Complementation of a yeast Δ*pkc1* mutant by the *Arabidopsis* protein ANT

Paola Vergani*, Piero Morandini, Carlo Soave

Department of Biology, Section of Plant Physiology and Biochemistry, University of Milano, Via Celoria, 26, 20133 Milano, Italy

Received 11 October 1996; revised version received 21 November 1996

Abstract The *Saccharomyces cerevisiae* protein kinase C homologue, PKC1, is involved in maintenance of cell integrity during polarized growth. We have used a mutant complementation approach to investigate related signal transduction pathways in higher plants. Here we report the isolation of a cDNA from *Arabidopsis thaliana* which partially suppresses the lytic defect of a Δ*pkc1* yeast strain. The encoded protein, ANT, belongs to the AP2-related gene family and is essential for ovule development. Expression in yeast of a LexA-ANT fusion protein activates transcription of a reporter gene from promoters containing *lexA* operators. Our results support the idea that ANT acts as transcriptional activator in planta.

Key words: Functional complementation; Plant; Protein kinase C; Signal transduction; Transcription factor; Yeast

1. Introduction

Bud emergence and growth in the yeast *S. cerevisiae* is a highly complex process. During well-defined phases of the cell cycle, the isotropic osmotic driving force becomes polarized through the delivery of wall-modifying enzymes and new cell surface material to specific sites [1–3]. Recent studies have shown that some of the molecular mechanisms underlying this morphogenetic process have been conserved during animal evolution and mammalian proteins can functionally substitute their yeast homologs [4–6].

The *PKC1* gene of *S. cerevisiae* encodes a homolog of the Ca^{2+}-dependent isotypes of the mammalian protein kinase C (PKC) [7]. PKC1 is thought to regulate a bifurcated pathway [8], one branch of which is a mitogen-activated protein (MAP) kinase activation cascade – a conserved functional module composed of three protein kinases acting sequentially [9,10]. This pathway has been shown to be activated in response to hypo-osmotic shock [11], during growth at high temperature [12], and in periods of polarized growth [13]. RHO1, a Ras-related G-protein which is probably involved in cytoskeleton polarization [14], has recently been shown to be necessary for PKC1 activation [15,16]. Finally, the pathway positively regulates the transcription of several genes involved in cell wall biosynthesis [3]. Thus, PKC1 is central to a complex network coordinating the correct spatial and temporal deposition of cell wall material at sites of growth.

In plants, the existence of PCK-like enzymes remains elusive but members of the ras-superfamily of GTP-binding proteins [17] and various kinases with homology to elements of MAP kinase activation cascades [18] have been identified. We performed a screen exploiting the growth defects (the cells are unviable unless grown on high osmolarity media) of a strain carrying a deletion in the *PKC1* gene (Δ*pkc1*) to identify plant proteins capable of functional interaction with the yeast signal transduction pathways. Here, we report that an *Arabidosis* protein, CKC1 (Complementing a protein Kinase C mutant) partially suppresses the lytic defect of the yeast mutant. CKC1 is identical to AINTEGUMENTA (ANT), a protein involved in ovule development and initiation of organ primordia [19,20]. We provide evidence suggesting that CKC1/ANT is an activator of transcription and discuss the possible relationships between ANT function and the yeast system.

