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Distinction and Relationship between Elongation Rate and Processivity of RNA Polymerase II In Vivo

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Summary

A number of proteins and drugs have been implicated in the process of transcriptional elongation by RNA polymerase (Pol) II, but the factors that govern the elongation rate (nucleotide additions per min) and processivity (nucleotide additions per initiation event) in vivo are poorly understood. Here, we show that a mutation in the Rpb2 subunit of Pol II reduces both the elongation rate and processivity in vivo. In contrast, none of the putative elongation factors tested affect the elongation rate, although mutations in the THO complex and in Spt4 significantly reduce processivity. The drugs 6-azauracil and mycophenolic acid reduce both the elongation rate and processivity, and this processivity defect is aggravated by mutations in Spt4, TFIIS, and CTDK-1. Our results suggest that, in vivo, a reduced rate of Pol II elongation leads to premature dissociation along the chromatin template and that Pol II processivity can be uncoupled from elongation rate.

Introduction

Transcriptional elongation is broadly defined as the process by which Pol II traverses the coding region after it initiates mRNA synthesis at the promoter. Pol II elongation factors have been defined by several criteria, and they include TFIIS, as well as the Spt4-Spt5, Spt6-Iws1, FACT, Paf, THO, Elongator, Set1, and Set2 complexes. Like Pol II itself, these elongation factors associate with coding regions in vivo in a manner that depends on the level of transcriptional activity (Krogan et al., 2002; Pokholok et al., 2002; Strasser et al., 2002; Krogan et al., 2003; Mason and Struhl, 2003; Ng et al., 2003; Saunders et al., 2003; Xiao et al., 2003; Gilbert et al., 2004); hence, they presumably travel with elongating Pol II. Some of these elongation factors interact with Pol II in vitro (Sopta et al., 1985; Shi et al., 1996; Wada et al., 1998; Otero et al., 1999; Krogan et al., 2002; Li et al., 2002; Krogan et al., 2003; Ng et al., 2003; Xiao et al., 2003) and/or increase Pol II elongation on naked and/or nucleosomal templates (Reinberg and Roeder, 1985; Orphanides et al., 1998; Wada et al., 1998; Rondon et al., 2003b; Endoh et al., 2004; Rondon et al., 2004). Mutations in many elongation factors cause hypersensitivity to 6-azauracil (6-AU) and/or mycophenolic acid (MPA) (Hampsey, 1997; Riles et al., 2004), drugs that are presumed to affect elongation in yeast cells by virtue of depleting the pools of UTP and GTP (Exinger and Lacroute, 1992) and affecting induction of *IMD2*, which encodes IMP dehydrogenase (Shaw and Reines, 2000).

Despite the information above, there have been no direct tests of how or even whether the various elongation factors and drugs affect Pol II elongation in vivo. As Pol II elongation is mechanistically linked to post-transcriptional processes such as 5′ capping, splicing, nuclear export, polyadenylation, and 3′-end formation (Bentley, 2002; Maniatis and Reed, 2002; Orphanides and Reinberg, 2002; Proudfoot et al., 2002; Shilatifard et al., 2003), many of these so-called elongation factors might not affect the process of elongation itself, but rather the linkage to subsequent events. In addition, previous in vitro and in vivo analyses use the collective term "elongation" to cover at least two fundamentally distinct aspects of the elongation process. Specifically, there is essentially no information on the relationship between the rate at which elongating Pol II traverses the gene and the ability of elongating Pol II to travel the entire length of the gene (processivity). Furthermore, the factors that govern elongation rate and/or processivity in vivo are unknown.

Here, we address the relationship between elongation rate and processivity in vivo by measuring the level of Pol II association at various positions within a large (8 kb) gene whose expression is driven by the rapidly regulated *GAL1* promoter (Strasser et al., 2002; Mason and Struhl, 2003; Schwabish and Struhl, 2004). We show that a mutation in the Rpb2 subunit of Pol II and the drugs 6-AU and MPA reduce both the elongation rate and processivity. None of the putative elongation factors tested affect the elongation rate, but mutations in the THO complex and in Spt4 significantly reduce processivity. TFIIS and CTDK-1, the kinase that phosphorylates the C-terminal domain of Pol II at serine 2, also affect processivity but only under conditions where elongation is inhibited by drugs. Our results demonstrate that elongation rate and processivity are functionally distinct and at least partially separable phenomena in vivo, and they suggest that most so-called elongation factors do not actually affect the rate at which Pol II travels down the gene.

Results

In Vivo Assays for Pol II Elongation Rate and Processivity

To address the relationship between elongation rate and processivity in vivo, we used an approach previously used to study other aspects of transcriptional elongation (Strasser et al., 2002; Mason and Struhl, 2003; Schwabish and Struhl, 2004). Specifically, we measured the level of Pol II association at various positions within the large (8 kb) and nonessential *YLR454* gene whose expression is driven by the rapidly regulated *GAL1* promoter. The elongation rate is determined by treating galactose-grown cells with glucose, and fol-

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lowing the kinetics of Pol II dissociation at different positions within the *YLR454* coding region during the "last wave" of transcription. Importantly, this elongation rate is a time- and cell-averaged metric inferred from snapshots of Pol II occupancy, a virtual kinetic movie; it does not measure elongation potential of individual Pol II molecules on the DNA template. Processivity is determined by measuring the level of Pol II association across the *YLR454* coding region in galactose-grown cells. As shown previously for *GAL1-YLR454* and other genes, the level of Pol II association is constant throughout coding regions (Strasser et al., 2002; Mason and Struhl, 2003); thus, Pol II elongation in vivo is highly processive, such that the vast majority of transcriptionally engaged Pol II molecules travel the entire length of the gene. Using this approach, we examined the functions of many factors and two drugs implicated in transcriptional elongation.

