DISSERTATION

NEW FUNCTIONS OF THE SAGA COMPLEX IN REGULATION OF TRANSCRIPTION BY RNA POLYMERASE II

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY XU CHEN ENTITLED NEW FUNCTION OF THE SAGA COMPLEX IN REGULATION OF TRANSCRIPTION BY RNA POLYMERASE II BE ACCEPTED AS FULLFILING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

NEW FUNCTIONS OF SAGA COMPLEX IN REGULATION OF TRANSCRIPTION BY RNA POLYMERASE II

The yeast SAGA (Spt-Ada-Gcn5-acetyltransferase) complex plays a role in Gal4-mediated transcriptional activation via delivery of TATA-binding protein (TBP) to Gal4-responsive promoters. Little is known about the impact of the sequence of the TATA element in the core promoter in this process. To investigate the SAGA complex regulatory function at different TATA element sequences, we compared a consensus element (TATA) to an off-consensus element (CATA) in the kinetics of Gal4-dependent gene activation, PIC occupancy, the requirement of SAGA components, and the histone acetylation state. We have found a new function of SAGA carried by subunits Gcn5, Ada2 and Spt8: TATA-element-censoring. This function enhances transcription driven by the consensus TATA element and represses transcription driven by off-consensus TATA elements. This functions works at both synthetic promoters and the endogenous *GAL* promoters. Via a genetic screen, Swi/Snf and RSC complexes were also identified with TATA-censoring function. Our study suggests that the new function involves TBP delivery, histone acetylation and histone eviction.

As an important part of transcription, histone eviction involves histone modification, chromatin remodeling and interaction with histone chaperones. We investigated the function of HATs and histone chaperones in *GAL* gene induction. We found the impacts of deletion of histone chaperones to the transcription of

endogenous *GAL* genes. Also deletions of Nap1 and Asf1 show different change to induction kinetics of *GAL1* and *GAL7* transcription, which suggests the different functions of these two chaperones and indicates that the histone chaperones at the promoters can affect transcription directly. Our ChIP data on recruitment and post-recruitment promoters show Nap1 occupancy is not related to transcription levels or TBP/RNAPII occupancy. However, Nap1 occupancy has same pattern as SAGA occupancy and the opposite pattern of histone occupancy, suggesting interaction between Nap1, SAGA and histone eviction during galactose induction.

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Chapter 1

Introduction

1.1 Classic recruitment-regulated promoter by RNAPII

Many transcription factors are involved in the expression of protein encoding genes by RNA polymerase II (RNAPII). TATA-binding protein (TBP) with TATA-associating proteins (TAFs) recognizes TATA-element at the promoter (Green, 2000). Other general transcription factors including TFIIA, TFIIB, TFIIE, TFIIF, and TFIIH are then recruited to the promoter and help recruit and stabilize RNAPII and form the pre-initiation complex (PIC) (Hampsey, 1998; Kornberg, 2001; Kuras and Struhl, 1999; Lee and Young, 2000). The formation of PIC at recruitment-regulated promoters is important for gene regulation (Colgan and Manley, 1992; Lee and Young, 2000). This step is the target of many gene specific regulatory factors such as activators, repressors, co-activators, mediators and chromatin remodeling complexes (Burley and Roeder, 1996; Starr and Hawley, 1991). Regulatory complexes work together to regulate the transcriptional efficiency of specific genes. The majority of the known genes transcribed by RNAPII are the recruitment-regulated promoters.

1.2 Activator Gal4 –regulated gene activation

One of the best-characterized yeast recruitment-regulated promoters is *GAL1,10* promoter. *GAL1* and *GAL10* are located next to each other on Chromosome II; have opposite transcription direction. They share one upstream activating sequence (UAS), which contain four binding sites for the

activator Gal4 (Johnston and Davis, 1984; Lohr and Hopper, 1985; West et al., 1984). *GAL* genes are repressed in the presence of glucose. Even though the activator Gal4 binds to UAS via its DNA-binding domain, Gal4 activation domain is blocked by the repressor Gal80 (figure 1.1.a). When the cells are grown under the inducing conditions (in which galactose is the only carbon source), the inducer Gal3 is activated and changes the interaction between Gal4 and Gal80 (figure 1.1.b). Gal80 no longer blocks the activation domain of Gal4 (Sil et al., 1999; Yano and Fukasawa, 1997). Thus Gal4 can recruit the co-activator complex SAGA to the promoter. The regulatory function of SAGA in transcription of yeast *GAL* genes has been well studied (Bhaumik and Green, 2001; Bhaumik and Green, 2002; Bhaumik et al., 2004; Dudley et al., 1999; Larschan and Winston, 2001; Roberts and Winston, 1997; Sterner et al., 1999b). Gal4 recruits SAGA to UAS via the interaction between its activation domain and the essential SAGA subunit Tra1 (Bhaumik et al., 2004). The SAGA subunit Gcn5, which carries histone acetyltransferase (HAT) activity, adds acetyl groups to the N-terminal tail of histone H3 at K9 and K14 (Grant et al., 1999; Kuo et al., 2000). The deubiquitylation activity of SAGA removes the ubiquitin from histone H2B K123 (Daniel et al., 2004; Henry et al., 2003; Ingvarsdottir et al., 2005; Lee et al., 2005b; Powell et al., 2004; Shukla et al., 2006b). Both of these histone modifications are important for transcription regulation (Berger, 2007). TBP associates with SAGA via the interaction with Spt8 and Spt3, gets recruited to the core promoter and starts the formation of preinitiation complex (Belotserkovskaya et al., 2000b; Bhaumik and Green, 2001; Bhaumik et al., 2004; Dudley et al., 1999; Larschan and Winston, 2001; Qiu et al., 2005). The modified nucleosomal histones are evicted from the

promoter DNA with the help of chromatin remodeling factors and histone chaperons. After TBP is recruited to the promoter, other general transcription factors and RNAPII are recruited, form the initiation complex and start transcription.

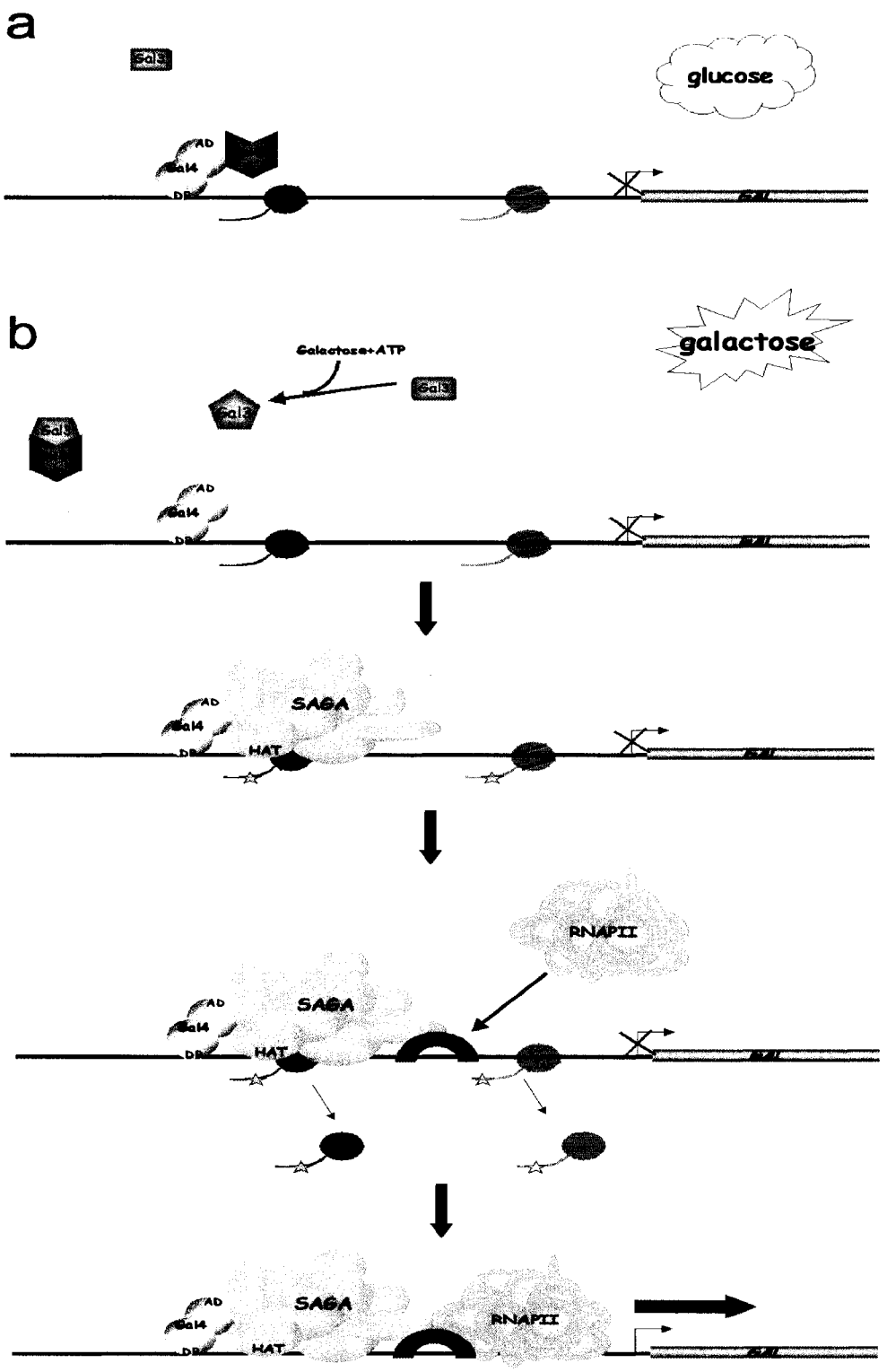


Figure 1.1 model of GAL gene galactose induction. (a) in the glucose condition, GAL genes are repressed. Gal4 bind to UAS with blocked activation domain. (b) in galactose condition, GAL genes are induced with SAGA, TBP and RNAPII recruited (context in 1.2)

1.3 The composition and functions of SAGA complex

1.3.1 The composition of SAGA complex

Yeast SAGA complex is a 1.8MDa multi-functional regulatory complex (Grant et al., 1997; Hampsey, 1997). There are a total of 20 subunits found in SAGA (Table1.1). Essential subunits, which include TAFs (TAF5, TAF6, TAF9, TAF10 and TAF12) and Tra1; nonessential subunits, include Spt proteins (Spt3, Spt7, Spt8 and Spt20); Ada proteins (Ada1, Ada2, Ada3, and Gcn5/Ada4); Sgf proteins (Sgf11, Sgf29 and Sgf73) and other proteins (Ubp8 and Chd1); and Sus1, of which essentiality is unknown. Some of SAGA subunits exist not only in SAGA, but also in other complexes (Table 1).

There are two complexes that are related to SAGA, the SLIK (SAGA-like) and ADA complexes. SLIK shares 15 subunits with SAGA. SLIK has a SLIK-specific subunit Rtg2 (Pray-Grant et al., 2002), a C-terminal truncated Spt7 (Sterner et al., 2002a), but does not contain Spt8, Sgf11 Sgf29 or Sus1. ADA complex contains only 5 subunits, Gcn5, Ada1, Ada2, Ada3, which are shared with SAGA and SLIK, and an ADA unique factor Ahc1 that is crucial for ADA complex integrity (Eberharter et al., 1999). Little is known about the unique functions of SLIK and ADA. Evidence suggests that SLIK is involved in the general amino acid control pathway (Pray-Grant et al., 2002).

Table1. Components of SAGA, SLIK and ADA complexes

Subunit	ADA	SLIK	SAGA	Essential	Description	Other location
Ada2	Y	Y	Y	N	Transcription coactivator, component of the ADA and SAGA transcriptional adaptor/HAT (histone acetyltransferase) complexes (Balasubramanian et al., 2002; Sterner et al., 2002b)	N/A
Gcn5	Y	Y	Y	N	Histone acetyltransferase, acetylates N-terminal lysines on histones H2B and H3; catalytic subunit of the ADA and SAGA histone acetyltransferase complexes; founding member of the Gcn5p-related N-acetyltransferase superfamily (Sterner and Berger, 2000)	N/A
Ada1	Y	Y	Y	N	Adaptor protein required for structural integrity of the SAGA complex, a histone acetyltransferase-coactivator complex that is involved in global regulation of gene expression through acetylation and transcription functions(Belotserkovskaya et al., 2000a; Bhaumik and Green, 2002; Grant et al., 1997; Horiuchi et al., 1997; Sterner and Berger, 2000; Sterner et al., 1999a)	N/A
Ada3	Y	Y	Y	N	Transcriptional regulator involved in glucose repression of Gal4p-regulated genes; component of transcriptional adaptor and histone acetyltransferase complexes, the ADA complex, the SAGA complex, and the SLIK complex (Brandl et al., 1993; Grant et al., 1998b; Pray-Grant et al., 2002; Saleh et al., 1997)	N/A
Ahc1	Y	N	N	N	Subunit of the Ada histone acetyltransferase complex, required for structural integrity of the complex (Eberharter et al., 1999)	N/A

Table 1.1 continued (2)

Subunit	ADA	SLIK	SAGA	Essential	Description	Other location
Chd1	N	Y	Y	N	Nucleosome remodeling factor that functions in regulation of transcription elongation; contains a chromo domain, a helicase domain and a DNA-binding domain; component of both the SAGA and SILK complexes (Pray-Grant et al., 2005; Simic et al., 2003; Tran et al., 2000; Woodage et al., 1997)	transcription elongation factor complex, mitochondrion
Sgf73	N	Y	Y	N	73 kDa subunit of SAGA histone acetyltransferase complex; involved in formation of the preinitiation complex assembly at promoters (McMahon et al., 2005; Sanders et al., 2002; Shukla et al., 2006a)	N/A
Spt20	N	Y	Y	N	Subunit of the SAGA transcriptional regulatory complex, involved in maintaining the integrity of the complex (Grant et al., 1998b)	N/A
Spt3	N	Y	Y	N	Subunit of the SAGA and SAGA-like transcriptional regulatory complexes, interacts with Spt15p to activate transcription of some RNA polymerase II-dependent genes, also functions to inhibit transcription at some promoters (Belotserkovskaya et al., 2000a; Eisenmann et al., 1992; Grant et al., 1997)	N/A
Spt7	N	Y	Y	N	Subunit of the SAGA transcriptional regulatory complex, involved in proper assembly of the complex; also present as a C-terminally truncated form in the SLIK/SALSA transcriptional regulatory complex (Grant et al., 1997; Pray-Grant et al., 2002; Sterner et al., 2002a)	mitochondrion

Table 1.1 continued (3)

Subunit	ADA	SLIK	SAGA	Essential	Description	Other location
TAF10	N	Y	Y	Y	Subunit (145 kDa) of TFIID and SAGA complexes, involved in RNA polymerase II transcription initiation and in chromatin modification (Grant et al., 1998b; Lee and Young, 2000; Tora, 2002)	TFIID
TAF12	N	Y	Y	Y	Subunit (61/68 kDa) of TFIID and SAGA complexes, involved in RNA polymerase II transcription initiation and in chromatin modification, similar to histone H2A (Grant et al., 1998b; Lee and Young, 2000; Tora, 2002)	TFIID
TAF5	N	Y	Y	Y	Subunit (90 kDa) of TFIID and SAGA complexes, involved in RNA polymerase II transcription initiation and in chromatin modification (Grant et al., 1998b; Lee and Young, 2000; Tora, 2002)	TFIID
TAF6	N	Y	Y	Y	Subunit (60 kDa) of TFIID and SAGA complexes, involved in transcription initiation of RNA polymerase II and in chromatin modification, similar to histone H4 (Grant et al., 1998b; Lee and Young, 2000; Tora, 2002)	TFIID
TAF9	N	Y	Y	Y	Subunit (17 kDa) of TFIID and SAGA complexes, involved in RNA polymerase II transcription initiation and in chromatin modification, similar to histone H3 (Grant et al., 1998b; Lee and Young, 2000; Tora, 2002)	TFIID
Tra1	N	Y	Y	Y	Subunit of SAGA and NuA4 histone acetyltransferase complexes; interacts with acidic activators (e.g., Gal4p) which leads to transcription activation; similar to human TRRAP, which is a cofactor for c-Myc mediated oncogenic transformation (Bhaumik et al., 2004; Brown et al., 2001; Grant et al., 1998c; Saleh et al., 1998)	NuA4 histone acetyltransferase complex

Table 1.1 continued (4)

Subunit	ADA	SLIK	SAGA	Essential	Description	Other location
Rtg2	N	Y	N	N	Sensor of mitochondrial dysfunction; regulates the subcellular location of Rtg1p and Rtg3p, transcriptional activators of the retrograde (RTG) and TOR pathways; Rtg2p is inhibited by the phosphorylated form of Mks1p (Sekito et al., 2002) (Liao and Butow, 1993) (Komeili et al., 2000; Liu et al., 2003; Sekito et al., 2000)	cytoplasm
Sgf11	N	N	Y	N	Integral subunit of SAGA histone acetyltransferase complex, regulates transcription of a subset of SAGA-regulated genes, required for the Ubp8p association with SAGA and for H2B deubiquitylation (Ingvarsdottir et al., 2005; Powell et al., 2004)	N/A
Sgf29	N	N	Y	N	Probable 29kKDa Subunit of SAGA histone acetyltransferase complex (Sanders et al., 2002)	N/A
Spt8	N	N	Y	N	Subunit of the SAGA transcriptional regulatory complex but not present in SAGA-like complex SLIK/SALSA, required for SAGA-mediated inhibition at some promoters (Bhaumik and Green, 2002; Sterner et al., 2002a)	N/A
Sus1	N	N	Y	not known	Protein involved in mRNA export coupled transcription activation; component of the SAGA histone acetylase complex (Rodriguez-Navarro et al., 2004)	nuclear pore
Ubp8	N	N	Y	N	Ubiquitin-specific protease that is a component of the SAGA complex; required for SAGA-mediated deubiquitylation of histone H2B (Hochstrasser, 1996) (Henry et al., 2003)	N/A

1.3.2 The functions of SAGA complex

1.3.2.a Activator interaction of SAGA

As a co-activator complex, SAGA is recruited to the promoter UAS via the interaction with the activator when a gene is in inducing condition. When *GAL* genes are induced in galactose, SAGA subunit Tra1 interacts with the activation domain of the activator Gal4 and recruits the SAGA complex to Gal4-regulated promoters (Bhaumik et al., 2004); (Brown et al., 2001).

1.3.2.b TBP delivery function of SAGA

TBP can be recruited to core promoter via the interaction with SAGA components Spt3 and Spt8 (Larschan and Winston, 2001; Sermwittayawong and Tan, 2006; Sterner et al., 1999a; Trievel et al., 1999). Spt3-TBP functional interaction is required for TBP recruitment to promoter (Bhaumik and Green, 2001). Spt8 can co-immunoprecipitate with TBP. *In vitro* study shows that Spt8 interacts with TBP directly and is involved in delivery of TBP to core promoter (Sermwittayawong and Tan, 2006).

1.3.2.c Nucleus export

Sus1 is a newly found SAGA subunit, which also exists at the nuclear pore (Rodriguez-Navarro et al., 2004). That links SAGA complex to the nuclear pore and facilitate the export of the mRNA product of SAGA-regulated genes out of the nucleus (Rodriguez-Navarro et al., 2004). Sus1 is among a few yeast proteins that undergo mRNA splicing. It is still not known whether Sus1 is essential for yeast survival in a normal environment.

1.3.2.d Deubiquitylation function of SAGA

Ubp8 is a ubiquitin-specific protease and is required for deubiquitylation of histone H2B at lysine 123. Sgf11 associates with Ubp8 and is required for the deubiquitylation activity of Ubp8. Ubp8 and Sgf11 form the deubiquitylation module of SAGA complex (Daniel et al., 2004; Henry et al., 2003; Ingvarsdottir et al., 2005; Lee et al., 2005b; Powell et al., 2004; Shukla et al., 2006b). The balance between ubiquintylation and deubiquitylation of histone is important for transcription regulation (Mutiu et al., 2007; Wyce et al., 2004).

