

Research article

Factors controlling internal initiation of transcription at *PRY3* in budding yeast

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Transcription initiation from within the coding regions of genes has been observed in budding yeast as a natural phenomenon induced by environmental changes. However, such 'internal initiation' events have also been observed in mutant yeast strains with aberrant chromatin structure. Failure to suppress initiation from cryptic internal promoters is thought to result from either loss of appropriate nucleosome density or loss of appropriate histone modifications in the coding region. *PRY3* is a gene previously shown to undergo internal initiation in response to mating pheromone. This project aimed to uncover which factors are involved in suppressing inappropriate transcription initiation from an internal TATA-box at *PRY3* in the absence of mating pheromone. This work demonstrates that factors controlling nucleosome density (Spt6, Spn1 and Spt10) but not histone deacetylation (Eaf3) are required to suppress internal initiation at *PRY3* in the absence of mating pheromone. In addition, TATA-box binding protein (TBP) is required for internal initiation at *PRY3*. Taken together, these results indicate that internal initiation at *PRY3* is predominantly suppressed by a nucleosome reassembly mechanism rather than histone modifications, and suggest that the transcriptional output at *PRY3* is controlled by competition between TBP and nucleosomes for binding an internal TATA-box.

Key words: chromatin, transcription, initiation, Spt6, *PRY3*, fidelity.

Introduction

The hereditary material in eukaryotes is organized as a deoxyribonucleoprotein complex known as chromatin.¹ Nucleosomes, the fundamental units of chromatin structure and function, not only compact the genome but are also intimately involved in regulating its expression.² Modification by ATP-dependent chromatin remodelling, histone post-translational modifications (such as acetylation, methylation and phosphorylation) and incorporation of histone variants renders chromatin a highly dynamic complex and these changes underlie all nuclear genomic processes that occur in its context.^{3–6}

During the process of transcription, modification of chromatin structure influences the accessibility and function of proteins that interact with chromatin and DNA. For example, nucleosomes generally impair accessibility of transcription factors to enhancers and promoters, and impose a physical barrier to the passage of elongating RNA polymerase II (RNAPII).^{7, 8} The increases in nucleosome mobility

and DNA accessibility that are mediated by chromatin modifications during transcription must be coordinated, however, with modifications that restore a repressive chromatin structure after the passage of RNAPII through a gene.⁹ Failure to maintain appropriate chromatin structure in the open-reading-frame (ORF) of certain genes can lead to an aberrant transcription phenotype known as 'internal initiation' where transcription initiates from a cryptic promoter located within the coding region of a gene.^{10–12} Studies using mutant yeast strains with aberrant chromatin structure have identified two mechanisms by which internal initiation is suppressed and thus 'transcriptional fidelity' maintained: (i) cotranscriptional nucleosome reassembly by histone chaperones, such as Spt6 and FACT, provides appropriate nucleosome density to conceal internal promoters and repress internal initiation;^{10, 11} and (ii) histone methylation directs deacetylation of histones to form a repressive chromatin structure.¹²

FLO8 is an example of gene where an internal TATA-box promoter is suppressed by reassembly of appropriate

nucleosome density in the coding region during transcription. Internal initiation at *FLO8* is observed in histone depletion strains, such as *spt10Δ*, *spt21Δ*, *bur1Δ*, *bur2Δ* (positive regulators of histone gene expression¹³) and *bur5Δ* (histone H3), and in strains that are defective in Spt6 and FACT, transcription elongation factors that function as cotranscriptional histone chaperones.^{7, 10, 11} Since *SPT6* is essential to growth and viability, the temperature-sensitive alleles, *spt6*¹⁴⁰ and *spt6*¹⁰⁰⁴, have been used to study Spt6 function.¹⁴ While the exact mutation in *spt6*¹⁴⁰ is unknown, the protein encoded by *spt6*¹⁰⁰⁴ contains a 63 amino-acid deletion (931–994) that removes its non-specific DNA-binding helix–hairpin–helix (HhH) domain. At 39°C (the non-permissive temperature) but not 30°C (the permissive temperature), these two alleles exhibit internal initiation at *FLO8* due to disruption of the cotranscriptional histone chaperone activity of Spt6 and exposure of the internal TATA-box.¹⁰ Further investigation of factors suppressing internal initiation at *FLO8* has identified Spn1, an essential protein that interacts with Spt6; and the SH2 (Src-homology 2) domain of Spt6 (M. Youdell, personal communication).

