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EXPRESSION PATTERNS OF CONVERGENTLY OVERLAPPING *ARABIDOPSIS THALIANA* GENE PAIRS *OHP-NDP1* AND *OHP2-MES14*

KAROL STAWSKI, MARIUSZ BANACH, AND ANNA GOC*

Department of Genetics, Nicolaus Copernicus University, Lwowska 1, 87-100 Toruń, Poland

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The function of one-helix proteins (OHPs) in the thylakoid membrane remains poorly understood but may be linked to plant photosystem protection. In *Arabidopsis*, the 3'UTRs of the genes encoding OHP and OHP2 partially overlap with *NDP1* and *MES14* respectively. Antisense orientation of genes has the potential to form double-stranded transcript (dsRNA) molecules which can be processed to siRNA and trigger RNA interference (RNAi). Natural siRNAs are induced by abiotic and biotic stresses. We examined whether the expression of the *OHP-NDP1* and *OHP2-MES14* gene pairs is regulated in this way. Both *OHP* genes, but neither *NDP1* nor *MES14*, were activated by light in etiolated seedlings, whereas cold and prolonged heat treatment elevated the *OHP* transcript level. Expression of *OHP2* was down-regulated after 2 h of osmotic and heat stress, while salt and osmotic stress increased *MES14* transcript levels. No inverse regulation of these overlapping gene pairs was observed, excluding RNAi as a regulatory mechanism in the tested conditions. The presence of alternatively polyadenylated transcripts of the studied genes raises the possibility of another regulatory mechanism of 3'UTR overlap.

Key words: Alternative polyadenylation, *Arabidopsis thaliana*, overlapping genes, 3'UTR.

INTRODUCTION

One-helix proteins (OHPs) are members of the early light-induced protein family (ELIPs) and are closely related to the chloroplast photosystem-associated chlorophyll *a*/*b*-binding (CAB) proteins. In higher plants OHPs are encoded in the nuclear genome, synthesized as precursors in the cytosol, and transported across the chloroplast envelope. OHPs have conserved chlorophyll binding residues and one transmembrane α-helix resembling the first of three helices of the LHC (light harvesting complex) proteins (Heddad and Adamska, 2002). Two OHP proteins have been reported in *Arabidopsis thaliana*: OHP (At5g02120; Jansson et al., 2000) and OHP2 (AY057393; Andersson et al., 2003). The OHP and OHP2 proteins are 69 and 130 amino acids long respectively. Expression of OHPs is induced by light stress (Jansson et al., 2000; Andersson et al., 2003) and it was suggested that they play a role in protective mechanisms against inactivation of photosystems within thylakoids under excess light. Under photoinhibitory conditions, OHPs might bind free chlorophyll molecules, preventing the formation of free radicals and/or might serve as a diffuser of light excitation energy (Montané and Kloppstech, 2000, Adamska et al., 2001). The *OHP* and *OHP2* genes are located on different *A. thaliana* chromosomes.

Little is known about the function of the proteins encoded by *NDP1* and *MES14*, two genes overlapping *OHPs*. The significance of this overlap also remains to be elucidated. The *NDP1* gene was originally isolated and sequenced from *Solanum tuberosum*. The *Arabidopsis NDP1* homologue is composed of 421 amino acids and shows similarity to kinesin light chain proteins (KLC), a class of microtubule motor proteins that can serve many essential cellular functions (Miki et al., 2005). Studies on the kinesins of higher plants indicate that they may be essential for chloroplast movement and anchorage to the plasma membrane (Suetsugu et al., 2010). All members of the *Arabidopsis* kinesin or kinesin-like superfamilies have been classified based on the presence of a conserved motor domain specific for the kinesin heavy chain but this domain is absent from the *NDP1* protein. The motor domain has nucleotide-dependent microtubule binding ability and microtubule-stimulated ATPase activity, and

*e-mail: goc@umk.pl

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TABLE 1. Primers used for gene expression analysis

Primer	Sequence (5'→3')	Gene specificity
ACT2_R	TGAAGCACAATCCAAGAGAG	*Act2*
ACT2_F	TCAACAGCAACAAAGGAGAG	
ACT2NN_R	TGAAGCACAATCCAAGAGAGCAC	
ACT2NN_F	TCAACAGCAACAAAGGAGAGTGC	
MES14_R	AAGATGGAGGAGGAGGAGGA	*Mes14*
MES14_F	GCCATTAGAAAACGGGTCAG	
MES14_OHP2_R	TGAAAACGAGAATACACTTTCCAT	
MES14_OHP2_F	TCAATCACTTTTATCTTCAAATGGT	
NDP1_R	ATCTTCAGGGTGTGGTTGCT	*Ndp1*
NDP1_F	CATCTCCTTTGCCAGTTCA	
OHP_R	CTCGTCGCCGTTATCTTCAT	*Ohp1*
OHP_F	TTTGGCACTATCACCCCTTC	
OHP2_R	ACCTGTTCCTCCATCACAGC	*Ohp2*
OHP2_F	AGCATACCAACTGCGAAACC	

KLCs are believed to interact with a variety of cargo (Reddy and Day, 2001).

