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## Cooperative processing of primary miRNAs by DUS16 and DCL3 in the unicellular green alga *Chlamydomonas reinhardtii*

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### ABSTRACT

We have previously reported that the RNA-binding protein Dull slicer 16 (DUS16) plays a key role in the processing of primary miRNAs (pri-miRNAs) in the unicellular green alga *Chlamydomonas reinhardtii*. In the present report, we elaborate on the interaction of DUS16 with Dicer-like 3 (DCL3) during pri-miRNA processing. Comprehensive analyses of small RNA libraries derived from mutant and wild-type algal strains allowed the de novo prediction of 35 pri-miRNA genes, including 9 previously unknown ones. The pri-miRNAs dependent on DUS16 for processing largely overlapped with those dependent on DCL3. Our findings suggest that DUS16 and DCL3 work cooperatively, presumably as components of a microprocessor complex, in the processing of the majority of pri-miRNAs in *C. reinhardtii*.

### ARTICLE HISTORY

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### KEYWORDS


Argonaute; *Chlamydomonas reinhardtii*; Dicer; miRNA; RNA-binding protein; small RNA-seq

MicroRNAs (miRNAs) are loaded into Argonaute (AGO) proteins during the formation of the RNA-induced silencing complex (RISC).<sup>1</sup> The main function of miRNAs in RNA silencing is guiding RISC to target transcripts for inducing endonucleolytic RNA cleavage and/or translational repression. In general, miRNAs are embedded in long primary miRNA (pri-miRNA) transcripts containing stem-loop structures and have to be processed to mature miRNAs with the assistance of RNase III Dicer and associated RNA-binding proteins.<sup>2,3</sup> We have recently reported that in the unicellular green alga *Chlamydomonas reinhardtii*, an RNA-binding protein, Dull slicer 16 (DUS16), is required for pri-miRNA processing and associates with Dicer-like 3 (DCL3), which in turn is involved in the biogenesis of the majority of miRNAs (Fig. 1).<sup>4,5</sup> We also reported that AGO3, which is one of the 3 AGOs encoded in the *C. reinhardtii* genome, predominantly binds to mature miRNAs and determines miRNA-mediated post-transcriptional gene silencing (Fig. 1).<sup>6</sup> The present report contains a comprehensive analysis of our previously published small RNA-seq (sRNA-seq) data [from the AGO3 mutant (*ago3-1*); the DUS16 mutant (*dus16-1*); the parental strain of these

mutants Gluc(1×), which expresses a reporter luciferase transgene in the wild-type background; and the wild-type strain CC-124] to predict de novo pri-miRNAs and gain insight into the functional coupling between DUS16 and DCL3.

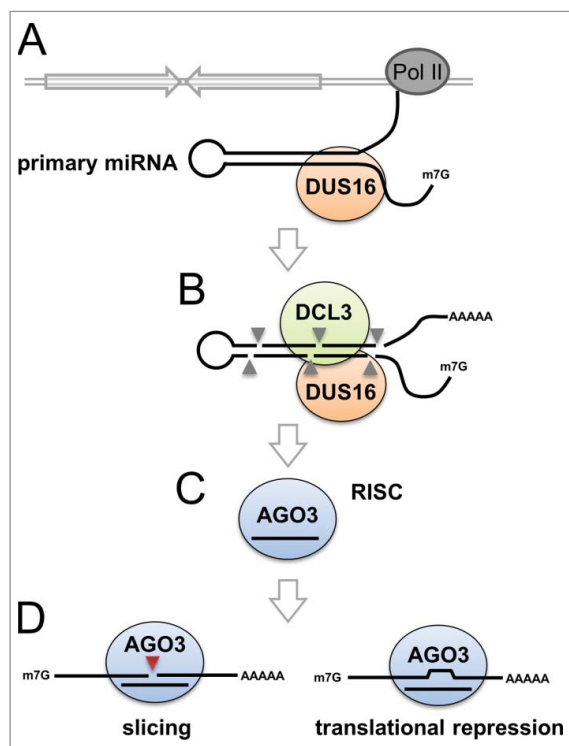
From the sRNA-seq raw data of CC-124, Gluc(1×), *ago3-1*, and *dus16-1*, adaptor sequences were removed and reads ranging from 17 to 25 nucleotides in length were selected for further analyses. The alignment of sorted sRNA reads from the Gluc(1×) sRNA library to the *C. reinhardtii* genome (Ch\_genome\_v5.0) using miRA,<sup>7</sup> an miRNA discovery tool for plants and algae, led to the identification of 1,062 inverted repeat loci encoding stem-loop RNAs. To stringently screen for genuine pri-miRNA genes, sRNA sequences with <10 read counts were excluded from the libraries, and the remaining redundant sRNA reads were aligned with *C. reinhardtii* gene models encompassing the inverted repeats using CLC genomic workbench (QIAGEN, <https://www.qiagenbioinformatics.com/products/clc-genomics-workbench/>). Gene models with <90 mapped-sRNA read counts in the sRNA libraries of CC-124 and Gluc(1×) and/or those without a predominant sRNA species on an arm of the predicted stem-loop structure were discarded.

