Daysleeper : from genomic parasite to indispensable gene

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Chapter 4

The influence of *DAYSLEEPER* on the *Arabidopsis thaliana* transcriptome

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Abstract

Background

DAYSLEEPER is a domesticated transposase in Arabidopsis that is essential for plant development [1]. Plants overexpressing the *DAYSLEEPER* gene display a severe phenotype that becomes more severe in subsequent generations [1]. DAYSLEEPER has a largely nuclear localization and possesses a DNA-binding domain [1]. Although *DAYSLEEPER* is essential for Arabidopsis, little is known about its mode of function. We shed light on *DAYSLEEPER* function by studying the effect of induced *DAYSLEEPER* overexpression with a SAGE (Serial Analysis of Gene Expression) approach, in combination with Illumina® GAIIx-Sequencing.

Results

We found that induction of 4 hours of tamoxifen (TAM) induced *DAYSLEEPER* overexpression, 13 genes were upregulated, whereas 55 were downregulated. Both up and downregulated genes are apparently functionally diverse and involved in various processes. Prolonged *DAYSLEEPER* overexpression causes differential regulation of a plethora of genes after 24 hours (413 up- and 622 downregulated genes). Our results partly overlap with a preliminary microarray analysis by Bundock and Hooykaas (2005) [1].

Conclusions

Our experiments show that *DAYSLEEPER* overexpression causes misregulation of genes in various functional realms. Ubiquitin-related genes and processes, such as chromatin remodeling, plant defense, flowering and circadian rhythm are strongly represented among both up and down regulated genes. This fact, together with coexpression analysis, leads us to suggest that DAYSLEEPER might be involved in protein degradation by regulating COP9-signalosome and cullin-RING-type E3 ubiquitin ligase-mediated ubiquitylation.

Introduction

DAYSLEEPER (DAYSLEEPER) is a domesticated transposase that is essential for Arabidopsis development [1]. Plants lacking *DAYSLEEPER* do not develop, rarely produce photosynthetic tissue and stall in the early seedling stage [1]. Plants overexpressing *DAYSLEEPER* also display aberrant phenotypic traits. These plants grow slower, have flower development defects, display fasciation, altered cauline leaves and partial or total sterility [1]. The severity of the overexpression phenotype is increased in the next generation, eventually leading to lethality [1]. The pleiotropic nature of both the overexpression and the null-mutant indicate that *DAYSLEEPER* is tightly regulated and most likely involved in fundamental processes. DAYSLEEPER has been shown to be able to bind to the DNA of the Ku-box *in vitro,* present in the promoter of the DNA damage repair gene *KU70* [1].

Daysleeper plantlets are phenotypically similar to mutants lacking subunits of the COP9-signalosome (*cop* mutants) [2]. Both *cop* mutants and *daysleeper* plantlets produce little or no photosynthetic tissue and do not progress beyond the young seedling stage [1, 3]. *Daysleeper* plantlets do not share the constitutively photomorphogenesis traits of *cop* mutants, however [1, 2]. The COP9 signalosome (CSN) regulates the ubiquitylation of proteins by Cullin-ring ubiquitin ligases (CRL's) [2, 4]. Different types of CRL's exist, based on various CULLIN proteins. SCF (for SKP/CUL/F-Box)-type CRL's are based on CULLIN1 (CUL1) [5], DCX (for DDB1/CUL4a/X-box)-type CRL's on CULLIN4 (CUL4) [6], BCR (for BTB-domain protein/CULLIN3 (CUL3)/RING protein)-type complexes on CULLIN3 [7]. The anaphase-promoting complex/cyclosome (APC/C) is based on the distant CULLIN-relative APC2, and can target PDS1/SECURIN and cyclin B for degradation, allowing sister chromatid separation and exit from mitosis [8, 9]. These complexes consist of multiple subunits and can specifically ubiquitylate proteins involved in a wide spectrum of cellular processes, which are thereby targeted for degradation by the 26S proteasome (reviewed by Hua *et al.* 2011 [10]).

Circadian rhythm regulation and light responses are one of the processes regulated by CRL complexes. ZEITLUPE (ZTL) and EMPFINDLICHER IM DUNKELROTEN LICHT 1 (EID1) are F-box proteins involved these processes [11, 12]. An SCF-complex containing ZTL (SCF^{ZTL}) regulates the degradation of the PSEUDO RESPONSE REGULATOR 5 protein (PRR5) [13], which is a component of the Arabidopsis central clock oscillator. Furthermore, CUL1 has been shown to be necessary for TIME FOR COFFEE 1 (TOC1) degradation and is thereby responsible for the establishment of the circadian period [14].

CRL's also play a role in regulating defense and stress-response related genes. For instance, the F-Box protein SUPPRESSOR OF NIM1-1 1 (SON1) regulates a plants defense response that is independent of systemic acquired resistance [15]. CONSTITUTIVE EXPRESSER OF PR GENES 30 (CPR30) is also an F-BOX protein that is a negative regulator of the defense response [16]. Furthermore, jasmonates are key regulators of plant responses to environmental stresses and biotic challenges [17]. It has been shown that the jasmonate ZIM-domain (JAZ) proteins, which are key-players in jasmonate signaling, are degraded by an SCF-complex containing the F-box protein CORONATINE INSENSITIVE 1 (COI1), SCF^{COI1} [18]. It has also been shown that silencing of CSN genes CSN4 or CSN5 leads to decreased expression of wounding response genes, but to an increased expression of the pathogenesis related genes during pathogen infection in tomato [19]. Another SCF-complex, namely SCF^{TIR1}, has been shown to mediate the auxin response in Arabidopsis. This SCF-complex is able to target AUX/IAA proteins for degradation by the 26S proteasome [20].

To determine which genes are influenced by *DAYSLEEPER*, we used a SAGE-approach coupled to Illumina® GAIIx-Sequencing to identify differentially regulated genes in *DAYSLEEPER* overexpressing plantlets.

Results

Seedlings transformed with tamoxifen (TAM)-inducible *DAYSLEEPER* or *GUSa* overexpression constructs [1], were incubated in liquid ½ MS medium or liquid ½ MS medium containing tamoxifen for induction of *DAYSLEEPER* or *GUSa* expression. We isolated RNA from this material and performed SAGE coupled to GAIIx-sequencing (Illumina®). To study the genes that are directly influenced by *DAYSLEEPER* overexpression, we incubated plantlets for 4 hours in medium with TAM. We also studied the effects of TAM-induced overexpression of *DAYSLEEPER* in plantlets that had been grown in liquid medium for 24 hours (Table 1). We used samples with *GUSa* constructs, non-induced samples (no TAM added) and samples treated with the protein-synthesis inhibitor cycloheximide (CHX) (only 4 hour-experiment) as control samples in our analysis (Table 1). As expected the *DAYSLEEPER* transcript itself was induced many-fold in all samples treated with TAM and not in untreated samples (Table 2). *DAYSLEEPER* expression was induced to a lesser extent in plantlets treated with both TAM and CHX (Table 2). This suggests that *DAYSLEEPER* may positively regulate its own expression.

Sample #	**Construct**	**Treatment**	**Raw sequencing reads**	**Processed tags**	**Index**	**DAYSLEEPER Expression***
1	pINDEX3::GUSa	4 hours	12,120,464	9,610,697	GGATAT	1
2	pINDEX3::At3g42170	4 hours	17,590,747	13,601,226	TGCACT	1.01
3	pINDEX3::GUSa	4 hours +TAM	14,192,381	10,457,094	CTATAG	1.55
4	pINDEX3::At3g42170	4 hours +TAM	19,276,475	14,683,962	GTCACG	80.75
5	pINDEX3::GUSa	4 hours +TAM +CHX	23,308,125	17,810,337	ACTTGA	0.53
6	pINDEX3::At3g42170	4 hours +TAM +CHX	55,163,405	37,927,603	CGATGT	12.34
7	pINDEX3::GUSa	24 hours +TAM	25,607,274	15,174,573	TTAGGC	0.80
8	pINDEX3::At3g42170	24 hours +TAM	14,109,223	10,958,513	TGACCA	102.27

Table 1. Overview and information of the sequenced samples and controls. (*) *DAYSLEEPER* (At3g42170) expression was compared to sample 1. as an internal control.

Transcriptional changes after 4 hours *DAYSLEEPER* overexpression

We found that after 4 hours of TAM-induced *DAYSLEEPER* overexpression 12 genes were upregulated, whereas 55 were downregulated (Figure 1 and Table 2). The upregulated genes were relatively mildly changed; only two genes were more than 5-fold altered in expression (Figure 1A). The gene *Qua Quine Starch* (*QQS*), does not contain any recognizable motifs and is unique to Arabidopisis [21]. The gene is predicted to encode a small protein that is thought to play a role in starch metabolism (Table 2A) [21]. The upregulated gene *AT4* plays a role in the plant's response to low phosphate conditions (Table 2A) [22]. This gene is suggested to be regulated by miRNA399b [22]. The upregulated gene *UPBEAT1* (*UPB1*) (Table 2A) encodes a basic Helix-loop-Helix-type (bHLH) transcription factor that regulates the transition from proliferation to differentiation in roots [23]. Another transcription factor that was upregulated is *WRKY23*, which is involved in the response to nematode infection and is inducible by auxin [24].

We found 55 downregulated genes, which appear to be quite diverse in function (Table 2B). The gene *ALLENE OXIDE CYCLASE 1* (*AOC1*) codes for a protein that is active in the jasmonate (JA)-synthesis pathway and is a target of the AP2-transcription factor *SEPALLATA3* (*SEP3*) [25, 26]. Besides *AOC1*, we found five other genes that are proposed

Rank	**Locus**	**Up regulated**	**Name/Description**	**A**
1	AT3G42170	78.38	DAYSLEEPER	
2	AT4G14480	8.24	Protein kinase	
3	AT3G30720	5.34	QUA-QUINE STARCH	
4	AT1G49530	3.83	GERANYLGERANYL PYROPHOSPHATE SYNTHASE 6	
5	AT5G03545	3.11	AT4	
6	AT2G17330	3.08	CYP51G2 (pseudogene)	
7	AT2G47270	2.85	UPBEAT1	
8	AT1G02430 /AT1G02440	2.65	ADP-RIBOSYLATION FACTOR D1A/B	
9	AT3G55910	2.48	Unknown protein	
10	AT5G10720	2.28	HISTIDINE KINASE 5	
11	AT2G47260	2.11	WRKY23	
12	AT1G53830	2.11	PECTIN METHYLESTERASE 2	
13	AT2G38823	2.09	Unknown protein	