The *MES14* gene of *A. thaliana* encodes a protein predicted to act as a carboxylesterase which catalyzes hydrolysis of carboxylic esters. The enzymes within this group possess a variety of substrate specificities, and *MES14* protein is most similar to the methyl salicylate esterase (SABP2) of tobacco, which catalyzes the conversion of methyl salicylic acid to salicylic acid (SA) (Forouhar et al., 2005). Derivatives of SA appear to be biologically inactive but can be readily converted back to free active SA. Activation of SA is important in the plant response to pathogen infection in both local resistance and systemic acquired resistance (Shulaev et al., 1997). However, none of the six methylated forms of SA appeared to be substrates of *MES14* (Yang et al., 2008; Vlot et al., 2008)

A large number of overlapping gene pairs are co-expressed in *Arabidopsis* but the physiological roles of antisense transcripts are largely unknown. As much as 4–8% of *A. thaliana* protein-coding genes form natural antisense transcripts (*cis*-NAT). Regarding their relative orientation the majority of overlapping genes can by classified as convergently overlapping gene pairs (Wang et al., 2005), tail-to-tail oriented *cis*-NATs, or type I pairs (Jen et al., 2005). Antiparallel transcription of sense and antisense genes in the same genomic loci leads to formation of natural antisense transcripts (*cis*-NATs), providing a source of dsRNA, which can be used as substrate for DICER-mediated biogenesis of small interfering RNAs (siRNAs) causing RNA interference (Borsani et al., 2005). NATs can also act as regulators in alternative splicing or polyadenylation (Jen et al., 2005), RNA editing (Sureau et al., 1997) and DNA methylation (Tufarelli et al., 2003), and have also been linked to X-chromosome inactivation (Lee et al., 1999).

This study examined two pairs of convergently overlapping genes of *Arabidopsis thaliana*: *OHP-NDP1* and *MES14-OHP2*. The *A. thaliana NDP1* gene (GenBank accession number At5g02130) located on chromosome 5 has a *cis*-antisense orientation towards the gene for a one-helix protein (*OHP*; At5g02120). According to NCBI data their simultaneous expression would allow for hybridization of 116 bp of their 3' regions to create dsRNA molecules. The overlap involves 24 nt of the *NDP1* coding sequence and 3'UTRs of their mRNA. *MES14* (AT1G33990) and *OHP2* (At1G34000) genes also have antisense orientation but on *A. thaliana* chromosome 1. *OHP2* and *MES14* NATs share 122 bp of their 3'UTRs.

We examined the co-expression pattern of the gene pairs *OHP-NDP1* and *MES14-OHP2* in *A. thaliana* under several stress conditions. Although no evidence for RNAi was identified, regulation by alternative polyadenylation can be suggested.

MATERIAL AND METHODS

PLANT MATERIAL AND GROWTH CONDITIONS

Arabidopsis thaliana (ecotype Columbia) seeds were surface-sterilized and sown on MS medium. Seedlings were grown at 25°C in a growth chamber under a 16 h photoperiod (~100 μmol m^{-2} s^{-1}a photon flux). Gene expression patterns in the light were assessed in 21-day-old plants harvested during the first 8 h of the light period. In another experiments,

21-day-old plants were at the end of night watered with half-strength MS liquid medium (control) or half-MS plus salt (0.3 M NaCl) or osmotic agent (0.3 M sorbitol), or were transferred to a different temperature regime (4°C or 37°C) and kept in darkness for another 8 h. Stressed plants were collected at 1, 2, 4 and 8 h post-treatment. Untreated seedlings collected according to the same time schedule were the controls. For the de-etiolation experiment, 21-day-old dark-grown seedlings were transferred to light and collected at 0 (control), 1, 2, 4 or 8 h. Samples of ~12 whole plants were frozen in liquid nitrogen and stored at –80°C.

RNA ISOLATION AND CDNA SYNTHESIS

Samples were ground in liquid nitrogen and total RNA was isolated using the GeneMATRIX Universal RNA/miRNA Purification Kit (Eurx) with DNAse I treatment, according to the manufacturer's instructions. Total RNA was quantified by UV spectrophotometry and cDNA was synthesized using 1 μg total RNA, anchored oligo(dT) primers and M-MuLV reverse transcriptase (Fermentas).

