Acta Biochimica Polonica

Vol. 48 No. 3/2001

637–646

QUARTERLY

Effect of nuclear matrix attachment regions on transgene expression in tobacco plants✪

Witold Nowak✉, Magdalena Gawłowska☼, Artur Jarmołowski and Jacek Augustyniak

Department of Biopolymer Biochemistry, Adam Mickiewicz University, Poznań, Poland

Received: 22 January, 2001; revised: 6 June, 2001; accepted: 16 August, 2001

Key words: matrix attachment region, plant transformation, transgene expression

Matrix attachment regions (MARs) are thought to participate in the organization and segregation of independent chromosomal loop domains. Although there are several reports on the action of natural MARs in the context of heterologous genes in transgenic plants, in our study we tested a synthetic MAR (sMAR) with the special property of unpairing when under superhelical strain, for its effect on reporter gene expression in tobacco plants. The synthetic MAR was a multimer of a short sequence from the MAR 3′ end of the immunoglobulin heavy chain (IgH) enhancer. This sMAR sequence was used to flank the β-glucuronidase (GUS) reporter gene within the T-DNA of the binary vector pBI121. Vectors with or without the sMARs were then used to transform tobacco plants by *Agrobacterium tumefaciens*. Transgenic plants containing the sMAR sequences flanking the GUS gene exhibited higher levels of transgene expression compared with transgenic plants which lacked the sMARs. This effect was observed independently of the position of the sMAR at the 5′ side of the reporter gene. However, variation of the detected transgene expression was significant in all transformed plant populations, irrespective of the construct used.

Most genes whose expression has been studied in transgenic plants are generally expressed in appropriate patterns. However, transgene expression can vary within an extremely wide range, often showing only a very low level [1, 2]. Variation in transgene expression is frequently attributed to corresponding variation in the transcription potential of different chromosomal insertion sites.

DNA sequences called scaffold/matrix attachment regions (S/MARs) are involved in the structural and functional organization of

✪Supported by the State Committee for Scientific Research (KBN, Poland) grant No. 6P04A03511

☼Present address: Institute of Plant Genetics, Polish Academy of Sciences, Strzeszyńska 34, 61-479 Poznań, Poland.

✉Corresponding author: Witold Nowak, Department of Biopolymer Biochemistry, Adam Mickiewicz University, Międzychodzka 5, 60-371 Poznań, Poland; e-mail: nowak@main.amu.edu.pl

Abbreviations: ARS, autonomously replicating sequence; BUR, base-unpairing region; GUS, β-glucuronidase; IgH, immunoglobulin heavy chain; MAR, matrix attachment region; sMAR, synthetic MAR sequence; S/MARs, scaffold/matrix attachment regions; SAR, scaffold attachment region.

all eukaryotic genomes. Evolutionarily, the structures of these sequences seem to be conserved. Typically, S/MARs are located every 5 to 200 kb of sequence and are known to bind specifically to components of the nuclear scaffold, therefore suggesting that they are responsible for loop domain base formation [3, 4].

Of the MAR elements reported, many do not display extensive sequence homology. It is therefore reasonable to assume that the scaffold probably recognizes some structural features of the MAR DNA rather than a specific sequence [5].

MARs appear to be functionally conserved, since animal MARs can bind to plant nuclear scaffolds and *vice versa* [6, 7].

Most MARs have been generally characterized as AT-rich sequences. However, AT-richness *per se* is not a sufficient criterion for specific sequence recognition of MARs by specific binding proteins [7]. Their capacity to bind to the nuclear matrix is determined by the specific structure of DNA. A prominent structural characteristic of different MARs is their strong potential for extensive unpairing when subjected to superhelical strain [8, 9]. The ability to assume a stably unpaired conformation has been described for several MARs. For example, within the MAR 3′ end of immunoglobulin heavy chain (IgH) enhancer there is an AATATATTT motif that is a nucleation site for DNA unwinding [10]. Concatamerized oligonucleotides containing seven repeats of this sequence exhibited a strong affinity for the nuclear scaffold and increased SV40 promoter activity in stably transformed mouse cells [11].

In this paper we present results of our studies that concern the effect of a synthetic MAR on transgene expression in tobacco plants. The synthetic MAR sequences were used to flank the β-glucuronidase (GUS) gene whose transcription was under control of the 35S CaMV promoter in the binary vector pBI121. This construct was introduced into tobacco plants and the GUS reporter gene expression was monitored in stably transformed plants.