2. Materials and methods

2.1. Strains and growth conditions

All strains used in this study are haploid derivatives of EG 123 (*Matα leu2-3,112 ura3-52 trp1-1 his4 can1r*). This wild type strain together with FL 102 [7] (*Matα EG123 Δpkc1::LEU2 (pGAL1::PKC1)*) were a gift from E. Martegani (University of Milano, Italy) and J. Thevelein (University of Leuven, Belgium). Δ*pkc1* (*Matα EG123 Δpkc1::LEU2*) was obtained from FL102: after several generations of growth under non-selective conditions (YPD+sorbitol), colonies which had lost the centromeric plasmid carrying the inducible, intact *PKC1* gene – i.e. which had lost both the ability to grow on hypo-osmotic, galactose-containing media and uracil prototrophy – were identified by replica plating. One of these colonies was used for further work. Yeast cultures were grown on either YPD (1% yeast extract, 2% bacto peptone, 2% dextrose) or YPGal (1% yeast extract, 2% bacto peptone, 2% galactose). Synthetic minimal medium (SD: 0.67% yeast nitrogen base w/o amino acids, 2% dextrose plus the appropriate supplements) was used when selection for plasmid maintenance was required. Addition of 1 M sorbitol to media provided osmotic support when necessary. All plasmids were propagated in *Escherichia coli* DH5α (except for pRI7Banti which was amplified in GM82, a *dam*$^-$ host), grown in Luria Broth+ampicillin (0.1 mg/ml) at 37°C, following standard procedures [21].

2.2. DNA manipulations and sequence analysis

The method described by Robzyk and Kassir [22] was used to rescue plasmids from yeast strains. *E. coli* transformations, PCR and cloning steps were carried out using standard molecular biology techniques [21,23]. Sequencing was performed – after subcloning the cDNA insert into the *Not*I site of pBSIISK (−) (Stratagene) – by the dideoxy chain termination method [24] using synthetic oligonucleotides, T7 DNA polymerase (Pharmacia) and denatured double-stranded DNA as template.

2.3. cDNA library and yeast transformations

The cDNA yeast expression library prepared by Minet et al. [25] was kindly sent to us by F. Lacroute (CNRS, Gif-sur-Yvette). pFL61, the library vector, is an episomic plasmid containing the *URA3* marker and in which expression of inserted cDNA is driven by the *PGK* promoter. Yeast transformations were performed following the improved high-efficiency lithium acetate method [26] with minor modifications: (i) unspecific carrier DNA addition was omitted; (ii) maximum speed pulse centrifugations were replaced by longer (5 min)

*Corresponding author (present address): Dept. Biological Sciences, Wye College, University of London, Wye, Ashford, Kent TN25 5AH, UK. Fax: (44) (1233) 813140. E-mail: pbs-pv@wye.ac.uk

PII S0014-5793(96)01399-3

centrifugations at 1200×*g* (see [27]); (iii) 1 M sorbitol was added to all solutions. Batches of competent Δ*pkc1* cells were transformed with 5 μg of library DNA each, and transformants were selected on SD plates lacking uracil but supplemented with sorbitol. After 3 days growth at 24°C, the cells were washed off the plates in 4 ml of 1 M sorbitol, 40 μl of this suspension were plated on SD lacking both uracil and sorbitol and the plates were further incubated at 24°C until osmoresistant colonies appeared.

2.4. Plasmid construction and sources

pLR1Δ1 [28] and pSH18-18 [29] were obtained from D. Stillman (University of Utah Medical Center, Salt Lake City). pBTM116, containing the complete LexA coding sequence under the control of the yeast *ADH* promoter and the *TRP1* gene as a selectable marker, was obtained from R. Mantovani (University of Milan).

The following procedure was used to construct plasmid pLAP, driving the expression of the LexA-ANT fusion protein. The insert excised from *pCKC1* was subcloned in the pBSIISK(−) *Not*I site in the 'anti' sense (relative to *lacZ* coding) obtaining plasmid p7Banti. To introduce an *Eco*RI site directly 5′ to the initial ATG, the first part of the coding sequence was amplified by PCR using primers RI (GGAATTCATGTCTTTTTG) and H1 (TTGAGTGCAGCAAGATC), complementary to nt 1075–1091 of the insert. The PCR product was cut with *Eco*RI and *Nco*I and the 393 bp fragment was then inserted back in p7Banti, between polylinker *Eco*RI site and the insert *Nco*I site (nt 474). The plasmid obtained (pRI7Banti) was amplified in the *dam*⁻ *E. coli* strain GM82, to avoid methylation at the *Bcl*I site (nt 1745). An *Eco*RI/*Bcl*I double digest yielded a 1665 bp fragment containing the entire coding region of *ANT* which was cloned into the *Eco*RI and *Bam*HI sites of pBMT116.

