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TFIIS Enhances Transcriptional Elongation through an Artificial Arrest Site In Vivo

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Transcriptional elongation by RNA polymerase II has been well studied in vitro, but understanding of this process in vivo has been limited by the lack of a direct and specific assay. Here, we designed a specific assay for transcriptional elongation in vivo that involves an artificial arrest (ARTAR) site designed from a thermodynamic theory of DNA-dependent transcriptional arrest in vitro. Transcriptional analysis and chromatin immunoprecipitation experiments indicate that the ARTAR site can arrest Pol II in vivo at a position far from the promoter. TFIIS can counteract this arrest, thereby demonstrating that it possesses transcriptional antiarrest activity in vivo. Unexpectedly, the ARTAR site does not function under conditions of high transcriptional activation unless cells are exposed to conditions (6-azauracil or reduced temperature) that are presumed to affect elongation in vivo. Conversely, TFIIS affects gene expression under conditions of high, but not low, transcriptional activation. Our results provide physical evidence for the discontinuity of transcription elongation in vivo, and they suggest that the functional importance of transcriptional arrest sites and TFIIS is strongly influenced by the level of transcriptional activation.

Transcriptional elongation is a distinct and important step in the transfer of genetic information from gene to protein. Transcriptional elongation by *Escherichia coli* RNA polymerase is discontinuous in vitro (24, 28). The ternary elongation complex, consisting of RNA polymerase, DNA, and nascent RNA, can isomerize into an "arrested" conformation that is stable but incompetent for further elongation. Importantly, such isomerization depends on the transcribed DNA sequence. Yeast RNA polymerase II (Pol II) also arrests transcription in vitro (19, 26), suggesting that discontinuous elongation is a property of eukaryotic RNA polymerases. In addition, transcript cleavage factor TFIIS significantly contributes to the fidelity of Pol II transcription in vitro (17, 36), perhaps as a consequence of its antiarrest activity. However, as these in vitro experiments were performed under artificial conditions, they do not address whether transcriptional arrest can occur in vivo. If transcriptional arrest occurs in vivo, the stalled RNA polymerase would prohibit any further transcription, and DNA in the transcription bubble might be susceptible to mutagenesis. In addition, a stalled elongation complex might be recognized as a damage signal, and it has been suggested that defects in elongation are associated with recombination between direct repeats (5, 33).

Discontinuity of transcription elongation in vivo has been suggested by the observation of transcriptionally engaged Pol II at the promoter-proximal regions of various eukaryotic genes (3). In yeast cells, Pol II can stall at the 5′ end of the *lacZ* gene (1, 5), and blocking Kin28-dependent phosphorylation of the C-terminal tail of Pol II does not preclude association of the preinitiation complex with promoters (20, 23). However, in all these situations Pol II is stalled at or near the promoter, suggesting that the transcriptional defect may occur at the level of promoter clearance and not at that of elongation per se. Furthermore, there is no evidence that the observed transcriptional defect depends on the DNA sequence around the block.

A number of proteins can affect eukaryotic transcriptional elongation in vitro (3, 38). The best-studied transcriptional elongation factor is TFIIS, which induces transcript cleavage in arrested elongation complexes, thereby permitting stalled RNA polymerase to proceed downstream (41). TFIIS is highly conserved among eukaryotes, and it has functional homologues in prokaryotes (22) and archaea (13). In yeast, TFIIS interacts genetically with several Pol II subunits (2, 14, 15, 25), components of the Spt4/Spt5 elongation complex (12, 40), the Cdc68 component of the FACT elongation complex (30), and the Swi/Snf nucleosome remodeling complex (7). Loss of TFIIS or mutations that interact genetically with TFIIS confer sensitivity to 6-azauracil (6-AU), a drug that inhibits GTP biosynthesis and hence decreases the concentration of nucleotides in vivo (8, 27). These observations have led to the hypothesis that TFIIS and 6-AU affect transcriptional elongation in vivo and that TFIIS is the physiologically relevant antiarrest factor. However, direct evidence for transcriptional arrest and for the role of TFIIS in transcription elongation in vivo is lacking.

Understanding of transcription elongation in vivo is significantly limited by the lack of a direct assay for this process. Here, we designed an artificial arrest (ARTAR) sequence, and we demonstrate that Pol II can be arrested in vivo at a position far from the promoter in a DNA sequence-dependent manner. TFIIS can counteract this arrest, thereby demonstrating its antiarrest activity in vivo. In addition, we show that the ARTAR sequence does not function under conditions of high transcriptional activation unless cells are exposed to conditions (6-AU or reduced temperature) that are presumed to affect

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elongation in vivo. Conversely, TFIIS affects gene expression under conditions of high, but not low, transcriptional activation. Our results provide physical evidence for the discontinuity of transcription elongation in vivo, and they suggest that the recovery from transcriptional arrest can be functionally distinguished from transcriptional elongation under conditions of high activation.

