Δ 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 *plc1*Δ cells than in wild-type cells (Fig. 7, *A*–*C*). Because the *ipk2*Δ strain also displays hypoacetylation of histones H3 and H4 (Fig. 1*A*) and Ipk1p and Kcs1p are required for synthesis of $InsP_6$ and PP-$InsP_4$/PP-$InsP_5$, respectively, we also analyzed expression of *HXT1*, *HXT3*, and *HXT4* genes in *ipk2*Δ, *ipk1*Δ, and *kcs1*Δ strains. The results show that similarly to *plc1*Δ cells, the *ipk2*Δ strain also displays decreased expression of the tested glucose transporters (Fig. 7, *A*–*C*). The expression pattern of *HXT1*, *HXT3*, and *HXT4* genes in *ipk1*Δ and *kcs1*Δ strains was similar to the pattern in the wild-type cells. The results suggest that the absence of $InsP_4$ and $InsP_5$ in *plc1*Δ and *ipk2*Δ cells hinders expression of glucose transporters. As a negative control we used *grr1*Δ 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 *plc1*Δ cells, one would expect a low intracellular level of pyruvate. Indeed, *plc1*Δ cells contain only 32% of the wild-type pyruvate level (Fig. 8*A*). This finding is in agreement with the lower level of acetyl-CoA found in *plc1*Δ cells (Fig. 6*A*). To elucidate the mechanism responsible for the altered glucose metabolism in *plc1*Δ 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 *plc1*Δ and *ipk2*Δ cells (Fig. 8*B*). 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 *plc1*Δ cells, we introduced the *mth1*Δ mutation in *plc1*Δ cells. Indeed, *mth1*Δ 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 *plc1*Δ cells (Fig. 8*E*). 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 *grr1*Δ cells also display histone hypoacetylation (Fig. 8*F*). Cumulatively, our results show that Plc1p and InsPs 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.