2.5. β-Galactosidase assays

Yeast strains were grown to late logarithmic phase in liquid SD (+sorbitol) lacking uracil and tryptophan to maintain selection for either pLR1Δ1 or pSH18-18 (bearing target genes) and for either pBMT116 or pLAP, respectively. Quantitative β-galactosidase activity measurements were performed on cell extracts as described in [23]. Three separate transformants were assayed, at least three times, for each genotype.

3. Results

3.1. Expression of the Arabidopsis ANT protein partially suppresses the osmotic fragility of a yeast pkc1 deletion mutant

A Δ*pkc1* mutant was transformed with a yeast expression library prepared from *A. thaliana* seedling mRNA ([25] 2μ-based, *URA3* as selectable marker). From an initial selection on medium without uracil, around 10^6 primary transformants were obtained. When these were subsequently screened for the ability to grow on media lacking osmotic stabilizer, we obtained 30 transformants which had become capable of forming colonies in hypoosmotic conditions. From each of these, plasmids were rescued in *E. coli* for further analysis. 23 plasmids failed to suppress the lytic phenotype when reintroduced in the Δ*pkc1* mutant with a second transformation. In contrast, 7 plasmids, deriving from at least four independent transformations, could reproducibly confer osmotic stability to Δ*pkc1*. Restriction analysis revealed that 6 of these plasmids were isolates of a similar (possibly identical) library element (*pCKC1*) containing a 1.9 kb insert. The remaining one contained a 0.8 kb insert derived from a different cDNA and further studies regarding it will be reported elsewhere.

CKC1 (genbank accession ATU44028) appeared to be identical to *AINTEGUMENTA (ANT)*. This gene was cloned by transposon and T-DNA tagging in *Arabidopsis* [19,20] (the library element we sequenced has a 38 bp longer 3′ untranslated leader and the poly(A) tail is inserted 13 bp downstream with respect to the cDNA5 sequence reported by Elliott et al.

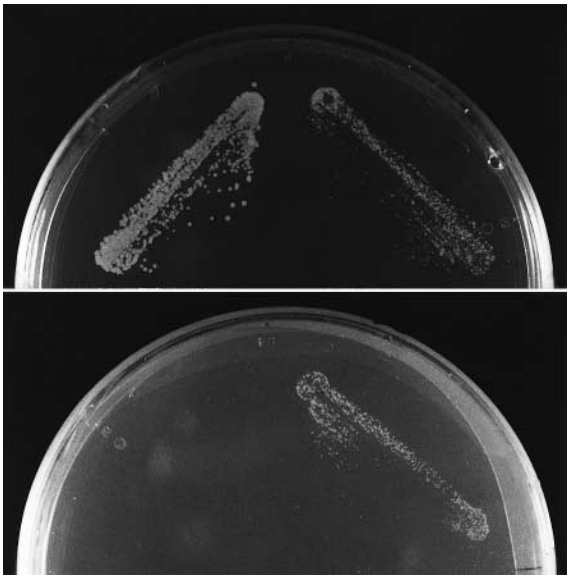
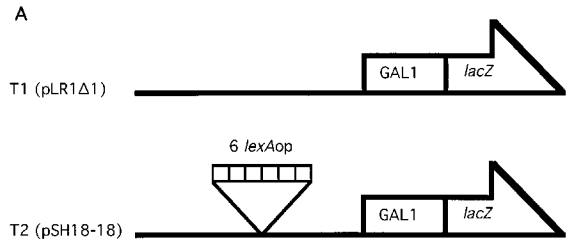


Fig. 1. Expression of *A. thaliana* ANT confers osmotic stability to a Δ*pkc1* yeast strain. Δ*pkc1 (pFL61)* (left) and Δ*pkc1 (pANT)* (right) were grown in minimal medium-uracil containing or lacking 1 M sorbitol, respectively. The late log cultures were then streaked on plates (top plate, SD lacking uracil plus 1 M sorbitol; bottom plate, as above but without sorbitol), grown for 3 days at 30°C and photographed.

