Sequence and Spacing of TATA Box Elements are Critical for Accurate Initiation from the β-Phaseolin Promoter

Margaret L. Grace¹, Mahesh B. Chandrasekharan², Timothy C. Hall²
and Alison J. Crowe¹*

¹Department of Biology, University of Washington, Seattle, Washington 98195
²Institute of Developmental and Molecular Biology and Department of Biology, Texas A&M University, College Station, Texas 77843

* To whom correspondence should be addressed

Corresponding Author:
Alison J. Crowe
Department of Biology
PO Box 351800
University of Washington 98195-1800
Tel: 206.685.8016
Fax: 206.543.3041
E-mail: acrowe@u.washington.edu

Running Title: Multiple TATA boxes direct β-phaseolin transcription
Summary

The β-phaseolin (phas) gene, which encodes one of the major seed storage proteins of P. vulgaris, is tightly regulated at the transcription level resulting in strict tissue-specific and spatial expression during embryonic development. The phas proximal promoter contains a complex arrangement of core promoter elements including three TATA boxes as well as several putative initiator elements. To delineate the respective contributions of the core promoter elements to transcription initiation we have performed site-directed mutagenesis of the phas promoter. In vivo expression studies were performed on transgenic Arabidopsis harboring phas promoter mutants driving expression of the β-glucuronidase (gus) reporter gene. Quantitative assessment of GUS activity in seeds bearing the promoter mutants indicated that both sequence and spacing of the TATA elements influenced the efficiency of transcription. Substitution, insertion or deletion mutations had no effect on histochemical staining patterns indicating that strict spacing requirements are not essential for correct spatial expression of phas during embryogenesis. Further evaluation of the phas promoter by in vitro transcription analysis revealed the presence of multiple TATA-dependent transcription initiation start sites. The distance between TATA elements and transcription start sites was maintained in insertion and deletion mutants through the creation of novel initiation sites, indicating that positioning of the TATA elements rather than DNA sequence was the primary determinant of start site location. We conclude that, while dispensable for proper spatial distribution, the complex architecture of the phas promoter is required to ensure high levels of accurate phas transcription initiation in the developing embryo.
Introduction

The proximal promoter region of a gene contains core promoter elements that determine the basal transcription activity of the gene and typically direct the positioning of the transcription initiation start site. Three different classes of transcription initiation elements have been identified in eukaryotes: TATA boxes, Initiator (Inr)\(^1\) elements and Downstream Promoter Elements (DPE). To date, only TATA boxes and Inr elements have been identified in plant promoters. All three elements utilize similar mechanisms of initiation requiring RNA polymerase II and sequence-specific binding of transcription factor IID (1). However, mechanistic differences exist between the different initiation elements. TATA-containing promoters can function in the absence of an Inr, whereas DPE promoters are Inr-dependent; TATA box directed transcription occurs on average 25-30 bp downstream of the TATA box while Inr driven transcription typically originates at the adenosine residue in the +1 position of the Inr element itself (2). Sequence analysis of the \textit{Drosophila} and human genomes indicates that only a minority of promoters contain the classical arrangement of one TATA box and one Inr element (3,4). The remaining promoters contain varying combinations of TATA, Inr and DPE elements. It remains unclear, however, what role this core promoter diversity plays in transcription regulation.

Originally core promoter elements were thought to mediate basal transcription whereas gene-specific upstream regulatory elements were responsible for directing regulated gene expression. However, recent studies with both plant and animal genes have demonstrated that core promoter elements can play an integral role in both environmentally-induced and developmentally-regulated gene expression (1). Recently, developmental stage-specific recruitment of the TATA binding protein (TBP) has been demonstrated for the human \(\gamma\)-globin gene (5) and light-induced expression of the TATA-less photosystem I gene \textit{psaDb} was found to be Inr-dependent (6). Several plant and animal promoters have been identified which contain multiple TATA boxes (7,8). Although the benefit, if any, of having more than one TBP binding site has not been well-documented, recent analysis of the tubulin promoter in soybean indicate that the two TATA boxes present in this promoter function additively to direct transcription in seedlings. Interestingly, these two TATA
boxes are differentially sensitive to light conditions (8), suggesting that, in the case of tubulin, the multiple TATA boxes play a role not only in directing high levels of transcription but also provide a mechanism for titrating gene activity in response to altered environmental conditions. Finally, recent enhancer-trapping studies in Drosophila (9,10) and analysis of upstream activating sequences (UAS) in yeast (11) indicate that many enhancers and UAS preferentially activate select groups of core promoters, suggesting that the composition of a core promoter may restrict its responsiveness to specific transcription regulators. These studies, along with the recent identification of several general transcription factors that regulate a distinct subset of genes in a developmental-specific manner (12,13) strongly suggest that core promoter elements play a more active role in determining gene expression patterns than previously expected. Core promoter diversity may thus provide an additional level of transcription control beyond that achieved with upstream regulatory elements.

We have employed the seed storage protein gene β–phaseolin (phas) as a model for understanding how core promoter elements participate in the regulation of plant gene expression. The phas gene is under strict developmental and tissue-specific control (14,15), primarily at the level of transcription initiation (16). The AT rich proximal promoter region of phas contains a complex array of transcription initiation regulatory elements within 45 bp of the transcription start site. These elements include two consensus TATA boxes (termed TATA1 and TATA2), a third non-canonical TATA box (TATA3) as well as two putative Inr elements (located at –9 and +1) based on sequence homology to a consensus mammalian Inr element (1,17). TATA1 and TATA2 conform to the consensus for dicot TATA boxes (TATAA/TA) and are predicted to be equally capable of directing high levels of transcription (18). The divergent TATA3 (TAATAT), however, would not be expected to participate significantly in transcription initiation (19,20). In plant promoters, as in animal promoters, there is strong conservation of spacing (25-30 bp) between the TATA box and the transcription initiation site; however, due to the paucity of plant promoter studies, the role of core element spacing in both location and intensity of transcription initiation remains uncertain.
Here, we have employed a combination of *in vivo* and *in vitro* transcription analyses to determine the respective roles of the *phas* core promoter elements in directing basal transcription. The detection of multiple *phas* mRNAs with variable 5’ ends *in vivo* (21) suggested the existence of several functional transcription initiation sites. We show here that both the sequence and spacing of the TATA elements is critical for high levels of accurately initiated transcription. Mutation analysis revealed that TATA1 is responsible for directing faithful initiation at the secondary transcription start site (-12) while TATA2 is required for transcription from the primary transcription start site (+1). TATA3 was found to be completely dispensable for transcription initiation. In the absence of a functional TATA box, the two putative Inr elements were insufficient to direct transcription suggesting either that these are not functional Inr elements or that they fall into the class of weak Inr elements which function solely to augment promoter strength of TATA-containing constructs. Further mutagenesis of the Inr elements themselves will be required to distinguish between these possibilities. Analysis of insertion and deletion mutants revealed a dependency on TATA spacing for accurate and efficient transcription initiation and suggested that the multiple TATA boxes function cooperatively to enhance *phas* transcription. The respective role of each TATA box was confirmed by parallel studies performed on *Arabidopsis* seeds harboring the TATA mutants. We conclude that the complex architecture of the TATA region of the *phas* promoter is not essential for maintaining spatially regulated expression of *phas* during embryogenesis, but is required to ensure high levels of *phas* message production during development.

