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# Identification and analysis of the *Arabidopsis thaliana BSH* gene, a member of the *SNF5* gene family

**Jan Brzeski[1], Wojciech Podstolski[1], Katarzyna Olczak[2] and Andrzej Jerzmanowski[1,2,*]**

[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences and [2]Laboratory of Plant Molecular Biology, Warsaw University, Pawinskiego 5A, 02-106 Warsaw, Poland

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## ABSTRACT

**The multiprotein complexes involved in active disruption of chromatin structure, homologous to yeast SWI/SNF complex, have been described for human and *Drosophila* cells. In all SWI/SNF-class complexes characterised so far, one of the key components is the SNF5-type protein. Here we describe the isolation of a plant (*Arabidopsis thaliana*) cDNA encoding a 27 kDa protein which we named BSH, with high homology to yeast SNF5p and its human (INI1) and *Drosophila* (SNR1) counterparts as well as to other putative SNF5-type proteins from *Caenorhabditis elegans*, fish and yeast. With 240 amino acids, the *Arabidopsis* BSH is the smallest SNF5-type protein so far identified. When expressed in *Saccharomyces cerevisiae*, the gene for BSH partially complements the *snf5* mutation. BSH is, however, unable to activate transcription in yeast when tethered to DNA. The gene for BSH occurs in single copy in the *Arabidopsis* genome and is ubiquitously expressed in the plant. Analysis of the whole cell and nuclear protein extracts with antibodies against recombinant BSH indicates that the protein is localised in nuclei. Transgenic *Arabidopsis* plants with markedly decreased physiological level of the *BSH* mRNA, resulting from the expression of antisense messenger, are viable but exhibit a distinctive phenotype characterised by bushy growth and flowers that are unable to produce seeds.**

## INTRODUCTION

The occurrence of the DNA in nucleosomes restricts the access of DNA-binding proteins to their specific sequences, a fact with potentially serious implications for the transcription process in eukaryotic nuclei (reviewed in 1). However, until recently there was no clear indication of how the impediment caused by the presence of nucleosomes is dealt with during transcription *in vivo*. The specialised transcription factors could themselves open up the chromatin structure, for example by competing with nucleosomes, without the need for any 'derepressing' factors or there could be unique mechanisms destabilising nucleosomal structure before the real transcriptional initiation could take place. While there is strong evidence that in the case of some sequences like the yeast *PH05* gene promoter, the recruitment of the RNA polymerase II holoenzyme is itself sufficient to remodel chromatin for efficient transcription (2), in the case of certain other genes, like yeast *HO* and *SUC2*, the presence of specialised chromatin remodelling complex is required for activation (3,4).

Of several protein complexes with reported chromatin remodelling activity, the SWI/SNF complex is by far the best characterised (reviewed in 5,6). In *Saccharomyces cerevisiae*, the products encoded by *SWI1*, *SWI2* (*SNF2*), *SWI3*, *SNF5* and *SNF6* genes were originally identified as positive regulators of *HO* and *SUC2* genes and later found to play the same role in the transcription of other diversely regulated genes. The functional interdependence between the five proteins strongly suggested that they act jointly as a complex. This was confirmed by the biochemical studies which led to the isolation of a large (~2 MDa) complex containing all five proteins plus additional polypeptide components. The ability of SWI/SNF complex to alter the structure of chromatin was demonstrated by *in vitro* experiments with reconstituted nucleosomes. The complex was capable of destabilising DNA–histone interactions in nucleosomes in an ATP-dependent manner. The destabilisation, the molecular basis of which is still not clear, led to the increased binding to specific sequences in nucleosomal DNA of transcription factors, such as GAL4 derivatives or the TATA-box binding protein. The genes homologous to those coding for yeast SWI/SNF proteins have been identified in *Drosophila* (7), mouse (8) and human (9,10). *Brahma* is a *Drosophila* homologue of *SWI2/SNF2*. Two human genes highly similar to *SWI2/SNF2* are *Brg1* and *hBrm*, named for their similarity to the *Drosophila Brahma*. Immunoprecipitation with antibodies to BRG1 and hBRM proteins enabled the isolation of two chromatographically distinct multiprotein complexes termed hSWI/SNF A and hSWI/SNF B, both of which were capable of destabilising nucleosome structure and facilitating the binding of GAL4 derivatives in an ATP-dependent manner (11,12). The other components of the human homologue of SWI/SNF complex are probably also similar to their yeast counterparts. The human *INI1* gene shows high similarity to yeast *SNF5p* and the INI1 protein cofractionates with hSWI/SNF and associates with BRG1 (13). In *Drosophila*, a protein homologous to yeast SNF5p is encoded by a *snr1* gene. Both the Brahma and SNR1 proteins are components of a large multiprotein complex and co-immunoprecipitate from *Drosophila* extracts (14).

