The SBF- and MBF-associated Protein Msa1 Is Required for Proper Timing of G₁-specific Transcription in Saccharomyces cerevisiae

Mabelle Ashe 1, Robertus A. M. de Bruin 1, Tatyana Kalashnikova 1, W. Hayes McDonald 1, John R. Yates III 1, and Curt Wittenberg 1,2

From the Departments of Molecular Biology and Cell Biology, The Scripps Research Institute, La Jolla, California 92037

In the budding yeast Saccharomyces cerevisiae, cell cycle initiation is prompted during G₁ phase by Cln3/cyclin-dependent protein kinase-mediated transcriptional activation of G₁-specific genes. A recent screening performed to reveal novel interactors of SCB-binding factor (SBF) and MCB-binding factor (MBF) identified, in addition to the SBF-specific repressor Whi5 and the MBF-specific corepressor Nrm1, a pair of homologous proteins, Msa1 and Msa2 (encoded by YOR066w and YKR077w), as interactors of SBF and MBF, respectively. MSA1 is expressed periodically during the cell cycle with peak mRNA levels occurring at the late M/early G₁ phase and peak protein levels occurring in early G₁. Msa1 associates with SBF- and MBF-regulated target promoters consistent with a role in G₁-specific transcriptional regulation. Msa1 affects cell cycle initiation by advancing the timing of transcription of G₁-specific genes. Msa1 binds to SBF- and MBF-regulated promoters and binding is maximal during the G₁ phase. Binding depends upon the cognate transcription factor. Msa1 overexpression advances the timing of SBF-dependent transcription and budding, whereas depletion delays both indicators of cell cycle initiation. Similar effects on MBF-regulated transcription are observed. Based upon these results, we conclude that Msa1 acts to advance the timing of G₁-specific transcription and cell cycle initiation.

Regulation of cell division is the means by which multicellular organisms ensure proper growth and differentiation and unicellular organisms achieve fidelity in duplication of genetic and cellular components in future generations. The primary mechanisms utilized by cells to control cell cycle progression are periodic transcription and proteolysis. Although there are distinctions between the metazoan cell cycle and that of budding yeast, such as apoptosis and cellular differentiation (11), several pRb-related molecular pathways pertaining to cell cycle regulation are conserved in yeast as well. For example, in metazoans, commitment of cells to transition through the G₁ to S phase of the cell cycle is known as the “restriction point” and involves phosphorylation and subsequent inactivation of pRb by cyclin D-Cdk4/Cdk6 to allow transcriptional activation by E2F1/DP, the pRb target transcription factor during G₁ (reviewed in Refs. 4, 12, and 13). Similarly, in yeast, commitment to transition from G₁ to S phase, known as “Start,” requires that Whi5 be inactivated by Cln3/CDK phosphorylation so that it no longer represses transcription via SBF, its target transcription factor during G₁ (reviewed in Refs. 4, 12, and 13). Therefore, the regulation of G₁ transcription had been identified (6–8). Recent work showing that Whi5 has an analogous function to pRb in the Cln3/CDK-mediated repression of G₁-specific transcription in budding yeast has addressed this apparent inconsistency (1, 9).

To date, over 110 pRb binding partners have been identified (10). Although pRb is associated with other functions besides cell cycle regulation, many of which have no obvious analogy in yeast, such as apoptosis and cellular differentiation (11), several pRb-related molecular pathways pertaining to cell cycle regulation are conserved in yeast as well. For example, in metazoans, commitment of cells to transition through the G₁, to S phase of the cell cycle is known as the “restriction point” and involves phosphorylation and subsequent inactivation of pRb by cyclin D-Cdk4/Cdk6 to allow transcriptional activation by E2F1/DP, the pRb target transcription factor during G₁ (reviewed in Refs. 4, 12, and 13). Similarly, in yeast, commitment to transition from G₁ to S phase, known as “Start,” requires that Whi5 be inactivated by Cln3/CDK phosphorylation so that it no longer represses transcription via SBF, its target transcription factor complex, thus allowing transcription of G₁-specific genes (1, 9). Also, recent studies suggest that analogous chromatin remodeling complexes in mammals and yeast are linked to G₁-specific transcriptional regulation. Specifically, both human BRG1 and hBRM chromatin remodeling complexes and their yeast transcription, are highly conserved (1). The pivotal role of the retinoblastoma (Rb)3 pocket protein family and, in particular, pRb, in the control of periodic transcription during the cell cycle and in cell cycle initiation (2–4), argued that its function would be conserved in yeast, as well as metazoan species. Like the E2F1/DP transcription factor complex in metazoans, two heterodimeric transcription factor complexes, known as SBF and MBF, are responsible for transcribing genes specific to the G₁ phase of the cell cycle (reviewed in Ref. 5). These complexes are comprised of a shared component, Swi6 and the DNA-binding component, Swi4 or Mbp1, respectively, that recognize specific DNA sequence elements, known as SCBs and MCBs, within the promoters of G₁-specific genes. Although it had been established that Cln3/CDK was the critical determinant of the timing of cell cycle initiation in the budding yeast, until recently no CDK-regulated target was functionally analogous to pRb in the regulation of G₁ transcription had been identified (6–8). Recent work showing that Whi5 has an analogous function to pRb in the Cln3/CDK-mediated repression of G₁-specific transcription in budding yeast has addressed this apparent inconsistency (1, 9).

