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RESEARCH ARTICLE

Differential expression of *Arabidopsis* EJC core proteins under short-day and long-day growth conditions

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ABSTRACT

Exon junction complexes (EJCs) associate with mRNAs, mediate the pre-mRNA splicing and eventually gets displaced by ribosomes during the initial phase of translation. EJCs are involved in several critical physiological pathways. The functional nature of EJCs and the underlying molecular mechanism(s) still needs to be elucidated particularly in case of plants. Here, we report that the putative core protein factors of the EJC differentially express under short-day and long-day conditions. Since, plants are constantly exposed to biotic and abiotic factor(s), it would be significant to see how the EJCs respond to different stress inducing conditions. The protein levels of EJC core proteins under short-day conditions were 1.25 times higher relative to the protein levels under long-day conditions. Similar results were observed for the mRNA transcripts of the EJC core protein factors. These results signify that under short-day conditions, the EJC proteins are more activated and might be involved in few events which are yet to be revealed.

Introduction

The exon junction complex (EJC) comprising of three core protein factors is a multi-protein complex and interacts with the spliced messenger RNA (mRNA) to form mRNA-ribonucleoproteins (mRNPs). EJC manipulates the post-transcriptional events (1-4). Several associating proteins bind to the core EJC factors, depending on the cellular event that they will mediate. Essentially in a sequence-independent manner, the EJCs assemble and bind to the mRNAs in the region, primarily in the upstream region of exon-exon junctions (5, 6). EJCs remain bound to the mRNA throughout their life cycle (7, 8). The central dogma is regulated by the EJC proteins, but the underlying molecular mechanism(s) in which they still remains blurred. The role of EJC in the splicing process is highly regarded and thought to play a central role in determining the fate of the mature mRNA (9). The EJC increases the fidelity, efficiency and productivity of the translation event (10, 11). Interestingly, how the EJC proteins respond to changes in plant growth conditions still remains to be revealed. Light, being an abiotic factor plays a significant role in regulating the growth of the plant. Flowering and other developmental stages are affected depending on the availability of light (12). It is intriguing to reveal the role of EJC when the plants are grown at different exposure to light. Plants invariably alter the key molecular processes when exposed to different stress conditions (13-15). Since, EJC is well involved in various physiological pathways; it would be significant to reveal the role of EJC when plants are grown at varied growth conditions (16, 17). Here, we provide evidence that the EJC core protein factors; Y14, MAGO and eIF4AIII gets differentially expressed under short-day and long-day growth conditions, which specifically highlights that the EJC proteins are more activated and get accumulated to respond to changed environmental conditions by modulating the key molecular events.

Materials and Methods

Plant materials and growth conditions

Arabidopsis thaliana ecotype Columbia-0 (Col-0) seeds were sown in soil. The plants were grown simultaneously under short-day (12/12 hr light/dark) and long-day (16/8 hr light/dark) conditions. Temperature was maintained at 22 °C with70-80% relative humidity. Western blotting and semi-quantitative reverse transcriptase polymerase chain reaction (sq-RT PCR) assays were done using the leaves of *Arabidopsis thaliana*. The primer sequence resembling to *ATGGCGAACA TAGAATCAGAAGCAGTC* (forward primer) and *GTAACGTCTTCTCGGACTTCTTGAAC*

(reverse primer) of the coding sequence of *Y14* were used for amplifying the open reading frame using cDNA as template and similarly for amplifying the open reading frame of *MAGO*, *ATGGCCGCGG AAGAAGCGACGGAGTTC* (forward primer) and *GATAGGCTTGATTTTGAAGTGCAG* (reverse primer) primer sequences were used. ATGGCGACATCTGAAGCGGAATATG (forward primer) and *CTTGCTAAAGGTCATCTCCGAGTAC* (reverse primer) were used to amplify the coding sequence of eIF4AIII. PCR was done up to 25 cycles to amplify the gene products.