*To whom correspondence should be addressed at: Laboratory of Plant Molecular Biology, Warsaw University, Pawinskiego 5A, 02-106 Warsaw, Poland. Tel: +48 22 659 60 72; Fax: +48 22 658 46 36; Email: andyj@ibb.waw.pl

In this paper we describe the isolation of a plant (*Arabidopsis thaliana*) cDNA encoding a protein with high homology to yeast SNF5p, human INI1 and *Drosophila* SNR1 proteins as well as to the other putative SNF5-type proteins. With 240 amino acids, the *Arabidopsis* homologue of yeast SNF5, which we named BSH (for bushy growth exhibited by mutants deficient in *BSH* mRNA), is the smallest SNF5-type protein so far identified. The BSH gene partially complements the *snf5* mutation in yeast. However, BSH, unlike yeast SNF5p and human INI1, is unable to activate transcription in yeast when tethered to DNA. We show that *BSH* mRNA is ubiquitously expressed in plant and that BSH protein is localised in nuclei.

## MATERIALS AND METHODS

### Cloning of BSH cDNA, Southern and northern blot analysis

The *A.thaliana* cDNA libraries λPRL-2 (15), λgt10 siliques library and λZAPII (16) were obtained from Arabidopsis Biological Resource Centre, OH, USA. The libraries were screened by plaque hybridisation using the probe generated with PCR with primers U1: GAA AGG TCC CGT CAA GTT and L1: TCA TAA GCC CGA AAG TCT, designed on the basis of the *A.thaliana* EST sequence found in GenBank. PCR conditions were as follows: 95°C for 5 min, 30× (52°C for 30 s, 72°C for 30 s, 95°C for 30 s), 52°C for 30 s, 72°C for 2 min. The PCR product obtained from cDNA generated with the Gibco BRL RT kit on the total RNA isolated from *A.thaliana*, was reamplified with the same set of primers and the PCR DIG labelling nucleotide mix (Boehringer) and used as a probe. After three rounds of screening of λgt10 siliques library a single positive clone was found. The cDNA was excised from this clone with *Sal*I and inserted into a Bluescript KS (Stratagene) plasmid resulting in the pS5c10 plasmid, and sequenced on both strands by the dideoxy method using ALF (Pharmacia) DNA sequencer. Total genomic DNA was prepared from leaves as described (17). Southern blot hybridisation with the DIG labelled probe used for screening cDNA libraries (see above) was according to (18). For northern blot analysis total RNA was extracted from flowers and siliques of *Arabidopsis* by the method of Verwoerd (19), electrophoresed in formaldehyde containing gel and transferred onto a membrane. The membrane was hybridised with DIG labelled (Boehringer labelling kit) antisense RNA probe corresponding to a full-length cDNA, by the method of Engler-Blum (18). To visualise the bound probe a CDP-Star chemiluminescent AP substrate (Boehringer) was used.

### Transcription activation assay

The PCR product amplified from pS5C10 with primers U2 (CGC CCT GAT AGA CGG TTT TTC GCC CTT TGA) and L2 (GGA TCC CTA GTG ATG GTG ATG GTG ATG TCT CTC TTC CCT GGC TTC AAG) comprised a full-length *BSH* coding sequence with six histidine codons and STOP codon added on the 3′ end by L2 primer and a part of the vector. The conditions of PCR were as follows: 95°C for 5 min, 30× (60°C for 30 s, 72°C for 1 min, 95°C for 30 s), 60°C for 30 s, 72°C for 5 min. The DNA fragment was cut with *Eco*RV and *Bam*HI and inserted into *Sma*I and *Bam*HI sites of the pGBT9 plasmid (Clontech) resulting in the pGBSH6 plasmid. The PCR was done with *Pfu* DNA polymerase (Stratagene) to ensure high fidelity. The correctness of the *BSH* sequence in the *GAL4$_{1–147}$–BSH* fusion was checked by DNA sequencing. The pGBSH6 plasmid was transformed into the Y190 (Clontech) yeast reporter strain. Expression of the GAL4$_{1–147}$–BSH fusion protein was checked by western blot with α-GAL4DB antibody (Santa Cruz Biotechnology). The level of β-galactosidase activity was measured by replica lift method and by colorimetric method as described in the Clontech yeast techniques manual. As controls, the Y190 strain was transformed with pGBT9 alone and with the pCL1 plasmid (20) to express the full-length GAL4 protein.

### Complementation of yeast *snf5* mutant strain

The PCR product amplified from the pS5c10 plasmid with a primer to the linker sequence of the pBluescript plasmid and the L2 primer was cut with *Sac*I and *Bam*HI and inserted into the pSI4 yeast multicopy expression vector resulting in the pSIBSH plasmid in which the expression of a cloned gene is driven by the CAT1 inducible promoter. The phenotypes of the yeast *snf5* strain MCY1991 (21) after transformation with the pSIBSH, empty pSI4 and pJW34 (4) derivative bearing the yeast *SNF5* gene, were checked on glucose and galactose.

### Analysis of *BSH* expression by RT–PCR

RNA was extracted from roots, stalks, pods, flowers and siliques of *A.thaliana* plants grown on the MS medium, by the method of Verwoerd (19). After treating with DNase I (Promega) the RNA was used for generation of cDNA using Gibco BRL RT kit according to the manufacturer's instructions. The PCR was done with L1 and U1 primers. As a control the fragment of *A.thaliana* protein kinase 1 (*atpk1*) cDNA (22) was amplified with primers KIN1 (AAA CAA CAA CCA AAG AAG) and KIN2 (AAA CCC GAA AAC ATA CTC), using the same conditions.