EXPRESSION ANALYSIS BY RT-PCR

Semiquantitative RT-PCR was used to determine expression of *OHP*, *OHP2*, *NDP1* and *MES14*. The cDNAs were amplified with a pair of gene-specific primers (10 pmol each, Tab. 1) and the constitutively expressed actin gene (*ACT2*) was the internal standard. To amplify *ACT2/MES14*, *ACT2/NDP1* and *ACT2/OHP2* in the same probe, both ACT2-specific primers (ACT2_R, ACT2_F) and competitive primers (ACT2NN_R, ACT2NN_F) were used (in 2:1 ratio) to generate unsaturated RT-PCR signals. All PCR reactions were performed using Paq5000 DNA polymerase (Stratagene) and 0.9 μl RT product. The thermal cycling conditions were as follows: 2 min at 94°C, 24–32 cycles of 30 s at 94°C, 40 s at 56.5°C, 45 s at 72°C, and final extension of 5 min at 72°C (25 cycles for ACT2/*MES14* and ACT2/OHP2, 32 for ACT2/*NDP1*, 24 for ACT2/OHP). Both number of cycles and annealing temperatures were optimized experimentally. Three rounds of RT-PCR were conducted with three independently isolated total RNA samples and 20 μl of each PCR reaction was fractionated by 1% agarose gel electrophoresis. Ethidium bromide stained gels were digitally photographed and the intensity of the stained DNA bands was analyzed using Fuji Image Guage ver. 3.46. The relative transcriptional activity of each gene is expressed as the ratio of the densitometric measurement of its RT-PCR product to the corresponding actin product normalized to the same ratio obtained for the probe from 21-day-old plants growing in a regular cycle and harvested at the end of the last night. To avoid decimal values the final score was multiplied by 10. Statistical analysis employed PAST software.

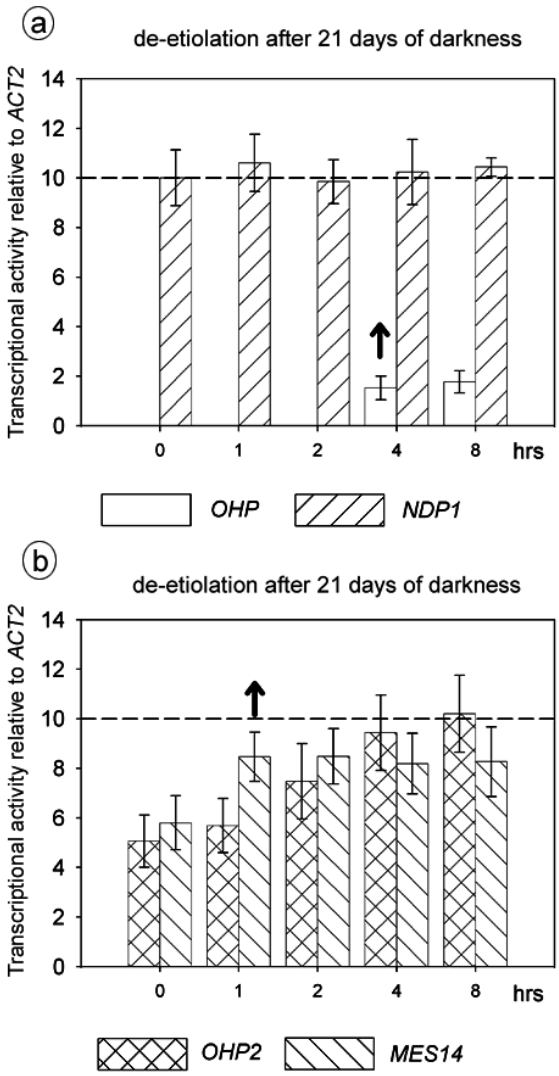

Fig. 1. Expression level of (**a**) *OHP* and *NDP1*, (**b**) *OHP2* and *MES14* in *Arabidopsis* seedlings during 8 h de-etiolation in ambient light conditions. Data are means ±SD of 3 independent experiments. Arrows indicate significant difference between etiolated and irradiated plants ($p < 0.05$). Dashed line demarcates normalization level.

RESULTS

LIGHT INDUCTION

To analyze the effect of moderate light conditions (100 μmol m^{-2} s^{-1}) on gene regulation, we assayed their mRNA levels in plants during 8 h of (i) de-etiolation (Fig. 1), (ii) additional darkness after the last

