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The seed dormancy defect of *Arabidopsis* mutants lacking the transcript elongation factor TFIIS is caused by reduced expression of the *DOG1* gene

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ABSTRACT

TFIIS is a transcript elongation factor that facilitates transcription by RNA polymerase II, as it assists the enzyme to bypass blocks to mRNA synthesis. Previously, we have reported that *Arabidopsis* plants lacking TFIIS exhibit reduced seed dormancy. Among the genes differentially expressed in *tfIIs* seeds, the *DOG1* gene was identified that is a known QTL for seed dormancy. Here we have analysed plants that overexpress *TFIIS* in wild type background, or that harbour an additional copy of *DOG1* in *tfIIs* mutant background. These experiments demonstrate that the down-regulation of *DOG1* expression causes the seed dormancy phenotype of *tfIIs* mutants.

1. Introduction

The elongation phase of RNA polymerase II (RNAPII) transcription is a dynamic and highly regulated step in gene expression. In line with that various transcript elongation factors were identified that modulate different aspects of RNAPII progression on chromatin templates. The concerted action of numerous transcript elongation factors ensures that RNAPII successfully reaches the end of the transcription unit [1–3]. Although mRNA synthesis is generally processive, transcript elongation can be blocked in various ways causing reverse translocation (backtracking) of RNAPII and displacement of the extendable 3′end of the nascent RNA from the polymerase active site. One of the regulators of transcript elongation is TFIIS, which modulates the catalytic properties of RNAPII, facilitating RNAPII read-through of various blocks to transcript elongation including arrest sites [4,5]. In complex with RNAPII, TFIIS extends from the polymerase surface via a pore to the internal active site of the enzyme, where it strongly enhances the intrinsic RNA nuclease activity of RNAPII. The endonucleolytic cleavage of the nascent transcript allows realigning of the transcript in the RNAPII active site to resume elongation [6,7].

We have previously characterised the nuclear 42-kDa TFIIS from *Arabidopsis* that shares 25% and 30% amino acid sequence identity with yeast and mouse TFIIS, respectively. When expressed in yeast cells, *Arabidopsis* TFIIS can partially complement the sensitivity to 6-azauridine of the cells lacking the endogenous TFIIS, indicating that the plant protein also acts as a transcript elongation factor. *Arabidopsis* plants harbouring T-DNA insertions in the *TFIIS* gene essentially have wild type appearance, but they are severely affected in seed dormancy [8]. Seed dormancy is defined as a block to complete germination of an intact viable seed under favourable conditions [9,10]. Fully developed seeds of freshly harvested siliques of *tfIIs* plants germinate efficiently (without after-ripening), while control seeds hardly germinate under these conditions [8]. In line with that, it was found that TFIIS is encoded by the *Arabidopsis RDO2* locus [11] that in previous analyses had been associated with reduced seed dormancy [12,13]. *TFIIS* transcript levels increase during seed development in both Col-0 and Ler seeds [11,14], suggesting that TFIIS may serve a critical function during seed development/germination. Consistent with the reduced dormancy, decreased transcript levels of the *DOG1* (*Delay Of Germination 1*) gene were detected in *tfIIs* seeds [11,14]. *DOG1* was characterised as a quantitative trait locus (QTL) for seed dormancy in *Arabidopsis* and it encodes a seed-specific protein of unknown function [15,16]. To examine whether the observed

Abbreviations: DAS, days after stratification; DAF, days after flowering; DOG1, delay of germination 1; QTL, quantitative trait locus; rtPCR, reverse transcription polymerase chain reaction

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down-regulation of *DOG1* expression in *tfIIs* seeds causes the reduced dormancy phenotype, we have analysed here *Arabidopsis* Col-0 plants that overexpress *TFIIS* as well as plants that express an additional copy of *DOG1* in a *tfIIs* mutant background.

2. Materials and methods

2.1. Plasmid construction and plant transformation

DNA fragments to be inserted into transformation vectors were generated by PCR using genomic Col-0 DNA as a template, KAPA HiFi DNA polymerase (PEQLAB) and gene-specific primers. The obtained *DOG1* gene fragment was inserted into plasmid pGreenII0229 and the *TFIIS* gene fragment in the overexpression construct was inserted into pGreenII0229-P35S [17] employing standard methods. All plasmid constructions were checked by DNA sequencing, and details of the plasmids generated in this work are summarised in Table S1. The pGreenII vectors were transformed into *Arabidopsis* Col-0 or *tfIIs-1* plants [8] by *Agrobacterium*-mediated transformation using the floral dip method as previously described [18–20].

