Volume 302, number 2, 145–150 FEBS 11008 May 1992

Characterization of the yeast *BMH1* gene encoding a putative protein homologous to mammalian protein kinase II activators and protein kinase C inhibitors

G. Paul H. van Heusden, Thibaut J. Wenzel, Ellen L. Lagendijk, H. Y. de Steensma and Johan A. van den Berg

Department of Cell Biology and Genetics, Leiden University, Clusius Laboratory, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

Received 26 March 1992

We describe the identification and characterization of the *BMH1* gene from the yeast *Saccharomyces cerevisiae*. The gene encodes a putative protein of 292 amino acids which is more than 50% identical with the bovine brain 14-3-3 protein and proteins isolated from sheep brain which are strong inhibitors of protein kinase C. Disruption mutants and strains with the *BMH1* gene on multicopy plasmids have impaired growth on minimal medium with glucose as carbon source, i.e. a 30–50% increase in generation time. These observations suggest a regulatory function of the bmh1 protein. In contrast to strains with an intact or a disrupted *BMH1* gene, strains with the *BMH1* gene on multicopy plasmids hardly grew on media with acetate or glycerol as carbon source.

Protein kinase C; Protein kinase II; *Saccharomyces cerevisiae*; 14-3-3 protein

1. INTRODUCTION

The 14-3-3 protein is a soluble acidic protein which is abundantly present in human and bovine brain tissue. This protein, originally identified by two-dimensional gel electrophoresis [1], is an activator of tryptophan 5-monooxygenase and tyrosine 3-monooxygenase in the presence of Ca^{2+}- and calmodulin-dependent protein kinase II [2]. This activation is required for neurotransmitter synthesis [3]. Biochemical analysis showed that the 14-3-3 protein is a dimer consisting of two subunits with a molecular weight between 25 and 32 kDa [4–6]. By reverse-phase chromatography the bovine brain 14-3-3 protein could be resolved into seven polypeptide components [7]. The amino acid sequences of three of these components were determined and appeared to be 75–85% identical [8]. Recently, acidic proteins were isolated from sheep brain which were strong inhibitors of the Ca^{2+}- and phospholipid-dependent protein kinase C [9]. Amino acid sequence analysis indicated that these proteins closely resemble the bovine brain 14-3-3 protein [10]. Recently, a cDNA clone has been isolated encoding a plant homologue of the mammalian 14-3-3 protein [11].

In the present paper we describe the isolation and analysis of the *BMH1* gene from the yeast, *Saccharomyces cerevisiae*. This gene encodes a putative protein with a molecular weight of 33 kDa which is more than 50% identical to the bovine brain 14-3-3 protein. The identification of this gene suggests that proteins like the brain 14-3-3 protein are not exclusively present in mammalian brain tissues but also in the evolutionarily distant organism, *Saccharomyces cerevisiae*. To obtain insight into the physiological function of the bmh1 protein, we disrupted the *BMH1* gene. In addition, we studied the phenotypes of yeast transformants with the *BMH1* gene on multi- and single-copy plasmids.

Correspondence address: G.P.H. van Heusden, Department of Cell Biology and Genetics, Leiden University, Clusius Laboratory, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands. Fax: (31) (71) 274 863.

2. MATERIALS AND METHODS

2.1. *Strains, plasmids and culture media*

The yeast strains used in this study are listed in Table I. The *Escherichia coli* strain JM107 [12] was used for plasmid amplification. The plasmids pEMBL8α10 and pEMBL9α10 were obtained by cloning the insert of the λE1α10 phage [13] into the *Eco*RI site of the plasmids pEMBL8 and pEMBL9, respectively [14] (Fig. 1A). The plasmids pEMBL8α10EB and pEMBL9α10EB were obtained from the latter plasmids by digestion with *Bam*HI and self-ligation of the linear molecules. The plasmids YEplac112, YEplac195, YCplac22 and YCplac33 were generous gifts from Dr. Gietz (National Institute of Environmental Health Sciences, Research Triangle Park, NC) [15]. *E. coli* was grown in LB [12]; yeast was grown in either YPD [16] or MY, supplemented as required with histidine (20 mg/l), uracil (20 mg/l) and tryptophan (20 mg/l) [17].

