RESEARCH ARTICLE

Phyllotactic regularity requires the Paf1 complex in Arabidopsis

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ABSTRACT

In plants, aerial organs are initiated at stereotyped intervals, both spatially (every 137° in a pattern called phyllotaxis) and temporally (at prescribed time intervals called plastochrons). To investigate the molecular basis of such regularity, mutants with altered architecture have been isolated. However, most of them only exhibit plastochron defects and/or produce a new, albeit equally reproducible, phyllotactic pattern. This leaves open the question of a molecular control of phyllotaxis regularity. Here, we show that phyllotaxis regularity depends on the function of VIP proteins, components of the RNA polymerase II-associated factor 1 complex (Paf1c). Divergence angles between successive organs along the stem exhibited increased variance in vip3-1 and vip3-2 compared with the wild type, in two different growth conditions. Similar results were obtained with the weak vip3-6 allele and in vip6, a mutant for another Paf1c subunit. Mathematical analysis confirmed that these defects could not be explained solely by plastochron defects. Instead, increased variance in phyllotaxis in vip3 was observed at the meristem and related to defects in spatial patterns of auxin activity. Thus, the regularity of spatial, auxin-dependent, patterning at the meristem requires Paf1c.

KEY WORDS: Phyllotaxis, Meristem, Paf1c, Auxin, Variability, Reproducibility

INTRODUCTION

‘Dreams apart, numerical precision is the very soul of science, and its attainment affords the best, perhaps, the only criterion of the truth of theories and the correctness of experiments’ (p. 2, Thompson, 1942).

Following in the footsteps of D’Arcy Thompson’s On Growth and Form, developmental biology is becoming increasingly quantitative. With the accumulation of fine-grained quantitative data, inherent variability in development is currently emerging as an instructional cue. Conversely, the analysis of mutants with excessive or reduced variability in particular features helps us unravel how developmental reproducibility arises (Singh et al., 2010; Wernet et al., 2006; Laslo et al., 2006; Gupta et al., 2011; Uyttewaal et al., 2012; Hong et al., 2016; Abley et al., 2016).

The pattern of organ position along the plant stem has fascinated scientists for centuries because of its stereotyped regularity. Both spatial and temporal factors contribute to the final architecture of a shoot. Aerial organs are initiated from the shoot apical meristem (SAM), where plant stem cells are located, and are called primordia at that stage. In Arabidopsis, successive primordia emerge from the SAM in a Fibonacci spiral, with a new primordium emerging every 137°. This angle between adjacent primordia is called the divergence angle, and is very regular in wild-type (WT) plants. In the strictest sense, phyllotaxis refers to this spatial meristematic pattern only. As stem growth occurs, the initial phyllotaxis pattern does not necessarily lead to an equally regular pattern along the stem. There are two main reasons for this. First, because successive organs are separated by an internode, stem twisting can increase or decrease the angle between successive organs as the stem grows (Landrein et al., 2013). Second, the plastochron, i.e. the time between the emergence of successive primordia, can affect the final divergence angles along the stem. Indeed, if the average plastochron is very small, it can locally reach negative values, and, as organs are initiated before internode grows, a permutation in the sequence of organ emergence is possible. How could this lead to an altered architecture in the end? Let’s take the example of a simple permutation: primordium 3 would emerge before primordium 2 at the meristem; later on, whereas organ 1 would originate from primordium 1, the contiguous organ along the stem would originate from primordium 3 instead of primordium 2, thus leading to a divergence angle of 137°+137°=274°. Such permutations are widespread and can be observed in WT Arabidopsis (Besnard et al., 2014; Landrein et al., 2015b; Guédon et al., 2013; Refahi et al., 2011).

Recently, the question of phyllotactic variability has emerged (Mirabet et al., 2012; Refahi et al., 2016; Besnard et al., 2014; Landrein et al., 2015b). However, all experimental work on the regularity of phyllotactic patterns in fact refers to reproducibility of plant architecture. In particular, none of the mutants with variable architecture exhibit a variable spatial pattern of organ initiation at the SAM. This is notably the case for mutants with defects in the plastochron, leading to permutations in the final organ positions along the stem: despite the increased variance in divergence angle between fully differentiated organs, primordia are still initiated along the stereotypical 137° divergence angle at the meristem (e.g. Couder, 1998; Mirabet et al., 2012; Besnard et al., 2014; Landrein et al., 2015b; Guédon et al., 2013). Genetic factors have been implicated in the control of phyllotactic modes, with mutants switching between spiral and alternate patterns for instance (Giulini et al., 2004; Prasad et al., 2011), but not in the maintenance of phyllotactic regularity.