Rank	**Locus**	**Down regulated**	**Name/Description**	**B**
1	AT2G17740	18.18	Cysteine/Histidine-rich C1 domain family protein	
2	AT2G05914	17.64	Potential natural antisense locus (AT2G05915)	
3	AT5G44575	13.36	Unknown protein	
4	AT4G31970	10.10	CYP82C2	
5	AT5G67290	10.04	FAD-dependent oxidoreductase family protein	
6	AT3G46080	9.81	C2H2-type zinc finger family protein	
7	AT3G25760	9.00	ALLENE OXIDE CYCLASE 1 (AOC1)	[44, 45]
8	AT2G27660	8.80	Cysteine/Histidine-rich C1 domain family protein	
9	AT4G23140	6.29	CYSTEINE-RICH RLK (RECEPTOR-LIKE PROTEIN KINASE) 6 (CRK6)	
10	AT2G39210	6.05	Major facilitator superfamily protein	
11	AT3G57240	5.98	AT3G57240	
12	AT3G46090	5.65	ZAT7	
13	AT1G53540	4.77	HSP20-like chaperones superfamily protein	
14	AT5G26690	4.65	Heavy metal transport/detoxification superfamily protein	
15	AT2G36770	4.49	UDP-Glycosyltransferase superfamily protein	
16	AT1G19610	4.14	PDF1.4	
17	AT4G11890	3.95	Protein kinase superfamily protein	
18	AT3G28500	3.89	60S acidic ribosomal protein family	
19	AT4G02330	3.61	ATPMEPCRB	
20	AT5G53110	3.58	RING/U-box superfamily protein	
21	AT5G57560	3.57	TOUCH 4	
22	AT2G40180	3.55	PHOSPHATASE 2C5	

23	AT4G30270	3.45	XYLOGLUCAN ENDOTRANSGLUCOSYLASE/ HYDROLASE 24 (XTH24)	
24	AT1G53490	3.36	RING/U-box superfamily protein	
25	AT4G19690	3.32	IRON-REGULATED TRANSPORTER 1 (IRT1)	
26	AT4G21680	3.20	NITRATE TRANSPORTER 1.8 (NRT1.8)	
27	AT3G28510	3.13	P-loop cont. nucleoside triphosphate hydrolases protein	
28	AT4G21390	3.10	B120	
29	AT5G57010	3.10	calmodulin-binding family protein	
30	AT5G51170	2.94	Unknown protein	
31	AT3G13950	2.90	Unknown protein	
32	AT3G54120	2.77	Reticulon family protein	
33	AT4G04220	2.59	RECEPTOR LIKE PROTEIN 46 (RLP46)	
34	AT3G28580	2.52	P-loop cont. nucleoside triphosphate hydrolases protein	
35	AT3G49620	2.48	DARK INDUCIBLE 11 (DIN11)	
36	AT2G34490	2.45	CYP710A2	
37	AT3G22121	2.43	Potential natural antisense gene (AT3G22120)	
38	AT1G16130	2.40	WALL ASSOCIATED KINASE-LIKE 2 (WAKL2)	
39	AT2G20142	2.38	Toll-Interleukin-Resistance (TIR) domain family protein	
40	AT1G13470	2.35	Unknown protein	
41	AT3G43832	2.35	pseudogene	
42	AT2G01660	2.28	PLASMODESMATA-LOCATED PROTEIN 6 (PDLP6)	
43	AT5G07950	2.28	Unknown protein	
44	AT1G20370	2.26	Pseudouridine synthase family protein	
45	AT1G70020	2.25	Unknown protein	
46	AT5G20230	2.24	BLUE-COPPER-BINDING PROTEIN (BCB)	
47	AT5G57220	2.19	CYP81F2	
48	AT2G39410	2.17	alpha/beta-Hydrolases superfamily protein	
49	AT3G28345	2.16	ATP-BINDING CASSETTE B15 (ABCB15)	
50	AT5G17450	2.15	HEAVY METAL ASS. ISOPRENYLATED PLANT PROTEIN 21 (HIPP21)	
51	AT3G63380	2.14	ATPase E1-E2type family protein	
52	AT4G09490	2.07	Polynucleotidyl transferase/ribonuclease H-like protein	
53	AT1G43160	2.04	RELATED TO AP2 6 (RAP2.6)	
54	AT3G09010	2.02	Protein kinase superfamily protein	
55	AT5G44740	2.00	Y-FAMILY DNA POLYMERASE H (POLH)	[46]

Table 2. Effects of 4 Hours of *DAYSLEEPER*-induction on gene expression. The genes that showed the largest differential expression after 4 hours of *DAYSLEEPER*-induction. (**A**) Lists the 13 upregulated genes and (**B**) the 56 downregulated genes with the fold induction or repression.

to be involved in defense responses (AT3G57240, AT2G46090 (*ZAT7*), AT1G19610 (*PDF1.4*), AT4G04220 (*RLP46*) and AT2G20142) (Table 2B). We identified two potential CRL-related RING/U-box motif genes (AT1G53490 and AT5G53110) and the DNA-polymerase *POLH* which functions together with *PCNA2* in translesion synthesis and is thought also to be regulated by CRL's (Table 2B) [27].

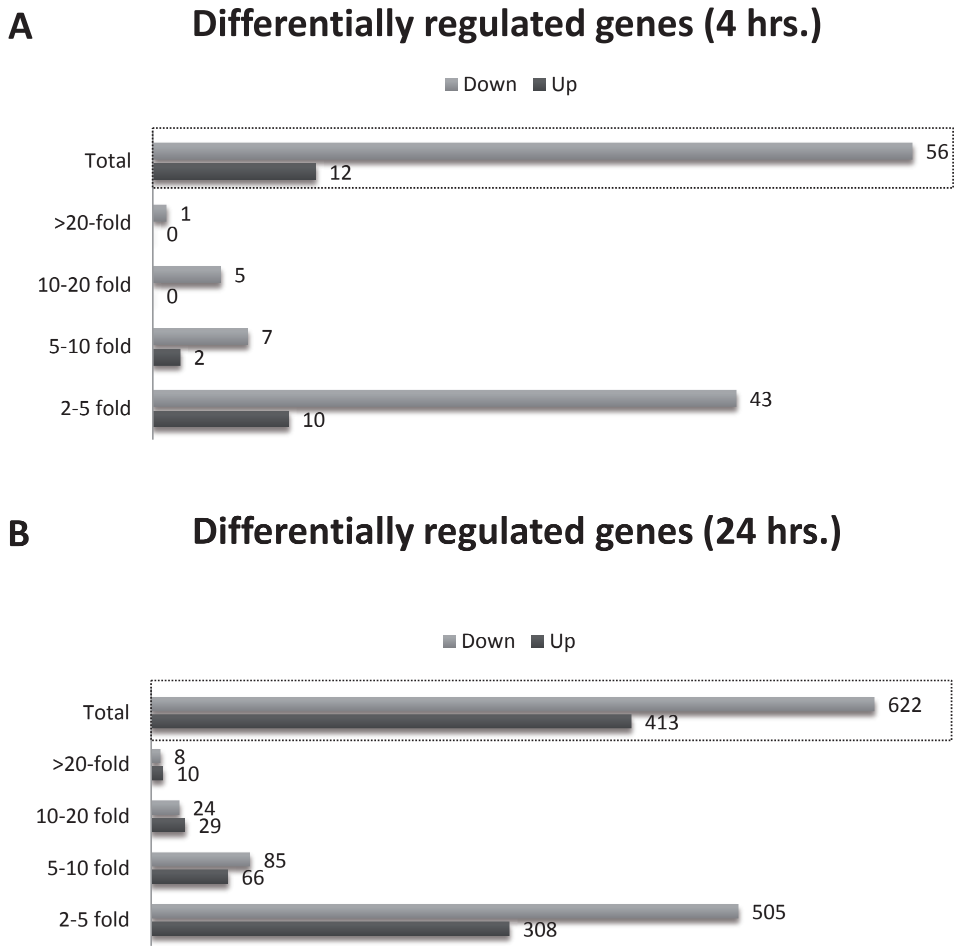

Figure 1. Graphical representation of genes up and down regulated in our datasets. Genes are visualized on the basis of their fold up- or downregulation (>20-fold, 10-20-fold, 5-10-fold and 2-5-fold). The number of genes that are differentially regulated compared to the control sample after 4 hours (**A**) and after 24 hours of *DAYSLEEPER*-induction (**B**).

DAYSLEEPER overexpression induces massive transcriptional changes after 24 hours

After 24 hours of induced *DAYSLEEPER* overexpression, many genes in various functional fields were up- or downregulated (Figure 1B, Figure 2). We listed the genes that were most severely differentially expressed (Table 3). Aided by the Classification SuperViewer [28], we sorted the differentially regulated genes into functional categories (Table 4). We found that a transcript which was found to be most upregulated after 24 hours, is the non-protein coding RNA 34 (npcRNA34, AT2G34655) (Table 3A). No function has been described for this npcRNA, but it was shown to be upregulated in a RNA-dependent RNA polymerase (RDR)2 mutant (*rdr2-6*), suggesting that it is likely dependent on RDR2 for its transcription or, alternatively, a target of RDR-dependent small RNAs such as the trans-acting siRNAs (tasiRNAs) [29]. Among the most upregulated genes were five more

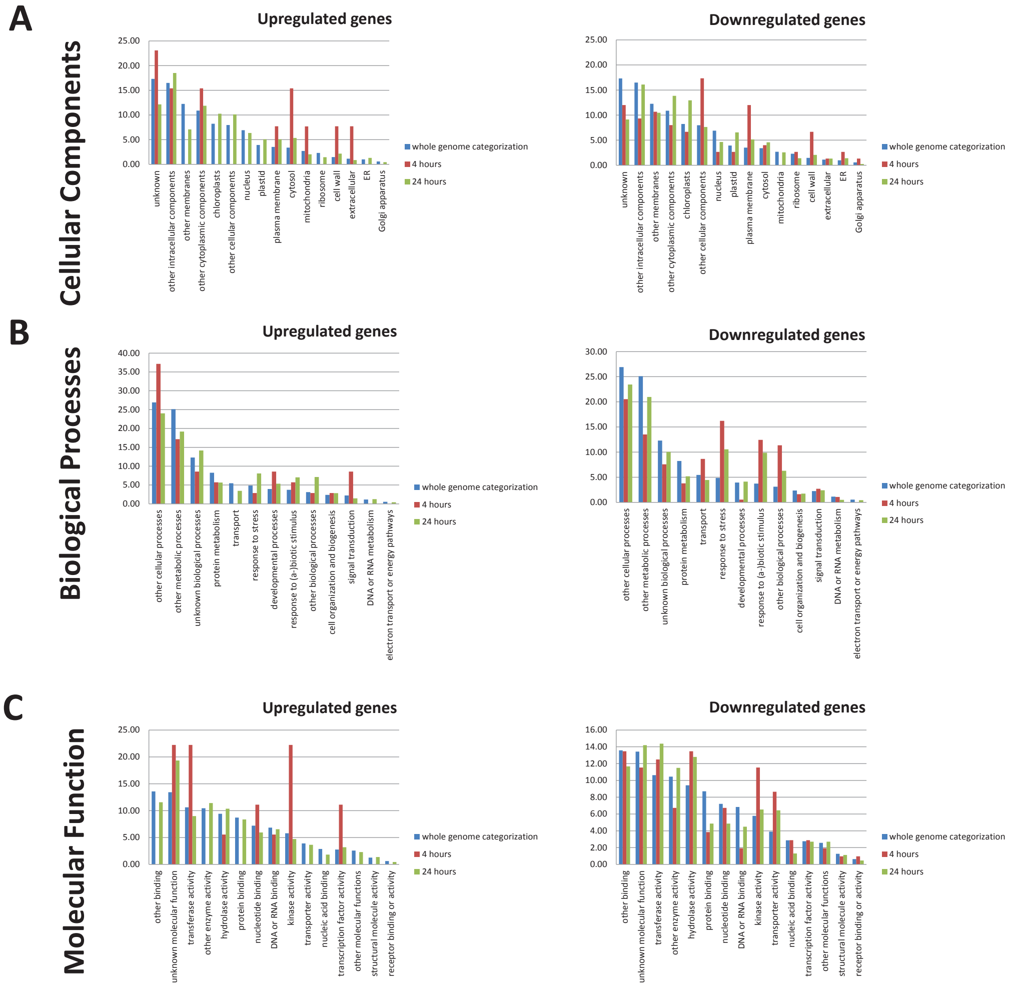

Figure 2. Categorization of differentially regulated genes, using a full-genome categorization as reference. These figures were created using “Gene Ontology Annotations”-tool [65]. Genes were categorized using cellular localization (**A**), involvement in biological processes (**B**) and molecular function (**C**).

genes that were shown to be involved with RNA-related processes (Table 3A). In Table 4 many RNA-related genes can be found that were either up- or downregulated. Genes involved in RNA transcription, processing, splicing, degradation and transport were found, but most noticeably several genes involved in the siRNA/miRNA pathways were identified. *ARGONAUTE1* (*AGO1*), responsible for cleavage of target mRNA's in the siRNA-pathway, was downregulated (Table 3B). The gene *TAS2* producing tasiRNAs was also downregulated. This gene produces at least 4 tasiRNAs, which control the expression of pentatricopeptide-proteins [30]. The upregulated gene *THO5* is a subunit of the THO/TREX mRNA export complex that is also involved in siRNA biosynthesis [31]. *NRPE3B* was also upregulated and is a subunit of the DNA-dependent RNA polymerase V. This complex is involved in siRNA mediated gene silencing by means of DNA methylation (RNA-directed DNA methylation (RdDM)) [32, 33]. Besides these 'RNA-related' genes, especially genes belonging to ubiquitin related pathways and defense responses were found to be overrepresented among both up and downregulated genes (Table 3 and 4).

