Role of Mediator in Regulating Pol II Elongation and Nucleosome Displacement in *Saccharomyces cerevisiae*

Selena B. Kremer, Sunyoung Kim,¹ Jeong Ok Jeon,¹ Yara W. Moustafa,¹ Apeng Chen,¹ Jing Zhao,
and David S. Gross²

Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, Shreveport, Louisiana 71130

ABSTRACT Mediator is a modular multisubunit complex that functions as a critical coregulator of RNA polymerase II (Pol II) transcription. While it is well accepted that Mediator plays important roles in the assembly and function of the preinitiation complex (PIC), less is known of its potential roles in regulating downstream steps of the transcription cycle. Here we use a combination of genetic and molecular approaches to investigate Mediator regulation of Pol II elongation in the model eukaryote, *Saccharomyces cerevisiae*. We find that *ewe* (*expression without heat shock element*) mutations in conserved Mediator subunits *Med7*, *Med14*, *Med19*, and *Med21*—all located within or adjacent to the middle module—severely diminish heat-shock-induced expression of the *Hsf1*-regulated *HSP82* gene. Interestingly, these mutations do not impede Pol II recruitment to the gene's promoter but instead impair its transit through the coding region. This implies that a normal function of Mediator is to regulate a postinitiation step at *HSP82*. In addition, displacement of histones from promoter and coding regions, a hallmark of activated heat-shock genes, is significantly impaired in the *med14* and *med21* mutants. Suggestive of a more general role, *ewe* mutations confer hypersensitivity to the anti-elongation drug 6-azauracil (6-AU) and one of them—*med21*—impairs Pol II processivity on a *GAL1*-regulated reporter gene. Taken together, our results suggest that yeast Mediator, acting principally through its middle module, can regulate Pol II elongation at both heat-shock and non-heat-shock genes.

In eukaryotes, transcription of the DNA template into pre-mRNA by RNA polymerase II (Pol II) occurs in a well-defined, stepwise fashion. First, chromatin, the nucleoprotein complex in which the DNA is packaged, must unfold into a 10 nm, beads-on-a-string filament, and for many genes a nucleosome-free region needs to be created over the core promoter (Venters and Pugh 2009). Both are achieved via activator-mediated recruitment of chromatin modification and remodeling enzymes (reviewed in Li *et al.* 2007). Once a permissive chromatin template has been created, Pol II and the other general transcription factors then bind the core promoter, where they are assembled into a preinitiation complex

(PIC; formally analogous to the closed RNA polymerase complex in prokaryotes). Next, Pol II forms an open complex concomitant with ATP-dependent melting of the DNA strands and initiates transcription following phosphorylation of its C-terminal repeat domain (CTD) at Ser5 residues by the TFIIF kinase, Cdk7. Following synthesis of ~25–30 nucleotides, Pol II pauses, allowing the nascent RNA to be capped. Finally, Pol II transitions to productive elongation, which requires Ser2 phosphorylation of the CTD. In metazoans, this is catalyzed by P-TEFb and in yeast by *Bur1* and *Ctk1* (reviewed in Saunders *et al.* 2006).

A key regulator of many of the above steps is Mediator, an evolutionarily conserved, modular multiprotein complex. Mediator acts as a signal transducer through its interaction with gene-specific regulatory proteins and Pol II to fine tune gene transcription levels. In organisms as diverse as budding yeast and mammals, Mediator adopts a similar overall architecture characterized by the presence of four modules termed head, middle, tail, and kinase (Cai *et al.* 2009). Although there exists only limited sequence homology between

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¹These authors contributed equally to this work.

²Corresponding author: Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71103.
E-mail: dgross@lsuhsc.edu

orthologous subunits in mammalian and yeast Mediator, their functions seem to be conserved (Bourbon 2008). The head module is essential for Mediator function as mutations within it abolish transcription *in vivo* (Thompson and Young 1995). Moreover, in yeast, the head subunit **Med17/Srb4** physically interacts with the Pol II subunit, **Rpb3**, and this interaction is important for genome-wide Pol II recruitment *in vivo* (Soutourina *et al.* 2011). The middle module confers structural integrity to Mediator (Baumli *et al.* 2005). It also facilitates activated transcription in purified systems (Baidooonso *et al.* 2007) and has a dual role in both activation and repression in cells (Tabtiang and Herskowitz 1998; Gromoller and Lehming 2000; Singh *et al.* 2006). The middle module also has been implicated in regulating transcriptional silencing (Zhu *et al.* 2011a) and interaction with histone tails (Zhu *et al.* 2011b). The tail module is the principal physical target of gene-specific activators and repressors. The strong conservation of function is illustrated by the fact that the tail subunit, **Med15/Gal11**, is a target of both mammalian sterol regulatory element-binding protein (SREBP), a sensor of cholesterol and regulator of lipid homeostasis, and a zinc finger-containing activator in yeast that senses fatty acid levels. Finally, the kinase (**Cdk8**) module is a separable subcomplex that reversibly associates with core Mediator and has been implicated in transcriptional repression (reviewed in Malik and Roeder 2010).

There is considerable evidence that Mediator plays roles in PIC assembly and function (reviewed in Malik and Roeder 2000; Myers and Kornberg 2000; Malik and Roeder 2010), and may serve as a scaffold around which the entire PIC assembles (Bernecky *et al.* 2011). However, less is known of its potential downstream roles. Several recent studies in mammalian cells implicate a role for Mediator in post-recruitment regulation. First, analysis of the ELK1-regulated *Egr1* gene revealed that despite a substantial reduction in *Egr1* transcript levels in *Med23*^{-/-} mouse embryonic stem cells, PIC recruitment was only moderately diminished. Pol II occupancy within the proximal coding region, on the other hand, was drastically reduced (Wang *et al.* 2005). Second, addition of Mediator facilitates Pol II release from a DRB sensitivity-inducing factor (DSIF)-imposed paused state *in vitro* (Malik *et al.* 2007). Third, p53-induced changes in the conformational state of Mediator were found to stimulate stalled, promoter-proximal Pol II molecules to productive elongation both *in vitro* and in cells (Meyer *et al.* 2010). Fourth, Cdk8 was observed to be a potent regulator of Pol II elongation at serum-responsive genes in human cells, as its knockdown impaired Pol II elongation and correlated with decreased levels of CTD phosphorylation at Ser2 and Ser5. Moreover, co-immunoprecipitation assays demonstrated the presence of the P-TEFb subunits Cdk9 and cyclin T1 in a Cdk8–Mediator complex isolated from these cells (Donner *et al.* 2010). Fifth, the N-terminal domain of Med26—located at the junction between middle and tail modules—was observed to act as a docking site for complexes that contain transcription elongation factors such

as P-TEFb and members of the ELL/EAF family in human cells (Takahashi *et al.* 2011). Consistent with this, siRNA-mediated depletion of Med26 resulted in defective recruitment of transcription elongation factors and Pol II CTD phosphorylation at several genes (Takahashi *et al.* 2011).

Taken together, these mammalian studies indicate that Mediator's role extends beyond PIC assembly and function, and that Mediator has roles at several postrecruitment steps. While less is known in this regard of yeast Mediator, genetic analyses have implicated a role for head (**Med18/Srb5**, **Med19/Rox3**, and **Med20/Srb2**), middle (**Med31/Soh1**), and tail subunits (**Med16/Sin4**, **Med15**, and **Med2**) in transcription elongation (Malagon *et al.* 2004; Gaillard *et al.* 2009). In addition, a recent genetic/ChIP analysis showed that Mediator plays a role in postrecruitment regulation of both a *lexA*–TBP-driven reporter gene and a natural gene, *CYC1* (Lee *et al.* 2010). Our laboratory has previously shown that Mediator plays an essential role in governing heat-shock (HS) gene expression in *Saccharomyces cerevisiae*. Genetic analysis demonstrated that six Mediator subunits, located principally in the middle module, can act as negative regulators of heat-shock protein (*HSP*) gene basal transcription, and positive regulators of induced *HSP* gene transcription (Singh *et al.* 2006). These results suggested that Mediator may collaborate with the evolutionarily conserved transcriptional activator, **Hsf1**, in controlling the dynamic range of *HSP* gene expression. In this study, we have extended our investigation into how Mediator regulates *HSP* gene transcription, with emphasis on its role in regulating Pol II elongation. We find that mutations in subunits identified in our earlier genetic screen severely diminish transcriptional output of the **Hsf1**-responsive gene, *HSP82*, yet typically do not impede Pol II recruitment to its promoter. Instead, these mutations impair either Pol II elongation rate or processivity at *HSP82*, and this effect appears to extend to non-heat-shock (NHS) genes as well.

