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Tye7 regulates yeast Ty1 retrotransposon sense and antisense transcription in response to adenylic nucleotides stress

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ABSTRACT

Transposable elements play a fundamental role in genome evolution. It is proposed that their mobility, activated under stress, induces mutations that could confer advantages to the host organism. Transcription of the Ty1 LTR-retrotransposon of *Saccharomyces cerevisiae* is activated in response to a severe deficiency in adenylic nucleotides. Here, we show that Ty2 and Ty3 are also stimulated under these stress conditions, revealing the simultaneous activation of three active Ty retrotransposon families. We demonstrate that Ty1 activation in response to adenylic nucleotide depletion requires the DNA-binding transcription factor Tye7. Ty1 is transcribed in both sense and antisense directions. We identify three Tye7 potential binding sites in the region of Ty1 DNA sequence where antisense transcription starts. We show that Tye7 binds to Ty1 DNA and regulates Ty1 antisense transcription. Altogether, our data suggest that, in response to adenylic nucleotide reduction, *TYE7* is induced and activates Ty1 mRNA transcription, possibly by controlling Ty1 antisense transcription. We also provide the first evidence that Ty1 antisense transcription can be regulated by environmental stress conditions, pointing to a new level of control of Ty1 activity by stress, as Ty1 antisense RNAs play an important role in regulating Ty1 mobility at both the transcriptional and post-transcriptional stages.

INTRODUCTION

Transposable elements constitute a large fraction of eukaryotic genomes (nearly half of the human genome, up to 85% of plant genomes and 3% of the compact genome of the yeast *Saccharomyces cerevisiae* as examples). Once seen as simple genomic parasites with potential mutagenic effects, they are currently believed to play a fundamental role in shaping genomes and triggering genetic innovations (1,2). Activation of transposable elements in response to stress conditions has been reported in a wide range of organisms (3–5) and has been proposed to promote genetic variability that could help the cell to adapt to environmental changes (6). Stress conditions generally stimulate transcription of the element, which is the first step of the transposition cycle, as shown with Tnt1A and TLC-1 in Solanaceae (3,7), Mutator in Maize (8,9), Tf2 in *Schizosaccharomyces Pombe* (10) and Ty1 in *S. cerevisiae* (11–18). Generally, this process involves regulatory sequences located in the promoter region of transposable elements, which are similar to the well-characterized motifs required for the

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induction of stress-responsive genes (3,4). Five Long Terminal Repeat (LTR)-retrotransposon families (Ty1–Ty5) reside in the genome of *S. cerevisiae* (19). They share the same basic structure, which consists of two direct LTRs and two open reading frames (ORFs), *TYA* and *TYB*, analogs of the retroviral *gag* and *pol* genes. They transpose through an RNA intermediate that is reverse-transcribed following encapsidation into a virus-like particle (VLP). Thereafter, the resulting cDNA copy is integrated into the yeast genome. With ~30 full-length copies per haploid genome, the Ty1 family is responsible for most of the mutagenic events associated with Ty elements (4). Different environmental stresses such as ionizing radiation, DNA damage and nutrient starvation activate Ty1 transcription and retrotransposition (11–18).

The full Ty1 promoter extends over 1 kb, both upstream and downstream of two TATA boxes, and includes the 5′ LTR and part of the *TYA* ORF (Figure 1A). Several transcription factors bind to the Ty1 promoter to regulate Ty1 transcription (4). The DNA-binding transcription factor Tye7, was originally identified as a multicopy activator of Ty1-adjacent gene transcription (20). More recently, *TYE7* was shown to be necessary for the up-regulation of Ty1 transcription in yeast cells lacking the adenylate kinase Adk1 (21). *TYE7* also contributes to the activation of several glycolytic genes (22–24).

In addition to Ty1 mRNA, Ty1 transcription produces antisense non-coding RNAs (25,26) whose transcription starts in *TYA* and encompasses Ty1 promoter sequences (Figure 1A: Ty1-AS RNAs). Transcription of non-coding sequences plays an important role in the regulation of gene expression (27). In *S. cerevisiae*, there are several examples of non-coding RNAs, or of their transcription, regulating the expression of genes in response to nutrient deprivation, such as the *IMD2* and *URA2* genes of the GMP and UMP biosynthesis pathways, respectively (28–30), the phosphate responsive *PHO84* and *PHO5* genes (31,32), the serine biosynthesis *SER3* gene (33) and the galactose-inducible *GAL1-GAL10* locus (34,35). Non-coding RNAs can either be stable (36) or be rapidly degraded by the nuclear exosome or the cytoplasmic Xrn1 5′–3′ exoribonuclease (referred as Cryptic Unstable Transcripts, CUTs and Xrn1-sensitive Unstable Transcripts, XUTs, respectively) (37–40). Ty1-AS RNAs (also named Ty1-RTL), which are stabilized in the absence of Xrn1 (25,41,42), repress Ty1 transcription by a trans-silencing mechanism requiring Set1-dependent histone H3 methylation (25). Ty1-AS RNAs are also detected in VLPs, where they interfere with the