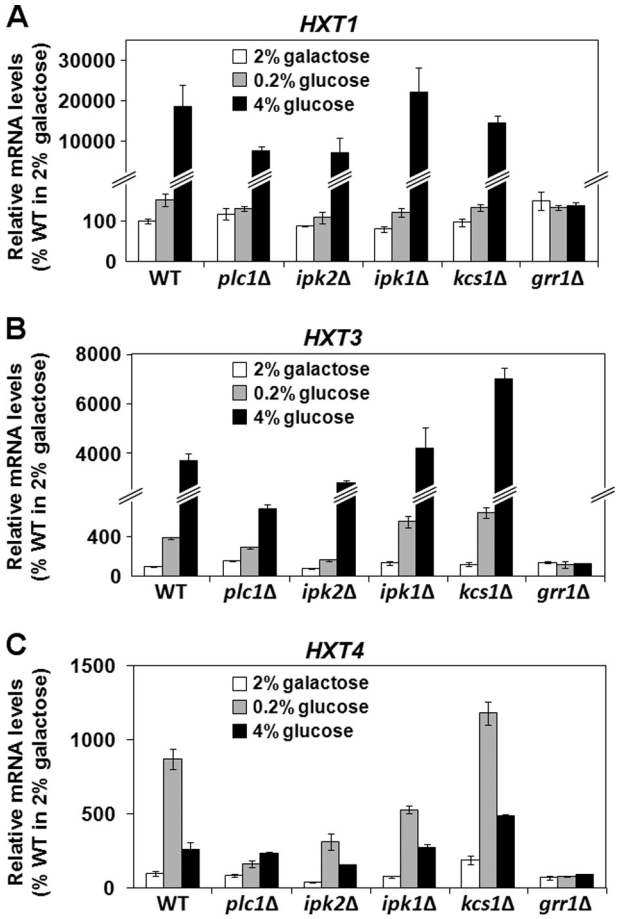

FIGURE 7. **Plc1p and Ipk2p are required for expression of glucose transporters upon glucose induction.** Wild-type, *plc1*Δ, *ipk2*Δ, *ipk1*Δ, *kcs1*Δ, and *grr1*Δ cells were grown in YP medium containing 2% galactose to an A_{600} = 0.5 at 30 °C. The culture was split and either left untreated or adjusted to a final concentration of 0.2% or 4% glucose and grown for an additional 2 h. Total RNA was isolated and assayed for *HXT1* (*A*), *HXT3* (*B*), and *HXT4* (*C*) transcripts by real-time RT-PCR. The results were normalized to *ACT1* RNA and expressed relative to the value for the WT strain grown in 2% galactose. The experiment was repeated three times, and the results are shown as mean ± S.D.

DISCUSSION

Our results show that Plc1p and InsPs 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 *plc1*Δ cells. Second, ChIP experiments show that different chromosomal loci are

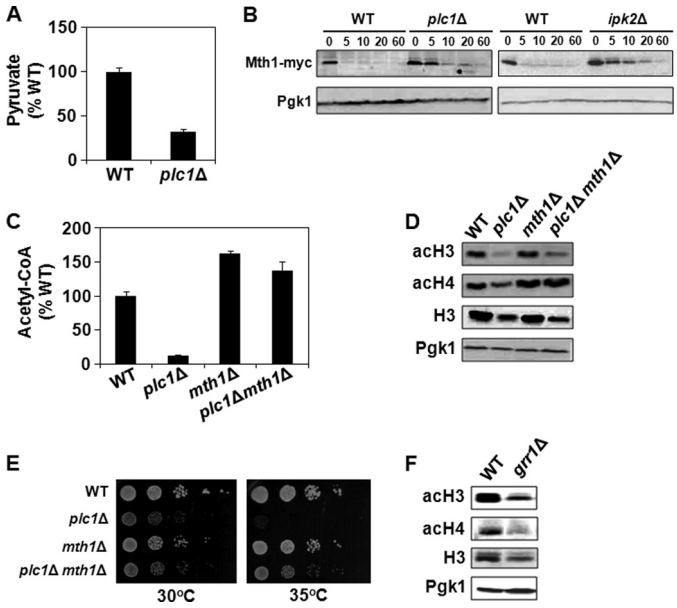

FIGURE 8. **Increased stability of Mth1p is responsible for the decreased level of acetyl-CoA and histone hypoacetylation in *plc1*Δ cells.** *A, plc1*Δ cells have low intracellular levels of pyruvate. Cells were grown in YPD medium, lysed, and pyruvate was assayed with colorimetric assay kit. The experiment was repeated three times, and the results are shown as mean ± S.D. *B,* samples from wild-type, *plc1*Δ, and *ipk2*Δ cells expressing Mth1-myc were grown in YP medium containing 2% galactose to an $A_{600} = 0.8$. 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 performed three times, and representative results are shown. *C,* low intracellular level of acetyl-CoA in *plc1*Δ cells is partially suppressed by the *mth1*Δ mutation. The indicated strains were grown in YPD medium to an $A_{600} = 0.8$. 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/10^7 cells. *D,* hypoacetylation of bulk histones in *plc1*Δ cells is suppressed by *mth1*Δ 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. *E,* temperature sensitivity of *plc1*Δ is partially suppressed by *mth1*Δ mutation. 10-Fold serial dilutions of the indicated strains were spotted onto YPD plates, and grown for 2 days at 30 and 35 °C. Typical results from three independent experiments are shown. *F, grr1*Δ cells display global hypoacetylation of histones. 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.

