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ORIGINAL PAPER

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The *Arabidopsis* PHD-finger protein SHL is required for proper development and fertility

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Abstract The *SHL* gene from *Arabidopsis thaliana* encodes a small nuclear protein that contains a BAH domain and a PHD finger. Both domains are found in numerous (putative) transcriptional regulators and chromatin-remodeling factors. Different sets of transgenic lines were established to analyze the physiological relevance of SHL. *SHL* expression driven by the CaMV 35S promoter results in reduced growth, early flowering, early senescence, and impaired flower and seed formation. Antisense inhibition of *SHL* expression gives rise to dwarfism and delayed development. In-frame N-terminal fusion of the SHL protein to β-glucuronidase (GUS) directs GUS to the nucleus of stably transformed *Arabidopsis* plants. Thus, *SHL* encodes a novel putative regulator of gene expression, which directly or indirectly influences a broad range of developmental processes.

Key words PHD finger · BAH domain · Transcription factor · Nuclear localization · *Arabidopsis*

Introduction

Plant transcriptional regulators are characterized by a multitude of structural motifs (Meshi and Iwabuchi 1995; Liu et al. 1999). These include various (putative) zinc-containing motifs, among them the PHD fingers (Aasland et al. 1995), which are characterized by a unique Cys_4-His-Cys_3 pattern, spanning approximately 50–80 amino acids. The arrangement of cysteine, histidine, and other residues distinguishes this motif from the similarly sized RING fingers (Saurin et al. 1996) and LIM domains (Sánchez-Garciá and Rabbitts 1994). The name "PHD finger" refers to the Cys-rich regions of the two *p*lant *h*omeo*d*omain proteins HAT3.1 of *Arabidopsis* and Zmhox1a of maize (Schindler et al. 1993). Other PHD finger-containing plant proteins are PRHP and PRHA (*p*athogenesis-*r*elated *h*omeodomain proteins from *p*arsley and *Arabidopsis*; Korfhage et al. 1994), which may be involved in the transcriptional regulation of pathogen defense-related genes. The function of another plant PHD finger protein, ES43 from barley (Speulman and Salamini 1995), however, is unknown. In animals PHD fingers are also found in transcriptional regulators, including members of the Polycomb and trithorax group of proteins (reviewed by Kennison 1995). The Polycomb group (Pc-G) gene products are transcriptional regulators which silence homeobox-containing (HOM) genes and thus contribute to ensuring the correct expression patterns at specific developmental stages (Pirrotta 1997). Trithorax group (trx-G) genes have been identified as suppressors of Pc-G mutants (Kennison 1995). They activate and maintain HOM gene transcription. The importance of plant homologs of Pc-G genes has recently been highlighted by the developmental abnormalities caused by mutations in the *CLF*, *MEDEA*, *PKL*, and *FIE* genes (Ohad et al. 1996; Goodrich et al. 1997; Grossniklaus et al. 1998; Ogas et al. 1999). In addition to PHD fingers, other domains, such as the chromodomain (e.g. in PC) and the bromodomain (e.g. in TRX), are frequently found in the products of Pc-G and trx-G genes. These domains are present in proteins which (putatively) regulate transcription by modulating chromatin structure (reviewed by Varga-Weisz and Becker 1995; Kingston et al. 1996). Accordingly, the WCRF180 protein from HeLa cells and the ACF protein of *Drosophila*, which contain PHD fingers and bromodomains, function in chromatin assembly (Ito et al. 1999; Bochar et al. 2000). Further-

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more, PHD fingers are found in the transcriptional corepressor KAP-1 (Friedman et al. 1996; Moosmann et al. 1996), the developmental regulator AF10 (e.g. Linder et al. 1998), and several other putative transcription factors. By analogy to classical zinc fingers (Takatsuji 1999), LIM domains, and RING fingers, the PHD finger has been proposed to mediate protein-protein, protein-DNA, or protein-RNA interactions (Aasland et al. 1995).

The BAH (bromo-adjacent homology) domain is found in eukaryotic DNA (cytosine-5) methyltransferases (Finnegan et al. 1998), the origin recognition complex 1 (Orc1) proteins (Gavin et al. 1995), and several proteins involved in transcriptional regulation. They are supposed to be involved in protein-protein interactions and mediate gene silencing (reviewed in: Callebaut et al. 1999). The BAH module can be associated with PHD fingers, and both domains are often found in close proximity (e.g. in ES43 of barley, or ASH1 of *Drosophila*). The functional relevance of this domain organization, however, is unknown.

As in animals, DNA modifications, such as methylation, are proposed to maintain transcriptional states in plants (reviewed in Richards 1997). Maintenance of cytosine methylation patterns in *Arabidopsis* is dependent on the *DDM1* gene product (Kakutani et al. 1995; Jeddeloh et al. 1999). Further (putative) chromatin-remodeling factors have been identified in plants (e.g., *BSH*, Brzeski et al. 1999; *PKL*, Ogas et al. 1999), as well as other transcription factors that act as developmental regulators (e.g., *KN1*, Sinha et al. 1993; *HAT4*, Schena et al. 1993).

