The influence of transcription factor (TF) IIE on mRNA synthesis in vivo was examined in a temperature-sensitive yeast mutant. A missense mutation in the conserved zinc finger domain severely weakened TFIIIE's transcription activity without appreciably affecting its quaternary structure, chromatographic properties, or cellular abundance. The mutation conferred recessive slow-growth and heat-sensitive phenotypes in yeast, but quantitative effects on promoter utilization by RNA polymerase II ranged from strongly negative to somewhat positive. Heat-induced activation of the HSP26, HSP104, and SSA4 genes was attenuated in the mutant, indicating dependence on TFIIIE for maximal rates of de novo synthesis. Constitutive Bsp expression in mutant cells was elevated, exposing a negative (likely indirect) influence by TFIIIE in the absence of heat stress. Our results corroborate and extend recent findings of differential dependence on TFIIIE activity for yeast promoters, but reveal an important counterpart to the notion that dependence is tied to TATA element structure (Sakurai, H., Ohishi, T., and Fukasawa, T. (1997) J. Biol. Chem. 272, 15936–15942). We also provide empirical evidence for conservation of structure-activity relationships in TFIIE's zinc finger domain, and establish a direct link between TFIIE's biochemical activity in reconstituted transcription and its function in cellular mRNA synthesis.

Transcription factor (TF) IIE is one of several general transcription factors (the others are TFIIB, TFIID, TFIIF, and TFIIH) that collectively enable RNA polymerase II to carry out promoter-dependent transcription in cell-free systems derived from a wide variety of eukaryotic organisms (Refs. 1–3; reviewed in Ref. 4). TFIIIE binds to RNA polymerase II (5, 6), TFIIH (6–9), and Gal11p, a subunit of various RNA polymerase II holoenzymes (10–13), and is thought to recruit TFIIH to preinitiation complexes in position to catalyze DNA unwinding and phosphorylation of the carboxyl-terminal repeat domain of the largest subunit of polymerase (4, 5, 7–9).

In mammalian reconstituted systems, TFIIE and TFIIH effect DNA strand separation at the start site prior to initiation (promoter melting) in an ATP-dependent manner (Refs. 14 and 15; and reviewed in Ref. 16). Heteroduplex “bubbles” upstream of and encompassing the start site, where TFIIIE closely contacts DNA (17), can bypass the need for both factors (15, 18). In reactions employing topologically relaxed DNA templates and limiting nucleoside triphosphate concentrations, TFIIE and TFIIH (with ATP or dATP as a co-factor) also help nascent transcription complexes overcome a barrier to elongation encountered close downstream of the start site (promoter escape; Refs. 19–21). In reconstituted systems comprising human proteins, the requirement for TFIIE and TFIIH depends on promoter stability and DNA superhelicity (19, 22, 23). In similar systems from yeast and flies, TFIIIE seems essential for productive initiation regardless of template topology (3, 24, 25).

TFIIE consists of two different polypeptide subunits (1–3). Genes encoding the TFIIIE subunits in Saccharomyces cerevisiae, designated TFA1 and TFA2, are essential for cell viability (2), and both gene products (Tfa1p and Tfa2p, respectively) are required for TFIIIE activity in vitro (25). The Tfa1p subunit has homology to the 56-kDa subunit of human TFIIE (2), most notably in a zinc finger domain (26) that is crucial for human TFIIE activity in vitro (27, 28). Conditional tfa1 mutations, including those that affect this domain, cause recessive growth defects associated with reductions in steady-state levels of total cytoplasmic polyadenylated RNA, consistent with a broad requirement for TFIIIE in mRNA synthesis (29, 30).

To help delineate the role of TFIIE in transcription more precisely, we assessed the effects of a missense mutation in its zinc finger domain on steady-state levels and de novo induction of specific mRNAs in vivo. The mutation conferred recessive phenotypes in yeast including slow growth at 30 °C and lethality at 38–39 °C. At each temperature, however, cellular mRNA levels were affected in disparate ways that suggested differential dependence on TFIIIE activity. We measured the expression of genes with TATA box promoter elements conforming to the consensus sequence 5'-TATA(A/T)A(A/T)-3' (31), a gene with a non-canonical TATA-like sequence, and genes lacking a recognizable TATA box. Contrary to a recent proposal (29), the quantitative effects of the tfa1 mutation argue against a simple correlation between TFIIIE dependence and promoter TATA box content. The TFIIIE variant was purified to apparent homogeneity, and the effects of the zinc finger substitution on its biochemical activity were examined in a minimal reconstituted system for promoter-dependent transcription.

