

Dissecting the Regulatory Circuitry of a Eukaryotic Genome

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Summary

Genome-wide expression analysis was used to identify genes whose expression depends on the functions of key components of the transcription initiation machinery in yeast. Components of the RNA polymerase II holoenzyme, the general transcription factor TFIID, and the SAGA chromatin modification complex were found to have roles in expression of distinct sets of genes. The results reveal an unanticipated level of regulation which is superimposed on that due to gene-specific transcription factors, a novel mechanism for coordinate regulation of specific sets of genes when cells encounter limiting nutrients, and evidence that the ultimate targets of signal transduction pathways can be identified within the initiation apparatus.

Introduction

Much of biological regulation occurs at the level of transcription initiation. Genes contain promoter sequences that are bound by transcriptional activators and repressors (Struhl, 1995; Ptashne and Gann, 1997). Activators recruit the transcription initiation machinery, which for protein-coding genes consists of RNA polymerase II and at least 50 additional components (Orphanides et al., 1996; Roeder, 1996; Greenblatt, 1997; Hampsey, 1998; Myer and Young, 1998). The transcription initiation machinery includes factors that bind to DNA, cyclin-dependent kinases that regulate polymerase activity, and acetylases and other enzymes that modify chromatin (Burley and Roeder, 1996; Kingston et al., 1996; Roth and Allis, 1996; Steger and Workman, 1996; Tsukiyama and Wu, 1997; Hengartner et al., 1998; Struhl, 1998).

Our understanding of eukaryotic gene expression remains limited in several ways. The complete set of transcriptional regulators has yet to be identified. How these

regulators interact with and regulate components of the transcriptional machinery is not yet clear. The functions of just a fraction of the components of the transcriptional machinery are understood, and then only with respect to a small set of genes. Cells must adjust genome expression to accommodate changes in their environment and in their programs of growth control and development, but precisely how coordinate remodeling of genome expression is accomplished for signal transduction pathways or for the cell cycle clock has yet to be learned.

Genome-wide expression monitoring has recently become feasible with the description of complete genome sequences and through the development of cDNA and high-density oligonucleotide array technology (Chee et al., 1996; Lockhart et al., 1996; DeRisi et al., 1997; Lashkari et al., 1997; Wodicka et al., 1997). Expression profiling has been used to examine differences in gene expression when yeast are grown in various media (Wodicka et al., 1997) and has revealed how yeast genome expression is remodeled during the metabolic shift from fermentation to respiration (DeRisi et al., 1997) and during the cell cycle (Cho et al., 1998). Expression profiling is also being used to improve our understanding of various aspects of human biology and disease (DeRisi et al., 1996; Schena et al., 1996) and to facilitate drug development (Gray et al., 1998). The data generated with genome-wide expression monitoring technology describes the level of each mRNA species in a population, but this data alone does not always produce significant new biological insights. Our knowledge of genome-wide transcriptional regulation is incomplete, making it difficult to understand how such genome-wide expression signatures transpire.

We are exploring the ability of genome-wide expression analysis to provide insights into the transcriptional regulatory circuitry of eukaryotic cells. Such study should provide the foundation and context for interpreting mechanistic studies in control of gene expression. One approach to this problem is to identify the set of genes regulated by each promoter-binding transcription factor. However, if the transcription initiation apparatus itself plays an important role in regulation of gene expression, then it is important to determine the extent to which each gene in the genome depends on the function of key components of the transcription machinery for its expression. We describe here the mRNA population of yeast cells, the requirement for key components of the transcriptional machinery in expression of this population, the observation that certain components of the general apparatus are themselves regulated when cells encounter limiting nutrients, evidence that the ultimate targets of signal transduction pathways can be identified within the initiation apparatus, and additional insights into genome-wide regulatory circuitry. It is well recognized that in eukaryotes transcriptional control is in large part due to the combinatorial action of promoter-specific activators at enhancers and promoters. Our results reveal that the general transcription factors add an additional level of combinatorial control of eukaryotic gene expression.

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Results

Features of the Study

The study described here was designed to assess the requirement for key components of the RNA polymerase II transcriptional machinery. This was accomplished by using high-density oligonucleotide arrays (HDAs) (Wodicka et al., 1997) to determine the effects of mutations in these components genome wide. Detailed information and databases supporting all aspects of this study can be found on the World Wide Web at <http://www.wi.mit.edu/young/expression.html>.

The Yeast Transcriptome

Knowledge of the levels of all detectable mRNA species in yeast is useful for evaluating the degree to which these levels depend on any one component of the transcription apparatus. To obtain this information and to assess the reproducibility of the HDA technology, RNA was harvested from two independent wild-type cultures and compared using two sets of HDAs on 2 separate days. The HDAs used here can score mRNA levels for up to 6181 genes. Of the 5460 genes whose mRNA levels were accurately determined and compared in both experiments, 99% of the mRNAs differed no more than 1.7-fold, and only 35 transcripts (0.65%) showed more than a 2-fold change. In order to prevent these minimal variations from influencing the results, all experiments were performed in duplicate. The levels determined for the 5460 transcripts in wild-type yeast cells and additional information derived from this experiment can be found under Yeast mRNA Population on the web site. The SAGE method has previously been used to determine values for 4465 transcripts, the result of which has been termed the yeast transcriptome (Velculescu et al., 1997). The sensitivity of the HDA technology permitted a determination of the levels of many additional gene products and revealed that transcripts from 80% of expressed yeast genes exist at levels of 0.1 to 2 molecules/cell.

Dependence of Genome Expression on Key Components of Transcriptional Machinery

At any one promoter, the transcriptional machinery might include the RNA polymerase II core enzyme, the general transcription factors (GTFs), the core Srb/mediator complex, the Srb10 CDK complex, the Swi/Snf complex, and the SAGA complex, among others (Figure 1). One or more subunits of each of these components has been investigated for its role in genome-wide gene expression through the use of mutations that affect either the function or the physical presence of the subunit (Table 1). Loss-of-function mutations in various components of the transcription apparatus were constructed or obtained from various investigators (see Study Design on the web site for details). Two types of mutations have proven to be useful in this study. For essential components of the apparatus, temperature-sensitive (ts) mutations are valuable because they allow the investigator to examine effects on gene expression at any point after inactivating the factor. For nonessential components, we have used either point mutations, which knock out

