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The Rts1 Regulatory Subunit of PP2A Phosphatase Controls Expression of the *HO* Endonuclease via Localization of the Ace2 Transcription Factor*[S]

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Background: The yeast *HO* gene is tightly repressed and expressed only in mother cells.
Results: Mutation of the PP2A^{Rts1} phosphatase results in decreased *HO* expression due to altered localization of the Ace2 transcription factor.
Conclusion: Altered Ace2 localization results in inappropriate Ash1 repressor expression in mothers.
Significance: Changes in transcription factor phosphorylation can affect localization and cause inappropriate expression of a repressor.

The *RTS1* gene encodes a subunit of the PP2A phosphatase that regulates cell cycle progression. Ace2 and Swi5 are cell cycle-regulated transcription factors, and we recently showed that phosphorylation of Ace2 and Swi5 is altered in an *rts1* mutant. Here we examine expression of Ace2 and Swi5 target genes and find that an *rts1* mutation markedly reduces expression of the *HO* gene. The decreased *HO* expression in an *rts1* mutant is significantly restored by an additional *ace2* mutation, a surprising result because *HO* is normally activated by Swi5 but not by Ace2. Ace2 normally accumulates only in daughter cells, and only activates transcription in daughters. However, in an *rts1* mutant, Ace2 is present in both mother and daughter cells. One of the genes activated by Ace2 is *ASH1*, a protein that normally accumulates mostly in daughter cells; Ash1 is a transcriptional repressor, and it blocks *HO* expression in daughters. We show that in the *rts1* mutant, Ace2 accumulation in mother cells results in Ash1 expression in mothers, and the Ash1 can now repress *HO* expression in mothers.

The yeast *HO* gene is under complex regulation, and has been an important model system for the study of transcriptional regulation (1). The *HO* gene encodes an endonuclease that initiates mating type interconversion by cleaving at the *MAT* locus. *HO* is cell cycle-regulated, with expression occurring in late G_1, after START. *HO* gene activation requires multiple activators and coactivators recruited in sequence during the cell cycle, and these factors induce waves of nucleosome eviction along the promoter as the cell cycle progresses. Inappropriate expression of an endonuclease could be toxic to cells, and the chromatin structure at *HO* ensures tight transcriptional repression.

The Ash1 protein contributes to the repressive character of *HO* chromatin (2). The Ash1 DNA-binding protein recruits the Rpd3(L) histone deacetylase complex to *HO* to facilitate transcriptional repression. Ash1 is cell cycle-regulated, and the protein is present at the promoter only transiently during the cell cycle. *ASH1* mRNA is expressed in late M phase, and the mRNA is transported to the bud tip in daughter cells (3). Consequently, most of the Ash1 protein is present in daughter cells, resulting in repression of *HO* expression in daughter cells. *HO* is normally expressed only in mother cells, but is expressed in both mother and daughter cells in an *ash1* mutant (4, 5). Transcriptional activation of the *ASH1* gene requires either of two transcription factors active in M and early G_1 phases, Swi5 or Ace2 (5). Swi5 and Ace2 have identical DNA-binding domains and recognize the same DNA sequence, but they activate different genes (6, 7). Some target genes, such as *EGT2* and *ASH1*, are known as Ace2-or-Swi5 genes because they can be activated by either factor; expression is sharply reduced only in the *ace2 swi5* double mutant (7). Other target genes are only activated by Ace2, or only by Swi5. One of these Swi5-only target genes is *HO*.

The Ace2 transcription factor is regulated in multiple ways. There is cell cycle regulation both for transcription of the gene and for nuclear accumulation of the protein (6, 8). In G_2 phase, the Ace2 protein is sequestered in the cytoplasm due to phosphorylated residues within the nuclear localization sequence (NLS),[2] and this masks the NLS (9). The Cdc14 phosphatase is released from sequestration at anaphase (10), and it can then dephosphorylate Ace2, allowing it to enter the nucleus (11).

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[2] The abbreviations used are: NLS, nuclear localization sequence; NES, nuclear export sequence.

