Arabidopsis DNA topoisomerase I alpha is required for adaptive response to light and flower development

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ABSTRACT

DNA topoisomerase I alpha (TOP1α) plays a specific role in Arabidopsis thaliana development and is required for stem cell regulation in shoot and floral meristems. Recently, a new role independent of meristem functioning has been described for TOP1α, namely flowering time regulation. The same feature had been detected by us earlier for fas5, a mutant allele of TOP1α. In this study we clarify the effects of fas5 on bolting initiation and analyze the molecular basis of its role on flowering time regulation. We show that fas5 mutation leads to a constitutive shade avoidance syndrome, accompanied by leaf hyponasty, petiole elongation, lighter leaf color and early bolting. Other alleles of TOP1α demonstrate the same shade avoidance response. RNA sequencing confirmed the activation of shade avoidance gene pathways in fas5 mutant plants. It also revealed the repression of many genes controlling floral meristem identity and organ morphogenesis. Our research further expands the knowledge of TOP1α function in plant development and reveals that besides stem cell maintenance TOP1α plays an important new role in regulating the adaptive plant response to light stimulus and flower development.

KEY WORDS: DNA topoisomerase, Light response, Shade avoidance, Floral morphogenesis, RNA sequencing

INTRODUCTION

DNA topoisomerases function to relieve torsional stress in the DNA helix by introducing transient breaks into the DNA molecule. These enzymes regulate genome stability and processes of DNA recombination, repair, replication and transcription. In Arabidopsis thaliana (hereafter Arabidopsis) topoisomerase I alpha (TOP1α) has recently been shown to regulate nucleosome density or positioning at regulatory gene regions, thus probably allowing transcription factors to bind to their target genes (Liu et al., 2014). Genetic studies show strong synergetic interactions between top1α alleles and mutations in Arabidopsis genes encoding Polycomb Group Protein (PcG) subunits, which suggest that TOP1α is required for PcG-mediated repression of gene expression (Graf et al., 2010; Liu et al., 2014). Overrepresentation of PcG targets among genes whose expression is altered in the top1α-2 mutant suggests that TOP1α affects PcG-mediated epigenetic regulation in Arabidopsis plants (Liu et al., 2014). These findings explain the specific developmental function of TOP1α, which has been actively studied.

To date several mutations in TOP1α have been described in Arabidopsis. The detailed study of mgo1-1, mgo1-4, top1α-1, top1α-2 and fas5 mutants showed that all of them display defects in specific developmental processes related to the function of apical shoot and floral meristems. In accordance, their main phenotypic features are an enlarged apical meristem and stem fasciation (Laufs et al., 1998; Takahashi et al., 2002; Graf et al., 2010; Liu et al., 2014; Albert et al., 2015a).

Unlike clv mutants, which also demonstrate larger meristems and fasciation of the inflorescence stem, mutations in TOP1α cause a continuous fragmentation of the shoot apex into multiple meristems, which leads to the formation of extra branches. Meristem enlargement in top1 and clv mutants are associated with an ectopic expression of stem cell maintenance gene WUS. However, in contrast to clv mutants, which maintained enlarged stem cell pool in apical and floral meristem throughout their life cycle, TOP1α mutants gradually lost the ability to maintain stem cells during development. The cells of the inflorescence apical meristem (IM) increased in size and lost the indeterminate state (Albert et al., 2015a). The progress of development is provided by ectopic formation of new meristems (Laufs et al., 1998; Albert et al., 2015a).

In addition to these conspicuous features, mutations in TOP1α cause other developmental changes. Some of them are related to meristem malfunction. Extra carpel development, for instance, is explained by the reduction of AG binding to WUS, which results in a prolonged WUS expression, and a consequent loss of floral determinacy (Graf et al., 2010; Liu et al., 2014). The relation between other features in TOP1α mutants and meristem functioning is less evident. Among these features we note the accelerated transition to the reproductive phase, and a delay in floral development, both described for the fas5 mutant (Albert et al., 2015b). Moreover, we demonstrated that the fas5 induced an accelerated bolting in lfy-10 mutant that had slightly delayed flowering on Dijon-M (Dj) background (wild type for fas5), but fully transformed flowers into branched vegetative structures in fas5 lfy-10 plants (Albert et al., 2015b). The fas5 also accelerated inflorescence characters in flowers of ap1-1, ap1-20 and ap2-1 mutants thus indicating that TOP1α could play important role in determining floral meristem development via mediating the expression levels of floral meristem identity genes (Albert et al., 2015b). Recently, an early flowering phenotype was demonstrated for top1α-10 and mgo1-7 mutants (Gong et al., 2017).

To clarify seemingly opposite effects of fas5 on bolting initiation and flower development, we studied the fas5 plant development in long day condition and performed RNA sequencing (RNA-seq) to

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investigate the molecular basis of TOP1α role in phase transition and flowering time.