MATERIALS AND METHODS

Strains and DNAs. pDK5, a multicopy *URA3* plasmid containing the *lacZ* structural gene expressed from the *GAL1* promoter, is a derivative of pRY131 (42). It contains a *Kpn*I-*Bgl*II cassette with two in-frame stop codons that is inserted between residues Pro^{686} and Gln^{687}, a position located in a functionally dispensable interdomain (domain IV) loop of β-galactosidase. The *Kpn*I-*Bgl*II cassette was inserted into pRY131 by a "nick-repair" procedure that employs homologous recombination in vivo. Specifically, *Sac*I-cleaved pRY131 and a 690-bp PCR product containing the *Kpn*I-*Bgl*II cassette flanked by corresponding *lacZ* homology regions (obtained by sequential PCRs using the product of the first reaction as a primer for the second reaction) were introduced into yeast, and Ura^+ transformants that were white in galactose-grown cells were selected. Double-stranded oligonucleotides carrying the full-length ARTAR sequence (UU*C) and a mutant derivative lacking the upstream-most segment (U*C) were obtained by PCR using appropriate oligonucleotides. Both the full-length and truncated versions of the ARTAR sequence were flanked by *Kpn*I and *Bgl*II and cloned between the *Kpn*I and *Bgl*II sites of pDK5. The resulting plasmids were partially digested with *Eco*RI to remove the 2μm origin of replication and then religated, thereby generating *URA3* integration plasmids.

The yeast strains used in these experiments were derived from a cross between Z280 (**a** *his3-Δ200 leu2-3,112 ura3-52 HA-RPB3::LEU2*), which was obtained from Rick Young (the hemagglutinin [HA] epitope is inserted directly after the *RPB3* start codon and *LEU2* is inserted downstream of the *RPB3* coding region), and GHY285 (α *his4-912δ lys2-128δ leu2-Δ1 ura3-52 ppr2::hisG*), which was obtained from Grant Hartzog. Plasmids containing the wild-type *GAL1-lacZ* reporter and the two ARTAR-containing derivatives were integrated into the *URA3* locus of wild-type and TFIIS deletion strains expressing the HA-tagged Rpb3 subunit of Pol II. Strains containing the ARTAR sequence are genetically stable under all growth conditions examined.

LacZ expression assays. For the β-galactosidase assays, cells were grown overnight at 30°C (unless otherwise indicated) in medium containing Casamino Acids and lacking uracil in the presence of 2% raffinose. In many experiments, glucose was added to 0.1%, a concentration that does not affect glucose repression but significantly increases cell growth, the galactose induction rate, and steady-state β-galactosidase levels. Upon reaching an optical density of approximately 1, galactose was added to the desired concentration and cells were incubated for an additional 4 h. β-Galactosidase values represent the average of at least six independent colonies and are accurate to ±20%. *lacZ* and *GAL1* RNA levels were determined by hybridization to oligonucleotide probes (located approximately 100 nucleotides downstream from the initiation site) and digestion with S1 nuclease as described previously (16).

Chromatin immunoprecipitation. Pol II occupancy was determined by chromatin immunoprecipitation assays, which were performed essentially as described previously (23). Cells (400 ml) were grown overnight in medium containing Casamino Acids without uracil in the presence of 2% of the appropriate carbon source (glucose, raffinose, or galactose) and then treated with 1% formaldehyde for 15 min. Chromatin from these cells was fragmented by sonication, and the resulting material was immunoprecipitated with anti-HA antibody bound to protein A-Sepharose beads. Quantitative PCR determinations were performed with five primer pairs covering various *lacZ* regions (see Fig. 5A) or with three primer pairs corresponding to the *GAL1* locus (one centered at the transcriptional initiation site, the others centered at +321 and +800).

RESULTS

Design of an ARTAR site. Our initial goal was to determine whether transcriptional arrest occurs in vivo. Towards this end, we decided to engineer an ARTAR site. To develop a simple genetic assay that specifically measures transcriptional elongation (as distinct from promoter clearance), we inserted the ARTAR site at a promoter-distal position of the *E. coli* β-galactosidase (*lacZ*) gene that is dispensable for enzymatic activity (4, 9).

