In synergy with various *cis*-acting elements, plant insterstitial telomere motifs regulate gene expression in *Arabidopsis* root meristems

Alexandra Manevski, Georges Bertoni[1], Claude Bardet, Dominique Tremousaygue, Bernard Lescure*

Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes, CNRS-INRA UMR 215, P.O. Box 27, 31326 Castanet-Tolosan, France

Received 24 July 2000; revised 8 September 2000; accepted 9 September 2000

Edited by Ned Mantei

Abstract The *telo*-box, an interstitial telomere motif, was shown to regulate gene expression in root meristems, in synergy with a *cis*-acting element involved in the activation of expression of plant *eEF1A* genes, encoding the translation elongation factor EF1A, and of several ribosomal protein genes. We demonstrate here that the *telo*-box is also required for transcription activation by two other *cis* elements present within the promoter of genes encoding the acidic ribosomal protein rp40 and the proliferating cell nuclear antigen respectively. The control of gene expression by *telo*-boxes during cell cycle progression in *Arabidopsis* root meristems is discussed. A parallel is drawn with the function of telomeric sequences in *Saccharomyces cerevisiae*.

Key words: Interstitial telomere motif; Plant; Cell cycle; G1/S phase; *Arabidopsis thaliana*

1. Introduction

In addition to telomere repeats found at the end of chromosomes, more or less degenerated telomere sequences are often observed at interstitial sites within the genome [1,2]. In *Arabidopsis*, plant interstitial telomere motifs, or *telo*-boxes (AAACCCTAA), found within the 5′ region of most plant genes encoding components of the translational apparatus, are involved in the activation of gene expression in root primordia [3]. This regulatory effect is observed when a *telo*-box is inserted upstream or downstream of the transcription initiation site and occurs synergistically with regulation by the *tef*-box, a *cis*-acting element involved in the activation of plant *eEF1A* genes, encoding the translation elongation factor EF1A, and of several ribosomal protein genes [4–6]. These results suggest that the regulation of this process in plants is similar to that achieved by Rap1p in *Saccharomyces cerevisiae*. In the yeast *S. cerevisiae*, the coordinated expression of ribosomal protein is primarily exerted at the level of transcription [7,8]. Rap1p is a DNA binding protein that has pleiotropic and context-dependent effects on chromosome functions [9]. Rap1p binds to a well-defined recognition sequence showing similarities with the yeast telomere repeat $[(C_{1-3}A)_n]$. The pleiotropic functions reported for the Rap1p protean regulator in yeast suggest that two of the major systems involved in growth and cellular division, namely ribosome formation and chromosome integrity during replication by telomere maintenance, may be connected. The function played by Rap1p in *S. cerevisiae* in telomere maintenance may be fulfilled by related proteins in other eukaryotic cells [10–12]. However, beside the role of these proteins in telomere metabolism, only Rap1p has been reported to participate in the regulation of the expression by interacting with interstitial telomere-like sequences in *S. cerevisiae*. A homology search on plant DNA sequences available in databases (D. Tremousaygue, unpublished data) indicated that the association of *telo*-boxes with promoter regions is not restricted to genes encoding components of the translational apparatus. Interestingly, a class of genes known to be overexpressed in cycling cells (e.g. PCNA (proliferating cell nuclear antigen), ribonucleotide reductase, etc.), a characteristic of genes encoding ribosomal proteins, also contain a *telo*-box (D. Tremousaygue, unpublished data, and Fig. 1B,C). Here we have used a set of characterized regulatory elements reported to be involved in the activation of gene expression in meristems in order to analyze whether these elements could act in synergy with a *telo*-box. Our results suggest that the *telo*-box is indeed involved in the potentiation of transactivation achieved by distinct transcriptional factors during the G1/S transition.