RESULTS

Mutations in TOP1α leads to constitutive shade avoidance syndrome

Plants homozygous for the fas5 mutant allele were grown under inductive photoperiods and compared with the wild-type Dj plants. Young mutant plants demonstrate slightly elevated leaf angles (hyponasty), elongated petioles and lighter leaf color associated with a slightly reduced chlorophyll and carotenoid level (Fig. 1A,B). The combination of these changes indicates that mutant plants display the shade avoidance phenotype (Franklin, 2008; Casal, 2012; Roig-Villanova and Martínez-García, 2016). This phenotype is detected in the ‘long days’ mutant plants in both the growth room (Fig. 1A) and glasshouse (Fig. S1A), but disappears in the growth chamber under higher light intensity.

After transferring the long days plants that had been grown in the growth room under 130 µmol·m⁻²·s⁻¹, to the conditions of reduced light intensity (60 µmol m⁻² s⁻¹) for 3 days, both Dj and fas5 show the shade avoidance syndrome (SAS). In these three days Dj plants lift up the leaves and demonstrate fast hypocotyl elongation. Under the same conditions, the length of fas5 hypocotyls remains unchanged, while the length of rosette internodes greatly

Fig. 1. Morphological features of LD fas5 and Dj plants. (A) Dj (left) and fas5 (right) plants at the age of 4 weeks; fas5 has elongated petioles, elevated leaf angles and develops inflorescence. (B) Chlorophyll (Chl) and carotenoid (Car) content in fas5 is lower than in Dj; values are the means of three biological replicates±s.d. (C) Dj (left) and two fas5 (right) 3-week-old plants after transition to low light for 3 days demonstrate SAS; arrows indicate elongated internodes in fas5. (D) Hypocotyl and first internode length in plants after 3 days in low light; mean of two biological replicates±s.d., n=33 plants per genotype. (E) Percentage of 4-week-old plants with inflorescences larger than 1 cm; mean of two biological replicates±s.d., n=50 plants per genotype. (F) Rosette (Ros) and cauline (Cal) leaf number. (G) Dynamics of flower opening (percent of plants with open flowers); the data represent one experiment, n=75 plants per genotype. Similar results are obtained in two independent experiments. Asterisks represent statistically significant differences of the mutant relative to wild-type plants (*P<0.05; **P<0.005; ***P<0.001 in Student’s t-test).
increases (Fig. 1C,D), thus resembling the phenotype of the double mutant phyA phyB (Devlin et al., 1996).

Mutant plants bolt earlier (Fig. 1A,E) and appear with fewer rosette leaves (Fig. 1F) than the wild-type plants, thus confirming the previously reported data for fas5 plants growing dynamics in a glasshouse (Albert et al., 2015b). This feature of fas5 is independent of light intensity. Under all conditions fas5 demonstrates earlier transition to the reproductive stage than Dj, although the exact time of inflorescence development varies. Despite early inflorescence initiation, the first open flower in fas5 plants developed later and after forming more cauline leaves (Fig. 1F,G). Consequently, after quick transition to the reproductive stage, fas5 mutant reduce the pace of development. The main features of flower morphology in fas5 were described by us earlier (Albert et al., 2015b).

Unexpectedly, light intensity has great influence on IM morphology and related degree of stem fasciation. Mutant plants growing in the growth room demonstrate apical meristem elongation after formation of several leaves (Fig. 2A,B) and fragments into multiple meristems (Fig. 2C-E). As the plant matured, these abnormalities became progressively more severe (Fig. 2C-E,G) and also strictly depended on growth conditions. At the end of the life cycle, IM in the growth room and glasshouse frequently looked like a meristem comb (Fig. 2G) and the stem usually terminated in a mass of carpelloid tissue (Fig. S1B), as in strong fhy mutants (Weigel et al., 1992). The percentage of such plants in the glasshouse was greater (up to 67%) than in the growth room (10%). In the growth chamber, IM maintains its integrity (Fig. 2F) and formation of carpelloid structures is not observed.

Thus, although the expressivity of fas5 depends on growth conditions, we conclude that TOP1a mutation shortens specifically the vegetative phase, although it also slightly delays the shoot apical meristem to IM transition. Considering that the expressivity of fas5 features depends on light intensity, we assume that the fas5 mutation affects light perception or response.