**EXPERIMENTAL PROCEDURES**

**Mutagenesis and Allele Replacement**—A double missense mutation in the TFA1 ORF changing cysteine 124 to serine and proline 125 to threonine (C124S/P125T) was created in the plasmid clone pBS/TFA1 (2) by oligonucleotide-directed mutagenesis (32) with the following primer (mutagenic bases underlined): 5'-GGTTACATGTCGACGATTT-
GTGTGACC-3'. *E. coli* strain BMH1-18 mutS (CLONTECH) was transformed to ampicillin resistance with mutagenesis reaction products and transformants were screened for plasmids bearing a new HindIII cleavage site created by the mutation. The resulting allelic was designated *tfa1-1*. This mutation was intended to disrupt the putative zinc coordination site (26, 27) in the Tfa1p polypeptide while creating a physical marker in the gene (the HindIII cleavage site) to facilitate its detection in yeast genomic DNA.

A pBS/TFA1 derivative (pTFA1::URA3) with the URA3 selectable marker cloned into a HindIII site 88 bp downstream of the TFA1 stop codon (2) was constructed for pop-in/pop-out gene replacement at the chromosomal TFA1 locus (33). The C124S/P125T mutation was placed in pTFA1::URA3 by swapping a wild-type *MunI* fragment encoding the zinc finger domain (260 bp) with the corresponding *MunI* fragment from the mutant phagemid clone. The resulting plasmid, pTFA1-1::URA3, was linearized with *ClaI* and integrated into the chromosomal TFA1 locus (2) in the diploid yeast strain 1788 (34). Ura+ transformants were selected on SC plates lacking uracil (35), followed by growth on YPD (35) to allow loss of URA3. Cells were then patched on SC plates containing chloramphenicol to select for ura revertants (33). Heterozygous 5-fluoroorotic acid survivors (TFA1/tfa1-1), which were identified by Southern blot hybridization to HindIII-digested genomic DNA, were patched on sporulation medium (35), and the resulting tetrads were dissected on YPD plates for haploid colony outgrowth at 30 °C.

**Cellular RNA and Protein Analysis**— Cultures were grown to mid-log phase at 28 or 30 °C in YPD medium. Half of each culture was transferred to a prewarmed flask in a 38 °C bath and shaking was continued. At subsequent times, cells were collected from culture aliquots by centrifugation for protein analysis (4 ml) or RNA analysis (10 ml) and immediately frozen in liquid nitrogen and stored at −80 °C. Cells were broken with glass beads to recover soluble protein (24). Total cellular RNA was recovered by extraction with hot acidic phenol and ethanol precipitation (36). Protein was quantified with the Bio-Rad Protein Assay using bovine serum albumin as a standard. Purified RNA was quantified by absorbance at 260 nm, and specific mRNA content was analyzed by Northern blot hybridization with 32P-labeled probes. Radioactivity hybridized to blots was quantified with a Fuji PhosphorImager and MacBAS software (Hitachi).

**Purification of Bacterially Expressed TFIIE**—The TFA1 and TFA2 ORFs were cloned in pET vectors (Novagen) for overexpression in *E. coli* strain BL21DE3 (37). pET21-TFA1 contains the wild-type TFA1 ORF and a 5′-flanking sequence (2) cloned into the NcoI site in pET21a (+). pET21-TFA2 contains the wild-type TFA2 ORF and 40 bp of 3′-flanking sequence (2) cloned between the NcoI and Xhol sites in pET21d (+). pET21-TFIIE, derived from pET21-TFA1 and pET21-TFA2, contains both ORFs, each transcribed from its own T7 promoter (construction details available on request). The 260-bp *MunI* fragment in pET21-TFIIE was replaced with the corresponding fragment from pTFA1::URA3 to yield the mutant co-expression plasmid pETIE-C124S. Co-expression plasmids were sequenced to ensure the absence of unwanted mutations in the TFA1 ORFs.