1.3.2.e HAT module of SAGA

Gcn5 is a 51 KDa component of yeast complexes ADA, SAGA and SLIK (Grant et al., 1997; Hampsey, 1997). It is highly conserved in eukaryotes (Georgakopoulos and Thireos, 1992; Inoue et al., 1996). Homologs of yeast Gcn5 can be found in *Tetrahymena, Drosophila*, mouse and human (figure 1.2a) (Brownell et al., 1996; Driessen et al., 1997; Mizzen et al., 1996; Smith et al., 1998). The domains of yeast Gcn5 have been fully characterized (figure 1.2b). The most significant characteristic of Gcn5 is highly conserved histone acetyltransferase (HAT) activity (Mizzen et al., 1996; Wang et al., 1997). The recombinant Gcn5 with the HAT domain of hGcn5 and the other domains of yGcn5 can fulfill the cell growth and transcription activation (Candau et al., 1996; Wang et al., 1997).Gcn5 preferentially acetylates lysine 14 (K14) and 9 (K9) of histone H3. Gcn5 can also acetylate K11 and K16 of histone H2B and K18, K23 and K27 of histone H3 *in vitro* (Grant et al., 1997; Ruiz-Garcia et al., 1997; Suka et al., 2001; Zhang et al., 1998). The HAT activity of Gcn5 is required for full activation of *GAL* genes, but is not required for TBP

recruitment to the core promoter (Brownell et al., 1996; Grant et al., 1997; Grant et al., 1998b; Utley et al., 1998). The minimal HAT domain of Gcn5 was already identified (Candau et al., 1997; Wang et al., 1998). Glutamate 173 is the essential catalytic residue of the HAT activity (Tanner et al., 1999). Besides the HAT catalytic domain, Gcn5 also contains a highly conserved bromodomain (Marcus et al., 1994). The bromodomain in Gcn5 is required for contact with histones. The bromodomain is also required for the HAT activity of Gcn5 in a complex, but not for HAT activity of free Gcn5 (Georgakopoulos et al., 1995; Sterner et al., 1999a). GST pull-down assays showed Gcn5 binds N-tail of H3 and H4 and the bromo-domain is involved in the interaction with N-terminal tail of H3 and H4 (Ornaghi et al., 1999). Ada2 Interaction domain is required for the interaction between Gcn5 and Ada2, which is important for Gcn5 HAT activity *in vivo* (Wang et al., 1998). The purified Gcn5 from yeast acetylates only free H3 histones, but not the nucleosomal histones. Gcn5 in SAGA or ADA complex can acetylate nucleosomal histones. Thus, the HAT activity of Gcn5 is within a complex that confers on it the ability to acetylate H3 in a nucleosome (Ruiz-Garcia et al., 1997).

The factors in the SAGA HAT module, Gcn5, Ada2 and Ada3 are the major components of the ADA complex (Grant et al., 1997). ADA and SAGA complexes have distinct but overlapping function (Grant et al., 1999). N-terminal domain of Ada3 can interact with the activation domain of transcription activators and the C-terminal domain is the Ada2-interacting domain (Horiuchi et al., 1995). N-terminal domain of Ada2 is Gcn5-interaction domain, and the middle domain of Ada2 is related with the Ada3 interaction

(Candau and Berger, 1996; Horiuchi et al., 1995; Marcus et al., 1994). The interaction between Gcn5 and Ada2 is required for transcription activation (Candau et al., 1997). The unique function of the ADA complex is still not clear.

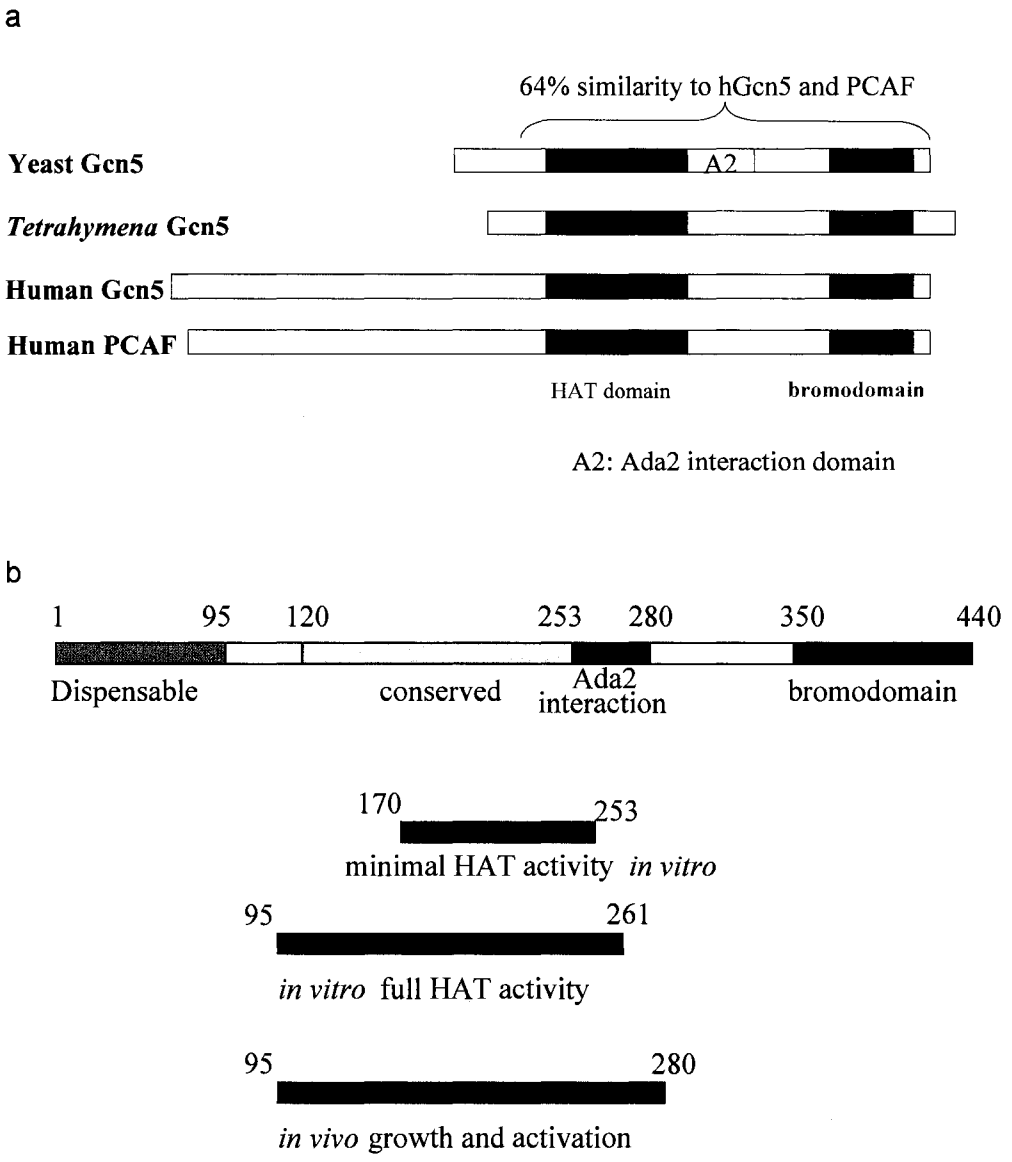


Figure 1.2 Gcn5 is evolutionary conserved with multiple functional domains. (a) Comparison of Gcn5 in yeast, *Tetrahymena* and human. All of these contain conserved HAT domain and bromodomain. (b) The functional domains in yeast Gcn5. The main functional domains of yeast Gcn5 are the conserved HAT domain, Ada2 interaction domain and C-terminal bromodomain.

1.3.3 The negative and positive functions of SAGA

As a multi-functional co-activator complex, SAGA was found with both negative and positive functions to same promoters. SAGA, especially HAT activity of Gcn5, represses *ARG1* in rich medium and is required for activation of *ARG1* in the minimal medium (Ricci et al., 2002). *In vitro* studies show that Spt8 and TFIIA compete for binding to TBP. Spt8 inhibits the binding of SAGA to PIC in the absence of activator (Warfield et al., 2004). With the presence of the activator, Spt8 enhances transcription.

1.4 Chromatin remodeling complexes and histone chaperones

Other than the SAGA complex, *GAL* genes are also regulated by the chromatin remodeling complex Swi/Snf (Lemieux and Gaudreau, 2004). In vivo, *GAL1,10* promoter DNA, wrapped into nucleosomes, is not open for the transcriptional machinery or regulatory complexes. Nucleosomes contain two copies of histones H2A, H2B, H3, and H4 (Kornberg, 1974; Luger et al., 1997). The histones of the nucleosome interact with the DNA phosphate backbone and the amino-terminal tails of the histones extend beyond the nucleosome in order to interact with other adjacent nucleosomes (Luger et al., 1997). The nucleosomes with the help of histone H1 and other factors form higher order structures of chromatin (Hampsey, 1997; Kim et al., 2002; Struhl, 1998). The Swi/Snf complex has been shown to remodel nucleosomes by either sliding the histone octomer on the same DNA template or by transferring the histone octomer to another adjacent DNA template (Lorch et al., 1999; Sudarsanam and Winston, 2000).

Swi/Snf is a 2MDa molecular weight protein complex composed of 11 subunits (Peterson and Tamkun, 1995; Smith et al., 2003; Sudarsanam and Winston, 2000). Snf2 is the ATPase subunit of Swi/Snf (Peterson and Herskowitz, 1992). In addition, proper function of Snf2 requires both the Snf5 and Snf6 subunits (Cairns et al., 1994; Geng et al., 2001; Laurent and Carlson, 1992). Swi/Snf recruitment to *GAL1,10* promoter upon galactose induction requires activator and Mediator, but not SAGA (Lemieux and Gaudreau, 2004). Swi/Snf replaces histones acetylated by SAGA (Chandy et al., 2006; Hassan et al., 2006).

Factors that are important in chromatin remodeling, histone assembly and disassembly, and chromosome formation are histone chaperones. Asf1 is a well-studied histone chaperone, with a universal function of histone assembly and disassembly (Adkins et al., 2004; Green, 2005; Robinson and Schultz, 2003; Schwabish and Struhl, 2006). Another well-studied histone chaperone is nucleosome assembly protein 1 (Nap1). Yeast Nap1 is 417 amino acids in length with an acidic C-terminal domain. The acidic carboxyl-terminal region of NAP1, although dispensable for nucleosome assembly *in vitro*, contributes to binding via structure-independent electrostatic interactions (McBryant et al., 2003). Nap1 has a preference for binding the $(H3\text{-}H4)_2$ tetramer over the (H2A-H2B) dimer. Nap1 interacts with the amino-terminal tails of H3 and H4 directly. Nap1 binds to one histone fold domain (McBryant et al., 2003; Park et al., 2005; Park et al., 2008). Nap1 is involved in the transport of H2A and H2B histones to the nucleus (Ishihama, 1981; Mosammaparast et al., 2002). *In*

vitro study showed that Nap1 cooperates with p300/CBP (a co-activator with HAT activity in higher eukaryotes) in HTLV-1 activation (unpublished data from Dr. Jennifer Nyborg and colleagues and Shikama et al., 2000). Nap1 is involved in eviction of histones that are acetylated by CBP from HTLV-1 promoter. Even though yeast does not have p300/CBP, P300/CBP proteins contain conserved HAT domain and bromodomain. Also the proteomics study shows the interaction between Nap1 and TBP by TAP-MS (Krogan et al., 2006). The connection between Nap1, HAT and TBP suggests that the histone chaperone Nap1 might be involved in transcription regulation by the SAGA complex.

1.5 TBP-delivery and core promoter sequences

For the recruitment-regulated promoters such as GAL gene promoters, TBP is recruited to the core promoter upon induction. The TBP binding site is a TA-rich DNA sequence called TATA element, and is about 20 bp to 30 bp upstream of the transcription initiation site in higher eukaryotes and in yeast it is variable at 50-100bp (Yang et al., 2007). The TATA element serves as the site where the assembly of the preinitiation complex (PIC) begins. Not all promoters contain TATA elements. Recent study shows in higher eukaryotes about 70% of genes have off consensus TATA elements (Yang et al., 2007). In yeast about 80% of genes do not have TATA elements (Basehoar et al., 2004). TBP is still delivered to core promoter with or without TATA elements. TBP can be recruited with two complexes TFIID or SAGA (Cabal et al., 2006; Daniel and Grant, 2007; Govind et al., 2007; Larschan and Winston, 2001; Lee et al., 2005a; Lemieux and Gaudreau, 2004; Roberts and Winston, 1997;

Sermwittayawong and Tan, 2006; Sterner et al., 1999b). The genomic studies show TATA-containing promoters tend to be regulated by SAGA, while TATA-less genes are regulated by TFIID (Huisinga and Pugh, 2004). In the yeast genome approximately 20% of the genes are TATA-containing (Basehoar et al., 2004). These TATA containing and SAGA-dependent genes are the genes that respond to stress and environmental changes such as heat shock, cold shock, oxidative stress and change in carbon source (Huisinga and Pugh, 2004).

Even for the TATA-containing promoters, the sequences of TATA elements vary and the efficiency of transcription from different TATA elements also varies (Hahn et al., 1989; Perier et al., 1999; Zhu and Zhang, 1999). The consensus sequence of TATA elements in yeast is TATAAA, from which transcription is more efficient than that from the off consensus ones (Stewart et al., 2006; Wobbe and Struhl, 1990). TBP binds to the minor groove of TATA element (Chasman et al., 1993) and the binding affinities of TBP at variant TATA elements are almost the same (Hahn et al., 1989). However in the step of TFIIA binding to TBP after TBP binding to TATA element, the stability of TFIIA-TBP complex varies a lot at different TATA elements, and the consensus element TATAAA has the most stable initiation complex (Stewart and Stargell, 2001). This indicates that *in vivo*, other factors are involved with core promoter sequence differentiate. Only a few of such regulatory factors are found, and SAGA may be involved. Spt3 with Mot1 can regulate redistribution of the limiting TBP from non-promoter sites to promoters (Collart, 1996; Madison and Winston, 1997; Muldrow et al., 1999).

There are some hints that SAGA may participate in the differential usage of consensus and off-consensus TATA elements at *HIS3* (Sterner et al., 1999a). There are two TATA elements in *HIS3* promoter, T_C and T_R (Struhl, 1982a; Struhl, 1982b; Struhl, 1982c). T_C is a 29 bp sequence containing off consensus TATA element. Transcriptional product from T_C starts at +1 site. T_R contains a consensus TATA element TATAAA and transcription product from T_R starts at +13. When *HIS3* is not activated, T_C is as strong as T_R, and when *HIS3* is activated transcription from T_R increases 5-10 folds, while transcription from T_C only increases 2 fold (Iyer and Struhl, 1995). That means some factors can help transcription from T_C or repress transcription from T_R when *HIS3* is not activated. Deletion of Gcn5 or gcn5 mutants with defective HAT activity causes the increased transcription from T_C of *HIS3* which has off-consensus core promoter sequence (Sterner et al., 1999a).

1.6. Gaps to be filled

Little is known about how SAGA differentiates core promoter sequences and the impact of the sequence of the TATA element in the core promoter in Gal4-mediated SAGA-dependent gene activation. SAGA seems involved in core promoter selection. Our hypothesis is the change of core promoter sequence (even only one base substitution from consensus sequence to off-consensus sequence) would affect the expression of SAGA-dependent genes significantly and SAGA with certain subunit deletion would have different impacts to the transcription consensus and off-consensus core promoter sequences. In this study, we use a reporter system to detect the different

impacts of SAGA subunit deletion to the core promoters containing consensus or off-consensus core promoter sequence. We found an interesting censoring function of SAGA to the off-consensus core promoter sequences not only to our reporter system but also to the well-studied endogenous *GAL* genes. The deletions of SAGA subunits have different impacts to the induction kinetics of the reporter and the endogenous genes with consensus and off-consensus core promoter sequences.

As an important part of transcription, histone eviction involves histone modification, chromatin remodeling and interaction with histone chaperones. We investigated the function of HATs and histone chaperones in *GAL* gene induction. We found an impact of deletion of histone chaperones to the transcription of endogenous *GAL* genes. Also deletions of Nap1 and Asf1 show different changes to induction kinetics of *GAL1* and *GAL7* transcription, which suggests the different functions of these two chaperones and indicates that the histone assembly at the promoters can affect transcription directly. Our ChIP data on recruitment and post-recruitment promoters show Nap1 occupancy is not related to transcription levels or RNAPII occupancy, but has the same pattern of SAGA occupancy and the opposite pattern of histone, suggesting interaction between Nap1, SAGA and histone eviction during galactose induction.

Chapter 2

Materials and Methods

2.1 Yeast strains and media

The deletion mutant strains and its parental strain (BY4741, *MATa his3*Δ1 *ura3*Δ0 *leu2*Δ0 *met15*Δ0) used in this study were purchased from Research Genetics Inc. Protein tagging via PCR amplification and genome recombination with either HA or Myc epitope was carried out according to literature (Longtine et al., 1998). All the strains used in this study are listed in table 2.1.

Glucose-repressed strains were grown in YP or selective media containing 2% glucose. Noninduced strains were grown in YP or selective media containing 2% raffinose. For galactose induction, cells were first grown in glucose or raffinose media, and then washed and transferred to YPGal or selective media containing 2% galactose. Cells were collected at different induction time points. For S1 nuclease protection assays to analyze the impacts of histone chaperone deletion to *GAL* genes, cells were first induced in galactose media for 1 hour and then were repressed for 30 minutes by adding glucose to final concentration to 2%.

Table 2.1 Yeast strains in this study

Strain	Parental strain	Relevant genotype	Reference or source
BY4741	N/A	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Research Genetics
ubp8Δ	BY4741	*ubp8::kanMX4*	Research Genetics
sgf11Δ	BY4741	*sgf11::kanMX4*	Research Genetics
sgf29Δ	BY4741	*sgf29::kanMX4*	Research Genetics
spt8Δ	BY4741	*spt8::kanMX4*	Research Genetics
spt3Δ	BY4741	*spt3::kanMX4*	Research Genetics
sgf73Δ	BY4741	*sgf73::kanMX4*	Research Genetics
chd1Δ	BY4741	*chd1::kanMX4*	Research Genetics
spt7Δ	BY4741	*spt7::kanMX4*	Research Genetics
spt20Δ	BY4741	*spt20::kanMX4*	Research Genetics
ada1Δ	BY4741	*ada1::kanMX4*	Research Genetics
gcn5Δ	BY4741	*gcn5::kanMX4*	Research Genetics
ada3Δ	BY4741	*ada3::kanMX4*	Research Genetics
ada2Δ	BY4741	*ada2::kanMX4*	Research Genetics
ahc1Δ	BY4741	*ahc1::kanMX4*	Research Genetics
rtg2Δ	BY4741	*rtg2::kanMX4*	Research Genetics
eaf3Δ	BY4741	*eaf3::kanMX4*	Research Genetics
elp3Δ	BY4741	*elp3::kanMX4*	Research Genetics
hpa3Δ	BY4741	*hpa3::kanMX4*	Research Genetics
spt10Δ	BY4741	*spt10::kanMX4*	Research Genetics
hda1Δ	BY4741	*hda1::kanMX4*	Research Genetics
hos1Δ	BY4741	*hos1::kanMX4*	Research Genetics
hos3Δ	BY4741	*hos3::kanMX4*	Research Genetics
pho23Δ	BY4741	*pho23::kanMX4*	Research Genetics
rpd3Δ	BY4741	*rpd3::kanMX4*	Research Genetics
gal4Δ	BY4741	*gal4::kanMX4*	Research Genetics

Table 2.1 (continued)

anc1Δ	BY4741	*anc1::kanMX4*	Research Genetics
bur2Δ	BY4741	*bur2::kanMX4*	Research Genetics
caf4Δ	BY4741	*caf4::kanMX4*	Research Genetics
ioc2Δ	BY4741	*ioc2::kanMX4*	Research Genetics
ioc3Δ	BY4741	*ioc3::kanMX4*	Research Genetics
ioc4Δ	BY4741	*ioc4::kanMX4*	Research Genetics
not3Δ	BY4741	*not3::kanMX4*	Research Genetics
nut1Δ	BY4741	*nut1::kanMX4*	Research Genetics
paf1Δ	BY4741	*paf1::kanMX4*	Research Genetics
pbp1Δ	BY4741	*pbp1::kanMX4*	Research Genetics
rsc1Δ	BY4741	*rsc1::kanMX4*	Research Genetics
rsc2Δ	BY4741	*rsc2::kanMX4*	Research Genetics
rtf1Δ	BY4741	*rtf1::kanMX4*	Research Genetics
sin4Δ	BY4741	*sin4::kanMX4*	Research Genetics
snf5Δ	BY4741	*snf5::kanMX4*	Research Genetics
snf6Δ	BY4741	*snf6::kanMX4*	Research Genetics
spt21Δ	BY4741	*spt21::kanMX4*	Research Genetics
spt23Δ	BY4741	*spt23::kanMX4*	Research Genetics
spt4Δ	BY4741	*spt4::kanMX4*	Research Genetics
srb2Δ	BY4741	*srb2::kanMX4*	Research Genetics
srb8Δ	BY4741	*srb8::kanMX4*	Research Genetics
nap1Δ	BY4741	*nap1::kanMX4*	Research Genetics
Myc-Gcn5	BY4741	*GCN5-myc13::HIS3*	(Fletcher et al., 2007)
HA-Ada2	BY4741	*ADA2-HA9::HIS3*	This study
HA-Spt8	BY4741	*SPT8-HA9::HIS3*	(Fletcher et al., 2007)
HA-Spt20	BY4741	*SPT20-HA9::HIS3*	(Fletcher et al., 2007)
HA-TAF1	BY4741	*TAF1-HA9::HIS3*	(Fletcher et al., 2007)
Myc-TBP	BY4741	*SPT15-myc13::HIS3*	This study

2.2. DNA constructs

The plasmids used for the *in vivo* expression analysis were derivatives of YCp86 containing the 2-kilobase fragment from pUC18 including the *bla* gene and the origin of replication, a 1.9-kilobase fragment of yeast DNA containing *cen3* and *ars1*, a 1.1-kilobase fragment containing the *URA3* gene, and a polylinker. The hybrid *HIS3* promoter YCp86-SC3801 has the wild type initiation and amino-terminal region of the *HIS3* gene fused in frame with a functional *Escherichia coli LacZ* gene and is used to detect levels of expression *in vivo*. The promoter region contains a 365-base pair *GAL1,10* fragment containing four Gal4 binding sites fused upstream of the *Eco*RI-*Sac*I restriction endonuclease sites, between which oligonucleotides to represent variant core promoter sequences could be inserted. The core promoter sequence TATAAA is used in this study to represent the consensus core promoter sequence. Variant off-consensus core promoter sequences were created with single base substitution in the sequence of TATAAA into C or G at each position. The two oligos (TATAAA and CATAAA) were cloned into this molecule it was renamed TATA and CATA in abbreviation. The plasmids with the other off-consensus core promoter sequences were named with the sequences in the core promoter (Singer et al., 1990; Stewart and Stargell, 2001; Stewart et al., 2006).