Reduced Elongation Rate and Processivity in a Strain Containing a Mutant Pol II

We first examined the effect of the *rpb2-10* mutation, which alters the second-largest Pol II subunit and reduces the Pol II elongation rate in vitro on naked DNA templates (Powell and Reines, 1996). Pol II density was monitored at five positions spanning the *GAL1-YLR454* gene (Figure 1A) at 1 min intervals following glucose addition. In the isogenic wild-type strain, the "last wave" of Pol II clears from the 5′ to 3′ ends of the gene as described previously (Strasser et al., 2002; Mason and Struhl, 2003), whereas the *rpb2-10* mutant strain yields a delayed pattern of Pol II dissociation (Figures 1B and 1C). Comparing the time needed for half-dissociation at each position within the coding region indicates that the elongation rate in the *rpb2-10* is approximately 1 kb/min, compared to the wild-type rate of 2 kb/min (Figure 1C). Thus, the *rpb2-10* mutation halves the Pol II elongation rate. Additionally, we noticed that key information about the elongation rate could be obtained by examination of the pattern of Pol II dissociation (in mutant versus isogenic wild-type strains) following a single 4 min glucose treatment (Figure 1C), and we exploited this feature of the assay in subsequent experiments.

Interestingly, the elongation rate defect is accompanied by reduced processivity (Figure 1D). In the *rpb2-10* strain, approximately 40% of the initiating Pol II molecules fail to translocate successfully to the 3′ end. A reduced elongation rate might lead to reduced processivity. If the RNA-DNA-Pol II complex dissociates at a constant rate, the increased time for Pol II translocation might result in an increased likelihood of Pol II dissociation from the template.

6-AU and MPA Reduce Elongation Rate and Processivity

The drugs 6-AU and MPA reduce intracellular GTP and UTP levels and are believed to affect transcriptional elongation by virtue of substrate limitation (Exinger and Lacroute, 1992; Shaw and Reines, 2000). The phenotype of sensitivity to 6-AU and/or MPA is commonly used as an indicator that a particular mutation affects a protein involved with Pol II elongation (Hampsey, 1997). However, there is no direct evidence that 6-AU and MPA actually affect some aspect of elongation, and the effects of these drugs on cell physiology are more broad (Riles et al., 2004).

We therefore examined the effects of these drugs on elongation rate and processivity. Pretreatment of cultures for 30 min with either 6-AU or MPA results in a delayed pattern of Pol II dissociation following glucose treatment (Figures 2A and 2B). Kinetic analysis of multiple time points of glucose treatment indicates an elongation rate that is approximately 1/3 that of the wild-type rate (Figure 2C). In addition to affecting the elongation rate, both drugs cause a processivity defect, as observed by progressive loss of steady-state Pol II density as a function of distance from the promoter (Figures 2A and 2B). We tested cells treated with lower levels of 6-AU, with the idea that this might separate the processivity and elongation rate defects. However, lower concentrations yielded intermediate phenotypes for both processivity and elongation rate (Figure 2D). This indicates that the elongation rate and processivity are linked phenomena, consistent with the idea that an elongation rate defect will cause a processivity defect.

Uracil Depletion Causes Elongation and Processivity Defects, but It Is Not Equivalent to 6-AU Treatment

To determine the effects of reduced concentrations of a nucleotide precursor on Pol II elongation, we shifted a Ura$^-$ strain to medium lacking uracil for 30 min (Figure 3A). Under these conditions, Pol II density is redistributed in a manner similar to that observed upon 6-AU treatment, indicating that uracil depletion is sufficient to cause a processivity defect. Additionally, like 6-AU, uracil depletion causes a disruption in the pattern of Pol II dissociation following glucose treatment, indicating that it significantly reduces the Pol II elongation rate (Figure 3B). As expected, the processivity defect caused by uracil depletion is rapidly corrected by readdition of uracil to the medium. In contrast, readdition of uracil does not reverse the processivity defect caused by 6-AU (Figure 3A), presumably at least partly because 6-AU inhibits IMP dehydrogenase activity and hence lowers GTP pools (Exinger and Lacroute, 1992). These results indicate that limiting the concentration of a single nucleotide precursor is sufficient to cause a significant defect in Pol II processivity and elongation rate in vivo.

Elongation Rate Is Not Affected in Mutant Strains Lacking Putative Elongation Factors

We next examined a collection of yeast strains lacking potential elongation factors that have one or more of the following properties: interaction with Pol II in vitro, association with coding regions in vivo, or sensitivity to 6-AU. These factors include Set1 and Set2 histone methylases, Elongator subunits (Elp1, 3, and 4), nucleosome-remodelling factor Chd1 (Simic et al., 2003), elongin A (*ELA1*) (Pan et al., 1997), transcription-coupled repair factor Rad26 (Lee et al., 2001), subunits of the Paf1 (Rtf1 and Cdc73) and THO (Hpr1, Thp2, and Mft1) complexes, Spt4, TFIIS, Bre1 (Hwang et al., 2003; Wood et al., 2003), and Bye1 (Wu et al., 2003). We did not examine Spt6 or FACT because mutations in these