2. Materials and methods

2.1. Histochemical analysis of GUS activity

Histochemical GUS staining was performed essentially according to [13]. Whole young seedlings were immersed in a 0.5 mg/ml 5-bromo-4-chloro-indolyl-β-D-glucuronic acid solution and infiltrated under vacuum conditions for 2 h. After 16 h incubation at 37°C, the plant material was cleared at high temperature in 70% (v/v) ethanol. For each construct, 10 independent transformants (F0) were selected and 10 day old F2 plants were observed for histochemical GUS activity using Nomarski optics.

2.2. Transgene construction and plant transformation

DNA constructions were performed according to [14] and verified by sequence analysis. A −43 *eEF1A* gene promoter lacking the 5′ IVS sequence fused to a *GUS* reporter gene encoding the β-glucuronidase at the ATG within the *Nco*I site was first introduced into pUC19. A *Pst*I–*Hin*dIII fragment corresponding to the *eEF1A* gene 3′ end was then introduced at the end of the GUS coding sequence. A *Pst*I–*Kpn*I synthetic oligonucleotide containing either a *telo*-box (in bold) (5′-TGCAG**AAACCCTAA**CTCG-3′) or a mutated sequence (in bold) (5′-TGCAG**GAATTCGAG**CTCG-3′) was added in position −43 to generate respectively the Telo and the ΔTelo (Fig. 2A) constructs. A *Xba*I–*Cla*I fragment containing a *trap40*-box (in bold) (5′-CTAGATTAAAAT**TTGGGTAACCGAGTTTGGGGGTAGAATAGACA**TTTTGCAAT-3′) from the *Arabidopsis thaliana Ap40* gene promoter [15] was introduced in the Telo and ΔTelo constructs, in position −77

*Corresponding author. Fax: (33)-561285061.
E-mail: lescure@toulouse.inra.fr

[1] Present address: Laboratoire de Biotechnologie et Amélioration des Plantes, INP-ENSAT, Chemin de Borte-Rouge, 31326 Castanet-Tolosan, France.

0014-5793/00/$20.00
PII: S0014-5793(00)02056-1

upstream of the transcription initiation site, to obtain the Trap-Telo and the Trap-ΔTelo constructs (Fig. 2B). A *Xba*I–*Cla*I fragment containing the regulatory elements driving the cell cycle-dependent expression of the *Arabidopsis H4* histone gene (5′-CTAGACGATTCTTAGTGGCTATCACTGCCATCACGCGGATCACTAATATGAACCGTCGATTAAAACAGATCGACGGTTTATACATCATTTTATGGTACACACGGATCGATATCTCAGCCGTTAGATTTAATAT-3′) [16] was introduced in the Telo and ΔTelo constructs to obtain the H4-Telo and the H4-ΔTelo constructs (Fig. 2C). This fragment was obtained by PCR using the oligonucleotides 5′-TCTAGACGATTCTTAGTGGCT-3′ and 5′-GAGTCGGCAATCTAAATTATAGCTA-3′ as primers and a plasmid containing the regulatory elements driving the cell cycle-dependent expression of the *Arabidopsis H4* histone gene provided by Nicole Chaubet-Gigot as a template. A *Xba*I–*Cla*I fragment containing a *IIa-IIb*-box (in bold) (5′-CTAGACTGCCAG**GTGGGCCCGT**ACTCCACC**ATGGTCCCAC**ATGAT-3′) from the rice *PCNA* gene promoter [17] was introduced in the Telo and ΔTelo constructs to obtain the IIa-IIb-Telo and the IIa-IIb-ΔTelo constructs (Fig. 2D). These constructs, shown in Fig. 2, were introduced into the *Bam*HI site of pDHB321.1 binary vector (obtained from D. Bouchez, INRA, Versailles), and *Agrobacterium* strain C58C1-pMP90 was transformed. In planta *Agrobacterium*-mediated gene transfer, by immersion of *Arabidopsis* inflorescences (adapted from [18]), was used to generate transgenic *Arabidopsis* from the Wassilevskija ecotype (Ws).