### Production of anti-BSH antibodies

Recombinant BSHis$^{6}_{EC}$ protein containing amino acids 1–238 of BSH with six histidines fused to the C-terminus was overproduced in *Escherichia coli* SG 13009 cells using the pQE60 plasmid of the QIAExpress system (Qiagen) and purified by selective binding to NiNTA (Qiagen) followed by elution according to manufacturer's protocol. The final preparation (~1 mg of protein) was separated by preparative SDS–PAGE. Gel fragments containing pure BSHis$^{6}_{EC}$ were used to immunise rabbits. Anti-BSHis$^{6}_{EC}$ rabbit serum was prepared by Eurogenetec (Seraing, Belgium).

### Protein extraction and immunoblot analyses

Total protein extracts were prepared from whole *A.thaliana* plants according to Foster *et al*. (23). Briefly, 50 g of frozen plant material was pulverised in a mortar and homogenised with 150 ml of extraction buffer (15 mM HEPES–KOH, pH 7.6, 40 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 0.1 mM PMSF). 4 M ammonium sulphate was then added dropwise with constant stirring. After 30 min the mixture was centrifuged in a Beckman SW 28 rotor at 19 000 r.p.m. and the supernatant was filtered through miracloth. Protein was precipitated from the filtrate by raising the concentration of ammonium sulphate to 0.33 g/ml and centrifuging the mixture for 30 min in a Beckman Ti 70 rotor at 19 000 r.p.m. The pellet was suspended in 2 ml of a buffer containing 20 mM HEPES–KOH, pH 7.6, 40 mm KCl, 1 mM DTT, 0.5 mM PMSF, 0.1 mM EDTA, 10% glycerol and dialysed for 3 h against 20 mM HEPES–KOH, pH 7.6, 40 mM KCl, 0.1 mM PMSF,

0.1 mM EDTA, 5 mM β-mercaptoethanol, 10% glycerol. All procedures were carried out at 4°C.

Extracts of nuclear proteins were obtained as follows. Plants were cut into small fragments and homogenised for 15 s in 5 vol of NIB buffer (0.33 M sucrose, 10 mM NaCl, 10 mM KCl, 10 mM β-mercaptoethanol, 2.5 mM EDTA, 0.1 mM spermine, 0.5 mM spermidine, 10 mM MES–KOH, pH 6.3). After filtration through four layers of gauze the subcellular organelles were pelleted from the filtrate by centrifugation for 10 min in an HB4 (Sorvall) rotor at 4000 r.p.m. The pellet was suspended in NIB buffer. In order to achieve the lysis of chloroplasts 10% Triton X-100 was slowly added to the suspension until the final concentration of 0.2%. After 5 min incubation the suspension was centrifuged as above and washed with NIB buffer. The final pellet was suspended in a small volume of NEB buffer (15 mM HEPES–KOH, pH 7.5, 110 mM KCl, 5 mM $MgCl_2$, 1 mM DTT) and incubated on ice for 30 min. During incubation NaCl was slowly added to the final concentration of 0.42 M. The suspension was then centrifuged for 30 min at 60 000 *g*. To the supernatant which contained the nuclear extract, ammonium sulphate was added to a final concentration of 80%. After centrifugation of the precipitate the pellet consisting of nuclear proteins was suspended in Z buffer (25 mM HEPES–KOH, pH 7.5, 70 mM KCl, 0.1 mM EDTA, 20% glycerol, 1 mM DTT) and dialysed for 2 h against 1000 vol of the same buffer with four changes. All procedures were at 4°C.

Protein samples were electrophoresed through 12% SDS–polyacrylamide gels according to (24), and transferred by electroblotting to PVDF membrane (Immobilon P, Milipore). Filters were blocked for 30 min at room temperature in Blotto A (10 mM Tris–HCl, pH 8.0, 150 mM NaCl, 5% non-fat dry milk). Incubation with rabbit anti-BSH serum was carried out in Blotto A blocking buffer for 45 min. The filters were washed twice with TBS (10 mM Tris–HCl, pH 8.0, 150 mM NaCl) and incubated for 1 h with alkaline phosphatase-conjugated goat anti-rabbit secondary antibody (DAKO) at a dilution of 1:1000 in Blotto A. The detection was with BCIP and NBT (Boehringer, Mannheim) colorimetric substrates.

### Construction and analysis of transgenic plants

The plant integration plasmid pROKBSH was constructed by inserting the *Sal*I fragment of pS5c10, in an antisense orientation, into the pROKF19 plasmid (25) in which the expression of the inserted gene is driven by an enhanced CaMV35S viral promoter. The transformation of *Arabidopsis* roots was according to Valvekens *et al*. (26). Transgenic plants were selected on the MS-kanamycine medium and regenerated on the MS medium. The RNA was extracted from plants obtained upon transformation with pROKBSH or with pROKF19 (control) by the method of Verwoerd (19). The RNA was subjected to DNase treatment and quantified spectrophotometrically. RNA samples (20 µg) were loaded into slots and the level of the *BSH* expression was analysed by slot-blot hybridisation with the DIG labelled antisense RNA probe specific for the *BSH* mRNA.