[20]). The *ANT* gene contains two copies of a motif known as the AP2 domain [30], which has so far been found only in a small family of plant proteins. These motifs are thought to act as DNA-binding domains since the single copies present in the EREBP members [31] have been shown to bind specific sequences within ethylene responsive elements. The sequence with highest homology to *ANT* is a maize cDNA (gb Z47554, 83% identical amino acids in a 166-residue-long stretch encompassing the two AP2 domains and the linker between them) which was isolated by complementing the heat shock and starvation sensitivity of an *E. coli* mutant lacking a functional L-isoaspartylmethyltransferase [32].

ANT complementation of Δ*pkc1* osmosensitivity (Fig. 1) was relatively weak: after growth to late logarithmic phase in the presence of osmotic support, only 5–10% of the vital cells (i.e. cells giving rise to colonies on sorbitol-supplemented plates) survived plating on media lacking sorbitol. In other words, expression of ANT was sufficient for survival to hypotonic shock only for a subset of the vital cells. Since activation of the pathway following hypo-osmotic shock is transient [11,33] and crosstalk between PKC1 pathway and regulation of cell cycle progression has been demonstrated [3,13,34], it is probable that the different degrees of susceptibility we observe are determined by the phase of the cell cycle each cell is in upon transfer to hypotonic media. Further work using synchronized cell cultures is needed to test this hypothesis. Overexpression of ANT was slightly toxic and this growth inhibitory effect was not dependent on the Δ*pkc1* background: also wild type or Δ*mpk1* cells transformed with multicopy plasmids containing the ANT coding sequence showed a slight growth inhibition when compared to control strains transformed with the empty vector (data not shown).

We also transformed a Δ*mpk1* strain (DL456-3B,[35]). This strain carries a disruption in the MAP kinase which acts downstream to PKC1 [12] (although activation through dif-



B

Relevant genotype	Expressed protein	β-Gal. activity T1	T2
wt	LexA	0.2 ± 0.2	1.1 ± 0.1
	LexA-ANT	0.5 ± 0.1	320 ± 15
Δpkc1	LexA	0.7 ± 0.4	2.6 ± 0.4
	LexA-ANT	1.3 ± 0.2	933 ± 200

Fig. 2. LexA-ANT activates transcription from *lexA* operator containing target genes. (A) Diagrams showing relevant features of target genes. In pLR1Δ1 (T1) the *lacZ* gene is under the control of the yeast *GAL1* promoter lacking its UAS_G; pSH18-18 (T2) is derived from pLR1Δ1 by insertion of six copies of the *lexA* operator, substituting for UAS_G. (B) wt (EG123) and Δ*pkc1* strains harbouring plasmids carrying one of the target genes (*URA3* as a selectable marker) were transformed with plasmids directing the expression of either LexA or LexA-ANT fusion (*TRP1* as a selectable marker). β-Galactosidase activity ± S.D. (units: $[1000\times(OD_{420}-OD_{550}\times1.75)]/[\text{time(min)}\times\text{volume of culture used (ml)}\times OD_{600}]$) was measured in cell lysates after growth on selective (lacking tryptophan and uracil) medium containing 1 M sorbitol.

ferent pathways has recently been reported [13]). ANT failed to restore growth at elevated temperatures or to suppress the increased caffeine sensitivity of the Δ*mpk1* strain (data not shown).