Cells harboring co-expression plasmids were grown to mid-log phase in LB medium (32) containing 0.1 mg/ml ampicillin, then isopropyl-β-D-thiogalactopyranoside was added (1 mM final concentration) and shaking was continued at 30 °C for 3 h. Cells were collected by centrifugation, frozen in liquid nitrogen, and stored at −80 °C. Subsequent steps were at 0–4 °C. Cells were resuspended in Buffer A (50 mM Tris acetate (pH 7.9 at 25 °C), 50 mM KCl, 10% (w/v) glycerol, 1 mM dithiothreitol, and protease inhibitors (0.6 μM leupeptin, 2 μM pepstatin A, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine hydrochloride)) and broken by sonication. Insoluble material was removed by centrifugation at 15,600 × g for 20 min. Supernatants were collected and dialyzed against a buffer containing 120 mM potassium acetate and chromatographed on a DEAE-Bioscale column (10 × 64 mm; Bio-Rad) as described (25).

**RESULTS**

**Phenotypes of a Heat-sensitive TFIIE Mutant**—To investigate the phenotypes of a substitution mutation in the TFIIE

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**Fig. 1.** TFIIE mutant phenotypes. A, upper: a TFA1/tfa1-1 heterozygote was sporulated and spor colonies (a, b, c, and d) from 10 tetrads were grown on YPD agar at 30 °C for 3 days. Lower, the resulting spore colonies were patched onto YPD agar and grown at 30 °C or 39 °C for 3 days. B, HindIII-digested genomic DNA from the TFA1/tfa1-1 heterozygote (left lane) and haploids from 5 tetrads in panel A was analyzed by Southern blotting with a TFA1-specific probe. The 2.5-kilobase band specific for the wild-type allele (TFA1) and the two bands resulting from the C124S/P125T mutation (tfa1-1) are marked with arrows at the left. C, a tfa1-1 mutant haploid was transformed with the centromeric (CEN) plasmid vector pRS315 (38) or a derivative (CEN-TFA1) containing the TFA1 gene on a 2.9-kilobase HindIII-HindIII fragment (2), or with the high-copy plasmid vector YEp13 (2 μ) or derivatives containing three different clones spanning the TFA1 locus (2.1, 4.1, and 5.1) recovered from a genomic DNA library (39) in a screen for high-copy suppressors (see text). Transformants were patched onto SC-ura plates (35) at 30 or 39 °C.

buffer containing 120 mM potassium acetate, and chromatographed on a TSK-heparin-5-PW column (75 × 7.5 mm; Supelco) as described (25).
zinc finger domain, heterozygous yeast bearing the recessive C124S/P125T mutation (TFA1/tfa1-1) were sporulated to obtain haploid progeny. Phenotypically normal or slow-growing haploid offspring were routinely recovered in a 2:2 ratio, indicating allele segregation at a single locus (Fig. 1A). As shown by Southern blot hybridization (Fig. 1B), the C124S/P125T mutation co-segregated with the slow-growth phenotype at 30 °C (Fig. 1A, upper panel) and with inviability at 38–39 °C (Fig. 1A, lower panel). Co-segregation was observed in three independently derived heterozygotes.2 Both of these phenotypes were reversed by transformation of tfa1-1 cells with an integrating plasmid containing the wild-type TFA1 gene (pTFA1::URA3), but not with its allelic derivative, ptfa1-1::URA3.

A centromeric TFA1 plasmid also complemented the tfa1-1 phenotypes, as did various TFA1 clones recovered by transformation with a YEpl-based genomic DNA library (39) in a screen for TFA1 complementation.

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2 P. Tijerina and M. H. Sayre, unpublished observations.
for high-copy suppressors (Fig. 1C). For the latter experiment, approximately 40,000 Leu+ tfa1-1 transformants were screened for the ability to form colonies at 39 °C. Among a total of 30 candidate suppressor isolates obtained, one suppressor candidate harbored the empty vector (YEp13) and was not analyzed further. The other 29 candidates contained one of four different TFA1 genomic clones distinguishable by their patterns of digestion with restriction endonucleases HindIII and SphI. Each TFA1 clone was recovered in at least 3 isolates. Representative inserts were partially sequenced from both ends using vector-specific primers. Clones 2.1 and 5.1 (Fig. 1C) represent contiguous segments of yeast chromosome XI spanning coordinates 385,077 to 399,275 or 380,005 to 386,338, respectively (40). Clone 2.1 contains the entire TFA1 ORF and 343 bp of 5'-flanking sequence. Clone 5.1 contains a truncated TFA1 ORF encoding a Tfa1p fragment (with an intact zinc finger) missing 167 C-proximal residues. Similar COOH-terminal TFA1 truncations have been shown by others to support normal growth in yeast (29, 30). Clone 10.1 (not shown) spans chromosome XI coordinates 383,112 to 391,111. Finally, clone 4.1 (Fig. 1C) has a chimeric insert with the TFA1 locus fused to part of chromosome III. Taken together, these co-segregation and complementation data demonstrate that the C124S/P125T mutation causes the recessive growth defects.