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 Supplemental data for this article can be accessed on the publisher's website.

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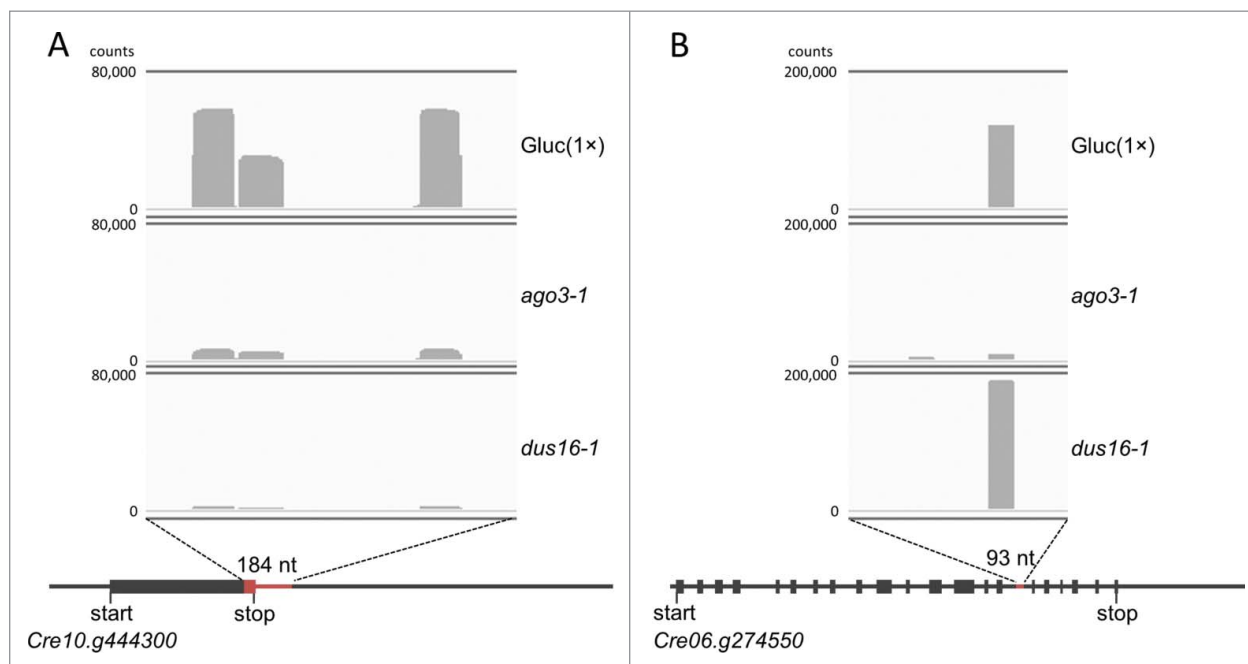


**Figure 1.** Model for miRNA biogenesis and action in *Chlamydomonas reinhardtii*. Dull slicer 16 (DUS16) recognizes nascent pri-miRNA transcripts (A). Dicer-like 3 (DCL3) mediates processing of most pri-miRNAs to miRNA duplexes with assistance of DUS16 (B). Argonaute 3 (AGO3) incorporates most *Chlamydomonas* mature miRNAs, having a U as their 5' nucleotide, and forms the RISC (C). AGO3-RISC recognizes target transcripts and induces slicing and/or translational repression (D).

Based on the above workflow, 35 gene models were annotated as pri-miRNA genes, including 9 previously unknown ones (Table 1).

