Promoter regulation by distinct mechanisms of functional interplay between lysine acetylase Rtt109 and histone chaperone Asf1

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The promoter activity of yeast genes can depend on lysine 56 (K56) acetylation of histone H3. This modification of H3 is performed by lysine acetylase Rtt109 acting in concert with histone chaperone Asf1. We have examined the contributions of Rtt109, Asf1, and H3 K56 acetylation to nutrient regulation of a well-studied metabolic gene, *ARG1*. As expected, Rtt109, Asf1, and H3 K56 acetylation are required for maximal transcription of *ARG1* under inducing conditions. However, Rtt109 and Asf1 also inhibit *ARG1* under repressing conditions. This inhibition requires Asf1 binding to H3-H4 and Rtt109 KAT activity, but not tail acetylation of H3-H4 or K56 acetylation of H3. These observations suggest the existence of a unique mechanism of transcriptional regulation by Rtt109. Indeed, chromatin immunoprecipitation and genetic interaction studies support a model in which promoter-targeted Rtt109 represses *ARG1* by silencing a pathway of transcriptional activation that depends on *ASF1*. Collectively, our results show that *ARG1* transcription intensity at its induced and repressed set points is controlled by different mechanisms of functional interplay between Rtt109 and Asf1.

amino acid biosynthesis | arginase | RNA polymerase II | transcription initiation

The acetylation state of nucleosomal histones has a profound influence on the initiation, elongation, and termination phases of transcription. Much of the regulation of transcription impinges on the proteins responsible for histone acetylation—the histone-directed lysine acetylases (KATs). One recently discovered KAT being intensively studied from the viewpoint of its regulation is Rtt109. This yeast protein catalyzes K9, K23, K27, and K56 acetylation of histone H3. All of these reactions depend, to a greater or lesser extent, on the conserved H3-H4 chaperone Asf1. Specifically, Asf1 stimulates H3 K9, K23, and K56 acetylation by Rtt109 on its own, and K27 acetylation by Rtt109 in complex with histone chaperone Vps75 (1–4). In current models, transcriptional regulation by Rtt109 is ascribed to its ability to acetylate H3, and functional interplay between Rtt109 and Asf1 in the regulation of transcription is limited to Asf1 stimulation of Rtt109 KAT activity.

Here we examine the role of Rtt109 and Asf1 in the regulation of *ARG1*, a well-studied metabolic gene of budding yeast. *ARG1* is repressed in arginine-replete cells by the ArgR/Mcm DNA binding complex consisting of Arg80, Arg81, Arg82, and Mcm1 (5–7). Upon arginine limitation, *ARG1* is activated by the transcription factor Gcn4 (8, 9). Chromatin reconfiguration, in particular, acetylation of residues in the amino-terminal tails of H3 and H4, makes an important contribution to the physiological regulation of *ARG1* promoter activity. The enzymes implicated in this regulation include the KATs Gcn5 and Esa1 (10, 11).

We extended these findings by exploring the contributions of Rtt109 and Asf1 to *ARG1* regulation. In part our results support the evidence that Asf1-dependent acetylation of H3 K56 by Rtt109 is important for high transcription (12–15). We also find that Asf1 and Rtt109 control *ARG1* promoter activity under repressive conditions by an unprecedented mechanism likely involving Rtt109 inhibition of transcription stimulation by Asf1.

Results and Discussion

H3 K56ac Favors High Transcription of *ARG1*. We studied the mechanism of *ARG1* transcriptional regulation under two steady-state conditions: repression in arginine-replete medium (yeast extract, bactopeptone, dextrose, YPD), and induction (or activation) in arginine-free minimal medium (composition in Table S1, M1D) (Fig. 1*A*). Compared to repression, the induced configuration of *ARG1* promoter chromatin is characterized by lower H3 content and enrichment of H3 K56ac (Fig. 1 *B* and *C*). H3 K56ac occupancy is sensitive to deletion of *RTT109* and *ASF1* in cells cultured in either arginine-replete or arginine-free medium (Fig. 1*D*), whereas H3 occupancy has little dependence on *ASF1* (Fig. 1*B*). Therefore, (*i*) *ARG1* promoter nucleosomes are marked by H3 K56ac whether the gene is active or repressed, (*ii*) high H3 K56 acetylation is a hallmark of the induced state, and (*iii*) Asf1 is not uniquely required to maintain H3 promoter occupancy under repressing or inducing conditions. Consistent with published evidence that H3 K56 acetylation is favorable for transcription, *ARG1* expression is dampened under inducing conditions by the H3 K56R mutation which mimics deacetylation (Fig. 1*E*). Conversely, repression is dampened (*ARG1* is "induced") by the K56Q and K56A mutations, which mimic the charge state conferred by lysine acetylation (Fig. 1*F*).

The effects of *RTT109* and *ASF1* deletion on activated transcription are consistent with regulation of *ARG1* by a mechanism that involves H3 K56 acetylation, as described for other genes (12, 13). Specifically, in arginine-free minimal medium *ARG1* transcription is lower in *rtt109*Δ and *asf1*Δ cells than wild type (Fig. 1*G*), and the Asf1 V94R mutation which compromises binding to H3-H4 and H3 K56 acetylation (16, 17) phenocopies the *ASF1* null (Fig. 1*H*). These results suggest that the shared function of Rtt109 and Asf1 in the regulation of H3 K56 acetylation is important for activated transcription of *ARG1*. Consistent with this interpretation, simultaneous deletion of *RTT109* and *ASF1* has no greater effect on activated transcription than either deletion alone (Fig. 1*G*), and the effect of *ASF1* deletion is similar in magnitude to, and nonadditive with, the H3 K56R mutation (Fig. 1*I*).