**Constitutive Transcription in tfa1-1 Yeast**—To test whether the tfa1-1 phenotypes coincided with defects in transcription in vivo, cellular mRNA levels were monitored at 30 and 38 °C. The mRNAs chosen for analysis are short-lived in normal cells and rapidly lost at the restrictive temperature in heat-sensitive mutants affecting TFIIH (41–44) or RNA polymerase II (45). Their steady-state levels are therefore closely tied to rates of synthesis (41–45). The genes contain a non-canonical TATA-like sequence (45). Their normalized steady-state levels, fell to 50% of normal within 20 or 30 min, but then gradually rebounded (Fig. 2B) to 70% of normal (Fig. 2B, right panel), clearly indicating new synthesis. By contrast, the relative decline in CDC7 mRNA was notably less severe, never falling much below 40% of wild-type levels (Fig. 2B, right panel). Moreover, steady CDC7 levels remained unchanged in going from 30 °C to 38 °C (Fig. 2A, despite the drop in TFIIE activity manifested by other genes.

**Transcriptional Activation in tfa1-1 Yeast**—To test whether the TFIIE mutation blocked transcriptional activation, as do mutations affecting TFIIH subunits Rad3p (41) or Ssl2p/Rad25p (42), we monitored mRNA levels for the heat-shock genes HSP26, HSP104, and SSA4 (46–48). In the absence of heat stress (28 °C), the tfa1-1 mutant expressed these genes at 2–3-fold higher levels than did wild-type cells (Fig. 3, zero time points, and data not shown). This could conceivably stem from low constitutive expression of SSA1 and SSA2, whose products negatively regulate the heat-shock transcription factor, HSF (46, 47), and from low levels of a repressor that works through negative cis-acting elements in the HSP26 promoter (48). Depression of HSP26 could also reflect chronic stress associated with a general transcription defect, such as from nutrient deprivation (see Ref. 48). (The doubling times for logarithmically growing cultures of wild-type and tfa1-1 cells were 1.5 and 2.5 h, respectively (see also Fig. 1A).) In any case, since wild-type TFIIE has never been shown to directly repress gene transcription in vitro, the elevated constitutive expression of HSP genes in mutant cells most likely derives from reduced expression of negative regulatory genes.

As expected (46–48), HSP26, HSP104, and SSA4 expression increased dramatically in wild-type cells within 15 min after a
shift from 28 °C to 38 °C (Fig. 3). By comparison, induction in the tfa1-1 mutant was much weaker and delayed. By 15 min after the temperature shift, levels of HSP26, HSP104, and SSA4 mRNA in the mutant rose 5-, 4-, and 14-fold, respectively (Fig. 3B), each accumulating to only 15–20% of wild-type levels. Taking into account the elevated levels of these mRNAs immediately prior to the shift (Fig. 3A), the initial transcriptional response to heat shock was attenuated by an order of magnitude in mutant cells (Fig. 3B), indicating a strong dependence on TFIIE. Reduced levels of HSF in tfa1-1 cells could also conceivably contribute to this induction defect (46, 47). However, an immunoblotting experiment revealed that levels of HSF protein were actually somewhat higher than normal in tfa1-1 cells immediately prior to and 45 min after the temperature shift, indicating that the observed activation defects did not stem from a paucity of HSF. Combined with the data for constitutively expressed genes (Fig. 2), these findings reveal negative effects of the tfa1-1 allele on mRNA accumulation ranging from 2-fold (CDC7) to 10-fold (HSP genes) reductions relative to wild-type cells. Last, heat shock gene induction was abnormally prolonged in the mutant as well. For instance, SSA4 mRNA was 3–4-fold more abundant than in wild-type cells at 1 or 2 h after the temperature shift, respectively (Fig. 3A). This presumably reflects tardy autorepression of the HSP and SSA4 genes (46–48) due to their abnormally slow, inefficient induction.

