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Christine Conesa
CEA Saclay

Roberta Ruotolo
University of Parma

Pascal Soularue
Commissariat a L'Energie Atomique CEA

Tiffany A. Simms
Louisiana State University

David Donze
Louisiana State University

See next page for additional authors

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Modulation of Yeast Genome Expression in Response to Defective RNA Polymerase III-Dependent Transcription†

Christine Conesa,[1]‡* Roberta Ruotolo,[2]‡ Pascal Soularue,[3] Tiffany A. Simms,[4] David Donze,[4] André Sentenac,[1] and Giorgio Dieci[2]*

Service de Biochimie et Génétique Moléculaire, Bâtiment 144, CEA/Saclay, 91191 Gif-sur-Yvette Cedex, France[1]; Dipartimento di Biochimica e Biologia Molecolare, Università degli Studi di Parma, Parco Area delle Scienze 23/A, 43100 Parma, Italy[2]; Service de Génomique Fonctionnelle, CEA/Evry, 2 rue Gaston Crémieux, CP22, 91057 Evry Cedex, France[3]; and Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803[4]

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We used genome-wide expression analysis in *Saccharomyces cerevisiae* to explore whether and how the expression of protein-coding, RNA polymerase (Pol) II-transcribed genes is influenced by a decrease in RNA Pol III-dependent transcription. The Pol II transcriptome was characterized in four thermosensitive, slow-growth mutants affected in different components of the RNA Pol III transcription machinery. Unexpectedly, we found only a modest correlation between altered expression of Pol II-transcribed genes and their proximity to class III genes, a result also confirmed by the analysis of single tRNA gene deletants. Instead, the transcriptome of all of the four mutants was characterized by increased expression of genes known to be under the control of the Gcn4p transcriptional activator. Indeed, *GCN4* was found to be translationally induced in the mutants, and deleting the *GCN4* gene eliminated the response. The Gcn4p-dependent expression changes did not require the Gcn2 protein kinase and could be specifically counteracted by an increased gene dosage of initiator $tRNA^{Met}$. Initiator $tRNA^{Met}$ depletion thus triggers a *GCN4*-dependent reprogramming of genome expression in response to decreased Pol III transcription. Such an effect might represent a key element in the coordinated transcriptional response of yeast cells to environmental changes.

In eukaryotic organisms, three forms of RNA polymerase (Pol) are involved in nuclear DNA transcription (48). Pol I transcribes a single essential gene to generate the rRNA precursor that is processed into the three largest rRNAs. Pol II synthesizes pre-mRNAs and a number of small RNAs involved in pre-mRNA splicing and rRNA modification. Pol III is devoted to the synthesis of tRNAs, 5S rRNA and a few other nontranslated RNAs participating in basic cellular functions such as pre-mRNA processing, tRNA maturation, or protein translocation. The triplication of the transcriptional machinery may provide a selective advantage to the eukaryotic cell, compared to the unique bacterial and archaeal RNA polymerases, by allowing independent or coordinated regulations of gene expression in response to environmental changes. Previous studies both in yeast and in higher eukaryotes have shown that Pol III transcription is coordinately regulated with Pol I transcription under many circumstances, e.g., upon nitrogen starvation or in response to a nutritional upshift (6, 9, 53, 58). Under other conditions, the regulatory pathways of Pol I and Pol III transcription are uncoupled. Upon amino acid starvation, for example, Pol I transcription was reported to diminish by 80%, while tRNA synthesis was only modestly affected (50). A coordinated repression of rRNA, tRNA, and ribosomal protein synthesis was observed in secretion-defective yeast cells or in response to rapamycin treatment (34, 41, 42). It is not known, however, whether such a coordinate regulation involves any direct cross talk between the transcription systems. In particular, the extent to which defects in Pol III transcription affect Pol II transcription has not been studied on a genomic scale yet. This issue deserves detailed investigation for two main reasons. First, the cellular levels of Pol III-synthesized RNAs might in principle exert a wide influence on genome expression, as recently demonstrated for the SINE-encoded mouse B2 RNA (1). Second, local Pol III-Pol II transcriptional interference phenomena have been described in *Saccharomyces cerevisiae*. An actively transcribed tRNA gene lying close to the silent *HMR* locus has been shown to serve as a barrier to heterochromatin spread and to prevent the silencing of the adjacent *GIT1* gene (15). Similarly, the *TRT2* $tRNA^{Thr}$ gene upstream of *STE6* has recently been shown to act as a barrier to repression in *MAT*α yeast cells (51). Actively transcribed tRNA genes can also negatively regulate adjacent Pol II-transcribed promoters (27, 31). This effect, also called tRNA gene-mediated silencing, has been observed mostly with selected artificial constructions, in which Pol II-transcribed reporter genes were found to be inhibited 2- to 60-fold by a neighboring tRNA gene (27, 31). Only two cases of negative tDNA position effects have been reported to operate at native chromosomal loci (4, 51). At this time, it is not known whether such a silencing effect operates on other Pol II-transcribed genes among those neighboring the 274 tRNA loci that are dispersed in the yeast genome, nor whether local silencing

* Corresponding author. Mailing address for Giorgio Dieci: Dipartimento di Biochimica e Biologia Molecolare, Università degli Studi di Parma. Parco Area delle Scienze 23A, 43100 Parma, Italy. Phone: 39-0521-905649. Fax: 39-0521-905151. E-mail: giorgio.dieci@unipr.it. Mailing address for Christine Conesa: Service de Biochimie et Génétique Moléculaire, Bâtiment 144, CEA/Saclay, 91191 Gif-sur-Yvette Cedex, France. Phone: 33-1-69083796. Fax: 33-1-69084712. E-mail: conesa@matthieu.saclay.cea.fr.

† Supplemental material for this article may be found at http://mcb.asm.org/.

‡ C.C. and R.R. contributed equally to this study.

TABLE 1. RNA Pol III transcription mutant and background strains used in this study

Strain	Genotype	Source or reference[a]
YNN282	*MAT*α *ade2-1 his3-Δ200 lys2-801 trp1-Δ ura3-52 gal mal* CUP^r	YGSC
YPH500	*MAT*α *ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52*	YGSC
rpc160-112	*MAT*α *ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 rpc160Δ::HIS3* pC160-112 (*TRP1 CEN4 rpc160-112*)	13
BRF1	*MAT***a** *leu2 ura3-52 brf1::HIS3* pRSM3 (*LEU2 CEN BRF1*)	10
brf1-II.6	*MAT***a** *leu2 ura3-52 brf1::HIS3* pRSM3*II.6 (*LEU2 CEN brf1-II.6*)	2
tfc3-G349E	*MAT*α *ade1 his3-Δ200 leu2Δ1 lys2-801 trp1-Δ63 ura3-52 tsv115*	33
tfc7-ΔN1	*MAT*α *ade2-101 his3-Δ200 lys2-801 trp1-Δ63 ura3-52 tfc7Δ::HIS3* pTFC7-ΔN1 (*TRP1 CEN4 tfc7-ΔN1*)	37

[a] YGSC, Yeast Genetic Stock Center.