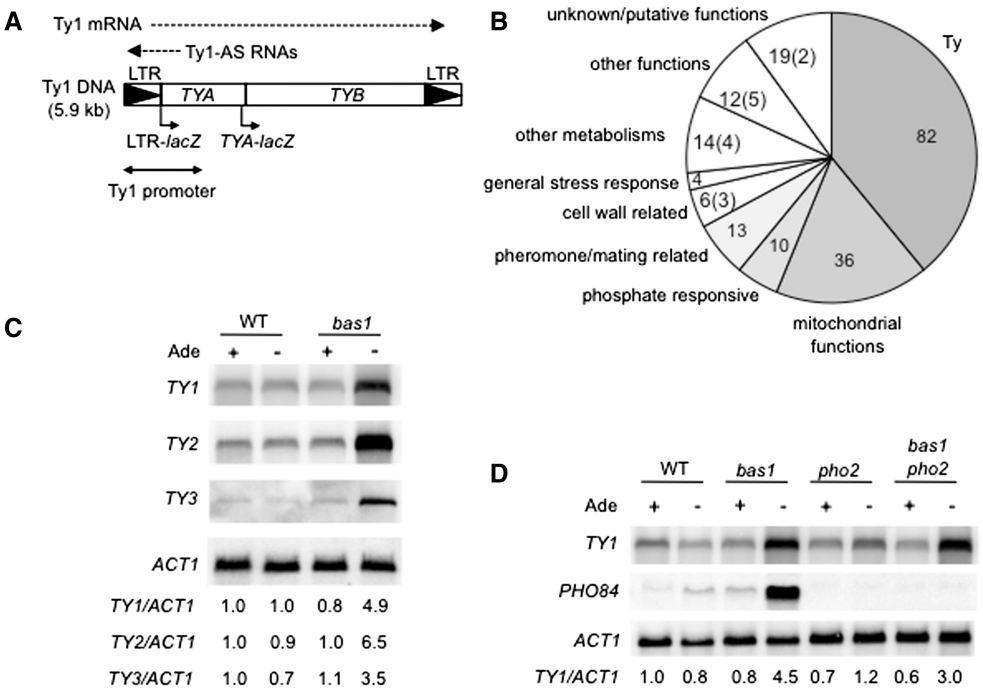

Figure 1. (**A**) Ty1 structure and transcription. Ty1 structure consists of two direct long terminal repeats (LTR, symbolized by black triangles) and two open reading frames, *TYA* and *TYB*, analogs of the retroviral *gag* and *pol* genes. Ty1 transcription regulatory sequences are located within the first kilobase of the retrotransposon. Dotted arrows indicate Ty1 mRNA and Ty1-AS RNA. LTR-*lacZ* and *TYA-lacZ* fusions are indicated by bent arrows under the box. (**B**) Functional classes of genes differentially expressed in adenine-deprived *bas1Δ* cells relative to adenine-supplied *bas1Δ* cells. The number of genes whose expression was up- or down-regulated (in parentheses) by at least a factor of 2.5 is indicated for each class. (**C**) Northern-blot analysis of Ty1, Ty2 and Ty3 mRNA levels in wild-type (FYBL1-23D) and *bas1Δ* (LV426) cells grown in SDc minimum medium supplemented or not with adenine. For each sample, ~15 µg of RNA were loaded onto the gel. The sizes of the mRNA molecules are 5.6 kb (*Ty1* and *Ty2*), 5.1 kb (*Ty3*) and 1.3 kb (*ACT1*). Ratios were determined on a Molecular Dynamics Phosphorimager with ImageQuant software and set as 1 for wild-type (WT) cells grown with adenine. (**D**) Northern-blot analysis of Ty1 mRNA in WT (FYBL1-23D), *bas1Δ* (LV426) *pho2Δ* (LV1010) and *bas1Δ pho2Δ* (LV1012) cells grown with and without adenine. *PHO84*, which is activated by *PHO2* served as a positive control (21). Growth conditions, the northern-blot experimental procedure and mRNA quantifications are described in the legend of Figure 1(C).

accumulation of Ty1-encoded integrase and reverse-transcriptase proteins, and inhibit post-transcriptional steps of Ty1 lifecycle (26). Ty1-AS RNAs could participate to transcriptional and post-transcriptional silencing of Ty1 (43,44), as *S. cerevisiae* lacks the classical RNAi machinery that usually silences transposable elements in other organisms (45).

We have previously shown that Ty1 transcription is activated in adenine-deprived cells defective in *de novo* AMP biosynthesis (referred as conditions of severe adenine starvation), the consequence of this activation being an increase in retrotransposition (18). This activation overcomes the absence of Ste12, a transcription factor which is required for basal levels of Ty1 transcription, is independent of the Bas1 transcription activator of the *de novo* AMP biosynthesis pathway and involves the Swi/Snf chromatin-remodeling complex (18,46). In this report, we used a global approach to characterize the transcriptome of adenine-deprived *bas1Δ* cells in order to get insights into the mechanism of activation of Ty1 transcription by severe adenine starvation. We found that Ty2 and Ty3 are also activated under these stress conditions. Genes involved in ATP regeneration are also up-regulated. Their stimulation is consistent with low ATP and ADP levels measured in adenine-deprived *bas1Δ* cells, which suggests that a decrease in adenylic nucleotide content might be a signal of activation of Ty1 transcription. Consistently, Ty1 transcription also increases in *adk1Δ* cells, which have low ATP and ADP levels (21). We found that expression of the Tye7 transcription factor is induced in response to adenylic nucleotide reduction and that Tye7 contributes to activation of Ty1 expression. Importantly, Ty1-AS RNA levels decrease in adenine-deprived *bas1Δ* cells. We provide evidence that Tye7 is implicated in the control of Ty1-AS RNA synthesis and that its action requires sequences located in *TYA*, where Ty1 antisense transcription starts. Based on these data, we propose a model in which the activation of *TYE7* in response to adenylic nucleotide depletion may contribute to the increase in Ty1 transcription by controlling Ty1-AS RNA synthesis.

MATERIALS AND METHODS

Yeast strains and plasmids

All strains used in this study are S288C derivatives, contain the same number of Ty retrotransposons in their genome and are described in Supplementary Table S1. Strains containing *TYA-lacZ* or LTR-*lacZ* fusions at the chromosomal locus of a native Ty1 element have already been described (14,46). All deletions were created in strains carrying *TYA-lacZ* or LTR-*lacZ* fusions, FYBL1-23D and BY4742, by one-step gene replacement, using polymerase chain reaction (PCR) fragment of *HIS3*, *hphMX* or *kanMX* cassettes, flanked with 5′ and 3′ sequences of the deleted gene. The 3′ end of *TYE7* was tagged with 13Myc sequences by cloning, as described (47).

To construct the pPL297 plasmid that expresses a *MYC-TYE7* allele, from the tetracyclin repressible promoter, *TYE7* coding sequence was amplified by PCR and cloned into a derivative of pCM189 (48) containing an *MYC* sequence at the BamHI site of the polylinker (p2717, *TETop-MYC*, *URA3*, centromeric). Details of constructions can be obtained upon request.

Growth conditions

Yeast strains were grown in rich YPD, Hartwell's synthetic complete (HC) and synthetic minimum (SDc: SD minimal medium containing arginine, isoleucine, tryptophan, leucine and valine) media, all supplemented with 2% glucose (49). Adenine, hypoxanthine or guanine was added to a final concentration of 0.3 mM.

Microarray analyses

Precultures of wild-type (BY4742) and *bas1Δ* mutant (Y2951) cells were grown in SDc medium containing adenine, at 30°C, and diluted 1:200 in 10 ml of the same medium either containing or not containing adenine to reach a concentration of 10^7 cells/ml the next day ($A_{600} = 1$). The cultures were then diluted again in the same media to grow for 3–4 generations and harvested at a concentration of 10^7 cells/ml. RNA extraction, purification and labeling and cDNA probing were performed as described at http://www.transcriptome.ens.fr/sgdb/protocols/. The arrays were read with a Genepix 4000 scanner. Two hybridizations were performed for each comparison using a dye-swap procedure. Normalization was done with the lowest global method (50). The complete datasets have been deposited at the GEO database (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=xpsxlqicaqyusrk&acc=GSE28579).

