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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

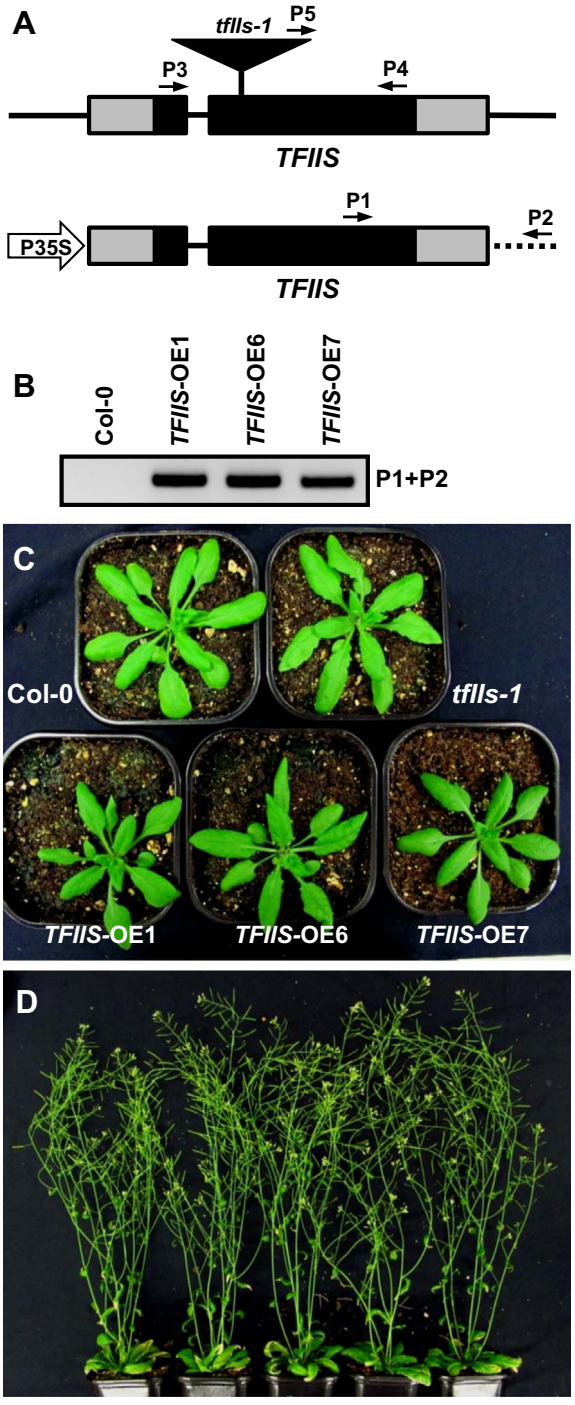

Fig. 1. Characterisation of *Arabidopsis* plants overexpressing *TFIIS*. (A) Schematic representation of the *TFIIS* gene (top) and the overexpression construct (bottom) introduced into Col-0 plants (P35S indicating the CaMV derived promoter and gray boxes representing UTRs and black boxes indicating 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).

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