**Experimental Procedures**

**Mutagenesis**

Except for TATA3, the mutations shown in Fig. 2 were introduced into the TATA elements within the -295 to +34 region of the *phas* promoter present in -295*phas/pPCR-Script* (14) using ‘Megaprimer’ PCR-based site-directed mutagenesis (22). Products obtained following two rounds of amplification were ligated to an *SrfI*-digested vector (pPCR-Script® (Amp) SK+: Stratagene
Inc.). The TATA3 mutant was generated using a QuikChange® mutagenesis kit (Stratagene Inc.) with -295phas/pPCR-Script as the DNA template. Incorporation of the mutations into the specific cis-elements and the integrity of the other regions of the promoter were verified by DNA sequencing.

**Plasmid constructions**

Promoter fragments bearing mutations in the TATA region were digested with HindIII and NcoI, and ligated to HindIII/NcoI-digested vector -295phas-gus-3'/pUC (14). Subsequently, the constructs were digested with HindIII and EcoRI to release fragments containing the mutant -295phas promoter, reporter gene and the 3' region. The fragments were fused to HindIII/EcoRI digested plant transformation vector pHM301K. The vector pHM301K is a derivative of pCB301 (23), modified to include a CaMV35S-nptII-nos3' cassette and a poly-cloning site in an inverted orientation to that of the parent vector. The control, -295phas-gus-3'phas fragment (3.4 kb) was excised from the vector -295phas-gus-3'phas/pUC19 using HindIII and EcoRI. The fragment was then mobilized into HindIII and EcoRI-digested pHM301K to obtain -295phas-gus-3'phas/HM301K.

**Agrobacterium-mediated transformation**

The promoter-gus fusions mtTATA1-gus, mtTATA2-gus, mtTATA123-gus, mtTATAΔ4-gus, mtTATA5AT-gus, mtTATA10GC-gus, and -295phas-gus in pHM301K were mobilized into Agrobacterium strain GV3101 and grown to saturation in LB medium containing gentamicin (30 mg/L) and kanamycin (30 mg/L) for plant transformation. Arabidopsis thaliana plants (ecotype Columbia) were transformed using vacuum infiltration (24). The T1 seeds were surface-sterilized using 50% bleach and plated on MS medium containing 1x B5 vitamins, kanamycin (50 mg/L) and Timentin (ticarcillin disodium and clavulananate potassium: SmithKline Beecham Pharmaceuticals, Philadelphia, PA; 150 mg/L). Two weeks after plating, putative transformants (kanamycin-resistant seedlings, T1 plants) were transferred to fresh MS medium containing 1x B5 vitamins and
antibiotics, and grown until they formed rosette leaves. The plants were subsequently transferred individually to soil in pots and grown to maturity in the greenhouse. The T2 seeds were collected from the dried siliques of individual transformants. Several individual homozygous or heterozygous lines were established for various phas/gus constructs. Genomic DNA blots were performed to determine the copy number of T-DNA insertions in the transgenic plants.

Assays for β-glucuronidase activity

For histochemical staining, Arabidopsis embryos were dissected from T2 seeds approximately 12-15 days after flowering. For each construct, embryos were dissected from at least 4 different lines bearing a single-copy insert of -295phas-gus and the mutant derivatives. For each line, approximately 30 embryos from 4-5 siliques were dissected. The embryos were stained in GUS staining solution at 37°C according to the method of Jefferson et al. (25). Incubation ranged from 15 min to overnight, depending on the promoter construct present. After staining, embryos were removed from the staining solution and immersed in 95% ethanol to remove chlorophyll. Images of the stained embryos were captured using a Zeiss SV11 microscope fitted with an AxioCam HRc camera.

GUS-specific fluorescence was determined according to (25) and activity was calculated as nmol 4-MU/h/µg of protein using a Microsoft Excel spreadsheet program. Protein estimation was performed as described by Bradford (26). Three independent fluorimetric assays were performed for dry T2 seeds from several individual lines of Arabidopsis for each construct (Table 1). Statistical analyses of the data were performed according to the method of Nap et al. (27).

In vitro transcription

DNA templates were in vitro transcribed upon addition of a RNA polymerase II-containing nuclear HeLa extract at a final protein concentration of 0.5 µg/µl and an NTP/salts/energy-generating mix to give final concentrations of 0.7mM CTP, UTP, GTP, ATP, 5mM MgCl₂, 66 mM KCl, 5mM creatine phosphate, 10U/ml of creatine kinase, 2.5 mM DTT, 9.0% glycerol and 18 mM
HEPES (pH7.9) as described previously (28). Unless otherwise noted, all *in vitro* transcription reactions were performed with 500 ng of template DNA. HeLa nuclear extracts were prepared exactly as described in Current Protocols in Molecular Biology (29). Final protein concentration ranged from 5 to 10 mg/ml. *Phas* promoter activity in HeLa extract was found to be dose-dependent on addition of human TBP (hTBP). All *in vitro* transcription reactions were supplemented with recombinant hTBP (ProteinOne Inc., College Park, MD) to a final concentration of 0.4 ng/µl. *In vitro* transcription reactions were incubated at 30°C for 1h.