effects are limited to the tRNA class of Pol III-transcribed genes. Several features of the RNA Pol III machinery might indeed favor positional effects on neighboring Pol II-transcribed genes. The core transcription apparatus acting on yeast class III genes comprises the 17-subunit RNA polymerase together with its transcription factors TFIIIA, TFIIIB, and TFIIIC (20). TFIIIA, a multi-zinc finger protein, is a 5S RNA gene-specific assembly factor for TFIIIC. TFIIIC is composed of six subunits, named τ138, τ131, τ95, τ91, τ60, and τ55. TFIIIC recognizes the promoter sequences of Pol III-transcribed genes and, once bound to DNA, acts as an assembly factor for TFIIIB, which is responsible for directing Pol III to its target genes. TFIIIB is composed of the TATA box-binding protein, the TFIIB-related component Brf1, and the Bdp1 protein. Using chromatin immunoprecipitation followed by microarray hybridization, recent studies have defined a complete inventory of the genes transcribed by Pol III in the yeast *Saccharomyces cerevisiae* and have strengthened the notion that these genes are persistently occupied by the transcription machinery during active growth (24, 38, 46). Such a tight occupancy state of class III transcriptional units, that are generally unclustered and thus interspersed throughout the chromosomes, has the potential to exert a genome-wide influence on the Pol II transcriptome through local positional effects. To address the issues of both Pol III-Pol II cross talk and positional interference, we analyzed by DNA microarray hybridization the genome-wide consequences, on Pol II transcription, of defects in Pol III transcription associated with mutations in four components of the Pol III machinery. We found very limited alteration in the expression of Pol II-transcribed genes lying close to class III genes, as if Pol II-transcribed genes were generally refractory to transcriptional interference by neighboring transcription units. Instead, we consistently found, as a consequence of defective Pol III transcription, a derepression of genes under the control of the Gcn4 transcription factor, and we demonstrate that such a response depends on depletion of initiator methionine tRNA.

MATERIALS AND METHODS

Yeast strains. The Pol III transcription mutant strains used in the present study are listed in Table 1. Well-characterized thermosensitive mutants were chosen. The *rpc160-112* mutant strain (YPH500 background) is affected in the largest subunit of Pol III (6, 13). The *brf1-II.6* strain harbors a double aspartate to alanine substitution in Brf1, one of the components of TFIIIB (2, 10). The *tfc3-G349E* strain (YPH500 background) corresponds to a point mutation in τ138, the largest subunit of TFIIIC, located in the τB domain and involved in DNA-binding activity (3, 33). The *tfc7-ΔN1*strain (YNN282 background) harbors a N-terminal deleted version of the τ55 subunit of TFIIIC (37). The four strains present a strong temperature-sensitive growth defect at 37°C. The *tfc3-G349EΔgcn2* strain was constructed by a one-step replacement strategy (35) integrating *kanMX* in the *GCN2* locus in the strain *tfc3-G349E*. The Δ*gcn2* control strain (AG207) was a gift from R. Serrano (22). The *tfc3-G349EΔgcn*4, *brf1-II.6Δgcn4*, and *YPH500Δgcn4* strains were constructed by one-step gene replacement integrating *kanMX* in the *GCN4* locus. To this end, a gene disruption cassette was PCR amplified from the Δ*gcn4* strain of the yeast gene knockout collection (Open Biosystems, Huntsville, AL) (55).

Analysis of *GCN4-lacZ* expression. Strains transformed with p180 (p*GCN4-LacZ URA3 CEN*), a gift from A. Hinnebusch (39), were grown at permissive temperatures (24 or 30°C) to an A_{600} of 0.5 under repressive conditions in standard rich medium (yeast extract-peptone-dextrose [YPD]) or under derepressive conditions (growth in YPD then shift to minimal medium with no amino acids for 3 h). The cells were collected and protein extracts were prepared with a glass bead procedure, and the β-galactosidase activity in the whole-cell extracts was measured as previously described (54).

RNA extraction. Overnight cultures of cells grown in YPD at the permissive temperatures (24 or 30°C) were diluted in fresh medium (A_{600} = 0.1) and incubated with shaking, either at the permissive temperature or at 37°C, to an A_{600} of 0.4 to 0.6. The cells were collected and total RNA (from 50-ml cultures) was extracted with the RNeasy Midi Kit (QIAGEN) according to the manufacturer's instructions.

Microarray analysis. The DNA microarrays used in the present study were manufactured by the Service de Génomique Fonctionnelle (CEA/Evry, France) as described by Fauchon et al. (17). Labeled cDNAs were synthesized by using an indirect labeling protocol adapted from P. Brown (http://cmgm.stanford.edu/pbrown/protocols/aadUTPCouplingProcedure.htm). Prehybridization and hybridization conditions were those described by P. Brown and coworkers (http://brownlab.stanford.edu/protocols.html). Hybridized arrays were scanned by using a GenePix 4000A scanner (Axon Instruments, Inc.), and fluorescence ratio measurements were determined with the GenePix Pro 4.0 software (Axon Instruments, Inc.). Spots were considered for analysis if more than 70% of the pixels fluoresced with intensity above the median fluorescence intensity of the background plus two standard deviations. The data were normalized assuming that the arithmetic median of the ratios for every considered spot is equal to 1. The data analysis was performed by using GeneSpring 6.0 software (Silicon Genetics) and Microsoft Excel. The results were averaged from at least three independent experiments with two batches of RNAs from wild-type or mutant strains. Each experiment consisted of two different hybridizations in which the fluorochromes were swapped in order to minimize changes due to technical variability. The complete datasets are available upon request.

Real-time PCR quantification. Total RNA (10 μg) was treated with DNase I (as described in the RiboPure-Yeast protocol; Ambion), followed by acid phenol-chloroform extraction and RNA precipitation. First-strand cDNA for each sample was synthesized by reverse transcription of total RNA using Superscript III reverse transcriptase (Invitrogen). The reaction was carried out at 50°C for 120 min, followed by thermal inactivation of the reverse transcriptase (70°C for 10 min). The reaction mixture (20-μl final volume) contained 1× reverse transcription buffer, 0.5 mM deoxynucleoside triphosphates, 1 μl of oligo(dT)$_{20}$ (50 mM), 10 mM dithiothreitol, 20 U of SUPERase-In (Ambion), 200 U of Superscript III, and 2 μg of total RNA.

Real-time PCR was performed with a TaqMan ABI Prism 7000 Sequence Detector System (PE Applied Biosystems) according to the manufacturer's instructions. Reaction mixtures (25-μl final volume) were assembled with the following components: 2.5 μl of 10-fold serial dilutions of cDNAs, optimized

amounts of each primer set (100 nM for all amplifications), 2× SYBR Green PCR Master Mix (PE Applied Biosystems). The housekeeping β-actin (*ACT1*) mRNA served as an independent internal standard. All primer pairs produced only one amplification band (ranging from 95 to 110 bp) when tested by conventional reverse transcription-PCR. The primer sequences are available upon request.

The specificity of individual real-time PCR products was assessed by melting-curve analysis (45) carried out immediately after PCR completion. Melting curves for individual PCR products displayed a single peak. The amplified products were also examined by agarose gel electrophoresis. Melting temperatures (T_m) were determined with the Dissociation Curve software (PE Applied Biosystems). All sets of reactions were conducted in triplicate, and each included a nontemplate control. The threshold cycle (C_T) was used to calculate relative gene expression levels using the "Comparative C_T Method" (PE Applied Biosystems, user bulletin number 2), which is based on the assumption that the efficiency of amplification of target and reference cDNAs are approximately the same. The validity of the above assumption was verified for each primer set using serial cDNA dilutions.

