Molecular genetic analysis of left–right handedness in plants

Takashi Hashimoto

Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0101, Japan (hasimoto@bs.aist-nara.ac.jp)

Handedness in plant growth may be most familiar to us when we think of tendrils or twining plants, which generally form consistent right- or left-handed helices as they climb. The petals of several species are sometimes arranged like fan blades that twist in the same direction. Another less conspicuous example is 'circumnutation', the oscillating growth of axial organs, which alternates between a clockwise and an anti-clockwise direction. To unravel molecular components and cellular determinants of handedness, we screened *Arabidopsis thaliana* seedlings for helical growth mutants with fixed handedness. Recessive *spiral1* and *spiral2* mutants show right-handed helical growth in roots, hypocotyls, petioles and petals; semi-dominant *lefty1* and *lefty2* mutants show opposite left-handed growth in these organs. *lefty* mutations are epistatic to *spiral* mutations. *Arabidopsis* helical growth mutants with fixed handedness may be impaired in certain aspects of cortical microtubule functions, and characterization of the mutated genes should lead us to a better understanding of how microtubules function in left–right handedness in plants.

Keywords: helical growth; handedness; *Arabidopsis* mutants; microtubule; circumnutation

1. INTRODUCTION

Left–right asymmetry in nature has always fascinated biologists. It is prominent in animals but also occurs in plants. During embryogenesis of vertebrates, a third axis of asymmetry, the left–right axis, is consistently orientated with reference to the other two primary axes of the body. Mouse and zebrafish mutants, and inherited human syndromes with distinct laterality defects, have helped us to link specific gene functions to particular processes during the development of the left–right axis (Capdevila *et al.* 2000). In several well-studied crustaceans that have asymmetrical claws, the paired claws are not consistently left- or right-handed and thus the direction of the asymmetry is apparently not controlled genetically, whereas the hermit crab has a dextrally coiled abdomen and an asymmetrical tail-fan with right-side reduction, suggesting that control over the direction of the asymmetry may be genetic (Harvey 1998).

In plants, left–right asymmetry can be either under genetic control or stochastic. The most conspicuous examples of the presumed genetic control are the direction of tendril or stem coiling of climbing plants. Twining plants generally show fixed handedness, either consistently forming right- or left-handed helices as they climb. Climbing plants such as hop and honeysuckle form left-handed helices, whereas *Convolvulus* and many other plants wind in the opposite, right-handed, direction (figure 1). Rare exceptions with random handedness are found in climbing pteridophytes belonging to the genus *Lygodium*—although the climbing organ of these plants is not a stem, as in other climbing plants, but is an indeterminately growing leaf.

More generally, helical growth movements of minute amplitude are found in all elongating axes and have been termed circumnutations (Darwin & Darwin 1880). Such rhythmic, elliptical movement has been observed in all plant species so far examined. Importantly, the direction of movement is often found to be constant in a given species, although the direction of movement is reported to change in some species. Two major models have been proposed to explain circumnutation: the gravitropic over-shoots model views circumnutation as a continuing gravitropic response, while the internal oscillation model postulates the existence of oscillators at the cellular or tissue level (Johnsson 1979). Space flight experiments have suggested that gravity is not required for the initiation or maintenance of circumnutation (Brown & Chapman 1984), thus disfavouring the gravitropic over-shoots model. The molecular nature of internal oscillators in the second model, however, remains speculative.

It should be pointed out that the term 'spiral', although often used interchangeably with the term 'helix', strictly refers to a two-dimensional discoid (such as a spiral galaxy) and is technically inappropriate for describing botanical structures. Nevertheless, the term spiral is frequently used in describing phyllotaxy and, indeed, as a taxonomic descriptor.

Contorted petal arrangement is another kind of left–right asymmetry in plants. When the edge of a petal is traced, the petals may have a clockwise or anti-clockwise twisted appearance. The direction of flower contortion is fixed in some species, such as *Datura stramonium* (anti-clockwise; figure 2) and oleander (clockwise), but not in others. In groups with fixed handedness, the direction of handedness can vary within the phylogeny of a family (Endress 2001).

Spiral phyllotaxy, on the other hand, is not controlled

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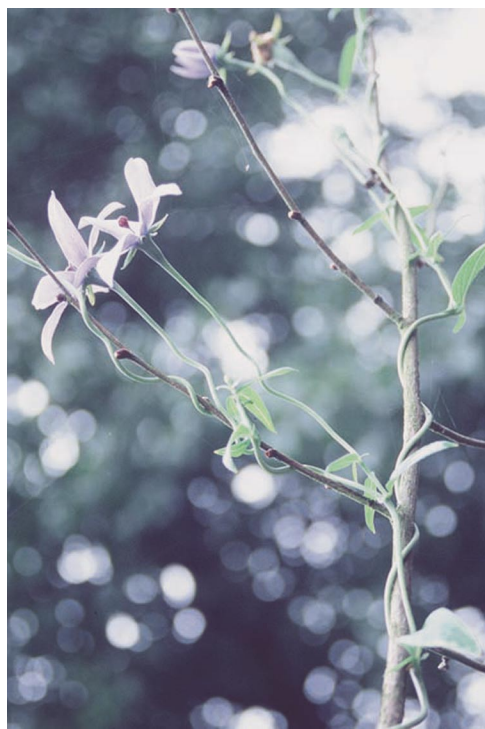DOI 10.1098/rstb.2002.1088

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Figure 1. *Codonopsis forrestii.* This twining species (Campanulaceae), which originated in south central China, is seen climbing up a woody plant. The stem of *C. forrestii* forms a right-handed helix.