***ARG1* Regulation by Rtt109 and Asf1 Under Repression.** The H3 K56R mutation which mimics deacetylation has no effect on *ARG1*

Author contributions: M.C.S. and L.-j.L. designed research; L.-j.L. performed research; L.-j.L. contributed new reagents/analytic tools; L.-j.L. and M.C.S. analyzed data; and M.C.S. wrote the paper.

The authors declare no conflict of interest.

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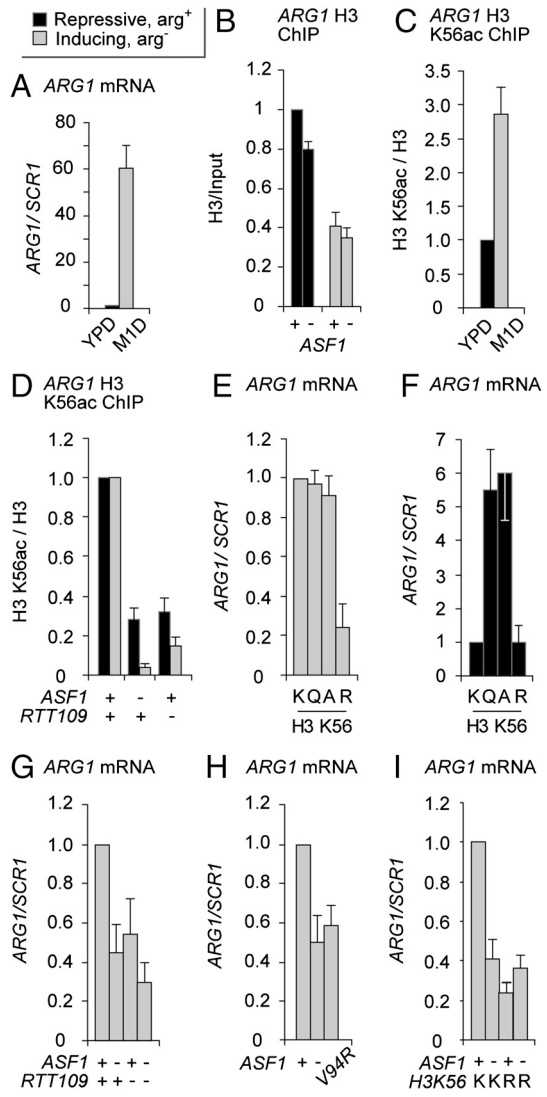

Fig. 1. *ARG1* regulation by H3 K56ac. (*A*) *ARG1* transcription in wild-type cells in inducing minimal medium, relative to transcription in repressive YPD medium (latter set to one). (*B*) ChIP analysis of H3 cross-linking to the promoter of *ARG1* in wild-type and *asf1*Δ cells, under repressing and inducing conditions. Occupancy in wild-type cells subject to repression is set to one. Average of two experiments; the error bar shows the range. (*C*) ChIP analysis of H3 K56ac at the promoter of *ARG1* under repressing and inducing conditions. All data points are normalized to H3 occupancy, and occupancy under repression is set to one. (*D*) ChIP analysis of H3 K56ac dependency on *RTT109* and *ASF1*. *ARG1* promoter chromatin was probed under repressing and inducing conditions. Analysis as in *C*. (*E*) *ARG1* transcription in H3 K56 mutants relative to wild type (H3 K56K), under inducing conditions. Average of two experiments; the error bar shows the range. (*F*) As in *E*, under repressing conditions. (*G*) Effect of *RTT109* and *ASF1* deletion (alone and in combination) on *ARG1* transcription under inducing conditions. (*H*) *ARG1* transcription in *asf1*Δ and *asf1*^{V94R} cells under inducing conditions. Wild-type transcription is set to one. (*I*) Effect of *ASF1* deletion and H3 K56 mutation on *ARG1* transcription under inducing conditions.