Overexpression of initiator and elongator tRNA^{Met}. The *IMT1* and *EMT3* genes, coding for initiator and elongator tRNA^{Met}, respectively, were PCR-amplified (together with ~70 bp of 5′-flanking and 30 bp of 3′-flanking regions) from yeast genomic DNA with two pairs of oligonucleotide primers—5′-TCCA AGATGAGAATTTTAAGTTTATGG (IMT1_fw) and 5′-CATTTCATTCTAT GTATTAACAATAATAG (IMT1_rev) for *IMT1* and 5′-TGCTATATCCTTT AATAACCATGG (EMT3_fw) and 5′-ATCTCAAAACATGAATGTTGAGC (EMT3_rev) for *EMT3*—and inserted into the SmaI site of YEp352 multicopy vector. The recombinant constructs (pIMT1 and pEMT3) were then transformed into the *BRF1* and the *brf1-II*.6 strains (see Table 1). The transformants were selected on the appropriate synthetic medium (lacking uracil). Total RNAs from transformed and untransformed strains were extracted from cells grown in YPD medium at the permissive temperature (retention of the YEp352-derived plasmids by the transformed strains was verified by replating on selective medium).

tDNA deletions. DNA fragments of approximately 250 to 500 bp flanking each tDNA were amplified from genomic DNA by PCR and directionally cloned into pBluescript SK(+) to flank the *URA3* gene. These *tdna*Δ::*URA3* constructs were transformed in the parent strains (*S. cerevisiae* W303 strain for *AMD2*, *ACO1*, *YEL033W*, and *YJL200C*, S288C strain for *ARO8* and *POR1*) and plated on minimal medium lacking uracil. Ura$^+$ transformants were screened by PCR for properly integrated constructs. To create strains containing specific deletions of the tDNAs, fragments of approximately 700 to 1,000 bp flanking the tDNAs were amplified by PCR and subcloned into pCR2.1-TOPO (Invitrogen). The tDNA sequences were deleted by site-directed mutagenesis (Stratagene QuikChange kit [oligonucleotide sequences used for deletions available on request]), and each *tdna*Δ construct was transformed into its corresponding *tdna*Δ::*URA3* strain and plated on 5-fluoorotic acid (5-FOA) media. FOA^r, uracil auxotrophic transformants were analyzed by PCR to verify the desired *tdna*Δ loci.

RESULTS

Global gene expression changes in mutants of the Pol III transcription machinery. To study the impact on Pol II-dependent gene expression of defects in Pol III transcription, we decided to focus on four well-characterized thermosensitive strains bearing mutations located in the C160 subunit of Pol III (*rpc160-112* mutant [13]) in the Brf1 component of TFIIIB (*brf1-II.6* mutant [2]) and in two different subunits of TFIIIC, τ138 (*tfc3-G349E* mutant [33]) and τ55 (*tfc7-ΔN1* mutant [37]). The four strains presented a strong growth defect at 37°C. Even at the permissive temperature (24°C for the *rpc160-112* and *brf1-II.6* strains and 30°C for the *tfc3-G349E* and *tfc7-ΔN1* strains), the mutant strains have a doubling time longer than that of the wild-type strains (150 to 190 min instead of 100 to 120 min), most likely reflecting defects in Pol III transcription. Indeed, we verified by real-time PCR amplification that, at the permissive temperature, the four mutants display a two- to threefold reduction of the steady-state levels of tRNA^{Leu}-(CAA) or tRNA^{Thr}(AGT) compared to wild type (data not shown; see also reference 6). A newly discovered, unexpected phenotype of these mutants was their ability to grow at the restrictive temperatures when the medium was supplemented with 1 M sorbitol, an osmotic stabilizer (data not shown). The mutants also grew at the restrictive temperatures in the presence of 500 mM KCl, suggesting that the rescue of growth was due to an osmotic effect rather than to a specific chemical property of the solute. An osmoremedial growth defect is often correlated with a weakened cell wall structure and has been found to be associated with several mutations in components of the Pkc1 mitogen-activated protein (MAP) kinase pathway, which mediates maintenance of cell integrity in yeast (23, 25). The mutants in the Pol III machinery, especially *tfc7-ΔN1*, displayed other phenotypes also reported for mutants in the Pkc1 MAP kinase pathway, such as sensitivity to caffeine and to Calcofluor White (data not shown) (36). These observations made it clear that defects in Pol III-mediated transcription can influence cell physiology in unpredictable ways and further motivated us to analyze on a genome-wide scale, by DNA microarray technology, the transcriptional response of *S. cerevisiae* to defective class III gene transcription. Yeast cultures of the mutants and of the corresponding background strains were exponentially grown in rich media at the permissive temperature. RNAs were extracted and fluorescently labeled cDNAs, synthesized from wild-type and mutant sample RNA by using an indirect labeling protocol, were hybridized to DNA microarrays that bear 6,144 yeast open reading frames (ORFs) (17). At least three independent experiments (technical replicates) were performed with two different batches of RNA from each strain (biological replicates). After spot quantification and normalization, the data sets were analyzed by using GeneSpring 6.0 software (Silicon Genetics). Differentially expressed genes were defined as at least twofold up- or downregulated in the mutant compared to the wild type (datasets available upon request). According to this criterion, more than 4% of the analyzed genes displayed significant changes in expression levels in each mutant, thus indicating that a significant remodeling of genome expression is elicited to allow yeast cells to adapt to defects in class III gene transcription. In all cases, the percentage of upregulated genes was significantly higher than the percentage of downregulated genes. The *rpc160-112*, *tfc3-G349E*, *tfc7-ΔN1*, and *brf1-II.6* mutants showed an at least threefold upregulation for 38, 78, 80, and 31 genes, respectively. Among all differentially expressed genes, only 2.5% increased their transcript levels by >10-fold, with a maximum of 30-fold. A large fraction of differentially expressed genes (including all downregulated genes) were specifically deregulated in some mutants and not in others. Only a small, but highly significant set of genes were coinduced by more than twofold in all of the four mutants (see below). We also conducted genome-wide expression studies after shift of the yeast cultures to the nonpermissive temperature (37°C). In this case, however, datasets were more difficult to interpret, likely due to the complicating effects of cell cycle arrest. Only data obtained at the permissive temperature were thus considered for the analyses described below.

Functional classification of the differentially expressed genes. The genes differentially expressed in each mutant were first categorized based on the FunCat annotation scheme, version 2.0 (47), available at the MIPS Web site (http://mips.gsf.de/projects/funcat). The number of genes in each category was