The second mechanism proposed to suppress internal initiation relies upon appropriate histone modifications in the coding region. Methylation of lysine 36 on histone H3 (H3K36me) by Set2 during transcription has previously been shown to recruit the repressive Rpd3(S) complex via its chromodomain-containing Eaf3 subunit to deacetylate histones in the coding region and suppress internal initiation from genes such as *STE11*.^{12, 15} Histone deacetylation may suppress internal initiation by promoting stable histone-DNA interactions and preventing the binding of bromodomain-containing transcription factors, such as Bdf1 in TF_{II}D.^{16–18} Internal initiation at *STE11* is thus observed in *set2Δ* and *eaf3Δ* strains, and also in *spt6*¹⁰⁰⁴ (but not *spt6*¹⁴⁰ or *spt6*^{SH2}—M. Youdell, personal communication) due to a H3K36 methylation defect at both the permissive temperature (30°C) and the non-permissive temperature (39°C).¹²

While internal initiation occurs at *FLO8* and *STE11* in certain mutant yeast strains with aberrant chromatin structure, biologically relevant internal initiation has been documented in wild-type yeast strains for numerous genes, as highlighted in Table 1.

Transcription from an internal promoter has the potential to produce mRNA that can be transcribed in the same ORF as the full-length transcript to produce an N-terminal truncated protein that may lack specific domains or be targeted to different cellular locations. For example, the Leu4 protein derived from full-length *LEU4* mRNA is targeted to mitochondria, while truncated Leu4, lacking a signal sequence, is cytoplasmic.²¹ Internal initiation may also confer different regulatory properties on gene expression; for example, Suc2 protein is constitutively expressed when

translated from an internal transcript, whereas in glucose, full-length *SUC2* transcription is repressed.²²

PRY3 is a budding yeast gene that undergoes different transcription events dependent on the presence of α -factor mating pheromone (Fig. 1).²³ In the absence of α -factor, transcription initiation occurs from the core TATA-box 1 promoter at –188 and a full-length transcript is produced. However, when cells are treated with α -factor, full-length transcription is repressed and internal initiation of transcription occurs from TATA-box 2, which lies 385 nt downstream of the initiating ATG codon. Although the significance of this internal initiation event is unknown, it is thought to arise from the activation of the transcription factor Ste12 by the pheromone kinase cascade. Its subsequent binding to upstream pheromone-response-elements (PREs) at –175 and –161 prevents TATA-box binding protein (TBP) from binding to TATA-box 1, and thus transcription initiates from TATA-box 2.²³

How transcription from TATA-box 2 is repressed in the absence of α -factor is currently unknown. Is the internal promoter repressed by appropriate nucleosome density in the coding region like *FLO8*, or by repressive histone modifications like *STE11*? This investigation aimed to address which factors are important in suppressing internal initiation at *PRY3* in the absence of α -factor mating pheromone.

Materials and methods

Yeast strains and culture

Yeast cultures from 0.5×10^7 cells/ml were grown in YPD medium (1% Difco yeast extract, 1% bacto-peptone, 2% glucose) at 30°C to exponential phase ($1\text{--}1.5 \times 10^7$ cell/ml). Cultures of the two *spt6*¹⁰⁰⁴ *spt15* strains were incubated at 30°C in leucine–glucose–CSM medium with 2% raffinose until OD₆₀₀ \approx 0.8. For galactose-induced samples, 30 ml cultures were washed, resuspended in leucine–glucose–CSM medium with 2% galactose, and incubated for a further 45 or 120 min. Heat shock was carried out at 39°C for a further 90 min where stated.

RNA extraction and northern blotting

Total RNA was prepared by the standard hot acid phenol method and separated on 1.2% formaldehyde/MOPS agarose gels for approximately 9 h at 80 V.²⁴ Gels were blotted by the standard method and RNA fixed by baking for 2 h.²⁴ Radioactive probes were made using a Stratagene Prime-It II random primer labelling kit and purified using Sephadex G-50. Probes correspond to positions relative to +1 at ATG: *PRY3* (+20 to +1600), *SER3* (0 to +1000), *VHT1* (+20 to +1700) and *RDN18* (0 to +400). Membranes were hybridized overnight at 65°C and washed twice in $1 \times \text{SSC}/0.1\%$ SDS for 20 min and twice in $0.2 \times \text{SSC}/0.1\%$ SDS for 20 min. Bands were detected by autoradiography.