TABLE 1
Strains used in this study

Fig. 1	
DY150	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*
DY3925	*MAT***a** *swi5::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY1993	*MAT***a** *ace2::HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY4653	*MAT***a** *ace2::HIS3 swi5::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY15688	*MAT***a** *rts1::KanMX ade2 can1 his3 leu2 lys2 trp1 ura3*
DY15724	*MAT***a** *rts1::KanMX swi5::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY15728	*MAT***a** *rts1::KanMX ace2::HIS3 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY15732	*MAT***a** *rts1::KanMX ace2::HIS3 swi5::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
Fig. 2	
DY16851	*MAT***a** *HO-GFP-NLS-PEST::NatMX4 MYO1-MYO1-mCherry::SpHis3 rts1::KanMX can1 his3 leu2 lys2 met15 trp1 ura3*
DY16852	*MAT***a** *HO-GFP-NLS-PEST::NatMX4 MYO1-MYO1-mCherry::SpHis3 rts1::KanMX ash1::TRP1 can1 his3 leu2 lys2 met15 trp1 ura3*
Fig. 3	
DK2148	*MAT***a** *bar1 ACE2-yeGFP::Kluv-TRP1 ade2 can1 his3 leu2 trp1 ura3*
DK2149	*MAT***a** *bar1 ACE2-yeGFP:: Kluv-TRP1 ade2 rts1::KanMX4 can1 his3 leu2 trp1 ura3*
Fig. 4	
DY17102	*MAT***a** *GALp::CDC20::ADE2 Ash1::EGFP::HIS3MX6 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY17098	*MAT***a** *GALp::CDC20::ADE2 Ash1::EGFP::HIS3MX6 rts1::KanMX ade2 can1 his3 leu2 lys2 trp1 ura3*
DY17099	*MAT***a** *GALp::CDC20::ADE2 Ash1::EGFP::HIS3MX6 rts1::KanMX ade2 can1 his3 leu2 lys2 trp1 ura3*
DY17100	*MAT***a** *GALp::CDC20::ADE2 Ash1::EGFP::HIS3MX6 ace2::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY17145	*MAT***a** *GALp::CDC20::ADE2 Ash1::EGFP::HIS3MX6 rts1::KanMX ace2::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY17146	*MAT***a** *GALp::CDC20::ADE2 Ash1::EGFP::HIS3MX6 rts1::KanMX ace2::TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY150	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*
DY16110	*MAT*α *rts1::HphMX4 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY8309	*MAT***a** *ASH1*-Myc *(13)::KanMX ade2 can1 his3 leu2 trp1 ura3*
DY16273	*MAT***a** *ASH1*-Myc *(13)::KanMX rts1:HphMX4 ade2 can1 his3 leu2 lys2 trp1 ura3*

However, Ace2 also has a nuclear export sequence (NES) (11, 12), which prevents Ace2 from accumulating in mother cell nuclei. In daughter cells, the NES is inactivated by the Cbk1 kinase, and Ace2 persists in the nucleus for a short period of the cell cycle (13). Thus, Ace2 only accumulates in daughter cell nuclei, and Ace2 target genes are only expressed in daughters (11, 14).

The *ASH1* gene is transcribed in two pulses in M and early G_1 phases. *ASH1* is first activated by Swi5, as Swi5 enters the nucleus slightly before Ace2 (8). *ASH1* is transcribed in both mothers and daughters (14), but the *ASH1* mRNA is transported to the daughter cell (3). Slightly later in the cell cycle, beginning at the time of cytokinesis, Ace2 accumulates in daughter cell nuclei (13) and activates *ASH1* expression. Most of this expression occurs after the completion of cytokinesis, so this mRNA is exclusively in daughters.

Rts1 is a subunit of the PP2A phosphatase (15). Disruption of the *RTS1* gene affects cell cycle progression and expression of G_1 cyclin genes (16). We recently used proteome-wide mass spectrometry to identify proteins whose phosphorylation is regulated by $PP2A^{Rts1}$, and we found that phosphorylation of both Swi5 and Ace2 are affected in an *rts1* mutant (17). In this study, we examine the effect of an *rts1* mutation on expression of genes activated by Swi5 and Ace2. Although there is little effect on most Swi5 and Ace2 target genes, we find that expression of *HO* is markedly reduced. Our analysis indicates that the *rts1* mutation, which alters Ace2 phosphorylation (17), also affects localization of Ace2. Ace2 now spends more time in the mother cell nucleus, where it can activate *ASH1* transcription, and this Ash1 blocks *HO* expression.