Materials and Methods

Yeast strain construction

Spontaneous *ewe* suppressors were derived from strain HS1004 (SLY101 background) and sorted into six complementation groups, termed EWE1–EWE6, as described previously (Singh *et al.* 2006). As HS1004 is a *MATa* strain that bears the mutated *hsp82-ΔHSE1* allele, we replaced *hsp82-ΔHSE1* with wild-type *HSP82* to permit RNA expression and ChIP analysis of this gene. This was achieved by mating strain SLY101 (*MATa*, *HSP82*) to representative suppressors in complementation groups EWE3–EWE6: J121 (bearing *med7-1002*); J20 (bearing *med21/srb7-1002*); J84 (bearing *med10/nut2-1002*); and B20 (bearing *med19/rox3-1002*). These suppressors were selected on the basis of the robustness of their *ewe* phenotype (*hsp82-ΔHSE1/lacZ* basal expression). Diploids were then sporulated and tetrads dissected; spores containing both *HSP82* and the *ewe* mutation were identified using molecular and genetic methods,

Table 1 Yeast strains

Strain	Genotype	Source or reference
SLY101	<i>MATα ade⁻ can1-100 cyh2^r his3-11,15 leu2-3,112 trp1-1 ura3</i>	Lee and Gross (1993)
JHD401	SLY101; <i>asf1Δ</i>	This study
EAS2001	SLY101; <i>hsp82-2001; sir4Δ2::HIS3</i>	Sekinger and Gross (1999)
JHD303	EAS2001; <i>ctk1Δ</i>	This study
DCY101	EAS2001; <i>dst1Δ</i>	This study
DCY102	EAS2001; <i>spt4Δ</i>	This study
HS1001	SLY101; <i>hsp82-ΔHSE1 hsp82-ΔHSE1-HIS3::ura3Δ hsp82-ΔHSE1/lacZ::LEU2 TRP1⁺</i>	Singh et al. (2006)
HS1004	HS1001; <i>MATα hsp82-ΔHSE1/HIS3::URA3 leu2::hsp82-ΔHSE1/lacZ::leu2::KAN-MX</i>	Singh et al. (2006)
B20	HS1004; <i>med19-1002</i>	Singh et al. (2006)
J20	HS1004; <i>med21-1002</i>	Singh et al. (2006)
J37	HS1004; <i>med14-1003</i>	Singh et al. (2006)
J44	HS1004; <i>med7-1002</i>	Singh et al. (2006)
J84	HS1004; <i>med10-1002</i>	Singh et al. (2006)
J121	HS1004; <i>med7-1002</i>	Singh et al. (2006)
DAD2	HS1001; <i>med20Δ::KAN-MX</i>	This study
YAH01	HS1001; <i>med12Δ::KAN-MX</i>	This study
RRG2	HS1001; <i>cdk8Δ::KAN-MX</i>	This study
HS1005	HS1001; <i>med16Δ::KAN-MX</i>	This study
JHD1	HS1001; <i>med5Δ::KAN-MX</i>	This study
SK-HS1004	SLY101; <i>hsp82-ΔHSE1-lacZ::leu2Δ::KAN-MX</i>	This study
SK-B20c	SK-HS1004; <i>MATα med19-1002</i>	This study
SK-J20b	SK-HS1004; <i>MATα med21-1002</i>	This study
SK-J84b	SK-HS1004; <i>med10-1002</i>	This study
SK-J121b	SK-HS1004; <i>MATα hsp82-ΔHSE1/HIS3::URA3 med7-1002</i>	This study
DY2694	<i>MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rgr1-Δ2::LEU2</i>	D. J. Stillman
SBK500	SK-HS1004; <i>MATα rgr1-Δ2::LEU2</i>	This study
BY4741	<i>MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 dst1Δ</i>	Research Genetics
	BY4741; <i>dst1Δ::KAN-MX</i>	Research Genetics

creating strains termed SK-J121b, SK-J20b, SK-J84b, and SK-B20c, respectively. The engineered mutation, *rgr1- Δ 2*, was crossed into the HS1004 background as previously described (Singh et al. 2006), creating strain SBK502. These strains were employed in RT-qPCR and ChIP assays.

For spot dilution and GLAM (gene length-dependent accumulation of mRNA) assays, the following HS1004 derivatives were used: J37 (*med14-1003*); J44 (*med7-1002*, bears the same mutation as J121); J20 (*med21-1002*); J84 (*med10-1002*); and B20 (*med19-1002*). In addition, the closely related *MAT α* strain, HS1001, was used for the following gene deletions (*KAN-MX* knockouts): *med5 Δ* , *med12 Δ* , *med16 Δ* , *med20 Δ* , and *cdk8 Δ* . Knockout DNA fragments were generated by PCR using as template genomic DNA isolated from strains in the Res-Gen strain collection (generously provided by Kelly Tatchell). Strain genotypes are presented in Table 1.

Sequence analysis of chromosomal mutations

To amplify the pertinent locus of each *ewe* suppressor, we employed 100 ng of genomic DNA as template and 1 unit of Phusion DNA polymerase (Finnzymes, F-530). All PCR products were purified using QIAquick gel extraction kit (Qiagen, 28704). Purified PCR products were sequenced on both upper and lower strands using primers spaced ~200–300 bp apart by Retrogen. For each *ewe* suppressor, two indepen-

dent genomic DNA samples were analyzed to obviate the possibility of a PCR-generated artifact. Amplicons and sequencing strategy were as follows: *med7*, amplified region encompassed –85 to +749 (where +1 = ATG initiation codon), two upper and two lower strand sequencing primers used; *med10*, –141 to +570, two upper, two lower strand sequencing primers; *med14*, –142 to +3420, six upper, six lower strand sequencing primers; *med19*, –91 to +733, two upper, two lower strand sequencing primers; and *med21*, –127 to +449, two upper, two lower strand sequencing primers.

β -Galactosidase liquid assays

β -Galactosidase liquid assays were performed as previously described (Singh et al. 2006) on cells either maintained at 30° (NHS) or shifted to 39° for 45 min (HS). The latter were returned to 30° for 20 min to permit efficient export and translation of *lacZ* mRNA.

RT-qPCR analysis

Cells were cultivated to an A_{600} of ~0.6 in a 100-ml culture at 30° and 20-ml aliquots were removed and subjected to an instantaneous 30° to 39° upshift through addition of an equivalent volume of prewarmed (51°) medium for 0, 2, 15, and 60 min. Heat-shock induction was terminated through addition of 20 mM sodium azide, and RNA was isolated as above. Contaminating genomic DNA was removed

from each RNA sample by digestion with TURBO DNA-free (Applied Biosystems, AM1907), followed by phenol/chloroform extraction. One half microgram of purified RNA was used in each cDNA synthesis with ProtoScript RT-PCR kit (New England Biolabs, E6400S). Random primers were used in cDNA synthesis. Two percent of the synthesized cDNA was added to each 20- μ l real-time PCR reaction, performed using RT² qPCR SYBR Green/ROX master mix (SABiosciences, 330529) on an Applied Biosystems 7900HT real-time PCR system. A standard curve specific for each amplicon was generated, the quantity of DNA present in each sample was determined, and background signal was subtracted. Primers were designed to target the coding regions of *HSP82*, *SSA4*, and *SCR1*. Their coordinates are: *HSP82*, +1248 to +1444; *SSA4*, +1092 to +1150; and *SCR1*, +21 to +76. For quantification, *SCR1* was used to normalize both *HSP82* and *SSA4* mRNA levels.

