The Conserved Foot Domain of RNA Pol II Associates with Proteins Involved in Transcriptional Initiation and/or Early Elongation

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ABSTRACT RNA polymerase (pol) II establishes many protein–protein interactions with transcriptional regulators to coordinate different steps of transcription. Although some of these interactions have been well described, little is known about the existence of RNA pol II regions involved in contact with transcriptional regulators. We hypothesize that conserved regions on the surface of RNA pol II contact transcriptional regulators. We identified such an RNA pol II conserved region that includes the majority of the "foot" domain and identified interactions of this region with Mvp1, a protein required for sorting proteins to the vacuole, and Spo14, a phospholipase D. Deletion of *MVP1* and *SPO14* affects the transcription of their target genes and increases phosphorylation of Ser5 in the carboxy-terminal domain (CTD). Genetic, phenotypic, and functional analyses point to a role for these proteins in transcriptional initiation and/or early elongation, consistent with their genetic interactions with *CEG1*, a guanylyltransferase subunit of the *Saccharomyces cerevisiae* capping enzyme.

IN eukaryotes as in archaea, bacteria, chloroplasts, some mitochondria, and nucleocytoplasmic DNA viruses, transcription is ensured by heteromultimeric DNA-dependent RNA polymerases (Thuriaux and Sentenac 1992; Vassylyev *et al.* 2002; Werner and Weinzierl 2002; Iyer *et al.* 2006). RNA polymerase II (RNA pol II) produces all mRNAs and many noncoding RNAs. Although it transcribes most of the nuclear genome, it contributes <10% of the total RNA present in growing cells (Hahn 2004). To transcribe a gene, RNA pol II requires the action of general transcription factors, coregulators, specific transcription activators, and repressors. In fact, the RNA pol II transcription machinery is the most complex of those associated with the three RNA polymerases, with a total of nearly 60 polypeptides (Hahn 2004).

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Knowledge of both the architecture making up this complex and the function of its different parts is essential to understand their role in the different transcription steps (Cramer 2006; Zaros *et al.* 2007; Venters and Pugh 2009). Structural data gathered over the last few years on *Saccharomyces cerevisiae* RNA pol II have provided a detailed map of the physical interactions between the different subunits, establishing regions that are important for transcription (Cramer *et al.* 2001; Bushnell *et al.* 2002; Armache *et al.* 2003; Meyer *et al.* 2009). Notably, recent work has contributed to the understanding of how RNA pol II amino acid regions or subunits are involved in the contact with transcriptional regulators such as TFIIS, TFIIB, TFIIE, TFIIF, or Mediator, among others, although the data are sometimes imprecise or controversial (Guglielmi *et al.* 2004; Chadick and Asturias 2005; Chen *et al.* 2007; Meyer *et al.* 2009; Kostrewa *et al.* 2009).

A major question that remains unexplored is the identification of domains of RNA pol II that could be involved in the interaction with elements of the transcriptional machinery and that could participate in coordinating with them.

The identification of new transcriptional regulators, how they assemble in the transcriptional machinery, and their contribution to these processes would be useful.

Here, we describe the existence of a conserved protein domain corresponding to the foot of Rpb1 of *S. cerevisiae* RNA pol II, located on the surface of the complex. We have identified interactions of this region with Mvp1 and Spo14 and demonstrate that a fraction of these proteins localizes in the nucleus. ChIP–chip analysis suggests that both Mvp1 and Spo14 associate with RNA pol II genes, but not with RNA pol I or III genes. Deletion of *MVP1* and *SP014* affects the expression of some of their target genes, as well as genes regulated by Mot1 and/or NC2 and increases phosphorylation of Ser5 in the carboxy-terminal domain (CTD), consistent with the genetic interactions between Δ*mvp1* or Δ*spo14* and the Δ*rtr1* mutation. Furthermore, these data together with phenotypic and functional analysis point to a role for these proteins in transcription initiation and/or early elongation, in accordance with the genetic interactions with *CEG1*. In addition, our data clearly agree with data from Suh and coworkers that have also defined the foot of the RNA pol II as a domain conserved among RNA pol IIs from different species and that contact the RNA capping enzyme (CE) in *S. cerevisiae* (Suh *et al.* 2010).

Materials and Methods

Yeast strains, plasmids, genetic manipulations, media, and genetic analysis

Common yeast media, growth conditions, and genetic techniques were used as described elsewhere (Garcia-Lopez *et al.* 2010).

Strains and plasmids are listed in Tables 1 and 2, respectively. MAY322 strain (Biomedal), expressing the C-LYTAG domain from the *NHP6A* gene promoter, was obtained from strain BY4742 by replacing the *NHP6A* ORF with the C-LytA ORF, through chromosomal integration of a PCR product from plasmid pUC19-lytAstop-cyc1term-His3MX6.

Two-hybrid screening and identification of interacting proteins

The FRYL genomic library (Fromont-Racine *et al.* 1997) contained randomly sheared genomic DNA fragments of 700-bp mean size in a modified pACT2 vector. Two-hybrid analyses were as described (Flores *et al.* 1999). The prey DNA were amplified by PCR and sequenced with 242 and 244 primers (Supporting Information, Table S2). The identity of the insert was determined by using the *Saccharomyces* Genome Database Blast service.

Protein tagging

Proteins were tagged with a C-LYTAG tag (Biomedal) as described in Longtine *et al.* (1998) amplified from the pUC19-LytA-Kan plasmid (gift from S. Chávez) by PCR, with primers MVP1lyt-501/301 and SPO14lyt-501/301. Positive colonies were analyzed by PCR with the Mvp1-501/301 and Spo14-501/301 primers (Table S2).

Protein immunoprecipitation

Immunoprecipitations (IPs) were carried out as described (Soutourina *et al.* 2006) with 100 μl of protein extracts (1500 μg) prepared from cells growing exponentially (A_{600} ~0.6–0.8) in yeast extract–peptone–dextrose (YPD) medium. An anti–C-LYTAG antibody (50 μl at 1 μg/μl) (Hernandez-Torres *et al.* 2008) was used. The affinity-purified proteins were released from the beads by boiling for 10 min. Eluted proteins were analyzed by Western blotting with anti–C-LYTAG and anti-Rpb1 (gift from P. Thuriaux) antibodies.

Immunolocalization

Cells were grown at 30° in SD medium (A_{600} ~0.8–1.0), fixed with 37% w/v formaldehyde at room temperature for 1 hr with slow shaking, and then centrifuged and washed twice with PBST (PBS 1× with 0.05% Tween-20). Cell wall was digested with 50 μg/ml zymolyase in PBST (USBiological) by incubation for 30 min at 37° without shaking. The spheroplasts were washed twice with PBST and then resuspended in the same solution. Cell suspension was added to an AAS (3-aminopropyltriethoxysilane; Sigma) slide, incubated at room temperature for 15 min and washed twice with PBST. A total of 50 μl of 1:50 dilution of the anti–C-LYTAG primary antibody in PBST–BSA (5 mg/ml BSA) were added and incubated overnight at 4°. The slides were then washed twice with PBST–BSA and incubated for 2 hr, in the dark, at room temperature, with 50 μl of 1:300 dilution of secondary antibody (anti-rabbit IgG conjugated with Cy3; The Jackson Laboratory). The slides were washed twice with PBST–BSA and finally covered with a Vectashield (Vectorlabs) mounting solution. The fluorescence intensity was scored with a fluorescence microscope (Olympus BX51).

Chromatin immunoprecipitation

For ChIP–chip experiments we followed the protocol described in Jimeno-Gonzalez *et al.* (2006) but using antibodies against C-LYTAG epitope. We included no-antibody samples (NA) as the negative controls of the immunoprecipitation process. Two independent biological replicates were made. Specificity for the candidate genes was reconfirmed by Q-PCR. Each PCR reaction was performed three times (Table S2 for oligonucleotides).

For Rpb1 (non–P-CTD), Ser5P, and Ser2P IPs, 8WG16 (Covance), CTD4H8 (Millipore), and ab5095 (Abcam) antibodies were used and chromatin immunoprecipitations were performed as previously described (Garcia *et al.* 2010). Genes were analyzed by quantitative real-time PCR in duplicate with at least three independent biological replicates. Values found for the immunoprecipitated PCR products were compared to those of the total input, and the ratio of values from each PCR product of transcribed genes to that of a nontranscribed region of CVII was calculated. The oligonucleotides used are listed in Table S2.