EXPERIMENTAL PROCEDURES

All of the yeast strains are in the W303 background, and they are listed in Table 1. Standard methods were used for strain constructions. The *ace2*, *ash1*, *rts1*, and *swi5* mutations are all gene deletions. RNA levels were measured by quantitative RT-PCR as described, using primers listed in Ref. 7. RNA values were normalized to an *RPR1* internal control, using primers F2430 (CACCTATGGGCGGGTTATCAG) and F2431 (CCTAGGCCGAACTCCGTGA). ChIPs were performed as described (7) using the 4A6 monoclonal antibody to the Myc epitope (Millipore) and antibody-coated magnetic beads (Pan Mouse IgG beads, Invitrogen). Each ChIP sample was first normalized to an input DNA sample and then to the ChIP signal for a *CDC2* control region. ChIP primers for *HO* were F2093 (AATGCTGGAGCAAAAATTTCAATCAG) and F2094 (GGAGCCCCTCAGACATTAGCC). ChIP primers for *CDC2* were F2903 (CAAGAAGAAGGTTGGCCGTTTAC) and F2904 (CCTGTAACACGAGCCATTTCTG).

Single cell measurements of *HO-GFP* were performed as described (18). Briefly, we grew yeast under an agar pad on a heated microscope and used an automated program to acquire phase and fluorescent images every 4 min for 8 h. Multiple movies of independent colonies can be recorded in a single experiment. One movie is included as supplemental Movie S1. We then used a MATLAB program to quantitate the fluorescence intensity of each cell, which was plotted as a function of time. In addition to *HO-GFP*, all the strains also contain Myo1-mCherry as a cell cycle marker. The "Peak expression level per cell cycle" was calculated as the difference between the peak and the trough of GFP intensity within each cell cycle.

The Ace2-GFP fusion uses a yeast enhanced GFP and was integrated at the *ACE2* locus, expressed under the control of the native *ACE2* promoter. For Ace2-GFP measurements, cells were grown in synthetic defined media containing 20 μg/ml adenine overnight at room temperature, and Ace2-GFP localization was visualized by live-cell imaging using a Leica TCS SP5 inverted confocal microscope (Leica Microsystems, PlanApo 63×). Time-lapse experiments were imaged in 35-mm glass-bottom culture dishes (P35-1.5-10-C; MatTek) coated with 10 mg ml^{-1} concanavalin A (Sigma-Aldrich) and