The abbreviations used are: Rb, retinoblastoma; CDK, cyclin-dependent protein kinase; SBF, SCB-binding factor; MBF, MCB-binding factor; MudPIT, multidimensional protein identification technology; TAP, tandem affinity purification; RT, reverse transcription.

1 Present address: Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831.
2 To whom correspondence should be addressed: Dept. of Molecular Biology, MB-3, The Scripps Research Institute, 10550 North Torrey Pines Rd., La Jolla, CA 92037. E-mail: curtw@scripps.edu.
homologs Swi/Snf have been shown to be involved in the recruitment and activation of their respective G₁-specific transcription factors (E2F1 and SBF, respectively) (14–16). Furthermore, these studies in human showed a direct correlation between the pattern of histone acetylation and deacetylation and E2F1 transcriptional activation, suggesting that these histone modification complexes may regulate the accessibility of G₁-specific transcription factors to their target promoters.

Taken together, these studies demonstrate the high level of conservation between species in the regulatory mechanisms that exist to control entry into the cell cycle. Understanding both the parallels and the distinctions that exist across species is critical to unraveling how abnormalities in these pathways deregulate the cell cycle and lead to human diseases including congenital malformations and cancer. Although the discovery of the parallels between Whi5 and Rb function has broadened our understanding of the molecular mechanisms that control G₁ transcription in yeast, much still remains to be learned about G₁-specific transcriptional regulation.

In an effort to broaden our understanding of G₁-mediated transcriptional regulation in budding yeast, we have used multidimensional protein identification technology (MudPIT) to screen interactors of known SBF and MBF components to identify novel regulators of these G₁-specific transcription factor complexes (1). This screen led to the identification of Whi5, an inhibitor of SBF-mediated transcription that is antagonized by Cln3/CDK, and Nrm1, an MBF-specific transcriptional corepressor required for the timely repression of MBF-regulated transcription during exit from the G₁ phase. In addition, two homologous proteins, Msa1 and Msa2, were identified. Further analysis of Msa1 revealed that it is expressed periodically during the cell cycle, displaying peak mRNA levels at late M/early G₁ and peak protein levels at early G₁, similar to Cln3. Msa1 interacts with both SBF and MBF at their target promoters. Furthermore, association of Msa1 with promoters is regulated during the cell cycle, with peak binding occurring during G₁. Msa1 promotes G₁-specific transcription and cell cycle initiation. Taken together, this study suggests that Msa1 is a novel regulator of G₁-specific transcription.

**MATERIALS AND METHODS**

**Strains and Methods**—All of the *Saccharomyces cerevisiae* strains used in this study were derived from 15Dub (*MATa ade1 leu2-3,112 his2 trp1-1 ura3Δα bar1Δ*). The relevant genotypes of the strains utilized in these studies are listed in Table 1. The PCR-based Longtine gene modification method (17) was utilized to create various *MSA1, SWI4, MBP1,* and *SWI6* alleles, including gene deletion, integration of the *GAL1* promoter, and epitope tagging with TAP, 3xHA, and 13xmyc. All of the strains including gene deletion, integration of the *GAL1-CLN3* as the only G₁ cyclin and one of the components of SBF or MBF, either *Swi6, Swi4,* or *Mbp1,* TAP-tagged at the endogenous locus. The cells were arrested in the G₁ phase by depletion of *CLN3* expression in the absence of galactose or released from arrest by induction of *CLN3* expression in the presence of galactose, at which time they were allowed to progress to maximal levels of transcription for analysis. All TAP-tagged proteins were checked by cell size analysis and Western blot to ensure proper function and expression of these proteins. Once purified, protein complexes were digested, and associated proteins were identified using MudPIT analysis as described by McDonald and Yates (19).