2.2. *Nucleic acid manipulations*

Plasmid DNA was isolated from *E. coli* using the alkaline lysis method [12]. Plasmid DNA was extracted from yeast by the same

method after preparation of spheroplasts [16]. Prior to analysis, this plasmid DNA was amplified in *E. coli*. Yeast chromosomal DNA was prepared as described by Sherman et al. [16]. Yeast RNA was isolated according to Schmitt et al. [18].

DNA sequence analysis was conducted by the dideoxy chain termination method [19] using single- or double-stranded DNA of pEMBL8α10EB and pEMBL9α10EB. Sequences were determined in both directions using oligonucleotide primers.

2.3. *Disruption of the* BMH1 *gene and transformation of spores*

Gene disruption in the diploid strain, YS60, was conducted according to Rothstein [20]. The plasmid pUT332 [21] was digested with *Bgl*II yielding a 1.1 kb fragment containing the *URA3* gene. This fragment was cloned into the *Bgl*II site of the plasmid, pEMBL9α10EB (Fig. 1B). This construct with the disrupted *BMH1* gene was digested with *Eco*RI and *Bam*HI and the incubation mixture was used without further purification to transform YS60 by electroporation [22]. Transformants were selected for uracil prototrophy. Sporulation was performed as described [16]. Plasmids were introduced into yeast by electroporation [22].

2.4. *Southern and Northern blot analysis*

Southern blot analysis was performed as described [12]. The *URA3* probe was obtained by digestion of pUT332 with *Bgl*II followed by isolation of the 1.1 kb fragment by agarose gel electrophoresis and the Gene Clean DNA isolation kit (Bio 101, La Jolla, CA). The *BMH1* probe was obtained by digestion of pEMBL9α10EB with *Eco*RI and *Bgl*II and isolation of the 1.5 kb fragment as described above. The probes were labeled with digoxigenine-dUTP and hybridization and detection were performed as suggested by the manufacturer (Boehringer, Mannheim, Germany).

Northern blot analysis was performed as described [12]. The *BMH1* probe was obtained by digestion of pEMBL9α10EB with *Bgl*II and *Hin*dIII and isolation of the 0.3 kb fragment. The probe was labeled with [α-^{32}P]dCTP (The Radiochemical Center, Amersham, UK) with random priming using a multiprime DNA labeling kit from The Radiochemical Center, Amersham.

2.5. *Growth rates*

MY growth medium (30 ml) containing 2% (w/v) glucose or 1% (w/v) sodium acetate (pH 5.0) supplemented with histidine was inoculated with an aliquot of an overnight culture of the yeast strain giving an OD at 620 nm of approximately 0.1. Media to culture the strains 2C, 4A, 4C and 8D were supplemented with histidine, uracil and tryptophan. The cultures were incubated at 30°C and at various times the OD at 620 nm was measured. The growth rate was determined using logaritmically growing cells.

3. RESULTS

3.1. *Identification of the* BMH1 *gene*

In a recent study we cloned the *PDA1* gene coding for the E1α subunit of the pyruvate dehydrogenase complex from *Saccharomyces cerevisiae* [13]. Nucleotide sequence analysis revealed an open reading frame upstream from the *PDA1* gene. In Fig. 1A a restriction map of the cloned fragment is shown with the position of the *PDA1* gene and this open reading frame. A search for the EMBL database revealed that the open reading frame encodes a putative protein of 292 amino acids which is more than 58% identical with the bovine brain 14-3-3 protein. Fig. 2 shows the nucleotide sequence of the part of the clone containing the open reading frame and the amino acid sequence of the putative protein encoded by this open reading frame. For comparison