We describe here the characterization of the plant gene *SHL* (for the ***sh****ort* ***l****ife* exhibited by plants that overexpress the *SHL* cDNA). Our interest in this gene was triggered by the interesting domain structure of its product. *SHL* encodes a short polypeptide with a putative nuclear localization signal (NLS), a BAH motif, and a C-terminal PHD finger. Reverse genetic approaches were used to elucidate the function of *SHL*. The pleiotropic phenotypic abnormalities observed in transgenic *Arabidopsis* plants in which the expression of the *SHL* gene was modulated indicate that *SHL* is necessary for normal plant growth and development. The primary structure of the protein, as well as the nuclear localization of a SHL-GUS fusion protein, point to a potential function as a transcriptional regulator.

Materials and methods

Plant material

A. thaliana cv. C24 and transgenic plants (see below) were grown for RNA isolation in half-strength MS medium (Murashige and Skoog 1962), supplemented with 1% sucrose and solidified with 0.7% agarose, under a 16 h day (140 μE, 22 °C)/8 h (22 °C) night regime. For phenotypic characterization, plants were grown in soil (Einheitserde Typ GS90; Gebrüder Patzer, Simtal-Jossa, Germany). After 7 days of stratification [16 h fluorescent light (120 μE), 20 °C/8 h dark, 6 °C, 75% relative humidity], established plants were grown in long days [16 h fluorescent light (60, 120, or 180 μE), 20 °C, 60% relative humidity/8 h dark, 16 °C, 75% relative humidity].

Generation of constructs and transgenic plants

The *SHL* cDNA was obtained as EST clone 181F3 (Accession No. H37427; Newman et al. 1994) from the Arabidopsis Biological Resource Center (Ohio State University, Columbus, Ohio). For sense or antisense expression (35S-SHL or α-SHL, respectively), a 1100-bp *Sma*I-*Hin*dIII cDNA fragment was inserted in either orientation into the *Sma*I site in the binary vector pBinAR (Höfgen and Willmitzer 1990), between the CaMV35S promoter and the OCS 3′ termination signal, after a fill-in reaction with the Klenow fragment. To generate the SHL-GUS fusion the *SHL* cDNA was inserted into the pBI121 vector (Clontech, Heidelberg, Germany). To facilitate this, PCR was performed to add *Xba*I and *Bam*HI sites using the primers sense (TCTAGAATGCCCAAGCAAAAAGCTCC) and antisense (TGGATCCACCTGGTCGCTTAGTGTG). The PCR was done with *Pfu* DNA polymerase (Stratagene, Heidelberg, Germany) to ensure high fidelity. Sequence analysis of the PCR product revealed 100% identity to the *SHL* cDNA sequence. After digestion of the PCR product and the vector with *Xba*I and *Bam*HI, the *SHL* fragment was inserted downstream of the CaMV35S promoter and in frame with the β-glucuronidase coding region (35S-SHL-GUS). Transgenic lines transformed with these constructs were selected using hygromycin (35S-SHL and α-SHL) or kanamycin (35S-SHL-GUS).

RNA extraction and Northern analysis

Total RNA was extracted from plants at indicated times, fractionated and blotted as previously described (Müssig et al. 2000). To generate sense or antisense *SHL* probes, linear PCRs were performed using the entire *SHL* cDNA as template together with antisense or sense primers, respectively (sequences given above), as well as [^{32}P]dCTP. The conditions for linear PCR were: 94 °C for 3 min, followed by 10 cycles of 57 °C for 20 s, 72 °C for 45 s, 94 °C for 20 s). Hybridizations were performed overnight at 65 °C in sodium phosphate buffer (0.25 M, pH 7.2) containing 7% SDS, 1% BSA, and 1 mM EDTA.

Histochemical analysis

X-gluc (β-D-5-bromo-4-chloro-3 indolyl glucuronide) was used to determine the localization of the enzyme activity of the SHL-GUS fusion protein. Tissue samples were incubated at 37 °C in GUS buffer (100 mM sodium phosphate buffer pH 7.2, 0.1% Triton X-100, 2 mM $K_3[Fe(CN)_6]$, 0.5 mg/ml X-gluc) for 2–15 h. After detection of the blue color, chlorophyll was extracted with 80% ethanol for 24 h at 37 °C. Nuclei were stained with DAPI according to the manufacturer's instructions (Boehringer, Mannheim, Germany). The location of the blue precipitate was compared with that of the DAPI-stained nuclei using bright-field or fluorescence optics.