As a conceptual framework for the design of the ARTAR site, we used the thermodynamic theory of DNA-dependent transcriptional arrest (39). In vitro, transcriptional arrest occurs due to the backward translocation (or "backtracking") of RNA polymerase along DNA and RNA (19, 21). Translocation is reversible and occurs in a DNA sequence-dependent manner, indicating that lateral stability of the RNA polymerase at the DNA is the key factor for a transcriptional arrest site. Lateral stability depends on total free energy of interactions at the RNA-DNA hybrid and at the two DNA-DNA duplexes flanking the transcription bubble (39). The strength of the RNA-DNA hybrid is particularly important because RNA-DNA hybrids are generally more stable than DNA duplexes. Indeed, weakening of the RNA-DNA hybrid increases backtracking in vitro (19, 29), and there is evidence that the strength of this hybrid affects the oscillations of the elongation complex in vivo (37). Finally, the design of the ARTAR site utilized biochemical observations about the dimensions of the elongation complex. Specifically, the length of hybrid is 8 nucleotides (19, 29), the distance between the active center and the front border of RNA polymerase is approximately 12 nucleotides (28), and the length of DNA covered by a single RNA polymerase molecule is approximately 30 nucleotides (24).

The thermodynamically ideal transcription-arresting sequence would (i) stop the elongating RNA polymerase, (ii) induce and allow backtracking, and (iii) stabilize the backtracked (arrested) conformation (Fig. 1). The first and second goals could be achieved by combining a weak 9-nucleotide RNA-DNA hybrid with a strong 9-nucleotide downstream duplex. The second goal could be facilitated by a weak upstream duplex, and the third goal could be achieved by providing a strong RNA-DNA hybrid in the backtracked conformation. Given the reversible nature of backtracking, another important consideration was to prevent displacement of the backtracked Pol II by the trailing Pol II molecule. We therefore designed the ARTAR site to have three similar arresting sites in tandem separated by 12-bp spacers to prevent mutual displacement of arrested polymerases.

The particular ARTAR sequence (UU*C; Fig. 2) was designed based on the following considerations. First, we employed the weakest possible RNA-DNA hybrid, namely an oligo(A) track in DNA hybridized with an oligo(U) track in RNA. Second, the weak DNA duplex was formed by an AT-rich sequence, whereas both the strongest RNA-DNA hybrid and DNA duplex would be formed by GC-rich sequences. Third, all sequences were diversified in their nucleotide composition to avoid homologous recombination. Fourth, the ARTAR site was placed in frame within the *lacZ* structural gene, and codons were biased to amino acids without chemically functional groups. Fifth, glycines were introduced at the ends of the sequence to facilitate flexibility of the loop. The first and second arresting sections of the ARTAR sequence correspond with the above description. The third section was modified in the RNA-DNA hybrid region to further promote stabilization of backtracked conformation based on the assumption that when the leading RNA polymerase backtracks, a hairpin might form at the 3′ end of the transcript and act as a clamp stabilizing the backtracked RNA polymerase (C-UH;

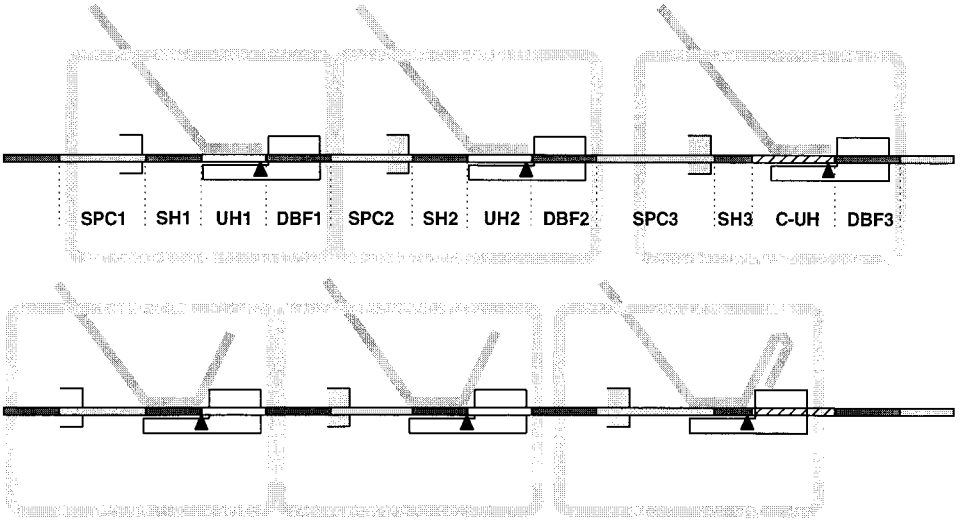

FIG. 1. Rationale for the artificial arresting DNA sequence design. The modules of the ARTAR site and three elongation complexes (gray boxes) occupying them are shown schematically. There are three similar arresting sections containing a spacer (SPC), a strong stabilizing hybrid (SH), a weak oligo(U) destabilizing hybrid (UH), and a strong stopping downstream bubble-flanking duplex (DBF). C-UH is an oligo(U) destabilizing hybrid that is modified by the clamp-forming element. The nascent transcript (dark grey line), DNA (open box in both duplex and RNA-DNA hybrid forms), active Pol II centers (triangles), and transcription bubble borders (brackets) are indicated. The upper panel shows three elongation complexes stalled by DBFs and prone to backtracking because of the weak UH and SPC. These complexes are shown to isomerize (backtrack) into the arrested conformations shown in the bottom panel that are stabilized by the SHs. The hairpin at the downstream ternary complex is predicted to form because of the C-UH structure.

