Acknowledgements We thank M. Kobayashi, D. Engel, M. Muramatsu and T. Honjo for discussion and comments on the manuscript, M. Busslinger for information on RT–PCR analysis, N. Kaneko for the preparation of tissue sections, and M. Kanno for the use of FACS machinery. This work was supported by Grants-in-aid from the Ministry of Education, Culture, Sport, Science and Technology of Japan and Hiroshima University 21st century COE programme. A.M. was initially supported by the Research Fellowships for Young Scientists from the Japanese Society for Promotion of Science.

Competing interests statement The authors declare that they have no competing financial interests.

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Intergenic transcription is required to repress the *Saccharomyces cerevisiae SER3* gene

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Transcription by RNA polymerase II in *Saccharomyces cerevisiae* and in humans is widespread, even in genomic regions that do not encode proteins[1–6]. The purpose of such intergenic transcription is largely unknown, although it can be regulatory[7,8]. We have discovered a role for one case of intergenic transcription by studying the *S. cerevisiae SER3* gene. Our previous results demonstrated that transcription of *SER3* is tightly repressed during growth in rich medium[9]. We now show that the regulatory region of this gene is highly transcribed under these conditions and produces a non-protein-coding RNA (*SRG1*). Expression of the *SRG1 RNA* is required for repression of *SER3*. Additional experiments have demonstrated that repression occurs by a transcription-interference mechanism in which *SRG1* transcription across the *SER3* promoter interferes with the binding of activators. This work identifies a previously unknown class of transcriptional regulatory genes.

The *S. cerevisiae SER3* gene encodes a phosphoglycerate dehydrogenase that catalyses a step in serine biosynthesis[10]. To investigate *SER3* repression in greater detail, we performed chromatin immunoprecipitation (ChIP) experiments to test for the association of general transcription factors with the *SER3* regulatory region *in vivo*. Surprisingly, our results showed that under repressing conditions, a significant level of both TATA-binding protein (TBP) and RNA polymerase II (Pol II) bind 5′ of the *SER3* TATA element (Fig. 1a). Additional ChIP experiments showed that other factors, including phosphorylated forms of Pol II and mRNA capping factors, are also associated with this region (unpublished data). Thus, when *SER3* is repressed, its regulatory region is associated with factors required for active transcription. As described below, this region contains a second TATA element (shown in Fig. 1a).

To determine whether transcription occurs in the *SER3* regulatory region, we performed transcription run-on experiments. These results showed that there is a high level of active transcription (Fig. 1b, probes 3–5). This transcription occurs on the same strand as *SER3* as determined by its hybridization specificity for single-stranded probes.

We gained additional insight into the transcription occurring 5′ of *SER3* by comparing the DNA sequence of this region to that of four yeasts closely related to *S. cerevisiae*[11,12]. This analysis (Supplementary Fig. 1) revealed that, in addition to the TATA element proximal to the SER3 coding region (−103 relative to the *SER3* ATG), there is a second TATA element at −558 that is perfectly conserved in all five yeasts. Short conserved sequences 5′ of this TATA element suggest the presence of regulatory sites. Within the transcribed region, there are three conserved sequence motifs between −262 and −156. Deletion analysis strongly suggests that this region functions as a *SER3* upstream activating sequence (UAS) (Supplementary Fig. 2). Outside of these sequence elements there is no significant conservation or open reading frame in this region, suggesting that it does not encode a protein. Because this region is transcribed and, as will be shown, has an identified function, we have designated it as the *SRG1* gene (*SER3* regulatory gene 1). We will refer to the TATA element at −558 as the *SRG1* TATA element.

To test for a role for *SRG1* in *SER3* repression, we constructed a mutation in the *SRG1* TATA sequence, from TATAAA to CCTAGG (*srg1-1*). By northern analysis, the *SRG1* transcript in a wild-type strain appears primarily as a diffuse band of approximately 550 bases (Fig. 2a). A longer RNA transcript is also present at a lower level whose length is consistent with initiation at the *SRG1* initiation site and readthrough across *SER3*. In the *srg1-1* TATA mutant, both RNAs are undetectable. Significantly, *SER3* transcription is derepressed to a high level in this mutant, demonstrating an