### Nucleotide sequence accession number

The cDNA sequence of *A.thaliana* BSH has been deposited in GenBank under accession no. U88061.

## RESULTS

### Identification of a plant homologue of yeast *SNF5* gene

In an attempt to identify plant counterparts to the genes coding for the components of yeast SWI/SNF complex we searched the *A.thaliana* dbEST database. This search revealed a single cDNA sequence of 340 nt with homology to *S.cerevisae SNF5*. Using a PCR amplified fragment of this cDNA as a probe, we screened the *A.thaliana* cDNA library. Screening of $2 \times 10^6$ plaques yielded a single positive clone containing an 876 nt cDNA insert, which we sequenced. The 876 nt cDNA contains an open reading frame (ORF) encoding a predicted 240-amino acid protein (DDBJ/EMBL/GenBank accession no. U88061). The end of the ORF is marked by the STOP codon followed by the 3′ untranslated region (UTR) that ends with a poly(A) tract. There is no typical AATAAA polyadenylation signal in the 3′ UTR. However, in plants the sequence requirement for polyadenylation is in general less stringent than in animal cells (27). The perfect copies of the AATAAA sequence are found upstream of the polyadenyl-ation site in less than one-half of all plant genes examined (28). Although in the cloned cDNA there is no in-frame translation termination codon preceding the first methionine, we have good reason to believe that the first methionine indeed marks the start of the complete ORF. The size of the cloned cDNA corresponds to that of the single mRNA band revealed by the specific probe on the northern blot performed with the total *A.thaliana* RNA (Fig. 2B), and the initial AUG resides in a sequence that conforms to the Kozak consensus (29). The sequence comparison of the predicted *A.thaliana* protein with *Drosophila*, human and fish homologues of SNF5 reveals significant similarity in all four proteins of the conserved N-terminal stretch (Fig. 1B). In addition, the comparison by BLAST of the ORF in the sequence upstream of the BSH putative AUG start codon revealed no homology with the Box C of the known members of the SNF5 family and in fact with no other protein sequence contained in databases. We named the *A.thaliana* protein BSH after the bushy phenotype of the *Arabidopsis* (described later in the Results) caused by the decreased level of the *BSH* transcript.

Comparison of the predicted sequence of the *Arabidopsis* BSH protein with known sequences in the databases revealed a significant match only to seven proteins: *S.cerevisiae* SNF5p and its homologues from human (INI1) and *Drosophila* (SNR1) cells, the second *S.cerevisiae* SNF5-type protein (ySNF5bp or SFH1) and the proteins from *Caenorhabditis elegans* (CeSNF5), fish (TfSNF5) and *Schizosaccharomyces pombe* (SpSNF5)—all members of the SNF5-type family. The alignment of all eight proteins reveals two regions of close similarity: the SNF5 domain and the B Box (Fig. 1A and B). The most significant is the similarity in the SNF5 domain, a characteristic distinction of the SNF5 family of proteins.

In BSH the SNF5 domain contains 137 residues. It spans from amino acid 21 to 158 and has two characteristic subregions similar to those occurring in SNF5 domains of other members of the family. The first subregion of BSH has 29.4% and the second 39% identical residues with the corresponding subregions of the *S.cerevisiae* SNF5p. The percentage of identical residues with corresponding subregions of INI1 is 39.2 and 34.4%; SNR1, 41.2 and 34.4%; and SNF5bp, 35.3 and 26.6%. The *Arabidopsis* BSH has no proline- and glutamine-rich sequences that flank the core SNF-5 domain in the *S.cerevisiae* SNF5p, but these sequences are