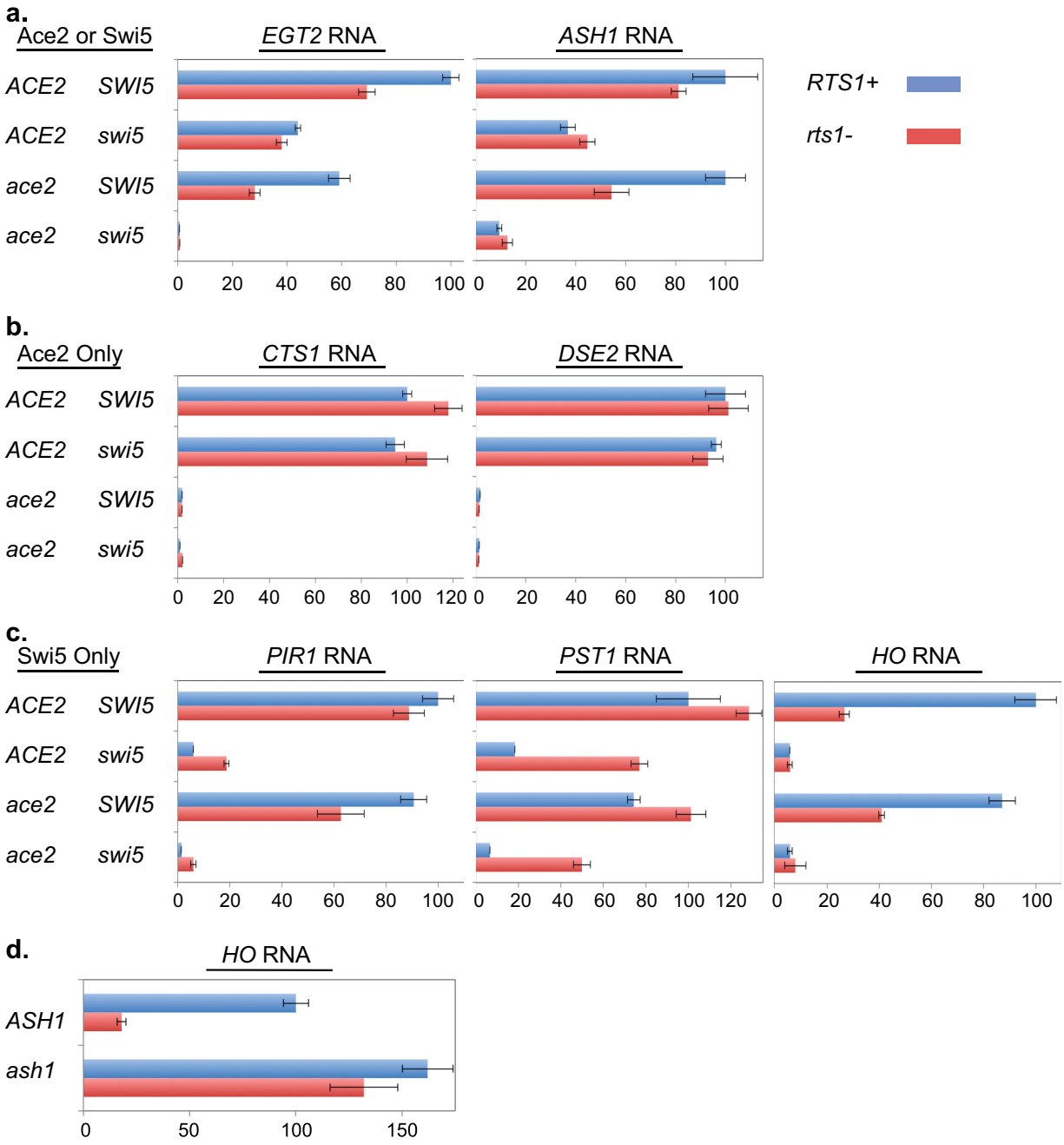


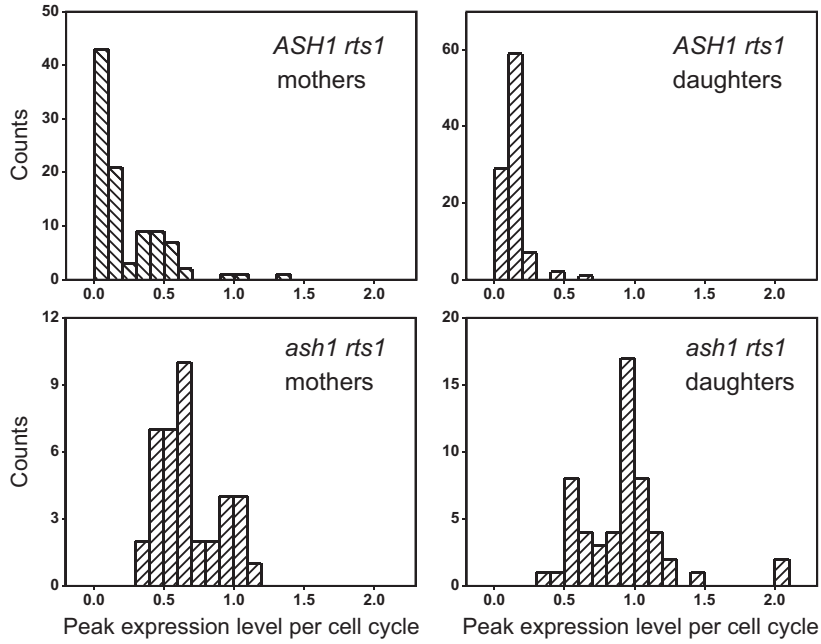
FIGURE 1. **An *rts1* mutant does not affect expression of most Ace2 and Swi5 target genes.** RNA was prepared from cells in mid-log phase, and specific mRNAs were measured by quantitative RT-PCR. *Error bars* indicate the standard deviation of two biological replicates. *a*, target genes activated by Ace2 or Swi5. *b*, Ace2-only target genes. *c*, Swi5-only target genes. *d*, *HO* expression in WT, *rts1*, *ash1*, and *rts1 ash1* strains.

immersed in 3 ml of synthetic defined medium lacking tryptophan. Images were acquired at 3-min intervals in eight z-series with a step size of 0.5 μm. Images were analyzed with ImageJ (National Institutes of Health) and Adobe Photoshop.

The Ash1-GFP fusion uses an enhanced GFP and was integrated at the *ASH1* locus, expressed under the control of the native *ASH1* promoter. For Ash1-GFP measurements, cells were grown in YP medium with 2% galactose and 2% raffinose until an optical density of 0.5. Cells were then transferred to YP medium with 2% raffinose to arrest cells at G_2/M. Then 2% galactose was added to release cells from the arrest, and images were acquired at 40 min following release using an Olympus BX51 fluorescence/differential interference contrast microscope and a MagnaFire SP S99810 camera. Images were analyzed with Adobe Photoshop.

