In order for RNA polymerase (pol) III to produce a sufficient quantity of RNAs of appropriate structure, initiation, termination, and reinitiation must be accurate and efficient. Termination-associated factors have been shown to facilitate reinitiation and regulate transcription in some species. Suppressor tRNA genes that differ in the dT(n) termination signal were examined for function in Schizosaccharomyces pombe. We also developed an S. pombe extract that is active for tRNA transcription that is described here for the first time. The ability of this tRNA gene to be transcribed in extracts from different species allowed us to compare termination in three model systems. Although human pol III terminates efficiently at 4 dTs and S. pombe at 5 dTs, Saccharomyces cerevisiae pol III requires 6 dTs to direct comparable but lower termination efficiency and also appears qualitatively distinct. Interestingly, this pattern of sensitivity to a minimal dT(n) termination signal was found to correlate with the sensitivity to α-amanitin, as S. pombe was intermediate between human and S. cerevisiae pols III. The results establish that the pols III of S. cerevisiae, S. pombe, and human exhibit distinctive properties and that termination occurs in S. pombe in a manner that is functionally more similar to human than is S. cerevisiae.

RNA polymerase (pol) III is a multisubunit enzyme that is directed to initiate RNA synthesis by transcription factors (TF) that bind to gene promoter elements. pol III transcripts comprise a large variety of small nuclear and cytoplasmic RNAs (1). Although there is diversity in the promoter structures of pol III-transcribed genes, three classes are responsible for the synthesis of most cellular pol III transcripts, tRNAs, 5 S rRNA, and U6 small nuclear RNA (2). Each of these represent a gene class that utilizes a characteristic promoter structure and specific set of TFs (3). 5 S tRNA genes comprise class I and contain a principal internal promoter that is recognized by TFIIIA. Class 3 genes utilize upstream TATA elements and in metazoans an upstream element recognized by a distinct multisubunit TF (4). Class 2 genes are represented by tRNA genes, which use an internal promoter comprised of proximal box A and distal box B elements. Distinct subunits of TFIIIC bind to the A box, B box, and terminator element of the class 2 genes and facilitate the assembly of this class of preinitiation complexes (3, 5). For each gene class, TFIIIB (or related activity) binds just upstream of the start site of transcription, and this in turn serves as the initiation factor proper as it recruits pol III (Refs. 6–8 and references therein). Subunits of TFIIIB as well as pol III have been conserved from Saccharomyces cerevisiae to human, as have two TFIIIC subunits that localize near the start site of transcription (9–14). By contrast, the downstream TFIIIC subunits in these organisms reveal no recognizable sequence homology (12, 15, 16).

Some evidence suggests that efficient transcription requires termination and associated activities that promote pol III recycling (17–22). Upon encountering the dT(n) tract that comprises the pol III termination signal, by pol III itself, the enzyme pauses and releases the transcript and template (23–26). Multiple subunits of pol III affect termination (Refs. 26 and 27 and also see Ref. 28). Evidence that TFIIIC and/or associated factors, as well as the -UUU-OH terminus binding protein, La, may facilitate pol III termination and reinitiation, exists for human-derived systems (19, 21, 22, 29, 30).

We modified a suppressor tRNA gene, sup3-e (31, 32), to serve as a reporter of pol III termination in the fission yeast Schizosaccharomyces pombe. To complement the results obtained, we developed an S. pombe-derived extract that is active for tRNA transcription. We took advantage of the extraordinary conservation of the tRNA gene promoter and the ability of the pol III machinery from different species to initiate transcription on it. Because sequence context can affect termination and, in some cases, associated activities of apparent regulatory significance, we also examined different pols III on the same template (17–19, 21, 27, 30, 33, 34). By this approach, a clear difference between S. cerevisiae and human pol III termination signal recognition was demonstrated with S. pombe pol III termination at an intermediate dT(n) length. Another difference supported the idea that S. pombe pol III termination may be mechanistically more similar to human than is S. cerevisiae. Finally, we examined transcription in the presence of α-amanitin, a small toxin that is known to inhibit certain eukaryotic RNA polymerases. This revealed a correlation between the response to a minimal dT(n) signal and sensitivity to α-amanitin by these pols III on the same template and further distinguished the enzymes.