Results

SHL-GUS localizes to the nucleus in transgenic *Arabidopsis* plants

The SHL protein contains amino acid motifs characteristic of nuclear proteins (Fig. 1). Several basic residues within the N-terminal region (aa 3–21) may form an NLS and direct SHL into the nucleus. The protein is composed of two domains, an N-terminal BAH domain

(aa 28–131) and a C-terminal PHD finger (aa 141–187). Their occurrence in specific classes of nuclear proteins points to a role in chromatin-mediated regulation of gene expression (Aasland et al. 1995; Callebaut et al. 1999).

To determine whether SHL is imported into the nucleus, a SHL-GUS fusion protein was expressed in transgenic *Arabidopsis* plants. The subcellular localization of the fusion protein was determined by incubating rosette and cauline leaf tissue with X-gluc. Nuclear staining was clearly detectable in tissue samples of several independent lines after 2–15 h exposure to the substrate (Fig. 2, and data not shown), while only minor activity was observed in the cytoplasm. Thus, SHL directs the GUS protein to the nucleus in *Arabidopsis* leaf tissue.

Pattern of *SHL* mRNA expression

Northern analysis revealed prominent *SHL* expression in roots, stems, rosette leaves, cauline leaves, and flowers (Fig. 3). Low levels of *SHL* transcript were detectable in siliques. Neither application of phytohormones, such as gibberellins (GA_3), 24-epibrassinolide or abscisic acid, nor various stress treatments, such as

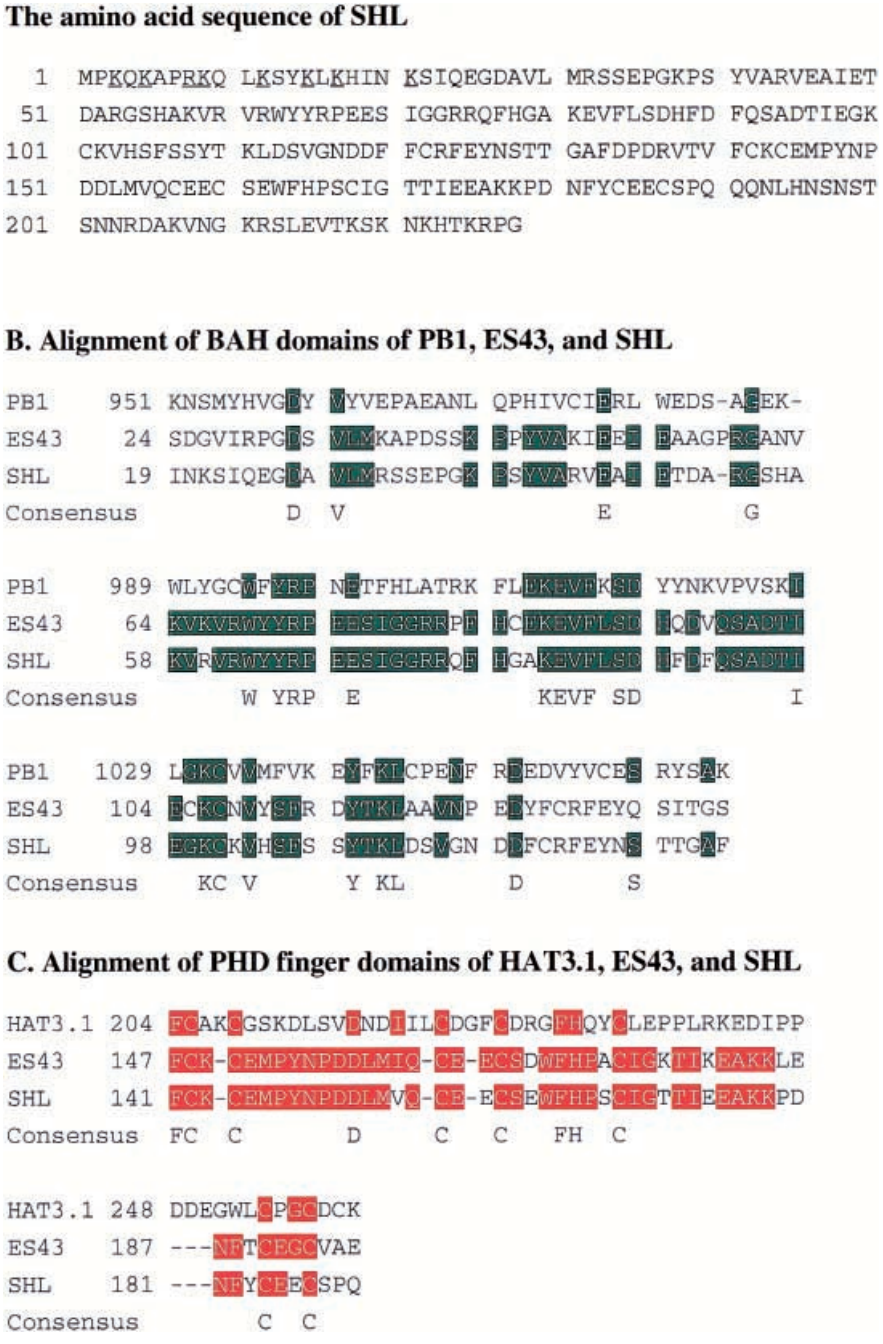

Fig. 1A–C Primary structure of the SHL protein. **A** The sequence encoded by the SHL cDNA. The basic amino acids of a putative nuclear localization signal are *underlined*. **B** Homology of SHL with the first BAH domain of PB1 (Nicolas et al. 1996) and the BAH domain of ES43 (Speulman and Salamini 1995). **C** Alignment of the PHD-finger domains of HAT3.1 (Schindler et al. 1993), ES43, and SHL