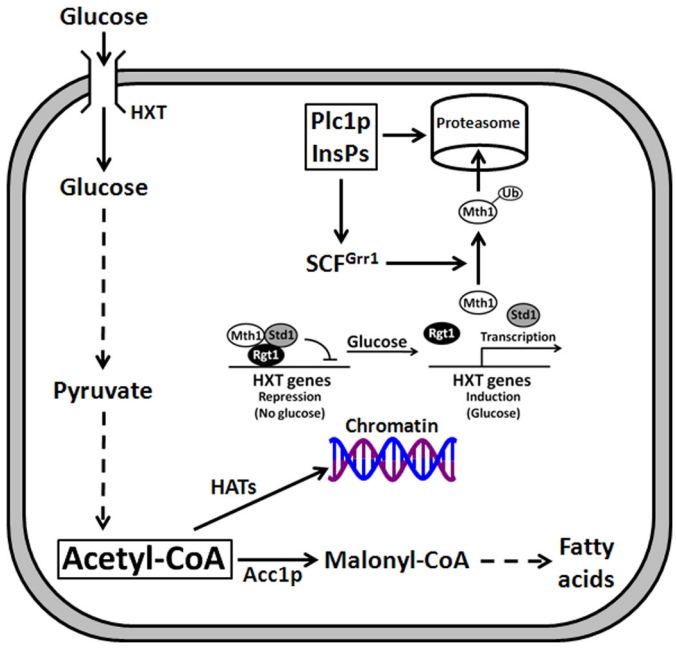

FIGURE 9. **Model for the role of Plc1p and InsPs in regulation of glucose catabolism and histone acetylation.** Glucose catabolism requires the expression of the glucose transporters, encoded by the *HXT* genes, and yields acetyl-CoA, a substrate for HATs. Plc1p and InsPs are important for Mth1p degradation, expression of the glucose transporters, and acetyl-CoA homeostasis. Histone hypoacetylation in *plc1*Δ cells is caused by a reduced cellular level of acetyl-CoA.

hypoacetylated in *plc1*Δ cells, suggesting that InsPs are important for untargeted, global histone acetylation. Third, genetic interactions strongly suggest that Plc1p and InsPs are important for histone acetylation. Deletions of genes encoding components of both the SAGA complex (*gcn5*Δ, *spt20*Δ, and *spt7*Δ) and NuA4 complex (*yng2*Δ and *eaf1*Δ) are synthetically lethal with *plc1*Δ mutation. Perhaps most importantly, the slow growth phenotype and overall fitness of *plc1*Δ cells is significantly improved by inactivation of the HDA complex by *hda1*Δ mutation.

The global untargeted histone hypoacetylation in *plc1*Δ cells prompted us to test whether this phenotype is a result of a decreased level of nucleocytosolic acetyl-CoA. Indeed, *plc1*Δ cells display only 12% of the acetyl-CoA level compared with wild-type cells and increasing the level of acetyl-CoA improves the growth rate and partially suppresses the temperature sensitivity of *plc1*Δ cells (Fig. 6). Our results implicate altered regulation of transcription of the *HXT* transporters and glucose metabolism as the mechanism responsible for the decreased cellular level of acetyl-CoA and histone hypoacetylation in *plc1*Δ cells (Fig. 9).

Our results show that *plc1*Δ cells do not degrade Mth1p efficiently. The defect in *HXT* transcription as the mechanism responsible for histone hypoacetylation in *plc1*Δ cells is supported by two findings. First, introducing the *mth1*Δ mutation in *plc1*Δ cells partially suppressed histone hypoacetylation, slow growth, and temperature sensitivity of *plc1*Δ cells (Fig. 8). Second, deletion of *GRR1*, a component of a Skp1p/Cullin/F box protein (SCF) E3 ubiquitin ligase complex, also resulted in histone hypoacetylation (Fig. 8).

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 an 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 *plc1*Δ cells is caused by increased recruitment of Rsp5p to the plasma membrane and increased ubiquitination of the hexose transporters (96). However, the *ipk2*Δ mutation is not expected to affect the level of phosphatidylinositol 4,5-bisphosphate and

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 *plc1*Δ and *ipk2*Δ 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 Caf130p (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 ($InsP_6$) 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 *plc1*Δ cells is due to the defect in Mth1p degradation, and consequently reduced synthesis of glucose-derived acetyl-CoA.

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