Mediator protein mutations that selectively abolish activated transcription

LAWRENCE C. MYERS*,†, CLAES M. GUSTAFSSON†‡, KATHLEEN C. HAYASHIBARA§, PATRICK O. BROWN§, and ROGER D. KORNBERG*¶

*Department of Structural Biology and †Howard Hughes Medical Institute and Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305; and §Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, Göteborg University, S-413 45 Göteborg, Sweden

CONTRIBUTED BY ROGER D. KORNBERG, OCTOBER 28, 1998

ABSTRACT Deletion of any one of three subunits of the yeast Mediator of transcriptional regulation, Med2, Pdg1 (Hrs1), and Sin4, abolished activation by Gal4–VP16 in vitro. By contrast, other Mediator functions, stimulation of basal transcription and of TFIIH kinase activity, were unaffected. A different but overlapping Mediator subunit dependence was found for activation by Gcn4. The genetic requirements for activation in vivo were closely coincident with those in vitro. A whole genome expression profile of a med2Δ strain showed diminished transcription of a subset of inducible genes but only minor effects on "basal" transcription. These findings make an important connection between transcriptional activation in vivo and in vitro, and identify Mediator as a "global" transcriptional coactivator.

Mediator was discovered as an activity in a crude yeast fraction able to relieve activator inhibition (1) and required for an activator response in a partially reconstituted RNA polymerase II transcription system (2). Mediator was initially resolved to homogeneity (3) by displacement from a complex with polymerase II ("holoenzyme"), and proved to contain the products of three groups of genes: SRBs, recovered from a genetic screen for CTD-activating proteins (4, 5); the SIN4/ RGR1 group, whose founding members were obtained from screens for mutations affecting transcription (5, 6); and the MED genes, not previously identified in any screen (7, 8). Functional analysis of purified Mediator in a transcription system reconstituted from essentially homogeneous proteins revealed three biochemical activities, stimulation of basal transcription, support of activated transcription, and stimulation of CTD phosphorylation by TFIIH (3, 8). Activated transcription occurred in the absence of TATA boxing-binding protein associated factors (TAFs), consistent with the lack of a TAF requirement for regulation of most yeast promoters in vivo (9–10). The outstanding question regarding Mediator has been whether it too might prove to be dispensable for regulation in vivo, or whether it plays a general role in activated transcription in vivo, in keeping with the biochemical results.

Work done to date has begun to address the physiologic relevance of Mediator and the relationship between its functions in vivo and in vitro. Cells harboring a temperature-sensitive mutation in SRB4 ceased transcription of all promoters analyzed at the restrictive temperature, indicating a widespread requirement for Mediator, though not distinguishing between roles in basal and activated transcription (11). A temperature-sensitive mutation in MED6 was shown to diminish activation by Gal4 in vivo and by VP16 in vitro, but because two different activators were used, the effects could not be correlated (7). Finally, CTD truncation has been shown to impair activation in vivo (12) and in vitro (8); the CTD interacts with Mediator in vitro (8, 13), enabling a correlation, but only an indirect one.

Here we use multiple activators and Mediator mutants in a combined biochemical and genetic analysis. The results define a consistent pattern of structure–function relationships, establish the fidelity of transcription control in the yeast system in vitro, and identify Mediator as an important conduit of regulatory information from enhancers to RNA polymerase II promoters in vivo.

MATERIALS AND METHODS

Protein Purification. Approximately 400 g of cells from yeast strains BJ926 (Mata/Mata trp1/1TRP1 Pre1–126/Pre1–126 pep4–3/pep4–3 prp1–1122/prp1–1122 can1/can1), MG107 (MATa ade2–1 can1–100 his3–11 15 leu2–2 3 112 trp1–1 ura3–1 med2Δ1::TRP1), SSAB-4A (MATa ura3 ade2 his3 2 leu2–2 11 hrs1 Δ::LEU2), or DY1707 (MATa Δsin4::URA3 ade2–1 can1–100 his3–11, 15 leu2–3 112 trp1–1) were used to purify wild-type, Δmed2, Δpdg1, or Δsin4 RNA polymerase II holoenzymes, respectively. The wild-type and mutant holoenzymes were purified through the Mono-Q fractionation step as described (14).

