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Intron-dependent transient expression of the maize *GapA1* gene

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Abstract

Transient expression experiments show that the maize *GapA1* promoter exhibits a requirement for sequences contained within intron 1 and surrounding exon border regions for expression in maize Black Mexican Sweet cells. Maize *GapA1*-promoter constructs lacking intron 1 are inactive. Intron 1 and its exon border sequences, when reintroduced into constructs lacking introns, restore gene activity whereas intron 2 and its exon borders to not. The minimal promoter so defined encompasses roughly 250 bp upstream of the *in vivo* transcription start and appears also to include intron 1. An octameric sequence was identified in intron 1 of maize *GapA1* which is similar to sequence motifs found in other maize introns known to increase transient expression. Partial restoration of gene expression in *GapA1* constructs lacking intron 1 was achieved through insertion of the identified octameric sequence.

Introduction

Nuclear genes in plants are primarily regulated at the transcriptional level and their promoters, as in other eukaryotes, consist of multiple sequence modules which respond to ubiquitous, developmentally regulated or inducible transcription factors [8, 16, 30]. Although regulatory elements are commonly located in the promoter and 5′-flanking region, sequences involved in transcriptional regulation of mammalian genomes can occasionally be found within introns, as in the case of the α2-collagen [25], troponin I [15] and *rpl* 32 [4] genes. The introns of many plant genes are known to have a marked influence upon expression. In dicots, several-fold increases in gene expression due to introns have been documented [24, 32] whereas in monocots, introns in transgenes can increase expression up to 100-fold [2, 17, 20, 35]. In maize, splicing efficiency of pre mRNA correlates with increased expression mediated by some introns, but enhancer-like elements, which have often been suspected to reside in plant intervening sequences [19, 20, 31], have not yet been identified.

The mere presence of introns in plant chimaeric genes does not neccessarily lead to higher levels of expression. This is evidenced by the findings that (1) different introns evoke different levels of stimulation in the context of the same promoter [2, 31], (2) one and the same intron may or may not evoke stimulation in the context of different promoters [17], and (3) introns in some cases can suppress rather than increase gene

expression [28, 31]. These observations also suggest that factors in addition to splicing, such as the presence of enhancer-like elements, may also be involved in intron-mediated expression in plants.

We wished to investigate promoter function of the maize nuclear *GapA1* gene [27] encoding the A-subunit of $NADP^+$-dependent chloroplast glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.13) which is of endosymbiotic origin and which catalyses the reductive step of the Calvin cycle [13, 22]. The *GapA1* promoter is relatively strong and generates about 0.5% of the total steady-state mRNA population in maize seedlings [11]. The first two introns of maize *GapA* genes are located in the transit peptide-coding region [26]. Here we show that *GapA1*-promoter constructs which lack introns are not expressed at detectable levels in transiently transformed Black Mexican Sweet (BMS) cells, but become active upon reintroduction of intron 1. We investigated the influence of intron 1 on transient *GapA1*-promoter function.

Materials and methods

Cultivation of plant cells

Black Mexican Sweet (BMS) cells were cultivated at 25 °C on a gyroshaker at 120 rpm on liquid medium as described [23] with 50 ml/l coconut milk, 1 mg/l 2,4-D and 6.5% sucrose. Fresh medium was added in 4–5-day intervals. Before bombardment, cells were placed for 2–4 h on the same medium containing 10% agar. For inhibition of methylases, cells were cultivated in the presence of 5-acacytidin (5, 20, and 100 mM).

Transformation of BMS cells

6 mg gold particles (BioRad, 1 μm diameter) were coated with 20 μl of DNA-mix (10 μg/μl of each plasmid) using the $CaCl_2$-spermidin protocol described [14]. 0.5 μg of coated gold particles were placed on flying discs, dried for 5 min under vacuum and used immediately for transformation. The plasmid pRT101LUC/Int [20] was used as the internal transformation control. Transformation was performed with a particle gun from Du Pont (model PDS-1000) at a vacuum of 3.3 kPa with a pressure of 8.3 MPa.

Expression analysis

48 h after transformation, 0.5 g of transformed BMS cells were washed in luciferase extraction buffer (0.1 M potassium phosphate, 1 mM DTT, pH 7.8), sonicated for 10 s in a volume of 500 μl and centrifuged for 5 min at 4 °C and 10000 rpm. Each supernatant was divided into two parts, one of which was used for luciferase assay, the other of which for GUS assay. Luciferase activity was measured as described [6] using 10 s assay reactions in a luminescence spectrometer (Berthold Lumat 9501). GUS activity was measured by the fluorometric assay over 3 h [12].