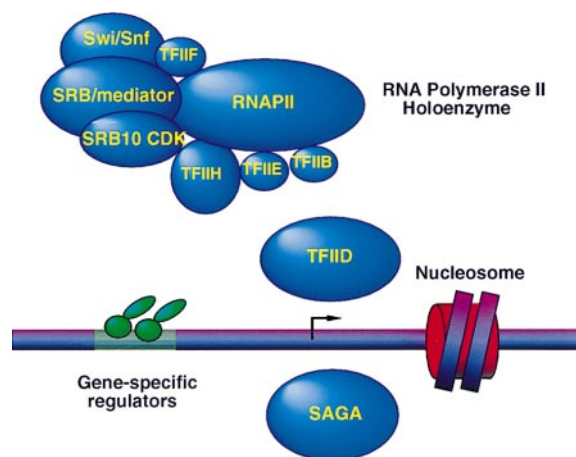


Figure 1. Model of RNA Polymerase II Transcription Initiation Machinery

The machinery depicted here encompasses over 85 polypeptides in ten (sub) complexes: core RNA polymerase II (RNAPII) consists of 12 subunits; TFIH, 9 subunits; TFIIE, 2 subunits; TFIIF, 3 subunits; TFIIIB, 1 subunit; TFIID, 14 subunits; core SRB/mediator, more than 16 subunits; Swi/Snf complex, 11 subunits; Srb10 kinase complex, 4 subunits; and SAGA, 13 subunits (see web site for more details). As detailed in Table 1, representative subunits of these complexes were chosen for analysis of genome-wide transcription dependence.

the catalytic function of known enzymatic activities, or complete deletion mutations. In each experiment, a mutant cell and its isogenic wild-type counterpart are grown to mid-log phase, the two populations are harvested, RNA is prepared, and hybridization to HDAs is carried out, all in duplicate.

Dependence on Core RNA Polymerase II

To determine the genome-wide dependence of gene expression on core RNA polymerase II, RNA was isolated from an *rpb1-1* ts cell and its wild-type counterpart 45 min after a shift to the nonpermissive temperature and was hybridized to HDAs. Because *rpb1-1* cells shut down transcription of protein-coding genes immediately after a temperature shift, these cells have been used by us and other investigators to determine the half-life of various yeast mRNAs (Nonet et al., 1987; Herrick et al., 1990). The 45 min time point was used for the analysis of all ts mutants in this study because it is sufficiently long to detect a significant (i.e., a 2-fold or more) loss of mRNA levels for 94% of detectable gene products without any loss of rRNA (Nonet et al., 1987). In addition, the 45 min time point is short enough to minimize the potentially complicating effects of cell cycle arrest and cell death.

The results of genome-wide expression analysis of the *rpb1-1* mutant as compared to an isogenic wild-type strain are shown in a grid format in Figure 2A. The grid shows the change in mRNA level for each gene, beginning with the left-most gene on chromosome I and proceeding in a linear fashion, left to right, through chromosome I, then II, then III, etc., until the last gene on the right arm of chromosome XVI is reached at the lower right-hand corner. Scored in this analysis were

Table 1. Transcriptional Machinery

Complex and Subunit	Features	Fraction of Genes Dependent on Subunit Function
RNA polymerase II		
Rpb1	Largest subunit, mRNA catalysis, contains CTD	100%
Srb/mediator (core)		
Srb4	Target of Gal4 activator	93%*
Srb5	Unknown function	16%
Med6	Role in activation of some genes	10%
Srb CDK complex		
Srb10	CTD kinase, negative regulator	3%
Swi/Snf		
Swi2	ATP-dependent chromatin remodeling	6%
General transcription factors		
TFIID (TAF _{II} 145)	Large TBP-associated factor, histone acetylase	16%
(TAF _{II} 17)	Component of both TFIID and SAGA	67%
TFIIE (Tfa1)	Promoter opening	54%
TFIIH (Kin28)	CTD kinase	87%*
SAGA		
Gcn5	Histone acetylase	5%
TAF _{II} 17	Component of both TFIID and SAGA	67%

* Srb4 and Kin28 results were essentially identical to Rpb1, but because of the stringency applied by the fit algorithm, a minimal estimate is produced.

5735 genes. The vast majority of mRNAs are reduced more than 2-fold in the mutant cells relative to wild-type cells, and this reduction provides an apparent half-life for each of the mRNA species (see Yeast mRNA Population on the web site). The value determined with this approach is an approximation but is useful for comparative purposes. Comparison of this data with that obtained for another ts factor identifies the set of genes whose expression is equivalently dependent on RNA polymerase II and the factor of interest.

There is a set of genes whose mRNAs are not significantly reduced in the mutant cells. These consist of genes that have stable messages as well as genes whose mRNA levels are slightly elevated in the mutant cells relative to wild type. In this latter group are many known heat shock or stress response genes (e.g., *SSA4*, *SSA3*, *HSP26*, *HSP30*, *HSP42*, and *SSL2*), plus additional ORFs of unknown but perhaps related function. Similar results were obtained using ts mutants in other general transcription factors.

Dependence on Srb/Mediator Core Subunits

The Srb/mediator complex is tightly associated with RNA polymerase II in a complex that has been termed the holoenzyme (Kim et al., 1994; Koleske and Young, 1994). Srb4 is an essential component of the Srb/mediator complex (Kim et al., 1994; Hengartner et al., 1995; Thompson and Young, 1995). A ts mutant in Srb4 (*srb4-138*) was previously used to obtain evidence that several protein-coding genes require the function of Srb4 and are thus likely to have the holoenzyme form of RNA polymerase II recruited to their promoters (Thompson and Young, 1995). Genome-wide expression analysis provides a more rigorous test of the model that expression of all protein-coding genes is dependent on Srb4. The experiment was carried out with the same protocol used with the Rpb1 ts mutant. Of the 5361 genes whose mRNA expression levels could be compared (i.e., those that had a greater than 2-fold decrease in the experiment with Rpb1 ts and were scored in the Srb4 ts experiment),

93% showed a decrease that closely fit the decrease observed in the Rpb1 ts experiment. Of the mRNAs that did not closely fit the Rpb1 ts decay, only two could be found that reproducibly showed large differences in their decay in the two experiments performed. Furthermore, the set of genes whose mRNAs are not significantly reduced in the Rpb1 ts mutant exhibit the same behavior in the Srb4 ts experiment. The results indicate that genome-wide expression is as dependent on Srb4 as it is on core RNA polymerase II (see Genome-Wide Expression Data on the web site for details). Because Srb4 is associated tightly and exclusively with the RNA polymerase II holoenzyme (Kim et al., 1994; Koleske and Young, 1994; Myers et al., 1998), we can infer that the Srb4-containing RNA polymerase II holoenzyme is generally required for transcription.