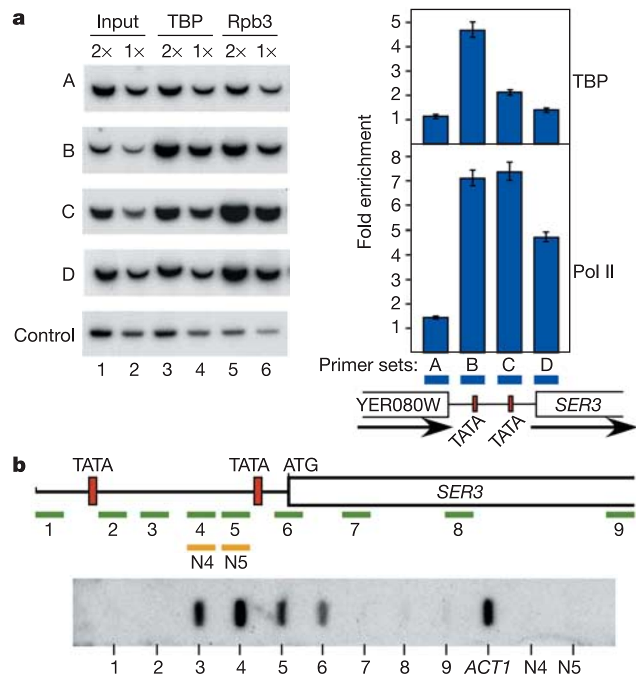

Figure 1 Evidence for active transcription 5′ of *SER3*. **a**, ChIP analysis at *SER3*. ChIP analysis of TBP and Pol II (Rpb3) was performed on wild-type strain FY2245 grown in repressing conditions (YPD medium). A representative set of PCR reactions from two-fold dilutions of chromatin is shown. The regions amplified by PCR (A–D) are marked by blue bars in the diagram of the *SER3* promoter on the right. The control primer set amplifies a region of chromosome V that lacks open reading frames[25]. The graphs summarize the results of three independent experiments, with each value representing the average and standard error of the fold enrichment (ratio of the percentage of *SER3* immunoprecipitated to the percentage of the control region immunoprecipitated). **b**, Transcription run-on analysis at *SER3*. Transcription run-on analysis was performed on wild-type strain FY2097 grown in repressing conditions. A schematic of *SER3* is shown, with the green bars representing antisense oligonucleotides that detect transcription from the Watson strand. The two yellow bars (N4, N5) represent sense oligonucleotides used as negative controls. An oligonucleotide that detects transcription of *ACT1* was included as a positive control.

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essential role for *SRG1* in the repression of *SER3*.

Characterization of the *SRG1* transcript was achieved through several experiments. First, primer-extension analysis showed that the *SRG1* RNA has discrete 5′ ends positioned 78 and 79 bases 3′ of the *SRG1* TATA (Fig. 2b). Second, to test whether the *SRG1* RNA is polyadenylated, we compared the levels of poly(A) enriched and total RNA for *SRG1*, *ACT1* (a polyadenylated control) and *SNR190* (a non-polyadenylated control[13]). Our results (Supplementary Fig. 3) show that *SRG1* is significantly enriched in the poly(A) sample, which strongly suggests that it is polyadenylated. Third, to test whether the presence of TBP and Pol II in the *SER3* 5′ region (Fig. 1a) is due to their association with the actively transcribed *SRG1* gene, we performed ChIP experiments, comparing wild-type and *srg1-1* strains. These results show that the level of both TBP and Pol II are significantly decreased over the *SRG1* TATA region (region B) in the *srg1-1* mutant (Fig. 2c). The levels are not reduced over regions C and D, owing to the derepression of *SER3* in the *srg1-1* mutant. Taken together, our results identify *SRG1* as a Pol-II-dependent, highly expressed, non-coding RNA (Fig. 2d).

We considered three models for the role of *SRG1* in *SER3* repression: an RNA-mediated model, a promoter-competition model and a transcription-interference model. In the RNA-mediated model, the *SRG1* RNA product itself is required for repression of *SER3*, perhaps by interacting with proteins. There are several precedents for RNA-mediated control of transcription, including X chromosome inactivation in mammals and dosage compensation in *Drosophila*[14]. In the promoter-competition model, the *SRG1* promoter outcompetes that of *SER3* for transcription factors, inhibiting activation of *SER3*. Evidence for this mechanism has been previously described[15,16]. In the transcription-interference model, transcription of *SRG1* across the *SER3* promoter region blocks the binding of transcription factors necessary for *SER3* activation. Transcription interference has previously been shown to occur either as a regulatory mechanism or as a consequence of deletion of the transcription terminator between genes[17–23].