Figure 2. *Datura stramonium.* The petals of *Datura* flowers twist in a right-handed direction, which appears anti-clockwise when viewed from a distal position. The right-handed coiling of petals is already seen in the flower bud (inset).

genetically. When the consecutive leaves of a tobacco or *Arabidopsis* plant are traced from the base of the stem to the top, they are arranged helically (figure 3). A helical arrangement of flowers is found, for example, on inflorescences of *Spiranthes australis*. When an individual with right- or left-handed phyllotaxy is self-pollinated, it gives
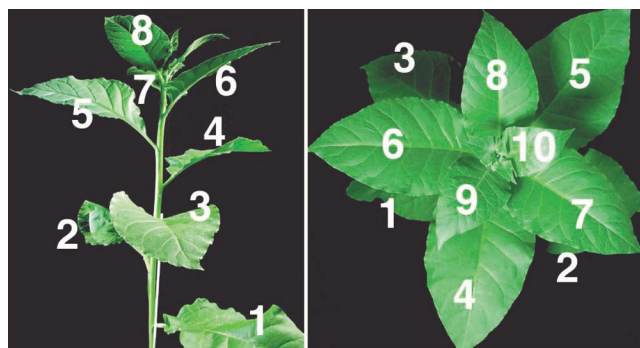

Figure 3. Spiral phyllotaxy seen in the leaf arrangement of *Nicotiana tabacum*. When the consecutive leaves (indicated by numbers) are followed from the base of the stem to the top, the trace forms either right- or left-handed helices. The tobacco plant shown has right-handed spiral phyllotaxy, but plants with either handedness occur with equal frequency.

right- and left-handed offspring in equal frequency (Callos & Medford 1994; Koriba 1914). The direction of phyllotaxy is therefore a developmental consequence; the handedness is determined where a new organ meristem is formed relative to the position of existing older organ meristems, on the flank of the central shoot apical meristem. In *Arabidopsis* embryo development, the phyllotactic handedness is stochastically determined when the two cotyledons are not formed entirely symmetrically (Woodrick *et al.* 2000).

In the old literature, there has been confusion over the terminology of 'right' and 'left'. One school of botanists once described that the direction of tendril twist in the morning glory is left-handed, while the other school defined it as right-handed. The debated issue was well covered in a monograph by Kihara (1972). Nowadays we categorize morning glory as a right-handed plant because the helix of the tendril matches the appearance of a right-handed corkscrew (figure 4*a*). The opposite terminology was once used because some botanists preferred to place the point of observation inside the observed helix. They reasoned that if an observer turns constantly to his left while ascending (or descending) a spiral staircase, the spiral should be described as left-handed (figure 4*b*). There is still some confusion remaining whether to call clockwise arrangement of petals or leaves 'left' or 'right'. For example, Kihara in his publications (e.g. Kihara 1972) adopted the terminology that described 'clockwise' as 'right' or 'right-handed'. However, because clockwise arrangements of petals and leaves are correlated with left-handed helical epidermal cell files, and anti-clockwise arrangement with right-handed epidermal helix (figure 5), I suggest that 'clockwise', when viewed from the distal end of the organ in question, be described as 'left-handed'. The key to resolving the confusion arising from the perspective of the viewer to the organ is the perceived direction of twist as the hand moves through the organ, in the way one would use a screwdriver.

2. GENETICS OF HANDEDNESS

The fact that the direction of tendril coiling and petal contortion in some flowers are fixed in given species suggests that asymmetry is under genetic control. Neverthe-
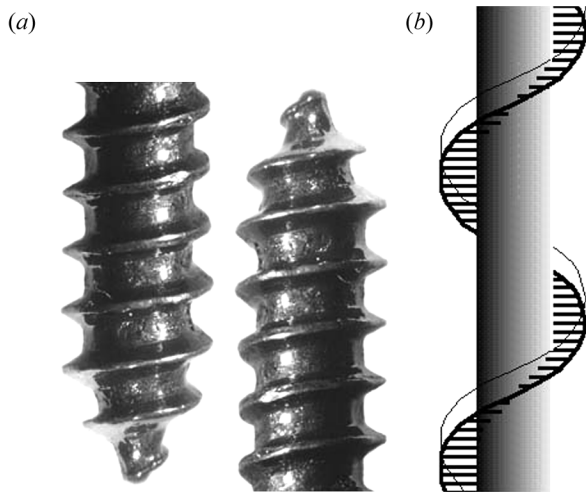
Figure 4. Definition of right- and left-handed helices.
(*a*) Right-handed screws. Note that whether the helix is right- or left-handed is intrinsic to a helix and does not depend on which end is up. (*b*) A right-handed spiral staircase.
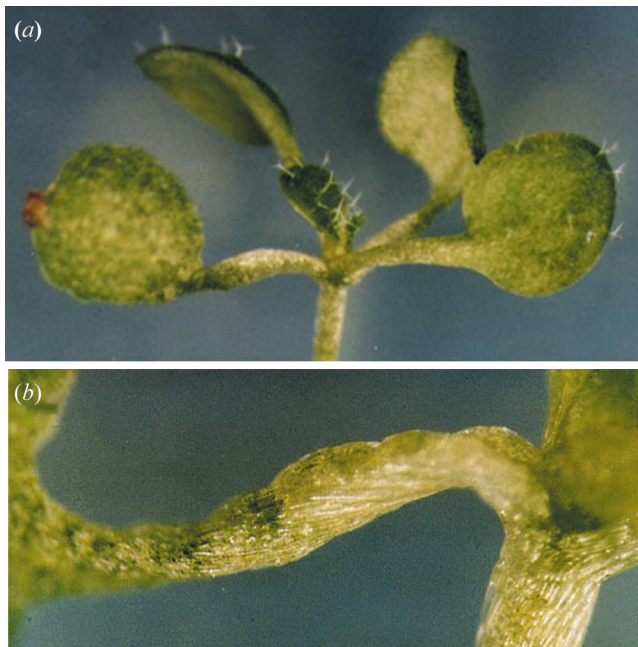

Figure 5. Twisting phenotypes of *spr2-1* seedlings.
(*a*) Cotyledons and leaves twist in an anti-clockwise direction. (*b*) Epidermal cell files of petioles form a right-handed helix.

less, few genetic studies or mutant isolation programs have been carried out until recently. The only classic examples may be on the twisted pods of *Medicago tuberculata* and *M. litoralis* (Lilienfeld & Kihara 1956). Genotypes that had consistently clockwise- or anti-clockwise-twisted pods were identified in these *Medicago* species. Reciprocal crosses between clockwise and anti-clockwise plants showed that clockwise-twisted pods are dominant over anti-clockwise-twisted pods, and that the trait is monogenic.