RESULTS

An rts1 Mutation Reduces HO Expression—A mutation affecting the Rts1 subunit of the PP2A phosphatase results in increased phosphorylation of both the Ace2 and the Swi5 transcription factors (17). This change in phosphorylation could affect the activity of these transcription factors, and we therefore examined expression of target genes known to be activated by Ace2 and Swi5 (7). Some target genes, such as *EGT2* and *ASH1*, are known as Ace2-or-Swi5 genes because they can be activated by either factor; an *rts1* mutation has only modest



		mothers			daughters		
		counted	% on	level	counted	% on	level
ASH1	*RTS1*	154	98% ± 1	1.00 ± 0.02	131	2% ± 1	NA
ash1	*RTS1*	127	98% ± 1	1.00 ± 0.02	62	94% ± 3	1.11 ± 0.06
ASH1	*rts1*	97	33% ± 5	0.50 + 0.04	98	5% ± 2	0.42 ± 0.07
ash1	*rts1*	39	100% ± 0	0.70 ± 0.04	55	100% ± 0	0.93 ± 0.04

FIGURE 2. **An *ash1* mutation suppresses the *rts1* defect in *HO* expression in both mother and daughter cells.** *Upper*, histograms show the amplitude of GFP expression in single mother and daughter cells for *rts1* and *ash1 rts1* strains, as described (18). For each panel, two independent time-lapse measurements were carried out, each containing two micro-colonies of the same strain. One movie of the *ASH1 rts1 HO-GFP* strain is in supplemental Movie S1. *Lower*, the table lists the number of cells counted, the percentage in which *HO-GFP* was active, and the relative level. Expression levels were normalized so that the average expression level in wild type mother cells is 1. The data for *ASH1 RTS1* and *ash1 RTS1* strains are from Ref. 18. *NA*, not available.

effects on expression of Ace2-or-Swi5 genes (Fig. 1*a*). Similarly, in the *rts1* mutant, few changes are seen in expression of *CTS1* or *DSE2*, Ace2-only genes (Fig. 1*b*). For two Swi5-only genes, *PIR1* and *PST1*, we see increased expression in the *ACE2 swi5 rts1* strain as compared with the *ACE2 swi5 RTS1* control, and also in the *ace2 swi5 rts1* strain as compared with the *ace2 swi5 RTS1* strain (Fig. 1*c*). This is particularly striking for *PST1* where expression in the *ace2 swi5 rts1* strain is at 50% of wild type, as compared with 7% for *ace2 swi5 RTS1*. It is surprising that these genes are expressed in the absence of both the Ace2 and the Swi5 activators, and we interpret the results as meaning that some other activator is now more capable at these promoters in the absence of the $PP2A^{Rts1}$ phosphatase.

The results with another Swi5-only target gene, *HO*, are quite different. *HO* expression is markedly reduced to 18–27% of wild type in the *rts1* mutant (Fig. 1, *c* and *d*). This decreased expression is unique to *HO* as the two other Swi5-only genes, *PIR1* and *PST1*, show expression of 89 and 128%, respectively, in the *rts1* mutant. *HO* is expressed in an *ace2 SWI5 rts1* mutant (41%), but at a lower level than in the *ace2 SWI5 RTS1* strain (87%). Importantly, Swi5 is still required for *HO* activation in the *rts1* mutant. What is special about *HO* that causes it to be expressed at lower levels in an *rts1* mutant? Additionally, why is the repression partially relieved in an *ace2 rts1* double mutant?

The HO Expression Defect in an rts1 Mutant Is Suppressed by an ash1 Mutation—One unique feature of *HO* regulation is that it is asymmetrically expressed, with expression in daughter cells strongly repressed by Ash1 (4, 5) (see also Fig. 2). We therefore examined *HO* expression in the *rts1 ash1* double mutant and found that the *ash1* mutation suppresses the defect in *HO* expression caused by *rts1* (Fig. 1*d*). This result raised the question of whether the additional *HO* expression in *rts1 ash1* cells is solely from expression in daughter cells, or whether there is also an increase in *HO* expression in mother cells. To address this question, we used an *HO-GFP* reporter, analyzing expression using single cell time-lapse fluorescence microscopy, as described (18). With this method the fluorescence intensity emanating from individual cells is quantitated, and this provides a measure of promoter activity in single mother and daughter cells. Single cell analysis shows that the *rts1* mutation reduces the fraction of mother cells that express *HO*, from 98% of wild type mother cells to 33% of *rts1* mothers (Fig. 2). Addi-