3. Results

The *trap40*-box is a *cis*-acting element involved in the activation of the *Arabidopsis Ap40* gene, encoding an acidic ribosomal protein [15]. This box presents similarities to the *tef*-box found in the *eEF1A* gene promoter. The promoter of the *Ap40* gene also contains two *telo*-boxes located upstream of the transcription initiation site (Fig. 1A). Here, we analyze the effect of the *telo*-box on the *trap40*-dependent expression of a GUS reporter gene in root primordia of transgenic *Arabidopsis*. A synthetic oligonucleotide containing the *trap40* DNA sequence was inserted, with or without a *telo*-box (Fig. 2B), upstream of a −43 *Arabidopsis eEF1A* minimal promoter deleted of its 5′ IVS located between the transcription initiation site and the translation initiation codon [3]. These chimeric promoters were fused upstream of the GUS reporter gene, inserted in a binary vector and used to obtain transgenic *Arabidopsis* plants. For each construct, seven independent transgenic plants were analyzed for the expression of GUS in root primordia. We observe, as already shown [3], that the construct with the *telo*-box alone is not able to direct GUS expression in root primordia (Fig. 3B, A). Then we show (Fig. 3A,B, B,C) that the *trap40* element is required for strong activation of GUS expression in root cycling cells and that this activation occurs in a cooperative manner with the *telo*-box.

In contrast to genes encoding components of the translational apparatus, none of the known plant histone genes contains a *telo*-box within its 5′ region (B. Lescure, unpublished data). These genes are specifically expressed during the S phase of cycling cells and regulatory elements involved in their expression have been characterized [16]. Using a similar approach we examined whether histone gene regulatory elements could act in a synergistic manner with a *telo*-box. A 133 bp DNA fragment containing the regulatory elements driving the cell cycle-dependent expression of the *Arabidopsis H4* histone gene [16] was cloned instead of the *trap40* sequences within the −43 minimum promoter (Fig. 2C). The cloned sequence was sufficient to activate the expression of GUS in root primordia of transgenic plants, in the presence or in the absence of a *telo*-box (Fig. 3A,B, D,E). However, we observed a slight positive effect of the *telo*-box on the activation of gene expression.

Among the *Arabidopsis* genes harboring a *telo*-box within their 5′ regions, the *PCNA* gene is of particular interest. First, in animals as in plants, PCNA is overexpressed in cycling cells during the G1/S phase transition, prior to DNA synthesis. Second, we observed that all known plant sequences (genomic or cDNA sequences) corresponding to a *PCNA* gene contain a *telo*-box found at approximately the same location between