Basal Transcription Activity in Vitro—To investigate the biochemical basis of these transcription phenotypes, TFIIE heteromers containing wild-type Tfa2p and wild-type or variant Tfa1p were purified from E. coli for analysis. In either case, the two subunits remained associated during ammonium sulfate fractionation and anion and cation exchange chromatography (Fig. 4A). The C124S/P125T substitution did not preclude interaction with the Tfa2p subunit, consistent with findings for similar substitutions in human (27, 28) or yeast TFIIE (30). Each heteromer eluted from a DEAE column in a symmetric peak at 760 mM potassium acetate, and from a heparin column at 280 mM potassium acetate (Fig. 4B). Both heteromers had identical subunit stoichiometries (Fig. 4A) and an apparent native molecular mass of 260 kDa, as judged by size exclusion chromatography (Fig. 4C). All of these properties were indistinguishable from those of TFIIE purified from yeast whole cell extract (25), strongly suggesting that the C124S/P125T mutation does not grossly perturb the overall structure of TFIIE.

We assayed recombinant TFIIE alongside native TFIIE (25) in a reconstituted transcription system (24) comprising near-homogeneous TATA box-binding protein, TFIIB, TFIIF, and polymerase, and a fraction enriched for TFIIH. The concentrations of TFIIE in recombinant and native preparations were checked by immunoblotting to ensure equivalence (Fig. 5A, 3 M. Deng and M. H. Sayre, unpublished observations.)

Fig. 4. Biochemical properties of the TFIIE zinc finger variant. A, samples from each step in the purification of recombinant TFIIE and TFIIE-C124S/P125T were analyzed by SDS-PAGE. A gel stained with Coomassie Blue displays crude cell extracts (CE), 35–45% ammonium sulfate pellets (AS), and peak fractions from DEAE-Bioscale (DEAE) and heparin (HEP) columns. Bands formed by Tfa1p and Tfa2p are marked with arrows. Molecular weights of marker proteins (M) are shown in kDa at the left. B, heparin column fractions containing bacterially expressed TFIIE and TFIIE-C124S/P125T were assayed for total protein (absorbance at 595 nm) and potassium acetate (conductivity in mS). Both heteromers eluted in symmetric peaks at 760 mM potassium acetate, and from a heparin column at 280 mM potassium acetate (Fig. 4B). Both heteromers had identical subunit stoichiometries (Fig. 4A) and an apparent native molecular mass of 260 kDa, as judged by size exclusion chromatography (Fig. 4C). All of these properties were indistinguishable from those of TFIIE purified from yeast whole cell extract (25), strongly suggesting that the C124S/P125T mutation does not grossly perturb the overall structure of TFIIE.

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Differential Effects of a TFIIE Mutation in Yeast

We have examined the effects of a temperature-sensitive mutation in the conserved zinc finger domain of TFIIE on mRNA accumulation in vivo. The effects of the mutation appear to be gene-specific, with different promoters affected to different extents. While it might be imagined that these effects do not truly gauge the dependence on TFIIE because tfa1-1 is a "weak" allele, the underlying premise that TFIIE's contribution to transcription is uniform and obligatory for all promoters is not entirely consistent with biochemical data (19, 22, 23). Moreover, Sakurai et al. (29) recently described a stronger TFIIE loss-of-function mutation (tfa1-21) bearing substitutions that destabilize the heteromer and, at the nonpermissive temperature, cause wholesale degradation of both subunits in vivo. ACT1 mRNA levels decay to zero in tfa1-21 cells after a temperature shift (29), indicating a strict dependence on TFIIE. Yet even in this seemingly null background, mRNA levels for HIS3, GAL4, and GAL80 are affected much less severely (29). The variegated effects on gene expression reported here for tfa1-1 are therefore not peculiar to a weak allele.