Biochemical Assays. The stimulation of basal transcription was measured in the system reconstituted with purified yeast proteins (8) by comparing a transcription reaction containing only core polymerase to a reaction in which 25% of the polymerase activity (measured in nonspecific assays) was supplied by holoenzyme. For measurement of activated transcription, purified Gal4–VP16 (2.5 ng) or Gcn4 (10 ng) was added to reactions containing two DNA templates (3) and either core polymerase or a mixture of core polymerase and holoenzyme as described above. The stimulation of TFIIH kinase activity was measured by comparing phosphorylation of core polymerase and holoenzyme, in amounts based on polymerase activity in nonspecific assays.

Assays of β-Galactosidase Activity in Vivo. Appropriate yeast strains were transformed with the pLGSD5 GAL-laCZ reporter (2 μm, URA3) and GAL4 fusion effector plasmids (ARS-CEN, LEU2) by using the lithium acetate procedure (15). Cells were grown in synthetic complete-Ura-Leu medium (16), and β-galactosidase assays were carried out by permeabilizing whole cells with chloroform and SDS (17).

Northern Analysis of Gene Induction. Total RNA from wild-type MG106 (MATa ade2–2 can1–100 his3–3 111 leu2–2 3 112 trp1–1 ura3–1) and Δmed2 MG107 mutant strain was prepared by hot phenol extraction (18). RNA samples (7 mg) were subjected to electrophoresis in 1% agarose-Mops-
formaldehyde gels and blotted onto nylon membranes (Hybond-N, Amersham) as described (19). Prehybridization and hybridization were performed in 0.25 M sodium phosphate (pH 7.0), 1 mM EDTA, 7% SDS, and 1% BSA at 65°C. The membranes were washed twice with 2× SSC plus 0.1% SDS for 15 min followed by a wash in 0.5× SSC plus 0.1% SDS for 15 min. DNA probes for the genes of interest were generated by PCR using the following synthetic oligonucleotides: GAL1, 5’-dGGCCGGCCATGGTGCAAGCAGGGGTG-3’ and 5’-dCCCGGCGGATCCCTTTTCTGTGGTGGACTTGGT-3’; DED1, 5’-dGGCCGGCCATGGGCGGCAATTGTGTATACTAGG-3’ and 5’-dCCCCGAGGATCCTTTCGCGGAA-3’; and ACT1, 5’-dATGGATTCTGAGGGCTGC-3’ and 5’-dTTAGAAACACTTGTGGTGAA-3’. The probes were labeled by random priming (United States Biochemical). Results were analyzed on a PhosphorImager (Molecular Dynamics).

DNA Microarray Analysis of Gene Expression. For gene expression analysis under galactose-inducing conditions, two total RNA samples were prepared from wild-type MG106 and Atmed2 MG107 mutant strains as described above for RNA blot analysis. Poly(A)+ mRNA was prepared from the total RNA by using an Oligotex mRNA Kit (Qiagen) according to the manufacturer’s protocols. The two mRNA samples were labeled individually, cohybridized to a single yeast whole genome microarray, and analyzed as described (20). For gene expression analysis under heat shock induction conditions, the wild-type MG106 and Atmed2 MG107 mutant strains were grown to OD600 = 0.6 at 23°C in yeast extract/peptone/dextrose media, warmed quickly to 37°C in a 42°C water bath, grown an additional 20 min at 37°C, and harvested. The two heat shock mRNA samples were prepared and analyzed by using a microarray as described above. Complete data sets and array images for both the galactose and heat shock experiments are available on the Internet at http://cmgm.stanford.edu/pbrown/med2.

RESULTS

Mediator Protein Mutations Specific for Activated Transcription in Vitro. We sought to identify Mediator mutations impairing one or more of the three activities measured in vitro. Deletions of the nonessential MED2, PGD1, and SIN4 genes proved effective in this regard. RNA polymerase II holoenzymes isolated from the three mutant strains were unresponsive to the activator Gal4–VP16 in transcription reconstituted with essentially pure transcription proteins (Table 1, Fig. 1). By contrast, stimulation of basal transcription and of TFIIH kinase activity remained within a factor of 2–3 of wild-type levels. Evidently the role of Mediator in transcriptional activation is distinct from those in basal transcription and CTD phosphorylation.