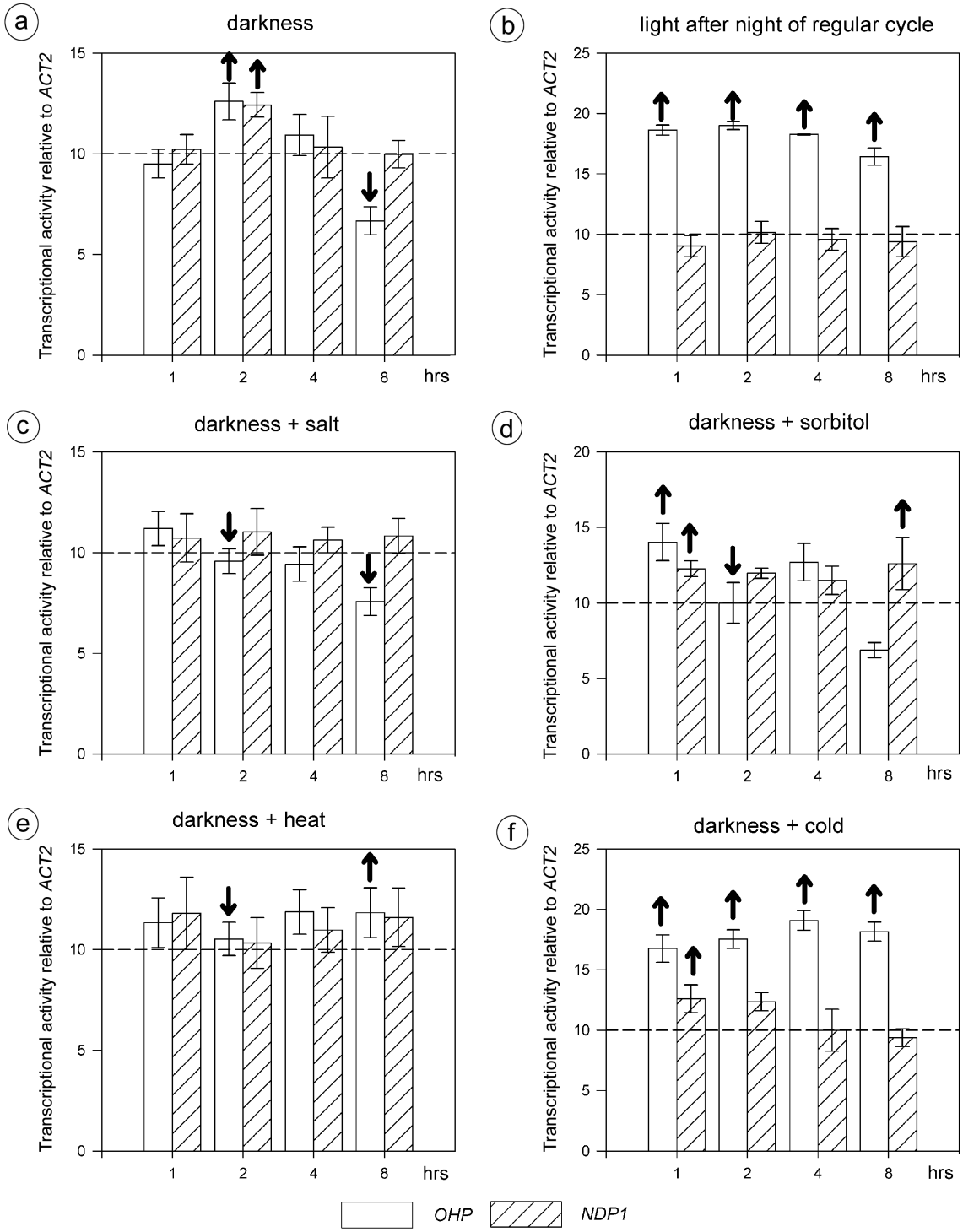

Fig. 2. Expression level of *OHP* and *NDP1* in *Arabidopsis* seedlings under different stress conditions: (**a**) Additional darkness after night of regular cycle (16 h day/8 h night), (**b**) Light after night of regular cycle, (**c**) Salt, (**d**) Sorbitol, (**e**) Heat, (**f**) Cold. Data are means ±SD of 3 independent experiments. Dashed line demarcates normalization level. In (a) and (b) arrows indicate significant differences ($p < 0.05$) between sample and probe from 0 time point to which results were normalized. In (c–f) arrows indicate significant differences between sample and untreated sample from the same time point shown in (a). Direction of change shown as up or down arrows.