Quantification of promoter activity

For determining the efficiency of transformation, luciferase activities of each bombardment were divided by the value of the highest luciferase activity measured in the particular experiment, so that the transformation efficiency lies between 0 and 1 rlu (relative light units). GUS activity was determined as 4-MUG turnover (pmol/h). The GUS activities were calibrated by dividing by the corresponding transformation efficiency. The relative GUS activity is thus expressed as GUS activity divided by time and transformation efficiency (pmol h^{-1} rlu). All measurements of transient expression are the mean value of at least six independent transformations.

Plasmid constructs

Starting point for plasmid constructs was the plasmid pGUS1 (Botterman, Plant Genetic Systems, Gent, personal communication) which contains the reporter gene β-glucuronidase and the 3′ polyadenylation signal of the octopin synthase

gene. The 1.5 kb *Eco* RI/*Nco* I fragment of maize *GapA1* promoter [27] was cloned into the *Eco* RI/*Nco* I site of pGUS1 to yield pGpaGus1. For convenience in these constructs, the A of the *GapA1* start codon was designated as position +1. The sequence 5′-CTAGCATCGTCATCTTGTCTTGATCCTCTGGTCCGTGGC-3′ at position −40 to −2 of the *in vivo* maize *GapA1* leader was replaced by the sequence 5′-CTAGCATCGTCATCTTGTCTTGATCCTCTGGTCCGAGGCCTGTGGC-3′ through exchange of the 43 bp *Nhe* I/*Nco* I fragment by a synthetic 50 bp *Nhe* I/*Nco* I fragment containing a *Stu* I cloning site (pGpaGusS) which was used for cloning of introns into the leader. Intron 1 of the maize *GapA1* gene was isolated from the genomic clone as a 217 bp *Nco* I/*Sac* I fragment (−1 to +216), the protruding ends of which were removed by mung bean nuclease. The resulting fragment (+4 to +212) was cloned into the *Stu* I site of pGpaGusS in both directions to yield pGpaSIE1 and pGpaSIE1R ('R' refers to the nonspliceable orientation). The same blunt fragment was also cloned in both orientations into the *Eco* RI site and into the *Sma* I site of pGpaGusS to yield plasmids pGpa1I3, pGpa1I3R, pGpa1I5 and pGpa1I5R respectively. Intron 2 of the maize *GapA1* gene was isolated from the same genomic clone as a 282 bp *Sac* I/*Sac* II fragment (+212 to +494), the protruding ends of which were removed by mung bean nuclease. The resulting fragment (+216 to +492) was cloned into the *Stu* I site of pGpaGusS in both directions to yield pGpaSIE2 and pGpaSIE2R. Deletion series of the plasmids pGpaGUS1 and pGpaSIE1 were generated by exonuclease III. Introns lacking exon border regions were PCR-amplified with appropriate primers and cloned into the *Stu* I site of pGpaGusS. The octameric sequence motif CGTGCCGC common to intron 1 of *GapA1*, *Adh1* and *Sh1* of maize was synthesized as dimer

5′-AATTCGTGCCGCGTCTCTCCGTGCCGCGTCTCTG-3′

3′-GCACGGCGCAGAGAGGCACGGCGCAGAGACTTAA-5′

and the resulting double-strand DNA was cloned as a dimeric dimer into the EcoRI site of pSK+ to yield pSKtGBM which thus contains four copies of the common motif. The 92 bp *Eco* RV/*Sma* I fragment of pSKtGBM was cloned into the StuI site of pGpaGusS to yield pGpaSB. Junctions of all plasmid constructs were verified by dideoxy sequencing prior to transformation. Standard DNA manipulations were performed as described [21].