**Yeast Cell Extract Preparation**—The cells growing in log phase were collected by centrifugation and washed with 0.5 ml of cold H₂O. The pellets were resuspended in lysis buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.1% Nonidet P-40, 10% glycerol) containing phosphate inhibitors (50 mM NaF, 0.1 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 5 mM EDTA, 5 mM EGTA), 0.4 mM phenylmethylsulfonyl fluoride, and leupeptin/aphroton (1 μg/ml). To lyse cells, the samples were vortexed with glass beads for four cycles of 40 s with 3-min rest intervals at 4 °C between cycles. To collect extracts, the samples were centrifuged twice at maximum speed for 15 min at 4 °C.

**Co-immunoprecipitation and Western Blotting**—0.5–1 μg of yeast protein extract was precleared with 20 μl of 50% protein A-Sepharose (Sigma); lysis buffer slurry for 20 min at 4 °C. The samples were spun down for 15 min at maximum speed. The supernatant was collected and incubated with 2.5–5 μg of appropriate primary antibody (α-HA [12CA5], α-Myc) for 1–2 h at 4 °C. The samples were spun at maximum speed for 15 min.

**TABLE 1**

| Yeast strain | Genotype | Source |
|--------------|----------|--------|
| CWY231 | MATa ade1 leu2-3, 112, his2 trp1-1 ura3Δα bar1Δ | 15Dub; Ref. 40 |
| CWY267 | cln1Δ | Ref. 1 |
| CWY391 | SWI4-6xmyc::KANr | Ref. 20 |
| CWY1410 | MBP1-TAP::KANr | Ref. 20 |
| CWY1415 | whi5::KANr | Ref. 1 |
| CWY1442 | SWI6-TAP::KANr | Ref. 20 |
| CWY1461 | TRP1::GAL1-CLN3 cln1Δ cln2Δα cln3Δα pep4Δ LEU2 SWI6-TAP::KANr | Ref. 1 |
| CWY1462 | TRP1::GAL1-CLN3 cln1Δ cln2Δα cln3Δα pep4Δ LEU2 SW14-TAP::KANr | Ref. 1 |
| CWY1463 | TRP1::GAL1-CLN3 cln1Δ cln2Δα cln3Δα pep4Δ LEU2 MBP1-TAP::KANr | Ref. 1 |
| CWY1480 | MSA1-HA::KANr | This study |
| CWY1482 | msa1::GAL1-MSA1::KANr | This study |
| CWY1501 | msa1::GAL1-MSA1-HA::KANr | This study |
| CWY1520 | MBP1-TAP::KANr | Ref. 20 |
| CWY1521 | SWI4-TAP::KANr | Ref. 20 |
| CWY1573 | MBP1-13xmyc::URA3 | Ref. 20 |
| CWY1580 | MSA1-13xmyc::URA3 | This study |
| CWY1581 | SWI4-TAP::KANr MSA1-13xmyc::URA3 | This study |
| CWY1583 | MBP1-TAP::KANr MSA1-13xmyc::URA3 | This study |
| CWY1588 | msa1::KANr | This study |
| CWY1590 | MSA1-TAP::KANr | This study |
| CWY1621 | MBP1-13xmyc::URA3 swi6::KANr | Ref. 20 |
| CWY1624 | swi6::KANr | Ref. 20 |
| CWY1633 | MSA1-TAP::KANr SWI4-13xmyc::URA3 | This study |
| CWY1657 | mbp1::URA3 | This study |
| CWY1658 | MBP1-13xmyc::URA3 | This study |
| CWY1660 | MSA1-TAP::KANr MBP1-13xmyc::URA3 | This study |
| CWY1665 | MSA1-TAP::KANr pep4Δ::URA3 | This study |
| CWY1667 | MSA1-TAP::KANr SWI4-6xMYC::URA3 | This study |
| CWY1780 | MSA1-3MYC::URA3 swi6::KANr | This study |

*All of the strains are in the 15Dub background and MATa (see CWY231) with the alterations shown.*
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The supernatant was again collected and incubated with 25 μl of 50% protein A-Sepharose slurry for 2–4 h at 4 °C. The samples were spun down briefly to remove supernatant. The remaining beads were washed three times with 1 ml of modified buffer solution (lysis buffer with 0.5% Triton X-100, 0.5 mM NaCl concentration, with phosphatase and protease inhibitors as described previously). 20 μl of 4× sample buffer was added to each sample in preparation for Western blot.