In total 58 ubiquitin-pathway related genes were found to be misregulated (Table 4). We found 9 Transducin/WD40-repeat containing genes to be upregulated and 4 to be downregulated. WD40-domain containing proteins often form CRL's in conjunction with CUL4 and DDB1. Other CULLINs partner with other proteins to form CRLs. CUL1 for instance, can bind to SKP1 and F (or U)-BOX proteins, whereas CUL3 binds to BTB-domain containing proteins (reviewed in Hua *et al.* (2011) [10]). CUL1 is upregulated in our data and is an integral part of the SCF-complex [34]. We also found two members of the APC/C-complex, which were differentially regulated, namely *APC8* and *APC11* (Table 4). The APC/C complex contains the CULLIN-related protein APC2 and consists of a large number of other subunits. In our data we identified several proteins that are involved with, or targets of, CRL's based on APC2, CULLINS 1, 3 and 4 (Table 4). It is worth mentioning that also genes for subunits of the proteasome were upregulated, and several other proteases downregulated.

We found 32 defense genes to be repressed and 14 genes to be upregulated (Table 4). We identified genes in various fields of plant defense, but found that in general, genes regulated by JA were repressed, whereas salicylic acid response (SA)-related genes were up regulated. *Pathogenesis Related 1 (PR1)*, which is normally transcribed in response to SA, is a noticeable exception. This gene is more than 70-fold repressed by *DAYSLEEPER* overexpression (Table 3b) [35]. We also identified several genes in control of the circadian clock and flowering timing, such as *LATE ELONGATED HYPOCOTYL* (*LHY*), *REVEILLE2* (*RVE2*) and *PSEUDO RESPONSE REGULATOR 5* (*PRR5*) of which expression was altered due to *DAYSLEEPER* overexpression. In preliminary experiments, we have also found that in plants that constitutively overexpress *DAYSLEEPER* at very high levels (*35S::DAYSLEEPER*), circadian-clock-related genes (e.g. CCA1/PRR5/PRR3) are also misregulated (data not shown).

Four chromatin genes were downregulated in our experiments, whereas 16 such genes were found to be upregulated (Table 4). Several DNA-damage repair genes were misregulated, which seems to correspond with the fact that we identified various cell-cycle-related genes to be either up- or downregulated (Table 4). An interesting observation is that among the upregulated genes we identified 3 *ALFIN-like* genes (*AL3*, *AL5* and *ING1*), which are known to code for proteins that bind to di- or tri methylated lysine 4 of histone H3 (H3K4) (Table 4A) [36] and are probably involved in chromatin remodeling.

***DAYSLEEPER* has a similar expression pattern as Polycomb-like Repressive Complex (PRC2)-related genes**

We used the Expression Angler tool at the Bio-Array Resource for plant biology [37] to identify genes that are co-expressed, or antagonistically expressed to *DAYSLEEPER* in a large set of micro-array experiments. We found that *DAYSLEEPER* is highly co-expressed with the genes *FIE* and *MSI1* (Table 5). These genes are subunits of the chromatin remodeling complex PRC2 [38, 39]. MSI-proteins are WD40-domain proteins and MSI1/MSI4 are thought to be part of a CUL4-based E3 Ubiquitin-ligase complex with CULLIN4 and DDB1 [40]. *E1 C-TERMINAL RELATED 1 (ECR1)* is also co-expressed with *DAYSLEEPER*. This gene is involved in activation of RUB, which is a ubiquitin-related protein that regulates the activity of CRL’s [41].

Rank	Locus	Up-regulated	Name/Description	A
1	AT3G42170	103.41	DAYSLEEPER	
2	AT2G34655	70.02	npcRNA	
3	AT1G65970	53.57	TPX2	
4	AT1G14960	44.81	Polyketide cyclase/dehydrase and lipid transport superfamily protein	
5	AT3G06145	41.10	Unknown protein	
6	AT1G21520	31.58	Unknown protein	
7	AT4G29340	23.16	PROFILIN 4	
8	AT5G58210	22.03	hydroxyproline-rich glycoprotein family protein	
9	AT2G37770	21.16	ChlAKR	
10	AT2G34020	20.38	Calcium-binding EF-hand family protein	
11	AT3G03650	20.02	EDA5	
12	AT3G26782	19.45	Tetratricopeptide repeat (TPR)-like superfamily protein	
13	AT5G51710	19.27	KEA5	
14	AT2G18600	18.99	Ubiquitin-conjugating enzyme family protein	
15	AT1G30990	18.29	Polyketide cyclase/dehydrase and lipid transport superfamily protein	
16	AT1G51580	17.21	RNA-binding KH domain-containing protein	
17	AT1G28560	17.06	SRD2	
18	AT1G71370	16.94	DEA(D/H)-box RNA helicase family protein	
19	AT3G24010	16.05	ING1	
20	AT2G32020	15.55	Acyl-CoA N-acyltransferases (NAT) superfamily protein	
21	AT3G13640	15.37	RNASE L INHIBITOR PROTEIN 1	
22	AT2G18193	15.11	P-loop containing nucleoside triphosphate hydrolases protein	
23	AT1G53850	14.78	20S PROTEASOME ALPHA SUBUNIT E1 (PAE1)	
24	AT3G57880	14.74	Ca$_2$-dep. lipid-binding plant phosphoribosyltransferase family protein	
25	AT3G30720	13.75	QUA-QUINE STARCH	
26	AT5G03470	13.44	ATB' ALPHA (PP2A subunit)	
27	AT2G48150	13.39	GPX4	
28	AT1G45165	12.07	Unknown protein	
29	AT1G13970	12.01	Unknown protein	
30	AT4G25020	11.90	D111/G-patch domain-containing protein	
31	AT3G49160	11.85	Unknown protein, downregulated by photoxidative stress	
32	AT5G43560	11.76	TRAF-like superfamily protein	
33	AT5G24200	11.71	alpha/beta-Hydrolases superfamily protein	
34	AT2G30060	11.51	Pleckstrin homology (PH) domain superfamily protein	
35	AT2G38823	11.51	Unknown protein	
36	AT4G37030	11.22	Unknown protein	
37	AT5G41280	10.70	Receptor-like protein kinase-related family protein	
38	AT2G39040	10.26	Peroxidase superfamily protein	
39	AT2G35680	10.23	Phosphotyrosine protein phosphatases superfamily protein	
40	AT1G65490	10.10	Unknown protein	
41	AT4G01960	9.89	Unknown protein	
42	AT4G37290	9.54	Unknown protein	
43	AT5G20510	9.29	ALFIN-LIKE 5	
44	AT1G19250	9.23	FMO1	
45	AT3G05090	9.22	LATERAL ROOT STIMULATOR 1	
46	AT2G41440	9.07	Unknown protein	
47	AT1G26360	9.04	MES13	
48	AT3G12680	8.95	ENHANCER OF AG-4 1 (HUA1)	
49	AT3G60550	8.92	CYCLIN P3 2	
50	AT1G51780	8.71	ILL5	

Rank	Locus	Down - regulated	Name/Description	B
1	AT2G14610	73.73	PATHOGENESIS-RELATED GENE 1 (PR1)	
2	AT1G14880	27.59	PLANT CADMIUM RESISTANCE 1 (PCR1)	
3	AT1G76080	26.38	CHLOROPLASTIC DROUGHT-INDUCED STRESS PROTEIN OF 32 KD	
4	AT3G44860	26.29	FARNESOIC ACID CARBOXYL-O-METHYLTRANSFERASE (FAMT)	
5	AT4G36670	21.28	POLYOL/MONOSACCHARIDE TRANSPORTER 6 (PMT6)	
6	AT3G08870	21.24	Concanavalin A-like lectin protein kinase family protein	
7	AT4G25810	20.33	XYLOGLUCAN ENDOTRANSGLYCOSYLASE 6 (XTR6)	
8	AT2G40340	19.49	DREB2C	
9	AT3G22740	18.09	HOMOCYSTEINE S-METHYLTRANSFERASE 3 (HMT3)	
10	AT2G41810	17.88	Unknown protein	
11	AT3G49620	17.71	DARK INDUCIBLE 11 (DIN11)	
12	AT5G62360	17.15	Plant invertase/pectin methylesterase inhibitor superfamily protein	
13	AT4G17470	16.93	alpha/beta-Hydrolases superfamily protein	
14	AT3G05660	16.75	RECEPTOR LIKE PROTEIN 33 (RLP33)	
15	AT2G15080	15.67	RECEPTOR LIKE PROTEIN 19 (RLP19)	
16	AT1G15040	15.43	Class I glutamine amidotransferase-like superfamily protein	
17	AT1G15790	15.43	Unknown protein	
18	AT4G31970	14.95	CYP82C2	
19	AT3G18080	14.05	B-S GLUCOSIDASE 44 (BGLU44)	
20	AT1G09240	13.36	NICOTIANAMINE SYNTHASE 3 (NAS3)	
21	AT5G62350	13.31	Plant invertase/pectin methylesterase inhibitor superfamily protein	
22	AT5G57220	12.65	CYP81F2	
23	AT2G36750	12.53	UDP-GLUCOSYL TRANSFERASE 73C1 (UGT73C1)	
24	AT4G15233	12.09	ATP-BINDING CASSETTE G42 (ABCG42)	
25	AT2G33830	11.69	Dormancy/auxin associated family protein	
26	AT5G48000	11.43	CYP708A2	
27	AT2G23010	11.19	SERINE CARBOXYPEPTIDASE-LIKE 9 (SCPL9)	
28	AT5G60050	11.12	BTB/POZ domain-containing protein	
29	AT2G28120	11.10	Major facilitator superfamily protein	
30	AT3G08860	10.18	PYRIMIDINE 4 (PYD4)	
31	AT1G73260	10.11	KUNITZ TRYPSIN INHIBITOR 1 (KTI1)	
32	AT4G00430	9.46	PLASMA MEMBRANE INTRINSIC PROTEIN 1;4 (PIP1;4)	
33	AT2G42530	9.41	COLD REGULATED 15B (COR15B)	
34	AT5G26600	8.99	Pyridoxal phosphate (PLP)-dependent transferases superfamily protein	
35	AT1G23390	8.99	Kelch repeat-containing F-box family protein	
36	AT3G25010	8.90	RECEPTOR LIKE PROTEIN 41 (RLP41)	
37	AT1G49720	8.90	ABSCISIC ACID RESPONSIVE ELEMENT-BINDING FACTOR 1 (ABF1)	
38	AT5G24770	8.43	VEGETATIVE STORAGE PROTEIN 2 (VSP2)	
39	AT4G09900	8.32	METHYL ESTERASE 12 (MES12)	
40	AT5G24470	8.22	PSEUDO-RESPONSE REGULATOR 5 (PRR5)	
41	AT3G07280	8.19	Unknown protein (pseudogene)	
42	AT1G75140	8.16	Unknown Protein	
43	AT1G72690	8.08	Unknown protein	
44	AT5G05090	7.94	Homeodomain-like superfamily protein	
45	AT3G45970	7.90	EXPANSIN-LIKE A1 (EXLA1)	
46	AT3G21520	7.63	Unknown membrane protein	
47	AT2G29460	7.60	GLUTATHIONE S-TRANSFERASE TAU 4 (GSTU4)	
48	AT5G24160	7.41	SQUALENE MONOXYGENASE 6 (SQE6)	
49	AT1G52190	7.05	Major facilitator superfamily protein	
50	AT1G69750	7.05	CYTOCHROME C OXIDASE 19-2 (COX19-2)	