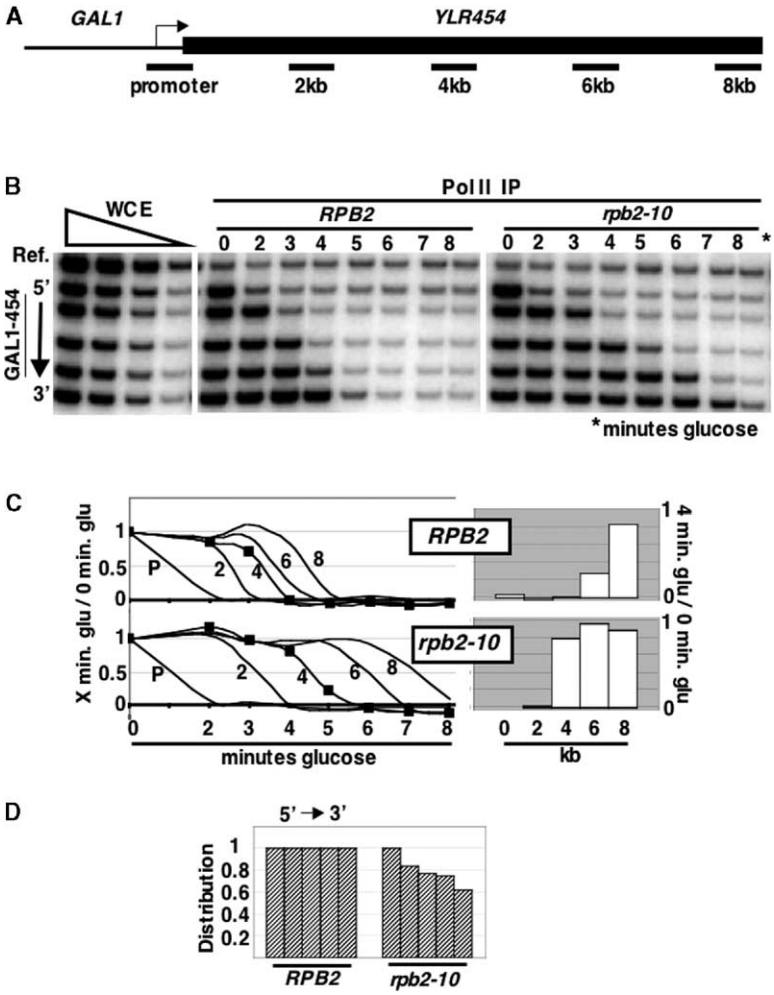


Figure 1. The *rpb2*-10 Mutation Affects Pol II Elongation Rate and Processivity In Vivo

(A) Diagram of the *GAL1-YLR454* gene with PCR primer pairs corresponding to the promoter and mRNA-coding region at the indicated positions downstream from the promoter.

(B) Pol II occupancy (8WG16 antibody) in strains containing *RPB2* or *rpb2-10* and *GAL1-YLR454* were induced with galactose and then treated with glucose for the indicated times.

(C) Relative Pol II occupancy at the indicated time point with respect to Pol II occupancy prior to glucose addition for the five indicated positions (left: data is shown as boxes for the 4 kb position and is not explicitly shown for the other genomic positions) with data for 4 min time point shown for all five positions tested (right).

(D) Relative Pol II occupancy at the five indicated positions in cells grown in galactose medium, with values in the wild-type strain normalized to 1.0 at each position.

factors cause internal initiation and increased Pol II density in coding regions (Strasser et al., 2002; Kaplan et al., 2003; Mason and Struhl, 2003), a property that interferes with the assay.

Following growth in galactose, each strain was treated either with glucose for 4 min or with 6-AU for 30 min. As judged by the pattern of Pol II dissociation following glucose treatment, none of the proteins tested have a detectable effect on the elongation rate (Figures 4A and 4B; data not shown). Thus, we have been unable to identify any protein factor that is required for achieving normal elongation rates in vivo, suggesting the possibility that elongation rate may be determined primarily by intrinsic enzymatic properties of Pol II. In addition, most of the factors tested do not detectably affect processivity (Figures 4A and 4C; data not shown). While these factors are likely involved in cotranscriptional processes, they appear to play a minimal (and perhaps no) role in the rate of elongation or processivity per se.

The THO Complex and Spt4 Are Important for Processivity, but Not Elongation Rate

In contrast to all the other factors tested, the THO complex and Spt4 are important for Pol II processivity. Mutant strains individually lacking Spt4 or three different subunits of the THO complex (Hpr1, Thp1, or Mtf1) do not show a constant level of Pol II density throughout the *YLR454* coding region (Figure 4C). Instead, there is a graded reduction in the level of Pol II density at positions increasingly downstream from the promoter, suggesting that only about half of the initiating Pol II molecules traverse a distance of 8 kb. Although Hpr1 can associate with the PAF complex (Chang et al., 1999), strains lacking the Cdc73 or Rtf1 (or Leo1; data not shown) components of the Paf complex show no defect in processivity. These results strongly suggest that the processivity-related function of Hpr1 occurs in the context of the THO complex. This observation is in agreement with the fact that disruption of the THO complex results in decreased abundance of RNA corresponding to the 3′ ends of long genes with little or no effect on 5′ RNA levels (Chavez and Aguilera, 1997; Chavez et al., 2000; Gallardo and Aguilera, 2001). However, our results indicate that this processivity defect is neither accompanied by, nor caused by, a defect in the elongation rate.

Interestingly, loss of Spt4 exacerbates the processivity defect caused by 6-AU to a significantly greater ex-

