

# **Sus1, a functional component of the SAGA transcription complex and the mRNA export machinery**

Susana Rodriguez-Navarro<sup>§</sup>, Tamás Fischer<sup>§</sup>, Ming-Juan Luo\*, Oreto Antunez<sup>§</sup>,  
Susanne Brettschneider<sup>§</sup>, Jose E. Perez-Ortin<sup>§</sup>, Robin Reed\*, and Ed Hurt<sup>§</sup>

\*Department of Cell Biology, Harvard Medical School, Boston, USA

<sup>§</sup>Department of, Biochemistry and Molecular Biology, University of Valencia, Valencia,  
Spain

<sup>§</sup>BZH, University of Heidelberg, BZH, Im Neuenheimer Feld 328, D-69120 Heidelberg,  
Germany

**Gene expression occurs via a network of coupled interactions involving components of the transcription, RNA processing, and mRNA export machineries<sup>1-3</sup>. Here we report the identification of a new protein, Sus1, that functions not only in transcription but also in mRNA export. Sus1 interacts physically and genetically with key components of the mRNA export machinery and *sus1* mutants inhibit bulk poly(A)<sup>+</sup> RNA export. Remarkably, Sus1 also associates stoichiometrically with the histone acetylase complex SAGA, which is required for the transcription of a subset of Pol II genes<sup>4-12</sup>. DNA macroarray analysis revealed that Sus1 is required for transcription regulation of ~ 9% of yeast genes. Moreover, chromatin immunoprecipitation showed that Sus1 is associated with the promoter of a SAGA-dependent gene during transcription activation. The functional interaction of Sus1 with both the SAGA complex and mRNA export components suggests the mRNA export machinery is specifically recruited to active genes at the earliest steps of gene expression.**

The Yra1 protein is an essential component of the conserved mRNA export machinery<sup>13-19</sup>. To identify new mRNA export factors, we performed a synthetic lethal (sl) screen using the *yra1-ΔRRM* mutant allele<sup>13</sup>. Three synthetic lethal mutants (sl9, sl15, sl302) were isolated in this screen, each of which was complemented by a yeast genomic DNA fragment (500 nts) located in an intergenic region between the *YSAI* and *SSN6* genes (Fig. 1a). Northern analyses using this DNA fragment as a probe detected an RNA of ~320 nts in both wild-type (Fig. 1b) and in the three sl mutant cells (data not shown). This RNA is absent in cells in which the chromosomal *YSAI-SSN6* locus is disrupted (Fig. 1b). The RNA from the *YSAI-SSN6* intergenic locus was cloned by RT-PCR and contains 2 introns and 3 exons (Fig. 1a). We designated the gene encoding this pre-mRNA *SUSI* (for sl gene upstream of Ysa1), which is located between the *YSAI* and *SSN6* genes on the “Watson” strand of chromosome 2 (Fig. 1a; GenBank accession AY278445). This gene had not been previously annotated in the yeast database. The *SUSI* gene encodes a conserved protein of 96 amino acids with orthologues in human, *Drosophila*, *Arabidopsis* and *Candida* (Fig. 1c).

To verify the genetic interaction between *YRAI* and *SUSI*, the *yra1-ΔRRM* allele was directly combined with the null allele of *SUSI* (*sus1Δ*) in a haploid yeast strain. The *sus1Δ* strain alone is viable, but exhibits slower growth at 23°C and 30°C and is temperature-sensitive for growth at 37°C (Fig. 1d). The *sus1Δ/yra1-ΔRRM* cells were not viable (Fig. 1e). In contrast, another mutant allele of *YRAI*, *yra1-ΔN*, is not synthetic lethal with *sus1Δ* suggesting an allele-specific interaction between *SUSI* and the RRM domain within *YRAI* (data not shown). To determine whether *SUSI* interacts genetically with other essential components of the conserved mRNA export machinery (Sub2, Mex67), pairwise combinations of mutant alleles were made in haploid strains. This analysis revealed that *sus1Δ* is synthetically lethal with *sub2-85* and *mex67-5* mutant alleles (Fig. 1e). Thus, Sus1 interacts genetically with all three known essential components of the conserved mRNA export machinery.

The observation that Sus1 interacts genetically with the mRNA export machinery suggests that Sus1 plays a role in mRNA export. To test this possibility, we carried out *in situ* poly(A)<sup>+</sup> RNA hybridization with *sus1Δ* cells. This analysis showed a significant defect in export of poly(A)<sup>+</sup> RNA after a 90 min shift to the

restrictive temperature (Fig. 2a). Moreover, mRNA export is defective in all three *sl* mutants *sl9*, *sl15*, *sl302* (Fig. 2a and data not shown), and the mRNA export defect of the *sus1* *sl* mutants is complemented by the recombinant *SUS1* gene (Fig. 2a). We conclude that *SUS1* is a newly identified component of the nuclear mRNA export machinery. Consistent with its role in nuclear export, Sus1-GFP has predominantly an intranuclear location with a detectable concentration around the nuclear periphery (Fig. 2b).

To identify proteins that associate with Sus1 in yeast, the *SUS1* gene was TAP-tagged at the 3' end by homologous integration. The Sus1-TAP cells grow normally and express a fusion protein of 30 kDa (data not shown). Sus1-TAP was affinity-purified from a whole cell lysate by two consecutive affinity columns (IgG-Sepharose and Calmodulin-Sepharose). Both the first (TEV) and second (EGTA) eluate were analyzed by SDS-PAGE and Coomassie staining. After the second purification, ~20 proteins were specifically enriched. Unexpectedly, mass spectrometry revealed that most of these proteins are components of the SAGA complex, which functions in histone acetylation, and is required for the expression of a subset of Pol II genes<sup>7</sup>. The SAGA proteins identified in the Sus1-TAP eluate include Spt3, Spt7, Spt8, Spt20, Ada1, Ada2, Ada3, Taf5, Taf6, Taf9, Taf10, Taf12, Gcn5, Sgf29, Sgf73 and Ubp8, which are essentially all of the reported SAGA components<sup>4,12,20,21</sup>. Moreover, histones H3 and H2B, which are specifically modified by SAGA, are present in the affinity-purified complex. Two other proteins detected in the Sus1-TAP pull-down were identified as Thp1 and Sac3 (Fig. 3a). Both of these proteins are required for mRNA export, and interact physically and genetically with the general mRNA export receptor Mex67<sup>22,23</sup>. In contrast to Thp1, most of the Sac3 dissociates from the Sus1-TAP complex during the second affinity-purification (Fig. 3a, TEV). Tra1, a well-defined subunit of SAGA<sup>4,24</sup>, also dissociates from the Sus1-TAP complex during the second purification step (Fig. 3a, TEV), indicating that Thp1 and components of the SAGA complex are more tightly associated with Sus1 than are Sac3 and Tra1.