Table 3. Effects of 24 Hours of *DAYSLEEPER*-induction on gene expression. The 50 genes that showed the largest differential expression after 24 hours of *DAYSLEEPER*-induction. (**A**) Lists the 50 upregulated genes and (**B**) the 50 downregulated genes with the fold induction or repression.

Ubiquitin-ligase complex related		**CRL**	**A**
AT4G15420	Ubiquitin fusion degradation UFD1 family protein		-
AT1G50490	Ubiquitin-conjugating enzyme (E2-C gene family)		UBC20
AT2G18600	Ubiquitin-conjugating enzyme family protein		-
AT4G10590	Ubiquitin-specific protease		UBC10
AT4G24690	UBQ-associated/TS-N domain-containing protein		NBR1
AT5G55160	*Small Ubiquitin-like Modifier*		*SUMO2*
AT1G20823	RING/U-box protein		-
AT3G10815	RING/U-box protein		-
AT3G29270	RING/U-box protein		-
AT2G45920	U-box domain-protein		-
AT4G36860	LIM-domain protein, ubiquitin interacting	??	-
AT1G71840	Transducin/WD40-repeat protein	??	-
AT5G54730	WD40-domain	??	G14F
AT4G30510	WD40-domain protein	??	G14D
AT5G05970	WD40-domain protein	??	NEDD1
AT5G21010	BTB-POZ and MATH domain	BCR	BPM5
AT2G47860	Phototropic-responsive NPH3 family protein (BTB/POZ-domain)	BCR	SETH6/NRL8
AT5G52250	Transducin protein	DCX	RUP1
AT3G05090	WD40-domain, CUL4 RING ubiquitin ligase complex	DCX	LRS1
AT2G46290	Transducin/WD40-repeat protein	DCX*	-
AT3G18140	Transducin/WD40-repeat protein	DCX*	-
AT5G56190	Transducin/WD40-repeat protein	DCX*	-
AT4G02570	Ubiquitin ligase complex	SCF	CULLIN1
AT1G63090	F-Box domain protein	SCF	PP2-A11
AT4G21510	F-Box protein	SCF	-
AT3G23050	SCF/Proteasome target	SCF	IAA7/AXR2
AT4G14365	Ubiquitin-ligase	SCF	XBAT34
Defense-related			
AT1G14960	response to biotic stimulus, defense response		
AT1G19250	Promotes resistance and cell-death at sites of infection		FMO1
AT1G20823	RING/U-box protein, response to chitin		-
AT1G30990	response to biotic stimulus, defense response		-
AT1G51780	IAA-Leu conjugates		ILL5
AT2G38870	PR-peptide, putative		-
AT2G44110	Mildew-resistance locus		MLO15
AT3G24020	Disease resistance-responsive (dirigent-like protein)		-
AT3G44260	Plays a role in plant defense response		CAF1a
AT3G52430	Salicylic acid signaling and resistance		PAD4
AT5G14180	Defense response to insects		MPL1
AT5G26920	Involved in SA-signaling		CBP60G
AT5G64750	AP2/ERF transcription factor		ABR1
AT5G64850	RPM1-interacting protein 4, defense response		-
Protein degradation			
AT1G66670	Caseinolytic protease		CLPP3
AT5G66140	20S Proteasome subunit		PAD2
AT1G53850	20S Proteasome		PAE1
AT2G05840	20S Proteasome		PAA2
AT4G14800	20S Proteasome		PBD2
AT5G66140	20S Proteasome		PAD2
At2g25240	Serine protease inhibitor (SERPIN) family protein		-
DNA and Chromatin-related			
AT1G01690	Double stranded DNA breaks in meiotic recombination		-
AT1G09200	Nucleosome assembly		-
AT1G18450	Chromatin organization (SWI/SNF-like)		ARP4
AT2G15400	DNA-dependent RNA polymerase V		NRPE3B
AT2G22475	Histone H3-K9 methylation		GEM
AT2G38810	Histone H2A protein		HTA8
AT3G01320	Transcriptional repression, telomere maintenance		SNL1
AT3G24010	Binds to H3K4 di or trimethylated DNA.		ING1

AT3G42790	Binds to H3K4 di or trimethylated DNA	AL3
AT3G57060	Chromosome condensation	-
AT4G13870	Exonuclease, interacts with Ku	WRNEXO
AT4G25540	Mismatch repair	MSH3
AT4G33925	Double-strand break repair via homologous recombination	SSN2
AT5G20510	Binds to H3K4 di or trimethylated DNA	AL5
AT5G22110	required for DNA replication	DPB2
AT5G62410	Chromosome organization	SMC2-1
Cell Cycle-related		
AT2G18040	Regulation of cell-cycle	PIN1AT
AT3G48150	Cell-cycle-related (APC/C complex)	APC8
AT3G60550	Regulation of cell-cycle, Cyclin	CYCP3;2
AT2G27960	Regulation of cell-cycle	CKS1
AT3G10525	Cell-cycle inhibitor-family protein	LGO
RNA-related		
AT1G28560	Mutant accumulates snRNA’s and other small RNA’s	SRD2
AT1G17370	mRNA 3’UTR binding	UBP1B
AT1G29550	Eukaryotic initiation factor 4E protein, RNA binding	-
AT3G48150	APC/C subunit involved in miRNA	APC8
AT1G51580	RNA-binding KH domain-containing protein	-
AT2G34655	Non protein coding RNA, expressed in leaves	npcRNA34
AT5G03495	RNA-binding (RRM/RBD/RNP motifs) family protein	-
AT3G06520	RNA-binding, Agenet domain-containing protein	-
AT3G12680	Processing of AGAMOUS pre-mRNA	HUA1
AT4G00420	Double-stranded RNA-binding domain	-
AT5G49030	tRNA-binding ovule development	OVA2
AT3G49601	mRNA splicing factor	-
AT1G53850	mRNA/protein degradation, 20S proteasome	PAE1
AT1G78882	uORF, transcriptional regulation	CPuORF56
AT2G15400	Subunit of Nuclear DNA-dependent RNA polymerase V	NRPE3B
AT2G29140	Regulates mRNA stability by binding 3’UTR	PUM3
AT2G42890	RNA-binding regulator of growth and meiosis	ML2
AT3G13640	RNASE inhibitor	ABCE1
AT3G44260	mRNA deadenylation-activity	CAF1a
AT5G16840	RNA-binding	BPA1
AT5G42920	Putative role in siRNA-process, transport of mRNA precursors	THO5
AT5G48870	mRNA splicing, export, and degradation	SAD1
Flowering and Circadian Rhythm		
AT1G01060	Clock gene, flowering time	LHY
AT1G18450	Mutant flowers early, chromatin remodeling complex	ARP4
AT3G21150	Regulates flowering time	BBX32/EIP6
AT5G02840	LHY/CCA1-homolog	LCL1
AT5G37260	Circadian regulation	RVE2
AT5G52250	Flowering and developmental regulator	RUP1
AT3G23050	SCF/Proteasome target, *axr2-1* mutant flowers late	IAA7/AXR2
AT2G47860	Phototropic-responsive NPH3 family protein	SETH6/NRL8
AT2G43010	Negatively regulates phyB mediated red light responses	PIF4
AT3G21150	Expression affected by monochromatic red-light	BBX32
AT5G64552	uORF, transcriptional regulation	CPuORF22
Flower and fruit development		
AT2G45190	Flower/fruit development	AFO
AT3G10525	Sepal development	LGO
AT3G12680	Member of the floral homeotic AGAMOUS pathway	HUA1
AT5G08335	Flower development	ATSTE14B
AT5G01370	Cell separation during fruit dehiscence	ACI1
Root development		
AT2G39830	Root system development	DAR2
AT2G22475	Patterning and differentiation of root epidermal cells	GEM
AT2G03720	root hair development	MRH6
AT4G16515	maintenance of the root stem cell niche	RGF6