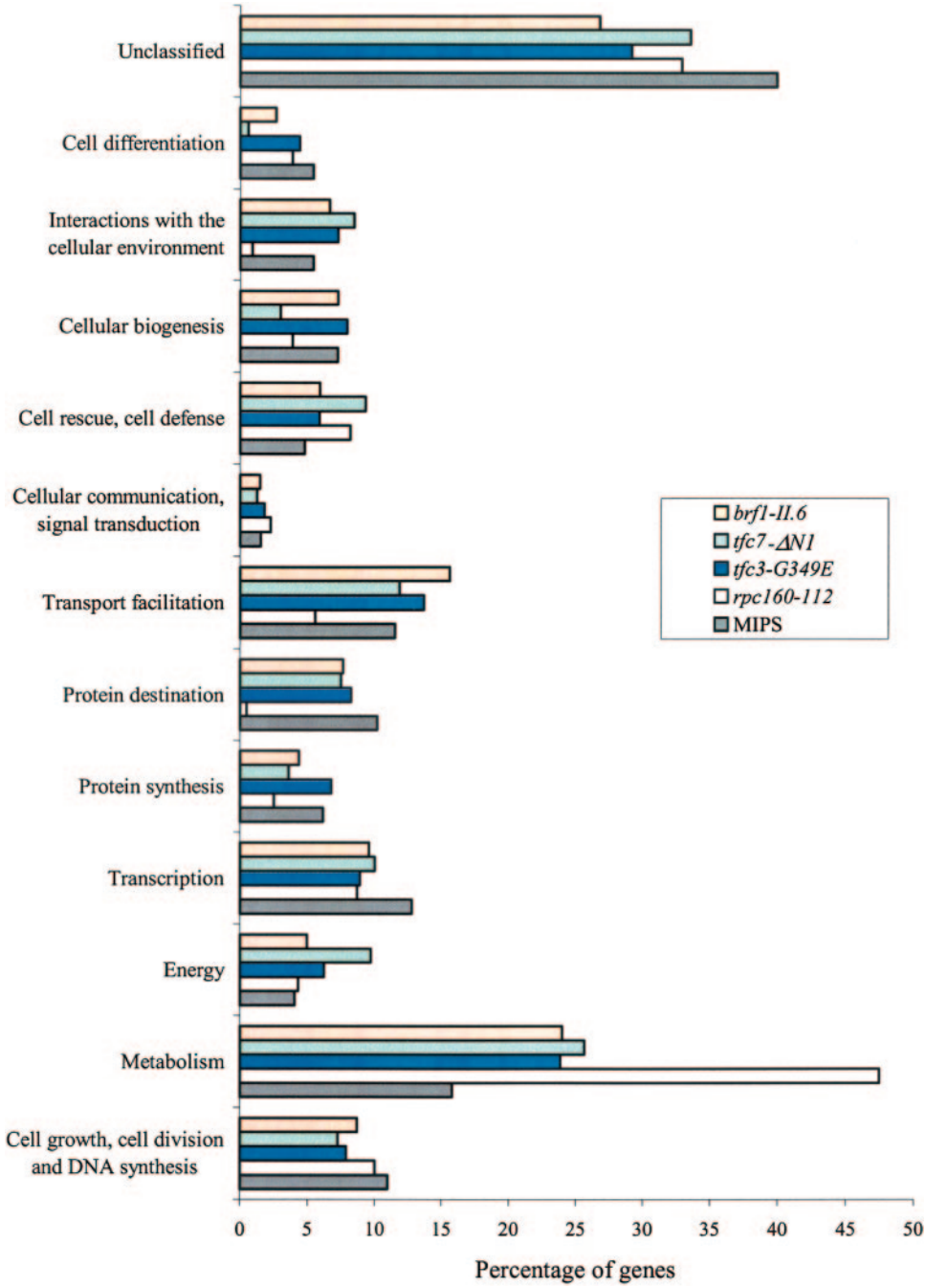

FIG. 1. Distribution of the differentially expressed genes in the four mutants according to functional categories. Gray bars represent the functional catalogue of 6,723 annotated genes according to the MIPS (http://mips.gsf.de/projects/funcat). The other bars represent the distributions in functional categories of the genes differentially expressed in the mutants.

expressed as a percentage of the total number of differentially expressed genes from each of the mutants. As shown in Fig. 1, Pol III transcriptional defects bring about a significant enrichment in transcripts of genes whose products are involved in central metabolism, generation of energy, and cell rescue/defense and, to a lesser extent, in interactions with the cellular environment. This reshaping of functional categories associated with defects in Pol III transcription is very similar to the remodeling of genome expression reported in previous studies analyzing the global gene expression changes in response to environmental stress, including for example, changes in temperature, osmolarity or salinity (8, 19, 44, 57). A similar functional remodeling has also been reported in a study analyzing the global expression changes induced by defects in the cell wall construction (32). Since we observed that mutants in the Pol III transcriptional machinery could share growth phenotypes with mutants of the Pkc1-MAP kinase pathway (see above), we were interested in comparing our data with global

analysis of gene expression changes constitutively induced by cell wall mutations, which have been shown to trigger three major transcriptional responses: the Pkc1-MAP kinase pathway, the global stress system mediated by Msn2/Msn4 and Hsf1 transcription factors, and the Ca^{2+}/calcineurin-mediated pathway (32). A significant overlap was found between the gene expression changes described previously (32) and those observed in the *tfc7-ΔN1* mutant of the Pol III transcriptional machinery. For example, 26 of the 79 genes coinduced (at least twofold) in five mutants of cell wall construction were also more than twofold upregulated in the *tfc7-ΔN1* mutant. Nine (*ASP3D*, *CWP1*, *ECM4*, *PIR3*, *PST1*, *SED1*, *SPI1*, *YGP1*, and *YNL158c*) of the thirty-three genes implicated in cell wall biogenesis whose expression was increased at least twofold in response to cell wall mutations were also upregulated more than twofold in *tfc7-ΔN1*. These results may explain why the *tfc7-ΔN1* strain was the most sensitive to low concentrations of Calcofluor White and caffeine, two cell wall-interfering drugs. In contrast, the expression of most of these genes was not significantly deregulated in the three other mutants of the Pol III transcriptional machinery. The statistical thresholds used in our data analyses did not allow the identification, in these three mutants, of genes whose deregulation could be correlated with the phenotypes they shared with mutants of the Pkc1-MAP kinase pathway, such as their osmotic remediability. The reasons for the peculiar deregulation profile of *tfc7-ΔN1* are unknown, but they might be related to the complex roles of the τ55 subunit of TFIIIC (encoded by *TFC7*) in the interplay between Pol III transcription and cell metabolism (37).

Mutations in the Pol III machinery are associated with a constitutive *GCN4* derepression. To better understand the biological meaning of gene expression remodeling associated with Pol III transcriptional defects, we used the GO::TermFinder software (5) available at the SGD website (http://db.yeastgenome.org/cgi-bin/GO/goTermFinder) to search for significantly enriched gene ontology (GO) terms associated with the four datasets of induced genes. Table 2 shows the results of such analysis. The listed GO terms are those considered to be significantly over-represented ($P < 10^{-4}$) in the four lists of upregulated genes (the same analysis conducted on the four lists of downregulated genes did not reveal any significantly enriched GO term). The most evident feature emerging from Table 2 is that a significant fraction of upregulated genes encodes proteins involved in amino acid metabolism. Importantly, such an enrichment is common to upregulated genes in all of the four mutants, and almost all of the genes that are coinduced in the four mutants (listed in Table 3) fall into this functional category. Previous studies on a genomic scale (28, 40) have shown that the expression of most of these genes is under the control of the Gcn4p transcriptional activator. Starvation for amino acids, purine, and glucose limitation stimulate *GCN4* mRNA translation and thus induce the synthesis of Gcn4p, which in turn controls the expression of at least 500 genes in yeast cells, including genes involved in amino acid biosynthetic pathways, cofactor biosynthesis, organelle biogenesis, mitochondrial transport, autophagy, and glycogen homeostasis (40). We found that more than 20% of all upregulated genes in each of the Pol III transcriptional machinery mutants corresponded to genes identified by Natarajan et al. (40) as being under the control of Gcn4p. Table 3 lists the genes that are consistently upmodulated in the Pol III transcription mutants. Among them, 14 genes were induced more than twofold in all of the four mutants (*ARG1*, *ARG3*, *ARG4*, *ARG5-6*, *CPA2*, *ECM40*, *HIS4*, *HIS5*, *HOM2*, *LEU4*, *MTG1*, *SNO1*, *SNZ1*, and *TMT1*, all reported to be Gcn4p regulated). The cells used for microarray analyses were grown in rich medium, i.e., under conditions in which *GCN4* mRNA translation is expected to be repressed (26). Since the levels of GCN4 mRNA were found to be substantially unchanged in the different Pol III mutants with respect to control strains, the activation of a large number of Gcn4p-responsive genes suggested that Pol III transcriptional defects result in constitutive derepression of *GCN4* mRNA translation.