A comparison of total sRNA read counts, mapped on the predicted pri-miRNA genes, from *dus16-1* and Gluc(1 $\times$ ) revealed that the production of mature sRNAs from 33 of the 35 pri-miRNAs is significantly lower in *dus16-1*, suggesting that these pri-miRNAs are mainly processed in a DUS16-dependent manner (Table 1, Fig. S1). Twenty-four of the 35 identified miRNA genes were previously annotated as pri-miRNAs by Valli *et al.* and are predominantly processed by DCL3 (annotated as “high confidence,” “medium confidence” and/or “upregulated” in Table 1, Fig. S1).<sup>5</sup> Furthermore, 22 of these 24 pri-miRNAs (91%) appear to require DUS16 for processing (Table 1; Fig. S1). This result suggests that, in addition to our previous finding of DUS16 physically interacting with DCL3,<sup>4</sup> DUS16 is functionally coupled to DCL3, presumably as part of a microprocessor complex involved in the processing of the majority of *C. reinhardtii* pri-miRNAs.

On the other hand, 2 pri-miRNA transcripts corresponding to *Cre04.g217925* and *Cre06.g274550*, which give rise to mature miR-1144 and miR-1162, respectively, are processed in a DCL3-dependent and DUS16-independent manner (Table 1). In the *ago3-1* mutant, the number of mature sRNAs generated from these pri-miRNAs is very low, indicating that most likely, they are authentic pri-miRNAs (Table 1, Fig. 2, Fig. S1). Some



**Figure 2.** Frequency (counts) of small RNA (sRNA) reads matching the inverted repeat regions of *Cre10.g444300* (A) and *Cre06.g274550* (B) in the AGO3 mutant (*ago3-1*), the DUS16 mutant (*dus16-1*), and their parental strain Gluc(1 $\times$ ). Schematic diagrams of gene structures, indicating predicted start and stop codons, are shown at the bottom of each panel. Inverted repeat regions are indicated in red. Gray bars represent the coverage of sRNA read counts on the corresponding sequences.

