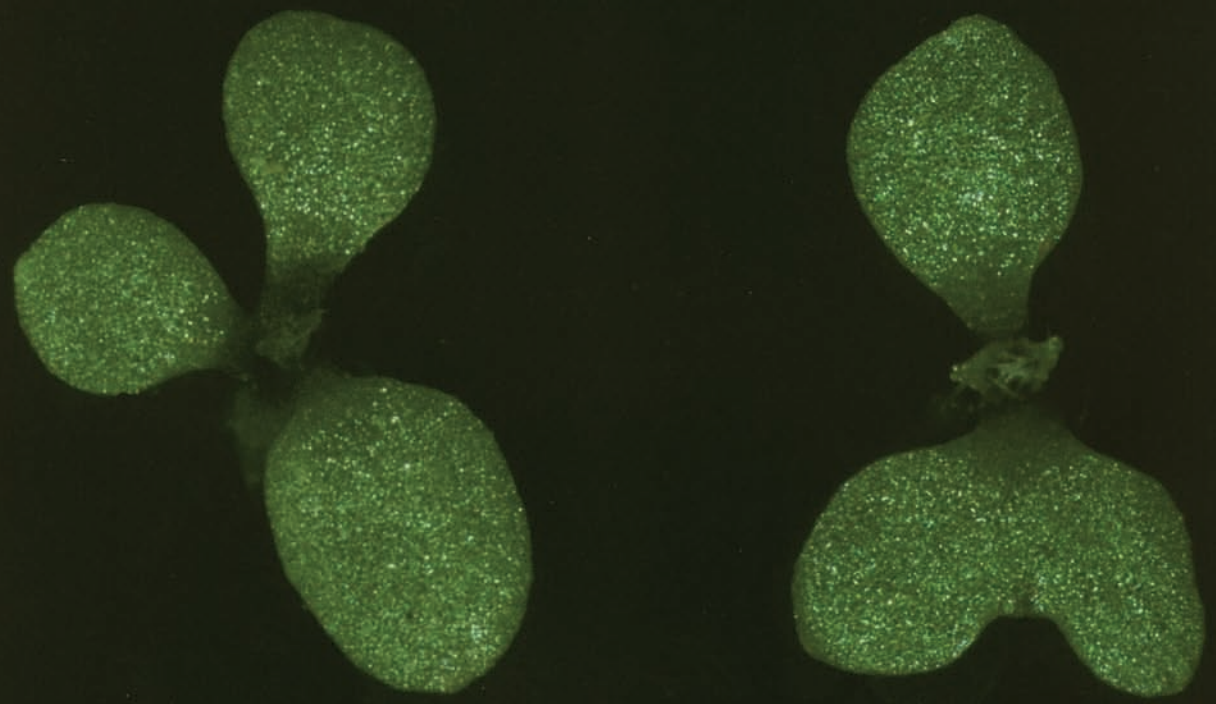
Volume 78, Nos. 1–2, January (I+II) 2012 ISSN 0167-4412

PLANT MOLECULAR BIOLOGY



Springer

Tricotyledons developed in a *nucleostemin-like 1* mutant

Plant Mol Biol (2012) 78:31–44
DOI 10.1007/s11103-011-9840-7

Nucleostemin-like 1 is required for embryogenesis and leaf development in *Arabidopsis*

Xiaomin Wang · Bo Xie · Maosheng Zhu · Zhongming Zhang · Zonglie Hong

Received: 1 December 2009 / Accepted: 19 October 2011 / Published online: 6 November 2011

Abstract *Arabidopsis NSN1* encodes a nucleolar GTP-binding protein and is required for flower development. Defective flowers were formed in heterozygous *nsn1/+* plants. Homozygous *nsn1* plants were dwarf and exhibited severe defects in reproduction. Arrests in embryo development in *nsn1* could occur at any stage of embryogenesis. Cotyledon initiation and development during embryogenesis were distorted in *nsn1* plants. At the seedling stage, cotyledons and leaves of *nsn1* formed upward curls. The curled leaves developed meristem-like outgrowths or hyperplasia tissues in the adaxial epidermis. Long and enlarged pavement cells, characteristic of the abaxial epidermis of wild type plants, were found in the adaxial epidermis in *nsn1* leaves, suggesting a disoriented leaf polarity in the mutant. The important role of *NSN1* in embryo development and leaf differentiation was consistent with the high level expression of the *NSN1* gene in the developing embryos and the primordia of cotyledons and leaves. The *CLAVATA 3* (*CLV3*) gene, a stem cell marker in the *Arabidopsis* shoot apical meristem (SAM), was expressed in expanded regions surrounding the SAM of *nsn1* plants, and induced ectopically in the meristem-like outgrowths in cotyledons and leaves. The *nsn1* mutation up-regulated the expression levels of several genes implicated in the meristem identity and the abaxial cell fate, and repressed the expression of other genes related to the specification of cotyledon boundary and abaxial identity. These results demonstrate that *NSN1* represents a novel GTPase required for embryogenesis, leaf development and leaf polarity establishment in *Arabidopsis*.

Keywords Nucleostemin · Embryogenesis · Stem cell · Leaf polarity · *Arabidopsis thaliana*

X. Wang · B. Xie · M. Zhu · Z. Hong (✉)
Department of Plant, Soil and Entomological Sciences and Program of Microbiology, Molecular Biology and Biochemistry, University of Idaho, Moscow, ID 83844-3052, USA
e-mail: zhong@uidaho.edu

M. Zhu · Z. Zhang
State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China

Introduction

Recent investigations on the stem cells in animals have been focused on the identification of stem cell-specific marker proteins and elucidation of regulatory pathways in stem cells (Bernardi and Pandolfi 2003). Nucleostemin (NS), initially identified in a differential screen for stem cell-specific markers, has proven to be an important regulator of stem cell proliferation in animals (Tsai and McKay 2002; Misteli 2005; Swaminathan 2005; Tsai and McKay 2005; Yaghoobi et al. 2005; Fan et al. 2006; Zhu et al. 2006; Cada et al. 2007; Ma and Pederson 2007; Yasumoto et al. 2007; Huang et al. 2008; Romanova et al. 2009a, b). NS is a GTP-binding protein with an N-terminal basic domain and a C-terminal acidic acid region (Tsai and McKay 2002). *NS* is expressed preferentially in the embryonic and neuronal stem cells, primitive cells of the bone marrow and several cancer cell lines. The expression of *NS* is turned off in cells undergoing differentiation and in differentiated cells of most adult tissues (Tsai and McKay 2002). Either depletion or overexpression of NS can result in the reduced cell proliferation rates of neuronal stem cells and transformed cells (Tsai and McKay 2002). At the molecular level, NS interacts with a spectrum of proteins implicated in the cell division control, DNA repair, telomere protection, maintenance of the nucleolar

structure, and pre-rRNA processing and transport (Beekman et al. 2006; Ma and Pederson 2007).

Nucleostemin is localized mainly in the nucleoli, and may shuttle between the nucleolus and nucleoplasm (Misteli 2005; Tsai and McKay 2005). When bound to GTP, NS enters the nucleolus. Its N-terminal basic domain interacts with the nucleolar components, allowing NS to accumulate in the nucleoli. After GTP hydrolysis, the protein rapidly diffuses back to the nucleoplasm. The intermediate (I)-domain of NS provides a nucleoplasmic anchor for the retention of the protein in the nucleoplasm (Tsai and McKay 2002, 2005). In addition, the interaction of NS with ribosomal protein RSL1D1 also affects its nucleolar localization (Meng et al. 2006). The mechanism of targeting NS to the nucleoli appears to be conserved for other homologs of the NS family of proteins (Dai et al. 2008).

Like in animals, stem cells in plants posses the ability to reproduce themselves and to differentiate into specific tissues (Lenhard et al. 2001; Lohmann et al. 2001; Parcy et al. 2002; Bernardi and Pandolfi 2003; Leibfried et al. 2005; Beekman et al. 2006; Krizek et al. 2006; Wurschum et al. 2006; Sablowski 2007). The shoot meristem in plants contains a pool of stem cells that occupy the center of the meristem and divide in a much slower rate than their neighboring cells in the growing apices (Weigel and Jurgens 2002). All aerial parts of a higher plant derive from the progeny of the stem cells (Stewart and Dermen 1970; Gallois et al. 2004).

Several genes required for the maintenance and regulation of stem cells in plants have been subject to intense research. *WUSCHEL* (*WUS*) and *CLAVATA3* (*CLV3*) forms a feedback loop that controls the size of the stem cell bank in the shoot meristem and inflorescence meristem (Schoof et al. 2000). In this feedback loop, *WUS* promotes stem cell identity and expression of stem cell marker *CLV3*, which, in return, represses the expression of *WUS* (Lenhard et al. 2001; Lohmann et al. 2001). In *wus-1*, the meristem is disrupted and fails to organize stem cells in the shoot meristem. The defective *wus* meristem contains larger and more vacuolated cells than those present in a normal shoot meristem (Laux et al. 1996). Ectopic expression of *WUS* can produce supernumerary stem cells, giving rise to supernumerary ectopic organs (Fletcher et al. 1999; Brand et al. 2000; Schoof et al. 2000; Lohmann et al. 2001). Enhanced expression of endogenous *WUS* results in accumulation of redundant cells with the stem cell identity (Xu et al. 2005). In *clv3-2* plants, *WUS* is expressed in an expanded domain in the meristem, leading to the development of enlarged SAM and defective flowers with extra floral organs (Fletcher et al. 1999; Brand et al. 2000). On contrary, the SAM of transgenic plants expressing *CaMV35S*::*CLV3* cannot develop beyond the two-leaf stage (Fletcher et al. 1999; Brand et al. 2000; Schoof et al. 2000). The development of the floral meristem is controlled by a different feedback loop involving *WUS*, *LEAFY* (*LFY*) and *AGAMOUS* (*AG*). The expression of *AG* in the central region of the floral meristems relies on the function of *WUS* and *LFY*. The activated *AG*, in return, down-regulates *WUS* expression (Lohmann et al. 2001). Our previous study has identified a plant homolog of nucleostemin, designated as NSN1 for nucleostemin-like 1 (Wang et al. 2011). *NSN1* plays an essential role in the maintenance of shoot and floral meristems in *Arabidopsis* (Wang et al. 2011). In this report, we provide further evidence for a novel function of *NSN1* in embryogenesis and leaf development in *Arabidopsis*.