The plasmids with Gcn5 mutants were gifts from Shelly Berger's lab, which contain wild type Gcn5 and Gcn5 mutant with point mutation or domain deletions (Candau et al., 1997; Sterner et al., 1999b; Trievel et al., 1999;

Wang et al., 1998). The ORFs of Gcn5 were subcloned into vectors between Gcn5 promoter and terminators.

2.3. β-galactosidase assay

Cells (1ml) in log phase after overnight galactose induction were collected and resuspended in 0.5ml Z-buffer (0.1M $NaPO_4$, 10mM KCl, 1mM $MgSO_4$, pH7.0). Then of 10µl of 0.1% SDS and 20µl of chloroform are added to lyse the cells followed by 30second of vortex. After 30-minute incubation at 30°C, 100µl of 4mg/ml ONPG (o-nitrophenyl-β-galactoside) was added as the substrate of the galactosidase. Stop solution (100µl of 1M $NaCO_3$) was added to stop the enzymatic reaction according to the change of the reaction color from clear to yellow. Each enzyme activity unit was determined by 4 repetitions and normalized to the A_{600} of the cultures and time (minutes) of reactions. The ratio of enzymatic unit in deletion strain to the unit in the wild type strain is used to represent the change of expression level of the reporter gene with certain gene deleted.

2.4. Primer extension

Total amount of 60µg of RNA was hybridized with oligos specific to the +233 to +240bp of *lacZ* gene ORF (sequences shown in table 2.2) at 55°C for 90minutes. The hybridized RNA-oligo products were precipitated with cold ethanol. 5U of AMW reverse transcriptase (Fisher Scientific) was added to transcribe DNA fragments, and RNA was removed by adding RNase. The reversed transcripts were precipitated and loaded in 10% sequencing gel and analyzed with ImageQuant.

2.5. S1 nuclease protection assays

S1 nuclease protection assays were performed as described in literature (Iyer and Struhl, 1996). Total amount of 40μg of RNA was hybridized with radiolabeled single-stranded *GAL1*, *GAL10*, *GAL7* and *tRNA*^{W} probes (sequences shown in table 2.2) overnight and digested by S1 nuclease (160U, Roche) at 37°C for 30 to 40 minutes. The digested products were ran on 10% sequencing gels and analyzed with ImageQuant. The RNA amounts in each reaction were normalized to the RNA amount from a probe to the intron of the tryptophan tRNA gene (*tRNA*^{W}).

2.6. Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assays were performed as described (Strahl-Bolsinger et al., 1997), with few modifications. Cells (50 ml) were grown in glucose media or galactose media for 3-hour induction at 30°C to OD_{600} of 0.8~1.0. Proteins and DNA were cross-linked by adding a final concentration of 1% formaldehyde for 15 minutes with occasional swirling of the flasks at intervals of 5 minutes. Glycine was added to a final concentration of 125mM at room temperature for 5 minutes to stop cross-linking. Cells were collected and washed twice in ice cold 1×TBS. Cells were resuspended in FA-lysis buffer (50mM Hepes pH 7.5, 140mM NaCl, 1mM EDTA, 1% Triton X-100, 0.1% Na-Deoxycholate, 1x Protease Inhibitor Cocktail: PMSF, beuzamidine, pepstatin, leupeptin, and chymostatin) and lysed by vortex with glass beads. Chromatin was sheared by sonication using a Branson W-350 model of sonifier (15 times at 10 seconds each on continuous pulse at a microtip power

setting of 6). 10% of the chromatin material used for the immunoprecipitation was processed as the input after reversing the cross-links and purifying the DNA. 500 µl of the chromatin material was incubated with approximately 5µl of either anti-RNAPII (8WG16, Covance Inc.), anti-HA (SC-7932, Santa Cruz), anti-Myc (05-419, Upstate), anti-histone H3 C-terminus (Abcam), anti-histone H3 acetyl-K14 (Upstate), anti-histone H3 acetyl-K9 (Upstate), and anti-Nap1 (Luger lab) antibodies by rotation overnight at 4°C. Protein-A sepharose beads (GE Healthcare, prepared as the manufacturers directions) was further incubated with the chromatin material for 3 hours at room temperature. The beads were spun down and washed with FA-lysis buffer, FA-Lysis (50mM NaCl), LiCl detergent and TE buffer. The Antigen-Antibody complexes bound to the beads were recovered and further treated with TE/SDS buffer for 15 minutes at 65°C to elute the complexes, followed by incubation with proteinase K for 1 hour at 37°C. Protein-DNA cross-links were reversed by incubation overnight at 65°C and the DNA was purified by phenol-chloroform extraction and used for the linear PCR analysis or Real Time PCR (RT-PCR) analysis signals. The occupancy of protein was determined via RT-PCR. Standard curves were generated using 10-fold serial dilutions of Input DNA. PCR efficiencies ranged from 90-100%, with a correlation coefficient of 0.990 or greater. Threshold cycle data were quantified relative to the input, as described (Frank et al., 2001). Background levels were calculated as the relative occupancy of untagged strains and these values were subtracted from that of the tagged strains. Each occupancy value of IP was set as 100×IP/input. The occupancy of the highest value in wild type strain was set as 100 in the normalized occupancy.

Table 2.2. Oligos in this study

S1 Oligos	
GAL1	5' CGG CCA ATG GTC TTG GTA ATT CCT TTG CGC TAG AAT TGA ACT CAG GTA CAA TCT GAA GA 3'
GAL10	5' CAG CAA AGT GAA TTA CCG AAT CAA TTT TAT ATT CTT TGA AAA CCT TTT CCA GAC CTT TTC GGT CAC ACA AAT CAA CCA GTA TC 3'
GAL7	5' GGG ATG GAT TCT AAG CAC CAA GCT TGG CCA TGT GGA TGT AAG TTG GAA CAA CCC ATG GCT GTA CCA AAC AA 3'
$tRNA^W$	5' GGA ATT TCC AAG ATT TAA TTG GAGG TCG AAA GCT CGC CTT 3'
Primer Extension Oligo	
lacZ ORF +233 to +240	5'- GCA CTC AAC GAT TAG CGA CCA GCC GG3'
ChIP Oligos	
Reporter promoter (–222 to +19)	(forward) 5' CAG GAA CGC GAC CGG TGA AGA 3'
	(reverse) 5' AGG GCT TTC TGC TCT GTC ATC 3'
lac Z ORF (+2001 to +2400)	(forward) 5' TGA AGT TCC TCT GGA TGT CGC 3'
	(reverse) 5' GGT CGC TTC ACT TAC GCC AAT 3'
GAL1,10 promoter (*GAL10* –388 to + 117)	(forward) 5' TAC GCT TAA CTG CTC ATT GCT 3'
	(reverse) 5' CGG AAT TCG ACA GGT TAT CAG CAA CA 3'
CYC1 promoter (–234 to + 79)	(forward) 5' AGG CGT GTA TAT ATA GCG TGG AT 3'
	(reverse) 5' CCA CGG TGT GGC ATT GTA GAC AT 3'

Chapter 3

A Role for SAGA in Core Promoter Quality Control

3.1 Introduction

One rate-limiting step of transcription initiation by RNAPII is recruitment of TBP (TATA binding protein) to core promoter (Colgan and Manley, 1992; Lee and Young, 2000). The sequence of core promoter is important in expression efficiency. The consensus TBP binding site sequence is TATAAA, which drives strong gene expression *in vivo* (Stewart et al., 2006; Wobbe and Struhl, 1990) However TBP can bind to different sequences with similar binding affinity *in intro* (Hahn et al., 1989). This indicates that *in vivo*, other factors are involved with core promoter sequence differentiate. TBP can be recruited by two complexes TFIID and SAGA (Cabal et al., 2006; Daniel and Grant, 2007; Govind et al., 2007; Larschan and Winston, 2001; Lee et al., 2005a; Lemieux and Gaudreau, 2004; Roberts and Winston, 1997; Sermwittayawong and Tan, 2006; Sterner et al., 1999b). SAGA complex has complicated functions (Cabal et al., 2006; Daniel and Grant, 2007; Govind et al., 2007; Larschan and Winston, 2001; Lee et al., 2005a; Lemieux and Gaudreau, 2004; Roberts and Winston, 1997; Sermwittayawong and Tan, 2006; Sterner et al., 1999b). The subunits of SAGA can be classified into groups according to their functions: essential TAFs, TBP-delivery, HAT, complex integrity and other functions.

The genomic studies show TATA-containing promoters tend to be regulated by SAGA, while TATA-less genes are regulated by TFIID. Approximately 20% of the genes in the yeast genome are TATA-containing (Basehoar et al., 2004;

Huisinga and Pugh, 2004). These TATA containing SAGA-dependent genes are the genes that respond to stress and environmental changes such as heat shock, cold shock, oxidative stress and change in carbon source (Huisinga and Pugh, 2004). Even for the TATA-containing promoters, the sequences of TATA elements vary (Hahn et al., 1989). Little is known about how SAGA differentiates core promoter sequences or the impact of the sequence of the TATA element on SAGA functions. In this study, we use a reporter system to detect the different impacts of SAGA subunit deletion to the core promoters containing consensus sequence TATAAA or off-consensus sequence CATAAA. We find that particular subunits play a critical role in distinguishing between consensus and off-consensus elements. We observe this phenomenon not only at a reporter gene system, but also at the endogenous GAL genes. In addition, the effect was observed for a number of off-consensus elements. Thus, we have identified a new role for the SAGA complex in core promoter quality control.

3.2 Results

3.2.1 Kinetics' of induction are similar at a consensus and off-consensus TATA elements although absolute levels of expression are different.

Yeast *GAL* responsive *GAL1,10* genes have been well characterized. *GAL1,10* induction is Gal4-mediated and SAGA-dependent (Bhaumik and Green, 2001; Bhaumik and Green, 2002; Bhaumik et al., 2004; Larschan and Winston, 2001). To study the regulatory functions of SAGA complex during *GAL* gene induction, a reporter system with synthetic promoters (Figure 3.1.a)

was utilized to measure the expression from different core promoters, which only have one base difference in core promoter sequence. The sequence TATAAA (TATA) is used to represent the consensus sequence, and sequence CATAAA (CATA) for off-consensus sequence. CATA is the most frequently used off-consensus TATA element sequence (Perier et al., 1999; Zhu and Zhang, 1999).

Gal4 is the activator of our reporter system with synthetic promoter. Deletion of Gal4 would damage the galactose induction. The expression levels from both core promoters were also measured in the *gal4* deletion strain. As expected, in *gal4Δ* there is no expression from both TATA and CATA. The unit driven by both core promoters is 0.59±0.05, at the same level as the background (wild type in YPD or YPRaffinose).

Previous i*n vivo* studies in our lab show that CATA drives a relatively lower expression than TATA and both TATA and CATA have strong TBP binding *in vitro* (Stewart and Stargell, 2001; Stewart et al., 2006). We wanted to know what makes CATA weak promoter *in vivo*. One possibility of why CATA is weak promoter *in vivo* is that the transcription driven by CATA initiates from a different site than transcription driven by TATA. Primer extension in time course (Figure 3.1.b) was performed to detect whether transcriptions from TATA and CATA start at the same position. Even though the sequence around the core promoter is TA-rich, the change of core promoter sequence from TATA to CATA does not affect the transcription start site. The reverse transcribed products from TATA and CATA have the same length, indicating

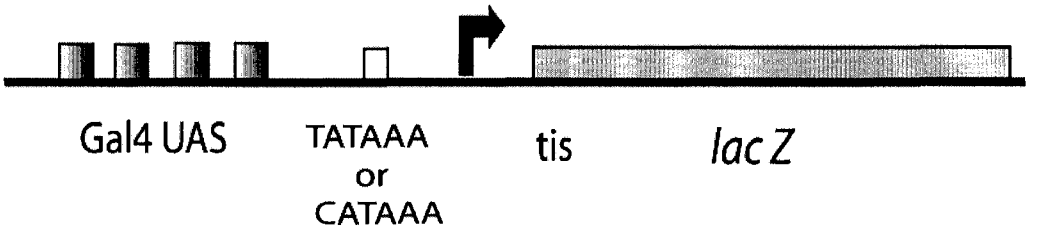
that transcription initiation driven by TATA or CATA starts at the same site. Also the kinetics of transcription from TATA and CATA show similarity, though the absolute levels are different. In the first ten minutes of galactose induction, the expression levels driven both TATA and CATA undergo a slow increase, between 10 minute and 30 minute the transcription from both core promoters has a rapid increase, after 30 minutes of induction mRNA levels decrease from TATA and CATA. The absolute maximal expression level from TATA is 3-fold higher than from CATA.

It is possible that the weak, off-consensus core promoter CATA simply saturates at a lower transcription level. If so, we would expect different induction kinetics compared to the consensus TATA. β-galactosidase assays in time course were performed to detect the induction kinetics of the reporter gene expression driven by TATA and CATA. In the first 3 hours the increase of the reporter gene products driven by TATA is slow (Figure 3.1.c). After that expression level of the reporter gene undergoes a rapid increase. After 6-hour induction, the expression enters a plateau stage. Expression driven by CATA has a very low level during the entire course of induction. The galactosidase analysis shows the maximal unit driven by CATA is about 13, while by TATA about 2500. Upon induction, the kinetic rate of expression for both core promoters is similar, the induction curve of CATA shown in an enlarged scale, though the absolute units driven by CATA are more than 100-fold lower than TATA. The similar induction kinetics of expression from TATA and CATA indicates that CATA is a weak promoter is not the result of early induction saturation.

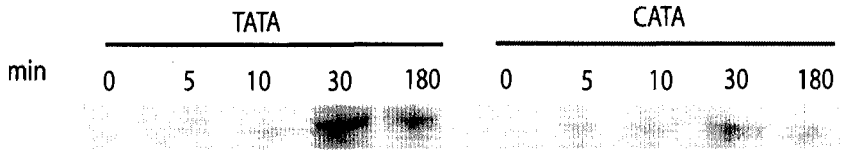
There might not be enough TBP or RNAPII recruited to the weak promoter CATA. We used ChIP assay to detect whether TBP and RNAPII are recruited to promoter CATA (Figure 3.1.d). We found that TBP and RNAPII are recruited to both promoters upon galactose induction. The levels of TBP and RNAPII occupancy at CATA is about half of those at TATA. The occupancy of TBP and RNAPII is correlated with the expression levels. This suggests that some factors can distinguish the difference between TATA and CATA prior to the step of TBP recruitment to the promoters.

Another possibility is that the single base change from consensus TATA to off-consensus sequence might make CATA induction TFIID dependent, which may result in a lower level of expression. Thus we tested the occupancy of TFIID during induction by ChIP assays. TAF1, which is exclusively in TFIID, was not found occupied at the either promoters before or after induction. The occupancy of TAF1 is as low as the background irrelevant antibody control (data not shown), which is about 1/1000 of RNAPII or TBP occupancy level. This indicates that induction of our reporter system is not TFIID-regulated, even though CATA is an off-consensus core promoter and drives a very low level of expression upon induction. These results suggest that some other factors play a role in core promoter sequence distinction and enhance the difference between consensus and off-consensus promoters *in vivo*.

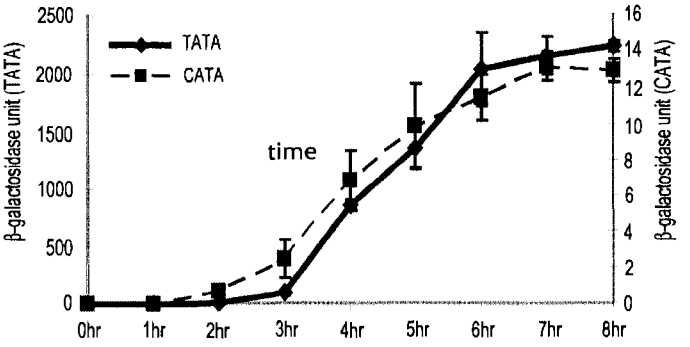
a


Gal4 UAS
TATAAA
or
CATAAA
tis
lac Z

b


TATA
CATA
min
0
5
10
30
180
0
5
10
30
180

c


TATA
CATA
time
β-galactosidase unit (TATA)
β-galactosidase unit (CATA)
2500
2000
1500
1000
500
0
16
14
12
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3hr
4hr
5hr
6hr
7hr
8hr

d

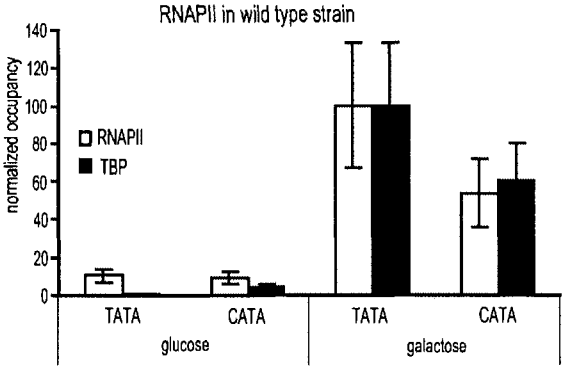

RNAPII in wild type strain
normalized occupancy
RNAPII
TBP
140
120
100
80
60
40
20
0
TATA
CATA
TATA
CATA
glucose
galactose

Figure 3.1 *In* vivo CATA drives low level of expression with the similar kinetics of expression as that driven by TATA. (a) The synthetic promoters of the reporter system. E. Coli. *Lac Z* gene is used as the reporter gene. Upstream of the transcription initiation site (*tis*) the *GAL1,10* UAS with 4 Gal4 binding sites is inserted for the galactose induction of the reporter gene. Between Gal UAS and tis, the sequence of TATAAA is used to represent the consensus core promoter. On the same reporter background, the sequence of CATAAA with single base change (from T to C) is used to represent as the off-consensus core promoter sequence. (b) Primer extension in time course was used to analyze the transcription initiation sites from the two synthetic promoters. (c) Yeast β-galactosidase activities at time points of 1hr to 8hr after galactose induction were measured for strains transformed with reporter plasmids containing TATA or CATA, shown in the absolute units. Left Y-axis: unit adjusted to expression driven by TATA (solid line). Right Y-axis: units adjusted to expression driven by CATA (dotted line). (d) ChIP assays show the occupancies of RNAPII and TBP increase upon galactose induction at the two core promoters.

3.2.2 Certain SAGA components repress expression from off-consensus TATA element.

Since the reporter system used above involves Gal4 UAS in the promoter region and as mentioned earlier, Gal4 induction is SAGA-dependent (Bhaumik and Green, 2001; Bhaumik and Green, 2002; Bhaumik et al., 2004; Dudley et al., 1999; Larschan and Winston, 2001; Roberts and Winston, 1997; Sterner et al., 1999b). It is possible that SAGA may have a role at expression from these reporter promoters. We next asked whether the SAGA complex affects expression from each core promoter equivalently. Strains were created containing a deletion of one non-essential subunit of the SAGA complex and the reporter constructs, and assayed for gene expression. We compared the expression levels in the wild type strain and in the strains with deletion of one SAGA subunit (Table.3.1). We present the data in ratio of expression reporter gene in the deletion strain over that in the wild type strain to reflex the change of expression caused by the SAGA subunit deletion. We find that a number of different patterns of gene expression profiles with regard to a comparison of the two core promoter elements in the SAGA deletion strains (Figure 3.2). It seems the consensus and off-consensus core promoters have different requirements of SAGA subunits for the galactose induction.

The most striking pattern was observed for deletions in *SPT8, GCN5*, and *ADA2*. Deletion of these genes diminished expression driven from the consensus TATA element, whereas expression from the off-consensus CATA element increased significantly. This indicates that in a wild type cell Spt8, Gcn5, and Ada2 play a role in TATA-element censoring by enhancing

expression from TATA while restricting expression from CATA. This pattern of sequence-dependent effects in which TATA-element-censoring is occurring is unique to these three gene products, although we also observed subunits that play a more important role at the consensus TATA promoter compared to the off-consensus CATA element. These TATA-specific subunits include gene products encoded by *SGF73*, *SPT20* and *ADA1*. The third pattern of expression is one in which the subunit tested is necessary for an increase in gene expression from either element. These commonly required subunits are encoded by *UBP8*, *SPT3* and *SPT7*. The final pattern is one in which deletion of the SAGA subunit had no significant change in expression from either TATA or CATA. These dispensable components are encoded by *SGF11*, *SGF73* and *CHD1*. Taken together, these results indicate that the requirement for SAGA subunits in the maintenance of normal patterns of gene expression depends on the particular subunit as well as on the sequence of the core promoter. Importantly, there are distinct SAGA subunits that function to magnifying the difference in expression levels from a canonical and non-canonical core promoter. This suggests that these particular gene products play a fundamental role in establishing the overall efficiency of the core promoter, which is an important aspect in core promoter quality control. For the consensus TATA sequence, expression reduces or remains unchanged in the deletion strains; while for the off-consensus sequence, expression even increases in three deletion strains.