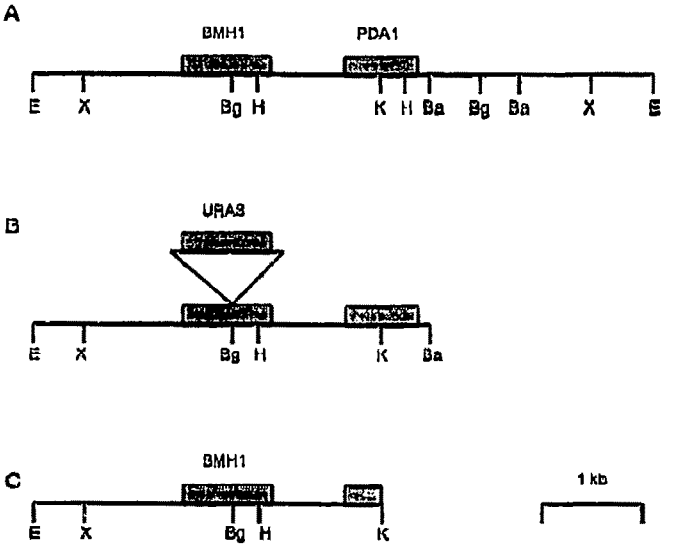


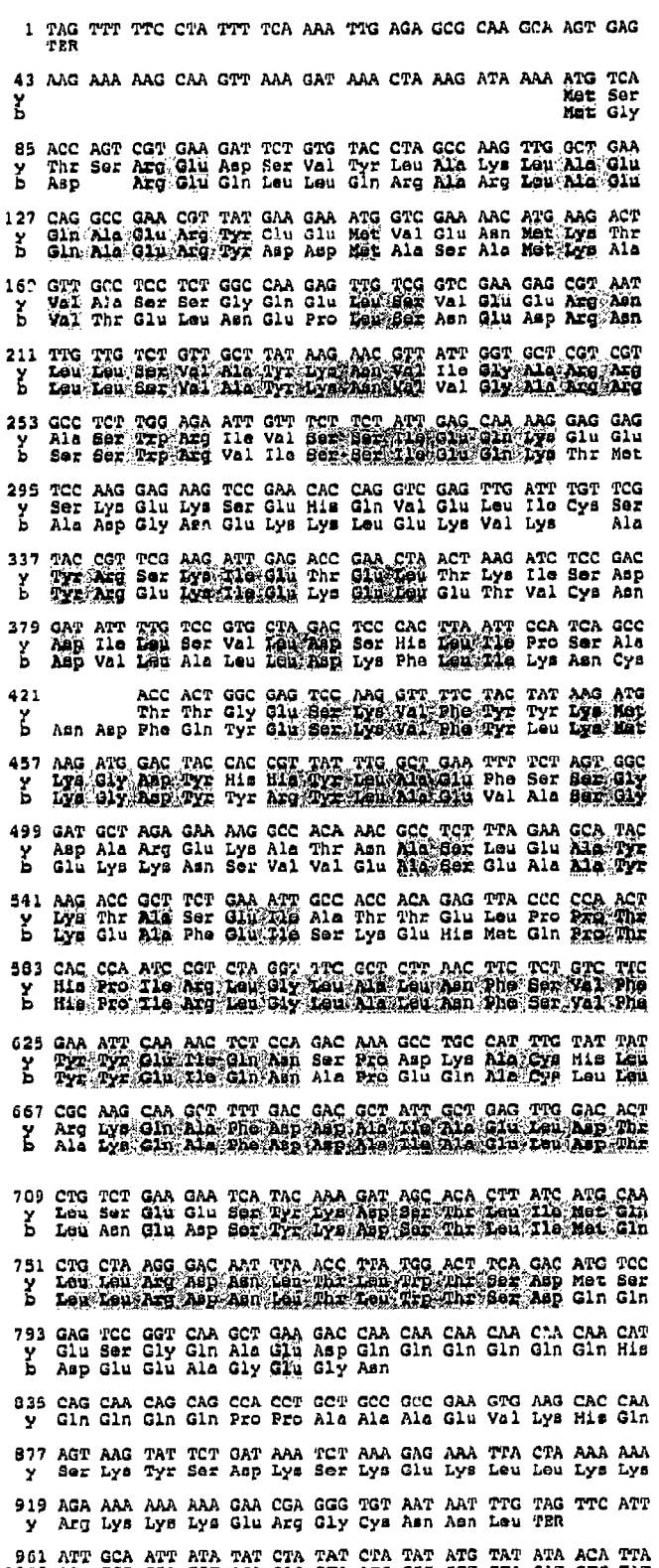
Fig. 1. Restriction map of the DNA fragment containing the *PDA1* and *BMH1* genes. (A) Restriction map of the fragment present in the λE1α10 phage. (B) Disruption of the *BMH1* gene by insertion of the *URA3* gene. (C) The *Eco*RI–*Kpn*I fragment used to clone into the plasmids YCplac22, YCplac33, YEplac 112 and YEplac 195. E, *Eco*RI; X, *Xho*I; Bg, *Bgl*II; H, *Hin*dIII; Ba, *Bam*HI; K, *Kpn*I.

