View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by DigitalCommons@The Texas Medical Center

EUKARYOTIC CELL, June 2008, p. 938–948 Vol. 7, No. 6
1535-9778/08/$08.00+0 doi:10.1128/EC.00042-08
Copyright © 2008, American Society for Microbiology. All Rights Reserved.

Rtr1 Is the *Saccharomyces cerevisiae* Homolog of a Novel Family of RNA Polymerase II-Binding Proteins[▽]

Patrick A. Gibney,[1] Thomas Fries,[2] Susanne M. Bailer,[2] and Kevin A. Morano[1]*

Department of Microbiology and Molecular Genetics, University of Texas Medical School, Houston, Texas 77030,[1] and Universität des Saarlandes, Medizinische Biochemie und Molekularbiologie, Gebäude 61.4, D-66421 Homburg/Saar, Germany[2]

Received 1 February 2008/Accepted 7 April 2008

Cells must rapidly sense and respond to a wide variety of potentially cytotoxic external stressors to survive in a constantly changing environment. In a search for novel genes required for stress tolerance in *Saccharomyces cerevisiae*, we identified the uncharacterized open reading frame YER139C as a gene required for growth at 37°C in the presence of the heat shock mimetic formamide. YER139C encodes the closest yeast homolog of the human RPAP2 protein, recently identified as a novel RNA polymerase II (RNAPII)-associated factor. Multiple lines of evidence support a role for this gene family in transcription, prompting us to rename YER139C *RTR1* (*r*egulator of *tr*anscription). The core RNAPII subunits *RPB5*, *RPB7*, and *RPB9* were isolated as potent high-copy-number suppressors of the *rtr1*Δ temperature-sensitive growth phenotype, and deletion of the nonessential subunits *RPB4* and *RPB9* hypersensitized cells to *RTR1* overexpression. Disruption of *RTR1* resulted in mycophenolic acid sensitivity and synthetic genetic interactions with a number of genes involved in multiple phases of transcription. Consistently, *rtr1*Δ cells are defective in inducible transcription from the *GAL1* promoter. Rtr1 constitutively shuttles between the cytoplasm and nucleus, where it physically associates with an active RNAPII transcriptional complex. Taken together, our data reveal a role for members of the RTR1/RPAP2 family as regulators of core RNAPII function.

Transcription of mRNA and most snRNAs in eukaryotic cells is carried out by the RNA polymerase II (RNAPII) enzyme, consisting of 12 protein subunits (Rpb1 to Rpb12). Five of these (Rpb1, Rpb2, Rpb3, Rpb6, and Rpb11) are homologous to counterparts in bacteria, and six others (Rpb4, Rpb5, Rpb7, Rpb9, Rpb10, and Rpb12) bear resemblance to archaeal RNA polymerase subunits (64). Many of these subunits are also represented by highly conserved homologs in the eukaryotic RNAPI and -III enzymes responsible for rRNA and tRNA synthesis, respectively (52). In addition to the core subunits, numerous additional protein cofactors are required for regulated and accurate gene expression, including the Mediator, Elongator, and SAGA complexes (39). Diverse cellular signals such as environmental stressors (heat, oxidative chemical), nutrient conditions, and proliferation state feed into the transcription machinery at multiple steps (44). One regulatory mechanism involves reversible phosphorylation of the carboxy-terminal domain (CTD) within RNAPII (51). The CTD contains multiple repeats of a conserved motif (YSPTSPS) subject to hyperphosphorylation by CTD kinases such as Kin28 and Srb10 (26). Phosphorylation of the Rpb1-CTD at serines in the second and fifth positions within the heptad repeat is associated with transition from preinitiation to a transcriptionally active elongation complex (17). With the scores of known signal inputs, multiple layers of regulation, and hundreds of described components, uncharacterized genes that may play important roles in modulating transcription undoubtedly still exist.

One facet of gene expression that remains largely unexplored is the ability of the transcription machinery to remain active under stress conditions. Both nonessential subunits of RNAPII, Rpb4 and Rpb9, are required for stress tolerance (14, 45, 47, 65, 66). Rpb4 is involved in mRNA export during cellular stress, transcription-coupled DNA repair, sporulation, pseudohyphal growth, recovery from stationary phase, and transcription at elevated temperatures (2, 20, 40, 53, 56). Rpb4 associates with an essential subunit, Rpb7, to form a readily dissociable heterodimer that interacts with the other subunits in a stress-responsive manner (13). This complex engenders tighter interaction between RNAPII and its DNA substrate and is thought to facilitate transcription during both normal and stress conditions. Not unexpectedly, deletion of *RPB4* results in a temperature-sensitive growth phenotype, likely due to cessation of transcription after shift to the nonpermissive temperature (47). Interestingly, Rpb4 is primarily nuclear in unstressed cells and translocates to the cytoplasm in response to some types of stress (20). Rpb9 has been shown to be involved in accurate start-site selection, transcription through arrest sites, regulation of transcription elongation, and transcription-coupled DNA repair (3, 25, 28, 40). Deletion of *RPB9* also results in temperature sensitivity, but the specific role of Rpb9 in transcription during periods of environmental stress has not been elucidated (65).

In an attempt to discover and characterize novel heat shock genes, we identified the open reading frame (ORF) YER139C. Deletion of YER139C results in a temperature-sensitive phenotype, indicating a putative role in heat stress homeostasis. YER139C and putative higher eukaryotic homologs contain a domain of unknown function with significant similarity to zinc-finger motifs, and mutation of conserved cysteine and histidine residues in this region abolished gene function. Initial charac-

* Corresponding author. Mailing address: Department of Microbiology and Molecular Genetics, University of Texas Medical School, 6431 Fannin Street, Houston, TX 77030. Phone: (713) 500-5890. Fax: (713) 500-5499. E-mail: Kevin.A.Morano@uth.tmc.edu.
▽ Published ahead of print on 11 April 2008.

TABLE 1. Strains

Strain	Genotype	Reference or source
BY4741	*MAT***a** *his3*Δ *leu2*Δ *met15*Δ *ura3*Δ	OpenBiosystems
RPB9-TAP	*rpb9*::*RPB9*-TAP	OpenBiosystems
RTR1-TAP	*rtr1*::*RTR1*-TAP	OpenBiosystems
PGY1	*rtr1*Δ::*LEU2*	This study
PGY2	*gal11*Δ::*kanMX rtr1*Δ::*LEU2*	This study
PGY3	*med1*Δ::*kanMX rtr1*Δ::*LEU2*	This study
PGY4	*soh1*Δ::*kanMX rtr1*Δ::*LEU2*	This study
PGY5	*srb5*Δ::*kanMX rtr1*Δ::*LEU2*	This study
PGY6	*ccr4*Δ::*kanMX rtr1*Δ::*LEU2*	This study
PGY7	*cdc73*Δ::*kanMX rtr1*Δ::*LEU2*	This study
PGY8	*dst1*Δ::*kanMX rtr1*Δ::*LEU2*	This study
PGY9	*elp2*Δ::*kanMX rtr1*Δ::*LEU2*	This study
PGY10	*spt4*Δ::*kanMX rtr1*Δ::*LEU2*	This study
PGY11	*rpb4*Δ::*kanMX rtr1*Δ::*LEU2*	This study
PGY12	*rpb9*Δ::*kanMX rtr1*Δ::*LEU2*	This study
PGY13	*ydr066c*Δ::*kanMX rtr1*Δ::*LEU2*	This study
PGY14	*rtr1*::*RTR1*-TAP *rtr1*Δ::*LEU2*	This study
RS453n	*MAT***a** *ade2, his3, leu2, trp1, ura3*	7
xpo1-1	*MAT***a** *ura3 trp1 his3 leu2 ade2 can1 xpo1*::*kanMX pASZ11-xpo1-1*	35

terization of this gene strongly links it to regulation of gene expression through genetic interaction with RNAPII and with associated transcriptional regulators. We show that Yer139c shuttles between the cytoplasm and nucleus and physically interacts with the transcriptionally active form of Rpb1. Cells lacking YER139C display a transcriptional defect in inducible expression from the *GAL1* promoter, demonstrating the physiological significance of the genetic and biochemical interactions. We have therefore named this gene *RTR1* (for "*r*egulator of *tr*anscription"). A recent proteomic analysis has identified the human homolog of *RTR1* among a group of novel RNAPII-associated proteins, supporting our proposed in vivo role for Rtr1 in transcription (30).

MATERIALS AND METHODS

***Saccharomyces cerevisiae* methodology.** *S. cerevisiae* strains were grown on media containing 2% dextrose, sucrose, or galactose as indicated. Unless otherwise noted, strains were grown at 30°C. Rich yeast extract-peptone-dextrose (YPD) growth medium was prepared containing 1% yeast extract, 2% peptone, and 2% dextrose (or another sugar, as indicated). Synthetic complete (SC) medium lacking the appropriate nutrient for plasmid selection was purchased from Sunrise Science Products (San Diego, CA). Other additions to media included formamide (2%), 6-azauracil (6AU) (100 μg/ml), and mycophenolic acid (MPA) (100 μg/ml) (Sigma Aldrich, St. Louis, MO). Standard yeast propagation and transformation procedures were employed (31). Plate growth assays were carried out by streaking or by serial dilution using 1/10 dilution steps with a starting culture optical density at 600 nm of 1.0, and the resulting culture was transferred with a multipronged replicating tool (Sigma). Strains used in this study are listed in Table 1. Haploid strains carrying *kanMX*-marked disruptions in *RTR1*, *RPB4*, *RPB9*, *GAL11*, *MEDI*, *SOH1*, *SRB5*, *CCR4*, *CDC73*, *DST1*, *ELP2*, *SPT4*, *RPB4*, and YDR066C were obtained from Open Biosystems (Huntsville, AL). An independent *RTR1* disruption cassette was generated by PCR amplification of the *LEU2* gene followed by cloning into pBlueScript II. Then, 250-bp regions flanking the 5′ and 3′ sides of *RTR1* were cloned upstream and downstream of the *LEU2* gene, respectively. The entire construct was excised by restriction digest and transformed into target strains. Disruption of *RTR1* by homologous recombination was selected by growth on media lacking leucine and confirmed by PCR. Sequences for all oligonucleotide primers used in this study are available upon request.