**Primer extension analysis**

RNA products were purified and analyzed by primer extension using a radiolabeled DNA primer (5’CAGACTGAATGCCACAGGCCG3’) complementary to bp +105 to +84 of the -295*phas-gus* DNA template as described previously (28). Annealing was performed in 250 mM KCl/0.2mM EDTA/2 mM Tris pH 8.0 at 58°C for 1h prior to reverse transcription with 10 U AMV reverse transcriptase (Life Sciences, St. Petersburg, Fl) in 10mM MgCl₂/0.33mM dNTPs/5mM DTT/100µg/ml Actinomycin D/20mM Tris-HCl pH 8.7 at 42°C for 1h. Primer extension products were purified and electrophoresed on an 8M urea/8% polyacrylamide gel (30) and detected by autoradiography. Each mutant was analyzed in at least 3 independent *in vitro* transcription reactions. Quantification was performed using a Storm 840 Phosphorimager with ImageQuant v1.2 software.

**Results**

*Multiple TATA elements are not essential for spatial expression but are required for high levels of transcriptional activity from the phas promoter*

The presence of three phased TATA elements within the *phas* promoter prompted an evaluation of their respective contributions to tissue-specificity and spatial distribution during embryogenesis. Substitution mutations were made in individual TATA elements (Fig. 1) and the ability of debilitated promoters (*mTATA1* and *mTATA2*) to drive expression of the reporter gene (*gus*) was
assessed in seeds and embryos of stably-transformed Arabidopsis plants. As shown in Fig. 2, histochemical staining patterns for embryos harboring $mTATA1$-gus (B) or $mTATA2$-gus (C) were similar to that obtained for embryos bearing the -295phasis-gus control (A). The absence of GUS expression in the radicle is consistent with earlier findings that expression in this region is mediated by distal regulatory elements upstream of bp-295 (14). As with -295phasis-gus control plants, no GUS expression could be detected in leaves of the stably transformed Arabidopsis plants harboring the TATA mutants (data not shown). Conservation of the wild-type staining pattern indicates that both tissue-specific expression and modular expression of phas within the embryo are not dependent on core promoter organization.

To identify any potential contributions from TATA-independent transcription, a mutant derivative of -295phasis promoter bearing mutations in all three TATA elements ($mTATA123$) was generated (Fig. 1). Given the importance of TATA elements in transcriptional initiation, we anticipated that debilitation of all three TATA elements would completely abrogate transcription from the phas promoter. This was essentially confirmed by the absence of GUS expression in embryos harboring $mTATA123$-gus, except for a faint staining in cotyledons upon prolonged incubation (>5 hr) in the X-gluc substrate (Fig. 2E). This faint staining was not observed for non-transgenic embryos (Fig. 2F). This qualitative assessment suggested that while tissue-specific and spatial positioning are maintained in the TATA mutants, high levels of phas expression require the presence of at least one functioning TATA box.

In order to assess the quantitative contribution of individual TATA elements to overall promoter activity, GUS activity was evaluated in seeds bearing each of the TATA mutants. As shown in Table 1, mutation of TATA1 or TATA2 yielded 33% and 54%, respectively, of the GUS activity obtained for the -295phasis-gus control. The statistically significant reduction in GUS activity due to these mutations indicates that both TATA1 and TATA2 contribute to the overall high level of expression from the phas promoter. As predicted from the histochemical analysis, mutation of all three TATA boxes essentially eliminated phas promoter activity. The minimal (~4%) activity shown for $mTATA123$-gus plants suggests that, in vivo, a cryptic cis-element or Inr site may be utilized in
the absence of TATA elements to initiate low levels of transcription. The 68% reduction in GUS activity for seeds of $mTATA1$-gus lines as compared to the 46% reduction in GUS activity observed for $mTATA2$-gus, suggested that TATA1 was functionally more important than TATA2. However, statistical analyses (two sample t-test) of the pair-wise comparison of GUS activity values for the seeds of transgenic lines bearing $mTATA1$-gus and $mTATA2$-gus yielded a $p$-value of 0.1 (data not shown), indicating that any ascribed functional hierarchy to the TATA elements is statistically insignificant.

**Spacing of TATA elements influences phas promoter activity**

The role of TATA element spacing relative to each other and to the transcription start site was evaluated by deletion of 4 bp between TATA2 and TATA3 ($mTATAA4$-gus; Fig. 1). As shown in Fig. 2D, the embryos harboring the deletion derivative displayed a staining pattern similar to that obtained for the embryos bearing the control -295phas-gus (Fig. 2A). However, quantitative fluorimetric MUG assays indicated that the juxtaposition of TATA2 and TATA3 caused a statistically significant 63% reduction in GUS activity relative to control. Thus, disruption of spacing between the TATA elements did not greatly affect spatial regulation during embryogenesis, but did severely inhibit overall promoter activity. To further explore how TATA box spacing contributed to phas promoter regulation, we generated two additional spacing mutants, $mTATA5AT$-gus and $mTATA10GC$-gus which contain insertions between TATA1 and TATA2 of 5 and 10 bp, respectively (Fig. 1). Interestingly, quantitative evaluation of GUS activity in embryos harboring these mutants (Table 1) suggests that the two insertion mutants have distinct effects on phas activity. Introduction of 10 bp in the $mTATA10GC$-gus mutant enhanced promoter activity relative to the -295phas-gus control whereas the 5 bp insertion ($mTATA5AT$-gus) caused a slight, although statistically insignificant, reduction in total activity. The relative positioning of TATA elements to each other and to the site of transcription initiation thus can have a substantial impact on phas promoter strength.
Reconstitution of phas transcription in vitro

To further analyze the role of phas promoter architecture on the accuracy and intensity of transcription initiation, we established phas transcription in vitro. As shown in Fig. 3A, in vitro transcription of increasing concentrations of –295phas-gus DNA in HeLa nuclear extract supplemented with hTBP demonstrated DNA template-dependent transcription. The observed sensitivity to low levels of α-amanitin (Fig. 3A, compare lanes 8 & 9) suggests that the transcription products are RNA polymerase II-specific (31). One major product (~105 bp) was detected, the expected size of a transcript originating from the +1 site previously identified as the primary transcription start site in vivo (21). This site conforms to the consensus for a mammalian Inr element (5’ PyPyCAPyPyPyPyPy-3’) with transcription initiating at the A (17), except that the phas +1 sequence contains a puridine in place of the penultimate underlined pyrimidine residue.