Ubiquitin-ligase complex related		**CRL**	**B**
AT1G02090	Subunit of the COP9-signalosome.		CSN7
AT1G14650	SWAP/Surp domain-containing protein/ ubiquitin family protein		-
AT5G21970	Ubiquitin carboxyl-terminal hydrolase family protein		-
AT4G32270	Ubiquitin-like superfamily protein		-
AT5G25270	Ubiquitin-like superfamily protein		-
AT5G42220	Ubiquitin-like superfamily protein		-
AT1G17970	Ubiquitin-protein ligase activity, Znc-finger; RING-type		-
AT1G53490	Ubiquitin-protein ligase activity, Znc-finger; RING-type		-
AT1G55530	Ubiquitin-protein ligase activity, Znc-finger; RING-type		-
AT2G23780	Ubiquitin-protein ligase activity, Znc-finger; RING-type		-
AT5G10380	Ubiquitin-protein ligase activity, Znc-finger; RING-type		ATRING1
AT5G49665	Ubiquitin-protein ligase activity, Znc-finger; RING-type		-
AT5G60710	Ubiquitin-protein ligase activity, Znc-finger; RING-type		MUP24.2
AT5G54200	Transducin/WD40 repeat-like superfamily protein	??	-
AT1G21670	WD40-like Beta Propeller containing protein	??	-
AT1G12910	WD40-protein. Circadian rhythm and flowering associated	??	LWD1
AT3G05870	A ubiquitin ligase complex that regulates progression through the cell cycle.	APC/C	APC11
AT3G16830	WD40-protein “TOPLESS-RELATED2”.	BCR	TPR2
AT3G44820	Phototropic-responsive NPH3 family protein (BTB/POZ-domain)	BCR	NRL16
AT5G63160	BTB AND TAZ DOMAIN PROTEIN 1	BCR	BT1
AT3G54850	Ubiquitin-protein ligase activity; U-box domain and Armadillo repeat	DCX	PUB14
AT1G76260	DDB1-binding WD40 protein	DCX	DWA2
AT5G24470	Photomorphogenesis, (far)red light signaling	SCF	PRR5
AT1G48410	siRNA related, involved in far/red light reception	SCF	AGO1
AT2G26860	FBD, F-box and Leucine Rich Repeat domains containing protein	SCF	-
AT3G23260	F-box and associated interaction domains-containing protein	SCF	-
AT3G61590	F-box domain, cyclin-like domain protein, involved in organ development	SCF	HWS
AT3G59210	F-box/RNI-like superfamily protein	SCF	-
AT1G23390	Kelch repeat-containing F-box family protein	SCF	-
AT1G72450	JASMONATE-ZIM-DOMAIN PROTEIN 6	SCF	JAZ6
AT1G77000	Ubiquitin-protein ligase, homolog of human SKP2	SCF	SKP2B
Plant defense response/disease-related			
At2g14610	Pathogenesis Related gene-1		PR1
At1g02920	Defense response to bacteria/fungus		GSTF7
At1g16540	Defense response to bacteria		ABA3
At1g19610	Pathogen Related protein. Plant defensin		PDF1.4
At1g52400	Defense response to fungi		BGLU18
At1g70890	Defense response		MLP43
At1g72450	JASMONATE-ZIM-DOMAIN PROTEIN 6		JAZ6
At1g75800	Pathogenesis-related thaumatin superfamily protein		-
At1g80840	Defense response, regulation of defense response		WRKY40
At2g04450	Positive regulation of salicylic acid mediated signaling pathway		NUDT6
At2g15080	Defense response		RLP19
At2g22330	Defense response. Cytochrome.		CYP79B3
At2g26010	Pathogen Related protein. Plant defensin		PDF1.3
At2g30860	Defense response to bacteria		GSTF9
At2g42530	Defense response to fungus		COR15B
At2g46370	Jasmonate-pathway. Pathogen response.		JAR1
At3g05660	Defense response		RLP33
At3g25010	Defense response		RLP41
At3g45140	Defense reponse and response to jasmonic acid.		LOX2
At3g50970	Defense response to fungus		LTI30
At3g54920	Defense response. Powdery mildew resistant 6		PMR6
At4g23670	defense response to bacterium		-
At4g30650	defense response to fungus		-
At4g34135	Response to other organism		UGT73B2
At5g10380	Ubiquitin E3 ligase, upregulated in response to fungal pathogen		RING1
At5g23400	Leucine-rich repeat (LRR) family protein, defense response		-
At5g24770	Defense response to insects		VSP2

At5g39580	Peroxidase, defense response to fungus	-
At5g40170	Defense response	RLP54
At5g46520	Innate immunity. Disease resistance protein (TIR-NBS-LRR class) family	-
At5g57220	Cytochrome. Defense response	CYP81F2
At5g61890	AP2/ERF transcription factor, defense response to fungus	-
Protein degradation		
At3g60820	20S Proteasome subunit	PBF1
At3g61540	alpha/beta-Hydrolases superfamily protein	-
At1g09730	Cysteine proteinases superfamily protein	-
At2g39710	Cysteine-rich peptide (CRP) family protein. SUMO-related.	-
At4g16563	Eukaryotic aspartyl protease family protein	-
At2g26140	Mitochondrial protease	ftsh4
At4g38220	Peptidase M20/M25/M40 family protein	AQI
At5g22860	Serine carboxypeptidase S28 family protein	-
At5g22860	Serine carboxypeptidase S28 family protein	-
At2g23010	Serine carboxypeptidase-like 9	SCPL9
At4g00690	UB-like protease 1B, SUMO-related	ULP1B
DNA and Chromatin-related		
At1g20693	Chromatin assembly or disassembly related protein	HMGB2
At2g28290	SWI/SNF-like protein.	SYD
At2g47620	SWI/SNF-like protein. Homologous to yeast SWI3	SWI3A
AT5G14170	Chromodomain chromatin remodeling complex	CHC1
Cell Cycle-related		
At3g05870	A ubiquitin ligase complex that regulates progression through the cell cycle.	APC11
At1g20930	Cyclin dependent kinase, expressed during M phase of mitotic cell cycle	CDKB2;2
At1g27630	Cyclin-protein, cell-cycle regulator	CYCT1;3
RNA-related		
At1g09140	Involved in the regulation of splicing	SR30
At1g09620	aminoacyl-tRNA ligase	-
At1g10320	Zinc finger C-x8-C-x5-C-x3-H type family protein, RNA binding	-
At1g14650	SWAP/Surp domain-containing protein/ ubiquitin family protein	-
At3g05870	APC/C subunit involved in miRNA	APC11
At1g48410	ARGONAUT, RNA Slicer that recruits siRNA’s and microRNA’s	AGO1
At1g72320	Regulates mRNA stability and translation through sequence-specific binding to the 3' UTR	PUM23
At1g79880	RNA recognition motif (RRM)-containing protein	-
At2g21660	mRNA export and DNA/RNA unwinding-activity	GRP7
At2g39681	RDR6-dependent trans-acting siRNA-generating locus. Regulated by miR173.	TAS2
At2g47250	RNA helicase family protein	-
At3g01540	RNA helicase	DRH1
At3g09650	Chloroplast RNA-binding protein, processing mRNA	HCF152
At3g11710	lysine-tRNA ligase	ATKRS-1
At3g53920	Chloroplast RNA polymerase subunit	SIGC
At3g61860	Splicing factor	RS31
At4g31010	RNA-binding CRS1 / YhbY (CRM) domain-containing protein	-
At5g13730	Plastid RNA polymerase subunit	-
Flowering and Circadian Rythm		
At1g12910	Clock protein regulating circadian period length and photoperiodic flowering	LWD1
At5g24470	Central clock gene	PRR5
At2g21660	Regulates the circadian oscillations of its own transcript; neg. feedback loop.	GRP7
At2g28290	SWI/SNF-like protein. Regulates flowering	SYD
Light response		
At1g09530	negative regulator of phyB signaling	PIF3
At3g49620	Induced by darkness	DIN11
At3g44820	NPH3 family protein	NRL16
At5g24470	Photomorphogenesis, (far)red light signaling	PRR5
At1g48410	siRNA related, involved in far/red light reception	AGO1
At1g02090	COP9-signalosome subunit, photomorphogenesis	COP7
At2g41430	Response to high light intensity	ERD15
At4g38170	FAR1/FHY-family protein, far-red light response	FRS9

At5g20230	Response to absence of light	BCB
At2g42540	Red or far red/light signaling	COR15A
Photosynthesis		
At2g30790	Photosystem II related	PSBP-2
At2g39470	Photosystem II related	PnsL1
At3g53920	Chloroplast RNA-polymerase, photosynthesis related	SIGC
Flower and fruit development		
At1g14720	Mutant has flower phenotype	XTH28
At5g05690	Positive regulation of flower development, Cytochrome protein	CPD
At1g26260	Positive regulation of flower development	CIB5
At2g28290	SWI/SNF-like protein. Regulator of floral homeotic gene expression	SYD
Root development		
At5g48000	Mutant has longer root than WT	CYP708A2
At5g14170	Altered root morphology and reduced root growth in mutant	CHC1
At2g39800	Biosynthetic gene involved in root development	P5CS1
At1g48410	Regulates genes involved in the auxin/light-signaling crosstalk during adventitious root dev.	AGO1
At1g23080	Auxin efflux carrier, root development	PIN7

Table 4. Functional categorization that was made with the help of the Classification Superviewer [28], of both up- (**A**) and downregulated genes (**B**) after 24 hours of *DAYSLEEPER* induction. Genes that are involved in multiple processes are mentioned several times in these lists. Genes under the header "Ubiquitin-ligase complex related" possess an extra column, indicating which type of CRL (BCR, DCX, SCF or APC/C) they are involved with. Genes marked with "??" are most likely part of a CRL, but have not been described yet. The *SUMO2* gene (Small Ubiquitin-like Modifier 2) has been depicted in italic, since it is strictly not ubiquitin-ligase complex related. The *NRL8* gene is underlined to indicate its product interacts with *DAYSLEEPER* (Chapter 5, this thesis).

Discussion

The effects of *DAYSLEEPER* overexpression are severe and widespread, which is in concordance with the severe *daysleeper* phenotype and the progressive pleiotropic phenotype of *35S::DAYSLEEPER* plants and their offspring [1]. Our data showed 67 genes to be misregulated after only 4 hours of TAM induction of *DAYSLEEPER* overexpression, which might suggest that an induction period of less than 4 hours (i.e. 2 hours) would have sufficed to find primary effects of *DAYSLEEPER* overexpression.

We identified many genes, differentially regulated in our dataset, that are involved in protein ubiquitylation in relation to the CSN and CRL complexes (Table 5). The genes that we classified as "ubiquitin-ligase complex related" are probably an underestimation of the total number of genes directly involved with these complexes, since we found several genes that code for proteins that contain domains with putative ubiquitin E3-ligase activity (e.g. LRR-domains and Tetratricopeptide-repeats [10], Table S5-S6). We did not include these genes in Table 5, because these protein-domains could be

Positive correlation with *DAYSLEEPER* expression pattern			
Rank	**R-Value**	**Gene**	**Description**
1	1	At3g42170	DAYSLEEPER
2	0.854	At3g20740	FIE
3	0.826	At5g08620	STRESS RESPONSE SUPPRESSOR 2 (STRS2)
4	0.823	At3g19790	Unknown protein
5	0.816	At5g47500	PECTIN METHYLESTERASE 5
6	0.815	At5g19180	E1 C-TERMINAL RELATED 1 (ECR1)
7	0.813	At2g03150	EMBRYO DEFECTIVE 1579
8	0.813	At5g58230	MULTICOPY SUPRESSOR OF IRA1 (MSI1)
9	0.811	At3g59650	mitochondrial ribosomal protein L51/S25/CI-B8 family protein
10	0.808	At5g57410	Afadin/alpha-actinin-binding protein
11	0.808	At3g51800	ATG2
12	0.806	At5g56590	O-Glycosyl hydrolases family 17 protein
13	0.805	At1g55170	Unknown protein
14	0.803	At1g55350	DEFECTIVE KERNEL 1 (DEK1)

Table 5. Genes with a strongly correlated expression pattern to the expression pattern of *DAYSLEEPER* over a large set of microarray data (NASCarrays 392), which was determined using the on-line Expression Angler tool [37].

involved in various processes and are not necessarily CRL-related.

SAGE-seq data comparison with microarray data from Bundock and Hooykaas (2005)

Bundock and Hooykaas (2005) [1] performed a micro array analysis comparing a control line (*pINDEX3::GUSa*), to plants with TAM induced *DAYSLEEPER* overexpression (*pINDEX3::AT3G42170*). Data from this experiment were compared to our data. We found an overlap in both up- and downregulated genes (Table 6). Genes related to circadian rhythm (e.g. *PRR5* and *LHY*), plant defense (e.g. a *PR*-peptide gene and *PMR5*) and ubiquitylation-related genes (e.g. two Kelch-repeat genes and *BT1*) were present in both datasets. Overall, a relatively small number of genes from our data-set are found in the data from this microarray analysis (Table 6).

