Recruitment of Gcn5-containing Complexes during c-Myc-dependent Gene Activation

STRUCTURE AND FUNCTION ASPECTS

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The N-terminal domain of c-Myc plays a key role in cellular transformation and is involved in both activation and repression of target genes as well as in modulated proteolysis of c-Myc via the proteasome. Given this functional complexity, it has been difficult to clarify the structures within the N terminus that contribute to these different processes as well as the mechanisms by which they function. We have used a simplified yeast model system to identify the primary determinants within the N terminus for (i) chromatin remodeling of a promoter, (ii) gene activation from a chromatin template in vivo, and (iii) interaction with highly purified Gcn5 complexes as well as other chromatin-remodeling complexes in vitro. The results identify two regions that contain autonomous chromatin opening and gene activation activity, but both regions are required for efficient interaction with chromatin-remodeling complexes in vitro. The conserved Myc boxes do not play a direct role in gene activation, and Myc box II is not generally required for in vitro interactions with remodeling complexes. The yeast SAGA complex, which is orthologous to the human GCN5-TRRAP complex that interacts with Myc in human cells, plays a role in Myc-mediated chromatin opening at the promoter but may also be involved in later steps of gene activation.

The c-Myc protein (Fig. 1) is a regulator of several important cellular processes. These include the control of normal cell growth, differentiation, and apoptosis. Elevated levels of c-Myc have been shown to play a role in tumorigenesis, probably through the altered control of genes required for cellular proliferation (1, 2). c-Myc is a member of a family of transcription factors characterized by leucine zipper and basic helix-loop-helix dimerization and DNA binding domains (3–6). c-Myc heterodimerizes with Max, another leucine zipper-basic helix-loop-helix protein. The Myc-Max heterodimer is a high affinity DNA binding complex, which binds to E boxes within promoters/enhancers and is a potent activator of gene expression and cellular transformation. Many studies have identified genes that are activated by c-Myc. These include genes encoding proteins required for cell cycle, growth, and apoptosis as well as proteins involved in stress response, amino acid transport, and cell adhesion (reviewed in Ref. 7). c-Myc can also repress target genes, and indeed c-MycS, which lacks most of the activation domain, is still able to repress genes and participate in cellular transformation (8).

Kato et al. (9) showed that the N-terminal 143 amino acids of c-Myc constitute the activation domain and play an important role in cellular transformation. However, the c-Myc activation domain appears to be a complex structure, since it mediates several other functions in addition to gene activation. Li et al. (10) showed that sequences within the N-terminal 143 amino acids are also involved in the transcriptional repression activity of c-Myc. Furthermore, the conserved Myc boxes within the activation domain have been implicated in targeted proteolysis of the Myc protein via the proteasome (11–14). The Myc boxes also play a key role in modulating c-Myc activity in response to cellular signaling systems. Consistent with this functional complexity, the N-terminal domain of c-Myc has been reported to interact with several other proteins, such as TATA-binding protein (15), p107 (16, 17), a-tubulin (18), Pam (19), MM-1 (20), Bin1 (21), AMY-1 (22), TRRAP (23), and Gcn5 (24). Binding to TATA-binding protein has been shown to be important for gene activation by c-Myc in vitro (25).

Interestingly, the TRRAP and Gcn5 proteins have been identified in mammalian protein complexes that display histone acetyltransferase (HAT) activity (26–28). Deletion of Myc box II causes loss of both interaction with TRRAP/Gcn5 and of cellular transformation potential (23, 24). These observations have led to an attractive model in which Gcn5 complexes are recruited to target promoters and help to remodel the promoter chromatin structure into an active form. Others have reported that Mad-Max heterodimers, which also bind to E boxes, recruit histone deacetylase complexes, leading to deacetylation of...

