

# Genome-wide location analysis reveals a role for Sub1 in RNA polymerase III transcription

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**Human PC4 and the yeast ortholog Sub1 have multiple functions in RNA polymerase II transcription. Genome-wide mapping revealed that Sub1 is present on Pol III-transcribed genes. Sub1 was found to interact with components of the Pol III transcription system and to stimulate the initiation and reinitiation steps in a system reconstituted with all recombinant factors. Sub1 was required for optimal Pol III gene transcription in exponentially growing cells.**

Chip chip | PC4 | reinitiation | TFIIB

**P**C4 plays an important role in various cellular processes, including transcription, DNA repair, and replication (1–3). First identified as a RNA polymerase (Pol) II coactivator (2, 4), PC4 was shown to interact with activators and components of the Pol II basal transcription machinery (2, 5) and to enhance activator-dependent transcription, stimulating both initiation and promoter escape (6). However, in the absence of TFIIF and TFIID, PC4 represses basal transcription (5, 7). Sub1, the yeast ortholog of PC4, was characterized biochemically as a coactivator required for activated transcription in vitro (8) and genetically as a suppressor of certain TFIIB mutations (9). Further investigations extended the role of PC4/Sub1 to transcription elongation and mRNA processing. Sub1 was shown to regulate enzymes modifying the CTD of the largest subunit of Pol II and might therefore enhance elongation (10). Functional interactions between not only PC4/Sub1 and Cstf-64/Rna15 (11) but also Pta1 and Sub1 (12) established additional connections between Sub1/PC4 and mRNA processing. PC4 has also been copurified with human TFIIC and was found to stimulate RNA Pol III transcription in vitro (13). Recently, PC4 was found to be associated with chromatin and to be important for chromatin organization, suggesting a more general role in transcription regulation (14).

Apart from its role in transcription, PC4 was implicated in other cellular processes, through its capacity to bind tightly to melted DNA and to single-stranded DNA (ssDNA, ref. 7). A direct interaction between PC4 and XPG, a subunit of the nucleotide excision factor, was correlated with the genetic interaction between their yeast counterparts, Sub1 and Rad2, suggesting a role for PC4/Sub1 in the repair of oxidative DNA damage (1). Furthermore, PC4 can form complexes with human HSSB protein on ssDNA and influences its replication function in vitro (3).

In this study, we identified the gene targets of Sub1 in exponentially growing yeast cells. Analysis of the genome-wide localization of Sub1 revealed its association to all of the genes transcribed by Pol III. We focused on the role of Sub1 in Pol III transcription. We present evidence that Sub1 is involved in Pol III transcription initiation and reinitiation processes in vitro and is required for optimal Pol III transcription in vivo.

## Results

**Genome-Wide Analysis of Sub1 Occupancy.** To define Sub1 gene targets in vivo, we performed a genome-wide analysis of Sub1 occupancy in exponentially growing cells. Chromatin immunoprecipitation (ChIP) assays were performed on epitope-tagged Sub1–3HA cross-linked chromatin and analyzed by hybridization to DNA microarrays harboring ORFs and intergenic regions similar to those