Chromatin immunoprecipitation ChIP

End point PCR: End point (gel-based) PCR ChIP assays (Figure 3, Figure S2, and Figure S3) were performed as previously described (Zhao *et al.* 2005; Kremer and Gross 2009). To effect immunoprecipitation (IP) of Pol II, an anti-CTD rabbit antiserum (Balakrishnan and Gross 2008) was used; to effect immunoprecipitation of Myc-tagged H4, the 9E10 mouse monoclonal antibody (Santa Cruz Biotechnology) was used. For Pol II ChIPs, net signal at *HSP82* was normalized to that at a nontranscribed locus on chromosome V (*ARS504*). For myc-H4 ChIPs, net signal at *HSP82* was normalized to that at the *PHO5* promoter.

Real-time PCR: For real-time ChIP analysis (Figure 4), 200-ml midlog cell cultures were used in heat-shock time course experiments, and 50-ml aliquots were removed for each time point. Cells were crosslinked with 1% formaldehyde and chromatin was sheared to a mean length of $\sim$ 0.5 kb with a Branson 250 sonifier equipped with a microtip. Approximately 2.6 ml of crosslinked chromatin was obtained for each time point and the equivalent of $\sim$ 600 μ g of chromatin protein (as quantified by the Bradford assay; typically 0.2 ml) was employed for each IP. Immunoprecipitations were conducted through addition of 55 μ l of CL-4B protein A sepharose beads and 2 μ l of an H3 globular domain-specific antibody (Abcam, ab1791) with incubation at 4° overnight. DNA was purified and dissolved in 50 μ l TE, and 2 μ l of immunoprecipitated DNA was added to each 20- μ l real-time PCR reaction, performed as described above. The quantity of DNA present in each IP was determined using a standard curve specific for each amplicon and background signal arising from beads alone was subtracted. To normalize for variation in sample recovery, abundance of H3 at the *PHO5* promoter (which is not activated by heat shock) was determined for each sample. Amplicon coordinates were: *HSP82* promoter, –157 to –88; *HSP82* ORF, +1248 to +1444; *HSP82* 3'-UTR, +2134 to +2228; and *PHO5* promoter, –197 to –124.

Spot dilution analysis

Spot dilution analysis was conducted essentially as described (Kim *et al.* 2011). Cultures were applied to SDC –Ura solid medium with or without 50 μ g/ml 6-azauracil.

GLAM assay

Wild-type (WT) and mutant strains were transformed with either long transcript plasmid (pSch212, containing *P_{GALI}-PHO5::lacZ*) or short transcript plasmid (pSch202, containing *P_{GALI}-PHO5*) (Morillo-Huesca *et al.* 2006) (gift of Daniel Ginsburg and Alan Hinnebusch). Cell growth, collection, and acid phosphatase activity were assayed as previously described (Kim *et al.* 2011).

Results

Previous work has shown that heat-shock-induced gene transcription in *S. cerevisiae* is resilient to mutations in TFIIA, TFIID, TFIIF, SAGA, the head module of Mediator, and even the Pol II CTD (Moqtaderi *et al.* 1996; Apone *et al.* 1998; Lee and Lis 1998; McNeil *et al.* 1998; Chou *et al.* 1999). By contrast, such *Hsf1*-mediated activation is sensitive to mutations in Mediator's middle and tail modules (Lee *et al.* 1999; Singh *et al.* 2006). Here we investigate the nature of the *Hsf1* transcriptional defect caused by mutations in the middle module and more generally, the role of Mediator in regulating Pol II elongation in *S. cerevisiae*. In a previous genetic analysis, we isolated spontaneous mutations in six Mediator subunits—*Med7*, *Med10/Nut2*, *Med14/Rgr1*, *Med16/Sin4*, *Med19/Rox3*, and *Med21/Srb7*—as extragenic suppressors (Singh *et al.* 2006). These suppressors were selected on the basis of their ability to increase the basal transcription of a chromosomal reporter gene, *hsp82- Δ HSE1/lacZ* (*HSP82* lacking its high-affinity *Hsf1* binding site, HSE1, fused to the *Escherichia coli lacZ* coding region), and were thus termed *expression without heat shock element (ewe)* mutants. In the present study, we have focused on representative members of complementation groups *EWE2*–*EWE6* that encode subunits located either within or adjacent to the middle module. The *EWE1* complementation group is composed of alleles of the nonessential tail subunit, *Med16* (Singh *et al.* 2006), and was not studied here.

We first wished to determine the nature of the mutations borne by these suppressors. As shown in Table 2, the alleles designated *med7-1002* and *med10-1002* (members of complementation groups *EWE3* and *EWE5*) contain stop codons that result in C-terminal truncations of 25 and 34 amino acids, respectively. Similarly, *med19-1002* (*EWE6*) bears a single nucleotide deletion, resulting in a frameshift and as a consequence, a novel, C-truncated terminus. The fourth spontaneous mutant, *med21-1002* (*EWE4*), bears a T227C point mutation that results in the substitution of proline for leucine at position 76. This mutation is located within the α 2 helix of *Med21* and a hydrophobic patch that serves as a *Med7* binding site (Baumli *et al.* 2005). Thus, the L76P

Table 2 Sequences and expression phenotypes of ewe mutations

EWE Complementation group	Mediator subunit (size)	Allele	Mediator module	Expression phenotype ^a (<i>hsp82-ΔHSE1/lacZ</i> reporter)		Mutation
				Basal expression	Fold induction	
Wild type	—	—	—	1.0	10.5	—
EWE2	Med14/Rgr1 (1082 aa)	<i>rgr1-Δ2</i>	Spans tail and middle	140	1.2	336-amino-acid C-terminal truncation ^b
EWE3	Med7 (223 aa)	<i>med7-1002</i>	Middle	58	1.0	25-amino-acid C-terminal truncation (C592T converts Q198 to a stop codon)
EWE4	Med21/Srb7 (140 aa)	<i>med21-1002</i>	Middle	45	1.6	L76P point mutation (T227C)
EWE5	Med10/Nut2 (158 aa)	<i>med10-1002</i>	Middle	130	0.84	34-amino-acid C-terminal truncation (C371A converts S124 to a stop codon)
EWE6	Med19/Rox3 (220 aa)	<i>med19-1002</i>	Spans head and middle	21	3.0	Deletion of A577 causes a frameshift at R193 resulting in a novel, truncated C terminus ^c

^a Basal expression levels (β-galactosidase units) were normalized to the wild-type strain whose expression was set to 1; fold induction represents the quotient of induced expression/basal expression.

^b Engineered mutation (gift of D. J. Stillman).

^c C termini: Med19*: KKRKNKSSGS SMATPTHSDS HEDMKRRRL - Stop. Med19-1002: KKGRNTLEVR WLQHIVTVM RI - Stop.

mutation is predicted to not only break the α2 helix but may also disrupt the Med7–Med21 interaction. Likewise, the stop codon in *med7-1002*, Q198*, lies within the α3 helix that contacts Med21 through the proteins' coiled-coil domains (Baumli *et al.* 2005), suggesting that it too may diminish the stability of the Med7–Med21 subcomplex. Finally, we employed the engineered mutation, *rgr1-Δ2*, as a representative of the EWE2/MED14 complementation group for ChIP and expression assays. *rgr1-Δ2* bears a 336-amino-acid deletion of its C terminus that results in the loss of tail subunits Med3, Med15, and Med16 from isolated Mediator complexes (Jiang *et al.* 1995; Li *et al.* 1995).

As summarized in Table 2, each ewe mutation has a profound effect on *hsp82-ΔHSE1/lacZ* basal expression, increasing it 20- to 140-fold. On the other hand, each mutation severely impairs the fold inducibility of *hsp82-ΔHSE1/lacZ*, consistent with previous observations of other ewe alleles of these genes (Singh *et al.* 2006). Each mutation also conferred marked temperature sensitivity, while several (*med7-1002*, *med10-1002*, and *med19-1002*) conferred a mild growth defect at 30°; the engineered *rgr1-Δ2* mutation caused severe slow growth (see Figure 1). Nonetheless, and important for work described below, a 60 min, 39° heat shock had no discernible effect on the viability of any mutant (see Supporting Information, Figure S1).