Northern blots and β-galactosidase assays

Growth conditions, β-galactosidase assays, RNA extraction and northern-blot assays were performed as described in ref. 46. Probes against Ty1 [coordinates 3137 to 3682 in the *TYB* sequence of Ty1-H3 (51)], Ty2 [coordinates 3194 to 3747 in the *TYB* sequence of YLRWTy2-1 (4), Ty3 (coordinates 3213 to 4216 in the *TYB* sequence of YGRWTy3-1 (4)] and *ACT1* mRNA were generated by random-priming (Roche). Ty1 and Ty2 probes were chosen with low homology to avoid cross-hybridization. A Ty1 antisense RNA probe [coordinates 686 to 340 in Ty1-H3 (51)] was synthesized by T7 RNA polymerase (Promega), following a procedure described in ref. 46. Northern blot assays were reproduced at least twice from independent cultures. Results were quantified on a Molecular Dynamics PhosphorImager with Image-Quant software.

Intracellular adenine derivatives content determination

Cellular extracts were prepared by an ethanol extraction method adapted from the one described by ref. (52), and metabolites were separated by high-performance liquid chromatography (HPLC), detected by UV-diode array detector and quantified as described (53). Cellular volume was determined by using a Multisizer 4 (Beckman Coulter).

Chromatin immunoprecipitation

To analyze Tye7 occupancy at the Ty1 promoter, the Tye7 protein was tagged at its C-terminus with a 13Myc-Tag, by cloning (54). The *TYE7-MYC* allele was expressed from *TYE7* native promoter at the chromosomal locus. Chromatin immunoprecipitations (ChIPs) and real-time PCR reactions were performed essentially as described in ref. 55. Yeast strains were grown to A_{600} = 0.8–1 in SDc medium either supplemented or not with adenine at 22°C, and cross-linked for 10 min by the addition of formaldehyde (1.2%). The cross-linking reaction was quenched by adding glycine (0.4 M). Chromatin was sonicated to yield average DNA size fragments of 400 bp (range 100–700 bp). The chromatin solution (1:3 of total chromatin) was immunoprecipitated with 1.4 mg of antibody against the Myc epitope (9E10, Santa Cruz Biotechnology) coupled to 3 mg of Dynabeads anti-mouse IgG (Invitrogen). Immunoprecipitated DNA was quantified by real-time PCR (Platinum SYBR green qPCR supermix-UDG, Invitrogen) using a Master Cycler Realplex (Eppendorf). Primers were designed to amplify Ty1 [coordinates 561–675 of Ty1-H3 (51), forward primer O-PL499 5′ATGAT GACCCAAAACCAAGC3′, reverse primer O-PL500 5′T GGATACTGCGGAAACTGTG3′], Ty2 [coordinates 559–687 of YLRWTy2-1 (4) forward primer O-PL505 5′ATGATGACCCCAAACAAAGC3′, reverse primer O-PL506 5′CTGTGGCAACGGATAGTGTG3′] and *ENO1* sequences [coordinates −497, −405, relative to ATG start codon (24), forward primer O-PL503 5′TCTA CTGATCCGAGCTTCCA3′, reverse primer O-PL504 5'GAGAGGCGAAAGTGGTTTTT3′] and an intergenic sequence on chromosome II (coordinates 408360–418469, O-GS55 5′GTCCCGAAGTAAGATGAGGTT3′, O-GS56 5′AGGTCTCGCAAATCAGAGG3′). For each pair of primers, a 10-fold dilution series of input DNA was used to calibrate the quantification. Real-time PCR reactions were done in triplicate in two independent experiments, using the following conditions of amplification: one cycle 2 min 95°C, 40 cycles 15 s 95°C, 15 s 55°C and 15 s 68°C.

RESULTS

The yeast transcriptome is strongly affected in adenine-deprived *bas1Δ* mutant

To examine the regulation of Ty1 transcription under conditions of severe adenine starvation, we compared the transcriptome of a *bas1Δ* strain grown with or without adenine. Expression of 197 genes was up-regulated, while that of 14 genes was down-regulated, in the absence of adenine (Supplementary Figure S1: activation/repression threshold of 2.5-fold). As a control, we compared the transcriptome of a wild-type strain grown with or without adenine and found that only 16 genes were up- or down-regulated (Supplementary Figure S1), most belonging to the AMP biosynthesis pathway (56). Strikingly, 82 out of the 197 (42%) up-regulated genes in *bas1Δ* cells grown in the absence of adenine matched with the *TYA* and *TYB* ORFs of Ty retrotransposons (Figure 1B): of these 82 genes, 51 corresponded to Ty1, 21 to Ty2 and five to Ty3 (Supplementary Figure S1), and the five remaining genes corresponded to Ty truncated sequences. The numbers of up-regulated Ty genes did not correspond to the numbers of Ty1, Ty2 and Ty3 elements present in the strain [31 Ty1, 12 Ty2 and 2 Ty3 (19)] because the probes that were designed to identify *TYA* and *TYB* ORFs are not specific within a Ty family, due to the strong sequence homology between the elements of each Ty family. Nevertheless, the up-regulation of Ty1, Ty2 and Ty3 ORFs in adenine-deprived *bas1Δ* cells indicated that the three families of Ty retrotransposons might be activated in these cells. We confirmed by northern-blot assay that steady-state levels of Ty2 and Ty3 mRNA increased in adenine-deprived *bas1Δ* cells (Figure 1C). Even though we already described the activation of Ty1 transcription by severe adenine starvation (18,46), these results establish that the mRNA level of Ty1, Ty2 and Ty3 elements increases simultaneously in cells defective in *de novo* AMP biosynthesis.

Besides Ty retrotransposons, 36 genes that were stimulated in adenine-deprived *bas1Δ* cells encoded proteins with mitochondrial functions (Figure 1B and Supplementary Figure S1). Among these proteins, 24 belong to oxidative phosphorylation chain complexes, such as ATP synthesis coupled proton transport, cytochrome *C* oxidase, cytochrome *C* reductase, cytochrome *C* and succinate dehydrogenase. Ten out of 14 genes of the *PHO* regulon (57), involved in phosphate uptake and storage, were also activated (Figure 1B and Supplementary Figure S1). Finally, several genes with functions in conjugation (13 genes), general stress response (four genes), and cell wall (nine genes) were also deregulated in adenine-deprived *bas1Δ* cells. We conclude from this analysis that conditions impairing *de novo* AMP biosynthesis activate the expression of Ty1, Ty2 and Ty3 retrotransposons, stress-related genes and a large number of genes related to energy production, such as the *PHO* and respiratory genes.

The *PHO* genes are activated in response to adenylic nucleotide variations by Pho2 (also known as Bas2), which is involved with Bas1 in the activation of the *de novo* AMP biosynthetic genes in adenine-depleted cells (21,58). This suggests that Pho2 could also be responsible for the activation of Ty1 transcription in adenine-deprived *bas1Δ* cells. However, Ty1 mRNA levels increased in adenine-deprived *bas1Δ pho2Δ* cells, indicating that Pho2 was dispensable for Ty1 activation (Figure 1D). As a control, *PHO84* was activated in adenine-deprived *bas1Δ* cells and the activation was dependent on *PHO2*. Additionally, the absence of adenine did not significantly activate Ty1 expression in *pho2Δ* cells (Figure 1D). Altogether, these observations rule out a role of Pho2 in the activation of Ty1 by severe adenine starvation.