First, if the *SRG1* RNA product functions in repression, it may act in *trans*. Therefore, we tested the ability of *SRG1* to repress when it is located either in *cis* or in *trans* to *SER3*. We constructed diploid strains in which one copy of the *SER3*-coding sequence was replaced by the sequence for orotidine-5′-phosphate decarboxylase (*URA3*) (*ser3Δ::URA3*), allowing expression from each *SER3* promoter to be assayed. One diploid had the *srg1-1* mutation adjacent to wild-type *SER3* (Fig. 3a, FY2254) and the other had *srg1-1* adjacent to *ser3Δ::URA3* (Fig. 3a, FY2255). Northern analysis of these strains demonstrates that wild-type *SRG1* represses the *SER3* promoter only when it is in *cis* (Fig. 3a). These results rule out the possibility that the *SRG1* RNA product represses transcription as a *trans*-acting factor, although they do not rule out an RNA-mediated model in which the RNA product acts in *cis*.

Next, we tested the promoter-competition model using two derivatives of *SRG1*. In each construct, we replaced 150 base pairs

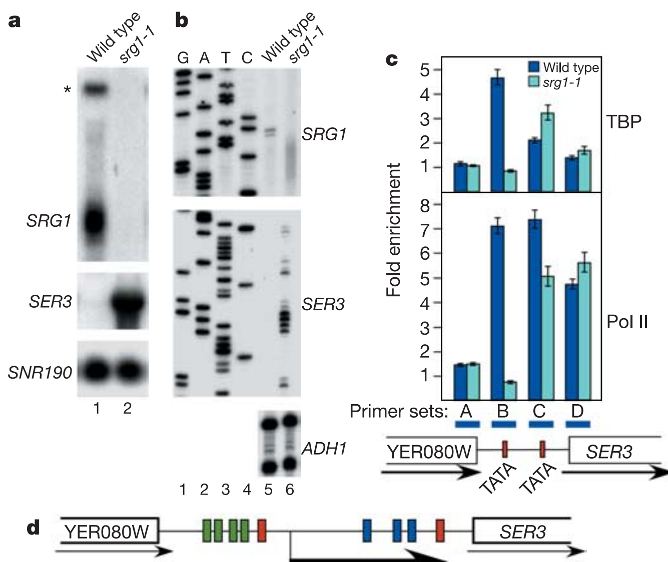

Figure 2 Characterization of *SRG1* transcription. **a**, Northern analysis of *SRG1* and *SER3* was performed on wild-type (FY2097) and *srg1-1* (FY2250) strains. *SNR190* was a loading control. The asterisk marks a minor RNA product of approximately 2 kb that is consistent with a readthrough product from the *SRG1* promoter to the end of *SER3*. This RNA is also detected with a *SER3* probe after long exposure (data not shown). **b**, Primer-extension analysis of *SRG1* and *SER3* was performed on wild-type (FY2256) and *srg1-1* (FY2247) strains. The two transcription start sites for *SRG1* (lane 5; top panel) map to 78 and 79 bases 3′ of the *SRG1* TATA. *SER3* transcription initiates at multiple sites within a 28 base region that begins 52 bases 3′ of the *SER3* TATA (lane 6, middle panel). The level of *ADH1* mRNA was measured as a control for the amount of RNA used in each reaction (bottom panel). **c**, ChIP analysis for TBP and Pol II (Rpb3) performed on wild-type (FY2245) and *srg1-1* (FY2247) strains. The values shown are calculated from three independent experiments as described in the legend of Fig. 1a. **d**, A diagram of *SRG1*. The dark arrow represents the approximate position of the *SRG1* transcript. The coloured boxes represent conserved sequences in this region. The red boxes are TATA elements, the green boxes are putative UAS elements for *SRG1*, and the blue boxes are putative UAS elements for *SER3* (see Supplementary Fig. 2).