Cell Size Analysis—For mating pheromone synchronization at G₁, the cells were grown in YEPD to log phase. α factor was added at a concentration of 100 ng/ml, and the cells were incubated at 30 °C for 1.5 h to synchronize. In addition to mating pheromone synchronization, G₁ cells were also obtained for synchronization studies by isolating small G₁ cell populations by centrifugal elutriation as described (20).

Real Time RT-PCR—Total RNA was isolated from cells using the RNeasy kit (Qiagen). The QuantiTech SYBR Green RT-PCR Kit (Qiagen) was used to prepare samples for analysis by RT-PCR. The reactions were then run on the Chromo-4 qPCR system (MJ Research) using standard RT-PCR conditions. The obtained data were subsequently analyzed using MJ Opticon Monitor Analysis Software 3.0 (MJ Research).

Chromatin Immunoprecipitation Analysis—Chromatin immunoprecipitation analysis was performed as previously described by Flick et al. (21).

Cell Size Analysis—Actively growing cell cultures were diluted and permitted to grow for three to four generations until they reached log phase. Once cells reached log phase, at an approximate A₂₆₀ of 0.4–0.7, 100 μl of culture were collected and subjected to sonication for 30 s at maximum power using a Sonicator 3000 Misonix Inc. sonicator. The samples were then diluted in 10 ml of Isoton® II Diluent and immediately subjected to cell size analysis using a Coulter Z2 Particle Cell Analyzer (Beckman-Coulter). For each analysis, the cell size of ~5–30 × 10³ was resolved and their distribution determined using Z2 AccuComp software (Beckman-Coulter).

RESULTS

Identification and Characterization of Msa1 and Msa2—In an effort to further our understanding of G₁-specific transcriptional regulation, we sought to identify novel components of SBF and MBF. To screen for novel components of these G₁-specific transcription factors, Swi4, Mbp1, and Swi6 were tagged with a carboxyl-terminal TAP epitope at their endogenous locus in a cln1Δcln2Δcln3Δ background containing a GAL1-regulated CLN3 as its only source of G₁ cyclin. TAP purification of associated protein complexes was performed in cells that were arrested in early G₁, prior to transcriptional initiation, and in cells in late G₁ phase at the peak of G₁ transcription, as described previously by de Bruin et al. (1).

Analyses of protein complexes obtained from these purifications were achieved using MudPIT to perform mass spectrophotometry-based identification of individual components. Along with known interactors of SBF and MBF, several novel proteins were also identified, including Whi5 and Nrm1, which function as negative regulators of SBF and MBF, respectively (1, 20). Among the novel interactors, two, encoded by the open reading frames YOR066w and YKR077w, were identified by MudPIT as interactors of MBF and SBF components, respectively. Because we later found that Msa1 interacts with SBF (shown below) and Msa2 interacts with MBF (data not shown), we have named the genes encoding these proteins MSA1 and MSA2, respectively, for “MBF and SBF associated.”

Msa1 was identified as a novel interactor of MBF components with a high degree of confidence based on sequence coverage and spectral quality (Fig. 1). Specifically, Msa1 was found to interact with Mbp1, demonstrating 4.3 and 14.1% sequence coverage in arrested and released cells, respectively, as well as Swi6, showing 10.5% coverage in released cells (Fig. 1A). Msa2 was identified during our MudPIT analysis as an interactor of Swi4 (Fig. 1A). It was also recently reported to associate with Swi6 based upon genome-wide protein-protein interaction analysis (22). Msa2, which is related to Msa1, sharing 28% amino acid identity and 43% similarity (Fig. 1B), awaits further investigation. Here we report the analysis of the role of Msa1 in the regulation of G₁-specific transcription.