**EXPERIMENTAL PROCEDURES**

A PstI-EcoRI fragment containing the sup3-e gene (35) was isolated from pRIP 1/s (36) and used for polymerase chain reaction mutagenesis, and a 0.4-kilobase pair fragment was cloned into the PstI and SacI sites of plasmid pK148 (37). The sequence following the mature tRNAfive 3′-end in the monomeric tRNAfive genes is GAGATC(TT)n. The cRT sequence, GAGCTC(G)C(A)G(A)G(C)GGCTGGAAAGGAGGAGGCT- TCCG(T)CACAAGCAGGATT(AG)GCAAACTCGAGTGAATACAGG- ATGGAAG(G)ACTAATTTTTTATGCT, immediately follows the test
Three substitutions were made in tRNAUGA as follows: C40T, T46.3C and C47.6T, numbered according to Ref. 31. One substitution resides in the anticodon stem and one in the variable arm stem, each converting a G:C to a G:U base pair, whereas the third resides in the loop of the extra arm (31). In the DNA, these substitutions reside between the A and B box promoter elements, in a region that is highly variable, that does not affect the transcription of other tRNA genes (38–40). The resulting plasmids of the series, tRNAUGA-M-2T (designated pMSer-2T), tRNAUGA_WT-2T (designated pSer-2T), -3T, -4T, etc. were linearized with NdeI and used to transform yAS50, h-S, leu1-32, ura4, ade6-704, obtained from ATCC (Manassas, VA) as strain SP1190 (41). After selection for leucine prototrophy, cells were grown in YEP. RNA was extracted with guanidinium thiocyanate and acid phenolchloroform, EtOH-precipitated, electrophoresed on 6% polyacrylamide-urea, blotted, and probed with 32P-labeled oligonucleotide DNAs (42). The tRNAUGA probe, 5’-TGCCCGGACAGCGCCGTTAAT-3’, “MSer,” is complementary to bases AAS-A51 of tRNAUGA, present in precursors and mature species. The probe, “5’-Ser”, 5’-CCACTCGGA-CATATGACCTTTAGC-3’, is directed to the common 5’-region of mature tRNAUGA (31, 43). The tRNAUGA probe was described (44).

S. pombe (yAS50) and S. cerevisiae (CG1945, CLONTECH) extracts used for in vitro transcription were prepared according to Nichols et al. (45) with the following modifications for S. pombe. After breaking cells with glass beads, the lysate was made 0.6 M NaCl by the slow addition of 5 M KCl. The extraction buffer contained antipain dihydrochloride (2.5 μg/ml), bestatin (0.35 μg/ml), chymostatin (2 μg/ml), leupeptin (0.5 μg/ml), pepstatin (0.4 μg/ml), and Pefabloc SC (0.1 mg/ml), all obtained from Roche Molecular Biochemicals. HeLa nuclear extract was prepared by a standard method (46).

Transcription reactions (25 μl) contained 60 mm KCl, 20 mm HEPES, pH 7.9, 10 mm MgCl₂, 0.2 mm EDTA, 500 ng of plasmid DNA, 0.6 mm CTP, ATP, UTP, 0.05 mm GTP, 0.5 μCi of [α-32P]GTP (ICN, Costa Mesa), and 0.5 μl of RNAase (Promega). α-Amanitin was from Sigma. Quantitation was performed on a PhosphorImager (Molecular Dynamics).

RESULTS

Development of a tRNA Reporter Gene Whose Activity in Fission Yeast Is Dependent on Accurate and Efficient Termination by pol III—To investigate the cis elements required for termination, we deleted the tRNAUGA sequence of sup3-ε and introduced test terminators consisting of varying lengths of dT residues, T(n), in place of the cryptic inefficient terminator (Fig. 1). Preliminary data suggested that constructs bearing inefficient terminators nonetheless produced functional tRNA, presumably because readthrough transcripts were correctly processed at their 3'-ends (47–49) (not shown). tRNA structure appears to be a major determinant of recognition by 3'-processing enzymes (50, 51), and although certain artificial sequences placed downstream of an inefficient terminator can interfere with 3'-processing, others support processing (47, 49). To ensure that tRNA expression would be dependent on accurate termination, we inserted a sequence downstream of the test terminator to interfere with tRNA formation if pol III was rate terminating, we inserted a sequence downstream of the tRNAUGA precursor to form an extended duplex that is not recognizable as a tRNA precursor (not shown). These changes do not alter the tRNAUGA sequence or the residues immediately surrounding the 3'-processing site. Upon accurate termination at the T1 test terminator, this gene, designated tRNAUGA_WT, would be expected to produce a nascent precursor of 112 nucleotides (Fig. 1). To distinguish tRNAUGA from tRNAUGA, the variable tRNAUGA and other tRNAUGAs, we introduced three “silent” substitutions (31) to allow specific detection by Northern blotting. The resulting gene was designated tRNAUGA_sup3-e (Fig. 1). These substitutions reside between the intron and box B, in a region of tRNA genes that is highly variable, such that they would not be expected to, nor do they, affect transcription (Refs. 31 and 38–40 and data not shown).