Materials and methods

Plant materials and growth condition

Wild type *Arabidopsis* and mutant plants (Columbia ecotype) were grown in long day conditions (16 h light/8 h dark) at 21 ± 1°C. T-DNA insertion mutant of *nsn1* (Salk_029201; Wang et al. 2011) was obtained from the *Arabidopsis* Biological Resources Center. Transgenic *Arabidopsis* line harboring *CLV3::GUS* was a gift of Dr. Thomas Laux (Gross-Hardt et al. 2002). Transgenic *Arabidopsis* line expressing NSN1-GFP fusion protein under the control of the CaMV 35S promoter was described previously (Wang et al. 2011).

Light microscopy and scanning electronic microscopy

Arabidopsis embryos were observed using the whole-mount method (Schoof et al. 2000). Embryos at different developmental stages were excised from siliques with 27-gauge needles. Materials were cleared in Hoyer's solution for 1–4 h, and observed under an Olympus BH-2 light microscope with differential interference contrast optics. Images were captured on Jenoptik ProgRes C12 plus digital camera using the Axiovision program (version 3.1).

Scanning electronic microscopy (SEM) was carried out as described previously (Wang et al. 2011). Plant tissues, fixed in 50 mM phosphate butter containing 2% glutaraldehyde and 2% paraformaldehyde, were rinsed three times with 50 mM phosphate buffer and incubated overnight in 1% osmium tetroxide at 4°C. After washing with 50 mM phosphate buffer, the tissues were dehydrated in an alcohol gradient of 30, 50, 70, 95 and 100%. After critical point drying, the tissues were coated with gold and observed under SEM.

Histology and GUS staining

Plant tissues were fixed in FAA solution (3.7% formaldehyde, 5% acetic acid and 50% ethanol) at room temperature for 16 h. The tissues were dehydrated in a series of ethanol (30, 50, 70, 85, 90 and 100%), cleared with xylene and embedded in Paraplast (Sigma) as described previously (Causier et al. 2005). Serial sections of 8 μm thickness were prepared with a glass knife on a Reichert Ultracut microtome. After de-waxing, the sections were stained with 0.01% toludine-blue for 5–10 min at room temperature.

For analysis of *CLV3* gene expression pattern in *nsn1* mutant background, pollen grains from transgenic plant harboring *CLV3::GUS* (Gross-Hardt et al. 2002) were used to pollinate the stigma of homozygous *nsn1* plants (Wang et al. 2011). F_2 plants with *nsn1* phenotypes and GUS activity were selected. After GUS staining (Schoof et al. 2000), plant tissues were treated in 70% ethanol overnight and images were taken under Jenoptik ProgRes C12 plus digital camera using the Axiovision program (version 3.1) with DIC lens.

Preparation of RNA probe and in situ RNA hybridization

For preparation of the *NSN1*-specific anti-sense mRNA probe, the 3′-terminal region (600 bp) of the *NSN1* cDNA was amplified using primers NSN1-LP and NSN1-RP (Table 1) and subcloned into pCRII (Invitrogen) to yield pNSN1. The plasmid was linearized with *Apa* I and transcribed with SP6 RNA polymerase in the presence of digoxigenin-labeled dUTP (Roche), producing the labeled anti-sense RNA probe of *NSN1*. For the *NSN1* sense probe control, pNSN1 was linearized with *BamH1* and transcribed with T7 RNA polymerase (Roche).

In situ hybridization was performed as previously described (Wang et al. 2011). Ovaries at different developmental stages were isolated by forceps and fixed in 4% paraformaldehyde. The fixed tissues were dehydrated in an ethanol series (30, 50, 70, 85, 90 and 100%), cleared with xylene and embedded in Paraplast (Sigma). Serial sections of 8 μm thickness were made and transferred to polylysine-coated slides (Sigma). After de-waxing, the sections were treated in proteinase K and hybridized with

Table 1 Primers used in this study

Name	Sequence from 5′ to 3′	Note
NSN1-LP	TATTGGGACTGCCTAATGTCGG	Amplification of a 600 bp *NSN1* 3′-UTR for mRNA probe
NSN1-RP	GAGTCTAAGACGCAGACGGAA	
WUS-F	ATGGAGCCGCCACAGCATCAGC	RT-PCR amplification of a *WUSCHEL*-specific cDNA
WUS-R	GCTAGTTCAGACGTAGCTCAAGAG	
CLV3-F	CTCATGTAAATGGATTCTTATAAGTCTC	RT-PCR of a *CLAVATA3*-specific cDNA
CLV3-R	TAGGTCAAGGGAGCTGAAAGTTG	
STM-F	TTAGGGAGCCTCAAGCAAGA	RT-PCR of a *SHOOT MERISTEMLESS*-specific cDNA
STM-R	TACAAACTGCATGTCCTCCG	
KNAT1-F	GCT CCA CCT GAT GTG GTT GA	RT-PCR of a *KNAT1*-specific cDNA
KNAT1-R	TGT TGA GGA TGT GAA TGG GA	
CUC2-F	CAGCCAATATCTTCCACCGGG	RT-PCR of a *CUP-SHAPED COTYLEDON2*-specific cDNA
CUC2-R	GGAGAGGTGGGAGTGAGACGGA	
AS2-F	ATT TCC CCT CTG AGC AAC AG	RT-PCR of a *ASYMMETRIC LEAVES2*-specific cDNA
AS2-R	AAG ACG GAT CAA CAG TAC GG	
PHB-F	TGATGGTCCATTCGATGAGC	RT-PCR of a *PHABULOSA*-specific cDNA
PHB-R	TCTAAACTCACGAGGCCGCA	
PHV-F	CCATGGACGATAGAGACTCTCC	RT-PCR of a *PHAVOLUTA* -specific cDNA
PHV-R	ACCACTTCCAAAACTTGGAAGA	
YAB5-F	ACGCCCTAATTTCCAGGCAAC	RT-PCR of a *YABBY5*-specific cDNA
YAB5-R	GTTGCTCAGTTATGGTACGAG	
FIL-F	AAACCAACATGCCCCAACAG	RT-PCR of a *FILAMENTOUS FLOWER*-specific cDNA
FIL-R	TCACACCAACGTTAGCAGCTG	
ACT-F	TGGTGTCATGGTTGGGATG	RT-PCR of an *ACTIN*-specific cDNA
ACT-R	CACCACTGAGCACAATGTTAC	

digoxigenin-labeled riboprobes overnight at 55°C. After washing with 0.2× SSC (30 mM NaCl, 3 mM trisodium citrate), the sections were blocked in 0.1% nonfat dry milk powder, and reacted with anti-digoxigenin antibody conjugated with the alkaline phosphatase (Roche). For color development, the sections were incubated in a commercial NBT/BCIP substrate (nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate, Roche) for 2 h and observed under a light microscope.

RNA isolation and RT-PCR

Total RNA from *Arabidopsis* rosette leaves was isolated using RNeasy Plant Mini Kit (Qiagen). cDNA was synthesized from 2 μg of total RNA with SuperScript First-Strand Synthesis system for RT-PCR (Invitrogen). One μl of the synthesized cDNA was used for PCR amplification. Gene-specific primers (Table 1) were used for amplification of *WUS*, *CLV3*, *STM*, *KNAT1*, *CUC2*, *AS2*, *PHB*, *PHV*, *YAB5*, *FIL*, and *ACTIN* cDNAs. After 35 cycles of amplification, PCR products were resolved on a 1% agarose gel and stained with ethidium bromide.

Results

Severe defects in seed setting in *nsn1*

As compared to the siliques of wild type *Arabidopsis* plants (14.46 ± 1.69 cm, n = 40), the mutant siliques were short (5.60 ± 1.96 cm, n = 40) in heterozygous *nsn1*/+ plants and very short (3.02 ± 0.67 cm, n = 40) in *nsn1* homozygote (Fig. 1a, b, e). To further investigate the potential effect of *nsn1* mutation on embryo development, we compared the seed numbers in *nsn1*/+ heterozygote, *nsn1* homozygote and wild type plants. The average number of seeds per silique was only 6.4 ± 3.8 (n = 15) per silique in *nsn1*/+ heterozygote and 0.2 ± 0.45 (n = 15) in *nsn1* homozygote, as compared to 60.2 ± 1.7 (n = 15) in wild type plants. Arrested embryos were present in early development stages in both *nsn1*/+ heterozygote and *nsn1* homozygote (red arrows, Fig. 1c–d). The fertility rate was only 9% in *nsn1*/+ heterozygote and 0.3% in *nsn1* homozygote, as opposed to the 100% fertility in wild type plants (Fig. 1f). These results indicate that *NSN1* may play an important role in ovule development and is required for embryogenesis in *Arabidopsis*.

Lethal embryos in *nsn1*

To investigate the time course of embryo lethality, we picked up white colored defective ovules from *nsn1*/+ and *nsn1* plants (arrowheads in Fig. 1c) for observations using light microscopy. Arrested embryos could be observed at the global, transition, heart, torpedo, bent cotyledon and mature embryo stages (Fig. 2h–n). These results show that embryo arrests could occur at different stages of embryo development in *nsn1* plants.

