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REVIEW

Enhancer–promoter interference and its prevention in transgenic plants

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Abstract Biotechnology has several advantages over conventional breeding for the precise engineering of gene function and provides a powerful tool for the genetic improvement of agronomically important traits in crops. In particular, it has been exploited for the improvement of multiple traits through the simultaneous introduction or stacking of several genes driven by distinct tissue-specific promoters. Since transcriptional enhancer elements have been shown to override the specificity of nearby promoters in a position- and orientation-independent manner, the co-existence of multiple enhancers/promoters within a single transgenic construct could be problematic as it has the potential to cause the mis-expression of transgene product(s). In order to develop strategies with, which to prevent such interference, a clear understanding of the mechanisms underlying enhancer-mediated activation of target promoters, as well as the identification of DNA sequences that function to block these interactions in plants, will be necessary. To date, little is known concerning enhancer function in plants and only a very limited number of enhancer-blocking insulators that operate in plant species have been identified. In this review, we discuss the current knowledge surrounding enhancer–promoter interactions, as well as possible means of minimizing such interference during plant transformation experiments.

Keywords Enhancer–promoter communication · Enhancer-blocking insulator · *35S* promoter/enhancer · Tissue-specific transgene expression · Plant biotechnology

Abbreviations

35S	*35S cauliflower mosaic virus* promoter/enhancer
AGIP	*Arabidopsis AGAMOUS* second intron-derived promoter
GUS	*β*-Glucuronidase
MAR	Matrix attachment region
TBS	Transformation booster sequence

Introduction

Transgenic plant technology, which often requires the use of tissue-, organ- or developmental stage-specific promoters to drive the expression of transgenes exclusively in targeted tissues, is central to both basic plant biological research and the improvement of agronomic traits in crop species. The majority of transgenic research that has been conducted thus far has involved the enhancement of a single trait despite the fact that crops in field conditions must cope with a range of biotic and abiotic challenges. To address this problem, researchers will no doubt begin to adopt a more comprehensive approach designed to increase the performance of several traits using transformation vectors that harbor multiple

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transcriptional gene units. Unfortunately, the presence of multiple enhancer/promoter elements within a single vector might, due to the position- and orientation-independent nature of enhancers, trigger inappropriate enhancer–promoter interactions, thereby disturbing the specificity and strength of discrete promoters in transgenic plants. This enhancer–promoter interference phenomenon is common in transgenic plants containing vectors in which the enhancer derived from the constitutive *cauliflower mosaic virus* (*CaMV*) *35S* promoter (Odell et al. 1988) is situated near a plant-derived promoter, resulting in both an increase and loss of specificity of their transcriptional activity (Zheng et al. 2007; Hily et al. 2009). Since the *35S* promoter is used to drive the expression of the selectable marker gene in a broad range of plant transformation vectors (for example Hajdukiewicz et al. 1994; Ouwerkerk et al. 2001), its strong enhancer function has the potential to be an impediment to transgenic research when the precise control of transgene expression is essential.

Enhancer–promoter crosstalk was first demonstrated for the *35S* enhancer when its presence near either the *Agrobacterium tumefaciens*-derived *nopaline synthase* (*nos*) or *gene 5* and *gene 7* promoters considerably augmented their transcriptional activities (Kay et al. 1987; Odell et al. 1988). Subsequently, this same enhancer was found to constitutively activate tissue-specific promoters, such as the vascular tissue-specific *AAP2*, carpel-specific *AGL5*, root-specific *LRP1*, stamen- and carpel-specific *AGIP*, tapetum-specific *TA29* and *A9*, and seed-specific *napin* promoters, resulting in their mis-expression in non-targeted tissues (Jagannath et al. 2001; Yoo et al. 2005; Zheng et al. 2007; Gudynaite-Savitch et al. 2009; Hily et al. 2009). Interestingly, the enhancer-mediated activation of nearby promoters is not restricted to the constitutive *35S* enhancer, but has also been observed with several tissue-specific enhancers. For example, insertion of the pollen-specific *LAT52* enhancer upstream of the stigma-specific *SLG* promoter has been shown to specifically activate the promoter in pollen without altering its inherent stigma-specific activity (Liu et al. 2008). Furthermore, the insertion of the carpel-specific *AGL5* enhancer upstream of the *LAT52* enhancer/*SLG* promoter resulted in a gain of carpel-specific activity while still retaining its pollen- and stigma-specific activity (Liu et al. 2008). Therefore, it seems that enhancer-mediated activation of adjacent promoters is not a rare occurrence, but instead is a common property of many enhancers; a potential roadblock to transgenic research that has sparked intense interest in both the nature of these interactions (Zheng et al. 2007; Singer et al. 2010a) and strategies with which to prevent them (Gudynaite-Savitch et al. 2009; Hily et al. 2009; Singer et al. 2010b).