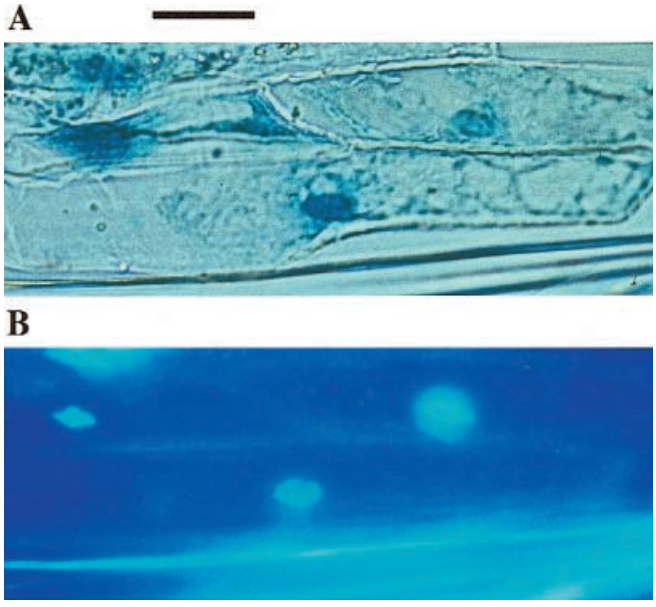

Fig. 2A, B Localization of a SHL-GUS fusion protein in epidermal peels of cauline leaves of transgenic *Arabidopsis* plants. **A** Detection of GUS activity under bright field (blue color). **B** Detection of the nuclei by DAPI staining and epifluorescence. The scale bar represents 10 μm

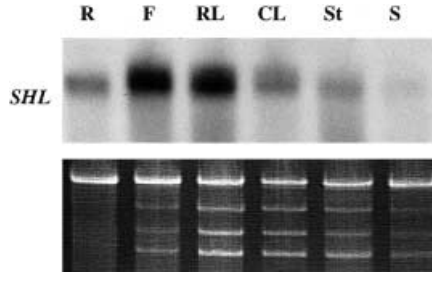

Fig. 3 Northern analysis of *SHL* expression. *SHL* expression in different tissues of soil-grown, wild-type plants. R, root; F, flower; RL, rosette leaf; CL, cauline leaf; St, stem; S, silique. The ethidium bromide-stained gel is shown to demonstrate that equal amounts of total RNA were loaded in each line

wounding, touch, wind, UV irradiation, heat, or cold, resulted in reproducible changes in *SHL* expression in wild-type plants (data not shown). The *SHL* mRNA is detectable in seedlings, and throughout the adult vegetative phase as well as the adult reproductive phase. This broad and constitutive expression pattern in different tissues is consistent with the pleiotropic alterations observed in plants in which *SHL* expression is modified (see below).

Modulation of *SHL* expression in transgenic plants

To investigate the physiological role of SHL, a series of transgenic plants was created. At least 100 independent first-generation (T1) plants per construct were obtained. Selected plants were propagated by selfing to obtain lines homozygous for the transgene. Third- (T3) or fourth-generation (T4) plants were examined in detail for phenotypic alterations.

SHL overexpression accelerates development but reduces growth and fertility

Plants overexpressing *SHL* differed clearly from wild-type plants. At least four out of a total of 20 independent T3 lines displayed reduced growth and accelerated development (Fig. 4). The severity of the phenotypic abnormalities was correlated with the relative increase in *SHL* mRNA levels. The highest transcript abundance was found in lines 12 and 18, which displayed the most dramatic alterations (see below). The leaves were reduced in number (Table 1) and size (Fig. 4). In addition to the reduction in the growth rates of aerial organs, root development was also retarded. The changes in vegetative characteristics were accompanied by alterations in reproductive traits. In the transgenic plants, inflorescences became visible earlier than in the wild-type plants (25.3 ± 1.3 days after germination for line #18 exposed to 180 μE illumination, 41.8 ± 3.2 days for wild-type plants; see also Fig. 4). Accordingly, siliques began to form about 6 weeks after germination in the transgenic line #18, in comparison to 9 weeks for the non-transformed plants. The development of fewer flowers resulted in reduced seed set in the transgenic plants. The flowers which were formed, however, were normal with respect to organ arrangement and number. Senescence of vegetative leaves started around 4 weeks earlier in the transgenic lines than in the wild-type. Overexpression of a SHL-GUS fusion protein resulted in developmental changes very similar to those observed in the 35S-SHL plants (Fig. 5). Also in these cases, the severity of the phenotypic alterations was correlated with the levels of SHL-GUS RNA (Fig. 6B).