the amino acid sequence of the bovine brain 14-3-3 protein (η-chain) is included. It is apparent that the putative yeast protein contains several stretches of up to 22 amino acids which are identical to the corresponding parts of the bovine brain protein. These stretches alternate with stretches with less similarity. The yeast protein contains an additional 45 amino acids at its C-terminal end. Comparison of the amino acid sequence of the yeast protein with that of the β- or γ-chain of the bovine brain 14-3-3 protein shows a similar homology (data not shown). Because of the similarity with the bovine brain 14-3-3 protein the open reading frame was named the *BMH1* (brain modulosignalin homologue 1) gene.

Table I

Yeast strains

Strain	Genotype	Source
YS60	*Mata/Matα FLO1/flo1 his4-519/his4-519 leu2-3,112/+trp1-789/+ ura3-52/ura3-52*	[13]
T1	*Mata/Matα* YS60 *bmh1::URA3/BMH1*	this study
2C	haploid *his4-519 ura3-52*	this study
4A	haploid *bmh1::URA3 his4-519 trp1-789 ura3-52*	this study
4C	haploid *his4-519 trp1-789 ura3-52*	this study
8D	haploid *bmh1::URA3 his4-519 ura3-52*	this study
22	haploid 4A (YCplac22)	this study
22B	haploid 4A (YCplac22[*BMH1*])	this study
33	haploid 2C (YCplac33)	this study
33B	haploid 2C (YCplac33[*BMH1*])	this study
112	haploid 4A (YEplac112)	this study
112B	haploid 4A (YEplac112[*BMH1*])	this study
195	haploid 2C (YEplac195)	this study
195B	haploid 2C (YEplac195[*BMH1*])	this study



3.2. *Disruption of the* BMH1 *gene*

To obtain information on the function of the *BMH1* gene we made disruption mutants. To this end, the *URA3* gene was cloned into the *Bgl*II site of the *BMH1* gene (Fig. 1B). Subsequently, the diploid strain, YS60, was transformed with a 4.7 kb *Eco*RI–*Bam*HI fragment containing the disrupted *BMH1* gene and yielded the uracil prototrophic transformant, T1. A proper integration of the *URA3* gene was confirmed by Southern blot analysis. In an *Xho*I digest of chromosomal DNA isolated from transformant T1 an extra 6 kb fragment hybridized to the *BMH1* probe in addition to the 5 kb fragment detected in DNA isolated from YS60 (data not shown). Similarly, a 6 kb fragment hybridized to the *URA3* probe. Similar results were obtained using *Hin*dIII digests of DNA isolated from T1 and YS60 (data not shown).

To investigate the effect of disruption of the *BMH1* gene, T1 was sporulated. Dissection of ten asci always yielded four viable spores with 2:2 segregation of uracil auxotrophy, indicating that the *BMH1* gene is not required for growth on rich media. For further studies non-flocculating and leucine prototrophic spore cultures were selected: 2C (*BMH1 ura3 his4*), 8D (*bmh1::URA3 ura3 his4*), 4C (*BMH1 trp1 ura3 his4*) and 4A (*bmh1::URA3 trp1 ura3 his4*). These spores were analyzed by Southern blot analysis as described above and the expected fragments hybridized to the *BMH1* and *URA3* probes (data not shown).

