miRNAs in the alga *Chlamydomonas reinhardtii* are not phylogenetically conserved and play a limited role in responses to nutrient deprivation

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TGCGGAACTACAGGCGACCCT	0.729	2.538	0.904
GCGGAACTACAGGCGACCCT	0.146	1.137	0.52
GCGACCCTCGACCAGAGCCGT	0.049	0.000	0.00
CGACCAGAGCCGTCCGCCGGCT	0.146	0.038	0.03
miRNAs identified in MS+N Only :			

miRNA: miR_t1 chromosome_9 Start: 5020265 End: 5020545 Condition: H

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miENA: miE t6 chromosome 17 Start: 5228339 End: 5228527 Condition: NS+N











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-TACCCGAACGTCGAGTTCAAGT	0.000	0.000	0.082
-TACCCGAACGTCGAGTTCAAG	0.808	0.800	0.897
TACCCGAACGTCGAGTTCAA	0.000	0.000	0.082
AGCTCTACCCGAACGTCGAG	0.000	0.000	0.082
- TCGAGCTCTACCCGAACGTCG	0.147	0.200	0.082
	0.220	0.000	0.163
-AAGACCTTCGAGCTCTACCCG	0.808	0.800	0.571
-AAGACCTTCGADCTCTACCCGA-	0.073	0.000	0.082
-AAGACCTTCGAGCTCTACC	0.000	0.200	0.082
	0.514	3.101	0.652
	0.073	0.300	0.082
-CCAAGACCTTCGADCTCTA-	0.000	0.100	0.082
	0.147	0.000	0.163
	0.147	0.400	0.326
TCCCMAGACCTTCGAGCCCTAC-	0.220	0.100	0.326
TCCCAAGACTTTCGAGETC	0.000	0.000	0.082
-TTCCCMAGACCTTCGAGETCT-	0.661	1.900	1.712
-TTCCCAAGACCTTCGAGETC	1.689	2.200	2.038
-TTCCCAAGACCTTCGAGCCTTAC	1.028	1.000	2.772
-TTCCCAAGACCTTCGAGETCTA	0.220	0.500	0.734
-TTCCCAAGACCTTCGAGC	0.073	0.100	0.245
-TTCCCMAGACCTTCGAGETCTACC	0.661	1.000	0.652
-TGACCOTCCTCCTTCCCAAG-	0.000	0.200	0.082
-GC07GACCGTCCTCCCTCCC	0.587	0.700	1.060
- OCOTGACCOTCCTCCCA-	1.028	0.500	0.408
	0.294	1.000	0.082
-GOCOTGACCGTCCTCCCCA	0.000	0.000	0.082
- GOODTGACCOTCCTCC	0.220	0.300	0.326
-GGGTTGACCOTCCTC	0.073	0.300	0.163
-GOODTGACCOTCCTCC	0.294	4.201	1.223
-GGGTTGACCOTCCTCCTCCC	0.808	1.900	0.978







0.126 0.2072 0.772 0.772 0.772 0.205 0.245 1.631 0.408 0.000 7.420 0.163 8.367 1.712

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INAX: MIK_S CEROMONOM_4 Start: 3694//3 End: 3695093 Condition: Ph	wpate-owricient					CTTAGTCGTCACAAGGCGTG	0.220	0.100	0.082
						-ACTTAGTCGTCACAAGGCGTG	24.376 0.220	27.706	63.111 0.245 0.245
						AACTTAGTCGTCACAAGGCGTG	0.147 0.220	0.300	0.978 0.245
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miRNA: miR_t13,miR_t109 chromosome_10 Start: 3399866 End: 3400007 Con	dition: Nutrient-replete						0.000	0.000	0.001
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TAAGAAGGAGCCGTAAGGT TAAGAAGGAGCCGTAAGGTAC	17.254 38.908 26.092 2.863 3.701 3.180								
TANGANGGCCGTANGG TANGANGGCCGTANGGTACC	2.717 7.602 2.691 0.073 0.100 0.000								
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-AGGTACCOGGCGTOGGGAGGGC	1.615 0.300 0.897 2.790 0.400 1.142								
	0.294 0.000 0.000 0.294 0.000 0.000								
GTACCGOGCGTGOOGAGGGCAG	2.570 0.900 1.468 0.073 0.000 0.000								
GTACCGGGCGTGGGGGGGGGGGGGGGGGGGGGGG	1.762 0.700 0.897 0.073 0.000 0.000 2.818 0.700 1.067								
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			0.147 0.000 0.073 0.000	0.000					
	GGAGCAGCTGCGCACAGCCCT		0.073 0.000	0.000					