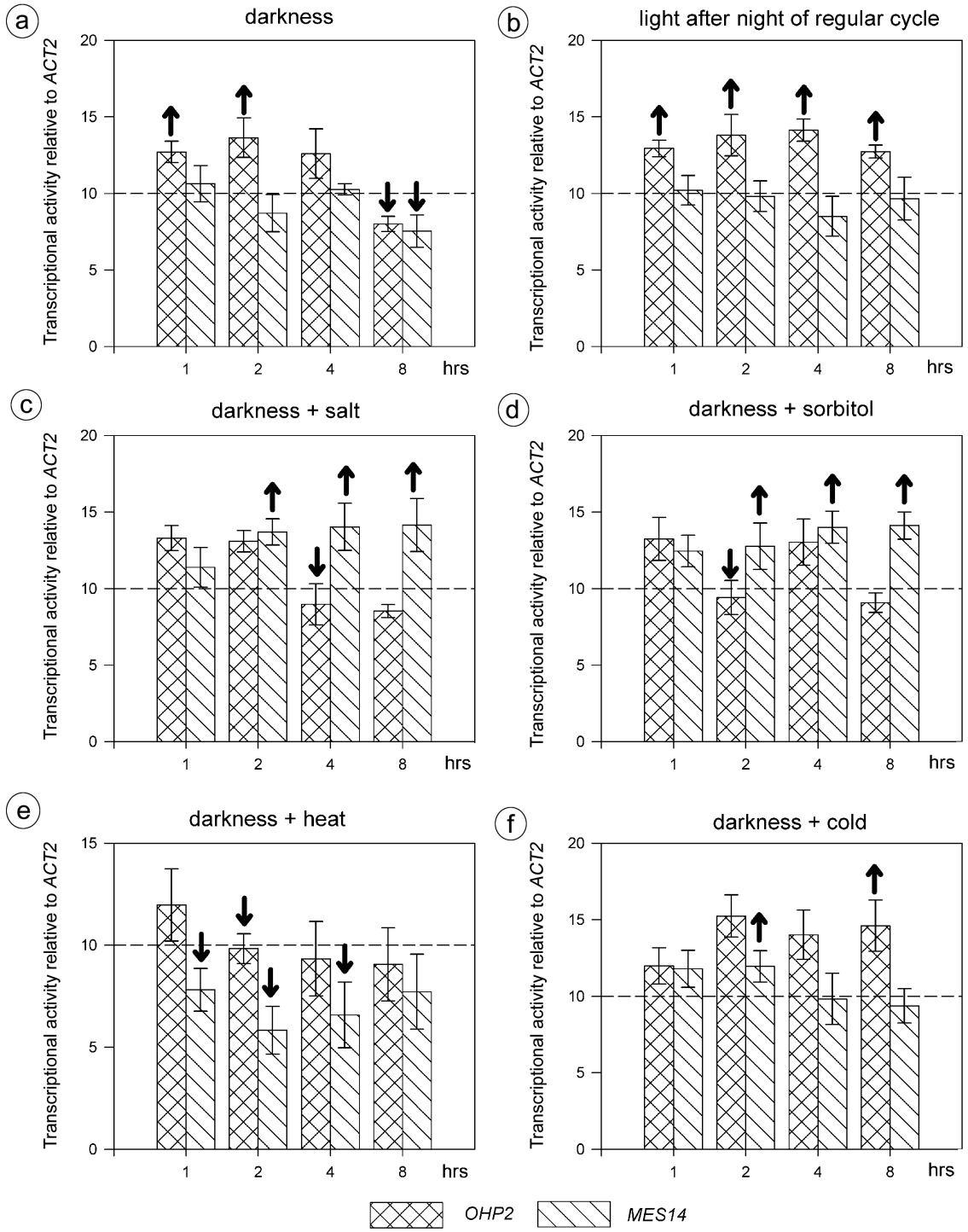

Fig. 3. Expression level of *OHP2* and *MES14* in *Arabidopsis* seedlings under different stress conditions. Descriptions as in Figure 2.

night of the regular cycle (16 h day/8 h night; Figs. 2a, 3a), and (iii) light after night of the regular cycle (Figs. 2b, 3b). Anticorrelated expression of overlapping gene pairs was not observed.

Both *OHP* genes are light-regulated. In long-etiolated plants *OHP* was completely silenced (Fig. 1a) and *OHP2* partially silenced (Fig. 1b), and their activation required 4 h illumination. The level of *OHPs*

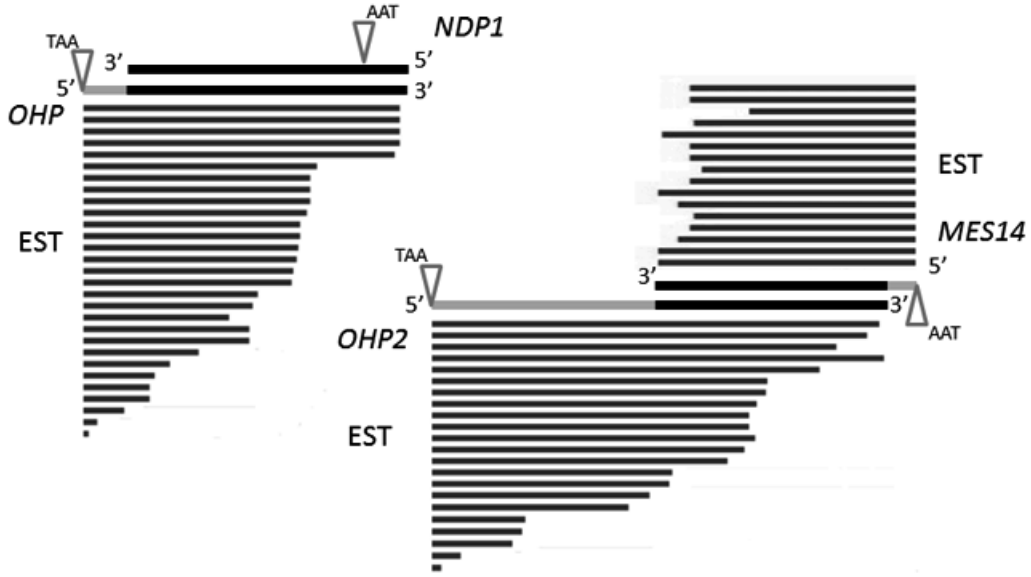

Fig. 4. EST database analysis of 3'UTRs of *OHP-NDP1* (left) and *OHP2-MES14* (right) transcripts. Arrowheads indicate stop codon (5'TAA3') positions, light grey lines represent nonoverlapping sequences, black lines represent overlapping sequences, dark grey lines show length of 3'UTRs.

mRNA was down-regulated by 8 h darkness (Figs. 2a, 3a). Their initial up-regulation in prolonged darkness probably reflects the diurnal cycle. The highest amount of *OHPs* transcript was found in plants exposed to light following a dark period (Figs. 2b, 3b). Light did not affect the level of *NDP1* or *MES14* mRNAs (Figs. 1, 2a,b, 3a,b).