Table 1 ***Saccharomyces cerevisiae* strains**

Strain	Genotype	Origin
Y190	*MAT***a** *gal4 gal80 his3 trp1-901 ade2-101 ura3-52 leu2-3*, *112 URA3*::*GAL1*::*lacZ LYS2*::*GAL4(UAS)*::*HIS3 cyh*^{R}	Flores *et al.* (1999)
Y187	*MAT*α *gal4 gal80 his3 trp1-901 ade2-101 ura3-52 leu2-3*, *112 met URA3*::*GAL1*::*lacZ LYS2*::*GAL4(UAS)*::*HIS3 cyh*^{R}	Flores *et al.* (1999)
JAY212	*MAT***a** *CAN1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1 rpo21-4*	Archambault *et al.* (1992)
DDT-Rt1	MATα *ura3-52 his3-Δ200 leu2-3,112 trp1-Δ63 rpb1-Δ187*::*HIS3 // pJA481-a (rpo21-24 TRP1 CEN6 ARSX)*	Archambault *et al.* (1992), Garcia-Lopez *et al.* (2010)
GR21-2d	*MAT*α *ura3-52 his3-Δ200 leu2-3,112 trp1-Δ63 rpb1-Δ187*::*HIS3* + pFL44-*RPB1* (2*μm URA3 RPB1*)	Garcia-Lopez *et al.* (2010)
Z102	*MAT*α *ura3-52 his3-Δ200 leu2-3,112 rpb2-Δ297*::*HIS3 /CEN LEU2 rpb2-6 (rpb2-R857K)*	Scafe *et al.* (1990)
BY4741	*MAT***a** *his3Δ1 leu2Δ0 met15 Δ 0 ura3Δ0*	Euroscarf
BY4742	*MAT*α *his3Δ1 leu2Δ0 lys2Δ 0 ura3Δ 0*	Euroscarf
BY4742-Mvp1-LytA	*MAT*α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 YMR004w-LytA-KanMX4*	This work
BY4742-Spo14-LytA	*MAT*α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 YKR031c-LytA-KanMX4*	This work
MAY322	*MAT*α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 nph6A Δ*::*c-LytA*::*HIS3MX6*	Biomedal
YPH499	*MAT***a** *ade2-101 Lys2-801 Ura3-52 Trp1- Δ63 His3-Δ200 Leu2Δ1*	Garcia-Lopez *et al.* (2010)
YFN291	*MAT***a** *ade2-101 Lys2-801 Ura3-52 Trp1-Δ63 His3-Δ200 Leu2Δ1 YMR004w*::*KanMX4 (mvp1Δ)*	This work
YFN292	*MAT***a** *ade2-101 Lys2-801 Ura3-52 Trp1-Δ63 His3-Δ200 Leu2Δ1 YKR031c*::*KanMX4 (spo14 Δ)*	This work
D334-1a	*MAT*α *ade2-1 lys2-801 ura3-52 trp1Δ63 his3Δ200 leu2Δ1 ppr2*::*hisG-URA3-hisG*	P. Thuriaux
YFN297	*MAT*α *ade2-1 lys2-801 ura3-52 trp1Δ63 his3Δ200 leu2Δ1 ppr2*::*hisG-URA3-hisG YMR004w*::*KanMX4 (mvp1Δ)*	This work
YFN293	*MAT*α *ade2-1 lys2-801 ura3-52 trp1Δ63 his3Δ200 leu2Δ1 ppr2*::*hisG-URA3-hisG YKR031c*::*KanMX4 (spo14 Δ)*	This work
SL21-3a	*MAT*α *rpb4-Δ*::*URA3(KI) ade2-1 lys2-801 ura3-52 trp1-d63 his3-Δ200 leu2-Δ1*	P. Thuriaux
YFN294	*MAT*α *rpb4-Δ*::*URA3(KI) ade2-1 lys2-801 ura3-52 trp1-d63 his3-Δ200 leu2-Δ1 YKR031c*::*KanMX4 (spo14 Δ)*	This work
YMR004w	*MAT*α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 YMR004w*::*KanMX4 (mvp1Δ)*	Euroscarf
Y06533	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 YMR004w*::*KanMX4(mvp1Δ)*	Euroscarf
Y16533	*MAT*α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 YKR031c*::*KanMX4 (spo14 Δ)*	Euroscarf
MSY465	*MAT*α ura3-52 leu2-Δ1 his3-Δ 200	Schmidt *et al.* (1999)
MSY467	*MAT*α *ura3-52 leu2-Δ1 his3-Δ 200 trp1Δ 63 std1*::*LEU2*	Schmidt *et al.* (1999)
AK152	*MAT*α *leu2 his3 ade2 trp1 ura3 can1-100 yra2Δ*::*TRP1*	Kashyap *et al.* (2005)
YSB517	*MAT*α *ura3-1 leu2,3-112 trp1-1 his3-11,15 ceg1-250 can1-100 ade3*::*hisG ade2-1*	Cho *et al.* (1997)
YF68	*MAT***a** *ura3-52 leu2-3,112 his3Δ200 rpb1Δ187*::*HIS3 pRP112* (*RPB1 URA3 CEN6 ARSX*)	Nonet *et al.* (1987)
YFN107	*MAT***a** *ura3Δ0 his3-Δ 200 leu2Δ0 trp1-Δ63 met2Δ0 rpb1-Δ187*::*HIS3 YMR004w*::*KanMX4 (Mvp1Δ)* pFL44-*RPB1* (2*μm URA3 RPB1*)	This work
CS41-4.3	*MAT*α *ura 3-52 leu2-3 his3-11 trp1-1 ade2-1 bur6*::*HIS3(pbur6-ts/CEN/LEU2)*	D. Reinberg
YFN187	*MAT*α *ura 3-52 leu2-3 his3-11 trp1-1 ade2-1 bur6*::*HIS3(pbur6-ts/CEN/LEU2) YMR004w*::*KanMX4 (mvp1Δ)*	This work
YFN194	*MAT*α *ura 3-52 leu2-3 his3-11 trp1-1 ade2-1 bur6*::*HIS3(pbur6-ts/CEN/LEU2) YKR031c*::*KanMX4 (spo14Δ)*	This work
GY236	*MAT*α *ura3-52 leu2Δ1 his 4-912d lys2-128d mot1-301*	Prelich (1997)
YFN185	*MAT***a** *ura3-52 leu2-Δ1 mot1-301 YMR004w*::*KanMX4 (mvp1Δ)*	This work
YFN186	*MAT***a** *leu2-3,112 his3-11,15 lys2-128d trp1-1 ura3-52 ade2 PLD1*::*Leu2 mot1-301*	This work
YFN231	*MAT*α *ura3-1 leu2,3-112 trp1-1 his3-11,15 ceg1-250 can1-100 ade3*::*hisG ade2-1 YKR031c*::*KanMX4 (spo14Δ)*	This work
YFN230	*MAT***a** *his3Δ1 leu2-3,11 ura3Δ0 trp1-1 YMR004w*::*KanMX4(mvp1Δ) ceg1-250 ade2-1*	This work
AB3	*MAT***a** *leu2-3,11 his3-11,15 ade2 trp1-1 ura3-1 can1-100 PLD1*::*Leu2*	A. L. Harkins
YFN226	*MAT***a** *his3-11,15 leu2-3,11 ura3Δ0 ade2 trp1 PLD1*::*Leu2 YER139c*::*kanMX4 (rtr1Δ)*	This work
YFN225	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 YMR004w*::*KanMX4 (mvp1Δ) YER139c*::*kanMX4 (rtr1Δ)*	This work

DNA amplification and array hybridization

Ligation-mediated PCR (LM-PCR) (Ren *et al.* 2000) was applied for DNA amplification and the PCR product labeled with ^{33}P-dCTP as described in Pelechano *et al.* (2009) with oligonucleotides Linker LE59 oJW102 and oJW103. Radioactive samples were hybridized onto macroarrays on which PCR products representing full-length ORFs for 6049 genes of *S. cerevisiae* were spotted (Ren *et al.* 2000) (Servei Central de Suport a la Investigació Experimental, Universitat de València, Spain).

Image analysis and data normalization

Image analysis and data normalization were undertaken as described (Pelechano *et al.* 2009). Images were quantified using the ArrayVision software 7.0 (Imaging Research). The signal intensity for each spot was the background subtracted ARM Density (artifact-removed median). Only enrichment values 1.35 times above the background were considered valid. Reproducibility of the replicates was checked using the ArrayStat software (Imaging Research). Normalization between conditions was performed by the global median method and the

Table 2 Plasmids

Name	Yeast markers and promoter	Origin
pGTB9- rpb1 (foot)	ORI (2 μm) *TRP1*	This work
pACT2- rpb1 (foot)	ORI (2 μm) *LEU2*	This work
pACT2-FRYL	ORI (2 μm) *LEU2*	Fromont-Racine *et al.* (1997)
pUC19 LytA kan	ORI (2 μm) *Kan*	S. Chávez (unpublished data)
Ycplac33	ORI (CEN) *URA3*	Morillo-Huesca *et al.* (2006)
pSCh202	ORI (CEN) *URA3*	Morillo-Huesca *et al.* (2006)
pSCh212	ORI (CEN) *URA3*	Morillo-Huesca *et al.* (2006)
pSCh209-*LAC4*	ORI (CEN) *URA3*	Morillo-Huesca *et al.* (2006)
pFL44L	ORI (2 μm) *URA3*	Bonneaud *et al.* (1991)
pFL44-*RPB1*	ORI (2 μm) *URA3*	Garcia-Lopez *et al.* (2010)
pRP101	ORI (CEN) *LEU2*	Nonet and Young (1989)
pRP103	ORI (CEN) *LEU2*	Nonet and Young (1989)
pRP104	ORI (CEN) *LEU2*	Nonet and Young (1989)

ratio between IP and whole cell extract (WCE) in each experiment was taken as the binding ratio. The functional analyses of the IP data were made using the Fatiscan application from Babelomics (Al-Shahrour *et al.* 2007). The genomic data are stored in Valencia Yeast (VYdBase; http://vydbase.uv.es/) and GEO databases (GSE16905).

Extraction of mRNA and reverse transcription

Total RNA from yeast cells and reverse transcribed RNAs were prepared as previously described (Garcia-Lopez *et al.* 2010).

Quantitative real-time PCR

To analyze gene expression, cDNA corresponding to 0.5 ng of total RNA was used. Each PCR reaction was performed at least three times, with three independent samples. The *18S rRNA* and the *ACT1* genes were used as the normalizers. The amplified PCR products were verified by agarose gel electrophoresis.