2.2. Plant material

To grow *Arabidopsis* plants, after sowing the seeds were stratified in darkness for 48 h at 4 °C prior to incubation in a plant growth chamber under long-day conditions (16 h light, 8 h darkness) as described previously [19,21], and plants were analysed at different days after stratification (DAS). Unless stated otherwise seeds of siliques (still green or just turning yellowish) harvested 15 days after flowering (DAF) were used for germination assays without prior storage (after-ripening) as described previously [8]. In brief, seeds were placed on Whatman 3 M paper soaked with water in Petri dishes and moved to a plant incubator (Percival Scientific; 16 h of light at 22 °C, 8 h of darkness at 19 °C) and germination was scored after seven days of incubation.

2.3. rtPCR

Total RNA was isolated from seeds using a described method [22]. cDNA synthesis using RevertAid H Minus M-MuLV reverse transcriptase (Fermentas) and random hexamer primers, and the following PCR analyses were performed as described previously [23]. All PCR primers used are listed in Table S1.

3. Results and discussion

3.1. Generation and analysis of plants overexpressing TFIIS

In view of the down-regulation of *DOG1* transcript levels in *tfIIs* plants [11,14], we generated plants overexpressing *TFIIS* to examine, whether elevated *TFIIS* levels possibly result in *DOG1* transcript levels elevated above the wild type level and accordingly in more pronounced seed dormancy. Therefore, a construct containing the genomic coding sequence of *TFIIS* (including 5′ and 3′ UTRs) under control of the CaMV 35S promoter was transformed into Col-0 plants. Transgenic plants containing the overexpression construct were identified by PCR-based genotyping with primer combinations (Fig. 1A) that allow discriminating the native *TFIIS* and that of the transgenic overexpression construct (Fig. 1B). Plants homozygous for the overexpression construct were grown under long-day conditions, revealing that they grow and develop similar to Col-0 plants except for a slightly smaller rosette diameter (Fig. 1C,D and Table S2). The transcript levels of *TFIIS* and *DOG1* in freshly harvested seeds were analysed by rtPCR. Three selected independent overexpression lines displayed clearly increased