GENE EXPRESSION UNDER STRESS

The semiquantitative RT-PCR profiles of *OHP*, *NDP1*, *MES14* and *OHP2* were also assessed in dark-grown *A. thaliana* plants exposed to abiotic stresses (high osmoticity, salinity, extreme temperature) and in untreated seedlings. Transcript levels were tested at 1, 2, 4 and 8 h of treatment.

All four genes were expressed under the studied conditions. The level of *NDP1* transcripts was stable, with the only significant changes (increases) observed under osmotic shock (Fig. 2d) and after 1 h of cold stress (Fig. 2f). Cold treatment elicited the strongest and most sustained *OHP* activation (Fig. 2f). *OHP* expression was also increased by prolonged (8 h) heat treatment (Fig. 2e). Elevated osmoticity caused fluctuation of *OHP* mRNA.

OHP2 transcript levels increased after 8 h at 4°C but decreased in response to 2 h elevated temperature and sorbitol (Fig. 3e,d). The transcript level of *MES14* significantly increased during salt and osmotic stress (2–8 h, Fig. 3c,d). Its down-regulation was observed under heat stress.

Sorbitol treatment for 2 h and salt stress for 4 h were the only instances in which the *OHP2-MES14* gene pair showed slightly different expression. No inverse expression of the *OHP-NDP1* pair occurred under the studied conditions.

POLYADENYLATION SITES OF ANALYZED GENE PAIRS

We examined the length of *OHP*, *NDP1*, *MES14* and *OHP2* 3'UTRs by aligning 3' sequences from an EST database. Several sets of *A. thaliana* 3'EST were selected for each of the analyzed mRNAs using criteria of >95% identity and length >300 bp (Fig. 4). The transcripts, especially *OHP* and *OHP2*, showed several alternative lengths of 3'UTR. The 3' ends of the *MES14* transcripts are not divergent and no *NDP1* 3'ESTs were found in the database.

Using the data generated by Sherstnev et al. (2012) gave a more detailed map of polyadenylation sites (Fig. 5). Using *OHP* alternative polyadenylation sites which take the 1st, 3rd, 4th, 6th and 8th place depending on their abundance creates transcripts that overlap the coding sequence of *NDP1* mRNA. Interestingly, some of the alternative polyadenylation sites of the *OHP2-MES14* pair are identical.

DISCUSSION

Small genes encoding a single transmembrane helix were first identified in cyanobacteria and termed high light-inducible proteins (HLIPs) (Dolganov et al., 1995) or small CAB-like proteins (SCPs) (Funk and Vermaas, 1999). It is accepted that during evolution some genes were transferred from the

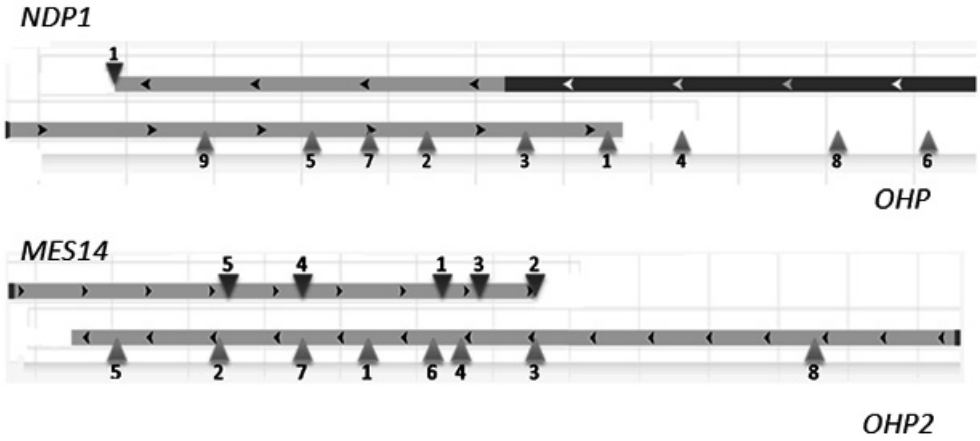

Fig. 5. Polyadenylation sites of *OHP-NDP1* (upper part, light grey arrowheads for *Ohp*, dark grey arrowheads for *NDP1*) and *MES14-Ohp2* (lower part, light grey arrowheads for *MES14*, dark grey arrowheads for *Ohp2*) transcripts according to data from Sherstnev et al. (2012). Dark grey lines denote coding sequences and light grey lines denote 3'UTR according to the gene database of NCBI (database does not include all polyadenylation sites found by Sherstnev et al., 2012). Horizontal arrowheads indicate transcription direction. Numbers reflect the frequency of site use.

cyanobacterial endosymbiont to the nucleus of the eukaryotic progenitor cells. The *A. thaliana* thylakoid membrane-localized homologous single membrane-spanning alpha-helix proteins were renamed one-helix proteins (OHPs) (Jansson et al., 2000; Andersson et al., 2003). Members of the *OHP* family have been found in the genomes of many photosynthetic eukaryotes (e.g., *Vitis vinifera, Oryza sativa, Populus trichocarpa, Zea mays, Ipomoea nil*) and cyanobacteria (Heddad and Adamska, 2002; Engelken et al., 2010), suggesting a primary function in photosynthesis.