Homology search

Sequence alignments were based on a saturating homology search with the standard default Psi-Blast and Multalin (Corpet 1988; Schaffer *et al.* 2001); see http://blast.ncbi.nlm.nig.gov/ and http://bioinfo.genotoul.fr/multalin/multalin.html. In some cases, they were improved by visual inspection, based on the following amino acid conservations: AG, ST, CS, DN, DE, EQ, MILV, KR, and FWY.

Statistical data analysis

Samples were compared by the Student's *t*-test using the Statgraphics Plus program.

Results

Identification of conserved domains of the RNA polymerase II

To identify conserved domains of the RNA pol II potentially involved in the interaction with transcriptional regulators, we searched for regions located on the surface of the structure of the complex that were also conserved among different species, but with poor or no conservation in their paralogs in RNA polymerases I (Rpa190) and III (Rpc160) or in their homologs in archaea and bacteria.

Using PSI-Blast and Multalin (Corpet 1988; Schaffer *et al.* 2001), we carried out an amino acid sequence alignment between the largest subunit of the RNA pol II from *S. cerevisiae*, Rpb1, its orthologs in different eukaryotic species, its paralogs Rpa190 and Rpc160, and its homologs in archaea and bacteria (see García-López and Navarro 2011).

We identified a region of 163 amino acids (residues 881–1044 of *S. cerevisiae* Rpb1) conserved in all Rpb1 sequences (Figure 1A). This region, designated as the "conserved domain of the foot," corresponds to the helix α27–α34, which includes most of the domain previously called the foot (Cramer *et al.* 2001; García-López and Navarro 2011) (Figure 1B). Consistently, the Rpb1 foot is poorly conserved in the structure of the RNA polymerase III from *S. cerevisiae* (Fernandez-Tornero *et al.* 2007).

Interactions with the conserved domain of the foot of the RNA pol II

The conserved domain of the foot was fused to the $Gal4p_{BD}$ in the pGBT9 vector and introduced into the tester strain Y190, which has two reporter genes for two-hybrid interaction, *GAL1*::*lacZ* and *GAL(UAS)*::*HIS3*. As a control, we also fused the same region to the $Gal4p_{AD}$ in the pACT2 vector and introduced it in the tester strain Y187.

The fusion protein did not confer resistance to 50 mM 3AT, indicating that it did not operate as transcriptional activators of Pol II, and thus could be used in the screen. In addition, no β-galactosidase activity was observed with any of the negative controls used in the experiment (Figure 2A).

We tested 2.5 10^7 transformants (at 50 mM 3AT) with a similar efficiency (37%) to other previous screens performed with the same library (Flores *et al.* 1999). Five $3AT^R$ βGal^+ clones were obtained (Figure 2). One of the interacting preys was a domain spanning the last 79 amino acids of Mvp1 (413–511) and the second, 258 amino acids in the C terminus of Spo14 (residues 1304–1562; the protein is 1683 amino acids long).

Mvp1, a protein required for sorting proteins to the vacuole (Ekena and Stevens 1995), physically interacts with three

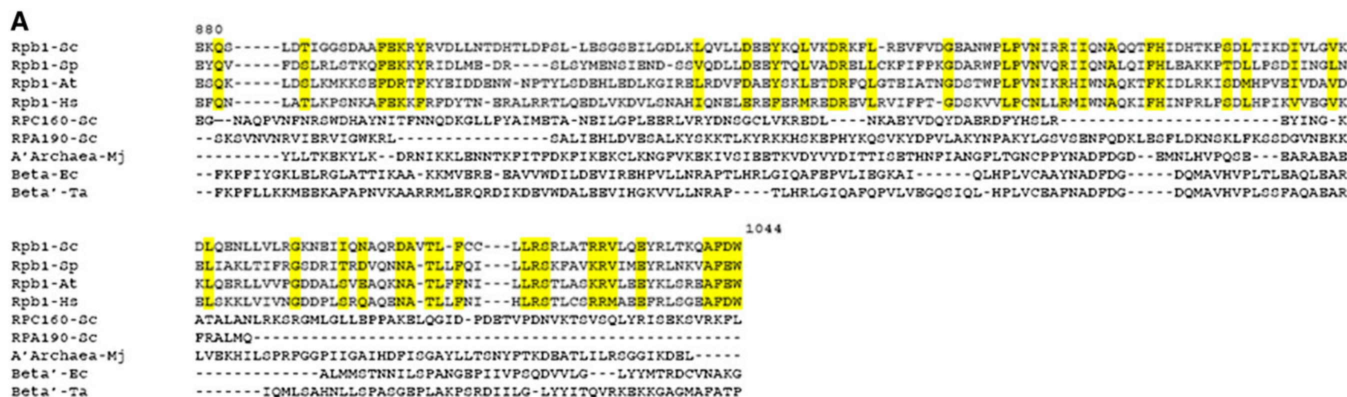


Figure 1 Identification of a domain of the foot as a conserved region of RNA pol II in eukaryotes. (A) Amino acid comparisons of Rpb1, Rpc160, Rpa190, and their homologs in archaea and bacteria. Amino acids were considered as conserved when they were present in at least half of the compared sequences. The following AG, ST, CS, DN, DE, EQ, MILV, KR, and FWY were grouped together. Highly conserved positions are shown in yellow. Species are indicated as follows: Sc (*Saccharomyces cerevisiae*), Sp (*Schizosaccharomyces pombe*), Hs (*Homo sapiens*), At (*Arabidopsis thaliana*), Mj (*Methanococcus jannaschii*), Ec (*Escherichia coli*), and Ta (*Thermus aquaticus*). The amino acid residue numbers indicated correspond to *S. cerevisiae* Rpb1 subunit. (B) Schematic view of the conserved region of the foot of the RNA pol II of *S. cerevisiae* on the structure of the RNA pol II. Blue and cyan: foot of RNA pol II where cyan corresponds to the conserved region of the foot. Magenta, Rpb5; green, Rpb8.

nuclear proteins, Std1, Yra2, and Srb7 (Schmidt *et al.* 1999; Hazbun *et al.* 2003; Vollert and Uetz 2004; Titz *et al.* 2006). Similarly, Spo14, a phospholipase D involved in Sec14p-independent secretion and required for meiosis and spore formation (Rudge *et al.* 2002; Nakanishi *et al.* 2006), physically interacts with Dcp2, (Fromont-Racine *et al.* 1997) and genetically interacts with the transcription factor Ste12 (Hairfield *et al.* 2001). The interactions of Mvp1 and Spo14 with the conserved domain of the foot might reveal connections between transcription and other aspects of the nuclear metabolism.

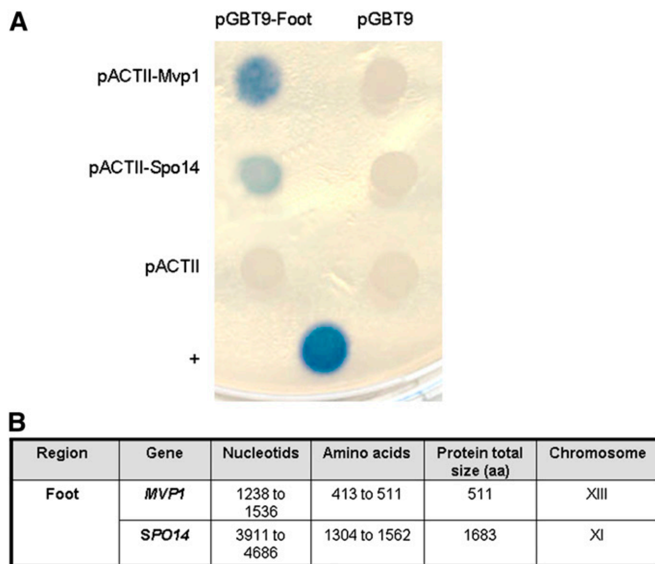
Mvp1 and Spo14 bind RNA pol II in vivo

Mvp1 and Spo14 were tagged at their C terminus by inserting a sequence encoding the C-LYTAG domain of the *lytA* gene from *Streptococcus pneumoniae* (~17 kDa). The addition of C-LYTAG tag to these nonessential proteins did not affect the growth of these strains.

Mvp1–C-LYTAG and Spo14–C-LYTAG were immunoprecipitated with anti–C-LYTAG antibody. In both cases, an Rpb1 reacting band was also revealed (Figure 3, right). No such band was observed when the IPs were performed in control strain MAY322 (Figure 3, right), indicating that Rpb1 does not interact with the C-LYTAG module and that no anti-Rpb1 reacting material was immunoprecipitated nonspecifically by anti–C-LYTAG antibody or adsorbed nonspecifically to the IgG magnetic beads. Similar results were found when C-LYTAG proteins were purified from Mvp1–C-LYTAG tagged and MAY322 strains by using a DEAE-cellulose matrix (data not shown). These observations suggest that interactions between RNA pol II and Mvp1 or Spo14 are specific.

Mvp1 and Spo14 localize in the nucleus of S. cerevisiae

We performed immunocytochemistry experiments with anti–C-LYTAG antibodies, using the Mvp1–C-LYTAG and Spo14–C-LYTAG version of the proteins. As shown in Figure 4, we detected mostly a cytosolic signal for both proteins. However, we also observed a nuclear staining. On the contrary, a control for C-LYTAG localization using the MAY322 strain showed only cytosolic signal (Figure 4), indicating that the nuclear localization of Mvp1 and Spo14 is not artifactual.