Fig. 1 and 2). We also designed a mutant form of the ARTAR site that lacked the upstream-most segment (U*C; Fig. 2).

The ARTAR sequence can arrest transcription in vivo. As we wished to assay elongation-specific events in vivo, the ARTAR sequence was inserted into the interdomain (domain IV) loop of β-galactosidase between Pro^{686} and Gln^{687}. This loop has minimal impact on *lacZ* activity (4, 9), and it is located more than 2,000 nucleotides downstream from the promoter. Wild-type and ARTAR site-containing *lacZ* derivatives expressed from the *GAL1* promoter were integrated into the *URA3* locus of wild-type and TFIIS deletion strains. For each of the resulting strains, we measured β-galactosidase activity in cells grown in glucose (repressed conditions; Fig. 3A), raffinose (noninducing conditions that permit low-level transcriptional activation; Fig. 3B), and galactose (inducing conditions; Fig. 3C).

Under conditions of weak activation (raffinose medium), TFIIS does not affect the level of wild-type *lacZ* expression and hence does not affect the function of the *GAL1* promoter. In the presence of TFIIS, the ARTAR sequence inhibits *lacZ* expression by a factor of 10. In the absence of TFIIS, *lacZ* expression is barely or not at all detectable, indicating that the ARTAR site causes at least a 30-fold inhibition. The U*C mutant form of the ARTAR site (designated artar in Fig. 3), which lacks the upstream-most segment, does not affect *lacZ* expression. Thus, the ARTAR site can have a significant inhibitory effect on *lacZ* expression, and this effect is more pronounced in the absence of TFIIS.

Unexpectedly, when cells are grown in galactose medium *lacZ* expression is insensitive to the ARTAR site, but it is reduced 10-fold in the absence of TFIIS. To determine whether TFIIS affects Gal4-dependent activation under these conditions, we measured RNA levels for the native *GAL1* gene as well as the *lacZ* reporter genes (Fig. 3D). In accord with the β-galactosidase assays, wild-type and ARTAR-containing *lacZ* RNA levels were reduced 10-fold in TFIIS deletion strains, even though *GAL1* RNA was reduced only twofold under these conditions. The observation that TFIIS does not significantly affect the level of *GAL1* activation indicates that the TFIIS-dependent effect on *lacZ* RNA levels reflects a transcriptional defect that occurs at a step after initiation. This postinitiation effect on *lacZ* transcription is similar to that observed for various proteins (Hpr1, Tho2, Mfp1, Thp2, and Thp1) that are presumed to be important for transcriptional elongation (5, 6, 10, 32, 33). The TFIIS- and ARTAR-dependent effects are clearly different in raffinose and galactose, and the shift between ARTAR dependence and TFIIS dependence occurs gradually as a function of galactose concentration, with both effects occurring at 0.2% galactose (Fig. 4).

Pol II can arrest at the ARTAR sequence in vivo. Although the above experiments suggest that the ARTAR sequence can

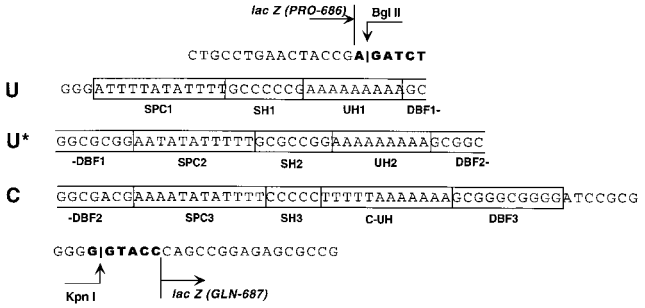

FIG. 2. The nucleotide sequence of the ARTAR site. The names of the ARTAR elements correspond to those described in the legend to Fig. 1.