that we used in a previous work to analyze the genomic location of the Pol III transcription machinery (15). A defined number of loci were significantly enriched (991 loci with a  $P$  value  $<0.01$ ) under active growth conditions. Approximately one-fourth of the enriched loci were located within ORF; the others corresponded to intergenic regions and to genes encoding nontranslated RNAs. We noted that the *ACT1*, *PMA1*, *PYK1*, *ADH1*, and snoRNA genes previously identified as DNA targets of Sub1 by ChIP and PCR amplification (10, 16, 17) were indeed enriched in our data. The distribution of the loci corresponding to snoRNA genes shown in Fig. 1A extended the binding of Sub1 to all of the H/ACA box or C/D box snoRNA genes. To further identify Pol II gene targets of Sub1, the enriched loci with a  $P$  value  $<0.01$  were analyzed by using the GoTermFinder software. Three overrepresented GO categories ( $P$  value  $<10^{-5}$ ) were identified, indicating that Sub1 was preferentially bound to a subset of Pol II-transcribed genes encoding constituents of the cell wall (30 genes), the nucleosome (H2A, H2B, H3, and H4 histone genes) and the ribosome (10% of the enriched loci, corresponding to genes encoding constituents of the translational apparatus, including *TEF1*, *TEF2*, and 50 ribosomal protein (RP) genes). The distribution of the loci corresponding to RP genes is shown in Fig. 1A. Because RP and histone genes are among the most highly transcribed genes in exponentially growing cells, we determined the relationship between transcription rates and Sub1 occupancy. Interestingly, although Sub1 was also localized on very poorly transcribed ORFs, Sub1-associated Pol II genes globally tended to be highly transcribed [supporting information (SI) Fig. S1]. Remarkably, one-third of Sub1 enriched loci corresponded to Pol III-transcribed genes present on the arrays or to the intergenic regions and ORFs adjacent to these genes. As shown in Fig. 1A, the Loci Enriched by the Pol III Transcription Machinery (LEPTM, ref. 15), comprising tRNA genes and other genes transcribed by Pol III, like 5S RNA gene, *SNR6*, *RPR1*, and *SCR1* were significantly overrepresented among the most enriched DNA regions, suggesting the association of Sub1 to all Pol III-transcribed genes in conditions of active growth.

To confirm the results obtained by microarray hybridizations, we performed conventional ChIP assays on a set of selected Pol III-transcribed genes. Using real-time PCR on DNA fragments

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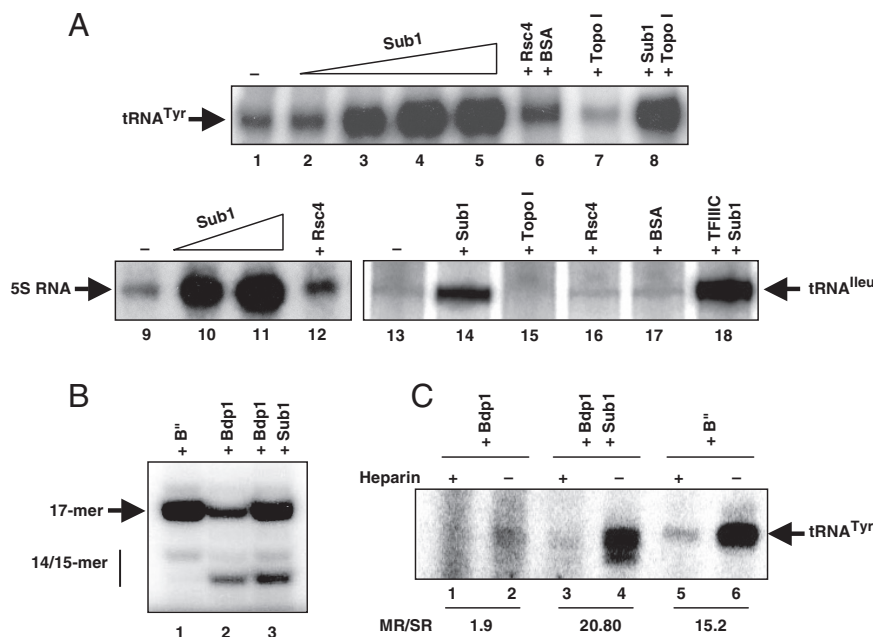
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**Fig. 2.** Sub1 is a Pol III transcription activator in vitro. (A) Sub1 stimulates the minimal Pol III transcription system. In vitro transcription of the *SUP4-tDNA<sup>Tyr</sup>* gene, 5S RNA gene or *tDNA<sup>Ileu</sup>* gene containing a TATA-box was carried out in the presence of rTBP, rBrf1, rBdp1, rTFIIIC (except for lanes 13–17), rTFIIIA (lanes 9–12 only), purified Pol III, varying amounts of purified rSub1 (lanes 2–5: 8, 24, 80, 240 ng; lane 8: 240 ng; lanes 10–11: 40, 160 ng; lanes 14, 18: 200 ng), or 200 ng of control proteins Rsc4, a recombinant protein purified under the same conditions than rSub1, BSA or Topo I, a single-stranded DNA binding protein like Sub1 that may play a role in human Pol III transcription (13). (B) Sub1 stimulates transcription initiation. Synthesis of the 17-mer RNA from the *SUP4-tDNA<sup>Tyr</sup>* template was carried out by using rTFIIIC, rTBP, rBrf1 and purified Pol III in the presence of rBdp1, B' or rSub1 (100 ng). The positions of the 14/15-mer and 17-mer transcripts are indicated. (C) Sub1 enhances Pol III transcription reinitiation. Preinitiation complexes were assembled on the *SUP4-tDNA<sup>Tyr</sup>* gene for 20 min in the presence of rTFIIIC, rTBP, rBrf1 and rBdp1 (lanes 1–4), or B' (lanes 5–6). rSub1 (100 ng) was added in lanes 3–4. Purified Pol III (10 ng) was then added together with a mixture lacking CTP and the incubation was continued for 20 min. Transcription was then resumed by the addition of CTP, either in the presence (+) or in the absence (–) of heparin, and the incubation was continued for 5 min. The ratios of multiple round (MR) versus single round (SR) of transcription are indicated.