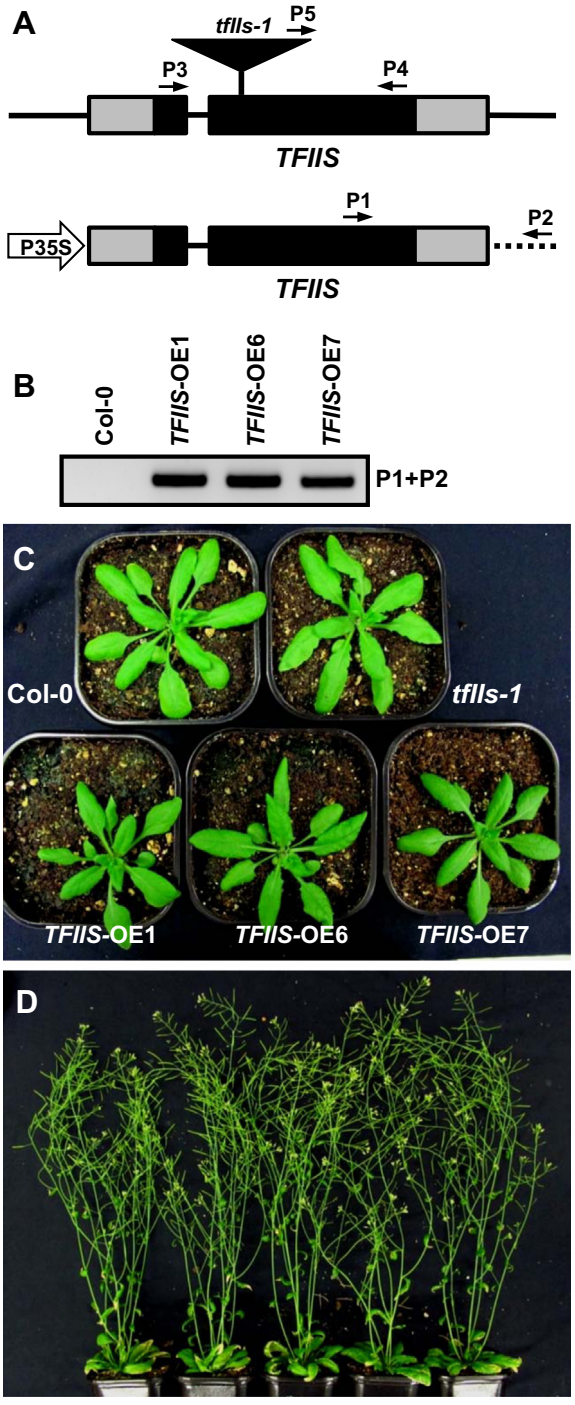

Fig. 1. Characterisation of *Arabidopsis* plants overexpressing *TFIIS*. (A) Schematic representation of the *TFIIS* gene (top) and the overexpression construct (bottom) introduced into Col-0 plants (P35S indicating the CaMV derived promoter and gray boxes representing UTRs and black boxes indicating exons). The position of the T-DNA inserted in the *TFIIS* gene of the *tfIIs-1* line is indicated by a triangle along with the primers (small arrows) used to examine the wild type/mutant genotype. Additional PCR primers used to analyse the *TFIIS* gene as well as the overexpression construct are also indicated (cf. Fig. S1). Primer P2 is specific for vector sequence (dotted line) and therefore was used to distinguish the overexpression construct and the native gene. (B) PCR-based genotyping of Col-0 plants transformed with the *TFIIS* overexpression construct. A primer combination specific for transgenic version of *TFIIS* revealed the presence of the construct in the transformed plants, but not in Col-0. (C) Appearance of typical individuals of the different plant lines grown under long-day conditions documented at 28 DAS. (D) Appearance of the plants grown under long-day conditions documented at 50 DAS showing plants in the same order as in (C).

levels of the *TFIIS* transcript relative to Col-0, while in these plants the transcript of the reference gene *UBQ5* was detected in wild type amounts (Fig. 2A). Despite the elevated *TFIIS* expression in these lines, the transcript levels of *DOG1* were in a similar range as in Col-0 and in one line (*TFIIS*-OE7) it even was reduced (Fig. 2B). When the seeds were examined in germination tests, the germination rate essentially correlated with the *DOG1* transcript levels.