Enhancer–promoter interactions

Enhancer elements have been found to promote the expression of a large number of eukaryotic genes independently of both their position and orientation in relation to the site of transcriptional initiation (for example Weterings et al. 1995) and can exert their control either locally or over long genomic distances. As a result, there is very little correlation between the location of enhancers and that of their target gene within a genomic context. For example, the wing margin enhancer of the *Drosophila β3-tubulin* and *Arabidopsis AGAMOUS* enhancers reside within introns of their respective transcriptional units (Hinz et al. 1992; Busch et al. 1999), whereas the *Drosophila cut* locus is positioned 85-kb upstream of its target promoter (Jack et al. 1991). In contrast, enhancers for the rat and human *myosin light chain 1/3* genes are situated downstream of their polyadenylation signals (Donoghue et al. 1988; Rosenthal et al. 1990) and in the case of certain enhancers, chromosome-pairing will even allow the enhancer to activate transcription from an allelic promoter on a separate chromosome (Morris et al. 1998).

Enhancers are thought to function by increasing the probability that a gene will be active in a given cell (Walters et al. 1995; Fiering et al. 2000) through the recruitment and delivery of protein factors required for the initiation of transcription to target promoters via protein–protein interactions (Ptashne and Gann 1997). However, only a small number of select metazoan enhancers and yeast upstream activating sequences (UASs), which are functional equivalents of enhancer elements, have been examined in any detail to date. Consequently, a general model of transcriptional regulation by enhancers is lacking and many questions remain regarding the mechanism by which they stimulate transcription.

It has been suggested that enhancers and promoters that are within approximately 1-kb of one another likely utilize a short-range mode of enhancer action whereby the enhancer and promoter communicate directly without the need for any facilitating mechanisms (Bondarenko et al. 2003; Fig. 1a). However, in the case of long-distance enhancer function, mechanisms are required to facilitate interactions between an enhancer and a remote target promoter (Fig. 1b). A number of different models have been proposed to explain this phenomenon (Blackwood and Kadonaga 1998; Bulger and Groudine 1999; Dorsett 1999), among which looping and/or scanning are foremost. The looping model suggests that the initiation of transcription is achieved through the direct interaction of an enhancer and promoter by means of DNA-bound proteins, with looping out of intervening chromatin. Conversely, the scanning model proposes that enhancers recruit RNA polymerase II, and possibly also other associated

transcription factors, which then migrate along the DNA until they encounter a competent promoter and subsequently activate the production of a transcription initiation complex. This tracking of RNA polymerase II from enhancer to promoter often results in the generation of transcripts derived from the intervening DNA. Finally, the facilitated tracking model includes features of both the looping and scanning models in that both the enhancer and its associated factors advance along the intervening chromatin until a stable interaction is established with promoter-bound proteins, resulting in a loop of intervening DNA that grows progressively during the scanning process (Fig. 1b). While there is evidence supporting each of these models, there does not appear to be unambiguous proof favoring any one of them. In fact, the existing data imply that one single model may not be generally applicable to all enhancers.