with DNA. This stimulation effect could not account, by itself, for the extent of stimulation by Sub1 in factor-dependent multiple round of transcription (Fig. 2A) where all the components were in saturating amounts.

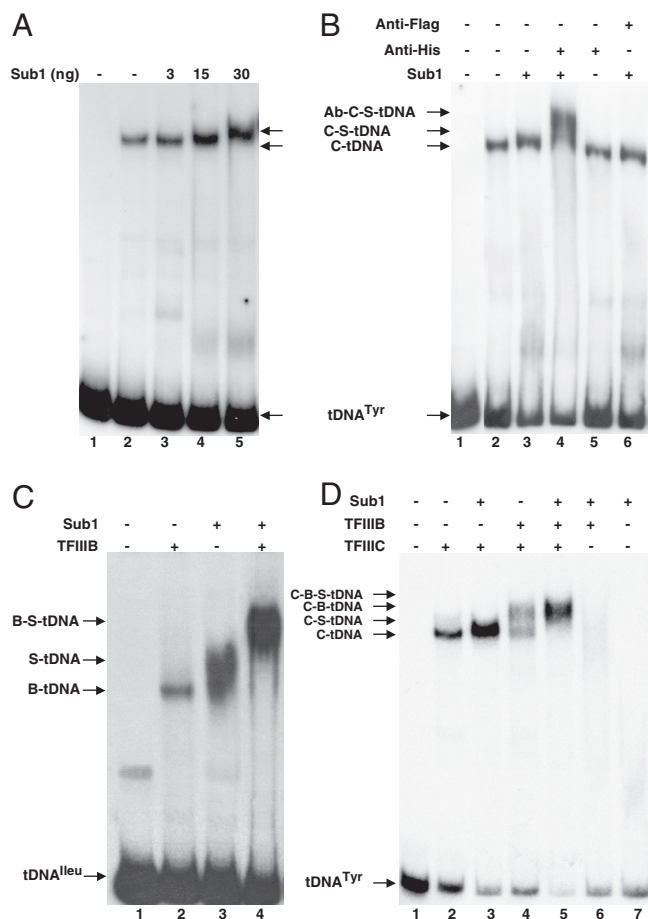
**Sub1 Stimulates Pol III Transcription Initiation in Vitro.** We examined the effect of Sub1 on transcription initiation in single round of transcription experiments using a *SUP4* tDNA template under conditions that yield a 17-mer RNA (Fig. 2B). As described, several transcription products were observed by using rTFIIIB. The 17- and 14-mer correspond to different start sites whereas the 15-mer results from the cleavage of the 17-mer product (29, 30). In the presence of B' fraction, only the 17-mer RNA was observed and the transcription level was stimulated up to 3 times. In the presence of rSub1, a similar stimulation of the transcription was observed but Sub1 had no effect on start site selection, indicating that Sub1 directly affected the initiation efficiency of Pol III transcription.