Results

A schematic structure of the region surrounding the maize *GapA1* promoter is shown in Fig. 1a. Since this promoter is quite strong *in vivo*, we expected at the onset of our studies high levels of expression in *GapA1* promoter fusions with GUS. pGpaGus1 contains 1.7 kb of the *GapA1* promoter and the 5′-UTR, the *in vivo GapA1* start codon is fused in frame to the GUS start codon as verified by sequencing. But surprisingly, pGpaGus1 yields no detectable GUS activity in transiently transformed maize cells (Fig. 1). Since both the *GapA1* promoter and coding regions are extremely rich in CpG dinucleotides [11, 27], we suspected that methylation might be responsible for promotor inactivation. Yet transformation of pGpaGus1 into BMS cells pretreated with 5-azacytidin, a potent methylase inhibitor [7], did not increase *GapA1* expression up to detectable levels, whereby luciferase expression from pRT101LUC/Int [20] and GUS expression of positive control plasmids under the CaMV 35S promoter (pRT103GUS) was easily detecteable in 5-azacytidin-treated cells (data not shown). This suggested that methylation was not the cause of lacking expression. Concentrations of 5-azacytidin in excess of 20 mM were apparently toxic to BMS cells and resulted in general decrease in gene expression in positive control plasmids.

Since silencing *cis* elements in the 5′ region can diminish gene expression [3, 16, 29, 34], we examined the *GapA1* promoter for the presence of such elements by construction and transformation of a deletion series in roughly 200 bp incre-