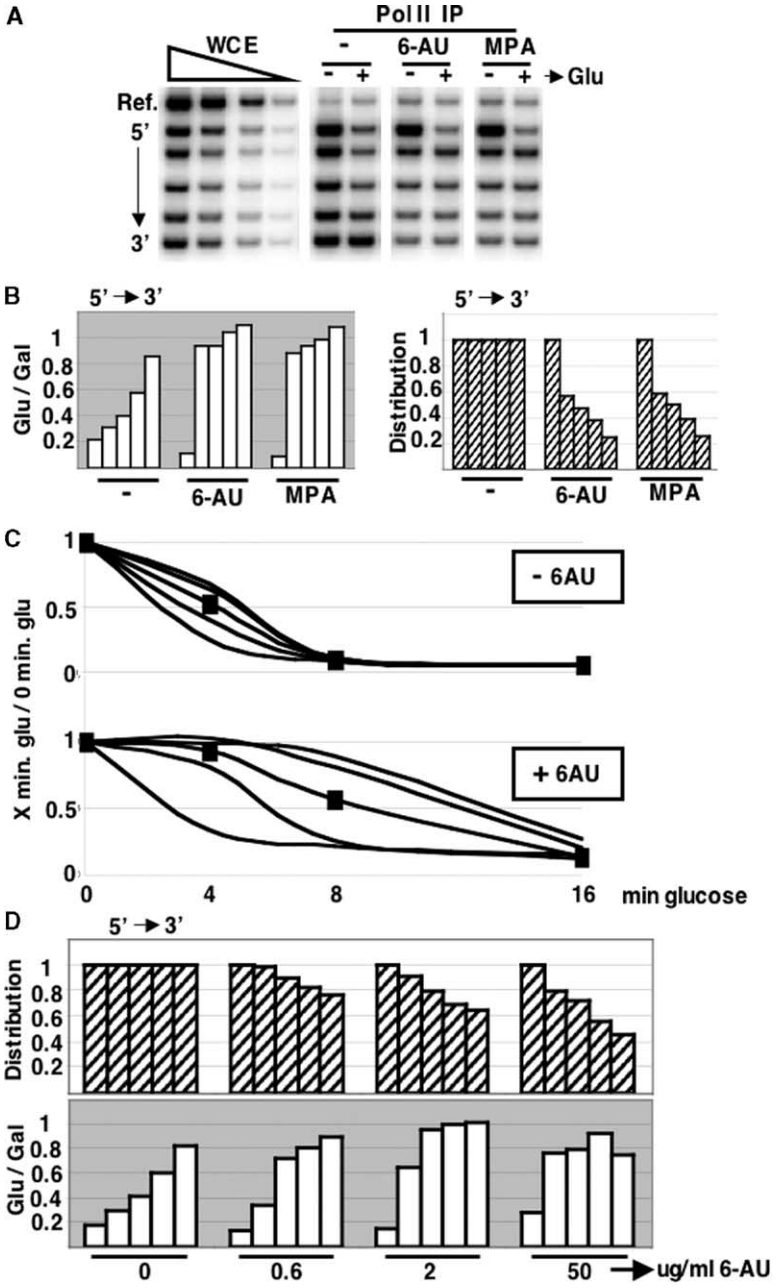


Figure 2. 6-AU and MPA Inhibit Pol II Elongation Rate and Processivity

(A) Pol II association at the five positions within *GAL1-YLR454* in wild-type cells induced with galactose and treated with 50 ug/ml 6-AU or 15 ug/ml MPA for 30 min prior to a 4 min treatment with glucose.

(B) The ratio of Pol II occupancy 4 min after glucose addition with respect to Pol II occupancy prior to glucose addition (left), and relative Pol II occupancy in cells grown in galactose medium are shown

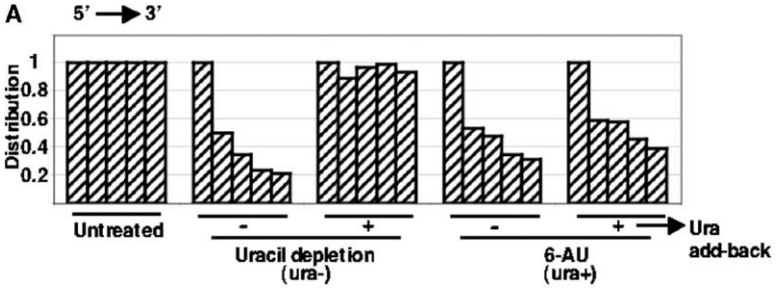
(C) Relative Pol II occupancy in galactose-induced wild-type cells that were untreated or treated with 6-AU for 30 min prior to treatment with glucose for the indicated times. Data are shown as described in Figure 1C.

(D) Galactose-grown cells were treated for 30 min with the indicated concentrations of 6-AU and analyzed for relative Pol II occupancy at the five positions before (top) or after (bottom) a 4 min treatment with glucose.

tent than does loss of the THO complex (Figures 4A and 4D). This observation suggests that Spt4 and the THO complex affect processivity of Pol II transcription by different mechanisms. Distinct roles for Spt4 and the THO complex in the overall process of Pol II elongation have been inferred from genetic and phenotypic analysis (Rondon et al., 2003a).

TFIIS and CTDK1 Affect Processivity, but Not Elongation Rate in the Presence of 6-AU

Although strains lacking a given elongation factor do not show a defect in elongation rate or processivity, they are often sensitive to 6-AU. This observation suggests that elongation factors may be functionally redundant in wild-type cells and that individual factors are important for Pol II function only under conditions where the Pol II elongation rate is inhibited. We therefore examined Pol II processivity in a variety of mutant strains grown in the presence of 6-AU (Figures 4A and 4D). The processivity defect caused by 6-AU is exacerbated in strains lacking TFIIS or either subunit of CTDK1, the kinase that phosphorylates the C-terminal domain of Pol II at serine 2, whereas these strains are essentially normal in the absence of 6-AU (Figure 4C). In the presence of 6-AU, Pol II density is significantly reduced 2 kb from the promoter and virtually absent at the distal end. Thus, TFIIS and CTDK1 can affect processivity, although this effect is observed only under



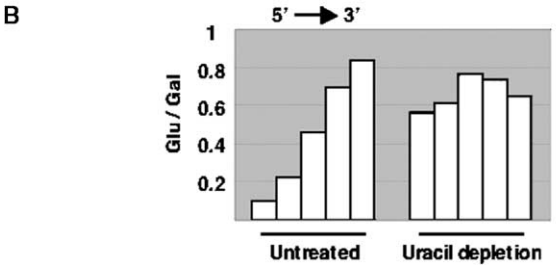


Figure 3. Uracil-Depleted Cells Show Reduced Pol II Elongation Rate and Processivity

(A) Pol II occupancy at the five positions within *GAL1-YLR454* in cells subjected to uracil depletion or 6-AU for 30 min, after which uracil was or was not added for 10 min.

(B) Pol II occupancy at the five positions within *GAL1-YLR454* in untreated or uracil-depleted cells that were induced with galactose and then treated with glucose for 4 min.

conditions where processivity is compromised by the presence of 6-AU.