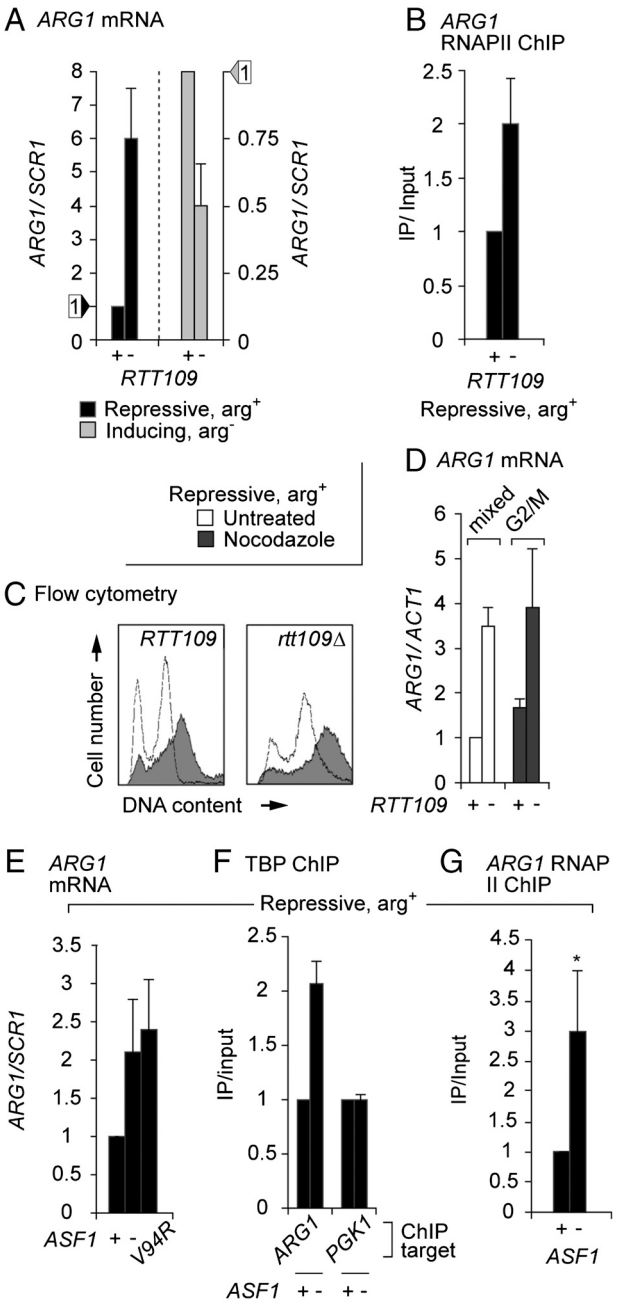

Fig. 2. *ARG1* regulation by Rtt109 and Asf1 under repressing conditions. (*A*) Effect of *RTT109* deletion on *ARG1* transcription under repressive and inducing conditions. Under each condition, transcription in the mutant is relative to wild type (set to one); this normalization highlights the fold-effect on steady-state transcription under each condition. (*B*) ChIP analysis of RNAP II cross-linking to the promoter of *ARG1* under repressing conditions. RNAP II occupancy in wild-type cells is set to one. Average of two experiments; the error bar shows the range. (*C*) Wild-type and *rtt109*Δ cell cycle profiles in repressive medium without or with nocodazole. (*D*) *ARG1* transcription in *rtt109*Δ cells relative to wild type in mixed populations of cells and G2/M-arrested cells. (*E*) *ARG1* transcription in *asf1*Δ and *asf1*^{V94R} cells under repressive conditions (wild-type transcription is set to one). (*F*) ChIP analysis of TBP cross-linking to the promoter of *ARG1* under repressing conditions. Occupancy in wild-type cells is set to one. *PGK1* is a control gene not regulated by arginine (10). (*G*) ChIP analysis of RNAP II cross-linking to the promoter of *ARG1* under repressing conditions in the presence and absence of Asf1. RNAP II occupancy in wild-type cells is set to one. Student's *t* test was used to assess significance ($^*P < 0.05$).

transcription under repressing conditions (Fig. 1*F*). Therefore, deletion of *RTT109* and the resulting global deacetylation of H3 K56 were not expected to affect *ARG1* transcription in YPD-grown cells. Surprisingly, however, deletion of *RTT109* causes sixfold induction of *ARG1* mRNA (Fig. 2*A*, *Left*; compare to dampening of induction under arginine limitation, *Right*). *ARG1* induction is likely due to elevated transcription initiation because RNA polymerase (RNAP) II cross-linking to its promoter is elevated in *rtt109*Δ in arginine-replete medium (Fig. 2*B*). Constitutive DNA damage signaling in *rtt109*Δ cells (12) cannot account for *ARG1* induction because *ARG1* is not controlled by the DNA

damage sensor kinase Mec1 (18). Possible induction by oxidative stress (19) is also unlikely. Deletion of *RTT109* does not confer sensitivity to exogenous oxidants (20). It follows that *rtt109*Δ cells are not under a higher than normal level of endogenous oxidative stress, and that oxidative stress signaling pathways are not constitutively activated in *rtt109*Δ cells. Although *ARG1* is normally induced in G2 (21) and *rtt109*Δ cells accumulate in G2/M (1), G2/M arrested *rtt109*Δ cells (Fig. 2*C*) support higher *ARG1* transcription than arrested wild-type cells (Fig. 2*D*). Overall, we conclude that Rtt109 can repress *ARG1* independently of cell cycle cues, by a mechanism that regulates the transcription process prior to elongation and does not involve Rtt109 acetylation of H3 K56.

Rtt109 function in transcriptional activation of *ARG1* requires Asf1 (Fig. 1*G*). Accordingly, we hypothesized that *ARG1* repression by Rtt109 also involves Asf1. Under this hypothesis, deletion of *ASF1* is expected to dampen *ARG1* repression. A microarray study provided evidence in favor of this possibility (5.9-fold relief of repression in *asf1*Δ cells) (12), and targeted mRNA analysis revealed twofold increased *ARG1* expression in *asf1*Δ and $asf1^{V94R}$ cells grown in YPD (Fig. 2*E*), associated with increased occupancy of the *ARG1* promoter by both the TATA binding protein and RNAP II (Fig. 2 *F* and *G*). We conclude that Rtt109 and Asf1 have a dual role at *ARG1*: They both promote transcription when steady-state physiological conditions trigger high *ARG1* expression and dampen promoter activity under physiological conditions of low steady-state transcription. Repression and activation of *ARG1* both require robust binding of Asf1 to H3-H4 (Figs. 1*H* and 2*E*). Overall, our results reveal an unprecedented role for Rtt109 in stimulation and inhibition of promoter activity, and demonstrate that the ability of Asf1 to promote functionally opposite states of chromatin architecture at an individual locus is not restricted to elongation-coupled events in coding regions (22): At an individual promoter, Asf1 can also exert positive and negative affects on chromatin that impact on transcription.