To extend our expression analysis to the wild-type *HSP82* gene, we conducted reverse transcription coupled with quantitative PCR (RT-qPCR) on newly constructed strains (see Materials and Methods). As shown in Figure 2A, the spontaneous ewe mutations have a modest effect on basal *HSP82* transcription, and may even decrease it. This contrasts with the stimulatory effect of these mutations on the *hsp82-ΔHSE1/lacZ* reporter, whose promoter is assembled into a repressive dinucleosome (Gross *et al.* 1993; Venturi *et al.* 2000) and whose coding region is of bacterial origin. Therefore, the basal expression state of *hsp82-ΔHSE1/lacZ*

may be sensitized to recessive mutations in genes encoding specific subunits of Mediator, possibly as a consequence of the reporter's unique chromatin structure (see Discussion). In addition, the ewe mutations significantly diminished heat-shock-induced *HSP82* mRNA levels at multiple time points (Figure 2B, left). The *rgr1-Δ2* mutation, in particular, reduced *HSP82* transcript levels (by 70–85%). Similar effects were seen with a second Hsf1-responsive gene, *SSA4* (Figure 2B, right).

To gain insight into the basis for the transcriptional defect, we used ChIP to measure Pol II occupancy of the *HSP82* promoter and coding region in the context of each ewe mutation. Formaldehyde crosslinking allowed us to take “snapshots” of Pol II occupancy at closely spaced time points during the early stages of heat shock (0.5, 0.75, 1.0, 1.25, 1.5, and 2.5 min) as well as at longer times (15 and 60 min). This analysis revealed that despite the reduction in transcript levels, Pol II recruitment to the promoter was not

Figure 1 Growth phenotypes of the MED⁺ strain SK-HS1004 and congenic ewe mutants employed in this study. Cells were grown on rich medium at the indicated temperature for 2 days.

Figure 2 *ewe* mutations diminish both noninduced and induced *HSP82* transcription. (A) RT-qPCR analysis of basal *HSP82* mRNA expression levels in the *MED*⁺ strain and *ewe* mutant derivatives. Cells were cultivated at 30° and RNA was isolated. *HSP82* mRNA/*SCR1* RNA quotients are shown. (*SCR1* is a Pol III transcript and served as an internal recovery control.) The *MED*⁺ quotient was set to 1.0, and all others were normalized to it. (B) RT-qPCR analysis as in A, except cells were heat-shocked for 2, 15, or 60 min and both *HSP82* mRNA/*SCR1* RNA and *SSA4* mRNA/*SCR1* RNA quotients were determined and then normalized to the *MED*⁺ *T* = 0 min sample. For both panels, means ± SEM are depicted (*N* = 3 or 4).

impeded in the *med7*, *med14* (*rgr1-Δ2*), and *med19* mutants at any time point (Figure 3A; see also Figure S2, black bars, for an alternative depiction of the data). In the *med7* mutant, Pol II promoter recruitment may be increased. In addition, Pol II occupancy within the *HSP82* coding region was also largely unaffected in the *rgr1-Δ2* and *med7-1002* mutants (Figure 3, B and C; Figure S2, white and purple bars), raising the possibility that their transcription phenotypes arise from reduced Pol II elongation rate (see Discussion). By contrast, in the *med19* and *med21* mutants, Pol II occupancy was diminished within the *HSP82* transcribed region, particularly toward its 3'-end, suggestive of a defect in polymerase processivity. Finally, in contrast to the others, the *med10-1002* mutation substantially reduced Pol II occupancy within the promoter with concomitant reduction within both ORF and 3'-UTR, consistent with a defect in Pol II recruitment. Therefore, *ewe* mutations affect more than one step in the transcription cycle, and several appear to elicit their effects at a postrecruitment stage.

We next asked whether the *ewe* mutations affected either the extent or kinetics of nucleosome eviction within *HSP82* following heat shock, using histone H3 ChIP as a measure of nucleosome density. Our previous work has shown that mutations in chromatin remodeling (*Swi/Snf*, *Isw1*, *Isw2*, and *Chd1*) and histone modification (*SAGA*, *NuA3*, *Set1*, *Set2*, and *Paf1*) enzymes have little or no effect on this process (Zhao *et al.* 2005; Kremer and Gross 2009; J. Zhao and D. S. Gross, unpublished observations). In addition, we found that depletion of the histone chaperone *Asf1* had no detectable effect (Figure S3A), despite the fact that this pro-

tein has previously been shown to play an important role in removing nucleosomes at *Pho4*- and *Gal4*-regulated genes (Adkins *et al.* 2004; Schwabish and Struhl 2006). Knockout of genes encoding transcription elongation factors *Ctk1*, *TFIIS*, and *Spt4* likewise did not impact histone eviction at *HSP82* (Figure S3B). By contrast, the *med14* and *med21* mutations in Mediator had a marked effect on H3 displacement, and not only at the *HSP82* promoter, but also within its ORF (Figure 4, A and C, asterisks). This could be due to impaired nucleosome disassembly, increased reassembly, or a combination of the two. The *rgr1-Δ2* chromatin phenotype is particularly notable given that Pol II density at *HSP82* is unaffected in this mutant. The *med7* and *med19* mutations had a lesser, yet discernible, effect on the extent of heat-shock-induced H3 depletion (Figure 4, B and E). Ironically, the *med10* mutation did not impair nucleosome eviction (Figure 4D), despite reduced Pol II occupancy within both *HSP82* promoter and coding regions. Differences in *ewe* phenotypes point to the fact that different lesions within Mediator, even those within its middle module, affect transcription, Pol II occupancy, and histone H3 density of activated *HSP82* in different ways.

We next investigated whether Mediator mutations affect transcription elongation more generally by comparing the growth of WT and mutant strains at 30° on medium either lacking or containing 6-AU, a drug known to reduce both the elongation rate and processivity of Pol II, secondary to the depletion of cellular UTP and GTP pools (Exinger and Lacroute 1992; Mason and Struhl 2005). As shown in Figure 5, two spontaneous *ewe* mutants, *med14-1003* (bearing a 179-

Figure 3 ewe mutations differentially affect RNA Pol II abundance at the heat-shock-induced *HSP82* gene. *In vivo* crosslinking analysis of Pol II at the *HSP82* promoter (A), ORF (B), and 3'-UTR (C) either prior to or for the indicated times following an instantaneous 39° heat shock. Pol II abundance at *HSP82*, quantified using an end-point PCR (gel-based) ChIP assay (Kremer and Gross 2009), was normalized to its abundance at a nontranscribed region (*ARS504*) evaluated in the same multiplex PCR. For each time point, data are presented for WT and the five congeneric ewe mutants in the order indicated in the key. For the WT strain, shown are means $\pm$ SEM ($N = 3$ biological samples; PCR = 12); for *rgr1-Δ2* and *med10-1002*, shown are means $\pm$ SEM ($N = 4$; PCR = 4); and for *med7-1002*, *med21-1002*, and *med19-1002*, shown are means $\pm$ SD ($N = 2$; PCR = 2).

amino-acid C-terminal truncation) and *med7-1002*, exhibited striking sensitivity to 6-AU (compare to the isogenic *MED*⁺ strain, row 9). (The *rgr1-Δ2* mutant grew too slowly on control medium to be usable in this assay.) These ewe mutants exceeded the 6-AU sensitivity of a strain lacking *TFIIS*, an elongation factor that facilitates the release of stalled Pol II molecules (*dst1Δ*; compare to the isogenic wild-type strain, row 1). Synthetic phenotypes were also observed for the other three ewe mutants, *med10-1002*, *med19-1002*, and *med21-1002*. Interestingly, deletions of several nonessential Mediator subunits, including those from the head (*med20Δ*), middle (*med5Δ*), tail (*med16Δ*), and kinase (*cdk8Δ* and *med12Δ*) modules had little or no effect on the 6-AU phenotype (Figure 5, compare rows 4–8 with row 3). These results are consistent with the idea that Ewe subunits of the Mediator middle module, more so than others, play an important role in regulating Pol II elongation in *S. cerevisiae*.