To further investigate this possibility, we first verified that the set of genes defined as induced by Gcn4p by Natarajan et al. (40) were also upregulated upon *GCN4* derepression in our background strains. To do this, we transformed one of our control strains (YNN282) with a plasmid harboring a modified version of *GCN4* that is expressed constitutively when the cells are grown in a rich medium [p238(*GCN4*^{c}) plasmid] (16). The genome-wide analysis of the expression changes induced by Gcn4p in our background (data not shown but available upon request) revealed a significant overlap (~65%) with the analysis by Natarajan et al. (40) with respect to upregulated genes. Importantly, as shown in Table 3, most of the genes found to be consistently upmodulated in the four Pol III transcription mutants were also responsive to constitutive *GCN4* activation in our background strain. We next directly measured the translational derepression of *GCN4* in the Pol III transcription mutants by using a β-galactosidase colorimetric assay. A *GCN4-lacZ* reporter plasmid, coding for a fusion transcript whose expression is under the translational control typical of *GCN4* mRNA (39), was introduced into the wild-type strain or in the *rpc160-112*, *tfc3-G349E*, *tfc7-ΔN1*, and *brfI-II.6* mutants. As shown in Table 4, a 4- to 5.5-fold induction of *GCN4-lacZ* expression, under nonstarvation conditions, was measured in the Pol III machinery mutants with respect to the wild type, indicating that *GCN4* translational induction was constitutively operating in the mutants. The derepression of *GCN4* in the mutants under nonstarvation conditions was, however, not at its maximum level since a further ~2-fold increase of *GCN4-lacZ* expression was observed when the cells were starved for amino acids for 3 h (Table 4). To conclusively prove that the induction of *GCN4* target genes in the Pol III mutants is dependent on Gcn4p, we sought to determine whether deleting *GCN4* in the mutants could eliminate the response. The *GCN4* gene was deleted in the *tfc3-G349E* and *brf1-II.6* mutant strains and in the YPH500 control strain, and the levels of several mRNAs were measured by real-time PCR. As shown in Table 5, the pronounced activation observed in the mutant strains for two *GCN4* target genes, *HIS4* and *ARG1* genes, was completely abolished upon deletion of *GCN4*, thus demonstrating that *GCN4* is necessary and sufficient for their upmodulation in the Pol III transcription mutants. A different behavior was observed for *PIC2*, a gene that we found only weakly (1.8-fold) upregulated in the presence of constitutive *GCN4* activation in the YNN282 strain (the same gene is completely unresponsive to *GCN4* in the analysis by Natarajan et al. [40]). *PIC2* was 2.2- and 2.9-fold upmodulated in the *tfc3-G349E* and *brf1-II.6* mutant strains, respectively. As shown in Table 5, *GCN4* deletion

TABLE 2. Functional classification of differentially expressed genes in the Pol III transcription mutants according to GO[a]

Biological process					Mutant(s)
Cellular process	Cell communication	Response to extracellular stimulus	Response to extracellular starvation	Cellular response to nitrogen starvation	tfc3
	Cellular physiological process	Cell growth and/or maintenance	Transport	Hexose transport	tfc3
					tfc3
Physiological process	Metabolism	Amine metabolism	Amino acid metabolism	Arginine biosynthesis	brf1, ΔN1, tfc3, rpc160
				Arginine metabolism	brf1, ΔN1, tfc3, rpc160
				Aromatic amino acid family biosynthesis	tfc3, rpc160
				Aromatic amino acid family metabolism	tfc3, rpc160
				Aromatic compound biosynthesis	tfc3, rpc160
				Aromatic compound metabolism	tfc3, rpc160
				Asparagine catabolism	tfc3
				Asparagine metabolism	tfc3
				Aspartate family amino acid catabolism	tfc3, rpc160
				Aspartate family amino acid metabolism	tfc3, rpc160
				Branched chain family amino acid biosynthesis	tfc3, rpc160
				Branched chain family amino acid metabolism	tfc3, rpc160
				Glutamate metabolism	tfc3
				Glutamine family amino acid biosynthesis	brf1, ΔN1, tfc3, rpc160
				Glutamine family amino acid metabolism	brf1, ΔN1, tfc3, rpc160
				Lysine biosynthesis	rpc160
				Lysine biosynthesis, aminoadipic pathway	rpc 160
				Lysine metabolism	rpc 160
				Nonprotein amino acid metabolism	brf1, ΔN1, tfc3, rpc160
		Alcohol metabolism	Monosaccharide metabolism	Glycolysis	rpc160
		Electron transport			Δ N1
		Energy pathways	Energy derivation by oxidation of organic compounds	Energy reserve metabolism	Δ N1
			Energy derivation by oxidation of organic compounds	Main pathways of carbohydrate metabolism	tfc3, rpc160
		Heterocycle metabolism			tfc3
		Macromolecule metabolism	Carbohydrate metabolism		Δ N1
			Protein metabolism	Protein folding	Δ N1
			Protein metabolism	Purine base metabolism	tfc3
		Nitrogen metabolism	Urea cycle intermediate metabolism		brf1, ΔN1, tfc3, rpc160
		Organic acid metabolism	Carboxylic acid metabolism		brf1, ΔN1, tfc3, rpc160
			Nucleobase biosynthesis	Purine base biosynthesis	tfc3
	Response to stimulus	Response to stress			brf1, ΔN1, tfc3, rpc160

[a] Only significantly enriched ($P < 0.0001$) GO terms (biological process) associated with at least one of the four datasets of induced genes are reported; the mutants whose modulated genes are significantly enriched in each category are reported in the last column on the right.

TABLE 3. Genes consistently upmodulated in the four Pol III transcription mutants[a]

Gene		Ratio				Gcn4 regulated[b]
Systematic name	Common name	*rpc160-112*	*tfc7-ΔN1*	*tfc3-G349E*	*brf1-II.6*	
YBR218C	*PYC2*	2.54	2.46	3.07	1.68	
YCL009C	*ILV6*	2.14	1.61	3.33	2.87	
YCL030C	*HIS4*	6.06	3.94	13.36	2.02	*
YDR035W	*ARO3*	3.28	2.21	6.81	1.54	*
YDR158W	*HOM2*	4.60	2.44	6.67	2.10	*
YER053C	*PIC2*	1.79	2.49	2.21	2.86	
YER069W	*ARG5,6*	4.13	4.22	4.86	2.38	*
YER175C	*TMT1*	4.43	4.49	12.03	2.89	*
YHR018C	*ARG4*	2.62	2.14	2.05	2.64	*
YHR208W	*BAT1*	6.77	2.10	2.15	1.76	*
YIL116W	*HIS5*	4.14	2.89	9.99	4.73	*
YJL088W	*ARG3*	6.36	5.36	4.46	4.69	*
YJR109C	*CPA2*	4.82	2.59	10.95	3.29	*
YKL218C	*SRY1*	3.51	2.00	6.49	1.91	*
YMR062C	*ECM40*	4.97	2.04	3.74	2.08	*
YMR094W	*CTF13*	2.07	1.53	5.83	3.44	*
YMR095C	*SNO1*	5.09	2.28	12.49	15.59	*
YMR096W	*SNZ1*	14.33	5.86	31.16	4.90	*
YMR097C	*MTG1*	4.46	2.12	6.41	2.13	*
YMR108W	*ILV2*	3.65	2.07	3.72	1.69	
YMR189W	*GCV2*	3.52	1.55	4.00	2.28	
YNL104C	*LEU4*	4.53	2.68	3.94	2.02	*
YOL058W	*ARG1*	8.28	3.39	7.87	4.60	*
YOL140W	*ARG8*	8.54	5.36	18.12	1.77	*

[a] The table lists the genes whose expression is increased more than 2-fold in at least three of the four mutants and more than 1.5-fold in the fourth mutant.
[b] An asterisk indicates that the gene was found to be at least twofold upregulated in the YNN282 background strain under conditions of constitutive *GCN4* derepression.

did not affect *PIC2* activation in the *brf1-II.6* mutant, whereas it abolished the response in the *tfc3-G349E* context, thus suggesting that *PIC2* upmodulation depends on different pathways in the two mutants. The modulation of *AVO2*, another gene that is not responsive to constitutive *GCN4* activation, also displays a complex, mutant-specific pattern. This gene is clearly upmodulated in the *brf1-II.6* mutant, whereas its expression is unchanged in the *tfc3-G349E* strain. Unexpectedly, the deletion of *GCN4* abolishes the response in *brf1-II.6*. Such a behavior might be explained by assuming that *GCN4* is necessary but not sufficient for *AVO2* activation in *brf1-II.6*. Reported in Table 5 are also the consequences of *GCN4* deletion on the response of three genes that are downmodulated in the Pol III mutants: *PHO11*, *HXT2*, and *GAL1*. *PHO11* and *HXT2* are unresponsive to constitutive *GCN4* activation and, accordingly, their downmodulation in the mutants was left unchanged by *GCN4* deletion. A different behavior was observed for *GAL1*. On the basis of our analysis and that of Natarajan et al. (40), *GAL1* is also unresponsive to constitutive *GCN4* activation. However, its marked downmodulation in the two Pol III mutants was abolished upon deletion of *GCN4*. Again, this result might be explained by assuming that *GAL1* repression in the mutants depends on the action of both *GCN4* and another regulator(s).