Data visualization

In order to visualize some of our data, we constructed a graphical overview that displays genes we found to be misregulated and their relation to each other (Figure 3). In Figure 3A, genes involved in plant defense-responses are grouped and their functional interactions visualized. We found that JA-regulated genes are generally downregulated, whereas SA response-related genes are upregulated, with the important exception of *PR1*. *ABR1* positively regulates SA abundance [42]. *PR1* is induced by SA [43], and so is *NUDT6*, which also positively influences *PR1* expression [44], but *PAD4* is negatively

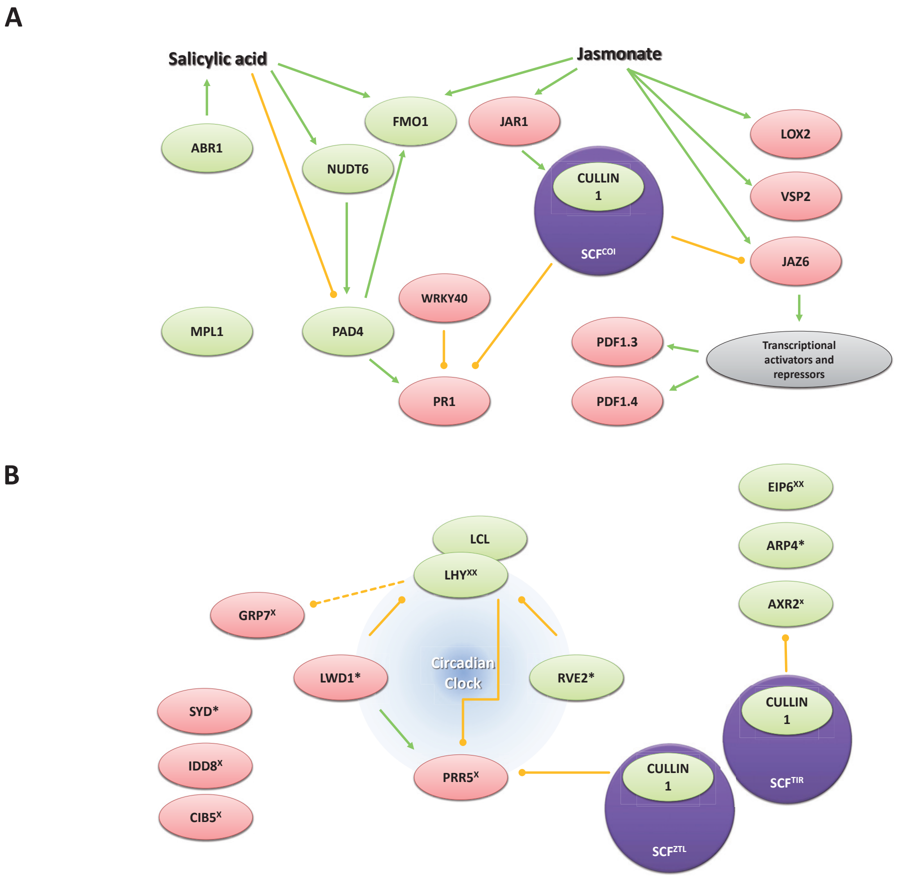

Figure 3. A. Genes involved in plant-defense related pathways, differentially expressed after 24 hours of DAYSLEEPER induction. SA-response-related genes were generally up-regulated, whereas JA responsive genes were downregulated. SA-responsive gene *PR1* was downregulated. *ABR1* positively regulates SA abundance [42]. *PR1* is induced by SA [43], and so is *NUDT6*, which also positively influences *PR1* expression [44], but *PAD4* is negatively regulated by high levels of SA [45]. *FMO1* is upregulated by both SA as JA, and is downregulated in a *pad4* background [46]. *PAD4* is involved with *MPL1* in the antibiosis response [47]. *JAR1* is essential for pathogen defense [48] and has been found to promote JAZ-protein degradation by stimulating SCF^{COI} activity [18]. *LOX2* and *VSP2* are both known to be jasmonate-inducible and to be upregulated by wounding [49, 50]. Wounding triggers JA and ethylene signaling, which culminates in the expression of defensins such as the *PDF1.3* and *PDF1.4* genes [51–53]. Genes depicted in red ovals were downregulated in our data, whereas genes in green ovals were found to be upregulated (24hrs of induction). Green arrows indicate a positive influence, whereas yellow arrows indicate negative regulation. The SCF-complexes are depicted in purple. **B.** Circadian rhythm and flowering timing related genes differentially regulated after 24 hours of *DAYSLEEPER* induction. Genes marked with an “^{X}” have described loss-of-function mutants that display late flowering. Genes marked with an “^{XX}” have been described to give rise to late flowering when overexpressed. Genes marked with an “*” have described loss-of-function mutants that display early flowering [56, 57, 66–75]. *LWD1* and *RVE2* negatively influence *LHY* expression [73, 76], whereas *LWD1* positively influences *PRR5* expression [76]. *LHY* is a negative regulator of *PRR5* [77]. *PRR5* and *AXR2* are both degraded by different SCF-type CRL’s, SCF^{ZTL} and SCF^{TIR}, respectively [13, 55].

regulated by high levels of SA [45]. *FMO1* is upregulated by both SA as JA, and is downregulated in a *pad4* background [46]. *PAD4* is involved with *MPL1* in the antibiosis response [47]. *JAR1* is essential for pathogen defense [48] and has been found to promote JAZ-protein degradation by stimulating SCF^{COI} activity [18]. *LOX2* and *VSP2* are both known to be jasmonate-inducible and to be upregulated by wounding [49, 50]. Wounding triggers JA and ethylene signaling, which culminates in the expression of defensins such as the *PDF1.3* and *PDF1.4* genes [51–53].

We made a similar schematic overview for genes involved in flowering timing and the circadian rhythm (Figure 3B). It seems that the MYB-transcription factors and central clock regulators *RVE2*, *LHY* and *LCL,* are induced by *DAYSLEEPER* overexpression. *PRR5*, which is negatively influenced by *LHY*, is also negatively regulated by a *ZEITLUPE*-containing CRL, namely SCF^{ZTL} (Figure 3B) [54]. Another SCF-complex, SCF^{TIR}, is responsible for the turnover of *IAA7/AXR2* [55]. Plants lacking functional *AXR2* display agravitropic shoot and root growth and display delayed flowering [55].

SPLAYED (*SYD*), which is a *SWI/SNF*-like gene, was found to be downregulated in our data-set. Mutant plants (*syd-1*) for this gene flower early [56]. Another *SWI/SNF*-like chromatin related factor we found to be upregulated was *ACTIN-RELATED PROTEIN 4* (*ARP4*) (Figure 3B, Table 4A). The *arp4-1* mutant also shows an early flowering phenotype [57]. The *SWI/SNF*-like gene *SWI3A*, also downregulated, is not involved in flowering, but is involved in development [58].

It is interesting to see that *DAYSLEEPER* overexpression apparently influences abnormal expression of many genes involved in plant defense and circadian rhythms and flowering timing, although our data does not point to single candidate genes that could be attributable for the observed misregulation of genes involved in the aforementioned processes.

DAYSLEEPER might be involved in epigenetic processes

DAYSLEEPER overexpression acts on a wide array of genes and causes a progressive misregulation of many important processes. The fact that several chromatin remodeling genes were identified and that *DAYSLEEPER* overexpressing plants display an increasingly severe phenotype in subsequent generations indicates that the misregulation induced by *DAYSLEEPER* might be caused by the disturbance of epigenetic imprints (Table 4) [1]. This is further substantiated by *DAYSLEEPER*'s co-expression with chromatin-related genes

A	**Up-regulated**	**Down-regulated**	**Up-regulated**	**Down-regulated**
	>2-fold	*>2-fold*	*>2-fold*	*>2-fold*
24 hrs. pINDEX3::At3g42170 +TAM	414	622	28	55
Bundock et al. (2005)	1022	638		

B		**Up-regulated**		
Rank	**Locus**	**SAGE-seq**	**Bundock et al.**	**Name/Description**
1	AT1G01060	3.78	29.62	LATE ELONGATED HYPOCOTYL (LHY)
2	AT1G07000	4.05	4.94	EXOCYST SUBUNIT EXO70 FAMILY PROTEIN B2 (EXO70B2)
3	AT1G14580	2.21	3.21	C2H2-like zinc finger protein
4	AT1G21240	2.43	4.24	WALL ASSOCIATED KINASE 3 (WAK3)
5	AT1G21520	31.58	3.26	Unknown protein
6	AT1G43910	5.07	3.92	P-loop containing nucleoside triphosphate hydrolases superfamily protein
7	AT1G65490	10.10	9.33	Unknown protein
8	AT1G65970	53.57	12.88	THIOREDOXIN-DEPENDENT PEROXIDASE 2 (TPX2)
9	AT1G77120	3.69	18.37	ALCOHOL DEHYDROGENASE 1 (ADH1)
10	AT2G05510	2.93	2.93	Glycine-rich protein family
11	AT2G05840	6.37	4.39	20S PROTEASOME SUBUNIT PAA2 (PAA2)
12	AT2G20340	2.60	5.44	Pyridoxal phosphate (PLP)-dependent transferases superfamily protein
13	AT2G24550	2.99	3.35	Unknown protein
14	AT2G31955	4.55	5.50	COFACTOR OF NITRATE REDUCTASE AND XANTHINE DEHYDROGENASE 2 (CNX2)
15	AT2G32260	2.74	3.65	PHOSPHORYLCHOLINE CYTIDYLYLTRANSFERASE (CCT1)
16	AT2G37760	3.68	9.63	ALDO-KETO REDUCTASE FAMILY 4 MEMBER C8 (AKR4C8)
17	AT2G37770	21.16	5.29	CHLOROPLASTIC ALDO-KETO REDUCTASE (ChlAKR)
18	AT2G38870	2.07	3.30	Predicted to encode a PR-peptide that belongs to the PR-6 proteinase inhibitor family
19	AT3G01820	2.29	5.99	P-loop containing nucleoside triphosphate hydrolases superfamily protein
20	AT3G20910	2.89	3.61	“NUCLEAR FACTOR Y, SUBUNIT A9” (NF-YA9)
21	AT3G24190	2.18	3.53	Protein kinase superfamily protein
22	AT3G45730	2.99	3.58	Unknown protein
23	AT3G49160	11.85	4.61	Downregulated in photoxidative stress
24	AT4G16155	2.41	3.76	dihydrolipoyl dehydrogenases
25	AT4G33070	4.01	4.02	Thiamine pyrophosphate dependent pyruvate decarboxylase family protein
26	AT5G02840	3.15	3.28	LHY/CCA1-LIKE 1 (LCL1)
27	AT5G58210	22.03	3.43	hydroxyproline-rich glycoprotein family protein
28	AT5G66140	3.23	4.53	PROTEASOME ALPHA SUBUNIT D2 (PAD2)