Finally, we wished to investigate more directly whether the ewe mutations impair Pol II elongation, in particular its processivity. To do so, we conducted an *in vivo* transcription elongation assay that compared the efficiency with which

a short, galactose-regulated episomal gene (~1.5 kb) is transcribed vs. a longer one (~4.5 kb). The assay is based on comparison of *Pho5* acid phosphatase expression arising from two genes sharing the same promoter (*GAL1*) as well as ORF and terminator (derived from *PHO5*) but differing in the length of their 3'-UTR (Morillo-Huesca *et al.* 2006). Previous work has shown that mutations in positive elongation factors (Morillo-Huesca *et al.* 2006; Ginsburg *et al.* 2009) or the ectopic expression of a negative elongation factor (Kim *et al.* 2011) diminish the steady-state levels of long vs. short transcript (termed the GLAM ratio). As shown in Figure 6, whereas the *dst1Δ* mutation elicited a significant decrease in the GLAM ratio, as expected (Mason and Struhl 2005), none of the spontaneous or engineered Mediator mutations did so, with the exception of *med21-1002*. Thus, although all ewe mutations elicit a strong 6-AU phenotype, only one has a corresponding effect on Pol II processivity at the episomal *PHO5* gene. This assay does not rule out processivity effects on chromosomal genes, whose chromatin might be more stable than the episomal reporter genes, nor does it rule out the possibility that ewe mutations affect Pol II elongation rate.

Figure 4 Mutations in the Med14/Rgr1 and Med21 subunits of Mediator diminish the extent of histone displacement at *HSP82*. Kinetics of histone H3 depletion over the promoter, ORF, and 3'-UTR of *HSP82* in WT and mutant cells as assayed by ChIP-qPCR at the indicated times following an instantaneous 30° to 39° temperature shift. H3 globular domain abundance at *HSP82* was normalized to its abundance at the *PHO5* promoter. This was determined by dividing each *HSP82* ChIP value (net nanograms DNA immunoprecipitated) by the corresponding *PHO5* ChIP value derived from the same biological sample. Note that H3 occupancy of the *PHO5* promoter is independent of both heat-shock and ewe strain background (data not shown and Sekinger and Gross 2001). Depicted are means $\pm$ SEM ($N = 2$; qPCR = 4). Asterisks signify a potentially significant difference between the indicated values ($P \leq 0.06$; two-tailed *t*-test; equal variance). (A–E) H3 occupancy in *MED*⁺ and the indicated ewe mutant strains. A, C, and E represent one set of immunoprecipitation reactions; B and D represent a second, independently conducted set.

Discussion

The Mediator middle module plays a role in regulating Pol II elongation

Here we have shown that mutations in conserved Mediator subunits located within or adjacent to the middle module impair the induced expression of the *Hsf1*-regulated *HSP82* heat-shock gene, and in some cases quite severely. Despite this, Pol II occupancy of the *HSP82* promoter is generally unaffected, and in the case of *med7-1002*, actually increased. Thus, a critical function of Mediator—to recruit Pol II to a gene's promoter (Soutourina *et al.* 2011)—is

typically unimpaired in *ewe* mutants, at least at *HSP82*. As Pol II occupancy within the *HSP82* coding region is likewise unaffected in the case of the *med7* and *med14* mutants, these mutations would appear to act by diminishing Pol II elongation rate. Their effect on Pol II elongation bears a strong resemblance to the effect of depleting *Cdk8* in HCT116 cells (Donner *et al.* 2010) since in both cases, impaired Mediator function results in a reduction in transcription without affecting Pol II occupancy. In the *med7* mutant, increased Pol II occupancy of the promoter relative to the coding region is consistent with an additional defect in promoter escape. With respect to *med19-1002* and *med21-*

Figure 5 Spontaneous ewe mutants exhibit growth defects on medium containing 6-azauracil. Fivefold serial dilutions of the indicated strains were spotted onto solid media deficient in uracil and containing either no drug (left) or 50 μ g/ml 6-AU (right). (Top) Isogenic *DST1*⁺ and *dst1* Δ strains; BY4741 strain background. (Middle) HS1001 derivatives (SLY101 strain background) transformed with plasmid pRS316 (*CEN-URA3*). The deletion strains *med16* Δ , *med5* Δ , *med20* Δ , *med12* Δ , and *cdk8* Δ represent the tail, middle, head, and kinase (twice) modules, respectively. (Bottom) HS1004 derivatives (spontaneous suppressors); SLY101 strain background. Plates were incubated for either 3 or 5 days at 30° (left and right, respectively).

1002, an effect on polymerase processivity could be occurring, given reduced Pol II density within the *HSP82* coding region relative to the promoter, which is particularly evident toward its 3'-UTR. Finally, *med10-1002* may represent an exception to the postrecruitment role of the *ewe* middle module subunits, since it appears to principally affect Pol II recruitment.

Integrity of the central Med7–Med21 heterodimer within the middle module (Baumli *et al.* 2005) may be important for yeast Mediator's postrecruitment function, given that both *med7-1002* and *med21-1002* exhibit elongation defects. Also consistent is the similar phenotype of *med19-1002*, given that Med19 has been implicated in stabilizing the association of the middle module with the rest of Mediator (Baidooobonso *et al.* 2007). A caveat to the above is that our data do not formally rule out the possibility that the Mediator mutations affect mRNA stability instead of, or in addition to, transcription. This consideration is particularly important in light of recent reports of a role for promoter elements in regulating cytoplasmic mRNA decay in *S. cerevisiae* (Bregman *et al.* 2011; Trcek *et al.* 2011). However, a primary role for *ewe* mutations in regulating *HSP82* mRNA stability seems unlikely. First, two *ewe* mutations, *rgr1-Δ2* and *med21-1002*, significantly diminish H3 eviction within both the promoter and ORF of *HSP82* following heat shock, while two others, *med7-1002* and *med19-1002*, elicit more minor, but nonetheless detectable defects in nucleosome

Figure 6 Pol II processivity assay reveals a potential role for Med21. Two sets of isogenic strains, derived from either HS1004 (*MED*⁺ cluster) or BY4741 (*DST1*⁺ and *dst1* Δ) and bearing the indicated mutations, were transformed with *P_{GAL1}-PHO5* and *P_{GAL1}-PHO5::lacZ* reporter plasmids, and grown in synthetic medium containing 1% galactose and 1% raffinose to log phase. Cells were harvested and Pho5 expression levels were determined by an acid phosphatase assay. The GLAM ratio represents the acid phosphatase activity of cells expressing the long transcript (*P_{GAL1}-PHO5::lacZ*) relative to those expressing the short transcript (*P_{GAL1}-PHO5*). Depicted are mean values $\pm$ SEM of three or six independent transformants of each indicated strain/plasmid combination ($N = 3$ for *med14-1003*, *med21-1002*, *med19-1002*, *cdk8* Δ , *DST1*, and *dst1* Δ ; $N = 6$ for *MED*⁺, *med7-1002*, *med10-1002*, and *med12* Δ). * $P \leq 0.05$; ** $P \leq 0.01$.

disassembly/reassembly. The chromatin phenotypes of these four mutants is consistent with a defect at the level of polymerase elongation (discussed more fully below). Second, with respect to *med10-1002*, we observed diminished Pol II occupancy within the *HSP82* promoter and coding region that paralleled diminished mRNA accumulation. This again is consistent with a transcription defect rather than one in mRNA stabilization.

Given their strong 6-AU phenotypes, the *ewe* mutations may affect RNA polymerase elongation more generally. And at least one—Med21—may play a role in Pol II processivity at non-heat-shock genes since the L76P mutation caused a reduction in the synthesis of long vs. short *PHO5*-containing transcripts as deduced from measurement of acid phosphatase levels. Consistent with the general idea that Mediator influences Pol II elongation, others have observed that deletion of the tail subunit Med15 affects Pol II processivity (Gaillard *et al.* 2009). Additionally, deletion of the head subunit Med18 was shown to reduce Pol II occupancy within the promoters of the *CHA1* and *INO1* genes yet increase Pol II abundance near their 3'-ends (Mukundan and Ansari 2011). Thus, in contrast to *ewe* mutations that seem to be linked primarily to Pol II elongation rate and/or processivity, loss of Med18 appears to affect late elongation or termination.