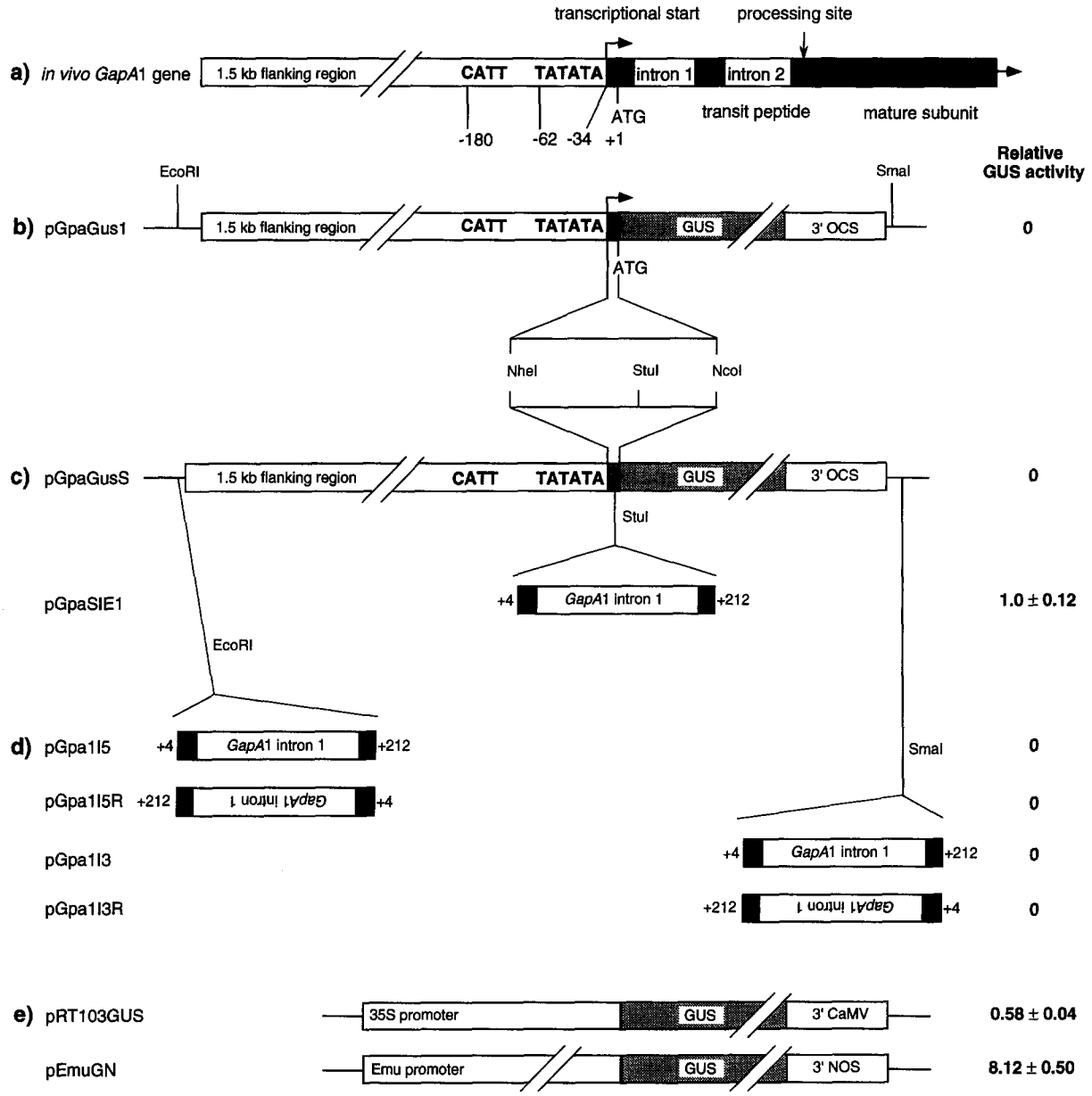

Fig. 1. Relative GUS activities in transiently transformed BMS cells for constructs derived from the maize *GapA1* gene. Means and standard errors (nmol/h) refer to six independent transformations except pGpaSIE1 (22 transformations); zero values indicate that GUS activity was not detectable in six independent transformations for the given construct. Relative GUS activity of pGpaSIE1 was arbitrarily set to 1.0 for reference. **a.** Schematic structure of the 5′ region of the gene. **b.** *GapA1*-promoter fusion with GUS of pGUS1 (Botterman, PGS, Gent). A 39 bp *Nhe* I/*Nco* I fragment of 5′-UTR was substituted by a 46 bp *Nhe* I/*Nco* I fragment containing a *Stu* I cloning site. **c.** Intron 1 of the maize *GapA1* gene was isolated as a 217 bp *Nco* I/*Sac* I fragment (−1 to +216), the protruding ends of which were removed by mung bean nuclease. The resulting fragment (+4 to +212) was cloned into the *Stu* I site of pGpaGus1S to yield pGpaSIE1. **d.** The same blunt *Nco* I/*Sac* I fragment of intron 1 was also cloned in both orientations into the *Eco* RI site and into the SmaI site of pGpaGus1 to yield plasmids pGpaSI5, pGpaSI5R, pGpaSI3, and pGpaSI3R, respectively. **e.** Control plasmids used and their relative GUS activities.

ments from pGpaGus1. None of these constructs produced an increase in GUS-expression up to detectable levels (data not shown), indicating that 5′-silencing elements are not responsible for the low level of gene expression.

Intron 1 is essential for maize GapA1 *gene expression*

The most straightforward interpretation of our unsuccessful efforts to obatin GUS activity from the *GapA1* promoter was simply that pGpaGus1 lacked *cis* elements required for *in vivo GapA1* expression. Since introns can have a stimulating effect upon maize gene expression [2, 9, 19, 20], we suspected that reintroduction of maize *GapA1* introns might restore promoter function. Introns 1 and 2 of the maize *GapA1* gene reside in the region encoding the N-terminal transit peptide of the cytosolic GapA precursor. pGpaGusS was constructed which contains a StuI cloning site in the 5′-UTR of the *GapA1* gene into which introns could be introduced. Transformation of pGpaGusS does not yield detectable GUS activity (Fig. 1c). Intron 1 from the *GapA1* gene was introduced into pGpaGusS to generate pGpaSIE1 which was tested in BMS cells (Fig. 1c). In contrast to pGpaGusS, pGpaSIE1 is expressed. By comparison, pGpaSIE1 yields roughly 2-fold higher levels of GUS activity than the CaMV 35S promoter-driven plasmid pRT103GUS [33] and roughly 8-fold lower activity than pEmuGN, in which GUS is under the control of the synthetic Emu promoter [17], one of the strongest monocot promoters currently available (Fig. 1e). In order to test for the presence of potential enhancer-like activity, intron 1 was cloned into pGpaGus1 in either orientation both 5′ of the promoter and 3′ of the OCS polyadenylation signal. None of these four constructs (pGpa1I3, pGpa1I3R, pGpa1I5 pGpa1I5R) yielded detectable levels of GUS activity (Fig. 1d), suggesting that intron 1 effects gene expression from the *GapA1* promoter in a position-dependent manner, rather than position independent, as would be expected for a general enhancer element.