Region	Gene	Nucleotids	Amino acids	Protein total size (aa)	Chromosome
Foot	*MVP1*	1238 to 1536	413 to 511	511	XIII
	SPO14	3911 to 4686	1304 to 1562	1683	XI

Figure 2 Two-hybrid interactions between the conserved region of Rpb1 foot with Mvp1 and Spo14. (A) Conserved region of Rpb1 foot fused to $Gal4_{BD}$ in plasmid pGBT9 was tested against pACT2–Mvp1 and pACT2–Spo14. β-Galactosidase was tested in an overlay assay (Flores *et al.* 1999). +, positive control for interaction (Rpb5 and a region of Rpa190). (B) Summary of the interactions.

These data together indicate that fractions of Mvp1 and Spo14 are localized inside the *S. cerevisiae* nucleus, probably according to their physical interaction with RNA pol II.

Mvp1 and Spo14 associate only with RNA pol II genes and regulate expression of their targets

ChIP–chip experiments were performed with Mvp1–C-LYTAG and Spo14–C-LYTAG. The signals were ordered by intensity after normalizing the results with total DNA. The global IP results as well as the genes with higher IP enrichment are shown in Table S1. The control probes for RNA pol I and RNA pol III genes were among the lowest intense spots, meaning that both proteins do not bind those kinds of genes. Given the difficulty of establishing a threshold separating bound and unbound genes, we looked for enriched Gene Ontology categories using a scanning algorithm (Fatiscan; Al-Shahrour *et al.* 2007). Spo14 was enriched on ribosomal protein genes (Figure 5A), whereas Mvp1 was not associated with any particular GO category. By performing Q-PCR ChIP assays [using probes encompassing the 5′ regions of the genes (surrounding ATG) or inside the genes], we confirmed independently the association of Mvp1 or Spo14 with those genes most strongly enriched in the ChIP–chip experiments (*MBF1* and *RPS15* for Mvp1 and *DAL3* and *LSM8* for Spo14) (Figure 5B).

We also compared the binding level of Mvp1 and Spo14 with the dataset of nascent transcription rates (TRs) calculated by genomic run-on (Pelechano *et al.* 2010). As can be seen in Figure 6A, there is a significant positive relationship between gene binding and TR for both proteins.

As the association of Mvp1 and Spo14 to specific genes suggested that they may act as transcription factors, we next investigated whether these proteins could regulate the expression of their target genes. The deletion of *MVP1* and *SPO14* clearly altered the expression of *MBF1* and also of *RPS15*, *LSM8*, and *PMA1*, although to a lesser extent (Figure 6B). However, this is not a general effect on gene expression, as *PYK1* or *ACT1* expression (used as internal control; data not shown) remains similar to those observed in a wild-type strain.

Phenotypic and genetic analyses indicate that Mvp1 and Spo14 have a role in transcription

Because Mvp1 and Spo14 were identified as interactors with RNA pol II, we considered the possibility that these proteins are functionally linked to each other. Figure 7, A–C show the most relevant phenotypes for the mutants analyzed and Figure 7D the whole genetic analysis. Deletion of *MVP1* but not of *SPO14* shows a synthetic growth defect when combined with the *rpo21-4* mutation in the conserved domain of the foot (Figure 7, A and D). This genetic interaction is specific to this *rpb1* mutant, since no differences in growth were detected when Δ*mvp1* was combined with another *rpb1* or *rpb2* mutation.

In an attempt to clarify the role of Mvp1 and Spo14 in transcription, we explored whether these proteins participated in transcription elongation. As opposed to other *S. cerevisiae* strains that are defective for transcription elongation and often sensitive to 6-azauracil (6AU) and mycophenolic

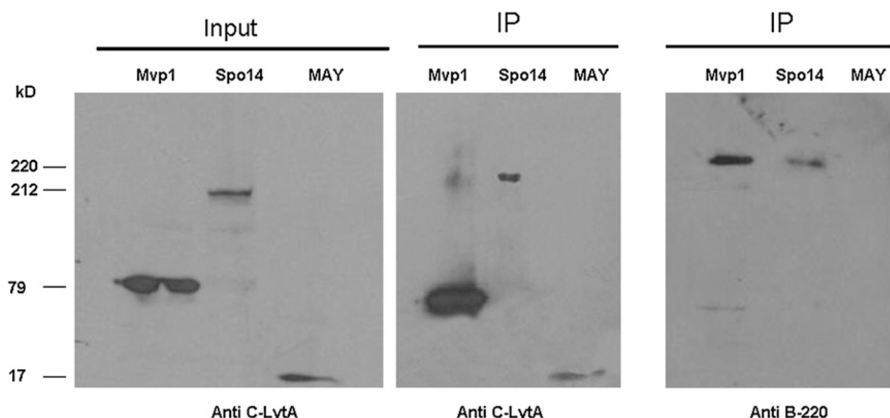


Figure 3 Mvp1 and Spo14 interact with Rpb1. Western blot of protein coinmunoprecipitation experiments from strains containing C-LYTAG tagged Mvp1 or Spo14. MAY, MAY322 strain was used as a control. Samples were immunoprecipitated with anti–C-LYTAG and then Western blotted with anti–C-LYTAG (center) or anti-Rpb1 (B220) antibodies (right). Input was Western blotted with anti–C-LYTAG antibodies (left).

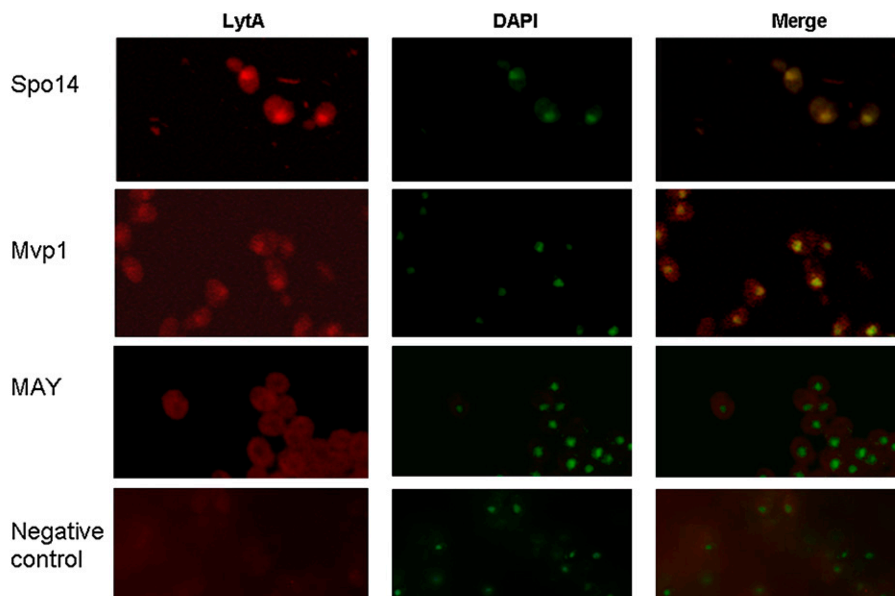


Figure 4 Mvp1 and Spo14 localize to the nuclei of *S. cerevisiae*. Immunolocalization of Mvp1 and Spo14 in *S. cerevisiae* strains expressing C-LYTAG tagged forms of the corresponding proteins, as well as in the control strain MAY322 (MAY). Anti–C-LYTAG primary and antirabbit IgG conjugated with Cy3 secondary antibodies were used. Nuclei were detected with DAPI (in green for better visualization). Negative control corresponds to a wild-type BY4741 strain.

acid (MPA) (Shaw *et al.* 2001; Garcia-Lopez *et al.* 2010), Δ*mvp1* and Δ*spo14* mutants were not sensitive to these drugs (see Figure 7B). In addition, the deletion of *MVP1* and *SPO14* does not affect mRNA biogenesis efficiency measured by GLAM, a method previously used as an indirect estimation of RNA pol II elongation (Morillo-Huesca *et al.* 2006). Although these negative results are not sufficiently strong to discard a relationship with transcription elongation, our data could suggest that these proteins are not involved in this process. However, the deletion of these genes corrected the growth and the sensitivity to 6AU and MPA of a Δ*dst1* mutant (for TFIIS elongation factor) affected in transcription elongation and initiation (Kim *et al.* 2007; Guglielmi *et al.* 2007; Ghavi-Helm *et al.* 2008) (Figure 7, A, B, and D).

Similarly, the deletion of *SPO14* suppressed the slow-growth phenotype and the MPA sensitivity of a mutant deleted for *RPB4*, a nonessential specific subunit of the RNA pol II participating in transcription initiation, elongation, or mRNA export (Choder 2004; Goler-Baron *et al.* 2008) (Figure 7, A, B, and D). *RPB4* and *DST1* genetically interact (Wery *et al.* 2004), as do *RBP9* and *DST1*, another RNA pol II nonessential subunit regulating transcription initiation and elongation. However, deletions of *MVP1* or *SPO14* did not alter the growth of cells lacking *RPB9*.

We also tested for conditional synthetic interactions between Δ*mvp1* and Δ*spo14* and mutations of the transcriptional initiation machinery. As shown in Figure 7, C and D, *MVP1* and *SPO14* genetically interact with *BUR6*, a component of the negative cofactor 2 (NC2) and *MOT1*, two conserved regulators of TATA-binding protein (TBP) function that cooperate to regulate gene expression on a global scale (Geisberg *et al.* 2001; Dasgupta *et al.* 2005; Masson *et al.* 2008; Van Werven *et al.* 2008). These data suggest that Mvp1 and Spo14 could be involved in any step of the transcriptional initiation or early elongation.