Activation of Ty1 transcription correlates with a decrease in intracellular ATP and ADP levels

In yeast, the activation of mitochondrial and *PHO* genes is correlated with decrease in ATP and/or ADP levels

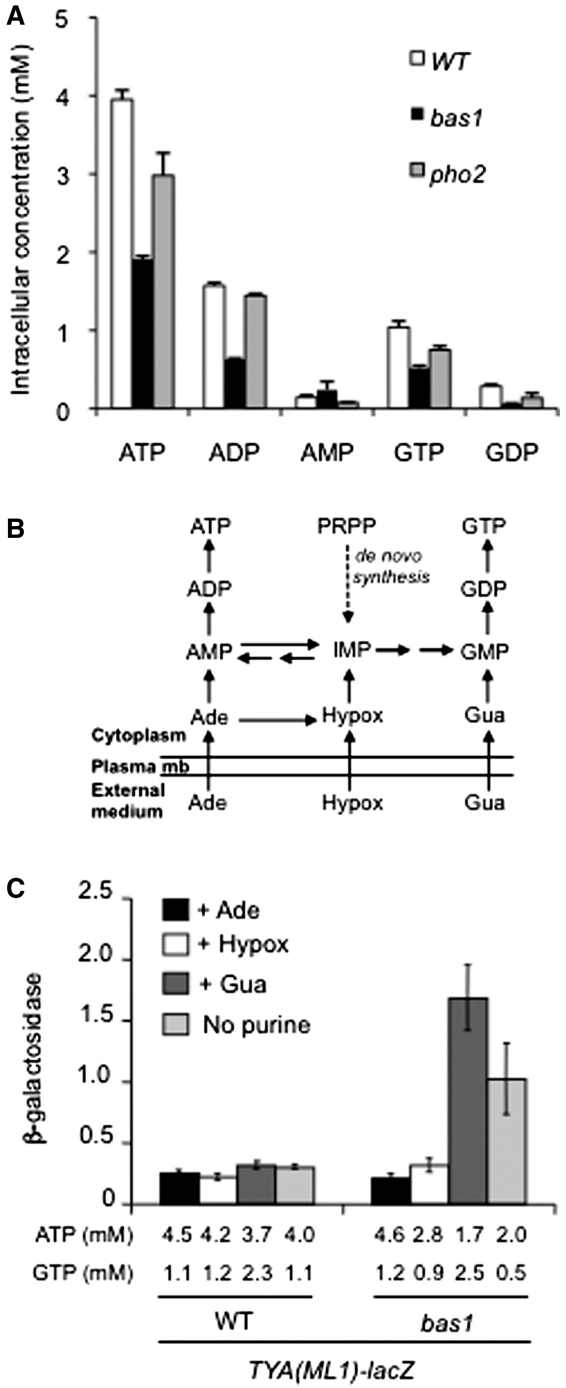

Figure 2. (**A**) Intracellular purine derivative contents from the WT (FYBL1-23D), *bas1Δ* (LV426) and *pho2Δ* (LV1010) strains. Cells were grown in SDc medium to mid-log phase, metabolites were extracted and intracellular nucleotide concentrations were determined by HPLC. (**B**) Schematic representation of *de novo* purine pathway in *S. cerevisiae*. Ade, adenine; Hypox, hypoxanthine; Gua, guanine ; IMP, inosine 5′-monophosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate. (**C**) β-Galactosidase activity of a *TYA-lacZ* fusion at Ty1-ML1 in WT (LV33 Ty1(ML1)-lacZ) and *bas1Δ* (LV436) cells grown in SDc minimum medium supplemented or not with adenine, hypoxanthine or guanine. β-Galactosidase specific activities are expressed in nanomoles of 2-nitrophenyl β-D-galactopyranoside hydrolyzed per minute per milligram of protein. Data represent the average and standard error of three independent cultures. Intracellular concentrations of ATP and GTP were determined by HPLC as in Figure 2A and are indicated for each culture below the bars.

(21,59,60). Thus, the up-regulation of mitochondrial and *PHO* genes in adenine-deprived *bas1Δ* cells indicated that intracellular adenylic nucleotide pools could be affected in these cells, leading to ATP deficiency. To search for depleted or accumulated metabolites in adenine-deprived *bas1Δ* cells that could account for Ty1 activation, we analyzed the intracellular concentration of purine nucleotides by HPLC in wild-type, *pho2Δ* and *bas1Δ* cells grown without adenine. We used *pho2Δ* cells as control, since these cells did not significantly activate Ty1 transcription in the absence of adenine (Figure 1D). Thus, we speculated that the comparison of adenine-deprived *bas1Δ* and *pho2Δ* cells would identify nucleotide variations specific to *bas1Δ* cells. Amounts of guanine nucleotides were also characterized, since they can be altered under certain conditions that affect *de novo* AMP biosynthesis (61). Quantification of the peaks indicated a significant reduction in intracellular ATP, ADP, GTP and GDP levels in adenine-deprived *bas1Δ* cells compared to wild-type and *pho2Δ* cells (Figure 2A).

We previously constructed a set of strains, each expressing *lacZ* from the full promoter sequence of a different chromosomal Ty1 copy (i.e. the 5′LTR and part of the *TYA* ORF, Figure 1A, *TYA-lacZ*), such that the β-galactosidase activity of these strains reflects the expression of each Ty1 element (14). To establish whether the variations in adenine or guanine derivatives could account for Ty1 activation, we compared the expression of a *TYA-lacZ* fusion expressed from the complete Ty1 promoter sequence of the Ty1-ML1 endogenous element, in wild-type and *bas1Δ* cells grown in the presence or absence of guanine, adenine or hypoxanthine, which is a precursor of adenine nucleotides (Figure 2B). The fusion was expressed at high levels when *bas1Δ* cells were grown in the absence of both adenine and guanine. The addition of adenine or hypoxanthine but not of guanine strongly decreased β-galactosidase activity (Figure 2C). HPLC determination of intracellular ATP and GTP concentrations in these cells indicated that the addition of adenine or hypoxanthine in adenine-deprived *bas1Δ* cells increased intracellular ATP and GTP levels, while guanine addition restored only high intracellular GTP levels (Figure 2C). Thus, there is a strong correlation between the decrease in adenylic nucleotides, but not guanylic nucleotides in the activation of Ty1 transcription. Of note, the levels of these nucleotides were much less affected in adenine-deprived *pho2Δ* cells (Figure 2A), which may explain why Ty1 transcription was less activated in these cells. From these experiments, we conclude that Ty1 transcription is activated under conditions that decrease intracellular ATP and ADP levels.