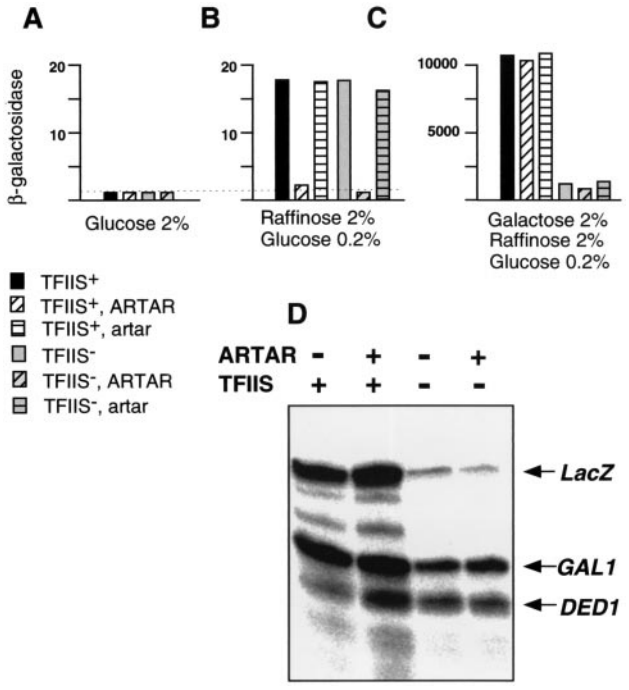

FIG. 3. Effect of the ARTAR site and TFIIS on *lacZ* expression. β-Galactosidase levels in the indicated strains (artar designates the mutated U*C derivative) grown in medium containing 2% glucose (A), 2% raffinose (B), and 2% galactose (C). Note the differences in scales for β-galactosidase levels in the various panels. (D) RNA levels in the indicated strains grown in 2% galactose medium.

affect transcriptional elongation in vivo, conclusive evidence requires direct observation of Pol II arrested at the ARTAR sequence. Although the transcriptional run-on assay has been widely used for measuring Pol II occupancy throughout the gene in vivo (3), this assay is based on the assumption that elongation-competent RNA polymerase becomes active after nuclei isolated from cells are treated with sarcosyl. As it is impossible to assess the validity of this assumption for the case of the ARTAR sequence, we instead measured Pol II occupancy in vivo using chromatin immunoprecipitation. Such experiments were facilitated by the fact that all the yeast strains described above contain an HA epitope-tagged version of the Rpb3 subunit of Pol II.

Chromatin was cross-linked with formaldehyde, sonicated, and immunoprecipitated with HA antibodies, and the resulting material was assayed by quantitative PCR for Pol II occupancy at five locations within *lacZ* (Fig. 5A), at the *GAL1* promoter and a site 800 bp downstream, and at the *PGK1* promoter. As expected, Pol II occupancy in wild-type and TFIIS-deficient strains containing the wild-type *lacZ* gene is very low at all positions tested when cells are grown in glucose or raffinose, and it increases approximately 40-fold when cells are grown in galactose (Fig. 5B). Indistinguishable results were observed for the ARTAR sequence-containing *lacZ* gene in the presence of TFIIS.

Interestingly, when cells lacking TFIIS but containing the ARTAR site are grown in raffinose medium, there is a sharp increase in Pol II occupancy just before the ARTAR site (15-fold increase at region C and 10-fold at region D). This increased Pol II occupancy at regions C and D depends on the ARTAR site. Pol II occupancy at region E, which lies about 500 bp downstream from the ARTAR sequence, is only threefold higher under these conditions and hence is significantly reduced in comparison to that observed at regions C and D. These observations strongly suggest that the ARTAR sequence mediates transcriptional arrest in vivo when TFIIS is not present. In fact, we suspect that much (and perhaps all) of the apparent increase at region E reflects Pol II molecules arrested at the ARTAR site, not elongation through the region. As the average length of the chromatin fragments is approximately 500 bp, some Pol II molecules arrested at the ARTAR site will be detected by the region E probe (11, 18). In addition, Pol II occupancy increases fourfold at region B, suggesting that Pol II molecules might accumulate prior to the ARTAR site due to the elongation block.

The above results suggest that the ARTAR sequence can mediate transcriptional arrest under conditions of low *GAL1* promoter activity (raffinose). However, Pol II occupancy is comparably high at all studied *lacZ* regions when cells are grown in galactose, suggesting that high levels of transcriptional activation can override the elongation block. In addition, TFIIS does not appear to affect Pol II occupancy of the *lacZ* gene when cells are grown in galactose, despite the fact that the level of *lacZ* RNA is 10-fold lower when TFIIS is deleted. This suggests that the overall rate of elongation at the *lacZ* gene is decreased (see Discussion).

Other phenotypes of the ARTAR sequence relevant to transcriptional elongation. We used three phenotypic assays to independently address the relevance of the ARTAR sequence and TFIIS to transcriptional elongation. First, we examined transcriptional elongation through the ARTAR sequence in the presence of 6-AU, a compound believed to inhibit elongation by reducing intracellular GTP and UTP levels (8, 27, 38). Interestingly, in galactose-grown strains containing TFIIS, 6-AU dramatically affects *lacZ* expression in an ARTAR-dependent manner (Fig. 6). As expected, the absence of TFIIS causes a dramatic reduction in *lacZ* expression, with the effect being somewhat more pronounced when the ARTAR site is present. Thus, 6-AU can enhance the effect of the ARTAR site in vivo, thereby strengthening their links to transcription elongation.