In plants, enhancers are typically found in close proximity to their cognate promoters, but there are also those that function from a distance (Sieburth and Meyerowitz 1997; Clark et al. 2006). While the mechanism behind enhancer function in plants has long been unclear because very little research has been conducted in this area, progress has been made recently through an analysis of both constitutive and tissue-specific enhancer-mediated activation of a target promoter in a plant system (Singer et al. 2010a). In this study, the flower-specific *AGI* enhancer (derived from the *AGAMOUS* second intron) fused to the *β-glucuronidase* (*GUS*) coding sequence in either the presence or absence of a target minimal promoter was found to confer the same *GUS* expression pattern in *Arabidopsis*, suggesting that the enhancer itself is able to autonomously activate transcription in the correct spatio-temporal pattern. 5′ RACE analyses indicated that this enhancer-mediated *GUS* transcription was initiated at multiple sites within and downstream of the enhancer element, which corresponds with a short-range mode of enhancer action as demonstrated in other organisms, and likely represents one of the mechanisms underlying enhancer–promoter interference in transgenic plants. In the same study, transgenic lines of *Arabidopsis* bearing the *35S* enhancer situated approximately 3-kb upstream of a target minimal promoter fused to the *GUS* coding sequence displayed constitutive *GUS* expression reminiscent of *35S* promoter-conferred activity, indicating that the *35S* enhancer was able to override a 3-kb distance barrier.

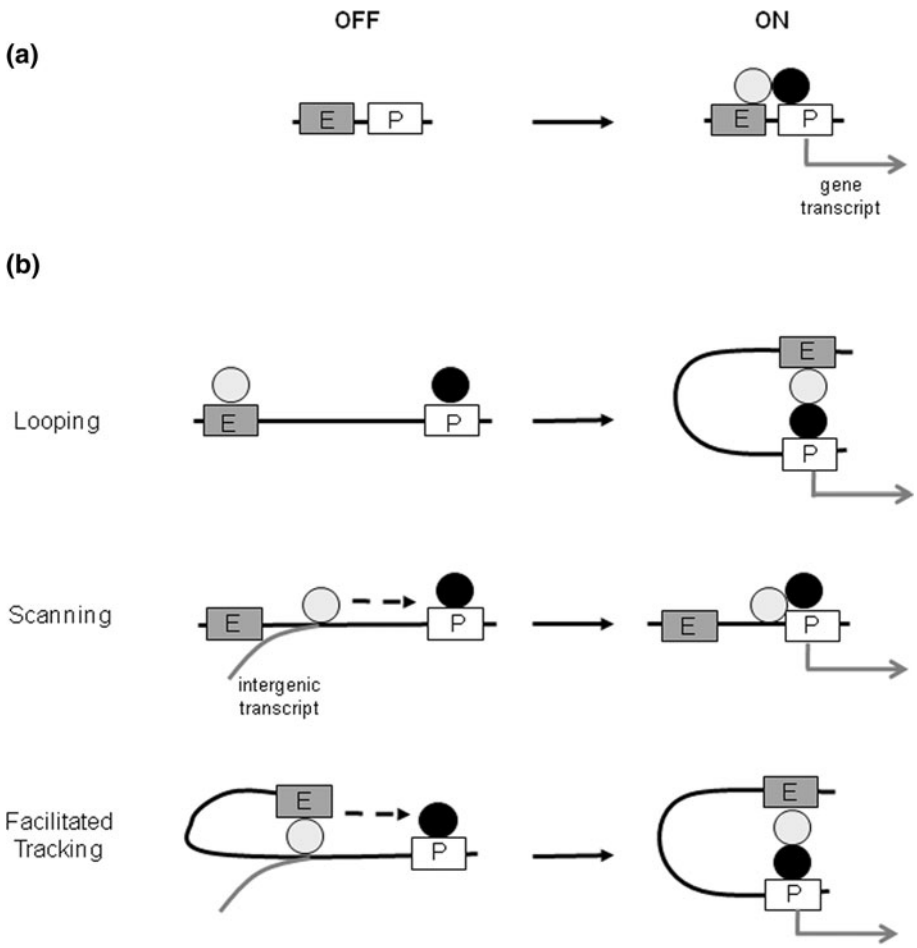

Fig. 1 Models of enhancer–promoter interaction. Mechanisms behind both short-distance **(a)** and long-distance **(b)** communication are shown. *Circles* denote protein factors, *boxes* indicate enhancer (*E*) and promoter (*P*) elements, respectively, *black lines* represent DNA sequence and *grey lines* indicate RNA transcripts. Where no interactions between enhancer-bound and promoter-bound proteins exist, gene transcription is 'off', whereas contact between factors causes gene transcription to turn 'on'. In the case of short-distance communication, enhancer- and promoter-bound proteins are in close enough proximity to interact directly without the need for any facilitating mechanisms. In the case of long-distance interactions, the three main models (looping, scanning and facilitated tracking) permitting communication are illustrated