C		Down-regulated		
Rank	Locus	SAGE-seq	Bundock et al.	Name/Description
1	AT1G08890	2,73	2,59	Major facilitator superfamily protein
2	AT1G10140	3,39	2,77	Uncharacterised conserved protein UCP031279
3	AT1G13340	4,54	2,96	Regulator of Vps4 activity in the MVB pathway protein
4	AT1G22400	4,06	5,46	UGT85A1
5	AT1G23390	8,99	2,85	Kelch repeat-containing F-box family protein
6	AT1G24280	6,87	2,21	GLUCOSE-6-PHOSPHATE DEHYDROGENASE 3 (G6PD3)
7	AT1G33170	2,17	2,21	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
8	AT1G49720	8,90	6,40	ABSCISIC ACID RESPONSIVE ELEMENT-BINDING FACTOR 1 (ABF1)
9	AT1G56300	6,29	26,31	Chaperone DnaJ-domain superfamily protein
10	AT1G67810	3,28	3,10	SULFUR E2 (SUFE2)
11	AT1G68500	2,53	2,66	Unknown protein
12	AT1G69830	4,01	2,10	ALPHA-AMYLASE-LIKE 3 (AMY3)
13	AT1G69890	4,34	16,30	Unknown protein
14	AT1G71030	4,98	2,20	MYB-LIKE 2 (MYBL2)
15	AT1G73810	2,83	2,35	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase
16	AT1G76680	3,62	2,38	12-OXOPHYTODIENOATE REDUCTASE 1 (OPR1)
17	AT1G80440	4,00	2,35	Galactose oxidase/kelch repeat superfamily protein
18	AT1G80840	2,39	3,80	WRKY DNA-BINDING PROTEIN 40 (WRKY40)
19	AT2G22330	4,23	2,63	“CYTOCHROME P450, FAMILY 79, SUBFAMILY B, POLYPEPTIDE 3” (CYP79B3)
20	AT2G23680	2,66	2,09	Cold acclimation protein WCOR413 family
21	AT2G29460	7,60	2,45	GLUTATHIONE S-TRANSFERASE TAU 4 (GSTU4)
22	AT2G29480	6,11	2,44	GLUTATHIONE S-TRANSFERASE TAU 2 (GSTU2)
23	AT2G38240	4,85	5,01	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
24	AT2G40340	19,49	9,72	DREB2C
25	AT2G40840	2,67	2,20	DISPROPORTIONATING ENZYME 2 (DPE2)
26	AT2G42530	9,41	2,67	COLD REGULATED 15B (COR15B)
27	AT3G13960	4,83	2,84	GROWTH-REGULATING FACTOR 5 (GRF5)
28	AT3G18080	14,05	2,44	B-S GLUCOSIDASE 44 (BGLU44)
29	AT3G44860	26,29	7,81	FARNESOIC ACID CARBOXYL-O-METHYLTRANSFERASE (FAMT)
30	AT3G45970	7,90	4,25	EXPANSIN-LIKE A1 (EXLA1)
31	AT3G46660	6,57	2,52	UDP-GLUCOSYL TRANSFERASE 76E12 (UGT76E12)
32	AT3G49620	17,71	26,96	DARK INDUCIBLE 11 (DIN11)
33	AT3G50970	4,97	3,01	LOW TEMPERATURE-INDUCED 30 (LTI30)
34	AT3G54150	3,15	6,34	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein

35	AT4G15680	2,10	18,39	Thioredoxin superfamily protein
36	AT4G16563	3,98	2,64	Eukaryotic aspartyl protease family protein
37	AT4G25810	20,33	5,94	XYLOGLUCAN ENDOTRANSGLYCOSYLASE 6 (XTR6)
38	AT4G26530	3,97	3,00	Aldolase superfamily protein
39	AT4G30270	2,99	3,66	XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE 24 (XTH24)
40	AT4G30650	2,72	12,08	Low temperature and salt responsive protein family
41	AT4G36670	21,28	2,65	POLYOL/MONOSACCHARIDE TRANSPORTER 6 (PMT6)
42	AT5G05410	5,37	3,33	DREB2A
43	AT5G13200	3,49	3,96	GRAM domain family protein
44	AT5G20230	4,35	5,05	BLUE-COPPER-BINDING PROTEIN (BCB)
45	AT5G22300	3,63	2,97	NITRILASE 4 (NIT4)
46	AT5G24470	8,22	7,00	PSEUDO-RESPONSE REGULATOR 5 (PRR5)
47	AT5G24660	2,51	2,16	RESPONSE TO LOW SULFUR 2 (LSU2)
48	AT5G39410	2,86	2,53	Saccharopine dehydrogenase
49	AT5G48000	11,43	3,27	“CYTOCHROME P450, FAMILY 708, SUBFAMILY A, POLYPEPTIDE 2” (CYP708A2)
50	AT5G51070	3,21	2,06	EARLY RESPONSIVE TO DEHYDRATION 1 (ERD1)
51	AT5G57220	12,65	3,97	“CYTOCHROME P450, FAMILY 81, SUBFAMILY F, POLYPEPTIDE 2” (CYP81F2)
52	AT5G57630	5,03	4,88	CBL-INTERACTING PROTEIN KINASE 21 (CIPK21)
53	AT5G58600	2,17	2,82	POWDERY MILDEW RESISTANT 5 (PMR5)
54	AT5G62360	17,15	3,36	Plant invertase/pectin methylesterase inhibitor superfamily protein
55	AT5G63160	2,74	2,97	BTB AND TAZ DOMAIN PROTEIN 1 (bt1)

Table 6. Comparison of differentially regulated genes found in this study and genes found by Bundock and Hooykaas (2005) [1]. The number of genes that were more than 2-fold differentially regulated after 24 hours of *DAYSLEEPER*-induction in both studies and the overlap between the studies is shown in (**A**). Overlapping genes, the fold of differential expression and functional description (**B**).

FIE and *MSI1* (Table 5). Our data also showed an overlap with results from a microarray experiment by Cartagena *et al.* (2008) [59], who analyzed expression in a mutant in the SET DOMAIN GROUP 4 (*SDG4*) gene, which encodes a histone methyltransferase [59]. From the 37 downregulated genes they identified in this mutant, 11 (~30%) were also downregulated in our data after *DAYSLEEPER* overexpression. Among the 80 identified upregulated genes, 20 (25%) were also upregulated in our data. Although it is difficult to envisage a direct functional relation between *DAYSLEEPER* and *SDG4,* and our data was obtained with induced *DAYSLEEPER* overexpression, versus a *sdg4* mutant in Cartagena et al. (2008)[59], it is at least noteworthy to see an overlap in differentially regulated genes in these data.

We have also found that *DAYSLEEPER* has a very similar expression pattern to *FIE* and *ECR-1* (Table 5). FIE is a subunit in a DCX-type CRL [40] and ECR-1 is responsible for the rubylation of CULLINs [60], and thereby regulating their activity. Complexes containing CUL4 are involved in many processes, such as development, hormone responses, light responses, chromatin remodeling and DNA-damage repair (reviewed in Biedermann et al. (2011)[61]). There is evidence that DAYSLEEPER can also interact with a CUL4-related protein (data not shown, personal communication Alexander Kenzior, Columbia, MI, USA). This suggests that DAYSLEEPER might be part of a chromatin remodeling related complex with FIE and CUL4 (Figure 4), similar to a complex recently described by Pazhouhandeh *et al.* (2011) [40]. DAYSLEEPER is able to bind to the Ku-box motif, which is present in the promoter of the *KU70* DNA damage repair gene [1]. We did not find *KU70*, or other genes with a Ku-box in their promoter, in the list of genes that were up or downregulated after 4 hours of *DAYSLEEPER* induction (Table 2), but complexes containing CUL4 are known to be involved in DNA damage repair [62, 63].

Conclusions

DAYSLEEPER has a broad influence on the transcription of genes, since many genes in several functional fields have altered levels of expression when *DAYSLEEPER* is overexpressed. *DAYSLEEPER* has a strong influence on defense related genes (e.g. *PR1* and *WRKY40*) and circadian clock genes (e.g. *PRR5* and *LHY*), and affects genes that are involved in RNA transport, processing and silencing processes (e.g. *AGO1* and *HUA1*). We argue that *DAYSLEEPER* modulates the ubiquitylation machinery in Arabidopsis, which enables its involvement in a plethora of mechanisms.

Material and Methods

Plant growth conditions and RNA extraction

Arabidopsis thaliana Col-0 plants were used, containing either *pINDEX3*::At3G42170 construct or *pINDEX3*::GUS [1, 64]. Seeds were plated on ½ MS solid medium containing 15 µg/ml Hygromycin (HYG) and grown under normal long-day conditions with 16 hours of light and 8 hours of darkness. After 12 days plantlets were transferred to 6-well plates containing 5 ml liquid ½ MS medium, ½ MS medium containing 10 µm Cycloheximide

(CHX), ½ MS medium containing 2 µm Tamoxifen (TAM), or ½ MS medium containing both (Table 1). Per well, 5 plantlets were treated for either 4 or 24 hours and flash frozen in liquid nitrogen. Treatment started 2.5 hours after the light was switched on in the climate chamber. The tissue was ground under liquid nitrogen in a TissueLyser II apparatus (Qiagen®). RNA was isolated with the RNeasy Mini Kit (Qiagen®), using the optional on-column DNAse treatment with the RNAse-free DNase kit (Qiagen®). RNA samples were stored at -80^{O}C and their integrity verified using an Agilent 2100 Bioanalyzer (Agilent Technologies®).

SAGE Sample prep and GAIIX sequencing

Total RNA samples were processed according to the protocol "Preparing Samples for Digital Gene Expression-Tag Profiling with *NlaIII*" (March 2008, Illumina®), with the following modifications. First-strand synthesis was altered by replacing Superscript II reverse-transcriptase with Superscript III reverse transcriptase (Illumina®) and preheating the sample at 50^{o}C. Before digesting the samples with *Nla*III, the reaction mix was incubated for 2 minutes at 37^{o}C without the enzyme. Before the second GEX Adapter was ligated the sample was preheated to 20^{o}C.

Data processing

Raw sequencing data were processed using CLC Genomic Workbench version 4.6 (CLC bio®). Processed reads were annotated to TAIR v10 annotated gene-models. For determination of transcription of individual genes, only TAGs mapped to the 3' end of genes were taken into account. Absolute TAG-numbers were normalized to TAGs/million reads to compensate for the difference in total TAGs obtained per sample. When multiple TAGs mapped to the 3' end of a single gene, the sum of these TAGs was used as the expression value for this gene.

Expression data were processed using Microsoft Excel (Microsoft®). For determining up and down regulated genes a cut-off value of 2-fold was set. Ratios were calculated for each sample in comparison to the 4hrs. *pINDEX::GUS* TAM CHX sample. To determine whether genes were differentially regulated by *DAYSLEEPER* induction, values were normalized by subtracting values from a control experiment, or in the case of multiple controls, the highest value (up-regulated), or lowest value (down-regulated) in the controls was subtracted. In this way, the data should have a minimum of false negative and positives, since the induced sample is effectively set against several controls, correcting for differences that were line-specific, induced by growth conditions or chemically-induced. For determining differential gene-expression after 4 hours, without the addition of CHX, for each gene present in all samples, the ratio of sample 4 and 1 was used and corrected by subtracting the highest value from ratios of samples 1 and 2, 1 and 3 and 1 and 5 (Table 1). As a control, we determined the differential gene-expression after 4 hours, despite the addition of CHX (control sample). The ratio of sample 6 and 1 was used and corrected by subtracting the highest value found in the ratios of sample1 and 2, 1 and 3, and 1 and 5 (Table 1). To correct for possible secondary effects of CHX on gene expression, other than blocking protein synthesis, we excluded genes that were more than 2-times up- or downregulated by CHX (ratio of samples 1 and 5), even if these genes met our other criteria (i.e. genes that were even more induced in sample 6 and still gave a value of more than 2-old after normalization). For determining differential gene-expression after 24 hours, without the addition of CHX, the ratio of samples 8 and 1 was used and corrected by subtracting the highest value from ratios of samples 1 and 7 and 2 and 7 (Table 1). In each analysis, cut-offs were set to remove transcripts that were found only rarely. Per analysis, the “TAGs per million” value for individual genes was used as a cut-off. Genes that had a value of less than 2 were excluded from the analysis.