How might the middle module (and in particular, Med7 and Med14) govern the rate with which Pol II traverses the gene? Estimates for the rate of Pol II elongation typically range from 25 to 40 nt/sec, although there are reports of

more rapid elongation in certain contexts (reviewed in Ardehali and Lis 2009). Although not tested here, one way Mediator could affect elongation rate is through its modulation of the phosphorylation state of the Pol II CTD, or of one or more elongation factors. This could be achieved directly, through its *Cdk8* subunit, or it could be mediated indirectly through other CTD kinases (Donner *et al.* 2010). The pattern of Ser2, Ser5, and Ser7 phosphorylation on the CTD specifies the cotranscriptional engagement of capping, RNA processing, and polyadenylation enzymes (Akhtar *et al.* 2009), as well as chromatin modification enzymes (Govind *et al.* 2010). Thus, Mediator may regulate the CTD phosphorylation pattern that dictates the timing and identity of factors that associate with the transcription elongation complex, and these in turn could affect elongation rate or processivity. Moreover, a collaborator of Mediator may be the SAGA complex. First, SAGA and Mediator exhibit interdependent recruitment at *Gcn4*-regulated promoters (Qiu *et al.* 2005) and may well share this interdependence at other Pol II promoters. Second, SAGA has been detected within the coding regions of several *Gal4*-, *Gcn4*-, and *Hsf1*-regulated genes (Govind *et al.* 2007; Wyce *et al.* 2007; Kremer and Gross 2009). Therefore, Mediator might control either the *Gcn5* histone acetyl transferase (HAT) or *Ubp8* ubiquitin protease activities within SAGA by regulating Pol II association of SAGA through CTD phosphorylation. In a similar manner, Mediator could control the *Esa1* HAT of NuA4 through Pol II association of NuA4 (Ginsburg *et al.* 2009).

A role for Mediator in nucleosome disassembly/reassembly

In addition to having a role in Pol II elongation, the *Med14* and *Med21* subunits facilitate nucleosomal loss at *HSP82* following heat shock. This may arise from their role in enhancing nucleosome disassembly or in impairing reassembly (or both). Rapid, gene-wide depletion of nucleosomes is characteristic of induced heat-shock genes from yeast to *Drosophila* (Zhao *et al.* 2005; Erkina and Erkinen 2006; Petesch and Lis 2008; Kremer and Gross 2009). Poly(ADP)-ribose polymerase (PARP) (or its activity) appears to be important in triggering histone eviction throughout the *Drosophila HSP70* gene (Petesch and Lis 2008). While a PARP ortholog has not been identified in yeast, other factors have been shown to be important in transcription-coupled nucleosome eviction in *S. cerevisiae*. One, the H3/H4 histone chaperone *Asf1*, mediates histone eviction from activated *PHO5* and *PHO8* promoters (Adkins *et al.* 2004) as well as from the galactose-induced *GAL1* and *GAL10* coding regions (Schwabish and Struhl 2006). Yet we show here that H4 is evicted efficiently at heat-shock-activated *HSP82* in an *asf1Δ* mutant (indistinguishable from the isogenic WT strain), mirroring the effects of *ctk1Δ*, *dst1Δ*, and *spt4Δ* mutations, as well as our earlier findings of *snf2Δ*, *swi1Δ*, *gcn5Δ*, *set1Δ*, *paf1Δ*, and *sas3Δ* mutants (Zhao *et al.* 2005; Kremer and Gross 2009). Particularly striking is the fact that while inactivation of the Swi/Snf remodeling complex

results in a sixfold reduction in *HSP82* transcription, only slightly less than that conferred by *rgr1-Δ2*, *rgr1-Δ2* strongly impairs nucleosomal depletion following heat shock, while Swi/Snf mutants have no detectable effect (Zhao *et al.* 2005). This may be the case because additional ATP-dependent remodeling complexes participate in chromatin remodeling at heat-shock genes (Erkina *et al.* 2010), while the role played by *Med14* (and to a lesser degree, *Med21*) in regulating nucleosome occupancy is unique.

The chromatin phenotypes of recessive mutations in *MED14* and *MED21* raise the question of how Mediator might regulate nucleosomal disassembly/reassembly. Particularly so, given that Pol II density is largely unaffected in *rgr1-Δ2* and *med21-1002* mutants and that Pol II transit has been implicated in mediating nucleosome disassembly within transcribed regions (Teves and Henikoff 2011). Clearly, Pol II density alone does not correlate with H3 depletion within the *HSP82* transcribed region. This is consistent with the Pol II and chromatin phenotypes of *med10-1002* reported here: substantial reduction in Pol II occupancy yet normal levels of histone displacement. It is also consistent with our earlier observation of an *hsp82* promoter mutant termed *hsp82-P2*, whose induced expression is reduced 85%, and whose nucleosomal displacement is obviated, yet whose Pol II density is unaffected (Zhao *et al.* 2005). Perhaps this mutant, bearing a double point mutation within HSE1, and the *rgr1-Δ2* mutant share a common defect in elongating Pol II. As discussed above, the phosphorylation state of the CTD governs association of various factors with elongating Pol II. These in turn may dictate the efficiency with which nucleosomes are evicted. Thus, in the case of the Mediator mutants *rgr1-Δ2* and *med21-1002*, the Pol II elongation complex might be deficient for nucleosome disassembly (or hyperactive in reassembly) that in turn contributes to the reduction in synthesis of full-length *HSP82* mRNA. It is possible that polymerase elongation rate and processivity are respectively impaired in these two mutants, as discussed above. Further work will be necessary to explore these and other potential mechanisms.

The expression phenotype of *ewe* mutants may stem from multiple factors

Although not the focus of this study, the question arises as to what might be the basis for the substantially increased expression of the *hsp82-ΔHSE1/lacZ* reporter observed in *ewe* mutants, as it is not observed at the wild-type *HSP82* gene (nor at *SSA4* or *HSP104*; J. Jeon, S. B. Kremer, and D. S. Gross, unpublished observations). It is possible that enhanced basal expression arises from some attribute of the chromatin structure of the fusion gene, whose promoter is assembled into a repressive dinucleosome (Gross *et al.* 1993; Venturi *et al.* 2000) and whose coding region is derived from a heterologous, prokaryotic gene. Others have observed that in the context of Mediator mutations (either *med16Δ* alone or in a *med5Δ nut2-1* double mutant), basal expression of *HO/lacZ* and *PHO5/lacZ* fusion genes is enhanced, while

expression of the intact *HO* and *PHO5* genes remains unaffected (Tabtiang and Herskowitz 1998). Likewise, we have found that, whereas *med16Δ* strongly derepresses *hsp82-ΔHSE1/lacZ*, it has no effect on the expression of *hsp82-ΔHSE1* itself (H. Singh and D. S. Gross, unpublished observations). This phenomenon is also seen with a second fusion, *hsp82-ΔHSE1/HIS3*, whose enhanced expression served as the basis for the selection of the *ewe* mutants (Singh *et al.* 2006), so it is not unique to *lacZ* gene fusions. While the basis for enhanced basal expression of fusion genes remains unclear, it could stem from increased transcription initiation secondary to enhanced accessibility of the nucleosomal promoter, or even arise from a post-transcriptional event such as increased mRNA stability. Whatever might be the basis for enhanced basal expression, the dramatic loss in inducibility of the *hsp82/lacZ* fusion gene in the *ewe* mutants likely arises from the same defects in Pol II elongation and chromatin remodeling described here for the wild-type gene.