Arabidopsis thaliana is a model plant ideally suited for molecular genetic studies. Although wild-type *Arabidopsis* plants do not have coiled organs, nor are their petals

arranged like fan blades, left–right asymmetry can be induced by mutations. The direction of helical growth can be fixed or random, depending on the genes disrupted (table 1). Those mutants with fixed handedness are discussed in this article. In contrast, mutations in the *LOPPED1* (allelic to *tornado1*) and *TORNADO2* loci give twisted roots, stems and flower organs, but the direction of the twist is not fixed (Carland & McHale 1996; Cnops *et al.* 2000). Vascular development and cell expansion are abnormal in *lopped1,* presumably because of defective basipetal auxin transport. Cellular mechanisms underlying the fixed and random handedness phenotypes may well be distinct.

(a) *Right-handed mutants*

When wild-type *Arabidopsis* seedlings are grown vertically on a hard agar surface, the direction of root growth deviates slightly to the left of vertical when the seedlings are viewed from the aerial (upper) side (figure 6). We first isolated right-handed *spiral* (*spr*) mutants whose roots bend to the right on the hard agar (figure 6; Furutani *et al.* 2000). The *spr1* and *spr2* mutants have epidermal cell files with right-handed helical growth. In *spr1* roots, bending to the right on the agar surface probably results from constitutive right-handed helical growth of epidermal cells (figure 7), which causes a rightward bend when roots are unable to penetrate the hard agar surface. Root gravitropism, however, is not affected in the *spr* mutants. In petioles and petals of *spr2*, right-handed twisting of the epidermis is apparently the cause of the anti-clockwise arrangement of cotyledons and petals when viewed from a distal aspect (figure 5). The *spr1* twisting phenotype is most apparent in the dark-grown central axis, including stem, hypocotyl and root, whereas the *spr2* phenotype is manifested mainly in lateral organs, such as petioles, cauline leaves and petals (figures 6–9). Shoot and root meristems appear to be normal in these mutants, but when cells enter the elongation zone they begin to skew to the right, indicating that the directional control of cell elongation is affected in *spr* mutants.

SPR1 maps to the top of chromosome 2, whereas *SPR2* is positioned in the mid-region of chromosome 4. *spr2* is allelic to *tortifolia1* (Bürger 1971) and *convoluta* (Relichova 1976). The *spr1spr2* double mutant has strong synergistic defects in all aspects of the *spr1* and *spr2* phenotypes (figure 10; Furutani *et al.* 2000), suggesting that SPR1 and SPR2 act on a similar process but in separate pathways in controlling the direction of cell elongation. Both *SPR1* and *SPR2* genes have now been identified by map-based approaches, and are found to encode novel proteins of distinct, plant-specific small gene families (T. Hashimoto, unpublished data).

(b) *Left-handed mutants*

Semi-dominant *sku* mutants have been reported that showed exaggerated leftward slanting on vertical agar surfaces (Rutherford & Masson 1996). We screened EMS-mutagenized M2 populations of the right-handed *spr1* for suppressors and identified mutant seedlings with opposite, leftward skewing roots. The resulting left-handed mutants, named *lefty1* and *lefty2,* display left-handed helical growth in roots, petals and petioles (figures 6–9). In the *spr1* background, *lefty1* and *lefty2* are semi-dominant;

Table 1. *Arabidopsis* helical growth mutants.

mutant	allelism	dominance	handedness	reference
spiral1		recessive	right	Furutani *et al.* (2000)
spiral2	*tortiforlia1, convoluta*	recessive	right	Furutani *et al.* (2000)
sku1		semi-dominant	left	Rutherford & Masson (1996)
sku2		semi-dominant	left	Rutherford & Masson (1996)
lefty1		recessive/semi-dominant[a]	left	Thitamadee *et al.* (2002)
lefty2		recessive/semi-dominant[a]	left	Thitamadee *et al.* (2002)
lopped1	*tornado1*	recessive	not fixed	Carland & McHale (1996)
tornado2		recessive	not fixed	Cnops *et al.* (2000)

[a] *lefty* mutants are recessive in wild-type background, but are semi-dominant in *spr1* background.
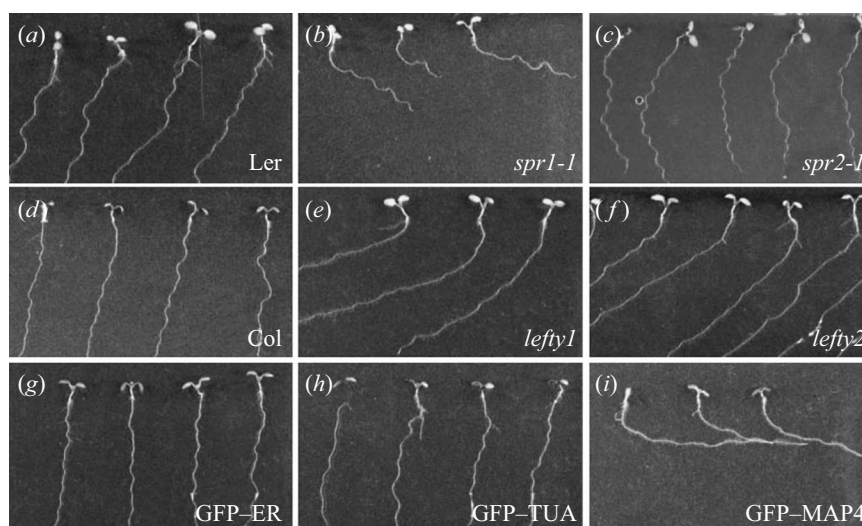

Figure 6. Slanting of seedling roots on hard-agar plates. Seven-day-old *Arabidopsis* seedlings grown on the 1.5% hard agar medium are viewed from above the agar surface. (*a*) Ler wild type; (*b*) *spr1-1*; (*c*) *spr2-1*; (*d*) Col wild-type; (*e*) *lefty1*; (*f*) *lefty2*; (*g*) transgenic plants expressing GFP with an ER retention signal (Ridge *et al.* 1999); (*h*) transgenic plants expressing α-tubulin fused to GFP, and (*i*) transgenic plants expressing MAP4 fused to GFP.