3.2. A LexA-ANT fusion protein activates transcription from lexA operator-containing promoters

On the basis of sequence homology to DNA-binding proteins, a role as transcription factor has been proposed for ANT and other members of the AP2 domain containing family. To verify this hypothesis a full-length, in-frame fusion of ANT to LexA was constructed. LexA is a bacterial protein which binds to a specific DNA sequence, the *lexA* operator. Appropriately constructed target genes [28,29] were used: in these the *lacZ* reporter gene was placed under a *GAL1* promoter lacking an endogenous upstream activating sequence (UAS) either with multiple (T2) or without (T1, control) *lexA* operator sequences in the upstream region (Fig. 2A). Yeast strains harbouring target genes were transformed with plasmids driving the expression of either LexA or the LexA-ANT fusion. The β-galactosidase activity measured in extracts was taken as an estimate of transcriptional activity. A high activity was measured only in extracts from strains containing LexA binding sites in the target gene and expressing the LexA-ANT fusion protein: as shown in Fig. 2B, (i) expression of the target gene containing binding sites was more than 600-fold higher than that from the corresponding target gene with no binding sites (320 vs. 0.5); (ii) the strong activation of transcription was dependent on the presence of the fusion protein since the binding of native LexA enhanced expression only marginally. Essentially the same stimulation factor was obtained when transcription activation was assayed in a Δ*pkc1* background although basal expression was higher in this strain.

4. Discussion

We have shown that expression of the *A. thaliana* protein ANT partially restores viability on hypo-osmotic media to a yeast Δ*pkc1* strain. Furthermore, we measured a strong transcriptional activation of the *lacZ* reporter gene due to expression of a lexA-ANT fusion. This result suggests that the ANT plant protein can act as a transcriptional activator in this heterologous system and supports the hypothesis, put forward by other authors [19,20], that ANT acts in planta as a transcription factor.

In metazoans, both RhoA (RHO1 homolog) and PKC isoforms [36,37] have been shown to control signal transduction pathways leading to the nucleus. In yeast, nuclear proteins possibly acting downstream of the PKC1-activated cascade have been identified in genetic screens [38,35]. Recently, the transcription of specific genes, involved in cell wall biosynthesis, has been shown to be positively regulated through the PKC1 pathway [3]. ANT probably inserts itself in the yeast signal transduction pathways at the nuclear level, activating the transcription of genes responsible for survival in hypo-osmotic media. However, the role ANT plays within the network of signal transduction pathways operating in yeast remains to be clarified. In this respect, we can give three possible interpretations of our results. First, ANT might substitute an endogenous downstream component of the PKC1-mediated pathway: the ANT-activated genes would normally be transcribed in a wt background upon PKC1 activation while, in the complemented strain, the need for upstream activation might be overcome by high levels of ANT expression (the cDNA is under the control of the strong PGK promoter, on a high copy number plasmid). Second, the expression of the heterologous protein might bypass PKC1 by acting in functionally redundant endogenous pathways which act in parallel to the PKC1 pathway in cell surface growth regulation. Various yeast genes act as dosage-dependent suppressors of PKC1-pathway mutant growth defects (*PPZ1* and *PPZ2* encoding phosphatases [8]; *BCK2* involved in regulation of G1 cyclin levels [8,39]; *SSD1*, which appears to take part in various stress responses [40,41]; *KRE6* involved in β(1–6)glucan synthesis [42]; *SKN7* encoding a transcriptional activator [43,44]). Because mutations in these genes in combination with a Δ*pkc1* deletion cause more severe defects than those observed in the single mutants, these proteins are thought to be part of pathways acting in parallel to the PKC1 cascade. Although we found no sequence homology between ANT and these yeast proteins, it is very likely that the plant protein functionally substitutes or interacts with these. Third, ANT might simply activate the transcription of gene(s), unrelated to PKC1, whose products strengthen the cell wall. The failure to suppress the growth defects of the Δ*mpk1* strain suggest that ANT is not playing the role of an endogenous factor normally acting downstream of the MAP kinase cascade arm of the PKC1 pathway. However, this is not an unambiguous conclusion since other interpretations are possible: e.g. (i) the balance between ANT-dependent toxicity and fragility suppression might be more favourable at lower temperature; (ii) ANT activity in the heterologous environment might be impaired at

higher temperature. These interpretations are consistent with the observation that expression of ANT did not modify the unconditional lethality (the strain is not rescued by osmotic support) of the Δ*pkc1* strain at 37°(data not shown). Among the endogenous suppressors of Δ*pkc1* defects also SKN7 [43], which is lethal when overexpressed [44], could not restore growth at elevated temperatures to cells lacking MPK1.