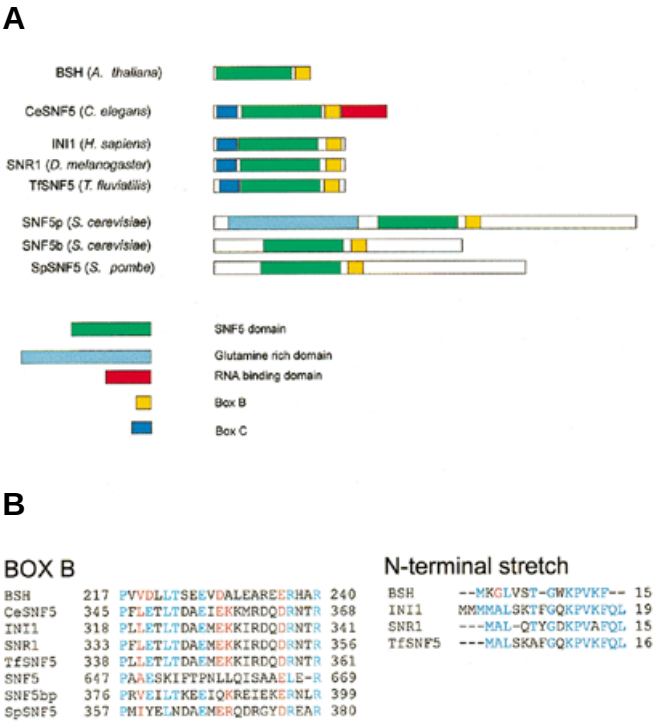


**Figure 1.** Comparison of the predicted *A.thaliana* BSH protein with SNF5-type proteins. (**A**) Schematic alignment of all known SNF5-type proteins. The regions of highest similarity are coloured in green (SNF5 domain), yellow (Box B) and dark blue (Box C). The glutamine- and proline-rich regions of yeast SNF5p (light blue) and RNA-binding domain of CeSNF5 (red) are indicated. (**B**) Alignment of amino acid sequences of Box B for all members of the SNF5 family and of the N-terminal amino acid stretch for BSH, INI1, SNR1 and TfSNF5. Residues that are identical in the majority of the proteins are marked in light blue. Residues of amino acids with closely similar properties are marked in red. The sequences were aligned using Clustal V program.

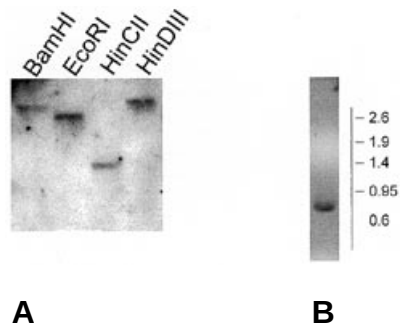


**Figure 2.** Southern and northern blot analysis of *BSH*. (**A**) Southern hybridisation of the *A.thaliana* genomic DNA digested with different restriction enzymes, with probe comprising the BSH SNF5 domain. (**B**) Northern blot analysis of *BSH*. The antisense RNA probe corresponding to the full size *BSH* cDNA was labelled with DIG and hybridised to a blot of total RNA from flowers and siliques of *A.thaliana*. The positions of RNA markers are indicated on the right.

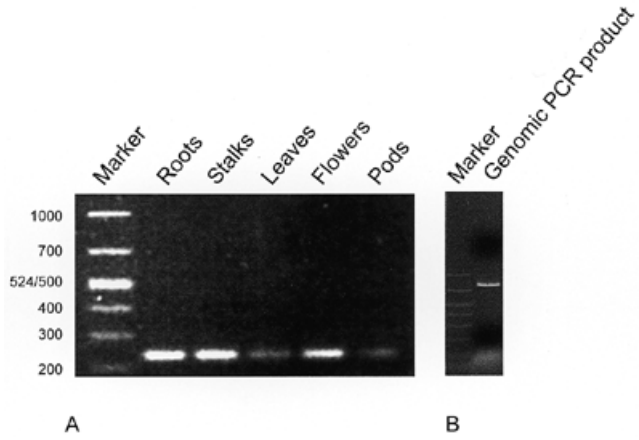


**Figure 3.** RT–PCR analysis of *BSH* expression in plant. (**A**) RNA was extracted from different organs of the plant and used for generation of the corresponding cDNAs. The presence of *BSH* mRNA was detected by PCR with primers to the conserved SNF5 domain. (**B**) PCR product obtained with the same primers on *A.thaliana* genomic DNA.

also absent in other representatives of the family. In addition, the BSH, INI1 and SNR1 have very similar stretches of amino acids in their N-terminal segments (Fig. 1B) with >50% of identical residues. On the basis of the very significant similarities in the core coding region between BSH and all the known representatives of the SNF5 family of proteins, we propose that *BSH* is an *A.thaliana* homologue of the *S.cerevisiae* gene encoding the SNF5 protein. With only 240 amino acids, BSH is the smallest of the known SNF5-type proteins.

## *BSH* is a single copy gene and is ubiquitously expressed in *Arabidopsis* plant

Southern hybridisation of the *BSH* cDNA probe with *Arabidopsis* genomic DNA cut with different restriction enzymes indicates that *BSH* is a single copy gene (Fig. 2A).

To study the expression of the *BSH* gene in *Arabidopsis*, the RNA isolated from different organs of the plant was reverse-transcribed into cDNA which was then used as template for the PCR reaction with primers specific for a region from the conserved SNF5 domain of the *BSH* gene. The PCR products of the expected size were detected for each of the tested organs, i.e. roots, stalks, leaves, flowers and pods (Fig. 3A). A control PCR using the same primers but performed on *Arabidopsis* genomic DNA resulted in a much larger product (Fig. 3B). This is consistent with our data showing that the *BSH* gene contains introns (results not shown). The occurrence of *BSH* mRNA in all tested plant organs could indicate that the BSH protein is involved in many different processes.