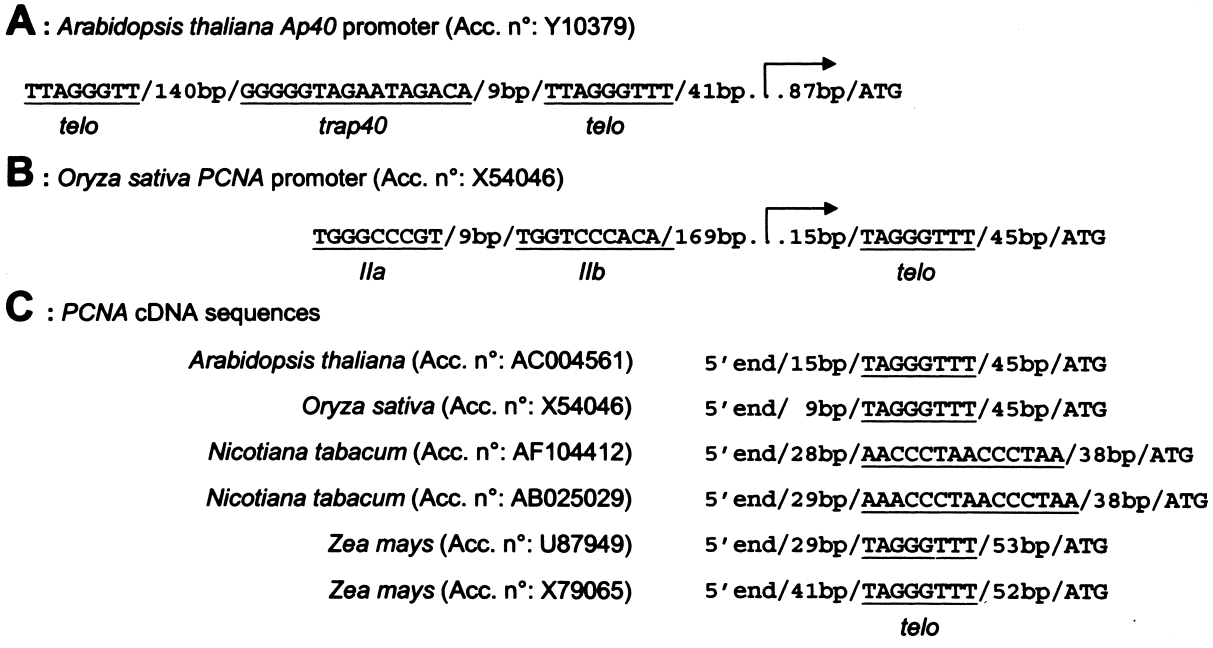

Fig. 1. *Telo*-boxes present in different promoters. A: Promoter fragment of the *A. thaliana Ap40* gene encoding an acidic ribosomal protein. B: Promoter fragment of the *Oryza sativa PCNA* gene encoding the proliferating cell nuclear antigen. C: cDNA fragments corresponding to the *PCNA* gene from various species. Acc. n°.: GenBank accession number.

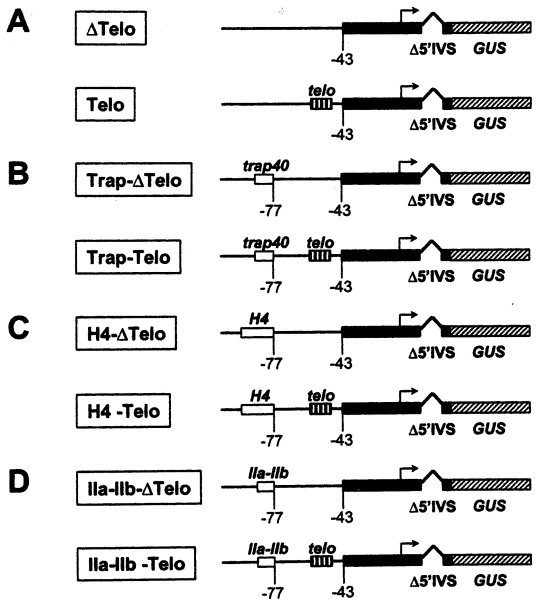

Fig. 2. Diagram of promoter fragments fused to the *GUS* reporter gene. A: Chimeric promoters with or without a *telo*-box (vertically striped box) used to obtain the different constructs. Lines represents synthetic sequences. Filled black boxes represent fragments of the wild-type promoter in which the deleted intron (Δ5′ IVS) is positioned. Hatched boxes represent the *GUS* gene encoding the β-glucuronidase. B–D: Synthetic sequences containing the *trap40* element, the regulatory elements driving the cell cycle-dependent expression of the *Arabidopsis H4* histone gene or the IIa-IIb element (white box) were inserted in the Telo and ΔTelo constructs at position −77 upstream of the transcription start site.