We also observed several minor products, the most prominent of which migrated at approximately 117 bp. A third minor product of approximately 85 bp was detected with varying efficiency, and is most likely the result of TATA-box independent transcription (Fig. 4A and data not shown). Mapping the 5’ ends of the primer extension products (Fig. 3B) confirmed that the primary transcription product (105 bp) initiated at the previously determined +1 in vivo transcription start site (21) while the 117 bp secondary product initiated at –12. After prolonged exposure, minor products could also be detected which initiated at –9, +4 and +16/+17. These findings are consistent with previous mapping studies in bean cotyledons which identified a complex pattern of phas messages predicted to originate between –12 and +5, with the predominant forms localizing around +1 and –11(21). Substitution of hTBP with purified recombinant wheat TBP (a gift of S. Ackerman, UMass) (32) had no effect on the location of transcription initiation, although the preference for +1 was slightly enhanced ². These data suggest that reconstitution of faithful basal phas transcription can be achieved with a human in vitro transcription system.
**TATA boxes determine preferred transcription initiation start site in vitro**

To assess the role of the TATA elements in determining both the efficiency and accuracy of transcription start site selection, TATA substitution mutants were transcribed *in vitro* and the resulting RNA products were analyzed by primer extension. Mutation of TATA1 eliminated transcription initiation at the secondary initiation site (-12) while only marginally reducing transcription efficiency from +1 (Fig. 4A, lane 2) resulting in an overall decrease of ~20% relative to control –295phas-gus values. Conversely, templates carrying a mutated TATA2 exhibited undetectable levels of the primary transcription product yet still maintained wild-type levels of initiation at –12 (lane 3). Thus, in agreement with the *in vivo* analysis, both TATA1 and TATA2 were found to contribute to *phas* promoter activity. These results further suggested that TATA1 mediates initiation at –12 while TATA2 is principally responsible for directing initiation at the primary transcription start site situated at +1. To determine if the non-canonical TATA3 element played a role in *phas* initiation we analyzed primer extension products from *in vitro* transcribed mTATA3-gus templates. Mutation of TATA3 caused no detectable changes in either level or start site location of transcription relative to –295phas-gus (lane 4) suggesting that TATA3 was not required for transcription initiation. To assess whether the TATA3 element could functionally substitute for TATA1 or TATA2, we generated a TATA12 double mutant (mTATA12-gus). Inactivation of both TATA1 and TATA2 severely inhibited transcription from all sites (lane 5) confirming that TATA3 is unable to function as an authentic TATA box. Consistent with the minimal GUS activity observed in mTATA123-gus embryos mutation of all three TATA boxes caused a similar overall reduction in transcription (lane 6). These data suggest that TATA3 is dispensable for *phas* promoter activity whereas TATA1 and TATA2 function in concert to direct initiation of multiple *phas* messages.

**TATA spacing is critical for start site selection**

To assess whether relative spacing of the *phas* core promoter elements influenced transcription start site selection, we analyzed the deletion and insertion mutants diagrammed in Fig. 1 by *in vitro*
transcription. Insertion of either five (mTATA5AT-gus; Fig. 4A, lane 9) or ten (mTATA10GC-gus; Fig. 4A, lane 10) nucleotides between TATA1 and TATA2 did not affect positioning of the primary transcription start site. Together with the mTATA2 results, the finding that both insertion mutants retain faithful positioning of the primary start site strongly suggests that TATA2, whose location 29 bp upstream of +1 remains unchanged in these mutants (Table 2), is responsible for directing positioning at this site. Quantification of the primer extension products revealed that the level of primary transcription product was reproducibly decreased in the 5AT insertion mutant by approx. 30%, while the 10GC insertion had only a minimal effect on the efficiency of transcription initiation at +1. This result suggests that although TATA1 is not essential for initiation at +1, the location of TATA1 relative to TATA2 may influence the efficiency of TATA2 directed transcription at the primary transcription start site.

As expected, if TATA1 is responsible for initiation at -12, the altered spacing between TATA1 and TATA2 caused a shift in location of the secondary start site. Thus, in vitro transcription of mTATA10GC-gus generated a novel, diffuse secondary product initiating at approximately –20 (Fig. 4A, lane 10); whereas mTATA5AT-gus transcription yielded two discrete secondary products equal to or smaller in size than the secondary product observed with the wild type promoter (lane 9). Mapping the 5’ end of the novel mTATA10GC-gus products confirmed that the new products initiated between bp –22 to –24, approximately 10 bp upstream of the wild type secondary product (Fig. 3C). The generation of a diffuse band suggests the use of multiple start sites situated near position -23, potentially due to the lack of a consensus Inr element in this region. By shifting the secondary start site upstream, spacing between TATA1 and the secondary start site of transcription was maintained in this mutant (Table 2). Similar analysis of mTATA5AT-gus primer extension products suggests that the most prominent product originated from the Inr element located at –9 (data not shown). This seemingly contradictory result, i.e. insertion of 5 bp resulting in a downstream shift rather than an upstream shift in the preferred start site, may be accounted for by the fortuitous introduction of a novel TATA box in the 5AT mutant (see Fig. 1). If the mTATA5AT-gus mutant utilizes this newly created TATA box (TATTTAA), which has been found
to function at moderate levels in human and yeast in vitro systems (20,33,34) the spacing between TATA and –12 would be maintained at a distance of 23 bp. Further mutational analysis will need to be performed to ascertain which TATA box is employed in this mutant. Regardless of the exact spacing, these data clearly demonstrate that insertions between TATA1 and TATA2 do not affect positioning of primary transcription initiation, but do have a marked effect on the location of the secondary transcription product.

To confirm whether positioning of TATA2 is responsible for the start site selection at +1, we analyzed the in vitro transcription products generated by mTATAΔ4-gus that contains a 4 bp deletion between TATA2 and TATA3. In order to maintain optimal spacing between the TATA boxes and their respective initiation sites, both the –12 and +1 sites would be predicted to shift downstream. Consistent with this prediction, both the primary and secondary transcripts generated by mTATAΔ4-gus exhibited a decrease in their apparent size (lane 8). Mapping the 5’ end of the mTATAΔ4-gus primer extension products indicated that transcription initiated at +4 and –9 (Fig. 4B). By relocating the start sites 3 bp downstream of their expected locations, a distance of 25-28 bp was maintained between the TATA boxes and their respective initiation sites in the deletion mutant (Table 2). These results are consistent with our initial prediction that TATA2 is critical for promoting transcription initiation at the primary start site, whereas TATA1 is primarily involved in directing transcription through the secondary start site located at -12.