3.3. *Transformation with the* BMH1 *gene on multicopy plasmids*

To study the effect of the *BMH1* gene on multicopy plasmids, a 3.2 kb *Eco*RI–*Kpn*I fragment containing the *BMH1* gene (Fig. 1C) was cloned into the multicloning site of the yeast/*E. coli* shuttle plasmids, YEplac112 and YEplac195. As a control, the same fragment was cloned into the centromeric yeast/*E. coli* shuttle plasmids, YCplac22 and YCplac33. The constructs with the *BMH1* gene on the YEplac195 and YCplac33, as well as the corresponding parent plasmids, were used to transform the strain 2C (*BMH1 ura3 his4*) to uracil prototrophy. The constructs with the YEplac112 and YCplac22 plasmids, as well as the parent plasmids, were used to transform the strain 4A (*bmh1::URA3 trp1 ura3 his4*) to tryptophan prototrophy. During all yeast transformations normal transformation frequencies were observed suggesting that the presence of the *BMH1* gene

←

Fig. 2. Nucleotide and predicted amino acid sequence of the *BMH1* gene and comparison with the amino acid sequence of the bovine brain 14-3-3 protein (η-chain). The predicted amino acid sequence starts with the first methionine codon in the open reading frame. Amino acids present in both the putative yeast protein and in the bovine brain 14-3-3 protein are shaded. y, predicted amino acid sequence of the yeast protein; b, amino acid sequence of the bovine brain protein [7].

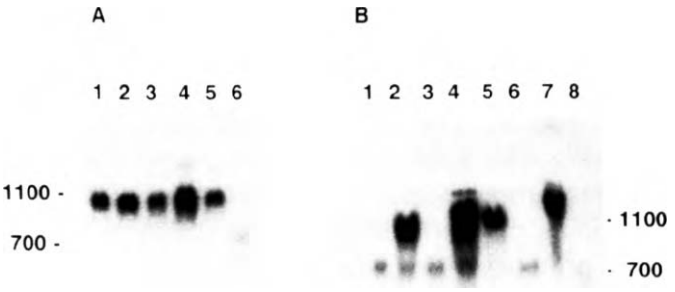


Fig. 3. Northern blot analysis. RNA was extracted from the yeast strains indicated and 30 μg was used for Northern blot analysis using the labeled 0.3 kb *Bgl*II–*Hin*dIII fragment of the *BMH1* gene as probe. (A) lane 1, 33; lane 2, 33B; lane 3, 195; lane 4, 195B; lane 5, 2C; lane 6, 8D. (B) lane 1, 22; lane 2, 22B; lane 3, 112; lane 4, 112B; lane 5, 2C; lane 6, 4A; lane 7, 4C; lane 8, 8D.

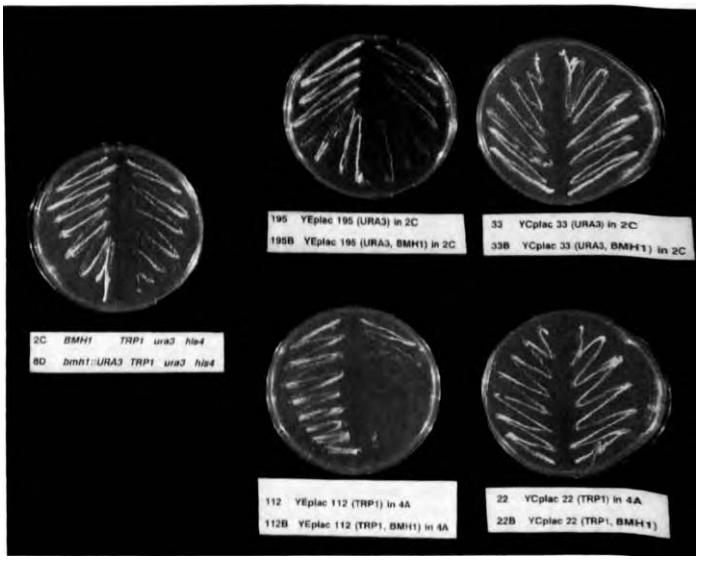


Fig. 4. Growth of the yeast strains on MY plates with acetate as carbon source. Strains were plated on MY plates containing 2% (w/v) acetate supplemented with histidine. Photos were taken after a 7 day incubation at 30°C. The medium used for strains 2C and 8D was supplemented with histidine, uracil and tryptophan.

on multicopy plasmids is not lethal to the yeast cells. From each transformation one transformant was selected after verification that these transformants indeed contained the expected plasmids. The genotypes of the various yeast transformants are summarized in Table I.

3.4. *RNA levels*

To verify the expression of the *BMH1* gene in the various strains, Northern blot analyses were performed. A single hybridizing band corresponding to RNA of approximately 1,100 nucleotides was observed in the *BMH1* strains, 2C and 4C, when an internal fragment of the *BMH1* gene, i.e. the 0.3 kb *Bgl*II–*Hin*dIII fragment, was used as hybridization probe (lanes 5 of Figs. 3A and 3B for strain 2C; lane 7 of Fig. 3B for strain 4C). This band was not detected in RNA isolated from the strains 8D and 4A containing a disrupted *BMH1* gene (Fig. 3A, lane 6 and Fig. 3B, lane 8 for strain 8D; Fig. 3B, lane 6 for strain 4A). This observation indicates that the gene disruption effectively blocks transcription of the *BMH1* gene. In the strains with a disrupted *BMH1* gene a weak hybridization was observed with RNA of approximately 700 nucleotides. However, this band was absent when the 1.5 kb *Eco*RI–*Bgl*II fragment, containing the N-terminal part of the *BMH1* gene, was used as hybridization probe. With this probe the same RNA of approximately 1,100 nucleotides was detected in all strains with an intact *BMH1* gene (data not shown). In RNA isolated from transformants of the strain 2C with the centromeric plasmid YCplac33 (strain 33) or with this plasmid containing the *BMH1* gene (strain 33B), the hybridization band at approximately 1,100 nucleotides was detected (Fig. 3A, lanes 1 and 2). Transformation of 2C with the multicopy plasmid YEplac195 containing the *BMH1* gene (strain 195B) resulted in an approximately 4-fold increase in the level of RNA hybridizing to the *BMH1* probe (Fig. 3A, lane 4). This was not observed in transformants containing the parent plasmid YEplac195 (strain 195; Fig. 3A, lane 3). Similarly, transformation of 4A with the centromeric plasmid YCplac22 containing the *BMH1* gene (strain 22B) resulted in restoration of the level of RNA hybridizing to the *BMH1* probe (Fig. 3B, lane 2). This was not observed in transformants containing the parental plasmid YCplac22 (strain 22; Fig. 3B, lane 1). Transformation of the spore 4A with the *BMH1* gene on the multicopy plasmid YEplac112 (strain 112B) resulted in an elevated level of RNA hybridizing with the *BMH1* probe (approximately 5-fold). This was not observed after transformation of the spore 4A with the YEplac112 plasmid (strain 112; Fig. 3B, lane 3). In conclusion, these results indicated that no *BMH1* mRNA was found in the disruption mutants and that the transformants with a multicopy plasmid contained elevated levels.

3.5. *Growth on glucose and acetate*

The properties of the strains obtained in this study were further investigated by growing them on various growth media. In Table II the generation times of the various strains relative to that of strain 33 are listed for growth on MY, i.e. a minimal medium with glucose as carbon source. The strains with one or two copies of the *BMH1* gene, i.e. strains 22B, 33, 33B and 195, have nearly equal generation times. On the other hand, strains lacking an intact copy of the *BMH1* gene, i.e. strains 22 and 112, and strains with the *BMH1* gene on a multicopy plasmid, i.e. strains 112B and 195B, grew slower (generation times 1.3- to 1.6-fold longer). As shown in Fig. 4, the strains with the *BMH1* gene on a multicopy plasmid (112B and 195B) hardly grew on plates containing minimal medium with acetate as carbon source. The other strains, both with an intact or a disrupted *BMH1* gene, grew normally. Growth of the

strains with the *BMH1* gene on a multicopy plasmid (112B and 195B) in liquid medium containing acetate indicated that the generation time of these two strains was approximately 5-fold longer than that of the other strains. No obvious difference was observed in generation times between strains with an intact or a disrupted *BMH1* gene. The strains 112B and 195B also hardly grew on media with glycerol as carbon source (data not shown).

4. DISCUSSION

The 14-3-3 protein is a soluble acidic protein which is abundantly present in mammalian brain tissues, i.e. it represents up to 1% of soluble brain proteins [4]. Within brain tissue the protein is mainly present in neurons and is subject to axonal transport [5]. By immunochemical methods this protein was also detected in other tissues, e.g. adrenal medulla and intestine, although at a much lower concentration [23]. In more recent studies the 14-3-3 protein was resolved into seven polypeptide components [7,8] and the amino acid sequence of three of these components was shown to be 75–85% identical. Northern blot analysis indicated that these three forms are expressed abundantly in bovine brain. One of the forms, the γ-chain, was expressed exclusively in the brain, whereas the two other forms, the β- and η-chains, were also expressed to some extent in other tissues [8]. In this study we identified a gene in the yeast *S. cerevisiae* which codes for a putative protein that is very similar to the 14-3-3 protein. Previously, we have shown that this gene is localized on chromosome V [13]. Comparison of the putative yeast protein with the bovine brain η-, β- and γ-chains indicates that these proteins are 51, 54 and 52% identical, respectively. The yeast protein contained stretches of up to 22 amino acids which were completely identical to the corresponding parts of the bovine brain protein. These stretches alternate with stretches with less identity (Fig. 2). The identification of a yeast gene coding for a putative protein with homology to the 14-3-3 protein indicates that proteins like the 14-3-3 protein are not exclusively present in mammalian tissues, but can also be found in evolutionarily distant organisms like *S. cerevisiae*.

Table II

Growth rates in minimal medium containing glucose

Strain	Copies of *BMH1* gene	Relative generation time[a]
22	0	1.4 ± 0.1 (n=3)[b]
22B	1	1.1 ± 0.1 (n=4)
112	0	1.4 ± 0.1 (n=4)
112B	multiple	1.6 ± 0.2 (n=4)
33	1	1.0 (n=4)
33B	2	1.1 ± 0.1 (n=4)
195	1	1.1 ± 0.1 (n=4)
195B	multiple	1.3 ± 0.1 (n=3)
2C	1	1.0 ± 0.2 (n=3)
8D	0	1.4 ± 0.3 (n=4)

[a] The generation time was determined in each individual experiment relative to the generation time of strain 33, i.e. 2.8 ± 0.8 h (n=4).
[b] mean ± S.D.
n, number of experiments

The observation that yeast contains a gene coding for a protein homologous to the 14-3-3 protein raises questions about the physiological function of this class of proteins. The protein isolated from bovine brain acts as an activator protein during the activation of tyrosine 3-monoxygenase and tryptophan 5-monooxygenase by Ca^{2+}- and calmodulin-dependent protein kinase II [3]. These two oxygenases are involved in neurotransmitter synthesis. Recently, proteins were isolated from sheep brain which were strong inhibitors of protein kinase C [9,10] and were more than 90% identical to the bovine brain 14-3-3 protein. At the moment it is still unclear whether this class of proteins is actually involved in regulation of neurotransmitter synthesis or protein kinase C function in vivo. Recently, a gene was identified in *S. cerevisiae* coding for a protein which is very similar to the mammalian protein kinases C [24]. In addition, protein kinase C activity was detected in yeast homogenates [25]. In this respect it is of interest that the bovine brain 14-3-3 protein contains a pseudo substrate site for protein kinase C, i.e. the sequence Gly-Ala-Arg-Arg (amino acids 54–57), which may play a key role in the inhibition of protein kinase C [26]. This same amino acid sequence is also present in the putative yeast bmh1 protein (amino acids 55–58 in Fig. 2), suggesting that the yeast protein may be involved in regulation of protein kinase C.

In an initial attempt to obtain insight into the physiological function of the bmh1 protein we made disruption mutants lacking an intact *BMH1* gene and made transformants with the *BMH1* gene on multicopy plasmids. We could show by Northern blot analysis that the disruption mutants completely lacked the mRNA of approximately 1,100 nucleotides hybridizing to the *BMH1* probe and that transformants with the *BMH1* gene on multicopy plasmids had an increased level of this RNA (Fig. 3). Whether this increased transcription resulted in an elevated level of the bmh1 protein is not yet known. However, we did show that the strains with the *BMH1* gene on multicopy plasmids grew slower than the corresponding wild-type strains on minimal medium containing glucose (Table II) and grew very poorly on media with acetate or glycerol as sole carbon source (Fig. 4). Strains lacking an intact *BMH1* gene grew slower than the corresponding wild-type strains on media containing glucose. This indicates that one or two copies of the *BMH1* gene is optimal for growth, suggesting that the *BMH1* gene has a regulatory function in vivo. However, its exact role is still unknown and a possible effect of the bmh1 protein on protein kinase C or other protein kinases has still to be proven. On the

other hand, the observations made in this study offer possibilities for future research to unravel the physiological function of the putative bmh1 protein and other members of this class of proteins.