Med6 is another essential component of the Srb/mediator complex and appears to be physically associated with Srb4 (Li et al., 1995; Lee et al., 1998; Myers et al., 1998). A Med6 ts mutant has been generated and used to demonstrate that Med6 is necessary for full induction of *GAL*, *SUC2*, *MFA1*, and *PYK1* genes but is not required for expression of several others (Lee et al., 1997). The genome-wide dependence of gene expression on Med6 was determined with this Med6 ts strain as described above for Rpb1. The results indicate that the expression of 10% of yeast genes are as dependent on Med6 as they are on Rpb1 (Figure 2B; see the web site for detailed information).

The reduction in mRNA levels observed in ts mutants soon after a temperature shift (i.e., 45 min) is likely a consequence of primary effects due to factor inactivation because the time required to produce most secondary effects involves a substantial reduction in both a transcript and its translation product. Nonetheless, the results obtained in this type of experiment must be regarded as the sum of primary and secondary effects. To identify the set of genes whose change in expression is most likely a direct consequence of the loss of function of the ts factor, we compare data from ts inactivation

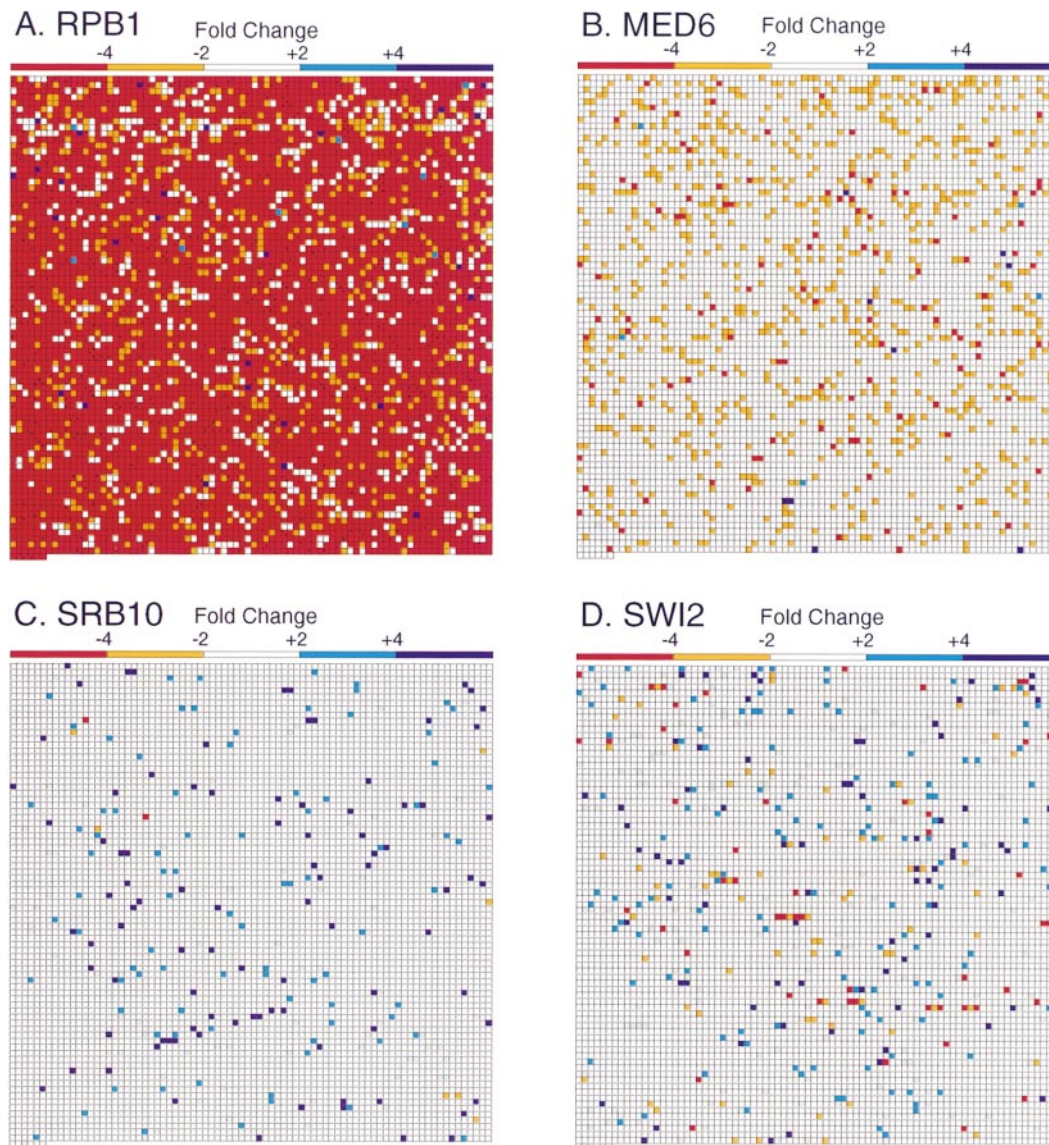


Figure 2. Genome-Wide Expression Data for Selected Components of the RNA Polymerase II Holoenzyme

Data reflecting the change in mRNA levels when a mutant is compared to its isogenic wild-type counterpart is presented in a grid format. In the grid, the upper left grid square represents the left-most gene on chromosome I, and the squares to its right represent adjacent genes, proceeding in a linear fashion through chromosome I, then II, then III, etc., until the last gene on the right arm of chromosome XVI is reached at the bottom of the grid. The results are shown for (A) Rpb1, (B) Med6, (C) Srb10, and (D) Swi2.

of RNA polymerase II with that obtained by its inactivation of any other factor. Comparison of the two data sets reveals the transcripts with equivalent decay kinetics in *rpb1-1* and the other *ts* mutant (see Technology, Protocols, and Data Analysis on the web site for details). For those genes affected by *ts* disruption of Med6 where such a comparison could be made, the mRNAs of 506 genes decreased with similar kinetics in the Med6 and Rpb1 experiments. Thus, the expression of 10% of yeast genes are as dependent on Med6 as they are on Rpb1. These 506 genes are most likely to have a direct requirement for Med6 function. The genes whose transcript levels do not fit the Rpb1 kinetics could have a direct, but partial, requirement for Med6 function, or the effects observed at these genes are a secondary consequence

of some other gene's altered mRNA levels. The 506 genes we have identified that require Med6 function to the same extent as Rpb1 function are those at which promoter-associated transcriptional regulators are most likely to function through interactions with Med6.