Plasmid construction. Unless otherwise noted, ORFs were amplified from BY4741 genomic DNA by use of standard PCR protocols and cloned into expression vectors by use of restriction endonuclease sites engineered into the amplifying oligonucleotide primers by use of standard DNA digestion and ligation protocols. The specific cloning sites and vectors used are identified together with the list of plasmids in Table 2. Vent DNA polymerase, T4 ligase, and restriction endonucleases were purchased from New England Biolabs (Beverly, MA). To construct both green fluorescent protein (GFP)-tagged and protein A-tagged versions of Rtr1, the coding region of *S. cerevisiae RTR1* (including 300 bp from the 3′ untranslated region) was amplified from *RS453n* genomic DNA by use of PCR and cloned into GFP and protein A expression vectors described elsewhere (6). Constructs were verified by DNA sequencing. Expression of the N-terminally GFP- or protein A-tagged Rtr1 fusion proteins was analyzed by immunoblotting. A triple hemagglutinin (HA)-tagged allele of *RTR1* was constructed using a PCR-based approach. Oligonucleotides containing sequences flanking the *RTR1* stop codon and containing additional homology to the template plasmid pFA6a-3HA-His3MX6 amplified a cassette used to modify p416CUP-RTR1 via homologous recombination in yeast cells. This construct was then used as a template for mutation of the conserved cysteine and histidine residues within the *RTR1* cysteine-rich domain. This was accomplished using overlap extension PCR. Complementary primers were designed to mutate the cysteine at position 73 to serine and the cysteine and histidine residues at positions 112 and 116, respectively, to serine. These primers were used in PCRs with primers flanking the start or stop codons to generate two products with an approximately 20-nucleotide overlap in the respective 3′ and 5′ ends. These products were used as templates in a third PCR that included only ORF-flanking primers to reconstruct a full-length ORF containing the mutation(s) of interest. V5-epitope coding sequence was included in the oligonucleotides for construction of all V5-tagged constructs. All constructs were verified by sequencing.

Protein extraction, affinity purification, and immunoblot analysis. Cells were harvested and resuspended in TEGN buffer (20 mM Tris [pH 7.9], 0.5 mM EDTA, 10% glycerol, 50 mM NaCl) with protease inhibitors (aprotinin, 2 μg/ml; pepstatin A, 2 μg/ml; leupeptin, 1 μg/ml; phenylmethylsulfonyl fluoride, 1 mM; chymostatin, 2 μg/ml; Sigma) followed by the addition of acid-washed glass beads. The samples were then lysed by agitation using a microtube mixer (Tomy MT-360) for five rounds of 1.5 min of lysis in each round followed by 1.5 min on ice. The lysate was then cleared by centrifugation at 4,500 × *g* at 4°C for 7 min. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose (Bio-Rad, Hercules, CA) for immunodetection. Purification and detection of protein A (tandem affinity purification [TAP])-tagged proteins expressed in yeast were performed as described previously (4). 12CA5 monoclonal antibody recognizing the HA epitope was purchased from Roche Diagnostics (Indianapolis, IN) and used at a dilution of 1:5,000. V5 monoclonal antibody recognizing the V5 epitope was purchased from Invitrogen and used at a dilution of 1:5,000. Mouse monoclonal antibodies 8WG16 (MMS-126R), H5 (MMS-129R), and H14 (MMS-134R), all recognizing RNAPII, were obtained from Covance (Princeton, NJ) (9, 51, 62). A slot-blot approach was used to react individual lanes with equivalent loads from the same blot with all three anti-RPB1 antibodies.

High-copy-number suppressor screening. The *rtr1*Δ strain was transformed with a genomic high-copy-number library in the yEP24 vector backbone (10). A total of 100,000 transformants were grown at 37°C in the presence of 2% formamide to select for suppressing colonies, and 23 were recovered. Plasmids responsible for suppression were rescued and placed into independent classes via restriction digestion with HindIII, resulting in three distinct groups. A representative member of each group was then sequenced using primers flanking the genomic fragment insertion site. Individual genes from each sequenced fragment were cloned into plasmid p423GPD and tested for suppression ability. After identification of *RPB5* and *RPB9* as high-copy-number suppressors, the remaining RNAPII subunits were also cloned into p423GPD as described above and examined for suppression ability. Overexpression plasmids containing *RPB1* and *RPB2* were kind gifts from Nancy Woychik (Robert Wood Johnson Medical School, New Brunswick, NJ).

Microscopy. Expression and localization of the GFP-Rtr1 fusion protein were done essentially as described previously (5). Specifically, the *rtr1*Δ::*kanMX4* deletion strain or yeast strains encoding the *xpo1-1* conditionally temperature-sensitive exportin mutant were transformed with a *CEN* plasmid encoding GFP-Rtr1. The cells were grown at 25°C to a optical density at 600 nm of 0.5 and incubated with 4′,6′-diamidino-2-phenylindole (DAPI) at a final concentration of 2.5 μg/ml for 45 min to stain nuclei. A fraction of the cell culture was shifted to the restrictive temperature of 35°C for 8 min and subsequently analyzed by fluorescence microscopy using a Nikon Eclipse TE2000-U fluorescence microscope, a Nikon DS-5Mc color camera, and NIS-Elements F2.30 software (Nikon Instruments Europe B.V., Düsseldorf, Germany) and processed in Adobe Photoshop 4 (Adobe Systems Inc., San Jose, CA).

Northern blot analysis of gene expression. Expression of galactose-inducible and constitutively expressed genes was determined by Northern analysis as fol-

TABLE 2. Plasmids

Plasmid	Description (cloning sites)	Reference or source
p416CUP1	Low copy number; weak constitutive promoter	36
p416CUP1-*RTR1*-HA	C-terminal HA-tag fusion (SpeI/XhoI)	This study
p416CUP1-*RTR1*^{C73S}-HA	C-terminal HA-tag fusion (SpeI/XhoI)	This study
p416CUP1-*RTR1*$^{C112S, H116S}$-HA	C-terminal HA-tag fusion (SpeI/XhoI)	This study
pFA6a-3HA-His3MX6	Contains both a triple HA epitope and a hexahistidine coding sequence	43
p416CUP1-GFP-RTR1	C-terminal GFP fusion (SpeI/XhoI)	This study
p423GPD	High copy number; strong constitutive promoter	48
p423GPD-RTR1-V5	C-terminal V5-tag fusion (SpeI/XhoI)	This study
p423GPD-V5-RTR1	N-terminal V5-tag fusion (SpeI/XhoI)	This study
p423GPD-V5-N-RTR1	N-terminal V5-tag fusion to N terminus of *RTR1* (amino acids 1–140) (SpeI/XhoI)	This study
P423GPD-RTR1-C-V5	C-terminal V5-tag fusion to C terminus of *RTR1* (amino acids 141–226) (SpeI/XhoI)	This study
p416GPD	Low copy number; strong constitutive promoter	48
p426GPD	High copy number; strong constitutive promoter	48
p416GPD-*RTR1*	ORF inserted into MCS[a] (SpeI/XhoI)	This study
p426GPD-*RTR1*	ORF inserted into MCS (SpeI/XhoI)	This study
p423GPD-RPB3	ORF inserted into MCS (SpeI/XhoI)	This study
p423GPD-RPB4	ORF inserted into MCS (SpeI/XhoI)	This study
p423GPD-RPB5	ORF inserted into MCS (SpeI/XhoI)	This study
p423GPD-RPB7	ORF inserted into MCS (SpeI/XhoI)	This study
p423GPD-RPB8	ORF inserted into MCS (SpeI/XhoI)	This study
p423GPD-RPB9	ORF inserted into MCS (SpeI/XhoI)	This study
p423GPD-RPB10	ORF inserted into MCS (SpeI/XhoI)	This study
p423GPD-RPB11	ORF inserted into MCS (SpeI/XhoI)	This study
p423GPD-RPB12	ORF inserted into MCS (SpeI/XhoI)	This study
pRPB1	RPB1 genomic clone	N. Woychik
pRPB2	RPB2 genomic clone	N. Woychik
pRS416	Low copy number; URA$^+$	59
p416-*RTR1*	Low copy number; native *RTR1* promoter	This study
pRS315 pNOP-GFP-*RTR1*	N-terminal GFP fusion (PstI/XhoI)	This study
pRS315 pNOP-ProtA-*RTR1*	N-terminal protein A fusion (PstI/XhoI)	This study

[a] MCS, multiple cloning site.

lows. Wild-type and *rtr1*Δ::*LEU2* cells were grown overnight in YPD medium containing 2% sucrose as the sole carbon source and were either maintained at 30°C or shifted to 39°C for 1 h. Cells were then harvested by centrifugation and resuspended in YP-galactose (2%) and grown for an additional hour at 30°C or 39°C prior to RNA extraction. Transcript levels for the RNAPII-dependent *GAL1*, *GAL7*, and *ACTI* genes were analyzed by Northern blotting as described previously (46a) by use of oligonucleotide probes (sequences available upon request). Levels of the RNAPIII-dependent *SCR1* RNA component of the signal recognition particle were assessed as a control.