In vitro reconstitution of in vivo transcription efficiency

Comparison of total phas message levels produced from both primary and secondary transcription start sites by TATA spacing mutants in vitro with GUS activity detected for the same mutants in vivo revealed several significant differences. Most noticeably, we observed wild-type levels of transcription from mTATAΔ4-gus and mTATA10GC-gus in vitro, while these same mutants exhibited significant differences in GUS activity relative to –295phas-gus in vivo (Table 1). We reasoned that the observed discrepancy might reflect differing requirements for basal transcription factor concentrations in vitro versus in vivo. To assess this possibility, we conducted titration experiments on each mutant, varying the level of HeLa extract in the in vitro transcription
reactions from 0.5 mg/ml, previously determined to be optimal for \(-295\text{phas-gus}\) expression (Fig. 4 and data not shown) up to 2.0 mg/ml. As shown in Fig. 5, transcription under conditions of excess HeLa (1.5 mg/ml) resulted in a significant decrease in transcription efficiency of \(mTATA\Delta 4\)-gus relative to wild-type (lane 2) equivalent to the 63% reduction in GUS levels observed in transgenic \(mTATA\Delta 4\)-gus embryos (Table 1). Concomitantly, \(mTATA10GC\)-gus exhibited a 2-fold increase in transcription (Fig. 5, lane 4), closely paralleling the 68% increase observed \textit{in vivo}.

Thus, by performing the \textit{in vitro} transcription reactions in the presence of super-optimal concentrations of the basal transcription machinery we were able to successfully reconstitute the requirements for core promoter spacing observed \textit{in vivo}.

Comparison of TATA substitution mutant transcription activity determined \textit{in vitro} (Fig. 4) and \textit{in vivo} (Table 1) revealed a significant difference in TATA box requirements. Specifically, the absolute requirement for a functioning TATA2 element in order to obtain high levels of transcription activity \textit{in vitro} (Fig. 4, lane 3) did not accurately reflect the moderate decrease of 46% in total GUS activity detected in \textit{Arabidopsis} seeds harboring \(mTATA2\)-gus (Table 1). However, the strict requirement for an intact TATA2 element \textit{in vitro} was abrogated when transcription was performed under excess HeLa conditions (Fig. 5, lane 6). The increase in overall \(mTATA2\)-gus transcription levels was due to a sharp increase in transcription from \(-12\), suggesting a shift in start site preference in the absence of TATA2. The relatively moderate effect of disrupting TATA2 in transgenic plants may similarly reflect the use of an alternate start site \textit{in vivo}.

Lastly, consistent with the very low level of expression detected upon disruption of all three TATA boxes \textit{in vivo} (\(~4\%)\), no detectable expression from \(-295mTATA12\) or \(-295mTATA123\) was observed under high HeLa concentrations (data not shown). These data suggest that although optimal expression of wild-type \(-295\text{phas-gus}\) is obtained under limiting HeLa conditions, analysis of the promoter mutants in the presence of excess HeLa more accurately reflects the effect of disrupting TATA elements \textit{in vivo}.
Discussion

The core promoter is the ultimate target through which all transcription is regulated. Transcription factors must communicate with the core promoter in order to either enhance or repress transcription (35). Mutational analysis of the phas core promoter has demonstrated a requirement for multiple phased TATA boxes to direct high levels of accurate basal transcription.

Maintenance of spatial distribution in TATA mutants

Sublocalization of phas message within the embryo was recently found to be under the combinatorial control of distal regulatory elements (14). The conservation of wild-type histochemical staining patterns observed for the TATA mutants (Fig. 2) indicates that module-specific expression of phas during embryogenesis is not dependent on core promoter organization but instead relies on a complex array of upstream regulatory elements (14).

Reconstitution of basal transcription in vitro

The reconstitution of faithful phas transcription initiation demonstrated here illustrates the utility of the HeLa in vitro transcription assay as a rapid means of assessing how core promoter architecture contributes to transcription initiation and supports previous findings that the function of basal transcription machinery is highly conserved in eukaryotes (20,34,36). By analyzing phas transcription activity in vitro we were able to assign specific roles for core promoter elements in transcription initiation in the absence of potential contributing effects on mRNA processing, transport or stability. In vitro and in vivo analysis of phas promoter activity demonstrated that the TATA box elements are required for basal transcription. Mapping of the primary and secondary phas transcription start sites confirmed that the HeLa in vitro transcription system accurately reconstituted the in vivo pattern of phas transcription initiation (21). The reconstitution of phas basal transcription regulation on naked DNA templates indicates that the observed requirement for core promoter organization is chromatin-independent. Comparison of in vitro transcription activity with GUS activity from transgenic Arabidopsis harboring phas TATA mutants confirmed that the
in vitro transcription assay accurately reflected the in vivo dependence on multiple phased TATA boxes for strong phas activity.

**TATA-dependent transcription**

In the majority of TATA-containing plant promoters, the TATA box lies 25-30 bp upstream of the transcription start site\(^3\) (A. Ackerman, personal comm. 18). The relative positioning of TATA elements within the phas promoter (Table 2) suggested that TATA2 and TATA1 function independently to direct transcription through the primary and secondary start sites, respectively, whereas the noncanonical TATA3 element played no role in phas transcription. Our in vitro transcription analysis of TATA substitution and spacing mutants confirmed this prediction, and also revealed an interdependence between TATA1 and TATA2. As such, mutation of TATA1 eliminated transcription from the secondary start site, while also reducing the efficiency of TATA2-directed transcription at +1. Conversely, the deleterious effect of eliminating transcription at the primary start site in mTATA2 was alleviated through a compensatory increase in the secondary transcription product (Fig. 5). Together these data suggest that TATA1 and TATA2 function in concert to direct start site selection and transcription efficiency at the phas promoter.

**Strict spacing requirement for core promoter elements**

Previous promoter studies of both animal and plant genes have demonstrated a strict spacing requirement between TATA box and Inr elements for accurate transcription initiation (2,18,37). Our data demonstrate that altering the distance between TATA elements and their respective sites of initiation has a significant effect both on accurate positioning and efficiency of transcription initiation at the phas promoter. Start site location was shifted in the phas promoter mutants in order to maintain a 25-30 bp distance between TATA box and start site. Our finding that both the primary and secondary start sites are relocated in the insertion and deletion mutants strongly suggests that it is the positioning of the TATA box that is critical for determining phas transcription start site selection. However, sequences surrounding the site of initiation may influence start site location as
deletion of 4 bp in the mTATA∆4-gus mutant caused the secondary transcription start site to shift 3 bp downstream to the putative Inr element located at −9 (PyPyCAPyPyPyPyPy) while the primary start site relocated from +1 to a weak consensus Inr element at +4 (PuPyCAPyPyPyPu). Although it remains unclear if this shift of 3 rather than 4 bp is due to the presence of functional Inr elements at these sites or simply a preference for initiation at adenosine residues, these data suggest that additional sequences at the site of initiation itself may contribute to TATA-directed start site positioning.