Previously, Sac3 was detected in a Thp1-TAP purification<sup>22</sup>. Sus1 was not detected in this purifications possibly due to its low molecular weight of ~10 kDa<sup>22</sup>. However, Sus1 is readily detected as a ~32 kDa band in a Thp1-TAP purification using a larger form of Sus1 tagged with a 13-mer tandem myc-cassette (Fig. 3b). We conclude that Sus1 is in a complex with Thp1 and Sac3.

Notably, low levels of the SAGA components Tra1 were detected in the Thp1-preparations<sup>22</sup>. This prompted us to test whether some of the substoichiometric bands seen in Thp1-TAP preparations could be other SAGA components. As shown in Fig. 3c, Western analysis using antibodies against SAGA components (Spt20, Taf12) revealed that they are also associated with Thp1. This association is specific as TAP-purified nucleoporin Nup82, which served as negative control, does not contain these SAGA components (Fig. 3c). We conclude that Thp1 is stoichiometrically associated with Sus1 and Sac3, and substoichiometrically associated with SAGA components.

In light of the unexpected observation that three mRNA export proteins (Sus1, Thp1 and Sac3) associate with the SAGA complex, we next asked whether SAGA mutants are impaired in poly(A)<sup>+</sup> RNA export. However, no nuclear accumulation of mRNA was observed in *gcn5*, *ada2*, *ada3* or *spt7* mutants (data not shown). Thus, SAGA components are not directly involved in mRNA export.

To determine whether Sus1, like other SAGA components, plays a role in transcription regulation of genes, we analyzed the expression profile of the ~6000 yeast genes by DNA macro-arrays in the *sus1Δ* strain. In a recent genome-wide analysis gene expression was found to be affected in several SAGA mutants<sup>7</sup>. Expression of ~7.5% of the genes is altered (416 decreased and 35 increased) in *spt20*, 1.8% in *gcn5* and 1.5% in *spt3* mutants (ChIP database; see <http://staffa.wi.mit.edu/chipdb/public/index.html>). Only a modest overlap is observed between genes that are repressed in several SAGA mutants<sup>7</sup>; see also Table 1b). Our genome-wide analysis showed that expression of ~9% of yeast genes is altered (341 decreased and 208 increased) in the *sus1Δ* strain (Table 1a and Supplementary information 2). As observed with other SAGA mutants, the overlap of genes whose expression is affected in both *sus1Δ* and SAGA mutants is moderate (see Table 1b). Thus, our data show that Sus1, like other SAGA components, is involved in a complex regulation of a subset of yeast genes.

To confirm these findings, we analyzed *PHO84* transcript levels in *sus1Δ* cells by Northern analyses. DNA macroarray revealed that *PHO84* is the most significantly decreased transcript (~50-fold) in *sus1Δ* cells (see Supplementary information 2). Consistent with these results, Northern analysis shows that *PHO84* transcript levels are dramatically reduced in *sus1Δ* cells, as well as in *spt7Δ* and *gcn5Δ* strains (Fig.

4a). The levels of *PGK1* mRNA, whose expression is not SAGA-dependent, was unaffected in the *sus1Δ*, *spt7Δ* and *gcn5* mutants (Fig. 4a).

To determine whether Sus1 is physically associated with SAGA-dependent genes, we carried out chromatin immunoprecipitation (ChIP) assays using a Sus1-Myc tagged strain and the SAGA-dependent *GALI* promoter<sup>8,10</sup>. This analysis revealed that Sus1 is specifically recruited to the *GALI* gene after induction with galactose (Fig. 4b, lane 1 - 3). A similar pattern of association with the *GALI* promoter was observed for PolIII (Fig. 4b, lane 4-6). To determine where Sus1 interacts along the gene, eight different regions of the *GALI* gene were used for ChIP assays. As shown in Fig. 4c, Sus1 is associated with the *GALI* promoter region, but not with the middle and 3' end of the gene. This association is characteristic of SAGA components<sup>10</sup>. These results, together with the DNA macroarray (Table 1) and the biochemical data (Fig. 3), strongly suggest that Sus1 functions in transcription together with the SAGA complex.

In this study, we report the identification of a protein Sus1 that is stoichiometrically associated with the SAGA transcription complex, and like the SAGA complex, functions in transcription of a subset of genes. Among the SAGA components associated with Sus1 is the histone-like Taf9. The *Drosophila* Sus1 homologue e(y)2 is a ubiquitous transcription factor that interacts with the counterpart of yeast Taf9 in a large complex<sup>25</sup>. Thus, the function of Sus1 in transcription is likely to be conserved. Remarkably, our data also show that Sus1 interacts physically and genetically with the mRNA export machinery and functions in mRNA export. In previous studies, we identified a complex designated TREX that couples mRNA export to transcription. The TREX complex contains the conserved mRNA export factors Yra1 and Sub2, as well as the THO complex (Tho2, Hpr1, Mft1, Thp2), which functions in transcription elongation<sup>26</sup>. ChIP analyses revealed that the TREX complex is recruited to the middle and 3' part of the gene, but not to the promoter<sup>26-29</sup>. In contrast, Sus1 is specifically associated with a transcription complex located at the promoter, and ChIP analysis shows that Sus1 is associated with the promoter but not the middle and 3' part of the gene. This reciprocal association of Sus1 and TREX raises the possibility that Sus1 is involved in recruiting the export components Sac3 and Thp1 during transcription activation, whereas TREX is involved in recruitment of Yra1 and Sub2 during transcription elongation. Previous studies showed that Mex67 and Sac3 interact with the nuclear pores and with each other<sup>22</sup>. Thus, all of the export

components that are recruited during transcription may associate in an mRNP that is targeted to the nuclear pore complex. Although we cannot rule out the possibility that Sus1 has a dual role in transcription and mRNA export, Sus1 and Thp1 pull-downs contain both export and SAGA components, suggesting the possibility that Sus1 is a bridging factor recruiting the export machinery to active genes at the earliest steps of gene expression.