Mvp1 and Spo14 regulate transcription from HSP12 and HSP26 promoters

Considering the genetic interactions between *MVP1*, *SPO14*, and elements of the transcription initiation machinery, we examined the transcriptional activity of the *HSP12* and *HSP26* promoters, that are regulated by NC2 and/or Mot1, respectively (Creton *et al.* 2002; Dasgupta *et al.* 2005; Peiro-Chova and Estruch 2007; Masson *et al.* 2008). Deletion of *MVP1* and *SPO14* decreased *HSP12* and *HSP26* mRNA accumulation by ~50% at 30° (Figure 8A) and significantly altered the induction levels of *HSP12* and *HSP26* promoters when cells were shifted to 37° for 30 min (Figure 8B). This effect is not a general consequence of the deletion of *MVP1* and *SPO14* on RNA pol II activity. Moreover, inducible gene expression of *GAL1* (not shown), or the constitutive expression of *PYK1* and *ACT1* genes (see above) were not altered. It is possible that *GAL1* is not a target for Mvp1 and Spo14, in concordance with the fact that we did not observed association of these proteins with *GAL1* promoter or coding region (data not shown).

Abnormal CTD Ser5P phosphorylation caused by Mvp1 and Spo14 inactivation

Our data account for a connection between Mvp1 and Spo14 and the transcription initiation or early elongation. The Rpb1 CTD is predominantly phosphorylated on serine 5 (Ser5) during promoter scape and early elongation (Komarnitsky *et al.* 2000; Gu *et al.* 2010; Mayer *et al.* 2010). Then we tested whether the deletion of *MVP1* and *SPO14* would have an effect on Ser5 CTD phosphorylation. We performed ChIP on wild-type, Δ*mvp1*, and Δ*spo14* cells using antibodies, which recognize unphosphorylated CTD, Ser5 phosphorylated, and Ser2 phosphorylated CTD. As shown in Figure 9A, Ser5P crosslinking increased at promoters of *MBF1*, *RPS15*, *PMA1*, *HSP26*, and *HSP12* genes in both mutants, while

A

Identifier and biological process	*P* value
GO:0005840: ribosome……………………………………………………..	2,46E-20
GO:0044445: cytosolic part……………………………………………...	2,46E-20
GO:0003735: structural constituent of ribosome………………………..	2,51E-19
GO:0015934: large ribosomal subunit ………………………………….	9,19E-16
GO:0006412: translation ………………………………………………...	1,46E-13
GO:0030529: ribonucleoprotein complex………………………………	1,49E-11
GO:0009059: macromolecule biosynthetic process ……………………	3,26E-10
GO:0005829: cytosol ……………………………………………………	5,05E-09
GO:0044249: cellular biosynthetic process ……………………………..	3,12E-08
GO:0016283: eukaryotic 48S initiation complex………………………..	5,40E-08
GO:0043228: non-membrane-bound organelle…………………………..	1,29E-07
GO:0043232: intracellular non-membrane-bound organelle ……………	1,29E-07
GO:0009058: biosynthetic process ……………………………………...	2,11E-07
GO:0016282: eukaryotic 43S preinitiation complex ……………………	5,91E-07

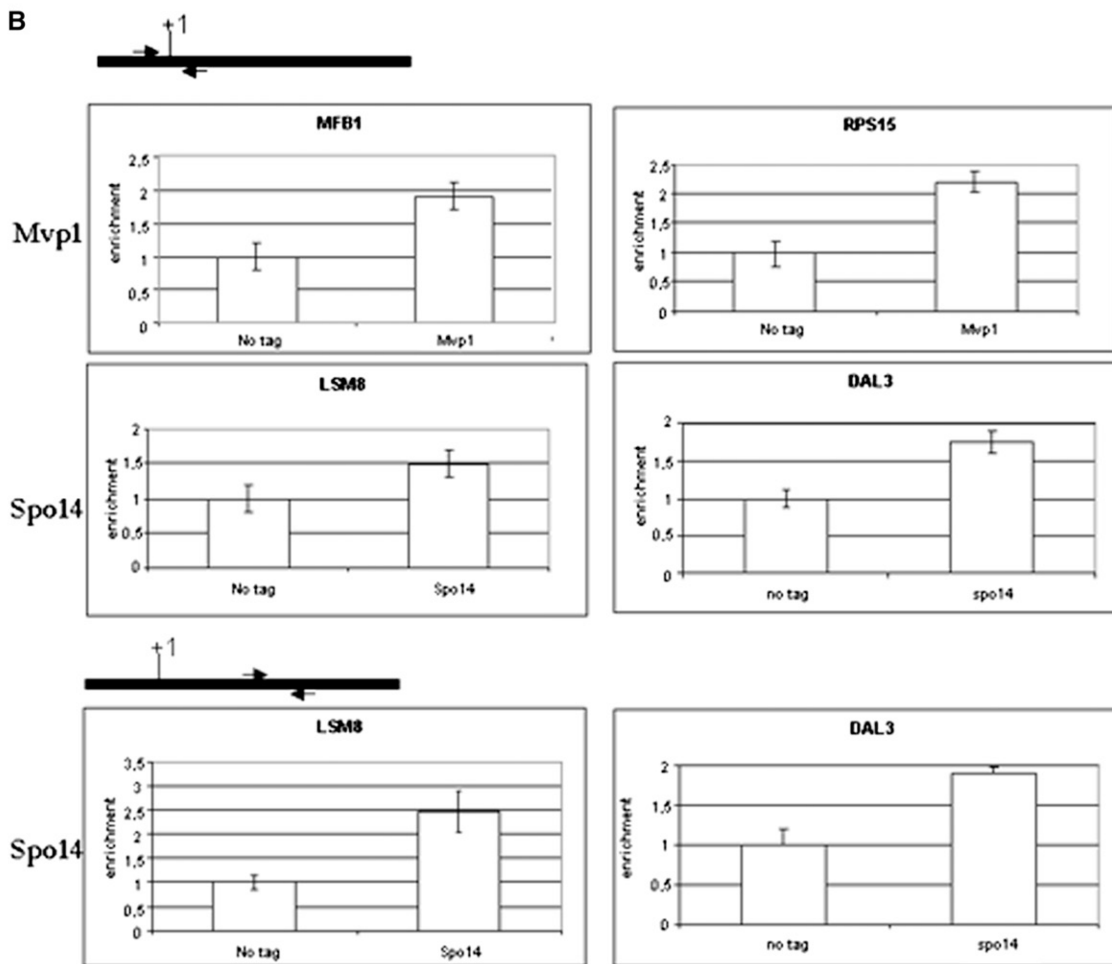


Figure 5 Mvp1 and Spo14 bind RNA pol II-transcribed genes in *S. cerevisiae*. (A) Summary of GO categories found with the Fatiscan algorithm enriched in ChIP–chip experiments performed with Spo14–LYTAG. (B) Q-PCR ChIP with samples from either the isogenic wild-type strain BY4741 (not tag) or Mvp1–LYTAG and Spo14–LYTAG cells. *MBF1* an *RPS15* for Mvp1 binding and *DAL3* and *LSM8* for Spo14 binding were analyzed. The fold enrichment of the indicated gene ChIP samples relative to WCE samples is plotted. Arrows show the position of the oligonucleotides used relative to each gene. +1 corresponds to the first nucleotide of each ORF.

no differences with respect to the wild-type strain was noted for the *PYK1* gene, which was not affected by the deletion of *MVP1* or *SPO14*. In addition, this phenomenon is specific for CTD Ser5P in the promoter region, since no significant differences for CTD Ser5P were generally observed at the 3′ region, nor for CTD Ser2P (Figure 9, B and C). These results also clearly agree with the genetic interactions found between Δ*mvp1* or Δ*spo14* strains and the Δ*rtr1* mutant