Other alleles also show constitutive shade avoidance response under long days. A strongest reaction is detected in top1a-2 (Ler background), which demonstrates leaf hyponasty from the early seedling stage (Fig. 3A). The TOP1a-I (Col wild type) shows weaker constitutive SAS (Fig. 3B), while mgo1-7 has intermediate phenotype (data not shown). After transferring the plants from 130 µmol m$^{-2}$ s$^{-1}$ to the reduced light intensity (60 µmol m$^{-2}$ s$^{-1}$) for 3 days, occasional top1a-1 plants increase their rosette internodes while most top1a-2

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**Fig. 2.** Scanning electron microscopy of apical inflorescence meristems (IM) in LD plants. (A,B) Shoot apical meristem of one-week-old Dj and fas5 plants, respectively. (C,D) IM of Dj and fas5 plants, respectively, at the flowering initiation phase; IM of fas5 fragmentizes into two parts. (E-G) IM of fas5 mature plants with weak (E) and strong (G) fasciation from the glasshouse and from the growth chamber (F). L, leaf primordium; F, floral primordium; *, IM. Scale bars: 10 µm in A-D; 100 µm in E-F; and 300 µm in G.
(Fig. S2) and more than half mgo1-7 studied plants do demonstrate this specific feature of phytochrome deficiency.

All previously studied mutant alleles of TOP1α demonstrate the accelerated transition to the reproductive phase in our experiments (Fig. 3C,D). All of them have reduced rosette leaf number and are characterized by an early bolting (Fig. S3). These observations provide evidence that phenotypic features revealed in fas5 mutant are common for other allelic mutants, though they differ by the expressivity of constitutive SAS.

**Mutation in TOP1α leads to a deeper and more extensive activation of genes, than to suppression**

We performed RNA-seq to determine the effects of the fas5 mutation on genome-wide gene expression in the apices of young inflorescences. This analysis shows that mutation in TOP1α causes changes in the expression level of 3901 genes: 2300 of the differentially expressed genes (DEGs) present higher expression in the mutant plants (Table S2), and 1601 present lower expression (Table S3). For the majority of genes only a slight change of the expression level is observed. If we omit DEGs with a log₂-fold change (FC) less than one, the number of DEGs falls to 550 for downregulated and 1389 for upregulated genes.

There is about 70% of genes with a less than twofold difference in the log₂FC-expression level in the group of activated genes, and 90% among repressed genes. The fraction of genes with a log₂FC between 2 and 4 is twice as high among activated DEGs (18.8%) as among repressed genes (8.7%). The number of DEGs with a more than fourfold difference in the log₂FC expression level is about 7%.
among activated genes, but only about 1% among repressed genes. Thus, the mutation in TOP1α leads to a deeper and more extensive activation of genes, than to suppression (Fig. 4).

It has been shown recently that genes with H3K27me3 in their chromatin preferentially represent PcG targets and require TOP1α for expression (Liu et al., 2014). We compare the list of fas5/DEGs with the list of H3K27me3 target genes in A. thaliana (Zhang et al., 2007). We find 16% and 18% of potential PcG target genes among the total list of repressed and activated genes in fas5 (Tables S4 and S5). These values are close to the total fraction of genes with H3K27me3 in the whole genome (Liu et al., 2014). However, a portion of potential PcG targets among DEGs in fas5 depends on FC in their expression level (Fig. 4). When considering the DEGs with less than onefold differences in log2FC, a portion of PcG targets is 9% for both upregulated and downregulated DEGs, but this portion increases in the group of DEGs with a greater elevation in log2FC. The largest number of PcG targets is found among activated genes, but only about 1% among repressed genes. Since most processes associated with chloroplasts depend on light stimulus, we suggest that genes regulated by light should be present among activated DEGs.

Overall, our analysis revealed significant qualitative differences between the annotation clusters of activated and repressed DEGs. Among activated genes, those associated with chloroplasts prevail, but the regulators of transcription and development are dominating among repressed genes. Among activated genes, those associated with chloroplasts prevail, but the regulators of transcription and development are dominating among repressed genes. Since most processes associated with chloroplasts depend on light stimulus, we suggest that genes regulated by light should be present among activated DEGs.

To obtain a comprehensive list of genes involved in light response and developmental processes among DEGs, we analyze Gene Ontologies for term enrichment using the AgriGO Single Enrichment Analysis tool. The full result of this analysis is presented in the Supplementary Information (Tables S8 and S9). This analysis confirms the results from DAVID. Among activated DEGs in fas5, a group of 267 genes (approximately 12% of activated DEGs) are associated with the term ‘plastids’ and 246 genes (11%) are associated with the term ‘chloroplast’. A group of 70 genes is related to response to light stimulus (Table S8, Fig. S4). Downregulated DEGs are enriched in genes involved in developmental processes (154 genes). This group includes the genes associated with flower (58 genes), shoot (43 genes), meristem (24 genes), root (28 genes), and leaf (23 genes) development (Table S9, Fig. S5). AgriGO also detected genes associated with the terms ‘response to light stimulus’ (42 genes) and ‘photoperiodism’ (9 genes, Table S9). Genes related to flowering, light response and photoperiodism are of particular interest. DEGs with an absolute value of FC≥2 and log2FC≥1 will be considered below.