Srb5 is a component of the Srb/mediator complex whose function is also not known (Thompson et al., 1993; Kim et al., 1994; Koleske and Young, 1994; Hengartner et al., 1995; Myers et al., 1998). To determine the genome-wide dependence of gene expression on Srb5, a strain lacking an *SRB5* gene and its wild-type counterpart were compared (see the web site for detailed information). The results indicate that 16% of all genes require Srb5 function for their expression. With the *SRB5* deletion strain and other constitutive mutants

analyzed here, it is not possible to distinguish between results that are a direct consequence of the loss of *Srb5* function and those that are due to a secondary effect such as the loss of another transcriptional regulator. Nonetheless, these results provide important information in that they reveal the complete set of genes that are directly or indirectly affected by loss of *Srb5* function. It was striking that expression of many genes central to the pheromone response pathway are dramatically affected by the loss of *Srb5*, as discussed below.

Dependence on *Srb10* CDK Complex

Srb10 is cyclin-dependent kinase that is part of a holoenzyme subcomplex containing *Srb8*, -9, -10, and -11 proteins (Hengartner et al., 1995; Liao et al., 1995). *Srb10* and its associated proteins have been proposed to form a negative regulatory complex that functions through phosphorylation of the RNA polymerase II CTD (Hengartner et al., 1998). To determine how gene expression depends on *Srb10*, RNA was isolated from an *Srb10* point mutant that lacks catalytic activity, and the expression profile was compared to that of its wild-type counterpart. The results are shown in a grid format in Figure 2C. Of the 5626 genes that were scored, 173 gene products showed 2-fold or greater increases in mRNA levels in the mutant relative to the wild type. This indicates that *Srb10* is normally a negative regulator of these 173 genes (approximately 3% of the genome). As discussed below, it is notable that nearly half of these genes are derepressed during nutrient deprivation.

Dependence on *Swi/Snf*

Swi2 ATPase activity plays an essential role in the ability of the *Swi/Snf* complex to remodel chromatin (Khavari et al., 1993; Laurent et al., 1993; Cote et al., 1994). This activity is thought to facilitate activator and transcription apparatus binding to promoter regions for a small number of genes, thereby overcoming repression by nucleosomes at those promoters (Cote et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994; Burns and Peterson, 1997). Consequently, we anticipated that a small number of genes would be reduced in expression levels in the *Swi2/Snf2* mutant. To determine the genome-wide dependence of gene expression on the *Swi/Snf* complex, RNA was isolated from a *Swi2/Snf2* point mutant that lacks ATPase activity and its wild-type counterpart, and the two RNA preparations were hybridized to HDAs. The surprising result was that a greater number of genes appear to be negatively regulated by *Swi/Snf* than are positively regulated (Figure 2D; see the web site for detailed information). The data show that 203 gene products were elevated 2-fold or more in the mutant relative to the wild type, while just 126 transcripts decreased 2-fold or more (see Genome-Wide Expression Data on the web site). As described below, this result may be explained by recent data indicating that the *Swi/Snf* complex can catalyze chromatin remodeling in either direction (Schnitzler et al., 1998).

Dependence on General Transcription Factors

The general transcription factors are necessary to reconstitute promoter-dependent transcription *in vitro*

with core RNA polymerase II. These factors include TFIID, TFIIB, TFIIF, TFIIE, and TFIIH. Among these factors, TFIIE and TFIIH are of particular interest because numerous reports have suggested that they are in fact not generally required for gene expression (Parvin et al., 1992; Serizawa et al., 1993; Timmers, 1994; Holstege et al., 1995; Kuldell and Buratowski, 1997; Sakurai et al., 1997; Tijerina and Sayre, 1998). Genome-wide expression analysis was carried out on a *Kin28* ts cell and its isogenic wild-type counterpart using the same experimental protocol used for the *Rpb1* ts mutant. *Kin28*, a CDK subunit of TFIIH, is an RNA polymerase II CTD kinase that is involved in the transition from initiation to elongation (Dahmus, 1996). The results reveal that *Kin28* is generally required for expression of protein-coding genes (see Genome-Wide Expression Data on the web site). TFIIE is thought to facilitate certain functions of TFIIH. In contrast to the results obtained with *Kin28*, analysis of genome-wide expression with a *Tfa1* ts mutant shows that only 54% of yeast genes require the largest subunit of TFIIE to the same extent as core RNA polymerase II (see Genome-Wide Expression Data on the web site).

The TBP-associated factors (TAF_{II}s) of TFIID are especially interesting because they have been postulated to play important roles in promoter selectivity and gene activation (Burley and Roeder, 1996; Verrijzer and Tjian, 1996; Lee and Young, 1998). A ts mutation in the TFIID subunit TAF_{II}145 (Walker et al., 1997) was used to determine the genome-wide dependence of gene expression on this TAF. Of the 5441 genes that were scored, 1618 gene products were reduced by 2-fold or greater on average in the two comparisons made 45 min after temperature shift. For those genes where a comparison with the *Rpb1* experiment could be made, 16% showed a dependence on TAF_{II}145 that was similar to their dependence on *Rpb1* (see Genome-Wide Expression Data on the web site for details). Interestingly, a large number of genes involved in functions associated with progression through the cell cycle are among the genes most likely to have a direct requirement for TAF_{II}145 function. The TAF_{II}145 ts mutant has a cell cycle phenotype: it arrests growth in G1-S after cells are shifted to the nonpermissive temperature. Previous studies showed that several G1-S cyclin genes are expressed at reduced levels in these cells, perhaps accounting for the cell cycle arrest phenotype (Walker et al., 1997). A subset of the genes that have a direct requirement for TAF_{II}145 function and that are involved in functions associated with progression through the cell cycle are listed in Table 2. For example, a significant decrease in mRNA levels was observed for *CTR9*, which is required for expression of G1 cyclins *CLN1* and *CLN2*. In addition, genes that are involved in DNA repair and DNA synthesis are dependent on TAF_{II}145 function. Thus, the G1/S arrest phenotype of TAF_{II}145 mutants may be due to multiple defects in cyclin and chromosome synthesis that occur during this period of the cell cycle.

We next analyzed which genes depend on TAF_{II}17, a histone H3-like TAF that is shared by TFIID and SAGA complexes, for their expression. RNA was isolated from a TAF_{II}17 temperature-sensitive cell (TAF17-ts) and its wild-type counterpart 45 min after a shift to the nonpermissive temperature and was hybridized to HDAs. Of

Table 2. Genes that Require Taf145 Function

Cell Cycle		
Gene	Description	Fold Reduction
DDC1*	DNA damage checkpoint protein	10
YER066W	Similar to CDC4, which degrades G1 cyclins	9
SPO1	Possible role in spindle pole body duplication	8
LTE1*	GDP/GTP exchange factor	8
MKK2*	Kinase involved in cell wall integrity	8
BIM1*	Possible role in early spindle pole body assembly	8
MDM1*	Involved in mitochondrial segregation	7
CTR9*	Required for normal expression of G1 cyclins	7
PAC1*	Possible role in spindle pole body orientation	6
SCP160*	Involved in control of chromosome transmission	6
CDC13	Telomere-binding protein	6
TOP3*	DNA topoisomerase III	5
TRX1*	Thioredoxin I	5
ARD1	N-acetyltransferase	5
SCC2*	Required for sister chromatid cohesion	5
CLB2*	G2/M cyclin	5
KIP2*	Kinesin-related protein	5
MEC1*	Cell cycle checkpoint protein	4
RAD9	DNA repair checkpoint protein	4
SPC98*	Spindle pole body component	4
BCK1*	Kinase involved in cell wall integrity	4
DNA Repair		
RAD3*	Involved in nucleotide excision repair	8
YHR031C*	Possible role in chromosome repair	7
RAD5*	Involved in DNA repair	6
HSM3*	Involved in mismatch repair	6
RAD50*	Involved in recombinational repair	5
EXO1*	Involved in mismatch repair	5
MSH3*	Involved in mismatch repair	5
YER041W	Similar to DNA repair protein Rad2	5
REV1	Involved in translesion DNA synthesis	4
HDF2	Involved in DNA end-joining repair pathway	4
MSH6	Involved in mismatch repair	4
DNA Synthesis		
MCM3*	Involved in replication initiation, MCM/P1 family	13
RLF2	Chromatin assembly complex, subunit 2	9
MCM6*	Involved in replication initiation, MCM/P1 family	9
REV7	DNA polymerase subunit zeta	7
MIP1*	Mitochondrial DNA-directed DNA polymerase	6
CDC47*	Involved in replication initiation, MCM/P1 family	6
CDC5*	Kinase	5
CDC46*	Involved in replication initiation, MCM/P1 family	5
RFC1*	DNA replication protein RFC large subunit	5
CAC2*	Chromatin assembly complex, subunit 1	5

* Gene exhibits equivalent dependence on Taf145 and Rpb1 for normal expression.

the yeast genes identified in the TAF_{II}17 experiment and appropriate for comparison, 67% are as dependent on TAF_{II}17 function as they are on Rpb1 and are thus most likely to have a direct requirement for TAF_{II}17 function (see Genome-Wide Expression Data on the web site for details). This indicates that TAF_{II}17 is critical for the expression of a much larger portion of the transcriptome than TAF_{II}145. The presence of TAF_{II}17 in two different complexes may account for this observation.

Dependence on Gcn5 Subunit of SAGA

The recent discovery that certain TAFs are components of both the TFIID general transcription factor and the SAGA complex (Grant et al., 1998) makes it particularly interesting to compare the effects of a mutation in a

component specific to each complex (TAF_{II}145 in the case of TFIID, and Gcn5 in the case of SAGA) with those of a mutation in a component shared by the two complexes (TAF_{II}17). The expression profile of a *GCN5* deletion mutant was compared with its isogenic counterpart (see Genome-Wide Expression Data on the web site for details). Of the 4912 genes that were scored, 185 transcripts were reduced by 2-fold or more and 83 increased by 2-fold or more.

The Gcn5 results indicate that this component of SAGA is necessary for normal expression of no more than 5% of yeast genes. Expression of 16% of protein-coding genes depends on the TAF_{II}145 subunit of TFIID to the same extent they depend on Rpb1. In contrast, the expression of 67% of yeast genes depends on the

function of the TAF_{II}17 subunit shared by SAGA and TFIID.

Distinct Requirements for Components of Transcriptional Machinery

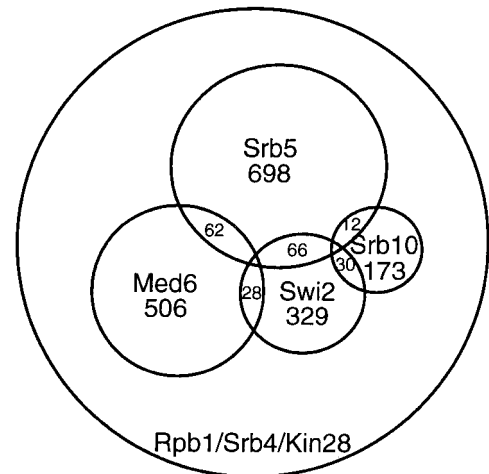
The analysis carried out thus far indicates that the Rpb1 subunit of core RNA polymerase II, the Srb4 subunit of the Srb/mediator complex, and the Kin28 subunit of the general transcription factor TFIID are generally required for transcription of protein-coding genes. In contrast, expression of only a subset of genes is dependent on Med5, Srb5, Srb10, Swi2, TAF_{II}145, TAF_{II}17, and Gcn5. The sets of genes whose expression requires various RNA polymerase II holoenzyme components are compared in the Venn diagram in Figure 3A. Similarly, the set of genes whose expression requires various TFIID and SAGA components are shown in a Venn diagram in Figure 3B. These diagrams show how distinct sets of genes require the function of distinct components of the transcription machinery. These data suggest that coordinate regulation of large sets of genes could be accomplished by affecting the function of specific components of the transcriptional machinery. If this is the case, then it would be expected that functional relationships exist among some genes within these sets, as has been observed with TAF_{II}145.

Srb5 Has Unexpected Roles in Pheromone Response

It was striking that many of the genes whose mRNA levels are most dramatically affected by the loss of Srb5 fall into the pheromone response pathway. The 15 genes involved in the pheromone response that are expressed at substantially lower levels in the absence of Srb5 are shown in Figure 4A. Dramatic effects are seen in genes involved in mating factor production and export; the expression of *MFA1* and *MFA2*, the two genes encoding mating pheromone α -factor, are down 28-fold and 11-fold, respectively. Additional genes involved in maturation (*STE13*) and export (*STE6*) of mating factor are expressed at substantially lower levels than in the cognate wild type. Furthermore, several components of the signal transduction pathway that responds to mating pheromone are expressed at reduced levels in the Srb5 mutant. These genes include the receptor for pheromone (*STE2*), subunits of the signaling G protein (*GPA1*), and the transcription factor that is itself the target of the signaling response and directly regulates subsequent gene expression (*STE12*).