MATERIALS AND METHODS

Synthetic MAR construction. All DNA manipulations were performed using standard techniques as described by Sambrook *et al.* [12]. The investigated MAR was constructed similarly to that described by Bode and colleagues [11]. Complementary oligonucleotides 5′-TCTTTAATTTCTAATATATTTAGAAttc-3′ and 5′-TTCTAAATATATTAGAAATTAAAGAgaa-3′ that contain the nucleation site of unwinding (underlined) were synthesized on a Gene Assembler Plus (Pharmacia), using the phosphite triester method. After deprotection and removal from the support, the synthesized oligonucleotides were desalted using NAP-25 columns (Pharmacia) and purified by gel electrophoresis. Following phosphorylation of their 5′-ends with polynucleotide kinase T4 (Promega), the oligonucleotides were annealed into double-stranded DNA, and concatamerized by hybridization of the overlapping single-stranded ends, indicated by small letters. Multimers were separated on a 4% agarose gel, and the decamer was eluted, incubated with Klenow polymerase (Sigma) to remove single-stranded ends and cloned into the *Eco*RV site of pBluescript II KS+. DNA sequencing of the insert in the recombinant vector was performed using the *Taq*-Track Sequencing System (Promega) according to the manufacturer's instructions.

Construction of binary vectors containing sMAR elements in the T-DNA. The pBluescript II KS+ construct containing the synthetic MAR, was partially digested with *Eco*RI to achieve a heptamer of the sMAR with appropriate cohesive ends. After purification by gel electrophoresis, the heptamer was ligated into the *Eco*RI site of the binary vector pBI121, that yielded the intermediate

vector pMGM0 with the sMAR at the 3′ side of the GUS gene (Fig. 1A).

In addition, the pBluescript II KS+ construct with the sMAR was also digested with *Pvu*II, and the fragment containing the synthetic MAR (decamer) was inserted into pBR322 (Fig. 1B). The *Hin*dIII fragment with the sMAR obtained from the recombinant pBR322 construct was then inserted into the *Hin*dIII site of the intermediate vector pMGM0. Depending upon the orientation of the insert, these generated two binary vectors which were named pMGM1 and pMGM2. In pMGM1 the decamer of the sMAR was located 27 bp upstream of the 35S CaMV promoter, while in pMGM2 the sMAR element was located 2242 bp upstream of the promoter (Fig. 1C, 2).

Plant material. Tobacco (*Nicotiana tabacum* cv Samsun) plants were grown on sterilized MS (Murashige & Skoog [13]) medium at 24°C, with a 16 h photoperiod. Rooted plants from the sterile cultures were transferred to pots containing sterilized garden soil and grown under a photoperiod of 11 h at 24°C. The plants were allowed to flower and self fertilize, with the achieved seeds being collected. Surface-sterilized seeds of the F1 generation were germinated on solid MS medium.

Plant transformation. The constructs pMGM1, pMGM2 or pBI121 were transferred from *Escherichia coli* strain HB101 into *Agrobacterium tumefaciens* strain LBA4404 *via* triparental mating with *E. coli* strain HB101 containing pRK2013 [14].