Mutation in TOP1α alters an expression level of key genes involved in light response and shade avoidance

We focus on genes associated with different aspects of light perception and shade response and reveal four interconnected groups of genes showing increased expression in fas5. The first group contains 19 genes (Fig. 5A) including: PHOT1 encoding a blue light (BL) photoreceptor phototropin, five paralogues NPY2, RPT2, NPH3/RPT3, At5G49970, and At5g67385 encoding BTB/POZ domain-containing proteins that might act in fine-tuning the photoreceptor PHOT1 activity, PRNI involved in BL signaling, and

![Fig. 4. DEGs distribution according to expression level.](http://bio.biologists.org/.../by-guest-on-January-4,-2018)

Several light-response DEGs are involved in phytochrome-mediated photomorphogenesis. Five of them encode bHLH phytochrome-interacting transcription factors, PIFs (PIF1, PIF4, and PIF5) or a PIF-like factor (PIL1), which participate in the phytochrome B signaling pathway. The genes PKS1 and PKS2 encode phytochrome kinase substrate (PKS) family proteins, which are involved in phyA and phyB and BL signaling. We also include in the first group the AFR gene, a part of the phyA-mediated signaling transduction pathway, the TZP gene that plays a critical role in phyB signaling. The genes BRC1 and BRC2 also increase their expression level in fas5. These genes contribute to reduced branching under shade and are negatively regulated by phytochrome (Aguilar-Martínez et al., 2007; González-Grandío et al., 2013). The gene COL7 is also involved in branching regulation and acts as an enhancer of the shade avoidance response via promotion the expression of PIL1 mRNA in response to shade (Wang et al., 2013).

The second group (Fig. 5B) contains seven upregulated genes involved in auxin synthesis (YUC8), transport (ABCB4, and ABCB13 genes encoding the ATP-binding cassette B family of transporter proteins, and PIN4 and PIN7 genes for auxin efflux transmembrane transporters) and auxin response (IAA5 and IAA7). The third group contains auxin-regulated genes of expansins and xyloglucan endotransglycosylase/hydrolases (Fig. 5C) playing an important role in loosening and extension of plant cell walls (Cosgrove, 2016). These genes provide rapid organ elongation during adaptive plant growth (Sasidharan et al., 2008; Casal, 2012). Among DFGs in fas5 with increased expression we find eight genes: three genes of expansins (EXPA1, EXPA10, and EXPA11), and five genes of xyloglucan endotransglycosylase/hydrolases (XTH7, XTH18, XTH19, XTH22, and XTH23). These three groups of genes contain a total of 33 upregulated genes that are an integral terminal part of adaptive growth responses known as SAS.

In addition to these three groups of activated genes we reveal light-regulated genes in fas5 with elevated expression involved in chloroplast development and functioning (Fig. 5D). Among them are the genes encoding RBCS-1B, RBCS-2B and RBCS-3B (ribulose bisphosphate carboxylase small chain family proteins), CAB2 (chlorophyll A/B binding proteins), LHB1B1 (light-harvesting chlorophyll-protein complex II subunit B1), ELIP1 (an early light-inducible chlorophyll A-B binding family protein), GNC AT5G56860 (GATA transcription factors regulating chlorophyll biosynthesis), PORA (protochlorophyllide oxidoreductase A), PSBO2 (photosystem II subunit O-2), and some other genes. Because PIFs regulate chlorophyll biosynthesis (Huq et al., 2004; Moon et al., 2008; Liu et al., 2013), the observed shift in expression of these genes is not surprising. By adjustment of growth and development plants can optimize light capture for photosynthetic utilization under shade conditions. Therefore, the revealed changes in chloroplast gene expression can also be considered as part of the SAS.

Among repressed light-regulated genes we find three genes controlling auxin transport (ABCB, PIN1, and PIN2) and three genes of Aux/IAA transcriptional factors (IAA2, IAA14, and IAA30) that function as repressors of early auxin response genes (Fig. 5B). Strongly repressed MADS-box gene MAF1 closely related to the negative regulator of flowering FLC is also found among light-regulated genes. This gene is simultaneously present both among
light-regulated genes and flowering genes, which will be discussed below.

In addition to light-regulated genes in fas5, we find DEGs, which are not regulated by light but may be related to the above-mentioned group of auxin-related genes. Three activated genes are involved in auxin synthesis and homeostasis: they are the nitrilase gene NIT1, which regulates auxin biosynthesis from indole-3-acetonitrile, and two auxin-induced GH3 family genes GH3.17 and DFL2 controlling auxin homeostasis via the synthesis of auxin conjugates with amino acids. We also find two other GH3 family genes GH3.3 and BRU6/GH3.2 among repressed genes in fas5 (Fig. 5B).