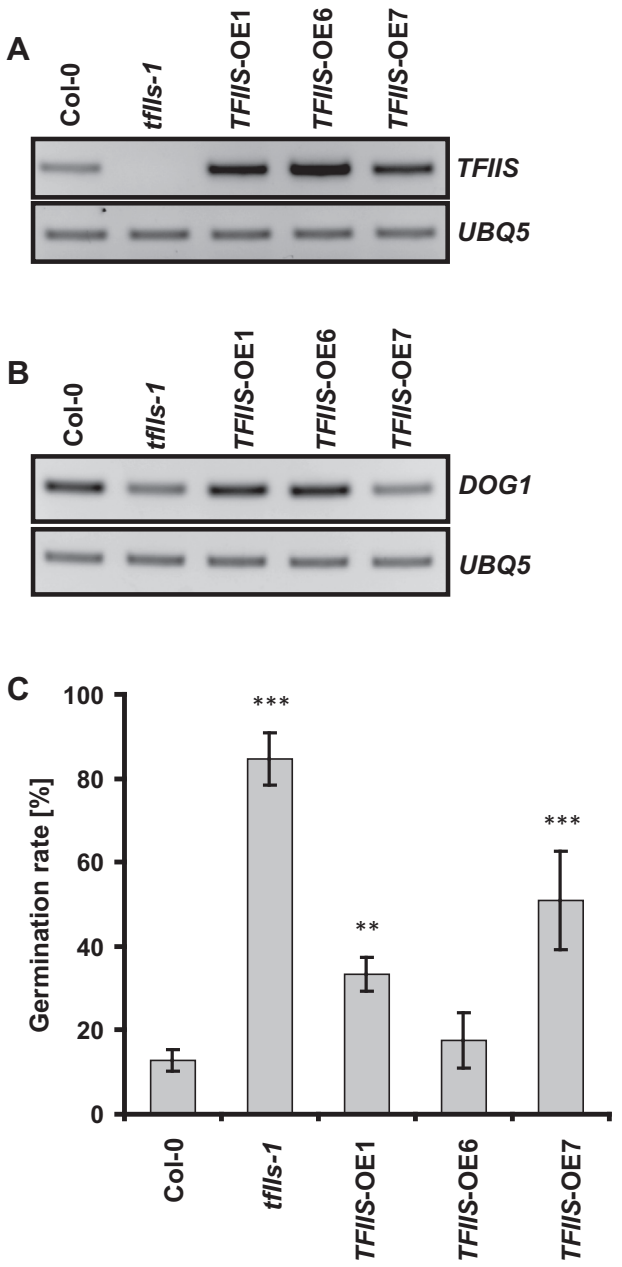

Fig. 2. Transcript levels of *TFIIS* and *DOG1* as well as germination rates of plants overexpressing *TFIIS*. (A) *TFIIS* transcript levels in seeds of three independent overexpression lines relative to Col-0 and *tfIIs-1*analysed by rtPCR. The transcript levels of the reference gene *UBQ5* were examined in parallel. (B) *DOG1* transcript levels in seeds of three independent overexpression lines relative to Col-0 and *tfIIs-1* analysed by rtPCR. The transcript levels of the reference gene *UBQ5* were examined in parallel, and a typical result of several repetitions is shown. (C) Seed germination rates of the overexpression plants, Col-0 and *tfIIs-1*. Freshly harvested 15-DAF seeds (250 seeds per genotype were used in two independent experiments) were sown and germination was scored after seven days. The histogram bars represent two pooled experiments, statistically evaluated with a one-way ANOVA and data sets marked with asterisks are significantly different from Col-0 ($^{**}P < 0.01$ and $^{***}P < 0.001$). The error bars indicate standard deviation.

tfIIs-1 seeds germinated efficiently, while those of Col-0 and *TFIIS*-OE1/6 germinated clearly less efficiently and *TFIIS*-OE7 showed intermediate germination rates (Fig. 2C). Although we observed a correlation between the *DOG1* transcript levels and the germination efficiency of the seeds of the different analysed plant lines, we used an additional approach (see Section 3.2) to further examine whether the seed dormancy phenotype of *tfIIs* plants is mediated by regulating *DOG1* transcript levels. The overexpression experiment also suggests that in Col-0 the amount of TFIIS is not limiting for expression of *DOG1* at normal levels. Moreover it demonstrates that the overexpression of *TFIIS* hardly affects the plant phenotype under normal growth conditions. Plants lacking TFIIS also develop normally and only show defects in seed dormancy [8]. Lack or overproduction of *TFIIS* has little effect on the growth of yeast cells under normal conditions [4], but inactivation of mouse *TFIIS* causes embryo lethality [24].

3.2. Analysis of tfIIs mutant plants harbouring an extra copy of the DOG1 gene

We used an alternative approach to test, whether seed dormancy in *tfIIs* plants is affected through down-regulation of *DOG1* expression. We intended to introduce an additional copy of the *DOG1* gene into *tfIIs* mutant background to examine whether higher *DOG1* transcript levels possibly revert the seed germination rates of *tfIIs* to wild type levels. Towards that goal the genomic sequence of the *DOG1* gene (from −2679 bp upstream of the translational start and including the 3′UTR) was transformed into *tfIIs-1* plants by *Agrobacterium*-mediated transformation. Plants harbouring the *DOG1*-construct were identified by PCR analysis of genomic DNA with primers (Fig. S1A) that allowed specifically amplifying the transgenic copy of the *DOG1* gene (Fig. S1B). As expected the transgenic plants having an extra copy of the seed-specifically expressed *DOG1* gene had wild type appearance (Fig. S1C). Seeds of three independent *tfIIs-1* plants lines homozygous for the *DOG1*-transgene were examined for their *DOG1* transcript levels by rt-PCR in comparison to seeds of the control plants Col-0 and *tfIIs-1*. In line *tfIIs*-DOG1-1, for unknown reasons the transcript level of *DOG1* was not increased and similar to *tfIIs-1* control plants (Fig. 3A). However, in the seeds of lines *tfIIs*-DOG1-2/3 clearly elevated *DOG1* transcript levels were detected, resembling the transcript level observed in Col-0. Germination tests performed with freshly harvested seeds of the different plant lines revealed that consistent with the low *DOG1* transcript levels, seeds of *tfIIs* and *tfIIs*-DOG1-1 germinated efficiently (Fig. 3B). In contrast to those, the seeds of Col-0 and *tfIIs*-DOG1-2/3 having higher *DOG1* transcript levels germinated inefficiently. *tfIIs* mutant plants and the *tfIIs* plants transformed with the *DOG1* expression cassette differ only in the presence of an extra copy of the *DOG1* gene leading in lines *tfIIs*-DOG1-2/3 to elevated *DOG1* transcript levels. The correlation of the *DOG1* transcript levels in the seeds and the germination efficiency argue that the seed dormancy indeed is controlled by TFIIS-mediated regulation of *DOG1* transcript levels. Therefore, the dormancy defect of *tfIIs* mutants is brought about by decreased *DOG1* transcript levels during seed maturation.