Functional Interplay Between Rtt109 and Asf1 in *ARG1* Repression. Our analysis of *ARG1* suggests previously unknown roles for Rtt109 and Asf1 in dampening of transcription. This unexpected outcome prompted us to consider conventional but indirect mechanisms that might account for the effects of *RTT109* and *ASF1* mutations on *ARG1* repression. We sought to explain three key observations, starting with dampening of *ARG1* repression in the absence of either Rtt109 or Asf1. Loss of Rtt109 or Asf1 could induce *ARG1* if these proteins normally support expression of the ArgR/Mcm repressor (an equivalent mechanism could explain *ARG1* induction in mutants of the SWI/SNF chromatin remodeling complex) (23). If this model is correct, then deletion of *ARG80* which encodes an essential subunit of ArgR/Mcm should have the same effect on *ARG1* transcription as deletion of either *RTT109* or *ASF1*. Our results do not support this prediction: *arg80*Δ has a stronger inducing effect on *ARG1* than either *rtt109*Δ or *asf1*Δ, and deletion of either *ASF1* or *RTT109* has an additive effect on depression caused by loss of *ARG80* (Fig. 3*A*). It follows that Rtt109 and Asf1 do not regulate *ARG1* under repressing conditions by modulating previously described mechanisms of *ARG1* regulation by ArgR/Mcm. Consistent with this interpretation, *ASF1* deletion is not associated with altered mRNA expression of any ArgR/Mcm component in budding yeast (microarray analysis of YPD-grown cells) (12) and *ARG1* induction in an *RTT109* null mutant of *Candida albicans* is not associated with misregulation of ArgR/Mcm subunits (24).

We next considered a straightforward explanation for the fact that *rtt109*Δ (Fig. 2*A*) more substantially induces *ARG1* transcription in arginine-replete medium than *asf1*Δ (Fig. 2*E*). Because histone chaperone Vps75 can regulate Rtt109 activity (25, 26), we hypothesized that Rtt109 repression of *ARG1* in wild-type cells is imposed by parallel nonredundant pathways which separately depend on Asf1 and Vps75. It follows that *asf1*Δ does not induce *ARG1* to the same extent as *rtt109*Δ because of residual Vps75-dependent Rtt109 repression in the *ASF1* null. If this hypothesis is correct, then *VPS75* deletion should be associated with partial induction of *ARG1* in arginine-replete medium. *ARG1* however is not induced in *vps75*Δ cells cultured under repressive conditions (27). Therefore functional redundancies between Asf1 and Vps75 do not account for the distinct effects of *rtt109*Δ and *asf1*Δ on *ARG1* repression, and the contribution of histone chaperones to *ARG1* regulation under repressing conditions is limited to Asf1.

Finally we sought to explain why we obtained two different answers to a straightforward question: How does H3 K56ac affect *ARG1* transcription under repressing conditions? That is, we sought to understand why *ARG1* is stimulated by mutations which mimic H3 K56ac (Fig. 1*F*, H3 K56Q and K56A), and by mutations which eliminate H3 K56ac (Fig. 2*A*, *rtt109*Δ; *E–G*, *asf1*Δ and $asf1^{V94R}$). This discordance suggests that Rtt109 and Asf1 control *ARG1* under repressing conditions by a mechanism unrelated to the control of H3 K56ac (installation of H3 K56 mutations which mimic acetylation presumably override another system of regulation by Rtt109 and Asf1).

A likely alternative to regulation of H3 K56ac by Rtt109-Asf1 is regulation of H3 K9ac. This alternative is likely in view of the evidence that tail acetylation of H3 is important for repression of *ARG1* (10), and that Rtt109 working in concert with Asf1 catalyzes H3 K9 (4) in addition to H3 K56 acetylation. If this

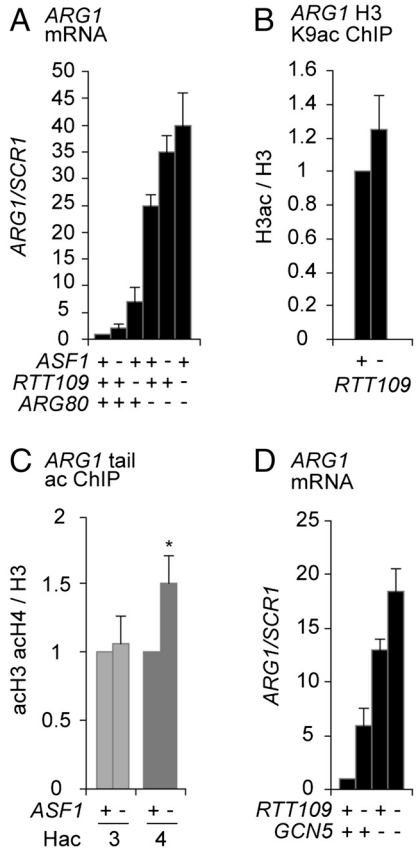

Fig. 3. Relationship of *ARG1* repression by Rtt109 and Asf1 to other pathways. (*A*) Effect of *ARG80* deletion on *ARG1* transcription in *rtt109*Δ and *asf1*Δ cells cultured under repressive conditions. (*B*) ChIP analysis of H3 K9ac at the promoter of *ARG1* in wild-type and *rtt109*Δ cells under repressing conditions. Occupancy in wild-type cells is set to one. (*C*) ChIP analysis of H3/H4 tail acetylation at the promoter of *ARG1* in wild-type and *asf1*Δ cells, under repressing conditions. Occupancy in wild-type cells is set to one. H4 acetylation in *asf1*Δ cells is significantly different from wild type (Student's *t* test, $^{*}P < 0.05$). (*D*) Effect of *RTT109* and *GCN5* deletion (alone and in combination) on *ARG1* transcription under repressive conditions.