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Literature Cited

- Adkins, M. W., S. R. Howar, and J. K. Tyler, 2004 Chromatin disassembly mediated by the histone chaperone Asf1 is essential for transcriptional activation of the yeast *PHO5* and *PHO8* genes. *Mol. Cell* 14: 657–666.
- Akhtar, M. S., M. Heidemann, J. R. Tietjen, D. W. Zhang, R. D. Chapman *et al.*, 2009 TFIIF kinase places bivalent marks on the carboxy-terminal domain of RNA polymerase II. *Mol. Cell* 34: 387–393.
- Apone, L. M., C. A. Virbasius, F. C. P. Holstege, J. Wang, R. A. Young *et al.*, 1998 Broad, but not universal, transcriptional requirement for yTAF_{II}17, a histone H3-like TAF_{II} present in TFIID and SAGA. *Mol. Cell* 2: 653–661.
- Ardehali, M. B., and J. T. Lis, 2009 Tracking rates of transcription and splicing in vivo. *Nat. Struct. Mol. Biol.* 16: 1123–1124.
- Baidooobonso, S. M., B. W. Guidi, and L. C. Myers, 2007 Med19 (Rox3) regulates intermodule interactions in the *Saccharomyces cerevisiae* Mediator complex. *J. Biol. Chem.* 282: 5551–5559.
- Balakrishnan, S. K., and D. S. Gross, 2008 The tumor suppressor p53 associates with gene coding regions and co-traverses with elongating RNA polymerase II in an in vivo model. *Oncogene* 27: 2661–2672.
- Baumli, S., S. Hoepfner, and P. Cramer, 2005 A conserved mediator hinge revealed in the structure of the MED7.MED21 (Med7.Srb7) heterodimer. *J. Biol. Chem.* 280: 18171–18178.
- Bernecky, C., P. Grob, C. C. Ebmeier, E. Nogales, and D. J. Taatjes, 2011 Molecular architecture of the human Mediator-RNA polymerase II-TFIIF assembly. *PLoS Biol.* 9: e1000603.
- Bourbon, H. M., 2008 Comparative genomics supports a deep evolutionary origin for the large, four-module transcriptional mediator complex. *Nucleic Acids Res.* 36: 3993–4008.
- Bregman, A., M. Avraham-Kelbert, O. Barkai, L. Duek, A. Guterman *et al.*, 2011 Promoter elements regulate cytoplasmic mRNA decay. *Cell* 147: 1473–1483.
- Cai, G., T. Imasaki, Y. Takagi, and F. Asturias, 2009 Mediator structural conservation and implications for the regulation mechanism. *Structure* 17: 559–567.
- Chou, S., S. Chatterjee, M. Lee, and K. Struhl, 1999 Transcriptional activation in yeast cells lacking transcription factor IIA. *Genetics* 153: 1573–1581.
- Donner, A. J., C. C. Ebmeier, D. J. Taatjes, and J. M. Espinosa, 2010 CDK8 is a positive regulator of transcriptional elongation within the serum response network. *Nat. Struct. Mol. Biol.* 17: 194–201.
- Erkina, T. Y., and A. M. Erkin, 2006 Displacement of histones at promoters of *Saccharomyces cerevisiae* heat shock genes is differentially associated with histone H3 acetylation. *Mol. Cell. Biol.* 26: 7587–7600.
- Erkina, T. Y., Y. Zou, S. Freeling, V. I. Vorobyev, and A. M. Erkin, 2010 Functional interplay between chromatin remodeling complexes RSC, SWI/SNF and ISWI in regulation of yeast heat shock genes. *Nucleic Acids Res.* 38: 1441–1449.
- Exinger, F., and F. Lacroute, 1992 6-Azauracil inhibition of GTP biosynthesis in *Saccharomyces cerevisiae*. *Curr. Genet.* 22: 9–11.
- Gaillard, H., C. Tous, J. Botet, C. Gonzalez-Aguilera, M. J. Quintero *et al.*, 2009 Genome-wide analysis of factors affecting transcription elongation and DNA repair: a new role for PAF and Ccr4-Not in transcription-coupled repair. *PLoS Genet.* 5: e1000364.
- Ginsburg, D. S., C. K. Govind, and A. G. Hinnebusch, 2009 NuA4 lysine acetyltransferase Esa1 is targeted to coding regions and stimulates transcription elongation with Gcn5. *Mol. Cell. Biol.* 29: 6473–6487.
- Govind, C. K., F. Zhang, H. Qiu, K. Hofmeyer, and A. G. Hinnebusch, 2007 Gcn5 promotes acetylation, eviction, and methylation of nucleosomes in transcribed coding regions. *Mol. Cell* 25: 31–42.
- Govind, C. K., H. Qiu, D. S. Ginsburg, C. Ruan, K. Hofmeyer *et al.*, 2010 Phosphorylated Pol II CTD recruits multiple HDACs, including Rpd3C(S), for methylation-dependent deacetylation of ORF nucleosomes. *Mol. Cell* 39: 234–246.
- Gromoller, A., and N. Lehming, 2000 Srb7p is a physical and physiological target of Tup1p. *EMBO J.* 19: 6845–6852.
- Gross, D. S., C. C. Adams, S. Lee, and B. Stentz, 1993 A critical role for heat shock transcription factor in establishing a nucleosome-free region over the TATA-initiation site of the yeast *HSP82* heat shock gene. *EMBO J.* 12: 3931–3945.
- Jiang, Y. W., P. R. Dohrmann, and D. J. Stillman, 1995 Genetic and physical interactions between yeast RGR1 and SIN4 in chromatin organization and transcriptional regulation. *Genetics* 140: 47–54.
- Kim, S., S. K. Balakrishnan, and D. S. Gross, 2011 p53 interacts with RNA polymerase II through its core domain and impairs Pol II processivity in vivo. *PLoS ONE* 6: e22183.
- Kremer, S. B., and D. S. Gross, 2009 SAGA and Rpd3 chromatin modification complexes dynamically regulate heat shock gene structure and expression. *J. Biol. Chem.* 284: 32914–32931.
- Lee, D., and J. T. Lis, 1998 Transcriptional activation independent of TFIIF kinase and the RNA polymerase II mediator in vivo. *Nature* 393: 389–392.
- Lee, D. K., S. Kim, and J. T. Lis, 1999 Different upstream transcriptional activators have distinct co-activator requirements. *Genes Dev.* 13: 2934–2939.
- Lee, S., and D. S. Gross, 1993 Conditional silencing: the *HMRE* mating-type silencer exerts a rapidly reversible position effect on the yeast *HSP82* heat shock gene. *Mol. Cell. Biol.* 13: 727–738.