## Methods

### Yeast analysis

The *yra1Δ ade2 ade3* strain used in the sl screen was transformed with the *ARS/CEN-TRP1* plasmid pRS314 that contained both the *yra1-ΔRRM* allele and the *SUB2* wild-type gene. The rationale of having an increased *SUB2* gene dosage was to counterselect for sl mutants that are complemented or suppressed by *SUB2*. It is known that *SUB2* can act as a high copy suppressor of *yra1* (K. Sträßer and E. Hurt, unpublished data) and *hpr1* mutants<sup>30</sup>. The sl screen was performed as previously described<sup>26</sup>. The *sus1* knock-out strain (*sus1Δ*) was generated by disrupting with a kanamycin (KanMX4) cassette the intergenic region (in total 550 nts) between *YSA1* (150 nts 5' of the ATG start codon) and *SSN6* (300 nts 3' of the stop codon) by homologous recombination. The *SUB2* and *MEX67* shuffle strains, and the *tho2Δ*, *thp2Δ*, *mft1Δ* and *hpr1Δ* null strains were described previously<sup>26,31</sup>. The *gcn5Δ*, *spt7Δ*, *ada2Δ* and *ada3Δ* strains were received from EUROSCARF. *SUS1* was cloned by PCR amplification of the *SUS1* gene locus from chromosomal DNA creating a *NotI* site 150 nts 5' upstream of the *SUS1* start codon and a *XhoI* site 180 nts 3' downstream of the stop codon. This PCR-amplified *SUS1* chromosomal DNA was inserted into the pRS315 vector. For identification of mutations in the 3 sl mutants causing lethality between *sus1* mutant alleles and *yra1-ΔRRM*, the PCR amplification was performed with the same primer set, but using genomic DNA isolated from sl9, sl15 and sl302. Sequencing revealed that sl9 has a point mutation in the branchpoint of the first intron (TACTGAC to CACTGAC), whereas sl15 and sl302 have point mutations, which generate pre-mature stop codons (S74→stop, S48→stop, respectively). *SUS1* cDNA was retrotranscribed from total yeast RNA using primers F-SUS1 (5'-ATGACTATGGATACTGCG-3') and R-SUS1 (5'-CCTTCATTGTGTGTATCT-3'). The obtained cDNA was amplified by PCR, cloned into the pCR2.1-TOPO vector (Invitrogen) and the inserts of two different clones were sequenced. The GenBank accession number of *SUS1* is AY278445.

Plasmids pUN100-*YRA1*, pUN100-*MEX67*, pRS314-*SUB2*, pRS314-*yra1-ΔRRM*, pRS314-*mex67-5* and pRS314-*sub2-85* were described previously<sup>26</sup>. The TAP-tag, GFP-tag or 13Myc-tag were integrated C-terminally into the genome of the *SUS1*, *THP1* and *NUP82* genes by homologous recombination<sup>22,32</sup>. The synthetic



lethal screen, oligo(dT) *in situ* hybridization and GFP localization were performed as described<sup>13</sup>.

### **Protein purification and mass spectrometry**

TAP-tagged proteins were purified essentially as described<sup>32</sup>. Briefly, yeast lysates were incubated first with IgG Sepharose, and bound proteins were eluted by cleavage with TEV protease. Next, these TEV-eluted proteins were bound to calmodulin beads and bound proteins were eluted with buffer containing 5 mM EGTA, precipitated with TCA, resuspended in SDS-sample buffer, and separated on a SDS 4-12% polyacrylamide gradient gel. Mass spectrometry was performed as described<sup>33</sup>. Western analysis was performed using polyclonal anti-Spt20, anti-Taf12 and anti-Myc antibodies. Sequence comparison was performed using ClustalW 1.8 (BCM Search Launcher; <http://dot.imgen.bcm.tmc.edu:9331/multi-align/multi-align.html>) and BOXSHADE 3.21 ([http://www.ch.embnet.org/software/BOX\\_form.html](http://www.ch.embnet.org/software/BOX_form.html)) for pretty printing and shading of multiple alignment files.

### **DNA macro-array and Northern analysis**

DNA macroarray construction will be described elsewhere (Alberola et al., in preparation). They were used as previously described<sup>34</sup>. Total RNA from logarithmically growing *sus1Δ* and isogenic wild-type cultures (for each strain three independent experiments were performed) was retrotranscribed into cDNA using <sup>33</sup>P-dCTP. Image and statistical analyses were done, respectively, with *ArrayVision* and *ArrayStat* softwares. A detailed description of the protocols is given in Supplementary information. Northern analysis was performed as described<sup>35</sup>.

### **Chromatin immunoprecipitation**

Cells containing Sus1-13Myc were grown in SC-trp medium containing 2% raffinose prior to galactose or glucose treatments as indicated. 40ml culture were treated with 1% formaldehyde and subjected to immunoprecipitation and PCR analysis essentially as described<sup>26,36</sup>, except that 15% of each whole cell extract (WCE) was used for immunoprecipitation with polyclonal Myc antibodies (Upstate Biotechnology), and 5% of each WCE was used for immunoprecipitation with anti-Rpb1 antibodies (8WG16, Covance). The *GALI* primers used in figure 4b amplify *GALI* promoter region #2 indicated in figure 4c. The reference primers amplify an intergenic region

around nt11000 on chromosome V. Other primers used for the *GAL1* gene are also indicated.

## Supplementary information

**Supplementary Table 1** Yeast strains used in this study.

Yeast strain	genotype
<i>yra1Δ ade2 ade3</i>	<i>Mat α, ade2, ade3, his3, leu2, trp1, ura3, yra1::HIS3 (pHT4467Δ-YRA1,pRS314-yra1-ΔRRM-SUB2)</i>
<i>sus1Δ</i>	<i>Mat α, ade2, ade3, his3, leu2, trp1, ura3, sus1::KanMX4</i>
<i>SUS1-TAP</i>	<i>Mat α, ade2, his3, leu2, trp1, ura3, SUS1-TAP::TRP1</i>
<i>SUS1-GFP</i>	<i>Mat α, ade2, ade3, his3, leu2, trp1, ura3, SUS1-GFP::KanMX4</i>
<i>SUS1-MYC</i>	<i>Mat α, his3, leu2, trp1, ura3, SUS1-MYC::TRP1</i>
<i>sus1Δyra1Δ</i>	<i>Mat α, ade2, his3, leu2, trp1, ura3, sus1::KanMX4, yra1::HIS3 (pRS316-YRA1)</i>
<i>sus1Δsub2Δ</i>	<i>Mat α, ade2, his3, leu2, trp1, ura3, sus1::KanMX4, sub2::KanMX4 (pRS316-SUB2)</i>
<i>sus1Δmex67Δ</i>	<i>Mat α, ade2, his3, leu2, trp1, ura3, sus1::KanMX4, mex67::HIS3 (pRS316-MEX67)</i>
<i>THP1-TAP SUS1-MYC</i>	<i>Mat α, his3, leu2, trp1, ura3, THP1-TAP::TRP1, SUS1-MYC::HIS3</i>
<i>NUP82-TAP SUS1-MYC</i>	<i>Mat α, leu2Δ1, his3Δ200, ura3-52, trp1Δ63, NUP82-TAP::TRP1, SUS1-MYC::KanMX4</i>

**Supplementary Table 2 (attached as Microsoft Excel file)** DNA marco-array analysis revealing yeast genes whose expression is affected in the *sus1Δ* strain. Ratios of expression levels between the *sus1Δ* mutant and an isogenic wild-type strain of the ~6000 yeast genes were obtained by dividing arbitrary expression units derived from three independent sets of experiments. Ratios >1 represent decreased transcript levels and ratios <1 increased transcript levels. Shown in *red* are the genes, which exhibit a statistically relevant decreased expression in *sus1Δ* cells (threshold level: 2.5-fold decrease). Shown in *green* are the genes, which exhibit a statistically relevant increased expression in *sus1Δ* cells (threshold level: 2.5-fold increase).