RESULTS

***rtr1*Δ cells are temperature sensitive, whereas overexpression of RTR1 causes a growth defect.** In an effort to uncover novel genes required for thermotolerance, we utilized published microarray data to identify unnamed genes induced in response to heat shock (11, 22). In our initial phenotypic characterization of haploid knockouts of these genes, cells carrying a disruption in the YER139C locus were exquisitely sensitive to the heat shock-mimetic formamide at 37°C but exhibited no additional stress sensitivities (Fig. 1A). Formamide has been used to exacerbate heat shock phenotypes, because it destabilizes noncovalent bonds in macromolecules (1). Further examination of this temperature sensitivity phenotype showed that *rtr1*Δ cells have no detectable growth defect at 30°C (doubling time of 1.9 h versus 1.8 h for the wild type), a mild growth defect at 37°C (4.2 h of doubling time), and a severe growth defect at 39°C (6.4 h of doubling time) compared to wild-type cells, which were capable of tolerating growth temperatures up to 41°C (Fig. 1B).

We next examined the consequences of elevated *RTR1* expression. Relative growth rates of wild-type cells transformed with an empty vector, a low-copy-number vector expressing *RTR1*, or a high-copy-number *RTR1* expression vector were assessed as shown in Fig. 1C. Increased expression of *RTR1* caused a dose-dependent reduction in growth rate: cells carrying an empty vector (low- or high-copy number) grew with a doubling time of approximately 2.1 h, those carrying an extra copy of *RTR1* on a *CEN* plasmid grew with a doubling time of approximately 2.7 h, and those from a 2-μ-based high-copy-number vector grew with a doubling time of approximately 2.7 h.

Rtr1 contains a conserved nonconsensus Zn-finger-like motif essential for function. Analysis of the primary amino acid sequence of Rtr1 revealed a cysteine-rich amino-terminal motif reminiscent of a Zn finger (C-x_4-C-x_{27}-C-x_3-H; Fig. 2A). However, the spacing and arrangement of the putative Zn-coordinating cysteine and histidine residues are novel, with no precise match to known Zn-finger modules. BLAST analysis revealed that this motif, including the intervening amino acids between the cysteine and histidine residues, is highly conserved in a wide range of eukaryotic species, including fission yeast (*Schizosaccharomyces pombe*), amoebae (*Dictyostelium discoideum*), mice, and humans. Homologs are notably absent from both the bacteria and archaea, suggesting that the *RTR1* gene family is unique to eukaryotes. The cysteine residues are invariant in these putative homologs, with a high (approximate) degree of sequence identity in the immediately adjacent regions. *RTR1* sequence similarity drops to negligible levels among the more distantly related species outside of this con-

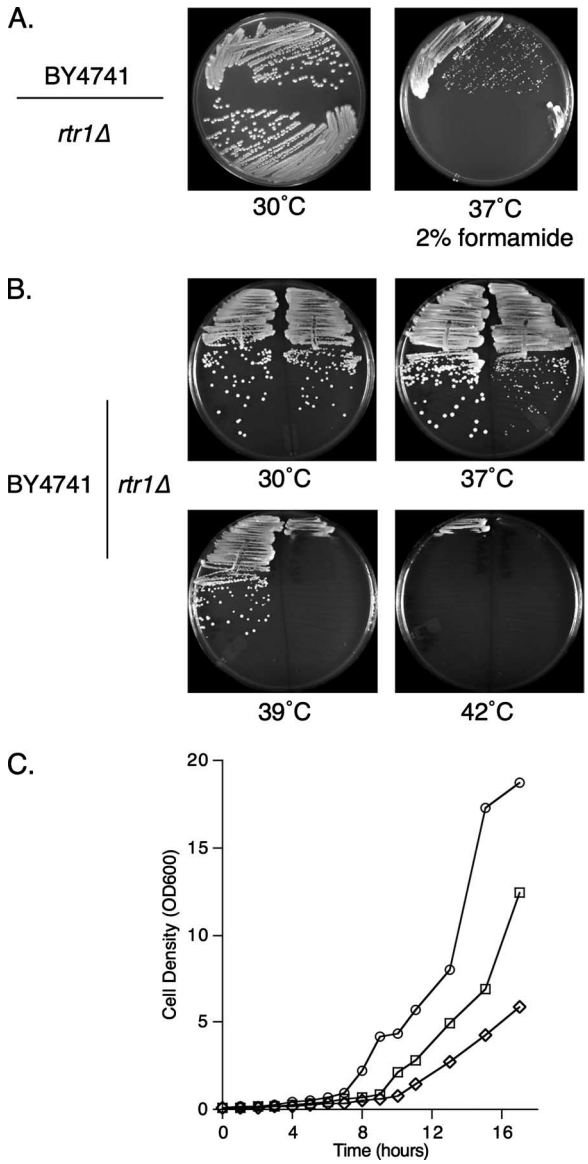

FIG. 1. Alteration of *RTR1* gene dosage causes conditional growth defects. (A) Wild-type (BY4741) and *rtr1*Δ yeast cells were streaked on rich media (YPD) with or without 2% formamide. Plates were incubated for 2 days at the indicated temperatures. (B) Wild-type (BY4741) and *rtr1*Δ yeast cells were streaked on rich media (YPD) and incubated at the indicated temperatures for 2 to 3 days. (C) Wild-type (BY4741) yeast cells were transformed with an empty vector (p416GPD), a low-copy-number vector expressing *RTR1* (p416GPD-*RTR1*), or a high-copy-number vector expressing *RTR1* (p426GPD-*RTR1*). These strains were grown in selective media (SC-URA), and the optical density at 600 nm (OD600) was read at the indicated time points (empty vector = circles, low-copy-number vector = squares, high-copy-number vector = diamonds).

served domain, implying that a conserved function likely requires the amino terminus. We therefore constructed point mutations within the *RTR1* gene containing either a C73S substitution or dual C112S-H116S substitutions to assess the functional requirement of the conserved cysteines. A triple influenza virus HA epitope tag was inserted at the carboxyl terminus to monitor production and stability of the mutant proteins. Replacement of either cysteine module completely blocked complementation of the *rtr1*Δ formamide-temperature sensitivity phenotype (Fig. 2B). To determine whether these *rtr1* mutant alleles were stably expressed, cells were grown to logarithmic phase, whole-cell extracts were isolated, and Rtr1 proteins were detected by immunoblot analysis. Both the *rtr1*(*C73S*) and the *rtr1*(*C112S*, *H116S*) mutants were produced at wild-type levels (Fig. 2C), demonstrating that lack of complementation was not due to destabilization. Moreover, these results suggest that the putative Zn finger is not required for Rtr1 structural integrity and may instead be involved in protein-protein or protein-nucleic acid interactions.

We next sought to determine whether overexpression toxicity likewise required the cysteine-rich domain. The conserved region comprises approximately half of the 226-amino-acid sequence of the Rtr1 protein. We therefore generated a series of constructs overexpressing the full-length protein or the amino- or carboxy-terminal halves of the protein. For convenience, the small (14-amino-acid) V5 epitope tag was engineered into the amplifying oligonucleotide primers to facilitate evaluation of protein expression. As a control, full-length Rtr1 was synthesized with either an amino- or a carboxy-terminal V5 tag, each of which was inhibitory to growth when overexpressed, as shown in Fig. 2D (see V5-Rtr1 and Rtr1-V5 data; doubling time, 2.7 and 3.1 h, respectively, versus 1.9 h for strains carrying the empty vector). In addition, the overexpressed nonconserved carboxy-terminal half of the protein was equally toxic ($Rtr1_{141-226}$-V5; 2.8 h doubling time). In contrast, the amino-terminal half of the protein containing the cysteine-rich domain (V5-$Rtr1_{1-140}$) did not result in a growth defect when overexpressed (doubling time, 2.1 h). This mutant protein was stably produced, as detected by immunoblotting (data not shown). Growth inhibition was further exacerbated at 37°C. Together, these data demonstrate that Rtr1 is the yeast homolog of members of a larger eukaryotic gene family that share a highly conserved cysteine-rich motif in the amino terminus that is required for function in vivo. Moreover, the nonconserved carboxy-terminal half of Rtr1 appears to dramatically hinder cell growth when overexpressed in yeast, suggesting the need for a stoichiometric balance of this protein with other cellular components essential for growth under normal and heat shock conditions.