- Lee, S. K., A. G. Fletcher, L. Zhang, X. Chen, J. A. Fischbeck *et al.*, 2010 Activation of a poised RNAPII-dependent promoter requires both SAGA and mediator. *Genetics* 184: 659–672.
- Li, B., M. Carey, and J. L. Workman, 2007 The role of chromatin during transcription. *Cell* 128: 707–719.
- Li, Y., S. Bjorklund, Y. W. Jiang, Y. J. Kim, W. S. Lane *et al.*, 1995 Yeast global transcriptional regulators Sin4 and Rgr1 are components of mediator complex/RNA polymerase II holoenzyme. *Proc. Natl. Acad. Sci. USA* 92: 10864–10868.
- Malagon, F., A. H. Tong, B. K. Shafer, and J. N. Strathern, 2004 Genetic interactions of DST1 in *Saccharomyces cerevisiae* suggest a role of TFIIS in the initiation-elongation transition. *Genetics* 166: 1215–1227.
- Malik, S., and R. G. Roeder, 2000 Transcriptional regulation through Mediator-like coactivators in yeast and metazoan cells. *Trends Biochem. Sci.* 25: 277–283.
- Malik, S., and R. G. Roeder, 2010 The metazoan Mediator co-activator complex as an integrative hub for transcriptional regulation. *Nat. Rev. Genet.* 11: 761–772.
- Malik, S., M. J. Barrero, and T. Jones, 2007 Identification of a regulator of transcription elongation as an accessory factor for the human Mediator coactivator. *Proc. Natl. Acad. Sci. USA* 104: 6182–6187.
- Mason, P. B., and K. Struhl, 2005 Distinction and relationship between elongation rate and processivity of RNA polymerase II in vivo. *Mol. Cell* 17: 831–840.
- McNeil, J. B., H. Agah, and D. Bentley, 1998 Activated transcription independent of the RNA polymerase II holoenzyme in budding yeast. *Genes Dev.* 12: 2510–2521.
- Meyer, K. D., S. C. Lin, C. Bernecky, Y. Gao, and D. J. Taatjes, 2010 p53 activates transcription by directing structural shifts in Mediator. *Nat. Struct. Mol. Biol.* 17: 753–760.
- Moqtaderi, Z., Y. Bai, D. Poon, P. A. Weil, and K. Struhl, 1996 TBP-associated factors are not generally required for transcriptional activation in yeast. *Nature* 382: 188–191.
- Morillo-Huesca, M., M. Vanti, and S. Chavez, 2006 A simple in vivo assay for measuring the efficiency of gene length-dependent processes in yeast mRNA biogenesis. *FEBS J.* 273: 756–769.
- Mukundan, B., and A. Ansari, 2011 A novel role for mediator subunit SRB5/MED18 in termination of transcription. *J. Biol. Chem.* 286: 37053–37057.
- Myers, L. C., and R. D. Kornberg, 2000 Mediator of transcriptional regulation. *Annu. Rev. Biochem.* 69: 729–749.
- Petes, S. J., and J. T. Lis, 2008 Rapid, transcription-independent loss of nucleosomes over a large chromatin domain at Hsp70 loci. *Cell* 134: 74–84.
- Qiu, H., C. Hu, F. Zhang, G. J. Hwang, M. J. Swanson *et al.*, 2005 Interdependent recruitment of SAGA and Srb mediator by transcriptional activator Gcn4p. *Mol. Cell. Biol.* 25: 3461–3474.
- Saunders, A., L. J. Core, and J. T. Lis, 2006 Breaking barriers to transcription elongation. *Nat. Rev. Mol. Cell Biol.* 7: 557–567.
- Schwabish, M. A., and K. Struhl, 2006 Asf1 mediates histone eviction and deposition during elongation by RNA polymerase II. *Mol. Cell* 22: 415–422.
- Sekinger, E. A., and D. S. Gross, 1999 *SIR* repression of a yeast heat shock gene: UAS and TATA footprints persist within heterochromatin. *EMBO J.* 18: 7041–7055.
- Sekinger, E. A., and D. S. Gross, 2001 Silenced chromatin is permissive to activator binding and PIC recruitment. *Cell* 105: 403–414.
- Singh, H., A. M. Erkin, S. B. Kremer, H. M. Duttweiler, D. A. Davis *et al.*, 2006 A functional module of yeast Mediator that governs the dynamic range of heat shock gene expression. *Genetics* 172: 2169–2184.
- Soutourina, J., S. Wyda, Y. Ambrose, C. Boschiero, and M. Werner, 2011 Direct interaction of RNA polymerase II and Mediator required for transcription in vivo. *Science* 331: 1451–1454.
- Tabtiang, R. K., and I. Herskowitz, 1998 Nuclear proteins Nut1p and Nut2p cooperate to negatively regulate a Swi4p-dependent *lacZ* reporter gene in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 18: 4707–4718.
- Takahashi, H., T. J. Parmely, S. Sato, C. Tomomori-Sato, C. A. Banks *et al.*, 2011 Human mediator subunit MED26 functions as a docking site for transcription elongation factors. *Cell* 146: 92–104.
- Teves, S. S., and S. Henikoff, 2011 Heat shock reduces stalled RNA polymerase II and nucleosome turnover genome-wide. *Genes Dev.* 25: 2387–2397.
- Thompson, C. M., and R. A. Young, 1995 General requirement for RNA polymerase II holoenzymes in vivo. *Proc. Natl. Acad. Sci. USA* 92: 4587–4590.
- Treck, T., D. R. Larson, A. Moldon, C. C. Query, and R. H. Singer, 2011 Single-molecule mRNA decay measurements reveal promoter-regulated mRNA stability in yeast. *Cell* 147: 1484–1497.
- Venters, B. J., and B. F. Pugh, 2009 A canonical promoter organization of the transcription machinery and its regulators in the *Saccharomyces* genome. *Genome Res.* 19: 360–371.
- Venturi, C. B., A. M. Erkin, and D. S. Gross, 2000 Cell cycle-dependent binding of yeast heat shock factor to nucleosomes. *Mol. Cell. Biol.* 20: 6435–6448.
- Wang, G., M. A. Balamotis, J. L. Stevens, Y. Yamaguchi, H. Handa *et al.*, 2005 Mediator requirement for both recruitment and postrecruitment steps in transcription initiation. *Mol. Cell* 17: 683–694.
- Wyce, A., T. Xiao, K. A. Whelan, C. Kosman, W. Walter *et al.*, 2007 H2B ubiquitylation acts as a barrier to Ctk1 nucleosomal recruitment prior to removal by Ubp8 within a SAGA-related complex. *Mol. Cell* 27: 275–288.
- Zhao, J., J. Herrera-Diaz, and D. S. Gross, 2005 Domain-wide displacement of histones by activated heat shock factor occurs independently of Swi/Snf and is not correlated with RNA polymerase II density. *Mol. Cell. Biol.* 25: 8985–8999.
- Zhu, X., B. Liu, J. O. Carlsten, J. Beve, T. Nystrom *et al.*, 2011a Mediator influences telomeric silencing and cellular life span. *Mol. Cell. Biol.* 31: 2413–2421.
- Zhu, X., Y. Zhang, G. Bjornsdottir, Z. Liu, A. Quan *et al.*, 2011b Histone modifications influence mediator interactions with chromatin. *Nucleic Acids Res.* 39: 8342–8354.

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Supporting Information

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Role of Mediator in Regulating Pol II Elongation and Nucleosome Displacement in *Saccharomyces cerevisiae*

Selena B. Kremer, Sunyoung Kim, Jeong Ok Jeon, Yara W. Moustafa, Apeng Chen, Jing Zhao,
and David S. Gross

Figure S1 Cell Viability Assay. Strains (as in Figure 1) were grown to an A_{600} of ~ 0.3 - 0.6 in a 50 ml culture at 30° and an aliquot was removed and subjected to a 39° heat shock for 1 hr. Control and heat shocked samples were then diluted 1:400 with fresh YPDA broth and 30 μ l from each were spread on YPDA plates.

C

med21-1002

D

med10-1002

Figure S2 *ewe* mutations alter RNA Pol II distribution within the *HSP82* heat shock gene. *In vivo* crosslinking analysis of Pol II at the *HSP82* promoter, ORF and 3'-UTR either prior to or for the indicated times following a 39° heat shock. Data are derived from same ChIP experiments as illustrated in Figure 3, but presented in an alternative format that allows direct comparison of promoter, ORF and 3'UTR Pol II occupancy at each time point for each strain. **A.- E.** Analysis of strain SK-HS1004 (WT) vs. SBK500 (*rgr1-Δ2*), SK-J121b (*med7-1002*), SK-J20b (*med21-1002*), SK-J84b (*med10-1002*) and SK-B20c (*med19-1002*), respectively.

A

B

Figure S3 Efficient histone displacement takes place at the activated *HSP82* gene in *asf1Δ* cells and in cells bearing deletions of transcription elongation factors. **A.** *In vivo* crosslinking analysis of myc-H4 to *HSP82* in isogenic WT and *asf1Δ* strains (SLY101 and JHD401, respectively) under either non-heat shock or 20 min heat shock states. Myc-H4 abundance, like that of the H3 globular domain (Figure 4), is considered a measure of bulk nucleosome occupancy. Myc-H4 was quantified as described for Pol II (Figure 3) and normalized to myc-H4 abundance at the *PHO5* promoter. Depicted are means $\pm$ SD; N=2. **B.** As in A, except *ctk1Δ*, *dst1Δ* and *spt4Δ* strains (JHD303, DCY101 and DCY102, respectively) were analyzed.