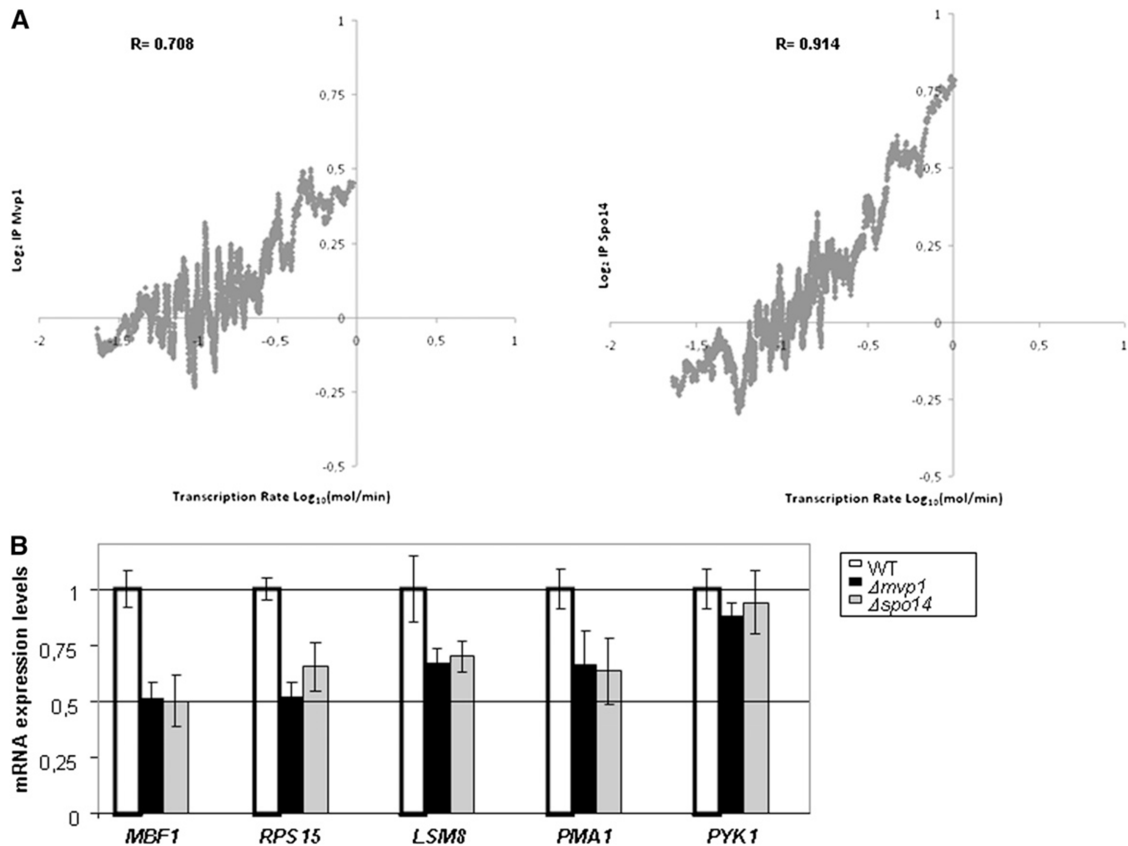


Figure 6 Transcriptional analysis. (A) Relationship between the presence of Mvp1 and Spo14 (in Log_2 of the immunoprecipitation enrichment) and the nascent transcription rate (in Log_{10} mRNA molecules/min) (Pelechano *et al.* 2010). All curves represent the smoothness of the data of IP using the averages values for a sliding window of 100 genes. The population median value for fold change of immunoprecipitate sample *vs.* whole cell extract has been arbitrarily set to 0. The data represent the merged values of two independent biological replicates. The Pearson correlation for the smoothed data is shown. (B) Quantitative RT–PCR analysis of mRNA levels for *MBF1*, *RPS15*, *LSM8*, *PMA1*, and *PYK1* in Δ*mvp1* and Δ*spo14* mutants and in the isogenic wild-type strain BY4741. Each PCR reaction was performed three times to make a representative average with two or three different samples. *18S* rRNA and *ACT1* were used as normalizers.

deleted for the gene coding for the recently described Ser5 CTD phosphatase Rtr1 (Mosley *et al.* 2009) (Figures 7D and 9D).

Ser5 phosphorylation occurs first in CTD in coordinated recruitment of the guanylyl-transferase subunit of the *S. cerevisiae* mRNA-capping enzyme (Ceg1) (Gu *et al.* 2010). As expected, *MVP1* and *SPO14* genetically interact with *CEG1* (Figure 9D). Furthermore *MVP1* deletion did not alter the growth of truncated mutants of the CTD (see Figure 7D), although these mutations are lethal when combined with a *ceg1* mutant (Cho *et al.* 1997).

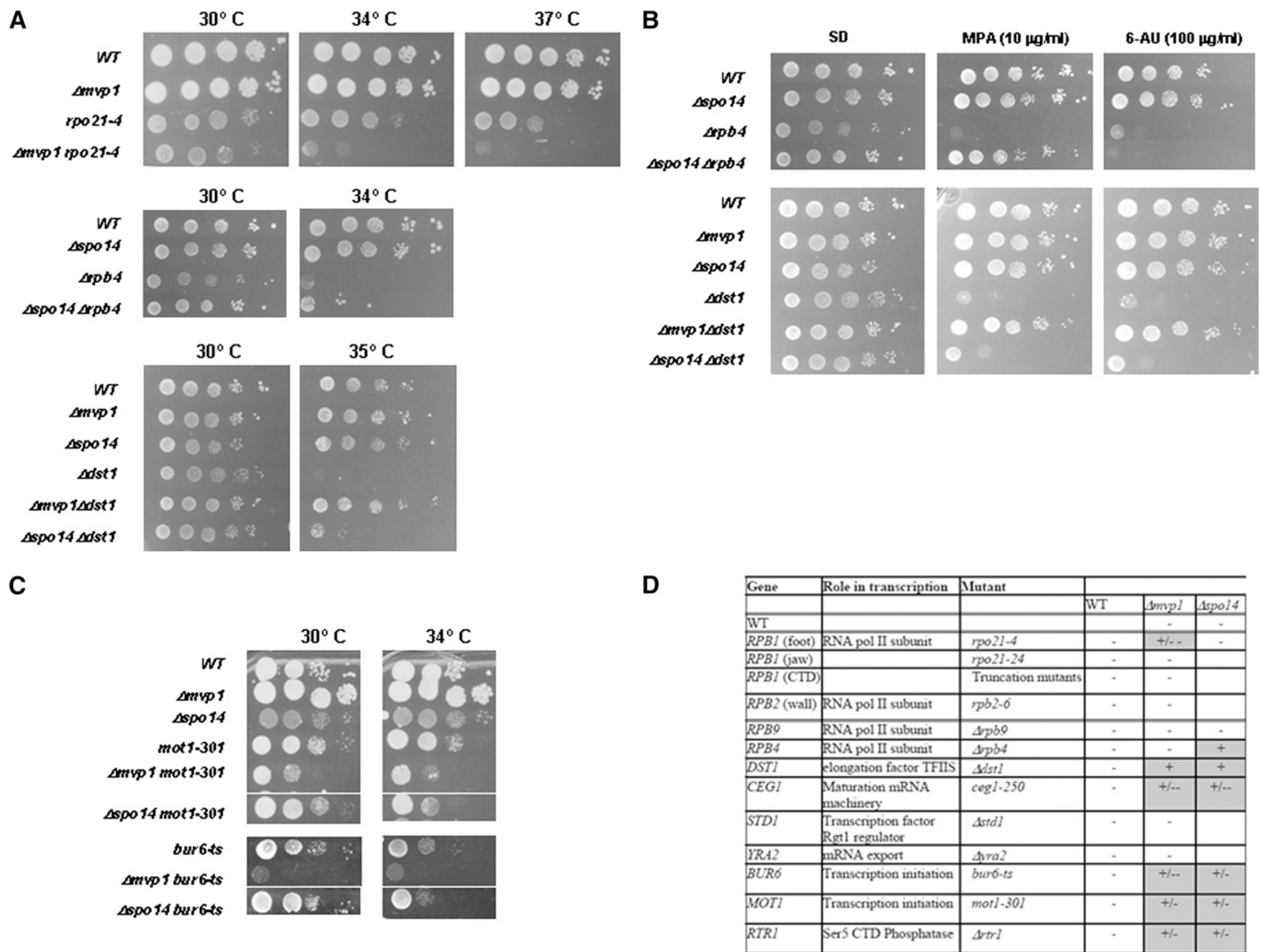
Discussion

In this work, we looked for conserved regions on the surface of the RNA pol II of *S. cerevisiae*, hypothesizing that they contact transcriptional regulators. We identified a domain at the foot of RNA pol II and demonstrated its interaction with Mvp1 and Spo14. Our study provides physical, genetic, and functional evidence that Mvp1 and Spo14 are associated with the transcriptional machinery and participate in transcription initiation and/or early elongation.

Mvp1 and Spo14 physically interact with the conserved domain of the foot of RNA pol II and localize in the nucleus

We identified a region of 163 amino acids (residues 881–1044 of *S. cerevisiae* Rpb1) with a significant conservation (28%) in all Rpb1 subunits, from yeast to human, but with low or no conservation in Rpb1 paralogs and in their homologs in archaea and bacteria. This region, designated as the conserved domain of the foot, corresponded to the majority of the RNA pol II "foot," which in cooperation with the "lower jaw," the "assembly" domain, and the "cleft" regions, constitute the "shelf" module of the RNA pol II that might contribute to the rotation of the DNA as it advances toward the active center (Cramer *et al.* 2001; Zaros *et al.* 2007). Consistently, the foot is poorly conserved in the structure of the RNA polymerase III from *S. cerevisiae* (Fernandez-Tornero *et al.* 2007). In accordance, Suh and coworkers have also defined the foot of the RNA pol II as an RNA pol II conserved domain (Suh *et al.* 2010).