Whereas increased variability in leaf positions has been reported in the rice mutants for the SHO1, 2 and 3 homeobox genes, qualitative observation of shoot meristems indicates that these defects might be correlated with variability in meristem shape rather than in phyllotaxis (Itoh et al., 2000). Aberrant architecture is also
observed in *clavata3* mutants, which exhibit meristems up to 1000 times bigger than the WT (Fletcher et al., 1999; Szczesny et al., 2009; Leyser and Furner, 1992). However, the extent to which morphogenetic defects in such mutant meristems are directly related to phyllotaxis is not yet clear. When meristems are larger, the average plastochron is also smaller and permutations between successive organs are more frequent, even in the WT (Landrein et al., 2015b), hampering the analysis of phyllotaxis regularity at the meristem. Therefore, mutants with normal or smaller meristems would be more useful for investigating the question of the control of phyllotactic variance at the meristem.

Auxin has been indirectly implicated in phyllotaxis both through genetics and through modeling approaches. *PIN1* encodes an auxin efflux carrier and is required to generate discrete auxin peaks where organs will later emerge in the SAM (Reinhardt et al., 2003). MP, an auxin response factor, was recently shown to control the polarity of the auxin efflux carrier PIN1, in a positive-feedback loop, reinforcing the spatial patterns of auxin peaks at the shoot meristem (Bhatia et al., 2016). Defective organogenesis has been recorded in severely affected mutants, such as *pin-formed 1* (*pin1*; Gälweiler et al., 1998) or *monopteros* (*mp*; Aida et al., 2002), in which no organs are generated. Although these important observations consolidate a role of auxin transport and transduction in organ initiation, and their periodic emergence, they do not formally demonstrate a role of auxin in the spatial regularity of phyllotaxis, notably because of the severity of the mutant phenotypes.

Computational simulations of auxin transport in the meristem suggest that a stable phyllotactic pattern can emerge from the local response to auxin flow or concentration, or a combination of both (Reinhardt et al., 2003; Heisler et al., 2005; de Reuille et al., 2006; Smith et al., 2006; Bayer et al., 2009; Heisler et al., 2010; Stoma et al., 2008; Sahlin et al., 2009). However, these theoretical results do not formally demonstrate that auxin is sufficient to generate stable patterns, notably because mutants with increased phyllotactic variance have not been isolated so far. Other models, involving geometrical features (namely the size of the stem cell niche in the meristem and the size of fields inhibiting outgrowth around emerging primordia) further show how phyllotaxis can, in principle, stably self-maintain or switch between equally reproducible patterns (Douady and Couder, 1992). We are thus left with a picture in which phyllotactic regularity emerges because it is heavily constrained, by geometry or auxin transport, leaving the question of a possible molecular control of phyllotactic variance unanswered.

The RNA polymerase-associated factor 1 complex (Paf1c) plays a role in transcription-related processes such as the facilitation of elongation, recruitment of chromatin remodeling factors (histone methylation) and polyadenylation in yeast, plants and animals (Koch et al., 1999; Nordick et al., 2008; Penheiter et al., 2005; Sheldon et al., 2005; Sadeghi et al., 2015; Jaehning, 2010; Dermody and Buratowski, 2010; Chu et al., 2013; Oh et al., 2008). Plant Paf1c contains several subunits that are functionally homologous to those present in animal cells, including VERNALIZATION INDEPENDENCE (VIP) 3, VIP4, VIP5, EARLY FLOWERING (ELF) 7, VIP6 (also known as ELF8) and PLANT HOMOLOGOUS TO PARAFIBROMIN (PHP) (He et al., 2004; Oh et al., 2004; Jaehning, 2010; Oh et al., 2008; Park et al., 2010; Zhang and van Nocker, 2002). Consistent with VIP3, VIP4, VIP5 and VIP6 contributing to a plant Paf1c homolog, these proteins co-immunoprecipitate (Oh et al., 2004) and the corresponding single mutants display similar growth defects, which include reduced plant size, severely affected fertility and early flowering (Zhang et al., 2003; Takagi and Ueguchi, 2012; Dorcey et al., 2012). Recently, a role for Paf1c in patterning has emerged in animals, notably in cell lineage specification (Akanuma et al., 2007; Nguyen et al., 2010; Langenbacher et al., 2011; Kim et al., 2012; Zhang et al., 2013; Kubota et al., 2014). Here, we provide evidence that the regularity of the spatial pattern of auxin activity and organ initiation at the SAM requires Paf1c.