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Fig. 1. Diagram locating the activation domain within the c-Myc protein. The evolutionarily conserved Myc boxes I and II (MB-I and MB-II) are shaded. MB-I is an important hot spot for cancer-related mutations.

promoters and gene repression (29). Thus, depending on the balance of E box occupancy between Myc-Max and Mad-Max, target genes will either be activated or repressed. The key role of Myc box II in this model has been extended by the observation that this region also plays a critical role in promoter recruitment of ATP-dependent chromatin-remodeling complexes by Myc (30). It has been shown that ATP-dependent complexes and histone acetyltransferases collaborate during gene activation (31).

Although this model remains attractive, there are two main problems with regard to the role of c-Myc. First, Myc box II, which has been suggested to be a key determinant for the interaction with Gen5-containing and ATP-dependent remodeling complexes and thus to play a key role in subsequent gene activation, has previously been associated with the gene repression activity of c-Myc (8, 10). Second, recent studies have shown that the acetylation status of some Myc-regulated promoters does not change in response to their activation by mitogen treatment (32). Therefore, since recruitment of histone acetyltransferase complexes is considered to be an important component of Myc-mediated gene activation, it is necessary to consider whether processes, other than promoter acetylation, might be modulated by recruited Gen5 complexes.

Gen5-containing complexes have been extensively purified from yeast, where their role in gene activation has been studied both in vitro and in vivo. Comparison with the mammalian complex shows that the yeast SAGA complex is highly homologous to the human complexes that have been described (33). Human c-Myc is an efficient activator of gene expression in yeast, and the N-terminal activation domain is active in yeast when fused to a heterologous DNA binding domain (34). Thus, biochemical and genetic approaches available for yeast offer the opportunity to study interaction of c-Myc with highly purified Gen5-containing complexes in vitro as well as the functional consequences of the interaction in vivo on chromatin templates in a relatively simple and well defined system. In this report, we define primary determinants within the c-Myc activation domain that are involved in chromatin remodeling, overall gene activation, and interaction with Gen5 complexes as well as other chromatin-remodeling complexes. Further, we suggest that Gen5 complexes and ATP-dependent remodeling complexes may effect transcriptional processes that lie downstream of the promoter chromatin remodeling step.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media

YS33 (MATa his3Δ11,15 leu2Δ2,3,112 ura3Δ5 Δpho80Δ HIS3 Δpho4Δ149I CanR) (35) was used for chromatin opening analysis. Pho80 is a high phosphate-dependent repressor of Pho4; by deleting it, the requirement for low phosphate conditions for Pho4 activity is avoided. YS33 is a derivative of YS33, with the GCN5 gene deleted. The Ada-SAGA complex components (ada1, ada2, and gen5) were deleted from the strain PSY316 (MATa ade2Δ101 ura3Δ5 leu2Δ2,3,112 his3ΔΔ00 ly2Δ) by insertion of the his5 gene. The Ada-specific component Ahi1 (36) was disrupted with Kan-MX from the strain BY4741 (MATa his3Δ1 leu2Δ2,3 met15Δ ura3Δ5 [Research Genetics]). FY60 (MATa his4–9178 ura3–52 leu2Δ1), FY295 (MATa his4–9178 ura3–52 leu2Δ1 lys2–173R2 sp3–202), and FY1291 (MATa ura3–52 leu2Δ1 lys2–173R2 trp1ΔΔ13 arg4–12 sp265ΔΔ000 ARG4) were used to investi-
of HaeIII in these assays. The intensity of hybridization to the PHO5 DNA fragments was measured using a phosphor imager (Fuji FLA-3000).

**Purification of HAT and SWI/SNF Complexes**

Yeast whole cell extracts and isolation of HAT complexes were performed as described by Grant et al. (48). Further purification of the SAGA, Ada, NuA4, and NuA3 complexes was as described by Grant et al. (49), except that the order of columns was modified. Each complex was purified over Ni²⁺-nitrilotriacetic acid-agarose (Qiagen), followed by MonoQ HR 5/5 (Amersham Biosciences), MonoS HR 5/5 (Amersham Biosciences), histone agarose (Sigma), and Superose 6 HR 10/30 (Amersham Biosciences) columns. The SWI/SNF complex, containing HA-tagged Snf2, was isolated from yeast extracts as described by Côté et al. (50).