It is important to note that in growth response, which is a final stage of SAS, more plant hormones are involved than just auxin (Martinez-Garcia et al., 2010; Stamm and Kumar, 2010). Among DEGs in fas5, we find activated genes related to abscisic acid (68 genes), jasmonic acid (29 genes) and gibberellin stimulus (27 genes) along with 9 repressed genes associated with the gibberellin signaling pathway (Tables S8 and S9). In this article we will not discuss these genes, although signaling pathways of these hormones not only actively interact with the auxin pathway but also regulate expression level and/or activity of PIF and other components of SAS pathway (Bou-Torrent et al., 2014; Leivar and Monte, 2014).

It is interesting that among the considered activated DEGs related to SAS, only 17% belong to potential PcG targets (8 genes out of 48); and vice versa, among downregulated DEGs associated with SAS (all of them regulate auxin associated processes) PcG targets (7 genes) prevail over non-targets (2 genes).

**Mutation in TOP1α changes an expression level of many genes involved in flowering initiation and development**

Among DEGs with an increased expression level in fas5 we find the UGT87A2 gene encoding a UDP-glycosyltransferase superfamily protein, which promotes flowering (Wang et al., 2012), and two genes encoding calmodulin-like proteins (CML23 and CML24) playing a role in the flowering transition (Tsai et al., 2007). We find two additional genes belonging to the same family of calmodulin-like genes (CLM41 and CLM37) among activated genes in fas5, however, their role in flowering time control is not defined (Fig. 6A).

In the group of downregulated genes associated with flowering we determine 26 genes with more than twofold decreased expression in fas5 (Fig. 6A,B). One gene from this list (MAF1) is mentioned above among repressed light-regulated genes. We also find two additional homologues of the FLC-clade among genes associated with flowering, the MAF3 and MAF5 genes, and the SVP gene, which function as a floral repressor together with members of the FLC-clade and encode MADS-domain transcription factors. Decrease in the expression level of these repressor genes as well as increased expression of the four above-mentioned positive regulators of flowering initiation may promote flowering in fas5 plants.

![Fig. 6. DEGs involved in flowering.](http://bio.biologists.org/Downloaded from http://bio.biologists.org/)
Most of revealed genes with decreased expression belong to positive flowering regulators. This group includes: four SBP-box genes SLP (SPL3, SPL5, SPL10 and SPL15), which define an endogenous flowering pathway in *A. thaliana*, and the FPF1 gene, which promotes flowering (Fig. 6A). Several DEGs regulate floral meristem identity and floral organ development (Fig. 6B). Among them are the major flower identity integrator gene *LFY* and its co-activator *UFO*, and MADS-box genes *FUL*, *SEP4*, and *CAL*. The latter gene is closely related to *AP1*, expression of which is downregulated slightly less than twofold. All 15 above-mentioned genes play important roles in flowering initiation and floral meristem identity.

Besides these genes, a group of genes associated with flower development contains 11 downregulated genes controlling different aspects of floral organ morphogenesis (Fig. 6B). The *HAN* gene encodes a GATA-3-type transcription factor with a single zinc finger domain and plays an important role in *Arabidopsis* flower meristem organization. A putative C2H2 zinc finger transcription factor *JAG* controls cell proliferation during organ growth by maintaining tissues in an actively dividing state (Dinneny et al., 2004). The *PRS*/ *WOX3* gene promotes cell proliferation in lateral floral primordium domains and is required for the formation of the margin cells of the first and second whorl organs. The MADS-box gene *AP3* is the main regulator of the second and third whorl organ identity. *RBE* and *ROXY1* are required for the early development of petal primordia and petal morphogenesis. The *STY2*/SRS2 and *EMS* genes regulate anther development. The *HAT1* gene is involved in floral meristem determinacy and is important for correct gynoecium and fruit development, and *AGO5* promotes the initiation of megagametogenesis. *REMI* may also play a role in flower development. This gene is required for proper integument development and specification of integument identity (Franco-Zorrilla et al., 2002). The downregulation of these above-listed genes may explain numerous alterations in floral morphogenesis that were described earlier for different mutant alleles of *TOP1α*.

Only one gene (*BEL1*) related to flower development is activated in *fas5* (Fig. 6B). It encodes a homeodomain protein required for ovule identity (Bencivenga et al., 2012). In total, only six genes somehow connected with flowering initiation and development are revealed among activated genes, but 26 important regulatory genes are found among repressed genes. Among 32 considered DEGs associated with flowering, 18 are potential PcG targets (56%). If we consider only the downregulated genes, the proportion of PcG targets is even higher (69%).