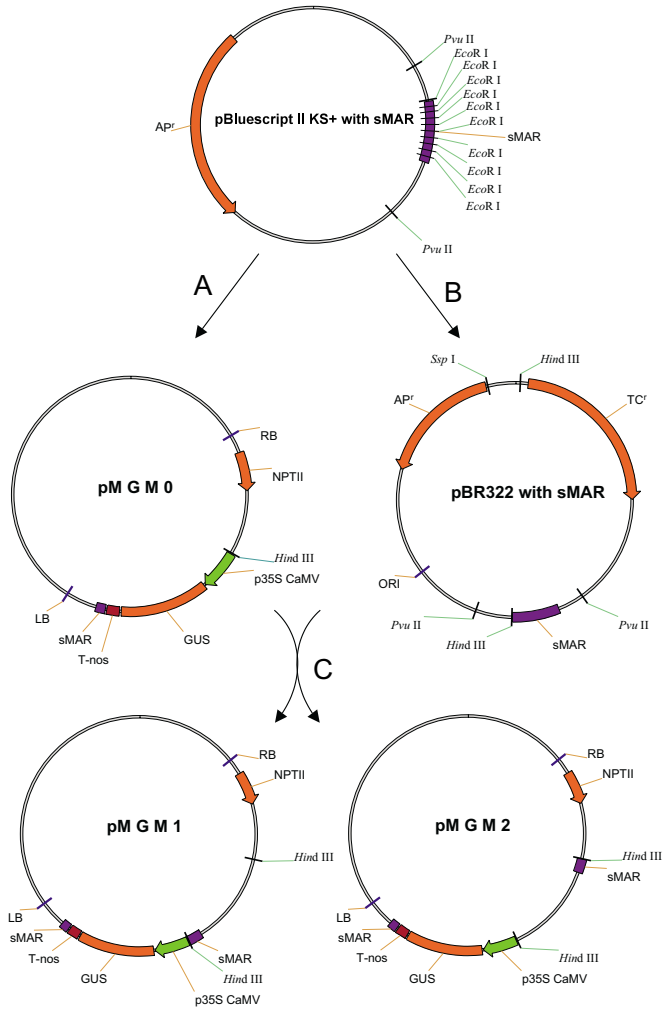

Figure 1. The construction scheme of the binary vectors used in this study.

Cloning steps of the respective constructs are described in details in Materials and Methods. Size proportions between the plasmids are not maintained.

Plasmid integrity was verified by restriction enzyme analysis. Plants were transformed by the leaf disc transformation procedure [15]. To ensure independent transformation events, regenerated shoots were taken from opposite sites of a leaf disc. Shoots that rooted in the presence of 50 μg/ml kanamycin were considered to be transgenic. The transformation proof was obtained by amplification of a 338 bp GUS gene fragment using genomic DNA isolated from the putative transgenic plant as a template. Tobacco genomic DNA used in this reaction was extracted from leaves according to the method of Chomczynski & Sacchi [16]. PCR reactions were carried out in a Biometra cycler under the following conditions: 20 s at 94°C, 20 s at 55°C and 20 s at 72°C for 36 cycles. Each PCR reaction (10 μl) contained 250 nM of each primer (5′-TGGAATTGATCAGCGTTGGTGGG-3′ and 5′-GCCAGTTCAGTTCGTTGTTCACA-3′), 0.2 mM dNTPs, 1x buffer DynaZyme (Finnzymes Oy), 0.2 U of thermostable polymerase DynaZyme (Finnzymes Oy) and 100 ng of DNA template.

Reporter gene assay. GUS activity was determined using the fluorometric assay described by Jefferson *et al.* [17] with methylumbelliferone glucuronide as substrate. GUS activity is expressed as pmoles of 4-methylumbelliferone (4-MU) formed per hour per microgram of total protein in the plant extract. Protein content was measured using the standard Bradford assay [18].

Statistical analysis. Statistical analysis was performed using Statistica 5 and Microsoft Excel 97.

RESULTS

Two complementary oligonucleotides containing an unwinding element (the AATATATTT motif) were synthesized. These oligonucleotides were hybridized to form double-stranded DNA and the obtained fragments were concatamerized. Such multimers were called synthetic MARs (sMARs). We used the sMAR concatamers to construct appropriate T-DNA vectors on the basis of the binary vector pBI121 carrying a selectable kanamycin resistance gene and the *uidA* reporter gene in the T-DNA. The reporter gene was flanked by synthetic MARs, as shown in Fig. 2. We constructed two types of vectors with sMAR sequences. In each case the *uidA* gene was flanked by the sMAR sequence in the same position at the 3′ side of the reporter gene, but the sMAR at the 5′ side of the *uidA* gene was placed either near the 35S CaMV promoter (pMGM1) or over 2 kb upstream of it (pMGM2). These two types of vectors were designated to test if the distance between the synthetic MARs or the sMAR localization relative to the promoter would change expression of the reporter gene. As a control, we used pBI121 without synthetic MARs.