mechanism underlies *ARG1* repression by Rtt109, then *RTT109* deletion should be associated with low H3 K9 acetylation of *ARG1* in YPD-grown cells. We observe no such association (Fig. 3*B*), and *RTT109* deletion does not affect H3 K14, K18, or K23 acetylation (28). Furthermore, we do not observe loss of overall H3 or H4 tail acetylation at *ARG1* in *asf1*Δ cells (Fig. 3*C*). It follows that neither Rtt109 nor Asf1 controls *ARG1* under repressing conditions by a mechanism that depends on H3 K9ac. By extension, we reasoned that repression by Rtt109 is not in the same pathway as repression that depends on Gcn5 acetylation of H3 K9. Consistent with this proposition, *rtt109*Δ and deletion of *GCN5* are additive in their stimulatory effect on *ARG1* transcription under the repressing condition (Fig. 3*D*). We conclude that Rtt109 and Asf1 act in parallel to Gcn5-dependent H3 acetylation to repress *ARG1* transcription in arginine-replete medium.

The absence of a compelling conventional explanation for *ARG1* repression by Rtt109 and Asf1 prompted our further characterization of this regulation. In current models, regulation of transcription by Rtt109 and Asf1 is mostly ascribed to their activities off chromatin, specifically their ability to collaborate in the acetylation of soluble H3. Because Rtt109-Asf1 acetylation of soluble H3 does not affect *ARG1* repression, we turned our attention to the possibility that Rtt109 and Asf1 control *ARG1* promoter activity as components of chromatin. We focused on Rtt109, because Asf1 occupancy of *ARG1* (Fig. S1) is likely to reflect global, nonspecific association with chromatin (12, 29). Rtt109 can be cross-linked to the upstream activating region, promoter, and coding region of *ARG1* under arginine-replete conditions (Fig. 4*A*). Importantly, Rtt109 promoter occupancy is (*i*) higher under repression than under induction (Fig. 4*B*; differential enrichment was not observed in the ORF—Fig. S2), and (*ii*) dependent under repression on sequence-specific transcription factors that modulate the strength of *ARG1* repression (Fig. 4*C Left*), namely, the leucine zipper protein Gcn4 and the zinc finger protein Arg81, which is assembled specifically on the *ARG1* promoter when arginine is not limiting (30). Deletion of *GCN4* and *ARG81* has little effect on Rtt109 occupancy of *POL1*, a gene not known to be regulated by arginine (Fig. 4*C*, *Right*). These relationships suggest that *ARG1* repression depends on promoter-targeted Rtt109 and perhaps Rtt109 regulation of a nonhistone protein directly involved in chromatin metabolism at the *ARG1* promoter.

To test if *ARG1* repression involves protein acetylation by Rtt109, transcription was compared in *rtt109*Δ cells expressing wild-type or catalytically inactive Rtt109 from a low-copy vector (28) (Fig. 4*D*). In repressing medium M2D, *ARG1* induction associated with deletion of *RTT109* is suppressed by wild-type but not catalytically dead rtt109^{DD287288AA}. Therefore protein acetylation by Rtt109 is important for repression of *ARG1*. Collectively, our results suggest that *ARG1* repression depends on KAT-dependent regulation of a nonhistone protein by chromatin-associated Rtt109.

Inhibition of transcription by Rtt109 and Asf1 is not restricted to *ARG1*; these proteins also inhibit transcriptional activation of stress response genes (31). There are however important differences between *ARG1* and the stress response genes in their regulation by Rtt109 and Asf1. First, Rtt109 and Asf1 control the steady-state set point of *ARG1* transcription under repressing conditions, but do not influence this phenotype of the stress response genes. Second, the *rtt109*Δ and *asf1*Δ mutations have the same effect on activation of the stress response genes, but significantly different effects on *ARG1* repression (Fig. 2 *A* and *E*). Third, Asf1 controls H3 dynamics at stress-induced genes but not the promoter of *ARG1* (Fig. 1*B*). The notion that *ARG1* and the stress response genes differ in their regulation by Rtt109 and Asf1 was confirmed by a genetic interaction experiment. In this experiment, mRNA expression was measured in *rtt109*Δ *asf1*Δ cells and the corresponding single mutants (Fig. 4*E*).