The severe deficiency of Pol II molecules associated with downstream portions of the *YLR454* coding region caused by 6-AU in strains lacking TFIIS or CTDK1 complicate the use of our standard assay for measuring elongation rate. However, the presence of Pol II molecules 2 kb downstream from the promoter (Figure 4D) excludes the possibility that Pol II is exclusively stalled at the promoter and unable to translocate. To measure elongation rates in TFIIS- or CTDK1-deficient cells treated with 6AU, we monitored Pol II density over shorter intervals located near the 5′ end of the gene (Figure 4E). The 5′-proximal Pol II density in the 6-AU-treated strains lacking TFIIS or CTDK1 decays upon glucose treatment (Figure 4E), directly demonstrating that Pol II elongation occurs under these conditions. Interestingly, the rate of elongation in the TFIIS and CTDK1 mutant strains is similar to, or only marginally reduced from, that occurring in the wild-type strain. Thus, TFIIS and CTDK1 are important for processivity under conditions where the elongation rate is reduced by 6-AU, but they have little if any affect on the elongation rate itself.

Given the pronounced hypersensitivity to 6-AU caused by TFIIS deletion, we tested a Ura$^-$ version of this strain for the response to uracil depletion. In contrast to the case of 6-AU treatment (Figure 4D), deletion of TFIIS did not cause hypersensitivity to uracil depletion (Figure 4F). This further suggests that the effects of 6-AU treatment are not exclusively the result of nucleotide depletion, and it indicates that TFIIS does not act solely at a step in Pol II translocation that is sensitive to substrate deprivation.

The correlation between the observed processivity defects in the presence of 6-AU with respect to growth defects on 6-AU plates (Figure 4C) is imperfect. For example, whereas growth of *cdc73* or *rtf1* deletion strains are inhibited by 6-AU, the processivity defect conferred by 6-AU in these strains is comparable to that of wild-type. In addition, while *ctk1* or *ctk2* deletion strains show 6-AU processivity defects similar to *ppr2* strains, which lack TFIIS, loss of TFIIS function causes a more pronounced 6-AU growth defect than loss of CTDK1. This suggests both that these factors have distinct modes of sensitivity to 6-AU and that 6-AU effects cell growth, and perhaps transcription, at multiple levels.

Fkh2 Does Not Appear to Affect Processivity at the *PMA1* Gene

It has been reported that loss of Fkh2, a forkhead transcription factor, causes a dramatic (30-fold) reduction of Pol II density at the 3′ end of the *PMA1* gene (Morillon et al., 2003). However, using similar primer pairs, our analysis of steady-state Pol II density at *PMA1* reveals no defect in either overall Pol II density or processivity upon deletion of Fkh2 (Figure 4G). For this reason, Fkh2 was omitted from the panel of factors tested in our *GAL1-YLR454* assay. We do not understand the basis for the discrepancy between our results and those published previously (Morillon et al., 2003).

6-AU Reduces the Association of Initiation Factors in a TFIIS-Deficient Strain

Given the pronounced concentration and narrow distribution of Pol II near the promoter in the TFIIS deletion strain treated with 6-AU, we examined the association of various transcription factors with the *GAL1-YLR454* gene (Figure 5). In the TFIIS-deleted strain, 6-AU treatment does not affect the association of Gal4, but it reduces association of the core-promoter factors TBP, TFIIB, and TFIIF (Rap74 subunit) by a factor of 2–3. In