![Fig. 1. Transcription assays of wild-type and Atmed2 holoenzymes.](https://example.com/fig1.png)

Two observations indicated that the effect of the Atmed2 mutation was exerted through Mediator and not by an alteration of polymerase, such as a modification, or by another indirect mechanism. First, results obtained with either the naturally occurring Mediator-RNA polymerase II complex or with highly purified transcription factors (see Fig. 1 for example of primary data). This ratio was normalized by division by the ratio obtained from a second template lacking the appropriate UAS. Stimulation of basal transcription was measured by the ratio of RPB1 CTD phosphorylation in a reaction containing TFIIH and holoenzyme to CTD phosphorylation in a reaction containing TFIIH and core polymerase. Stimulation of basal transcription and TFIIH kinase activity was highly dependent on the ratio of Mediator to core polymerase in the holoenzyme fraction. This ratio, and thus the stimulatory effect, varied a few-fold for different holoenzyme preparations, even from the same strain. In contrast, VP16- and Gcn4-activated transcription was relatively unaffected by the ratio of Mediator to core polymerase.

Table 1. Functional analysis of wild-type and mutant Mediators in the purified yeast transcription system

<table>
<thead>
<tr>
<th></th>
<th>Core Pol II</th>
<th>Wild-type holoenzyme</th>
<th>Atmed2 holoenzyme</th>
<th>Gcn4-VP16 holoenzyme</th>
<th>Atmed2 holoenzyme</th>
<th>Atmed2 holoenzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation by VP16 (fold)</td>
<td>1.7</td>
<td>31</td>
<td>1.7</td>
<td>1.8</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Activation by Gcn4 (fold)</td>
<td>1.3</td>
<td>8.2</td>
<td>6.4</td>
<td>6.9</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Stimulation of basal transcription (fold)</td>
<td>—</td>
<td>18</td>
<td>6.9</td>
<td>6.6</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>Stimulation of TFIIH CTD-kinase activity (fold)</td>
<td>—</td>
<td>31</td>
<td>17</td>
<td>9</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

Fold activation by VP16 and Gcn4 was the ratio of full-length transcripts in presence of activator from a template bearing the appropriate activator-binding sequence (UAS) to transcripts in the absence of the activator (see Fig. 1 for example of primary data). This ratio was normalized by division by the ratio obtained from a second template lacking the appropriate UAS. Stimulation of basal transcription was measured by the ratio of transcripts produced by holoenzyme and core polymerase under identical reaction conditions. Stimulation of kinase activity was measured by the ratio of RPB1 CTD phosphorylation in a reaction containing TFIIH and holoenzyme to CTD phosphorylation in a reaction containing TFIIH and core polymerase.
Structure–Function Relationships of Yeast Mediator. Pgd1 is associated with Mediator through Sin4, as shown by the loss of Pgd1 from holoenzymes isolated from SIN4 deletion and RGR1 truncation mutants (6, 8). Pgd1 was also absent from the Δmed2 holoenzyme isolated here (Fig. 2). Conversely, Med2 was not retained in the purified Δpgd1 holoenzyme (Fig. 2). Med2 and Pgd1 therefore must interact, either directly or indirectly, to stabilize their mutual association with the holoenzyme. SDS/PAGE and silver staining (not shown) revealed the presence of all other Mediator polypeptides in the mutant holoenzymes (the presence of Rox3 and Gal11 could not be conclusively confirmed because they comigrate exactly with Med8 and Rpb2, respectively), so Med2 and Pgd1 are likely to occupy peripheral locations. The picture of Mediator subunit organization that emerges conforms well with the results of functional studies (Fig. 3). The mutual association of Pgd1 and Med2 with the holoenzyme is reflected in their joint requirement for Gal4–VP16 activation. The interaction of these two proteins through Sin4 leads to the requirement for Sin4 as well. Finally, the peripheral location of Pgd1 and Med2 explains why they may be dispensable for Gcn4 activation whereas Sin4 is not.

Similar Mediator Mechanism in Vitro and in Vivo. Having identified Mediator mutations specific for transcriptional activation in vitro, we investigated the effects of the same mutations on transcription in vivo. The Δmed2 mutation diminished Gal4–VP16 activation of a lacZ reporter gene downstream of Gal4-binding sites by ~10-fold (Table 2). This effect clearly involved VP16, because activation with a VP16 mutant was lower and was similarly impaired by the Δmed2 mutation (Table 2). As noted above, activation by Gal4–VP16 of transcription in vitro was diminished by the Δmed2 mutation (Table 1), also by an order of magnitude, establishing a parallel between effects of Mediator mutations in vivo and in vitro.