**GST Pull-down and HAT Assays**

Each HAT complex was incubated in PDB (150 mM NaCl, 50 mM HEPES, pH 7.5, 10% glycerol, 0.1% Tween 20, 0.5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride) with the indicated GST fusion protein for 2 h at 4 °C while rotating on a wheel. The supernatant was removed, and beads were washed four times in PDB. For the HAT complex assays, equal fractions of both supernatants and beads were directly assayed for nucleosomal acetyltransferase activity as described previously (49). The SWI/SNF complex was detected by immunoblotting using anti-HA antibodies (51). Expression and purification of the GST-Myc143 fusion protein has been described previously (44).

**RESULTS**

**Mapping of Activation Domain Regions Involved in c-Myc-dependent Gene Activation**—To determine which regions of the c-Myc activation domain are involved in gene activation, a number of derivatives of the activation domain were fused to the oligomerization/DNA-binding domain of the yeast Pho4 protein as described in Ref. 12. The activity of the different fusion proteins was then measured by their ability to activate a lacZ reporter gene, which was fused to the Pho4-responsive PHO5 promoter. The experiments were performed in a yeast strain that lacks the PHO4 gene, and thus lacks the normal Pho4 protein. Fig. 2A shows that gene activation potential lies within the regions outside the conserved Myc boxes, namely residues 1–41 and 66–127. Both of these fragments have an autonomous activation potential, whereas, in contrast, the construct containing only the Myc boxes has no measurable activity. Furthermore, the reduced activity of the 94–127 fragment shows that full activity of 66–127 requires sequences spanning residue 94. All of the constructs needed to draw these conclusions are expressed at similarly high levels in yeast, while the remaining constructs, containing one or both Myc boxes, are expressed at much lower levels due to the role of the Myc boxes in targeting proteasome-mediated degradation (12). The results from these latter constructs further support the importance of the 1–41 and 66–127 fragments in gene activation, since there is little or no change in activation potential when either of the Myc boxes are deleted compared with constructs containing both.

The predominant activation potential lies in the 66–127 fragment. The importance of this fragment could be confirmed in the context of the intact c-Myc protein, which can bind to the Pho4 binding sites in the PHO5 upstream activating sequence in the context of a CYC1-lacZ reporter gene as a heterodimer with the Max protein (42, 52). As shown in Fig. 2A, the construct lacking this region has a 6-fold lower activation potential compared with the intact c-Myc protein. In contrast, deletion of one or both Myc boxes does not significantly reduce activation potential, either in the context of intact c-Myc or the derivative lacking residues 66–127.

We chose to use a reporter gene based on the PHO5 promoter because chromatin remodeling within this promoter during gene activation has been extensively studied (53). This is appropriate in the light of previous reports showing recruitment of chromatin remodeling activities by the c-Myc activation domain (23, 24, 30). The status of the PHO5 promoter can be monitored quantitatively by isolating chromatin and measuring the proportion of promoters that can be cleaved by ClaI. The ClaI site is occluded by a nucleosome in the repressed promoter but becomes available for cleavage in the remodeled promoter. Fig. 2B shows that the intact c-Myc activation domain is able to mediate opening of the PHO5 promoter and further that this occurs equally well using a nonexpressed PHO5 reporter gene from which the TATA box has been deleted. Thus, the observed remodeling is not an indirect consequence of reporter gene transcription. This assay was used to measure the chromatin remodeling potential of the different c-Myc fusion proteins described above. The results, shown in Fig. 2A, generally reflect the potential of the same constructs to activate the reporter gene. They further support the predominant role of residues 1–41 and 66–127 as well as the view that the Myc boxes do not appear to play a direct role in chromatin remodeling, at least at this promoter.