the transcription initiation site and the translation initiation codon (Fig. 1C). Third, among the plant genes that are expressed during the G1/S transition, the rice *PCNA* gene is one of the best characterized in studies of transcriptional control [17,19,20]. The redundant *IIa* and *IIb cis*-acting elements identified in the rice *PCNA* promoter are essential for meristematic tissue-specific expression and functional in transgenic tobacco, suggesting conservation of the *IIa-IIb*-dependent regulation process in monocots and dicots. These promoter elements are specifically recognized by bHLH transcriptional factors (PCF factors) [20] and are clearly distinct from the *tef cis*-acting element [3–6]. Using the same experimental approach as described above for the *trap40* and *H4* regulatory elements, we introduced the rice *IIa-IIb* element within the −43 minimum promoter with or without a *telo*-box. The *IIa-IIb* sequence is able to activate the expression in root primordia only in the presence of a *telo*-box (Fig. 3A,B, F,G), as observed in the case of *tef*- and *trap40*-dependent expression [3].

4. Discussion

Our initial studies on the frequency of appearance of *telo*-boxes, together with their mode of distribution within the *Arabidopsis* genome and the characterization of a plant DNA binding activity specifically interacting with these motifs [2], suggested a biological function for these elements. We recently reported that the *telo*-box indeed acts synergistically with *tef*-dependent activation in cycling cells of *Arabidopsis* root primordia [3]. In the present work we show that this synergistic effect can be extended to other genes whose expression is under the control of a related (*trap40*-box) or a distinct *cis*-acting element (*IIa-IIb*-box) [15,17]. As for ribosomal genes, the expression of *PCNA* genes is correlated with the proliferative state of cells and is induced by earlier events at the G1/S boundary of the cell cycle [21]. The expression of histone H4 genes is also correlated with the proliferative state but their activation occurs later, at the beginning of the S phase [16]. Since, in contrast to what is observed for regulatory elements of r-protein and *PCNA* genes, the activation of a minimum promoter by *Arabidopsis H4 cis*-acting element is not dependent on a *telo*-box (Fig. 3A,B, D,E), it is tempting to speculate that the role of *telo*-boxes in the activation of expression during the cell cycle specifically concerns a subset of genes activated during the G1/S transition. This assumption is reinforced by the observation that the association of one or several *telo*-boxes with the 5′ region of plant genes

A

Constructs	Transgenic plant 1	2	3	4	5	6	7
Trap-ΔTelo	-	+	-	-	-	++	+
Trap-Telo	+++	+++	+++	++++	+++	++++	++
H4-ΔTelo	++++	+++	+++	+++	++	++	++
H4-Telo	++++	++++	+++	++++	++++	++++	++++
IIa-IIb-ΔTelo	-	-	-	-	-	-	-
IIa-IIb-Telo	+	+	+	++	+	+	++

B

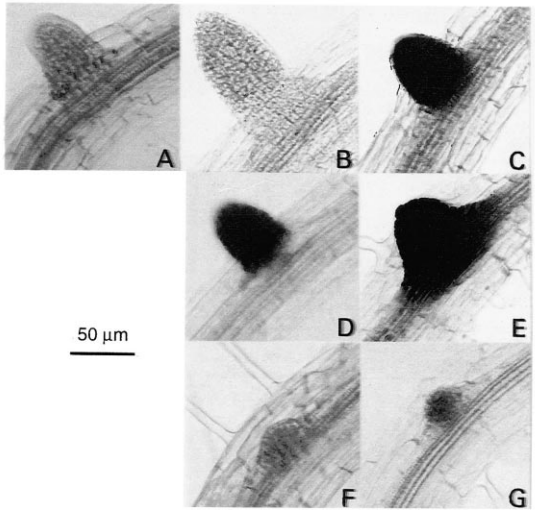

Fig. 3. A: Relative GUS activity of transformants. Seven independent transformants of each construct were observed. + denotes GUS activity (the number of pluses reflects the intensity of activity), − denotes the absence of GUS activity. B: Histochemical GUS activity in transgenic *Arabidopsis* secondary root primordia. A developing primordium is presented for each construct. A: A Telo construct (Fig. 2A) transformant is shown as negative control. B: Trap-ΔTelo transformant. C: Trap-Telo transformant. D: H4-ΔTelo transformant. E: H4-Telo transformant. F: IIa-IIb-ΔTelo transformant. G: IIa-IIb-Telo transformant.