Northern blot and 5′ RACE analyses implied that two major transcription initiation regions existed in these lines: the first appeared to initiate near the *35S* enhancer and was transcribed through the intervening 3-kb sequence and at least a portion of the *GUS* coding sequence, generating a product that was almost certainly not translated into functional GUS protein, and the second initiated at the minimal promoter just upstream of the *GUS* coding region (Singer et al. 2010a). These results were very similar to those observed in animal cells during long-range transcriptional activation by the HS2 enhancer from the human *globin* locus in an artificial vector. In this case, non-coding intergenic RNAs were initiated from multiple sites within the HS2 enhancer and elongated through the intervening DNA, target promoter and reporter gene, while functional reporter gene mRNAs were initiated at a single site within the target promoter (Ling et al. 2005). Interestingly, the ability of enhancers to initiate transcription autonomously in a promiscuous fashion appears to be an inherent property of these elements in a broad range of eukaryotic organisms, especially in artificial systems which do not possess the mechanisms present in a genomic environment to avert interference between enhancers and non-target genes (Ling et al. 2005; Dobi and Winston 2007; Singer et al. 2010a).

A number of studies in animal systems have also found that non-coding RNA derived from the intervening sequence separating an enhancer and a distant promoter are generated in addition to functional gene transcripts (Ho et al. 2006; Dobi and Winston 2007; Zhu et al. 2007; Tchurikov et al. 2009). These intergenic transcripts have been proposed to be the consequence of a facilitated tracking mechanism in which RNA polymerase II and a bound enhancer track along the DNA from enhancer to target promoter to ultimately form a loop. This migration of RNA polymerase II along the intervening DNA, and the associated synthesis of intergenic RNAs, has been suggested to supply enhancer-bound proteins to the target promoter and/or 'open' the nucleosomal structure of the gene domain through the association of histone acetyltransferases (Zhu et al. 2007).

However, while scanning/facilitated tracking seems to be a rather widespread means of establishing communication between regulatory elements, the mechanism by which this is accomplished may vary as the characteristics of derived intergenic RNAs have been found to be quite diverse depending on the system analyzed (for example Broders and Scherrer 1987; Razin et al. 2004; Ling et al. 2005; Wang et al. 2005; Zhu et al 2007). As a result, it is still debated whether enhancer-initiated transcripts are required for target gene expression (for example Müller et al. 1990) despite the fact that the majority of studies provide evidence for the importance of intergenic transcripts to enhancer–promoter communication and the initiation of functional gene transcripts (Drewell et al. 2002; Ling et al. 2004; Ho et al. 2006; Dobi and Winston 2007). Thus, while *35S* enhancer-mediated long-range activation in plants appears to utilize a scanning-based mechanism, there is still a lack of evidence that the tracking of RNA polymerase along the intervening DNA towards a target promoter ultimately results in the formation of a direct link between enhancer and promoter via looping as would be expected for facilitated tracking.

Taken together, it seems that enhancers may exploit at least two different modes of action to exert their activation function in transgenic plants. Short-range activation appears to involve transcription initiation events instigated by the enhancer itself, while long-range activation involves transcription initiation at both the enhancer and the promoter. In any case, the seemingly broad presence of enhancer–promoter interference in transgenic plants demands the development of tactics to effectively remedy the resulting mis-expression of introduced genes.