Termination-dependent Expression of Suppressor tRNAUGA

Species-specific Differences in pol III Termination

FIG. 1. Derivation of the opal suppressor tRNA reporter genes used here. Schematic representation of the dimeric sup3-ε gene and the modifications that led to the monomeric tRNAUGA genes. The A and B box promoter and terminator elements are represented by diagonally hatched rectangles. The spacer between the two tRNA sequences in sup3-ε is represented by a cross-hatched rectangle denoted Tc. First, test terminators T(n) were inserted downstream of the tRNAUGA, in place of Tc. Second, tRNAUGA was deleted, and a sequence complementary to the 3'-half of tRNAUGA (designated cRT) was inserted downstream of Tn. Third, three "silent" substitutions (31) (indicated by ) were introduced between the intron (shaded) and B box, to distinguish the transcript from related endogenous tRNAs on Northern blots (see text). The primary transcripts produced from the tRNAUGA construct are indicated as 112 nucleotides (n1) for the test terminator, T1, or 210 nucleotides for the default 8T terminator, T2.

S. pombe cells carrying the ade6-704 allele accumulate red pigment in limiting adenine but grow as white colonies when ade6-704 is efficiently suppressed by tRNAUGA (31). An ade6-704, leu1-32 strain, yAS50, was transformed with tRNAUGAWT constructs harboring various test terminators in a vector that undergoes homologous integration at the leu1 locus (37). Colonies were selected for leucine prototrophy, and the percentage of suppressed colonies was scored. No suppression was observed in cells transformed with the tRNAUGAWT gene harboring 2 Ts in its terminator (Fig. 2A). This suggested that readthrough transcription beyond the 2T test terminator did not lead to suppression. The tRNAUGAWT gene bearing a 3T test terminator reproducibly resulted in a significant number of partially suppressed and fewer fully suppressed colonies (Fig. 2A). The 4T terminator supported more suppression than the 3T terminator but significantly less than 5T or longer T tracts. Suppressor activity from the 5T, 6T, and 7T terminators yielded comparable, near-saturating amounts of suppression. Leucine prototrophs that have lost tRNAUGAWT upon homologous recombination at the leu1 locus, as monitored by polymerase chain reaction, typically account for 10–15% of the colonies that yield no suppression in this assay (not shown). Multiple integrated copies can also contribute to color heterogeneity (not shown (37)). Notwithstanding these limitations, the only variable in the experimental design is the length of the dT tract of the test terminator, and the data indicated that a significant and functional increase in termination occurred between 3 Ts and 4 Ts (Fig. 2A). At 5 T tracts of 5 Ts or longer, suppressor activity appeared saturated. From this we conclude that highly efficient termination appeared to occur at 5 Ts.
notype and RNA expression from the same gene prompted us to focus on tRNAUGA<sub>M</sub> for the remainder of this study. Suppression was not detected unless the tRNAUGA<sub>M</sub> test terminator harbored at least 5 Ts (Fig. 2B). This gene yielded limited suppressor activity even with an 8T terminator, which, as will be shown below, directs highly efficient termination. Although a minimum of 5 Ts produced efficient suppression, longer T tracts led to only a moderate increase in suppression. These data suggested that the limited activity of the tRNAUGA<sub>M</sub> gene was not due to limited termination efficiency and that a large increase in termination occurred between 4T and 5T terminators, with only moderate increases at longer dT tracts.