The expression of nuclear-encoded chloroplast genes is regulated at multiple levels. Coordinated expression of chloroplast and nuclear genes is essential for proper chloroplast development, maintenance and function. In higher plants, plastid-derived signals can affect the expression of nuclear genes that encode chloroplast proteins (Nott et al., 2006), and changes in the expression of *Arabidopsis OHP* and *OHP2* may depend not only on the chloroplast redox state, tetrapyrrole biosynthesis and organellar protein synthesis (Pesaresi et al., 2006, 2007); they may also result from overlap of their sequences with other genes. siRNA-mediated regulation has been suggested for some overlapping genes (e.g., the *Arabidopsis SRO5* and *P5CDH* gene pair). According to Borsani et al. (2005), induction of SRO5 transcription in response to salt stress resulted in the formation of 24 bp NAT-siRNA which caused cleavage of constitutively expressed P5CDH, resulting in proline accumulation and increased salt stress tolerance. However, a recent finding by Sherstnev et al. (2012) suggests a more minor role for NAT-siRNA-mediated regulation of gene pairs with anticorrelated expression.

Many proteins of the *LHC* family undergo diurnal fluctuation mediated by circadian signals (Millar and Kay, 1996). Analysis of the databases of several diurnal and circadian genome-wide expression array experiments conducted on *Arabidopsis* genes (http://diurnal.cgrb.oregonstate.edu/; Mockler et al., 2007) confirms that *OHP* genes are regulated by a self-sustained oscillator with a period of about 24 hours. In light of this, we compared all our expression data obtained for treated plants and control plants at the same point of the darkness cycle.

It has been shown that the expression of *OHP* family members in *Arabidopsis* is induced by light stress (Jansson et al., 2000; Anderson et al., 2003). In our study the accumulation of *OHP* and *OHP2* mRNAs occurred in ambient light conditions during de-etiolation in which the amount of *NDP1* and *MES14* transcripts was unaffected. All studied ELIP family members have been found to be transiently induced during photomorphogenesis of etiolated seedlings (Adamska, 2001). Our analyses indicated that the *A. thaliana OHPs* transcripts are repressed during darkness (Figs. 1a, 3a) and activated by light (Figs. 1b, 3b) but that OHP2 is less sensitive to light conditions.

As many plant siRNAs are specific to certain stress conditions or developmental stages, we studied the expression profiles of overlapping genes after osmotic and salt shock and during cold and heat treatment. We observed down-regulation of *OHP2* at hour 2 of heat and osmotic stress, and its up-regulation by prolonged cold treatment. Expression of *OHP2* homologs is also induced by cold stress in the absence of high light in cyanobacterium, wheat, bar-

ley and green algae (He et al., 2001; Adamska, 2001). These observations are not in accord with Andersson et al.'s (2003) finding of reduction of *OHP2* mRNA by cold or high salt and no response under heat stress, but they examined the stress response of detached *Arabidopsis* leaves treated for 3 hours. In our study *OHP* expression was also up-regulated by cold treatment. We also noted an increase in *OHP* transcripts in hour 8 of heat stress. It seems that OHP proteins may have photosystem protective functions beyond photoprotection.

RNA interference (RNAi) between sense and antisense genes should result in anticorrelated transcription. We detected no such correlation between *OHPs* and their convergently overlapping genes (*NDP1* and *MES14*). This is not surprising in view of Jen et al.'s (2005) demonstration that most *Arabidopsis* NATs show a pattern of co-expression and that an RNAi mechanism might be limited to certain developmental stages, stresses and/or specific cell types. Using data from Jin et al. (2008), we identified some short RNAs complementary to each of our genes of interest but their target sequences are not located in the overlapping regions. Taking these findings together, it seems unlikely that RNAi acts as a regulatory mechanism for the *OHP-NDP1* and *OHP2-MES14* gene pairs. This is consistent with the findings of Zhan and Lukens (2013), who reported limited siRNA production from *cis*-NATs.

Overlapping 3'UTRs have been shown to affect alternative splicing or polyadenylation of many plant NAT genes (Manen and Simon, 1993; Jen et al., 2005; Wang et al., 2005; Zubko et al., 2011). If *cis*-NAT genes overlap near the last intron-exon boundary it might favor splicing of the last intron (Jan et al., 2005; Zubko et al. 2011). However, even though the *OHP*, *NDP1*, *MES14* and *OHP2* genes contain introns, there is no evidence for their differentially spliced mRNA forms in the NCBI database.