The above results indicate that Gcn5, Ada2 and Spt8 play a role in differentiating between core promoter sequences and the result is to expand the difference in output between the two core promoter sequences. Gcn5 and Ada2 are in the HAT module of SAGA. Gcn5 and Ada2 also exist in ADA and SLIK (SAGA-like) complexes (Sterner et al., 2002a). Spt8 only exists in SAGA and is involved in TBP delivery to core promoter. Thus it is possible that ADA and SLIK complexes are also involved in core promoter quality control. We tested the expression change in the strains with deletion of *ACH1* (integrity factor only in ADA) and *RTG2* (SLIK-specific). The deletion of these factors does not affect expression from either TATA or CATA. Therefore, ADA is not involved in this TATA-element-censoring function. Rtg2 is not the integrity subunit of SLIK. It is possible that SLIK, like its homologue SAGA, has the TATA-element-censoring function. Little is known about SLIK –specific function. It is possible that SAGA/SLIK censor core promoter sequence and SAGA regulates transcription from consensus core promoter; while SLIK regulates transcription driven by off-consensus TATA elements.

Table 3.1 Comparison of activity fro TATA and CATA driven constructs in a variety of strains.

Strain[a]	TATA[b]			CATA[d]		
	units	ratio[c]		units	ratio	
Wild type	2500.00±454.31	1.00±0.18	=	9.91±4.14	1.00±0.42	=
ubp8Δ	3.87±0.36	0.00±0.00	↓	1.07±0.13	0.11±0.01	↓
sgf11Δ	2162.31±83.59	0.86±0.03	=	12.02±0.44	1.21±0.04	=
sgf29Δ	2209.70±89.15	0.88±0.04	=	8.03±0.51	0.81±0.05	=
spt8Δ	208.54±34.03	0.08±0.01	↓	29.65±12.01	2.99±1.21	↑
spt3Δ	1.05±1.39	0.00±0.00	↓	0.00±0.00	0.00±0.00	↓
sgf73Δ	921.80±21.28	0.37±0.01	↓	9.34±0.28	0.94±0.03	=
chd1Δ	2908.59±77.31	1.16±0.03	=	13.02±0.44	1.31±0.04	=
spt7Δ	3.91±2.62	0.00±0.00	↓	0.03±0.05	0.00±0.01	↓
spt20Δ	731.43±36.96	0.29±0.01	↓	7.84±0.64	0.79±0.06	=
ada1Δ	603.70±148.90	0.24±0.06	↓	11.92±2.76	1.20±0.28	=
gcn5Δ	1318.18±229.05	0.53±0.09	↓	55.74±7.62	5.62±0.77	↑
ada2Δ	1142.40±244.50	0.46±0.10	↓	56.50±13.10	5.70±1.32	↑
ada3Δ	88.20±43.10	0.04±0.02	↓	9.94±5.70	1.00±0.58	=
ahc1Δ	2557.12±89.33	1.02±0.04	=	11.20±0.93	1.13±0.09	=
rtg2Δ	2193.74±89.82	0.88±0.04	=	16.41±0.55	1.66±0.06	=

a. The order of strains is consistent with that of Figure 3.2

b. Units of activity for the gene product from reporter core promoter containing TATA.

c. The fold change of units in deletion strains (Δ/wt).

=: no change, ↑: increase, ↓: decrease.

d. Units of activity for the gene product from reporter core promoter containing CATA.

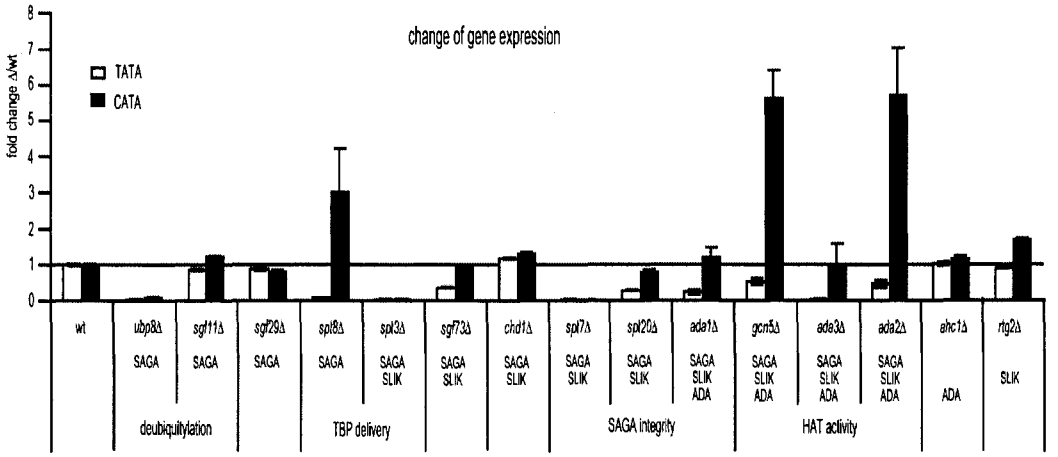


Figure 3.2 The expression driven by CATA has different requirements for SAGA subunits. The activities of β-galactosidase driven by TATA or CATA were measured in wild type strain and the deletion strains of SAGA subunits (Upb8, Sgf11, Sgf29, Spt8, Spt3, Chd1, Sgf73, Spt7, Spt20, Ada1, Gcn5, Ada3 and Ada2), gene products specific in ADA complex (Ahc1) or SLIK complex (Rtg2). The ratios of the expression from TATA (white columns) or CATA (black columns) in the deletion strains over the expression level from the same core promoter in the wild type strain were used to represent the change of the expression affected by deletion of certain SAGA subunit (Table 3.1). For the deletion strains, the complexes (ADA, SAGA or SLIK) in which the deleted subunit exists and the function of some subunits are listed.

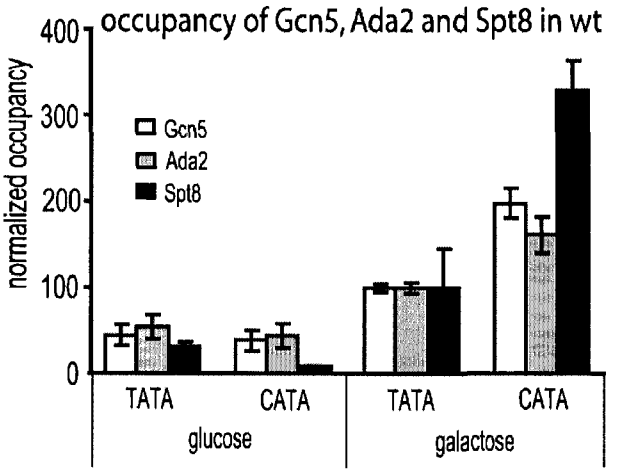
3.2.3 The off-consensus core promoter CATA remains SAGA-dependent.

Our study shows that the SAGA subunits Gcn5, Ada2 and Spt8 have repressive function to the expression from off-consensus core promoter sequence. Previous study shows that SAGA complex is recruited to promoter of the endogenous *GAL* gene via the interaction with the activator Gal4 upon galactose induction (Bhaumik and Green, 2001; Bhaumik and Green, 2002; Bhaumik et al., 2004; Dudley et al., 1999; Larschan and Winston, 2001; Roberts and Winston, 1997; Sterner et al., 1999b). Although we show that TFIID does not occupy at either promoter in uninduced or inducted condition, it is not known whether SAGA is still recruited to the promoter in the induced condition if the core promoter sequence is changed from consensus one to off-consensus one. To determine the occupancy of these three SAGA subunits Gcn5, Ada2 and Spt8 at the two promoters upon induction, ChIP assays were performed in the wild type strain with tagged Gcn5, Ada2 and Spt8. Upon galactose induction, these three SAGA subunits are recruited to both TATA and CATA. These three SAGA subunits have higher occupancy at CATA than TATA, which is opposite to the expression levels (Figure 3.3.a).

In the strains of *gcn5Δ*, *ada2Δ* and *spt8Δ*, expression from CATA increases. The level of RNAPII occupancy usually is correlated with expression level (Kim and Iyer, 2004). We next tested whether for either promoter RNAPII occupancy would change in SAGA deletion strains. We used ChIP assay to detect RNAPII occupancy in the wild strain and some SAGA deletion strains of *gcn5Δ*, *ada2Δ* and *spt8Δ*. For the consensus core promoter TATA, deletion of Gcn5, Ada2 or Spt8 causes significant decreased RNAPII occupancy upon induction (Figure 3.3.b), which is correlated with the decreased expression.

However for the off-consensus core promoter CATA, deletion of Gcn5 or Ada2 does not result in a significant drop in RNAPII occupancy in galactose condition. Interestingly, deletion of Spt8 reduces RNAPII occupancy dramatically in the induced condition to the same level as in the uninduced condition, even though the expression driven by CATA is about 3-fold higher in *spt8Δ* than in the wild type strain. These results suggest that in *spt8Δ* upon induction the activity of RNAPII is changing, while the occupancy of RNAPII is not.

a



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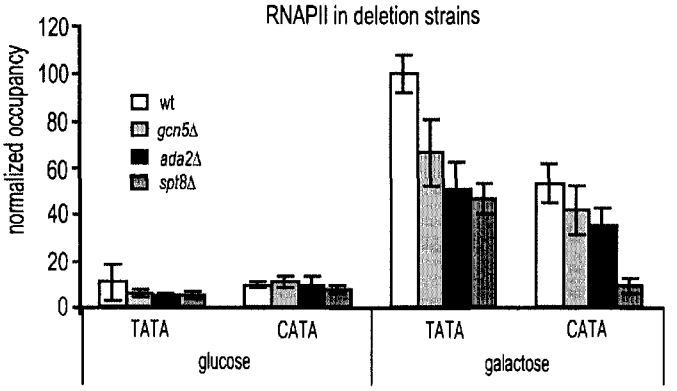


Figure 3.3. In the wild type strain the weak core promoter CATA has less RNAPII occupancy and more SAGA occupancy than TATA (a) ChIP assays measured the occupancy of SAGA subunits Gcn5, Ada2 and Spt8 at the promoters containing TATA and CATA, upon galactose induction in the wild type strain. Anti-Myc and anti-HA were use to immuno-precipitate Myc-tagged Gcn5, HA-tagged Ada2 and HA-tagged Spt8. (b) Anti-Rpb1 antibody were used in ChIP assays to detect occupancy of RNAPII at the core promoter containing TATA or CATA in the uninduced condition and the induction condition in the wild type strain and the deletion strains of Gcn5, Ada2 and Spt8. Data with error bars in this paper are mean ± S.D. from three independent experiments.

3.2.4 HAT activity in Gcn5 is involved in core promoter sequence differentiation.

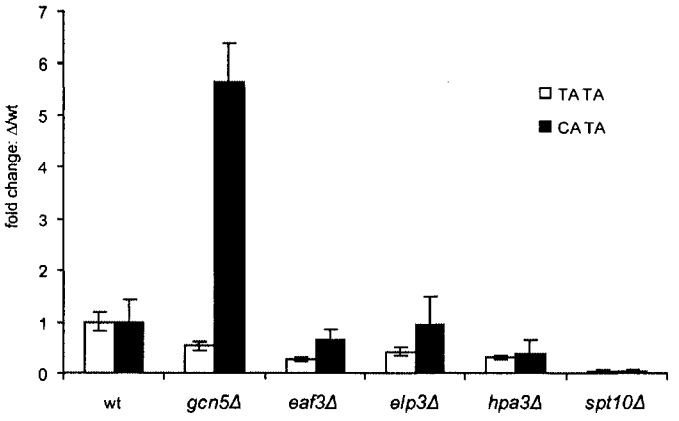
Gcn5, Ada2 and Spt8were found involved in SAGA new function: TATA-element-censoring. Gcn5 and Ada2 are in the HAT module of SAGA complex. To test whether other factors, which also carry HAT activity, can differentiate the two core promoter sequences we performed the galactosidase assays in the deletion strains of *eaf3Δ*, *elp3Δ hap3Δ* and *spt10Δ*, shown in figure 3.4.a. None of these deletion strains show the same pattern in the galactosidase assay as *gcn5Δ*. The expression from TATA decreases at least to half in all the deletion strain of HATs. While only two strains (*hap3Δ* and *spt10Δ*) have decreased expression from CATA, in other deletion strains expression from CATA does not change significantly. This suggests the universal function of HATs. It HAT activity is involved in transcription differentiation from consensus and off-consensus core promoters, this specific HAT activity is carried only in Gcn5.

The yeast Gcn5 contains 4 important domains: the N-terminal domain is dispensable for Gcn5, the conserved domain contains the catalytic residue E173 of the HAT activity, the Ada2-interaction domains can help Gcn5 interact with Ada2 in vitro, and the C-terminal domain is the bromodomain which can help Gcn5 recognize the partially acetylated histone tail and add acetyl groups to other lysine residues (Candau et al., 1997; Sterner et al., 1999b; Trievel et al., 1999; Wang et al., 1998). We used wild type Gcn5 and four Gcn5 mutants on plasmids to cover deleted Gcn5 in the strain of *gcn5Δ*. The 4 mutants are: (1) E173Q that is the point mutation of the HAT catalytic residue, (2) the HAT

domain deletion, (3) the bromodomain deletion and (4) the Ada2-interaction domain deletion (A-intΔ). We used plasmids containing the wild type or the mutants were transformed into the reporter system to recover the deleted Gcn5. The galactosidase assays shows the patterns of expression from consensus and off-consensus TATA elements with the wild type Gcn5 or the Gcn5 mutants (figure 3.4.b).

Gcn5 mutants of HAT domain deletion and the bromodomain deletion show the similar expressional pattern as the Gcn5 deletion strain. The two mutants are related to the HAT activity of Gcn5, suggesting that HAT activity of Gcn5 is involved in TATA-element-censoring. However the Gcn5 mutant with point mutation E173Q shows no affect to expression from TATA, and increased expression from CATA, suggesting that Gcn5 HAT activity represses expression from CATA and non-HAT function of Gcn5 facilitates expression from TATA. The mutant of Ada2-interaction domain deletion (A-intΔ) shows the expression pattern as the wild type. This suggests that interaction between Gcn5 and Ade2 is not required for the core promoter differentiation. However the previous genetic screen showed that the Ada2 deletion strain has the same expression pattern as the Gcn5 deletion strain, which means Ada2 in also important to such regulation.

a



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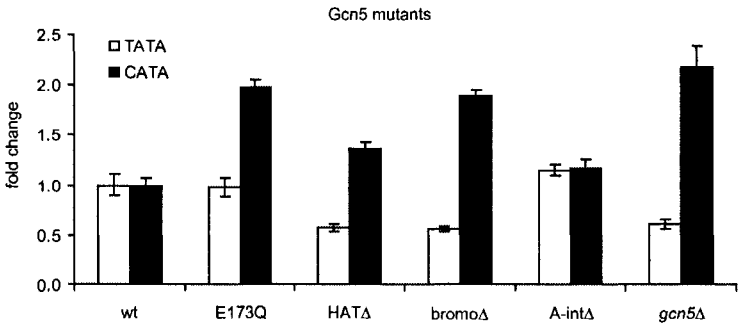


Figure 3.4 HAT activity carried by Gcn5 is involved in core promoter sequence differentiation. (a) In the deletion strains of factors that also carry HAT activity, expression from neither TATA nor CATA shows increase. (b) β-galactosidase assays show the change of expression from TATA and CATA in the strains with Gcn5 mutants.

3.2.5 Gcn5-related histone acetylation is important in the induction, but there is no difference of acetylation levels between TATA and CATA.

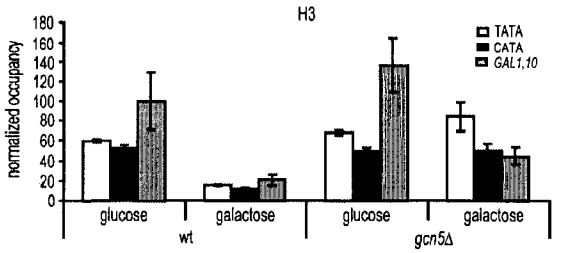
HAT activity of gcn5 seems involved in TATA-element-censoring function of SAGA. The main target Gcn5 HAT is histone H3 lysine14. We used ChIP assays to test whether the histone H3K14 acetylation state would be different between TATA and CATA. We check the occupancy of whole H3 (acetylated plus unacetylated) and acetyl-H3K14 in the wild type strain and *gcn5Δ* at reporter promoters and the endogenous *GAL1,10* promoter. In the wild type strain, in the uninduced condition histone H3 has the same occupancy at the reporter promoters containing either TATA or CATA, the genomic *GAL10* promoter has higher level of H3 occupancy (Figure 3.5.a). Upon induction histone occupancy decreases dramatically at all the tested promoters, indicating that histones are evicted from all the promoters. While in *gcn5Δ*, the reporter promoters have the same high level of H3 occupancy in both conditions, suggesting histone eviction does not happen in the reporter promoters. For the endogenous *GAL1,10* in each condition deletion of Gcn5 does not change H3 occupancy, suggesting that for endogenous promoter acetylation at H3k14 is not required for histone eviction.

Then we tested the acetylated histone H3K14 (H3Ac) (Figure 3.5.b) In the wild type strain, at all the promoters, we found the same levels of increased H3Ac occupancy upon induction (3-fold increase). While in the strain of *gcn5Δ*, in the uninduced condition the acetyl-H3 levels are as low as that in the wild type strain; upon induction the occupancy of acetylated-histones shows no increase. Deletion of Gcn5 totally diminishes acetylation of H3K14 at both

reporter promoters and *GAL1,10* promoter. This indicates that Gcn5 is the only HAT responsible for the histone acetylation of H3K14 at the reporter promoters and the endogenous *GAL1,10* promoter during induction. The ratios of the H3Ac occupancy to the H3 occupancy were calculated and represent as the histone acetylation levels (Figure 3.5.c). Histone acetylation levels increase during induction in the wild type strain. There is no difference between TATA and CATA, while the endogenous *GAL10* promoter has a lower level. The higher level of histone acetylation at the reporter promoter than at the endogenous *GAL10* promoter is probably due to lack of other regulatory elements than Gal4 UAS at the synthetic reporter promoters. The deletion of *GCN5* diminishes the increase of the histone acetylation at both synthetic and endogenous promoters.

For the reporter promoters, HAT activity of Gcn5 is required for histone acetylation and eviction from the promoters upon galactose induction, but acetylation of H3K14 by Gcn5 HAT is not required for the distinction between core promoters, suggesting that acetylation of other Gcn5 HAT targets or non-HAT function of Gcn5 is involved in this core promoter differentiation.

a



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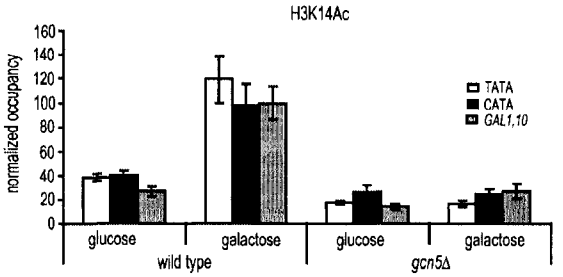

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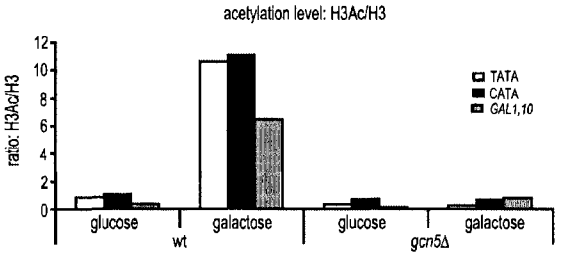


Figure 3.5. Between TATA and CATA, there is no difference of histone acetylation catalyzed by Gcn5. (a) Antibody to histone H3 tail and to acetylated histone H3 lysine 14 was used in ChIP assays to detect the occupancy of whole histone H3 the (b) Antibody specific to acctyl-H3 lysine 14(H3Ac) was used in ChIP to detect the occupancy of Gcn5- acetylated H3 at the two promoters in the uninduced and induced condition. (c) The ratios of H3Ac to the whole histone H3 are used to represent the histone acetylation state. White columns: promoter containing TATA. Black columns: promoter containing CATA. Grey columns: endogenous *GAL10* promoter.

3.2.6 Endogenous genes with core promoter consensus or off-consensus core promoters also have different requirements for SAGA subunits.