## Acknowledgements

We are grateful to Dr. F. Winston (Harvard Medical School, Boston, Massachusetts) for Spt20 and Dr. C. Fry (University of Massachusetts Medical School) for Taf12 and Taf6 antibodies. The excellent technical assistance of Sabine Merker and Petra Ihrig under the supervision of Dr. J. Lechner (Mass Spectrometry Unit, BZH, Heidelberg)

is acknowledged. We are grateful to Dr. Tom Maniatis for critical reading of the manuscript. E. C. H. was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 352, Leibniz-Programm) and Fonds der Chemischen Industrie, and R.R. by a grant from NIH. S. R-N. is a holder of a Marie Curie fellowship.

**Correspondence and requests for materials should be addressed to E. H. (cg5@ix.urz.uni-heidelberg.de).**

## References

1. Maniatis, T. and Reed, R. An extensive network of coupling between gene expression machines. *Nature* **416**, 499-506 (2002).
2. Proudfoot, N.J., Furger, A., and Dye, M.J. Integrating mRNA processing with transcription. *Cell* **108**, 501-512 (2002).
3. Orphanides, G. and Reinberg, D. A unified theory of gene expression. *Cell* **108**, 439-451 (2002).
4. Grant, P.A. *et al.* Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev.* **11**, 1640-1650 (1997).
5. Holstege, F.C. *et al.* Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* **95**, 717-728 (1998).
6. Moqtaderi, Z., Keaveney, M., and Struhl, K. The histone H3-like TAF is broadly required for transcription in yeast. *Mol. Cell* **2**, 675-682 (1998).
7. Lee, T.I. *et al.* Redundant roles for the TFIID and SAGA complexes in global transcription. *Nature* **405**, 701-704 (2000).
8. Bhaumik, S.R. and Green, M.R. SAGA is an essential *in vivo* target of the yeast acidic activator Gal4p. *Genes Dev.* **15**, 1935-1945 (2001).
9. Brown, C.E. *et al.* Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. *Science* **292**, 2333-2337 (2001).
10. Larschan, E. and Winston, F. The *S. cerevisiae* SAGA complex functions *in vivo* as a coactivator for transcriptional activation by Gal4. *Genes Dev.* **15**, 1946-1956 (2001).
11. Reinke, H., Gregory, P.D., and Horz, W. A transient histone hyperacetylation signal marks nucleosomes for remodeling at the PHO8 promoter *in vivo*. *Mol. Cell* **7**, 529-538 (2001).
12. Wu, P.Y. and Winston, F. Analysis of Spt7 function in the *Saccharomyces cerevisiae* SAGA coactivator complex. *Mol. Cell Biol.* **22**, 5367-5379 (2002).
13. Sträßer, K. and Hurt, E.C. Yra1p, a Conserved Nuclear RNA Binding Protein, Interacts Directly with Mex67p and is Required for mRNA Export. *EMBO J.* **19**, 410-420 (2000).
14. Stutz, F. *et al.* REF, an evolutionarily conserved family of hnRNP-like proteins, interacts with TAP/Mex67p and participates in mRNA nuclear export. *RNA* **6**, 638-650 (2000).

15. Zhou, Z.L. *et al.* The protein Aly links pre-messenger-RNA splicing to nuclear export in metazoans. *Nature* **407**, 401-405 (2000).
16. Luo, M.-J. *et al.* Pre-mRNA splicing and mRNA export linked by direct interactions between UAP56 and Aly. *Nature* **413**, 644-647 (2001).
17. Rodrigues, J.P. *et al.* REF proteins mediate the export of spliced and unspliced mRNAs from the nucleus. *Proc. Natl. Acad. Sci. USA* **98**, 1030-1035 (2001).
18. Sträßer, K. and Hurt, E.C. Splicing factor Sub2p is required for nuclear export through its interaction with Yra1p. *Nature* **413**, 648-652 (2001).
19. Reed, R. and Hurt, E.C. A conserved mRNA export machinery coupled to pre-mRNA splicing. *Cell* **108**, 523-531 (2002).
20. Gavin, A.-C. *et al.* Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* **415**, 141-147 (2002).
21. Sanders, S.L., Jennings, J., Canutescu, A., Link, A.J., and Weil, P.A. Proteomics of the eukaryotic transcription machinery: identification of proteins associated with components of yeast TFIID by multidimensional mass spectrometry. *Mol. Cell Biol.* **22**, 4723-4738 (2002).
22. Fischer, T. *et al.* The mRNA export machinery requires the novel Sac3p-Thp1p complex to dock at the nucleoplasmic entrance of the nuclear pores. *EMBO J.* **21**, 5843-5852 (2002).
23. Lei, P. *et al.* Sac3 Is an mRNA Export Factor That Localizes to Cytoplasmic Fibrils of Nuclear Pore Complex. *Mol. Biol. Cell* **14**, 836-847 (2003).
24. Grant, P.A., Schieltz, D., Pray-Grant, M.G., Yates, J.R., and Workman, J.L. The ATM-related cofactor Tra1 is a component of the purified SAGA complex. *Mol. Cell* **2**, 863-867 (1998).
25. Georgieva, S. *et al.* The novel transcription factor e(y)2 interacts with TAF(II)40 and potentiates transcription activation on chromatin templates. *Mol. Cell Biol.* **21**, 5223-5231 (2001).
26. Sträßer, K. *et al.* TREX is a conserved complex coupling transcription with messenger RNA export. *Nature* **417**, 304-308 (2002).
27. Lei, E.P., Krebber, H., and Silver, P.A. Messenger RNAs are recruited for nuclear export during transcription. *Genes Dev.* **15**, 1771-1782 (2001).
28. Lei, E.P. and Silver, P.A. Intron status and 3'-end formation control cotranscriptional export of mRNA. *Genes Dev.* **16**, 2761-2766 (2002).
29. Zenklusen, D., Vinciguerra, P., Wyss, J.C., and Stutz, F. Stable mRNP Formation and Export Require Cotranscriptional Recruitment of the mRNA Export Factors Yra1p and Sub2p by Hpr1p. *Mol. Cell Biol.* **22**, 8241-8253 (2002).