Variable dependence on TFIIE is well documented for human promoters in vitro (22, 23). Our results, together with those of Sakurai et al. (29), provide evidence for this phenomenon in living cells. A total of 18 different genes have been examined in tfa1 mutants (Ref. 29 and this report), with effects ranging from negligible (the +1 start site in the HIS3 transcription unit; Ref. 29) to modest (GAL80, CDC7) to severe (MFa2, MET19). How might these differences arise? Differential effects on HIS3 and GAL80 start site utilization in the tfa1-21 mutant led Sakurai et al. (29) to postulate a link between TFIIE dependence and canonical TATA box promoter elements. While TATA elements may somehow influence the degree of dependence TFIIE for those genes and others such as ACT1 (29), mRNAs for the TATA-less genes CDC9 and RAD23 suffer equally sharp declines in the tfa1-1 mutant (Fig. 2), even though this allele is weaker than the tfa1-21 allele, at least with regard to effects on cellular TFIIE levels and ACT1 expression (Fig. 2 and Ref. 29). These results clearly indicate that TATA elements are not necessary for establishing a strong dependence on TFIIE activity in vivo.

Since ACT1 mRNA synthesis evidently depends on TFIIE (29), its persistence at 38°C in tfa1-1 cells (Fig. 2) probably reflects the residual activity exhibited by the purified TFIIE variant, TFIIE-C124S/P125T (Fig. 5B). While, in a quantitative sense, the transcription activity of this variant seems more severely defective in vitro than in vivo, interactions with other macromolecules in the cell, like RNA polymerase II (5, 6), TFIIH (6–9), Gal11p (10), and/or molecular chaperones, may ameliorate the mutation's possible effects on the conformational stability of the zinc finger domain (26), effects that could be exacerbated by the relatively harsh conditions of purification. In any case, our results establish a direct (albeit qualitative) connection between TFIIE's activity in reconstituted "basal" transcription systems (1–3, 24, 25) and its function in cellular mRNA synthesis (Refs. 29 and 30, and this work). In addition, since an analogous cysteine-to-alanine substitution in the zinc finger of human TFIIE affects its biochemical properties in similar ways (27), our findings provide empirical evi-
dence for a functionally significant and phylogenetically conserved structure-activity relationship in the TFIIIE zinc finger domain.

Each step in the initiation pathway for RNA polymerase II (reviewed in Ref. 4) may potentially pose a kinetic barrier to transcription (51). Recruitment of polymerase to the promoter can be rate-limiting for transcription in vitro (13). Promoter utilization in such cases should be relatively insensitive to fluctuations in TFIIIE activity if polymerase recruitment occurs independent of TFIIIE, as shown in vitro (4). If TFIIIE's role is limited to promoter melting or promoter escape (14, 15, 19–21), the sensitivities of promoters to changes in levels of TFIIIE activity should be proportional to the energetic cost of these isomerizations, which need not be the same for all promoters (51). The degree of dependence on TFIIIE in vitro may thus be tied to the helical stability of DNA in the vicinity of the start site, as seen for human TFIIIE on "naked" DNA templates (15, 19, 22, 23). This stability may be intrinsic to DNA sequence, but could also be influenced in a conditional way by the topological effects of chromatin structure (13). In Drosophila cells, heat-shock gene activation appears to hinge on the release of transcriptionally engaged polymerases stalled 20–40 bp downstream of the start site (52, 53). If this represents a general case in which promoter escape is rate-limiting, one might predict dependence on TFIIIE and TFIIH for maximal rates of HSF transcription, as reported here for TFIIIE (Fig. 3B) and elsewhere for TFIIH (41). Further work is needed to fully delineate TFIIIE's promoter specificity in vivo, and to try to recapitulate it in vitro so that dynamic effects of promoter structure can be correlated to gene activity in the cell.