Altered spacing had only a negligible effect on transcription efficiency through the secondary site; however, transcription from the primary start site displayed marked sensitivity to promoter architecture. Thus, deletion of 4 bp between TATA2 and the +1 transcription start site in mTATA∆4-gus dramatically reduced efficiency of transcription both in vivo and under excess HeLa conditions in vitro indicating that the function of TATA2 is highly dependent on its position relative to the initiation site. Further site-directed mutagenesis analysis will be needed to determine if the putative Inr which overlaps the primary transcription start site is indeed a functional Inr element. Interestingly, in vitro transcription efficiency at +1 was also lowered upon insertion of one-half of a helical turn upstream of TATA2 (mTATA5AT-gus). This decrease was not due solely to increased spacing between TATA1 and TATA2 as insertion of a full helical turn at the same location (mTATA10GC-gus) enhanced transcription activity of the primary message. Therefore, the relative positioning of TATA1 and TATA2 appears to play a role in TATA2-directed initiation at the primary transcription start site, even though these insertions do not alter the distance between TATA2 and +1 (Table 2). The higher level of expression observed in mTATA10GC-gus may reflect an increased binding affinity for TBP. Based on the observations of Bruckner and coworkers, it can be suggested that insertion of a series of GC residues induces intrinsic DNA curvature (38). Bent DNA exhibits a significantly higher binding affinity for TBP (39), presumably through increased exposure of the minor groove (40,41), and thus may lead to an upregulation of gene expression (42). Alternatively, the observed impact of TATA box spacing on transcription efficiency may reflect a requirement for TBP to bind to TATA1 and TATA2 in a
cooperative manner. This hypothesis is consistent with our finding that transcription from the *phas* promoter displays a strong dose-dependency for TBP. Thus, the enhanced transcription efficiency realized by having multiple TATA boxes might be achieved through cooperative binding of TBP molecules to adjacent binding sites on the same surface of the DNA helix. Crippling of TATA1/TATA2 cooperativity in the TATA1 and TATA2 mutants may cause the observed decrease in transcription at the primary start site. Regardless of mechanism, it is clear from our data that the relative positioning of TATA boxes within the *phas* core promoter strongly influences promoter activity.

*Nucleosome positioning at the phas promoter*

Chromatin structural analysis of the *phas* promoter has implicated the TATA region in positioning a nucleosome over the start site of transcription (43). Screening of existing genomic sequences for their competitive ability to bind nucleosomes identified phased TATA boxes, similar to those found at the *phas* promoter, as one of the most stable nucleosome positioning sequences yet characterized (44). Preliminary Micrococcal nuclease analysis of the *phas* promoter suggest that the TATA elements themselves are essential for maintaining a positioned nucleosome over the transcription start site in vitro. Potentially TATA3, which appears to play no significant role in transcription initiation, still contributes to *phas* gene regulation by influencing the local chromatin structure of the TATA region. Histone modification, including acetylation, is a key modulator of chromatin structure and gene activation (45,46). Interestingly, a role for the TATA box in mediating targeted histone acetylation has recently been identified at the CUP1 promoter in yeast (47). This study suggests a direct requirement for sequence-specific TBP binding in the localized recruitment of histone acetyl transferases. We are currently exploring these and other potential mechanisms for TATA-directed chromatin regulation of the *phas* promoter.

Our data suggest that the complex core promoter architecture of the *phas* promoter is required for the robust production of multiple messages observed in vivo. As correct patterning of GUS activity was maintained in the absence of multiple functioning TATA elements, cooperativity
between TATA boxes is not essential for proper spatial distribution or tissue-specificity of phas messages. Taken together, our data support a model in which phas start site location is directed by individual TATA boxes, while efficiency of initiation requires the combined presence of multiple phased TATA boxes.

Footnotes
1 The abbreviations used are: Inr, initiator; DPE, downstream promoter element; phas, β-phaseolin; TBP, TATA binding protein; hTBP, human TATA binding protein; UAS, upstream activating sequence; bp, base pair; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.
2 A. J. Crowe, unpublished observation.
3 S. Ackerman, Univ. Mass, personal communication.

Acknowledgements
We thank Wang Kit Ng, Xiangyu Shi and James Townsend for technical assistance. This work was supported in part by National Science Foundation grants MCB-9974706 to TCH and MCB-0139958 to AJC.
References

