Daysleeper : from genomic parasite to indispensable gene

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Chapter 6

DAYSLEEPER plays an important role in several plant processes, through its involvement in the ubiquitylation machinery

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Summarizing discussion

DAYSLEEPER is essential in Arabidopsis [1] and we have shown that it is conserved in higher plants. We identified and cloned homologous genes from the grapevine (*Vitis vinifera*) and rice (*Oryza sativa*) genomes and obtained evidence of expression of homologous genes in many other angiosperms, including lower angiosperms (Chapter 2, this thesis). *DAYSLEEPER* is widely expressed throughout the plant, although this is most pronounced in meristems and developing flowers. DAYSLEEPER is located both within the nucleus and in vesicular structures of plant cells (Chapter 3, this thesis).

We have found that overexpression of *DAYSLEEPER* disturbs the regulation of many genes (Chapter 4, this thesis) and also that the DAYSLEEPER protein interacts with several other proteins and seems to influence the abundance of CULLIN1 (Chapter 5, this thesis). In this discussion we try to place our experimental data in perspective and put forward a model of the functional context we think *DAYSLEEPER* is involved in (Figure 1).

DAYSLEEPER and the Endosomal Sorting Complex Required for Transport-machinery III (ESCRT-III)

DAYSLEEPER is able to interact with the proteins VPS2.1, VPS2.2 and VPS2.3 (chapter 5, this thesis). These proteins are homologs of the canonical VPS2 subunit of the Endosomal Sorting Complex Required for Transport-machinery III (ESCRT-III) [2, 3]. The ESCRT-III machinery is involved in sorting ubiquitylated cargo proteins into vesicles before degradation [4]. *Daysleeper* mutants display a disrupted cellular organization in the root, a problem which also occurs in *vps2.2* and *vps2.3* plantlets, albeit less severe (Chapter 5, this thesis) [5, 6]. This might suggest that *DAYSLEEPER* and *VPS2.2* and *VPS2.3* are involved in cytokinesis in the root, although *VPS2.1 Vps2.1* plants are embryo lethal [6, 7]. has the most overlapping expression pattern with *DAYSLEEPER* in the root.

It was shown that that ESCRT-III machinery is necessary for the establishment of proper auxin maxima in Arabidopsis [8]. It is thought that this is caused by the fact that auxin transporters, such as PIN1, need vesicular transport to be targeted to specific sites of the cellular membrane [8, 9]. *Daysleeper* mutants also display disturbed auxin maxima (Chapter 5, this thesis). We found that RFP:DAYSLEEPER co-localizes in vesicular

structures with PIN1:GFP in protoplasts (Chapter 5, this thesis). However, although maxima are irregularly shaped in *daysleeper* plants, they are formed, which indicates that PIN1 transport does not require DAYSLEEPER. It seems therefore more likely that the aberrant auxin maxima observed in *daysleeper* mutants might be the result of problems with cellular organization, possibly caused by disturbance of a functional connection of DAYSLEEPER to (one of the) VPS2 proteins.

In our transcriptomics data, we found the downregulation of AT1G13340 (Chapter 4, this thesis) [1]. This gene is a putative negative regulator of *VPS4*, which is a subunit of the ESCRT-III machinery [10]. AT1G13340 contains an InterPro domain IPR005061 [11], which is also found in the negative regulator of VPS4 in yeast, Ist1 [11, 12]. Although this data does not provide a direct functional link, it is a further indication of the involvement of DAYSLEEPER with ESCRT-III related proteins.

The ESCRT-III machinery has also been shown to be involved in membrane budding and cytokinesis and there are also indications that proteins in this complex might fulfill a nuclear function [2, 3]. ESCRT-related protein CHMP1/VPS46 has been reported to interact with Polycomb-like factor Pcl on condensed chromatin in human cells [13]. Furthermore, CHMP2 overexpression induces condensed chromatin regions, to which Polycomb group protein Rac1 and phosphorylated histone H3 are recruited [14]. Recently it was also described that ESCRTIII protein CHMP4C in human cells (homologous to SNF7 in Arabidopsis) has a role in maintaining genomic stability by regulating the phosphorylation of chromatin associated protein Aurora B [15]. An interesting observation is therefore the nuclear localization of the interaction of the VPS2-proteins and DAYSLEEPER (Chapter 5, this thesis). From a proteomic analysis it has become apparent that VPS2.2 in Arabidopsis may play a role in the nucleus[16], which is in line with our finding that DAYSLEEPER has been shown to interact with VPS2-proteins in the nucleus (Chapter 3 and 5, this thesis).