Another point to be discussed concerns the ANT AP2 domain. This domain is thought to be responsible for DNA binding and so far has been found only in plant proteins. Our experiments do not give information on whether the *Arabidopsis* protein recognizes specific sequences in the yeast genome: the complementation could be explained also with recruitment by a yeast DNA-binding protein. But, unless a totally unspecific activation is occurring, the *Arabidopsis* protein ANT is interacting functionally with a yeast macromolecule (either DNA or another protein) implying at least a conserved interaction surface.

A last consideration regards possible relationships between the physiological basis of the yeast Δ*pkc1* and *Arabidopsis ant* phenotypes. Some cells in ovules of the *ant-9 Arabidopsis* mutant show an abnormal callose deposition. Interestingly, two yeast genes, *FKS1* and *FKS2*, activated through PKC1 signalling [3,5], encode redundant catalytic subunits of β(1–3) glucan synthase, an enzyme catalyzing the same UDP-glucose transfer as plant callose synthase. It would be interesting to study whether ANT can directly or indirectly interact with these promoters

Finally, studies of the spatial and temporal regulation of ANT gene expression in the plant suggest that its product might play a role in organ primordia initiation and growth [20]. Since plant cells possess a rigid cell wall and no cell migration occurs during organogenesis, the establishment and maintenance of cell polarity in the dividing cells of meristems must play a crucial role in plant morphogenesis. It is therefore intriguing that our screen pulled out the ANT protein, PKC1 being central in coordination of polarized growth in yeast.

Acknowledgements: The authors thank Enzo Martegani, Davide Baroni (University of Milan, Italy), Chrystoph Schueller (University of Vienna, Austria), Tom Miosga (University of Darmstadt, Germany) and Hanns Frohnmeyer (University of Freiburg, Germany) for very helpful discussions; Enzo Martegani, Johan Thevelein (University of Leuven, Belgium) and Kenji Irie (Nagoya University, Japan) for yeast strains; David Stillman (University of Utah Medical Center, Salt Lake City, USA) and Roberto Mantovani (University of Milan) for plasmids; Francois Lacroute (CNRS, Gif-sur-Yvette, France) for the cDNA library and finally Isabella Mauri and Marco Foiani (University of Milan) who got us started with yeast.