Acknowledgements: The authors wish to thank Mrs. A. Bergmans and Mrs. Q.J.M. van der Aart for their contributions in the nucleotide sequence determinations and Drs. A.W.R.H. Teunissen for his help in dissecting tetrads. Dr. J.G. Sgouros (Martinsried Institute for Protein Sequences, Munchen, Germany) is acknowledged for screening the MIPS data base and Dr. T. Isobe (Department of Chemistry, Tokyo Metropolitan University, Japan) for his helpful collaboration.

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 Cliff, NJ.
[2] Ichimura, T., Isobe, T., Okuyama, T., Yamauchi, T. and Fujisawa, H. (1987) FEBS Lett. 219, 79–82.
[3] Yamauchi, T., Nakata, H. and Fujisawa, H. (1981) J. Biol. Chem. 256, 5404–5409.
[4] Boston, P.F., Jackson, P., Kynoch, P.A.M. and Thompson, R.J. (1982) J. Neurochem. 38, 1466–1474.
[5] Erickson, P.F. and Moore, B.W. (1980) J. Neurochem. 35, 232–241.
[6] Grasso, A., Roda, G., Hogue-Angeletti, R.A., Moore, B.W. and Perez, V.J. (1977) Brain Res. 124, 497–507.
[7] Ichimura, T., Isobe, T., Okuyama, T., Takahashi, N., Araki, K., Kuwano, R. and Takahashi, Y. (1988) Proc. Natl. Acad. Sci. USA 85, 7084–7088.
[8] Isobe, T., Ichimura, T., Sunaya, T., Okuyama, T., Takahashi, N., Kuwano, R. and Takahashi, Y. (1991) J. Mol. Biol. 217, 125–132.
[9] Toker, A., Ellis, C.A., Sellers, L.A. and Aitken, A. (1990) Eur. J. Biochem. 191, 421–429.
[10] Aitken, A., Ellis, C.A., Ha, A., Sellers, L.A. and Toker, A. (1991) Nature 344, 594.
[11] Hirsch, S., Aitken, A., Bentsch, U. and Soll, J. (1992) FEBS Lett. 296, 222–224.
[12] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) in: Molecular Cloning, second edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[13] Steensma, H.Y., Holterman, L., Dekker, I., van Sluis, C.A. and Wenzel, T.J. (1990) Eur. J. Biochem. 191, 769–774.
[14] Dente, L., Cesareni, G. and Cortese, R. (1983) Nucleic Acids Res. 11, 1645–1655.
[15] Gietz, R.D. and Sugino, A. (1988) Gene 74, 527–534.
[16] Sherman, F., Fink, G.R. and Hicks, J.B. (1986) Laboratory Course Manual for Methods in Yeast Gene Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[17] Zonneveld, B.J.M. (1986) J. Microbiol. Methods 4, 287–291.
[18] Schmitt, M.E., Brown, T.A. and Trumpower, B.L. (1990) Nucleic Acids Res. 18, 3091–3092.
[19] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 83, 5463–5467.
[20] Rothstein, R. (1983) Methods Enzymol. 101, 202–211.
[21] Gatignol, A., Dassein, M. and Tiraby, G. (1990) Gene 91, 35–41.
[22] Becker, D.M. and Guarente, L. (1991) Methods Enzymol. 194, 182–187.
[23] Boston, P.F., Jackson, P. and Thompson, R.J. (1982) J. Neurochem. 38, 1475–1482.
[24] Levin, D.E., Fields, F.O., Kunisawa, R., Bishop, J.M. and Thorner, J. (1990) Cell 62, 213–224.
[25] Ogita, K., Miyamoto, S., Koide, H., Iwai, T., Oka, M., Ando, K., Kishimoto, K., Ikeda, K., Fukami, Y. and Nishizuka, Y. (1990) Proc. Natl. Acad. Sci. USA 87, 5011–5015.
[26] Hardie, D.G. (1988) Nature 335, 592–593.