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REFERENCES

1. Peterson, M. G., Inostroza, J., Maxen, M. E., Flores, O., Admon, A., Reinberg, D., and Tjian, R. (1993) Nature 364, 369–373
2. Weaver, W. J., Henry, N. L., Bushnell, D. A., Sayre, M. H., Brickner, J. H., Gileadi, O., and Kornberg, R. D. (1994) J. Biol. Chem. 269, 27459–27553
3. Wang, X., Hansen, S. K., Ratts, R., Zhou, S., Snook, A. J., and Zehring, W. (1994) J. Biol. Chem. 269, 535–544
4. Orphanides, G., Lagrange, T., and Reinberg, D. (1996) J. Biol. Chem. 271, 20170–20174
5. Lehter, K. E., Bushnell D. A., and Kornberg, R. D. (1996) Cell 85, 733–729
6. Bushnell, D. A., Bamdad, C., and Kornberg, R. D. (1996) J. Biol. Chem. 271, 20170–20174
7. Maxon, M. E., Goodrich, J. A., and Tjian, R. (1994) Genes Dev. 8, 515–524
8. Okuhma, Y., and Roeder, R. (1994) Nature 368, 160–163
9. Drapkin, R., Reardon, J. T., Ansari, A., Huang, J. C., Zawel, L., Ahn, K., Sanac, A., and Reinberg, D. (1994) Nature 368, 769–772
10. Sakurui, H., Kim, Y.-J., Ohishi, T., Kornberg, R. D., and Fukusawa, T. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9488–9492
11. Kim, Y.-J., Bjorklund, S., Li, Y., Sayre, M. H., and Kornberg, R. D. (1994) Cell 77, 599–608
12. Shi, X., Chang, M., Wolf, A. J., Chang, C.-H., Frazer-Abel, A. A., Wade, P. A., Burton, Z. F., and Jaehning, J. A. (1997) Mol. Cell. Biol. 17, 1160–1169
13. Plushne, M., and Gatm, A. (1997) Nature 386, 569–577
14. Dvor, A., Garrett, K. P., Chalot, C., Egly, J. M., Conaway, J. W., and Conaway, R. C. (1996) J. Biol. Chem. 271, 7245–7248
15. Holstege, F. C. P., van der Vliet, P. C., and Timmers, H. T. M. (1996) EMBO J. 15, 1668–1677
16. Svejstrup, J. Q., Vichi, P., and Egly, J.-M. (1996) Trends Biochem. Sci. 21, 346–350
17. Robert, F., Forget, D., Li, J., Greenblatt, J., and Coulombe, B. (1996) J. Biol. Chem. 271, 8517–8520
18. Pan, G., and Greenblatt, J. (1994) J. Biol. Chem. 269, 30101–30104
19. Goodrich, J. A., and Tjian, R. (1994) Cell 77, 145–156
20. Dvor, A., Conaway, R. C., and Conaway, J. W. (1996) J. Biol. Chem. 271, 23392–23396
21. Dvor, A., Conaway, R. C., and Conaway, J. W. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9006–9010
22. Parvin, J. D., Shykind, B. M., Meyers, R. E., Kim, J., and Sharp, P. A. (1994) J. Biol. Chem. 269, 18144–18142
23. Holstege, F. C. P., Tantin, D., Carey, M., van der Vliet, P. C., and Timmers, H. T. M. (1995) EMBO J. 14, 810–819
24. Sayre, M. H., Tschochner, H., and Kornberg, R. D. (1992) J. Biol. Chem. 267, 23376–23382
25. Sayre, M. H., Tschochner, H., and Kornberg, R. D. (1992) J. Biol. Chem. 267, 23383–23387
26. Qian, X., Gozani, S. N., Youn, H., Joon, C., Agarwal, K., and Weiss, M. A. (1993) Biochemistry 32, 9944–9959
27. Maxon, M. E., and Tjian, R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5529–5533
28. Ohkuma, Y., Hashimoto, S., Wang, C. K., Horikoshi, M., and Roeder, R. G. (1995) Mol. Cell. Biol. 15, 4586–4586
29. Sakurai, H., Ohishi, T., and Fukusawa, T. (1997) J. Biol. Chem. 272, 15906–15912
30. Kulski, N. H., and Buratowski, S. (1997) Mol. Cell. Biol. 17, 5288–5298
31. Burton, Z. F., and Jaehning, J. A. (1997) Methods Enzymol. 289, 2338–23392
32. Studier, W. F., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Methods Enzymol. 185, 60–89
33. Svejstrup, J. Q., and Lis, J. T. (1995) J. Biol. Chem. 270, 5232–5237
34. Kaiser, C., Michaelis, S., and Mitchell, A. (1994) Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
35. Rothstein, R. (1991) Methods Enzymol. 194, 251–301
36. Svejstrup, J. Q., and Lis, J. T. (1995) J. Biol. Chem. 270, 20170–20174
37. Halladay, J. T., and Craig, E. A. (1995) Mol. Cell. Biol. 15, 5433–5443
38. Sakurai, H., Ohishi, T., and Fukusawa, T. (1997) J. Biol. Chem. 272, 15906–15912
39. Svejstrup, J. Q., and Lis, J. T. (1995) J. Biol. Chem. 270, 20170–20174
40. Burton, Z. F., and Jaehning, J. A. (1997) Methods Enzymol. 289, 2338–23392