TABLE 4. Expression of *GCN4-lacZ* in Pol III transcription mutants

Strain	β-Galactosidase activity (U)[a]	
	Nonstarvation	Starvation
Expt 1		
WT (YPH500)	45	400
rpc160-112	250	530
tfc3-G349E	190	450
tfc7-ΔN1	180	445
brf1-II.6	270	490
Expt 2		
WT (YPH500)	40	425
Δ*gcn2*	15	25
tfc3-G349E	180	450
*tfc3-G349E*Δ*gcn2*	150	140

[a] The indicated strains, transformed with plasmid p180 (*GCN4-lacZ-*, *CEN*, *URA*), were grown at the permissive temperature under the nonstarvation or starvation conditions as described in Materials and Methods. Extracts were prepared and assayed for β-galactosidase activity (expressed as nanomoles of *o*-nitrophenol-β-D-galactopyranoside hydrolyzed per minute per microgram of total protein). The reported values are averages of at least three independent measurements, performed in duplicate, that differed by no more than 20% of the mean. The upper and lower parts of the table refer to two independent experiments in which different groups of strains were analyzed. WT, wild type.

We finally wondered whether *GCN4* constitutive derepression is a general feature of the mutants in the Pol III transcriptional machinery. To this purpose, *GCN4-lacZ* expression was measured in six other thermosensitive mutants grown under nonstarvation conditions at the permissive temperature, and it was found to be increased in all mutants with the exception of the *tfc1-E447K* mutant (29) and a *brf1-HA*-tagged strain (24) (data not shown).

The *GCN4* derepression in Pol III transcription mutants is *GCN2* independent. Previous studies showed that *GCN4* expression is stimulated under conditions producing a decrease in the levels of the ternary complex composed of eIF2, GTP, and Met-tRNA$_i^{Met}$ (26). In response to amino acid or purine starvation, the reduced formation of the ternary complex is

TABLE 5. Effect of *GCN4* deletion on transcriptional responses in the *brf1-II.6* and *tfc3-G349E* mutants

Gene name		Ratio[a]					
		brf1-II.6		*brf1-II.6Δgcn4* (qPCR)	*tfc3-G349E*		*tfc3-G349EΔgcn4* (qPCR)
Systematic	Common	Microarray	qPCR		Microarray	qPCR	
YHR018C	*ARG1*	2.64	11.63	1.39	2.05	4.01	0.78
YCL030C	*HIS4*	2.02	2.10	1.17	13.36	3.90	0.77
YER053C	*PIC2*	2.86	ND	2.69	2.21	ND	1.22
YAR071W	*PHO11*	0.16	ND	0.26	0.41	ND	0.27
YMR011W	*HXT2*	0.62	ND	0.37	0.19	ND	0.25
YBR020W	*GAL1*	0.02	0.31	1.31	0.11	ND	1.36
YMR068W	*AVO2*	2.10	3.23	1.01	0.78	ND	0.61

[a] The reported values are the ratios between mRNA levels in the indicated strains and in the corresponding background strains. qPCR, real-time quantitative PCR. ND, not determined.

controlled by the Gcn2 protein kinase. There are also several examples where *GCN4* is derepressed in a manner dependent on the reduced levels of the ternary complex but independent of Gcn2 (26, 43). To test the *GCN2*-dependence of *GCN4* derepression in the Pol III transcription mutants, the *GCN2* gene was deleted in *tfc3-G349E* strain, and *GCN4-lacZ* expression was measured in *tfc3-G349E* and *tfc3-G349EΔgcn2* strains and a Δ*gcn2* control strain (22). As shown in Table 4, similar low levels of β-galactosidase activity were obtained when the Δ*gcn2* cells were grown under repressing or derepressing conditions, as expected. The ~4-fold derepression of *GCN4-lacZ* in *tfc3-G349E* versus wild-type cells grown in rich medium was also observed in *tfc3-G349EΔgcn2* cells, thus indicating that *GCN4* derepression in the Pol III transcription mutant is independent of *GCN2*. In contrast, the 2.5-fold increase in *GCN4-lacZ* expression observed in the mutant upon amino acid starvation is *GCN2*-dependent.

The *GCN4* derepression in Pol III transcription mutants depends on initiator tRNA^{Met} depletion. A reduced gene dosage of initiator tRNA^{Met}, or defects in its maturation, have previously been shown to increase Gcn4p expression by reducing the concentration of eIF2 · GTP · Met-tRNA$_i^{Met}$ ternary complex (7, 11). By real-time PCR analysis, the steady-state levels of tRNA$_i^{Met}$ were found to be ~2-fold reduced in the four Pol III mutant strains with respect to control strains at the permissive temperature (data not shown). To see whether increased *GCN4* expression under conditions of defective Pol III transcription could be due to such a reduction of tRNA$_i^{Met}$ levels, the *brf1-II.6* mutant strain was transformed with multicopy plasmids carrying either the *IMT1* gene, coding for the initiator tRNA^{Met}, or the *EMT3* gene, coding for the elongator tRNA^{Met}. We verified by both real-time PCR and Northern analysis that overexpression of *IMT1* in the mutant restored levels of initiator tRNA^{Met} that were ~1.5-fold higher than in the wild type; we also observed that the growth defect of *brf1-II.6* mutant was partially corrected by increased *IMT1*, but not *EMT3*, gene dosage (data not shown). The expression levels of *ARG1* and *HIS4*, two Gcn4p-responsive genes whose mRNAs were found to be increased ~5- and ~2-fold, respectively, in *brf1-II.6* by microarray analysis, were analyzed in nontransformed and transformed *brf1-II.6* and wt cells by real-time PCR. As shown in Table 6, the upregulation of both *ARG1* and *HIS4* genes typically observed in *brf1-II.6* was abolished by increased *IMT1* gene dosage, whereas it was unaffected (or even slightly exacerbated) by increased *EMT3* dosage. Initiator tRNA^{Met} depletion is thus specifically responsible for the Gcn4p-dependent transcriptional response in the *brf1-II.6* mutant.

Positional effects of tRNA genes on neighboring Pol II transcription units. It has previously been proposed that the expression of a number of chromosomal, Pol II-transcribed genes might be influenced by tDNA position effects (4). Based on computational evidence, such effects would be mainly inhibitory, and two cases have been reported of chromosomal genes whose expression is slightly increased when the neighboring tRNA genes are transcriptionally inactivated or deleted (4, 51). Cases of tRNA genes with a protective function against transcriptional repression have also been reported (15, 51). To establish how widespread are the tDNA proximity effects on Pol II-directed transcription in the yeast genome, we compared the average expression changes, at the permissive temperature, of the subset of Pol II-transcribed genes that are nearest neighbors of tDNAs with those of the other genes (the complete data set for modulation of the tDNA nearest-neighbor ORFs is available in the supplemental material). We considered as significantly modulated the genes whose expression varied at least twofold in the mutant with respect to control strains. As shown in Fig. 2, the tDNA-flanked genes appear to be slightly more modulated than the other genes in the *tfc7-DN1* and *brf1-II.6* mutants, but not in the case of the *tfc3-G349E* and *rpc160-112* mutants. The data in Fig. 2 also take into account the distance between the tDNA and its nearest neighbor, since it is not known how far tDNA positioneffects may reach. In the case of *brf1-II.6*, the tDNA-flanked genes located at less than 1,000 bp

TABLE 6. Increased *IMT1* gene dosage specifically overrides the *GCN4*-dependent transcriptional response in the *brf1-II.6* mutant

Analysis	Strain	Ratio[a]	
		ARG1	*HIS4*
Microarray	*brf1-II.6*	4.60	2.02
Real-time PCR	*brf1-II.6*	11.63	2.10
	brf1-II.6 + pIMT1	1.04	0.94
	brf1-II.6 + pEMT3	17.27	2.47

[a] The reported values are the ratios between *ARG1* or *HIS4* expression in the indicated strains and in the corresponding background strains (*BRF1*, either untransformed or transformed with pIMT1 or pEMT3).