**RESULTS**

**Paf1c mutants display cotyledon number defects**

As reported previously, we observed that *vip3* mutants exhibit growth defects (Zhang et al., 2003; Takagi and Ueguchi, 2012; Dorcey et al., 2012). However, we also noticed that *vip3* seedlings frequently exhibit three cotyledons instead of two. Approximately 9% of *vip3-2* (112/1235; n=8 independent populations; T-DNA insertion in the first exon of *VIP3*) and nearly 2% of *vip3-1* (14/766; n=7 independent populations; T-DNA insertion in the 2nd exon of *VIP3*) 8-day-old seedlings displayed an altered cotyledon number, compared with a very low frequency in the WT (Fig. 1); no tricotyledons were observed in 1742 plants examined; n=8 independent populations). Similar cotyledon number defects were observed for seedlings deficient in VIP6, another component of the *Arabidopsis* Paf1 complex (Oh et al., 2004): around 6% of vip6 8-day-old seedlings displayed three cotyledons (Fig. 1; 52/805; n=8 independent populations). Note that the presence of tricot seedlings was previously described for another allele of *vip3* (*bouquet-1* allele); however, it was thought to be associated with the dominant-negative effect of the point mutation in this allele (Takagi and Ueguchi, 2012). Such defects had also been observed in several mutants affected in organogenesis and auxin distribution, such as *pinoid* or *pin1*, prompting us to search for other architectural defects in the *vip3* and *vip6* mutants.

**Paf1c mutants display architecture defects**

As previously reported (e.g. Zhang et al., 2003), *vip3* mutant plants display a semi-dwarf phenotype and strong male sterility when grown in long-day conditions (i.e. 16 h light/8 h dark, 21°C). Under these conditions, both the average number of siliques and the stem length decreased in *vip* mutants compared with WT plants (Fig. S1). Qualitatively, we observed aberrant angles between successive siliques along the stem, but these can also, though rarely, be observed in the WT (Fig. 2B,D). As described above, in principle, these defects could be caused by stem twisting, changes in phyllotaxis, or changes in plastochron.

Changes in plastochron are likely to contribute to architecture defects in *vip* mutants. Irregular timing of organ outgrowth is frequent even in the WT, leading to permutations between the final positions of successive organs, generating detectable signatures in divergence angle sequences along the stem (Besnard et al., 2014; Sheldon et al., 2005; Takagi and Ueguchi, 2012; Dorcey et al., 2012). Recently, a role for Paf1c in patterning has emerged in animals, notably in cell lineage specification (Akanuma et al., 2007; Nguyen et al., 2010; Langenbacher et al., 2011; Kim et al., 2012; Zhang et al., 2013; Kubota et al., 2014). Here, we provide evidence that the regularity of the spatial pattern of auxin activity and organ initiation at the SAM requires Paf1c.
Landrein et al., 2015b): typically, a 274°-223°-274° sequence between successive organs along the stem instead of 137°-137°-137° marks one permutation event involving two organs (Guédon et al., 2013; Refahi et al., 2011). Depending on the number of organs involved in a permutation and the chaining of such events, the distribution of divergence angles in a population of plants with plastochron defects will show a characteristic set of peaks centered around multiples of the canonical angle 137° (Guédon et al., 2013; Refahi et al., 2011). For instance, in the WS-4 ecotype, the presence of a larger meristem is associated with more frequent organ permutations, leading to peaks around 274° (2α), 223° (−α) and 51°(3α) and thus a reduced peak at 137° (α) (Landrein et al., 2015b).