1. Smale, S. T., and Kadonaga, J. T. (2003) Annu Rev Biochem 19, 19
2. O'Shea-Greenfield, A., and Smale, S. T. (1992) J. Biol. Chem. 267, 1391-402.
3. Kutach, A. K., and Kadonaga, J. T. (2000) Mol. Cell. Biol. 20, 4754-64.
4. Burke, T. W., Willy, P. J., Kutach, A. K., Butler, J. E., and Kadonaga, J. T. (1998) Cold Spring Harb. Symp. Quant. Biol. 63, 75-82.
5. Duan, Z. J., Fang, X., Rohde, A., Han, H., Stamatoyannopoulos, G., and Li, Q. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 5509-14.
6. Nakamura, M., Tsunoda, T., and Obokata, J. (2002) Plant J. 29, 1-10.
7. Yamada, M., Ozawa, A., Ishii, S., Shibusawa, N., Hashida, T., Ishizuka, T., Hosoya, T., Monden, T., Satoh, T., and Mori, M. (2001) Biochem. Biophys. Res. Commun. 281, 53-6.
8. Doyle, M. C., and Han, I. S. (2001) Mol. Cells 12, 197-203.
9. Kadonaga, J. T. (2002) Exp. Mol. Med. 34, 259-64.
10. Butler, J. E., and Kadonaga, J. T. (2001) Genes Dev. 15, 2515-9.
11. Li, X. Y., Bhaumik, S. R., Zhu, X., Li, L., Shen, W. C., Dixit, B. L., and Green, M. R. (2002) Curr. Biol. 12, 1240-4.
12. Holmes, M. C., and Tjian, R. (2000) Science 288, 867-70.
13. Dikstein, R., Zhou, S., and Tjian, R. (1996) Cell 87, 137-46.
14. Chandrasekharan, M. B., Bishop, K. J., and Hall, T. C. (2003) Plant J. 33, 853-66.
15. Bustos, M. M., Begum, D., Kalkan, F. A., Battraw, M. J., and Hall, T. C. (1991) Embo J 10, 1469-79
16. Li, G., Chandrasekharan, M. B., Wolfe, A. P., and Hall, T. C. (2001) Plant Mol. Biol. 46, 121-9.
17. Smale, S. T., and Baltimore, D. (1989) Cell 57, 103-13.
18. Joshi, C. P. (1987) Nuc. Acids Res. 15, 6643-53.
19. Juo, Z. S., Chiu, T. K., Leiberman, P. M., Baikalov, I., Berk, A. J., and Dickerson, R. E. (1996) J. Mol. Biol. 261, 239-54
20. Wobbe, C. R., and Struhl, K. (1990) *Mol. Cell. Biol.* **10**, 3859-67.
21. Slightom, J. L., Drong, R. F., Klasy, R. C., and Hoffman, L. M. (1985) *Nuc. Acids Res.* **13**, 6483-98
22. Sarkar, G., and Sommer, S. S. (1990) *Biotechniques* **8**, 404-7.
23. Xiang, C., Han, P., Lutziger, I., Wang, K., and Oliver, D. J. (1999) *Plant Mol. Biol.* **40**, 711-717
24. Bechtold, N., and Pelletier, G. (1998) *Methods Mol. Biol.* **82**, 259-266
25. Jefferson, R. A., Kavanaugh, T. A., and Bevan, M. W. (1987) *EMBO J.* **6**, 3901-3908
26. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248-254
27. Nap, J.-P., Keizer, P., and Jansen, R. (1993) *Plant Mol. Biol. Rep.* **11**, 156-164
28. Crowe, A. J., Sang, L., Li, K. K., Lee, K. C., Spear, B. T., and Barton, M. C. (1999) *J. Biol. Chem.* **274**, 25113-20
29. Workman, J. L., and Abmayr, S. (1987) in *Preparation of nuclear and cytoplasmic extracts from mammalian cells* (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds) Vol. 2, pp. 12.1.1-12.1.9, Greene publishing associates and Wiley interscience
30. Waterman, M. L., Fischer, W. H., and Jones, K. A. (1991) *Genes Dev.* **5**, 656-69
31. Lindell, T. J., Weinberg, F., Morris, P. W., Roeder, R. G., and Rutter, W. J. (1970) *Science* **170**, 447-9.
32. Apsit, V., Freeberg, J. A., Chase, M. R., Davis, E. A., and Ackerman, S. (1993) *Nuc. Acids Res.* **21**, 1494.
33. Singer, V. L., Wobbe, C. R., and Struhl, K. (1990) *Genes Dev.* **4**, 636-45.
34. Mukumoto, F., Hirose, S., Imaseki, H., and Yamazaki, K. (1993) *Plant Mol. Biol.* **23**, 995-1003
35. Smale, S. T. (2001) *Genes Dev.* **15**, 2503-8.
36. Gasch, A., Hoffmann, A., Horikoshi, M., Roeder, R. G., and Chua, N. H. (1990) *Nature* **346**, 390-4.
37. Zhu, Q., Dabi, T., and Lamb, C. (1995) *Plant Cell* **7**, 1681-9.
38. Brukner, I., Dlakic, M., Savic, A., Susic, S., Pongor, S., and Suck, D. (1993) *Nuc. Acids Res.* **21**, 1025-9.
39. Parvin, J. D., McCormick, R. J., Sharp, P. A., and Fisher, D. E. (1995) *Nature* **373**, 724-7.
40. Kim, J. L., and Burley, S. K. (1994) *Nat. Struct. Biol.* **1**, 638-53.
41. Kim, J. L., Nikolov, D. B., and Burley, S. K. (1993) *Nature* **365**, 520-7.
42. Wu, J., Parkhurst, K. M., Powell, R. M., Brenowitz, M., and Parkhurst, L. J. (2001) *J. Biol. Chem.* **276**, 14614-22.
43. Li, G., Chandler, S. P., Wolff, A. P., and Hall, T. C. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 4772-7.
44. Thastrom, A., Lowary, P. T., Widlund, H. R., Cao, H., Kubista, M., and Widom, J. (1999) *J. Mol. Biol.* **288**, 213-29.
45. Berger, S. L. (2002) *Curr. Opin. Genet. Dev.* **12**, 142-8.
46. Eberharter, A., and Becker, P. B. (2002) *EMBO Rep.* **3**, 224-9.
47. Shen, C. H., Leblanc, B. P., Neal, C., Akhavan, R., and Clark, D. J. (2002) *Mol. Cell. Biol.* **22**, 6406-16.
Figure legends

**Figure 1.** Position and mutations of the TATA region within the -295phas promoter. The black bar (top) represents the proximal -295 to +34 region of the phas promoter (P-295phas). The 34 bp of the 5’ untranslated (UTR) region are indicated by a thin black line and the hatched box represents the upstream part of the reporter gene (gus). T1, T2 and T3 (ovals) denote the locations of the three TATA elements. The primary and secondary transcription start sites, +1 and –12, respectively, are also indicated. A list of wild type and mutant sequences studied is shown. The -295phas promoter derivatives bearing the mutations in cis-elements are denoted by the prefix ‘m’. TATA regions are denoted by bold letters and the introduced mutations are represented by lower case letters. The 4 bp deletion (Δ) is indicated by ‘-’. The position and sequence of the insertions are also shown (boxes). Putative Inr elements are underlined.

**Figure 2. Spatial expression patterns for GUS driven by wild type or mutant -295phas promoters.** GUS expression is shown for embryos transgenic for -295phas-gus (A); mTATA1-gus (B); mTATA2-gus (C); mTATAΔ4-gus (D) and mTATA123-gus (E). As a control, an embryo from a non-transformed Arabidopsis plant subjected to histochemical staining is shown in panel F. Scale bars denote 20 μm.

**Figure 3. Reconstitution of phas transcription in vitro.** (A) Template-dependent transcription of phas in vitro. Increasing concentrations of -295phas-gus DNA template (250-4000 ng DNA as indicated) was in vitro transcribed in HeLa nuclear extract supplemented with recombinant hTBP. Transcription reactions were performed in the presence (+, lane 8) or absence (-, lanes 1-7) of 2 ng/μl α–amanitin. Transcripts were detected by primer extension followed by autoradiography. The primary 105 bp and secondary 117 bp phas primer extension products are indicated by arrows. (B) Mapping of phas transcription initiation start sites. Primer extension products obtained exactly as in Fig.1A lane 4, were electrophoresed on a 6% denaturing polyacrylamide gel alongside a
dideoxy sequencing ladder. The predominant transcription initiation sites are indicated by arrows. Minor sites (detected on longer exposures) are designated with closed circles. Primer extension products are indicated with arrows. Radiolabeled φX 174 DNA digested with HaeIII (Life technologies, Inc.) was used as a molecular weight marker (MW).