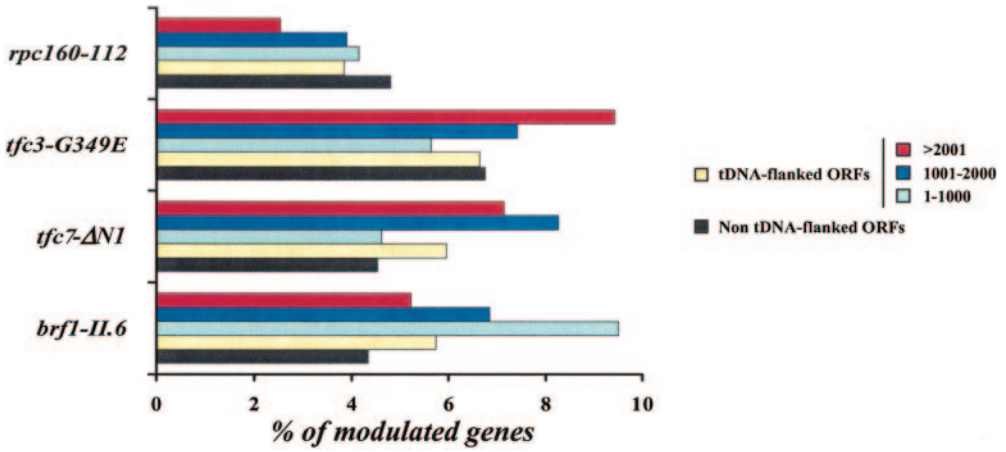

FIG. 2. Modulation of tDNA-flanked genes in mutants of the Pol III transcription machinery. The bar plot reports, for each of the four mutants, the percentages of significantly (more than twofold) modulated genes within the entire set of genes that are nearest neighbors of tDNAs (yellow bars), within the entire set of genes that are not tDNA-neighbors (black bars), within the set of tDNA-flanked genes located at less than 1,000 bp (green bars), at distances between 1,000 and 2,000 bp (blue bars), and at distances larger that 2,000 bp (red bars). The set of tDNA-flanking Pol II genes, as well as their distances to the neighbor tDNA, were derived from the tRNA gene loci database reported in reference 4.

from the tDNA were significantly more deregulated than those located at larger distances. The situation was reversed in the case of the two TFIIIC mutants, for which the genes located at more that 1,000 bp from the tDNA were more deregulated. The modulation of tDNA neighbor genes was also separately analyzed for the subset of genes that are not regulated by *GCN4*. The results of such analysis were almost identical to those in Fig. 2. These results argue in favor of the existence of some transcriptional interference between tDNAs and neighboring Pol II-transcribed genes (see Discussion). However, it is also evident that the expression of most (>90%) of the tDNA-flanked genes was not affected significantly in the Pol III transcriptional mutants. To gain more insight into tDNA position effects, we thus decided to undertake a tDNA deletion approach by focusing on a small number of loci in which the expression of a tDNA-flanked gene was found to be consistently modulated in at least three of four Pol III transcriptional mutants. Of the ORFs considered for this analysis (Fig. 3), five were upmodulated >1.5-fold in at least three of the four mutants, whereas only one, *YEL033W*, was consistently downmodulated in the mutants. The corresponding tDNAs were removed by a two-step homologous recombination strategy, first replacing each tDNA with *URA3* and then transforming the *tdna*Δ::*URA3* strains with genomic fragments lacking the tDNA and selecting them on 5-FOA media. As shown in Fig. 3, only two of the five genes upregulated in the Pol III mutant microarray analysis showed a consistent increase in transcript level by Northern blot analysis upon deletion of the adjacent tDNA (*ACO1* and *ARO8*). The observed upregulation was modest, however, at best a 1.4-fold increase upon deletion of the tDNA. The other three genes (*AMD2*, *POR1*, and *YJL200C*) showed no or inconsistent changes in transcript levels upon tDNA deletion compared to their respective parent strains. Interestingly, deletion of the tDNA immediately downstream of *YEL033W*, a downregulated gene in the microarray analysis, resulted in a significant downregulation of *YEL033W* transcript level. Both the weak upregulation of *ACO1* and *ARO8* and the downregulation of *YEL033W* upon deletion of

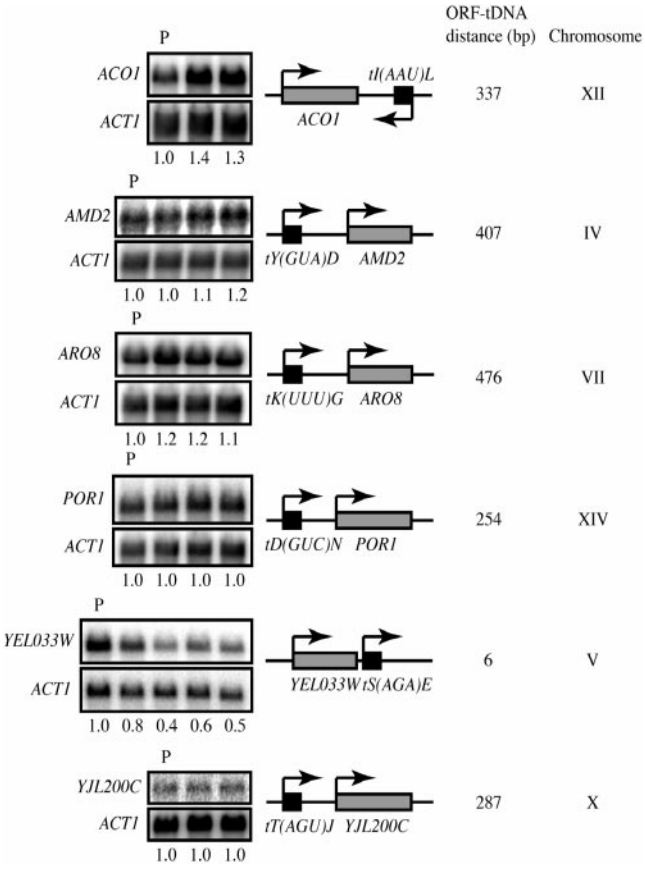

FIG. 3. Effects of tDNA deletion on the expression of adjacent Pol II-transcribed genes. The tDNA adjacent to each of the six ORFs (the modified loci are schematically illustrated on the right) was deleted from the chromosome. At least two independent tDNAΔ recombinant strains were isolated in each case. Northern blot analysis of the expression of each Pol II gene is shown compared to the corresponding parent strain (P). Band intensities were determined by phosphorimager analysis, and normalized to the *ACT1* signal for each lane. The values under each lane represent the fold difference of the normalized signals relative to that of the parent strain.

the nearest tDNA were not a result of *GCN4* derepression, possibly triggered by tDNA deletion, because the expression of the *GCN4*-responsive *ARG1* and *HIS4* genes was not affected in the tDNA deletants, as revealed by Northern blot analysis (data not shown). A conclusion of this analysis is that the upmodulation of *ACO1*, *AMD2*, *ARO8*, *POR1*, and *YJL200C*, observed in the Pol III transcription mutants, cannot be accounted for by a tDNA proximity effect (upmodulation *ACO1* and *YJL200C* might instead be attributed to the *GCN4* response, because both of these genes were more than twofold activated in response to constitutive *GCN4* derepression).