Delayed embryonic leaf initiation and expansion in *nsn1*

In wild type *Arabidopsis* plants, embryonic leaves were initiated from both sides of the SAM at the early heart stage, became rapidly expanding at the bent embryo stage

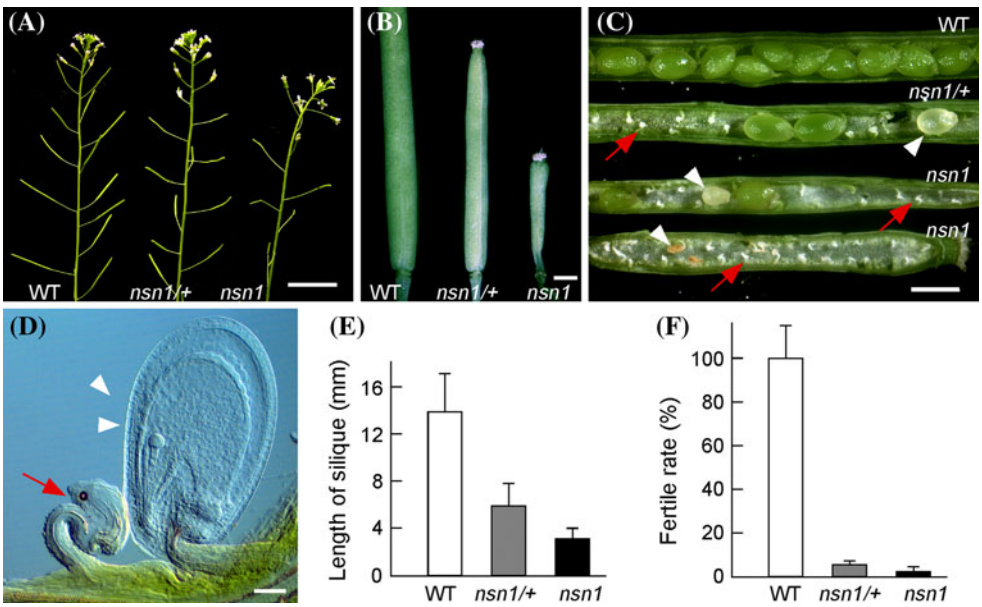


Fig. 1 Fertility phenotypes of *nsn1* mutant. **a** Heterozygote *nsn1*/+ plants produced short siliques, while homozygote *nsn1* plants were dwarf and formed very short siliques. **b** and **e** Phenotype of short siliques formed in the *nsn1*/+ and *nsn1* plants. **c** and **f** Presence of aborted embryos (*white arrowheads*) and ovules (*red arrows*) in opened siliques of *nsn1* mutant plants. **d** Image of an aborted ovule (*red arrow*) and a fertilized developing embryo (*white arrowhead*). *Scale bar* 5 mm in **a**, 1 mm in **b**, 2 mm in **c** and 25 μm in **d**

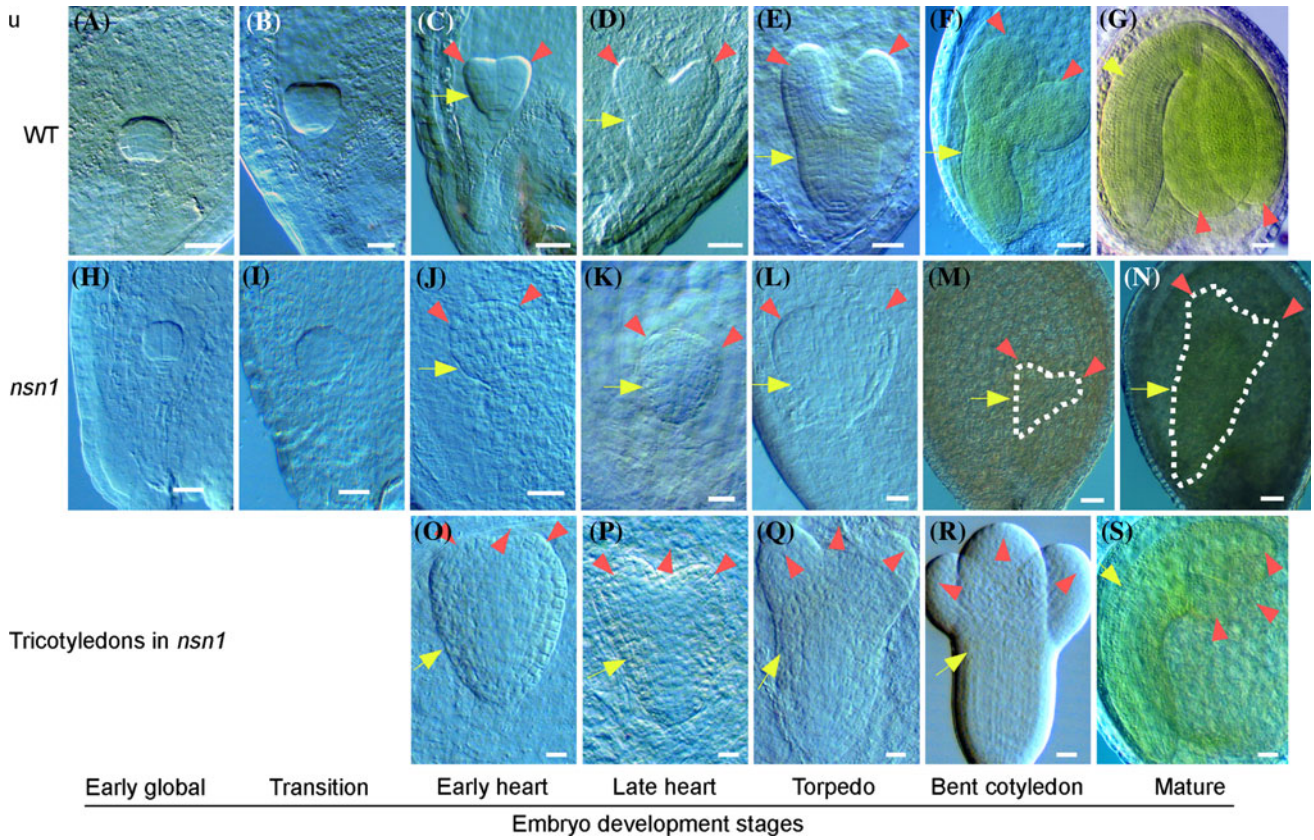


Fig. 2 Arrested embryos at different embryogenesis stages in homozygote *nsn1* plants. **a–g** Embryogenesis in wild type (WT) *Arabidopsis* plants. **h–n** Phenotypes of aborted embryos in homozygote *nsn1* plants. *Dotted lines* (**m–n**) indicate the blurred embryo boundaries in aborted ovules of *nsn1* plants at the bent cotyledon and mature stages, which contained accumulated pigments. **o–s** Development of tricotyledons in *nsn1*. Embryos of *nsn1* at early stages of embryogenesis were indistinguishable from those of the wild type plant (**a–b**). The initiation of the tricotyledon primordia (*red arrowheads*) in *nsn1* embryos was overall delayed while the basal part of embryos was apparently normal (*yellow arrows*). The tricotyledons became recognizable at the late heart stage (**p**) and could be clearly identified at torpedo and bent cotyledon stages. The boundaries of tricotyledon embryos were blurred, and pigments were distributed in other parts of the embryo as well (**s**). *Scale bar* 50 μm in **a–s**

and grew into cotyledons at the mature embryo stage (Fig. 2c–g). During this process, the top part and basal portion of the embryo elongated simultaneously with the initiation of cotyledons (Fig. 2c–g). In *nsn1*, embryos lost their distinct shapes at their corresponding stages, suggesting that cell differentiation in *nsn1* mutant is defective. The *nsn1* embryos were filled with less differentiated cells at their corresponding anatomic positions. This difference in embryo shape between the wild type plants and *nsn1* mutant were less significant before the early global stage (Fig. 2a, h). From the transition stage (Fig. 2b, i), especially from the early heart stage (Fig. 2c, j, o), the shape of embryonic leaf primordia (red arrowheads, Fig. 2) in *nsn1* became morphologically distinct from that of the wild type control. The initiation and expansion of embryonic leaves in *nsn1* were delayed and impeded (Fig. 2o–s). A characteristic defect of an embryo with three cotyledons was observed in *nsn1* embryos at a frequency of 7% (Figs. 2o–s, 4h). These results suggest that *NSN1* is essential for the organization of the embryonic leaf primordia and for the initiation and development of cotyledons in *Arabidopsis*. In *nsn1*, embryo development could be arrested at any stage of embryogenesis, and both cotyledon initiation and expansion could be blocked or delayed. We, thus, conclude that *NSN1* may act as a general regulator of embryonic development, instead of an embryogenesis stage-special regulator.

Loss-of-function phenotypes of embryogenesis in *NSN1*-overexpressing plants

In transgenic plants expressing *NSN1* under the control of the CaMV 35S promoter ($NSN1^{OX}$), embryo arrests could be found at each stage of embryogenesis (Fig. 3e–h) as in the loss-of-function *nsn1* mutant plants (Fig. 2h–m). The $NSN1^{OX}$ embryos also lost the characteristic shapes and patterns at various embryogenesis stages (Fig. 3e–h). The major difference in embryo defects between $NSN1^{OX}$ and *nsn1* was that no tricotyledon embryo was ever observed in $NSN1^{OX}$. These results indicate that *NSN1* may act in a gene dosage-sensitive manner. This conclusion is

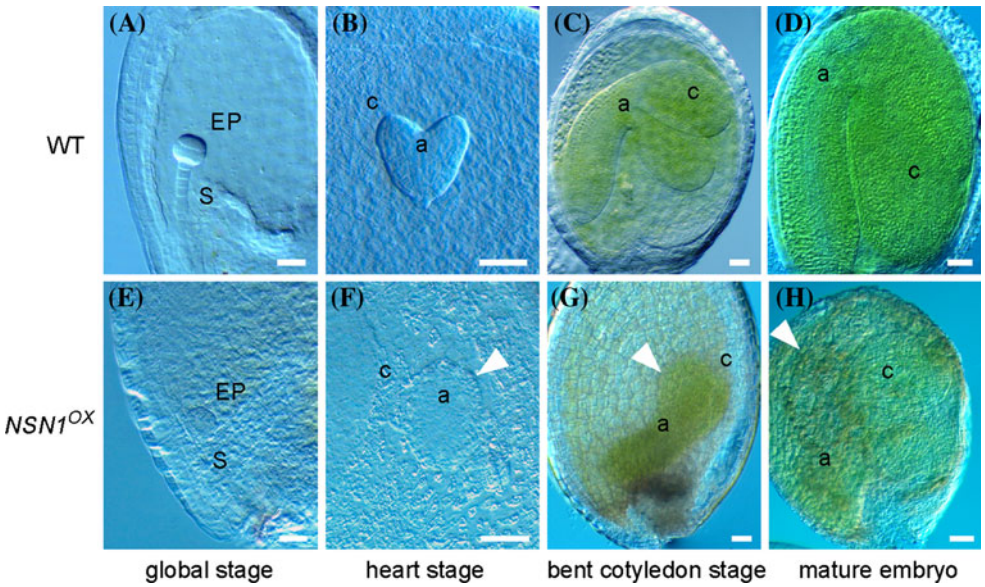


Fig. 3 Arrested embryos and retarded embryonic leaf initiation in plants overexpressing *NSN1* (*NSN1*^{OX}). **a**–**d** Wild type embryos; **e**–**h** Embryos of transgenic *NSN1*^{OX} plants. Arrest of embryo development in *NSN1*^{OX} could be seen at the global stage (**e**). Leaf initiation in *NSN1*^{OX} was repressed (**f**–**h**). *a* axis; *c* cotyledon; *EP* embryo proper; *S* suspensor. *Scale bar* 50 μm in **a**–**h**

consistent with the observation that heterozygous *nsn1/+* plants exhibited characteristics of a semidominant mutation, having growth and reproduction defects as compared to the wild type plants, but less severe phenotypes than those of the *nsn1* homozygotes (Fig. 1). Taken together, the expression level of the *NSN1* gene appears to be critical for embryo development in *Arabidopsis*.