Fig. 4. Core promoter architecture determines transcription start site selection. (A) In vitro transcription of phas promoter mutants. -295phas-gus and the indicated TATA mutant templates were in vitro transcribed in HeLa extract supplemented with hTBP. Transcripts were detected by primer extension followed by autoradiography. Radiolabeled φX 174 DNA digested with HaeIII (Life technologies, Inc.) was used as a molecular weight marker (MW). Numbers below the figure indicate levels of expression relative to wild-type as determined by ImageQuant analysis of the scanned autoradiogram. (B) Mapping of TATA mutant transcription start sites. Primer extension products obtained as in (A), were electrophoresed on a 6% denaturing polyacrylamide gel alongside a dideoxy sequencing ladder. Primer extension products initiating from +1 and –12 are indicated by arrows. Asterisks denote novel primer extension products resulting from aberrant transcription initiation.

Fig. 5. In vitro reconstitution of in vivo phas expression levels. -295phas-gus and the indicated TATA mutant templates were in vitro transcribed in excess HeLa extract (1.5 μg/μl) supplemented with hTBP. Transcripts were detected by primer extension followed by autoradiography. Radiolabeled φX 174 DNA digested with HaeIII (Life technologies, Inc.) was used as a molecular weight marker (MW). Numbers below the figure indicate level of expression relative to wild-type as determined by ImageQuant analysis of the scanned autoradiogram.
### Table 1. GUS activity in seeds of plants transformed with gus fusions to the wild type and TATA-mutated -295phas promoter.

(1) Construct names reflect the size of the promoter (bp) for the control (-295phas/gus) and mutated (m) derivatives; the mutant bearing the 4 bp deletion between TATA2 and TATA3 is denoted by ‘Δ4’; the mutants bearing either an insertion of 5 bp AT-rich sequence or an insertion of a 10 bp GC-stretch (Fig. 1) between TATA1 and TATA2 are denoted by ‘5AT’ and ‘10GC’, respectively.

(2) N, the total number of independent transformants analyzed for each construct.

(3) Mean, (4) Standard deviation and (5) Standard error of the mean were calculated using a natural logarithmic (ln) scale of GUS activities.

(6) Standard error of the mean obtained using the two sample t-test for each pair-wise comparison involving a given mutant construct compared to the control.

(7) P, indicates the p-value obtained for each comparison using the two sample t-test at 95% confidence interval; na, not applicable in the control.

(8) Mean GUS activity was calculated by back transforming the mean ln values to the actual scale of measurement (nmol 4-MU/h/µg of protein).

(9) Percentage (%) GUS activity was calculated using the mean GUS activity for each construct to depict the change in GUS activity due to the mutation compared to the control.

| Construct          | N | Mean | SD  | SE  | Pairwise SE | P   | Mean GUS activity | %GUS activity |
|--------------------|---|------|-----|-----|-------------|-----|------------------|--------------|
| -295phas-gus       | 19| 13.74| 0.45| 0.10| na          | na  | 935.02           | 100.00       |
| mTATA1-gus         | 13| 12.63| 0.84| 0.23| 0.23        | 0   | 308.27           | 32.96        |
| mTATA2-gus         | 14| 13.13| 0.61| 0.16| 0.18        | 0   | 505.14           | 54.02        |
| mTATAA4-gus        | 15| 12.69| 0.68| 0.17| 0.19        | 0   | 345.46           | 37.39        |
| mTATA123-gus       | 14| 10.39| 0.40| 0.10| 0.15        | 0   | 32.75            | 3.50         |
| mTATA5AT-gus       | 14| 13.42| 0.85| 0.23| 0.22        | 0.16| 671.42           | 71.81        |
| mTATA10GC-gus      | 11| 14.26| 0.65| 0.19| 0.20        | 0.01| 1561.63          | 167.02       |
| DNA template   | Core Elements | Distance (bp) |
|---------------|---------------|---------------|
| -295phas-gus  | TATA1/+1      | 38            |
| -295phas-gus  | TATA1/-12     | 26            |
| -295phas-gus  | TATA2/+1      | 29            |
| -295phas-gus  | TATA2/-12     | 17            |
| -295phas-gus  | TATA3/+1      | 17            |
| -295phas-gus  | TATA3/-12     | 5             |
| mTATA5AT-gus  | TATA1/-9²     | 34³ or 29⁴    |
| mTATA5AT-gus  | TATA2/+1      | 29            |
| mTATA10GC-gus | TATA1/-22 to -24 | 24-26        |
| mTATA10GC-gus | TATA2/+1      | 29            |
| mTATAΔ4-gus   | TATA1/-9²     | 25            |
| mTATAΔ4-gus   | TATA2/+4²     | 28            |

**Table 2.** Conservation of spacing between TATA elements and transcription initiation sites in TATA mutants.

(1) Distance in base pairs (bp) was measured from the bp immediately following the TATA element up to and including the bp located at the site of transcription initiation.
(2) Novel transcription initiation site detected in promoter mutant
(3) Distance if the original TATA1 is utilized.
(4) Distance if the novel TATA1 element (TATTTAA) is utilized.
Wild type  CTTATATAATACTTATAATAACCTCTAAATACCTCACTTCTTTCA
mTATA1    CTTgTcTgcTACCTATAATAACCTCTAAATACCTCACTTCTTTCA
mTATA2    CTTATATAATACTgTgcTACCTCTATAATACCTCACTTCTTTCA
mTATA3    CTTATATAATACTTATAATAACCTCTgTgTACCTCACTTCTTTCA
mTATA123  CTTgTcTgcTACCTgTgcTACCTCTgTgTACCTCACTTCTTTCA
mTATAΔ4   CTTATATAATACTTATAATAAACCTCTAAATACCTCACTTCTTTCA
mTATA5AT  CTTATATAATACTTATAATAACCTCTAAATACCTCACTTCTTTCA
mTATA10GC CTTATATAATACTTATAATAACCTCTAAATACCTCACTTCTTTCA
Sequence and spacing of TATA box elements are critical for accurate initiation from the β-phaseolin promoter

Margaret L. Grace, Mahesh B. Chandrasekharan, Timothy C. Hall and Alison J. Crowe

J. Biol. Chem. published online December 4, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M309376200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts