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# Four *Arabidopsis thaliana* 14-3-3 protein isoforms can complement the lethal yeast *bmh1 bmh2* double disruption

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**Abstract The 14-3-3 proteins comprise a family of highly conserved proteins with multiple functions, most of which are related to signal transduction. Four isoforms from the plant *Arabidopsis thaliana* were able to complement the lethal disruption of the two *Saccharomyces cerevisiae* genes encoding 14-3-3 proteins; one complemented very poorly and one did not complement. However, the expression of the latter two isoforms was very low. These results show that at least four of the six *A. thaliana* isoforms are able to perform the same function(s) as the yeast 14-3-3 proteins.**

*Key words:* 14-3-3 Protein; *BMH1*; *BMH2*; GF14; *Arabidopsis thaliana*; *Saccharomyces cerevisiae*

## 1. Introduction

The 14-3-3 proteins comprise a family of acidic dimeric proteins with a subunit molecular mass of approx. 30 kDa [1–3]. These proteins are found in a wide variety of organisms including mammals [1–6], plants [7–9], *Xenopus* [10], *Drosophila* [11], *Dictyostelium discoideum* (M. Knetch et al., manuscript in preparation) and yeast [12]. Most organisms contain multiple isoforms of these proteins. The amino acid sequences are well conserved throughout evolution.

Many different activities have been ascribed to the 14-3-3 proteins. The first reported function of the 14-3-3 proteins was a role as activator of tryptophan 5-monooxygenase and tyrosine 3-monooxygenase in the presence of $Ca^{2+}$- and calmodulin-dependent protein kinase II [13]. This activation is required for neurotransmitter synthesis [14]. Later, 14-3-3 proteins which strongly inhibited protein kinase C were isolated from sheep brain [4,15]. Other groups observed a stimulatory effect of 14-3-3 proteins on protein kinase C [16,17]. An arachidonic acid specific phospholipase $A_2$, isolated from human platelets, appeared to be a 14-3-3 protein [18]. A cytosolic protein (exo1) required for $Ca^{2+}$-dependent exocytosis in permeabilized adrenal chromaffin cells also belongs to the 14-3-3 protein family [19]. A 14-3-3 protein from bovine brain activates exoenzyme S (ExoS) of *Pseudomonas aeruginosa*, which ADP-ribosylates Ras and other host GTP-binding proteins [20]. 14-3-3 proteins from *Arabidopsis thaliana* and maize [9] were found associated with the G-box DNA/protein complex. In recent years, evidence has been provided that 14-3-3 proteins bind to Raf protein kinases [21–25], BCR kinase [25,26], polyoma middle tumor antigen [27], a Ras-dependent MAPKKK from bovine brain [28] and human cdc25 phosphatases [29]. In the yeast *Schizosaccharomyces pombe*, 14-3-3 proteins appear to be involved in DNA damage control during the cell cycle [30]. Finally, the plant receptor for the fungal toxin fusicoccin was shown to be a 14-3-3 protein [31,32].

Fractionation of a 14-3-3 protein fraction isolated from mammalian brain tissue revealed the existence of at least seven isoforms of these proteins [5,33–35]. Two of the isoforms were shown to be a phosphorylated form of other isoforms [36]. From the plant *A. thaliana* six different cDNAs encoding 14-3-3 protein isoforms have been isolated [37,38]. The yeast *Saccharomyces cerevisiae* has two genes, *BMH1* and *BMH2*, coding for 14-3-3 proteins [12,39,40]. Previously, we showed that disruption of the *BMH1* or the *BMH2* gene alone had hardly any effect [12,39]. On the other hand, disruption of both genes simultaneously is a lethal event [39]. Expression of a 14-3-3 isoform from *A. thaliana* (GF14-14) was able to complement the lethal double disruption.

Despite the high conservation, it is not clear whether all isoforms perform all functions mentioned above or that specific isoforms exist for the various activities. To investigate this question, we made use of the lethality of yeast mutants devoid of 14-3-3 proteins and tested whether all known *A. thaliana* isoforms were able to complement these mutations. In addition, the ability of the human theta isoform and the unique 14-3-3 protein from *D. discoideum* to complement was investigated.

## 2. Materials and methods

### 2.1. Strains, plasmids and culture media

The yeast strains used in this study are: GG580, *MATa/MATα leu2-3,112/leu2-3,112 ura3-52/ura3-52 trp1-92/trp1-92 his4/+ bmh1::LEU2/+* [39]; GG576, *MATa/MATα leu2-3,112/leu2-3,112 ura3-52/ura3-52 trp1-92/trp1-92 his4/+ bmh1::LEU2/+ bmh2::APT1/+*; GG1305, *MATa leu2-3,112 ura3-52 trp1-92 his4 bmh1::LEU2 bmh2::APT1* (YCplac33[*BMH1*]); and GG1306, *MATa leu2-3,112 ura3-52 trp1-92 his4 bmh1::LEU2 bmh2::APT1* (YCplac33[*BMH2*]). The *Escherichia coli* strain XL1-blue [41] was used for plasmid amplification. Culture media for yeast and *E. coli* were described previously [39].

### 2.2. Nucleic acid manipulations

DNA isolations were performed as described previously [39]. DNA was sequenced using an ALF DNA sequencer (Pharmacia). Double stranded DNA isolated with a Nucleobond DNA isolation kit (Machery-Nagel, Dueren, Germany) was used for sequencing.

### 2.3. Construction of the strains GG1305 and GG1306

An internal 0.5 kb *Xmn*I-*Bcl*I fragment of the *BMH2* gene in pBlue[*BMH2*] was removed as described previously [39] and replaced by a 2.4 kb *Hin*cII-*Bgl*II fragment from pBEJ24 [42]. The latter fragment contains the *APT1* gene under control of the *PGK1* promoter and codes for resistance to geneticin (G418). The resulting plasmid was digested with *Eco*RI, yielding a 5.1 kb fragment containing the

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disrupted *BMH2* gene. This fragment was used to transform the diploid strain GG580 heterozygous for the *BMH1* disruption [39] and G418 resistant colonies were selected. The correct disruption of the *BMH2* gene was verified by Southern blot analysis. One of the transformants was selected (GG576) and transformed with the plasmid YCplac33[*BMH1*] [12] or YCplac33[*BMH2*]. The latter plasmid contains a 3.2 kb *Eco*RI fragment with the *BMH2* gene [39]. From both transformations, one of the transformants was sporulated and leucine and uracil prototrophic G418 resistant haploids were selected. This resulted in the isolation of a haploid strain with a disrupted *BMH1* gene, a disrupted *BMH2* gene carrying either the plasmid YCplac33[*BMH1*], strain GG1305, or the plasmid YCplac33[*BMH2*], strain GG1306. The correct genotype was verified by Southern blot analysis.

### 2.4. Construction of pYES-TRP plasmids encoding 14-3-3 protein isoforms

A 1.0 kb DNA fragment with the coding sequences of the *BMH1* gene was obtained from the plasmid pRS306[*BMH1*] [39] after digestion with *Eco*RI and *Xho*I. This fragment was ligated into the plasmid pYES2 (Invitrogen Corp.) digested with the same enzymes. The *URA3* gene was replaced by the *TRP1* gene as described previously [39] resulting in the plasmid pYES-TRP[*BMH1*]. Similarly, pYES-TRP was obtained from pYES2 by replacing the *URA3* gene of pYES2 by the *TRP1* gene. The coding sequences of the *BMH2* gene were amplified by the PCR reaction using primers with a *Kpn*I and *Xho*I site, respectively, and ligated into the pYES-TRP plasmid after digestion with *Kpn*I and *Xho*I. pYES-TRP[GF14-14] was constructed as described previously [39]. pCRII plasmids containing the cDNAs encoding the GF14-5 and GF14-10 isoforms [37] were digested with *Kpn*I and *Xho*I and the cDNAs were ligated into pYES-TRP after digestion with the same enzymes. The cDNA encoding the GF14-4 isoform [37] was released from the pCRII plasmid by digestion with *Pst*I and *Spe*I and ligated into pRS305. Subsequently, it was ligated into pYES-TRP after digestion with *Kpn*I and *Sac*I. The cDNA encoding the GF13-19 isoform was obtained by the PCR reaction on a plasmid preparation of an *A. thaliana* cDNA library in the vector pFL61 [43] using the primers: 5'AAGTCGACAAGATGTCTTCT-GATTC3' and 5'ATGTCGACAAATCACCCCATGGATC3'. These primers contain a *Sal*I restriction site. The PCR product obtained was digested with *Sal*I and ligated into pYES-TRP digested with *Xho*I. pBluescript containing the cDNA encoding the RCl1B isoform [38] was digested with *Kpn*I and *Bam*HI and the cDNA was ligated into pUC18 after digestion with the same enzymes. The resulting plasmid was digested with *Kpn*I and *Sal*I and the cDNA was ligated into pYES-TRP digested with *Kpn*I and *Xho*I. The cDNA encoding the human theta isoform [6] was obtained from Dr. A.M. Carr (MRC, Brighton, UK). It was ligated into the pYES-TRP plasmid after digestion with *Sac*I and *Xho*I. All clones amplified by the PCR reaction were verified by sequencing.

### 2.5. Complementation of the double disruptants

Strains GG1305 and GG1306 were transformed [44] with the various pYES-TRP plasmids and tryptophan prototrophic colonies were selected. For all transformations at least 100 transformants were obtained. Six colonies from each transformation were selected and after purification streaked on MYZ medium [39] containing galactose (1.5%, w/v), uracil (20 mg/l), histidine (20 mg/l) and 5-fluoroorotic acid (5-FOA) (1 mg/ml) and incubated for 5 days at 30°C. At least five colonies from each transformation gave identical results; one of these was selected for further studies.

After selection for loss of the YCplac33[*BMH2*] plasmid on MYZ medium with galactose, histidine, uracil and 5-FOA, one colony was selected and after purification used for further studies. The presence of the correct plasmid was verified by isolation of the plasmid, followed by amplification in *E. coli*.

## 3. Results

The cDNAs encoding the *A. thaliana* GF14-14, GF14-10, GF14-5, GF14-4, GF13-19 and RCL1B 14-3-3 protein isoforms as well as the human theta isoform and the yeast *BMH1* and *BMH2* genes are placed under control of the yeast *GAL1* promoter on the plasmid pYES-TRP (see Section 2). In order to investigate whether they can complement the lethal *bmh1 bmh2* double disruption, the plasmid shuffling technique was used. A haploid yeast strain (GG1305) was constructed in which the *BMH1* gene was disrupted with the *LEU2* gene and the *BMH2* gene with the *APT1* gene, carrying the plasmid YCplac33[*BMH1*] with an active *BMH1* gene and the *URA3* selection marker (Fig. 1A). A similar strain (GG1306) was constructed carrying the plasmid YCplac33[*BMH2*] with an active *BMH2* gene. These strains were transformed with the various pYES-TRP plasmids (Fig. 1B). The transformants were streaked on a plate containing minimal medium (MYZ) with galactose, 5-FOA, histidine and uracil. If the foreign 14-3-3 isoform can take over the function of the *BMH1* or *BMH2* gene on the plasmid, the yeast cells can lose the plasmid (with the *URA3* gene) and form colonies on this medium (Fig. 1C). The results of a typical experiment using strains derived from GG1305 or from GG1306 are shown in Fig. 2A and B, respectively. It is clear that the *BMH1* and *BMH2* genes under control of the *GAL1* promoter can complement the double disruption. Furthermore, the *A. thaliana* GF14-14, GF14-4, GF14-5 and RCL1B isoforms as well as the human theta isoform gave complementation. Also, the *D. discoideum* isoform complemented well (data not shown). For reasons unknown, the GF14-5 isoform gave smaller and a lower number of colonies (only visible after prolonged incubation) when expressed in the strain GG1306 compared to expression in GG1305. The *A. thaliana* GF13-19 isoform gave colonies only after very long incubation (at least 14 days at 30°C). GF14-10 appeared not to complement under these conditions.

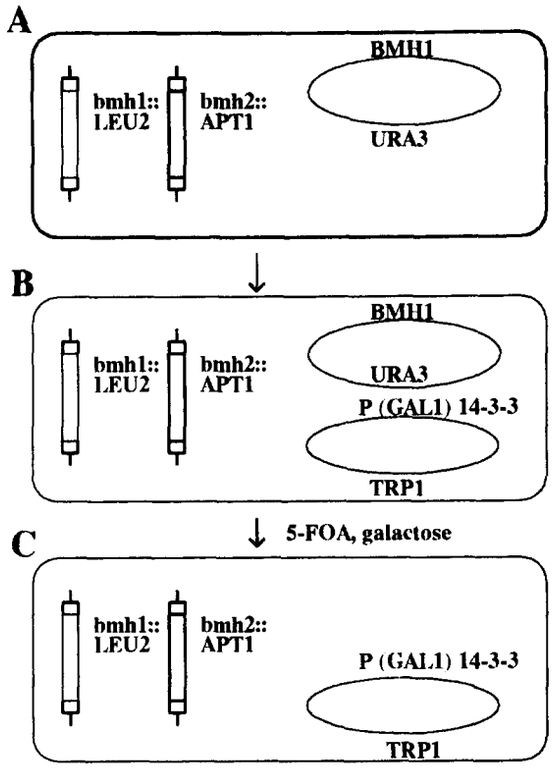


Fig. 1. Complementation of the *bmh1 bmh2* double disruptant by foreign 14-3-3 protein isoforms.

After selection on MYZ medium with galactose, 5-FOA, uracil and histidine one colony was selected from each of

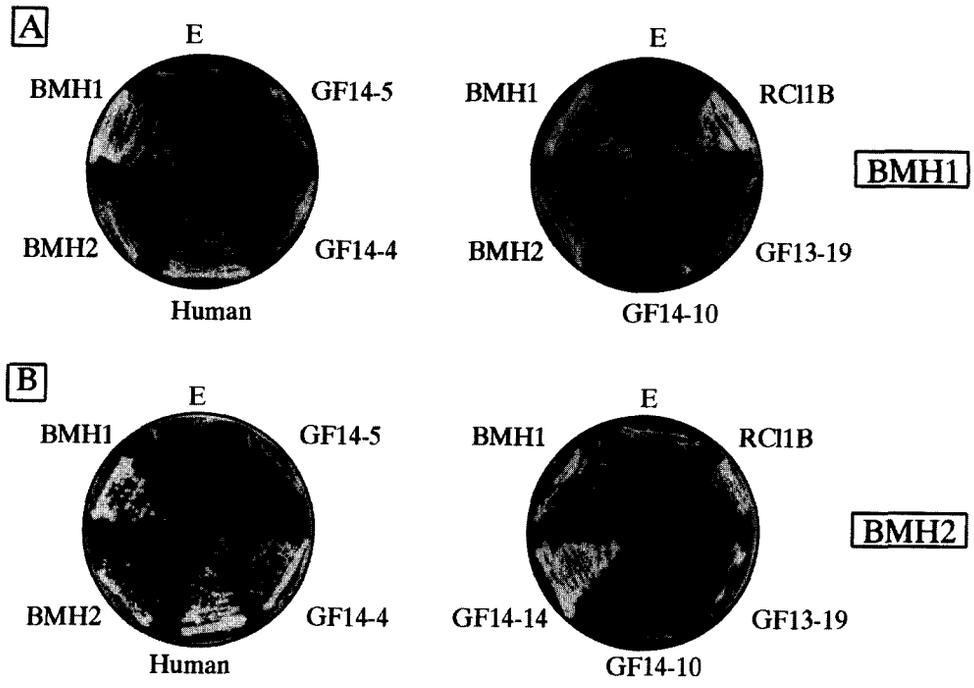


Fig. 2. Selection for growth on media with galactose in the presence of 5-FOA. Strains derived from GG1305 (A) or from GG1306 (B) by transformation with pYES-TRP plasmids encoding the isoforms indicated are streaked on plates containing MYZ medium with galactose, uracil, histidine and 5-FOA. E, pYES-TRP plasmid lacking a 14-3-3 protein isoform.

the strains derived from GG1306. The colony was purified and the presence of the proper pYES-TRP plasmid was verified by isolation of the plasmid after amplification in *E. coli*. All strains grew much slower on media with glucose instead of galactose (data not shown), indicating that the inducible *GAL1* promoter is involved. To investigate the expression of the foreign 14-3-3 proteins, the various strains were grown in YP medium with galactose and the protein extracts were analyzed by Western blotting (Fig. 3). Probed with the antiserum against the Bmh1 protein, clear bands are visible in extracts from the strains containing the pYES-TRP plasmids with the

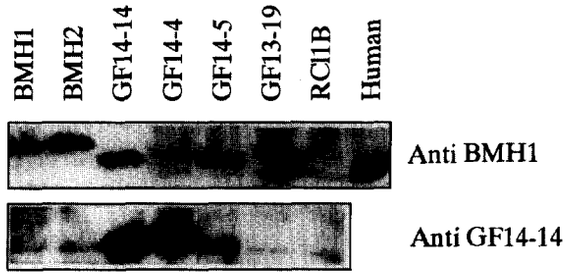


Fig. 3. Western blot analysis of the complemented double disruptants. Strains obtained after loss of the YCplac33[*BMH2*] plasmid were grown in YP medium containing galactose (1.5%, w/v) until an $OD_{620}$ between 0.4 and 0.7. Cells were harvested by centrifugation, resuspended in water until a concentration of 500 $OD_{620}$ units/ml. 5 $OD_{620}$ units were used for each lane. For the blot probed with the anti-BMH1 antiserum 0.05, 0.006 and 0.25 $OD_{620}$ units of cells expressing *BMH1*, *BMH2* or GF14-14, respectively, were used. Western blot analysis was performed as described previously [39] using a chemiluminescence kit from Boehringer. Blots were probed with either the rabbit anti-BMH1 antiserum [39] or a mouse monoclonal anti GF14-14 antiserum [8].

yeast *BMH1* or *BMH2* genes, with the *A. thaliana* GF14-14 and GF14-5 cDNAs and with the human theta cDNA. As the Bmh1 and Bmh2 proteins (31 and 32 kDa) are larger than the foreign 14-3-3 proteins (approx. 27 kDa) they can be distinguished. It is clear that the Bmh1 and Bmh2 proteins are absent from the strains expressing the foreign 14-3-3 isoforms. On a Western blot probed with a monoclonal antiserum raised against the GF14-14 protein no bands are visible in extracts from strains expressing the *BMH1* or *BMH2* gene. This confirms our previous observations that this antiserum does not detect the yeast 14-3-3 proteins [45]. Clear bands are visible after expression of the GF14-14, GF14-4 and GF14-5 isoforms. Very weak bands are visible in extracts from strains expressing the GF13-19 and RCL1B isoform. The combined Western blot experiments indicate that the yeast *BMH1* and *BMH2* genes, the plant GF14-14, GF14-4 and GF14-5 cDNAs and the human theta cDNA are expressed well in the *bmh1 bmh2* double disruptant. The plant GF13-19 cDNA is probably expressed very poorly. As the RCl1B isoform is more distinct from the other isoforms, it may be less reactive with the antisera used.

## 4. Discussion

A long list of activities has been ascribed to the 14-3-3 proteins. As most eukaryotic organisms contain multiple isoforms of the 14-3-3 proteins, the question arises whether each isoform has its own function or only a limited number of functions or whether a single isoform can fulfil all functions ascribed to the 14-3-3 proteins. In order to address this question, we investigated whether the six *A. thaliana* isoforms can complement the lethal yeast *bmh1 bmh2* double disruption. Four isoforms (GF14-14, GF14-4, GF14-5 and RCl1B)

clearly complemented the double disruption. One isoform (GF13-19) complemented very poorly. Only after a 2 weeks incubation are small colonies visible on plates with MYZ medium with galactose and 5-FOA. Growth rates in liquid media were very low and a large number of cells were impaired in cytokinesis as we showed previously for cells lacking all 14-3-3 proteins [39]. Two independent cDNA clones encoding the GF13-19 isoform were used which were ligated differently in the pYES-TRP plasmid and similar results were obtained (data not shown). No complementation was found for the GF14-10 isoform. Western blot experiments suggested that the expression of the GF13-19 and GF14-10 isoforms is very low. It is still unclear why levels of these proteins are so low. Either expression is very low or, because the proteins are non-functional, proteins and/or RNAs are rapidly degraded. The most obvious differences of the GF14-10 isoform compared to the other isoforms are its hydrophobic N-terminus, and it contains a serine residue at position 125 whereas all other isoforms have an alanine residue at this position). The GF13-19 isoform has a serine residue at position 217, whereas the other isoforms have an alanine at this position. However, the significance of these sequence differences remains to be established. As complementation was found for the human theta and the *A. thaliana* RC11B isoform which are more distinct from the other isoforms, quite a variation in amino acid sequences of the non-conserved regions are tolerated.

This study shows that at least four *A. thaliana* isoforms can fulfil the same function(s) as the yeast Bmh1 and Bmh2 proteins. This indicates that these isoforms are not highly specialized. However, it is still very well possible that in the plant when several different isoforms are present, the isoforms have more specialized functions due to subtle differences in affinity and/or selectivity of the different isoforms for their cellular targets. Previously, it was demonstrated that the GF14-14 isoform was able to fulfil a number of activities ascribed to the 14-3-3 proteins [45]. It was able to activate rat brain tryptophan hydroxylase, protein kinase C and exoenzyme S from *Pseudomonas aeruginosa*, it binds $Ca^{2+}$ and it is present in the G-box binding factor. The other isoforms have been studied less thoroughly. Also, the human theta isoform and a *D. discoideum* isoform (data not shown) can complement the double disruption, suggesting that these isoforms are not very specialized as well.

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## References

[1] Moore, B.W. and Perez, V.J. (1967) in: Physiological and Biochemical Aspects of Nervous Integration (Carlson, F.D. ed.) pp. 343–359, Prentice-Hall, Englewood Cliffs, NJ.
[2] Aitken, A., Collinge, D.B., Van Heusden, G.P.H., Isobe, T., Roseboom, P.H., Rosenfeld, G. and Soll, J. (1992) Trends Biochem. Sci. 17, 498–501.
[3] Aitken, A. (1995) Trends Biochem. Sci. 20, 95–97.
[4] Aitken, A., Ellis, C.A., Ha, A., Sellers, L.A. and Toker, A. (1991) Nature 344, 594.
[5] Ichimura, T., Isobe, T., Okuyama, T., Takahashi, N., Araki, K., Kuwano, R. and Takahashi, Y. (1988) Proc. Natl. Acad. Sci. USA 85, 7084–7088.
[6] Nielsen, P.J. (1991) Biochim. Biophys. Acta 1088, 425–428.
[7] Hirsch, S., Aitken, A., Bentch, U. and Soll, J. (1992) FEBS Lett. 296, 222–224.
[8] Lu, G., DeLisle, A.J., De Vetten, N.C. and Ferl, R.J. (1992) Proc. Natl. Acad. Sci. USA 89, 11490–11494.
[9] Brandt, J., Thordal-Christensen, H., Vad, K., Gregersen, P.L. and Collinge, D.B. (1992) Plant J. 2, 815–820.
[10] Martens, G.J.M., Piosik, P.A. and Danen, E.H.J. (1992) Biochem. Biophys. Res. Commun. 184, 1456–1459.
[11] Swanson, K.D. and Ganguly, R. (1992) Gene 113, 183–190.
[12] Van Heusden, G.P.H., Wenzel, T.J., Lagendijk, E.L., Steensma, H.Y. and Van den Berg, J.A. (1992) FEBS Lett. 302, 145–150.
[13] Ichimura, T., Isobe, T., Okuyama, T., Yamauchi, T. and Fujisawa, H. (1987) FEBS Lett. 219, 79–82.
[14] Yamauchi, T., Nakata, H. and Fujisawa, H. (1981) J. Biol. Chem. 256, 5404–5409.
[15] Toker, A., Ellis, C.A., Sellers, L.A. and Aitken, A. (1990) Eur. J. Biochem. 191, 421–429.
[16] Isobe, T., Hiyane, Y., Ichimura, T., Okuyama, T., Takahashi, N., Nakajo, S. and Nakaya, K. (1992) FEBS Lett. 308, 121–124.
[17] Morgan, A. and Burgoyne, R.D. (1992) Biochem. J. 286, 807–811.
[18] Zupan, L.A., Steffens, D.L., Berry, C.A., Landt, M. and Gross, R.W. (1992) J. Biol. Chem. 267, 8707–8710.
[19] Morgan, A. and Burgoyne, R.D. (1992) Nature 355, 833–836.
[20] Fu, H. and Collier, R.J. (1993) Proc. Natl. Acad. Sci. USA 90, 2320–2324.
[21] Freed, E., Symons, M., Macdonald, S.G., McCormick, F. and Ruggieri, R. (1994) Science 265, 1713–1716.
[22] Irie, K., Gotoh, Y., Yashar, B.M., Errede, B., Nishida, E. and Matsumoto, K. (1994) Science 265, 1716–1719.
[23] Fantl, W.J., Muslin, A.J., Kikuchi, A., Martin, J.A., MacNicol, A.M., Gross, R.W. and Williams, L.T. (1994) Nature 371, 612–614.
[24] Fu, H., Xia, K., Pallas, D.C., Cui, C., Conroy, K., Narsimhan, R.P., Mamon, H., Collier, R.J and Roberts, T.M. (1994) Science 266, 126–129.
[25] Brasselmann, S. and McCormick, F. (1995) EMBO J. 14, 4839–4848.
[26] Reuther, G.W., Fu, H., Cripe, L.D., Colier, R.J. and Pendergast, A.M. (1994) Science 266, 129–133.
[27] Pallas, C.D., Fu, H., Haehnel, L.C., Weller, W., Collier, R.J. and Roberts, T.M. (1994) Science 265, 535–537.
[28] Yamamori, B., Kuroda, S., Shimizu, K., Fukui, K., Ohtsuka, T. and Yoshima, T. (1995) J. Biol. Chem. 270, 11723–11726.
[29] Conklin, D.S., Galaktionov, K. and Beach, D. (1995) Proc. Natl. Acad. Sci. USA 92, 7892–7896.
[30] Ford, J.C., Al-Khdairy, F., Fotou, E., Sheldrick, K.S., Griffith, D.J.F. and Carr, A.M. (1994) Science 265, 533–535.
[31] Korthout, H.A.A.J. and De Boer, A.H. (1994) Plant Cell. 6, 1681–1692.
[32] Oecking, C., Eckerskorn, C. and Weiler, E.W. (1994) FEBS Lett. 352, 163–166.
[33] Isobe, T., Ichimura, T., Sunaya, T., Okuyama, T., Takahashi, N., Kuwano, R. and Takahashi, Y. (1991) J. Mol. Biol. 217, 125–132.
[34] Toker, A., Sellers, L.A., Amess, B., Patel, Y., Harris, A. and Aitken, A. (1992) Eur. J. Biochem. 206, 453–461.
[35] Leffers, H., Madsen, P., Rasmussen, H.H., Honore, B., Andersen, A.H., Walbum, E., Vandekerckhoven, J. and Celis, J.E. (1993) J. Mol. Biol. 231, 982–998.
[36] Aitken, A., Howell, S., Jones, D.,Madrazo, J. and Patel, Y. (1995) J. Biol. Chem. 270, 5706–5709.
[37] Ferl, R.J., Lu, G.H. and Bowen, B.W. (1994) Genetica 92, 129–132.
[38] Jarillo, J.A., Capel, J., Leyva, A., Martinez-Zapater, J.M. and Salinas, J. (1994) Plant Mol. Biol. 25, 693–704.
[39] Van Heusden, G.P.H., Griffiths, D.J.F., Ford, J.C., Chin-A-Woeng, T.F.C., Schrader, P.A.T., Carr, A.M. and Steensma, H.Y. (1995) Eur. J. Biochem. 229, 45–53.
[40] Gelperin, D., Weigle, J., Nelson, K., Roseboom, P., Irie, K.,

Matsumoto, K. and Lemmon, S. (1995) Proc. Natl. Acad. Sci. USA 92, 11539–11543.
[41] Bullock, W.O., Fernandez, J.M. and Short, J.M. (1987) Biotechniques 5, 376–380.
[42] Hadfield, C., Jordan, B.E., Mount, R.I., Pretorius, G.H.J. and Burak, E. (1990) Curr. Genet. 18, 303,313.
[43] Minet, M., Dufour, M.-E. and Lacroute, F. (1992) Plant J. 2, 417–422.
[44] Gietz, R.D., Schiestl, R.H., Willems, A.R. and Woods, R.A. (1995) Yeast 11, 355–360.
[45] Lu, G., De Vetten, N.C., Sehnke, P.C., Isobe, T., Ichimura, T., Fu, H., Van Heusden, G.P.H. and Ferl, R.J. (1994) Plant Mol. Biol. 25, 659–667.