TYE7 contributes to the activation of Ty1 mRNA transcription in adenine-deprived *bas1Δ* mutant

The DNA-binding protein Tye7 is required for the activation of Ty1 transcription in yeast cells lacking the adenylate kinase (Adk1p) responsible for the conversion of AMP into ADP (21). Interestingly, as for adenine-deprived *bas1Δ* cells, *adk1Δ* mutants have low ATP and ADP content and their transcriptome is characterized by an

increase in the expression of Ty1 elements and genes of the energetic and *PHO* pathways (21). However, *BAS1*-activated genes are up-regulated in *adk1Δ* cells. We reproduced the activation of Ty1 transcription in *adk1*Δ cells using our conditions of culture and the expression of *TYA-lacZ* fusions introduced at three Ty1 elements (Supplementary Figure S2). Cells lacking Adk1 activated Ty1 transcription with the same preference as adenine-deprived *bas1Δ* cells for weakly expressed Ty1 elements (18). Our transcriptome analysis indicated that *TYE7* was up-regulated 3-fold in adenine-deprived *bas1Δ* cells (Supplementary Figure S1). Therefore, Ty1 transcription might also be activated by a mechanism involving *TYE7* in *bas1Δ* cells.

To test this hypothesis, we compared the activation of *TYA-lacZ* fusions introduced at three Ty1 elements, Ty1-ML1, Ty1-OR and Ty1-ML2, in *bas1Δ* and *bas1Δ tye7Δ* cells grown with or without adenine. Adenine deprivation in *bas1Δ* cells stimulated *lacZ* transcription from the three fusions 6.7-, 4.7- and 2.5-fold respectively, but activation was abolished for the three elements when *TYE7* was deleted (Figure 3A). This indicates that *TYE7* is essential for *TYA-lacZ* activation in adenine-deprived *bas1Δ* cells. Northern-blot analysis showed that the increase of Ty1 mRNA levels was also 3-fold lower in *tye7Δ bas1Δ* cells than in *bas1Δ* cells, under adenine deprivation (Figure 3B). Note that *TYE7* deletion alone had no effect on Ty1 mRNA levels (Figure 5A) and a weak effect on expression of *TYA-lacZ* fusion at Ty1-DR3 and Ty1-ML2 (Supplementary Figure S3). Together, these findings indicate that *TYE7* is required for the activation of Ty1 transcription under conditions of severe adenine

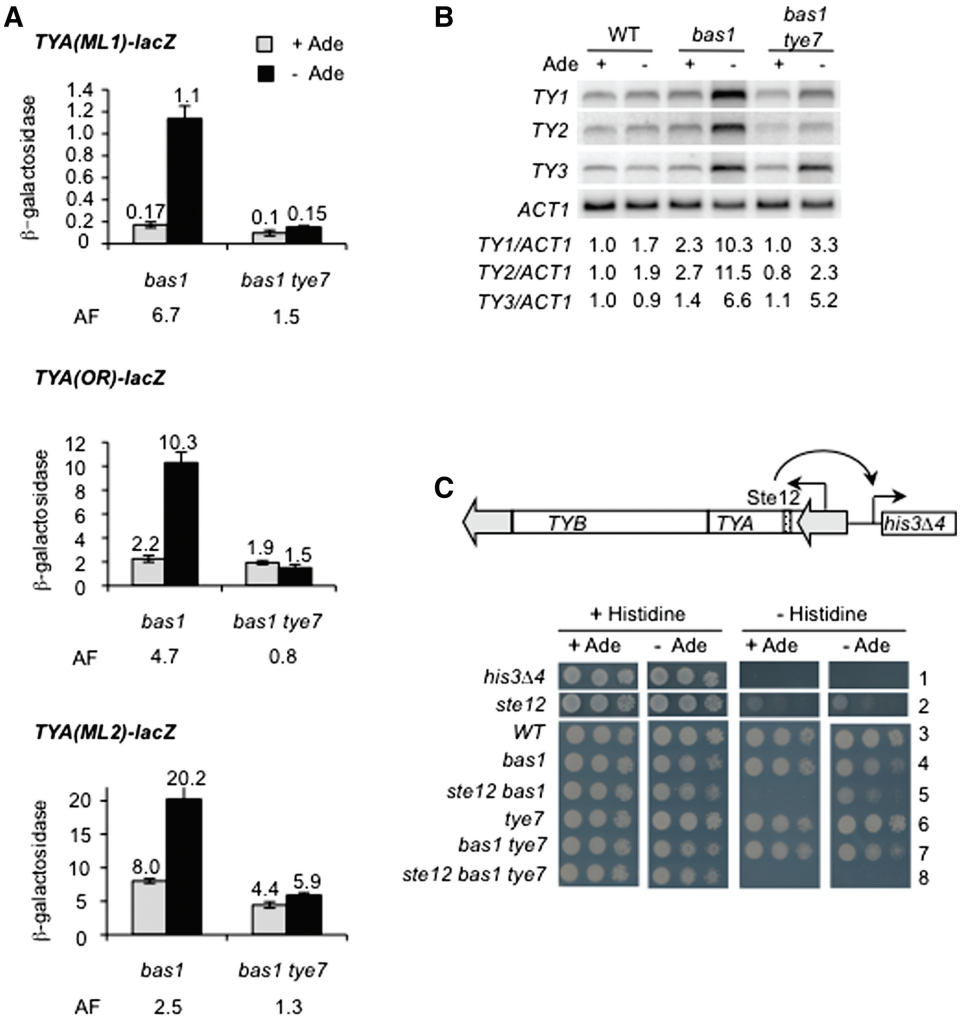

Figure 3. (**A**) β-Galactosidase activity of *TYA-lacZ* fusions at Ty1-ML1, Ty1-OR and Ty1-ML2 in *bas1Δ* cells (LV436, LV658 and LV500, respectively) and *bas1Δ tye7Δ* cells (LV1213, LV1287 and LV1285, respectively). Growth conditions and data representations are described in the legend of Figure 2C. Exact averages of β-galactosidase specific activities are given above the bars. AF, activation factor (No adenine versus + adenine). (**B**) Northern-blot analysis of Ty1, Ty2 and Ty3 mRNA levels in WT (FYBL1-23D), *bas1Δ* (LV426) and *bas1Δ tye7Δ* (LV1234) cells. Growth conditions, northern-blot experimental procedure and mRNA quantifications are described in the legend of Figure 1C. (**C**) Growth assay of WT cells carrying a *his3Δ4* allele (LV69, row 1) and WT (LV150, row 3), *ste12Δ* (LV993, row 2), *bas1Δ* (LV922, row 4), *ste12Δ bas1Δ* (LV926, row 5), *tye7Δ* (LV1370, row 6), *bas1Δ tye7Δ* (LV1372, row 7) and *ste12Δ bas1Δ tye7Δ* (LV1374, row 8) cells, carrying a Ty1-*his3Δ4* allele. Cells were spotted onto plates in a series of 10-fold dilutions of 1 A_{600} of overnight precultures. All plates were incubated at 30°C for four days. Rows are numbered for clarity.

starvation. Interestingly, *TYE7* was also necessary for the activation of Ty2, but not of Ty3, in adenine-deprived *bas1Δ* cells, as shown in Figure 3B.