encoding components of the translational apparatus is the rule rather than the exception [3] and that all known plant genes encoding PCNA contain a *telo*-box (Fig. 1B,C). In contrast, none of the plant genes encoding histones contains this element (B. Lescure, unpublished data). As *PCNA* genes should be induced at each successive cell cycle in cycling cells whereas ribosomal genes are certainly induced at the G1/S transition when quiescent cells re-enter the cell cycle, one point could be raised concerning the implication of the *telo*-box in both types of transition. The observation of older root meristems (data not shown) that express the *gus* gene in constructs also having the *IIa-IIb/telo* promoter or the *trap40/telo* promoter made us think that in the promoter configurations we used the *telo*-box acts as a regulator at each cycle of the cell. The molecular process involved in the synergistic activation of expression by the *telo*-box remains to be elucidated. One way to progress in the understanding of this process is by the characterization of *trans*-acting elements interacting with a *telo*-box. Plant cellular extracts contain a DNA binding component which specifically interacts with the motif AAACCCTAA [2] and the screening of an *Arabidopsis* expression cDNA library with this multimerized sequence has allowed a cDNA encoding a protein related to the animal Purα protein (AtPurα) to be isolated [3]. Recent studies have implicated animal Purα in the control of promoter activity of different cellular and viral genes. In several cases this control occurs in cooperation with transcriptional factors ([22] and references therein), a process similar to that reported for Rap1p in yeast [9]. The Rap1p binding sites are not themselves sufficient for a high level of transcription of ribosomal protein or glycolytic genes; additional *cis*-acting elements appear to be required. Rap1p participates in the activation of expression by recruiting transcriptional activators at promoters by a direct protein–protein interaction [23] or by opening chromatin [24]. It is noteworthy that several reports suggest that animal Purα is involved in the control of the cell cycle progression by interacting with pRb [25] and E2F-1 [26]. The hypophosphorylated form of the retinoblastoma protein, Rb, associates with the human Purα protein and negatively regulates the binding to its DNA recognition element [25]. The Rb family of proteins play an important role in the mammalian cell cycle by controlling transit of G1 [27,28]. The presence of a Rb homologue in plants [29] leads us to imagine that the AtPurα interaction with Rb could modulate the functionality of Purα in late G1. Comparison of the regulatory system we are studying here with animal systems reveals interesting avenues for further work. Another interesting question is whether AtPurα interacts with plant telomeres. Studies are in progress to examine such possibilities. Whatever the answer, the data reported in the present work confirm and extend the observation that plant interstitial telomere motifs are involved in the regulation of plant gene expression in cycling cells. This leads to the possibility that, like Rap1p in yeast, common molecular elements could participate in both regulation of gene expression and telomere metabolism in plants. Could this observation be extended to animal organisms? Searches for telomere motifs associated with genes encoding components of the translational apparatus in *Homo sapiens* and *Caenorhabditis elegans* (B. Lescure, data not shown) do not reveal a significant statistical association of corresponding telomere motifs with this class of genes. However, the results of this analysis do not exclude the possibility of a similar functional relationship between control of gene expression and telomere functions in animals. In this respect, it must be noted that the consensus sequence of Rap1p binding sites can significantly diverge from the yeast telomere repeat [30].

Acknowledgements: We are grateful to Dr. Nicole Chaubet-Gigot for providing a plasmid containing the regulatory elements of the *Arabidopsis H4* histone gene promoter [16]. We would like to thank Dr. Nigel Grimsley for critical reading of the manuscript. A.M. holds a grant from the Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche.