The effect of the Δmed2 mutation on Gcn4 activation in vivo was also consistent with the results obtained in vitro. The Δmed2 strain displayed wild-type levels of Gcn4-dependent HIS4 transcription (Fig. 4A), in keeping with the lack of requirement of Med2 protein for Gcn4 activation in vitro (Table 1). A similar parallel can be drawn for Sin4, because a Δsin4 strain previously was shown to be defective in the activation of HIS4 transcription (24) and, as mentioned, Sin4 is essential for Gcn4 activation in the reconstituted transcription system. The correlation breaks down, however, for HIS3, whose level of Gcn4-dependent transcription increases in a Δsin4 strain (24). Various mechanisms, mostly indirect, may be involved in this case.

Fig. 2. Immunoblotting analysis of wild-type and mutant holoenzymes. Mono-Q fractions of wild-type, Δmed2, and Δpgd1 holoenzymes were subjected to immunoblot analysis by using antibodies directed against Mediator components Med2, Pgd1, Med4, and Med7 (8). The amounts of the Δmed2 and Δpgd1 holoenzymes loaded on the gel were approximately three times greater than the amount of wild-type holoenzyme, to demonstrate the absence of Med2 and Pgd1 subunits.

Table 2. Gal4–VP16 activation in wild-type, Δmed2, and Δsin4 strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Vector</th>
<th>Gal4–VP16</th>
<th>Gal4–VP16FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MED2 wild type (MG106)</td>
<td>&lt;1</td>
<td>1156</td>
<td>568</td>
</tr>
<tr>
<td>Δmed2 (MG107)</td>
<td>&lt;1</td>
<td>138</td>
<td>35</td>
</tr>
<tr>
<td>SIN4 wild type (DY1880)</td>
<td>&lt;1</td>
<td>2806</td>
<td>660</td>
</tr>
<tr>
<td>Δsin4 (DY1876)</td>
<td>&lt;1</td>
<td>1763</td>
<td>342</td>
</tr>
</tbody>
</table>

Levels of β-galactosidase activity were assayed in strains with an ARS–CEN plasmid containing Gal4–VP16 or Gal4–VP16 bearing the Phc–442 to AAl mutations (Gal4–VP16FA) under control of the ADH1 promoter, and the pGSD5 GAL (lacZ) reporter plasmid (23). The units of activity are normalized to cell OD600 and results shown are the means from at least three replicate assays.
number of genes showed a >2-fold decrease in expression in the mutant strain under heat shock induction conditions. On examination of the specific genes affected, certain patterns emerged. First, the genes most dependent on Med2 were, in general, highly transcribed under the conditions tested. The majority of abundant transcript levels did not change, however, showing specificity of the Med2 effect. Second, although there was some overlap between the sets of genes dependent on Med2 under the two different growth conditions, these sets were largely distinct. For example, deletion of MED2 diminished expression of MFA1, STE3, CDC19, and MFα2, which share common regulatory sequences and which were shown previously to require the Mediator components Med6 (7) and Gal11 (27) for optimal transcription. Of these four genes, only CDC19 was Med2-dependent under both galactose induction and heat shock growth conditions. Third, two cell cycle-related genes, CTSI and EGT2, were less well transcribed under both growth conditions, which may relate to neither galactose nor heat shock induction, but rather reflect a requirement of Med2 for temporal induction of transcription. Defective expression of CTSI also has been noted in a Δsin4 strain (28), consistent with the structure–function relationships described above (Fig. 3). Fourth, as anticipated from the results of blot hybridization (Fig. 4B), galactose induction of GAL genes was defective in the mutant strain, and transcription of some heat shock promoters, including HSP12, HXT6, HSP30, and YRO2, decreased as well (Fig. 6). GAL4 and GAL80 transcript levels were unchanged in the mutant, arguing against secondary effects on GAL gene transcription arising from altered expression of these regulatory proteins. An almost 2-fold defect in expression of GAL3, however, could have played a role, because gal3 mutants show a diminished rate of galactose induction of transcription (29). Finally, the effect of the MED2