## Nuclear localisation of the BSH and its unusual behaviour in SDS–PAGE

To determine whether BSH is a cytoplasmic or nuclear protein we prepared protein extracts from whole cells and from isolated nuclei (Materials and Methods). Identical amounts (20 µg) of proteins from each type of extract were separated by SDS–PAGE, blotted to membrane and probed with polyclonal antibodies raised against recombinant BSH (Materials and Methods). BSH was detected only in the nuclear extract (Fig. 4) indicating that *in vivo* it is localised in nuclei. Both the recombinant and the native BSH migrate in SDS–PAGE as a 40 kDa protein, i.e. considerably

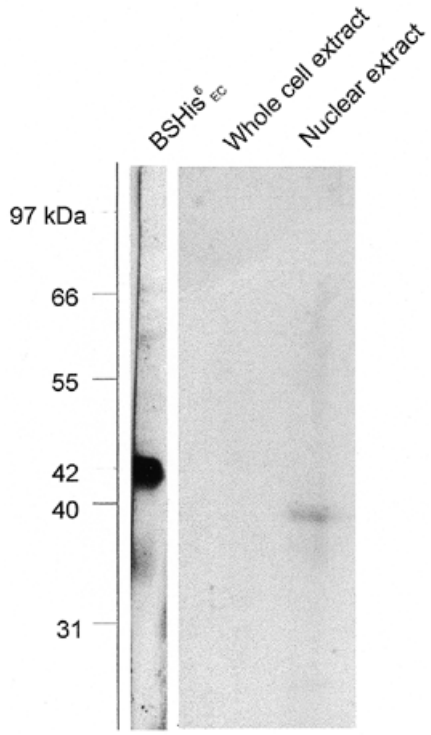


**Figure 4.** BSH protein is localised in nuclei. Identical amounts of protein extracts prepared from whole cells or isolated nuclei of *A.thaliana* plants (Materials and Methods) were separated by SDS–PAGE and immunoblotted for detection of BSH. Lane marked $BSHis^{6}_{EC}$ shows the migration of the recombinant BSH protein. The positions of molecular size markers are shown on the left.

slower than protein of the predicted molecular mass of 27 kDa. This concerns both the recombinant and the native BSH protein (the small difference in mass between the recombinant and native BSH seen on the gel is fully accounted for by the presence of six histidines in the *E.coli* made protein). We have checked by overexpression in *E.coli* of the BSH SNF5 domain that the slower mobility of the protein results from the anomalous behaviour of its SNF5 domain (results not shown). In order to eliminate any possibility of an error we confirmed the identity of the overexpressed BSH SNF5 domain by extracting the protein band from SDS gel and performing N-terminal microsequencing. Thus, the characteristic feature of *Arabidopsis* BSH protein is a anomalous behaviour in SDS–PAGE. Whether this reflects some specific steric properties remains to be checked.

### BSH partially complements yeast *snf5* mutation but is unable to activate transcription in yeast when tethered to DNA

In order to determine if BSH can functionally replace the yeast SNF5 *in vivo*, we tested the ability of the *Arabidopsis* gene to complement the *snf5* mutation in *S.cerevisiae*. The characteristic phenotype caused by *snf5* mutation is a weak growth on media with a carbon source other than glucose (4). The yeast strain with the *snf5* mutation (MCY 1991) was transformed with the multicopy expression vectors (Materials and Methods) bearing either complete *S.cerevisiae SNF5* or *Arabidopsis BSH* gene. The mutant strain was also transformed with the empty pSI4 vector to serve as control for the *snf5* phenotypes. The growth of

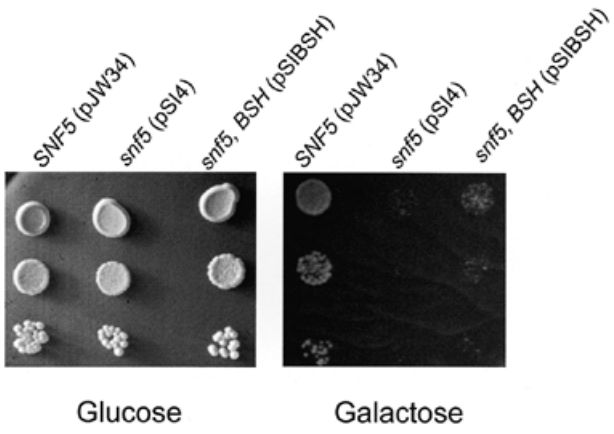


**Figure 5.** *BSH* partially complements the SNF5$^{-}$ phenotype of the *S.cerevisiae snf5* mutant. Yeast *snf5* strain MCY1991 was transformed with pJW34 (*SNF5*), pSI4 (vector) or pSIBSH (*BSH*). Five-fold serial dilutions of the transformants were transferred to plates with minimal medium lacking uracil and containing either glucose or galactose. The plates were incubated for 5 days at 30°C and photographed.

transformed strains on media containing glucose or galactose as a carbon source was then examined (Fig. 5). All strains grew normally on glucose. On galactose the construct bearing no *SNF5*-type gene failed to complement the *snf5* mutant phenotype. As expected, the ability to grow on galactose was readily restored upon transformation of the tested strain with plasmid bearing the yeast *SNF5* gene. Transformation with the plasmid bearing the *BSH* gene resulted in a partial restoration of the ability of the mutant strain to use galactose (Fig. 5). In conclusion, *BSH* is capable of partial complementation of the *snf5* phenotype in *S.cerevisiae*.