Previous studies of intron-mediated increase in gene expression in plants implicated the splicing process itself as a potential mechanism since spliced transcripts may be more efficiently transported to the cytosol for translation [1, 10]. To investigate this possibility for *GapA1*, we cloned intron 2 of maize *GapA* with sufficient exon border regions for efficient splicing into the StuI site of pGpaGusS. This plasmid, pGpaSIE2 does not generate detectable levels of GUS expression in transiently transformed maize (Fig. 2a), indicating that the mere presence of an intron is not sufficient to restore activity to the *GapA1* promoter. Neither pGpaSIE1R nor pGpaSIE2R, which contain intron 1 and 2 of *GapA1* in inverse orientation, respectively, confer GUS activity to maize cells (Fig. 2a). These results suggest that intron 1 in the proper orientation restores *GapA1* gene expression in a position-dependent manner and is required for proper expression of the gene.

Analysis of other GapA1 *constructs*

The functional promoter construct pGpaSIE1 was used to generate a series of 5′ deletion mutants from the original 1.4 kb *GapA1* promoter fragment in order to define the minimal promoter. GUS activity measured in the transient expression assay for these mutants is given in Fig. 3. Plasmids containing greater than 250 bases of the *GapA1* promoter do not differ markedly in expression from pGpaSIE1. Only after removal of the CAAT box was a significant decrease in GUS activity measurable, removal of the TATA box resulted in a decrease of expression below limits of detection. The minimal promoter so defined encompasses roughly 250 bp upstream of the *in vivo* transcription start and appears also to include intron 1.

In order to determine whether intron 1 can stimulate expression of other promoters, we introduced it into the 5′-UTR of pRT103GUS to yield p35SIE1. p35SIE1-transformed cells contain only about 7% greater GUS activity than the pRT103GUS control (Fig. 2b), suggesting that intron 1 does not contain a general enhancer of transcription, but leaving open the possibility that

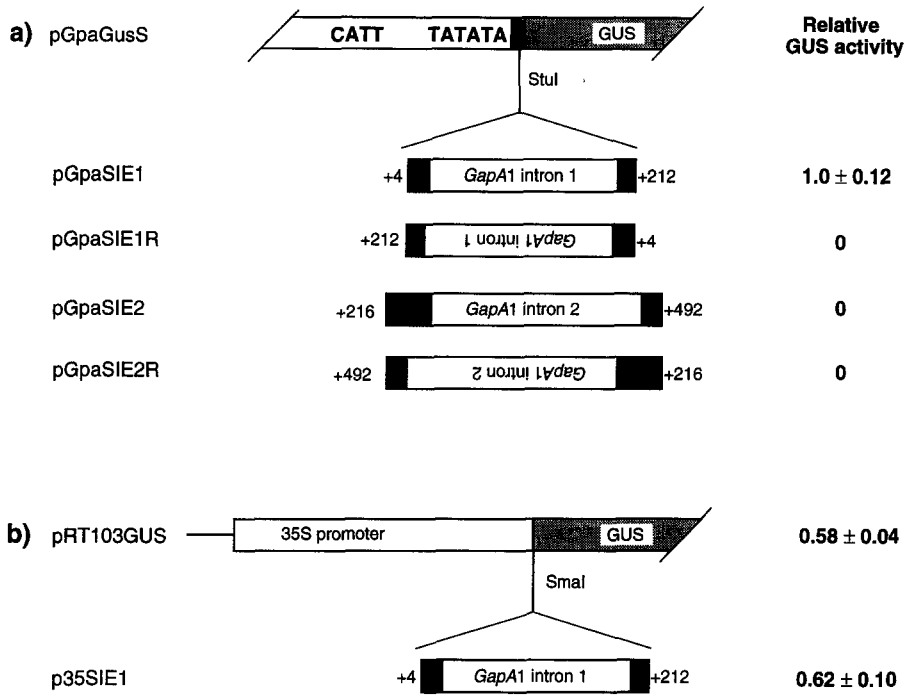

Fig. 2. Transient expression levels of *GapA1* plasmid constructs. **a.** Intron 1 of the maize *GapA1* gene was isolated as a 217 bp *Nco* I/*Sac* I fragment (−1 to +216), the protruding ends of which were removed by mung bean nuclease. The resulting fragment (+4 to +212) was cloned in both orientation into the *Stu* I site of pGpaGusS to yield pGpaSIE1 and pGpaSIE1R. Intron 2 of the maize *GapA1* gene was isolated as a 282 bp *Sac* I/*Sac* II fragment (+212 to +494), the protruding ends of which were removed by mung bean nuclease. The resulting fragment (+216 to +492) was cloned into the *Stu* I site of pGpaGusS in both directions to yield pGpaSIE2 and pGpaSIE2R. **b.** The same blunt *Nco* I/*Sac* I fragment of intron 1 as described in a was also cloned in spliceable orientation into the SmaI site of pRT103GUS to yield p35SIE1. Relative GUS activity is expressed as nmol/h representing the mean and standard error for six individual transformations (Except pGpaSIE1, 22 transformations).

it contains enhancer-like elements which attain function in the context of their natural promoter.