The genome-wide expression profile for the Srb5 mutant suggests that these cells should exhibit a defect in mating efficiency, a phenotype we had not previously suspected or investigated. Indeed, quantitative mating assays show that the Srb5 mutant does have a significant defect in mating (Figure 4B). The mating defect was more pronounced than that due to mutations in Fus3, a MAP kinase required for cell cycle arrest and cell fusion during mating, but less pronounced than that due to mutations in *STE12*. The defect in mating deficiency may reflect SRB5-dependent coordinate regulation of the set of pheromone response genes identified through genome-wide expression analysis.

A. RNAP II Holoenzyme Components



B. TFIID and SAGA Components

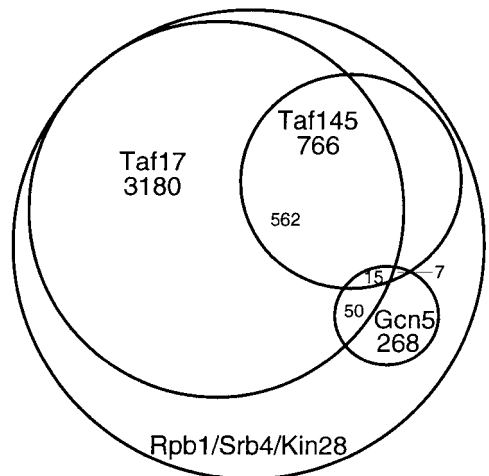


Figure 3. Genome-Wide Dependence on Key Components of the Transcription Machinery

(A) RNA polymerase II holoenzyme components show distinct patterns of genome control. Venn diagram depicting Srb5-, Swi2-, Srb10-, and Med6-dependent genes (small circles) in relation to the whole transcriptome (Rpb1-, Srb4-, and Kin28-dependent; large circle). The numbers under each subunit name are the sum of genes whose expression depends on that subunit.

(B) Genome control patterns of components of TFIID and SAGA.

Coordinate Regulation of Nutrient Starvation Response Genes via Srb10

The analysis revealed that Srb10 is a negative regulator of 173 genes. It is notable that nearly half of these genes are derepressed during the nutrient deprivation that occurs during the diauxic shift (DeRisi et al., 1997) (Figure 5). Yeast cells undergo a diauxic shift as nutrients are depleted in culture, and a variety of genes that enable the cell to survive nutrient-limiting conditions are derepressed (Yin et al., 1996). These include genes involved in dimorphic morphology (nutrient-starved cells alter

A.	Gene	Description	Fold-reduction
	MFA1	Mating pheromone a-factor	28
	STE2	Alpha factor receptor	12
	MFA2	Mating pheromone a-factor	11
	BAR1	Protease that degrades alpha factor	10
	SST2	Involved in desensitization to alpha factor	9
	FAR1	Inhibitor of CDKs involved in cell-cycle arrest for mating	8
	FUS2	Protein required for cell fusion during mating	6
	STE6	Membrane transporter; exports a-factor	6
	AGA2	a-agglutinin binding subunit	6
	AGA1	a-agglutinin anchor subunit	5
	STE12	Transcription factor binds to pheromone response element	4
	GPA1	GTP-binding subunit of pheromone response pathway	4
	STE13	Involved in maturation of alpha factor	4
	KAR4	Required for pheromone induction of karyogamy genes	4

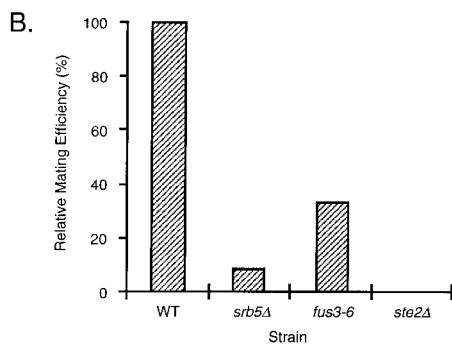


Figure 4. *Srb5* Is Required for Expression of Pheromone Response Genes

(A) Pheromone response genes whose expression levels are reduced in the absence of *Srb5*.

(B) Cells lacking *Srb5* are defective in mating. The mating efficiencies for mutant strains are expressed as a percentage of the mating efficiency of an isogenic wild-type strain. For comparison, strains with mutations in two components of the mating signal transduction pathway (*Fus3* and *ste2*) are included.

their morphology to permit foraging for nutrients) and stress responses (starved cells are apparently better able to survive nutrient deprivation when stress proteins are elevated). *Srb10* in wild-type cells is most likely responsible for repressing this set of genes when cells are in exponential growth on glucose, but no longer performs this function as cells enter the diauxic shift. Coordinate regulation of this set of genes could be accomplished by eliminating the function of *Srb10* as cells enter the diauxic shift.

To determine whether *Srb10* is physically lost from cells as they enter the diauxic shift, cells containing an epitope-tagged *Srb10* protein were grown in YPD media and sampled at various times during the growth curve (Figure 5C). Cell lysates were prepared from each sample, and the levels of *Srb10* were assayed by Western blot. The data in Figure 5C show that *Srb10* is physically depleted as cells enter the diauxic phase of growth. This result is consistent with evidence that the levels of *Srb11*, the cyclin partner of *Srb10*, are reduced when cells are exposed to the limiting nutrient environment in sporulation media (Cooper et al., 1997). It may also explain why a form of yeast holoenzyme purified from commercially available yeast cells lacks the *Srb10*/*Srb11* kinase/cyclin pair (Li et al., 1995; Myers et al.,

1998), as these cells are typically grown past mid-log phase. The results thus indicate that the nutrient starvation response is mediated, in part, through the physical loss of the *Srb10* CDK from the holoenzyme. This novel mechanism provides one example of how coordinate regulation of gene expression can be accomplished through regulation of components of the general initiation machinery.

FLO11, which encodes a cell wall protein that is highly expressed in pseudohyphal cells, is expressed at 15-fold higher levels when *Srb10* function is lost (Figure 5A). The dramatic increase in the expression of *FLO11* and other genes whose products are involved in the dimorphic shift led us to determine whether the absence of *Srb10* function produces a pseudohyphal phenotype. Both copies of the *SRB10* gene were deleted from a diploid strain that is generally used to assay this phenotype, and colony morphology was examined under the microscope. The results in Figure 5D demonstrate that the loss of *Srb10* causes cells to grow preferentially in a pseudohyphal form. This again shows that expression analysis is useful for predicting unexpected phenotypes. More importantly, specific signal transduction pathways control the dimorphic shift (Madhani and Fink, 1998), and these results suggest that one of the ultimate targets of these pathways is the *Srb10* kinase.