Semi-quantitative reverse transcriptase polymerase chain reaction (sq-RT PCR)

Full-length cDNA of Y14, MAGO and eIF4AIII was obtained by RT-PCR using Col-0 RNA. For RNA extraction, twenty-one-day-old plants were used. Twenty-one-old plants display optimum development and most importantly bolting process does not get initiated, which would otherwise may affect the synthesis of desired transcripts. According to the manufacturer's instruction (TRIzol® reagent, Thermo Fisher Scientific) total RNA was extracted. Genomic DNA were removed using 2 units of DNase (TURBO DNase™, Thermo Fisher Scientific). The purified RNA after phenol/chloroform/isoamyl alcohol extraction, DEPC-treated water was used to solubilise the RNA. Gene-specific primers were used for polymerase chain reactions. The primer sequence resembling to *ATGGCGAACA TAGAATCAGAAGCAGTC* (forward primer) and *GTAACGTCTTCTCGGACTTCTTGAAC* (reverse primer) of the coding sequence of *Y14* were used for amplifying the open reading frame using cDNA as template and similarly for amplifying the open reading frame of *MAGO*, *ATGGCCGCGG AAGAAGCGACGGAGTTC* (forward primer) and *GATAGGCTTGATTTTGAAGTGCAG* (reverse primer) primer sequences were used. ATGGCGACATCTGAAGCGGAATATG (forward primer) and *CTTGCTAAAGGTCATCTCCGAGTAC* (reverse primer) were used to amplify the coding sequence of eIF4AIII. *ATGGGTAAAGAGAAGTTTCACATC* (forward primer) and *CTTGGCACCCTTCTTGACTGCAGCCTT* (reverse primer) were used to amplify the coding sequence of EF1α. PCR was done up to 25 cycles to amplify the gene products. Reactions were performed in triplicate and the relative levels of the transcripts were normalized to the expression levels of genes used as internal controls, which included EF1α. Since EF1α is one of the constitutively expressed genes, it was used as an experimental control.

Protein extraction and Western blotting

Total proteins were isolated from 0.1 gm leaf of the twenty-one-day-old *Arabidopsis thaliana* Col-0 plants. The leaves were harvested from plants which were grown simultaneously under short-day (12/12 hr light/dark) and long-day (16/8 hr light/dark) conditions at 22 °C. The composition of the extraction buffer was 150 mM of NaCl, 30 mM of Tris-Cl (pH 8.0), 1 mM of PMSF, 1 mM of EDTA, and 1X proteinase inhibitor. Western blot analysis was done using protein-specific antibodies.

Results and Discussion

Y14, MAGO and eIF4AIII are highly conserved proteins.

The core EJC proteins are highly conserved in several plant species as evident from the similarities they share for their protein sequences (Fig. 1, Fig. 2, Fig. 3). The web-based tool (UniProt) was used to generate the multalign images indicating the proteins across various plant species. The *Arabidopsis* EJC proteins harbour utmost number of fully conserved amino acid residues as compared to other plant species. It is also revealed that considerable fraction of amino acid residues of EJC core proteins of *Arabidopsis* share resemblance>70%. A small amount of amino acid residues represent non-conserved segment. Since, the *Arabidopsis* proteins,