Table 1 Leaf numbers of 56-day-old transgenic plants

Line	Leaf number[a]	
	180 μE	60 μE
α-SHL		
#20	68.2 ± 3.3	17 ± 1.9
#25	63.8 ± 12.5	24.8 ± 1.6
#33	72.0 ± 10.8	28.6 ± 1.4
#39	87.3 ± 6.8	27.8 ± 3.1
35S-SHL		
#8	46.2 ± 3.8	14.3 ± 1.9
#12	40.0 ± 2.8	11.8 ± 1.5
#18	12.0 ± 2.4	9.4 ± 1.4
#20	61.4 ± 12.6	22.5 ± 3.2
Wild type	90.5 ± 10.2	30.0 ± 4.1

[a] Leaf number refers to the total number of leaves in the rosette, including those formed from axillary buds. Cotyledons and cauline leaves were excluded. Values (means ± SD) were calculated from a minimum of five individual plants from each line, grown under medium (180 μE) or low (60 μE) light levels

Fig. 4A–C Morphology of plants with modified *SHL* expression. Wild-type, *SHL*-antisense, and *SHL*-overexpressing *Arabidopsis* plants were grown in soil for 38 days (**A**), 53 days (**B**), or 36 days (**C**) under low (60 µE) or medium (180 µE) light conditions. **A, B** *Top left to bottom right*: plants from the overexpressing lines 8, 20, 12 and 18, wild-type, and antisense lines 20, 25, 33, and 39. **C** *Left to right*: plants from antisense line 20, overexpressing line 18, and wild type

Antisense inhibition of *SHL* expression results in delayed development and impaired growth

SHL antisense expression resulted in a clear reduction in *SHL* transcript levels in all lines analyzed (Fig. 6A). Plants of strongly affected antisense lines exhibited a stunted axis, small, dark-green leaves, a compact rosette structure, reduced leaf expansion and lower leaf number (Fig. 4 and Table 1). Although the organs were slightly

Fig. 5 Morphology of 45-day-old *Arabidopsis* plants overexpressing the SHL-GUS fusion construct. *Top left to bottom right*: wild type, and transgenic SHL-GUS lines 1, 3, 12, 18, 13, 16 and 17

reduced in length, root development was essentially normal. Furthermore, antisense *SHL* expression provokes late flowering (49.4 ± 2.9 days after germination under light levels of 180 μE for line #20, in comparison to 41.8 ± 3.2 day for the wild type), and the transgenic plants produced fewer flowers and seeds. The most dramatically affected antisense lines failed to flower and could not be propagated.

In summary, modulation of *SHL* expression affects both vegetative and reproductive traits, indicating that the gene product is necessary for wild-type ontogenesis.

Discussion

The SHL protein is largely composed of a BAH motif and a PHD finger domain. A close homolog from barley, ES43 (Speulman and Salamini 1995), has been