Table 1. Genes that undergo biologically relevant internal initiation in budding yeast

Gene	Protein function	Transcriptional output
<i>ASP3</i>	Cell wall asparaginase II	Full-length transcript occurs under nitrogen starvation, otherwise internal transcript produced. ¹⁹
<i>AQY1</i>	Spore-specific water channel	Full-length transcript occurs under osmotic stress, otherwise internal transcript is produced. ¹⁹
<i>CRH1</i>	Putative cell-wall glycosylase	Internal initiation occurs on treatment with α -factor. ¹⁹
<i>KAR4</i>	Transcription factor required for pheromone response and meiosis	Protein derived from pheromone-inducible internal initiation is fast-migrating and turned over rapidly in vegetative cells, while the protein derived from constitutive full-length transcription migrates slowly and is turned over at a slower rate. ²⁰
<i>KAR5</i>	Involved in nuclear membrane fusion	Internal initiation in the absence of α -factor. ¹⁹
<i>LEU4</i>	α -Isopropylmalate synthase	Protein translated from full-length mRNA is targeted to mitochondria while that derived from the internal transcript is located in the cytoplasm. ²¹
<i>SUC2</i>	Sucrose invertase	Full-length transcript is repressed by glucose and encodes a secreted glycosylated protein, while the internal transcript is constitutively produced and encodes a cytoplasmic non-glycosylated protein. ²²
<i>PRM2</i>	Unknown function, regulated by Ste12 in response to pheromone	Internal initiation in the absence of α -factor mating pheromone. ¹⁹
<i>PRP39</i>	U1 snRNP splicing factor	Internal initiation occurs in the presence of α -factor. ¹⁹
<i>PRY3</i>	Unknown function	Internal initiation on α -factor mating pheromone treatment. ²³

Protein extraction and western blotting

All cultures were harvested at 4°C and 100 μ l acid-washed glass beads, 300 μ l 6 M urea, 40 μ l 100 mM dithiothreitol and 260 μ l loading buffer (50 mM Tris pH 6.8, 2% SDS, 0.05% bromophenol blue, 10% glycerol) were added to the pellet before vortexing for 3 min and boiling for 5 min.

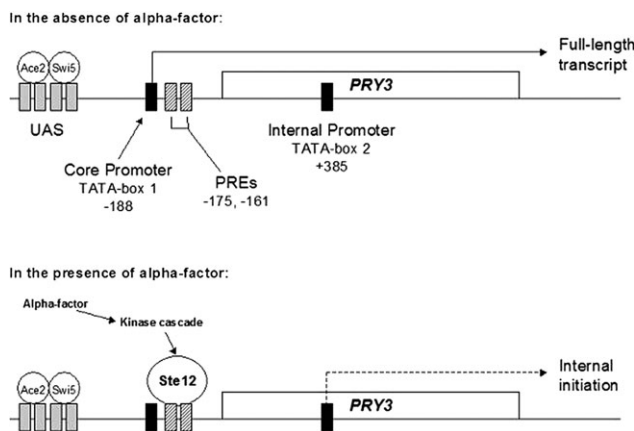


Figure 1. Transcriptional output at *PRY3* is regulated by α -factor mating pheromone. Ace2 and Swi5 are transcription factors that bind an upstream activation sequence (UAS) and activate gene expression in early G₁ phase and M/G₁ phase, respectively. In the absence of α -factor, TBP is recruited to TATA-box 1 and full-length transcription from -76 occurs. α -factor binding to cell-surface receptors initiates a mitogen-activated protein kinase (MAPK) cascade that phosphorylates and activates the Ste12 transcription factor. Ste12 binding to pheromone response elements (PREs) at -175 and -161 prevents TBP from binding TATA-box 1 and thus it binds TATA-box 2 at $+385$ and directs internal initiation at *PRY3*.²³

SDS-PAGE and western blotting were carried out by standard methods using anti-haemagglutinin (HA) and anti- β -actin monoclonal antibodies at a dilution of 1/1000, and probing with an anti-mouse IgG secondary antibody conjugated to horseradish peroxidase.²⁴

Results

Suppression of internal initiation at *PRY3* in the absence of α -factor requires Spt10, Spt6 and Spn1, but not Eaf3

Transcription initiation from the cryptic internal promoters at *FLO8* occurs when factors controlling nucleosome reassembly are disrupted. In contrast, factors controlling H3K36me and histone deacetylation repress the internal promoters at *STE11*.^{10, 12} Transcription from the internal TATA-box of *PRY3* occurs only in the presence of α -factor.²³ Northern blotting was used to investigate which factors required for transcriptional fidelity at *FLO8* and *STE11* are also needed to suppress internal initiation at *PRY3* in the absence of α -factor.