**In Vivo Evidence of Highly Efficient Termination at 5 Ts by S. pombe pol III**—Northern blot analysis of RNAs from cells expressing tRNAUGA<sub>M</sub> constructs is shown in Fig. 3A. Background signal in lanes U and C revealed limited cross-reactivity of our probe with endogenous tRNA species (Fig. 3A). Constructs with 2–4 Ts produced low levels of mature tRNAUGA<sub>M</sub>, consistent with their unsuppressed phenotypes, in addition to longer transcripts (indicated as RT, Fig. 3A). A probe specific for cRT confirmed that the long transcripts represented readthrough beyond the test terminator (not shown; the multiple bands may reflect various conformers of the self-complementary RNA). Constructs with 5 or more Ts produced mostly mature tRNAUGA<sub>M</sub> with relatively little RT, consistent with their suppressed phenotype. The size and reactivity with oligonucleotide probes directed to the intron, 5’-leader, and 3’-trailer of the nascent transcript that would be expected from tRNAUGA<sub>M</sub> were not due to limited termination efficiency and that a large increase in termination occurred between 4T and 5T terminators, with only moderate increases at longer dT tracts.

**Fig. 2.** Termination-dependent suppression in *S. pombe*. A, tRNA<sup>Ser<sub>WT</sub></sup> constructs with test terminators of varying length dT tracts as depicted in Fig. 1 were assayed for suppressor activity. Partial suppression (pink) and full suppression (white) are represented as percent suppressed colonies (vertical axis) as indicated in the inset; typically, ~200 colonies of each are represented. B, activity of the tRNA<sup>Ser<sub>M</sub></sup> gene constructs as in A. Note that leucine prototrophs that have lost the tRNA<sup>Ser<sub>M</sub></sup> gene upon homologous recombination at the leu<sup>1</sup> locus typically account for 10–15% of the colonies that yield no suppression in this assay, as determined by polymerase chain reaction (not shown, see text).

**Fig. 3.** Correlation between suppression and efficient termination by pol III as monitored by tRNA<sup>Ser</sup><sub>UGA</sub> transcripts in *vivo*. Northern blots of RNAs prepared from cells bearing integrated tRNA<sup>Ser</sup><sub>UGA</sub> genes with the test terminators indicated above the lanes. Lane U represents RNA from an untransformed cell line, and lane C represents a *leu<sup>1</sup>* transformant that lacks a tRNA<sup>Ser</sup><sub>UGA</sub> gene. The blot was probed sequentially to detect tRNA<sup>Ser</sup><sub>UGA</sub> and other rRNAs. The presence of a band representing termination at the test terminator (Fig. 3A) corresponds to a unprocessed pre-tRNA whose synthesis was terminated at the test terminator, whereas the bands between this and the mature tRNA are processing intermediates (52). Quantitation revealed that the amount of mature tRNA in the 4T sample was significantly higher than in the 3T, 2T, and control samples, although this nonetheless represented low termination efficiency (not shown). The relatively high ratio of mature tRNA to RT from constructs with 5 or more Ts indicated high termination efficiency (Fig. 3A). A control probe that detects tRNA<sup>Lys</sup><sub>UCU</sub> (Fig. 3C) indicated that the difference in tRNA<sup>Ser</sup><sub>UGA</sub> levels in the suppressed and unsuppressed cells was significant.

We also examined the steady state levels of tRNA<sup>Ser</sup><sub>UGA</sub> that are related to tRNA<sup>Ser</sub><sub>UGA</sub><sub>M</sub>. Fig. 3B shows the blot in Fig. 3A after stripping and rehybridization with an oligonucleotide probe complementary to an invariant region of three tRNA<sup>Ser</sup> sequences as well as tRNA<sup>Ser</sup><sub>UGA</sub><sub>M</sub> using the same conditions for both hybridizations. Quantitation revealed that tRNA<sup>Ser</sup><sub>UGA</sub><sub>M</sub> contributed to the level of total tRNA<sup>Ser</sup> by ~50% in these cells and therefore that it accumulated to a level similar to or greater than the tRNA<sup>Ser</sup> expressed from individual endogenous genes. This indicated that the limited activity of tRNA<sup>Ser</sup><sub>UGA</sub><sub>M</sub> is not due to a deficiency in accumulation but is instead more consistent with a decrease in the specific activity of the mature tRNA<sup>Ser</sup><sub>UGA</sub><sub>M</sub> as a suppressor.