30. Fan, H.Y., Merker, R.J., and Klein, H.L. High-Copy-Number Expression of Sub2p, a Member of the RNA Helicase Superfamily, Suppresses hpr1-Mediated Genomic Instability. *Mol. Cell. Biol.* **21**, 5459-5470 (2001).
31. Piruat, J.I. and Aguilera, A. A novel yeast gene, THO2, is involved in RNA pol II transcription and provides new evidence for transcriptional elongation-associated recombination. *EMBO J.* **17**, 4859-4872 (1998).
32. Rigaut, G. *et al.* A generic protein purification method for protein complex characterization and proteome exploration. *Nature Biotech.* **17**, 1030-1032 (1999).
33. Baßler, J. *et al.* Identification of a 60S pre-ribosomal particle that is closely linked to nuclear export. *Mol. Cell* **8**, 517-529 (2001).
34. Hauser, N.C. *et al.* Transcriptional profiling on all open reading frames of *Saccharomyces cerevisiae*. *Yeast* **14**, 1209-1221 (1998).
35. Rodriguez-Navarro, S., Sträßer, K., and Hurt, E. An intron in the YRA1 gene is required to control Yra1 protein expression and mRNA export in yeast. *EMBO Rep.* **3**, 438-442 (2002).
36. Kuras, L. and Struhl, K. Binding of TBP to promoters in vivo is stimulated by activators and requires Pol II holoenzyme. *Nature* **399**, 609-613 (1999).

**Table 1** Genes repressed or induced in *sus1Δ* and SAGA mutants. **a**, number of genes, whose expression is increased or decreased in the *sus1Δ* strain (see Supplementary information 2) and in several SAGA mutants. Except for *sus1Δ*, the numbers were obtained from the ChipDB (<http://staffa.wi.mit.edu/chipdb/public/index.html>). **b**, Overlap between genes, which are commonly repressed in *sus1Δ* and several SAGA mutants. Numbers were obtained by using the “compare set of genes” tool from <http://staffa.wi.mit.edu/chipdb/public/index.html> for *spt3*, *spt20* and *gcn5* mutants and comparing them with our *sus1Δ* DNA macro-array data (for a detailed list see Supplementary information 2).

**a**

<b>Mutant</b>	<b>sus1</b>	<b>spt20</b>	<b>spt3</b>	<b>gcn5</b>	<b>taf9</b>	<b>taf6</b>	<b>taf12</b>	<b>taf5</b>
<b>Number of genes increased</b>	208	35	8	11	22	42	51	164
<b>Number of genes decreased</b>	341	416	79	108	1050	1574	661	269
<b>% of genes regulated</b>	9	7.5	1.5	1.8	18	26	12	7.2

**b**

<i>Mutant</i>	<b>Overlapping genes</b>
spt3+gcn5	13
spt3+spt20	45
spt20+gcn5	26
sus1+spt3	9
sus1+gcn5	17
sus1+spt20	34
spt3+spt20+gcn5	8
sus1+spt20+spt3+gcn5	3

## Figure legends

**Figure 1** *SUS1* interacts genetically with genes encoding components of the conserved mRNA export machinery. **a**, A drawing of the *SUS1* gene locus on the “Watson strand” of chromosome 2 between the *YSA1* and *SSN6* genes. *SUS1* pre-mRNA consists of 3 exons (E1, 2,3) and 2 introns (I1,2), and is spliced to generate *SUS1* mRNA (E1-E2-E3). **b**, Detection of the *SUS1* mRNA by Northern analysis. Total RNA from wild-type and *sus1Δ* was analyzed by agarose-formaldehyde gel electrophoresis. The oligonucleotides used for the Northern were specific for either *SUS1* or actin (*ACT1*) mRNA. The *SUS1* RNA has an apparent size of ~320 nts. **c**, Amino acid sequence of Sus1 and alignment with orthologues from *Arabidopsis* (arabidop), human (human), *Drosophila* (ey2dros) and *Candida tropicalis* (candida). **d**, Growth of the *sus1Δ* and wild-type yeast cells. Cells were diluted in 10<sup>-1</sup> steps, and equivalent amount of cells were spotted onto YPD plates. It was grown for 3 days at the temperatures indicated. **e**, Synthetic lethality of *sus1Δ* with *yra1-ΔRRM*, *sub2-85* and *mex67-5*. Double shuffle strains *sus1Δ/yra1Δ*, *sus1Δ/sub2Δ* and *sus1Δ/mex67Δ* were transformed with an empty plasmid (pUN100) or plasmids encoding *SUS1*, *YRA1*, *SUB2* and *MEX67*, respectively. Transformants were streaked onto 5-FOA containing plates, which were incubated at 23°C for 5 days. No growth indicates synthetic lethality.

**Figure 2** *sus1* mutants are defective in mRNA export. **a**, Wild-type yeast (wt), *sus1Δ* and sl9 strains, transformed with an empty or *SUS1*-containing plasmid were shifted for 90 minutes to 37°C. The localization of poly(A)<sup>+</sup> RNA was assessed by *in situ* hybridization with a Cy3-labeled oligo(dT) probe. DNA was stained with DAPI. **b**, Sus1 is localized to the nucleus. The *in vivo* location of the indicated Sus1-GFP fusion protein was analyzed in the fluorescence microscope.

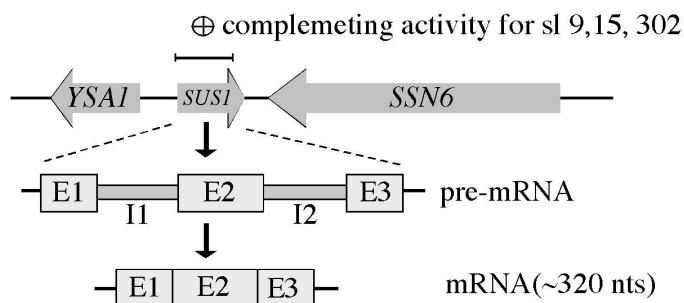
**Figure 3** Association of Sus1 with the SAGA complex and Sac3-Thp1. **a**, TAP-tagged Sus1 was isolated from yeast by two step-affinity purification. The first TEV-protease eluate and the second EGTA eluate are shown. Purified proteins were separated on an 4-12% gradient SDS-polyacrylamide gel and visualized by Coomassie staining. The Sus1 protein migrates at ~15 kDa on the gel (note that Sus1 carries the ~5 kDa long CBP-tag). Co-purifying proteins were identified by mass