Fig. 4. RNA blot analysis of HIS4 and GAL1 induction in wild-type and Δmed2 strains. (A) Wild-type (MG106) and Δmed2 (MG107) mutant cells transformed with pRS313 (HIS3) (22) were grown in synthetic minimal medium (16) supplemented with 0.2 mM inositol, 2.0 mM leucine, 0.5 mM isoleucine, 0.5 mM valine, 0.4 mM tryptophan, 0.25 mM arginine, 0.1 mM adenine, 0.2 mM lysine, and 0.2 mM uracil to OD600 = 0.8. For starvation conditions, 3-aminitriazol (3-AT) was added to 100 mM, and the cultures were harvested 3 hr later. The RNA blot was hybridized to radioactively labeled probes for HIS4 and ACT1. (B) Wild-type (MG106) and Δmed2 (MG107) mutant cells were grown in yeast extract/peptone/raffinose medium overnight, washed with water and transferred to yeast extract/peptone/glucose (Glu) or yeast extract/peptone/galactose (Gal) media at a density of OD600 = 0.15, followed by harvest at OD600 = 0.6. The RNA blot was hybridized to radioactively labeled probes for GAL1 and DED1.

Whole Genome Analysis of MED2-Dependent Transcription. We used whole genome DNA microarrays (20) to investigate the generality of the Med2 requirement for activated transcription. Differences in specific transcript levels between Δmed2 and wild-type strains were determined under galactose and heat shock growth conditions (Fig. 5). Approximately 200 of the ∼6,000 genes analyzed showed a >2-fold decrease in expression in the Δmed2 strain grown in galactose. A similar
transcription defects in a med2 strain. (A) Well characterized galactose-induced genes that suffer a >2-fold defect in expression in the ∆med2 strain grown on galactose medium are shown. For comparison, fold differences in expression of these same genes under heat shock conditions (in glucose) are also indicated. (B) Well characterized heat shock-induced genes that suffer a >2-fold defect in expression in the ∆med2 strain under heat shock (37°C) growth conditions are shown. For comparison, fold differences in expression of these same genes under galactose growth conditions (30°C) are also indicated. “Fold Decrease (∆med2)” and “Fold Increase (∆med2)” refer to ratios (wild-type/∆med2 and ∆med2/wild-type, respectively) of normalized transcript levels.

deletion on galactose induction was selective: transcript levels of several genes shown by previous microarray analysis to be induced by galactose (30), such as COX12, QCR6, COR1, PET9, COX8, ATP5, ATP3, COX9, and MCR1, were essentially unchanged.

The picture that emerges from the microarray analysis is one of gene-specific involvement of Med2 in transcriptional induction in vivo. There appears to be no general requirement of the protein for constitutive (“basal”) transcription. Although the array analysis also revealed increased expression of some genes in the Δmed2 strain, these increases seem likely to reflect an adaptive response rather than a direct consequence of the genetic deficiency.

**DISCUSSION**

The chief import of this work lies in the validation of transcriptional activation in the yeast system in vitro and the implications for the role of Mediator in vivo. The work must be considered in the context of other efforts to elucidate transcriptional activation mechanisms. Dissection of human and Drosophila systems led to the discovery of TAFs and to evidence for their requirement for activation in vitro. It now appears that the function of TAFs is to augment the sequence specificity of TATA-binding protein, rather than to facilitate enhancer–promoter communication (31). Other factors, similar or equivalent to yeast Mediator, are more important than TAFs for activation in the human system in vitro (32). The same questions arise for yeast Mediator, whether its requirement for activation in vitro holds true in vivo, and whether it conveys the regulatory influence of enhancers in vivo.

Pursuit of these questions was facilitated by our finding of Mediator mutations that abolish activated transcription in vitro with only minimal effects (at most 2- to 3-fold) on basal transcription or TFIIH kinase activity. Study of the same mutations in vivo revealed selective effects on activated transcription as well. The number of inducible genes whose expression was diminished by the mutations should be regarded as a lower estimate, because only one Med protein mutation and two inducing conditions have so far been investigated by microarray analysis. Selectivity was shown by a lack of effect of the mutations on DEDI and ACTI transcript levels in RNA blots and by a lack of effect on expression of the majority of yeast ORFs in microarrays. Although the cellular equivalent of basal transcription in vitro has not been defined, the diminished expression of inducible genes in the med2 deletion strain stands out against the broad background of genes that are unaffected. It can be said that Med2 protein plays a positive role in the transcription of many genes but that it is not generally required, so its function in vivo is in transcriptional activation. Evidence that this role is direct and relates to upstream regulatory sequences comes from the results obtained for induction of GAL gene transcription and for Gal4–VP16 activation of a Gal4-binding reporter construct. Mediator thus provides a functional connection between upstream sequences and promoters. Evidence for related complexes in mammalian cells (33, 34) suggests the Mediator connection is conserved across species from yeast to man.