Our studies with the synthetic reporters show that SAGA subunits Gcn5, Ada2 and Spt8 carry a new function of SAGA: TATA-element-censoring, which facilitates expression driven by the consensus TATA sequence and represses expression driven by the off-consensus sequence (CATAAA in the reporter system). Given that our reporter system is a simplified model, we wanted to know whether the TATA-element-censoring function of these three SAGA subunits still occurs to endogenous SAGA-regulated genes. *GAL1,10* core promoter has the perfect consensus TATA sequence TATAAA, while for GAL10, the sequence of putative TBP binding site is CATAAA in *Saccharomyces cerevisiae*. S1 nuclease protection assays were performed to detect the RNA levels of *GAL1* and *GAL10* in a 7-hour galactose induction (Figure 3.6.a). Even though *GAL1* and *GAL10* share the same UAS for Gal4 binding and have the same induction kinetics, the absolute expression levels of the two genes upon galactose induction are significantly different. *GAL1* has higher transcription level than *GAL10*. Probably the higher expression level of *GAL1* results from the consensus core promoter sequence.

We measured the mRNA levels of *GAL1* and *GAL10* after 7-hour induction in the strains of wt, *gcn5Δ*, *ada2Δ*, *spt8Δ*, *spt3Δ* and *spt20Δ* (figure 3.6.b). Also expression levels in *gal4Δ* were used as the negative control to investigate the impact of SAGA subunit deletion to transcription of *GAL* genes. Deletion of Gcn5, Ada2 or Spt8 increases the expression of *GAL10* (which has off-

consensus TATA element sequence) upon a long time galactose induction, and the expression of *GAL1* does not change in the strains of *gcn5Δ*, *ada2Δ*, and *spt8Δ*. SAGA integrity factor Spt20 is required for transcription of both *GAL10* and *GAL1*. In *spt20Δ*, transcription levels of *GAL10* and *GAL1* decrease to half as in wild type. The transcription levels of both genes do not change significantly in *spt3Δ*. The activator Gal4 is crucial for *GAL* gene induction. Deletion of Gal4, expression of both *GAL1* and *GAL10* is abolished. As in the reporter system, the SAGA subunits Gcn5, Ada2 and Spt8 have a repressive function to transcription of endogenous gene with off-consensus core prompter sequence.

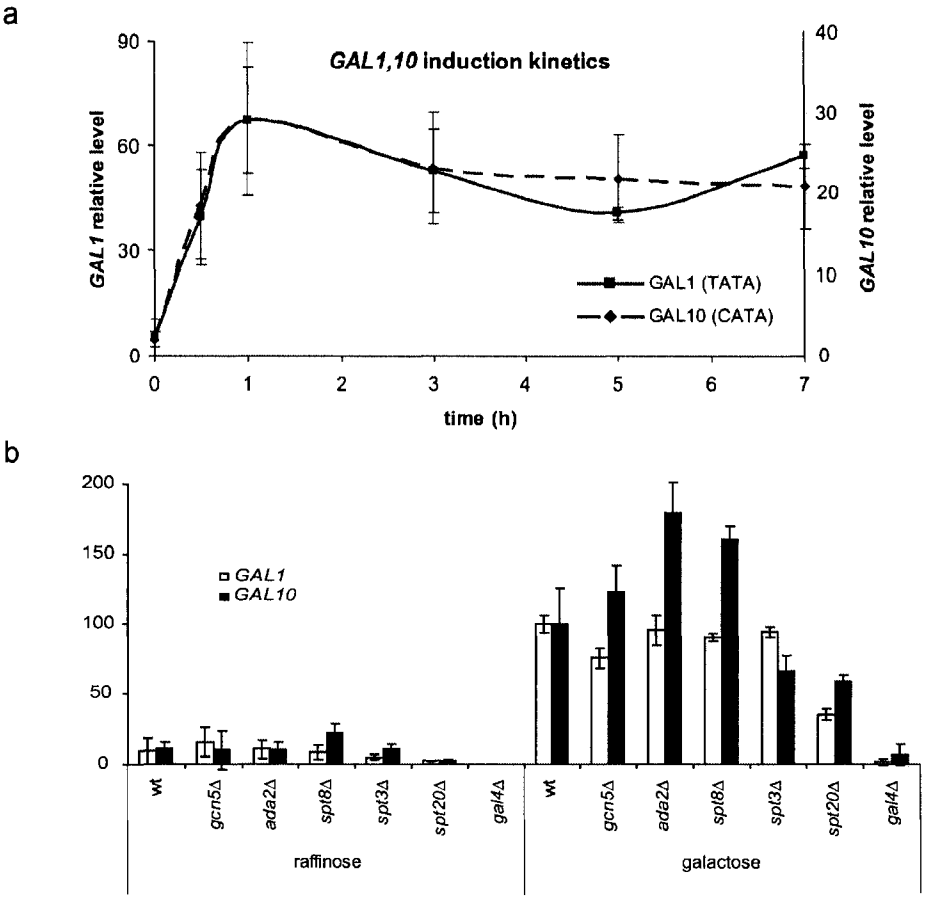


Figure 3.6. Deletion of SAGA subunits has different effects on the endogenous *GAL1* and *GAL10* genes. (a) S1 nuclease protection assay in time course was used to analyze the expression levels of *GAL1* and *GAL10* gene in wild type and SAGA deletion strains. *GAL1*, which has the consensus TATA sequence, has higher expression (left Y-axis); *GAL10*, which has the putative off-consensus core promoter, has lower expression level (right Y-axis). (b) Normalized quantification of mRNA levels of GAL1 and GAL10 after 7-hour galactose induction in wt, *gcn5Δ*, *ada2Δ* and *spt7Δ* *spt3Δ*, *spt20Δ* and *gal4Δ* is shown. The RNA amounts in each reaction are normalized to the RNA amount from a probe to the intron of the tryptophan tRNA gene ($tRNA^{W}$). The change of transcription levels in the deletion strains is shown as percentage to the expression of *GAL1* or *GAL10* in wt strain in galactose media.

3.2.7 Majority of other TATA variants also drive higher expression in the deletion strain of *GCN5*

Our study on the synthetic promoters and the endogenous promoters shows that some SAGA complex subunits are involved in transcriptional regulation from different sequences of the core promoters. In the yeast genome, the sequences of the core promoters vary other than TATAAA and CATAAA. We wanted to rule out the possibility that SAGA only regulates transcription driven by the specific core promoter sequences TATAAA and CATAAA, not by other core promoter sequences. To test the hypothesis that the repressive function of SAGA is not only to the promoter containing the exact sequence CATA, but also to the promoters containing other off-consensus sequences. We investigated the change of expression levels from other off consensus sequences that have C or G to substitute the base in the sequence of TATAAA (Stewart and Stargell, 2001; Stewart et al., 2006). In Gcn5 deletion strain, 9 out of 12 off-consensus elements drive increased expression; two elements (TATAAC and TAGAAA) did not show any change compared to the canonical element TATA; only one off-consensus element TCTAAA drives reduced expression (Figure 3.7). The repressive function of SAGA to expression from off-consensus core promoter sequences is not specific to certain sequence. It is a relatively universal phenomenon.

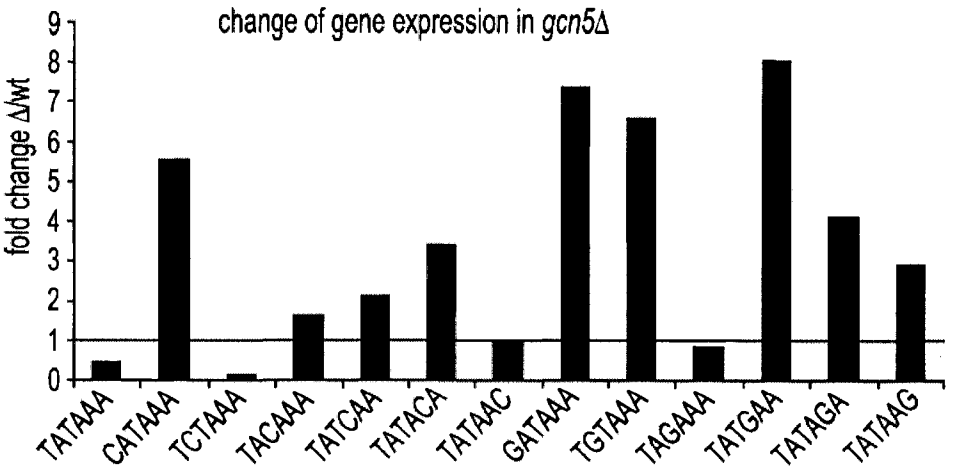


Figure 3.7 Majority of the tested off-consensus core promoter sequences are de-repressed in the *GCN5* deletion strain. β-Galactosidase activities were measured for wild type strain and *gcn5Δ* transformed with the reporter plasmids containing either the consensus TATAAA element or the indicated off-consensus element. The ratios of expression driven by off-consensus element over the expression level driven by the consensus core promoter are used to represent the change of expression affected by deletion of *GCN5*.

3.3 Discussion

In this study, we used a reporter system with synthetic core promoter sequences to test the impact of SAGA deletion to consensus and off-consensus core promoters. We have found a new function of SAGA carried by subunits Spt8, Gcn5 and Ada2: TATA-element-censoring. This function enhances transcription driven by the consensus TATA element and represses transcription driven by off-consensus TATA elements. This function works at both synthetic promoters with variant TATA-element sequences and the endogenous *GAL* promoters. The HAT activity carried by Gcn5 is required for this TATA-element censoring function. Our study suggests that the new function involves TBP delivery, histone acetylation and histone eviction.

3.3.1 The role of Spt8 in TATA-element-censoring function

Spt8 is one of SAGA subunits that can interact with TBP directly and is involved in TBP delivery to the promoter. Spt8 has both positive and negative function in transcription initiation (Belotserkovskaya et al., 2000b; Warfield et al., 2004). Spt8 prevents TBP binding to the promoter sequences in the uninduced condition. Results show the competition between Spt8 and TFIIA to bind TBP. The results from previous studies in our lab found TFIIA can help TBP to distinguish strong or weak TATA elements. Tan's handoff model explains the competition between Spt8 and TATA elements to bind TBP. Spt8 competes with TATA sequence to bind to TBP. In the uninduced condition, Spt8 is a strong competitor. Spt8 binds to TBP and prevents TBP from binding DNA sequence. Upon induction, Spt8 becomes a weak competitor and loses the competition with promoter TATA sequence (TATATAAA), and releases

TBP to promoter. Tan's data also showed that off-consensus TATA sequence (CAGATAAA) is always an off-consensus weak competitor. It could not bind to TBP in the presence of Spt8. GATA is one of the off-consensus TATA sequences used in this study. Expression driven by GATAAA has about 6-fold increase in the deletion strains of Gcn5, Spt8 and Ada2 compared with the wild type strain. Our *in vivo* results also showed that with the deletion of Spt8, the promoters containing off-consensus TATA sequences would be able to drive higher expression. This indicated without competition of Spt8, TBP would have a chance to be released from SAGA complex to the weak TATA sequences.

The other TBP interaction factor in SAGA is Spt3. Our results show that Spt3 is important for expression driven by both consensus and off-consensus core promoter sequences, suggesting that for TBP delivery function of SAGA, Spt3 is more important than Spt8. In vivo, Spt3 may be the one factor that is crucial for TBP delivery, and Spt8 may help TBP recruitment to correct place by distinction of core promoter sequences.

Our results suggest that complex lacking Gcn5, Ada2 or Spt8 would enhance expression from off-consensus core promoter. Interestingly, SLIK complex shares 9 subunits with SAGA, but lack of Spt8. It is possible that SLIK, like SAGA, has the TATA-element-censoring function. Little is known about SLIK–specific function. It is possible that SAGA/SLIK censor core promoter sequence: SAGA regulates transcription from consensus core promoter, and SLIK regulates transcription driven by off-consensus TATA elements.

3.3.2 HAT activity of Gcn5 is involved in TATA-element-censoring functions.

In this study we found that Gcn5 could differentiate the consensus and off-consensus core promoters in the reporter system and endogenous *GAL* genes. Previous studies show that deletion of *GCN5* or *gcn5* mutants with defective HAT activity increases the usage of *HIS3* T_C (off-consensus TATA) in the induced condition (Sterner et al., 1999a).

Targets of Gcn5 HAT are H3 K9 and K14. *In vitro*, Gcn5 can also acetylate H2B K11/K16 and H3 K18/K23/K27. Gcn5 HAT activity to acetylate histone H3 K9 and K14 is not required for this distinction between consensus and off-consensus core promoter sequences (Balasubramanian et al., 2002; Bu et al., 2007b; Govind et al., 2007; Hassan et al., 2001; Jacobson and Pillus, 2004; Shukla et al., 2006a; Syntichaki and Thireos, 1998; Syntichaki et al., 2000; Wang et al., 1998; Yoon et al., 2003), suggesting the acetylation level of other Gcn5 HAT targets is involved in TATA-element-censoring function or even some function of Gcn5 other than HAT activity is involved in the core promoter quality control by SAGA complex, which might be related with change of SAGA conformation (Imoberdorf et al., 2006). Mouse Gcn5 has function other than HAT activity. This non-HAT function of mouse Gcn5 is important in mouse embryo development (Bu et al., 2007a; Lin et al., 2008). Another possibility is that Gcn5 can acetylate other factors than histone. Although it is not clear whether yeast Gcn5 acetylate non-histone proteins, recent study found that Drosophila Gcn5 could acetylate ATP-dependent histone remodeling factor ISW1 at lysine 753 (Ferreira et al., 2007).

3.3.3 SAGA recruitment, stability at the core promoter, and core promoter quality control

The recruitment of SAGA depends on the activator. SAGA is recruited to the promoter by the interaction between SAGA and the activator Gal4. However the ChIP assays showed that SAGA has higher occupancy at CATA than at TATA, while PIC has less occupancy at CATA. It suggests that SAGA might get trapped at the off consensus promoter. Once SAGA is recruited at the promoters, if the core promoter is not perfectly consensus, SAGA might linger at the promoter for a longer time, reduce RNAPII recruitment efficiency and block the activity of RNAPII, resulting in a low transcription. With certain subunit deleted, this core promoter quality control function of SAGA is impaired to some extent.

3.3.4 Importance of this study for yeast and higher eukaryotes

In yeast genome approximately 20% of the genes contain TATA element in their promoters (Basehoar et al., 2004; Huisinga and Pugh, 2004). These TATA containing genes respond to stress and environmental changes such as heat shock, cold shock, oxidative stress and change in carbon source and they are regulated by SAGA complex (Huisinga and Pugh, 2004). The newly found TATA-element-censoring function of SAGA in our study gives hints of how SAGA regulates these stress-responsive genes that contains variant core sequences. The human genomic study also found in human genome only 30% of genes have TATA elements (Yang et al., 2007). The human SAGA homologues may have the CATA-censoring function in transcriptional

regulation. Transcription from variant TATA element sequences has more options for regulation. HIV-1 promoter contains CATA instead of TATA for optimal transcription regulation and replication (van Opijnen, 2004).

Chapter 4

Detailed examination of the important parameters in SAGA-dependent core promoter functions

4.1 Introduction

CATA-censoring function of SAGA complex carried by Gcn5, Ada2 and Spt8 was identified using a reporter system and at endogenous genes (Chapter 3). These three SAGA subunits are involved in distinguishing of the difference between consensus and off-consensus core promoter sequences and amplification of the difference by facilitating expression from the consensus core promoter and repressing that from the off-consensus promoter sequences. We show the kinetics of expression driven by consensus and off-consensus core promoter sequences are the same, both in the synthetic promoters of the reporter system and with the endogenous promoters. In this chapter, we describe the impacts on the induction kinetics of both endogenous *GAL* genes and the reporter system with deletion of CATA-censoring SAGA subunits. Other than SAGA, *GAL* genes are also regulated by other complexes such as Swi/Snf and Mediator complexes. These regulatory complexes might be also involved in core promoter sequence censoring directly or indirectly. Using the reporter system we identified other regulatory factors that can differentiate TATA and CATA.

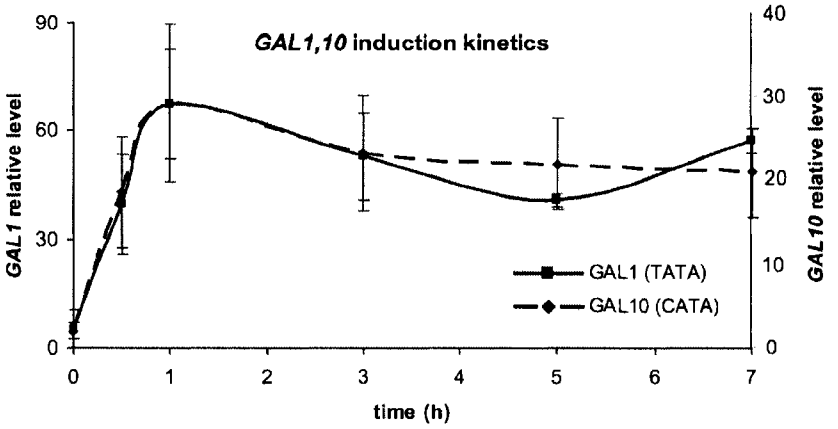
4.2 Results

4.2.1 Deletions of SAGA subunits have different impacts to the induction kinetics of endogenous *GAL1* and *GAL10* genes.

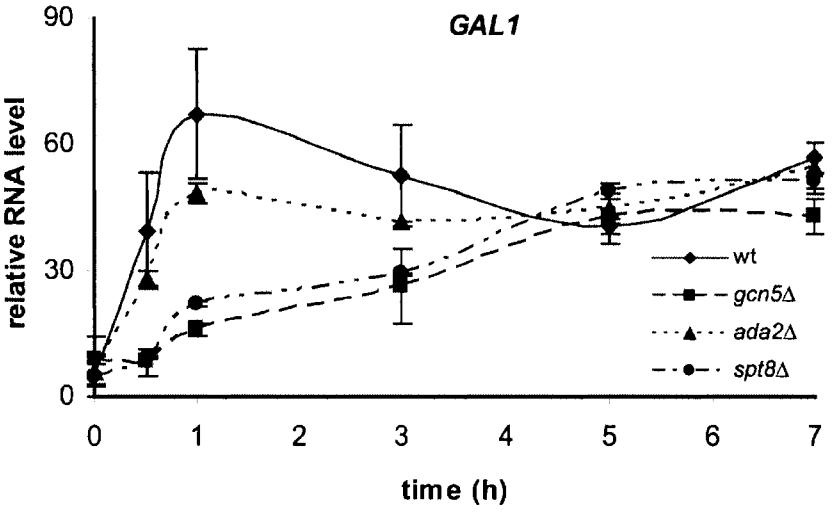
Upon galactose induction, *GAL1* (consensus TATA sequence) and *GAL10* (off-consensus TATA sequence) undergo a rapid expression increase in 1 hour (figure 4.1.a). Then the mRNA levels of *GAL1* and *GAL10* enter a plateau phase with slow decrease through the entire 7 hours induction time in this study. Even though the absolute peak level of *GAL1* is about 2-fold higher as *GAL10*, the relative induction kinetics of the two genes is the same. Also SAGA subunit deletion changes mRNA levels of *GAL1* and *GAL10* after 7-hour induction (shown in Chapter 3). It is possible that deletion of SAGA subunit changes not only the absolute mRNA levels of *GAL* genes, but also the induction kinetics. Then the mRNA levels of *GAL1* and *GAL10* during the whole 7-hour induction in SAGA deletion strains were detected via S1 nuclease protection assays. The deletion of Ada2, Gcn5 or Spt8 causes a different pattern of *GAL1* expression than *GAL10*. Deletion strain of *ada2Δ* has the similar *GAL1* transcription level as the wild type (Figure 4.1.b). Deletion strains of g*cn5Δ* and *spt8Δ* have a very slow induction of *GAL1* in the first 5 hours, and later end up with the same levels as in wild type. For *GAL10* (off-consensus core promoter), *ada2* deletion has the same expression levels in the first 3 hours as the wild type and has higher level after (Figure 4.1.c.). Deletion strains of *spt8* and *gcn5* show slower induction than wild type in the first 3 hours, and after 3 hours *GAL10* has higher expression in these two deletion strains. As control we also detected the induction kinetics of GAL genes in *spt20Δ* and *spt3Δ*. Both Spt20 and Spt3 are required for TBP recruitment to *GAL* promoter (Bhaumik and Green, 2001; Bhaumik and Green,

2002). Strain of *spt20Δ* has slow induction and a low expression of both *GAL1* and *GAL10* through the whole induced period (Figure 4.1.d,). Spt20, the core subunit for SAGA integrity, is required for induction of *GAL* genes at any time point. As expected, neither *GAL10* nor *GAL1* shows induction in the strain of *gal4Δ* (figure 4.1.e). We also found a different requirement of Spt3 for *GAL10* and *GAL1* induction. In *spt3Δ*, strain *GAL10* expression level reduces to half of that in wild type through the whole 7-hour induction, while *GAL1* expression has the very similar level as in wild type. Spt3 seems required only for *GAL10*, but not for *GAL1* induction. Even though *GAL1* and *GAL10* share the same Gal4 UAS, deletion of certain SAGA subunit has different impacts to transcription levels of *GAL1* (containing consensus core promoter sequence) and *GAL10* (containing off-consensus core promoter sequence). Deletion of Gcn5, Ada2 or Spt8 increases the expression of *GAL10* upon a long time galactose induction, suggesting that upon induction, SAGA seems to prefer the consensus *GAL1* core promoter to initiate transcription.