The length of bordering exon sequences is known to influence intron-mediated stimulation of gene expression at the level of splicing [31]. In order to determine whether this also holds true for *GapA1* intron 1, we amplified fragments which contained shorter or no *GapA1* exon border sequences and introduced these into the *Stu* I site of pGpaGusS (Fig. 4a). By reducing the length of the *GapA1* exon 1 and exon 2 border sequences from 42 and 33 bp in pGpaSIE1 to below 14 and 23 bp, respectively, GUS expression fell below detectable levels, suggesting that for *GapA1* intron 1, as for other stimulating introns in maize, exon border sequences were critical to intron-mediated expression increase. Non-spliceable intron 1 derivatives lacking terminal guanosine residues at the acceptor and donor sites, respectively, were also constructed and tested, but yielded no GUS activity (data not shown). By analogy to other similar experiments on maize introns [18, 31], it seems likely that the decrease in expression observed results from decreased splicing efficiency, although the possibility cannot be fully excluded that *cis* elements exist in the deleted exon border regions which are involved in transcriptional activation. These results, in the light of previous studies on exon border length requirements in maize, suggest that intron 1 of *GapA1* has minimum border requirements for proper splicing and that the loss of expression observed for pGpaSIE1.35, pGpaSIE1.26 and pGpaSIE1.33 (see Fig. 4) may be due to decreased splicing efficiency as the result of lacking exon borders.

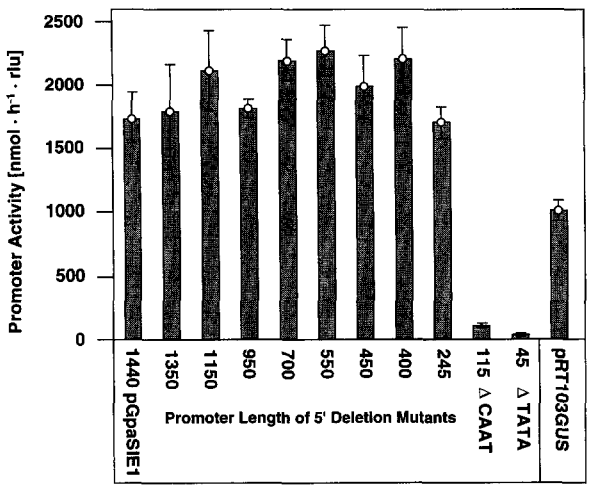

Fig. 3. Deletion series of pGpaSIE1. Lengths of remaining promoter sequences are given in bp. Promoter lengths greater than 250 bp were determined on agarose gels, those shorter than 250 bp were determined by sequencing. For comparison, the relative strengths of pRT103GUS and pGpaSB are shown (see text). Relative promoter strength (GUS activity standardized to internal luciferase control) is given by grey columns, the standard error attached to each value is indicated. Columns represent the mean and standard error for six individual transformations except for the initial 1.4 kb pGpaSIE1 clone (22 transformations) from which deletions were generated.

The first intron of two other maize genes, *Adh1* [2] and *Sh1* [20], were previously shown to stimulate gene expression in a manner similar to that of *GapA1*, since increase of promoter activity for *GapA1*, *Adh1* and *Sh1* through their respective first introns is, in each case, much greater than any increase conferred by the introns upon other promoters such as CaMV 35S. Also, the activating properties of *GapA1*, *Adh1* and *Sh1* introns 1 are only observed when these are located 3′ of the TATA box [20, 31]. This prompted us to examine the first introns and surrounding exon borders of *Adh1* and *Sh1* for the presence of sequence similarities to *GapA1*. Intron 1 of maize *GapA1* was found to contain the octameric sequence motif 5′-CGTGCCGC starting at position 10 of the intron. This motif has one mismatch each relative to the motifs in maize *Adh1* 5′AGTGCCGC, starting at position 102 of *Adh1* intron 1, and 5′-CGTGCAG^C, which spans its 3′ splice acceptor junction. The *GapA1* motif also has one mismatch to the sequence 5′-CGTGCCGT found starting at position 413 within intron 1 of maize *Sh1* [20]. pGpaSB contains four copies of the CGTGCCGC motif 16, 19 and 16 bp apart in the 5′-UTR of *GapA1* and reveals promotor activity at a level roughly 20% of that of pGpaSIE1 (Fig. 4b).