Three constructs: pMGM1, pMGM2, pBI121 were used to transform tobacco plants using *Agrobacterium tumefaciens*. The independently transformed kanamycin-resistant pMGM1, pMGM2 and pBI121 plants were regenerated *in vitro* and then transferred to soil. There is some probability that primary transformants can be chimeras consisting of transformed and non-transformed tissues, thus resulting in irregular transgene expression patterns [19]. Therefore, we decided to examine the reporter gene expression in transgenic progeny to avoid the potential chimeric character of transformed parent plants and contamination of transformants by *Agrobacterium*. Following a selfing of flowering parent plants we collected seeds of six plants transformed with pBI121, eight plants transformed with pMGM1 and eight plants transformed with pMGM2. The seeding was performed on selective medium and transgenity of the obtained plants was confirmed by PCR. We obtained five progeny plants from each transformed parent plant. GUS activity was determined in leaves of all five F1 generation plants, thus in total 30 plants for the pBI121 construct and 40 plants

for each of the pMGM1 and pMGM2 constructs were analyzed. Five plants from the pMGM1 population, five plants from the pMGM2 population and seven plants from the pBI121 population contained the *uidA* transgene integrated in the genome, but a distribution free statistical test to find if a statistical difference between GUS activity levels in different populations of transgenic plants existed. The Mann-Whitney U test supports the assumption that sMARs have an effect on transgene expression in plants

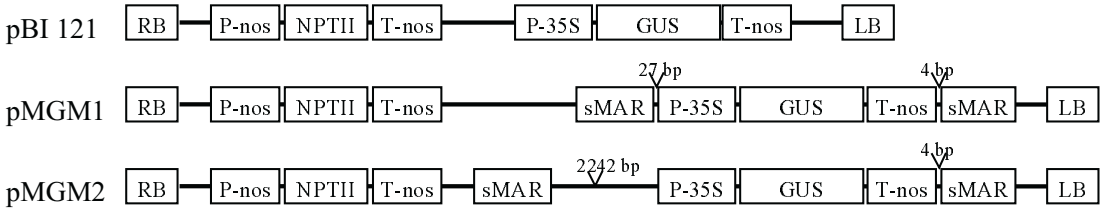

Figure 2. Schematic maps of the T-DNA region within each of the binary vectors.

The relative positions of the GUS gene and the NPTII gene are shown with respect to the left border (LB) and the right border (RB) of the T-DNA. The T-DNA regions are not drawn to scale. The numbers indicate distances (bp) between sMARs and the 35S promoter (P-35S) or sMARs and the NOS terminator (T-nos).

showed no GUS expression. The results concerning these individual plants were omitted from the statistical evaluations shown in Table 1. A wide range of GUS activities was observed in each population of transformed plants. Compared with the pBI121 transformed population, the pMGM1 and pMGM2 transformants showed no significant reduction in the variation of the *uidA* gene expression levels.

Because our raw data did not follow a normal distribution (according to Lilliefors' test and Shapiro-Wilk test), it was inappropriate to apply statistical tests dependent on a normal distribution [20]. For this reason, we used (Table 1). However, the Mann-Whitney U test showed no significant difference between populations of plants transformed with pMGM1 and pMGM2. Nap *et al.* [20] suggested that in the case of non-normal distribution it was more appropriate to use the median rather than the mean GUS activities to assess quantitatively the effect of sMARs on transgene expression. The groups of transgenic plants containing constructs with sMARs showed the median GUS activities 2- to 2.5-fold higher than the median of the control population (Table 1).

As shown in Fig. 3, frequency distribution of GUS activities in all three populations of

Table 1. Overall statistics of three populations

Gene population[a]	Mean[b]	SD[c]	CV[d]	P[e]	Median
pBI121	440	330	0.75	–	333
pMGM1	1012	1528	1.5	0.032	675
pMGM2	1655	1989	1	0.0126	819

[a]Populations are named according to the T-DNA construct the plants were transformed with; [b]pmoles 4-methyl-umbelliferone/h per μg total protein; [c]Standard deviation; [d]Coefficient of variation; [e] Probability according to the Mann-Whitney U test compared with the corresponding control population pBI121.

transformants exhibited a wide range. The variability in GUS activity in all three groups of transformants was high, although plants with sMAR elements flanking the GUS gene had a higher level of variability compared with the control group (Table 1). In addition, the plants transformed with the sMAR constructs displayed higher levels of reporter gene expression compared with plants transformed with pBI121. The GUS level ranged from 2.5 to 1046 pmoles of 4-methyl-umbelliferone/h per μg total protein in non-sMAR transgenic plant extracts, whereas up to 8373 pmoles of 4-methyl-umbelliferone/h per μg total protein was achieved in sMAR-based transgenic plant extracts.