We identified interactions between the conserved domain of the foot and Mvp1 and Spo14. It is important to note that



Gene	Role in transcription	Mutant			
			WT	*Δmvp1*	*Δspo14*
WT				-	-
RPB1 (foot)	RNA pol II subunit	*rpo21-4*	-	+/- -	-
RPB1 (jaw)		*rpo21-24*	-	-	
RPB1 (CTD)		Truncation mutants	-	-	
RPB2 (wall)	RNA pol II subunit	*rpb2-6*	-	-	
RPB9	RNA pol II subunit	*Δrpb9*	-	-	-
RPB4	RNA pol II subunit	*Δrpb4*	-	-	+
DST1	elongation factor TFIIS	*Δdst1*	-	+	+
CEG1	Maturation mRNA machinery	*ceg1-250*	-	+/--	+/--
STD1	Transcription factor Rgt1 regulator	*Δstd1*	-	-	
YRA2	mRNA export	*Δyra2*	-	-	
BUR6	Transcription initiation	*bur6-ts*	-	+/--	+/-
MOT1	Transcription initiation	*mot1-301*	-	+/-	+/-
RTR1	Ser5 CTD Phosphatase	*Δrtr1*	-	+/-	+/-

Figure 7 Δ*mvp1* and Δ*spo14* mutants synthetically interact with components of the transcription machinery. (A and C) Growth of single and double mutants at different temperatures. (B) Growth of single and double mutants at 30° in media containing mycophenolic acid (MPA) or 6-azauracil (6AU). (D) Summary of the genetic interactions between *MVP1*, *SPO14*, and components of the transcription machinery. Shading represents synthetic interactions where + indicates suppression and +/− and +/−− growth slightly or strongly aggravated. −, no synthetic interaction; foot, jaw, wall, and CTD are the different domains of RNA pol II where mutations are located.

these interactions have never before been found in other genomic screens. Interestingly, Suh and coworkers have recently demonstrated that the foot of the RNA pol II contacts the RNA CE in *S. cerevisiae* (Suh *et al.* 2010).

Mvp1, a protein required for vacuolar protein sorting in yeast (Ekena and Stevens 1995) and Spo14, a phospholipase D (Rudge *et al.* 2002; Nakanishi *et al.* 2006), have never been reported before as proteins participating in transcription. However, physical interactions have been observed between Mvp1 and three nuclear proteins, Std1, Yra2, and Srb7 (Schmidt *et al.* 1999; Hazbun *et al.* 2003; Vollert and Uetz 2004; Titz *et al.* 2006). Similarly, Spo14 physically interacts with Dcp2 (Fromont-Racine *et al.* 1997) and genetically with the transcription factor Ste12 (Hairfield *et al.* 2001).

Curiously, Mvp1 and Spo14 are two of 15 proteins containing phox homology (PX) domain in yeast, which is involved in protein–protein interactions (Sato *et al.* 2001; Vollert and Uetz 2004). However, the regions of Mvp1 and Spo14 in contact with Rpb1 do not contain the PX domains. In addition, no homology between the regions of interaction was detected, thus ruling out the possibility of unspecific interactions due to a similar protein domain in both Mvp1 and Spo14 peptides. In addition, in no case were interactions between these 15 PX domain proteins and Rpb1 observed (Vollert and Uetz 2004).

In agreement with the interactions of Mvp1 and Spo14 with the RNA pol II, we observed that a fraction of Mvp1 and Spo14 localized in the nucleus of *S. cerevisiae*, although previous data have indicated a cytoplasmic localization of both proteins (Ekena and Stevens 1995; Rudge *et al.* 1998, 2001). Moreover, the GFP-tagged yeast strain collection show that these proteins give cytoplasmic signals (Huh *et al.* 2003). Hence, it is possible that the tag we used (C-Lytag from *S. pneumoniae*) gave a better and clearer signal, allowing us to detect Mvp1 and Spo14 proteins in the nucleus. This nuclear localization seems not to be artifactual,

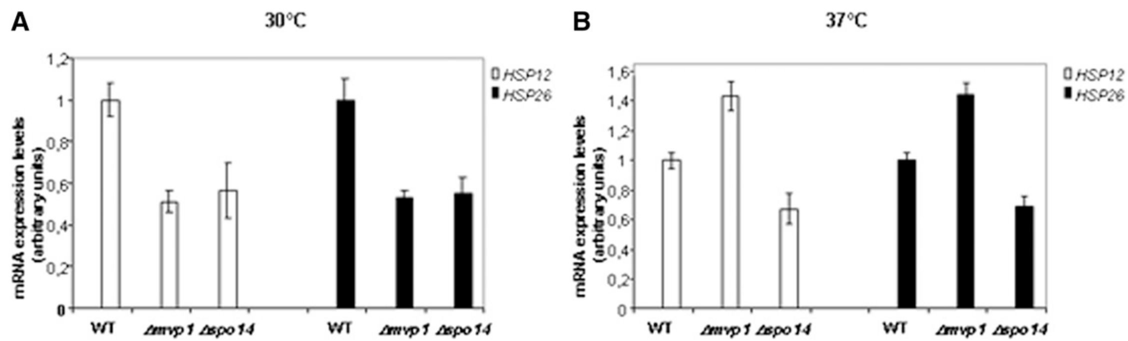


Figure 8 Deletion of *MVP1* or *SPO14* affects *HSP12* and *HSP26* expression levels. Quantitative RT–PCR analysis of mRNA levels for *HSP12* and *HSP26* in mutant strain Δ*mvp1*, Δ*spo14*, and the isogenic wild-type strain BY4741 at 30° (A) and under a shift to 37° for 30 min (B). Four independent experiments were performed and each PCR reaction was carried out three times to provide a representative average. *18S* rRNA and *ACT1* were used as normalizers.

since a control for C-LYTAG localization using the MAY322 strain in which the C-LYTAG tag is expressed under the control of the *NHP6A* promoter, shows only cytosolic signal. In addition, the C-LYTAG domain does not contain any NLS or NES motifs, as revealed by using different prediction servers. These data also agree with the interactions of Mvp1 and Spo14 with proteins of the transcriptional machinery (see above). Other proteins that participate in transcription have been also shown to shuttle between the cytoplasm and nucleus in yeast or mammalian cells, such as Rpb4, RMP, or Iwr1, among others (Delgermaa *et al.* 2004; Peiro-Chova and Estruch 2009; Harel-Sharvit *et al.* 2010) or even the two Mvp1 interactors Yra2 and Std1 (Vollert and Uetz 2004).

Mvp1 and Spo14 associate with RNA pol II genes and affect gene expression of their target genes

Mvp1 and Spo14 associate with the DNA *in vivo*, as shown in our ChIp–chip analysis, probably through their interactions with RNA pol II. This occupancy is observed only in RNA pol II genes and, while Spo14 is selectively bound to ribosomal protein genes, Mvp1 does not bind any particular GO category. The fact that only a small fraction of these proteins is detected in the nucleus and the possibility that Mvp1 and Spo14 could associate only transiently with Rpb1 *in vivo* could account for the low levels of these two proteins detected by ChIP, as observed for other proteins known to play important roles in transcription, such as Mediator (Andrau *et al.* 2006; Fan *et al.* 2006; Zhu *et al.* 2006; Soutourina *et al.* 2011). According to these data, the deletion of *MVP1* and *SPO14* affect mRNA accumulation of some of their target genes, although this is not a general feature, so that the expression of other genes such as *PYK1* or *ACT1* is not altered.

Phenotypic and genetic analyses suggest a role for Mvp1 and Spo14 in any step of transcription initiation and/or early elongation

The genetic interactions with *BUR6*, a component of the negative cofactor 2 (NC2) and *MOT1*, two conserved regulators of TBP function that cooperate to regulate gene expression on a global scale (Van Werven *et al.* 2008), suggest a link between these two proteins and the transcription initiation machinery. These data also agree with the fact that deletion of *MVP1* and *SPO14* affects the transcription of *HSP12* and *HSP26* genes regulated by NC2 and Mot1 (Creton *et al.* 2002; Dasgupta *et al.* 2005; Peiro-Chova and Estruch 2007; Masson *et al.* 2008).

In addition, both *MVP1* and *SPO14* genetically interact with *DST1* (TFIIS) and RPB4, two transcriptional elements that also interact genetically (Wery *et al.* 2004) and that have been shown to participate in transcription initiation (Armache *et al.* 2003; Choder 2004; Guglielmi *et al.* 2007; Kim *et al.* 2007; Goler-Baron *et al.* 2008; Ghavi-Helm *et al.* 2008; Garcia-Lopez *et al.* 2010). All these data together and the fact that *MVP1* and *SPO14* disruptions do not affect transcription elongation, nor CTD Ser2P, which is associated mainly with elongating RNAPII (Kim *et al.* 2009; Garcia *et al.* 2010), argue for a connection between Mvp1 and Spo14 with the transcription initiation machinery, although we cannot rule out a role in early elongation.

Both possibilities are also supported by our data showing that deletion of *MVP1* and *SPO14* specifically increase CTD Ser5P phosphorylation, and by the genetic interactions between *MVP1*, *SPO14*, and *RTR1*, the gene coding for the recently reported CTD Ser5P phosphatase Rtr1 required for the Ser5-to-Ser2P transition (Mosley *et al.* 2009). In fact, RNA pol II with CTD Ser5P is generally found in the 5′ region of genes at transcription initiation (Kim *et al.* 2009), but also during promoter escape and early elongation (Mayer *et al.* 2010).