In the past years, studies of mutants defective in various transcript elongation factors in different eukaryotic model systems have revealed that these factors play critical roles in development, illustrating their importance in maintaining proper gene expression programs.

It emerged that transcript elongation factors can control various aspects of development, most likely by regulating the expression of genes that play key roles in higher eukaryote developmental processes [25,26]. Since some of the known transcript elongation factors are relatively conserved, *Arabidopsis* is a promising model to study the role these proteins play in the development of

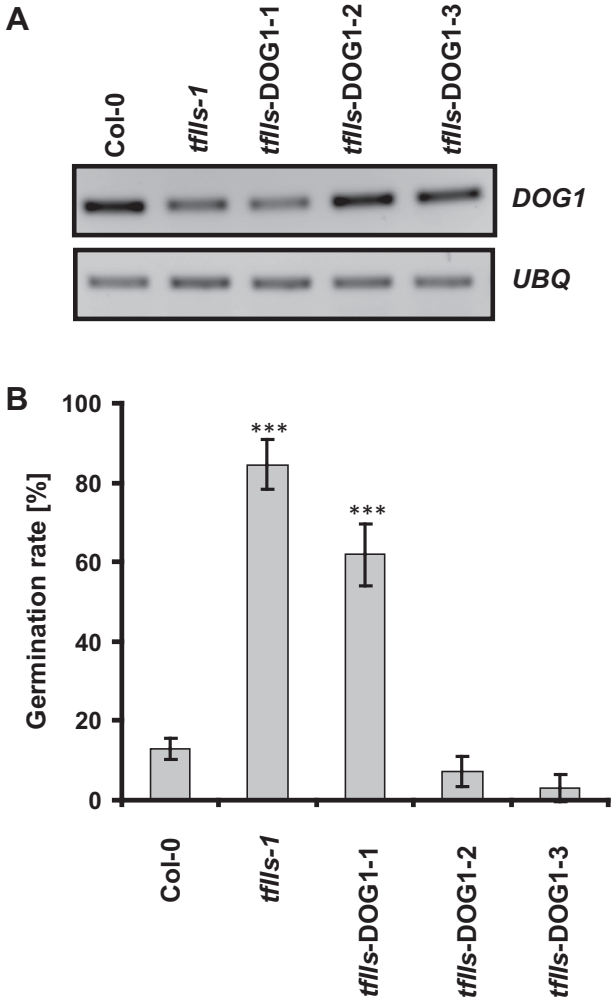

Fig. 3. Transcript levels of *DOG1* and germination rates of *tfIIs-1* plants harbouring an additional copy of *DOG1*. (A) *DOG1* transcript levels in seeds of three independent *tfIIs-1* plant lines harbouring an additional copy of *DOG1* relative to Col-0 and *tfIIs-1*analysed by rtPCR. The transcript levels of the reference gene *UBQ5* were examined in parallel, and a typical result of several repetitions is shown. (B) Seed germination rates of Col-0, *tfIIs*-1 and the *tfIIs-1* plants harbouring an additional copy of *DOG1*. Freshly harvested 15-DAF seeds (250 seeds per genotype were used in two independent experiments) were sown and germination was scored after seven days. The histogram bars represent two pooled experiments, statistically evaluated with a one-way ANOVA and data sets marked with asterisks are significantly different from Col-0 (***$P < 0.001$). The error bars indicate standard deviation.

multicellular organisms [27,28]. The experiments presented here show that the seed dormancy phenotype of *Arabidopsis tfIIs* mutants [8,11] is caused by down-regulation of the transcript levels of *DOG1* that was previously identified as a QTL with a strong effect on *Arabidopsis* seed dormancy [15]. Genome-wide chromatin association studies in yeast imply that various transcript elongation factors are found at all transcribed genes [29]. However, transcript profiling experiments in mutants defective in different transcript elongation factors (including *Arabidopsis* TFIIS) have shown that typically only a relatively small subset of genes is differentially expressed [8,30,31]. The finding that only some genes are incorrectly expressed in the absence of a certain transcript elongation factor, indicates that these genes appear to be more sensitive (than the majority of genes) to the loss or depletion of a specific transcript elongation factor [27,32]. Currently, it is still unclear to which extent different characteristics of a gene (e.g., DNA sequence, inducibility, expression level, RNAPII density, chromatin structure, co-transcriptional mRNA processing) determine RNAPII elongation rate and the requirement of certain transcript elongation factors for proper transcription [33,34]. Therefore, it will be attractive for future experiments to identify the feature(s) of the *DOG1* gene, which determine that its proper expression is dependent on TFIIS.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2013.10.047.

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