The yeast SNF5p and human INI1 proteins, when tethered to DNA by fusion to the LexA or GAL4 DNA-binding (DB) domain, are capable of activating a reporter gene in yeast cells (30,31). To determine whether *Arabidopsis* BSH was capable of similar activation in yeast cells, we prepared a yeast expression plasmid encoding the GAL4DB–BSH fusion protein. Transformation with this plasmid of the yeast Y190 strain (Clontech) containing the *GAL*-dependent *lacZ* reporter did not result in any activation of the *lacZ* transcription as assayed by measurement of the β-galactosidase activity (results not shown). We confirmed (by western blot with α-GAL4DB antibody) the expression in yeast Y190 strain of the GAL4DB–BSH fusion protein. We also checked that the expression in the reporter strain of a full GAL4 protein resulted in considerable β-galactosidase activity. BSH is thus unable to activate transcription in yeast cells.

### Transgenic *Arabidopsis* plants with significantly reduced physiological level of *BSH* mRNA display characteristic phenotype

To investigate the possible function of BSH we used the antisense strategy in order to eliminate or considerably reduce the *BSH* mRNA in plants *in vivo*. To this end we constructed transgenic plants containing a complete *BSH* cDNA placed in a reversed orientation under strong non-specific viral promoter. The effect of the expression of this construct was monitored by measuring the level of *BSH* mRNA in individual transgenic plants. This was

done by a slot-blot hybridisation of identical amounts of the total RNA from each plant with the labelled antisense RNA probe specific for *BSH* mRNA. The level of *BSH* mRNA was identically determined for the non-transformed plants and for plants transformed with the plasmid not containing the fragment of *BSH* cDNA (Table 1). In ~25% of plants transformed with the 'antisense' construct the level of *BSH* mRNA was not considerably changed compared to the control transformed with empty vector (Table 1, plants 2–4). In the remaining transgenic plants (Table 1, plants 5–12) the level of *BSH* mRNA was markedly decreased (5–15% of that in control plants). The decreased level of *BSH* mRNA was correlated with a bushy phenotype (Fig. 6) characterised by reduction in internode length and a decrease in apical dominance. In addition, the flowers of the plants with low level of *BSH* mRNA had no seeds. An identical correlation between the phenotype of the plants and the decreased level of the *BSH* mRNA occurred in two independent transformation experiments.

**Table 1.** Correlation between the level of *BSH* mRNA and the phenotype in transgenic *A.thaliana* plants[a]

| Plant | BSH mRNA level (% of control) | Phenotype |
|---|---|---|
| 1 | 100 (Control) | WT |
| 2 | 85 | WT |
| 3 | 75 | WT |
| 4 | 80 | WT |
| 5 | 10 | Bsh |
| 6 | 15 | Bsh |
| 7 | 12 | Bsh |
| 8 | 12 | Bsh |
| 9 | 10 | Bsh |
| 10 | 8 | Bsh |
| 11 | 5 | Bsh |
| 12 | 8 | Bsh |

[a]Plants were regenerated from root tissue transformed with vector containing *BSH* cDNA in antisense orientation (Materials and Methods). Control was a plant obtained upon transformation with an empty vector. Total RNA isolated from plants was quantified spectrophotometrically and analysed by slot-blot hybridisation with DIG labelled antisense RNA probe specific for *BSH* mRNA. The quantitative analysis of the hybridisation signal was done with IMAGEQUANT (Molecular Dynamics). WT, wild type (right plant in Fig. 6); Bsh, bushy growth (left plant in Fig. 6).

## DISCUSSION

We have identified in dbEST database and cloned the *A.thaliana BSH* gene by virtue of its homology to the yeast *SNF5* gene. The predicted 27 kDa BSH protein has a distinctive SNF5 domain which exhibits two characteristic subregions, also present in SNF5 domains of *S.cerevisiae* SNF5p and SNF5bp, human INI1, *Drosophila* SNR1, *C.elegans* CeSNF5, fish TfSNF5 and *S.pombe* SpSNF5—all members of the unique SNF5 family of proteins. The similarity of BSH (as judged by comparison of the characteristic subregions of the SNF5 domains) is highest to *Drosophila* SNR1 and then in decreasing order: to fish, human, *C.elegans*, *S.cerevisiae* SNF5bp and SNF5a and *S.pombe* SNF5-type proteins. In addition to the SNF5 domain the BSH has a characteristic small C-terminal domain (Fig. 1B, box B) which is also present in all other members of the SNF5 family. Neither the BSH nor the other six SNF5-type proteins have long glutamine-

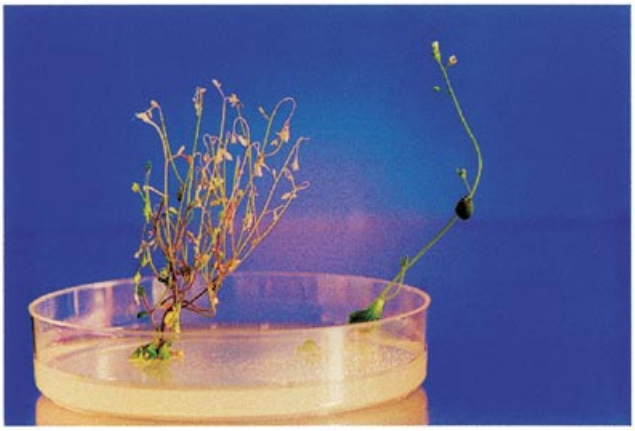

**Figure 6.** *Arabidopsis* plants with considerably decreased level of *BSH* mRNA have a characteristic bushy appearance. Transgenic *A.thaliana* plants regenerated from root tissue transformed with expression vector bearing the *BSH* cDNA in antisense orientation. On the left is a typical phenotype of a plant with drastically reduced level of *BSH* mRNA, on the right a plant transformed with an empty vector and exhibiting a normal level of *BSH* mRNA.