Rtr1 constitutively shuttles between the cytoplasm and nucleus. As part of our characterization of the Rtr1 protein, we determined the subcellular localization of Rtr1 by use of a functional GFP-tagged allele expressed from a low-copy-number vector. Under both normal growth conditions (25°C) and elevated temperatures (35°C), GFP-tagged Rtr1 localized within the cytoplasm, in agreement with a previously published proteome-wide localization study (Fig. 3A) (27). We were unable to detect changes in the fluorescence localization pattern upon exposure to other environmental stresses (data not shown). Yeast two-hybrid analysis suggested that Rtr1 may gain access to the nucleocytoplasmic transport system via interaction with Ran (8). Therefore, to test for nuclear localization of Rtr1, GFP-Rtr1 was expressed in cells lacking Xpo1 (Crm1), the major nuclear export factor that shuttles NES-containing proteins out of the nucleus (60). Inactivation of this protein has been shown to lead to accumulation of substrates with rapid transit kinetics (7, 60). Using the temperature-sen-

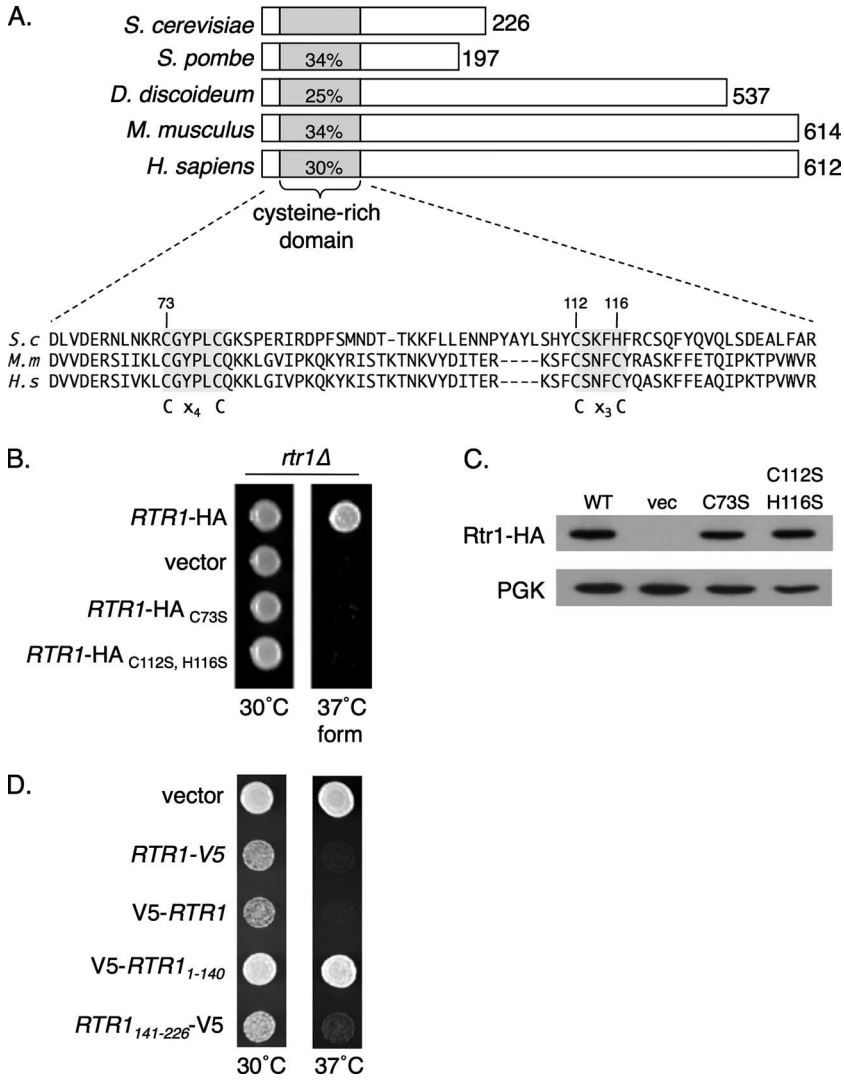

FIG. 2. *RTR1* contains an essential, conserved zinc-finger-like motif. (A) Homologs of *RTR1* from the indicated species are shown in the diagram. Percent identity with *RTR1* cysteine-rich domain is indicated, along with total amino acid length. Clustal alignment of the *RTR1* zinc-finger-like motif and its mouse (*M.m.*; NCBI accession BAE36627) and human (*H.s.*; NCBI accession NP_079089) homologues (shown at the bottom of the panel) are shown, with Zn-finger-like residues highlighted and their positions in the *S. cerevisiae* sequence indicated. (B) *rtr1*Δ yeast cells were transformed with vectors containing HA-tagged *RTR1* or the mutant alleles constructed as described in Materials and Methods (empty parent vector p416CUP1 was included as a negative control). These strains were spotted onto selective media (SC-URA) with or without 2% formamide (form) and incubated at the indicated temperatures for 3 days. (C) The strains described for panel B were grown and protein was extracted as described in Materials and Methods. Western blot analysis was used to detect stably produced protein with anti-HA and anti-PGK. WT, wild type; vec, vector. (D) V5-epitope-tagged versions of Rtr1 were constructed as described in Materials and Methods, all by using the high-copy-number plasmid p423GPD as the backbone. These constructs were transformed into wild-type (BY4741) yeast and plated by spot dilution onto selective media (SC-HIS). The plates were incubated for 2 days at 30°C.

sitive allele *xpo1-1*, we observed prominent nuclear localization within 8 min after shifting to the nonpermissive temperature (Fig. 3B). These data demonstrate that Rtr1 constitutively shuttles between cytoplasm and the nucleus and is actively transported out of the nucleus via the Xpo1 system.

Suppression of *rtr1*Δ temperature sensitivity by overexpression of RNAPII core subunits. In an effort to understand the cellular role of Rtr1, we undertook a high-copy-number suppressor screening to identify genes whose overexpression could repair the temperature-sensitive growth defect caused by the loss of *RTR1*. To accomplish this, we transformed a high-copy-number yEP24-based genomic library into the *rtr1*Δ background and selected for growth at 37°C in the presence of 2% formamide. Suppressors were then identified and characterized as described in Materials and Methods. From 100,000 independent transformants, we isolated only two independent suppressors of *rtr1*Δ, *RPB5* and *RPB9* (Fig. 4). Strikingly, the proteins encoded by these genes are both core subunits of eukaryotic RNAPII. To ask whether this phenomenon was a general property of RNAPII subunit overexpression, we tested 9 of the remaining 10 subunit-encoding genes for high-copy-number suppression of the *rtr1*Δ phenotype. *RPB7* was identified as yet another RNAPII subunit suppressor of *rtr1*Δ, while the remaining subunits were unable to confer growth when

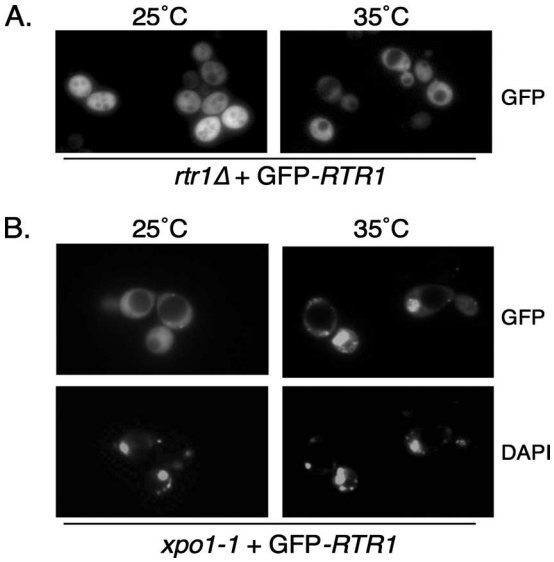

FIG. 3. Rtr1 shuttles constitutively between the nucleus and cytoplasm. Localization of GFP-Rtr1 in the *rtr1*Δ (A) or *xpo1-1* (B) strains at the permissive temperature of 25°C or the restrictive temperature of 35°C was determined by fluorescence microscopy. The cells shown in panel B were stained with DAPI as described in Materials and Methods to facilitate assessment of nuclear colocalization.

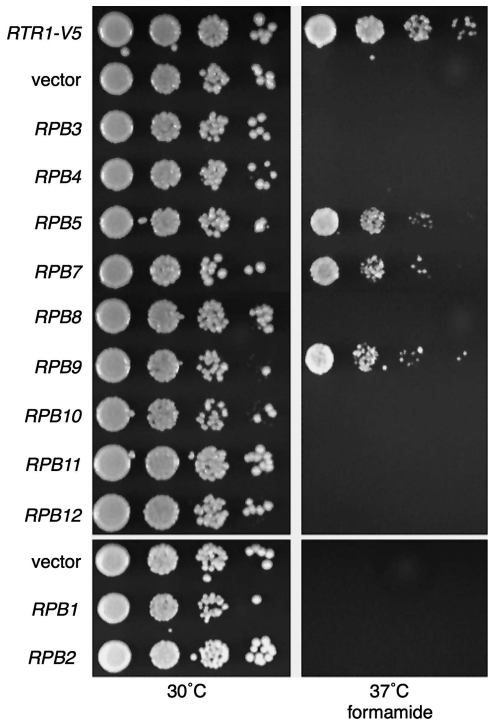

FIG. 4. A high-copy-number suppressor screening for genes that can compensate for the loss of *RTR1* identified *RPB5*, *RPB7*, and *RPB9*. A high-copy-number suppressor screening was done as described in Materials and Methods. *rtr1*Δ cells containing high-copy-number plasmids of RNAPII subunits were grown on selective media with or without 2% formamide at the indicated temperatures for 2 days. See Table 2 for relevant plasmid data.

overexpressed (Fig. 4). These results suggest that *RPB5*, *RPB7*, and *RPB9* share a common but unknown functional characteristic that permits overexpression of any one of those genes alone to overcome loss of the Rtr1 protein.

Phenotypic and genetic analyses indicate that Rtr1 has a role in transcription. Because overexpression of *RPB9* complements the formamide sensitivity phenotype of *rtr1*Δ, we considered the possibility that these two proteins are functionally linked. *rpb9*Δ cells are defective in start-site selection, utilizing secondary transcription start sites for a number of genes. We performed primer extension analysis using the *ADH1* gene and observed the reported defects of an *rpb9*Δ mutant but did not detect obvious transcriptional defects in *rtr1*Δ cells (data not shown). *RTR1* is therefore not required for at least this role of *RPB9*. To further probe the genetic relationship between *RTR1* and RNAPII, we tested whether strains lacking *RPB9* or the only other nonessential subunit, *RPB4*, could be sensitized to moderate overexpression of *RTR1* by use of the uninduced *CUP1* promoter (36). Surprisingly, at a normal growth temperature of 30°C, both *rpb9*Δ and *rpb4*Δ cells, but not wild-type cells, were exquisitely sensitive to heightened *RTR1* levels (Fig. 5). This effect was completely abrogated when either *rtr1*(*C73S*) or *rtr1*(*C112S*, *H116S*), the nonfunctional mutant alleles, was likewise overexpressed. These data, along with the results of the overexpression studies represented in Fig. 2, suggest that Rtr1 exists in an optimal stoichiometric balance with functional RNAPII.