Discussion

Previous studies have shown that the presence of introns can affect expression by influencing steady-state plant mRNA levels [9, 19, 20, 31]. Although we did not directly assess spliced *vs.* unspliced mRNA levels here, the data obtained in transient expression experiments for *GapA1* constructs are consistent with the view that proper splicing is required for expression of *GapA1*. This is because neither pGpaSIE1R, which contains intron 1 and exon border regions in reverse orientation (Fig. 2a), nor derivatives of pGpaSIE1 which contain shortened or no exon border regions (Fig. 4) yield detectable levels of expression. The latter finding is not surprising, since exon border regions are known to be required for intron-mediated expression increase. Sinibaldi and Mettler [31] found that the optimal 5′ exon border length for maize is in the order of 60–90 bp and that increases in expression correlate with spliced mRNA levels, but their finding that different introns bordered by highly homologus exon regions can give rise to markedly different levels of expression led them to suspect that, in addition to splicing effects, intron-internal sequences might be involved in enhancer-like function. Furthermore, it was shown that one and the same intron can elicit either positive or negative effects upon expression, depending upon the promoter behind which it is inserted. For example, intron 1 of maize *Adh1* evokes a 10- to 20-fold increase in expression when placed 3′ of a truncated *Adh1* promoter, yet evokes no increase when located 3′ the CaMV 35S promoter [17], similar to results observed here for maize *GapA1* intron 1 (Fig. 2). Such findings are difficult to reconcile with the view that splicing may be the

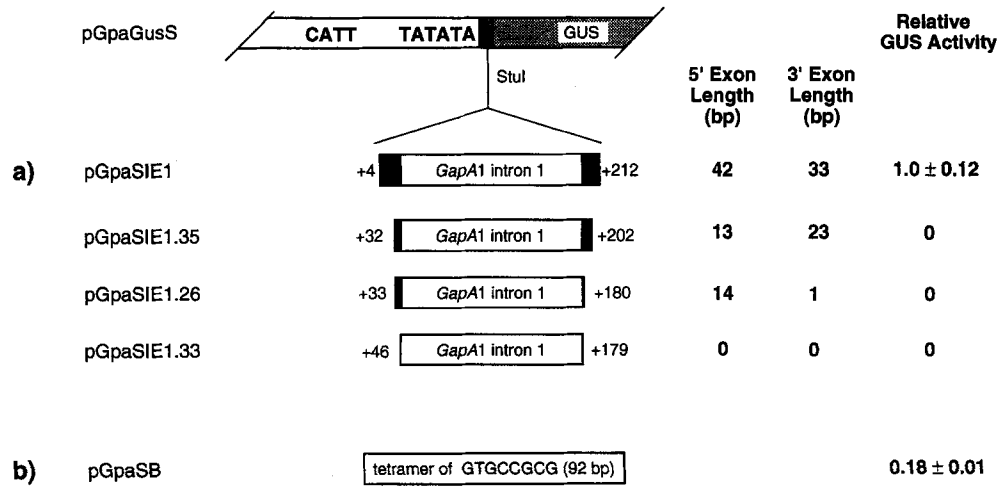

Fig. 4. Transient expression levels of *GapA1* plasmid constructs. Relative GUS activities are expressed as in the legend to Fig. 1 and represent the mean and standard error for six individual transformations except pGpaSB (ten transformations) and pGpaSIE1 (22 transformations). **a.** Constructs containing intron 1 with varying exon border regions. Constructs pGpaSIE1.35, pGpaSIE1.26 and pGpaSIE1.33 were generated via PCR. Amplifications products were cloned blunt into the *Stu* I site of pGpaGusS and verified by sequencing. Numbering coordinates of the *GapA1* sequence are as given in the legend to Fig. 1. Lengths of exon border sequences are given. **b.** Construct pGpaSB containing a synthetic tetramer of the conserved sequence motif 5′-GTGCCGCG in the *Stu* I site instead of intron 1.

sole source of intron-mediated expression increase in maize.