**Interaction of the c-Myc Activation Domain with Chromatin-remodeling Complexes**—It has been reported that the c-Myc activation domain interacts with Gcn5-containing histone acetyltransferase complexes in order to recruit them to Myc-activated target genes (23, 24). Since Gcn5 complexes are highly conserved between yeast and mammalian cells, we tested the activation potential of Pho4 fusion proteins containing the intact activation domain (1–149) as well as its isolated 1–41 and 66–127 deletion derivatives in ada2, ada3, and gcn5 mutant yeast strains that lack functional Gcn5 complexes (49). The activity of all constructs was severely reduced in the mutant strains, indicating that both the 1–41 and the 66–127 activation domain modules require Gcn5 complexes for full activity, irrespective of the presence or absence of the Myc boxes (Fig. 3A). In light of previous reports showing that Myc box II is required for interaction with Gcn5 containing complexes in mammalian cell-free extracts, we decided to investigate the interaction of purified Myc activation domain derivatives with purified Gcn5-containing complexes in vitro. In yeast, two main Gcn5-containing complexes have been identified, the SAGA complex and the Ada complex. c-Myc might be expected to interact with the SAGA complex, since this complex contains the Tra1 protein, the yeast homologue of the mammalian TRRAP protein that has been implicated in Gcn5 recruitment by Myc in mammalian cells (23). Fig. 3B shows that both complexes interact with the intact c-Myc activation domain in vitro. However, under the same conditions, interaction of the 66–127 fragment with the SAGA complex is greatly reduced, and interaction with the Ada complex is not detectable. This is consistent with the recent observation that a larger c-Myc fragment is also required for efficient interaction with the TATA-binding protein (44). Interestingly, Myc box II (residues 128–143) is required for interaction with the Ada complex but not the Tra1-containing SAGA complex. To permit interpretation of the specificity and significance of these results in a broader context, we also measured interactions with some other purified chromatin-remodeling complexes. The NuA3 complex has not previously been shown to interact with activator proteins, and it did not interact with the c-Myc activation domain in our experiments. The NuA4 complex is a histone H4 HAT, which also contains the Tra1 protein and can be recruited to promoters by several activators during gene activation (54, 55). NuA4 interacts with the c-Myc activation domain in a way similar to the SAGA complex. (Note that the NuA4 fraction used is contaminated with a histone H3 HAT activity but that NuA4 interaction can be unambiguously measured by its hist-
FIG. 2. Identification of determinants for gene activation and chromatin remodeling within the c-Myc activation domain. A, the gene activation and chromatin opening potency of deletion derivatives of the c-Myc activation domain are shown ± S.D. The values are shown relative to the activity of the 1–149P construct (upper panel) or the full-length construct (lower panel). The activation domain derivatives are fused to the DNA binding and oligomerization domains of the Pho4 protein (upper panel) or are assayed in the context of the full-length protein in cells expressing the Max protein (lower panel). The residues included in each construct are evident from the construct name, and each construct is also shown in diagram form. The shaded regions represent the conserved Myc boxes I and II (residues 42–65 and 128–149, respectively).

B, the c-Myc activation domain is needed for efficient chromatin remodeling of the PHO5 promoter, and remodeling is not dependent on transcription of the PHO5 gene. The diagram shows the repressed PHO5 promoter. The four positioned nucleosomes covering the promoter that are subject to remodeling are shown (gray circles). The small black and white circles represent binding sites for the Pho4 protein and the c-Myc/Max heterodimer. The arrow indicates a ClaI recognition site that is occluded in the repressed promoter but that is available for cleavage in chromatin from derepressed cells. The amount of cleavage by 120 and 400 units of ClaI shows the proportion of promoters that have been remodeled (open) in relation to those that have not been (closed). The amount of remodeling is shown for each construct ± S.D. (n = 4–6). The ΔTATA strain contains a PHO5 promoter from which the TATA box has been deleted, leading to a lack of measurable transcription from the promoter under derepressed conditions.
The SAGA Gcn5-containing complex is specifically important for Myc-mediated gene activation in vivo. Shown is the ability of the 1–149P Myc-Pho4 fusion protein to activate a PHO5-lacZ reporter gene in wild type and mutant yeast strains. The ada2, ada3, and gcn5 mutations cause defects in both the SAGA and Ada complexes. Defects resulting from the spt3 and spt20 mutants are specific for the SAGA complex. The ahc1 and snf6 mutations each cause specific defects in the Ada and SWI/SNF complexes, respectively.