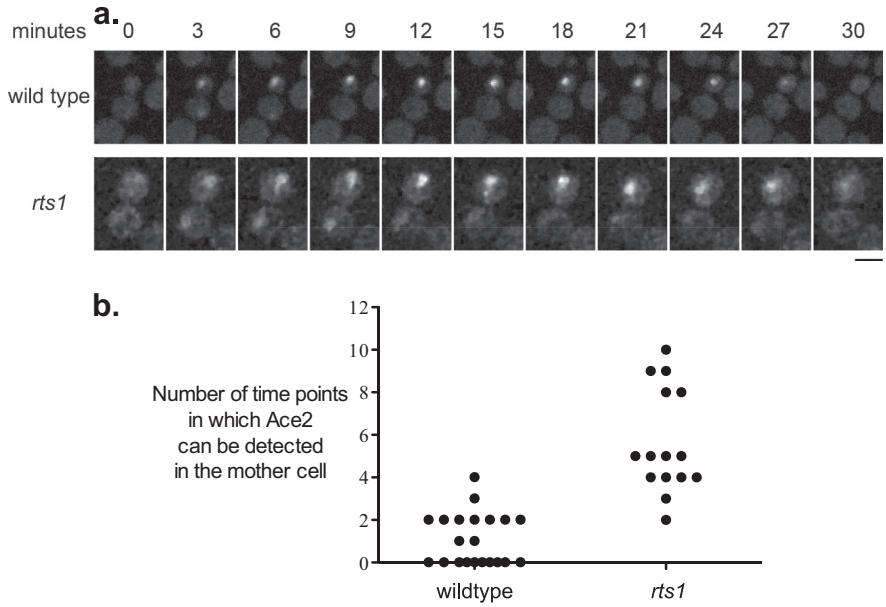


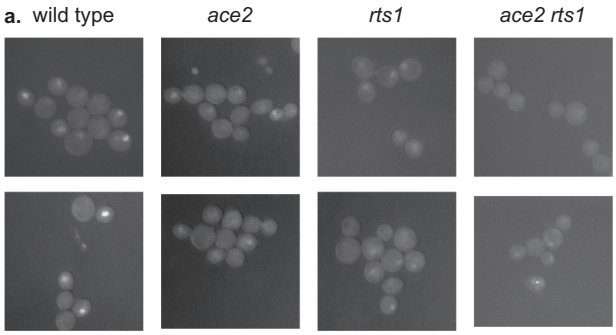
FIGURE 3. **Ace2 accumulation in mother cells in an *rts1* mutant.** *a*, *Ace2-GFP* and *Ace2-GFP rts1* cells were grown to log phase, and Ace2 localization was visualized by time-lapse confocal microscopy. Time points indicate minutes after first appearance of Ace2 in the nucleus. The cell cycle delay in the *rts1* mutant results in larger cell size. *Bar*, 5 μm. Sample movies of the *Ace2-GFP* and *Ace2-GFP rts1* cells are in supplemental Movie S2 and supplemental Movie S3, respectively. *b*, cells in *a* were quantified, and the number of time points in which Ace2 signal was detected in the mother cell was plotted for wild type and *rts1*.

tionally, in *rts1* mothers that do express *HO*, the level of expression is reduced by half. Our previous studies (18) with *ash1* single mutants showed that *HO* expression is low in *ASH1* daughters and high in *ash1* daughters. Most importantly, the *ash1* mutation restores *HO* expression frequency to 100% in *rts1* mothers. This suggests that the decreased *HO* expression in the *rts1* mutant is largely mediated by Ash1.

Altered Ace2 Localization in an rts1 Mutant—We considered the possibility that Ash1 accumulation is altered in the *rts1* mutant, allowing Ash1 in mother cells to block *HO* expression. The observation that the repression of *HO* observed in an *rts1* mutant is partly relieved by an *ace2 rts1* double mutant (Fig. 1*c*) suggests that Ace2 contributes in some way to the repression. Because Ace2 activates *ASH1* transcription, we surmised that a change in Ace2 localization could alter Ash1 localization, thereby reducing *HO* expression. Ace2 normally shows significant accumulation only in daughter cells, with a very transient appearance in mothers (11, 14). It is possible that the increased phosphorylation of Ace2 seen in the *rts1* mutant could change Ace2 localization, resulting in inappropriate Ace2 accumulation in mother cells. A second round of *ASH1* transcription could then be induced in mother cells by Ace2, but because cell division has already occurred, the *ASH1* mRNA could not be transported to daughters. This would result in much higher levels of Ash1 protein in mother cells, as compared with wild type. To test this hypothesis, we used an Ace2-GFP fusion protein to assess protein localization in wild type and *rts1* cells (Fig. 3). In wild type cells, Ace2 was localized to both mother and daughter cells briefly before accumulating exclusively in the daughter cell, as described previously (11, 14). Ace2 could be clearly seen in both mother and daughter nuclei at 3 min in wild type, and trace signals were visible at the 6- and 9-min time points. In contrast, in *rts1* cells, strong Ace2 signals could be seen in both mother and daughter cells for an extended period (from 3 to 9 min) before becoming asymmetrically localized in the daughter cell. Additionally, the level of Ace2-GFP fluorescence in daughter cells is greater in the *rts1* mutant. One interpretation of these results is that in *rts1* cells, the hyperphosphorylation of Ace2 by the Cbk1 kinase causes increased nuclear retention of Ace2, and thus increased Ace2 activity, in both mother and daughter nuclei. The prolonged nuclear localization of Ace2 in *rts1* mother cells could also be caused by the cell cycle delay seen in *rts1* mutants because Ace2 represses transcription of G_1 cyclins (16, 17).