F2 progeny between *lefty1spr1* (or *lefty2spr1*) and *spr1* segregate in a ratio of 1 leftward slanting: 2 wild-type: 1 rightward slanting roots. However, when the *spr1* mutation was segregated out, *lefty* single mutant phenotypes were found to be recessive. Thus, *lefty1* and *lefty2* are cryptic semi-dominant and are epistatic to *spr1*. The *spr1* mutation appears to increase the cellular sensitivity to *lefty* mutations. Null mutant alleles of *LEFTY* genes were next isolated, by screening intragenic suppressors of the original *lefty* mutations. Molecular analyses of *LEFTY1* and *LEFTY2* genes revealed that the initial *LEFTY* mutants are (semi-)dominant negative alleles, and that null alleles are wild-type. Both *LEFTY* genes are involved in MT organization (Thitamadee *et al.* 2002).

3. HELICAL GROWTH INDUCED IN TRANSGENIC PLANTS

Helical growth in roots, petioles, and petals can be induced by overexpression of modified transgenes involved in MT organization or stability. When *Arabidopsis* TUA6 was fused to the C-terminus of GFP and expressed under control of the strong CaMV35S promoter in transgenic *Arabidopsis* plants, the chimeric fluorescent protein was incorporated into, and labelled *in situ*, MT polymers

in many, but not all, cell types (Ueda *et al.* 1999). In moderately fluorescent transgenic lines, plant growth and development were nearly normal, except that epidermal cell files in the petiole twisted in a right-handed helix, and cotyledons, when viewed from their outer edge, appeared to have an anti-clockwise twist (figures 6–9). Stronger skewing phenotypes were observed in a strongly fluorescent line; petals, in addition to the cotyledons, twisted in a right-handed direction. Even when the N-terminal GFP tag was replaced with a small c-myc epitope tag of only 18 amino acid residues, the transgenic *Arabidopsis* plants constitutively expressing the chimeric tubulin gene still showed anti-clockwise arrangement of cotyledons (T. Hashimoto, unpublished data).

In another strategy, plant MTs were visualized with MAP4 tagged with GFP at the N-terminus. MAP4 is a ubiquitous MT-associated protein thought to play a role in the polymerization and stability of MTs in interphase and mitotic animal cells (Olson *et al.* 1995). In transgenic lines generating moderate to strong GFP fluorescence, roots had right-handed helical epidermal cell files and grew strongly skewed to the right on the hard agar medium, and rosette leaves were arranged in an anti-clockwise orientation (figures 6–9).

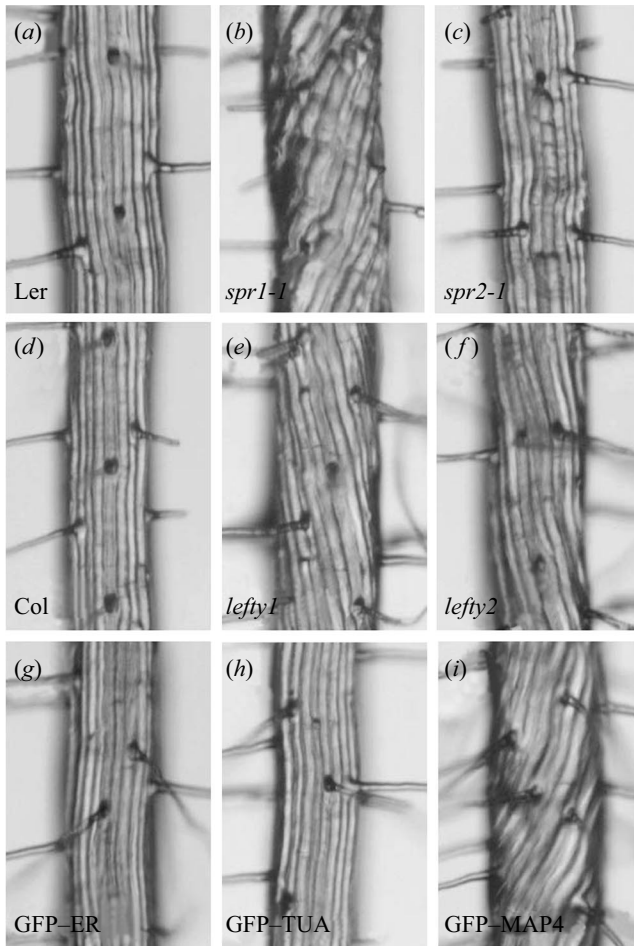These right-handed helical growth phenotypes induced

Figure 7. Epidermal cell files of seedling roots in the differentiated zone. The labels are the same as in figure 6.

by ectopic expression of chimeric tubulin and MAP4 proteins resemble the mutant phenotypes of *spr1* and *spr2*.

4. DRUG-INDUCED HELICAL GROWTH

Chemicals that affect polymerization or stability of plant MTs are effective in inducing left-handed helical growth in wild-type *Arabidopsis* seedlings when applied to the growth medium in low concentrations (Furutani *et al.* 2000). Helical growth can be caused either by the depolymerizing drug propyzamide (also known as pronamid) at 3 μM or the stabilizing drug Taxol at 1 μM. At these concentrations, cortical MTs in the root epidermal cells were neither depolymerized nor bundled, but instead formed right-handed helical arrays. Higher concentrations of propyzamide or Taxol were required to induce depolymerization or bundling of cortical MTs in the same cells.