Aberrant postembryonic meristem and leaf development in *nsn1* and *NSN1*^{OX}

In wild type *Arabidopsis* seedlings, leaf initiation could be observed 5 days after germination (Fig. 4b) and rapid leaf growth occurred 9 days after germination (Fig. 4c). In *nsn1*, cotyledons were small and asymmetric, and leaf

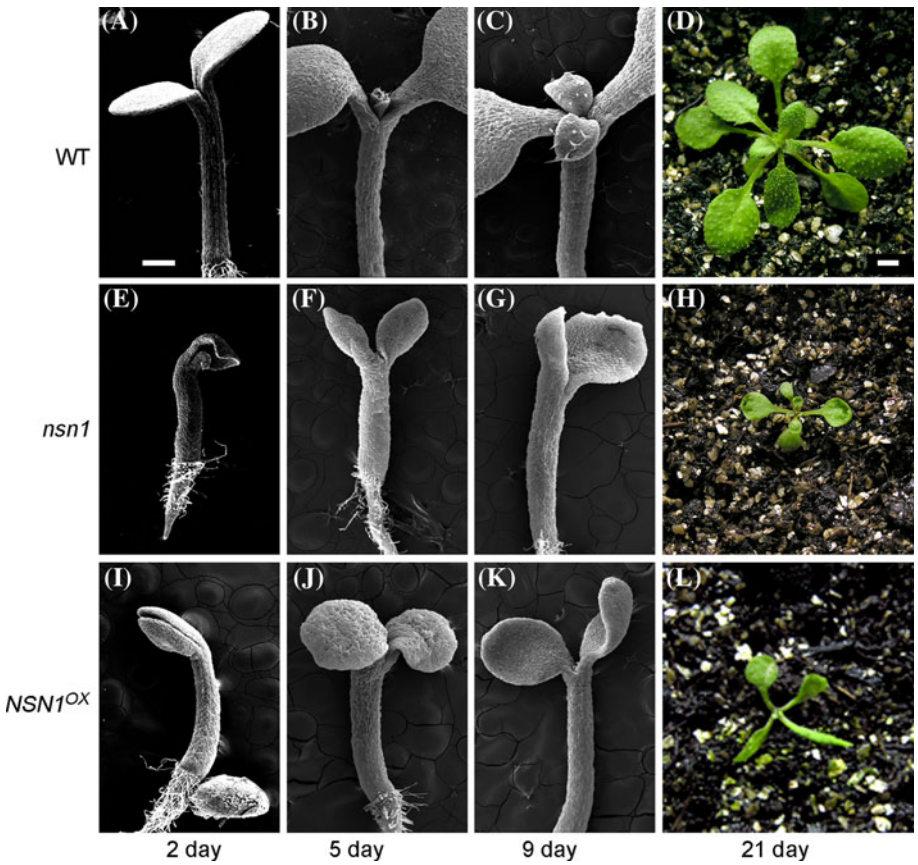


Fig. 4 Retardation of seedling growth in homozygous *nsn1* mutant and in *NSN1*^{OX} transgenic plants. **a**–**d** Control of wild type seedlings. **e**–**h** *nsn1* homozygote plants. **i**–*l* *NSN1*^{OX} transgenic plants. The opening and expansion of cotyledons in *nsn1* (**e**) and *NSN1*^{OX} (**i**) were delayed as compared to those of the wild type seedling (**a**). The first pair of leaves was initiated in wild type seedlings 5 days after germination (**b**) and expanded rapidly in 9 days after germination (**c**). No leaf initiation and expansion was observed in *nsn1* (**f**–**g**) and *NSN1*^{OX} (**j**–**k**) 9 days after germination. Seedlings of *nsn1* (**h**) and *NSN1*^{OX} (**l**) 21 days after germination were much smaller than the wild type (**d**) and developed small and curled leaves. *Scale bar* 1 mm in **a**–**c**, **e**–**g**, **i**–**k**; 3 mm in **d**, **h**, **l**

initiation and expansion were greatly delayed (Fig. 4e–h). Similar phenotypes of delayed leaf initiation and reduced leaf growth were also observed in *NSN1*^{OX} seedlings (Fig. 4i–l). These results suggest that *NSN1* is required for leaf initiation and expansion.

Wild type *Arabidopsis* leaves were slightly curled downward and had shallow serrations on the leaf edge (Fig. 5i). In *nsn1,* seedlings with three cotyledons (Fig. 2q–s, Fig. 5c), serrated cotyledons or monocotyledon (Fig. 5d–e) could be found at certain frequencies, which were not observed in *NSN1*^{OX} seedlings. Leaves of *nsn1* and *NSN1*^{OX} also lacked serrations on their edge. Both *nsn1* and *NSN1*^{OX} seedlings developed characteristic curls on the cotyledons and leaves (Figs. 4h, 5b, e, j–m). Interestingly, leaves of *nsn1* often exhibited upward curls (Fig. 5j), whereas downward curls were found in aberrant leaves of *NSN1*^{OX} seedlings (Fig. 5k–m). The molecular mechanism underlying those differences in cotyledon morphology and leaf curling remains to be studied. These observations suggest an essential role of *NSN1* in cotyledon and leaf development in *Arabidopsis*.

Ectopic meristem-like outgrowths on the surface of *nsn1* cotyledons and leaves

Curled cotyledons and leaves in *nsn1* were associated with the outgrowths on the adaxial epidermal surface. Under a scanning electronic microscope, these outgrowths were found to consist of 4–5 cells around one stoma (Fig. 5p), and in some cases, contained only one drastically enlarged epidermal cell (Fig. 5q). These outgrowths were apparently different from the ectopic meristems observed previously on leaves overexpressing the *WUS* gene (Gallois et al. 2002; Lenhard et al. 2002; Xu et al. 2005). Such ectopic meristems could develop into leaf- and flower-like structures, whereas the adaxial outgrowths of *nsn1* could not produce leaf- or flower-like organs. This result suggests that the ectopic meristem-like structures of *nsn1* do not have the capability of growth and differentiation as the real meristem. Taken together, our observations indicate that *NSN1* is involved in the differentiation of epidermal cells.

Disoriented leaf polarity in *nsn1* and *NSN*^{ox}

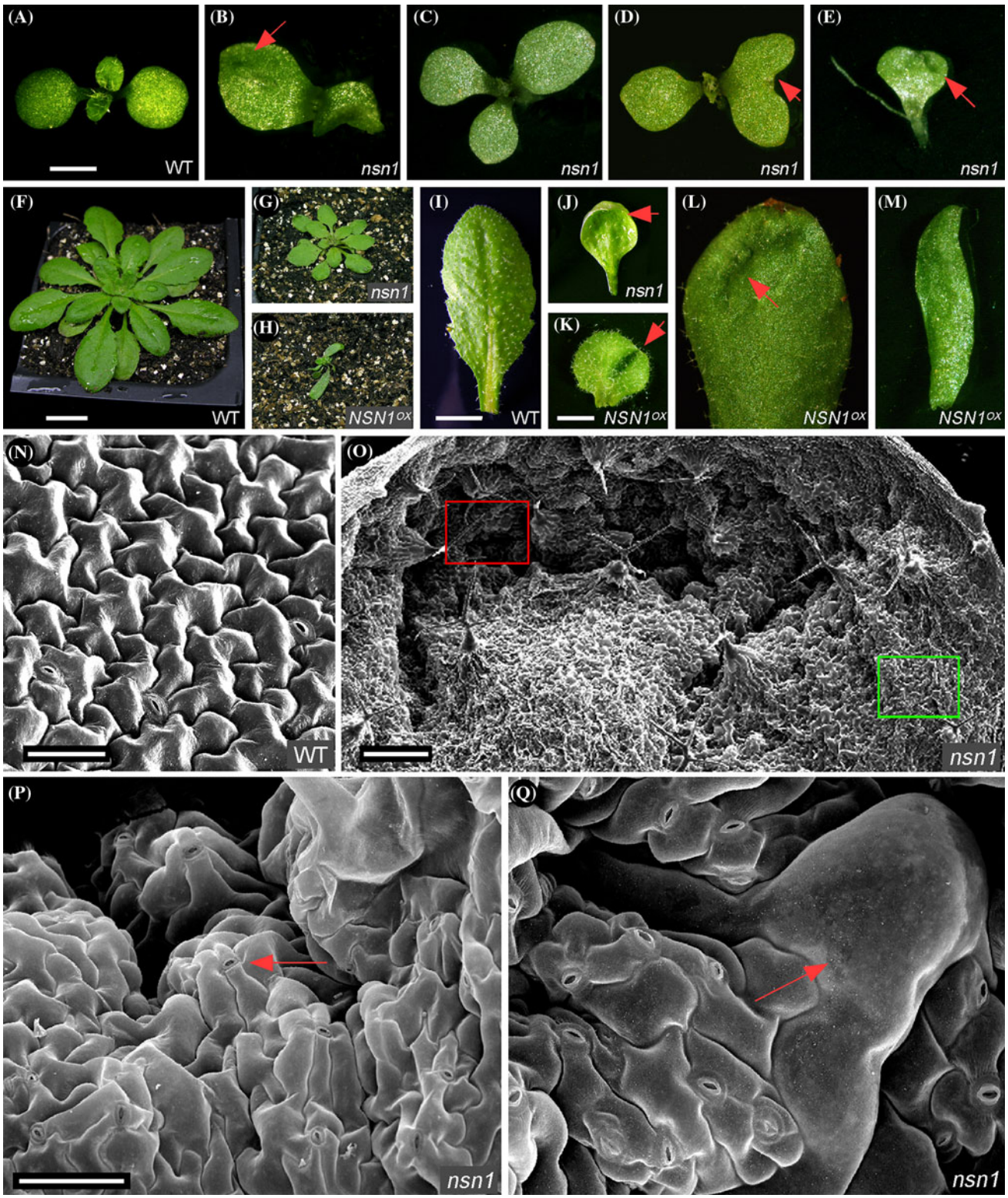
In leaves of wild type *Arabidopsis* plants, the adaxial epidermis comprised uniformed cells in size, whereas the abaxial epidermis was mosaic with smaller and less uniformed cells mixed with longer and narrow cells (Fig. 6a, d; McConnell and Barton 1998). In contrast, the adaxial epidermis of *nsn1* contained both small and large cells (Fig. 6b), a mosaic pattern similar to that of the abaxial epidermis (Fig. 6d, e). Similar alterations in cell pattern were also observed in the leaf adaxial epidermis of *NSN1*^{ox} transgenic plants (Fig. 6c). This type of disoriented leaf polarity has been reported previously in the *rev-6 as2-101* double mutant and *phantastica* in *Antirrhinum* (Waites et al. 1998; Fu et al. 2007). These results indicate that *NSN1* is involved in the control of leaf adaxial identity.