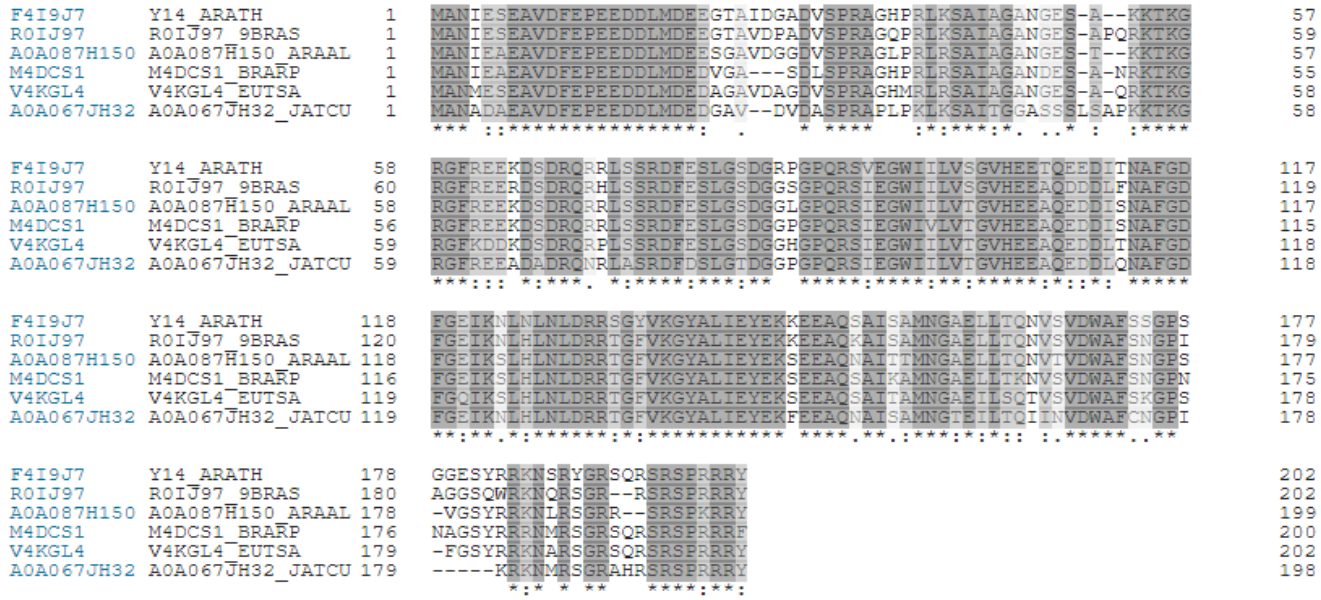

Fig. 1. Protein sequence alignment of the YI4 of *Arabidopsis thaliana*. Residues that are conserved are represented in box shades. Figure was generated with UniProt. (F4I9J7: *Arabidopsis thaliana*; R0IJ97: *Capsella rubella*; A0A087H150: *Arabis alpina*; M4DCS1: *Brassica rapa*; V4KGL4: *Eutrema salsugineum*; A0A067JH32: *Jatropha curcas*).

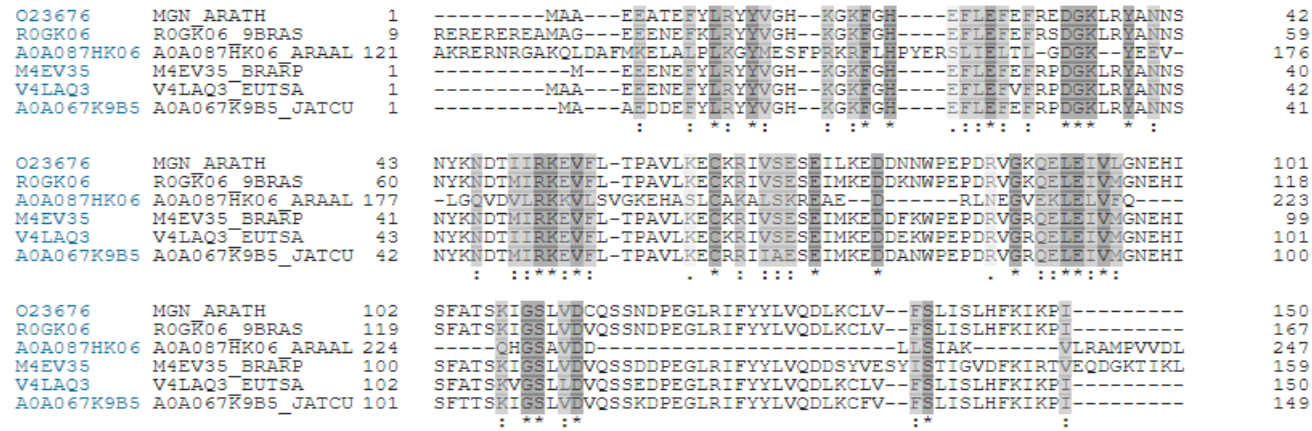

Fig. 2. Protein sequence alignment of the MAGO of *Arabidopsis thaliana*. Residues that are conserved are represented in box shades. Figure was generated with UniProt. (O23676: *Arabidopsis thaliana*; R0GKQ6: *Capsella rubella*; A0A087HK06: *Arabis alpina*; M4EV35: *Brassica rapa*; V4LAQ3: *Eutrema salsugineum*; A0A067K9B5: *Jatropha curcas*).