To determine whether changes in plastochron could explain the observed phenotype in vip3, we used a 3D protractor (Peaucelle et al., 2007) to report divergence angles in WT and vip3-2, grown in long-day conditions (WT: 304 angles measured from 15 plants; vip3-2: 343 angles measured from 21 plants; Fig. 2A). Although the average divergence angle was close to 137° in vip3-2, as in the WT, the distribution of angles in the vip3-2 was flatter and wider. The presence of secondary peaks around 275° and 223° could reflect the presence of organ permutations (Fig. 2C) (Guédon et al., 2013; Besnard et al., 2014; Landrein et al., 2015b), but these angle signatures were not prominent, suggesting that defects in plastochron might not be sufficient to explain the vip3 phenotype.

We next analyzed every divergence angle sequence individually using the same mathematical method as described by Besnard et al. (2014) to test whether the increased variability in the vip3-2 divergence angles could be entirely explained by two-organ or more complex permutations. We found that the number of permutations involving two or three organs was higher in vip3-2 than in the WT (17% and 3.3% of permutated organs, respectively) (Table 1). However, the presence of permutations involving up to five organs did not fully explain the observed variability in vip3-2 angle distribution: 22% of the vip3-2 divergence angle sequences could not be explained by plastochron defects and associated organ permutations, compared with 3.5% in the WT (see Table 1). To confirm this conclusion, we performed the same analysis in vip3-1 and in vip6 and we obtained similar results, with up to 74% of angles in vip6 unexplained by plastochron defects (vip3-1: 201 angles, 15 plants; vip6: 360 angles, 17 plants; Fig. S2, Table 1). Thus, these data suggest that the aberrant divergence angles found on vip3 and
Continuous light and low temperature partially restore growth and enhance patterning defects in Paf1c mutants

To test whether aberrant divergence angles can be detected in vip3 and vip6 mutant meristems, organ initiation patterns were analyzed by meristem dissection. Although the previously used long-day conditions are the most commonly used conditions in Arabidopsis research worldwide, these conditions were not appropriate to pursue our analysis of vip3 and vip6 mutants, as apices were so small and fragile that dissection was close to impossible. We thus tested new growth conditions in which plants could be more vigorous. This also allowed us to test whether the increased variance in divergence angle remained when growth conditions were more favorable to stem growth.

In line with a previous report that the floral phenotype of vip3 plants can be attenuated by lowering growth temperature (Zhang et al., 2003), we grew vip3-2 plants for 3 weeks in short-day conditions at 21°C, and then transferred them to continuous light at 16°C (this scheme is referred as ‘continuous-light conditions’ thereafter). Under these conditions, WT plants and their apices were bigger, and stem length as well as average silique number in vip3-1, vip3-2 and vip6 mutants were closer to the WT than were those of plants grown in long days (Fig. S1). The average stem internode length of WT plants in continuous-light conditions was increased (because inhibitory fields around each organ necessarily lead to phyllotactic defects: in WT plants, different growth conditions produce different meristem sizes, yet a stereotypical spatial pattern of organ initiation is conserved. In maize and Arabidopsis, in which a switch in phyllotactic mode is observed (Giulini et al., 2004; Prasad et al., 2011). Interestingly, none of these mutants exhibits a variable phyllotactic pattern, but instead exhibit a reproducible, albeit different, phyllotactic mode. Given the observed divergence angle distribution along the stem, vip3 and vip6 mutant alleles might thus be specifically affected in phyllotactic regularity at the meristem."

vip3 mutants exhibit small meristems and altered MONOPTEROS expression

To investigate the role of VIP3 in patterning organ initiation at the SAM, we first checked whether VIP3 is expressed in this tissue at the inflorescence stage. VIP3 was previously reported to be ubiquitously expressed in Arabidopsis tissues (Zhang et al., 2003) and its expression was detected in the shoot apex 10 days after germination (Takagi and Ueguchi, 2012). Here, we confirmed by in situ hybridization that VIP3 mRNA is indeed enriched in the inflorescence meristem (Fig. 3).