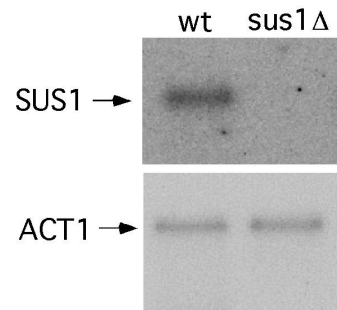
spectrometry and are indicated. Ribosomal protein contaminants were also found and are labeled with closed circles. **b**, TAP-tagged Thp1 (lane 1 and 2) and Nup82 (lane 3 and 4) were isolated from yeast expressing Sus1-13myc (lane 1 and 3) or Sus1 (lane 2 and 4) by two step-affinity purification. Purified proteins were separated on an 4-12% gradient gel and visualized by Coomassie staining. The co-purifying protein band at ~32kDa was identified by mass spectrometry to be Sus1-13myc. The star (\*) indicates a contaminating ribosomal protein band in TAP-purifications. **c**, Western analysis of the TAP-purified Thp1 (lane 1 and 2) and Nup82 (lane 3 and 4) using antibodies against the SAGA components Spt20 and Taf12.

**Figure 4** Sus1 is required for gene expression. **a**, Northern analysis of total RNA to detect *PHO84* transcripts in *sus1Δ*, *gcn5Δ*, *spt7Δ* and wild-type cells in comparison to the *PGK1* signal. RNA loading was controlled by showing rRNA levels. **b**, Sus1 associates with the *GALI* promoter upon transcription activation. Cells containing Sus1-13myc were grown in raffinose (lanes 1, 4, 7), raffinose followed by 2 hour galactose induction (lanes 2, 5, 8), or after galactose induction followed by 15 min of glucose incubation (lanes 3, 6, 9). Chomatin immunoprecipitations were then carried out with Myc or Pol II antibodies, and PCR analysis was performed using the indicated primer sets. Ref, reference (see Methods). **c**, Sus1 specifically associates with the *GALI* promoter region. Cells containing Sus1-13myc were grown in raffinose followed by 3 hours of galactose induction. Chomatin immunoprecipitations were then carried out with a Myc antibody and PCR was performed with the indicated primers. Immunoprecipitations were quantified and displayed as the fold enrichment of Sus1 association with the *GALI* gene relative to that with an intergenic region.

a



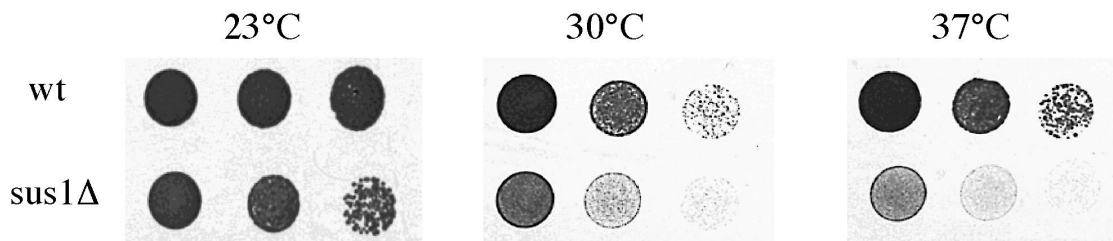
b



c

arabidop	1	----	VADGF	EKDKVT	LREI	I	NV	KL	VES	GE	KEN	I	MEL	V	R	D	R	L	V	E	C	G	W	K	D	E	M	R	I	A	C	R	E	H	V	K	K																							
human	1	----	MV	SK	M	N	K	D	A	Q	M	R	A	I	N	Q	K	L	I	E	T	G	E	R	E	R	K	E	L	L	R	A	K	L	I	E	C	G	W	K	D	Q	L	K	A	H	C	K	E	V	I	K								
ey2dros	1	-----	M	S	T	S	G	A	V	D	Q	Y	T	V	L	T	G	D	R	S	K	I	K	D	L	L	C	S	R	L	T	E	C	G	W	R	D	E	V	R	L	M	C	R	N	I	L	M												
candida	1	M	S	T	N	N	S	T	Q	Q	Q	D	E	L	D	Q	I	K	S	K	I	Q	D	N	L	I	S	S	G	N	Y	D	I	I	N	K	Q	L	K	L	Q	L	Y	E	S	G	W	Y	D	K	V	S	Q	I	A	S	R	E	L	M
Sus1	1	-----	M	T	M	D	T	A	Q	L	K	S	Q	I	Q	Q	Y	L	V	E	S	G	N	Y	E	L	I	S	N	E	L	K	A	R	L	L	Q	E	G	W	V	D	K	V	K	D	L	T	K	S	E	M	N							
arabidop	57	K	G	R	K	D	-----	-----	V	T	V	D	E	L	I	R	V	I	T	P	K	G	R	A	S	V	P	D	S	V	K	A	E	L	L	N	R	I	Q	N	F	I	V	S	A	A	L													
human	57	K	G	L	E	H	-----	-----	V	T	V	D	D	L	V	A	E	I	T	P	K	G	R	A	L	V	P	D	S	V	K	K	E	L	L	Q	R	I	R	T	F	L	A	Q	H	A	S	L												
ey2dros	48	K	G	T	N	N	S	-----	-----	F	T	V	E	Q	L	I	A	E	V	T	P	K	A	R	T	L	V	P	D	A	V	K	K	E	L	L	M	K	I	R	T	I	L	T	E	I	E	E	P	D	E	P	E	D	E					
candida	61	H	Q	Q	E	V	N	S	S	N	S	N	S	N	S	N	S	N	K	K	N	E	L	T	F	D	Q	L	E	A	F	V	K	P	K	A	E	E	L	V	P	N	E	V	K	Q	D	I	L	N	R	I	T	K	Y	L	D	D	I	Q
Sus1	53	N	E	S	T	N	-----	-----	F	T	Q	I	L	S	T	V	E	P	K	A	L	E	M	V	S	D	S	T	R	E	T	V	L	K	Q	I	R	E	F	L	E	E	I	V	D	T	Q													

d



e

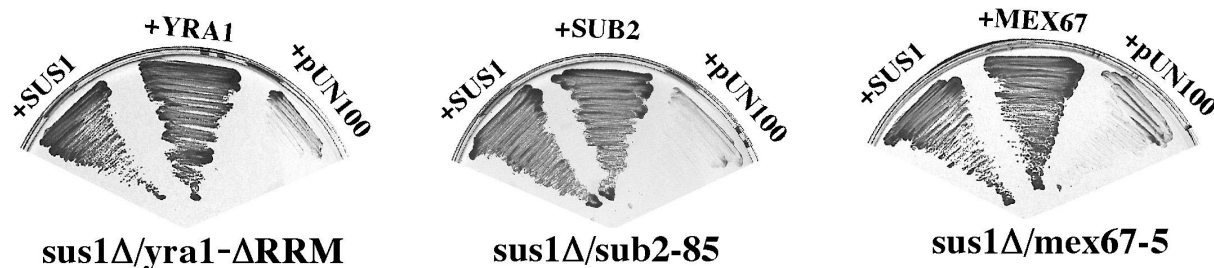


Fig. 1 (Rodriguez-Navarro et al., 2003)