Expression pattern of *NSN1* in embryos and leaves

Expression of the *NSN1* mRNA was detected by in situ RNA hybridization in embryos at the global, transition, heart, torpedo, bent cotyledon and mature stages (Fig. 7a–l). The *NSN1* transcript was concentrated in the central part of embryos at the early stages of embryogenesis (Fig. 7a, c–d). As embryos continued to develop, the highest level of *NSN1* mRNA was found in the embryonic leaf primordia (Fig. 7e, g, i). At the mature stage, *NSN1* was expressed throughout the embryos (Fig. 7k). After seed germination, strong signals of *NSN1* expression were detected in the seedling leaves and the SAM (blue arrowheads). It is interesting to note that the expression level of *NSN1* was higher in the adaxial epidermis (green arrow, Fig. 7m) than the abaxial side (light blue arrow, Fig. 7m), which is consistent with the observation of the outgrowths found on the adaxial surface (Figs. 5, 6). These results clearly indicate that *NSN1* plays a role in the leaf growth and polarity determination in *Arabidopsis*.

Ectopic expression of meristem-related genes in *nsn1* plants

Our previous study showed that stem cell marker genes *CLV3* and *WUS* are expressed in an expanded region in the inflorescence and floral meristems in *nsn1* plants (Wang et al. 2011). To investigate the expression pattern of *CLV3* and *WUS* in the shoot meristem of embryos and seedlings, we introduced *CLV3::GUS* construct into the *nsn1* plants. In the wild type plants, *CLV3::GUS* was expressed in the stem cells in embryos at the heart stage (Fig. 8b). In *nsn1* plants, *CLV3::GUS* was expressed in a much expanded region surrounding the shoot meristem in the heart stage embryo (Fig. 8b–c). At the bent cotyledon stage, the GUS reporter signal was restricted to the SAM in WT embryos, whereas *CLV3* gene expression was expanded from the SAM to the petioles of the three cotyledons in *nsn1* (Fig. 8d–e). In the developing cotyledons of *nsn1* embryos, the highest level of *CLV3* expression was found in the cotyledon primordia and outgrowths (Fig. 8f). In the seedling stage, *CLV3* expression was confined to the SAM in WT plants (Fig. 8g), but could be detected in the SAM as well as in the cotyledons and their petioles in *nsn1* (Fig. 8h). These results are consistent with the expanded expression pattern of *CLV3* and *WUS* observed in the inflorescence meristem (Wang et al. 2011). Taken together, our observations suggest that the outgrowths (Fig. 5) found on the *nsn1* leaves might consist



of stem cell-like cells that arose from the de-differentiation of epidermal cells as a result of ectopic expression of *CLV3* in the *nsn1* mutant.

We also investigated the expression patterns of other meristem-related genes, *STM* and *KNAT1*, by semi-quantitative RT-PCR. The expression levels of both genes were up-regulated in *nsn1* seedlings (Fig. 8i). The *CUC2* (*CUP-SHAPED COTYLEDON2*) gene has been implicated in the regulation of embryonic shoot meristem formation and cotyledon boundary specification (Aida et al. 1999).

◀**Fig. 5** Aberrant cotyledon and leaf morphology in *nsn1* and *NSN1*^{OX} plants. **a–b** 10-day-old seedlings of wild type (**a**) and *nsn1* (**b**). *Red arrow* indicates the upward curled leaf edge of the *nsn1* plant. **c–e** Two-week-old seedlings of *nsn1* with an extra cotyledon (**c**), a serrated cotyledon (**d**) or only one cotyledon (**e**). **f–h** Three-week-old seedlings of wild type (**f**), *nsn1* (**g**) and *NSN1*^{OX} (**h**). **i–m** Leaves of three-week-old seedlings of wild type (**i**), *nsn1* (**j**) and *NSN1*^{OX} (**k–m**). Upward curled leaves (*arrow* in **j**) were typical in *nsn1* plants, whereas down-curled leaves (*arrows* in **k–m**) were developed in *NSN1*^{OX} plants. **n** The adaxial epidermis of wild type cotyledons. **o** The adaxial surface of the upward curled leaf of *nsn1* shown in (**j**). **p–q** Magnified areas of the *nsn1* leaf shown in (**o**) indicated by the red frame (**p**) and green frame (**q**). *Arrows* indicate an outgrowth structure consisting of 4–5 cells with a stoma on the *top* (**p**) or an enlarged epidermal cell on the adaxial surface of *nsn1* leaves (**q**). *Scale bar* 3 mm in **a–e**; 10 mm in **f–h**; 10 mm in **i–j**; 5 mm in **k–m**; 1 mm in **o;** 50 μm in **n**, **p**, **q**

Expression of *CUC2* was down-regulated in *nsn1* mutant (Fig. 8i). This might explain the aberrant cotyledon phenotypes found in *nsn1* (Fig. 5). All these results suggest that the aberrant cotyledon and leaf morphology of *nsn1* plants might be caused by the alteration of expression of the meristem-related genes. In *NSN1*^{OX} plants, the expression levels of *WUS, CLV3, STM* and *KNAT1* were down-regulated (Fig. 8i). These results shed light on the termination of SAM in *NSN1*^{OX} plants. It remains to be explained why *CUC2* expression was down-regulated in both *nsn1* and *NSN1*^{OX}.

Altered expression patterns of leaf polarity-related genes in *nsn1*

We investigated the expression patterns of genes implicated in the leaf polarity determination. Genes known to determine the adaxial cell fate, including *AS2, PHB* and *PHV*, were found to be down-regulated in *nsn1* plants (Fig. 8j), whereas genes that control the abaxial cell fate, including *YAB5* and *FIL* (Sawa et al. 1999), were up-regulated by the *nsn1* knockout (Fig. 8j). These results imply that the upward curling of leaves and disoriented leaf polarity in *nsn1* seedlings could be related to the abnormal expression of leaf polarity genes. We also investigated the expression levels of these genes in *NSN1*^{OX}. Results showed that *AS1, PHB* and *PHV* expression levels were also down-regulated in *NSN1*^{OX}, while *YAB5* and *FIL* expression levels were up-regulated in *NSN1*^{OX} compared to those in wide type plants**.** These results suggest that the abaxialized cell identity of adaxial leaf surface of *NSN1*^{OX} may be related to the ectopic expression of these leaf polarity genes. Detailed molecular mechanisms underlying