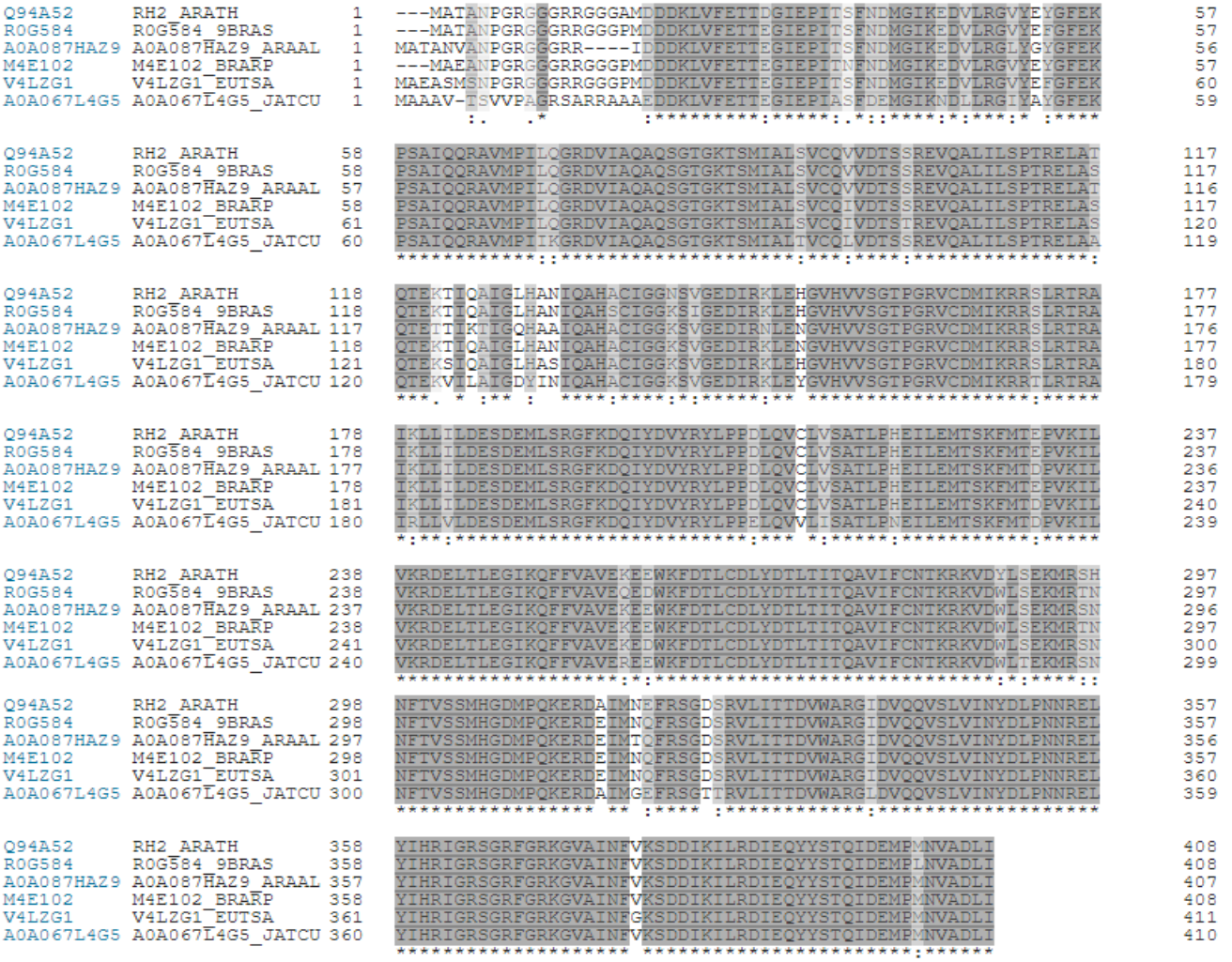

Fig. 3. Protein sequence alignment of the eIF4AIII of *Arabidopsis thaliana*. Residues that are conserved are represented in box shades. Figure was generated with UniProt. (Q94A52: *Arabidopsis thaliana*; R0G584: *Capsella rubella*; A0A087HAZ9: *Arabis alpina*; M4E102: *Brassica rapa*; V4LZG1: *Eutrema salsugineum*; A0A067L4G5: *Jatropha curcas*).

Y14, MAGO and eIF4AIII are extremely conserved in diverse plant species; it suggests that they all are equipped with these essential protein factors. The EJC core proteins are engaged in several numbers of physiological events and are thus indispensable in nature. Essentially The EJC maintains a stable, sequence-independent, hold on the mRNA until its removal during translation in the cytoplasm (18).

The Y14, MAGO and eIF4AIII transcripts are differentially expressed under long-day and short-day conditions.