**Table 1. De novo prediction of primary and mature miRNAs.**

| microRNA precursor | Gluc(1X)           |                    |                   |                    | dus16-1            |                   |                    |                                     | Position of stem-loop (strand) | Length of stem-loop (nt) <sup>a</sup> | Location of stem-loop | MIR gene <sup>b</sup> | Voshall et al. <sup>9</sup> | Predicted as miRNA precursor with <sup>c</sup> confidence | Upregulated in the DCL3 mutant <sup>d</sup> | Mature miRNA sequences | Length (nt) | agg3-1 /Gluc (1x) <sup>e</sup> |
|--------------------|--------------------|--------------------|-------------------|--------------------|--------------------|-------------------|--------------------|-------------------------------------|--------------------------------|---------------------------------------|-----------------------|-----------------------|-----------------------------|---|---|------------------------|-------------|--------------------------------|
|                    | rep#1 <sup>b</sup> | rep#2 <sup>b</sup> | mean <sup>c</sup> | rep#1 <sup>b</sup> | rep#2 <sup>b</sup> | mean <sup>c</sup> | rep#1 <sup>b</sup> | rep#2 <sup>b</sup>                  |                                |                                       |                       |                       |                             |   |   |                        |             |                                |
| Cre01.g011500      | 13,070             | 12,102             | 12,586            | 2,202              | 1,741              | 1,972             | 0.16               | chromosome_1:2125423..2125712       | 290                            | intron                                | MIR906                |                       |                             |   | CGGTTGGTGGGGGTGATCAGC                       | 21                     | 2.10        |                                |
| Cre01.g023913      | 2,050              | 1,944              | 1,997             | 117                | 61                 | 89                | 0.04               | chromosome_1:37724948..37725125 (-) | 178                            | 3'UTR                                 |                       |                       | medium confidence           | upregulated   | TGACACATGGAACACACACACA                      | 22                     | 0.50        |                                |
| Cre01.g038350      | 3,881              | 3,515              | 3,698             | 84                 | 54                 | 69                | 0.02               | chromosome_1:5449977..5451159       | 1,183                          | 5'UTR-exon-intron                     |                       | Cluster14712          |                             | medium confidence   | upregulated                                 | TCATTGTGACAGGTTGGAG    | 21          | 0.27                           |
| Cre02.g143427      | 2,353              | 2,190              | 2,272             | 93                 | 40                 | 67                | 0.03               | chromosome_2:9129472..9129630       | 159                            | 3'UTR                                 |                       | Cluster16411          |                             | medium confidence   | upregulated                                 | TGCGTCTTCCGCCCTCTAGC   | 21          | 1.70                           |
| Cre03.g195950      | 7,435              | 6,963              | 7,199             | 128                | 89                 | 109               | 0.02               | chromosome_3:6573882..6574002       | 121                            | 3'UTR                                 |                       |                       |                             | medium confidence   | upregulated                                 | TGACATGGCGGTGAATGTGAAT | 21          | 0.51                           |
| Cre03.g206250      | 2,279              | 2,071              | 2,175             | 327                | 166                | 247               | 0.11               | chromosome_3:7376018..7376645       | 728                            | exon-intron                           |                       |                       |                             | medium confidence   | upregulated                                 | TACGGGCTCGTCTTCGGAGACA | 22          | 0.20                           |
| Cre04.g217925      | 2,154              | 2,159              | 2,157             | 4,616              | 3,668              | 4,142             | 1.92               | chromosome_4:457731..458006 (+)     | 276                            | intron-exon                           | MIR1144               |                       |                             | medium confidence   |   | 21                     | 0.71        |                                |
| Cre04.g220461      | 3,896              | 3,279              | 3,588             | 253                | 116                | 185               | 0.05               | chromosome_4:2304022..230586 (-)    | 1,565                          | 3 different model                     |                       |                       |                             | medium confidence   | upregulated                                 | AGAAAGCAGCTGGAATGATG   | 21          | 0.14                           |
| Cre04.g225700      | 55,579             | 52,177             | 53,878            | 2,535              | 2,010              | 2,273             | 0.04               | chromosome_4:3100596..3100778       | 183                            | intron                                | MIR1153               | Cluster17620          |                             | high confidence   |   | 22                     | 0.16        |                                |
| Cre05.g238343      | 17,163             | 15,132             | 16,148            | 206                | 117                | 162               | 0.01               | chromosome_5:2985422..2986713       | 1,293                          | exon-3'UTR-intron                     |                       |                       |                             | high confidence   | upregulated                                 | TGCCATCTTGGGACTCCTGG   | 21          | 0.09                           |
| Cre05.g239950      | 57,037             | 54,236             | 55,637            | 790                | 595                | 693               | 0.01               | chromosome_5:3227648..3227768 (-)   | 121                            | exon                                  |                       |                       |                             | high confidence   | upregulated                                 | AGGGGTGAAAAGTGTGGAATG  | 21          | 1.32                           |