Increased Ash1 Localization in rts1 Mothers Requires Ace2—We next evaluated Ash1 localization using Ash1-GFP fusions. The Ash1-GFP signal was weaker in the three mutant strains as compared with wild type, and in many cells, the signal was too weak to observe. To enrich for cells in which the Ash1 signal was visible, we made *pGAL::CDC20* versions of these strains, allowing for G_2/M arrest and synchronous release by withdrawal and reintroduction of galactose, respectively. Cells were arrested and released, and at 40 min after release, many photographs were taken at random positions throughout the slide. The photographs were examined subsequently, and each mother-daughter pair of cells was scored for Ash1 localization as daughter only, both mother and daughter, or mother only. Selected images are shown in Fig. 4*a*, and quantitation is shown in Fig. 4*b*. For the mutant strains (particularly the *ace2 rts1* strain), fewer cells were able to be scored due to an extremely weak signal.

In wild type cells, Ash1-GFP fluorescence was seen nearly exclusively in daughter cells; among mother-daughter pairs, 93% showed daughter-only fluorescence, 7% showed mother and daughter fluorescence, and none showed a signal only in mothers. (Fig. 4*b*). These results are consistent with previous quantitation (4). There was decreased fluorescence signal in the *ace2* mutant as compared with wild type, and quantitation of



b.

	counted	D only	M + D	M only
wild type	161	93%	7%	0%
ace2	72	85%	11%	4%
rts1	161	18%	68%	14%
ace2 rts1 *	49	31%	53%	16%

* most cells have no visible signal

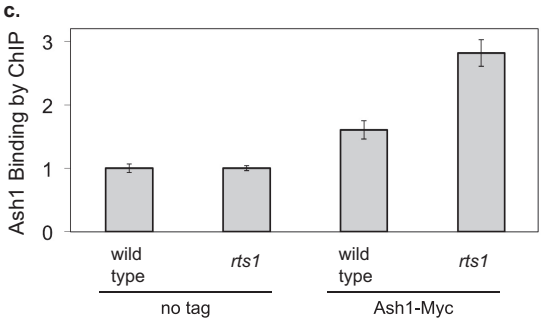


FIGURE 4. **Ash1 accumulation in mother cells in an *rts1* mutant.** Cells with the Ash1-GFP reporter were arrested at G_2/M, and samples were examined by fluorescence microscopy at 40 min following release from the arrest. *a*, representative images. *b*, individual mother-daughter cell pairs were examined and classified as having daughter-only expression (*D only*), mother and daughter expression (*M + D*), or mother-only expression (*M only*). Ash1-GFP signals were extremely weak in the *ace2 rts1* strain, and most cells had no visible signal. *c*, ChIP experiments were performed with wild type and *rts1* cells containing Ash1-Myc. Control ChIP experiments were performed with untagged wild type and mutant strains. Ash1 binding to *HO* is significantly increased in an *rts1* mutant. *Error bars* in ChIP assays reflect the standard deviation of ChIP assays from two biological replicates.

localization showed a slight increase in mother cell fluorescence. The *rts1* mutant was notable because Ash1-GFP showed equivalent localization in mothers and daughters. The Ash1-GFP fluorescence signal was weaker in *rts1* cells than in wild type, and the Ash1-GFP signal was barely detectable in the *ace2 rts1* double mutants. Ash1-GFP could not be detected at all for most of the *ace2 rts1* cells. Although the localization pattern could be discerned for some of the *ace2 rts1* cells, the quantitation of these *ace2 rts1* cells should be interpreted cautiously.