Author’s contribution

MK performed the experiments and data analysis. MK, SdP and PJJH contributed to the study design and writing of the manuscript. All authors have read and approved the final manuscript.

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Rank	Locus	Upregulated	Name/Description A
1	AT3G42170	22.49	DAYSLEEPER
2	AT3G49580	16.12	RESPONSE TO LOW SULFUR 1 (LSU1)
3	AT3G09960	11.53	Calcineurin-like metallo-phosphoesterase superfamily protein
4	AT1G62422	11.18	Unknown Protein
5	AT1G49030	9.97	PLAC8 family protein
6	AT1G08310	9.91	alpha/beta-Hydrolases superfamily protein
7	AT1G07630	9.35	POL-LIKE 5 (PLL5)
8	AT2G32235	9.34	Unknown protein
9	AT5G18270	7.74	ARABIDOPSIS NAC DOMAIN CONTAINING PROTEIN 87 (ANAC087)
10	AT3G02140	7.37	TWO OR MORE ABRES-CONTAINING GENE 2 (TMAC2)
11	AT3G27950	7.16	GDSL-like Lipase/Acylhydrolase superfamily protein
12	AT5G24210	6.48	alpha/beta-Hydrolases superfamily protein
13	AT5G56980	6.31	Unknown protein
14	AT5G53830	6.27	VQ motif-containing protein
15	AT3G60140	5.62	DARK INDUCIBLE 2 (DIN2)
16	AT3G48780	5.49	SERINE PALMITOYLTRANSFERASE 1 (SPT1)
17	AT1G27020	5.47	Unknown protein
18	AT3G53232	5.40	ROTUNDIFOLIA LIKE 1 (RTFL1)
19	AT1G12420	5.31	ACT DOMAIN REPEAT 8 (ACR8)
20	AT5G58787	4.92	RING/U-box superfamily protein
21	AT2G22860	4.87	PHYTOSULFOKINE 2 PRECURSOR (PSK2)
22	AT5G04720	4.80	ADR1-LIKE 2 (ADR1-L2)
23	AT2G33585	4.72	Unknown protein
24	AT4G21865	4.64	Unknown protein
25	AT2G40270	4.61	Protein kinase family protein
26	AT1G02660	4.49	alpha/beta-Hydrolases superfamily protein
27	AT1G06160	4.46	OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF 59 (ORA59)
28	AT4G02410	4.42	Concanavalin A-like lectin protein kinase family protein
29	AT3G45860	4.40	CYSTEINE-RICH RLK (RECEPTOR-LIKE PROTEIN KINASE) 4 (CRK4)
30	AT4G39740	4.39	HOMOLOGUE OF COPPER CHAPERONE SCO1 2 (HCC2)
31	AT3G23030	4.38	INDOLE-3-ACETIC ACID INDUCIBLE 2 (IAA2)
32	AT1G76690	4.17	12-OXOPHYTODIENOATE REDUCTASE 2 (OPR2)
33	AT5G46510	4.10	Disease resistance protein (TIR-NBS-LRR class) family
34	AT5G40470	4.04	RNI-like superfamily protein
35	AT1G50400	3.94	Eukaryotic porin family protein
36	AT3G19940	3.93	Major facilitator superfamily protein
37	AT1G22400	3.90	UGT85A1
38	AT1G62840	3.81	Unknown protein
39	AT5G44565	3.69	Unknown protein

40	AT2G34500	3.67	CYP710A1
41	AT1G51760	3.67	IAA-ALANINE RESISTANT 3 (IAR3)
42	AT2G30870	3.59	GLUTATHIONE S-TRANSFERASE PHI 10 (GSTF10)
43	AT1G12950	3.59	ROOT HAIR SPECIFIC 2 (RSH2)
44	AT5G64300	3.52	GTP CYCLOHYDROLASE II (GCH)
45	AT3G57550	3.50	GUANYLATE KINASE (AGK2)
46	AT4G29950	3.47	Ypt/Rab-GAP domain of gyp1p superfamily protein
47	AT5G39360	3.45	EID1-LIKE 2 (EDL2), F-BOX
48	AT2G30990	3.40	Unknown protein
49	AT1G62200	3.34	PEPTIDE TRANSPORTER 6 (PTR6)
50	AT5G63850	3.28	AMINO ACID PERMEASE 4 (AAP4)

Rank	**Locus**	**Down-regulated**	**Name/Description** **B**
1	AT3G15356	18.63	Legume lectin family protein
2	AT2G03760	12.12	SULPHOTRANSFERASE 12 (SOT12)
3	AT1G52100	5.80	Mannose-binding lectin superfamily protein
4	AT1G20030	4.99	Pathogenesis-related thaumatin superfamily protein
5	AT5G66330	4.74	Leucine-rich repeat (LRR) family protein
6	AT2G37640	4.69	ARABIDOPSIS THALIANA EXPANSIN A3 (ATEXPA3)
7	AT1G80050	4.48	ADENINE PHOSPHORIBOSYL TRANSFERASE 2 (APT2)
8	AT1G33040	4.01	NASCENT POLYPEPTIDE-ASS. COMPLEX SUBUNIT ALPHA-LIKE PROTEIN5 (NACA5)
9	AT4G22505	3.84	Bifunctional inhib./lipid-transfer prot./seed stor. 2S albumin superfamily protein
10	AT3G05727	3.73	Encodes a defensin-like (DEFL) family protein
11	AT4G29310	3.66	Unknown protein
12	AT5G03170	3.60	FASCICLIN-LIKE ARABINOGALACTAN-PROTEIN 11 (FLA11)
13	AT3G57830	3.58	Leucine-rich repeat protein kinase family protein
14	AT2G23985	3.57	Unknown protein
15	AT1G17100	3.47	SOUL heme-binding family protein
16	AT2G42840	3.41	PROTODERMAL FACTOR 1 (PDF1)
17	AT5G53980	3.41	HOMEOBOX PROTEIN 52 (HB52)
18	AT1G65900	3.40	Unknown protein
19	AT5G11550	3.35	ARM repeat superfamily protein
20	AT4G38430	3.26	RHO GUANYL-NUCLEOTIDE EXCHANGE FACTOR 1 (ROPGEF1)
21	AT5G01920	3.20	STATE TRANSITION 8 (STN8)
22	AT4G00430	3.19	PLASMA MEMBRANE INTRINSIC PROTEIN 1;4 (PIP1;4)
23	AT5G07030	3.11	Eukaryotic aspartyl protease family protein
24	AT3G62390	3.09	TRICHOME BIREFRINGENCE-LIKE 6 (TBL6)
25	AT5G37020	3.06	AUXIN RESPONSE FACTOR 8 (ARF8)

26	AT5G39210	2.97	CHLORORESPIRATORY REDUCTION 7 (CRR7)
27	AT5G15740	2.94	O-fucosyltransferase family protein
28	AT4G02800	2.92	Unknown Protein
29	AT4G03150	2.92	Unknown Protein
30	AT2G23010	2.87	SERINE CARBOXYPEPTIDASE-LIKE 9 (SCPL9)
31	AT4G00100	2.86	RIBOSOMAL PROTEIN S13A (RPS13A)
32	AT2G21045	2.85	Rhodanese/Cell cycle control phosphatase superfamily protein
33	AT5G16250	2.83	Unknown Protein
34	AT3G55660	2.83	ROP (RHO OF PLANTS) GUANINE NUCLEOTIDE EXCHANGE FACTOR 6 (ROPGEF6)
35	AT3G02690	2.81	nodulin MtN21 /EamA-like transporter family protein
36	AT3G26520	2.79	TONOPLAST INTRINSIC PROTEIN 2 (TIP2)
37	AT2G30080	2.79	(ZIP6)
38	AT1G19570	2.76	DEHYDROASCORBATE REDUCTASE (DHAR1)
39	AT2G22510	2.73	hydroxyproline-rich glycoprotein family protein
40	AT3G20820	2.72	Leucine-rich repeat (LRR) family protein
41	AT2G46690	2.70	SAUR-like auxin-responsive protein family
42	AT4G29905	2.68	Unknown Protein
43	AT5G37010	2.68	Unknown protein
44	AT4G22490	2.68	Bifunct. inhib./lipid-transfer prot./seed storage 2S albumin superfamily protein
45	AT5G51720	2.65	2 iron, 2 sulfur cluster binding
46	AT2G25060	2.65	EARLY NODULIN-LIKE PROTEIN 14 (ENODL14)
47	AT1G05835	2.65	PHD finger protein
48	AT3G24240	2.63	Leucine-rich repeat receptor-like protein kinase family protein
49	AT1G70210	2.63	CYCLIN D1;1
50	AT5G05890	2.62	UDP-Glycosyltransferase

TF-family	**Locus**	**Upregulated**	**Name** **C**
AP2-EREBP	AT1G06160	4.46	ORA59
AP2-EREBP	AT1G22985	2.11	-
bZIP	AT3G54620	2.14	AtbZIP25
C2H2	AT3G28210	2.32	PMZ
C2H2	AT3G53600	2.37	-
C2H2-CO-like	AT2G47890	3.14	COL13, CO-like, B-box gene
Homeobox	AT4G16780	2.41	HAT4,ATHB2
Homeobox	AT4G37790	2.81	HAT22
MADS	AT5G13790	2.76	AGL15
NAC	AT5G18270	7.74	ANAC087
WRKY	AT2G40740	2.71	WRKY55
WRKY	AT3G56400	3.23	WRKY70
TF-family	**Locus**	**Downregulated**	**Name**
AP2-EREBP	AT4G37750	2.06	AINTEGUMENTA/DRAGON, (ANT/ DRG)
AP2-EREBP	AT5G60120	2.39	TOE2
ARF	AT2G33860	2.45	ARF3/ETT
ARF	AT5G37020	3.06	ARF8
bHLH	AT2G46810	2.13	-
Homeobox	AT5G53980	3.41	ATHB52
MYB	AT3G46130	2.38	ATMYB48, PFG3
WRKY	AT1G30650	2.15	WRKY14
ZF-HD	AT5G65410	2.01	ATHB25, ZFHD2

Supplemental Table 1. 4 Hours of *DAYSLEEPER*-induction with the presence of CHX. The 50 genes that showed the largest differential expression after 4 hours of *DAYSLEEPER*-induction with the presence of CHX. (**A**) Lists the upregulated genes and (**B**) the downregulated genes. (**C**) Depicts transcription factors that were found to be differentially expressed.

Supplementary data

Supplemental Data S1.-S6 Excel-sheets of the up- down related genes (Figure 1.), including a classification of the found genes using the Classification Superviewer [28]. Due to the size of the Supplementary data files, as well as the raw sequencing data, these data will be made available upon request.

S1.-S2 Genes that are up- or down regulated after 4 hours of *DAYSLEEPER*-induction with the presence of CHX.

S3.-S4 Genes that are up- or down regulated after 4 hours of *DAYSLEEPER*-induction.

S5.-S6 Genes that are up- or down regulated after 24 hours of *DAYSLEEPER*-induction.