Expression of Msa1 Is Regulated during the Cell Cycle—Identifying Msa1 as a novel component of MBF implicated it in the regulation of cell cycle initiation events during G₁. This led to the prediction that Msa1, similar to other regulators of G₁-specific transcription, including Whi5 and Cln3 (1, 8), would accumulate periodically during the cell cycle. In fact, the MSA1 transcript was shown to accumulate during the early G₁ phase (23) and has more recently been assigned to the Yox1/Mcm1 target cluster, which is expressed during the M/G₁ phase (24). To look at accumulation of the Msa1 protein during the cell cycle, we synchronized cells during the G₁ phase with mating pheromone and then released them into the cell cycle. The samples were taken every 15 min and subjected to both Western blot analysis to evaluate protein levels and analysis of budding index to establish the cell cycle position and degree of synchrony. Our results demonstrate that the Msa1 protein accumulates periodically during the cell cycle and achieves peak abundance between 0 and 15 min after release from pheromone arrest, followed by a rapid decrease after 30 min (Fig. 2). This pattern is reinitiated at 75 min after release as cells enter a new cell cycle. The highest level of accumulation of Msa1 occurs during G₁, prior to budding, and decreases rapidly as cells enter into the S phase consistent with the pattern of gene expression. These studies reveal a cell cycle expression pattern for Msa1 that is similar to that observed for other regulators of G₁-specific transcription and is consistent with a role for Msa1 in regulation of transcriptional activation at the time of cell cycle initiation.

Msa1 Interacts with both SBF and MBF Components—To confirm the interactions revealed by MudPIT analysis, we performed co-immunoprecipitations of Msa1 with individual components of the SBF and MBF transcription factors. Msa1 was epitope-tagged at its genomic locus along with either Swi4, Mbp1, or Swi6. Whole cell lysates were collected from these strains, and protein complexes were immunoprecipitated using antibody specific for the epitope on Msa1 or the G₁ transcription factor components, as indicated (Fig. 3). Analysis of the constituents of these protein complexes by Western blot revealed that Msa1 co-precipitated with all of the G₁ transcription factor components. Although it was not apparent from the
MudPIT analysis of G₁ transcription factor complexes, which revealed an interaction only with MBF, this analysis demonstrates that Msa1 interacts with SBF and MBF. This is not an entirely surprising result because, considering its qualitative nature, the MudPIT technique is an insufficient basis upon which to exclude an interaction between proteins.

Msa1 Associates with SBF- and MBF-regulated Promoters—We have shown that Msa1 interacts with both SBF and MBF transcription factors. Furthermore, we observed that its periodic expression is similar to some of the established regulators of G₁-specific transcriptional activation. We therefore postulated that Msa1 is involved in the regulation of SBF- and/or MBF-regulated gene expression. If so, then we expect Msa1 to be present at SBF- and MBF-regulated promoters. To analyze whether Msa1 is recruited to SBF- and MCB-regulated promoters, we performed chromatin immunoprecipitation assays of Myc-tagged Msa1 along with Swi4 and Mbp1, the DNA-binding components for SBF and MBF, respectively, for comparison. Our results demonstrate that Msa1 associates with both MBF-regulated (RNR1 and CDC21) and SBF-regulated G₁-specific promoters (CLN2 and SVS1) (Fig. 4). Furthermore, inactivation of Swi4 strongly reduces Msa1 binding to SBF target promoters, whereas inactivation of Mbp1 reduces binding to MBF target promoters, without appreciable effects on binding of the
other transcription factor to its target promoters. We conclude that Msa1 binds to both SBF and MBF target promoters.

Binding of Msa1 to Promoters Is Regulated during the Cell Cycle—Our finding that Msa1 binds to G1-specific promoter elements suggests that this protein functions at promoters to regulate transcription during the G1 phase of the cell cycle. To test this hypothesis, we analyzed whether changes in binding status occurred during the cell cycle and whether binding was affected by the absence of SBF. To monitor periodic changes in binding status of Msa1 at promoters, we performed chromatin immunoprecipitation analysis of Myc-tagged Msa1 on cells that had been synchronized at G1 by pheromone arrest and released to allow progression through the cell cycle. Binding of Msa1 to both SBF- and MBF-dependent promoters was observed to fluctuate during the cell cycle (Fig. 5A). The level of binding was maximal during early G1 prior to maximum budding, after which Msa1 rapidly dissociated from the promoter as cells entered the S phase.