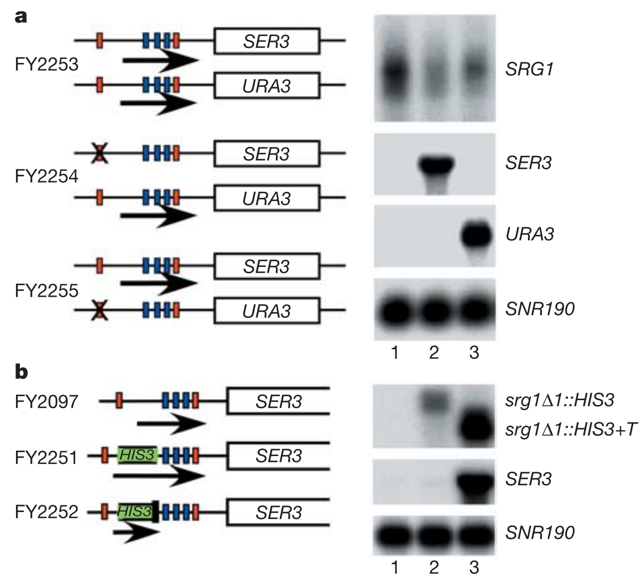

Figure 3 Tests of models for *SRG1* function. **a**, A *cis/trans* test for *SRG1* function. The left part of the figure shows a diagram of the *SER3* alleles of the three diploids used in the experiment (FY2253, FY2254 and FY2255). The alleles that contain the *srg1-1* TATA mutation are indicated by an X over the *SRG1* TATA box. The black arrows represent *SRG1* transcripts. The right part is a northern analysis of each diploid for *SRG1*, *SER3* and *URA3* RNA levels, with *SNR190* as a loading control. **b**, A test of the promoter-competition model. The left part shows a diagram of the *SRG1* allele (FY2097) and two different *HIS3* insertions (the green boxes): one without (FY2251) and one with its terminator sequence (black box; FY2252). The red boxes represent the *SRG1* and *SER3* TATAs. The blue boxes represent three sequence motifs conserved between multiple yeast strains that may serve as a *SER3* UAS (see Supplementary Fig. 1). The black arrows represent transcripts initiated from the *SRG1* promoter. On the right is a northern analysis of *SRG1*, *SER3* and *SNR190* (loading control) from the strains shown on the left. The transcripts generated from the *SRG1* promoter were detected using a probe for *HIS3* (lanes 2 and 3; top panel).

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(bp) of *SRG1* sequence with the *HIS3* reporter gene coding sequence such that the putative *SER3* UAS remained intact (Fig. 3b). In one construct (FY2251), the *HIS3* transcription-termination region was not included, allowing *SRG1* transcription to elongate across the *SER3* UAS as in a wild-type strain. In the other construct (FY2252), the *HIS3* transcription-termination sequence was included, thereby terminating *SRG1* transcription 5′ of the *SER3* promoter. Northern analysis confirmed that *SRG1* transcription behaved as expected in each *HIS3* insertion (Fig. 3b). When *SER3* mRNA levels were examined, a striking difference was observed between the two. For the construct lacking the *HIS3* terminator, normal *SER3* repression was observed, showing that the substitution of 150 bp of *SRG1* with *HIS3* did not impair repression (Fig. 3b, lane 2). By contrast, when the *HIS3* terminator was included, *SER3* repression was abolished (Fig. 3b, lane 3). Because the *SRG1* promoter is fully functional in the latter construct, but *SER3* is derepressed, we conclude that repression does not occur by promoter competition. These results also support the transcription-interference model, because termination of *SRG1* transcription 5′ of the *SER3* UAS causes derepression of *SER3*.

Finally, we performed experiments to test directly the transcription-interference model. Because the direct activators of *SER3* have not been identified, we constructed strains in which the *SER3* UAS was replaced by Gal4-binding sites (Fig. 4a). In such strains we could assay the binding of a known transcription factor, Gal4. Our results show that in these constructs, *SER3* transcription is repressed even when cells are grown under inducing conditions for Gal4 activation, demonstrating that *SRG1*-dependent repression is intact (Fig. 4b, lane 2). By contrast, in an *srg1-1* mutant, *SER3* transcription is strongly galactose inducible (Fig. 4b, lane 4). The *GAL1* gene serves as a control for galactose induction. We then used ChIP to measure the level of Gal4 bound *in vivo* to the *SER3* promoter in these constructs. Our results show that Gal4 binding is low when it is adjacent to wild-type *SRG1* (Fig. 4c, lanes 3 and 4; Supplementary Fig. 5a), but it is high when adjacent to *srg1-1* (Fig. 4c, lanes 7 and 8; Supplementary Fig. 5a). Thus, *SRG1* transcription inhibits Gal4 binding, providing direct support for a transcription-interference model.