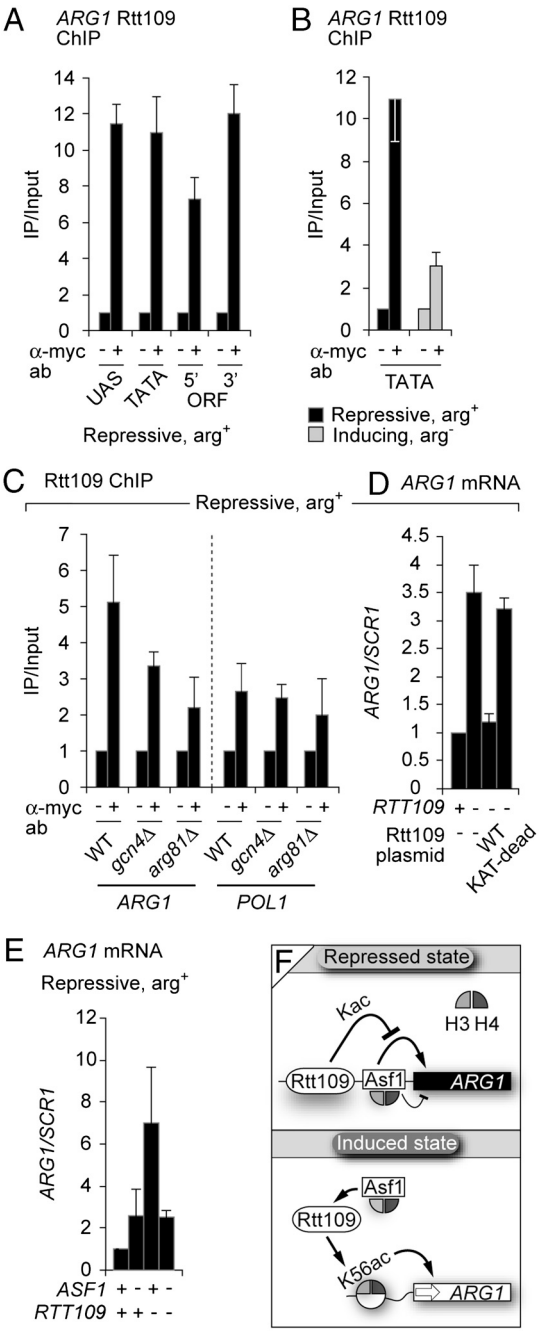

Fig. 4. Interplay between Rtt109 and Asf1 in the repression of *ARG1*. (*A*) ChIP analysis of Rtt109 cross-linking at the *ARG1* locus under repressing conditions. The occupancy measurement obtained in no-antibody control ChIPs is set to one. Average of two experiments; the error bar shows the range. (*B*) ChIP analysis of Rtt109 cross-linking to the promoter of *ARG1* under repressing and inducing conditions. Analysis as in *A*. (*C*) Effect of *GCN4* and *ARG81* deletion on Rtt109 cross-linking to the promoter of *ARG1*, and coding region of *POL1*, under repressing conditions. Analysis as in *A*; results are for three independent experiments. (*D*) Rtt109 KAT activity is required for *ARG1* repression. The averages are for three (*RTT109* + and – at left; both strains harbor the empty vector) or two ("WT" and "KAT-dead") experiments; the error bars respectively show the standard error and range. (*E*) Effect of *RTT109* and *ASF1* deletion (alone and in combination) on *ARG1* transcription under repressive conditions. (*F*) Model of *ARG1* regulation by Rtt109 and Asf1. See text for details.

The individual mutations have identical and nonadditive effects on activation of the stress response genes (31). The same mutations have strikingly different effects on *ARG1*. First, *rtt109*Δ

more strongly affects *ARG1* repression than *asf1*Δ (Fig. 4*E*; also compare Fig. 2 *A* and *E*). Second, deletion of *ASF1* suppresses transcription induction associated with deletion of *RTT109*, such that *ARG1* expression in the double mutant is identical to expression in *asf1*Δ (Fig. 4*E*). Based on these results, we suggest that Rtt109 and Asf1 are components of a unique system that represses *ARG1* independently of mechanisms that control H3 acetylation. Below we refer to this system of regulation using Asf1 not H3Kac yes Rtt109 (ANKYR).

The most important contribution of this work is the description of previously undiscovered modes of functional interplay between Rtt109 and Asf1 in a system which contributes to *ARG1* regulation under repressing conditions. We propose the following working model to explain the architecture of this ANKYR system (Fig. 4*F*; Fig. S3 explains this model in the context of the transcription phenotypes of the mutants examined). As originally suggested by Tyler and coworkers, we envisage that a population of Asf1 molecules interacts nonspecifically with chromatin throughout the genome (ref. 29; see also ref. 12). This nonspecific interaction accounts for the presence of Asf1 at *ARG1* (Fig. S1). When associated with chromatin, Asf1 can potentially reconfigure nucleosomes by one of two mechanisms—one that favors transcription, and one that disfavors transcription (22). We suggest that the pathway of transcription stimulation by *ARG1*-associated Asf1 is intrinsically more potent than the Asf1-dependent pathway of transcription inhibition. The notion that alternative pathways of promoter regulation by Asf1 can have different strengths is well established for *PHO5* (13). In our model, deletion of *RTT109* has a stronger inducing effect than deletion of *ASF1* because, in wild-type cells, Rtt109 inhibits the positive effect of Asf1 on *ARG1* transcription. The latter regulation is likely to occur on chromatin, because (*i*) both proteins occupy the promoter of *ARG1*, (*ii*) Rtt109 occupancy is higher under repressing conditions than under inducing conditions (Fig. 4*B*), and (*iii*) *ARG1* regulation does not involve H3 K56 acetylation (a reaction that Rtt109 can only perform on soluble H3) (32). Because Rtt109 is present in the promoters of numerous genes (12, 13, 33), and Asf1 is a promiscuous chromatin-binding protein, it is possible that the ANKYR system operates at multiple loci throughout the genome.