DISCUSSION

The interplay between the three nuclear RNA polymerase systems in eukaryotes is presumably a key aspect of growth control, yet it remains largely uncharacterized. In the present study, we have addressed this issue by analyzing, on a genome-wide scale, the influence of RNA Pol III-directed transcription on the expression of RNA Pol II-transcribed genes in *S. cerevisiae*. The problem of interplay between Pol III and Pol II systems is twofold. First, class III genes are characterized by an extremely high rate of transcription and transcription factor occupancy during active cell growth (14, 18, 24), a feature that might favor repressive or antirepressive position effects on neighboring RNA Pol II promoters. Second, Pol III synthesizes very abundant, essential RNAs whose levels might influence in a complex way the expression pattern of protein-coding genes. The results of our study provide insight into both of these aspects of Pol III-Pol II interplay. As for the positional interference exerted by class III genes on neighboring Pol II promoters, we have shown that it operates only to a limited extent and thus probably does not represent a key feature of yeast genome expression. As for the effects on the Pol II transcriptome of reduced levels of class III gene products, we have revealed an extensive, *GCN4*-mediated reprogramming of yeast genome expression that entirely depends on initiator $tRNA^{Met}$ depletion.

The general lack of positional effects exerted by tDNAs and the other class III genes was somehow unexpected, because actively transcribed class III genes have been shown to strongly repress adjacent Pol II promoters in some cases (27, 30, 31) and to protect neighboring genes from the propagation of repressive chromatin in other cases (15, 51). Although information on tRNA gene-mediated repression mostly comes from studies in which tRNA genes and Pol II reporter genes were artificially juxtaposed in plasmid constructs, repressive tDNA positional effects have also been reported to operate at native chromosomal loci (4, 27, 51). The general lack of such effects emerging from our microarray data might be due to the fact that the transcriptional defects associated with the Pol III system mutations are not sufficiently strong to cause a loss of the positional effect. Transcriptional interference might require nothing more than the presence of TFIIIB and/or TFIIIC on class III genes, regardless of their actual transcription. In the Pol III catalytic mutant *rpc160-112*, for example, TFIIIC and TFIIIB are likely to be assembled on tDNAs as they are in the wild type and thus to produce the same level of transcriptional interference. On the other hand, tDNA occupancy by TFIIIC and/or TFIIIB is expected to be decreased in the other three mutants with respect to wild type. In these mutants, however, reduced transcription complex assembly might not be sufficient to impair transcriptional interference. These considerations prompted us to test the Pol III-Pol II interference at selected loci by a tDNA deletion approach. With the interesting exception of *YEL033w*, whose expression was clearly reduced upon deletion of the adjacent S(AGA)E tDNA, the results of this analysis confirmed the conclusion that positional interference produces small magnitude effects and probably operates on a very small set of Pol II-transcribed genes. This in turn implies that the majority of Pol II transcription units in *S. cerevisiae* are well insulated and thus refractory to in *cis* perturbation by nearby tDNAs. The observation that, in the *brf1-II.6* mutant, the tDNA-neighbor genes closer to tDNAs were more deregulated than those located at larger distances argues in favor of the presence of tDNA position effect at the subset of loci very close to tRNA genes, in agreement with the conclusion of a previous bioinformatic analysis of the tRNA gene loci database (4). The reversal of the distance dependence of modulation in the two TFIIIC mutants, however, suggests the existence of more subtle and less predictable effects of tDNA transcription on neighboring genes, perhaps related to the extensive presence of Ty elements around tRNA genes. Given this complexity, we believe that the systematic deletion of all yeast tDNAs will be required in order to conclude about the extent of tDNA position effects in yeast.

Based on transcriptomic data, the *GCN4*-dependent transcriptional response is the major consequence of defects in Pol III transcription. The activation of several Gcn4p-regulated genes was invariably observed in all of the Pol III mutants analyzed, thus suggesting that a reduced level of Pol III transcription products is the primary intracellular signal eliciting such a response. The molecular pathway leading from Pol III transcript depletion to *GCN4* derepression turned out to be unexpectedly straightforward. An increase in initiator $tRNA^{Met}$ gene copy number was indeed found to be sufficient to abolish *GCN4* derepression in the Pol III mutant context, thus indicating that $tRNA_i^{Met}$ depletion is the main (if not the sole) factor responsible for eliciting the Gcn4p-mediated response. It has previously been shown that *GCN4* expression can be translationally induced by reducing the $tRNA_i^{Met}$ gene dosage, in agreement with a model inversely coupling *GCN4* translation to the level of eIF2 · GTP · Met-$tRNA_i^{Met}$ ternary complex (11). What our data add to this picture is that a reduction in ternary complex levels can occur as a direct (and perhaps, for cell physiology, the most important) consequence of a decrease in Pol III transcription. Interestingly, a genome expression remodeling reminiscent of the general amino acid control response has recently been observed in response to treatment of yeast cells with a small molecule inhibitor (UK-118005) of RNA Pol III (56). Such a response was also found to be *GCN2* independent; however, in disagreement with our observations, *GCN4* mRNA translation was not found to be increased in UK-118005-treated cells. The reasons for such a discrepancy are presently unclear but might include side effects of the drug on other cellular processes.

Recent genome-wide studies have shown that Gcn4p induces an unexpectedly large set of genes, encompassing as much as 1/10 or more of the yeast genome, and that it does so in response not only to amino acid deprivation but also to

purine and glucose starvation, high salinity, and treatment with UV, methyl methanesulfonate, and rapamycin (reviewed in reference 26). Gcn4p thus appears to be a key regulatory protein in the global remodeling of RNA Pol II-dependent genome expression elicited by diverse signals of starvation and stress. Several environmentally stressful conditions, such as nutrient limitation, secretion defects, and treatment with DNA-damaging agents, are known to severely downregulate RNA Pol III-dependent transcription in yeast (9, 12, 21, 34, 49, 52, 58). The reduction in the level of initiator $tRNA^{Met}$ resulting from such perturbations might contribute to *GCN4* translational activation and the consequent, genome-wide reprogramming of RNA Pol II-dependent transcription. Together with the still largely unknown molecular strategies allowing for coregulation of Pol I and Pol III transcription, the $tRNA_i^{Met}$-mediated cross talk between Pol III and Pol II transcription might represent a key element of the coordinated regulation of the three eukaryotic transcription systems in response to environmental changes.

The involvement of other regulatory pathways, besides *GCN4* activation, in the genome-wide response to defects in the Pol III machinery is made evident by the high number of genes that are significantly (at least twofold) up- or downregulated in the mutants but that are not responsive to constitutive *GCN4* activation. These genes represent ~80% of all modulated genes in each of the mutant strains. It is important to note, however, that the up- or downregulation of most of these genes was not found to be conserved in the different mutants. As already mentioned, the general remodeling of genome expression in the Pol III mutants, illustrated in Fig. 1, is reminiscent of the previously described common environmental response (8) and environmental stress response (19). For each of the mutants, however, a search for significantly enriched GO terms associated with the sets of modulated, *GCN4*-independent genes did not produce any reliable result. The complexity and heterogeneity of responses in the different mutants is probably related to the wide impact exerted on cell physiology by a reduced rate of protein synthesis (consequent to reduced levels of class III gene products) and to the fact that the different mutations in the Pol III system might affect the rate of protein synthesis to different extents.

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