Figure 2 shows that internal initiation occurs in both *spt6*¹⁰⁰⁴ and *spt6*¹⁴⁰ strains at both 30°C and 39°C; in the two temperature-sensitive *spn1* strains at 39°C; and in *spt10* Δ . Internal initiation also occurs in *spt6*^{SH2}, a strain that lacks the 100 C-terminal amino acids of Spt6 that contain a Src-homology 2 (SH2) domain. Furthermore, these same strains have an increased amount of full-length transcript coincident with the production of the short internally initiated transcript. Notably, deletion of *EAF3* does not lead to internal initiation nor to increased full-length

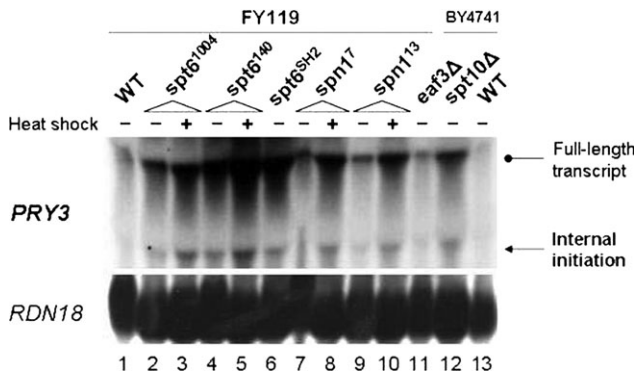


Figure 2. Northern blot analysis of transcription at *PRY3*. Total RNA was extracted from cultures grown in YPD at 30°C until exponential phase and heat shock was carried out at 39°C for an additional 90 min where stated. FY119 and BY4741 indicate the genetic backgrounds of wild-type (WT) and mutant strains, as detailed in Table 2. The protein encoded by *spt6*¹⁰⁰⁴ contains a 63 amino-acid deletion (931–994) that removes its non-specific DNA-binding helix-hairpin-helix (HhH) domain; *spt6*¹⁴⁰ is a temperature-sensitive allele of *SPT6*; and *spt6*^{SH2} encodes a protein that lacks the 100 C-terminal amino acids containing an SH2 domain. *spn1*⁷ and *spn1*¹³ encode temperature-sensitive versions of Spn1, an Spt6-interacting protein. The *eaf3*Δ strain lacks the histone deacetylase Eaf3 while *spt10*Δ lacks the Spt10 protein, a positive regulator of histone gene expression. The membrane was reprobed for *RDN18* (encoding 18S ribosomal RNA) to control for RNA loading.

transcript. This experiment was repeated a further two times with comparable results (data not shown).

These results show that Spt10, Spn1 and Spt6 (but not Eaf3) are required to suppress internal initiation at *PRY3*

Table 2. Yeast strains

Strain	Parent	Genotype	Reference
FY119 (WT)	FY119	<i>MATα, his4-912δ, leu2Δ1, ura3-52, trp1Δ63, lys2-128δ</i>	F. Winston ^a
BY4741 (WT)	BY4741	<i>MATα, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0</i>	EUROSCARF
<i>spt6</i> ¹⁰⁰⁴	FY119	<i>spt6</i> ¹⁰⁰⁴	F. Winston
<i>spt6</i> ¹⁴⁰	FY119	<i>spt6</i> ¹⁴⁰	F. Winston
<i>spt6</i> ^{SH2}	FY119	<i>SPT6SH2::ADH1, KanMX</i>	M. Youdell ^b
<i>spn1</i> ⁷	FY119	<i>spn1</i> ⁷	G. Hartzog ^c
<i>spn1</i> ¹³	FY119	<i>spn1</i> ¹³	G. Hartzog
<i>eaf3</i> Δ	FY119	<i>eaf3Δ::Trp1</i>	M. Youdell
<i>spt10</i> Δ	BY4741	<i>spt10Δ::Trp1</i>	EUROSCARF
<i>spt15</i> ^{V71E}	<i>spt6</i> ¹⁰⁰⁴	<i>pCALF-leu-pGal-spt15</i> ^{V71E} -HA	This study
<i>spt6</i> ¹⁰⁰⁴			
<i>spt15</i> ^{V161E}	<i>spt6</i> ¹⁰⁰⁴	<i>pCALF-leu-pGal-spt15</i> ^{V161E} -HA	This study
<i>spt6</i> ¹⁰⁰⁴			