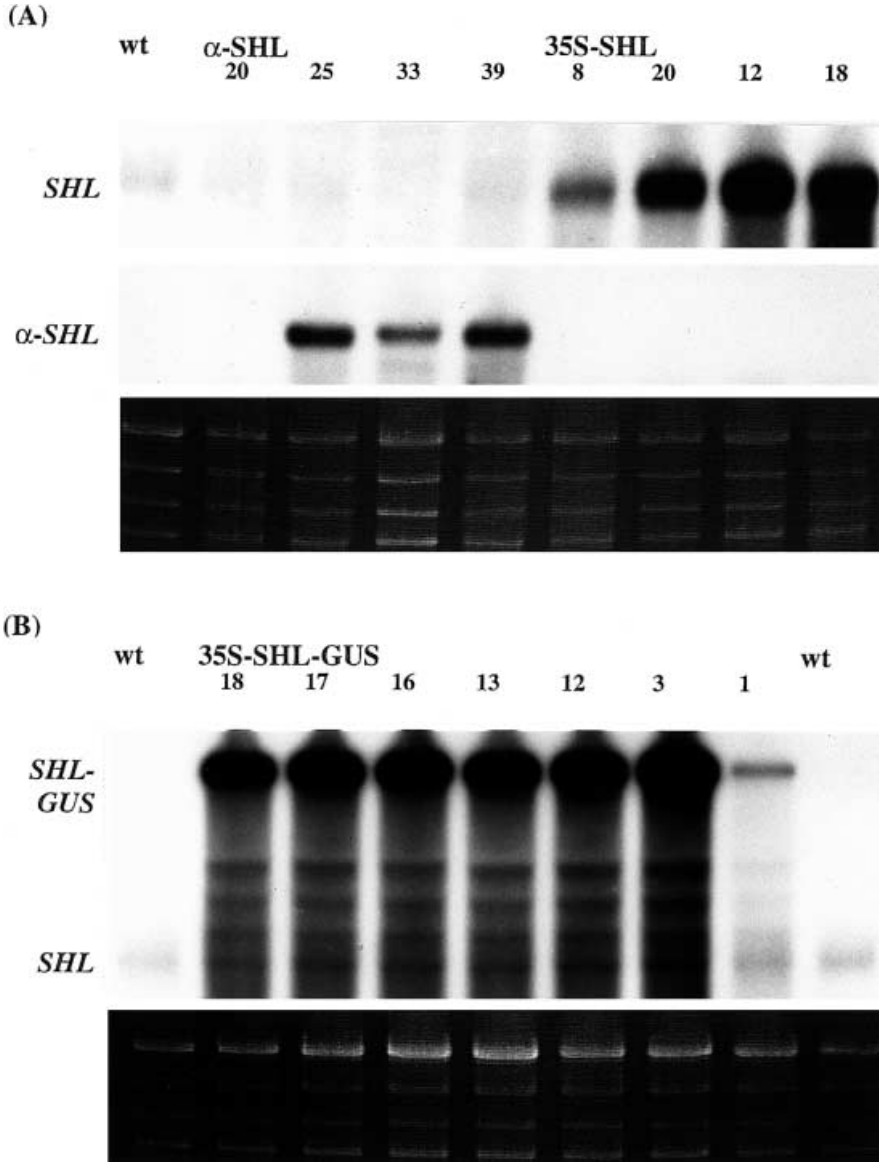

Fig. 6A, B Northern analysis of transgenic plants. **A** Northern analysis of antisense (α-SHL) and overexpressing (35S-SHL) lines. Total RNA was isolated from 21-day-old plants. *SHL*, SHL mRNA; *α-SHL*, SHL antisense RNA. The ethidium bromide-stained gel is shown to demonstrate that equal amounts of total RNA were loaded in each line. **B** Northern analysis of 35S-SHL-GUS transgenic plants. Total RNA was isolated from 14-day-old seedlings. The ethidium bromide-stained gel is shown to demonstrate that equal amounts of total RNA were loaded in each line. *SHL*, SHL mRNA; *SHL-GUS*, SHL-GUS mRNA

identified previously and displays 57.5% sequence identity to SHL. Further SHL homologs in several other higher plants have been identified as ESTs (e.g. from tomato, Accession No. AW035325; cotton, AI726030; rice, AF074797). SHL and its homologs are of special interest due to the joint presence of the PHD finger and the BAH domain. The function of this domain combination is unknown, even in the case of animal proteins such as ASH1 of *Drosophila* (Tripoulas et al. 1994). Furthermore, no information was hitherto available on the role of those genes in plants. SHL is a nuclear protein, as demonstrated by its ability to redirect the GUS protein into the nucleus. Correspondingly, the BAH- and PHD-finger domains are found in numerous (putative) transcriptional regulators and proteins involved in chromatin modification (Aasland et al. 1995; Callebaut et al. 1999). To analyze the function of SHL, different sets of transgenic *Arabidopsis* lines with modified *SHL* expression were created. These lines revealed a requirement for SHL for proper vegetative and reproductive development. *SHL*-overexpressing lines showed reduced growth, early flowering and senescence, whereas antisense lines showed delayed development. Similar changes have been observed in plants with altered DNA methylation (Kakutani et al. 1995; Finnegan et al. 1996; Ronemus et al. 1996). In contrast to plants with modified *SHL* expression, however, *MET1* antisense plants, in which methylation levels are reduced (Finnegan et al. 1996), as well as the hypomethylation mutant *ddm1* (Kakutani et al. 1995), show homeotic transformations of floral organs. This suggests that the plants with modified *SHL* expression do not suffer from gross hypomethylation of DNA. Correspondingly, no change in cytosine methylation of *Hpa*II and *Msp*I sites (CCGG) in the centromeric repeat (Martinez-Zapater et al. 1986) has been observed in α-SHL or 35S-SHL plants (data not shown). However, it is possible that SHL plays a role in the regulation of sequence-specific DNA methylation or in the recognition of the methylation status of the DNA.