References

[1] Lew, D.J. and Reed, S.I. (1993) J. Cell Biol. 120, 1305–1320.
[2] Chant, J. (1994) Trends Genet. 10, 328–333.
[3] Igual, J.C., Johnson, A.L. and Johnston, L.H. (1996) EMBO J. 15, 5001–5013.
[4] Bussey, H. (1996) Science 272, 224–225.
[5] Machesky, L.M. and Hall A. (1996) Trends Cell Biol. 6, 304–310.
[6] Quadota, H., Anraku, Y., Botstein, D. and Ohya, Y. (1994) Proc. Natl. Acad. Sci. USA 91, 9317–9321.
[7] Levin, D.E., Owen-Fields, F., Kunisawa, R., Bishop, J.M. and Thorner, J. (1990) Cell 62, 213–224.
[8] Lee, K.S., Hines, L.K. and Levin D.E. (1993) Mol. Cell. Biol. 13, 5843–5853.
[9] Neiman, A.M. (1993) Trends Genet. 9, 390–394.
[10] Herskowitz, I. (1995) Cell 80, 187–197.
[11] Davenport, K.R., Sohaskey, M., Kamada, Y., Levin, D.E. and Gustin, M.C. (1995) J. Biol. Chem. 270, 30157–30161.
[12] Kamada, Y., Jung, U.S., Piotrowski, J. and Levin, D.E. (1995) Genes Dev. 9, 1559–1571.
[13] Zarzov, P., Mazzoni, C. and Mann, C. (1996) EMBO J. 15, 83–91.
[14] Bussey, H. (1996) Science 273, 203.
[15] Kamada, Y., Quadota, H., Python, C.P., Anraku, Y., Ohya, Y. and Levin, D.E. (1996) J. Biol. Chem. 271, 9193–9196.
[16] Nonaka, H., Tanaka, K., Hirano, H., Fujiwara, T., Kohno, H., Umikawa, M., Mino, A. and Takai, Y. (1995) EMBO J. 14, 5931–5938.
[17] Yang, Z. and Watson, J.C. (1993) Proc. Natl. Acad. Sci. USA 90, 8732–8736.
[18] Nishihama, R., Banno, H., Shibata, W., Hirano, K., Nakashima, M., Usami, S. and Machida, Y. (1995) Plant Cell. Physiol. 36, 749–757.
[19] Klucher, K.M., Chow, H., Reiser, L. and Fischer R. (1996) Plant Cell 8, 137–153.
[20] Elliott, R.C., Betzner, A.S., Huttner, E., Oakes, M.P., Tucker, W., Gerentes, D., Perez, P. and Smyth, D.R. (1996) Plant Cell 8, 155–168.
[21] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[22] Robzyk, K. and Kassir, Y. (1992) Nucl. Acids Res. 20, 3790.
[23] Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidman, J.S. and Struhl, K (1987–1994) Current Protocols in Molecular Biology, Wiley, New York.
[24] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 83, 5463–5467.
[25] Minet, M. Dufour, M.E. and Lacroute, F. (1992) Plant J. 2, 417–422.
[26] Gietz, D., St. Jean, A., Woods, R.A. and Schiestl, R.H. (1992) Nucl. Acids Res. 20, 1425.
[27] Paravicini, G., Cooper, M., Friedli, L., Smith, D.J., Carpentier, J.L., Klig, L.S. and Payton, M.A. (1992) Mol. Cell. Biol. 12, 4896–4905.
[28] West, R.W., Jr., Yocum, R.R. and Ptashne, M. (1984) Mol. Cell. Biol. 4, 2467–2478.
[29] Hanes, S.D. and Brent, R. (1989) Cell 57, 1275–1283.
[30] Weigel, D. (1995) Plant Cell 7, 388–389.
[31] Ohme-Takagi, M. and Shinshi, H. (1995) Plant Cell 7, 173–182.
[32] Daniell, T., Fordham-Skelton, A., Vergani, P. and Edwards, R. (1996) Plant Physiol. 110, 1435.
[33] Levin, D.E. and Errede, B. (1995) Curr. Opin. Cell Biol. 7, 197–202.
[34] Mazzoni, C., Zarzov, P., Rambourg, A. and Mann, C. (1993) J. Cell Biol. 123, 1821–1833.
[35] Watanabe, Y., Irie, K. and Matsumoto, K. (1995) Mol. Cell. Biol. 15, 5740–5749.
[36] Hill, C.S., Wynne, J. and Treisman, R. (1995) Cell 81, 1159–1170.
[37] Hill, C.S. and Treisman, R. (1995) Cell 80, 199–211.
[38] Costigan, C., Kolodrubetz, D. and Snyder, M. (1994) Mol. Cell. Biol. 14, 2391–2403.
[39] Di Como, C., Chang, H. and Arndt, K.T. (1995) Mol. Cell. Biol. 15, 1835–1846.
[40] Costigan, C., Gehrung, S. and Snyder, M. (1992) Mol. Cell. Biol. 12, 1162–1178.
[41] Kikuchi, Y., Oka, Y., Kobayashi, M., Uesono, Y., Toh-e, A. and Kikuchi, A. (1994) Mol. Gen. Genet. 245, 107–116.
[42] Roemer, T., Parravicini, G., Payton, M.A. and Bussey, H. (1994) J. Cell Biol.127, 567–579.
[43] Brown, J.L., Bussey, H. and Stewart, R.C. (1994) EMBO J. 13, 5186–5194.
[44] Morgan, B.A., Bouquin, N., Merrill, G.F. and Johnston, L.H. (1995) EMBO J. 14, 5679–5689.