DAYSLEEPER, the COP9-signalosome (CSN) and CULLIN-ring ligases (CRL's)

We found that DAYSLEEPER interacts with the COP9-signalosome (CSN) subunit CSN5A (Chapter 3, this thesis). *Daysleeper* plantlets display similarities to *cop* mutants [1, 17, 18]. *Cop* mutants are similar to *daysleeper* mutants in that they do not progress past the seedling stage and accumulate DNA-damage (Chapter 5, this thesis)[19]. The light-grown phenotype of *cop* mutants is not shared by *daysleeper* plantlets and also the accumulation of anthocyanin is not observed [1].

We found that *daysleeper* seedlings have an accumulation of CULLIN1 (Chapter 5, this thesis). Furthermore, we found that induced *DAYSLEEPER* overexpression prompts the differential regulation of several genes, among which many CRL-related genes and COP9-signalosome subunits, as well as genes linked to the Anaphase Promoting Complex/Cyclosome (APC/C) (Chapter 4, this thesis).

We have found that *DAYSLEEPER* has a very similar expression pattern to chromatin related genes *MSI1* and *FIE* (Chapter 4, this thesis). FIE is a subunit in a CUL4-containing DCX-type CRL [20]. Furthermore, there is evidence that DAYSLEEPER can also interact with a CUL4-related protein *in vivo* (personal communication Alexander Kenzior, Columbia, MI, USA). DCX complexes have been known to play a role in DNA damage repair, chromatin remodeling and several other processes [21–23].

The abovementioned findings lead us to conclude that DAYSLEEPER functions in concert with both CSN and CRL complexes. DAYSLEEPER's precise mode of activity remains unknown, but the increased amount of CUL1 in *daysleeper* mutants could point to a role in regulating CULLIN expression or stability. However, we did not find an effect of *DAYSLEEPER* overexpression on CULLIN transcript abundance (Chapter 4, this thesis). Therefore, it is likely that DAYSLEEPER has an effect in CULLIN translation or stability. Also *DAYSLEEPER* has a highly correlated expression pattern to *ECR-1* (Chapter 4, this thesis). *ECR-1* is responsible for the rubylation of CULLINs, which is thought to regulate their activity [24]. It is interesting that DAYSLEEPER was first identified as a protein binding to the promoter of the DNA damage repair gene *KU70* and that both *cop* and *daysleeper* mutants accumulate DNA damage [18, 19] (Chapter 5, this thesis). DAYSLEEPER may therefore be associated with DCX-type CRL's, possibly in a complex associated with DNA damage repair or maintenance of genomic stability [22, 23]. Alternatively, DAYSLEEPER might be part of a chromatin remodeling related complex with FIE, which is highly coexpressed with *DAYSLEEPER* (Chapter 5), and CUL4, similar to a complex recently described by Pazhouhandeh *et al.* (2011) [20]. Furthermore, we found that several chromatin-related genes are misregulated when *DAYSLEEPER* is overexpressed and that overlap exists between our expression data set and a data set obtained by studying gene expression in the histone methyltransferase *sdg4* [25] mutant (Chapter 4, this thesis).