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in the absence of α-factor. Complete loss of Spt6 function enhances the production of the internal transcript suggesting that, similar to *FLO8*, transcriptional fidelity at *PRY3* is maintained by a nucleosome reassembly mechanism requiring the Spt6 chaperone function. Significant levels of the internal transcript, however, are evident at the permissive temperature (30°C) for the *spt6*¹⁰⁰⁴ allele (no defect in histone chaperone activity) and the *spt6*¹⁴⁰ allele (no defect in H3K36me or histone chaperone activity), suggesting a potentially novel mechanism for suppression of internal transcripts at *PRY3*. The increase in levels of full-length transcript in the strains defective in histone assembly suggests that the upstream promoter is also restrained by nucleosomes.

The ability of TBP to bind a TATA-box is required for internal initiation at *PRY3*

Both the upstream and the internal promoters at *PRY3* have canonical TATA-boxes. I tested whether internal initiation at *PRY3* is dependent on the ability of TATA-box binding protein (TBP) to bind to a TATA-box. The *spt6*¹⁰⁰⁴ strain was transformed with plasmids that over-express substituted versions of TBP (*Spt15*) under the control of a galactose-inducible promoter. The two mutants, *spt15*^{V71E} and *spt15*^{V161E}, contain single valine-to-glutamate substitutions of different amino acids along the concave DNA-binding surface of TBP that specifically abolish DNA binding in a dominant-negative fashion, without affecting functions independent of DNA binding.^{25, 26} The two plasmid constructs produce TBP tagged with a haemagglutinin (HA) epitope, allowing analysis of mutant TBP expression under inducing (galactose) and non-inducing (raffinose) conditions by western blot. Figure 3 shows that both *spt6*¹⁰⁰⁴ *spt15* strains over-express TBP only in the presence of galactose.

Microarray analysis of genome-wide transcription in *spt15*^{V71E} and *spt15*^{V161E} strains has revealed the effects of over-expression of the substituted TBP proteins on TATA-dependent transcription.²⁵ Figure 4 shows that the effect of disruption of TATA-box dependent transcription on two control genes, *VHT1* and *SER3*, in an *spt6*¹⁰⁰⁴ background is consistent with the results of the microarray study. *VHT1* transcription is highly dependent on its TATA-box promoter, and preventing the TBP-TATA interaction by over-expression of TBP^{V71E} or TBP^{V161E} abolishes *VHT1* transcription. *SER3* transcription is repressed by a non-coding interfering transcript (*SRG1*) produced from a TATA-TBP dependent promoter. Loss of the interfering transcript upon over-expression of TBP^{V71E} and TBP^{V161E} relieves the transcriptional interference-mediated repression of downstream *SER3* transcription.²⁷ These two results are consistent with the microarray data, demonstrating that the mutant TBP in both *spt15* *spt6*¹⁰⁰⁴ strains is disrupting TATA-box-dependent transcription.

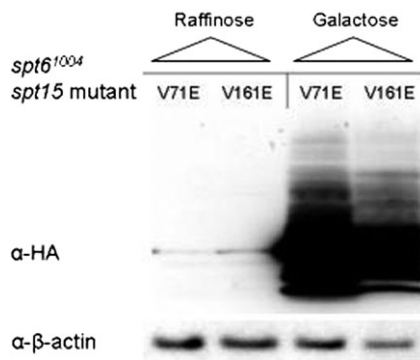


Figure 3. Western blot confirming overexpression of TBP^{V71E}-HA and TBP^{V161E}-HA in *spt15 spt6¹⁰⁰⁴* strains under inducing conditions (galactose) but not non-inducing (raffinose) conditions. Cultures were incubated at 30°C in leucine-glucose-SM medium with 2% raffinose until OD₆₀₀ ≈ 0.8. For galactose-induced samples, 30 ml cultures were washed, resuspended in leucine-glucose-CSM medium with 2% galactose and incubated for a further 2h. Total protein extracts were run on a 15% SDS-polyacrylamide gel. α-HA is a monoclonal antibody against haemagglutinin. α-β-actin is a monoclonal antibody against β-actin – used as a loading control.