A number of transcription mutants are sensitive to the IMP dehydrogenase inhibitors 6AU and MPA (29, 46, 57). These compounds are thought to decrease the nucleotide pool, thereby inhibiting transcription elongation (54). We tested wild type, *rpb9*Δ, and *rtr1*Δ cells for their relative resistances to these inhibitors and, given our previous results, also included formamide. When cells were plated onto media containing no drug, 6AU, MPA, or formamide, we observed differential sensitivity results, as shown in Fig. 6. At drug concentrations that were permissive for the growth of wild-type cells, *rtr1*Δ cells were sensitive only to MPA at 37°C and were resistant to 6AU. *rpb9*Δ cells exhibited more severe phenotypes, including sensitivity to 6AU at 37°C and to MPA at both 30°C and 37°C. Interestingly, *rpb9*Δ and *rtr1*Δ cells exhibited identical sensitivities to formamide only at 37°C. Loss of *RTR1* therefore recapitulates only a subset of the phenotypes exhibited by cells lacking *RBP9*, specifically in combination with elevated temperature.

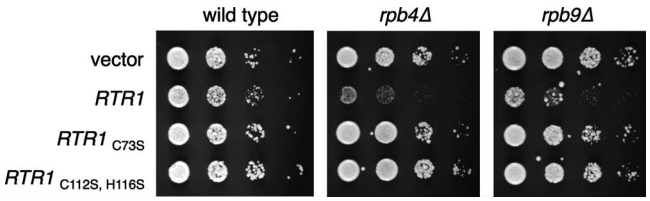

FIG. 5. Cells lacking *RPB4* and *RPB9* are sensitive to moderate *RTR1* overexpression. (A) Wild-type (BY4741), *rpb4*Δ, and *rpb9*Δ yeast cells were transformed with vectors expressing *RTR1*, *rtr1*(*C73S*), or *rtr1*(*C112S*, *H116S*) (including an empty parent vector control, p416CUP1). These strains were serially diluted, spotted onto selective media containing 50 μM copper sulfate, and incubated for 2 days at 30°C.

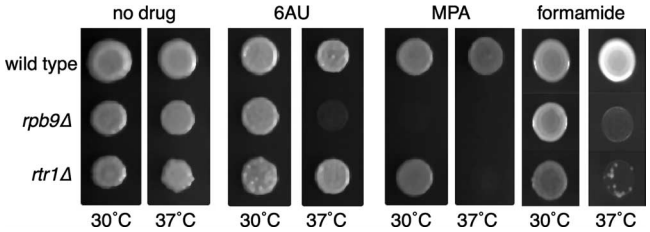

FIG. 6. *rtr1*Δ and *rpb9*Δ cells show differing sensitivities to transcription elongation inhibitors. Wild-type (BY4741), *rpb9*Δ, and *rtr1*Δ yeast cells were spotted onto YPD plates containing no drug, 100 μg/ml 6AU, 100 μg/ml MPA, or 2% formamide and incubated at the indicated temperatures for 2 days.

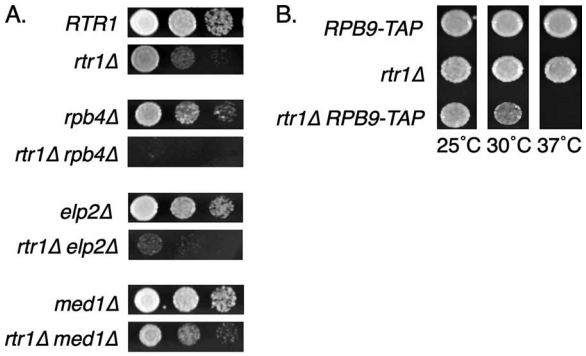

FIG. 7. *rtr1*Δ synthetically interacts with components of the transcription machinery. (A) Representative primary data from the synthetic interaction data presented in Table 3. Wild-type (BY4741), *rtr1*Δ (PGY1), or the indicated double-deletion cells were serially diluted and spotted onto plates containing 100 μg/ml MPA. Plates were incubated at 37°C for 3 days. (B) *RPB9*-TAP (Open Biosystems), *rtr1*Δ, and *RPB9*-TAP *rtr1*Δ (PGY14) yeast cells were spotted onto YPD medium and incubated at the indicated temperatures for 2 days.

To further delineate the transcriptional roles of Rtr1, we tested for conditional synthetic interactions between *rtr1*Δ and deletion of genes involved in various stages of transcription. We observed a number of synthetic growth defects (SGDs), including inviability in the presence of MPA for *rtr1*Δ *rpb4*Δ and *rtr1*Δ *elp2*Δ double mutants (Fig. 7A and Table 3). *ELP2* encodes a subunit of the Elongator complex, which associates with actively transcribing RNAPII and also possesses histone acetyltransferase activity (21). These interactions were not a general result of transcriptional impairment, as *RTR1* exhibited no SGDs with the initiation factors *MED1* and *GAL11*. *RTR1* also exhibited substantial interaction with the uncharacterized ORF YDR066C, which, based on the strong level of homology (89% sequence similarity) and the presence of the *RTR1* cysteine-rich domain, may be a recently diverged paralog. Two additional initiation factors, *SOH1* and *SRB5*, and the elongation factors *CCR4*, *CDC73*, *SPT4*, and *DST1* exhibited modest SGDs. The strongest interactions were observed with the RNAPII core subunits *RPB4* and *RPB9*. In the course of another line of investigation, we also observed a strong temperature-dependent synthetic interaction between *rtr1*Δ and an allele of Rpb9 that included the TAP tag *RPB9*-TAP (Fig. 7B). While *RPB9*-TAP and *rtr1*Δ were both viable at temperatures of up to 37°C, deletion of *RTR1* in the context of *RPB9*-TAP resulted in an inability to grow at 37°C. These results suggest that the *RPB9-TAP* allele may be cryptically hypomorphic and further underscore the close relationship between these two proteins.

Rtr1 physically interacts with active Rpb1. Recent large-scale proteomic studies of both yeast and human cells have indicated that Rtr1 (RPAP2 in humans) physically interacts with multiple components of the core RNAPII enzyme (15, 23, 30). Given the extensive genetic interactions we demonstrated between Rtr1 and RNAPII, we sought to validate and extend these findings in more detail. Protein A-tagged Rtr1 was purified as described in Materials and Methods, and copurifying proteins were visualized using Coomassie brilliant blue staining. Rpb1 and Rpb2 were associated with Rtr1, as evaluated by gel migration investigations (Fig. 8A). Immunoblot analysis of the protein A-Rtr1 affinity purification results by use of the specific monoclonal antibody 8WG16 positively identified Rpb1. Additional proteins were coisolated with Rtr1, including a prominent component of about 180 kDa that currently remains unidentified. We noted that Rpb1 migrated as a doublet, as is consistent with previously documented populations of the protein, one unphosphorylated and the other phosphorylated at serines in the second and fifth positions within the highly conserved heptad repeats of the regulatory CTD. Both protein bands were recognized by the 8WG16 antibody that interacts with nonphosphorylated heptad repeats (Fig. 8B) (9, 51, 62). We also tested reactivity of equivalent amounts of the coimmunoprecipitation mixtures with antibodies specific for phosphorylated CTD. The slower-migrating band reacted strongly with the H5 antibody specific for the CTD phosphorylated at serine 5 in the heptad repeat and weakly with the 8WG16 antibody H14 that recognizes phosphorylation at serine 2. These results are consistent with association of Rtr1 and actively transcribing RNAPII. None of the anti-RNAPII antibodies recognized bands from a parallel control TAP purification (Fig. 8B), and Rpb1 and Rpb2 were not detected by Coomassie staining (data not shown), demonstrating the specificity of the Rtr1-TAP · RNAPII interaction.

Rtr1 regulates transcription from the GAL1 promoter. Considering the genetic and biochemical interactions between *RTR1* and components of the transcription machinery, we were interested to determine the transcriptional consequences of *RTR1* disruption. We examined both inducible gene expression from the *GAL* regulon and constitutive expression of a standard "housekeeping" gene, *ACT1*. Because we anticipated that the requirement for RTR1 might be limited to transcription during heat shock, we also examined gene expression after a 1-h shift to 39°C. As shown in Fig. 8C, expression of *GAL1* and *GAL7* was strongly induced by a shift from sucrose to galactose in wild-type cells but not in *rtr1*Δ cells at either normal or heat shock temperatures, indicating a significant role for *RTR1* in expression from these loci. Expression of *ACT1* and the RNAPIII-dependent RNA component of *SCR1*, the signal recognition particle, was unaffected in *rtr1*Δ cells. Complementation of *rtr1*Δ restored *GAL1* induction, verifying that loss of transcriptional activity was due to loss of Rtr1 function (data not shown). Interestingly, RNAPII- but not RNAPIII-dependent gene expression appeared to be inhibited at high temperatures, as is consistent with a previous report (67).