Discussion

We have characterized the mRNA population of yeast cells and the requirement for key components of the transcriptional machinery in expression of this population using HDA technology. The insights obtained from this analysis include the following. Genome-wide expression is equivalently dependent on *Srb4* and *Rpb1*, suggesting that the *Srb4*-containing RNA polymerase II holoenzyme is generally recruited to promoters of protein-coding genes. Distinct expression signatures are obtained when a wide variety of components of the transcription apparatus are inactivated, revealing a level of genome regulation that can be superimposed on that due to gene-specific transcription factors. Coordinate regulation of functionally related genes can be effected by regulating a component of the initiation machinery, as exemplified by the regulation of *Srb10* and the role of this kinase in the response to nutrient deprivation. The ultimate targets of certain signal transduction pathways can be identified by comparing genome expression signatures from these experiments and those that modify the cellular environment.

Transcriptome

We have estimated the number of mRNA molecules present for all genes in a single wild-type haploid cell using HDA data (see Yeast mRNA Population on the web site). This is a more accurate representation of the transcriptome than that previously determined because it is better able to score mRNA species that are expressed at very low levels (5460 genes were scored using HDAs, whereas 4465 genes were scored with SAGE). It is particularly valuable to have information on transcripts from genes expressed at low levels because many of the

A.	Gene	Description	Fold Up
	FLO1	Flocculence cell wall protein	102
	SIP18	Induced by osmotic stress	74
	YBR116C	Induced by diauxic shift	61
	YMR107W	Induced by diauxic shift	32
	ALD3	Induced by diauxic shift	28
	HSP26	Induced by osmotic stress, diauxic shift	26
	GRE1	Induced by osmotic stress, diauxic shift	25
	YER150W	Induced by diauxic shift	24
	HSP12	Induced by numerous stresses	18
	RCK1	Serine/threonine protein kinase	18
	FLO11	Flocculence	15
	RTA1	Involved in 7-aminosterol resistance	15
	YDR070C	Induced by diauxic shift	13
	YBR147W	Induced by diauxic shift	10
	CTT1	Induced by osmotic stress, diauxic shift	10
	YDL204W	Induced by diauxic shift	10
	TKL2	Induced by diauxic shift	10
	YGR043C	Induced by diauxic shift	9
	YNL194C	Induced by diauxic shift	9
	SOL4	Induced by diauxic shift	8
	CYC7	Induced by numerous stresses, diauxic shift	8
	PUT4	Proline permease, nitrogen induced	8
	YKL187C	Induced by diauxic shift	8
	NCA3	Life-span determination	8
	YML128C	Induced by diauxic shift	8
	GPH1	Induced by diauxic shift	8
	POT1	Induced by diauxic shift	7

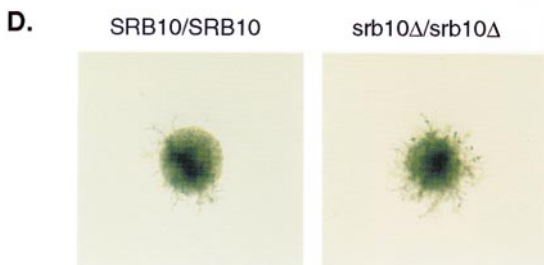
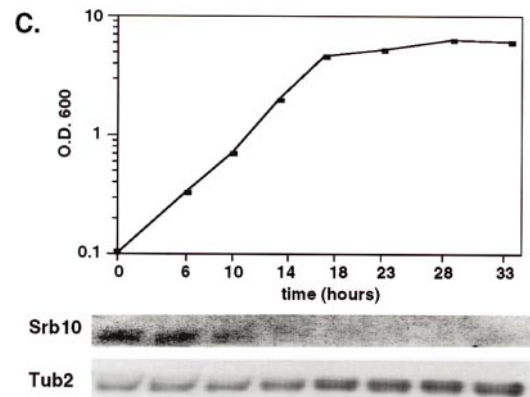
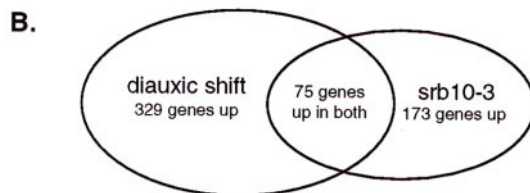


Figure 5. Srb10 CDK Represses Genes Elevated during Response to Nutrient Starvation
(A) Subset of 173 genes whose expression levels are derepressed in cells lacking Srb10 kinase activity.

regulatory components of the cell are expressed at low levels.

Gene-Specific Regulation via the General Transcription Machinery

The textbook models describe regulation of eukaryotic gene expression as the recruitment of the general transcription machinery to genes by gene-specific activators. Our results demonstrate that the function of certain key components of the general transcription machinery is required for the expression of distinct sets of genes, as illustrated in Figure 3. It is possible that these components of the transcription machinery are necessary targets for a specific set of gene-specific activators, and the loss of such a component produces a dramatic effect at only those genes under the control of such activators. In this case, the data presented here provide candidate targets within the initiation machinery for activators at most yeast genes.

The results described here also reveal that a layer of regulation is available to the cell in addition to that provided by gene-specific regulators: the expression of specific sets of genes can be regulated by affecting the availability or function of a specific component of the general machinery. Since various components of the general machinery can be acetylated and phosphorylated (Kitajima et al., 1994; Imhof et al., 1997), it is possible that these modifications serve to regulate these components, and thus the genes that require their functions.

Insights into Roles of Transcriptional Complexes

The components of the transcription apparatus that were the focus of this study were selected because they are among the key subunits of the major multiprotein complexes that have roles in transcription of protein-coding genes. These complexes include the RNA polymerase II core enzyme, the general transcription factors (GTFs), the core Srb/mediator complex, the Srb10 CDK complex, the Swi/Snf complex, and the SAGA complex. We found that three components were generally required for transcription of protein-coding genes (Rpb1, Kin28, Srb4). Two were found to be required for more than half, but not all genes (Tfa1, Taf17). Most components investigated thus far were necessary for transcription of less than a fifth of the genome (Srb5, Med6, Srb10, Swi2, Taf145, Gcn5). In this latter group, the evidence

(B) Venn diagram showing the number of genes that are derepressed during the nutrient deprivation that occurs during the diauxic shift and the fraction of these that are derepressed in cells lacking Srb10 kinase activity.