Fig. 4 shows that mutations affecting the Spt3 and Spt20 proteins that are specific for the SAGA complex cause similar reductions in activity to the ada2, ada3, and gcn5 mutations that affect both complexes. Furthermore, deletion of the AHC1 gene, which causes defects that are specific for the Ada complex, has little effect on the activation potential of the c-Myc activation domain. If anything, activation is increased in this strain, consistent with recent studies of another activator in an ahc1 mutant strain (56). We thus conclude that the SAGA complex is the Gcn5-containing complex that is responsible for Myc-dependent gene activation, at least for this promoter. ATP-dependent chromatin-remodeling complexes have also been implicated in Myc-dependent gene activation (30). Since we observed a direct interaction of the c-Myc activation domain with the SWI/SNF complex (Fig. 3C), we used a yeast mutant strain defective in the Snf6 subunit of the SWI/SNF complex to determine whether ATP-dependent complexes might be important in our model system. Fig. 4 shows that the Snf6 protein is important for efficient gene activation by the c-Myc activation domain.

Requirement for the SAGA and SWI/SNF Complexes for Myc-mediated Remodeling of the PHO5 Promoter—We then looked to see whether the SAGA and SWI/SNF complexes were needed for c-Myc to remodel chromatin structure at the PHO5 promoter. Surprisingly, deletion of GCN5 caused only a mild reduction in chromatin remodeling (to ~80%), whereas deletion of the SNF2 encoding a subunit of SWI/SNF had no reproducible effect at all. Furthermore, a double mutant lacking both complexes (Δsnf2, Δada2) was not further reduced in PHO5 promoter remodeling compared with the single gen5 mutant (Fig. 5A). Thus, defects in chromatin remodeling of the PHO5 promoter can only account for part of the severe defects observed for c-Myc in mutants strains defective in Gcn5-containing complexes (Fig. 4).

The results in Fig. 4, showing that the SAGA and SWI/SNF complexes are required for gene activation, were performed using a plasmid-borne reporter gene, whereas Fig. 5A shows the behavior at the chromosomal PHO5 locus. Although previous analysis indicates that the PHO5 promoter behaves similarly in both contexts (41), a formal possibility is that there are differences in the dependence of chromosomal and episomal PHO5 promoters. To test this, we repeated the chromatin analysis on the episomal PHO5-lacZ reporter gene. The amount of opening measured for the plasmid-borne promoter was generally lower than for genomic PHO5, but comparison of the val-
FIG. 5. Effect of mutants defective in the SAGA and SWI/SNF complexes on c-Myc-mediated opening of the yeast PHO5 promoter. A, the ability of the 1–149P Myc-Pho4 fusion protein to remodel the genomic PHO5 promoter in wild type and mutant strains lacking the SAGA complex (gcn5), the SWI/SNF complex (snf2), and both complexes (snf2, ada2). Annotation is as for Fig. 2B. B, the ability of the 1–149P Myc-Pho4 fusion protein to remodel the plasmid-borne PHO5 promoter fused to the lacZ reporter gene in wild type, gcn5, and snf6 mutant strains. Annotation is as for Fig. 2B. C, the amount of acid phosphatase induced by the 1–149P Myc-Pho4 fusion protein from the PHO5 gene in wild type, gcn5, and snf6 strains.

ues for mutant and wild type strains showed the same trend as previously. The gcn5 mutant showed a greater reduction in opening (to ~30%), whereas no significant effect could be attributed to the SWI/SNF defect (Fig. 5B). Although the remodeling defect in the gcn5 strain was increased for the plasmid-borne reporter gene, we conclude that the magnitude of the remodeling defects in the mutant strains cannot account for the severe gene activation defects measured in Fig. 4.