Second, we examined the kinetics of *lacZ* expression based on the observation that transcriptional activation is often delayed in strains lacking Elongator, a complex that mediates transcriptional elongation in vitro (31). Indeed, the presence of the ARTAR sequence delayed induction in cells induced by galactose (Fig. 7). At 30 min after induction, there was con-

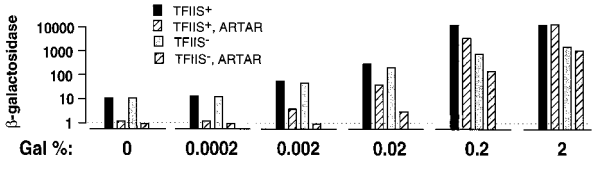

FIG. 4. Inverse effects of the ARTAR site and TFIIS as a function of galactose (gal) concentration. β-Galactosidase levels in the indicated strains grown in medium containing 2% raffinose, 0.1% glucose, and the indicated concentrations of galactose. The scale for β-galactosidase levels is exponential.

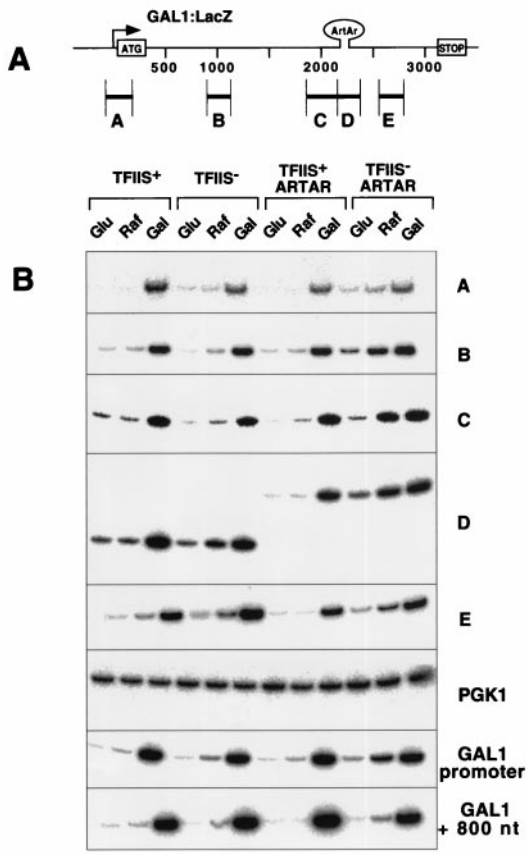

FIG. 5. Analysis of Pol II occupancy by chromatin immunoprecipitation. (A) Diagram showing the location of primer pairs for analysis of Pol II occupancy over the *lacZ* region. (B) Cross-linked chromatin from the indicated HA-Rpb3-containing strains grown in glucose (glu), raffinose (raf), or galactose (gal) medium immunoprecipitated with an HA-specific antibody and analyzed by quantitative PCR primers to the indicated regions.

siderable expression of the wild-type *lacZ* allele, whereas the ARTAR sequence-containing *lacZ* allele essentially was not expressed. This delayed induction effect of the ARTAR sequence occurs in both wild-type and TFIIS deletion strains.

Third, we analyzed transcription at a low temperature (16°C) based on the observation that presumed elongation-defective *spt5* alleles are cold sensitive (12). Most significantly, the ARTAR sequence has a strong effect on *lacZ* expression under conditions of high activation (0.2 and 2% galactose; Fig.

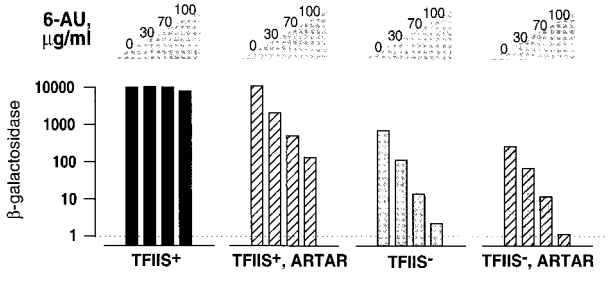

FIG. 6. ARTAR and TFIIS dependence of *lacZ* expression in the presence of the indicated concentrations of 6-AU.