Transcription of *PRY3* was subsequently analysed in *spt15^{V71E} spt6¹⁰⁰⁴* and *spt15^{V161E} spt6¹⁰⁰⁴* strains under non-inducing (raffinose) and inducing conditions (galactose). Figure 4 shows that internal initiation occurs in both *spt15 spt6¹⁰⁰⁴* strains in raffinose, but after galactose induction for 45 and 120 min both full-length and internal initiation are substantially abolished at *PRY3* in both *spt15 spt6¹⁰⁰⁴* strains. This result suggests that the full-length transcript

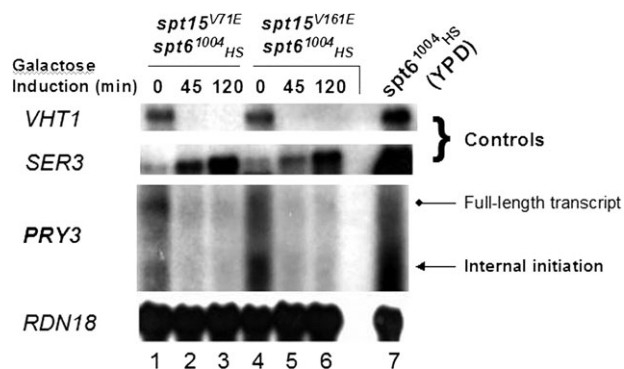


Figure 4. The effect of *spt15^{V71E} spt6¹⁰⁰⁴* and *spt15^{V161E} spt6¹⁰⁰⁴* mutants on transcription and internal initiation at *PRY3*. Cultures were incubated at 30°C in leucine-glucose-CSM medium with 2% raffinose until OD₆₀₀ ≈ 0.8. For galactose-induced samples, 30 ml cultures were washed, resuspended in leucine-glucose-CSM medium with 2% galactose and incubated for a further 45 or 120 min. For both galactose-induced and non-induced cultures, heat shock was carried out at 39°C for a further 90 min. The *spt6¹⁰⁰⁴* (YPD) culture was grown in YPD medium as described in Material and methods, and heat-shocked at 39°C for 90 min. The membrane was re-probed for *RDN18* (encoding 18S ribosomal RNA) to control for RNA loading.

and internal initiated transcript at *PRY3* are dependent upon TBP binding to a TATA-box, and suggests the internal transcript observed arises from TATA-box 2.

Discussion

PRY3 undergoes internal initiation in response to α-factor mating pheromone due to binding of activated Ste12 to PREs, consequential occlusion of TATA-box 1 at -188 and selection of TATA-box 2 at +385. In the absence of α-factor, the utilization of TATA-box 2 is suppressed and transcription initiation occurs at TATA-box 1 to produce a full-length transcript.²³

Internal initiation is observed, however, when factors controlling nucleosome reassembly are disrupted in *spt6¹⁰⁰⁴*, *spt6¹⁴⁰*, *spn1⁷* and *spn1¹³* strains at 39°C; and in *spt10Δ* and *spt6^{SH2}*. Together with the absence of internal initiation in an *eaf3Δ* strain, these results suggest that, like *FLO8*, a mechanism predominantly involving nucleosome reassembly rather than histone methylation and deacetylation is important for maintenance of transcriptional fidelity at *PRY3*. Due to disruption of the cotranscriptional histone chaperone function of Spt6 in *spt6¹⁰⁰⁴* and *spt6¹⁴⁰* at 39°C, or depletion of histone levels in *spt10Δ*, the inability to restore adequate nucleosome density over the coding region after the passage of elongating RNAPII allows transcription initiation from an internal promoter.

Since over-expression of substituted TBP that is unable to direct TATA-dependent transcription in the *spt6¹⁰⁰⁴ spt15^{V71E}* and *spt6¹⁰⁰⁴ spt15^{V161E}* strains leads to appropriate changes in the *VHT1* and *SER3* controls, the loss of internal initiation at *PRY3* upon over-expression of TBP^{V71E} and TBP^{V161E} shows that the internal transcript is dependent upon the ability of TBP to bind to a TATA-box (Fig. 4). Although this suggests that the internal transcript may arise from TATA-box 2, this needs to be supported by mapping of its transcriptional start site by primer extension analysis or rapid amplification of cDNA ends (RACE).