| Cre05.g242180      | 6,071              | 5,467              | 5,769             | 0                  | 0                  | 0                 | 0.00               | chromosome_5:1813823..1814182 (-)   | 360                            | exon-3'UTR                            |                       |                       |                             | high confidence   |   | 22                     | 0.08        |                                |
| Cre05.g242301      | 6,514              | 5,970              | 6,242             | 14                 | 0                  | 7                 | 0.00               | chromosome_5:1814195..1814341 (-)   | 147                            | 5'UTR                                 | MIR913                |                       |                             | medium confidence   |   | 21                     | 0.23        |                                |
| Cre05.g247100      | 4,209              | 3,519              | 3,864             | 760                | 492                | 626               | 0.16               | chromosome_5:1790617..1790877       | 261                            | 3'UTR                                 | MIR918/919            | Cluster18100          |                             | medium confidence   |   | 21                     | 0.98        |                                |
| Cre06.g266052      | 19,750             | 18,386             | 19,068            | 349                | 202                | 276               | 0.01               | chromosome_6:2201552..2201759 (-)   | 208                            | intron-exon                           |                       | Cluster19166          |                             | high confidence   | upregulated                                 | TCTTGGGACGCTGTAGAGC    | 21          | 0.06                           |
| Cre06.g274550      | 123,480            | 117,542            | 120,511           | 19,0817            | 141,689            | 166,253           | 1.38               | chromosome_6:3067367..3067459       | 93                             | intron                                | MIR1162               | Cluster19538          |                             | high confidence   |   | 20                     | 0.32        |                                |
| Cre06.g278206      | 71,633             | 65,283             | 68,458            | 2,312              | 1,338              | 1,825             | 0.03               | chromosome_6:4031321..4031518       | 198                            | 5'UTR-exon                            | MIR907                |                       |                             | medium confidence   | upregulated                                 | TGTTGTAGTAGTTAGCCCTCC  | 22          | 0.08                           |
| Cre06.g295350      | 3,314              | 2,618              | 2,966             | 103                | 64                 | 84                | 0.03               | chromosome_6:6854015..6854278       | 264                            | exon-3'UTR                            |                       |                       |                             | medium confidence   |   | 20                     | 0.37        |                                |
| Cre07.g312650      | 1,933              | 1,875              | 1,904             | 96                 | 58                 | 77                | 0.04               | chromosome_7:77597..78113 (+)       | 517                            | exon-intron-3'UTR                     |                       |                       |                             | high confidence   | upregulated                                 | TCTTCCGAGCGGTCCGAGC    | 21          | 0.25                           |
| Cre08.g358535      | 12,763             | 12,226             | 12,495            | 412                | 269                | 341               | 0.03               | chromosome_8:121841..121961 (+)     | 121                            | 3'UTR                                 |                       | Cluster22587          |                             | high confidence   | upregulated                                 | TACAGACCTGATGAGGATG    | 21          | 0.22                           |
| Cre10.g444300      | 87,015             | 81,861             | 84,438            | 2,858              | 1,766              | 2,312             | 0.03               | chromosome_10:339862..340009        | 148                            | exon-3'UTR                            | MIR9897               | Cluster2675           |                             | medium confidence   | upregulated                                 | TGGCTTTCGTCGTCCTAGG    | 20          | 0.33                           |
| Cre10.g452700      | 33,037             | 28,233             | 30,635            | 1,841              | 1,280              | 1,561             | 0.05               | chromosome_10:459837..4598830       | 194                            | intron-exon                           |                       |                       |                             | medium confidence   |   | 20                     | 0.41        |                                |
|                    |                    |                    |                   |                    |                    |                   |                    |                                     |                                |                                       |                       |                       |                             |   | TACCGCGGTGGGAGGGCAGG                        | 22                     | 0.16        |                                |
|                    |                    |                    |                   |                    |                    |                   |                    |                                     |                                |                                       |                       |                       |                             |   | TTACGGCTCTTCTATCGGC                         | 21                     | 0.13        |                                |
|                    |                    |                    |                   |                    |                    |                   |                    |                                     |                                |                                       |                       |                       |                             |   | AGCGGATGATGATGAGAG                          | 21                     | 0.56        |                                |
|                    |                    |                    |                   |                    |                    |                   |                    |                                     |                                |                                       |                       |                       |                             |   | CTTGGCGGCTGAAGACATAG                        | 21                     | 0.52        |                                |