**DISCUSSION**

Analysis of *Arabidopsis* mutants uncovered an important role of *TOP1α* in specific developmental processes. These mutants are characterized by defects in stem cell homeostasis and phyllotaxy (Laufs et al., 1998; Takahashi et al., 2002; Graf et al., 2010). Here we demonstrate that the *fas5* mutation displays novel features. It causes clear SAS features including accelerated bolting, but slightly delays flower development (Fig. 1). Expressivity of shade-associated characteristics depends on light intensity. Most of them disappeared in high light, although accelerated *fas5* bolting was detected under all studied conditions. Our study of phenotype of *top1α-2*, *top1α-1*, and *mgol*-7 mutants shows that constitutive SAS is a common feature for other allelic mutants (Fig. 3). Most of these alleles are thought to be the null alleles and so their phenotypic similarity is expected. Moreover, the fact that in high light many *fas5* malfunctions become minimal (including apical meristem morphology, Fig. 2F) indicates that the discovered change in sensitivity to light is one of the key *fas5* (and probably of other alleles) characteristics that affect other developmental processes, including stem cell homeostasis.

SAS is an adaptive growth response activated by a reduced ratio of red to far-red (R:FR) light and reduced BL intensity (Casal, 2012; Pierik and de Wit, 2014; Roig-Villanova and Martinez-García, 2016). One possible explanation for constitutive SAS in *fas5* mutant is an alteration in the gene expression network involved in SAS. RNA-seq confirms an activation of many genes regulating response to low BL and R:FR ratio (Figs 5A and 7). In the *fas5* mutant, we detect upregulation of *PHOT1* mRNA. *PHOT1* is the primary receptor of BL controlling phototropism, regulating leaf blade expansion, flattening, and positioning under low BL intensity (Ohtsuki et al., 2004; Takemiya et al., 2005; Han et al., 2013). We find five activated homologues of *RPT2* and *NPH3*/RPT3. Proteins encoded by *NPY2*, *RPT2*, and *NPH3*/RPT3 modify *PHOT1* and...
might act in fine-tuning the photoreceptor activity through modulation of PHOT1 subcellular trafficking and desensitization by degradation (Sakai and Haga, 2012; Liscum et al., 2014).

In fas5, we also detect activation of PKS2 and PKS4, important components of the signaling cascade that function as positive regulators of hypocotyl phototropism (Pedmale et al., 2010; Sakai and Haga, 2012; Holm et al., 2013). Several studies have demonstrated that PKSs serve as a molecular link between phytochrome and phototropin-mediated responses through interaction with phytochromes and PHOT1 (Lariguet et al., 2006; Demarsy et al., 2012; Kami et al., 2014). PKSs may contribute to the phototropic response regulation through modulation of local auxin signaling or transport (Kami et al., 2014). Hence, light can effectively coordinate the activity of major auxin transporters and therefore auxin distribution to control phototropic responses in different organs (Žádníková et al., 2015). The observed changes in the expression of seven auxin transport genes (Fig. 5B) indicate activation of the phototropic response in fas5 plants, although the opposite changes that we also observe complicate the interpretation.

Several genes activated in fas5 are involved in phytochrome-mediated photomorphogenesis. We detected activation of phytochrome-interacting factors (PIFs) that play the central role in SAS and optimization of plant development to multiple internal and external signals (Casal, 2012; Leivar and Monte, 2014). In an FR-enriched environment, PIF proteins are stabilized and induce transcription of the YUC8 gene regulating auxin synthesis (Homitschek et al., 2012), thus directly linking the perception of a low R/FR signal to changes in free auxin required for shade-induced growth (Li et al., 2012). On the other hand, PIFs regulate transcription of IAA genes belonging to the auxin signaling repressor family, which mediate the attenuation of auxin signaling at the illuminated side of plant organs (Sun et al., 2013). In fas5 we reveal upregulation of YUC8, IAA5, and IAA7. At the same time, IAA2, IAA14, and IAA30 are repressed in fas5 (Fig. 5B). Changes in the mRNA level of all these genes may trigger a transient increase in auxin levels and tissue-specific growth response, which is an integral part of plant tropisms and SAS (de Wit et al., 2015).

Expansins (EXP) and xyloglucan endotransglucosylase/hydrolases (XTH) are two well-characterized cell wall modifying proteins that are implicated in cellular expansion (Cosgrove, 2016). In fas5 plants activation of five XTH and three EXP genes are revealed (Fig. 5C). The upregulation of these genes can play an important role in loosening and extension of the plant cell walls during SAS.

Most shade-avoiding plants display reduced branching and enhanced apical growth that help them compete for incident light (Franklin, 2008). Therefore, upregulation of BRC1 and BRC2, which suppress axillary bud outgrowth (González-Grandío et al., 2013), represents an additional evidence in favor of SAS activation in fas5 (Fig. 5A).