Averting enhancer–promoter crosstalk in transgenic plants

While mechanisms are in place within eukaryotic genomes to prevent the inappropriate activation of promoters by nearby enhancers (reviewed by Kadauke and Blobel 2009), this is not the case in transgenic constructs. One of the main factors influencing the onset and extent of enhancer–promoter interference that occurs within a transgenic system is the strength of the enhancer itself. For example, all enhancers may not have the same activation potential, as is indicated by the observation that the weak *nos* and strong *supP* (Ni et al. 1995) enhancers show differential activation of the seed-specific *napin* promoter in vegetative tissues (Gudynaite-Savitch et al. 2009). Therefore, a simple strategy to avoid this type of crosstalk is to utilize promoters that do not affect the tissue-specificity of nearby promoters within the transgene construct. To date, a number of promoters derived from *A. tumefaciens*, such as *nos* (Kim et al. 1993), *octopine synthase* (Kononowicz et al. 1992) and *mannopine synthase* (Fox et al. 1992), as well as the *ubiquitin-1* promoter from maize (Brinch-Pedersen et al. 2003), *actin-1* promoter from rice (Datta et al. 1998), and tCUP from tobacco (Foster et al. 1999), have successfully been used to drive selectable marker expression in lieu of the *35S* promoter (for example Gudynaite-Savitch et al. 2009). However, it seems that there is not one single enhancer that will solve the problem entirely, as different target promoters vary in their responsiveness to the activation potential of each particular enhancer.

It has also been suggested that enhancers are only able to act at a relatively close range and that virtually any

sequence of sufficient length could be utilized to block enhancer activation (van der Geest and Hall 1997; Jagannath et al. 2001). Indeed, several studies have shown that increasing the distance between enhancer and promoter in a transgenic construct can reduce interference. For example, positioning the *AGL5* enhancer 1.8-kb from the *SLG* promoter resulted in failure of the enhancer to activate expression in carpel tissue (Liu et al. 2008), while *35S* enhancer-mediated activation of the tapetum-specific *TA29* promoter in vegetative tissues was substantially reduced when a 5-kb spacer fragment was inserted between them (Jagannath et al. 2001). However, we have found that the partially duplicated *35S* promoter, which contains two enhancer elements and has been estimated to induce expression levels that are at least tenfold higher than its unduplicated counterpart (Kay et al. 1987), is able to overcome a 4-kb spacer fragment and activate the flower-specific *AGIP* promoter in *Arabidopsis* vegetative tissues (Hily et al. 2009; Singer et al. 2010b). Furthermore, while both the seed-specific *napin* and *prx* promoters are activated by a nearby *35S* enhancer in transgenic plants, when a 2.7-kb fragment was inserted between the enhancer and promoters, respectively, enhancer activation was only impeded in the case of the *napin* promoter (Gudynaite-Savitch et al. 2009). This indicates that both the strength of the enhancer and the sensitivity of the target promoter influence the precise length of the distance barrier required to prevent enhancer–promoter communication. Therefore, the exact length required to completely obstruct enhancer-mediated mis-expression will vary by construct. Moreover, the incorporation of large spacer fragments into plant transformation vectors could pose technical difficulties for cloning and vector stability (for example Park et al. 2000).

Enhancer–promoter interference can also be averted through the use of enhancer-blocking insulators, which have been widely used during mammalian cell transfection experiments (Steinwaerder and Lieber 2000; Ye et al. 2003). Genetic insulators have been studied extensively in metazoan systems, the most well-characterized of which include the *gypsy* retrotransposon element (Geyer et al. 1986) and scs/scs' paired elements (Kellum and Schedl 1992) from *Drosophila*, as well as the cHS4 insulator from the chicken *β-globin* locus (Chung et al. 1993). These elements have been classified into two general groups based on their function: whereas barrier insulators prevent the spreading of a heterochromatin state and its mediated gene silencing, enhancer-blocking insulators impede enhancer–promoter interactions when placed between an enhancer and promoter. While some insulators function as either barrier or enhancer-blocking elements, others are able to serve as both (reviewed by Gaszner and Felsenfeld 2006).

Unfortunately, few sequences with enhancer-blocking activity have been characterized in plants to date (van der Geest and Hall 1997; Jagannath et al. 2001; Gudynaite-Savitch et al. 2009; Hily et al. 2009; Singer et al. 2010b; Table 1) and the acquirement of such information is of some urgency, as it may provide a novel means for minimizing enhancer–promoter interference during plant transformation experiments with composite vectors. While there has been a report suggesting that a 16-bp palindromic sequence from *Arabidopsis* (*NI29*) functions as an enhancer-blocking insulator in plants (Gan and Xie 2002), which would be ideal due to its small size, further studies indicated that *NI29* failed to block *35S* enhancer-mediated activation of adjacent promoters (Gudynaite-Savitch et al.