In our model of the ANKYR system, under repressing conditions Rtt109 KAT activity dampens transcriptional stimulation by Asf1. How protein acetylation by Rtt109 (Fig. 4*D*) might control this putative Asf1-dependent stimulation of *ARG1* remains a matter of speculation. There are numerous precedents for cellular regulation of nonhistone targets by KATs that also modify histones (34, 35). Although acetylation of cellular Asf1 has not been reported, Rtt109 has weak KAT activity toward Asf1 in vitro (1) and a complex containing Rtt109 and Asf1 can be recovered from yeast cells after chemical cross-linking (36). Perhaps then Rtt109 acetylation directly modulates Asf1 activity. Alternatively, autoacetylation of Rtt109 (37, 38) could affect the function of Asf1. For example, Asf1 stimulation of transcription could be inhibited when Asf1 comes into contact with autoacetylated Rtt109. Finally, Rtt109 might regulate another nonhistone protein involved in Asf1-dependent stimulation of *ARG1*.

Adkins et al. (29) have proposed that the Asf1 associated with repressed promoters is not able to drive chromatin toward a configuration that is permissive for transcription. How is Asf1 prevented from doing so when it is fully capable of promoting chromatin opening in the context of transcription elongation? This problem remains unsolved. For example, whether the positive pathway of chromatin reconfiguration that depends on Asf1 has a default state of low activity, or a default state of high activity that is restrained under some conditions, has not been studied. Our results suggest that Asf1 stimulation of transcription (at *ARG1*) is restrained by a mechanism that involves Rtt109. This finding raises the possibility that functional switching of Asf1 between states that close and open chromatin is under physiological control by a pathway that depends on Rtt109.

Materials and Methods

Yeast Strains and Culture. Yeast strains (Table S2) were obtained from published sources or constructed by methods outlined in Minard et al. (12). The standard repressing medium was yeast extract, bactopeptone, dextrose (YPD). The standard inducing medium (M1D) was based on yeast nitrogen base without amino acids. Strains harboring centromere-based plasmids were cultured in a similar repressing medium with arginine (M2D). Formulations are given in Table S1. Cells were arrested in G2/M with 10 μg/mL nocodazole (Sigma) for 2–4 h.

Cell Cycle Profiling. Cellular DNA content was measured by flow cytometry (39)

Chromatin Immunoprecipitation. ChIP was performed as published (12, 40) using antibodies against the indicated proteins or epitopes. Preferential recognition of H3 K9ac by the antibody used for the experiment in Fig. 3*B* has been reported elsewhere (41). Primers for PCR and antibody suppliers are listed in Tables S3 and S4, respectively. Quantification of PCR data has been described elsewhere (12, 39).

RNA Analysis. Total RNA isolation and quantification by real-time RT-PCR were performed as described previously (12). Primers including loading controls (*SCR1* and *ACT1*) are listed in Table S5.

Except where indicated, all graphs show the average result of at least three independent experiments and the error bar represents the standard error.

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1. Driscoll R, Hudson A, Jackson SP (2007) Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. *Science* 315:649–652.
2. Collins SR, et al. (2007) Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. *Nature* 446:806–810.
3. Burgess RJ, Zhou H, Han J, Zhang Z (2010) A role for Gcn5 in replication-coupled nucleosome assembly. *Mol Cell* 37:469–480.
4. Fillingham J, et al. (2008) Chaperone control of the activity and specificity of the histone H3 acetyltransferase Rtt109. *Mol Cell Biol* 28:4342–4353.
5. Dubois E, Bercy J, Messenguy F (1987) Characterization of two genes, ARGRI and ARGRIII required for specific regulation of arginine metabolism in yeast. *Mol Gen Genet* 207:142–148.
6. Messenguy F, Dubois E (1993) Genetic evidence for a role for MCM1 in the regulation of arginine metabolism in Saccharomyces cerevisiae. *Mol Cell Biol* 13:2586–2592.
7. Qui HF, Dubois E, Messenguy F (1991) Dissection of the bifunctional ARGRII protein involved in the regulation of arginine anabolic and catabolic pathways. *Mol Cell Biol* 11:2169–2179.
8. Crabeel M, et al. (1995) Further definition of the sequence and position requirements of the arginine control element that mediates repression and induction by arginine in Saccharomyces cerevisiae. *Yeast* 11:1367–1380.
9. Natarajan K, et al. (2001) Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. *Mol Cell Biol* 21:4347–4368.
10. Ricci AR, Genereaux J, Brandl CJ (2002) Components of the SAGA histone acetyltransferase complex are required for repressed transcription of ARG1 in rich medium. *Mol Cell Biol* 22:4033–4042.
11. Ginsburg DS, Govind CK, Hinnebusch AG (2009) NuA4 lysine acetyltransferase Esa1 is targeted to coding regions and stimulates transcription elongation with Gcn5. *Mol Cell Biol* 29:6473–6487.
12. Minard LV, Williams JS, Walker AC, Schultz MC (2011) Transcriptional regulation by Asf1: New mechanistic insights from studies of the DNA damage response to replication stress. *J Biol Chem* 286:7082–7092.
13. Williams SK, Truong D, Tyler JK (2008) Acetylation in the globular core of histone H3 on lysine-56 promotes chromatin disassembly during transcriptional activation. *Proc Natl Acad Sci USA* 105:9000–9005.
14. Rufiange A, Jacques PE, Bhat W, Robert F, Nourani A (2007) Genome-wide replication-independent histone H3 exchange occurs predominantly at promoters and implicates H3 K56 acetylation and Asf1. *Mol Cell* 27:393–405.