Thus, RNA-seq analysis reveals changes in expression level of key SAS pathway genes. This finding can explain constitutive SAS in fas5 mutant, including early plant bolting. It was shown that PIF4 and PIF5 transcription factors promote flowering by at least two means: inducing FT expression and acting independently of FT through an unknown mechanism (Kumar et al., 2012; Thines et al., 2014). FT was not revealed among DEGs in fas5, since we extracted RNA from inflorescence apices when flowering initiation had already taken place. Nevertheless, fas5 is characterized by a reduced expression of SVP and genes of the FLC-clade MAF1, MAF3, and MAF5 (Figs 6A and 7). Proteins encoded by these genes may form nuclear MADS-domain complexes (Gu et al., 2013; Mateos et al., 2015). Regulation of flowering time by these complexes appears to be achieved via diverse pathways, including photoperiod or circadian clock, since MAF1 (Rateliffe et al., 2003) and SVP (Fujiiwara et al., 2008; Andrés et al., 2014) participate in the photoperiod pathway. Some indication of MAF3 involvement in the circadian regulation was also obtained (Mateos et al., 2015). Hence, we cannot exclude that these genes (at least the strongly repressed MAF1) are the main component of early flowering in shade and their repression is the main reason for the early phase transition in fas5 plants. Our datum is in a good agreement with downregulation of FLC, MAF4 and MAF5 in top1α-10 seedlings demonstrated by real-time PCR analysis (Gong et al., 2017).

Besides repressed MAFs we reveal three activated DEGs, which act early and may promote flowering. UGT87A2 promotes flowering by repressing FLC. An ugt87a2 mutant exhibited late flowering under both long day (LD) and short day (SD), and its flowering was promoted by vernalization and gibberellin (Wang et al., 2012). Some other activated genes such as genes encoding calmodulin like proteins can promote flowering of fas5. The impact of CML23 and CML24 in flowering transition via FLC repression (Tsai et al., 2007) allows speculating that these genes and probably CML35 and CML41 are involved in the repression of close FLC homologues MAF1, MAF3 and MAF5.

Many other light-regulated genes are upregulated in fas5, thus demonstrating essential changes in the light response gene network. These changes together with the SAS phenotype demonstrate that TOP1α plays an important role in the regulation of light perception and light response. Apparently an alteration of the initial stages of light perception may explain the activation of the whole set of key SAS genes, most of which are not PcG targets. To identify the primary cause of the constitutive shade response, directed studies of light receptors activity in the fas5 mutant are required. The similarity between the fas5 phenotype and the phyA phyB double mutant phenotype indicates possible changes in the phytochrome system.

We determine important positive regulators of flowering and floral meristem identity that are repressed in fas5 (Fig. 6B and 7). Four flower-promoting SPL genes (Yamaguchi et al., 2009) are repressed in fas5, as well as their targets FUL, LFY and AP1. Repression of the UFO, a LFY co-regulator, and the CAL gene, which functions redundantly with AP1, are also detected in fas5. These data are in a good agreement with the phenotype of the double mutants fas5 lfy-10 and fas5 ap1, for which a great enhancement of shoot-like features in flowers was described (Albert et al., 2015b). The downregulation of these key positive regulators of floral meristem identity explains delayed flower development in fas5 mutant.

Decrease in the expression of genes regulating floral organ development can also affect flower morphology (Fig. 6B). For instance, reduced expression of at least three genes RBE, HAN and JAG can explain the reduced number of petals, which is the most easily detected feature in flowers of fas5 and other allelic mutants. In fas5 we find 3.3 log2FC repression of RBE. The effect of this repression is comparable to the loss-of-function rbe mutants exhibiting a loss of or aberrant petals (Takeda et al., 2004; Krizek et al., 2006). Flowers of the double mutant han-2 jag-3 have a reduced number of petals (Ding et al., 2015). A simultaneous decrease in HAN and JAG expression is exactly what we have seen in the fas5 transcriptome.

In conclusion, our study explains two seemingly opposite features of the fas5 mutation in the Arabidopsis gene TOP1α, i.e. an early transition to the reproductive stage and quick initiation of inflorescence growth on the one hand, and, on the other hand,
slowing the pace of flower development (timing of the first flower opening). The first feature is the consequence of the constitutive activation of the shade avoidance gene pathway in fas5, which accelerates bolting. We find activation of all key regulators of this pathway including BL photoreceptor phototropin (PHOT1), phytochrome-interacting transcription factors (PIFs and PIL), phytochrome kinase substrate family proteins (PKS) and downstream auxin-dependent components, which adapts plant growth to low BL and low R:FR light conditions. The delayed flower development in fas5 is the effect of the downregulation of key genes controlling floral meristem identity and floral organ morphogenesis, most of which are PcG targets. The so-called floral integrator gene LFY, as well as FUL, SEP4, CAL, AP3, RBE and many other genes are among them. It is rather doubtful that revealed coordinated changes in the expression level of the whole set of interconnected genes within shade response and floral development networks are random. Given the general role of topoisomerase in maintaining proper DNA topology and its involvement in chromatin remodeling, it is more likely that the fas5 mutation may cause the direct effect on some of the genes among these networks. The changes in expression level of these target genes, in turn, can affect the expression level of other interacting genes and cause secondary pleiotropic effect on plant morphology. The complete correspondence of the revealed changes in the transcriptome with the phenotype of fas5 and other allelic mutants leaves no doubt that the gene TOP1α is involved in regulation of the shade response gene network.