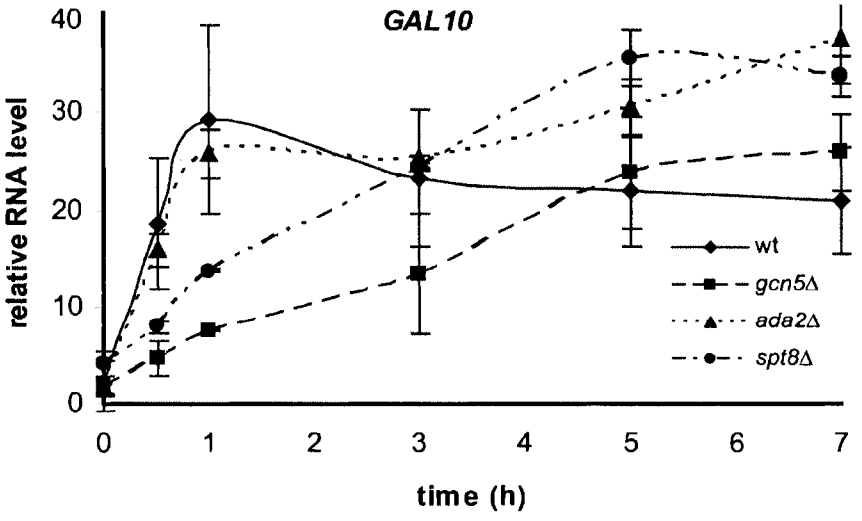
a


GAL1,10 induction kinetics
GAL1 relative level
GAL10 relative level
GAL1 (TATA)
GAL10 (CATA)
time (h)

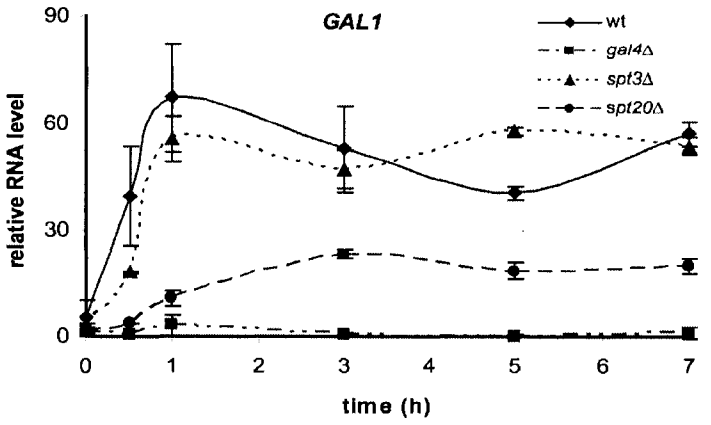
b


GAL1
relative RNA level
wt
gcn5Δ
ada2Δ
spt8Δ
time (h)

c


GAL10
relative RNA level
wt
gcn5Δ
ada2Δ
spt8Δ
time (h)

d



e

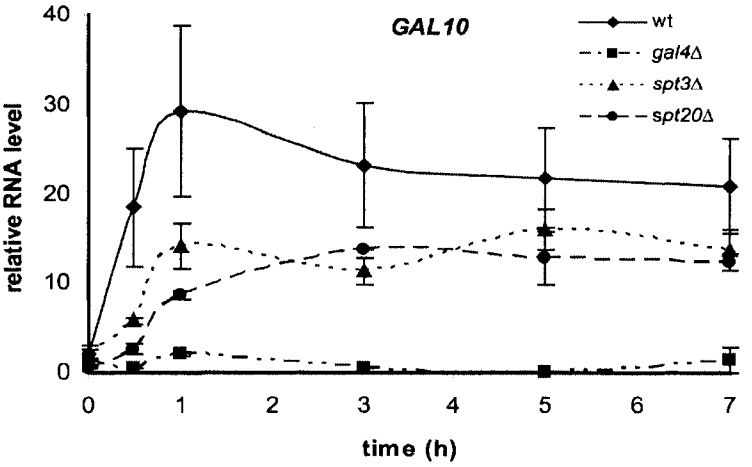


Figure 4.1. Deletion of SAGA subunits has different effects on the endogenous *GAL1* and GAL10 genes. (a) S1 nuclease protection assay in time course was used to analyze the expression levels of *GAL1* and *GAL10* gene in wild type and SAGA deletion strains. Even *GAL1* and *GAL10* share the same Gal4 UAS, the absolute expression levels of the two genes are different in the wild type strain. *GAL1*, which has the consensus TATA sequence, has higher expression (left Y-axis); *GAL10*, which has the putative off-consensus core promoter, has lower expression level (right Y-axis). (b) and (c) show the *GAL10* and *GAL1* expression levels in wt and *gcn5Δ*, *ada2Δ* and *spt7Δ*. (d) and (e) show the expression levels of *GAL10* and *GAL1* in wt, *spt3Δ*, *spt20Δ* and *gal4Δ* as control. The RNA amounts in each reaction were normalized to the RNA amount from a probe to the intron of the tryptophan tRNA gene ($tRNA^{W}$).

4.2.2 The kinetics of reporter gene expression in 16-hour induction

Then we wanted to know whether deletion of SAGA subunit changed the induction kinetics of reporter gene. β-galactosidase assays in 16-hour time course were used to determine the expression kinetics from the consensus and off-consensus core promoter on the reporter plasmids in wild type and SAGA deletion strains (figure 4.2.2). For the consensus core promoter TATA, in wild type strain, protein level of lac Z product increase significantly in the first 6-hour induction and then enters a plateau state after. For the SAGA deletion strains, gcn5Δ and spt8 have lower expression levels (about 1/3 of wild type strain) through all the induction. The expression level in *ada2Δ* arrives to the plateau state at about 4-hour induction, earlier than the wild type, but the absolute level is also about 1/3 of wild type strain. For the off-consensus core promoter CATA, all the strains arrive at the maximal expression levels at about 10-hour. All the deletion strains have higher expression levels than wild type strain through out the whole induction. Compared to the kinetics of endogenous *GAL* transcription, the kinetics of *LacZ* of the report system more represent the end-point levels.

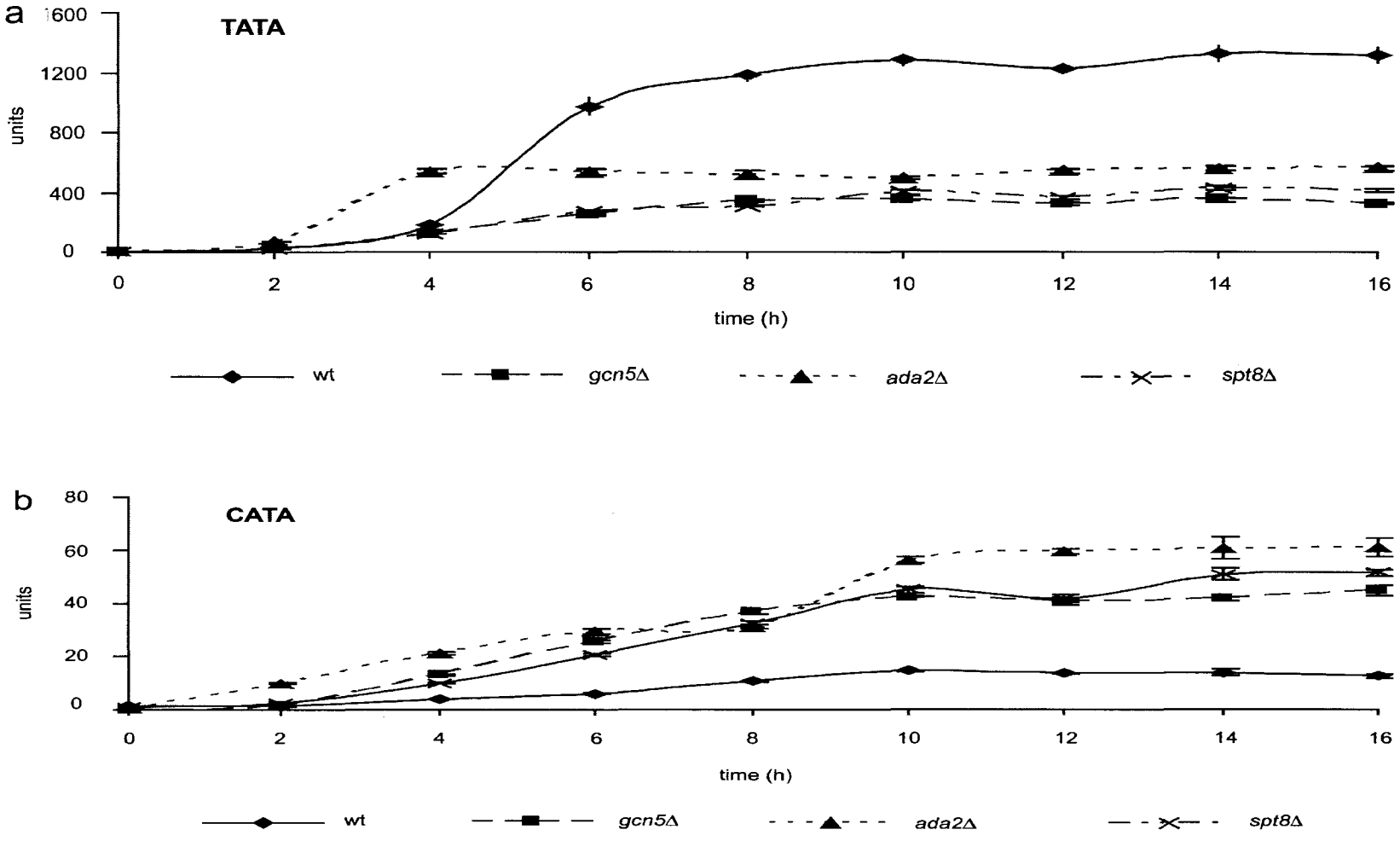
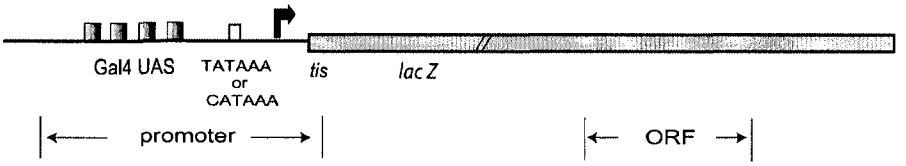

a
TATA
1600
1200
800
400
0
units
0
2
4
6
8
10
12
14
16
time (h)
wt
gcn5Δ
ada2Δ
spt8Δ
b
CATA
80
60
40
20
0
units
0
2
4
6
8
10
12
14
16
time (h)
wt
gcn5Δ
ada2Δ
spt8Δ

Figure 4.2 Kinetics of expression from reporter promoters during 16-hour galactose induction in wt, *gcn5Δ*, *ada2Δ* and *spt8Δ* strains via galactosidase assays. Galactosidase assays in 16-hour time course were performed to detect the expression levels driven by TATA and CATA in the wild type strain and SAGA deletion strains (a) Expression kinetics from the consensus sequence TATA. (b) Expression kinetics from the off-consensus sequence CATA.

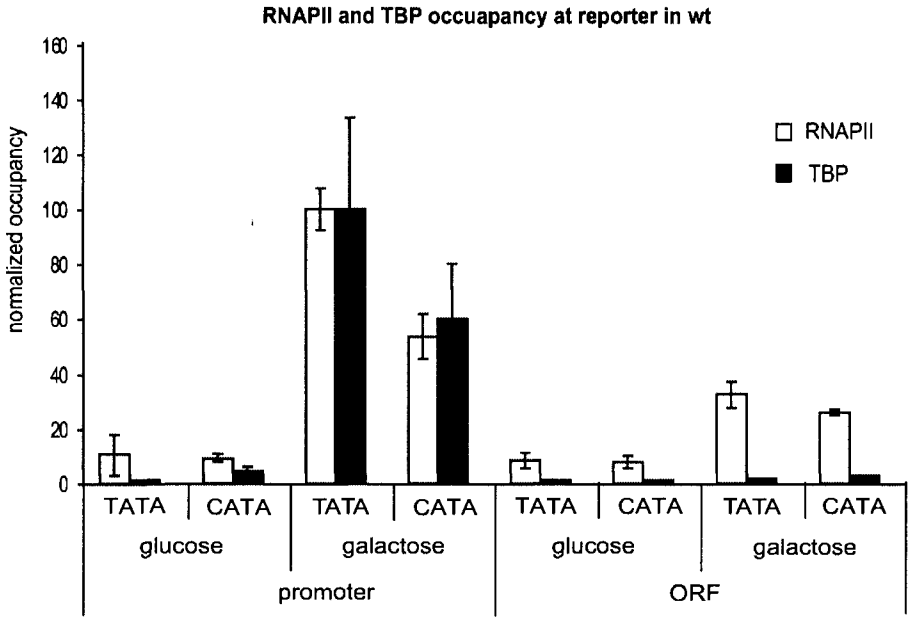
4.2.3 TBP and Gcn5 do not move from promoter into ORF with RNAPII.

As shown in Chapter 3, TBP, RANPII and SAGA subunits (Gcn5, Ada2 and Spt8) are recruited to the reporter promoters both TATA and CATA and acetylation level of histone H3K14 increases upon galactose induction. Next we wanted to know whether TATA-element-censoring function of SAGA occurs at ORF. ChIP assays were preformed to detect the occupancy of RNAPII TBP and Gcn5, and the acetylation state of H3K14 at the reporter promoter and ORF (Figure 4.3.a). In the wild type strain upon induction, TBP is recruited only to the promoter and TBP has no occupancy at ORF (Figure 4.3.b). RNAPII has occupancy at both promoter and ORF. At ORF RNAPII has relative lower occupancy than at promoter, which indicates that RNAPII is recruited to promoter and moves downstream into ORF. Gcn5 (figure 4.3.c) has the highest level of occupancy at CATA promoter. Even before induction, there is some Gcn5 occupancy at both promoters and ORFs, and upon induction Gcn5 occupancy at ORF does not change at either reporter, which suggests universal function of Gcn5 other than transcription regulation of Gal-dependent genes. SAGA can binds to the genomic DNA and acetylates nucleosomal histones randomly. The acetylation state of H3K14 at ORF has similar level no matter form TATA or CATA, or in glucose or galactose condition. Without Gcn5, the histone acetylation at H3K14 is totally diminished at promoter and ORF (figure 4.3.d).

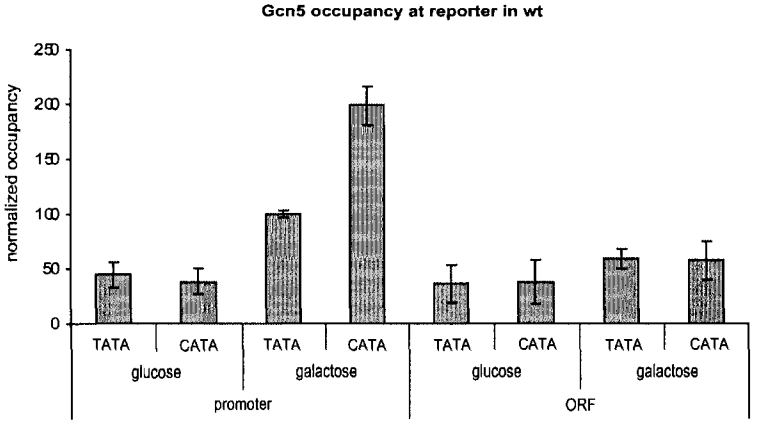
a


Gal4 UAS
TATAAA
or
CATAAA
tis
lac Z
promoter
ORF

b


RNAPII and TBP occuapancy at reporter in wt
normalized occupancy
RNAPII
TBP
TATA
CATA
glucose
galactose
promoter
ORF

c


Gcn5 occupancy at reporter in wt
normalized occupancy
TATA
CATA
glucose
galactose
promoter
ORF

d

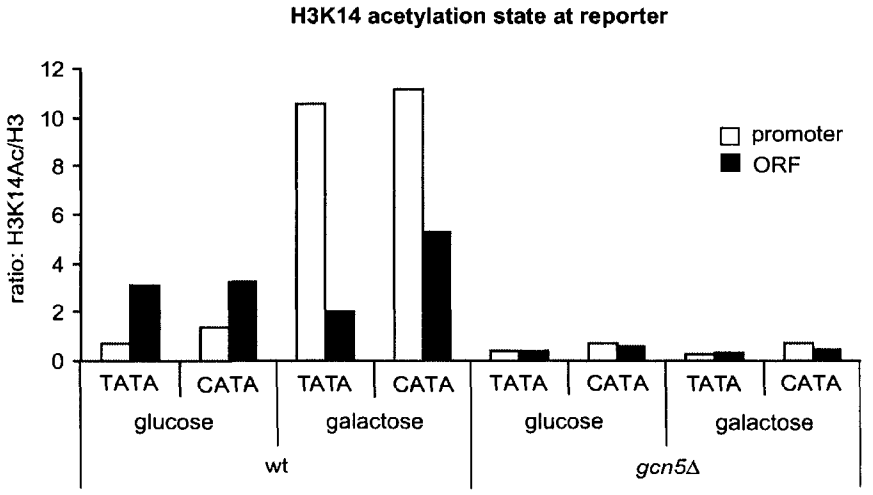


Figure 4.3 RNAPII has occupancy at both the promoter and the ORF, while TBP and SAGA only have increased occupancy at promoter. (a) Representation of the amplified fragments as promoter and ORF on reporter. (b) RNAPII and TBP occupancy. (c) Gcn5 occupancy. (d) Histone H3K14 acetylation state at promoter and ORF in wt and *gcn5Δ*.

4.2.4 Other factors are involved in core promoter differentiation.

We wanted to know whether other transcription regulatory factors also carry TATA-element-censoring function like SAGA. Additional deletion strains of certain factors that are involved in transcription regulation were tested for the change of expression from TATA and CATA. We found the Mediator complex is required for expression from TATA and CATA. Some deletion strains of Swi/Snf and RSC complexes both have the same expression pattern as *gcn5* deletion strain. Both Swi/Snf and RSC complexes are ATP-dependent chromatin remodeling complex and are involved in transcription regulation of the endogenous *GAL* genes. All these results suggest that the change of chromatin structure is involved in core promoter sequence censoring.

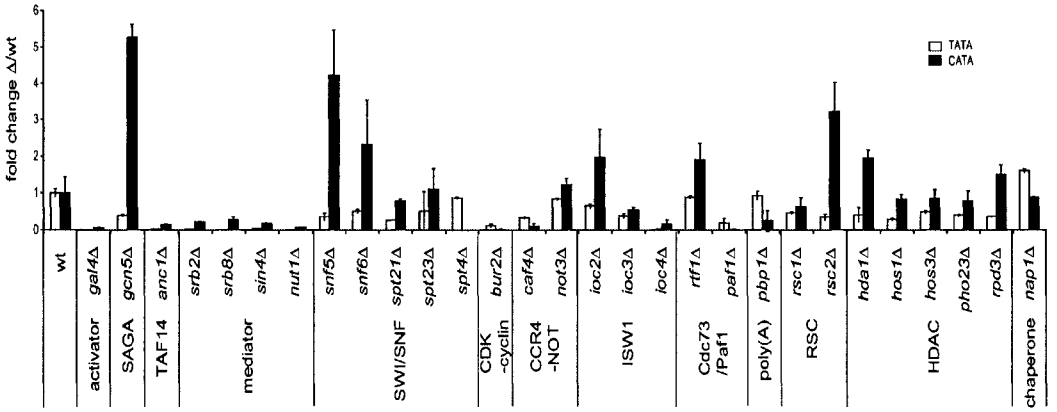


Figure 4.4 Genetic screen with the reporter system to identify other factors that are involved in core promoter sequence differentiation. Change of expression from TATA and CATA on the reporter in the deletion strains was abstained through galactosidase assays. The deleted factors are involved in transcription initiation regulation. Which complex the deleted factors belong is shown in the bottom of the panel.

4.3. Discussion

SAGA is required for *GAL* gene induction. Deletion of some SAGA subunits causes less recruitment of PIC to the promoter. Our transcription analyses show that in a short time period deletion of some SAGA subunits does lower *GAL* transcription, but in a long run the transcription levels with SAGA subunit deletion will get to the extend as in the wild type. Deletion of certain SAGA subunits only delays the induction of *GAL* genes.

Previous studies show some controversy observation about whether Gcn5 is required for TBP recruitment to the *GAL* promoters upon induction. Michael Green and colleagues showed that in gcn5Δ TBP has the same level of occupancy as in wild type strain upon galactose induction (Bhaumik and Green, 2002). Fred Winston and colleagues showed that with deletion of Gcn5, both the transcription level of *GAL1* and TBP occupancy at *GAL* promoter reduce to half of those in wild type upon galactose induction (Dudley et al., 1999). These can be explained with our kinetics results. Both of the labs only tested the TBP occupancy or transcription levels at single time points. Green lab used time point of 20 minutes after galactose induction and Winston lab used the time point of 3 hours. Our transcription assays in time course show the deletions of Gcn5, Ada2 or Spt8 only delay the transcription in the first 2 hours and after 2-hour galactose induction the transcription levels in these three deletion strains will reach the same or even higher levels than in wild type strain. Deletion of these three SAGA subunits only delay the recruitment of TBP to the core promoter, and TBP would be recruited to the

promoter to the same levels in the deletion strains as in the wild type strain eventually.