The core EJC protein factors are engaged in several key molecular events. Plants when exposed to varied environmental conditions acclimatize themselves according to the prevailing stimulus. We intended to see the expression pattern of the core EJC proteins at

the transcript level under long-day and short-day growth conditions. Interestingly, it was observed that the expression levels of *Y14*, *MAGO*, *eIF4AIII* were upregulated for short-day grown plants relative to the long-day plants to 1.26, 1.25 and 1.09 times respectively (Fig. 4A, Fig. 4B). The transcript level of *ACTIN* represents the control of the experiment. Heat map of differential expression patterns of the EJC core genes under long-day and short-day growth conditions are represented in Fig. 4C.

The protein products of Y14, MAGO and eIF4AIIIare higher at short-day growth conditions

The protein levels of the core EJC protein factors are differentially regulated under long-day and short-day growth conditions. Concurrently, it was observed that the protein levels of Y14, MAGO, eIF4AIII were upregulated for short-day grown plants relative to the long-day plants to 1.38, 1.21 and 1.1 times respectively (Fig. 5A, Fig. 5B). The protein level of ACTIN represents the internal control of the experiment. Ponceau S staining represents RuBisCo and confrms equal protein loading. This result signifies that the EJC core proteins might accumulate under short-day growth conditions so that the plants can respond at a molecular level. The increase in protein levels of the Y14, MAGO, eIF4AIII also highlights that under short-day growth conditions these protein factors may be involved in molecular events which are yet to be exposed.

Conclusion

Revealing the functional and physiological role of the EJC is of great interest may unravel many hidden molecular mechanism(s). For instance, the EJC is indispensable for the growth of plants as the knockout of the core EJC protein factors results in embryo lethality conditions in *Arabidopsis*. Similarly in animals, knockout of both the core and peripheral EJC proteins significantly affects the

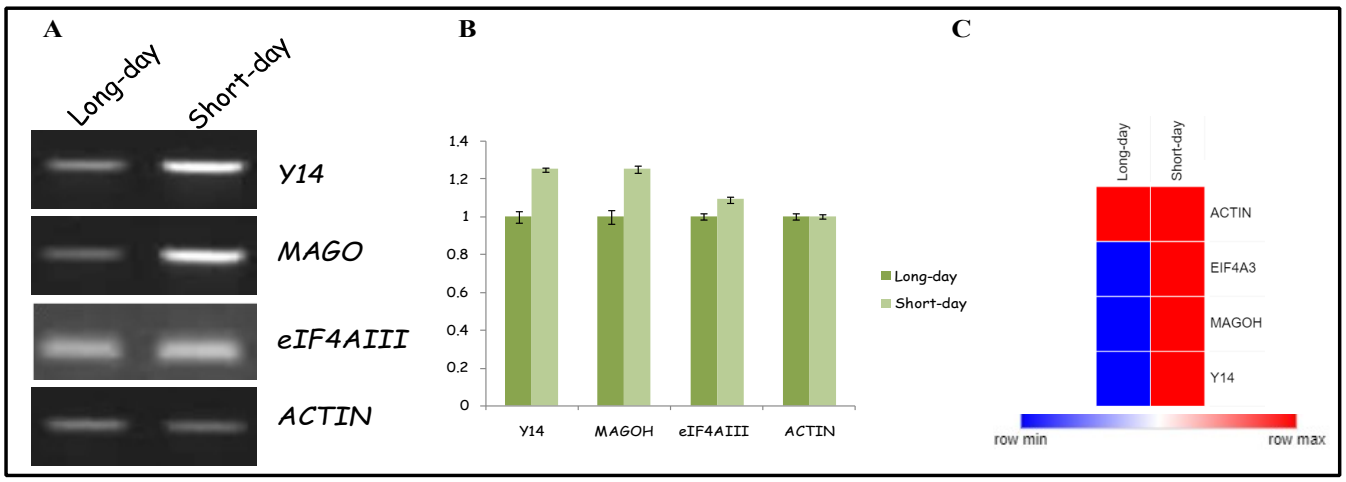

Fig. 4. Differential RNA level expression of the *Y14, MAGO, eIF4AIII* under long-day and short-day conditions. (A) The agarose-gel images were generated using primers specific for *Y14*, *MAGO* and *eIF4AIII*. *ACTIN* amplification represents the internal control of the experiment. (B) The expression levels of *Y14*, *MAGO*, *eIF4AIII* were upregulated for short-day grown plants relative to the long-day plants to 1.26, 1.25 and 1.09 times respectively. (C) Heat map of differential expression patterns of the EJC core genes under long-day and short-day growth conditions. Web-based tool Morpheus was used to generate the heat map.