Large-scale studies of alternative polyadenylation (APA) have suggested that it affects as much as 25% of *Arabidopsis* genes (Meyers et al., 2004). APA sites may be located at internal intron/exon boundaries but are most common inside 3'UTRs (Wu et al., 2011). We identified alternatively 3'-terminated forms of *OHP*, *OHP2* and *MES14* (but not of *NDP1*) mRNAs in the *Arabidopsis* EST database. These results, combined with data of Sherstnev et al. (2012), revealed transcripts with 3'UTRs of different length coding the same protein (Fig. 5) but even the longest identified 3'UTRs did not harbor known (Jin et al., 2008) siRNA binding sites. The *OHP2* and *MES14* genes have so many APA sites that some of them generate nonoverlapping transcripts, and few APA sites are shared between the genes. Shared APA regions have been documented by Zubko et al. (2011) for some *Arabidopsis* gene pairs in which partners differed in expression levels, but all the genes in our study had similar levels of expression. The most frequent polyadenylation sites of the *OHP2-MES14* gene pair (Fig. 5) create mRNAs with 21 nt overlap. Five of nine *OHP* APA sites fall inside the *NDP1* coding sequence (Fig. 5) and their mRNAs always overlap. These features of both gene pairs can influence their mRNA stability, localization or translatability. This is done through a wide variety of processes: regulation of translation alone, mediated by 3'UTR-binding proteins, has at least eight potential mechanisms (Szostak and Gebauer, 2013). One class of such RNA binding proteins, TZF, is encoded by 11 genes in *Arabidopsis*, and their expression is mediated by developmental and environmental stimuli, including light, salinity and osmoticity (Pomeranz et al., 2011). However, Zhan and Lukens (2013) postulated reciprocal transcriptional regulation of *cis*-NAT-encoding genes.

Some known regulatory functions executed by 3'UTRs are unlikely in the studied genes. Their 3'UTRs do not reach the length (>300 nt) documented to induce mRNA instability (Schwartz et al., 2006) and they do not contain the AU-rich sites for ARE-binding proteins that also influence mRNA stability (Barreau et al., 2005). The more likely roles played by overlapping 3'UTRs with APA sites include induction of ribosome binding and translation by 3'UTR shortening, as hypothesized by Andreassi and Riccio (2009).

That the gene-encoded proteins are in different locations in the cell (OHPs in plastids, *MES14* and *NDP1* putatively in cytoplasm) raises the possibility that their mRNAs must be properly localized for efficient translation. At least in animals, *cis*-elements found within the 3'UTR, including APA sites, can influence mRNA localization (Andreassi and Riccio, 2009).

If *OHP-NDP1* and *MES14-Ohp2* gene overlap is shared between species it may suggest a conserved functional relationship. We used the NCBI nucleotide database to search for homologues of *OHP*, *NDP1*, *OHP2* and *MES14* in other plant genomes. In the closely related *Arabidopsis lyrata*, homologues of both gene pairs neighbor each other in a tail-to-tail orientation but it is unclear whether these genes overlap. Orthologous genes of *OHP2* and *MES14* do not adjoin or are located on different chromosomes in *Brachypodium distachyon, Cucumis sativus, Oryza sativa, Fragaria vesca, Populus trichocarpa, Ricinus communis, Solanum lycopersicum, Sorghum bicolor* and *Vitis vinifera*. In the case of *OHP-NDP1* genes a 96 and 77 bp 3'UTR overlap exists in the genomes of *S. lycopersicum* and *C. sativus* respectively. Although *OHP-NDP1* are also proximal (tail-to-tail) in the *F. vesca, R. communis* and *V. vinifera* genomes, these genes map to different chromosomes of *S. bicolor, B. distachyon* and *P. trichocarpa*.

The investigated gene pairs differ in many ways. For example, though the OHP and OHP2 proteins share similar structure, plastid localization and photoprotective function, their genes differ in length and sequence. Their expression levels are also different, forcing us to use competitive ACT2 primers. Moreover, the overlap of the *OHP2-MES14* gene pair showed no conservation across species, and there was only partial evolutionary preservation of the *OHP-NDP1* overlap, the latter having only 3'UTR overlap and the former including a coding region overlap of one partner. APA sites may generate variability in gene pairs: from 0 to 159 nt overlap of 3'UTRs and common sites for the *OHP2-MES14* partners, and a consistent 19–184 nt overhang occasionally encompassing the coding sequence of one member in *OHP-NDP1*. The possible functions of their overlap may also diverge.

We suggest that more work on the association between APA and developmental states or stress conditions will yield a better understanding of how the 3'UTR length of *OHP*, *OHP2*, *MES14* mRNAs influences their expression profiles.

Our analysis of the *OHP*, *NDP1*, *OHP2* and *MES14* genes in other plant genomes demonstrates the high rates of evolutionary change between overlapping and nonoverlapping genes. Sanna et al. (2008) drew a similar conclusion from research on conservation of overlap in the human and mouse genomes. In mammals, conservation of antiparallel overlaps is higher than for single-strand overlaps, and the overlapping/nonoverlapping state transition is subject to high rates of evolution in 3'UTRs (Sanna et al., 2008). The closely related species *Arabidopsis thaliana* and *A. lyrata* present dramatic changes in chromosome rearrangements (Yogeeswaran et al., 2005) but the relative position of *OHP* vs. *NDP1* and *OHP2* vs. *MES14* is conserved. It remains to be determined whether plant 3'UTRs are evolutionarily labile.

AUTHORS' CONTRIBUTIONS

KS, AG study conception and design; MB, KS acquisition of data; KS, MB, AG analysis and interpretation of data; KS, AG drafting of manuscript; MB, AG critical revision of manuscript. The authors declare that there are no conflicts of interests.

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