We have previously shown that transcription of a gene adjacent to a full-length Ty1 element is activated in adenine-deprived *bas1Δ* cells (46). Since *TYE7* has been identified as a gene involved in Ty1-mediated gene expression (20), we asked whether it could be responsible for activating expression of a Ty1-adjacent gene under conditions of severe adenine starvation. To address this point, we used yeast cells, which are unable to grow in the absence of histidine, unless a Ty1 element is present upstream of the promoterless *his3Δ4* allele, such that *his3Δ4* expression is driven from Ty1 promoter sequences (Figure 3C, rows 1 and 3). The growth of *Ty1-his3Δ4* cells depends on the Ste12 transcriptional activator, which binds to the Ty1 promoter and is required for Ty1 transcription (Figure 3C, row 2 and ref. 46). We have previously shown that the histidine prototrophy of Ty1-*his3Δ4 ste12Δ* cells is recovered in adenine-deprived Ty1-*his3Δ4 ste12Δ bas1Δ* cells (Figure 3C, row 5 and ref. 46). As shown in Figure 3C row 8, this activation was dependent on *TYE7*. As expected, *TYE7* deletion alone had no impact on the growth of *Ty1-his3Δ4* in wild-type and *bas1Δ* cells (Figure 3C rows 6 and 7).

Altogether, these results indicate that the increase in *TYE7* expression in adenine-deprived *bas1Δ* cells could be part of the mechanism of activation of Ty1 and Ty1-adjacent gene transcription.

TYE7 requires sequences located in *TYA* to activate Ty1 expression

In vivo, the Tye7 protein binds to the CACCTG E-box of the *ENO1* gene promoter and activates its transcription (22–24). Genome-wide analyses of Tye7-binding sites have established an extended but slightly different TCACGTG A consensus sequence (62–65). Three conserved DNA motifs identical to the *ENO1* E-box are present in the *TYA* portion of Ty1 promoter and are conserved in the promoter of Ty2 elements (Figure 4A). By ChIP experiments, we confirmed the presence of the Tye7 protein at the Ty1 and Ty2 promoter in *bas1Δ* cells carrying a chromosomal Myc-tagged *TYE7* allele, expressed from *TYE7* promoter sequences (Figure 4B). There was no difference in the relative Tye7–Myc occupancy whether cells were grown with or without adenine. However, we could not establish whether Tye7–Myc bound to a subset of sites under normal growth conditions or to the three sites simultaneously in the absence of adenine. Indeed, the three potential E-boxes are located within a window of 269 bp, which is smaller than the average size of DNA fragments obtained upon chromatin sonication and required for the experiment. Moreover, ChIP experiment gives an average of Tye7-binding to all *TYA* sequences in the genome (from Ty1 or Ty2), but provides no information on Tye7-binding to individual elements, within a family.

To further analyze whether *TYE7* activates Ty1 transcription through *TYA* sequences where the three potential E-boxes are located, we compared the expression of LTR-*lacZ* and *TYA-lacZ* fusions (Figure 1A), at the

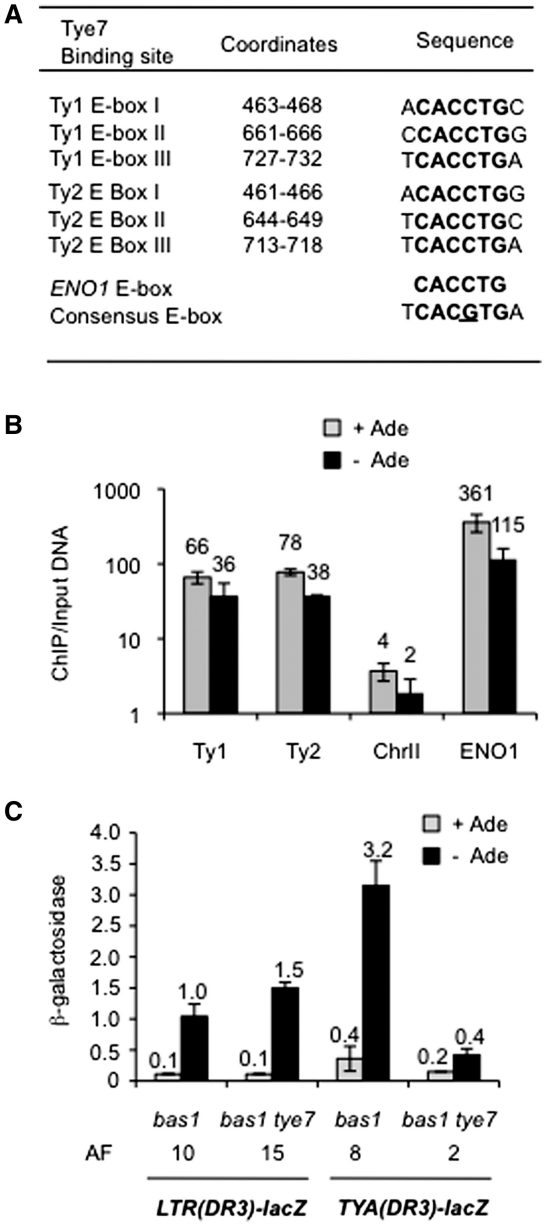

Figure 4. (**A**) Coordinates and sequences of potential Tye7 binding sites in Ty1 and Ty2 (E-box I to III). Coordinates are given relative to the numbering of Ty1-H3 (51) and YLRWTy2-1 (4). *ENO1* E-box (24) and Tye7p consensus binding site (62) are indicated. (**B**) Chromatin immunoprecipitation (ChIP) analysis of Tye7 occupancy at the Ty1 promoter in *bas1Δ* (LV1058) and *bas1Δ TYE7-MYC* (LV1368) strain. Signals are expressed as ratios of ChIP/Total DNA and are set as 1 for untagged *TYE7 bas1Δ* cells grown with adenine. Data represent the average and standard error of two independent real-time PCR amplifications. (**C**) β-Galactosidase activity of LTR-*lacZ* and *TYA-lacZ* fusions at Ty1-DR3 in *bas1Δ* cells (LV1013 and LV722, respectively) and *bas1Δ tye7Δ* cells (LV1320 and LV1341, respectively). Growth conditions and data representations are described in the legend of Figure 2C. Exact averages of β-galactosidase specific activities are given above the bars. AF, activation factor (versus adenine).