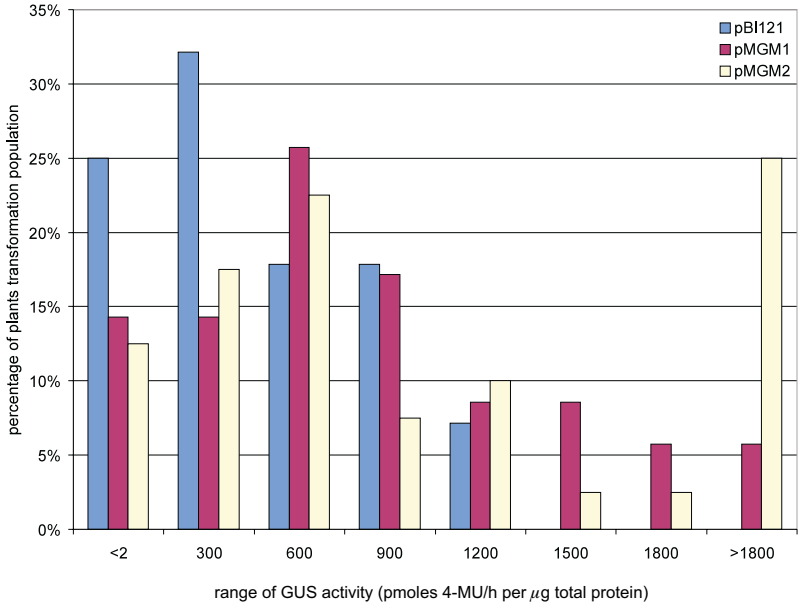

Figure 3. Frequency distribution patterns of GUS activity.

Plant populations are named according to the T-DNA that was inserted. For each population the total number of transgenic plants is given as 100%. The percent distribution of transgenic plant number of each population is plotted in the succeeding ranges of GUS activity, i.e. the GUS activities in the class labeled 300 contain all plants (in percentage) with the activity ranging between 2 and 300 pmoles of 4-methyl-umbelliferone/h per μg total protein.

DISCUSSION

The interaction of chromatin with the nuclear matrix *via* matrix attachment regions (MARs) on DNA is considered to be of fundamental importance for higher order chromatin organization and regulation of gene expression. Matrix attachment regions (MARs) are thought to participate in the organization and segregation of independent chromosomal loop domains [21, 22]. MARs as the boundary elements can define domains of independent gene regulation. Besides mediating chromatin higher-order organization, MARs may also have functional roles in gene expression. Regulatory MARs may facilitate the binding and action of regulatory proteins, perhaps providing a critical discontinuity in compacted chromatin. The location of some MARs within transcription regulatory elements suggests that MARs may serve to bring these DNA sequences into close proximity to the scaffold, thereby promoting enhancer and promoter activity by facilitating the interaction with *trans*-acting and/or transcription factors, which would assemble on the nuclear scaffold [23].

MARs may contain AT-rich sequences (called base-unpairing regions, BURs) that are capable of readily relieving negative superhelical strain by base unpairing or unwinding. BURs contain a core unwinding element and a cluster of sequences in which one of the strands consists exclusively of As, Ts, and Cs (ATC sequence context) [9]. BURs have been identified in animal as well as in plant MAR sequences [9, 24, 25]. A synthetic MAR sequence enriched in these unwinding core elements appears to increase biological activity of a linked gene in animal cells *in vivo* [11]. Considering the functional conservation of MAR elements and the fact that the same

sequence motif was found in a scaffold-associated DNA region located downstream of the pea plastocyanin gene, the goal of our research was to investigate the usefulness of a synthetic MAR for enhancing transgene expression in tobacco plants.