Consistent with its expression pattern, VIP3 also controls meristem size: we found that vip3 and vip6 mutant meristems are about half the size of WT meristems (rs38 µm in vip3-2, rs34 µm in vip3-1, rs36 µm in vip6 versus rs61 µm for WT and vip3-6; Fig. 4A, Fig. S3B). However, altered meristem size does not necessarily lead to phyllotactic defects: in WT plants, different growth conditions produce different meristem sizes, yet a stereotypical spatial pattern of organ initiation is conserved. In fact, reduction in meristem size has been associated with an increased plastochron (because inhibitory fields around each organ would encompass a proportionally larger domain when meristems are smaller), leading to a more clear-cut separation between successive primordia, reduced number of organ permutations and thus a more regular architecture (Landrein et al., 2015b). Consistent with these observations, the CLV3- and WUS-expressing zones of the meristem have recently been shown to scale to meristem size through a geometrical feedback involving cytokinin diffusion from the meristem L1 layer (Gruel et al., 2016), consistent with the maintenance of phyllotaxis regularity in smaller meristems. To check whether such scaling occurs in vip3 mutants, we next analyzed the expression of STM (a whole meristem marker; Long

VIP3 and VIP6 Mutants Exhibit Small Meristems and Altered MONOPTEROS Expression

Table 1. Identification of divergence angle permutations between successive siliques on the stems

| Angle/sequence number | WT | vip3-1 | vip3-2 | vip6 | Continuous light | WT | vip3-6 | vip3-1 | vip3-2 | vip6 |
|-----------------------|----|--------|--------|------|-----------------|----|--------|--------|--------|------|
| Unexplained angles    | 3.5% | 17% | 22% | 74% | 5.6% | 12.9% | 71% | 57% | 58% |
| Two permutations      | 5 (3.3%) | 4 (3.7%) | 15 (8.2%) | 2 (1%) | 53 (3.7%) | 66 (13.3%) | 23 (5.4%) | 65 (7.1%) | 37 (6.9%) |
| Three permutations    | 0 | 8 (11.1%) | 7 (5.8%) | 4 (3.2%) | 16 (2.3%) | 30 (9.1%) | 16 (5.7%) | 51 (8.4%) | 16 (4.5%) |
| Percentage of permutated organs | 3.3% | 19% | 17% | 4.2% | 10% | 22.4% | 11% | 15% | 12% |

vip6 mutants stem are caused by defects both in the temporal sequence of organ initiation (plastochron) and in the spatial pattern of organ initiation at the meristem (phyllotaxis).

Table 1. Identification of divergence angle permutations between successive siliques on the stems

| Angle/sequence number | Long days | Continuous light |
|-----------------------|-----------|-----------------|
| Unexplained angles    | 289/13    | 201/15           |
| Two permutations      | 5 (3.3%)  | 4 (3.7%)         |
| Three permutations    | 0 | 8 (11.1%)        |
| Percentage of permutated organs | 3.3% | 19% |
et al., 1996), CLV3 (a central zone marker; Fletcher et al., 1999) and
MONOPTEROS (MP; also known as ARF5) (a peripheral zone
marker; Rademacher et al., 2011) (Fig. 3). A genetic interaction had
previously been reported for VIP3 and STM (Takagi and Ueguchi,
2012). Using in situ hybridization, we found that the expression
pattern of STM was not qualitatively different in vip3 than in the
WT, and confirmed that the domain with meristem identity, marked
with STM signal, is smaller in vip3 shoot apices. Surprisingly, we
observed no major modification in the size of the CLV3 expression
domain, even though meristems were half the size of the WT (as
shown above). Thus, the reduction in size is probably due to a
reduction in the size of the peripheral zone. In contrast, the pattern
for MP mRNA, a peripheral zone marker encoding an auxin
response factor, appeared to be affected in vip3 SAMs, with a
marked reduction of MP signal in vip3 meristem (Fig. 3). Using a
pMP::3xGFP reporter line, we confirmed that MP expression is
reduced in the meristem, consistent with our MP in situ
hybridization results (Fig. S4D).

**vip3 exhibits increased phyllotactic variance at the SAM**

We next measured the divergence angles between successive
primordia in vip3-1, vip3-2, vip3-6, vip6 and WT meristems from
plants grown in continuous-light conditions, as described by
Besnard et al. (2014). We found divergence angles in the WT to
be at 137±2.8°, consistent with previous reports (Fig. 4B,C)
(Besnard et al., 2014; Landrein et al., 2015b, 2013). Although the
average divergence angle was also around 137° in vip3 and vip6
mutants, the variance was significantly increased (Fig. 4B,C; vip3-
6: 137±9.2°, vip3-1: 137±11°, vip3-2: 136±17°, vip6: 136±14°). Furthermore, the number of angle outliers was much higher in vip3 and vip6 mutants than in the WT, in which divergence angles very
rarely deviated from the canonical angle. The vip3 and vip6 mutants are also different from mutants affected in plastochron such as ahp6, which exhibit no defect in the variance of divergence angle at the
meristem like the WT (Besnard et al., 2014), consistent with a
specific defect in phyllotaxis in vip3 and vip6.