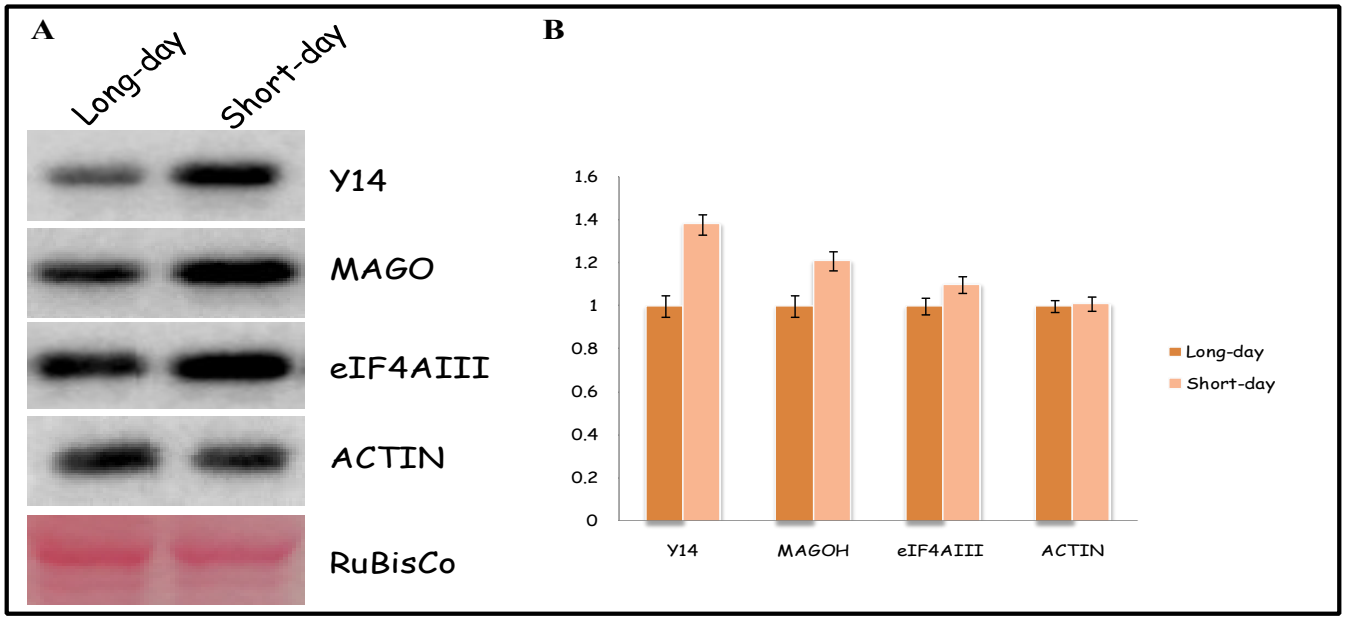

Fig. 5. Differential protein level expression of the Y14, MAGO, eIF4AIII under long-day and short-day conditions. (A) The Western blots were generated using α-Y14, α-MAGO and α-EIF4AIII antibodies. Protein loading is shown by Ponceau S staining for RuBisCo. The protein level of ACTIN is used as an internal control. (B) The protein levels of Y14, MAGO, eIF4AIII were upregulated for short-day grown plants relative to the long-day plants to 1.38, 1.21 and 1.1 times respectively.

growth of the cell and mostly lead to hereditary diseases. It seems worthwhile to focus on the underlying molecular mechanism(s) through which EJC mediates a wide range of physiological events. The detailed mechanism(s) of the EJC-mediated events remains to be elucidated. Safeguarding the accurate spatial and temporal structure of nuclear compartments is critical for efficient mRNA processing and maintenance of genome integrity. The EJC links the different aspects of mRNA biogenesis, such as transcription, splicing, export and translation. The parallel exposition of the function of the EJC and its position with respect to nuclear architecture is essential to realize how the cell responds internal and external stimuli that they encounter. How EJC-mediated response is triggered in plants in response to stress conditions is not yet fully understood. Our results gives a prima facie evidence that the EJC core proteins are differentially expressed under short-day growth conditions in *Arabidopsis* and may be well involved in modulating different physiological processes necessary for the plant to resist the environmental changes. Future research on understanding the role of EJC would be significant.

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Authors' contributions

AS conceived the idea, performed the experiments, analyzed the results, wrote the manuscript, have read and approved the final manuscript before submission. KBS conceived the idea, analyzed the results, have read and approved the final manuscript before submission.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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