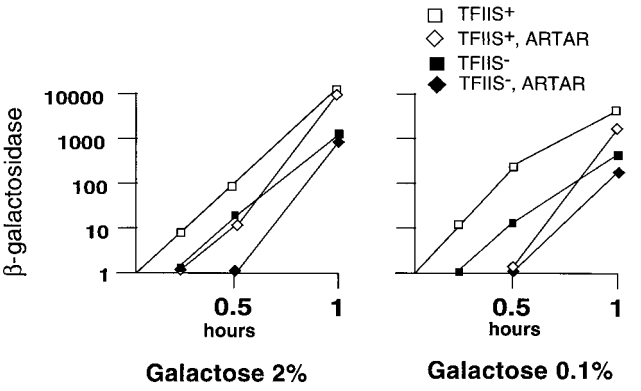

FIG. 7. ARTAR and TFIIS dependence of the kinetics of *lacZ* expression after induction with 2 or 0.1% galactose.

8). This result is in contrast to the situation at 30°C, in which the ARTAR sequence has essentially no effect on *lacZ* expression (Fig. 3). In addition, under conditions of high activation TFIIS has no effect on wild-type *lacZ* expression at 16°C (Fig. 6C), whereas TFIIS is important for *lacZ* expression at 30°C (Fig. 3). Thus, at 16°C *lacZ* expression is more significantly affected by the ARTAR sequence and less significantly affected by TFIIS.

DISCUSSION

A specific assay for transcriptional elongation in vivo. Although there are various biochemical assays for transcriptional elongation, it has been difficult to specifically assay elongation in vivo. Nuclear run-on assays, which are often used to analyze elongation in vivo, are actually performed under artificial biochemical conditions, and extrapolation of such results to the physiological state requires a number of assumptions about the properties of Pol II. In yeast, elongation has been analyzed by measuring *lacZ* RNA (or protein) levels based on the observation that various mutations can decrease *lacZ* expression at a step after transcriptional activation (5, 6, 10, 32). However, it is unclear whether the specific effect on *lacZ* expression is due to transcriptional elongation per se or rather to promoter clearance or some other process. Lastly, 6-AU sensitivity has been used as a genetic assay for elongation, although the mechanistic basis for this is unclear (8, 27, 34, 35).

Here, we describe a specific assay for transcriptional elongation in vivo that employs an ARTAR sequence. Unlike previous elongation assays in vivo, our assay depends on a specific DNA sequence within a structural gene. Moreover, as the ARTAR sequence is located more than 2 kb from the promoter, ARTAR-dependent effects cannot be due to promoter

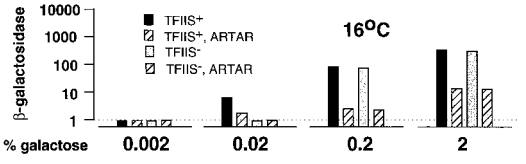

FIG. 8. ARTAR and TFIIS dependence of *lacZ* expression in cells grown at 16°C.

clearance or other promoter-proximal events but rather must be due to elongation. Finally, the ARTAR-dependent assay utilizes *lacZ* as a reporter gene and hence should be useful for genetic screens to identify new genes influencing transcriptional elongation and for further studies of elongation-relevant proteins described previously. We note that although the ARTAR sequence was designed based on a thermodynamic theory of backtracking, we have not proven that ARTAR-dependent effects in vivo are due to classical backtracking as defined in vitro.

The ARTAR sequence can arrest transcription in vivo. Five lines of evidence indicate that the ARTAR sequence can, under appropriate conditions, affect transcription in vivo in a manner that is promoter distal and sequence dependent. First, in the absence of TFIIS, there is a striking ARTAR-dependent increase in Pol II occupancy just prior to the ARTAR sequence (Fig. 5; regions C and D). Importantly, Pol II occupancy 500 bp downstream from the ARTAR site (region E) is reduced at least fivefold in comparison to that observed at region C. These observations provide direct physical evidence for transcriptional arrest in vivo. Second, in wild-type cells grown under conditions of low activation (raffinose medium), there is a 10-fold reduction in *lacZ* expression that depends on the ARTAR sequence (Fig. 3). Third, under conditions of high activation (galactose medium), 6-AU dramatically affects *lacZ* expression in an ARTAR-dependent manner (Fig. 6). Fourth, the ARTAR sequence delays galactose-inducible *lacZ* expression (Fig. 7). Fifth, the ARTAR sequence has a strong effect on *lacZ* expression under conditions of high activation and low temperature (16°C) (Fig. 8). Although these ARTAR-dependent effects occur under specific experimental conditions, their existence provides conclusive evidence for the discontinuity of transcription elongation in vivo.