As the initiation of new organs at the SAM is associated with
increased auxin content and activity, the pDR5::GFP (DR5) auxin
activity reporter line (Ulmasov et al., 1997) was introgressed in vip3-
2 and its pattern of expression analyzed by confocal microscopy. In
WT meristems, we observed peaks of DR5 expression marking the
early pattern of organ initiation, as previously reported (Fig. 5A)
(e.g. Smith et al., 2006; Heisler et al., 2005; Vernoux et al., 2011). In
vip3-2 meristems, DR5 expression peaks were detected at the sites of
incipient primordia but signal was also present outside these
regions, and sometimes close to the central zone (Fig. 5A, Fig. S4A;
n=30 for vip3-2, n=18 for WT). The presence of abnormal organ
positions in the SAM could also be confirmed in this genetic
background, with the occasional presence of adjacent organs at
similar age (Fig. 5A, white dots). Together with the altered pattern
of MP expression in vip3, these data demonstrate that the pattern of
auxin activity is disturbed in vip3 mutant apices, indicating that
VIP3 is required for the spatial regularity of auxin peaks, and thus of
organ initiation, at the SAM (Fig. 5C). This also suggests that self-
organizing processes are not robust enough to generate stable
phyllotaxis, and thus that phyllotaxis order requires a fully
functional peripheral zone, via VIP3 activity.

We also analyzed the expression pattern of STM, which is
downregulated in incipient primordia (Long et al., 1996) and
induced in organ boundaries (Landrein et al., 2015a); AHP6, which
is induced in emerging organs (Besnard et al., 2014); and CUC3,
which is more highly expressed in organ boundaries (Hibara et al.,
All three genes have been related to phyllotaxis indirectly through their gene expression pattern (Burian et al., 2015; Peaucelle et al., 2007; Besnard et al., 2014; Gallois et al., 2002). In vip3, STM, AHP6 and CUC3 reporters were expressed at the same location as in the WT, i.e. downregulated in incipient organs (STM), induced in boundaries (STM, CUC3) and induced in emerging organs (AHP6). However, their expression pattern was consistent with the abnormal spatial organ initiation pattern observed in vip3 (Fig. 5B, Fig. S4B, C,E). Together with the fact that these factors spatially mark organs later than DR5, these results are consistent with a role of VIP3 in controlling the regularity of phyllotaxis early on during the specification of organ initiation sites, and through the auxin pathway rather than other pathways.

**DISCUSSION**

The molecular mechanisms behind patterning in multicellular organisms are becoming unraveled in all species, from stomata and trichome patterning in plants to hairs and somites in animals (Oates et al., 2009). Here, we explored whether regulators of pattern variance exist, in parallel with pattern effectors. To do so, we focus on one of the
most striking mathematical patterns in biology, phyllotaxis, the ordered pattern of organ initiation at the SAM, in Arabidopsis.

The mathematical precision of phyllotaxis has fascinated scientists for centuries. D’Arcy Thompson, in his chapter ‘On leaf-arrangement, or phyllotaxis’, quoting Nehemiah Grew, writes: ‘From the contemplation of plants, men might first be invited to mathematical enquiries’ (p. 912, Thompson, 1942). Consistently, most theoretical work on phyllotaxis emphasizes its regularity (e.g. Douady and Couder, 1992). Interestingly, finding mutants with irregular phyllotaxis has proven extremely difficult: mutants with irregular architecture, and thus possibly defects in phyllotaxis, turned out to have defects in stem growth (e.g. Landrein et al., 2013) or in plastochron, i.e. in the temporal sequence, but not in the initial position, of organ emergence (e.g. Besnard et al., 2014). Here, we identify the Paf1 complex as a regulator of phyllotactic regularity at the meristem. Our study thus demonstrates that variability in phyllotaxis can arise from internal noise due to genetic background (WT versus vip3 mutants; Fig. 5C).