Our studies complement the expanding amount of data that illustrate that MARs are useful tools for enhancing transgene expression in plants and animals. In order to evaluate the synthetic MAR for functional properties in gene expression, two chimaeric gene constructs were prepared. Figure 2 shows structures of these two constructs containing the sMAR regions. We assumed that if the sMARs are responsible for loop formation, then the base pair sequence between the sMARs should be of an appropriate length. Therefore, we tested two distances between the sMAR sequences flanking the GUS gene: 5 kb and 2 kb in the pMGM2 and pMGM1 constructs, respectively. Because sMARs, independent of their arrangement in the T-DNA, showed no reduction of transgene expression variation, these sMAR elements probably did not play an insulating role for the boundaries of the loop domain. Independently of the localization of the sMAR in the T-DNA, the level of β-glucuronidase expression was increased in comparison with the control construct. This confirms that BURs are important for gene expression in plants, but perhaps more interestingly suggests that the unwinding property of MARs is not an exclusive determinant for creating independent domains. It could be that the sMAR acted as an enhancer in our experiments. This is supported by research conducted elsewhere, the results of which have shown that MARs can often stimulate transgene expression, but are not sufficient to completely over-ride genomic position effects in plants (reviewed in [26]). The enhancement of transgene expression by synthetic MARs used in our study seems to have an additive character. One could hypothesize that this sMAR augmented the positive effect of endogenous *cis*-elements on transgene transcription. Van der Geest *et al.* [27] reported that the β-phaseolin 5′ MAR placed between an enhancer and promoter of a reporter gene acted as an enhancer facilitator and not an enhancer blocker. Furthermore, other studies revealed that insertion of the T-DNA of *Agrobacterium* was usually within or near transcriptionally active loci [28] and that natural MARs located in these regions were somewhat effective in promoting high and stable transgene expression [29, 30]. Our data and results that have been reported previously by others, suggest that MARs, having the ability to unwind, have a common positive effect on transcription. It is plausible that the synthetic MAR is able to function as a topological switch by storing and releasing supercoil-generated energy during transcription. The transcriptional process generates local negative supercoiling 5′ of a transcribed gene and positive supercoiling 3′ of it.

Other studies on MAR function in plant transgene expression have provided interesting but unequivocal conclusions. The effect of any particular MAR on transgene expression is observed only after integration of the transgene with the plant genome. According to the loop model, MARs play a role in increasing transgene expression and reducing variability of that expression between independent transformants. Enhancement of transgene expression in stably transformed plant cell lines or whole plants has been reported for the soybean Gmhsp17.6.L [31], yeast ARS1 [32, 33], tobacco Rb7 [34–36], tomato HSC80 [37], bean β-phaseolin [24], chicken lysozyme [38], pea vicilin and *Arabidopsis thaliana* [39] MARs. In a few cases only a very slight or no positive effect of a MAR element on transgene expression levels in plants has been reported [6, 39]. In the majority of studies, MARs increased expression levels two- to nine-fold in whole plants and up to 60-fold in tobacco cell cultures [24, 31–39, 40]. There are several possible explanations for the reported differences in the magnitude of the MAR-mediated augmentation of transgene ex-

pression. These include: the transformation method, vector design, inheritance features of the MAR and the type of tissue that is used for transformation. The second effect of MARs as boundary elements that has been reported is the normalization of transgene expression. Boundary elements placed in a gene-flanking arrangement should insulate the gene from regulatory elements, such as enhancers or silencers, that reside outside the MAR-defined domain. This should result in the MAR being able to decrease the variability of transgene expression and even lead to a copy number-dependent expression. In the majority of studies to date, the decrease of the variability of transgene expression was rather moderate. Furthermore, the expression was rarely dependent on the copy number of the transgene. Only the presence of the chicken lysozyme A element MAR in transgene constructs significantly, up to 7-fold, reduced variability between transformation events by eliminating low-level expressing plants, but without affecting maximal transgene expression levels [38, 40, 41]. Although a normalization in reporter gene expression was obtained, variation was still evident among independent plants containing transgenes flanked by MARs. This was attributed mostly to environmental or developmental influences. On the other hand, any MAR element may play various functions. A lack of reduction in variation that has been reported for some MARs illustrates that not all of these elements have boundary functions or the ability to eliminate transgene silencing.

In summary, the results from different studies are not unequivocal. Some MARs have both general and specific properties. This heterogeneity can complicate functional analysis, because results obtained with different MAR sequences may not be comparable. In this study a synthetic MAR element was used that exhibited only one of the structural features characteristic for this group of DNA elements. Therefore, our results can only be associated with the potential of MARs for unpairing and the effects that this feature of MARs may have on plant transgene expression.

The authors wish to especially thank Jason A. Able, Agata Rapińska and Artur Rogowski for the helpful and critical review of the manuscript.

REFERENCES

1. Peach, C. & Velten, J. (1991) Transgene expression variability (position effect) of CAT and GUS reporter genes driven by linked divergent T-DNA promoters. *Plant Mol. Biol.* **17**, 49–60.

2. Walters, D.A., Vetsch, C.S., Potts, D.E. & Lundquist, R.C. (1992) Transformation and inheritance of a hygromycin phosphotransferase gene in maize plants. *Plant Mol. Biol.* **18**, 189–200.