CTD Ser5P phosphorylation achieved by Kin28 mediates cotranscriptional recruitment of Ceg1 (Gu *et al.* 2010). In accordance, *MVP1* and, *SPO14* genetically interact with *CEG1*. Curiously, the foot of the RNA pol II contacts the RNA CE in *S. cerevisiae* (Suh *et al.* 2010). In addition, the mRNA cap-binding complex stimulates the formation of PIC via its interaction with Mot1 *in vivo* (Lahudkar *et al.* 2010). It bears noting that the human homolog of Rtr1, RPAP2, has been found as part of the RNA pol II assembly intermediates (Boulon *et al.* 2010). Consequently, we cannot rule out the possibility that the genetic interactions between *MVP1*, *SPO14*, and *RTR1* could be associated with a defect in

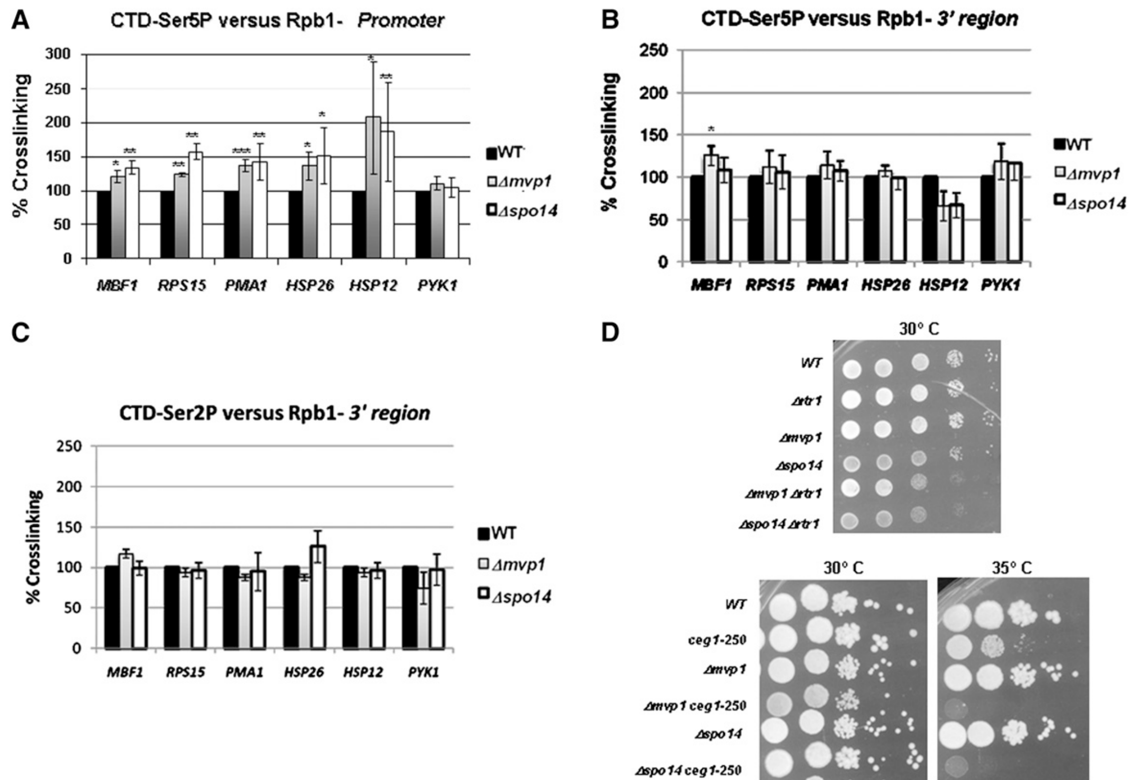


Figure 9 Deletion of *MVP1* or *SPO14* affects the amount of phosphorylated RNA pol II *in vivo*. ChIP analysis of Rpb1, CTD Ser5P, and CTD Ser2P was performed in wild-type (WT) and Δ*mvp1* and Δ*spo14*. Binding to promoter or 5′ region (A) and 3′ region (B and C) of *MBF1*, *RPS15*, *PMA1*, *HSP26*, *HSP12*, and *PYK1* genes were analyzed by quantitative RT–PCR. Numbers on the *Y*-axis represent the percentage of Rpb1 and Rpb1–CTDSer5P or Rpb1–CTDSer2P cross-linked to the DNA region in Δ*mvp1* and Δ*spo14* cells relative to WT cells, where cross-linking is considered 100%. (D) Analysis of genetic interactions between Δ*mvp1*, Δ*spo14*, and Δ*rtr1* mutants (top) or Δ*mvp1*, Δ*spo14*, *and ceg1-250* mutants (bottom). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

RNA pol II assembly, although this seems unlikely since the deletion of *MVP1* and *SPO14* do not affect the amount of Rpb1 (data not shown).

In conclusion, all together these data constitute the first experimental evidence pointing to a role of Mvp1 and Spo14 in transcription initiation and/or early elongation. In any case, we cannot rule out that Mvp1 and Spo14 could modulate or allow the access of the Ser5 kinase or Ser5 phosphatases to the transcription complex, as is the case for Abd1 (a capping enzyme in budding yeast) for which inactivation causes a defect in promoter clearance and/or early elongation, which correlates with failure to dephosphorylate Ser5 residues normally (Schroeder *et al.* 2004). In addition, we cannot dismiss the possibility that the genetic interaction observed between *MVP1*, *SPO14*, and *DST1* could be related to the fact that TFIIS binds adjacent to the foot domain in the RNA Pol II–TFIIS complex crystal structure (Kettenberger *et al.* 2003). Finally, we cannot rule out the possibility that Mvp1 and/or Spo14 could participate also in other steps of transcription.

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The Conserved Foot Domain of RNA Pol II Associates with Proteins Involved in Transcriptional Initiation and/or Early Elongation

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Table S1   Genome-wide localization data for Mvp1 and Spo14.

Data prepared for the immunoprecipitation experiments in Log2 fold change (immunoprecipitate / whole-cell extract) for the two independent biological replicates.

Table S1 is available for download at http://www.genetics.org/content/suppl/2011/09/27/genetics.111.133215.DC1 as an Excel file.

Table S2 Oligonucleotides

The primers used were as follows (all 5’-3’):

242		CTATTCGATGATGAAGATACCCCACC
244		CAGTTGAAGTGAACTTGCG
Rpb1	504	CCCGGATCCGCCAATCGTAG
	307	CCCCTGCAGCCAATCGAATGC
Rpb1	504	CCCGGATCCGCCAATCGTAG
	308	CCCCTCGAGCCAATCGAATGC
MVP1lyt	501	GAAAAGCTCGTCACAAGCATCATGGATATGCCGATTTCTCGTGAAGACATGATGGGCATTAGC
	301	ATATGGGAACCTATAATATCCGAATTAGGCTTCATACACTGTATTTCTTCGCTATTACGCCAG
SPO14lyt	501	TGGATTTTTAACTCGGATAGACTTTCTCCAATGGAAATCTACAATGACATGATGGGCATTAGC
	301	ATAAAGCACATAAATTACATAATGATATTATTATTATATATATTTTCTTCGCTATTACGCCAG
Mvp1	501	GAACTCCAACCATGCCGGTC
	301	TCTTCGCTATTACGCCAG
Mvp1	502	CCAGATCATCTCGATATTGCCG
	302	CCGAATTAGGCTTCATACACTG
Spo14	501	AACCGAATGGTTAGCTAAAG
	301	TCTTCGCTATTACGCCAG
Spo14	502	CTCGTTATCAACGACAAACGGG
	302	ATTTGGCATCGACTTATCCAAG
Linker LE59	oJW102	GCGGTGACCCGGGAGATCTGAATTC
	oJW103	GAATTCAGATC
MBF1	501	TTAGGTAGCTGGAAAGGCGC
	301	GTTGTCACCCCTCGTATTGG
MBF1	502	CGTGCCAGAACAGACAAGAA
	302	GCGAACCGATGTTGTTACCT
RPS15	501	GCAGAGTTTCACATTATGGTATGG
	301	TAGCTGGGGCCAACTTGAC
RPS15	502	GGTTCCGTCGTCGGTATCTA
	302	TGGGATGAAACGGGAAGTAG
LSM8	501	CCTTGTTTTGCTTGTTTTCC
	301	AGTCTTTCAAGGTGGCTGAC
LSM8	502	AATCAAAGTTGACGGCGAAT
	302	CTGCATCTATGAGGCCAACA
DAL3	501	TATGCGGTCTATTCATGTGC
	301	GGTTTTGCATCCTTGAAATC
DAL3	503	AACGTCAGCGGAAGTAGCAT
	303	CCTGTCAGGGTCCAGACTTT
HSP12	501	CTTCCAAGGTGTCCACGACT
	301	TTGGTTGGGTCTTCTTCACC

HSP12	502	CGCAGGTAGAAAAGGATTCG
	303	CACCTTGGAAGACACCCTTG
HSP26	501	AAGTCGTGGTTCCTGGTGTC
	301	TGTTGTCTGCATCCACACCT
HSP26p	503	CAACGGTTCTTTTTCACCCTTA
	303	CTTCGTTGTTGATGTTGTCAAA
PMA1-P	forw	GGTACCGCTTATGCTCCCCTCCAT
	rev	ATTTTTTTTCTTTCTTTTGAATGTGTG
PMA1-6	forw	ATATTGTTACTGTCGTCCGTGTCTGGAT
	rev	ATTAGGTTTCCTTTTCGTGTTGAGTAGA
ACT1	501	GCCTTCTACGTTTCCATCCA
	301	GGCCAAATCGATTCTCAAAA
PYK1-P	forw	GAATGCTTGTGATGTCT TCCAAGT
	rev	TGATTGGTGTCTTGTAAATAGAAACA
PYK1-4	forw	CTATGGCTGAAACCGCTGTCATTG
	rev	CAGCTCTTGGGCATCTGGTAAC
GAL1	501	TGGTGTTAACAATGGCGGTA
	301	GGGCGGTTTCAAACTTGTTA
18S	501	CATGGCCGTTCTTAGTTGGT
	301	ATTGCCTCAAACTTCCATCG
Intergenic VII-1	forw	CCCACCACCGATAACGACAAG
Intergenic VII-2	rev	CCAACAAATGAGGCGGAACC