A model for suppression of internal initiation at *PRY3* in the absence of α-factor is presented in Fig. 5. Spt6-mediated cotranscriptional nucleosome reassembly over the coding region during transcription that initiates from TATA-box 1 is predicted to conceal TATA-box 2 and prevent internal initiation. A further investigation of factors suppressing internal initiation at *PRY3* could analyse the role of components of FACT, such as Spt16, which have been shown to be required to suppress internal initiation at *FLO8*.¹⁰

In addition, however, internal initiation at *PRY3* is observed in *spt6¹⁴⁰* and *spt6¹⁰⁰⁴* strains at 30°C. The function disrupted here is probably independent of the histone modification pathway since: (i) *spt6¹⁴⁰* is not defective in H3K36me (M. Youdell, personal communication); and (ii) though *spt6¹⁰⁰⁴* is defective in H3K36me, *eaf3Δ* significantly does not display internal initiation. Furthermore, since the

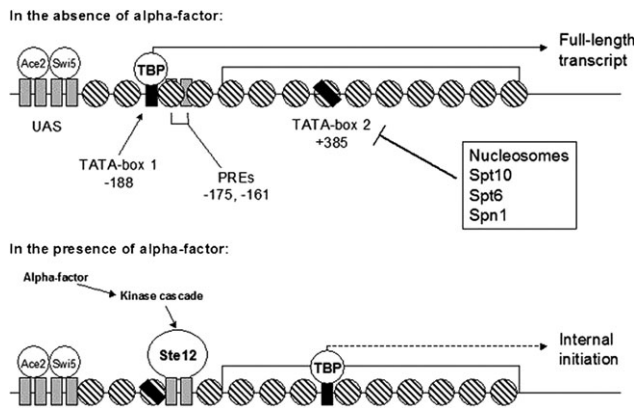


Figure 5. Suppression of internal initiation at *PRY3* in the absence of α -factor mating pheromone. Cotranscriptional reassembly of nucleosomes over the coding region of *PRY3* prevents transcription initiation from TATA-box 2 in the absence of α -factor. Internal initiation arises when Spt10, Spt6 or Spn1 is disrupted.

cotranscriptional histone chaperone function of *spt6*¹⁴⁰ and *spt6*¹⁰⁰⁴ is not abolished at 30°C, the disrupted function may also be independent of nucleosome reassembly.¹⁰ However, this novel observation could in fact be explained in accordance with a nucleosome reassembly model if the kinetics of nucleosome reassembly are altered in these mutants at 30°C. If nucleosomes compete with Ace2/Swi5-promoted TBP for binding to the internal TATA-box, then a decreased speed of nucleosome reassembly in *spt6*¹⁴⁰ or *spt6*¹⁰⁰⁴ at 30°C may allow TBP to out-compete nucleosomes for TATA-box binding. To further investigate this nucleosome/TBP competition hypothesis, the chromatin structure over *PRY3* in *spt6*¹⁴⁰ and *spt6*¹⁰⁰⁴ at 30°C could be analysed by micrococcal nuclease digestion and the kinetics of nucleosome reassembly by chromatin immunoprecipitation.

Increases in full-length transcription are also observed under the same conditions that induce internal initiation at *PRY3* (Fig. 2). This may indicate that nucleosome reassembly at TATA-box 1 is also required to regulate full-length transcription. Nucleosome occlusion of TATA-boxes is a common mechanism of repression, and a requirement for Spt6-mediated nucleosome reassembly in transcriptional repression has previously been shown for *PHO5*, *PHO8*, *ADH2*, *ADY2* and *SUC2*.²⁸

Conclusion

This investigation has demonstrated that: (i) maintenance of transcriptional fidelity at *PRY3* in the absence of α -factor requires Spt6, Spn1 and Spt10, but not Eaf3. This suggests that appropriate nucleosome density in the coding region is important to suppress internal initiation; (ii) internal

initiation at *PRY3* is dependent upon the ability of TBP to bind to a TATA-box, suggesting that internal initiation arises from TATA-box 2. Taken together, these results suggest a model for transcriptional output at *PRY3* that is dependent upon competition between TBP and nucleosomes for binding an internal TATA-box. Transcription factors may promote TBP binding while Spt6 may control the kinetics of nucleosome reassembly.

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