Previous work of others showed the involvement of Sin4 and Rgr1 in the negative regulation of many genes, leading to their designation as “global” repressors (35). The discovery of Mediator united these diverse molecules in a common biochemical entity (3, 6, 8). It was further shown that Sin4, Rgr1, Gal11, and Pgd1 (Hrs1) interact in the same subcomplex of the Mediator assembly, thus accounting for their involvement in regulation of the same set of genes (6). Our findings extend the characterization of this Sin4/Rgr1 module in two respects. First, Med2 is identified as an additional component of this module, occupying a peripheral location, interdependent in its association with Pgd1 (Hrs1). Second, the module is required for activation of many genes in vitro and in vivo. Using previously defined terminology, Mediator may be described as a global coactivator and corepressor.

It is noteworthy that the involvement of Mediator in both activation and repression is brought about by the same subcomplex of the larger assembly. Biochemical and genetic findings thus converge on the notion of a common activation/repression mechanism. This idea is nicely compatible with a suggestion that repression occurs through the same complex of RNA polymerase II and general factors as the initiation of transcription (36).

Our finding that VP16 and Gcn4 differ in their Mediator subunit requirements for activation was unexpected in view of the common classification of these proteins in a single group of “acidic” activators. It will be instructive to determine the Mediator subunit requirements for other “acidic” activators. Genetic analyses of VP16 (37) and Gcn4 (38), examining the consequences of amino acid substitutions in the activation domains of these proteins for function in vivo, have questioned the importance of acidic amino acids, and our findings indicate that the two activators may differ in regard to mechanism.

Ultimately, activator proteins must be categorized on the basis of mechanism. Although this study does not directly address the activation mechanism, some findings are pertinent. Binding studies in vitro and functional studies in vivo have suggested that a direct physical interaction between the activation domain of Gal4 and Srb4, a mediator component, is critical for Gal4-stimulated transcription (39). Holoenzymes purified from the ∆med2, ∆pgd1, and med6Δ strains all retain Srb4 but support neither Gal4 activation in vivo nor VP16 activation in vitro. Despite the presence of Srb4, preliminary studies indicate a diminished interaction between the ∆med2 and ∆pgd1 holoenzymes and both VP16 and Gcn4 (L.C.M., C.M.G., and R.D.K., unpublished results). We investigated the possibility of activator–Med2 or –Pgd1 interaction with the use of recombinant proteins and obtained only negative results (data not shown). Others have reported that Med6 also is required for a VP16 response in vitro, that Med6 alone is lost from isolated med6Δ Mediator, and that no activator–Med6 interaction can be detected (7). The separate requirements for Med2/Pgd1 and Med6, and the lack of activator interaction, are not immediately compatible with the
“recruitment model” (40) of activation, but no definitive statement regarding the activation mechanism can be made at the present time.

We thank B. Cairns for assistance in the phenotypic analysis of the Δmed2 mutant and D. Bushnell for purified proteins. We also thank Y.-W. Jiang for the Δsin⁴ strain and helpful discussions. Whole genome yeast DNA microarrays were kindly provided by J. DeRisi and V. Iyer. The Δpfd1Δ(Jhrs1) strain and anti-Pgd1(Hrs1) antibodies were gifts from A. Aguilera and the DY1880 and DY1876 (Δsin⁴) strains were gifts from D. J. Stillman. L.C.M. was recipient of a Cancer Research Fund of the Damon Runyon–Walter Winchell Foundation Fellowship, DRG-1361. C.M.G. was a recipient of a Swedish Cancer Society postdoctoral fellowship. This research was supported by Swedish Cancer Society Grant 3947 (to C.M.G.) and National Institutes of Health Grant GM36659 (to R.D.K.).