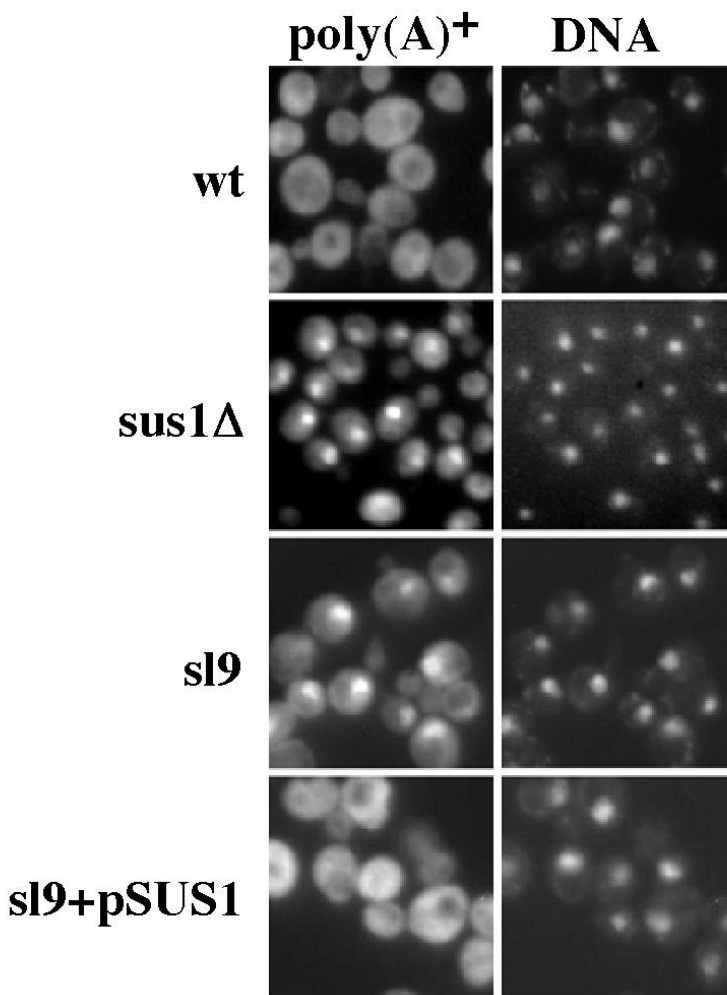
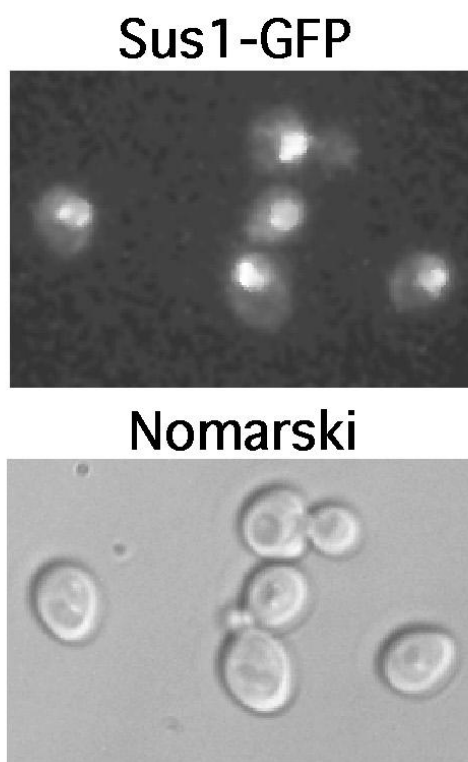
**a****b**

Fig. 2 (Rodriguez-Navarro et al., 2003)