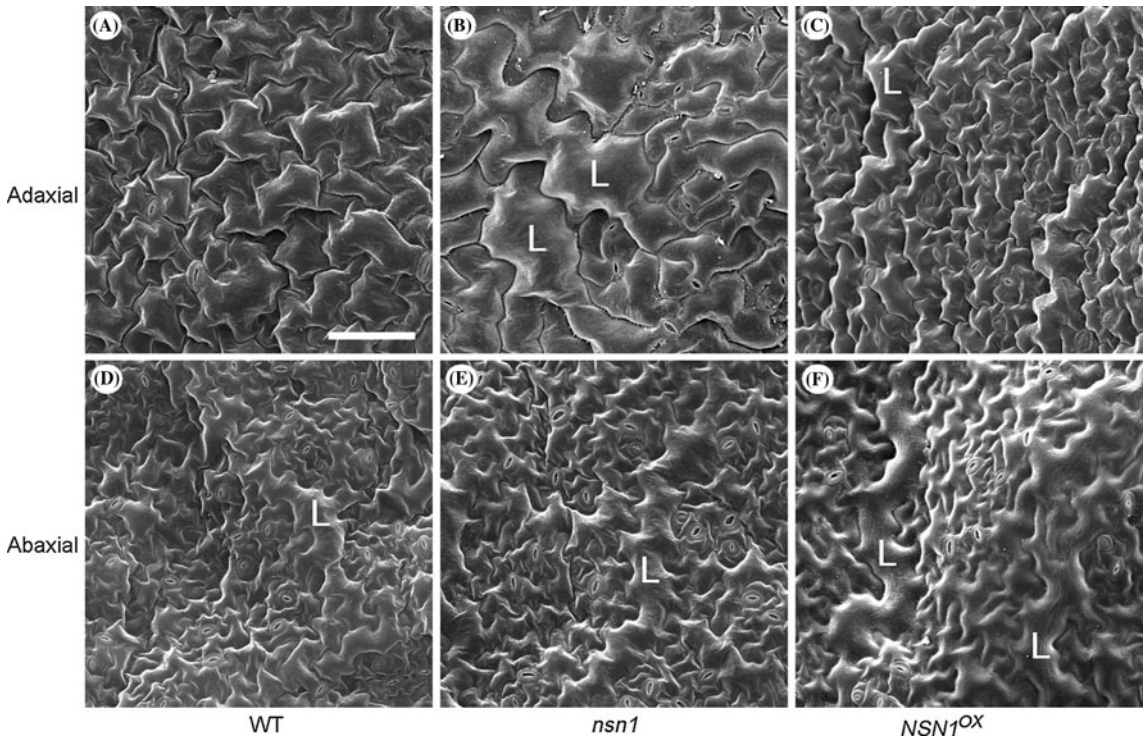


Fig. 6 Altered adaxial-abaxial polarity of leaves in *nsn1* and *NSN1*^{OX}. **a–c** Scanning electronic microscopy (SEM) images of the adaxial leaf epidermis of wild type (**a**), *nsn1* (**b**) and *NSN1*^{OX} (**c**). Sinusoid-shaped pavement cells in relatively uniformed sizes were present in mature wild type leaves (**a**). Long and enlarged cells (**L**) were found in *nsn1* (**b**) and *NSN1*^{OX} (**c**) leaves. **d–f** SEM images of the abaxial leaf epidermis of wild type (**d**), *nsn1* (**e**) and *NSN1*^{OX} (**f**). Long and enlarged cells (**L**) were present in mature wild type leaves (**d**). However, this type of cells were even longer and larger in *nsn1* (**e**) and *NSN1*^{OX} (**f**) than in the wild type (**d**). *Scale bar* 50 μm in **a–h**

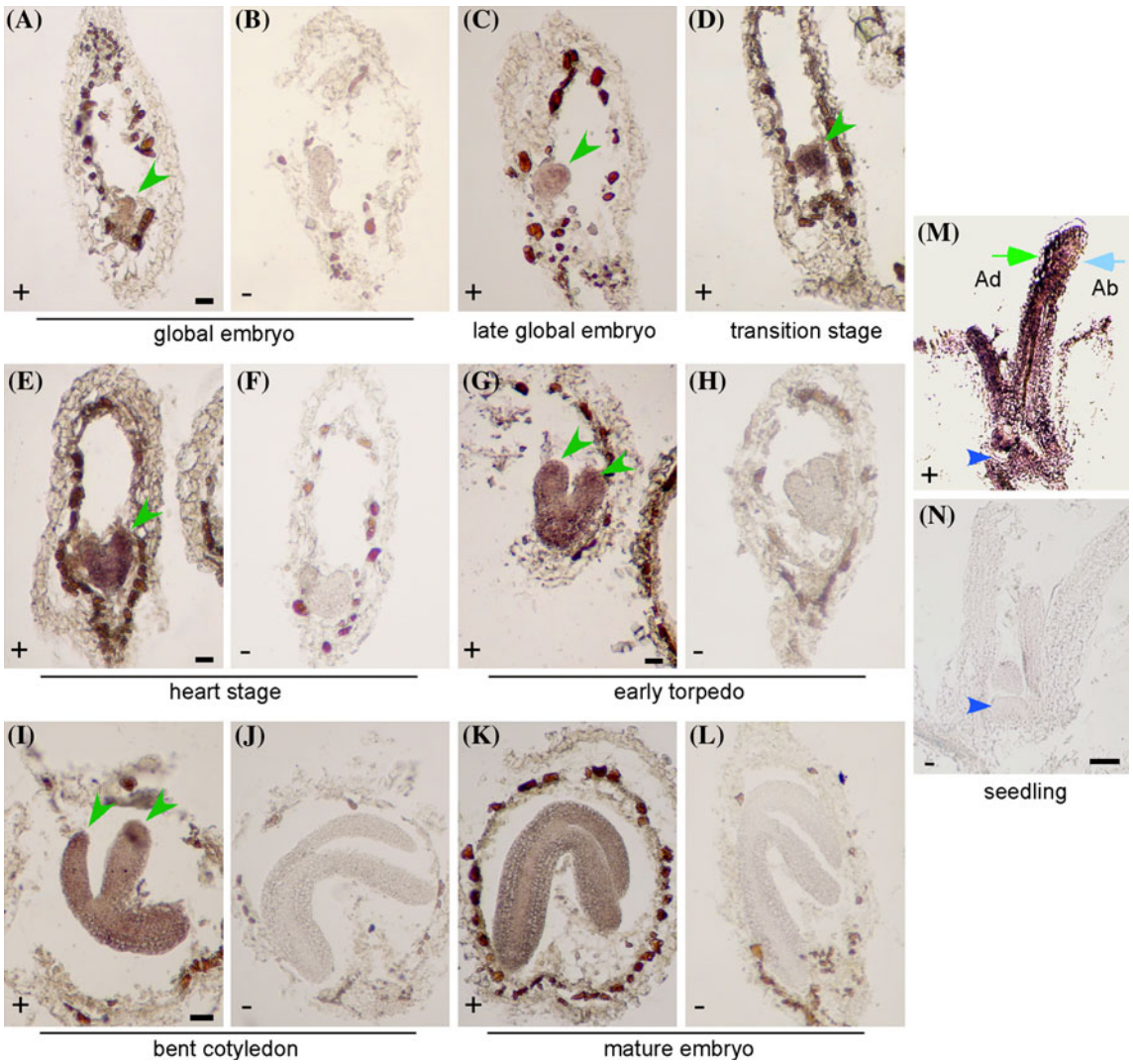


Fig. 7 Expression pattern of the *NSN1* gene in WT embryos at different stages of embryogenesis. **a**, **c**, **d**, **e**, **g**, **i**, **k** Embryo sections hybridized with the *NSN1* antisense RNA probe (+). **b**, **f**, **h**, **j**, **l** Negative control of similar embryo sections hybridized with the *NSN1* sense RNA probe (−). *NSN1* expression was detected in the whole embryo at the global embryo (**a**), late global embryo (**c**), transition (**d**), heart (**e**), early torpedo (**g**), bent cotyledon (**i**) and mature embryo stages (**k**). The highest level of *NSN1* expression was observed in the cotyledon primordia (*arrowheads*) at the heart (**e**), early torpedo (**g**) and bent cotyledon stages (**i**). **m**, **n** At the seedling stage, *NSN1* expression was detected in the SAM (*blue arrowhead*) and leaves. The expression level of *NSN1* was higher in the adaxial (*Ad*) epidermis than the abaxial (*Ab*) side of the leaf (**m**). No signal was detected using the *NSN1* sense probe (**n**). *Scale bar* 20 μm in **a–d**; **e–f**; **g–h**; **i–l**; 100 μm in **m–n**

the distorted expression of leaf polarity genes by *NSN1* overexpression remain to be investigated.

Discussion

Higher plants offer a unique multicellular system to study the integration of cell division and differentiation, and the regulation of stem cells. The molecular basis governing the fine balance between stem cell reproduction and the initiation of cell differentiation within the meristem is poorly understood. In our previous study (Wang et al. 2011), we demonstrated that *Arabidopsis NSN1* plays a pivotal role in defining the boundary of stem cells in the shoot and inflorescence meristems. In this report, we show that *NSN1* is required for embryo development and for the initiation and expansion of cotyledons and postembryonic leaves in *Arabidopsis*. Genetic and molecular evidence presented in this report suggests that *NSN1* exerts its regulatory function by altering the expression of key genes implicated in the control of meristem maintenance and leaf differentiation. Our data show that *NSN1* acts as a repressor of *WUS*,