5. MODELS

(a) *Helical growth model*

The helical arrangement of organs, such as petals, cotyledons and leaves, is apparently caused by twisting of the organ axis, which manifests as helical growth of epidermal cell files. As geometric constraints would predict, the pitch angle of cell files in etiolated *spiral* hypocotyls decreased in the order of epidermis, outer cortex, inner cortex, endodermis and pericycle. Anisotropic growth of epidermal
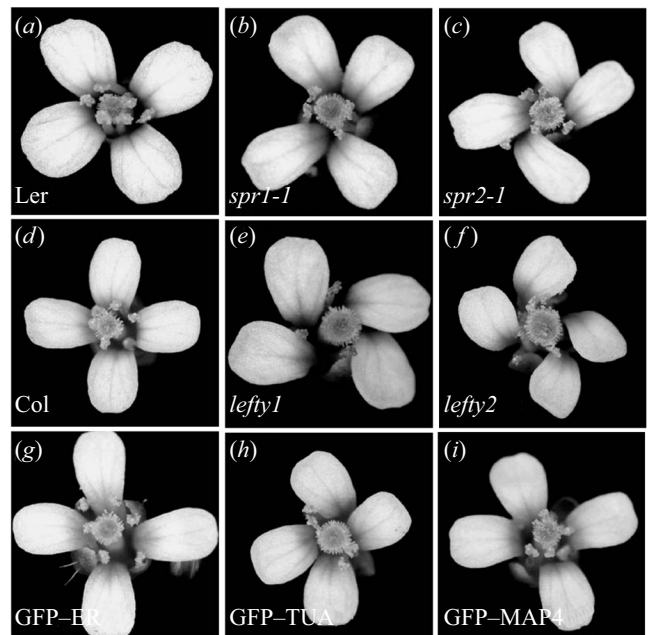
Figure 8. Petal twisting. The labels are the same as in figure 6. The petals of the GFP–TUA line (number 13) shown in (*h*) contort slightly anti-clockwise, while the GFP–TUA line (number 4) with stronger twisting phenotypes gives clear anti-clockwise contortion.

cells, although skewed to the right, appeared essentially normal, but ground tissue (cortex and endodermis) showed highly reduced longitudinal expansion and increased radial expansion (figure 11). The cells in the ground tissue of *spiral* mutants were shorter and wider than equivalent wild-type cells and lacked the cylindrical shape typical of wild-type cells (figure 12). Reduced anisotropy of *spr1* mutant cells starts as soon as cells enter the rapid elongation zone of roots and etiolated hypocotyls; cell division at the meristematic region appears to be unaffected in the mutant. Although cell elongation is characterized in detail in the *spr1* mutant, growth anisotropy in the ground tissue of *spr2* and *lefty* mutants also appeared to be reduced.

The above observation suggests that reduced longitudinal growth of inner cell layers compared with outer cell layers causes twisting of plant organs (figure 13). In the wild-type organ axis, the sum of inner cell length is equal to the sum of epidermal cell length, whereas inner cells of twisting mutant organs are defective in anisotropic growth and the total longitudinal length is shorter than that of epidermal cells. To compensate for this difference, the mutant epidermal cell files skew. This model also applies to the helical growth induced in transgenic plants and drug-treated wild-type seedlings.

(b) *Cortical microtubles and growth anisotropy*

Several lines of evidence demonstrate that reduced anisotropy is caused by defects in stability or proper orientation of cortical MTs. First, MT-interacting drugs reduce anisotropic growth in wild-type ground tissue. Second, interfering with normal MT properties by expression of chimeric tubulin or *MAP4* genes causes helical growth. Third, in etiolated *spr1* hypocotyls, cortical MT arrays are irregularly orientated in cortex cells that have greatly
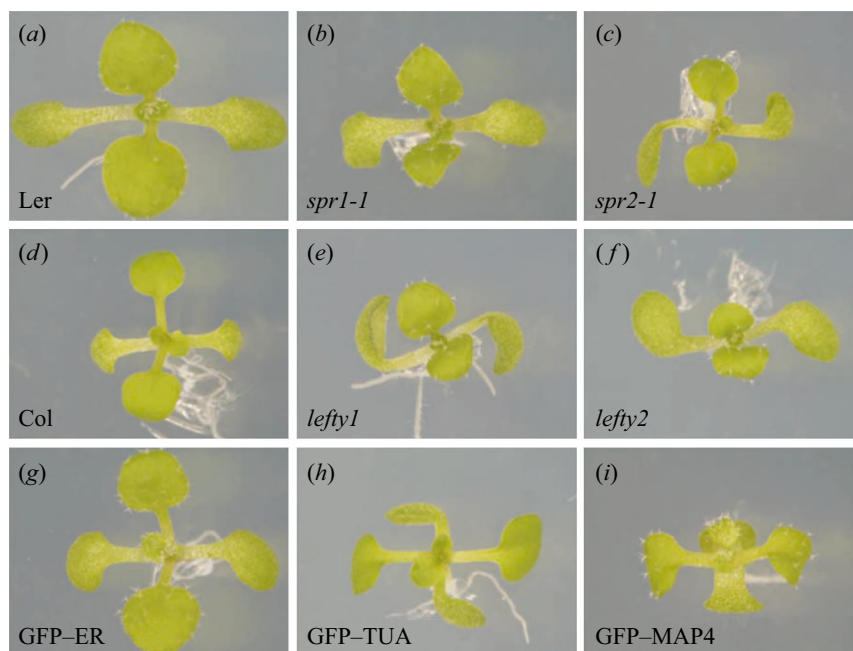
Figure 9. Twisting of cotyledons. The labels are the same as in figure 6. Note that since Col wild-type (*d*) and GFP–TUA (*h*) are in the *gl1* background, no trichomes are seen on the true leaves. Petioles of GFP–MAP4 cotyledons (*i*) are wider and shorter than those of other plants.
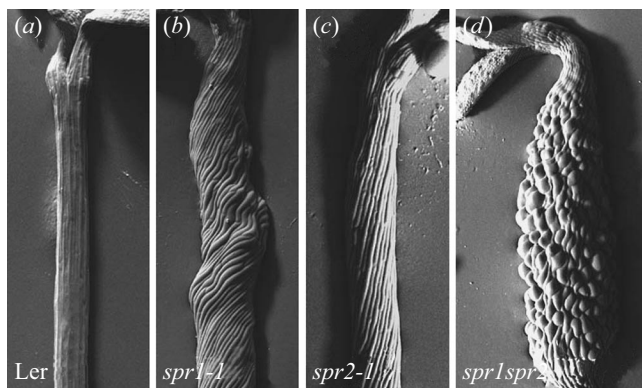

Figure 10. Helical growth phenotypes of 5-day-old etiolated seedlings. Upper regions of etiolated hypocotyls are shown. (*a*) Ler wild-type; (*b*) *spr1-1*; (*c*) *spr2-1* and (*d*) *spr1-1-spr2-1* double mutant. Helical pitch angles are stronger in *spr1* (*b*) than in *spr2* (*c*), whereas epidermal cells swell in the double mutant (*d*).