As a second test of the interference model, we integrated the *SRG1* and *srg1-1* promoters at the *GAL7* locus, 5′ of the *GAL7* UAS (Fig. 4d), and analysed both *GAL7* mRNA levels and the binding of Gal4. Transcription from the *SRG1* promoter, as detected by a *GAL7* 5′ probe, appears to extend across *GAL7*, probably because the *SRG1* terminator is not present (Fig. 4e, lanes 3 and 4). Northern analysis shows that the *SRG1* promoter represses *GAL7* transcription (Fig. 4e, lane 4), consistent with the Gal$^-$ phenotype observed for this strain. By contrast, the *srg1-1* mutant promoter has no detectable effect on *GAL7* expression (Fig. 4e, lane 6). ChIP analysis demonstrates that binding of Gal4 to the *GAL7* UAS is significantly reduced in the presence of the wild-type *SRG1* promoter but not in the presence of *srg1-1* (Fig. 4f, compare lanes 7 and 8 with lanes 11 and 12; Supplementary Fig. 5b). Thus, transcription from the *SRG1* promoter is sufficient to interfere with the binding of Gal4. Because only 31 bases of the *SRG1* transcript are included in this construct, these results also provide strong evidence against the RNA-mediated model. These results are similar to previous studies that demonstrated that interference with Gal4 binding at *GAL7* was caused by aberrant transcription readthrough from the *GAL10* gene[20].