We found that *GapA1* intron 1 and exon border sequences increase (and may be essential for) expression of the maize *GapA1* promoter and that the position of intron 1 relative to the TATA box is crucial. We identified a motif (CGTGCCGC) in maize *GapA1* intron 1 which is found (allowing for one mismatch) twice in *Adh1* intron 1 and once in *Sh1* intron 1, both of which are known to stimulate transient expression in maize. Interestingly, expression stimulation for internal deletion mutants of *Adh1* intron 1 was reported and it was concluded that 'if an enhancer is present, it is very near the splice junctions' [19]. Indeed, one of the CGTGCCGC-like motifs in *Adh1* intron 1 spans the 3′ splice site. In addition, more recently reported internal deletion mutants of *Adh1* intron 1 which do not stimulate expression, although they are shown here to still contain the second CGTGCCGC-like motif, are not efficiently spliced because the intron is too short [17]. Construct pGpaSB, which contains four copies of this motif instead of intron 1, restores about 20% activity to the *GapA1* promoter relative to pGpaSIE1, suggesting that the octameric motif itself may play a role in intron-mediated enhancement. Yet, as for all constructs studied here, the effects of mRNA stability and splicing efficiency for pGpaSB still have to be determined. Thus, although the octameric motifs in intron 1 of *GapA1*, *Sh1* and *Adh1* have not been directly shown to be functional, available data do not exclude the possibility that they may also be directly involved in gene expression. Congruent with this view is the finding that sequences which do not elicit a marked increase in expression, such as intron 2 of *GapA1* (Fig. 2) or introns 2 and 3 of *Adh1* and their respective exon border regions [2], also do not contain similarly conserved copies of the octameric motif. In preliminary DNA-footprint and gel-retardation experiments, we found that the octameric sequence (5′-CGTGCCGC-3′) within intron 1 appears to bind a maize nuclear factor (data not shown).

The general pattern of activation exerted by *GapA1* intron 1 is quite similar to that shown for the first intron of the mouse *rp132* gene. That vertebrate intron contains a sequence motif (the δ-factor binding element) which influences tran-

scription only when located 3′ of the promoter and which furthermore specifically binds a nuclear (zinc-finger) factor involved in regulation of vertebrate ribosomal protein genes [4, 5]. We have shown that the increase in expression mediated by the *GapA1* intron is also position-dependent, leaving open the possibility that intron 1 does not contain an enhancer *per se* but might contain an enhancer-like element. The mouse δ-factor-binding element increases expression about 10-fold in *rp132*, but represses expression in other genes, suggesting that the function of the element depends upon interaction within the context of other transcriptional activators. The same may hold true for *GapA1* intron 1, since it clearly does not stimulate the CaMV 35S promoter.

The *GapA1* minimal promoter encompasses the CAAT box and is not longer than 245 bp. This is congruent with the finding that upstream of the CAAT box, no sequence similarity is observed between the active *GapA1* gene and its two pseudogene counterparts in the maize genome, ΨGapA1 ΨGapA2, both of which were functional in the maize genome long before their evolutionarily recent inactivation [26]. Notably, in both ΨGapA1 and ΨGapA2 the first two nucleotides of the octameric motif within intron 1 are deleted. We could not reliably determine the absolute degree of expression stimulation achieved by reintroduction of intron 1 and its exon borders because constructs lacking the intron do not yield expression levels significantly greater than background. Yet since background GUS activity in our BMS cells is less than 8% of that measured observed with pGpaSIE1 containing intron 1, the level of stimulation is at least 10-fold, although histochemical assays which we performed in parallel to activity measurements in transformation experiments suggest that the degree of stimulation may be 20-fold or greater (data not shown).

Numerous cases of intron-stimulated plant gene expression are known [2, 9, 19, 20, 24, 28, 31, 32, 35], but the possible role of protein-DNA interactions in intron-stimulated is still unclear. In mammalian cells, introns can exert an influence upon gene expression by carrying enhancer-like elements which regulate transcription as in the case of a2-collagen [25], troponin I [15] or *rp132* [5]. Intron 1 of maize *GapA1* may carry such an enhancer-like element. Increases in gene expression conferred by introns may involve transcriptional activation in addition to such splicing and mRNA stability effects as are necessary to observe elevated promoter strength.

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