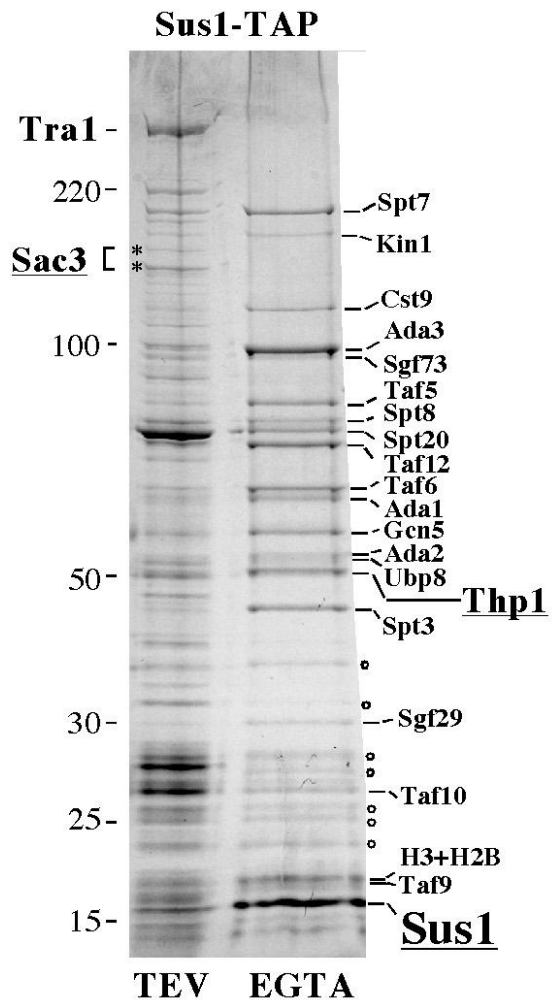
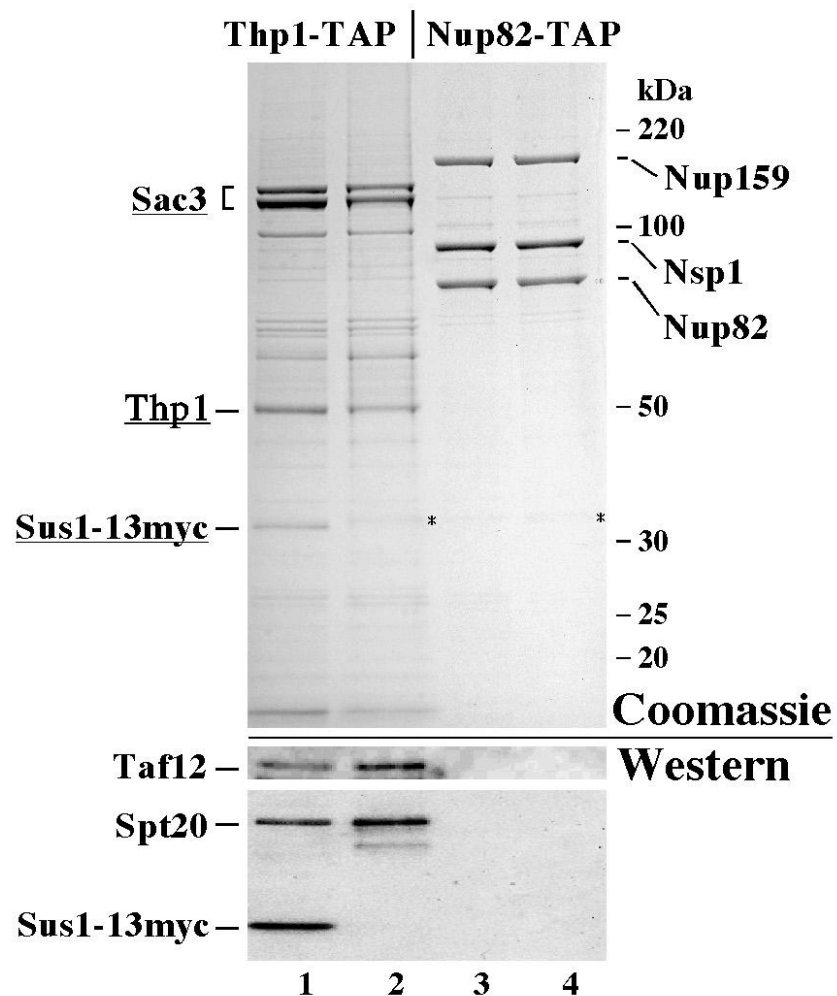
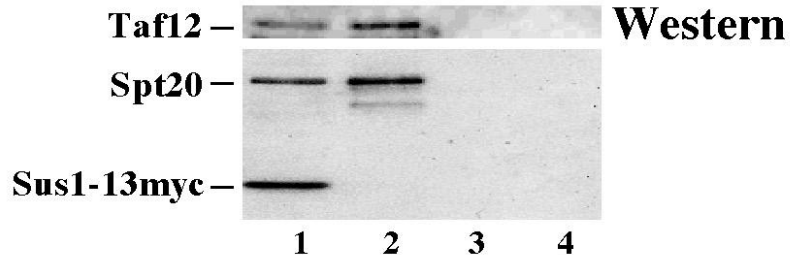
**a****b****c**

Fig. 3 (Rodriguez-Navarro et al., 2003)

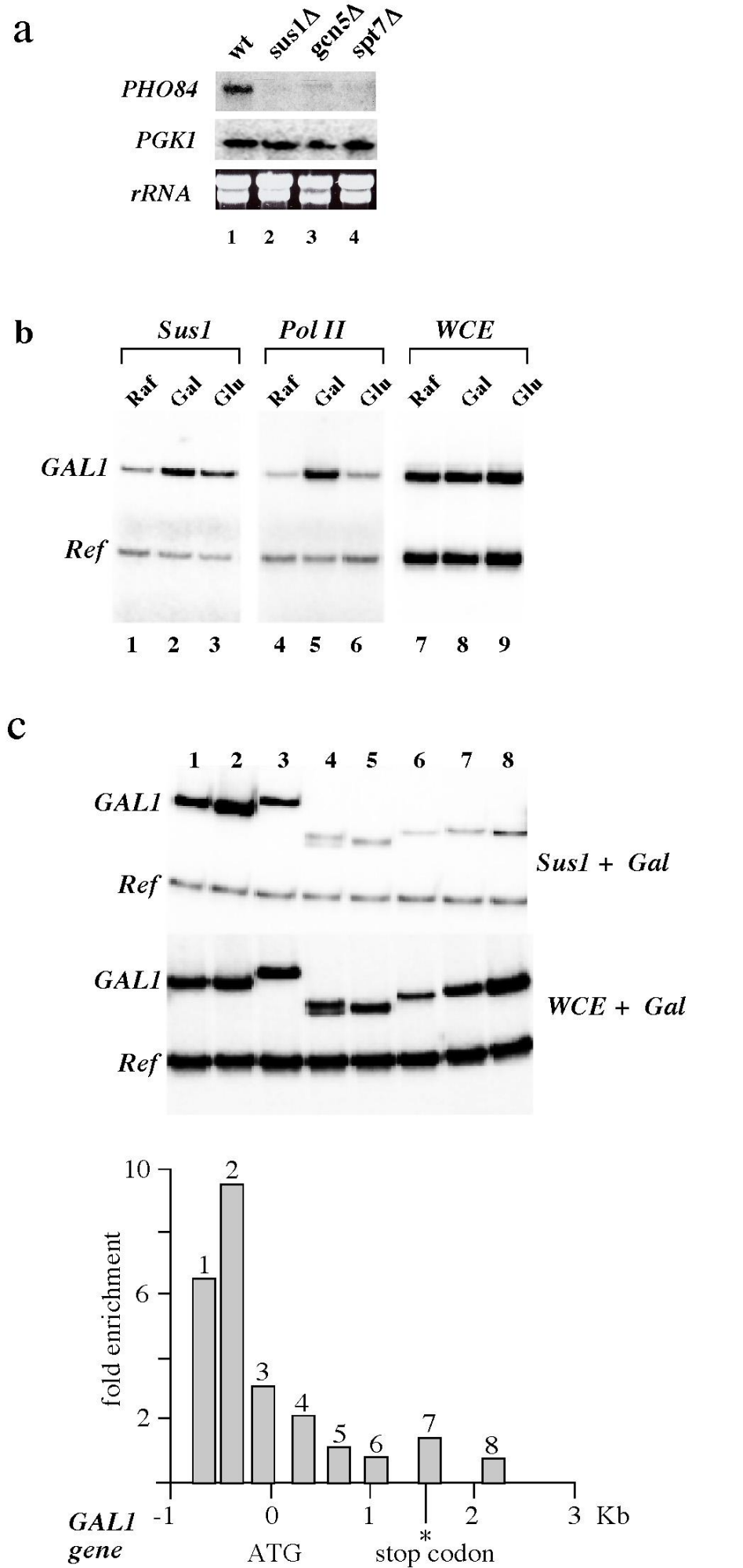


Fig. 4 (Rodríguez-Navarro et al., 2003)