Ty1-DR3 element, in *bas1Δ* and *bas1Δ tye7Δ* cells. We previously showed that the 5′LTR of several endogenous Ty1 elements is sufficient to activate transcription of *lacZ* fusions in adenine-deprived *bas1Δ* cells, although

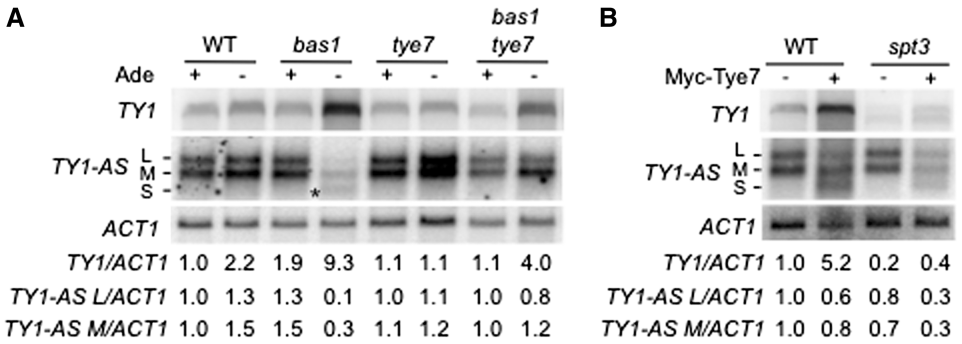

Figure 5. (**A**) Northern-blot analysis of RNA extracted from WT (FYBL1-23D), *bas1Δ* (LV426), *tye7Δ* (LV1232) and *bas1Δ tye7Δ* (LV1234) cells. L, M and S stand for large, medium and short Ty1-AS RNA species, respectively. (**B**) Northern-blot analysis of Ty1 mRNA and Ty1-AS RNA levels in WT (FYBL1-23D) and *spt3Δ* (LV940) cells transformed with pPL297 (*TETop-Myc-TYE7*, *URA3*, *CEN*) or the empty vector p2717. Growth conditions, northern-blot experimental procedure and mRNA quantifications are described in the legend of Figure 1C.

additional sequences in *TYA* are necessary to optimize such activation (18,46). As expected, expression of both fusions increased significantly in *bas1Δ* cells grown without adenine (Figure 4C). However, in the double mutant *tye7Δ bas1Δ* and in the absence of adenine, the activation of the *TYA-lacZ* fusion was abolished, whereas the LTR-*lacZ* fusion was still fully activated. This result indicates that *TYE7* does not stimulate Ty1 transcription through promoter sequences located in the 5′LTR but rather through sequences located in *TYA* ORF, and is consistent with the binding of Tye7 to potential E-boxes located in *TYA*.

TYE7 regulates Ty1 antisense transcription in adenine-deprived *bas1Δ* mutant

Ty1-AS RNAs have been reported to repress Ty1 at both transcriptional and post-transcriptional levels (25,26). Since the accumulation of Ty1-AS RNAs in an *xrn1* mutant represses Ty1 transcription (25), we asked whether, conversely, adenine-deprived *bas1Δ* cells could prevent Ty1-AS RNA synthesis as part of the mechanism of activation of Ty1 transcription. Northern-blot analysis showed two different species of Ty1-AS RNA in wild-type cells [Figure 5A, lanes 1 and 2, Large (L) and Medium (M) species], with an estimated size comprised between 0.5 and 1-kb, as described in ref. (26). Remarkably, this pattern was altered in adenine-deprived *bas1Δ* cells, since these two species decreased in intensity, whereas a species of lower molecular weight appeared [Figure 5A, lane 4, Short (S) species]. Interestingly, the alteration of Ty1-AS RNA pattern correlated with an increase in Ty1 mRNA levels (Figure 5A). This suggests that the mechanism of activation of Ty1 transcription is linked to a change in Ty1-AS RNA expression in adenine-deprived *bas1Δ* cells. Strikingly, the Ty1-AS RNA profile was not modified in adenine-deprived *tye7Δ bas1Δ* cells (Figure 5A, lane 8). On the other hand, *TYE7* overexpression from the tetracycline-repressed operator in wild-type and adenine-supplied *bas1Δ* cells reproduced the increase in Ty1 mRNA levels concomitantly to the decrease in the L and M Ty1-AS RNA and the increase in the S species, observed in adenine-deprived *bas1Δ* cells (Figure 5B lane 2 and Supplementary Figure S4). In adenine-deprived *bas1Δ* cells, *TYE7* overexpression enhanced this phenotype (Supplementary Figure S4). These observations reveal the implication of *TYE7* in the alteration of Ty1-AS RNA expression in adenine-deprived *bas1Δ* cells.

TYE7 could repress transcription of Ty1-AS RNA directly or indirectly, by activating Ty1 mRNA transcription. If Tye7 directly affects Ty1-AS RNA synthesis, down-regulation of Ty1-AS RNAs should be observed even in the absence of Ty1 mRNA transcription. To address this point, *TYE7* was overexpressed from the tetracycline-repressed operator in an *spt3Δ* mutant, which is defective in Ty1 mRNA transcription, but has elevated levels of Ty1-AS RNAs (41,42). In the *spt3Δ* mutant, a similar change in the Ty1-AS RNA profile to that observed in wild-type cells was detected upon *TYE7* overexpression, although Ty1 mRNA transcription did not increase significantly. These findings strongly suggest that Tye7 directly controls Ty1-AS RNA synthesis. In the *spt3Δ* mutant, a smaller transcript running below the full-length Ty1 transcript was detected and corresponds to an already described 5′-end truncated Ty1 transcript (66). Altogether, these results indicate that the synthesis of Ty1-AS RNAs is modified in adenine-deprived *bas1Δ* cells and that *TYE7* is involved in the control of Ty1-AS transcription.

DISCUSSION

This work shows that a decrease in intracellular ATP and ADP levels correlates with an increase in Ty1, Ty2 and Ty3 mRNA transcription. Activation of Ty1 requires the Tye7 transcription factor, which binds to the *TYA* portion of Ty1 promoter, where Ty1-AS transcription occurs. We provide evidence that Tye7 also regulates Ty1-AS transcription. These data support a model in which activation of *TYE7* in response to adenylic nucleotide depletion contributes to the increase in Ty1 transcription by controlling Ty1-AS RNA synthesis.

The data presented here establish that the transcription of three of the five Ty families in the yeast genome,

i.e. Ty1, Ty2 and Ty3, are activated in adenine-deprived *bas1Δ* cells. Activation of Ty4 and Ty5 was not detected; however, Ty4 expression is extremely low and gives rise to truncated transcripts, and no functional Ty5 elements are present in *S. cerevisiae* laboratory strains (67,68). Although Ty1 and Ty2 are both *copia*-like elements sharing a high level of sequence similarity (19), the regulation of their transcription presents some differences. For instance, the transcription of Ty1 but not of Ty2 strongly depends on transcription factors Ste12 and Tec1 (13,69,70). Ty3 is a *gypsy*-like element with a weak homology with Ty1 and its transcription is differently regulated (71,72). Our results indicate that activation of transcription of Ty1 and Ty2 but not Ty3 depends on the Tye7 transcription factor in adenine-deprived *bas1Δ* cells (see below), suggesting that the transcription of Ty1 and Ty2 is regulated by a similar mechanism. Simultaneous activation of several active mobile elements, which are structurally different, has already been described in *Drosophila virilis* upon hybrid dysgenesis and in Maize in response to chromosome breakage (6,73). However, simultaneous transcriptional activation of different families of Ty elements by nutrient starvation is a novel finding in *S. cerevisiae*, although Ty1 and Ty3 share many host factors that control their transposition, mostly at post-transcriptional levels (74).

Several lines of evidence indicate that suboptimal ATP and ADP intracellular concentrations could be a signal for the activation of Ty1, Ty2 and Ty3 transcription. First, adenine-deprived *bas1Δ* cells contain abnormally low ATP and ADP levels and activate mitochondrial and *PHO* genes, which is consistent with a deficit in ATP (21,59). Second, cells lacking the major adenylate kinase Adk1 also display low ATP and ADP levels and activate Ty1 transcription. Third, although the amounts of other purine metabolites are affected in adenine-deprived *bas1Δ* cells (i.e. GTP and GDP), only the deficiency in ATP and ADP is consistently associated with the induction of Ty1 transcription. In the case of Ty1, we have shown that the increase in transcription is accompanied by an increase in retrotransposition (18) and activation of the expression of genes adjacent to Ty1 insertions (46). We could not identify laboratory conditions that would decrease ATP and ADP concentrations in wild-type cells, to the same extent as in adenine deprived *bas1Δ* cells. However, limited nutrient availability is a common situation in nature, and microorganisms are able to decrease their rate of metabolism and to survive using rare nutrient sources (75). Thus, adenine-deprived *bas1Δ* cells might reproduce a metabolic state of yeasts in their natural environment. Noteworthy, 'domestication' of yeast cells isolated from nature to grow on rich medium in the laboratory is accompanied by a decrease in Ty1 mRNA levels (76).

We provide strong evidence that the Tye7 transcription factor is involved in the mechanism of activation of Ty1 transcription. First, *TYE7* transcription is activated in adenine-deprived *bas1Δ* cells as in *adk1Δ* cells (21). Second, *TYE7* deletion abolishes the activation of several *TYA-lacZ* fusions containing the full Ty1 promoter and reduces Ty1 mRNA levels in adenine-deprived *bas1Δ* cells. Third, *TYE7* overexpression activates Ty1 mRNA transcription in wild-type cells. We have previously shown that transcription of genes adjacent to Ty1 insertions is stimulated in adenine-deprived *ste12Δ bas1Δ* cells (46). Here, we demonstrate that *TYE7* is necessary for this activation to occur. Fourth, Tye7 protein is present at the Ty1 promoter. We have identified three consecutive potential Tye7 binding sites (E-boxes), downstream of Ty1 transcription start site. Their location is consistent with our result indicating that *TYE7* does not stimulate Ty1 transcription through promoter sequences located in the 5′LTR but rather through sequences located in *TYA* ORF. However, there is no evidence that all or a subset of the E-boxes are involved in Tye7-dependent regulation and we cannot exclude that other sequences in *TYA* could be involved in this regulation. Nevertheless, it is noteworthy that Ty1 and Ty2 contain three E-boxes and are regulated by Tye7, while Ty3 does not contain E-boxes and is not regulated by Tye7. Importantly, *TYE7* deletion does not alter basal Ty1 transcription, indicating that the Tye7 protein might be essential for the full activation of Ty1 transcription under certain environmental stress conditions, only. Since the LTR-*lacZ* fusion at Ty1-DR3 is activated and there is a residual activation of Ty1 mRNA transcription, in adenine-deprived *bas1Δ* cells independently of Tye7, additional transcription factor(s) could participate in the activation of Ty1 transcription by interacting with the 5′LTR.

TYE7 alters Ty1-AS RNA synthesis in adenine-deprived *bas1Δ* cells or in response to Tye7 overexpression in wild-type cells. The alteration is characterized by a decrease in the levels of two Ty1-AS RNA species present under normal growth conditions and already described in ref. 26 and the appearance of a new species of lower molecular weight, whose synthesis might interfere with the synthesis of the two other Ty1-AS species. One possible model is that the alteration of Ty1 antisense transcription is the consequence of the activation of Ty1 transcription by Tye7. Supporting this hypothesis, the activation of Ty1 and Ty2 expression from sequences located downstream of the transcription start has already been described (13,69,77). However, Tye7 also down-regulates the already described Ty1 antisense transcription in a *spt3Δ* mutant, which is defective in Ty1 mRNA transcription. Thus, it is likely that Tye7 directly controls Ty1 antisense transcription. An attractive hypothesis is that the Tye7 protein represses Ty1 antisense transcription by binding to one or more of the three E-boxes. In support of a potential repressive role of *TYE7*, a previous study has reported that it could repress the transcription of the E-box-containing *CIT2* gene (78). In a first model, Ty1 sense and antisense transcription could interfere *in cis*. A reduction in Ty1-AS RNA synthesis would therefore increase Ty1 mRNA transcription. Such a mechanism has already been described for the control of the stress-responsive *SER3* gene by the *SRG1* non-coding gene although, in contrast to the Ty1 situation, these two genes are adjacent and transcribed in the same direction (33). It is noteworthy that Tye7 is required for the activation of adjacent gene transcription under adenine starvation, while it down-regulates

Ty1 antisense transcription, since both transcription occur in the same direction. This discrepancy could be explained by the fact that the transcription of genes adjacent to Ty1 starts from cryptic sites located in the 5′LTR (46). Thus, reducing Ty1 antisense transcription would stimulate RNA synthesis from the 5′LTR, bi-directionally. In a second model, the decrease in Ty1-AS RNA levels could relieve the basal level of Ty1 trans-silencing, since antisense Ty1-RTL RNA, which accumulate in *xrn1Δ* cells, has been reported to inhibit Ty1 transcription *in trans*, by helping to install repressive chromatin over the Ty1 promoter (25). Although we cannot discriminate between the *cis* and *trans* models, the reduction of Ty1-AS RNA levels in cells severely depleted in ATP and ADP provides evidence that Ty1-AS RNA can be regulated by environmental stress conditions.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Table 1, Supplementary Figures 1–4 and Supplementary References [14,18,79].

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