Table 1 DNA sequences found to exhibit enhancer-blocking insulator activity in transgenic plants to date

Insulator	Length[a] (kb)	Enhancer[b]	Promoter[b]	Organism[c]	% efficiency[d]	Reference
phas 3′ MAR	1.3	*35S*	*phas* basal	Tobacco	80	van der Geest and Hall (1997)
phas genomic sequence	1.2	*35S*	*phas* basal	Tobacco	93	van der Geest and Hall (1997)
phas coding sequence	0.9	*35S*	*phas* basal	Tobacco	91	van der Geest and Hall (1997)
3-kb pea *topoisomerase*/2-kb *Arabidopsis acetolactate synthase* genomic sequences	5.0	*35S*	*TA29*	*Brassica juncea*	100	Jagannath et al. (2001)
BEAD-1	1.0	*35S*	*napin*	*Arabidopsis*	77	Gudynaite-Savitch et al. (2009)
BEAD-1C	0.6	*35S*	*napin*	*Arabidopsis*	87	Gudynaite-Savitch et al. (2009)
UAS_{rpg}	0.25	*35S*	*napin*	*Arabidopsis*	92	Gudynaite-Savitch et al. (2009)
TBS	2.0	*35S*	*AGIP*	*Arabidopsis*	69	Hily et al. (2009)
λ EXOB	1.0	*35S*	*AGIP*	*Arabidopsis*	93	Singer et al. (2010a, b)

[a] The length of the insulating sequence

[b] The enhancer and promoter between which the insulating sequence was inserted

[c] The transgenic plant in which the insulating sequence was tested

[d] The percentage of transgenic plants in which enhancer–promoter interference was completely blocked by the insulating sequence

2009; Yang et al. 2010). Recently, two DNA fragments including a 2-kb transformation booster sequence (*TBS*) from petunia and a 1-kb *EcoR*I/*Sal*I fragment (*EXOB*) from bacteriophage lambda were found to impede activation of the flower-specific *AGIP* promoter in vegetative tissues by the partially duplicated *35S* enhancer when situated between the two in *Arabidopsis* (Hily et al. 2009; Singer et al. 2010b). It seems that neither of these fragments possesses silencing activity, and therefore functions as true enhancer-blocking insulators in this system. Furthermore, preliminary evidence suggests that a 1.3-kb 3′ matrix attachment region (MAR) from the *β-phaseolin* gene of *Phaseolus vulgaris*, as well as 1.2-kb genomic and 0.9-kb coding sequence fragments from the same gene, inhibit *35S* enhancer-mediated activation of a nearby promoter (van der Geest and Hall 1997). While the authors attributed the enhancer-blocking activity of these sequences solely to their length, there is evidence that the *35S* enhancer can exert its effects over distances as large as 78-kb (Ren et al. 2004), which suggests that relatively small lengths of spacer sequence would likely not impede interference. Therefore, it is tempting to surmise that these fragments possess inherent, as of yet unidentified, properties that prevent interactions between an enhancer and promoter when situated between them.

Several heterologous enhancer-blocking insulators from various organisms have also recently been tested in plants (Nagaya et al. 2001; Gudynaite-Savitch et al. 2009), but the majority were not effective or functioned differently in plant cells. For example, the *Fab7PRE* enhancer-blocking insulator from *Drosophila* (Barges et al. 2000) acted as a silencer rather than an insulator in plants (Gudynaite-Savitch et al. 2009). Nevertheless, a minority of the sequences, such as the BEAD-1 and BEAD-1C insulators from the human T-cell receptor α/δ locus (Zhong and Krangel 1997), as well as the UAS_{rpg} insulator from *Ashbya gossypii* (Bi and Broach 2006), were found to reduce non-specific enhancer–promoter interactions (Gudynaite-Savitch et al. 2009), which hints at the possibility of a conserved mechanism between a wide range of eukaryotic organisms.