In the genetic screen, we found other factors in SAGA, Swi/Snf and RSC complexes can differentiate the two core promoter sequences. These 3 complexes are all involved in chromatin remodeling and modification and all these 3 complexes contain factors that have conserved bromo-domains (Hassan et al., 2006; Kasten et al., 2004; Syntichaki et al., 2000; VanDemark et al., 2007), which recognize acetylated histones and facilitate the complex to bind the nucleosomes. This suggests the nucleosome structure and histone position play a role in the TATA-element-censoring function carried by SAGA, Swi/Snf and RSC complexes.

Chapter 5

The role of Nap1 in Gal4-dependent transcriptional activation

5.1 Introduction

Histone chaperones play an important role in chromatin remodeling, histone assembly and disassembly, and chromosome formation. One of the well-studied histone chaperones is nucleosome assembly protein 1 (Nap1). Nap1 is 417 amino acids in length with an acidic C-terminal domain. The acidic carboxyl-terminal region of yNAP1, although dispensable for nucleosome assembly *in vitro*, contributes to binding via structure-independent electrostatic interactions. NAP1 has a preference for binding the (H3-H4)2 tetramer over the (H2A-H2B) dimer (McBryant et al., 2003). Yeast NAP1 interacts with the amino-terminal tails of H3 and H4 directly. Nap1 binds to one histone fold domain (McBryant et al., 2003; Park et al., 2005; Park et al., 2008). Nap1 is involved in the transport of H2A and H2B histones to the nucleus (Ishihama, 1981; Mosammaparast et al., 2002). Nap1 can bind core histones and p300. An *in vitro* study showed that Nap1 cooperates with CBP, a HAT in higher eukaryotes, in HTLV-1 activation (unpublished data from Dr Jennifer Nyborg and colleagues). Even though yeast does not have p300/CBP, the HAT domain and bromodomain in p300/CBP are highly conserved. Nap1 is involved in eviction of histones that are acetylated by CBP from HTLV-1 promoter. Also A proteomics study shows the interaction

between Nap1 and TBP by TAP-MS (Krogan et al., 2006). The new genomic study shows that Nap1 genetically interacts with SAGA subunits Sus1 and TAF12 (Collins et al., 2007). The connection between Nap1, HAT, TBP and SAGA suggests that the histone chaperone Nap1 might be involved in transcription regulation by SAGA complex.

SAGA regulates many genes in the yeast genome (Huisinga and Pugh, 2004; Lee et al., 2000). It plays important functions at both recruitment-regulated promoters (like the *GAL* gene promoters) and post-recruitment regulated genes (like *CYC1*). In the presence of glucose, *CYC1* gene has basal levels of transcription and is referred to as partially repressed and unlike *GAL* genes, TBP and RNAPII are present on the *CYC1* gene (Kuras and Struhl, 1999; Martens et al., 2001). The gene is fully activated when the cell is grown on a non-fermentable carbon source. TBP and RNAPII occupancy increases marginally (less than two-fold) with ten-fold higher transcription level (Guarente et al., 1984; Guarente and Mason, 1983). Studies in our lab also show that *CYC1* promoter is occupied by SAGA before induction, and the occupancy does not change in the induced condition (Fletcher et al., 2007; Zhang et al., 2008).

In the previous chapters and other studies (Govind et al., 2007), it has been shown that histone eviction from promoters is part of yeast *GAL* gene activation process. It is possible for Nap1 to be involved in histone deposition and eviction at the *GAL* gene promoter during galactose induction. In this chapter, I describe the investigation of Nap1 function at *GAL* genes and the

relationship of Nap1, RNAPII, histone, and SAGA occupancies at the recruitment-regulated promoter and post-recruitment promoter.

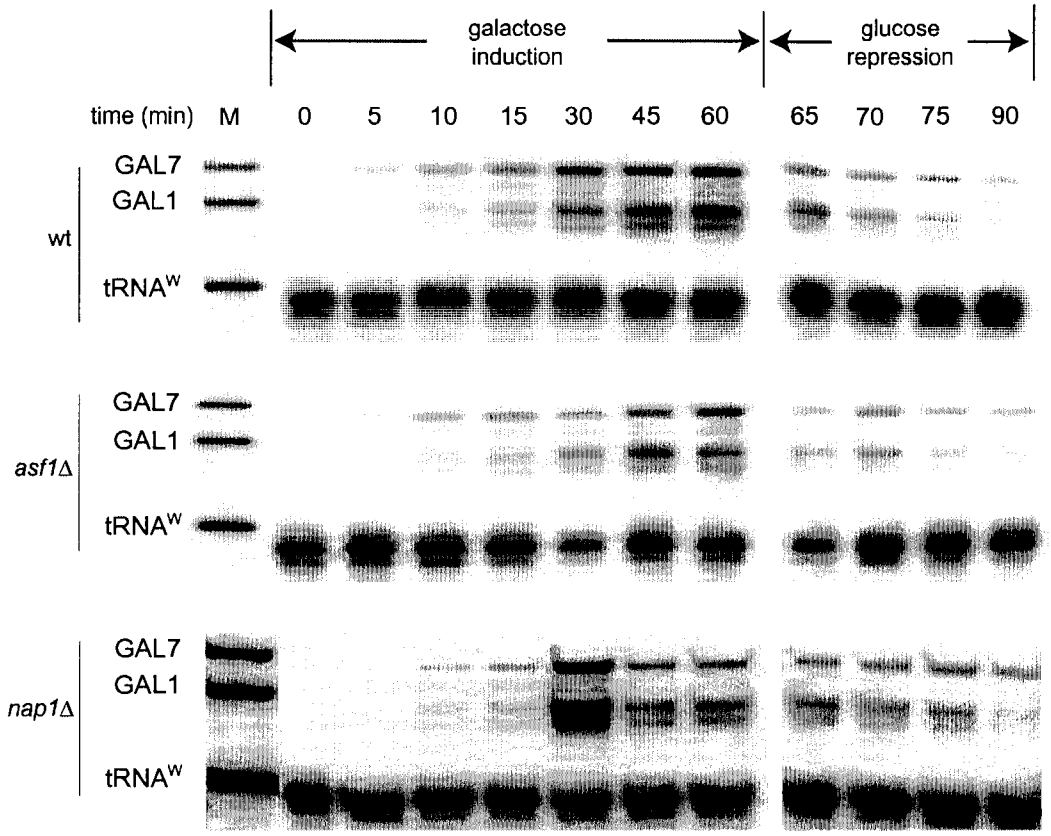
5.2 Results

5.2.1 Deletion of Nap1 changes the induction kinetics of *GAL* genes.

The ChIP result of histone occupancy in Chapter 3 and Chapter 4 indicate that histones are evicted from promoters in the process of Gal4-dependent gene activation. Histone chaperone may be involved in this process. We wanted to test whether deletion of the histone chaperone would affect the histone occupancy at promoters. S1 nuclease protection assays were performed to measure the impacts of histone chaperone deletion on the induction and repression transcription kinetics of *GAL1* and *GAL7*. We measured the mRNA levels of wild type strain, *nap1Δ* and *asf1Δ* during 1-hour galactose induction followed by 30-minute glucose repression. We chose *asf1Δ*, because Asf1 is another well-studied histone chaperone, which has a universal function of histone assembly and disassembly (Adkins et al., 2004; Green, 2005; Robinson and Schultz, 2003; Schwabish and Struhl, 2006). Deletion of Nap1 and deletion of Asf1 change *GAL1* and *GAL7* induction and repression kinetics in different patterns (Figure 5.1). In the wild type strain upon galactose induction, expression of both *GAL7* and *GAL1* reaches the maximal level at 45 min and remains at this level. In the glucose repression, the RNA levels of both *GAL7* and *GAL1* decrease rapidly within 5 minutes. In *asf1Δ, GAL* gene induction has a slower kinetics and a lower level of transcription compared to the wild type strain. In *nap1Δ, GAL* genes have a fast induction between 15 to 30 minutes. The expression levels reach the

peak at 30 min and then soon drop later in galactose condition to the same level as in *asf1Δ*. In glucose repression, transcription level in *nap1Δ* also drops rapidly but has a higher than in wild type strain and *asf1Δ*. Within each strain, *GAL7* and *GAL1* behave the same. The change of *GAL* gene transcription kinetics with histone chaperone deletion suggests that histone chaperones are involved in the process of gene activation. The different kinetics in *nap1Δ* and *asf1Δ* indicates that Nap1 and Asf1 have different functions in the *GAL* gene induction and repression.

a


galactose induction
glucose repression
time (min)
M
0
5
10
15
30
45
60
65
70
75
90
wt
GAL7
GAL1
tRNA^W
asf1Δ
GAL7
GAL1
tRNA^W
nap1Δ
GAL7
GAL1
tRNA^W

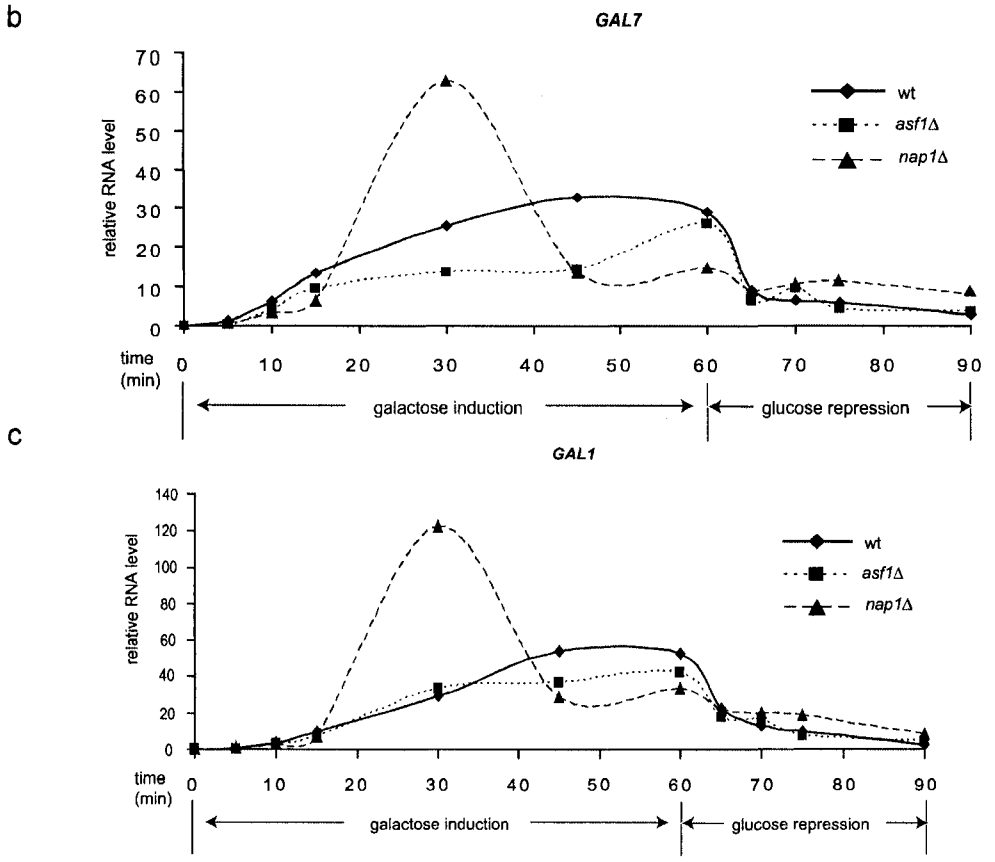
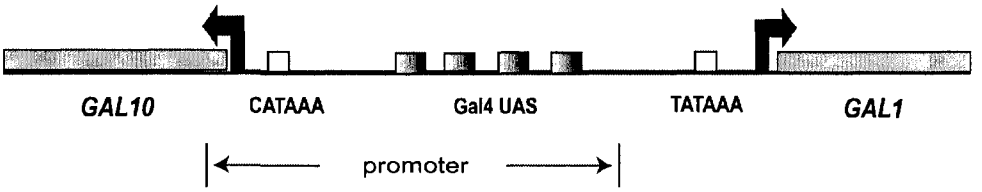


Figure 5.1 Deletion of histone chaperone changes the induction kinetics of *GAL7* and *GAL1*. (a) S1 nuclease protection assay in time course was used to analyze the expression levels of *GAL7* and *GAL1* in wild type, *asf1Δ* and *nap1Δ* during 1-hour galactose induction followed with glucose repression. (b) and (c) Quantification of normalized RNA levels of *GAL7* and *GAL1*.

5.2.2 Nap1 has increased occupancy at *GAL1,10* promoter upon galactose induction.

Our data suggest that Nap1 is involved in *GAL* gene activation. It was not known whether Nap1 interacts directly with promoter DNA. Therefore, we used the ChIP assays to detect Nap1, PIC and histone occupancy at *GAL1,10* promoter in wild type cells. The occupancies of TBP, RNAPII and acetylated histone H3 (K9 and K14) at *GAL* promoter increase significantly upon galactose induction and the occupancy of H3 drops upon induction due to histone eviction (Figure 5.2). In the uninduced condition, *GAL* promoter has some Nap1 occupied and upon galactose induction Nap1 occupancy doubles. Thus like TBP and RNAPII, as well as SAGA, Nap1 occupancy increases at Gal4-dependent promoters upon induction. This suggests that upon induction, the density of Nap1 with promoter increases, and there are more Nap1-related histone exchanges upon induction, which correlates with histone eviction from the promoter.

a



b

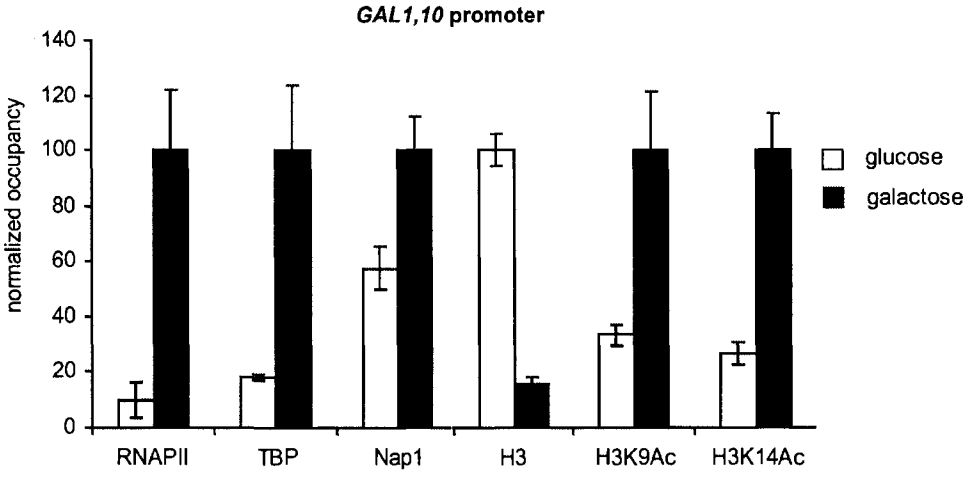
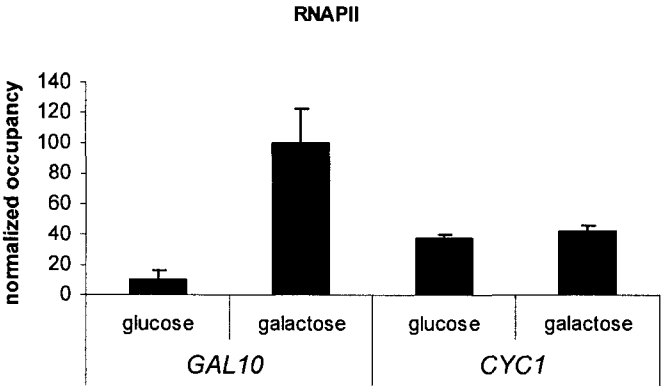


Figure 5.2 Nap1 has increased occupancy at *GAL1,10* promoter with histone acetylation and eviction upon induction. (a) Representation of *GAL1,10* locus. The amplified region of the *GAL1,10* promoter in ChIP assay is shown. (b) ChIP analysis of RNAPII, TBP, Nap1, H3K9Ac, H3K14Ac and H3 occupancy during galactose induction.

5.2.3 Nap1 occupancy correlates with SAGA occupancy and diminishes histone occupancy.

It is still not known which complex causes the increased Nap1 occupancy upon induction. The connections between Nap1, TBP and HAT suggest the interaction of Nap1 and SAGA. Also at the *GAL 1,10* promoter, Nap1 occupancy increases upon induction with histone eviction. We wondered whether the occupancy of Nap1 still increased if there was no histone at the promoter before full induction. As mention before, *CYC1* is the post-recruitment regulated promoter and is nucleosome free. Then we used ChIP analysis to detect Nap1 occupancy at *CYC1* promoter in galactose induction (Figure. 5.3). IN contrast to the recruitment promoter *GAL1,10*, upon glucose induction, the occupancies of RNAPII, histone H3 and Nap1 do not change. Nap1 occupancy does not correlate with transcription or RNAPII recruitment. Nap1, like SAGA, maintains unchanged high-level occupancy before and after induction. This suggests interaction between Nap1 and SAGA. At *CYC1* and *GAL1,10* promoters, Nap1 and histone have opposite patterns at both recruitment and post-recruitment promoters. High levels of Nap1 occupancy accompany with low level of histone occupancy,

a



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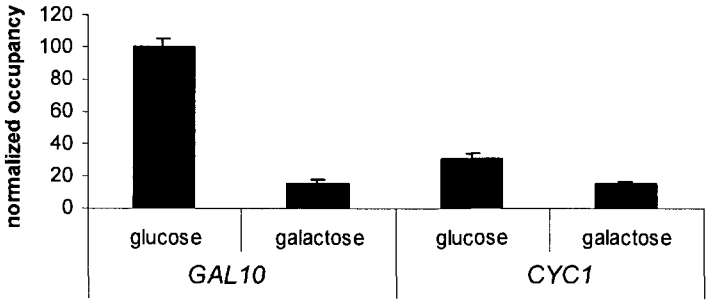

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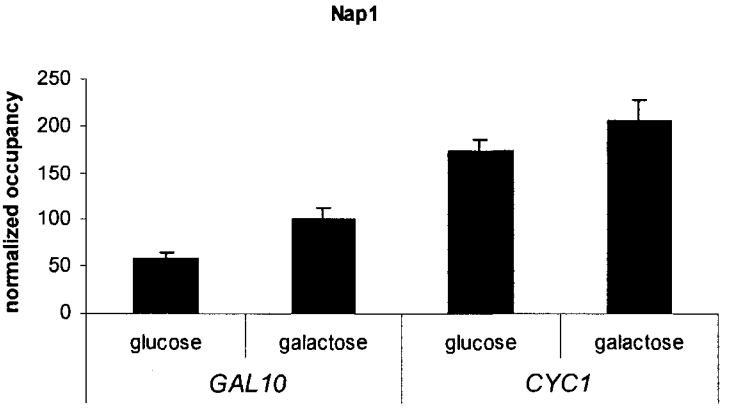
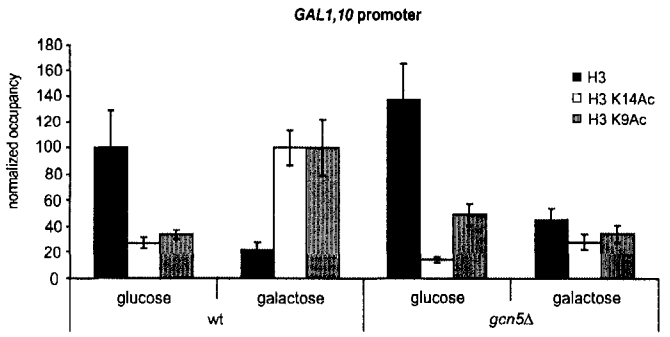


Figure 5.3 Comparison of occupancy of RNAPII, histone and Nap1 at *GAL1,10* promoter and *CYC1* promoter. ChIP analysis of RNAPII (a), histone H3 (b) and Nap1 (c) occupancy at *GAL1,10* and *CYC1* promoters in glucose condition and 3-hour galactose induction.

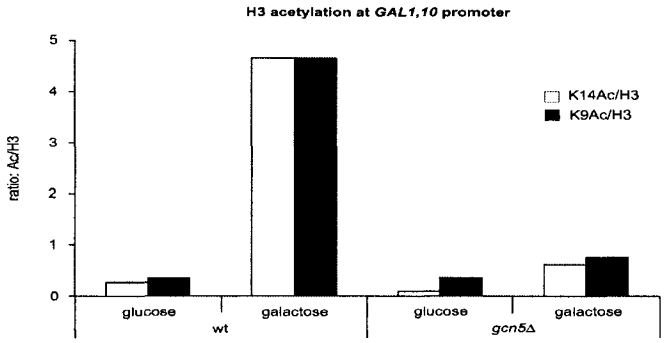
5.2.4 Gcn5 is not required for histone eviction from *GAL1,10* promoter.