The presence of a band representing termination at the test terminator (−Term) was detectable from constructs containing 3 or more dTs but less clear in 2T samples as illustrated in Fig. 2A.
3D. This provided evidence that a small amount of termination indeed occurs at a 3T terminator but not a 2T terminator. We conclude that highly efficient termination occurs at 5 Ts in S. pombe and that tRNAUGA is a reporter of pol III termination in vitro only if termination is highly efficient and accurate.

Faithful tRNA Transcription in a High Salt Extract of S. pombe; Pol III Termination Signal Recognition Is More Similar to Human Than Is S. cerevisiae—After several failed attempts to develop extracts of S. pombe cells that were active for tRNA transcription using buffers containing 0.15–0.4 M salts, we determined that activity could be obtained by increasing the concentration of salt present during the extraction. 0.6 M NaCl yielded extracts that were active for tRNA transcription. The following criteria indicated that the transcripts were synthesized de novo by pol III as follows: (i) synthesis required the presence of all four NTPs (not shown); (ii) transcript size varied expectedly, depending on the length of the dT tract (below); (iii) transcript production was decreased by tagetitoxin, an inhibitor of pol III (data not shown), and α-amanitin at an intermediate concentration (below). Transcription efficiency was monitored at varying concentrations of KCl and MgCl₂ and was found to be optimal at 60 and 10 mm, respectively (not shown), similar to other in vitro pol III systems, including the ones used below.

To compare termination on the same template and therefore control for sequence context, transcription complexes were assembled on tRNAUGA gene constructs using human, S. cerevisiae, and S. pombe cell extracts as the source of TFs and pol III. We examined tRNAUGA gene constructs that differed in the T1 test terminator, the dT tract length of which is indicated by the numbers above the lanes in Fig. 4. In this assay, promoter-initiated transcripts that are not terminated at T1 are extended to the default terminator, T2 (see Fig. 1). Human pol III terminated at 4 Ts with a low but significant amount of transcription to T2 (Fig. 4A). S. pombe pol III terminated at 5 Ts with a low but significant amount of the T2 transcript produced (Fig. 4B). S. cerevisiae pol III terminated at 5 Ts but with significantly lower efficiency than S. pombe at 5 Ts (Fig. 4C and D). The bands in the T1 region of the gel are the size expected for termination at the test terminator, T1. Note that the multiple species in the T1 region in Fig. 4B (and Fig. 4C) correspond to the nascent transcript and the processing intermediates identified on Northern blots (Fig. 3A). This suggested that this extract is competent for pre-tRNA processing as are S. cerevisiae extracts (45). The lack of intermediates in the HeLa system (Fig. 4A) probably reflects the inhibitory effect of the human La protein on pre-tRNA processing (see Fig. 1C in Ref. 53).

Quantitation of the T1 and T2 bands was performed and termination efficiency (T1) was calculated according to the formula T1 = (T1/T1 + T2) × 100. This revealed that termination by S. cerevisiae pol III was lower at 6 Ts than was S. pombe at 5 Ts (Fig. 4D). Human and S. pombe pols III terminated with >95% efficiency at 6 Ts, whereas this degree of efficiency was not attained by S. cerevisiae pol III until it encountered 8 Ts (Fig. 4D). We conclude that human pol III is the most sensitive to termination at a minimal dTn tract length, whereas S. cerevisiae was the least sensitive, and S. pombe was intermediate.

Another distinguishing feature of the termination pattern was reproducibly apparent in these experiments. Although human and S. pombe pol III require 4 and 5 Ts, respectively, for efficient termination, both exhibit a relatively distinct transition thereafter, as revealed by a sharp decline in T2 transcripts at the subsequent longer dTTracts (Fig. 4, A and B). The decline is not as sharp for S. cerevisiae pol III, as the intensity of T2 transcripts instead taper off more gradually (Fig. 4C). Thus, in Fig. 4C, about half of the amount of T2 that appears in lane 5 is seen in lane 6 and about half as much again appears in lane 7, whereas the decline was clearly more sharp for S. pombe (Fig. 4B, quantitation not shown). This is consistent with the idea that termination by S. cerevisiae pol III within a dT(n) tract may be a more of a stochastic process than occurs in the other pol III complexes (24).