However, a further formal possibility was that small defects in chromatin remodeling are sufficient to cause severe defects in gene activation. To test this, we measured the activation of the genomic PHO5 gene, encoding acid phosphatase, by measuring acid phosphatase activity. Relative to wild type strains, the levels of acid phosphatase activity were reduced to about 50% in the gcn5 strain, and they were not significantly reduced in the snf2 mutant (Fig. 5C). Thus, for the genomic PHO5 gene, there is a very good correlation in the SAGA and SWI/SNF requirement both for remodeling of the promoter chromatin structure and for activation of the gene. Putative roles for recruited remodeling complexes at post-promoter opening steps during activation of the PHO5-lacZ reporter gene are discussed below.

DISCUSSION

Architecture of the c-Myc Activation Domain—As discussed in the Introduction, the c-Myc activation domain (residues 1–143) is critical for the role of c-Myc in cellular transformation during the development of cancer. Mechanistically, the activation domain functions as both an activator and a repressor of target gene expression in addition to its role in regulating the stability of the c-Myc protein itself. To understand how Myc participates in cellular transformation at the molecular level, it is necessary to characterize the contribution of regions within the activation domain to these different molecular functions. Our results in yeast clearly map the primary determinants for chromatin remodeling and gene activation to residues 1–41 and 66–127 of the c-Myc activation domain. These regions correspond to parts of the activation domain that are poorly conserved in different members of the Myc protein family. The Myc boxes that are conserved between Myc family members and which have been implicated in gene repression and proteolytic targeting of c-Myc did not have any measurable direct effect on chromatin remodeling or gene activation potential. These results are consistent with previous observations showing that the naturally occurring c-MycS form of c-Myc, which lacks most of the sequences N-terminal of Myc box II, is severely reduced in its activation potential (8).

It is more difficult to reconcile our results with previous studies showing that Myc box II plays a key role in promoter recruitment of Gcn5-containing histone acetyltransferase complexes that are normally associated with chromatin remodeling and gene activation (24). In these studies, Myc box II is clearly important for co-immunoprecipitation of c-Myc together with Gcn5 or its associated protein, TRRAP, from lysates prepared from 293 cells. To clarify the primary determinants required for protein interaction, we studied purified recombinant derivatives of the c-Myc activation domain and highly purified preparations of Gcn5-containing complexes from yeast in vitro. The results clearly show that Myc box II is not required for efficient interaction with the SAGA complex, which is the yeast homologue of the mammalian Gcn5 complex that is thought to interact with c-Myc. The SAGA complex is also the Gcn5 complex that functions in association with c-Myc in yeast (Fig. 4). Myc box II is, however, important for interaction with the yeast Ada complex. To our knowledge, this is the first report of an activator interacting with the Ada complex, although it is unclear whether the interaction is of functional significance in vivo (Fig. 4). Nonetheless, this observation indicates that Myc box II can play an important role in direct protein interactions in some cases. However, our observation that neither the NuA4 nor the SWI/SNF complex require Myc box II for efficient interaction argues that Myc box II is not generally a key part of the interaction surface. Given the previous characterization of Myc box II as a repression domain (10), an attractive possibility is that Myc box II could be a modulator domain that helps to determine whether c-Myc adopts a repressing or an activating conformation. Interestingly, a possible role of Myc box II in protein folding has been suggested previously (57). Such a model would suggest that in mammalian cell extracts the primary interaction surface of Myc box II-deleted Myc could be locked into a nonactive conformation as a result of associated proteins and/or post-translational modifications. Such a model has clear parallels to Myc box I function, which is regulated by phosphorylation (58–61), and several proteins have been identified that bind to Myc fragments containing Myc box II (57). This model is also consistent with previous reports suggesting that Myc box II is a key region for activation of c-Myc’s activation potential by Ras signaling pathways (62).