reduced anisotropic growth. As described in our recent study, the arrays were often parallel in localized regions of a cell, but most cells contained a mixed population of longitudinal, transverse and oblique arrays (Furutani *et al.* 2000). It should be stressed that cortical MT arrays were still present in the *spr1* cortex cells but that their proper orientation was defective. Fourth, helical growth and reduced anisotropy in *spr* seedlings can be suppressed efficiently by addition of low concentrations of a MT-interacting drug (propyzamide or Taxol) in the culture medium (figures 12 and 14; Furutani *et al.* 2000). Together, these observations suggest that it is not simply polymerization/depolymerization *per se* but more subtle properties, such as dynamic instability, that are affected in cortical MTs of expanding ground-tissue cells with reduced anisotropy. Apparently normal meristematic

regions of skewing organs indicate that MT structures used in cell division are less sensitive to twisting mutations and treatment with MT-interacting drugs than cortical MTs in the interphase cells.

(c) Control of fixed handedness

Helices of epidermal cell files in *spr* mutants and the transgenic plants expressing MT-related transgenes are invariably right-handed, whereas helices in *lefty* mutants and drug-induced twisting seedlings are always left-handed. Thus, the control of handedness must be incorporated into the helical growth model.

According to figure 13, epidermal cells are predicted to exert a strong influence on the handedness. Although cellular defects in *spr1* were most obvious in ground tissues, epidermal cells under certain conditions showed moderate radial swelling or local outgrowth of bulges, suggesting that the mutant epidermis has a compromised physical property and becomes partially defective in anisotropic growth under conditions that enhance the mutant phenotype (Furutani *et al.* 2000). Likewise, low concentrations of propyzamide or Taxol induced similar local bulges on the hypocotyl epidermis of light-grown wild-type seedlings. Here we refer to the compromised state of epidermal cells that potentially give rise to a right-handed helical cell file as 'R', the opposite state as 'L', and the state that results in straight elongation parallel to the organ's long axis as 'N' (figure 15). The R and L states may correspond to the epidermal cell states during rapid elongation in which anisotropic cell expansion is partially impaired, or the ground-tissue states that give rise to a substantial loss of anisotropic expansion. Ground tissue might be more sensitive to the disruption of MT functions than epidermis. The helical handedness induced by propyzamide or Taxol, or by *lefty* mutations, suggests that changes in MT stability pushes the state from L to N.
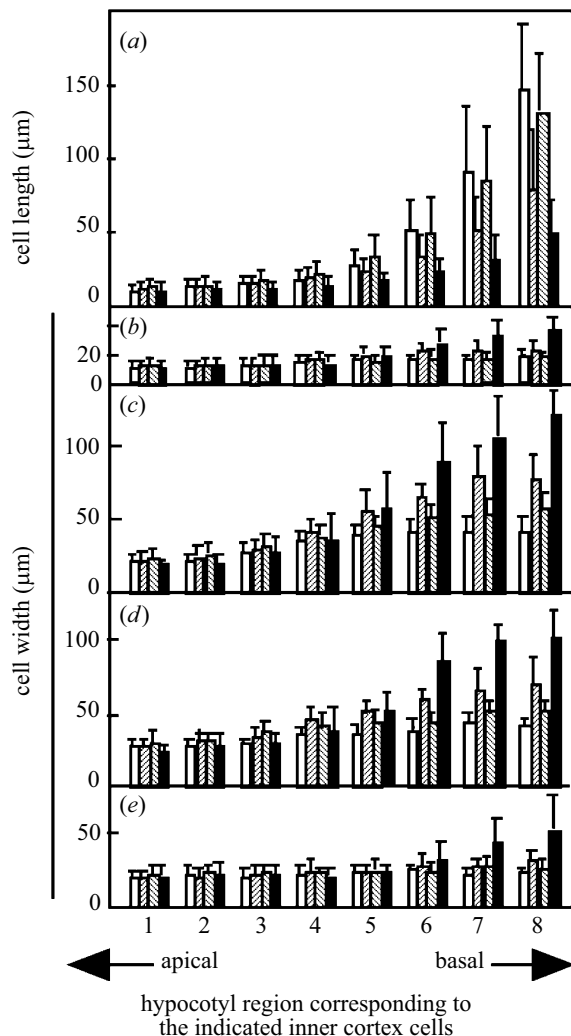
Figure 11. Cell sizes of 5-day-old etiolated hypocotyls. More than 20 cells for each cell layer of each genotype were measured for a given hypocotyl location on longitudinal sections cut through the middle of vascular cylinders. (*a*) Cell length of endodermis. The endodermal cell layers of *spr1* and *spr2* skewed to the right from the longitudinal hypocotyl axis by only $10.1° \pm 1.5°$ and $5.1° \pm 2.1°$, respectively. The longitudinal cell length is expected to be underestimated by less than 4%. (*b–e*) Cell width of (*b*) endodermis, (*c*) inner cortex, (*d*) outer cortex and (*e*) epidermis. The bars indicate standard deviations. White bars, Ler; forward leaning diagonal, *spr1-1*; backward leaning diagonal, *spr2-1*; black, *spr1spr2*.

Partial disruption of MT function by these drugs or by *lefty* mutations overrides the *spr* mutations, indicating that SPR1 and SPR2 function in the processes that prevent cells from proceeding further from N to R in a MT- and LEFTY-dependent pathway. SPR1 and SPR2 may represent a R-to-L reverse pathway, may act to suppress the L-to-R pathway, or may stabilize the N state to prevent overshooting to R. The driving force for helical growth may come from radial swelling of inner cells, and the elongating epidermis may function passively just to determine the direction of skewing.