(Continued on next page)



**Table 1. (Continued)**

| microRNA precursor | Gluc(1X)                                   |                    |                    |                   |                    |                    |                   |                    |                                |                                | dus16-1                        |                          |                       |                       |                             |  |   |                        |             |                  | Valli et al. |  |  |  |  |  |  |  |  |  |
|--------------------|--|--------------------|--------------------|-------------------|--------------------|--------------------|-------------------|--------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------|-----------------------|-----------------------|-----------------------------|--|---|------------------------|-------------|------------------|--------------|--|--|--|--|--|--|--|--|--|
|                    | encoded proteins/domains                   | rep# <sup>1b</sup> | rep# <sup>2b</sup> | mean <sup>c</sup> | rep# <sup>1b</sup> | rep# <sup>2b</sup> | mean <sup>c</sup> | rep# <sup>2b</sup> | mean <sup>c</sup>              | dus16-1 /Gluc(1X) <sup>d</sup> | Position of stem-loop (strand) | Length (nt) <sup>e</sup> | Location of stem-loop | MIR gene <sup>f</sup> | Voshall et al. <sup>g</sup> | Predicted as miRNA precursor with <sup>h</sup> | Upregulated in the DCL3 mutant <sup>i</sup> | Mature miRNA sequences | Length (nt) | ago3-1 /Gluc(1X) |              |  |  |  |  |  |  |  |  |  |
| Cre10.g464300      | no putative conserved proteins/ domains    | 35,962             | 33,490             | 34,726            | 2,437              | 1,685              | 2,061             | 0.06               | chromosome_10:6199729..6199816 | (+)                            | 88                             | intron                   |                       |                       |                             | upregulated                                    | ATCTCGTCGTCAGGCTTG                          | 21                     | 0.61        |                  |              |  |  |  |  |  |  |  |  |  |
| Cre11.g467650      | conserved hypothetical protein             | 12,488             | 10,717             | 11,603            | 2,461              | 1,773              | 2,117             | 0.18               | chromosome_11:1824675..1824782 | (+)                            | 108                            | 3'UTR                    |                       |                       |                             | upregulated                                    | AAGGACGGCTCTGTACTGACG                       | 22                     | 0.63        |                  |              |  |  |  |  |  |  |  |  |  |
| Cre12.g536301      | no putative conserved proteins/ domains    | 28,770             | 24,661             | 26,716            | 272                | 134                | 203               | 0.01               | chromosome_12:6166877..6167231 | (+)                            | 355                            | intron-3 UTR             |                       |                       |                             | upregulated                                    | TGGCAAAAGAGAAAGCGGAGC                       | 21                     | 0.29        |                  |              |  |  |  |  |  |  |  |  |  |
| Cre13.g576700      | conserved hypothetical protein             | 17,130             | 15,285             | 16,208            | 152                | 95                 | 124               | 0.01               | chromosome_13:2001062..2001207 | (-)                            | 146                            | 3'UTR                    | MIR1159               | Cluster7085           | medium confidence           | upregulated                                    | AAGCAGTCAGGTAGAAAGCC                        | 20                     | 0.66        |                  |              |  |  |  |  |  |  |  |  |  |
| Cre13.g579050      | anaphase promoting complex subunit 1       | 105061             | 90,219             | 97,640            | 3,332              | 2,426              | 2,879             | 0.03               | chromosome_13:2301400..2301727 | (-)                            | 328                            | 3'UTR                    |                       |                       |                             | upregulated                                    | TGACTCTACTCTACTCTGGC                        | 21                     | 0.23        |                  |              |  |  |  |  |  |  |  |  |  |
| Cre14.g615950      | translation elongation factor 3            | 40,709             | 36,425             | 38,567            | 1,913              | 1,337              | 1,625             | 0.04               | chromosome_14:1191293..1192047 | (-)                            | 755                            | intron                   | MIR1159               |                       |                             | upregulated                                    | TGTTTGTGTGACGTGGTCTT                        | 21                     | 0.25        |                  |              |  |  |  |  |  |  |  |  |  |
| Cre16.g686203      | no putative conserved proteins/ domains    | 2,430              | 2,412              | 2,421             | 0                  | 0                  | 0                 | 0.00               | chromosome_16:4838378..4838521 | (+)                            | 144                            | intron-exon              |                       |                       |                             | upregulated                                    | CGGGCAGTCCGGGCACTGTGGC                      | 21                     | 0.57        |                  |              |  |  |  |  |  |  |  |  |  |
| Cre16.g686398      | no putative conserved proteins/ domains    | 1,133              | 1,008              | 1,071             | 21                 | 0                  | 11                | 0.01               | chromosome_16:7434870..7435158 | (+)                            | 289                            | 5' UTR-exon-intron       |                       |                       |                             | upregulated                                    | TCCTTGTGGCTAGGGCCCTTG                       | 21                     | 0.07        |                  |              |  |  |  |  |  |  |  |  |  |
| Cre17.g697550      | no putative conserved proteins/ domains    | 32,592             | 28,227             | 30,410            | 6,170              | 4,240              | 5,205             | 0.17               | chromosome_17:194516..194869   | (+)                            | 354                            | exon-intron-exon         |                       |                       |                             | upregulated                                    | TGCACGCTGTGACTGTCTAGC                       | 21                     | 1.12        |                  |              |  |  |  |  |  |  |  |  |  |
| Cre17.g697800      | chromosome segregation protein             | 1,527              | 1,308              | 1,418             | 458                | 330                | 394               | 0.28               | chromosome_17:228757..228889   | (+)                            | 133                            | intron                   |                       |                       |                             | upregulated                                    | ATGCACGGCACGGGCGCGGT                        | 21                     | 0.62        |                  |              |  |  |  |  |  |  |  |  |  |
| Cre17.g735375      | FAP164, flagellar A-associated protein 164 | 60,726             | 54,267             | 57,497            | 28,321             | 21,351             | 24,836            | 0.43               | chromosome_17:5152751..5152951 | (-)                            | 201                            | 5'UTR                    |                       | Cluster12364          | medium confidence           | upregulated                                    | CGGTCTGTAGCATCAAAACG                        | 22                     | 0.95        |                  |              |  |  |  |  |  |  |  |  |  |
| Cre17.g741601      | no putative conserved proteins/ domains    | 12,965             | 11,544             | 12,255            | 369                | 223                | 296               | 0.02               | chromosome_17:6144100..6144226 | (-)                            | 127                            | 3'UTR                    |                       | Cluster12551          | high confidence             | upregulated                                    | TCGGAGAAGCGGGTAGCTGAGG                      | 22                     | 0.41        |                  |              |  |  |  |  |  |  |  |  |  |
| Cre24.g755697      | conserved hypothetical protein             | 3,094              | 2,846              | 2,970             | 276                | 211                | 244               | 0.08               | scaffold_24:82169..82327       | (+)                            | 159                            | 3'UTR                    | MIR1172               |                       | medium confidence           | upregulated                                    | TAAACAGACAAGGGACCGGACA                      | 22                     | 0.42        |                  |              |  |  |  |  |  |  |  |  |  |
|                    |  |                    |                    |                   |                    |                    |                   |                    |                                |                                |                                |                          |                       |                       |                             | upregulated                                    | AGGATTGCAGCAACACGGGGC                       | 22                     | 0.44        |                  |              |  |  |  |  |  |  |  |  |  |