α-Amanitin Sensitivity Distinguishes S. cerevisiae from S. pombe and Human polys III and Correlates with Termination Signal Recognition by These Enzymes—A tRNAUGA gene bearing a 3T test terminator was used to examine the sensitivity to α-amanitin of the three pols III (Fig. 5A–C). Human pol III was clearly the most sensitive to α-amanitin; S. pombe was less sensitive, and S. cerevisiae was insensitive. Quantitation revealed that S. pombe pol III was ~50% inhibited by 400 μg/ml α-amanitin (Fig. 5D) as previously reported for the S. pombe 7SL RNA gene (54) (note that the concentrations used here may not reveal an accurate curve for the human enzyme). Since S. pombe and human pols III are sensitive to α-amanitin and S. cerevisiae is not, inhibition by this toxin provides another criterion that distinguishes these enzymes (55).

**DISCUSSION**

We have developed a tRNA suppressor gene whose biological function in S. pombe is dependent on accurate and efficient termination by pol III, and we used it to study transcriptional termination in vivo and in vitro. We described for the first time...
FIG. 5. Species-specific differences in pol III sensitivity to α-amanitin. The tRNA<sub>185M</sub> gene construct with an inefficient (3T) test terminator at T1 was transcribed by human (A), S. pombe (B), and S. cerevisiae (C) pol III transcription complexes in the presence of α-amanitin as indicated above the lanes in μg/ml. The position of the T2-terminated band is indicated. Quantitation using a recovery marker as an internal control was performed and plotted in D. We note that since intermediate points between 1 and 200 μg/ml were not included, the curve for human pol III is approximate, and in actuality may be steeper than shown but is plotted here for comparison only.

extract derived from S. pombe that is active for faithful tRNA transcription, confirming the results obtained in vivo and establishing that pol III termination occurs efficiently at 5 Ts in S. pombe. Results obtained on the same templates demonstrated that S. cerevisiae pol III requires a longer dT tract than human or S. pombe pols III for termination and supported the idea that this enzyme terminates more stochastically than vertebrate or S. pombe pols III in a dT tract (24).

During the final stages of this work, after our in vitro transcription extract was developed, a paper appeared that described transcription of 7SL RNA in a low salt extract of S. pombe (54). The authors noted that 7SL RNA synthesis was sensitive to α-amanitin, and they concluded that the enzyme responsible was pol III because tRNA and 5 S rRNA synthesized in nuclei exhibited the same sensitivity. However, since RNA transcription in vitro was not reported, it is unclear whether the S. pombe low salt extract would be active for tRNA transcription. In this regard, it is interesting to note that an upstream deletion mutant of the S. pombe 7SL RNA gene, slr1, was transcribed efficiently in HeLa extract but not in the S. pombe low salt extract (54). This suggests that the internal tRNA-like promoter of slr1 was recognized by factors in HeLa extract and that a comparable activity was not present in the S. pombe low salt extract. Our results indicate that high salt extraction is a critical determinant in obtaining tRNA transcription activity from S. pombe cells.

A conclusion that we wish to draw from our experiments with α-amanitin is that differential sensitivity to this compound provides clear biochemical evidence of the distinct nature of the pols III examined here. Mutations in eukaryotic RNA polymerases that confer resistance to α-amanitin have been reported to affect termination and recycling by pol III (22). Other lines of investigation also indicate that pol III termination is a complex multistep process (17, 26–28, 63). The system described here can be used in parallel with other systems to dissect further these important aspects of pol III termination in yeast and humans.

Acknowledgments—We thank I. Willis and members of H. Levin’s Laboratory for advice; and D. Jin, Y. Huang, and an anonymous reviewer for critical comments.