We then determined whether association of Msa1 with promoter elements was dependent on G1-specific transcription factors. Binding was analyzed in swi4Δ strains to determine whether Msa1 association with promoter elements required SBF. In the absence of SWI4, the binding of Msa1 to SBF-specific promoter elements is severely disrupted, whereas binding to MBF-specific promoter elements is not affected (Fig. 5B). This is consistent with the behavior of Msa1 observed in asynchronous populations of swi4Δ cells (Fig. 4). Furthermore, it appears that, although the level of association of Msa1 to SBF and MBF target promoters is compromised, the periodicity of binding during the cell cycle is unaffected (Fig. 5B). These studies demonstrate that Msa1 associates periodically with SBF and MBF target promoter elements with the highest levels of association occurring at early G1. Furthermore, association Msa1 with SBF is dependent on Swi4. Taken together, these results are consistent with a role for Msa1 in G1 transcriptional regulation.

Msa1 Promotes Cell Cycle Initiation—Because our findings were consistent with a role for Msa1 in G1 transcriptional acti-
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To determine whether the changes observed in the relative size of cells misexpressing sMSA1 are a reflection of changes occurring in the timing of cell cycle initiation or of other factors that can affect cell size outside of G₁, we synchronized wild type cells and cells having GAL1-MSA1 as their sole source of Msa1 protein. Strains were grown under inducing conditions to cause a small cell size, and small daughter cells were isolated by centrifugal elutriation and inoculated into fresh medium under either repressing (glucose) or inducing (galactose) conditions. Small G₁ daughter cells progressing synchronously into the cell cycle were analyzed for cell size and budding. We found that overexpression of Msa1 advances the size at which purified G₁ cells and cells having GAL1-MSA1 in glucose led to an increase in cell size (Fig. 6A, bottom panel). A similar result was obtained comparing glucose-grown msa1Δ mutants to wild type cells growing under the same conditions (data not shown). Taken together, these observations suggest that Msa1 promotes cell cycle initiation. Together with our finding that it interacts with SBF and MBF promoter elements, our results suggest that the effect of Msa1 on cell size may reflect a role in activating G₁-specific transcription.

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tightly correlated the timing of G1-specific transcriptional activation (6, 7), we followed the expression of the G1-specific transcriptional targets CLN2, a target for SBF and primary indicator of Start (4, 12, 26) and CDC21, a target of MBF encoding thymidylate synthase (20, 27). The same wild type and GAL-MSA1-containing populations of synchronized G1 daughter cells that were analyzed in Fig. 6 were used to analyze expression of the SBF and MBF target genes. Cultures grown in galactose (Fig. 8, left panels) or glucose (Fig. 8, right panels) to induce or repress GAL1-MSA1 expression, respectively, were used to analyze expression of the SBF and MBF target genes. As expected, CLN2 and CDC21 mRNA accumulation precedes budding emergence in both wild type and MSA1 overexpressing cells. However, in cells misexpressing MSA1, the effect on budding reflects the effect on G1-specific gene expression (Fig. 8, solid lines). In MSA1 overexpressing cells, peak CLN2 and CDC21 mRNA levels occur at a smaller mean cell size compared with wild type cells (Fig. 8, left panels). Similarly, peak CLN2 and CDC21 mRNA levels for MSA1 depleted cells occur at a larger cell size than wild type cells (Fig. 8, left panels). Thus, the
changes in critical cell size in MSA1 misexpressing cells are associated with a complementary change in the timing of expression of G1-specific genes. We show that the altered level of Msa1 expression can lead to advancement of the timing of G1-specific gene expression without affecting the level of expression. Because Cln2 drives cells through Start (4, 28), the earlier accumulation of CLN2 is expected to drive cells through Start at a smaller size. A similar, but more pronounced, effect on cell size is observed in cells overexpressing CLN3, a known activator of G1-specific transcription (29, 30). Again, the timing of transcriptional induction of CLN2 and CDC21 is delayed in Msa1-depleted cells without a substantial effect on the maximal level of expression (Fig. 8, right panels). These results suggest that the MSA1 acts to regulate transcription by affecting activation of G1-specific transcription factors, SBF and MBF, to which it binds. Together these data suggest that the primary activity of Msa1 is as an activator of SBF and MBF that primarily affects the timing, rather than the extent, of transcriptional activation. However, Msa1 can apparently interfere with another as yet unknown pathway affecting the timing of transcriptional activation in the absence of SBF.