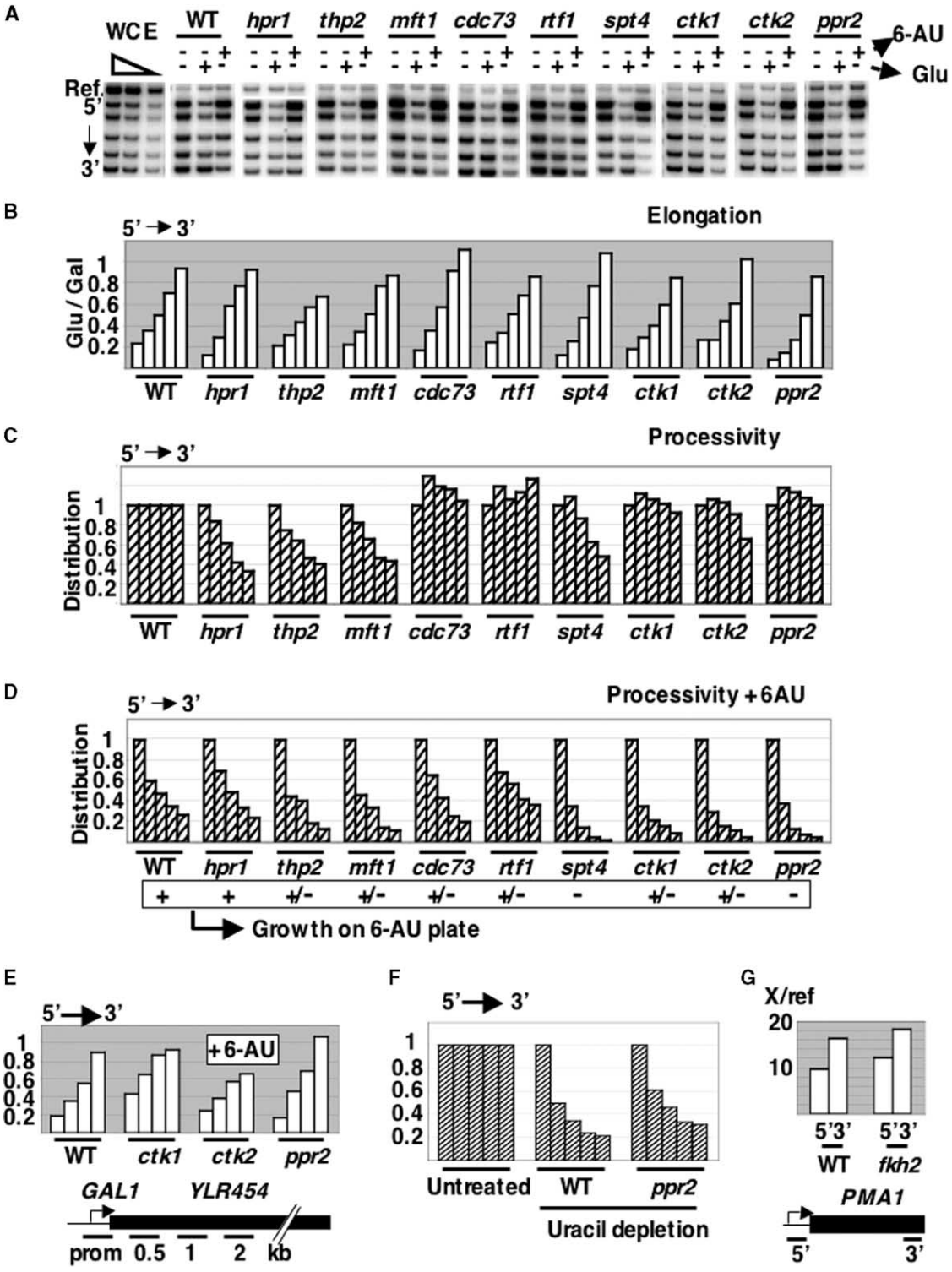


Figure 4. Analysis of Pol II Elongation Rate and Processivity in Strains Mutated for Elongation Factors

(A) Pol II occupancy at the five positions within *GAL1-YLR454* in wild-type and the indicated mutant strains that were induced with galactose and either left untreated, treated for 4 min with glucose, or treated for 30 min with 6-AU.

(B) Ratio of Pol II occupancy 4 min after glucose addition with respect to Pol II occupancy prior to glucose addition.

(C) Relative Pol II occupancy within the *GAL454* locus in cells grown in galactose medium.

(D) Relative Pol II occupancy within the *GAL454* locus in cells grown in the presence of 6-AU. Growth of strains on plates containing various 6-AU concentrations (0, 6, 12, 25, and 50 ug/ml) were defined as follows: (+), wild-type growth; (±), moderate growth inhibition; and (–) severe growth inhibition.

(E) Ratio of Pol II occupancy at the indicated four positions at the 5′ proximal mRNA coding region in 6-AU-treated wild-type and mutant strains 4 min after glucose addition with respect to Pol II occupancy prior to glucose addition.

(F) Relative Pol II occupancy in wild-type and TFIIS-deficient strains subjected to uracil depletion.

(G) Pol II occupancy at the indicated positions of *PMA1* in wild-type and *fkh2* deletion strains (alleles confirmed by genomic analysis) grown in YPD medium.

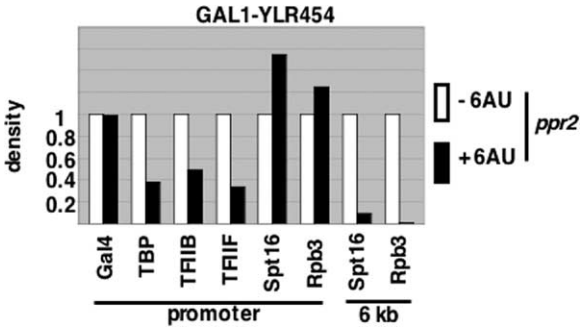


Figure 5. 6-AU Affects Association of Transcriptional Initiation Factors

Occupancy of the indicated transcription factors at the indicated positions of *GAL1-YLR454* in wild-type or *ppr2* strains that were induced with galactose and were or were not treated with 6-AU. Values are normalized to the occupancy in the untreated cells, which are defined as 1.0.

contrast, association of Pol II and the Spt16 subunit of the FACT complex at or near the promoter is not reduced, and is perhaps slightly increased, by 6-AU treatment. Similar results are observed at the proximal or distal segments of the *PYK1* and *PMA1* genes (data not shown). This potential initiation defect does not occur in the wild-type strain treated with 6-AU, suggesting that 6-AU per se is insufficient to cause a defect in association of initiation factors at the promoter. A simple, but unproven, explanation for these observations is that reduced translocation of Pol II caused by 6-AU in the absence of TFIIS increases the time for clearance of Pol II from the promoter, thereby increasing the probability of dissociation of the preinitiation complex and/or inhibiting subsequent initiation events. However, we cannot exclude the possibility that the reduced association of initiation factors in TFIIS-deficient strains treated with 6-AU is an indirect consequence of the severe elongation defect.

Discussion

Transcriptional elongation is a complex process that is mechanistically linked to posttranscriptional processes such as 5′-capping, splicing, nuclear export, polyadenylation, and 3′-end formation (Bentley, 2002; Maniatis and Reed, 2002; Orphanides and Reinberg, 2002; Proudfoot et al., 2002; Shilatifard et al., 2003). As such, many elongation factors might not affect the process of elongation itself but rather the linkage to subsequent events. Here, we address a fundamental but poorly understood issue of the Pol II elongation process, namely the relationship between the elongation rate (the speed at which Pol II traverses the gene) and the ability of elongating Pol II to travel the entire length of the gene (processivity).

A Reduced Rate of Pol II Elongation Results in Premature Dissociation of Pol II from the Chromatin Template In Vivo

The *rpb2*-10 mutation of Pol II itself or treatment with nucleotide depleting drugs 6-AU and MPA causes a reduction in the Pol II elongation rate. In addition, these conditions render Pol II more prone toward premature disassociation from the template. In all cases, an elongation-rate defect is accompanied by a processivity defect of corresponding magnitude. Our observations made in vivo are consistent with observations made in vitro—that reduction of elongation rate by the *rpb2*-10 allele or by nucleotide depletion results in increased transcriptional arrest (Powell and Reines, 1996). However, our observations indicate that Pol II does not simply arrest transcription but rather dissociates from the chromatin template in vivo. In this regard, transcriptional arrest at a defined site results in significantly increased Pol II density at the site of arrest (Kulish and Struhl, 2001), a pattern that is distinct from the gradual reduction in Pol II density observed in the *rpb2*-10 strain or in the wild-type strain treated with 6-AU or MPA.