3. Gasser, S.M., Amati, B.B., Cardenas, M.E. & Hofmann, J.F.-X. (1989) Studies on scaffold attachment sites and their relation to genome function. *Int. Rev. Cytol.* **119**, 57–96.

4. Boulikas, T. (1993) Nature of DNA sequences at the attachment regions of genes to the nuclear matrix. *J. Cell Biochem.* **52**, 14–22.

5. Laemmli, U.K., Käs, E., Poljak, L. & Adachi, Y. (1992) Scaffold-associated regions: *cis*-acting determinants of chromatin structural loops and functional domains. *Curr. Opin. Genet. Dev.* **2**, 275–285.

6. Breyne, P., Van Montagu, M., Depicker, A. & Gheysen, G. (1992) Chracterization of a plant scaffold attachment region in a DNA fragment that normalizes transgene expression in tobacco. *Plant Cell* **4**, 463–471.

7. Mielke, C., Kohwi, Y., Kohwi-Shigematsu, T., & Bode, J. (1990) Hierarchical binding of DNA fragments derived from scaffold-attached regions: Correlation of properties *in vitro* and function *in vivo*. *Biochemistry* **29**, 7475–7485.

8. Benham, C., Kohwishigematsu, T. & Bode, J. (1997) Stress-induced duplex DNA destabilization in scaffold/matrix attachment regions. *J. Mol. Biol.* **274**, 181–196.

9. Kohwi-Shigematsu, T. & Kohwi, Y. (1997) High unwinding capability of matrix attachment regions and ATC-sequence context-specific MAR-binding proteins; in *Nuclear Structure and Gene Expression* (Bird, R.C., ed.) pp. 111–144, Academic Press, San Diego.

10. Kohwi-Shigematsu, T. & Kohwi, Y. (1990) Torsional stress stabilizes extended base unpairing in supressor sites flanking immunoglobin heavy chain enhancer. *Biochemistry* **29**, 9551–9560.

11. Bode, J., Kohwi, Y., Dickinson, L., Joh, T., Klehr, D., Mielke, C. & Kohwi-Shigematsu, T. (1992) Biological significance of unwinding capability of nuclear matrix-associating DNAs. *Science* **255**, 195–197.

12. Sambrook, J., Maniatis T. & Fritsch, E.F. (1989) *Molecular Cloning. A Laboratory Manual.* 2nd Edn., Cold Spring Harbor Laboratory.

13. Murashige, T. & Skoog, F. (1962) A revised medium for rapid growth and bio-essays with tobacco tissue cultures. *Physiol. Plant.* **15**, 473–497.

14. Bevan, M.W. (1984) Binary *Agrobacterium* vectors for plant transformation. *Nucleic Acids Res.* **12**, 8711–8721.

15. Horsh, R.B., Fry, J., Hoffmann, N., Niedermeyer, J., Rogers, S.G. & Fraley, T.F. (1988) Leaf disc transformation; in *Plant Molecular Biology Manual*, pp. 1–9, Kluwer Academic Publisher, London.

16. Chomczynski, P. & Sacchi, N. (1987) Single step method of RNA isolation by acid guanidinium thiocyanate – phenol – chloroform extraction. *Anal. Biochem.* **162**, 156–159.

17. Jefferson, R., Kavanagh, T. & Bevan, M. (1987) GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* **13**, 3901–3907.

18. Bradford, M.M. (1976) A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

19. Dong, J.-Z. & McHughen, A. (1993) Transgenic flax plants from *Agrobacterium* mediated transformation: Incidence of chimeric regenerants and inheritance of transgenic plants. *Plant Cell* **91**, 139–148.

20. Nap, J.-P., Kiezer, P. & Jansen, R. (1993) First-generation transgenic plants and statistics. *Plant Mol. Biol. Rep.* **11**, 156–164.

21. Razin, S.V. (1999) Chromosomal DNA loops may constitute basic units of the eukaryotic genome organization and evolution. *Crit. Rev. Eukaryot. Gene Expr.* **9**, 279–283.

22. Paul, A.L. & Ferl, R.J. (1999) Higher-order chromatin structure: Looping long molecules (Review). *Plant Mol.* **41**, 713–720.

23. Nardozza, T.A., Quigley, M.M. & Getzenberg, R.H. (1996) Association of transcription factors with the nuclear matrix. *J. Cell Biochem.* **61**, 467–477.