REFERENCES

1. Willis, I. M. (1993) Eur. J. Biochem. 212, 1–11
2. Baserga, S. J., and Steitz, J. A. (1983) in The RNA World (Gesteland, R. F., and Atkins, J. F., eds) pp. 359–81, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
3. Kassavetis, G. A., Bardeleben, C., Bartholomew, B., Braun, B. R., Soares, C. A. P., Pisu, M., and Geiduschek, E. P. (1994) in Transcription: Mechanisms and Regulation (Conaway, R. C., and Conaway, J. W., eds) pp. 107–126, Raven Press, Ltd., New York
4. Lobo, S. M., and Hernandez, N. T. (1994) in Transcription: Mechanisms and Regulation (Conaway, R. C. and Conaway, J. W., ed) pp. 127–159, Raven Press Ltd., New York
5. Arrebola, A., Manaia, N., Reizenfeld, S., Marsolier, M.-C., Lefebvre, O., Carles, G., Guex, P., Conesa, C., and Sentenac, A. (1998) Mol. Cell. Biol. 18, 1–9
6. Kassavetis, G. A., Braun, B. R., Nguyen, L. H., and Geiduschek, E. P. (1999) Cell 90, 235–245
7. Hernandez, N. (1999) in Transcriptional Regulation (McKnight, S. L., and Yamamoto, K. R., eds) pp. 281–313, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
8. McFreyant, S. J., Baird, E. E., Travers, J. W., Dervan, P. B., and Gottesfeld, J. M. (1999) J. Mol. Biol. 286, 973–981
9. Taggart, A. K., Fisher, T. S., and Pugh, B. F. (1992) Cell 71, 1015–1028
10. Lopez-De-Leon, A., Librizzi, M., Puglia, K., and Willis, I. M. (1992) Cell 71, 211–220
11. Wang, Z., and Roeder, R. G. (1995) Proc. Natl. Acad. Sci. U. S. A. 82, 7026–7030
12. Hsieh, Y. J., Wang, Z., Kovelman, R., and Roeder, R. G. (1999) Mol. Cell. Biol. 19, 4944–4952
13. Colbert, T., and Hahn, S. (1992) Genes Dev. 6, 1940–1949
14. Wang, Z., and Roeder, R. G. (1997) Genes Dev. 11, 1315–1326
15. Lagraa, G., Kovelman, R., Suwaekawa, J., and Roeder, R. G. (1994) Mol. Cell. Biol. 14, 3053–3064
16. Sinn, E., Wang, Z., Kovelman, R., and Roeder, R. G. (1995) Genes Dev. 9, 675–685
17. Dieci, G., and Sentenac, A. (1996) *Cell* **84**, 245–252
18. Fan, H., Sakulich, A. L., Goodier, J. L., Zhang, X., Qin, J., and Maraia, R. J. (1997) *Cell* **88**, 707–715
19. Goodier, J. L., and Maraia, R. J. (1998) *J. Biol. Chem.* **273**, 26110–26116
20. Maraia, R. J. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 3383–3387
21. Maraia, R. J., Kenan, D. J., and Keene, J. D. (1994) *Mol. Cell. Biol.* **14**, 2147–2158
22. Wang, Z., and Roeder, R. G. (1998) *Cell* **84**, 749–757
23. Bogenhagen, D. F., and Brown, D. D. (1981) *Cell. Biol.* **27**, 2260–2272
24. Geiduschek, E. P., and Tocchini-Valentini, G. P. (1988) *Annu. Rev. Biochem.* **57**, 873–914
25. Campbell, F. E., and Setzer, D. R. (1992) *Mol. Cell. Biol.* **12**, 165–174
26. Shaaban, S. A., Bobkova, E. V., Chudzik, D. M., and Hall, B. D. (1996) *Mol. Gen. Genet.* **251**, 260–270
27. Chedin, S., Riva, M., Schultz, P., Sentenac, A., and Carles, C. (1998) *Genes Dev.* **12**, 3857–3871
28. Thuillier, V., Brun, I., Sentenac, A., and Werner, M. (1996) *EMBO J.* **15**, 618–629
29. Gottlieb, E., and Steitz, J. A. (1989) *EMBO J.* **8**, 851–861
30. Chu, W. M., Ballard, R. E., and Schmid, C. W. (1997) *Nucleic Acids Res.* **25**, 2077–2082
31. Kohli, J., Munz, P., and Soll, D. (1989) in *Molecular Biology of the Fission Yeast* (Nasm, A., Young, P., and Johnson, B. F., eds) pp. 75–96, Academic Press, Inc., San Diego