In summary, in *rts1* mutant cells, Ash1 is present in both mother and daughter cells, as opposed to the pattern of exclusively daughter cell localization seen in wild type. Notably, the fluorescence data suggest that Ace2 is required to see strong Ash1-GFP signals in mother cells in the *rts1* mutant. This agrees with the RNA expression data, in which the *HO* repression in an *rts1* mutant is at least partly dependent upon Ace2.

Increased Ash1 Binding to HO in an rts1 Mutant—Increased Ash1 accumulation in mother cells should result in more Ash1 bound to promoters in mother cells. To address this question, we performed ChIP experiments to measure Ash1 binding to *HO* (Fig. 4*c*). In the wild type strain, Ash1 binding is at 1.6, as compared with the wild type strain. Importantly, Ash1 binding is much higher, at 2.8, in the *rts1* mutant. Thus, the *rts1* mutation results in increased Ash1 binding at *HO*.

DISCUSSION

The Rts1 subunit of PP2A phosphatase has important roles in regulating cell cycle progression (16), and we have shown that an *rts1* mutation results in increased phosphorylation of two cell cycle-regulated transcription factors, Ace2 and Swi5 (17). Here we show that expression of only one of the target genes activated by Ace2 or Swi5, *HO*, is reduced in an *rts1* mutant. Normally, *HO* is expressed only in mother cells because the Ash1 repressor accumulates preferentially in daughter cells. In contrast, Ash1 accumulation is equivalent in mother and daughter cells in an *rts1* mutant. The presence of Ash1 in mothers is sufficient to reduce *HO* expression.

HO is activated by the Swi5 transcription factor, but not by Ace2 (6). Phosphorylation of Swi5 is affected by an *rts1* mutation (17), and one possibility is that this altered phosphorylation affects the function of Swi5 as a transcriptional activator, leading to reduced *HO* expression. However the *rts1* mutation does not affect expression of *PIR1* and *PST1*, suggesting that Swi5 continues to be a robust activator. The major distinction is that *HO* is strongly repressed by Ash1, whereas *PIR1* and *PST1* are not. Ace2 and Swi5 contain the same DNA-binding domain and recognize the same DNA sequence, but they activate different target genes (7). Although Ace2 can bind to the *HO* promoter *in vitro* (6), chromatin immunoprecipitation experiments show that Ace2 does not bind to *HO in vivo* (7). Nonetheless, Ace2 is essential for the loss of *HO* expression in the *rts1* mutant (Fig. 1*c*). Ace2 normally accumulates only in daughter cell nuclei (14), but in an *rts1* strain, Ace2 is present in mother cell nuclei. This Ace2 present in mother cells activates transcription of *ASH1*; as cytokinesis has occurred, the *ASH1* mRNA cannot be exported to daughter cells, and the Ash1 protein produced represses *HO* in mother cells.

Ace2 localization is regulated by both nuclear import and export sequences, but the details of how it accumulates exclusively in daughter cells remains an important question (19). The Cbk1 kinase phosphorylates Ace2 at multiple positions, and it is believed that one of these phosphorylation events inactivates the NES. The dominant *ACE2* mutations that suppress a *cbk1* mutation are present within the NES, and these mutant Ace2 proteins show some accumulation in mother cells (11, 20). Additionally, Cbk1 accumulates preferentially in wild type daughter cells (21). These results suggest that the presence of Cbk1 specifically in daughters is important for the daughter-only pattern of Ace2 accumulation.

How does an *rts1* mutation allow Ace2 accumulation in mother cells? One possibility is that in the absence of the $PP2A^{Rts1}$ phosphatase, the hyperphosphorylation of Ace2 by the Cbk1 kinase changes its localization pattern. Alternatively, Ace2 localization could be controlled by other proteins whose

activity is controlled by dephosphorylation by Rts1. We note that Lre1 has recently shown to be an inhibitor of the Cbk1 kinase that is required for nuclear entry by Ace2 (22). Lre1 is phosphorylated, and it is a substrate of the Cdk1 kinase and the Cdc14 phosphatase (22). Lre1 phosphorylation is also regulated by the Rts1 phosphatase, either directly or indirectly, as an *rts1* mutation affects Lre1 phosphorylation (17). Thus, one possible explanation is that an *rts1* mutation results in increased Lre1 phosphorylation, and hyperphosphorylated Lre1 no longer inhibits Cbk1. Cbk1 could be hyperactive in the *rts1* mutant, perhaps facilitating accumulation of Ace2 in mother cell nuclei. Further studies will be needed to test this hypothesis.

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Gene Regulation:

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