24. Van der Geest, A.H.M., Hall, G.E., Spiker, S. & Hall, T.C. (1994) The beta-phaseolin gene is flanked by matrix attachment regions. *Plant J.* **6**, 413–423.

25. Paul, A.L. & Ferl, R.J. (1993) Osmium tetroxide footprinting of a scaffold attachment region in the maize Adh1 promoter. *Plant Mol. Biol.* **22**, 1145–1151.

26. Holmes-Davis, R. & Comai, L. (1998) Nuclear matrix attachment regions and plant gene expression (review). *Trends Plant Sci.* **3**, 91–97.

27. Van der Geest, A.H.M. & Hall, T.C. (1997) The beta-phaseolin 5′ matrix attachment region

acts as an enhancer facilitator. *Plant Mol. Biol.* **33**, 553–557.

28. Koncz, C., Martini, N., Mayerhofer, R., Koncz-Kalman, Z., Korber, H., Redei, G.P. & Schell, J. (1989) High-frequency T-DNA-mediated gene tagging in plants. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8467–8471.

29. Dietz, A., Kay, V., Schlake, T., Landsmann, J. & Bode, J. (1994) A plant scaffold attached region detected close to a T-DNA integration site is active in mammalian cells. *Nucleic Acids Res.* **22**, 2744–2751.

30. Iglesias, V.A., Moscone, E.A., Papp, I., Neuhuber, F., Michalowski, S., Phelan, T., Spiker, S., Matzke, M. & Matzke, A.J.M. (1997) Molecular and cytogenetic analyses of stably and unstably expressed transgene loci in tobacco. *Plant Cell* **9**, 1251–1264.

31. Schöffl, F., Schroder, G., Kliem, M. & Rieping, M. (1993) An SAR-sequence containing 395 bp-DNA fragment mediates enhanced, gene-dosage-correlated expression of a chimaeric heat shock gene in transgenic tobacco plants. *Transgenic Res.* **2**, 93–100.

32. Allen, G.C., Hall, G.E., Childs, L.C., Weissinger, A.K., Spiker, S. & Thompson, W.F. (1993) Scaffold attachment regions increase reporter gene expression in stably transformed plant cells. *Plant Cell* **5**, 603–613.

33. Vain, P., Worland, B., Kohli, A., Snape, J.W., Christou, P., Allen, G.C. & Thompson, W.F. (1999) Matrix attachment regions increase transgene expression levels and stability in transgenic rice plants and their progeny. *Plant J.* **18**, 233–242.

34. Allen, G.C., Hall, G., Michalowski, S., Newman, W., Spiker, S., Weissinger, A.K. & Thompson, W.F. (1996) High-level transgene expression in plant cells: Effects of a strong scaffold attachment region from tobacco. *Plant Cell* **8**, 899–913.

35. Ulker, B., Allen, G.C., Thompson, W.F., Spiker, S. & Weissinger, A.K. (1999) A tobacco matrix attachment region reduces the loss of transgene expression in the progeny of transgenic tobacco plants. *Plant J.* **18**, 253–263.

36. Able, J.A. & Godwin, I.D. (2001) 'Sorghum transformation using the Rb7 matrix attachment region (MAR): *In vitro* and in planta analysis'. *Field Crops Res.* in press.

37. Chinn, A.M. & Comai, L. (1996) The heat shock cognate 80 gene of tomato is flanked by matrix attachment regions. *Plant Mol. Biol.* **32**, 959–968.

38. Mlynarova, L., Loonen, A., Heldens, J., Jansen, R.C., Keizer, P., Stiekema, W.J. & Nap, J.P. (1994) Reduced position effect in mature transgenic plants conferred by the chicken lysozyme matrix-associated region. *Plant Cell* **6**, 417–426.

39. Liu, J.W. & Tabe, L.M. (1998) The influences of two plant nuclear matrix attachment regions (MARs) on gene expression in transgenic plants. *Plant Cell Physiol.* **39**, 115–123.

40. Mlynarova, L., Jansen, R.C., Conner, A.J., Stiekema, W.J. & Nap, J.P. (1995) The MAR-mediated reduction in position effect can be uncoupled from copy number-dependent expression in transgenic plants. *Plant Cell* **7**, 599–609.

41. Mlynarova, L., Keizer, L.C.P., Stiekema, W.J. & Nap, J.-P. (1996) Approaching the lower limits of transgene variability. *Plant Cell* **8**, 1589–1599.