Notes. <sup>a</sup>Phytozome ([https://phytozome.jgi.doe.gov/pz/portal.html#info?alias=Org\\_Creihardtii](https://phytozome.jgi.doe.gov/pz/portal.html#info?alias=Org_Creihardtii)).

<sup>b</sup>Absolute sRNA read counts from the individual sRNA libraries that align to each gene model.

<sup>c</sup>Mean values of 2 replicates.

<sup>d</sup>Ratio of the means of abundant mature miRNAs in *dus16-1* over *Gluc(1X)*.

<sup>e</sup>Length of the sequences corresponding to a stem-loop RNA.

<sup>f</sup>miRBASE (<http://www.mirbase.org/>).

<sup>g</sup>Previously annotated pri-miRNA genes published by Voshall et al.<sup>12</sup>

<sup>h</sup>Previously annotated pri-miRNA genes with high or medium confidence interval published by Valli et al.<sup>5</sup>

<sup>i</sup>Putative pri-miRNA genes with abundant upregulated transcripts in the *DCL3* mutant (Valli et al.).<sup>5</sup>

<sup>j</sup>Ratio of the means of abundant mature miRNAs in *ago3-1* over *Gluc(1X)*.

sRNAs are also produced from the transcripts of inverted repeats in a DCL3-independent manner.<sup>5</sup> These results imply the presence of minor DUS16- and/or DCL3-independent pri-miRNA-processing pathways in *C. reinhardtii*.

*C. reinhardtii* appears to possess canonical miRNA biogenesis pathways and miRNA-mediated post-transcriptional gene regulation with certain similarities to those in animals and plants<sup>8,9,10,11,12</sup> Mutant analyses revealed that the initial processing of the majority of pri-miRNAs relies on a putative microprocessor complex comprising both DUS16 and DCL3.<sup>4,5</sup> In addition, our analyses also uncovered a minor set of pri-miRNAs that are likely processed in a DUS16 and/or DCL3-independent manner.

### Accession numbers

Small RNA-seq raw data has been deposited in the DDBJ sequence read archive (DRA) under accession numbers DRA003930 and DRA004107 (CC-124 replicate #1, DRX040414; CC-124 replicate #2, CDRX040415; Gluc1(×) replicate #1, DRX040416; Gluc1(×) replicate #2, DRX040417; *ago3-1* replicate#1, DRR045098; *ago3-1* replicate#2 DRR045099; *dus16-1* replicate #1, DRX043778; and *dus16-1* replicate #2, DRX043779).

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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