The simplest explanation for the connection between elongation rate and processivity is that the longer a Pol II molecule stays on the gene, the more likely it is to dissociate and give rise to a prematurely terminated transcript. In principle, Pol II might simply dissociate from the chromatin template as a function of time spent at a particular location. However, elongating Pol II complexes are stable in vitro, suggesting that Pol II dissociation in vivo might require an active process. Interestingly, mutations in Rbp2, TFIIS, Spt5, and Spt6 can lead to increased utilization of upstream poly(A) signals (Cui and Denis, 2003; Kaplan et al., 2004). Thus, a reduced elongation rate and hence increased Pol II dwell time on the chromatin template might lead to increased probability of 3′-processing and transcriptional termination, thereby resulting in Pol II dissociation as a function of distance from the promoter.

Although not exhaustive, our search thus far has revealed no protein factors other than Pol II that detectably regulate the Pol II elongation rate. We did not directly analyze FACT or Spt6, due to the complications of internally initiated transcripts, but mutations in these factors do not appear to affect processivity (Kaplan et al., 2003; Mason and Struhl, 2003) and, hence, may not affect elongation rate. These observations prompt the suggestion that the Pol II elongation rate in vivo may be governed in large part by the inherent properties of Pol II itself. In this regard, our measurements of the Pol II elongation rate in vivo are consistent with previous in vivo measurements made using other methods (Ucker and Yamamoto, 1984; O'Brien and Lis, 1993; Iyer and Struhl, 1996) and with in vitro measurements made using purified Pol II and naked DNA templates. However, nucleosomes strongly block Pol II elongation in vitro (Izban and Luse, 1991; Izban and Luse, 1992) and actively transcribed genes display low histone density across the entire mRNA coding region in vivo (Kristjuhan and Svejstrup, 2004; Lee et al., 2004; Schwabish and Struhl, 2004). Kinetic analysis suggests that histones are rapidly evicted upon Pol II elongation in vivo (Schwabish and Struhl, 2004), strongly suggesting that chromatin-modifying activities that evict nucleosomes are required for elongation. Presumably, such nucleosome-evicting activities have not been analyzed in our current study, and/or there is functional redundancy such that multiple activities (including one or more as-

sayed here) are capable of nucleosome eviction upon Pol II elongation.

Pol II Processivity Can Be Uncoupled from Elongation Rate: Role of the THO and Spt4-Spt5 Complexes

Although defects in elongation rate appear to cause defects in processivity, we demonstrate that the THO complex and Spt4 (and presumably the Spt4-Spt5 complex) affect Pol II processivity, but not elongation rate. This suggests that the THO and Spt4-Spt5 complexes are important for the stability of the Pol II elongation machinery on the chromatin template but not for the inherent ability of this machinery to translocate down the gene. However, the THO and Spt4-Spt5 complexes appear to affect processivity by different mechanisms because loss of Spt4, but not the THO complex, significantly exacerbates the processivity defect caused by 6-AU. Distinct roles for Spt4 and the THO complex have been inferred from genetic and phenotypic analysis, and it has been suggested that Spt4 acts prior to the THO complex in the overall elongation process (Rondon et al., 2003a).

The mechanisms by which the Spt4-Spt5 and THO complexes affect processivity are unknown. In vitro, Spt5 cooperates with Tat to reduce Pol II pausing at arrest sites and to prevent premature dissociation of RNA from the transcription complex at terminators (Bourgeois et al., 2002). Spt5 resembles bacterial NusG (Hartzog et al., 1998), which contacts and enhances RNA polymerase stability on DNA templates (Li et al., 1992; Sullivan and Gottesman, 1992). These biochemical observations are consistent with the observation that *spt4* mutants exacerbate processivity defects under conditions where elongation rate is inhibited by 6-AU. The THO complex associates with RNA and mRNA export proteins Sub2 and Yra1 in the larger TREX complex, and THO mutants accumulate mRNA in the nucleus (Jimeno et al., 2002; Strasser et al., 2002). Loss of Hpr1 increases cotranscriptionally formed RNA:DNA hybrids, suggesting that the THO complex is important for stabilizing or processing nascent RNA in the Pol II elongation complex (Huertas and Aguilera, 2003; Jensen et al., 2004). This effect at the level of nascent RNA may be important for the stability of the elongating Pol II machinery on DNA and hence for the processivity, but not for the elongation rate of Pol II. This suggestion also explains why mutational disruption of the THO complex does not exacerbate the processivity defect when the elongation rate is inhibited by 6-AU.

TFIIS and CTDK1 also affect Pol II processivity without affecting the elongation rate, but this processivity defect is only observed in the presence of 6-AU. We presume that the level of Pol II pausing or at arrest in wild-type cells is sufficiently low such that the minor contribution of TFIIS and CTDK1 to processivity is not observed. In contrast, Pol II pausing and/or arrest increases upon 6-AU treatment, thereby causing a processivity defect that is exacerbated in the absence of TFIIS or CTDK1. Consistent with this suggestion, TFIIS is required for Pol II elongation through an artificial arrest site in vivo, and this arrest site does not function under conditions of high transcriptional activity unless elongation is inhibited by 6-AU or other treatments (Kulish and Struhl, 2001).