32. Willis, I., Nichols, M., Chisholm, V., Soll, D., Heyer, W. D., Szankasi, P., Amstutz, H., Munz, P., and Kohli, J. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 7680–7684
33. Maraia, R. J., Chang, D. Y., Wolffe, A. P., Vorce, R. L., and Hsu, K. (1992) *Mol. Cell. Biol.* **12**, 1500–1506
34. O’Neill, C., Ma, Y., and Mathews, M. B. (1999) *J. Mol. Biol.* **286**, 745–757
35. Hottinger, H., Pearson, D., Yamao, F., Gamulin, V., Cooley, L., Cooper, T., and Soll, D. (1982) *Mol. Gen. Genet.* **188**, 219–224
36. Maudrell, K. (1993) *Gene* (Amst.) **123**, 127–130
37. Keene, J. B., and Boeke, J. D. (1994) *Genetics* **136**, 849–856
38. Hestetrup, H., Kressman, A., and Birnstiel, M. L. (1981) *Cell* **24**, 573–585
39. Joazeiro, C. A. P., Kassavets, G. A., and Geiduschek, E. P. (1996) *Genes Dev.* **10**, 768–776
40. Murphy, M. H., and Baralle, F. E. (1983) *Nucleic Acids Res.* **11**, 7695–7700
41. Connolly, T., and Beach, D. (1994) *Mol. Cell. Biol.* **14**, 768–776
42. Maraia, R. (1991) *Nucleic Acids Res.* **19**, 5695–5702
43. Mao, J., Schmidt, O., and Soll, D. (1980) *Cell* **21**, 509–516
44. Van Horn, D. J., Yoo, C. J., Xue, D., Shi, H., and Wolin, S. L. (1997) *RNA* **3**, 1434–1443
45. Nichols, M., Willis, I., and Soll, D. (1990) *Methods Enzymol.* **181**, 377–394
46. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) *Nucleic Acids Res.* **11**, 1475–1489
47. Adeniyi-Jones, S., Rome, P. H., and Zaslavoff, M. (1984) *Nucleic Acids Res.* **12**, 1101–1115
48. Venenak-Jones, C. L., Wahah, S. Z., Zehner, Z. E., and Holmes, W. M. (1987) *Mol. Cell. Biol.* **7**, 4134–4138
49. Furter, R., Staith, M., Gillespie, D. E., and Hall, B. D. (1992) *Biochemistry* **31**, 10817–10824
50. Castano, J. G., Tobian, J. A., and Zaslavoff, M. (1985) *J. Biol. Chem.* **260**, 9002–9008
51. Levinger, L., Visitsht, V., Greene, V., Bourne, R., Birk, A., and Kolla, S. (1995) *J. Biol. Chem.* **270**, 18903–18909
52. Intine, R. V. A., Sakulich, A. L., Koduru, S. B., Huang, Y., Pierstorff, E., Goodier, J. L., Phan, L., and Maraia, R. J. (2000) *Cell* in press
53. Fan, H., Goodier, J. L., Chamberlain, J., Engelke, D. R., and Maraia, R. J. (1998) *Mol. Cell. Biol.* **18**, 3201–3211
54. Rodicker, F., Ossenbuhl, F., Michels, D., and Benecke, B. J. (1999) *Gene Expr.* **8**, 165–174
55. Valenzuela, P., Hager, G. L., Weinberg, F., and Rutter, W. J. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73**, 1024–1028
56. Yang, X., and Price, C. W. (1995) *J. Biol. Chem.* **270**, 23930–23933
57. Weibaelcher, R., Hebron, C., Feng, G., and Landick, R. (1994) *Genes Dev.* **8**, 2913–2927
58. Severinov, K., Markov, D., Severinova, E., Nikiforov, V., Landick, R., Darst, S. A., and Goldfarb, A. (1995) *J. Biol. Chem.* **270**, 23926–23929
59. Jin, D. J., Burgess, R. R., Richardson, J. P., and Gross, C. A. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 1453–1457
60. Johnson, T. L., and Chamberlin, M. J. (1994) *Cell* **77**, 217–224
61. Chaifin, D. R., Guo, H., and Price, D. H. (1995) *J. Biol. Chem.* **270**, 19114–19119
62. Allison, D. S., and Hall, B. D. (1985) *EMBO J.* **4**, 2657–2664
63. Bobkova, E. V., and Hall, B. D. (1997) *J. Biol. Chem.* **272**, 22832–22839