PC4 was found to enhance and extend the interactions of TFIIIC with tDNA (13, 18). We explored a possible role of Sub1 in the assembly of preinitiation complexes using gel shift assays. The addition of 3 or 15 ng of Sub1 was sufficient to promote the binding of a limiting amount of rTFIIIC (1–2 ng) to the *SUP4* tRNA<sup>Tyr</sup> gene, as evidenced by the clear increase in signal intensity (Fig. 3A). With larger amounts of Sub1, the mobility of the TFIIIC-DNA complexes decreased. Because the amounts of unbound DNA remained largely unchanged, a specific association between TFIIIC and at least one molecule of Sub1 was indicated. As shown in Fig. 3B, the addition of antibodies raised against the histidine epitope further decreased the mobility of the DNA complexes, showing the presence of histidine-tagged Sub1 in the supershifted TFIIIC-DNA complexes.

To test whether Sub1 could also influence the assembly of

rTFIIIB into preinitiation complexes, we took advantage of the *tRNA<sup>Ileu</sup>* (TAT) gene that can be transcribed in a TFIIIC-independent manner (31) and bound by rTFIIIB in gel shift assays (Fig. 3C). We also studied the recruitment of TFIIIB by TFIIIC to the *SUP4* tRNA<sup>Tyr</sup> gene that results in the formation of TFIIIB-TFIIIC-DNA complexes of slower electrophoretic mobility as shown in Fig. 3D. In both cases, the addition of Sub1 strongly stimulated the binding of TFIIIB resulting in the formation of a larger amount of slow-migrating complexes clearly distinct from the nonspecific protein-DNA complexes obtained with Sub1 alone. The stimulation of TFIIIB assembly on tDNA<sup>Ileu</sup> by Sub1 (Fig. 3C) was correlated with an at least 4-fold increase in the transcription levels observed in the presence of Sub1 using a minimal transcription system composed of rTFIIIB and Pol III (Fig. 2A, lanes 13–14). Furthermore, TFIIIC and Sub1 had additive stimulatory effects on transcription (Fig. 2A, lanes 14 and 18).

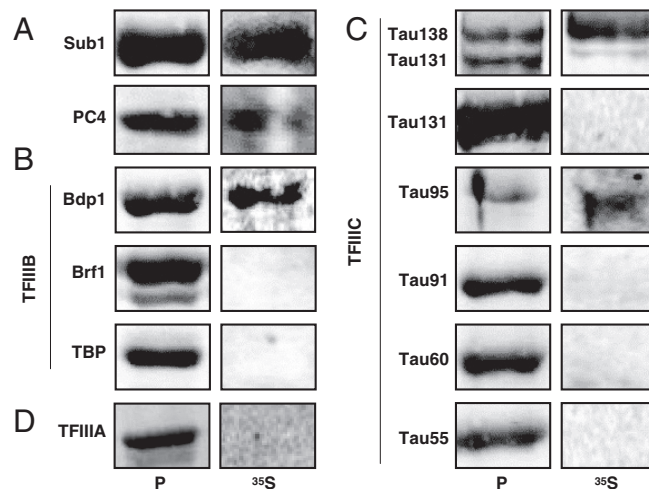
**Sub1 Stimulates Pol III Transcription Reinitiation in Vitro.** We next wondered whether the effects of Sub1 on Pol III transcription resulted only from the stimulation of preinitiation complexes assembly. To address this question, we carried out a time course analysis of *tRNA<sup>Ileu</sup>* synthesis (Fig. S4E) using our recombinant system in the presence or absence of Sub1. In both conditions, similar amounts of full-length transcripts could be visualized very rapidly (5 min), suggesting that the effects of Sub1 on transcription factors recruitment were not sufficient to explain the strong stimulation observed in multiple round of transcription (Fig. 2A). After 30 min of incubation, a strong accumulation of *tRNA<sup>Ileu</sup>* transcripts was observed only when Sub1 was present. Under these conditions, both de novo complex formation and reinitiation were combined. Because the high levels of Pol III transcription come mainly from reinitiation efficiency (32), we thus examined whether Sub1 could promote Pol III transcription reinitiation.