Possible mechanisms for enhancer-blocking insulator function

Several DNA-binding proteins have been found to be sufficient to block enhancer–promoter interactions in animals (Parkhurst et al. 1988; Bell et al. 1999; Gaszner et al. 1999). However, no significant similarities exist either in the DNA-binding sites or in the proteins themselves, which hints at the possibility that a number of molecular mechanisms may be exploited to obstruct enhancer function. In vertebrates, the CTCF factor (Filippova et al. 1996), which is highly conserved and ubiquitously expressed, binds diverse sequences that have been consistently identified in almost all insulators analyzed to date in these organisms (Kim et al. 2007). The fact that vertebrate CTCF binding sites have been found to function in *Drosophila* (Namciu et al. 1998) and the identification of CTCF-like factors in invertebrates (Zhou et al. 1999; Barges et al. 2000; Moon et al. 2005) indicate that this protein factor is a highly conserved element of at least a proportion of insulator systems. However, whether similar CTCF and/or invertebrate-specific (e.g. *gypsy* and scs/scs′) insulators have evolved in plants remains unclear. It is difficult to address this question because insulator sequences appear to have diverged significantly, even within the vertebrate genome. As of yet, no functional equivalents of CTCF binding sites have been identified in plants; however, a number of plant zinc-finger gene families collectively share at least some degree of identity at the amino acid level with the zinc-finger domains of vertebrate CTCF proteins (Engelbrecht et al. 2004). This suggests that a CTCF-dependent insulator system might also exist in plants. Furthermore, the fact that insulators from humans and fungi provide at least partial blocking of *35S* enhancer-mediated activation in plants (Gudynaite-Savitch et al. 2009) insinuates that the insulator machinery in these organisms may be evolutionarily conserved (reviewed by Wallace and Felsenfeld 2007).

Perhaps the most accepted model describing the mechanism behind enhancer-blocking insulator function is one suggesting that they provide a steric effect by dividing chromatin into topologically distinct domains across which enhancer–promoter interactions are unable to occur (Gaszner and Felsenfeld 2006). This model is based on the observation that proteins which bind enhancer-blocking insulators often come together to form clusters that are localized at the nuclear periphery, resulting in loops of DNA. However, it has recently been suggested that aggregates of the Su(Hw) protein, which bind the *gypsy* insulator element, are not, in fact, related to enhancer-blocking function (Golovnin et al. 2008). Alternatively, a subset of these proteins may act through the physical blockage of an activating signal, such as histone modification or intergenic transcription that is initiated at the enhancer and progresses towards the target promoter (reviewed by Wallace and Felsenfeld 2007).

Yet another model of enhancer-blocking insulator function proposes that insulators act as a decoy by directly interacting with the enhancer, or through attenuation of the putative signal that travels from enhancer to promoter, thereby preventing interactions between the enhancer and target promoter. Indeed, a number of similarities have been found to exist between insulators and promoters, including specific chromatin-modification signatures, the binding of

particular transcription factors and localization to distinct nuclear regions (reviewed by Raab and Kamakaka 2010), and several *Drosophila* insulators have been shown to contain promoters (Geyer 1997; Bae et al. 2002; Drewell et al. 2002). It has also been demonstrated recently that stalled promoters are more likely to impede enhancer-mediated activation of a target promoter when situated between the two than non-stalled promoters (Chopra et al. 2009). This might result from either the greater window of opportunity for enhancer–promoter interactions due to the increased dwell time of RNA polymerase II at stalled promoters or a preference by the enhancer for components of the stalled transcriptional complex. Alternatively, this phenomenon may simply be a case of the inherent selectivity of enhancers for certain types of promoters, such as those containing a downstream promoter element (Butler and Kadonaga 2001; Juven-Gershon et al. 2008), which are typical of stalled promoters.

Conclusions

The number of studies reporting mis-expression of transgenes because of enhancer–promoter interference is increasing rapidly, which highlights an imminent need for effective tools to block inappropriate interactions between enhancers and promoters in plant transformation vectors. While further research will be required to elucidate the exact mechanisms behind both enhancer–promoter interactions and enhancer-blocking insulators in plants, the identification of several sequences that exhibit potential as enhancer-blocking insulators, such as the *TBS* fragment from petunia and the *EcoR*I/*Sal*I *EXOB* fragment from bacteriophage lambda, may be of immediate practical use for minimizing enhancer–promoter interference in transgenic plants. Furthermore, since insulators are often able to function in a broad range of species (Chung et al. 1993; Gudynaite-Savitch et al. 2009), these enhancer-blocking insulators have the potential to be of use in a variety of crop species.

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