The orientation of cortical MT arrays in epidermal cells of L, N and R states showed a right-handed helix (Z-helix), transverse and a left-handed helix (S-helix), respectively (Furutani *et al.* 2000; T. Hashimoto, unpub-

lished data). The helical pitch was not steep but of consistent handedness in a population of elongating cells. Assuming that the load-bearing innermost cellulose microfibrils are deposited along the helical MT arrays, the resulting microfibril arrangement on the outer tangential wall would then generate a polar cell growth in the direction perpendicular to the helical pitch of the arrays. Thus, a right-handed MT helical array in L is expected to give rise to left-handed helical growth, and a left-handed array in R would produce right-handed helical growth (figure 15). If epidermal cells and neighbouring cortex cells possess the same handedness of the MT array, a criss-cross cellulose microfibril pattern would be formed on the wall between them. The criss-cross pattern would seem to reduce the skewing force generated on that wall. Thus, the helical pattern of the newly synthesized microfibrils on the outer wall of the epidermis presumably has the strongest influence on the handedness of helical growth.

The model in figure 15 may be interpreted as a concept that the configurations of the cortical MT array can change from an S-helix to a Z-helix via transverse orientation, and that SPR1 and SPR2 act to suppress this transition partially, whereas LEFTY1 and LEFTY2 contribute to MT assembly dynamics. This proposed transition of the MT array configurations does not necessarily imply continuous movement of whole helical MT arrays with numerous intermediary configurations. It seems geometrically impossible to change a left-handed helix to a right-handed helix without disassembling MT arrays. Indeed, microinjection of pea epidermal cells with rhodamine-labelled tubulin revealed that discordant MT arrays appeared in particular locations of a cell and neighbouring MTs then adopted the new alignment when cortical MTs reorientated from transverse to steeply oblique configuration (Yuan *et al.* 1994).

6. PERSPECTIVES

By characterizing *Arabidopsis* helical growth mutants and transgenic plants with fixed handedness, we found that compromised MT properties may underlie reduced anisotropic growth of ground tissue in helically growing axial organs. Depending on how MT properties are affected, cortical MT arrays may adopt either a right-handed or a left-handed helix. Although several mutant loci for helical growth have been cloned by map-based approaches, we still do not understand how the mutated gene products affect MT function or which properties are affected, or how individual MT polymers with compromised functions are organized into the three-dimensional MT arrays that surround the cell cortex with distinct handedness. The affected properties are probably more subtle than simply the polymerization/depolymerization status, and may include the MT behaviour described as dynamic instability.

Alternatively, but not mutually exclusively, MT structure itself might be slightly altered. Such altered structures might favour curved polymer geometry with distinct handedness, which, by assembling into a higher level of bundled polymer structure, imparts biased handedness (orientation) to the cortical MT array. An example of a biopolymer alternating two helical forms can be found in bacterial flagellar filaments (Samatey *et al.* 2001). The
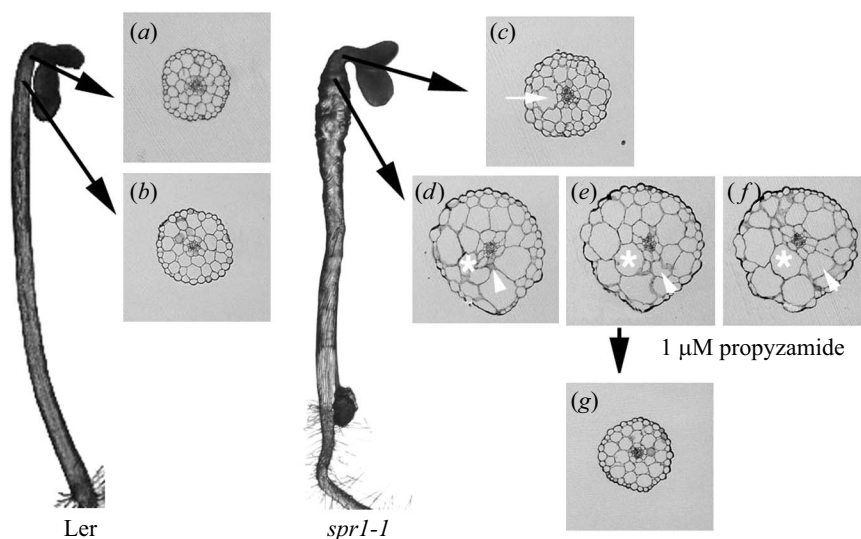
Figure 12. Anisotropic cell expansion in 5-day-old etiolated hypocotyls. (*a*,*b*) In Ler wild-type hypocotyls, cells of epidermis and ground tissue were cylindrical throughout the hypocotyl axis. (*c*–*f*) In contrast, *spr1* cells of ground tissue, but not of epidermis, showed highly reduced anisotropic expansion, as seen in three consecutive cross-sections 40 μm apart (*d*–*f*). The same two cells in three sections are indicated by an asterisk and an arrowhead. In a more apical region where cell elongation was not extensive (*c*), *spr1* ground tissue appeared mostly wild-type but occasionally contained somewhat deformed cells (arrow). When grown in the presence of 1 μM propyzamide, both the reduced anisotropy and the helical growth of *spr1* seedlings were efficiently suppressed (*g*).
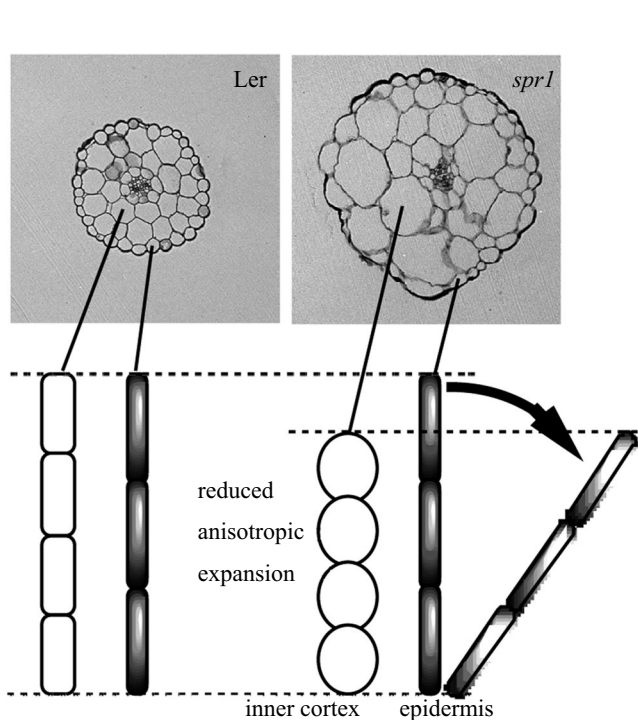