**Fig. 3.** Sub1 enhances the assembly of TFIIB-TFIIC-tDNA complexes. The positions of protein-tDNA complexes visualized by gel shift assays are indicated. Lane 1: control without protein. (A) Sub1 promotes the binding of TFIIC to tDNA. Limiting amounts of rTFIIC (C, 2 ng) were incubated in the presence of the indicated amounts of Sub1 (5) with the *SUP4*-tDNA<sup>Tyr</sup> probe. (B) Sub1 is recruited with TFIIC on tDNA. Endogenous yeast TFIIC was preincubated with the *SUP4*-tDNA<sup>Tyr</sup> probe for 10 min at 25 °C, alone or in the presence of 30 ng of Sub1 and then further incubated for 20 min at 25 °C with anti-histidine or anti-Flag monoclonal antibodies. (C) Sub1 stimulates the assembly of TFIIB on tDNA<sup>leu</sup>. rTFIIB (B, 10 ng) and Sub1 (100 ng) were incubated as indicated with the tDNA<sup>leu</sup> probe containing a TATA-box. (D) Sub1 helps TFIIC to recruit TFIIB on tDNA. rTFIIC (10 ng), rTFIIB (10 ng) or Sub1 (100 ng) were incubated as indicated with the *SUP4*-tDNA<sup>Tyr</sup> probe.

The transcription initiation frequency was determined for the *SUP4* tRNA<sup>Tyr</sup> gene (Fig. 2C) by comparing the output of multiple round versus single round transcription cycles performed with limiting amounts of Pol III, as described (33). The basal transcription system reconstituted with rTFIIC could support only 1.9 cycle of transcription in 5 min. In contrast, more than 20 transcription cycles were obtained within 5 min in the presence of Sub1 or B'' fraction. Similar results were obtained with the tRNA<sup>leu</sup> gene (ratio of 1.2 and 19, Fig. S4D) showing that Sub1 played a critical role in the transcription reinitiation processes. Note that under limiting amounts of Pol III, part of the transcription stimulation could be due to a factor-independent effect of Sub1 on Pol III (Fig. S5).

**Sub1 Interacts with TFIIB and TFIIC Components.** Based on gel shift assays (Fig. 3), our results suggested that Sub1 helps TFIIB and TFIIC to assemble on tRNA genes, possibly through direct protein-protein interactions with basal factors, at least in a DNA-dependent manner. Far Western experiments were performed as described (34) using <sup>35</sup>S-labeled Sub1 as a probe (Fig. 4). As



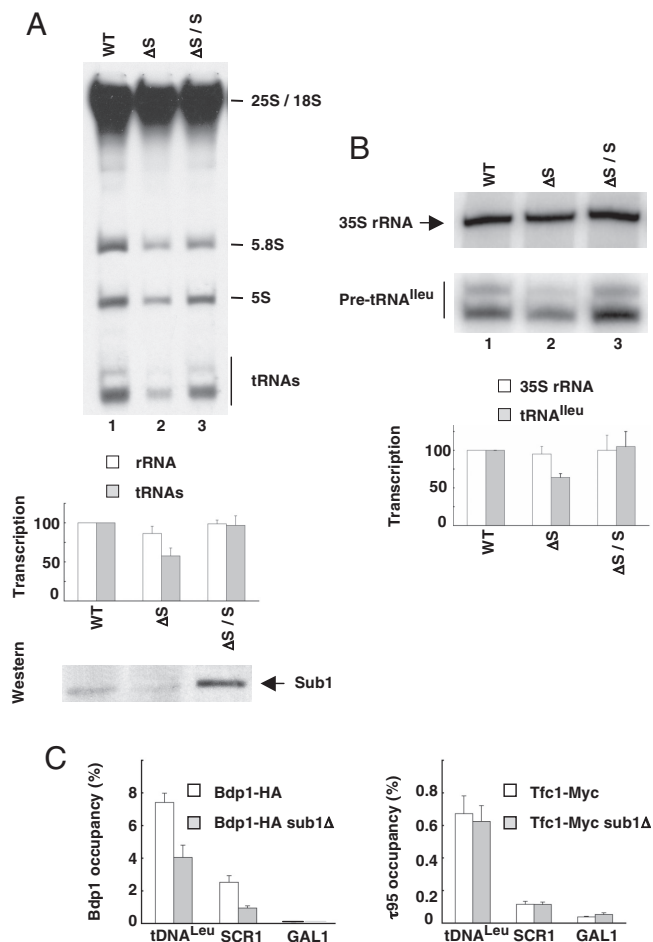
**Fig. 4.** Sub1 interacts with components of the Pol III transcription machinery. rSub1 or rPC4 (A), subunits of rTFIIB (B) or of rTFIIC (C), or rTFIIIA (D) were subjected to SDS/PAGE, transferred onto a membrane, stained with Ponceau S (lanes P) and then probed with <sup>35</sup>S-labeled Sub1 (lanes <sup>35</sup>S). Labeled protein complexes were revealed by autoradiography.