TABLE 3. Genetic interactions between *rtr1*Δ and other transcription genes

Gene	Role (reference)	Result of interaction with *rtr1*Δ under indicated conditions[a]							
		YPD		Form		MPA		6AU	
		30°C	37°C	30°C	37°C	30°C	37°C	30°C	37°C
GAL11	Initiation (49)	—	—	—	ND	—	—	—	—
MED1	Initiation (49)	—	—	—	ND	—	—	—	—
SOH1	Initiation (41)	—	SGD	SGD	ND	—	SL	—	SGD
SRB5	Initiation (61)	—	—	ND	ND	—	—	—	SGD
CCR4	Elongation (18)	—	—	—	ND	—	—	—	—
CDC73	Elongation (34, 58)	—	—	—	ND	—	SGD	—	—
DST1	Elongation (50)	—	—	—	ND	—	SGD	—	—
ELP2	Elongation (21)	SGD	SGD	SGD	ND	SGD	SGD	SGD	SGD
SPT4	Elongation (42, 55)	—	—	SGD	ND	—	—	—	—
RPB4	Core (16)	—	SGD	SGD	ND	SGD	SL	—	SGD
RPB9	Core (16)	—	SGD	SGD	ND	ND	ND	—	ND
YDR066C	Unknown	SGD	SGD	SGD	ND	SGD	SGD	SGD	SGD

[a] YPD, rich medium, no stress; Form, 1% formamide; —, no synthetic interaction; ND, not determined (due to single-gene lethality); SL, synthetic lethality.

DISCUSSION

This report constitutes the first in vivo characterization of the *RTR1* gene and its protein product. We have elucidated multiple phenotypes associated with loss of *RTR1* and identified a highly conserved amino-terminal motif essential for function. We have further defined multiple genetic and biochemical interactions between *RTR1* and components of the transcription machinery—specifically, subunits of the core RNAPII enzyme. Finally, we observed a defect in transcription from the *GAL1* promoter in *rtr1*Δ cells, demonstrating a functional transcriptional consequence associated with loss of this novel protein. Our analyses indicate that Rtr1 functions in modulating RNAPII-based transcription, specifically via interactions with RNAPII core subunits. These findings are further bolstered by the identification of C1ORF82, the closest human *RTR1* homolog, as an RNAPII-associated factor. This ORF has been renamed RPAP2 (for "*R*NA*P*II-*a*ssociated *p*olypeptide") and is located within an expansive network of interacting

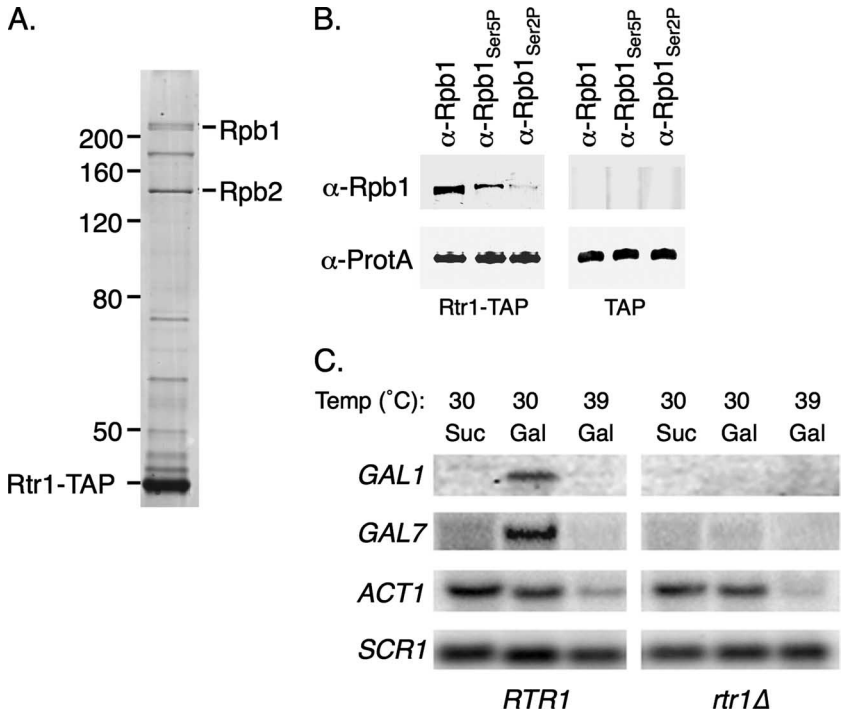

FIG. 8. Rtr1 physically interacts with the active form of Rpb1 and regulates transcription from the *GAL1* promoter. (A) Coomassie brilliant blue staining of a sodium dodecyl sulfate-polyacrylamide electrophoresis gel containing protein A-Rtr1 for affinity purification (described in Materials and Methods). The positions of Rbp1 and Rpb2 are indicated. (B) Immunoblot analysis of Rtr1-TAP and control TAP purifications using anti-Rpb1, anti-Rpb1(Ser5P), and anti-Rpb1(Ser2P) antibodies. (C) Northern blot analysis of wild-type (BY4741) and *rtr1*Δ cells grown to logarithmic phase in sucrose (Suc) medium and either maintained at 30°C or shifted to 39°C for 1 h followed by a shift to galactose (Gal)-containing medium at the indicated temperatures.

protein complexes, as identified by large-scale affinity purification with other transcriptional components (30). However, no functional insights into the roles of RPAP2 have emerged, underscoring the need for further study of the yeast homolog in its in vivo context. For example, comprehensive cataloging of transcriptional defects in *rtr1*Δ cells would aid the assignment of a specific functional role within the RNAPII multiprotein transcriptional complex.

We believe that the Rtr1/RPAP2 proteins may play a unique role in transcription because of the array of genetic interactions and specific physical interactions with core subunits of RNAPII. While many accessory factors and complexes have been identified (Mediator, Elongator, general and specific transcription factors, etc.), few interact robustly with the core subunits of RNAPII. Eight affinity capture interactions with Rtr1 examined in yeast proteome-wide analyses have been described previously—among them, interactions with the nucleolar protein Rpf2, with the microtubule-associated protein Bik1, and with the enzyme Ura2, an apparently promiscuous binding protein with 43 unconfirmed affinity interactions (23, 24, 33). The remaining four Rtr1 interactors are RNAPII subunits: Rpb1, Rpb2, Rpb3, and Rpb8 (15, 23). Strikingly, RPAP2 likewise interacts with at least eight core subunits (30). We confirmed the interaction of Rtr1 with Rpb1 in yeast cells and showed that Rtr1-associated Rpb1 is transcriptionally active. Further, *RTR1* genetically interacts with transcriptional components acting at multiple stages in transcription, including initiation and elongation. Core RNAPII subunits *RPB4* and *RPB9* exhibited some of the strongest genetic interactions with *rtr1*Δ. In addition, the *RPB9*-TAP allele also rendered cells temperature sensitive in the context of *rtr1*Δ. The heat shock/formamide sensitivity phenotype is shared by a number of other transcription mutants, including those defective in the Paf1 complex (Ctr9, Cdc73, Ccr4, Hpr1, Rtf1, Leo1) (12). RPAP2 also appears to associate with a number of additional polypeptides in one or more complexes, many of which have yeast counterparts (30). When taken together, these numerous genetic and biochemical interactions place Rtr1/RPAP2 in close proximity to the actively transcribing RNAPII with an as-yet-undescribed molecular role. Interestingly, under all the growth conditions we tested, Rtr1 was largely present in the cytoplasm. The finding that Rtr1 accumulated in the nucleus upon inactivation of the Xpo1-dependent export pathway indicates that nuclear shuttling is constitutive and may be an important aspect of its function. In contrast, nearly all RNAPII subunits are exclusively nuclear. Cytoplasmic localization of a core RNAPII-associated protein has been shown previously for the Rpb4 subunit in both *S. cerevisiae* and *Schizosaccharomyces pombe*, but the significance of this dual localization is not clear (20, 32).

The presence of the essential Zn-finger-like motif defines a eukaryotic lineage for Rtr1 and its homologous counterparts. This motif is restricted to a single putative homolog in each of the higher eukaryotic species examined. Interestingly, the carboxyl terminus is highly divergent in these putative *RTR1* homologs and is of variable length. This suggests either that this region serves no obvious cellular role or that it mediates interaction with disparate components. The former scenario is unlikely, as we found this portion of the protein to be responsible for growth inhibition upon protein overexpression in wild-type cells. We considered the possibility that Rtr1 interacts with a fungus-specific protein via this region, which could have resulted in titration of a cellular component critical for growth. We therefore undertook an additional high-copy-number suppressor screening to identify genes that would reverse the growth inhibition caused by *RTR1* overexpression but failed to isolate candidates capable of doing so (data not shown). *S. cerevisiae* is unique in that it contains two ORFs with the conserved cysteine-rich motif—*RTR1* and YDR066C. This is most likely due to the whole-genome duplication proposed to have occurred approximately 150 million years ago, given the high level of amino acid sequence similarity (89%) that extends throughout the protein (38). Despite the fact that we could not detect any phenotypes associated with deletion of YDR066C, we observed substantial synthetic interactions between *rtr1*Δ and *ydr066c*Δ, indicating possible functional redundancy between the two proteins (Table 3). We therefore propose renaming the uncharacterized ORF YDR066C *RTR2*, although at this time we do not have evidence for direct interaction with RNAPII.