(C) Srb10 protein is depleted from cells as they enter the diauxic shift. The graph shows the growth curve of a yeast strain allowed to grow to stationary phase (33 hr). At specified time points, aliquots from a culture were measured for cell density, and equal amounts of cells were harvested for Western blot analysis. Western blots against epitope-tagged Srb10p and a control protein, Tub2p (tubulin), show that Srb10 levels decrease substantially as cells enter the diauxic shift (14 to 18 hr).

(D) Cells lacking Srb10 kinase activity exhibit increased pseudohyphal growth. The strains used to assay pseudohyphal growth were derived from L5978 and are congenic to the E1278b genetic background (Liu et al., 1993).

indicates that *Srb5*, *Med6*, and *Taf145* have predominantly positive roles, *Srb10* has an almost exclusively negative role, and *Swi2* and *Gcn5* can have either a positive or a negative role in gene expression.

General Factors

Because *Rpb1* and *Srb4* proteins are generally required for expression of protein-coding genes, and they are both associated tightly and exclusively with RNA polymerase II and the mediator complex, respectively (Kim et al., 1994; Koleske and Young, 1994; Myers et al., 1998), we can infer that RNA polymerase II and the core mediator complex are generally required for transcription. Assuming that the function of *Kin28* is restricted to *TFIIH*, the data obtained with the *Kin28* mutant demonstrates that *TFIIH* is a general factor. Since 54% of yeast genes are as dependent on *Tfa1* as they are on *Rpb1*, we infer that *TFIIE* is directly involved in expression of at least 54% of protein-coding genes, but without knowing the contribution of *Tfa2*, the other subunit of *TFIIE*, we cannot eliminate the possibility that *TFIIE* has roles at additional genes.

SRB/Mediator Complex

The SRB/mediator core complex is essential for general transcription, as evidenced by the requirement for *Srb4*, but components such as *Srb5* and *Med6* have roles at specific subsets of genes. These results are consistent with the proposal that the SRB/mediator complex is recruited to promoters of most genes together with RNA polymerase II, where it acts in a manner analogous to a signal processor with the capacity to integrate the combinatorial effects of multiple inputs from gene-specific transcriptional activators and repressors (Kim et al., 1994; Koleske and Young, 1994; Koh et al., 1998; Myers et al., 1998; Sun et al., 1998).

Srb10 CDK Complex

The function of the *Srb10* CDK complex can be defined by the kinase itself, since loss-of-function mutations in any of the four components of this complex produce identical phenotypes (Hengartner et al., 1995; Carlson, 1997). The *Srb10* kinase is a negative regulator of a substantial fraction of genes that are repressed when cells grow vegetatively in rich media and are induced as cells experience nutrient deprivation. The genes regulated by *Srb10* are involved in the nutrient stress response and in the morphological change that permits foraging for nutrients. *Srb10* is physically depleted from cells as they enter the diauxic shift, providing a mechanism for derepression of this set of genes. *Srb10* in wild-type cells is thus responsible for repressing this set of genes when cells are in exponential growth on glucose, but no longer performs this function as cells enter the diauxic shift.

Swi/Snf Complex

If the function of the Swi/Snf complex is ATP-dependent remodeling of chromatin (Laurent et al., 1993; Cote et al., 1994), then the effects we observe due to the *Swi2* ATPase mutation should represent the dependence of genome-wide expression on the entire Swi/Snf complex. The results indicate that a greater number of genes

are negatively regulated by Swi/Snf than are positively regulated. This is surprising in view of the model that Swi/Snf-catalyzed remodeling of chromatin facilitates activator binding. It is possible that chromatin remodeling may facilitate binding of negative factors as well as positive factors. An alternative possibility is suggested by recent data indicating that the Swi/Snf complex can remodel chromatin in both directions: it can convert a repressive nucleosome structure toward a more accessible state, and vice versa (Schnitzler et al., 1998). It is thus possible that Swi/Snf helps produce a nucleosome structure conducive to transcription at some promoters, and a structure that is repressive at others.

TFIID and SAGA

The general transcription factor TFIID and the SAGA complex share two features: they both contain a subunit capable of histone acetylation (*TAF_{II}145* in the case of TFIID and *Gcn5* in the case of SAGA) and they share multiple subunits, among which is the histone H3-like *TAF_{II}17* (Grant et al., 1998). As summarized in Figure 4, the results indicate that *Gcn5*, *TAF_{II}145*, and *TAF_{II}17* are necessary for expression of 5%, 16%, and 67% of yeast genes, respectively. Two models can account for this data: one posits that *TAF_{II}17* functions exclusively within the TFIID and SAGA complexes, and the other that *TAF_{II}17* is a component of one or more additional complexes. If *TAF_{II}17* functions exclusively within TFIID and SAGA, then *TAF_{II}145* and *Gcn5* do not fully represent the functions of the two complexes, since the sum of genes that require *TAF_{II}145* and *Gcn5* function is much smaller than the number of genes that require *TAF_{II}17*. In this model, one or both complexes contain subunits that make different contributions to gene expression, as might be expected if different subunits are targets of different transcriptional activators and repressors. The results can also be accommodated in a second model, in which *TAF_{II}17* is a component of one or more complexes in addition to TFIID and SAGA. The results described here lay a useful foundation for the additional experiments necessary to gain a fuller understanding of the roles of TFIID and SAGA subunits in gene expression.

Our data, in conjunction with that of previous studies, reveal several striking similarities between *TAF_{II}145* and prokaryotic sigma factors. First, both sigma factors and *TAF_{II}145* are components of the general transcription machinery. Second, many sigma factors are required for the expression of a related subset of genes; likewise, we have shown that *TAF_{II}145* appears to be required for expression of a set of genes involved in chromosomal synthesis and G1/S progression. Finally, both sigma factors and *TAF_{II}145* act through core promoter elements by direct DNA contacts.

Development of a Genome Control Map

The results described here support the feasibility of dissecting the regulatory circuitry of the yeast genome by using genome-wide expression analysis on cells with mutations in the transcription apparatus. The set of yeast genes whose expression depends on the functions of key components of the transcriptional machinery has

been identified. The genome-wide expression signatures produced by lesions in specific components of the transcription apparatus are quite distinct, making it possible to envision a genome control map. Such a map would identify all the components of the transcriptional machinery that have roles at any particular promoter and the contribution that specific components make to coordinate regulation of genes. The map will facilitate modeling of the molecular mechanisms that regulate gene expression and implicate components of the transcription apparatus in functional interactions with gene-specific regulators.

Experimental Procedures

Detailed information on experimental procedures, genetic reagents, HDA technology, and data analysis can be found on the World Web at <http://www.wi.mit.edu/young/expression.html> in the section titled Study Design.

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