expected, because PC4 forms tightly associated homodimers (35), we found that Sub1 interacted with itself and with PC4 (Fig. 4A). Sub1 was also found to interact with Bdp1, a component of TFIIB and with  $\tau$ 138 and  $\tau$ 95, 2 subunits of TFIIC, in good agreement with the coimmunoprecipitation of PC4 with human TFIIC (13). No interaction was detected with TFIIIA. According to these data, the shifts seen in EMSA experiments (Fig. 3) after the addition of Sub1 may likely reflect both the binding of Sub1 to DNA in addition to direct interactions with TFIIB and TFIIC.

**Sub1 Is Required for Optimal Pol III Transcription in Vivo.** Because Sub1 acts as an activator of Pol III transcription in vitro, we next investigated whether the presence of Sub1 was important for Pol III transcription in vivo. There was no difference in the cell growth rate of exponentially growing *sub1* $\Delta$  cells as compared with wild type strain (8). Consistently, we could not detect any significant difference between both strains in the steady state levels of Pol III transcripts in exponentially growing cells (Fig. S6B Right). However, we did observe that *sub1* $\Delta$  crude extracts were systematically less efficient than wild type extracts in Pol III transcription as exemplified in Fig. S6A for the *SUP4* tRNA<sup>Tyr</sup> gene ( $\approx$ 20% less RNA transcripts). The addition of Sub1 stimulated the transcription in both *sub1* $\Delta$  and wild type extracts. However, the 20% lower activity of the *sub1* $\Delta$  extracts was too modest for further analysis.

In good accordance with these results, pulse labeling experiments performed on exponentially growing cells revealed that Pol III transcription was decreased in vivo in the absence of Sub1. The level of tRNA gene transcription was  $\approx$ 40% lower in *sub1* $\Delta$  cells as compared with wild type whereas the level of Pol I transcription measured by the neosynthesis of 25S and 18S RNA was not significantly changed (Figs. 5A and S6B Left). The defect in Pol III transcription was due to the absence of Sub1, because wild type levels of Pol III transcription were restored when Sub1 was expressed in *sub1* $\Delta$  cells from a centromeric plasmid (Fig. 5A Bottom). Primer extension analysis was performed on total RNA prepared from *sub1* $\Delta$  cells (Fig. 5B). Once again, whereas the levels of 35S RNA neosynthesis were not significantly altered, a  $\approx$ 35% decrease of tRNA<sup>leu</sup> (TAT) neosynthesis was observed in *sub1* $\Delta$  cells and wild type transcription levels were restored in *sub1* $\Delta$  cells expressing Sub1 from a centromeric plasmid.

We next wondered whether the decrease in Pol III transcription in *sub1* $\Delta$  cells was correlated to a lower occupancy of Pol III-