Zn-finger motifs are predominantly involved in nucleic acid binding; as a result, the spacing between cysteine and histidine residues contributes to binding specificity (37). However, unlike many Zn fingers, the residues between the putative metal chelating cysteines and histidine in Rtr1 are also highly conserved. It may also be noteworthy that *RPB5* and *RPB9*, two of the three high-copy-number suppressors, are themselves Zn-containing proteins (19, 63). In fact, *RPB9* is a small 122-amino-acid subunit that harbors two distinct Zn-binding domains organized into what has been termed a "zinc ribbon." Because *RPB5* and *RPB9* are both located at the "jaws" of RNAPII and function as part of the DNA clamping mechanism, it is tempting to speculate that Rtr1 may function as an accessory DNA binding factor for RNAPII in a mechanism requiring its cysteine-rich motif. Further analysis of the precise protein-protein interactions of Rtr1/RPAP2 with RNAPII, of its presence or absence on the actively transcribing enzyme, and of gene-specific transcriptional requirements are needed to provide a full understanding of this protein family.

ACKNOWLEDGMENTS

Work in the K.A.M. laboratory was supported by grants from the American Heart Association (0160113Y) and the National Institutes of Health (GM074696). The S.M.B. laboratory was supported by grants from the Deutsche Forschungsgemeinschaft (Ba 1165/3-2), the Forschungsausschuβ (61 CL/TG84), and the HOMFOR 2005 program of the Universität des Saarlandes.

We thank Amy Trott, Cana Ross, Reginald Hence, and Carmen Galvan for early contributions to the study. We also thank Nancy Woychik and Ambro van Hoof for reagents and advice.

REFERENCES

1. **Aguilera, A.** 1994. Formamide sensitivity: a novel conditional phenotype in yeast. Genetics **136:**87–91.
2. **Armache, K. J., S. Mitterweger, A. Meinhart, and P. Cramer.** 2005. Structures of complete RNA polymerase II and its subcomplex, Rpb4/7. J. Biol. Chem. **280:**7131–7134.
3. **Awrey, D. E., R. G. Weilbaecher, S. A. Hemming, S. M. Orlicky, C. M. Kane, and A. M. Edwards.** 1997. Transcription elongation through DNA arrest sites. A multistep process involving both RNA polymerase II subunit Rpb9 and TFIIS. J. Biol. Chem. **272:**14747–14754.
4. **Bailer, S. M., C. Balduf, and E. Hurt.** 2001. The Nsp1p carboxy-terminal domain is organized into functionally distinct coiled-coil regions required for assembly of nucleoporin subcomplexes and nucleocytoplasmic transport. Mol. Cell. Biol. **21:**7944–7955.

5. **Bailer, S. M., C. Balduf, J. Katahira, A. Podtelejnikov, C. Rollenhagen, M. Mann, N. Pante, and E. Hurt.** 2000. Nup116p associates with the Nup82p-Nsp1p-Nup159p nucleoporin complex. J. Biol. Chem. **275:**23540–23548.
6. **Bailer, S. M., S. Siniossoglou, A. Podtelejnikov, A. Hellwig, M. Mann, and E. Hurt.** 1998. Nup116p and Nup100p are interchangeable through a conserved motif which constitutes a docking site for the mRNA transport factor Gle2p. EMBO J. **17:**1107–1119.
7. **Betz, C., G. Schlenstedt, and S. M. Bailer.** 2004. Asr1p, a novel yeast RING/PHD finger protein, signals alcohol stress to the nucleus. J. Biol. Chem. **279:**28174–28181.
8. **Braunwarth, A., M. Fromont-Racine, P. Legrain, F. R. Bischoff, T. Gerstberger, E. Hurt, and M. Kunzler.** 2003. Identification and characterization of a novel RanGTP-binding protein in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **278:**15397–15405.
9. **Bregman, D. B., L. Du, S. van der Zee, and S. L. Warren.** 1995. Transcription-dependent redistribution of the large subunit of RNA polymerase II to discrete nuclear domains. J. Cell Biol. **129:**287–298.
10. **Carlson, M., and D. Botstein.** 1982. Two differentially regulated mRNAs with different 5′ ends encode secreted with intracellular forms of yeast invertase. Cell **28:**145–154.
11. **Causton, H. C., B. Ren, S. S. Koh, C. T. Harbison, E. Kanin, E. G. Jennings, T. I. Lee, H. L. True, E. S. Lander, and R. A. Young.** 2001. Remodeling of yeast genome expression in response to environmental changes. Mol. Biol. Cell **12:**323–337.
12. **Chang, M., D. French-Cornay, H. Y. Fan, H. Klein, C. L. Denis, and J. A. Jaehning.** 1999. A complex containing RNA polymerase II, Paf1p, Cdc73p, Hpr1p, and Ccr4p plays a role in protein kinase C signaling. Mol. Cell. Biol. **19:**1056–1067.
13. **Choder, M.** 2004. Rpb4 and Rpb7: subunits of RNA polymerase II and beyond. Trends Biochem. Sci. **29:**674–681.
14. **Choder, M., and R. A. Young.** 1993. A portion of RNA polymerase II molecules has a component essential for stress responses and stress survival. Mol. Cell. Biol. **13:**6984–6991.
15. **Collins, M. O., L. Yu, and J. S. Choudhary.** 2007. Analysis of protein phosphorylation on a proteome-scale. Proteomics **7:**2751–2768.
16. **Cramer, P., D. A. Bushnell, and R. D. Kornberg.** 2001. Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. Science **292:** 1863–1876.
17. **Dahmus, M. E.** 1996. Reversible phosphorylation of the C-terminal domain of RNA polymerase II. J. Biol. Chem. **271:**19009–19012.
18. **Denis, C. L., Y. C. Chiang, Y. Cui, and J. Chen.** 2001. Genetic evidence supports a role for the yeast Ccr4-Not complex in transcriptional elongation. Genetics **158:**627–634.
19. **Donaldson, I. M., and J. D. Friesen.** 2000. Zinc stoichiometry of yeast RNA polymerase II and characterization of mutations in the zinc-binding domain of the largest subunit. J. Biol. Chem. **275:**13780–13788.
20. **Farago, M., T. Nahari, C. Hammel, C. N. Cole, and M. Choder.** 2003. Rpb4p, a subunit of RNA polymerase II, mediates mRNA export during stress. Mol. Biol. Cell **14:**2744–2755.
21. **Fellows, J., H. Erdjument-Bromage, P. Tempst, and J. Q. Svejstrup.** 2000. The Elp2 subunit of elongator and elongating RNA polymerase II holoenzyme is a WD40 repeat protein. J. Biol. Chem. **275:**12896–12899.
22. **Gasch, A. P., P. T. Spellman, C. M. Kao, O. Carmel-Harel, M. B. Eisen, G. Storz, D. Botstein, and P. O. Brown.** 2000. Genomic expression programs in the response of yeast cells to environmental changes. Mol. Biol. Cell **11:** 4241–4257.
23. **Gavin, A. C., P. Aloy, P. Grandi, R. Krause, M. Boesche, M. Marzioch, C. Rau, L. J. Jensen, S. Bastuck, B. Dumpelfeld, A. Edelmann, M. A. Heurtier, V. Hoffman, C. Hoefert, K. Klein, M. Hudak, A. M. Michon, M. Schelder, M. Schirle, M. Remor, T. Rudi, S. Hooper, A. Bauer, T. Bouwmeester, G. Casari, G. Drewes, G. Neubauer, J. M. Rick, B. Kuster, P. Bork, R. B. Russell, and G. Superti-Furga.** 2006. Proteome survey reveals modularity of the yeast cell machinery. Nature **440:**631–636.
24. **Gavin, A. C., M. Bosche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J. M. Rick, A. M. Michon, C. M. Cruciat, M. Remor, C. Hofert, M. Schelder, M. Brajenovic, H. Ruffner, A. Merino, K. Klein, M. Hudak, D. Dickson, T. Rudi, V. Gnau, A. Bauch, S. Bastuck, B. Huhse, C. Leutwein, M. A. Heurtier, R. R. Copley, A. Edelmann, E. Querfurth, V. Rybin, G. Drewes, M. Raida, T. Bouwmeester, P. Bork, B. Seraphin, B. Kuster, G. Neubauer, and G. Superti-Furga.** 2002. Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature **415:**141–147.
25. **Hemming, S. A., D. B. Jansma, P. F. Macgregor, A. Goryachev, J. D. Friesen, and A. M. Edwards.** 2000. RNA polymerase II subunit Rpb9 regulates transcription elongation in vivo. J. Biol. Chem. **275:**35506–35511.
26. **Hengartner, C. J., V. E. Myer, S. M. Liao, C. J. Wilson, S. S. Koh, and R. A. Young.** 1998. Temporal regulation of RNA polymerase II by Srb10 and Kin28 cyclin-dependent kinases. Mol. Cell **2:**43–53.
27. **Huh, W. K., J. V. Falvo, L. C. Gerke, A. S. Carroll, R. W. Howson, J. S. Weissman, and E. K. O'Shea.** 2003. Global analysis of protein localization in budding yeast. Nature **425:**686–691.
28. **Hull, M. W., K. McKune, and N. A. Woychik.** 1995. RNA polymerase II subunit Rpb9 is required for accurate start site selection. Genes Dev. **9:**481–490.
29. **Jenks, M. H., and D. Reines.** 2005. Dissection of the molecular basis of mycophenolate resistance in *Saccharomyces cerevisiae*. Yeast **22:**1181–1190.
30. **Jeronimo, C., D. Forget, A. Bouchard, Q. Li, G. Chua, C. Poitras, C. Therien, D. Bergeron, S. Bourassa, J. Greenblatt, B. Chabot, G. G. Poirier, T. R. Hughes, M. Blanchette, D. H. Price, and B. Coulombe.** 2007. Systematic analysis of the protein interaction network for the human transcription machinery reveals the identity of the 7SK capping enzyme. Mol. Cell **27:** 262–274.
31. **Kaiser, C., S. Michaelis, and A. Mitchell (ed.).** 1994. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, New York, NY.
32. **Kimura, M., H. Sakurai, and A. Ishihama.** 2001. Intracellular contents and assembly states of all 12 subunits of the RNA polymerase II in the fission yeast *Schizosaccharomyces pombe*. Eur. J. Biochem. **268:**612–619.
33. **Krogan, N. J., G. Cagney, H. Yu, G. Zhong, X. Guo, A. Ignatchenko, J. Li, S. Pu, N. Datta, A. P. Tikuisis, T. Punna, J. M. Peregrin-Alvarez, M. Shales, X. Zhang, M. Davey, M. D. Robinson, A. Paccanaro, J. E. Bray, A. Sheung, B. Beattie, D. P. Richards, V. Canadien, A. Lalev, F. Mena, P. Wong, A. Starostine, M. M. Canete, J. Vlasblom, S. Wu, C. Orsi, S. R. Collins, S. Chandran, R. Haw, J. J. Rilstone, K. Gandi, N. J. Thompson, G. Musso, P. St. Onge, S. Ghanny, M. H. Lam, G. Butland, A. M. Altaf-Ul, S. Kanaya, A. Shilatifard, E. O'Shea, J. S. Weissman, C. J. Ingles, T. R. Hughes, J. Parkinson, M. Gerstein, S. J. Wodak, A. Emili, and J. F. Greenblatt.** 2006. Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. Nature **440:**637–643.
34. **Krogan, N. J., M. Kim, S. H. Ahn, G. Zhong, M. S. Kobor, G. Cagney, A. Emili, A. Shilatifard, S. Buratowski, and J. F. Greenblatt.** 2002. RNA polymerase II elongation factors of *Saccharomyces cerevisiae*: a targeted proteomics approach. Mol. Cell. Biol. **22:**6979–6992.
35. **Künzler, M., T. Gerstberger, F. Stutz, F. R. Bischoff, and E. Hurt.** 2000. Yeast Ran-binding protein 1 (Yrb1) shuttles between the nucleus and cytoplasm and is exported from the nucleus via a Crm1 (Xpo1)-dependent pathway. Mol. Cell. Biol. **20:**4295–4308.
36. **Labbé, S., and D. J. Thiele.** 1999. Copper ion inducible and repressible promoter systems in yeast. Methods Enzymol. **306:**145–153.
37. **Laity, J. H., B. M. Lee, and P. E. Wright.** 2001. Zinc finger proteins: new insights into structural and functional diversity. Curr. Opin. Struct. Biol. **11:**39–46.
38. **Langkjaer, R. B., P. F. Cliften, M. Johnston, and J. Piskur.** 2003. Yeast genome duplication was followed by asynchronous differentiation of duplicated genes. Nature **421:**848–852.
39. **Lee, T. I., and R. A. Young.** 2000. Transcription of eukaryotic protein-coding genes. Annu. Rev. Genet. **34:**77–137.
40. **Li, S., and M. J. Smerdon.** 2002. Rpb4 and Rpb9 mediate subpathways of transcription-coupled DNA repair in *Saccharomyces cerevisiae*. EMBO J. **21:**5921–5929.
41. **Linder, T., and C. M. Gustafsson.** 2004. The Soh1/Med31 protein is an ancient component of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* Mediator. J. Biol. Chem. **279:**49455–49459.
42. **Lindstrom, D. L., S. L. Squazzo, N. Muster, T. A. Burckin, K. C. Wachter, C. A. Emigh, J. A. McCleery, J. R. Yates 3rd, and G. A. Hartzog.** 2003. Dual roles for Spt5 in pre-mRNA processing and transcription elongation revealed by identification of Spt5-associated proteins. Mol. Cell. Biol. **23:**1368–1378.
43. **Longtine, M. S., A. McKenzie 3rd, D. J. Demarini, N. G. Shah, A. Wach, A. Brachat, P. Philippsen, and J. R. Pringle.** 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast **14:**953–961.
44. **Mager, W. H., and A. J. De Kruijff.** 1995. Stress-induced transcriptional activation. Microbiol. Rev. **59:**506–531.
45. **Maillet, I., J. M. Buhler, A. Sentenac, and J. Labarre.** 1999. Rpb4p is necessary for RNA polymerase II activity at high temperature. J. Biol. Chem. **274:**22586–22590.
46. **Malagon, F., M. L. Kireeva, B. K. Shafer, L. Lubkowska, M. Kashlev, and J. N. Strathern.** 2006. Mutations in the *Saccharomyces cerevisiae RPB1* gene conferring hypersensitivity to 6-azauracil. Genetics **172:**2201–2209.