15. Xu F, Zhang K, Grunstein M (2005) Acetylation in histone H3 globular domain regulates gene expression in yeast. *Cell* 121:375–385.
16. Mousson F, et al. (2005) Structural basis for the interaction of Asf1 with histone H3 and its functional implications. *Proc Natl Acad Sci USA* 102:5975–5980.
17. Recht J, et al. (2006) Histone chaperone Asf1 is required for histone H3 lysine 56 acetylation, a modification associated with S phase in mitosis and meiosis. *Proc Natl Acad Sci USA* 103:6988–6993.
18. Gasch AP, et al. (2001) Genomic expression responses to DNA-damaging agents and the regulatory role of the yeast ATR homolog Mec1p. *Mol Biol Cell* 12:2987–3003.
19. Milgrom E, Diab H, Middleton F, Kane PM (2007) Loss of vacuolar proton-translocating ATPase activity in yeast results in chronic oxidative stress. *J Biol Chem* 282:7125–7136.
20. Hillenmeyer ME, et al. (2008) The chemical genomic portrait of yeast: Uncovering a phenotype for all genes. *Science* 320:362–365.
21. Spellman PT, et al. (1998) Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization. *Mol Biol Cell* 9:3273–3297.
22. Schwabish MA, Struhl K (2006) Asf1 mediates histone eviction and deposition during elongation by RNA polymerase II. *Mol Cell* 22:415–422.
23. Sudarsanam P, Iyer VR, Brown PO, Winston F (2000) Whole-genome expression analysis of snf/swi mutants of Saccharomyces cerevisiae. *Proc Natl Acad Sci USA* 97:3364–3369.
24. Lopes da Rosa J, Boyartchuk VL, Zhu LJ, Kaufman PD (2011) Histone acetyltransferase Rtt109 is required for Candida albicans pathogenesis. *Proc Natl Acad Sci USA* 107:1594–1599.
25. Tsubota T, et al. (2007) Histone H3-K56 acetylation is catalyzed by histone chaperone-dependent complexes. *Mol Cell* 25:703–712.
26. Han J, Zhou H, Li Z, Xu RM, Zhang Z (2007) The Rtt109-Vps75 histone acetyltransferase complex acetylates non-nucleosomal histone H3. *J Biol Chem* 282:14158–14164.
27. Selth LA, et al. (2009) An Rtt109-independent role for Vps75 in transcription-associated nucleosome dynamics. *Mol Cell Biol* 29:4220–4234.
28. Han J, et al. (2007) Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. *Science* 315:653–655.
29. Adkins MW, Williams SK, Linger J, Tyler JK (2007) Chromatin disassembly from the PHO5 promoter is essential for the recruitment of the general transcription machinery and coactivators. *Mol Cell Biol* 27:6372–6382.
30. Yoon S, et al. (2004) Recruitment of the ArgR/Mcm1p repressor is stimulated by the activator Gcn4p: A self-checking activation mechanism. *Proc Natl Acad Sci USA* 101:11713–11718.
31. Klopf E, et al. (2009) Cooperation between the INO80 complex and histone chaperones determines adaptation of stress gene transcription in the yeast Saccharomyces cerevisiae. *Mol Cell Biol* 29:4994–5007.
32. Rocha W, Verreault A (2008) Clothing up DNA for all seasons: Histone chaperones and nucleosome assembly pathways. *FEBS Lett* 582:1938–1949.
33. Schneider J, Bajwa P, Johnson FC, Bhaumik SR, Shilatifard A (2006) Rtt109 is required for proper H3K56 acetylation: A chromatin mark associated with the elongating RNA polymerase II. *J Biol Chem* 281:37270–37274.
34. Lin YY, et al. (2009) Protein acetylation microarray reveals that NuA4 controls key metabolic target regulating gluconeogenesis. *Cell* 136:1073–1084.
35. Gu W, Roeder RG (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* 90:595–606.
36. Han J, Zhou H, Li Z, Xu RM, Zhang Z (2007) Acetylation of lysine 56 of histone H3 catalyzed by RTT109 and regulated by ASF1 is required for replisome integrity. *J Biol Chem* 282:28587–28596.
37. Tang Y, et al. (2008) Fungal Rtt109 histone acetyltransferase is an unexpected structural homolog of metazoan p300/CBP. *Nat Struct Mol Biol* 15:738–745.
38. Albaugh BN, Arnold KM, Lee S, Denu JM (2011) Autoacetylation of the histone acetyltransferase Rtt109. *J Biol Chem* 286:24694–24701.
39. Lin LJ, Minard LV, Johnston GC, Singer RA, Schultz MC (2010) Asf1 can promote trimethylation of H3 K36 by Set2. *Mol Cell Biol* 30:1116–1129.
40. Geisberg JV, Struhl K (2005) Analysis of protein co-occupancy by quantitative sequential chromatin immunoprecipitation. *Curr Protoc Mol Biol* Chap 21:21.8.1–21.8.7.
41. Edmondson DG, et al. (2002) Site-specific loss of acetylation upon phosphorylation of histone H3. *J Biol Chem* 277:29496–29502.