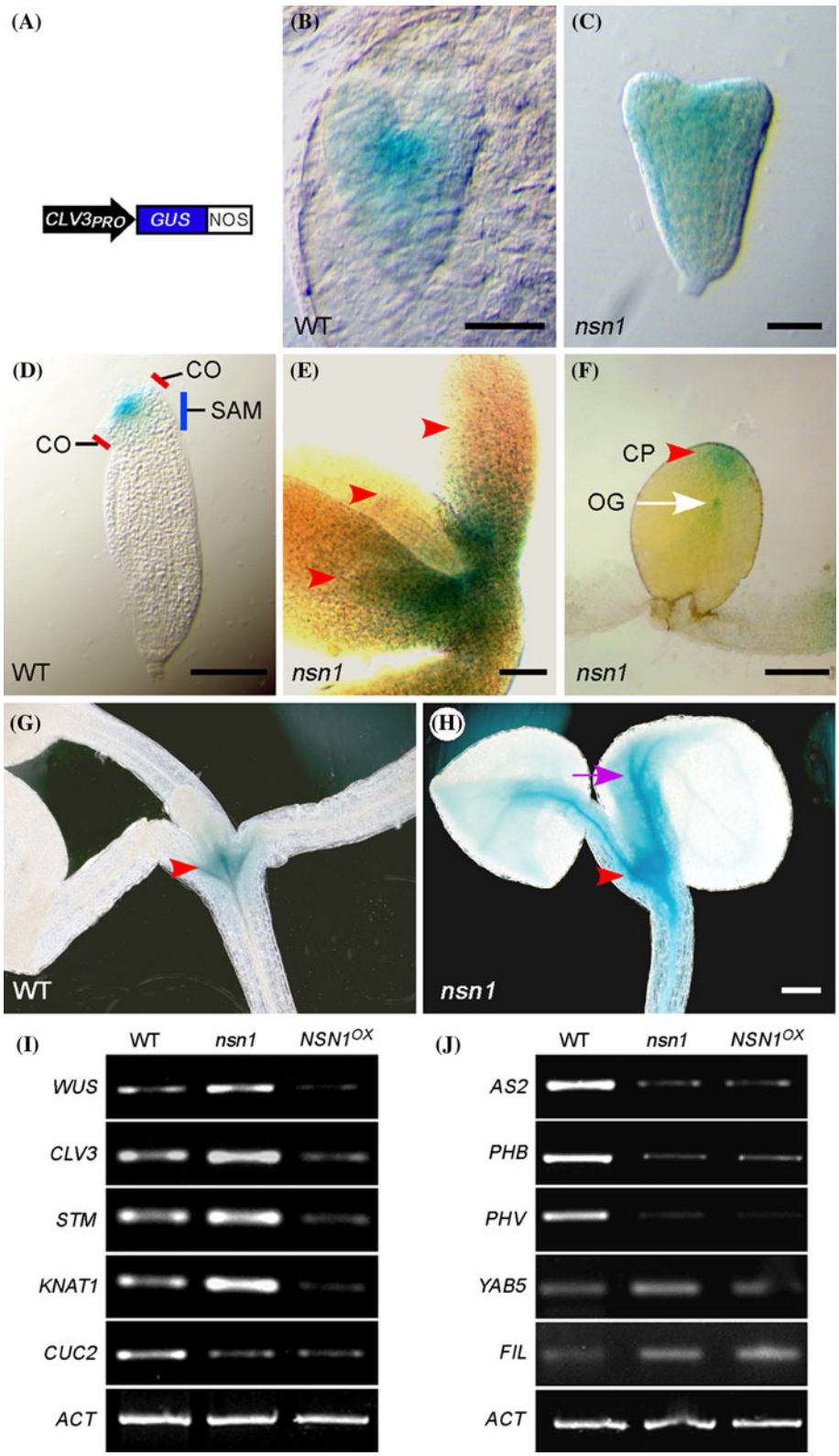


Fig. 8 Alteration in expression of the genes implicated in the regulation of shoot meristem and leaf polarity. **a–h** Expression of *CLV3::GUS* in wild type and *nsn1* plants. The expression of the *CLV3* gene at the heart stage was detected in a much expanded region of the shoot apical meristem of the *nsn1* embryo (**c**) than in a wild type (WT) embryo (**b**). At the bent cotyledon stage, the expression of *CLV3* was restricted to the apical meristem (SAM, indicated by a *blue line*) in a WT embryo with two detached cotyledons (*CO*) indicated by *red lines* (**d**). *CLV3* gene expression was expanded from SAM to the petioles of the three cotyledons (*red arrowheads*) in *nsn1* (**e**). Ectopic gene expression of *CLV3* was also found in the cotyledon primordium (CP, *red arrowhead*) and the adaxial outgrowths (OG, *white arrow*) of *nsn1* embryos stained briefly in GUS solution (**f**). In the seedling stage, *CLV3* was expressed in the cotyledons and their petioles (*red arrow* in **g**) as compared to the limited staining of GUS in the SAM of the WT seedling (**h**). **i–j** RT-PCR products showing the expression levels of meristem-related genes, *WUS, CLV3, STM* and *KANT1,* lateral organ boundary gene *CUC2*, and polarity-related genes *AS2, PHB, PHV, YAB5* and *FIL*. Note that *WUS, CLV3, STM and KNAT1* were up-regulated in *nsn1*, and down-regulated in $NSN1^{ox}$. *CUC2, AS2, PHB,* and *PHV* were down-regulated, while *YAB5* and *FIL* were up-regulated in both *nsn1* and $NSN1^{ox}$ plants. *ACTIN* (*ACT*) gene expression served as an internal control. *Scale bar* 1 mm in **a–h**

CLV3, STM, KNAT1, *YAB5* and *FIL* genes, and promotes the expression of *CUC2, AS2, PHB* and *PHV* genes during embryo development and seedling growth. How NSN1, a nucleolar GTP-binding protein, achieves its control over the expression of these genes at the biochemical level remains to be further investigated.

Interaction between *NSN1* and meristem genes

The stem cells are a small group of cells located in the center of the meristem. They can renew themselves via cell division. They can also generate progeny cells for differentiation into lateral organs like cotyledons and leaves (Weigel and Jurgens 2002). Maintaining the homeostasis of self-renewing and differentiation of stem cells is vitally important in higher plants. Several key genes involved in the stem cell regulation have recently been subject to intense studies. Among them are *WUS* and *CLV3*, encoding a mighty homeodomain transcription factor and a 96-amino acid hormone peptide, respectively (Veit 2006; Clark et al. 1997; Jeong et al. 1999). *WUS* acts to activate *CLV3*, whereas *CLV3* represses *WUS* expression. They regulate each other and together, they form a negative feedback loop governing the homeostasis of the stem cell pool and tissue differentiation (Fletcher et al. 1999; Brand et al. 2000; Schoof et al. 2000). In *wus* mutant, the meristem is depleted of stem cells and filled with differentiated cells (Laux et al. 1996). On contrary, the meristem of *clv3* mutant is fantastic and produces extra flowers (Fletcher et al. 1999; Brand et al. 2000). When *WUS* is overexpressed, ectopic meristems can be produced on the hypocotyl, leaves, chalazor and roots (Gallois et al. 2002; Gross-Hardt et al. 2002; Lenhard et al. 2002; Gallois et al. 2004). A growing list of genes is known to regulate the *WUS-CLV3* loop directly or indirectly. SPY and BARD1 can bind directly to the promoter of the *WUS* gene (Kwon et al. 2005; Han et al. 2008).

NSN1 has been shown to repress *WUS* and *CLV3* expression (Wang et al. 2011). In *nsn1* mutant, *WUS* and *CLV3* are expressed in an expanded domain in the inflorescence meristem, resulting in a reduced size of the meristem. In this report, we showed that the *nsn1* mutation could arrest embryo development at any stage of embryogenesis, and disrupt the process of cotyledon initiation and expansion at early development stages (Figs. 2, 4). This suggests that *NSN1* is involved in organizing the meristem during embryogenesis and in controlling the cotyledon development in *Arabidopsis*. This conclusion is supported by the in situ RNA hybridization data of the *NSN1* gene, which show that *NSN1* is expressed throughout the embryo with the highest activity in the cotyledon primordia (Fig. 7). Using the *CLV3::GUS* construct, we were able to demonstrate that *NSN1* functions to repress the expression of *CLV3* in the base of cotyledons, petioles of cotyledons and cotyledon primordia (Fig. 8).

We identified meristem-like outgrowths on the adaxial surface of upward curled cotyledons and leaves in the *nsn1* plants (Fig. 5). Each of the outgrowths consisted of several cells around one stoma, or a highly enlarged cell in some occasions (Fig. 5). Using the *CLV3::GUS* construct, we demonstrated that to the expression of the *CLV3* gene is up-regulated in the outgrowths (Fig. 8). These results indicate that *NSN1* functions to repress *CLV3* expression in the leaves. The fact that these meristem-like outgrowths lacked the ability to develop into certain organs suggests that activating the *CLV3* alone is not sufficient to create a stem cell niche and other gene activities that are not altered in these outgrowths may be required for organ differentiation. Thus, *NSN1* is likely to act in concert with other genes in regulating that the meristem activity and cotyledon/leaf differentiation.

NSN1 and leaf development

During embryo development, the initiation and expansion of embryonic leaves were severely retarded by the *nsn1* mutation (Fig. 2). Seedlings with three cotyledons, defective cotyledons and leaves were produced frequently in *nsn1* plants (Figs. 2, 5). Leaf defects exhibited in *nsn1* plants included reduced leaf sizes, elongated leaves, lack of serrations on the leaf edge, upward curling and outgrowths on the adaxial epidermis (Figs. 4, 5). The function of *NSN1* in the leaf development is supported by the gene expression data, showing the high level of *NSN1* expression in the leaf primordia and on the adaxial side of expanding leaves (Fig. 7).

Genes including *STM, KNAT1, KNAT2*, *AS2*, *AS1*, *CUC1* and *CUC2* are known to be involved in leaf development and organ boundary separation (Aida et al. 1997; Aida et al. 1999; Byrne et al. 2000; Ori et al. 2000; Takada et al. 2001; Byrne et al. 2002; Ueno et al. 2007; Guo et al. 2008). We showed that in addition to *WUS* and *CLV3*, two other meristem-related genes, *STM,* and *KNAT1*, and two genes regulating the abaxial cell fate, *YAB5* and *FIL*, were induced by the *nsn1* mutation. The cotyledon-related gene, *CUC2,* as well as three adxial genes, *AS2, PHB* and *PHV*, were depressed in the leaves of *nsn1* plants (Fig. 8). These data suggests that *NSN1* regulates leaf development probably via altering the expression of these genes during seedling growth.

The characteristic phenotypes of leaf curls and disoriented adaxial-abaxial polarity in the *nsn1* plants indicate that *NSN1* plays an important role in the leaf polarity determination. Several genes have been implicated in the adaxial-abaxial polarity determination (Eshed et al. 2001; Kerstetter et al. 2001; McConnell et al. 2001). The fact that the expression of adaxial-related genes *PHB* and *PHV* was up-regulated while abaxial-related genes *FIL* and *YAB5* was repressed by the *nsn1* mutation (Fig. 8) suggests that *NSN1* function in concert with these genes in leaf polarity determination. Whether NSN1 regulates these genes in a direct or indirect manner remains to a challenging task for future investigations.

Acknowledgments We thank ABRC (the Ohio State University) and Dr. Thomas Laux (University of Freiburg, Germany) for providing *Arabidopsis* seeds. This work was supported by NSF grants (MCB 0548525 and IOB 0543923) to Z.H. and a fellowship from the Chinese Scholarship Council (CSC) to M.Z.