Table 1. Primer Sequences for Quantitative PCR Analysis

GAL1-YLR454
–194/+35 (promoter)
5′-CTGGGGTAATTAATCAGCGAAGCGATG-3′
5′-CACTTGTACAGTAGAACATTAATCGGAAAC-3′
+392/+605 (0.5 kb)
5′-CGCAATTAGTCAACAACGATATCACGATTG-3′
5′-CCTACTTGAAGTCCATCCTTCAGAGG-3′
+945/+1147 (1 kb)
5′-CAATACCAACAGGTTCAGAAATGAGATGC-3′
5′-GAGAGAACAAATTGGTTTCGCCAAATATCG-3′
+1986/+2199 (2 kb)
5′-CATATCATCCACCCTAGGTGCTAGGTCGG-3′
5′-GAGCTGACCAGACCTAACCATAGTAGCGTG-3′
+4069/+4268 (4 kb)
5′-AG ATATTACTCG TTGTTCGTGC CCAG-3′
5′-AG ATATTACTCG TTGTTCGTGC CCAG-3′
+5904/+6074 (6 kb)
5′-CGTACTGTTGAAATGGAACGAGGACGC-3′
5′-ATCGCTTCCATACTCGTTGTATCATCAGTC-3′
+7701/+7850 (8 kb)
5′-GAGGGTCACA GATCTATTAC TTGCCC-3′
5′-GTTGTGAGTTGCTTCAGTGGTGAAGTG-3′
ORF-Free Chr1
position 214193/214477
5′-GTTTATAGCGGGCATTATGCGTAGATCAG-3′
5′-GTTCCTCTAGAATTTTTCCACTCGCACATTC-3′
SSA4
–354/–52
5′-TGTACTCTCCCACCATTGGGTATTA-3′
5′-AGTGGTTTTTATTCGAAAGTTGTGGA-3′
PMA1
–356/–82
5′-CCCCTCCATTAGTTTCGATTATATAAAAAGGCC-3′
5′-TTTCTTTCTTTTGAATGTGTGTATAAAAGAGAG-3′
+2609/+2756
5′-GTGGTTTCTACTACGAAATGTCCACTTC-3′
5′-TAGGTTTCCTTTTCGTGTTGAGTAGAGAC-3′

Biological Significance of Defects in Pol II Processivity

The processivity defects observed in the absence of 6-AU, while readily detectable, are quantitatively modest. Moreover, the 8 kb *YLR454w* gene is significantly longer than a typical 1–2 kb yeast gene. Assuming that Pol II processivity defects are not gene specific, approximately 80%–90% of initiating Pol II elongate through a typical yeast gene in the mutant strains. Thus, it is not surprising that strains lacking Spt4 or THO complex members grow normally. However, higher eukaryotes contain much longer, intron-rich genes, and it is highly likely that subtle defects in Pol II processivity would have catastrophic phenotypic effects. In addition, the connection between Pol II elongation rate and processivity suggests that genetic or environmental perturbations that subtly reduce elongation rate will also have major biological consequences. These considerations underscore the biological importance of factors such as the THO and Spt4-Spt5 complexes in permitting Pol

II molecules to travel very long distances through a gene without dissociating from the template.

Experimental Procedures

Yeast Strains and Media

Test strains were prepared by single-step integration of a *TRP1* or *URA3* plasmid containing the *GAL1* promoter fused to the 5′-most 300 bp of the *YLR454w* open-reading frame at the *YLR454w* locus. Strains *RPB2* and *rpb2-10* (Z24 and Z428, respectively) were provided by Richard Young, and strain *hpr1::HIS3* was provided by Katja Strasser. Other deletion strains, in the BY4741 background, wherein relevant open-reading frames were replaced by *KAN*r, were obtained from Research Genetics. In Figures 2, 3, and 5, W303.1A containing *GAL1-YLR454* was converted to Ura+ by single-step integration of linearized YIplac211 or by integration of pJD3, as described (Alani et al., 1987), which simultaneously confers uracil prototrophy and replaces *PPR2* with *hisG::URA3::hisG*. Where appropriate, both strains were returned to uracil auxotrophy by FOA selection.

Strains were grown in synthetic complete medium lacking uracil and/or tryptophan with 2% raffinose as the carbon source at 30°C to early log phase, followed by a 2.5 hr session of galactose induction, wherein cells were untreated or treated with 6-AU for the last 30 min of the session, prior to treatment with 4% glucose. 6-AU and MPA were purchased from Sigma.

Chromatin Immunoprecipitation

Chromatin immunoprecipitation with antibodies against the C-terminal domain of the largest subunit of Pol II (8WG16 from Covance) were performed as described previously (Mason and Struhl, 2003). Control immunoprecipitations of diagnostic extracts using Rpb3 antibodies (Neoclone) yielded similar results, as did various combinations of primers in the PCR mixtures (data not shown). All experiments shown were performed multiple times using independently grown cultures.

PCRs on the input and immunoprecipitated samples were performed using ^{32}P-dATP and primers as in Table 1, and the resulting products were separated by acrylamide gel electrophoresis and quantitated by PhosphorImage analysis (Mason and Struhl, 2003). Background and uniformity of immunoprecipitation were monitored with reference DNA segments consisting of the *SSA4* promoter and/or an ORF-free region of Chromosome 1, which gave similar results. Immunoprecipitation efficiency was calculated as the amount of PCR product in the immunoprecipitated sample divided by the amount of PCR product in the whole cell extract sample, and specific occupancy was determined by subtracting the apparent immunoprecipitation efficiency of the reference from the apparent immunoprecipitation efficiency of the relevant gene segment. In Figure 4G, occupancy is expressed as the ratio of gene-specific signal over the reference background. For evaluation of elongation rate, data is expressed as the ratio of specific occupancy following glucose treatment, divided by specific occupancy in galactose. For evaluation of processivity, data is expressed as the ratio (distribution) of Pol II occupancy in a given strain (or drug condition), relative to the 5′-most (promoter) position, followed by normalization of each value to the corresponding position in wild-type (or untreated) strain, which was defined as "1."

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