Several aspects of *SRG1* repression remain to be elucidated,

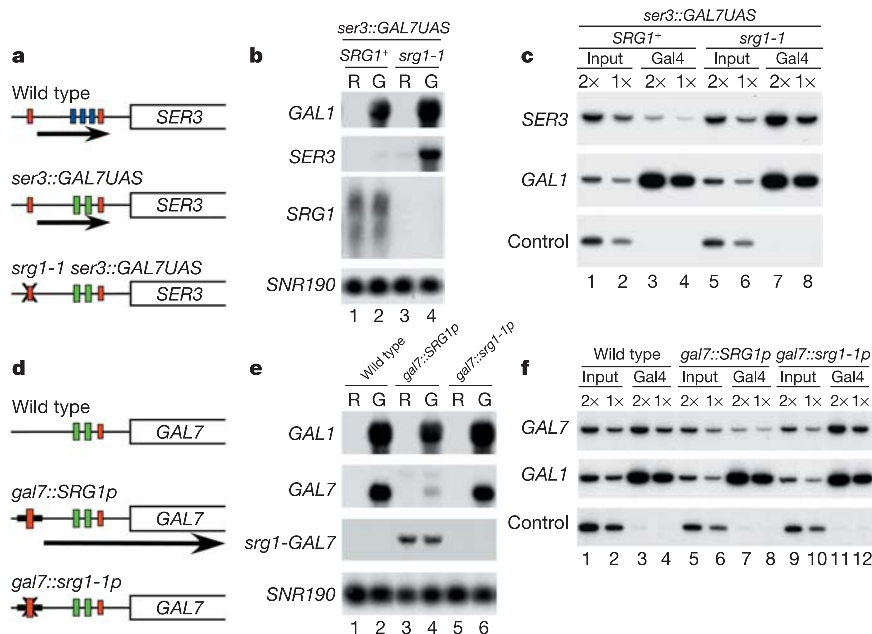

Figure 4 Two tests for transcription interference by *SRG1*. **a**, A diagram of a wild-type *SER3* allele (FY2097) and two *ser3::GAL7UAS* alleles in which the *GAL7* UAS region (with two Gal4-binding sites (green boxes)) has replaced the putative *SER3* UAS region (blue boxes) 3′ of either the wild-type *SRG1* promoter (FY2259) or the *srg1-1* TATA mutant (FY2260). **b**, Northern analysis of *GAL1* (control for Gal4 activation), *SRG1*, *SER3* and *SNR190* (loading control) from the strains described in **a**. Strains were grown in media that is non-inducing (raffinose, R) and inducing (galactose, G) for Gal4 activity. **c**, ChIP analysis of Gal4 in the strains described in panel **a** that were grown in media containing galactose. Gal4 binding was measured at the Gal4-binding sites inserted at *SER3* (top panel), at *GAL1* as a positive control (middle panel) and at a region of chromosome V that lacks open reading frames as a negative control (bottom panel). Quantification of the results is shown in Supplementary Fig. 5. **d**, A diagram of a wild-type *GAL7* allele (FY2256) and two *gal7* mutants in which either the wild-type *SRG1* promoter (FY2257; *gal7::SRG1p*) or the *srg1-1* TATA mutant promoter (FY2258; *gal7::srg1-1p*) is integrated 5′ of the Gal4-binding sites (green boxes) in *GAL7*. **e**, Northern analysis of *GAL1* (a control for Gal4 activation), *GAL7*, *srg1-GAL7* and *SNR190* (loading control) from the strains described in panel **d**. Strains were grown in media that are non-inducing (raffinose, R) and inducing (galactose, G) for Gal4 activity. **f**, ChIP analysis of Gal4 binding at *GAL7* in the strains described in panel **d** that were grown in media containing galactose. Gal4 binding was measured at *GAL7* (top panel), at *GAL1* as a positive control (middle panel) and at a region of chromosome V that lacks open reading frames as a negative control (bottom panel). Quantification of the results is shown in Supplementary Fig. 5.

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including its relationship with other *SER3* regulatory factors. Previous studies demonstrated that the Swi/Snf nucleosome-remodelling complex is also required for repression of *SER3*[9]. Thus, one possibility is that Swi/Snf activates *SRG1* transcription, conferring *SER3* repression. However, northern analysis (Supplementary Fig. 4) shows that *SRG1* RNA levels are only reduced two-fold in a *snf2Δ* mutant, indicating that the roles of Swi/Snf and *SRG1* in *SER3* repression may be independent. In addition, the expression and role of *SRG1* during *SER3* derepression by amino-acid starvation awaits further analysis.

Our studies have identified the first case of an intergenic transcript that represses by transcription interference. Given the large number of non-coding RNAs present in many organisms, including *Escherichia coli, S. cerevisiae* and humans[1–6,24], this mechanism of transcriptional control may be widespread. □

Methods

S. cerevisiae strains and media

All *S. cerevisiae* strains used (Supplementary Table 1) are isogenic with a $GAL2^+$ derivative of S288C. Strains were constructed by standard methods, either by crosses or by transformation. Details are available upon request. The *ser33Δ::kanMX* allele has been previously described[9]. The *srg1-1* mutation changes the *SRG1* TATA sequence at position −558 (relative to the *SER3* ATG) from TATAAA to CCTAGG. The *srg1Δ1::HIS3* and *srg1Δ1::HIS3 + T* mutations replace *SRG1* sequence from −451 to −300 by either the HIS3 ORF (+1 to +663 with +1 at the *HIS3* ATG) or the *HIS3* ORF and its transcription terminator (+1 to +817). The *ser3Δ::URA3* mutation replaces the *SER3*-coding sequence (+1 to +1410) with the coding sequence of *URA3* (+1 to +804). The *gal7::SRG1p* mutation inserts a sequence that includes the *SRG1* promoter and the two *SRG1* transcription-initiation sites (−713 to −445 relative to the *SER3* ATG) at position −378 of *GAL7*. Similar to *gal7::SRG1p*, the *gal7::srg1-1p* mutation inserts the *srg1-1* promoter sequence containing the mutant TATA sequence. The *ser3::GAL7UAS* mutation replaces *SER3* promoter sequences from −245 to −150 with *GAL7* sequences from −289 to −164, which includes two Gal4-binding sites. The *ser3Δ150::3MYC*, *ser3Δ250::3MYC* and *ser3Δ350::3MYC* mutations are internal deletions within *SRG1* that replace sequences from −660 to −150, −250 or -350 (relative to *SER3* ATG) with three copies of the Myc epitope tag. Unless otherwise noted, strains were grown in rich YPD medium, which is a repressing condition for *SER3* transcription.