We have shown that the c-Myc activation domain interacts with four well characterized chromatin-remodeling complexes from yeast. Interestingly, Myc has been shown to interact with subunits from mammalian SWI/SNF complexes previously via...
its C-terminal DNA-binding/dimerization domain (63). Our observation that the activation domain interacts directly with the SWI/SNF complex combined with previous reports that it makes interactions with mammalian ATP-dependent remodeling complexes (30) suggests that Myc may make multiple interactions, at least with the ATP-dependent class of chromatin-remodeling complexes. The association of c-Myc with Gen5 complexes was originally discovered by co-immunoprecipitation of c-Myc with the TRRAP protein. The highly conserved yeast homologue of TRRAP, Tra1, is found in both the SAGA complex and the NuA4 complex. Our observation that c-Myc interacts efficiently with the NuA4, which is otherwise thought to be structurally unrelated to the SAGA complex, is thus of interest. A potential mammalian equivalent of the NuA4 complex becomes associated with RNA polymerase II first during transcriptional elongation through transcriptional pause sites (77). The same function has recently been reported for the highly conserved Elongator histone acetyltransferase complex (75). Further, Myc target genes including the ornithine decarboxylase gene and the myc gene itself, have been shown to be regulated at the level of transcriptional elongation (78–82). Finally, the transcriptional attenuation of myc gene elongation seen in normal cells is abrogated in Burkitt’s lymphoma cells, clearly implicating regulation of elongation as an important component in the ontogeny of cancer (83).

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**Recruitment of Gen5-containing Complexes by c-Myc**

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Defects in promoter opening do not provide a complete explanation for the role of Gen5-containing complexes and SWI/SNF complexes in activation of the PHO5–lacZ reporter gene. Activation of this gene is reduced 50-fold in gen5 strains, whereas chromatin opening was only reduced 3-fold. Defects in the SWI/SNF complex caused a reduction of over 5-fold in gene activation, but we were unable to show any reproducible reduction in chromatin opening at the promoter. Our results show that the different requirements for activation of endogenous PHO5 and PHO5–lacZ are associated with the lacZ coding sequence. Thus, for the PHO5–lacZ gene, both Gen5 complexes and SWI/SNF complexes appear to play a role in activation subsequent to chromatin opening of the promoter. Interestingly, it has been reported previously that in yeast, transcriptional elongation of lacZ reporter genes as well as some endogenous yeast genes is inefficient (72–74). These studies conclude that transcriptional elongation of long and/or GC-rich genes is inefficient and specifically dependent on a protein complex containing the Hpr1, Tho2, Mf1, and Thp2 proteins. Our results suggest that the SAGA and SWI/SNF complexes may also play a direct role in the transcriptional elongation of a subset of genes, including lacZ. Consistent with this, a recent report has provided genetic evidence for coupling between the SAGA complex and transcriptional elongation (75). That report shows that mutations causing defects in the histone acetyltransferase activity of the Elongator complex show synthetic lethality with mutations defective in the SAGA complex. Since the Elongator complex becomes associated with RNA polymerase II first during elongation, this provides strong evidence for involvement of the SAGA complex in the transcriptional elongation of some important yeast mRNAs in vivo.

Our results suggest that whereas promoter opening by chromatin-modifying and -remodeling factors recruited by c-Myc may represent their main role during activation of some genes, they may also contribute at later steps during the activation of other genes. Consistent with this, the histone acetylation status in the promoters of some Myc target genes does not change upon mitogen stimulation, leading to the conclusion that recruited HATs must function later in gene activation (32). Whereas direct support for such a role will require extensive further study, there is already substantial supportive evidence in the literature. For example, artificial activator proteins have been shown to work at the elongation level previously (76), and human SWI/SNF complex has been implicated in efficient elongation through transcriptional pause sites in vitro (77). The same function has recently been reported for the highly conserved Elongator histone acetyltransferase complex (75). Furthermore, Myc target genes including the ornithine decarboxylase gene and the myc gene itself, have been shown to be regulated at the level of transcriptional elongation (78–82). Finally, the transcriptional attenuation of myc gene elongation seen in normal cells is abrogated in Burkitt’s lymphoma cells, clearly implicating regulation of elongation as an important component in the ontogeny of cancer (83).
Recruitment of Gcn5-containing Complexes by c-Myc

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