References

[1] Stoll, S., Zirlich, T., Maerker, C. and Lipps, H.J. (1993) Nucleic Acids Res. 21, 1783–1788.
[2] Regad, F., Lebas, M. and Lescure, B. (1994) J. Mol. Biol. 239, 163–169.
[3] Tremousaygue, D., Manevski, A., Bardet, C., Lescure, N. and Lescure, B. (1999) Plant J. 20, 1–9.
[4] Curie, C., Axelos, M., Bardet, C., Atanassova, R., Chaubet, N. and Lescure, B. (1993) Mol. Gen. Genet. 238, 428–436.
[5] Regad, F., Hervé, C., Marinx, O., Bergounioux, C., Tremousaygue, D. and Lescure, B. (1995) Mol. Gen. Genet. 248, 703–711.
[6] Manevski, A., Bardet, C., Tremousaygue, D. and Lescure, B. (1999) Mol. Gen. Genet. 261, 892–900.
[7] Planta, R.J., Gonçalves, P.M. and Mager, W.H. (1995) Biochem. Cell. Biol. 73, 825–834.
[8] Kressler, D., Linder, P. and de la Cruz (1999) J. Mol. Cell. Biol. 19, 7897–7912.
[9] Shore, D. (1994) Trends Genet. 10, 408–412.
[10] Shore, D. (1997) Trends Biochem. Sci. 22, 233–235.
[11] Van Steensel, B. and de Lange, T. (1997) Nature 385, 740–743.
[12] Cooper, J.P., Nimmo, E.R., Allshire, R.C. and Cech, T.R. (1997) Nature 385, 744–747.
[13] Jefferson, R.A. (1987) Plant Mol. Biol. Rep. 5, 387–405.
[14] Sambrook, J., Fritsch E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[15] Scheer, I., Ludevid, M.D., Regad, F., Lescure, B. and Pont-Lezica, R.F. (1997) Plant Mol. Biol. 35, 905–913.
[16] Chaubet, N., Flénet, M., Clément, B., Brignon, P. and Gigot, C. (1996) Plant J. 10, 425–435.
[17] Kosugi, S., Suzuka, I. and Ohashi, Y. (1995) Plant J. 7, 877–886.
[18] Bechtold, N., Ellis, J. and Pelletier, G. (1993) C. R. Acad. Sci. Paris 316, 1194–1199.
[19] Kosugi, S., Suzuka, I., Ohashi, Y., Mukarami, T. and Arai, Y. (1991) Nucleic Acids Res. 19, 1571–1579.
[20] Kosugi, S. and Ohashi, Y. (1997) Plant Cell 9, 1607–1619.
[21] Kosugi, S., Suzuka, I. and Ohashi, Y. (1995) Plant J. 7, 877–886.
[22] Safak, M., Gallia, G.L. and Khalili, K. (1999) Mol. Cell. Biol. 19, 2712–2723.
[23] Tornow, J., Zeng, X., Gao, W. and Santangelo, G.M. (1993) EMBO J. 12, 2431–2437.
[24] Yu, L. and Morse, R.H. (1999) Mol. Cell. Biol. 19, 5279–5288.
[25] Johnson, E.M., Chen, P.L., Krachmarov, C.P., Barr, S.M., Kanovski, M., Ma, Z.W. and Lee, W.H. (1995) J. Biol. Chem. 270, 24352–24360.
[26] Darbinian, N., Gallia, G.L., Kundu, M., Shcherbik, M., Tretiakova, N., Giordano, A. and Khalili, K. (1999) Oncogene 18, 6398–6402.
[27] Goodrich, D.W., Wang, N.P., Quian, Y.W., Lee, E.Y. and Lee, W.H. (1991) Cell 67, 293–302.
[28] Weinberg, R.A. (1995) Cell 81, 323–330.
[29] Ach, R.A., Durfee, T., Miller, A.B., Taranto, P., Hanley-Bowdoin, L., Zambryski, P.C. and Gruissem, W. (1997) Mol. Cell. Biol. 17, 5077–5086.
[30] Idrissi, F.-Z. and Pina, B. (1999) Biochem. J. 1, 341 and 477–482.