**Fig. 5.** Sub1 is an activator of Pol III transcription in exponentially growing cells. (A) Pol III transcription is less efficient in *sub1* $\Delta$  strain. Three micrograms of total labeled RNA prepared from WT, *sub1* $\Delta$  ( $\Delta S$ ) or *sub1* $\Delta$ /pCM-*SUB1* cells (*sub1* $\Delta$  transformed with a centromeric plasmid harbouring *SUB1*,  $\Delta S/S$ ) were analyzed by electrophoresis and fluorography. (Top) The positions of labeled 25S, 18S and 5.8S RNA transcribed by Pol I, and of 5S RNA and tRNAs transcribed by Pol III are indicated. (Middle) Quantitation of Pol I (25S + 18S rRNA) and Pol III (tRNAs) labeled transcripts in  $\Delta S$  or  $\Delta S/S$  cells are represented as histograms and as a percentage of their transcription in WT cells. Error bars represent the standard deviation between three independent experiments. (Bottom) The amounts of Sub1 in crude extracts (5  $\mu$ g) were analyzed by Western blot analysis with antibodies directed to Sub1. (B) The neosynthesis of tRNA<sup>leu</sup> is decreased in *sub1* $\Delta$  cells. (Upper) The amounts of neosynthesized 35S rRNA or pre-tRNA<sup>leu</sup> were determined by primer extension analysis on 4  $\mu$ g of total RNA. (Lower) Quantitation of neosynthesized 35S rRNA or pre-tRNA<sup>leu</sup> in *sub1* $\Delta$  or *sub1* $\Delta$ /pCM-*SUB1* cells is represented as histograms and as a percentage of their transcription in WT cells. Error bars represent the standard deviation between three independent experiments. (C) Less TFIIB factor is associated to its gene targets in *sub1* $\Delta$  cells. ChIP assays were performed on WT or *sub1* $\Delta$  cells by using antibodies against HA (for Bdp1-3HA) or Myc (for Tfc1-13Myc) epitope and analyzed by real-time PCR on selected genes. Error bars represent the standard deviation between at least three independent replicates.

transcribed genes by the transcription machinery. To address this question, cross-linked chromatin extracts were prepared from *sub1* $\Delta$  strains expressing  $\tau 95$ -13myc or Bdp1-3HA. ChIP experiments were performed by using antibodies directed to the epitopes and analyzed by real-time PCR on selected Pol III-transcribed genes. As shown Fig. 5C, whereas TFIIC binding was unchanged, less TFIIB was present on the *SCR1* or tRNA<sup>Leu</sup> genes when *SUB1* was deleted. In good agreement with our in vitro data, these results suggested that Sub1 may help TFIIC to efficiently recruit TFIIB on DNA.

## Discussion

In this work, we demonstrate that Sub1 is present on Pol III-transcribed genes. We find that Sub1 stimulates in vitro Pol III transcription at two discrete steps of the transcription cycle. Although Sub1 is not an essential protein in exponentially growing cells, we show that its absence in vivo correlates with a decrease in Pol III transcription efficiency and with lower levels of TFIIB associated with two of its target genes.

**Sub1 Stimulates Two Steps of the Pol III Transcription Cycle.** In this work, we provide biochemical evidence that Sub1 strongly stimulates in vitro transcription by Pol III, in agreement with the stimulation of human Pol III transcription by PC4 (13, 18). Using a transcription system reconstituted with all recombinant factors and highly purified Pol III, we showed that recombinant Sub1 stimulated both the initiation and reinitiation steps of transcription. Sub1 was determined to promote the binding of both TFIIB and TFIIC to their cognate sites, likely through direct TFIIB and TFIIC interactions, resulting in increased levels of Pol III transcription. However, the main effect of Sub1 was to relieve the Pol III reinitiation defect observed when using recombinant factors. Therefore, Sub1 can be considered as a Pol III reinitiation factor. Although its exact function in that transcription step remains to be determined, one may hypothesize that Sub1 could help Pol III to be directly transferred from the terminator to the start site of transcription as it has been proposed in the facilitated recycling pathway (36).

In all our in vitro assays, optimal stimulations required higher molar amounts of Sub1 relative to that of TFIIC, TFIIB or Pol III. Several characteristics of Sub1 might contribute to this phenomenon. First, incomplete or inappropriate modification of Sub1 residues because of its expression in insect cells might decrease its activity (for example, acetylation of PC4 has been shown to interfere with its DNA binding activity, ref. 37). Second, because Sub1 was found to bind both single- and double-stranded DNA (8), a substantial amount of the protein could be sequestered by the nonspecific DNA present in the reaction mixtures. Last, Sub1 might bind to DNA as a dimer or even multimerize along the DNA as suggested for PC4 (35), as a prerequisite for productive interaction with the components of the Pol III transcription machinery on DNA.