Other (putative) chromatin-remodeling factors from plants include MEDEA, FIE, CLF, PKL, and BSH. *MEDEA* and *FIE* are Polycomb-group genes involved in embryo development (Grossniklaus et al. 1998; Ohad et al. 1999) and may interact to silence the transcription of genes needed to initiate endosperm development (Kiyosue et al. 1999; Ohad et al. 1999). CLF is required for stable repression of a floral homeotic gene in leaves and stems, and shows homology to the product of the Pc-G gene *Enhancer of zeste* (Goodrich et al. 1997). PKL belongs to the group of CHD3 proteins which are characterized by PHD-finger domains. PKL regulates the transition from embryonic to vegetative development and might mediate gibberellin effects (Ogas et al. 1999). *BSH* is a member of the *SNF5* gene family. SNF5 proteins are components of SWI/SNF complexes, which may serve to destabilise DNA-histone interactions and allow binding of transcription factors. Expression of *BSH* in antisense orientation results in a bushy growth habit and in the development of infertile flowers (Brzeski et al. 1999). Further proteins involved in DNA modifications, such as cytosine methylation and histone acetylation, as well as factors involved in the recognition of specific DNA sites, remain to be identified. Taking into account the primary structure of the SHL protein, the multiple developmental abnormalities seen in the *SHL* antisense and overexpression lines, and the nuclear localization of the SHL-GUS fusion protein, it is conceivable that SHL belongs to this group of nuclear factors and may exert its effects through interaction with, or modification of, chromatin. However, the phenotypic alterations observed in the transgenic plants may also be due to defects in functions that are independent of chromatin modification (e.g. biosynthesis of, or responses to phytohormones) and may thus be of secondary nature.

To elucidate further the function of *SHL*, gene expression profiling experiments using cDNA arrays are in progress for wild-type, α-SHL, and 35S-SHL plants. The information thus gained should provide more detailed insight into SHL-dependent gene expression and provide a means to place SHL in the context of other regulatory pathways.

References

Aasland R, Gibson TJ, Stewart AF (1995) The PHD finger: implications for chromatin-mediated transcriptional regulation. Trends Biochem Sci 20: 56–59

Bochar DA, Savard J, Wang W, Lafleur DW, Moore P, Côte J, Shiekhattar R (2000) A family of chromatin remodeling factors related to Williams syndrome transcription factor. Proc Natl Acad Sci USA 97: 1038–1043

Brzeski J, Podstolski W, Olczak K, Jerzmanowski A (1999) Identification and analysis of the *Arabidopsis thaliana BSH* gene, a member of the *SNF5* gene family. Nucleic Acids Res 27: 2393–2399

Callebaut I, Courvalin J-C, Mornon J-P (1999) The BAH (bromo-adjacent homology) domain: a link between DNA methylation, replication and transcriptional regulation. FEBS Lett 446: 189–193

Finnegan EJ, Peacock WJ, Dennis ES (1996) Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development. Proc Natl Acad Sci 93: 8449–8454

Finnegan EJ, Genger RK, Peacock WJ, Dennis ES (1998) DNA methylation in plants. Annu Rev Plant Physiol Plant Mol Biol 49: 223–247

Friedman JR, Fredericks WJ, Jensen DE, Speicher DW, Huang X-P, Neilson EG, Rauscher FJR (1996) KAP-1, a novel corepressor for the highly conserved KRAB repression domain. Genes Dev 10: 2067–2078

Gavin KA, Hidaka M, Stillman B (1995) Conserved initiator proteins in eukaryotes. Science 270: 1667–1671

Goodrich J, Puangsomlee P, Martin M, Long D, Meyerowitz EM, Coupland G (1997) A Polycomb-group gene regulates homeotic gene expression in *Arabidopsis*. Nature 386: 44–51

Grossniklaus U, Vielle-Calzada J-P, Hoeppner MA, Gagliano WB (1998) Maternal control of embryogenesis by *MEDEA*, a Polycomb group gene in *Arabidopsis*. Science 280: 446–450

Höfgen R, Willmitzer L (1990) Biochemical and genetic analysis of different patatin isoforms expressed in various organs of potato (*Solanum tuberosum*). Plant Sci 66: 221–230

Ito T, Levenstein ME, Fyodorov DV, Kutach AK, Kobayashi R, Kadonaga JT (1999) ACF consists of two subunits, ACF1 and