46a. **Meaux, S., and A. van Hoof.** 2006. Yeast transcripts cleaved by an internal ribozyme provide new insight into the role of the cap and poly(A) tail in translation and mRNA decay. RNA **12:**1323–1327.

47. **Miyao, T., J. D. Barnett, and N. A. Woychik.** 2001. Deletion of the RNA polymerase subunit Rpb4 acts as a global, not stress-specific, shut-off switch for RNA polymerase II transcription at high temperatures. J. Biol. Chem. **276:**46408–46413.
48. **Mumberg, D., R. Muller, and M. Funk.** 1995. Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Gene **156:**119–122.
49. **Myers, L. C., C. M. Gustafsson, D. A. Bushnell, M. Lui, H. Erdjument-Bromage, P. Tempst, and R. D. Kornberg.** 1998. The MED proteins of yeast and their function through the RNA polymerase II carboxy-terminal domain. Genes Dev. **12:**45–54.
50. **Nakanishi, T., A. Nakano, K. Nomura, K. Sekimizu, and S. Natori.** 1992.

Purification, gene cloning, and gene disruption of the transcription elongation factor S-II in *Saccharomyces cerevisiae*. J. Biol. Chem. **267:**13200–13204.
51. **Patturajan, M., R. J. Schulte, B. M. Sefton, R. Berezney, M. Vincent, O. Bensaude, S. L. Warren, and J. L. Corden.** 1998. Growth-related changes in phosphorylation of yeast RNA polymerase II. J. Biol. Chem. **273:**4689–4694.
52. **Paule, M. R., and R. J. White.** 2000. Survey and summary: transcription by RNA polymerases I and III. Nucleic Acids Res. **28:**1283–1298.
53. **Pillai, B., J. Verma, A. Abraham, P. Francis, Y. Kumar, U. Tatu, S. K. Brahmachari, and P. P. Sadhale.** 2003. Whole genome expression profiles of yeast RNA polymerase II core subunit, Rpb4, in stress and nonstress conditions. J. Biol. Chem. **278:**3339–3346.
54. **Reines, D.** 2003. Use of RNA yeast polymerase II mutants in studying transcription elongation. Methods Enzymol. **371:**284–292.
55. **Rondón, A. G., M. Garcia-Rubio, S. Gonzalez-Barrera, and A. Aguilera.** 2003. Molecular evidence for a positive role of Spt4 in transcription elongation. EMBO J. **22:**612–620.
56. **Rosenheck, S., and M. Choder.** 1998. Rpb4, a subunit of RNA polymerase II, enables the enzyme to transcribe at temperature extremes in vitro. J. Bacteriol. **180:**6187–6192.
57. **Shaw, R. J., J. L. Wilson, K. T. Smith, and D. Reines.** 2001. Regulation of an IMP-dehydrogenase gene and its overexpression in drug-sensitive transcription elongation mutants of yeast. J. Biol. Chem. **276:**32905–32916.
58. **Shi, X., M. Chang, A. J. Wolf, C. H. Chang, A. A. Frazer-Abel, P. A. Wade, Z. F. Burton, and J. A. Jaehning.** 1997. Cdc73p and Paf1p are found in a novel RNA polymerase II-containing complex distinct from the Srbp-containing holoenzyme. Mol. Cell. Biol. **17:**1160–1169.
59. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
60. **Stade, K., C. S. Ford, C. Guthrie, and K. Weis.** 1997. Exportin 1 (Crm1p) is an essential nuclear export factor. Cell **90:**1041–1050.
61. **Thompson, C. M., A. J. Koleske, D. M. Chao, and R. A. Young.** 1993. A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell **73:**1361–1375.
62. **Thompson, N. E., T. H. Steinberg, D. B. Aronson, and R. R. Burgess.** 1989. Inhibition of in vivo and in vitro transcription by monoclonal antibodies prepared against wheat germ RNA polymerase II that react with the heptapeptide repeat of eukaryotic RNA polymerase II. J. Biol. Chem. **264:** 11511–11520.
63. **Treich, I., M. Riva, and A. Sentenac.** 1991. Zinc-binding subunits of yeast RNA polymerases. J. Biol. Chem. **266:**21971–21976.
64. **Woychik, N. A., and M. Hampsey.** 2002. The RNA polymerase II machinery: structure illuminates function. Cell **108:**453–463.
65. **Woychik, N. A., W. S. Lane, and R. A. Young.** 1991. Yeast RNA polymerase II subunit Rpb9 is essential for growth at temperature extremes. J. Biol. Chem. **266:**19053–19055.
66. **Woychik, N. A., and R. A. Young.** 1989. RNA polymerase II subunit Rpb4 is essential for high- and low-temperature yeast cell growth. Mol. Cell. Biol. **9:**2854–2859.
67. **Yamamoto, N., Y. Maeda, A. Ikeda, and H. Sakurai.** 2008. Regulation of thermotolerance by stress-induced transcription factors in *Saccharomyces cerevisiae*. Eukaryot. Cell. ePUB March 21.