References

Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M (1997) Genes involved in organ separation in *Arabidopsis*: an analysis of the *cup-shaped cotyledon* mutant. Plant Cell 9:841–857

Aida M, Ishida T, Tasaka M (1999) Shoot apical meristem and cotyledon formation during Arabidopsis embryogenesis: interaction among the *CUP-SHAPED COTYLEDON* and *SHOOT MERISTEMLESS* genes. Development 126:1563–1570

Beekman C, Nichane M, De Clercq S, Maetens M, Floss T, Wurst W, Bellefroid E, Marine JC (2006) Evolutionarily conserved role of nucleostemin: controlling proliferation of stem/progenitor cells during early vertebrate development. Mol Cell Biol 26:9291–9301

Bernardi R, Pandolfi PP (2003) The nucleolus: at the stem of immortality. Nat Med 9:24–25

Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R (2000) Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by *CLV3* activity. Science 289:617–619

Byrne ME, Barley R, Curtis M, Arroyo JM, Dunham M, Hudson A, Martienssen RA (2000) *ASYMMETRIC Leaves1* mediates leaf patterning and stem cell function in *Arabidopsis*. Nature 408:967–971

Byrne ME, Simorowski J, Martienssen RA (2002) *ASYMMETRIC LEAVES1* reveals Knox gene redundancy in *Arabidopsis*. Development 129:1957–1965

Cada Z, Boucek J, Dvorankova B, Chovanec M, Plzak J, Kodets R, Betka J, Pinot GL, Gabius HJ, Smetana K Jr (2007) *Nucleostemin* expression in squamous cell carcinoma of the head and neck. Anticancer Res 27:3279–3284

Causier B, Castillo R, Zhou J, Ingram R, Xue Y, Schwarz-Sommer Z, Davies B (2005) Evolution in action: following function in duplicated floral homeotic genes. Curr Biol 15:1508–1512

Clark SE, Williams RW, Meyerowitz EM (1997) The *CLAVATA1* gene encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis*. Cell 89:575–585

Dai MS, Sun XX, Lu H (2008) Aberrant expression of *nucleostemin* activates *p53* and induces cell cycle arrest via inhibition of *MDM2*. Mol Cell Biol 28:4365–4376

Eshed Y, Baum SF, Perea JV, Bowman JL (2001) Establishment of polarity in lateral organs of plants. Curr Biol 11:1251–1260

Fan Y, Liu Z, Zhao S, Lou F, Nilsson S, Ekman P, Xu D, Fang X (2006) *Nucleostemin* mRNA is expressed in both normal and malignant renal tissues. Br J Cancer 94:1658–1662

Fletcher JC, Brand U, Running MP, Simon R, Meyerowitz EM (1999) Signaling of cell fate decisions by *CLAVATA3* in *Arabidopsis* shoot meristems. Science 283:1911–1914

Fu Y, Xu L, Xu B, Yang L, Ling Q, Wang H, Huang H (2007) Genetic interactions between leaf polarity-controlling genes and *ASYMMETRIC LEAVES1* and 2 in *Arabidopsis* leaf patterning. Plant Cell Physiol 48:724–735

Gallois JL, Woodward C, Reddy GV, Sablowski R (2002) Combined *SHOOT MERISTEMLESS* and *WUSCHEL* trigger ectopic organogenesis in *Arabidopsis*. Development 129:3207–3217

Gallois JL, Nora FR, Muzukami Y, Sablowski R (2004) *WUSCHEL* induces shoot stem cell activity and development plasticity in the root meristem. Genes Dev 18:375–380

Gross-Hardt R, Lenhard M, Laux T (2002) *WUSCHEL* signaling functions in interregional communication during *Arabidopsis* ovule development. Genes Dev 16:1129–1138

Guo M, Thomas J, Collins G, Timmermans MC (2008) Direct repression of KNOX loci by the *ASYMMETRIC LEAVES1* complex of *Arabidopsis*. Plant Cell 20:48–58

Han P, Li Q, Zhu YX (2008) Mutation of *Arabidopsis BARD1* causes meristem defects by failing to confine *WUSCHEL* expression to the organizing center. Plant Cell 20:1482–1493

Huang M, Ji Y, Itahana K, Zhang Y, Mitchell B (2008) Guanine nucleotide depletion inhibits pre-ribosomal RNA synthesis and causes nucleolar disruption. Leuk Res 32:131–141

Jeong S, Trotochaud AE, Clark SE (1999) The *Arabidopsis CLAVATA2* gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase. Plant Cell 11:1925–1934

Kerstetter RA, Bollman K, Taylor RA, Bomblies K, Poethig RS (2001) *KANADI* regulates organ polarity in *Arabidopsis*. Nature 411:706–709

Krizek BA, Lewis MW, Fletcher JC (2006) *RABBIT EARS* is a second-whorl repressor of *AGAMOUS* that maintains spatial boundaries in *Arabidopsis* flowers. Plant J 45:369–383

Kwon CS, Chen C, Wagner D (2005) *WUSCHEL* is a primary target for transcriptional regulation by *SPLAYED* in dynamic control of stem cell fate in *Arabidopsis*. Genes Dev 19:992–1003

Laux T, Mayer KF, Berger J, Jurgens G (1996) The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. Development 122:87–96

Leibfried A, To JP, Busch W, Stehling S, Kehle A, Demar M, Kieber JJ, Lohmann JU (2005) *WUSCHEL* controls meristem function by direct regulation of cytokinin-inducible response regulators. Nature 438:1172–1175

Lenhard M, Bohnert A, Jurgens G, Laux T (2001) Termination of stem cell maintenance in *Arabidopsis* floral meristems by interactions between *WUSCHEL* and *AGAMOUS*. Cell 105:805–814

Lenhard M, Jurgens G, Laux T (2002) The *WUSCHEL* and *SHOOTMERISTEMLESS* genes fulfil complementary roles in *Arabidopsis* shoot meristem regulation. Development 129:3195–3206

Lohmann JU, Hong RL, Hobe M, Busch MA, Parcy F, Simon R, Weigel D (2001) A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. Cell 105:793–803

Ma H, Pederson T (2007) Depletion of the nucleolar protein *nucleostemin* causes G1 cell cycle arrest via the *p53* pathway. Mol Biol Cell 18:2630–2635

McConnell JR, Barton MK (1998) Leaf polarity and meristem formation in *Arabidopsis*. Development 125:2935–2942

McConnell JR, Emery J, Eshed Y, Bao N, Bowman J, Barton MK (2001) Role of *PHABULOSA* and *PHAVOLUTA* in determining radial patterning in shoots. Nature 411:709–713

Meng L, Yasumoto H, Tsai RY (2006) Multiple controls regulate nucleostemin partitioning between nucleolus and nucleoplasm. J Cell Sci 119:5124–5136

Misteli T (2005) Going in GTP cycles in the nucleolus. J Cell Biol 168:177–178

Ori N, Eshed Y, Chuck G, Bowman JL, Hake S (2000) Mechanisms that control *knox* gene expression in the *Arabidopsis* shoot. Development 127:5523–5532

Parcy F, Bomblies K, Weigel D (2002) Interaction of *LEAFY AGAMOUS* and *TERMINAL FLOWER1* in maintaining floral meristem identity in *Arabidopsis*. Development 129:2519–2527

Romanova L, Grand A, Zhang L, Rayner S, Katoku-Kikyo N, Kellner S, Kikyo N (2009a) Critical role of nucleostemin in pre-rRNA processing. J Biol Chem 284:4968–4977

Romanova L, Kellner S, Katoku-Kikyo N, Kikyo N (2009b) Novel role of nucleostemin in the maintenance of nucleolar architecture and integrity of small nucleolar ribonucleoproteins and the telomerase complex. J Biol Chem 284:26685–26694

Sablowski R (2007) The dynamic plant stem cell niches. Curr Opin Plant Biol 10:639–644

Sawa S, Watanabe K, Goto K, Liu YG, Shibata D, Kanaya E, Morita EH, Okada K (1999) *FILAMENTOUS FLOWER*, a meristem and organ identity gene of *Arabidopsis*, encodes a protein with a zinc finger and HMG-related domains. Genes Dev 13:1079–1088

Schoof H, Lenhard M, Haecker A, Mayer KF, Jurgens G, Laux T (2000) The stem cell population of *Arabidopsis* shoot meristems in maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. Cell 100:635–644

Stewart RN, Dermen H (1970) Determination of number and mitotic activity of shoot apical initial cells by analysis of mericlinal chimeras. Am J Bot 57:816–826

Swaminathan S (2005) Nucleolar targeting runs on GTP cycles. Nat Cell Biol 7:217

Takada S, Hibara K, Ishida T, Tasaka M (2001) The *CUP-SHAPED COTYLEDON1* gene of *Arabidopsis* regulates shoot apical meristem formation. Development 128:1127–1135

Tsai RY, McKay RD (2002) A nucleolar mechanism controlling cell proliferation in stem cells and cancer cells. Genes Dev 16:2991–3003

Tsai RY, McKay RD (2005) A multistep GTP-driven mechanism controlling the dynamic cycling of nucleostemin. J Cell Biol 168:179–184

Ueno Y, Ishikawa T, Watanabe K, Terakura S, Iwakawa H, Okada K, Machida C, Machida Y (2007) Histone deacetylases and *ASYMMETRIC LEAVES2* are involved in the establishment of polarity in leaves of *Arabidopsis*. Plant Cell 19:445–457

Veit B (2006) Stem cell signalling networks in plants. Plant Mol Biol 60:793–810

Waites R, Selvadurai HR, Oliver IR, Hudson A (1998) The *PHANTASTICA* gene encodes a MYB transcription factor involved in growth and dorsoventrality of lateral organs in *Antirrhinum*. Cell 93:779–789

Wang X, Gingrich DK, Deng Y, Hong Z (2011) A nucleostemin-like GTPase required for apical and floral meristem development in *Arabidopsis*. Mol Biol Cell (in press)

Weigel D, Jurgens G (2002) Stem cells that make stems. Nature 415:751–754

Wurschum T, Gross-Hardt R, Laux T (2006) APETALA2 regulates the stem cell niche in the Arabidopsis shoot meristem. Plant Cell 18:295–307

Xu YY, Wang XM, Li J, Li JH, Wu JS, Walker JC, Xu ZH, Chong K (2005) Activation of the *WUS* gene induces ectopic initiation of floral meristems on mature stem surface in *Arabidopsis thaliana*. Plant Mol Biol 57:773–784

Yaghoobi MM, Mowla SJ, Tiraihi T (2005) Nucleostemin, a coordinator of self-renewal, is expressed in rat marrow stromal cells and turns off after induction of neural differentiation. Neurosci Lett 390:81–86

Yasumoto H, Meng L, Lin T, Zhu Q, Tsai RY (2007) GNL3L inhibits activity of estrogen-related receptor gamma by competing for coactivator binding. J Cell Sci 120:2532–2543

Zhu Q, Yasumoto H, Tsai RY (2006) Nucleostemin delays cellular senescence and negatively regulates TRF1 protein stability. Mol Cell Biol 26:9279–9290