Chromatin immunoprecipitation analysis

ChIP analysis was performed as previously described[9]. Antibodies used for immunoprecipitation were rabbit polyclonal anti-TBP serum (5 μl per immunoprecipitation; a gift of S. Buratowski), mouse monoclonal 12CA5 anti-HA ascites fluid (2 μl per immunoprecipitation; gift of E. Harlow), and rabbit polyclonal anti-Gal4(DBD) serum (1 μl per immunoprecipitation; Santa Cruz Biotechnology). Dilutions of input DNA (1:500 and 1:1000) and immunoprecipitated DNA (1:5 and 1:10 for Rpb3-HA, 1:2.5 and 1:5 for TBP, and 1:10 and 1:20 for Gal4) were analysed by quantitative polymerase chain reaction (PCR) and the products were separated on a 6% non-denaturing polyacrylamide gel. The PCR primers amplify the following regions whose coordinates are given relative to the ATG (+1): *SER3* A primers amplify a 312-bp product from −1157 to −846; *SER3* B primers amplify a 315-bp product from −644 to −329; *SER3* C primers amplify a 298-bp product from −234 to +64; *SER3* D primers amplify a 318-bp fragment from +111 to +429; *GAL7* UAS primers amplify a 283-bp product from −370 to −88; and *ser3::GAL7* UAS primers amplify a 332-bp product from −454 to −123. The PCR primers used to amplify the *GAL1* UAS and the control region from chromosome V that lacks open reading frames have been previously described[25,26].

Transcription run-on analysis

The transcription run-on assay was performed as previously described[27], with the following modifications. YPD cultures of 200 ml were grown to approximately $3–5 \times 10^6$ cells ml^{-1}. Transcription was allowed to proceed for 5 min at 30 °C after addition of 200 μCi of [α-^{32}P] rUTP. Total RNA was isolated using the hot phenol method[9]. After partial RNA hydrolysis with 0.2 M NaOH, labelled RNA was hybridized to oligonucleotides immobilized on GeneScreen (PerkinElmer). Membranes were washed with 2 × SSC/0.1% SDS for 30 min, twice at 37 °C and once at 42 °C. The nine probes used were antisense oligonucleotides corresponding to the following positions relative to the *SER3* ATG: −650 to −724 (1), −467 to −541 (2), −341 to −415 (3), −217 to −291 (4), −118 to −192 (5), +39 to −36 (6), +223 to +149 (7), +530 to +456 (8), +891 to +817 (9). See the gene map in Fig. 1c. Two sense oligonucleotides that correspond to positions −291 to −217 (N4) and −192 to −118 (N5) of *SER3* were used as negative controls. An antisense oligonucleotide corresponding to positions + 437 to +363 of *ACT1* was included as a positive control.

RNA analysis

Northern hybridization analysis was performed as previously described[9]. Probes for *SER3*, *SRG1*, *SNR190*, *URA3*, *HIS3*, *GAL7*, *GAL7* 5′ UTR, *GAL1* and *ACT1* were generated by randomly labelling PCR products that were amplified from genomic DNA. The coordinates of these probes relative to the ATG of each gene are as follows: *SER3* from +111 to +1342, *SRG1* from −454 to −123 (relative to SER3 ATG), *URA3* from +206 to +680, *HIS3* from −27 to +376, *GAL7* from +22 to +706, *GAL7* 5′ UTR from −370 to −88, *GAL1* from +252 to +1329, and *ACT1* from −376 to +1015. Probes for *SNR190* and *TPI1* have been previously described[28,29]. Primer-extension analysis was performed as previously described[30]. The *SER3* primer corresponds to bases +26 to +3 of the *SER3* coding sequence. The *SRG1* primer corresponds to bases −414 to −439 relative to the *SER3* ATG. The *ADH1* primer corresponds to bases + 32 to +10 of the *ADH1*-coding sequence.

Received 2 February; accepted 2 April 2004; doi:10.1038/nature02538.

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Supplementary Information accompanies this paper on **www.nature.com/nature**.

Acknowledgements We thank A. Dudley, A. Duina and J. Wu for comments on the manuscript. We also thank P. Cliften, P. Sudarsanam and M. Johnston for sharing unpublished results, and J. O'Sullivan and N. Proudfoot for advice on transcription run-on experiments. This work was supported by a grant from the National Institutes of Health to F.W. and by a postdoctoral fellowship from the Canadian Institute for Health Research to J.A.M.

Competing interests statement The authors declare that they have no competing financial interests.

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