and proline-rich regions present in yeast SNF5p. However, the *Arabidopsis*, human, *Drosophila* and fish proteins all have a similar small N-terminal stretch of amino acids (Fig. 1B) which is not present in yeast SNF5. On the basis of the above similarities we conclude that *BSH* is a member of the *SNF5* family. The fact that we found only one positive clone after screening $2 \times 10^6$ plaques of the cDNA library might indicate that *in vivo* the amount of *BSH* mRNA per cell is rather low. We have shown that BSH is a nuclear protein, similarity to the SNF5-type proteins of yeast, human and *Drosophila*. The migration of BSH in SDS–PAGE (corresponding to that of a 40 kDa protein) is slower than anticipated for a predicted 27 kDa protein. We determined that this is due to the anomalous electrophoretic properties of the BSH SNF-5 domain. The slower electrophoretic mobilities in SDS–PAGE than predicted from sequence analysis was also reported for human and *Drosophila* homologues of yeast SWI3, another member of SWI/SNF complex (32,33).

The finding that *Arabidopsis BSH* gene partially complements the *snf5* mutation in *S.cerevisiae* (Fig. 5) is a strong indication of the functional homology between BSH and yeast SNF5. In contrast to INI1, the BSH, when tethered to DNA, was unable to activate transcription of a reporter gene in yeast. The reason for this is not clear. The transcriptional activation by native SNF5p in such a system could be through recruitment of other members of SWI/SNF complex. However, in yeast cells used to test the BSH (and INI1) activity there was a native SNF5p protein which could make the formation of the heterologous complex rather difficult. It is also possible that the transcription results from the complex-independent activation by SNF5p alone. In this case BSH, in contrast to INI1, would lack such an activity in yeast. It should be noted that the DNA-tethered INI1 could not activate transcription of a reporter gene in human cells (31).

Human INI1 was identified as a component of a large multiprotein complex homologous to the yeast SWI/SNF complex (13). Both yeast and human complexes are capable of disrupting nucleosomes and facilitate the binding of transcriptional activators to a nucleosomal template. As regards *Drosophila* SNR1, it too had been shown to be a component of a large protein complex that

also contained the brm protein, a homologue of the yeast SWI2/SNF2 protein (14).

The human INI1 protein has been shown to interact directly with hbrm (a human homologue of the yeast SWI2/SNF protein) through its conserved SNF5 domain (31). As there are strong indications of the homology between SWI/SNF-type nucleosome remodelling complexes in yeast, human and *Drosophila* it is plausible that the SNF5 domains of yeast SNF5 and *Drosophila* SNR1 are similarly involved in the interactions with SWI2/SNF2 and brm protein, respectively. If this was a general feature of the SNF5 domain one could predict the existence of the *Arabidopsis* homologue of the yeast SWI2/SNF2 protein. While our search of the *Arabidopsis* dbEST database (which is now considered to be >70% saturated) for the homologues of yeast SWI2/SNF2 protein rendered no scores, we identified and sequenced (results not shown) a clone with a rare domain characteristic for the SNF2L subfamily of the SNF2 family of proteins (34). To this subfamily belongs the ISWI protein (35), a component of a different chromatin remodelling complex called NURF, identified in *Drosophila* embryo extract (36). We also identified in the database of the *A.thaliana* genomic sequences two separate genes with high homology to yeast SWI3 protein, another component of the SWI/SNF complex.

The data accumulated so far indicate that in eukaryotic cells, in addition to multiple forms of the SWI/SNF-type complexes (6), there can exist other complexes with chromatin remodelling activity, like the NURF complex in *Drosophila* or a recently discovered RSC complex in yeast (37). It is also evident that in different evolutionary lines the SWI/SNF complexes can be involved in different functions. The human SWI/SNF-type complexes containing either BRG1 or hbrm (homologues of SWI2/SNF2) are not essential for cell viability (38). However, the *Drosophila* homologue of SWI/SNF complex seems to be indispensable for normal embryonic development. The homozygosity in mutated *snr1* gene encoding the *Drosophila* homologue of SNF5 was shown to be lethal (14). In contrast to the spatially and temporary selective expression of snr1 in *Drosophila*, in *Arabidopsis* the *BSH* gene is expressed ubiquitously (Fig. 3A), a pattern more resembling that reported for expression of the *INI1* gene in human tissues (13). Despite the ubiquitous expression in plant of the *BSH* mRNA, a considerable decrease of its level achieved by the use of 'antisense' strategy did not cause lethality. However, it resulted in a distinctive change in the morphological appearance (a bushy growth) and in flowers that were unable to produce seeds. This phenotype bears strong resemblance to that of the *aux1* mutants of *Arabidopsis* characterised by resistance to the plant hormone auxin (39). This may indicate a possible involvement of *Arabidopsis BSH* in the control of some hormone-responsive genes.

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