Unexpectedly, the ARTAR sequence does not appear to function under conditions of high transcriptional activation (galactose medium) and optimal growth conditions (30°C in the absence of 6-AU). We consider two possible explanations for this observation. In one model, a powerful transcriptional activator (and its associated coactivators) alters the Pol II elongation machinery such that it does not stall at the ARTAR sequence. Potential alterations might include the recruitment of an antiarrest factor other than TFIIS or modification of Pol II or an associated factor. Alternatively, conditions of high transcriptional activation might create a "bumper-to-bumper" situation in which Pol II molecules "push" each other down the gene. Such bumper-to-bumper conditions are likely to inhibit any backtracking and therefore should decrease the probability of transcriptional arrest.

TFIIS can function as an antiarrest factor in vivo. Chromatin immunoprecipitation experiments conclusively demonstrate that TFIIS can relieve transcriptional arrest at the ARTAR site. Specifically, under conditions of low activation (raffinose or glucose) there is a sharp increase in Pol II occupancy just before the ARTAR site in cells lacking TFIIS but not in wild-type cells. Under low-activation conditions, we presume that only a single Pol II molecule transverses the gene at any given time, such that transcriptional arrest results in a traffic jam of Pol II molecules just before the ARTAR sequence. Such transcriptional arrest is clearly observed in TFIIS deletion cells but not in wild-type cells, therefore demonstrating that TFIIS can function as an antiarrest factor in vivo. To our knowledge, these results represent the first physical evidence for an elongation-specific function of TFIIS in vivo.

Although the buildup of Pol II molecules upstream of the ARTAR site is not observed in raffinose-grown wild-type cells, the ARTAR site nevertheless inhibits *lacZ* transcription in this situation. We presume that ARTAR-dependent arrest is occurring in such cells but that the antiarrest of activity of TFIIS is sufficient to reduce the length of time an individual Pol II molecule remains blocked at the ARTAR site. In addition, the antiarrest activity of TFIIS should also reduce the length of the Pol II traffic jam. These considerations are relevant because measurements of Pol II occupancy by chromatin immunoprecipitation are influenced both by the occupancy time of an individual Pol II molecule as well as by the number of Pol II molecules associated with a given region of the gene. Our results suggest that although TFIIS functions as an antiarrest factor in vivo, the physiological concentration of TFIIS is insufficient to completely override the transcriptional block at the ARTAR site. In this regard, TFIIS dosage is very important for its antiarrest function in vitro.

Effect of TFIIS on transcriptional elongation in vivo. In addition to its ability to mediate antiarrest at the ARTAR site, TFIIS is important for *lacZ* expression under conditions of strong Gal4-dependent activation. This decreased *lacZ* expression in the absence of TFIIS is presumably due to a transcriptional elongation defect, because the level of *GAL1* mRNA is not significantly affected. By this assay, TFIIS behaves similarly to the Hpr1, Tho2, Mfp1, Thp2, and Thp1 proteins that have been implicated in transcriptional elongation (5, 6, 10, 32, 33). As such, our results provide additional evidence that TFIIS is important for transcriptional elongation in vivo. This presumed elongation activity of TFIIS on *lacZ* may or may not be similar to the activity that allows TFIIS to read through the ARTAR sequence (see below).

Two aspects of the above elongation function of TFIIS are surprising. First, TFIIS is not required for *GAL1-lacZ* expression at a reduced temperature (16°C) or under conditions of low activation (raffinose medium). Second, even under conditions where TFIIS has a 10-fold effect on *lacZ* mRNA levels, Pol II occupancy throughout the *lacZ* gene is unaffected by TFIIS. This latter observation suggests that under conditions of high transcriptional activation, Pol II molecules are tightly packed in a bumper-to-bumper manner over the *lacZ* gene in both wild-type and TFIIS deletion cells and TFIIS increases the speed of the Pol II traffic through the gene. The hypothesis that TFIIS increases the rate of transcriptional elongation in vivo explains why TFIIS is important under conditions of high transcriptional activation (where elongation is limiting) but not under conditions of low activation (where initiation is limiting).

Transcriptional elongation and recovery from transcriptional arrest are distinguishable functions in vivo. It is generally believed that transcriptional elongation and the relief of transcriptional arrest are mechanistically related and that both processes involve TFIIS. However, our results indicate that the ARTAR sequence and TFIIS differently affect transcription in response to particular experimental conditions. At 30°C, ARTAR is important for *lacZ* expression under conditions of low activation (raffinose), whereas TFIIS is not. Conversely,

TFIIS is important under conditions of high activation (galactose), whereas ARTAR is not. At 16°C, *lacZ* expression is more significantly affected by the ARTAR sequence and less significantly affected by TFIIS. These observations suggest that the ARTAR sequence imposes a transcriptional block that is distinct from the ones hindering transcriptional elongation at high activation levels. This distinction might involve different functions of TFIIS and/or might arise from kinetic effects related to which parameters of the overall transcriptional process are rate limiting under particular experimental conditions.

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