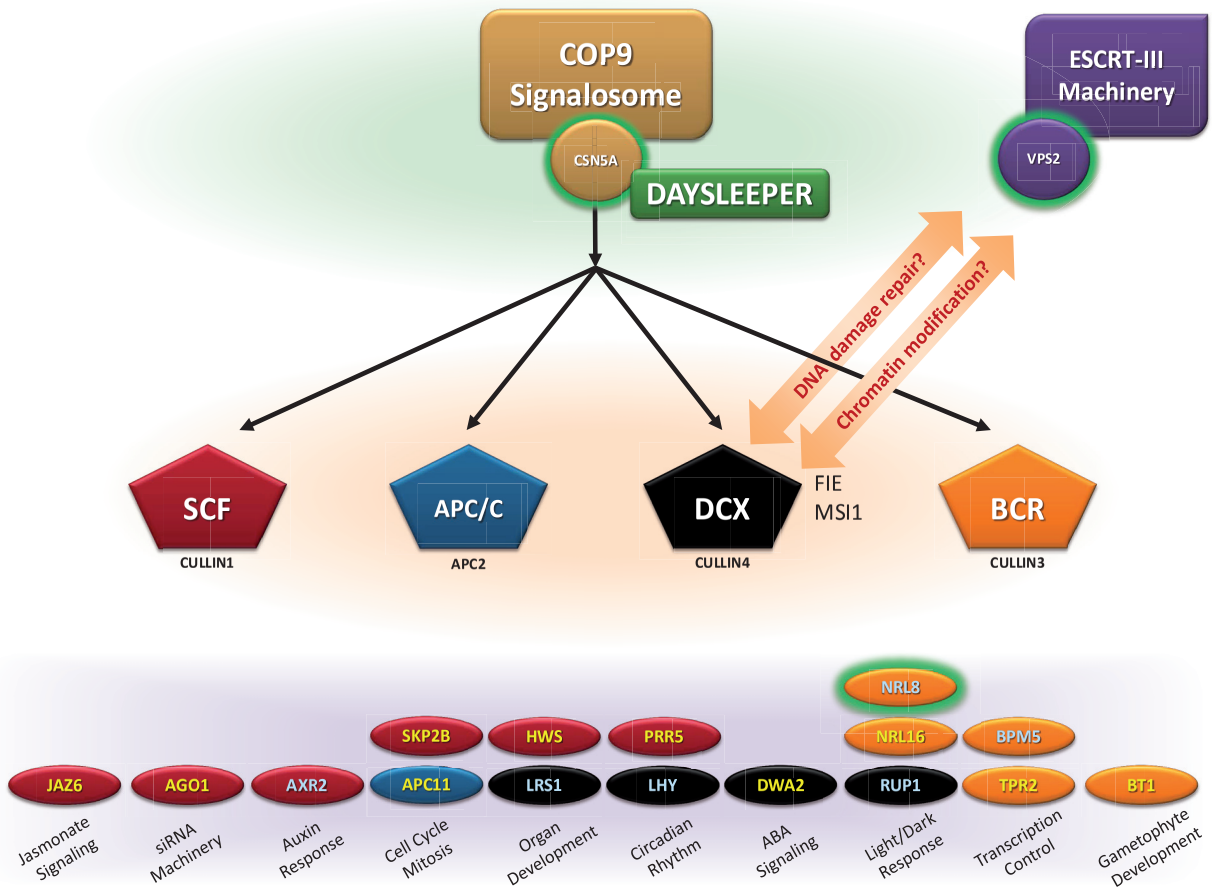

Figure 1. Model of *DAYSLEEPER*'s influence on the regulation of CRL's and the CSN. The CSN derubylates CRL's, thereby regulating their activity (reviewed in Hotton *et al.* (2008) [26]). Genes are depicted in ovals with colors referring to the type of CRL that regulates them. Burgundy = SCF, Black = DCX, APC/C = Blue and BCR = orange. The color of the text of the gene refers to the up- or downregulation in the SAGE-seq dataset (Chapter 4, this thesis; 24 hours of *DAYSLEEPER* induction). Genes written with blue letters are downregulated and yellow-written genes are upregulated. Ovals that have a green glow (i.e. *NRL8* and *CSN5A*) are genes that code for proteins that have been shown to interact with DAYSLEEPER (Chapter 5, this thesis). Genes that have a role in chromatin modifying complexes and are found to have a highly correlated expression pattern to *DAYSLEEPER* are placed to the right from DCX (i.e. MSI1 and FIE) (Chapter 5, this thesis). The ESCRT-III machinery (purple box) is involved in sorting ubiquitylated cargo, before degradation [3]. DAYSLEEPER also interacts with one of the components (VPS2) of this complex (Chapter 5, this thesis).

Conclusion: A graphical representation of DAYSLEEPER's field of function

We created a schematic representation of the interactions of DAYSLEEPER based on the interactions and influence of induced DAYSLEEPER overexpression discussed in the paragraphs above. *DAYSLEEPER* is likely to have a role in the interplay between the CSN and CRL complexes and the related APC/C complex, in connection to protein ubiquitylation (Figure 1). We have found many genes that are part of CRL's or genes that are regulated by CRL's (Chapter 4, this thesis) that are misregulated when *DAYSLEEPER* is overexpressed. This indicates that DAYSLEEPER plays a role in the fundamental processes of the interplay between the CSN and CRL complexes, which is substantiated by the severe phenotype of both the *daysleeper* and the overexpression mutants.

We speculate that the main functionality of the interaction between DAYSLEEPER and the ESCRTIII-complex through its interaction partner VPS2 could be the involvement of DAYSLEEPER in DCX-type CRL's, involved in DNA damage repair or chromatin modifying (PRC2-related) complexes (Figure 1).

In this work we have made steps towards understanding *DAYSLEEPER* evolution and function. Although the exact molecular role of DAYSLEEPER remains elusive, our findings suggest a role in elemental cellular processes and offers an enticing starting point for further understanding the role of *DAYSLEEPER* in Arabidopsis, and *SLEEPER* genes in other plant species.

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