DISCUSSION

Coordination of events of the cell division cycle is governed in part by the periodic expression of genes required for the execution of the events that define cell cycle phases. Cell cycle initiation in many eukaryotic cells is defined by the coordinate transcription of family of G1-specific genes that leads to the accumulation of activities required for DNA replication and cell growth. Faithful regulation of those genes is paramount for the coordination of cell growth and proliferation.

Here we describe Msa1, a novel regulator of G1-specific transcription in the budding yeast S. cerevisiae that is required for the proper timing of cell cycle initiation. Although Msa1 was identified as a protein associated with Mbp1 and Swi6 by MudPIT analysis, we show by co-immunoprecipitation that it binds to both MBF and SBF. Chromatin immunoprecipitation experiments show that it binds both MBF and SBF target promoters. In line with these observations both MBF and SBF target genes are affected by overexpression and depletion of Msa1. Consistent with its role in G1-specific transcriptional regulation, the MSA1 gene is expressed early in the G1 phase and accumulates during arrest by mating pheromone consistent with its importance for timely activation of G1-specific gene expression.

Altering Msa1 abundance affects the size at which cells activate transcription of G1-specific targets and, as a consequence, the size at which cells produce a new bud, the first overt indicator of the initiation of a new cell cycle (31). Overexpression of Msa1 promotes early transcription and budding. Conversely, depletion of Msa1 leads to a delay in those events. Consistent with a role of Msa1 in the pathway by which Cln3 activates G1-specific transcription, inactivating Cln3 abrogated the effect of Msa1 inactivation on cell cycle initiation. This suggests that Msa1 acts downstream in a Cln3-dependent pathway. However, inactivation of the Cln3/CDK target, Whi5, still prompts a cell cycle advance in cells lacking Msa1, suggesting that Whi5 and Msa1 lie, at least in part, on separate pathways.
Together, these findings define Msa1 as a size-sensitive component of cell cycle initiation prior to transcriptional activation (32).

Cln3-CDK has multiple substrates in the G1-specific transcriptional apparatus including Whi5 and Swi6. In addition to its effect on the activity and localization of Whi5, Cln3/CDK also directly phosphorylates Swi6, a component of both SBF and MBF. Although it is possible that Msa1 affects the interaction of Cln3/CDK with either of those targets, Msa1 may also act as a substrate. Msa1 contains multiple consensus sites for CDK phosphorylation. Thus, Msa1 may act as a substrate. Msa1 contains multiple consensus sites for CDK phosphorylation. However, when and to what extent transcription remains poorly characterized.

Msa1 is just one of a number of proteins that affect the timing of transcription initiation or magnitude of expression of G1-specific genes. In addition to Cln3 and Whi5, which are the primary determinants of the timing of transcriptional activation of SBF and MBF target genes, other factors play more subtle roles. Notably, Bck2 can promote transcriptional activation of G1-specific genes via a mechanism that is independent of CLN3 (30). Although it clearly acts via a pathway that is independent of Msa1 (data not shown), its mechanism of action is unknown. In addition, Stb1 promotes transcriptional activation of SBF- and MBF-regulated genes. Like Msa1, Stb1 binds to both transcription factors at promoters and is a target for CDK-dependent phosphorylation. However, unlike Msa1, it primarily affects the magnitude, rather than the timing, of G1-specific transcription. Together, these proteins contribute to fine tuning of activation of the G1-specific genes at the start of the cell cycle and, perhaps, participate in the integration of nutritional and stress signals with the cell cycle transcription program.

Similarly, G1-specific transcription in mammalian cells is subject to a broad array of environmental and internal influences. Consistent with the greater complexity of those cells and genomes, the E2F family of transcription factors is diverse and subject to many types of regulatory signals. We speculate that, like in yeast where G1 transcription is subject to modulation by multiple factors, the many Rb-associated proteins provide for that regulatory complexity. We are hopeful that understanding the mechanisms by which those yeast modulators, including Msa1, influence G1-specific transcription will facilitate the analysis of proteins having similar roles in mammals.

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