Figure 13. A model for helical growth. In the wild-type organ axis, the sum of inner cell length is equal to the sum of epidermal cell length, whereas inner cells of *spiral* mutant organs are defective in anisotropic growth and the total longitudinal length is shorter than that of epidermal cells. To compensate for this difference, the mutant epidermal cell files must skew. Note that the circumference of the inner tissues also increases, which will alter the pitch of the epidermal cells. The model also applies to the helical growth in *lefty* mutants and the helical growth induced in wild-type seedlings by MT-interacting drugs. Cross-sections of etiolated wild-type (Ler) and *spr1-1* hypocotyls are shown.
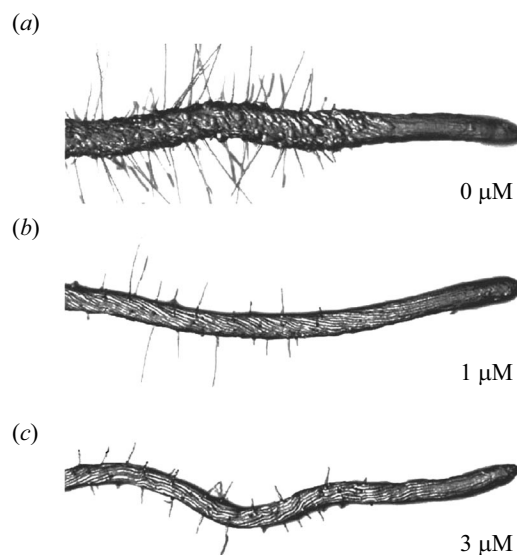

Figure 14. Suppression of *spr* phenotypes by propyzamide. The *spr1spr2* double mutant was grown in the absence (*a*) or presence of propyzamide at 1 μM (*b*) or 3 μM (*c*). Epidermal cells of the double mutant were radially swollen and skewed strongly to the right (*a*). Propyzamide at 1 μM suppressed the radial expansion of epidermis and the skewing pitch became weaker (*b*), while at 3 μM, epidermal cell files were mostly parallel to the main axis of the root (*c*).

filament is a tubular structure comprising 11 strands of protofilaments and can take right- or left-handed helical forms by supercoiling. Switching the conformation or packing interactions of the flagellin subunits between two distinct states and their non- and quasi-equivalent inter-subunit interactions is proposed to cause two different helical forms. When bacteria swim, several left-handed helical flagellar filaments form a bundle to propel the cell. Quick reversal of the motor to a clockwise rotation pro-
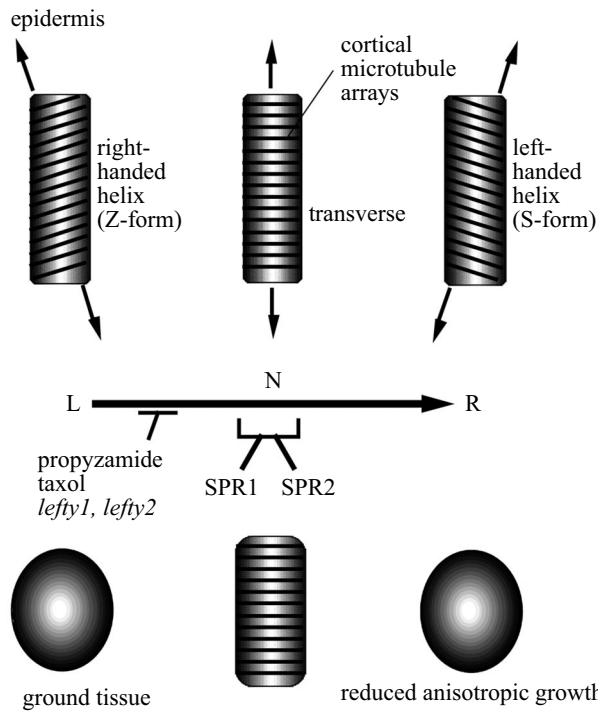
Figure 15. A model for helical handedness. Wild-type elongating epidermal cells are balanced in a neutral (N) state by the action between a pathway that shifts from a potential left-handed (L) state to a potential right-handed (R) state, and stabilizing (shown) or antagonistic pathways involving SPR1 and SPR2. The R-bound pathway is quantitatively suppressed by propyzamide, Taxol or *lefty* mutations. L and R states in the epidermis may represent elongating cells with compromised cortical MT arrays of right- or left-handed helices, respectively. The N cell with transverse arrays elongates parallel to the long axis but L and R epidermal cells skew respectively to the left and right during elongation. Ground tissue may be more sensitive to the compromised functions of cortical MTs, and cortex and endodermis cells of L and R states may expand with reduced anisotropy.

duces a twisting force that transforms the left-handed helical form of the filament into a right-handed one, causing the bundle to fall apart rapidly, halting (tumbling) the bacteria. Fine structures of cortical MTs in helically growing organs must be analysed to understand the structural basis of fixed handedness.

Our proposal that cortical MT arrays determine the handedness of twisting organs as depicted in figure 15 is based mostly on the reverse correlation between helical handedness of cortical MT arrays and skewing direction of elongating epidermal cells. Because physical strains on bending plant cells could reorientate cortical MT arrays (Fischer & Schopfer 1998), it remains possible that organ twisting itself causes distinct helical array arrangement in strained epidermal cells. Further experiments are needed to address critically whether the helical MT arrays are the cause or the consequence of organ twisting.

The availability of increasing numbers of *Arabidopsis* twisting mutants with fixed or random handedness will undoubtedly advance our understanding of the molecular and cellular mechanisms of symmetry breaking in this model plant. It may not be too long before we have mol-

ecular explanations for the natural left–right asymmetries that have fascinated botanists for many years.

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GLOSSARY

EMS: ethyl methane sulphonate
GFP: green fluorescent protein
ER: endoplasmic reticulum
MAP4: microtubule-associated protein 4
MT: microtubule
TUA6: α-tubulin 6