In chapter 3 we showed that histone acetylation by Gcn5 occurs upon induction at the Gal4-dependent reporter promoter. Our ChIP results also show that histone H3 acetylation at K9 and K14 by Gcn5 and histone eviction also occur at the endogenous *GAL1,10* promoter (Figure. 5.4. a and b). Upon induction, the occupancy of total H3 decreases in wild type and *gcn5Δ*. The occupancies of acetylated H3K14 and K9 increase in wild type strain. The acetylation of H3K14 and K9 is abolished in *gcn5Δ* strain. However with Gcn5 deletion, histones are still evicted from *GAL* promoter, suggesting that acetylation at H3K9 and K14 is not required for histone eviction, and other factors are involved. Galactosidase assays in the report promoter show that other HATs are required for high levels of expression from the reporter promoter (Figure 5.4.c.). This indicates that histone acetylation at lysine residues other than K9 and K14 may be involved in histone eviction upon induction.

a



b



c

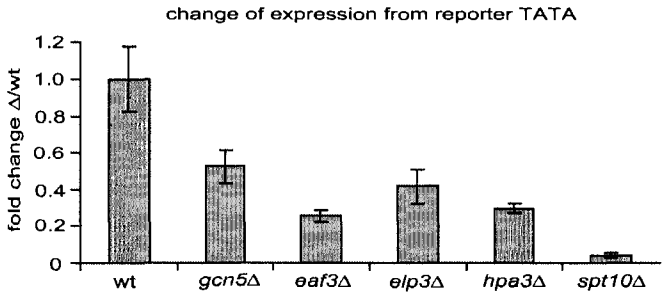


Figure 5.4 HAT activity carried by factors other than Gcn5 is important for Gal4-dependent transcriptional induction. (a) ChIP assays of histone H3 and to acetylated histone H3 K14 and K9 occupancy during galactose induction in wild type strain and *gcn5Δ*. (b) Ratios of H3K14Ac and H3K9Ac to the total histone H3 are used to represent the histone acetylation state. (c) Galactosidase analysis in other HAT deletion strains show a reduced expression from the consensus core promoter sequence TATA of the reporter.

5.3 Discussion

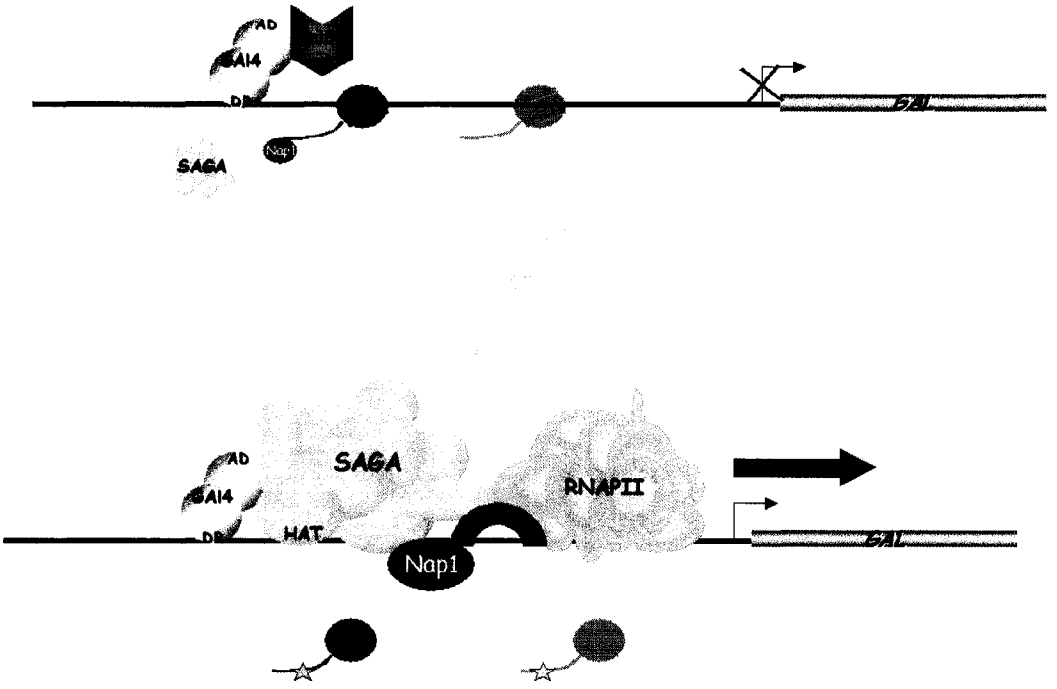
As an important part of transcription, histone eviction involves histone modification, chromatin remodeling and interaction with histone chaperones. We investigated the function of HATs and histone chaperones in *GAL* gene induction. We identified impact on deletion of histone chaperones to the transcription of endogenous *GAL* genes. Deletion of Asf1 reduces the expression level of *GAL1* and *GAL7*. Deletion of Nap1 makes GAL genes super-activated between the time points of 15 minute and 30 minute. Also deletions of *NAP1* and *ASF1* show different change to induction kinetics of *GAL1* and *GAL7* transcription, which suggests the different functions of these two chaperones and indicates that the histone assembly at the promoters can affect transcription directly. A recent study also shows that Nap1 is also involved in *PHO5* transcription regulation. The expression level of *PHO5* also increases in *nap1Δ* strain(Del Rosario and Pemberton, 2008).

Our ChIP data on recruitment and post-recruitment promoters show Nap1 occupancy is not related to transcription levels or RNAPII occupancy, but has same pattern of SAGA occupancy and opposite pattern of histone, suggesting interaction between Nap1, SAGA and histone eviction during galactose induction. The possible functions of Nap1 in *GAL* gene induction and repression the wild type strains are listed in table 5.1. The model of the change of occupancy or SAGA, NAP1, TBP and RNAPII is shown in figure 5.5.

Table 5.1 The possible functions of Nap1 in *GAL* gene induction and repression.

Condition	Wild type strain	*nap1Δ*
Uninduced	Histones (in nucleosomes) are positioned at promoter and ORF. **Nap1 function: histone deposition**	Histones are not position correctly or even not positioned at promoter an ORF.
Induced	Nap1 has increased occupancy, accelerating histone exchange. Histones are evicted from DNA Transcription machinery can be recruited to promoter and move along ORF. **Nap1 function: histone eviction**	Promoters are "naked" or "open", more accessible for transcriptional machinery. Genes are easier to get activated (super activated).
Repressed	Histones get assembled into nucleosomes at promoter and ORF. **Nap1 function: histone deposition**	Without the chaperone Nap1, deposition of histones back to DNA is impaired.

a


AD
GAL4
DB
Nap1
SAGA
GAL
AD
GAL4
DB
SAGA
HAT
RNAPII
Nap1
GAL

b

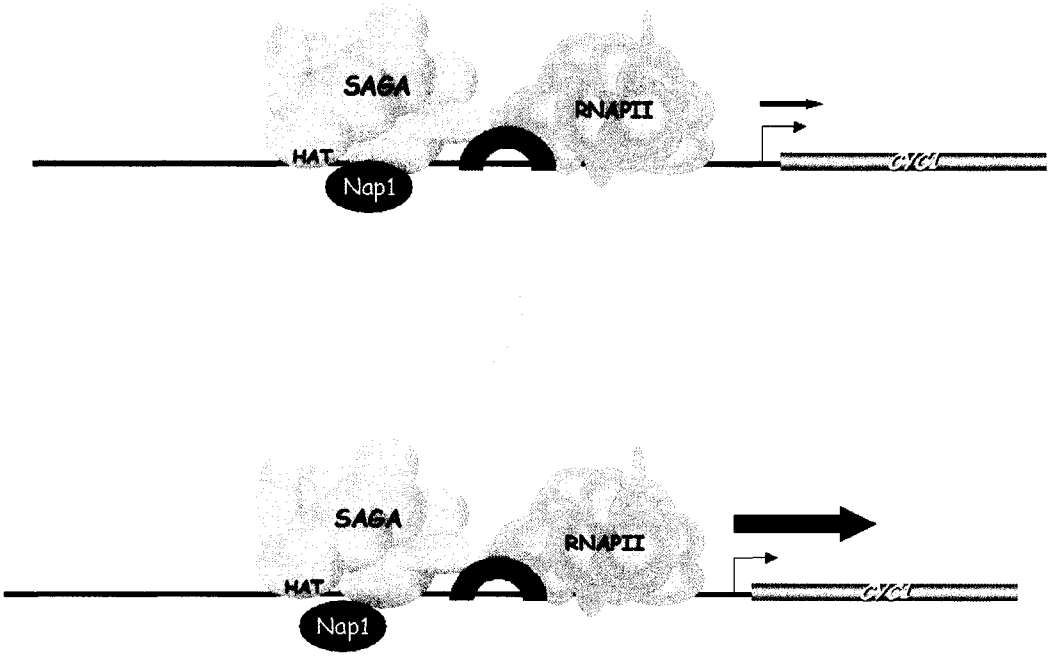

SAGA
HAT
RNAPII
Nap1
CYC1
SAGA
HAT
RNAPII
Nap1
CYC1

Figure 5.5 the model of factor occupancies at the recruitment-regulated promoter *GAL1,10* and the post-recruitment regulated promoter *CYC1*. (a) At the GAL1,10 promoter, before induction, there is no expression of the gene. The activator Gal4 binds to UAS with it activation domains blocked by Gal80. Promoter DNA is wrapped into nucleosomes. TBP and RNAPII do not have occupancy. SAGA and Nap1 have background occupancy, which is related to their global (non-induction- related) functions. Upon induction, the activation domain of Gal4 is exposed and recruits SAGA to the promoter. Nap1 has increased occupancy. Then TBP and RNAPII are recruited. The histones in the nucleosomes first are acetylated by SAGA, and then get evicted from the promoter with the help of other factors. The *GAL* genes undergo a high level of expression. (b) For the post-recruitment regulated *CYC1* promoter, before fully induction, all of SAGA, Nap1, TBP and RNAPII have high levels of occupancy at the promoter. There is only a minimal level of transcription of *CYC1*. Upon induction, the occupancy of SAGA, Nap1, TBP and RNAPII remains almost the same levels, while there is a huge increase of the transcription of *CYC1*.

In the future we will perform in vivo and in vitro experiments to further characterize the interaction between Nap1 and SAGA in transcription regulation. The genomic study shows that Nap1 genetically interacts with SAGA subunits Sus1 and TAF12 (Collins et al., 2007). We find that the occupancy of Nap1 is similar to SAGA occupancy on both recruitment and post-recruitment promoters, suggesting that Nap1 might be recruited to promoter by SAGA. To test this, we can perform ChIP assay to check Nap1 occupancy at *GAL1,10* and *CYC1* promoters in deletion strains of *spt3Δ* and *spt20Δ*, in which the whole SAGA complex is destroyed. Also we can perform ChIP assay to detect Nap1 occupancy and acetylation levels of histone at different lysine residues in HAT deletion strains (especially *gcn5Δ*). Thus we will know whether Nap1 function required histone acetylation, and if so, which lysine residue is important. Since we find that Nap1 occupies the *CYC1* promoter, it is possible that Nap1 is involved in *CYC1* transcription activation. We can investigate the impact of Nap1 deletion to *CYC1* induction kinetics via S1 nuclease protection assay in time course as we did on *GAL* genes described in this chapter. We are also very interested to determine which of the above functions are maintained in Nap1 mutants already generated and characterized in vitro by Luger and colleagues (McBryant et al., 2003; Park et al., 2005; Park et al., 2008). Thus, we may be able to separate the multi-functions of Nap1 to its domains.

We can also use co-immunoprecipitation assays to check the physical interaction between Nap1 and SAGA *in vivo*. We will also perform pull-down assays in vitro with purified SAGA, recombinant Nap1, and histones. This will

allow us to test the affinity of the interactions, as well as the role of histones and acetylation in the interactions. We can also examine whether the interactions are DNA dependent by using the *GAL* promoter and purified Gal4 with the above components (Nap1, SAGA) in a gel shift assay to investigate whether DNA is required for the interactions. The yeast whole cell extract *in vitro* transcription system in our lab can be tested with Nap1, Gcn5 (or SAGA), and histones can be used to detect whether yeast Nap1 cooperates with Gcn5 to regulate transcription activation, as Dr. Jennifer Nyborg and colleagues did in HTLV-1 system.

Chapter 6

Significance of the work and future perspectives

In this study a new function of SAGA complex, CATA censoring was found. Multiple subunits of SAGA complex (Spt8, Gcn5 and Ada2) can differentiate the consensus and off-consensus core promoter sequences and enlarge the difference of the strength of promoters with variant sequences. SAGA complex enhances the expression from the consensus core promoter sequence and represses that from the off consensus sequences. The CATA-censoring function of SAGA complex is not only specific to the core promoter with CATAAA sequence, also to the other off consensus sequences in the reporter system. Also this new function of SAGA can be found to the endogenous *GAL* genes.

Our results suggest that complex lacking Gcn5, Ada2 or Spt8 would enhance expression from off-consensus core promoter. Interestingly, SLIK complex shares 9 subunits with SAGA, but lack of Spt8. It is possible that SLIK, like SAGA, has the TATA-element-censoring function. Little is known about SLIK–specific function. It is possible that SAGA/SLIK censor core promoter sequence: SAGA regulates transcription from consensus core promoter, and SLIK regulates transcription driven by off-consensus TATA elements. This can be tested by comparing the TATA-element sequences of SLIK-regulated and SAGA-regulated genes. Rtg2 chip-on-ChIP assays will allow us to find

SLIK-regulated genes, which have promoters occupied with Rtg2 (SLIK-specific subunit).

In our study HAT activity of Gcn5 is required for the CATA-censoring function. However the acetylation levels of the prime Gcn5 HAT targets H3 K9 and K14 have no differences between the consensus and off consensus core prompters. This suggests that non-HAT function of Gcn5, or other Gcn5 target histone H2B K11/K16, H3 K18/K23/K27 or even non-histone proteins are involved in this CATA-censoring function of SAGA complex. Although it is not clear whether yeast Gcn5 acetylate non-histone proteins, recent study found that Drosophila Gcn5 could acetylate ATP-dependent histone remodeling factor ISW1 at lysine 753 (Ferreira et al., 2007).

Through the genetic screen other complexes (Swi/Snf and RSC) were also found with the CATA-censoring function. Both of these two complexes are involved in ATP-dependent chromatin remodeling. This suggests that the chromatin structure and histone assembly are related to the CATA-censoring function.

As mentioned in the previous chapters, in the whole yeast genome, about 20% of genes contain TATA element in their promoters and these genes respond to the changes of environment and are regulated by SAGA complex instead of TFIID (Huisinga and Pugh, 2004) (Basehoar et al., 2004). The newly found CATA-censoring function of SAGA complex in this study explains how SAGA tells the differences between the variant core promoter sequences

and regulates the expression from these core promoter sequences with priority of the consensus one. The human genomic study also found in human genome only 30% of gene contains TATA elements (Yang et al., 2007). The human SAGA complex and it homologues may also have the CATA-censoring function in transcriptional regulation. The new genomic study shows that human gene promoters share a lot similarity with the yeast promoters. In human genome about 24% of core promoters contain TATA elements (Yang et al., 2007). These TATA-containing genes are highly regulated, by biotic or stress stimuli. Only 8% of the promoters in the genome are TATA-only promoters, which contain only TATA element, but not INR. The products of the TATA-only genes tend to be localized in the extracellular space. The other 76% of the core promoter lack the AT-rich TATA-like sequences (Tirosh et al., 2007; Yang et al., 2007). HIV-1 promoter contains CATA instead of TATA for optimal transcription regulation and replication (van Opijnen, 2004). Off-consensus core promoter sequences probably have higher percentage than the results from the genomic promoter studies. Whether in higher eukaryotes, these TATA-containing, highly regulated genes are regulated by SAGA or other similar transcriptional regulatory complexes is not known.

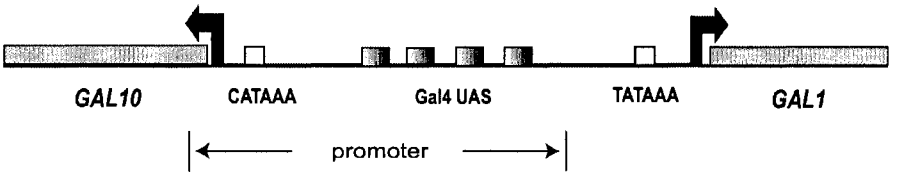
In chapter 5, it is shown the impacts of deletion of histone chaperones to the transcription of endogenous *GAL* genes. Also deletions of Nap1 and Asf1 show different change to induction kinetics of *GAL1* and *GAL7* transcription, which suggests the different functions of these two chaperones and indicates that the histone assembly at the promoters can affect transcription directly. Our ChIP data on recruitment and post-recruitment promoters show Nap1

occupancy is not related to transcription levels or RNAPII occupancy, but has same pattern of SAGA occupancy and opposite pattern of histone, suggesting interaction between Nap1, SAGA and histone eviction during galactose induction. In the future we will perform in vivo and in vitro experiments proposed in Chapter 5 to further characterize the interaction between Nap1, SAGA and histones in transcription regulation.

Appendix I

In previous chapters it is shown that deletion of SAGA subunits affect induction kinetic of the endogenous *GAL1* and *GAL10* genes. A lot of literature also have shown that SAGA subunits are required for TBP and RNAPII recruitment to *GAL1,10* promoter. Thus with our system, we also did ChIP assays to check the occupancy of TBP and RNAPII at endogenous *GAL* promoter as a positive control. As expected, both TBP and RNAPII occupancy reduces in the SAGA deletion strains (figure A).

a



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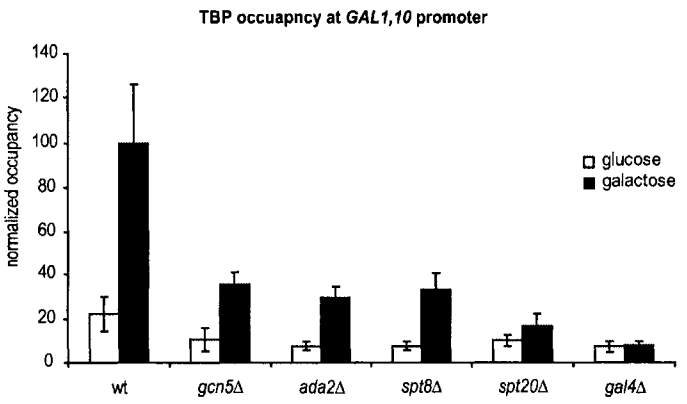

C

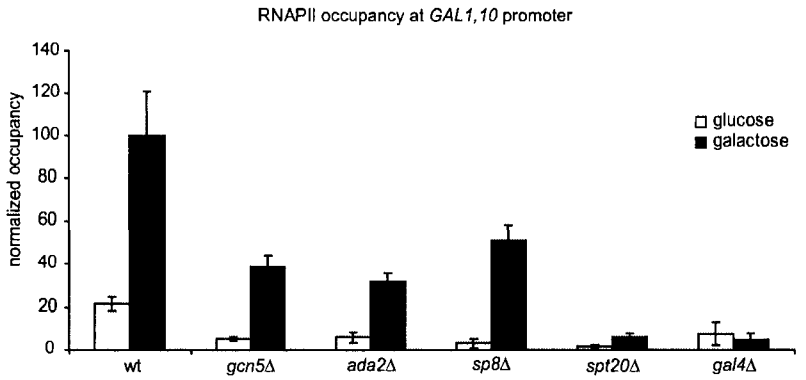

Figure A **Deletion of SAGA subunits reduces RNAPII and TBP occupancy at endogenous *GAL1,10* promoter at the time point of 3-hour galactose induction.** (a) The represent of *GAL1,10* promoter and the amplified fragment in ChIP. (b) TBP and (c) RNAPII occupancy in wt and SAGA deletion strains.

Appendix II

Non-optimal TATA Elements Exhibit Diverse Mechanistic Consequences

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Abstract:

To reveal mechanistic differences in transcription initiation between variant TATA elements, *in vivo* and *in vitro* assays of the functional activity of 14 different sequences were compared. Variant elements exhibited particular degrees of activation *in vivo* but universally were unable to support the -fold activation observed for an element consisting of TATAAA. Each element was classified by its functional activity for *in vitro* interaction with TATA-binding protein (TBP), TFIIA, and TFIIB. Certain off-consensus TATA elements form poor binding sites for TBP and this compromised interaction interferes with higher order complex formation with TFIIA and/or TFIIB. Other elements are only modestly decreased for TBP binding but dramatically affected for higher order complex formation. Another distinct category is comprised of two elements (CATAAA and TATAAG), which are not affected in the initial formation of the TBP, TFIIA-TBP, or TFIIB-TBP complexes. However, CATAAA and TATAAG are unable to form a stable TFIIA-TBP-DNA complex *in vitro*. Moreover, fusion of TFIIA to TBP specifically restores activity from

these two elements *in vivo*. Taken together, these results indicate that the interplay between the sequence of the TATA element and the components of the general transcription machinery can lead to variations in the formation of functional complexes and/or the stability of these complexes. These differences offer distinct opportunities for an organism to exploit diverse steps in the regulation of gene expression depending on the precise TATA element sequence at a given gene.

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