ISWI, that function cooperatively in the ATP-dependent catalysis of chromatin assembly. Genes Dev 13: 1529–1539
Jeddeloh JA, Stokes TL, Richards EJ (1999) Maintenance of genomic methylation requires a SWI2/SNF2-like protein. Nat Genet 22: 94–97
Kakutani T, Jeddeloh JA, Richards EJ (1995) Characterization of an *Arabidopsis thaliana* DNA hypomethylation mutant. Nucleic Acids Res 23: 130–137
Kennison JA (1995) The Polycomb and trithorax group proteins of *Drosophila*: trans-regulators of homeotic gene function. Annu Rev Genet 29: 289–303
Kingston RE, Bunker CA, Imbalzano AN (1996) Repression and activation by multiprotein complexes that alter chromatin structure. Genes Dev 10: 905–920
Kiyosue T, Ohad N, Yadegari R, Hannon M, Dinneny J, Wells D, Katz A, Margossian L, Harada JJ, Goldberg RB, Fischer RL (1999) Control of fertilization-independent endosperm development by the *MEDEA* Polycomb gene in *Arabidopsis*. Proc Natl Acad Sci USA 96: 4186–4191
Korfhage U, Trezzini G, Meier I, Hahlbrock K, Somssich IE (1994) Plant homeodomain protein involved in transcriptional regulation of a pathogen defense-related gene. Plant Cell 6: 695–708
Linder B, Jones LK, Chaplin T, Mohd-Sarip A, Heinlein UAO, Young BD, Vaskar S (1998) Expression pattern and cellular distribution of the murine homologue of AF10. Biochim Biophys Acta 1443: 285–296
Liu L, White MJ, MacRae TH (1999) Transcription factors and their genes in higher plants. Eur J Biochem 262: 247–257
Martinez-Zapater JM, Estelle MA, Somerville CR (1986) A highly repeated DNA sequence in *Arabidopsis thaliana*. Mol Gen Genet 204: 417–423
Meshi T, Iwabuchi M (1995) Plant transcription factors. Plant Cell Physiol 36: 1405–1420
Moosmann P, Georgiev O, Le Douarin B, Bourquin J-P, Schaffner W (1996) Transcriptional repression by RING finger protein TIF1β that interacts with the KRAB repressor domain of KOX1. Nucleic Acids Res 24: 4859–4867
Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15: 473–497
Müssig C, Biesgen C, Lisso J, Uwer U, Weiler EW, Altmann T (2000) A novel stress-inducible 12-oxophytodienoate reductase from *Arabidopsis thaliana* provides a potential link between brassinosteroid-action and jasmonic-acid synthesis. J Plant Physiol, in press
Newman T, de Bruijn FJ, Green P, Keegstra K, Kende H, McIntosh L, Ohlrogge J, Raikhel N, Somerville S, Thomashow M, Retzel E, Somerville C (1994) Genes galore: a summary of methods for accessing results from large-scale partial sequencing of anonymous *Arabidopsis* cDNA clones. Plant Physiol 106: 1241–1255
Nicolas RH, Goodwin GH (1996) Molecular cloning of polybromo, a nuclear protein containing multiple domains including five bromodomains, a truncated HMG-box, and two repeats of a novel domain. Gene 175: 233–240
Ogas J, Kaufmann S, Henderson J, Somerville C (1999) PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. Proc Natl Acad Sci USA 96: 13839–13844
Ohad N, Margossian L, Hsu Y-C, Williams C, Repetti P, Fischer RL (1996) A mutation that allows endosperm development without fertilization. Proc Natl Acad Sci USA 93: 5319–5324
Ohad N, Yadegari R, Margossian L, Hannon M, Michaeli D, Harada JJ, Goldberg RB, Fischer RL (1999) Mutations in *FIE*, a WD polycomb group gene, allow endosperm development without fertilization. Plant Cell 11: 407–415
Pirrotta V (1997) Chromatin-silencing mechanisms in *Drosophila* maintain patterns of gene expression. Trends Genet 13: 314–318
Richards EJ (1997) DNA methylation and plant development. Trends Genet 13: 319–329
Ronemus MJ, Galbiati M, Ticknor C, Chen J, Dellaporta SL (1996) Demethylation-induced developmental pleiotropy in *Arabidopsis*. Science 273: 654–657
Sánchez-Garciá I, Rabbitts TH (1994) The LIM domain: a new structural motif found in zinc-finger-like proteins. Trends Genet 10: 315–320
Saurin AJ, Borden KLB, Boddy MN, Freemont PS (1996) Does this have a familiar RING? Trends Biochem Sci 21: 208–214
Schena M, Lloyd AM, Davis RW (1993) The *HAT4* gene of *Arabidopsis* encodes a developmental regulator. Genes Dev 7: 367–379
Schindler U, Beckmann H, Cashmore AR (1993) HAT3.1, a novel *Arabidopsis* homeodomain protein containing a conserved cysteine-rich region. Plant J 4: 137–150
Sinha NR, Williams RE, Hake S (1993) Overexpression of the maize homeo box gene, *KNOTTED-1*, causes a switch from determinate to indeterminate cell fates. Genes Dev 7: 787–795
Speulman E, Salamini F (1995) A barley cDNA clone with homology to the DNA-binding domain of the steroid hormone receptors. Plant Sci 106: 91–98
Takatsuji H (1999) Zinc-finger proteins: the classical zinc finger emerges in contemporary plant science. Plant Mol Biol 39: 1073–1078
Tripoulas NA, Hersperger E, La Jeunesse D, Shearn A (1994) Molecular genetic analysis of the *Drosophila melanogaster* gene *absent*, *small or homeotic discs1* (*ash1*). Genetics 137: 1027–1038
Varga-Weisz PD, Becker PB (1995) Transcription factor-mediated chromatin remodelling: mechanisms and models. FEBS Lett 369: 118–121