From the literature, evidence for a role of auxin in phyllotaxis regularity is indirect: it is well-established that auxin drives organ outgrowth very early on at the SAM (Besnard et al., 2011); yet, because the auxin-related mutants (mp, pin1, pid) do not produce organs, the role of auxin in phyllotaxis regularity could be questioned. Because auxin activity is disturbed in the vip3 mutant, this work further consolidates auxin as a central player in phyllotaxis regularity, consistent with predictions from computational models and experimental work on organogenesis so far.

Our data also show that plant architecture can be influenced by environmental conditions (long days at 21°C versus continuous light at 16°C), confirming that growth conditions leading to more vigorous plants also increase the probability of affecting the temporal sequence of organ emergence (Landrein et al., 2015b), in parallel with phyllotaxis defects, at the shoot apex.

Paf1c might also contribute to the reproducibility of other morphogenetic events in animals (Akannah et al., 2007; Nguyen et al., 2010; Langenbacher et al., 2011; Kubota et al., 2014). Previous studies have suggested that variability at the local scale marks cell identity (Singh et al., 2010; Laslo et al., 2006), primes organogenesis (Uyttewaal et al., 2012), contributes to reproducible organ shapes (Hong et al., 2016) and is actively maintained (Abley et al., 2016; Uyttewaal et al., 2012). Because the Paf1 complex has a rather pleiotropic biochemical role, from transcriptional regulation to the control of histone modification and mRNA stability, revisiting the role of Paf1c in relation to phenotypic variance could help us understand how genome-wide variability is buffered to generate reproducible shapes.

**Materials and Methods**

**Plant lines and growth conditions**

All procedures were performed on plants from the Col-0 ecotype. The pDRC5::GFP (Veroux et al., 2011), pSTM::CPP-N7 (Landrein et al., 2015a), pCUC3::CPP (Landrein et al., 2015a), pAHFP6::GFP (Besnard et al., 2014), pMP::3xGFP (Radermacher et al., 2011) reporter lines and T-DNA insertion lines vip3-1 (salk139885), vip3-2 (salk033664), vip3-6 (Jensen et al., 2017) and vip6 (salk065364) were used for this study (genotyping primers are listed in Table S1). In ‘long-day’ conditions, plants were continuously grown at 16 h/8 h light/dark period at 21°C. In ‘continuous-light’ conditions, plants were first grown for 3 weeks at 8 h/ 16 h light/dark at 21°C and then transferred to continuous light at 16°C.

**Phyllotaxis measurement**

Internode distances were measured on fully elongated main stems, from the insertion site of the last secondary branch to the last silique. The measurement of divergence angles between the successive siliques, and the mathematical analysis of permutations in individual angle sequences was performed as described by Besnard et al. (2014).

**Meristem dissection**

Semi-wet and the SAM was dissected when it switched to an inflorescence meristem identity, i.e. between the appearance of the first flower to the appearance of first silique (stages 13 to 17; Smyth et al., 1990) and transferred onto a half MS medium with vitamins and 0.125 μg/μl benzylaminopurine for imaging as described by Hamant et al. (2014).

**Confocal laser scanning microscopy and image analysis**

Dissected meristems and plants grown in vitro were imaged with a water-dipping lens (×25, NA=0.8) using an SP8 confocal microscope (Leica, Germany) to generate a stack of optical sections with an interval of 0.25 μm between slices. The membranes were stained with FM4-64. Meristem size and divergence angles between successive primordia from the confocal stack images, as described by Besnard et al. (2014). Statistical analysis was performed using either Microsoft Excel or R software. Two-tailed Student’s t-tests were performed to compare means of independent biological replicates.

**In situ hybridization**

**In situ** hybridization on paraplast-embedded tissues was performed as described by Vernoux et al. (2011). Shoot apices were sectioned into 7-μm-thick slices. The probes for the coding regions of VIP3, STM, CLV3, MP, and GFP were amplified with specific primers (listed in Table S1) using the GoTaq G2 Polymerase (Promega, 9PIM784). In vitro transcription and digoxigenin (DIG) labeling of the probes were performed using T7 RNA polymerase (Promega, P2077) and DIG RNA Labeling Mix (Roche, 11277073910).

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**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**

Conceptualization: K.F., O.H.; Methodology: K.F., M.L., A.D.; Formal analysis: K.F., A.D.; Writing - original draft: K.F., O.H.; Writing - review & editing: K.F., Y.R., E.S.H., O.H.; Supervision: O.H.; Project administration: O.H.; Funding acquisition: O.H.

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**Supplementary information**

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