While we still do not know which of the identified genes with altered expression are the direct targets of topoisomerase activity and what kind of marks do attract TOP1α to these particular genes, answers to these questions is important for elucidation of the possible role of the gene TOP1α and other chromatin regulators in the origin and functioning of gene networks which provide coordination between multiple developmental processes and environmental variation.

MATERIALS AND METHODS

Plant material and growth conditions

Arabidopsis thaliana (L.) Heynh. fas5 mutant was isolated from an EMS-mutagenized Dijon-M (Dj) seed population and was backcrossed with wild-type Dj plants three times prior to further analysis. The mutation was mapped by whole genome sequencing as described (Leshchiner et al., 2012) using DNA pools of wild-type and fas5 plants from the F2 fas5 × Columbia cross. The fas5 mutation localized to the 9th exon and represented the C to T transition, leading to the replacement of the CAG codon (glutamine 701) by the stop codon TAG. This mutation leads to a premature termination of transcription and loss of a functionally significant C-terminal domain of topoisomerase I (Albert et al., 2014). Plants were grown in a growth room with 130 µmol m−2 s−1 light exposure at 23°C under long day (LD) conditions (16 h light: 8 h dark cycle). All measurements, unless otherwise stated, were carried out in a growth room. Some measurements under LD were performed in a growth room with 60 µmol m−2 s−1 light exposure, a glasshouse with about 100 µmol m−2 s−1 light intensity (90-135 µmol m−2 s−1) and varied day-night temperature, or in a growth chamber with 150 µmol m−2 s−1 light intensity at 22°C. Plants and apical meristem morphology documentation was performed using light microscopy and scanning electron microscopy as described previously (Albert et al., 2015a). To provide evidence that phenotypic features revealed in fas5 mutant are not the effect of genetic background, rather they are the cause of the TOP1α gene disfunction, phenotype of some other mutant alleles were studied: top1α-2 on the Ler background and three mutations top1a-1, mgo1-7 on the Columbia (Col) ecotype.

Pigment assay

Chlorophyll and carotenoid content was measured for 3-week-old plants (before bolting) using three biological repeats. Pigment content was measured spectrophotometrically in acetone extracts of rosette leaves (Lichtenthaler, 1987).

RNA extraction and sequencing

RNA was isolated from 25- and 20-day-old Dj and fas5 plants, correspondingly, grown under LD in a growth room. Hand-dissected apices of young inflorescences from 25 wild-type and 25 fas5 plants were fixed in RNA later (Qiagen, Germany) in two biological replicates. Total RNA extraction and sequencing of the cDNA libraries were performed in the laboratory of Evolutionary Genomics of Moscow State University using the Illumina Hiseq2000 platform with adapter ligation and single-end 50 bp reads length.

Quality control, mapping of RNA-seq reads and bioinformatic analysis

A quality control analysis of raw reads was accomplished by FastQC 0.11.2 (Anders and Huber, 2010). Adapters and low-quality reads were trimmed before data analysis using Trimmomatic 0.32 (Bolger et al., 2014). A total of 150,184,519 reads after trimming were mapped to the A. thaliana genome (TAIR10; Table S1) using the BWA package 0.7.1 (Li and Durbin, 2009) and processed by HTSeq (Anders et al., 2015) to get the total gene reads count. Approximately 87% of all reads were mapped back, 95% of which were uniquely mapped to only one location and could be assigned to a single annotated TAIR10 gene. A comparison of the samples shows a very high correlation (Pearson’s Correlation Coefficient R^2>0.92) between wild-type and fas5 samples (Table S1). Gene counts were normalized and analyzed with the DESeq R package (Anders and Huber, 2010) with a false discovery rate (FDR) of 0.05 as the threshold for differentially expressed genes (DEGs) detection. An enrichment analysis was performed using the DAVID Bioinformatics Resources 6.7 (Huang et al., 2007) and AgiGO Single Enrichment Analysis tool (Du et al., 2010) with an FDR value of 0.05 as the threshold of significance.

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Competing interests

The authors declare no competing or financial interests.

Author contributions


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Data availability

The Illumina sequence reads have been deposited to the NCBI Sequence Read Archive (accession no. PRJNA318497).

Supplementary information

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