**Sub1 Is Required for Optimal Pol III Transcription in Exponentially Growing Cells.** Our data imply that Sub1 is a novel Pol III transcriptional activator in vivo. The modest but reproducible decreased efficiency of Pol III transcription in *sub1* $\Delta$  cells suggests that Sub1 is necessary to sustain wild type levels of Pol III transcription under normal growth conditions. The lower transcription efficiency was correlated to a reduced association of Bdp1, a TFIIB subunit, with Pol III-transcribed genes. These data are in good agreements with a direct role of Sub1 on transcription initiation in vivo through the efficient recruitment of TFIIB by TFIIC on DNA.

However, we could not detect any significant difference in the steady-state levels of Pol III transcripts in the absence of Sub1 (Fig. S6B). Consistently, *SUB1* is a nonessential gene and its deletion does not interfere with the cell growth rate in exponentially growing cells (1, 8). All these results suggest the existence of regulatory mechanisms that compensate for the decreased neosynthesis of Pol III transcripts caused by the absence of Sub1 to maintain a wild type pool of tRNA in the cell.

Our genome-wide occupancy studies suggest that Sub1 could play a more general role in transcription than anticipated. Our data demonstrated that Sub1 is not restricted to the Pol II transcription system, but is also involved in Pol III transcription. A function of Sub1 in Pol I transcription remains an open possibility but our preliminary in vivo pulse labeling and primer

extension analysis provided no evidence for such a role, at least in exponentially growing cells.

The role of Sub1 is also likely to be more complex than a straightforward activation function. Both PC4 and Sub1 were recently shown to play a negative role in Pol II transcription in vivo. Knocking down PC4 expression using siRNA altered the expression of less than 200 genes, most of them being up-regulated (14), whereas disruption of *SUB1* increased *IMD2* transcription (38) showing that PC4/Sub1 could also repress the expression of some genes under normal growth conditions. Like the NC2 cofactor (39) or Mot1 (40), Sub1 may thus play both a positive and a negative role in transcription.

Finally, previous studies suggested that Sub1 may have some physiological importance under suboptimal growth conditions. For instance, it has been reported that the expression of Sub1 mRNA rapidly increases after transfer of quiescent yeast cells in rich medium (20). It would be interesting to analyze the possible role of Sub1 in reactivating transcription during recovery from poor growth conditions that were found to repress transcription (nutrients or serum starvation, mitotic repression, secretory pathway defects, oxidative stress, DNA damages, chemical treatments with diverse drugs). Furthermore, Sub1 has been shown to be required for resistance to the oxidizing agents *tert*-butyl hydroperoxide or hydrogen peroxide (1, 41) suggesting a role of Sub1 in the stress response. Further investigations should be performed to characterize conditions where Sub1 is necessary for cellular growth and to determine whether and by which molecular mechanisms Pol III transcription is regulated by Sub1 in such conditions.

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## Materials and Methods

**Yeast Strains.** All strains used in this study are described in *SI Text* and *Table S1*.

**ChIP, Microarray Hybridization, and Data Analysis.** ChIP and PCR were performed as described (15). The sequences of the oligonucleotides used in this study are available upon request. For ChIP on chip experiments, DNA from wild type or 3HA-Sub1 strains were competitively hybridized to DNA microarrays harbouring 14,172 yeast ORF and intergenic regions (15, 21). Data from 3 independent experiments were compiled. Data analysis are described in *SI Text*. The complete raw dataset and analyzed data are available at [www.ncbi.nlm.nih.gov/geo](http://www.ncbi.nlm.nih.gov/geo) (GEO accession no. GSE11054) in *SI, Datasets S1 and S2*.

**In Vitro Transcription Assays.** Transcriptions were performed as described in Ducrot et al. (26) with 10 ng of rTFIIC, 20 ng of rTBP, 10 ng of rBrf1, 10 ng of rBdp1 or 0.5  $\mu$ g of partially purified B'' fraction, 100 ng of highly purified Pol III and 40 ng of rTFIIIA when 5S DNA template was used. Facilitated transcription reinitiation assays were performed as described (33). Specific transcripts were visualized with a Typhoon 9200 Imager (Amersham Biosciences). Quantitation was performed by using the Quantity one software (Bio-Rad). See also *SI Text*.

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