Figure S1. Secondary structure plots of predicted miRNA precursor loci and frequency of reads from the Chlamydomonas AGO3-associated sRNA libraries or from the total sRNA libraries prepared by Chávez Montes et al.²⁵. For AGO3-associated sRNAs, the first column indicates read abundance (Counts Per Million mapped reads) in libraries prepared from cells grown mixotrophically (TAP), the second column indicates read abundance in libraries prepared from cells grown photoautotrophically in nitrogen depleted minimal medium (HS-N) and the third column indicates read abundance in libraries prepared from cells grown photoautotrophically in nutrient replete minimal medium (HS+N). For the miRNAs identified in the libraries prepared by Chávez Montes et al.²⁵, the first column indicates read abundance (CPM) in the libraries from cells grown in nutrient replete medium, the second column indicates read abundance in the libraries from cells grown in sulfur deprived medium and the third column indicates read abundance in the libraries from cells grown in phosphate deprived medium. Mature miRNAs are indicated in red and passenger strands (miRNA*), when detected, are indicated in blue. Each plot is annotated with the locus ID (miR t#), the chromosome, the chromosomal position (start and end), and the trophic condition(s) under which the miRNA was preferentially identified. Loci with long hairpins may code for more than one candidate miRNA. Additionally, some loci code for more than one hairpin in a tandem arrangement (clustered miRNA precursor loci) and, in these cases, the folding of each hairpin is shown separately.



Figure S2. U6 small nuclear RNA abundance in Chlamydomonas cells grown under the denoted trophic conditions. Transcript abundance was examined by semi-quantitative RT-PCR. The panels show representative reverse images of agarose resolved RT-PCR products stained with ethidium bromide. CC-124, wild type strain (mt⁻); Maa7-IR44s, CC-124 containing a transgene expressing FLAG-tagged AGO3; Mut-20, *TSN1* deletion mutant, in the Maa7-IR44s background, defective in sRNA biogenesis¹⁴. Amplification of the mRNA corresponding to *ACT1* (encoding actin) was used as a control for equivalent amounts of input RNA and for the efficiency of the RT-PCRs [Msanne, J. *et al. Phytochemistry* **75**, 50-59 (2012)].



Figure S3. Northern blot analysis of miRNA expression in Chlamydomonas cells grown under the denoted trophic conditions. (A) Small RNAs were detected with probes specific for the indicated miRNAs. The same filters were reprobed with the U6 small nuclear RNA sequence as a control for lane loading. CC-124, wild type strain (mt⁻); CC-125, wild type strain (mt⁺); Maa7-IR44s, CC-124 containing a transgene expressing FLAG-tagged AGO3; Mut-20, *TSN1* deletion mutant, in the Maa7-IR44s background, defective in sRNA biogenesis¹⁴. (B) Relative miRNA levels in the indicated strains under the different trophic conditions. Values shown are the average of two independent experiments and are normalized to those of the CC-124 strain (the CC-125 strain for read counts) grown mixotrophically in nutrient replete medium (TAP). The relative standard deviation, as percentage of the mean, was in no case higher than 34.7%. Data corresponds to phosphorimager measurements of sRNA signals on northern blots (gray bars) or normalized read counts from the total sRNA libraries prepared by Chávez Montes *et al.*²⁵ (white bars).



Figure S4. Gene expression in Chlamydomonas cells grown under the denoted trophic conditions. Transcript abundance was examined by semi-quantitative RT-PCR. The panels show representative reverse images of agarose resolved RT-PCR products stained with ethidium bromide. CC-124, wild type strain (mt); Maa7-IR44s, CC-124 containing a transgene expressing FLAG-tagged AGO3; Mut-20, *TSN1* deletion mutant, in the Maa7-IR44s background, defective in sRNA biogenesis¹⁴. Examined genes included *PHO5*, encoding a phosphate-repressible alkaline phosphatase, *SLT1* (SAC1-*LIKE TRANSPORTER1*), encoding a sodium/sulfate cotransporter, and the *U6* small nuclear RNA. Amplification of the mRNA corresponding to *CBLP* (also known as *RECEPTOR OF ACTIVATED PROTEIN KINASE C1, RCK1*) was used as a control for equivalent amounts of input RNA and for the efficiency of the RT-PCRs [Aksov, M. *et al. Plant Physiol* **162**, 195-211 (2013)].



Figure S5. Quantitative RT-PCR analysis of predicted miRNA-target transcript abundance. (A) Relative transcript levels of *Cre04.g227600* (target of c12364), *Cre06.g249550* (target of c18100a) and *Cre12.g552950* (target of miR_t70) in the indicated strains. Maa7-IR44s, CC-124 strain containing a transgene expressing FLAG-tagged AGO3; Mut-20, *TSN1* deletion mutant, in the Maa7-IR44s background, defective in sRNA biogenesis¹⁴; Gluc(1x), wild type strain derived from CC-124; ago3-1, *AGO3* disrupted mutant, in the Gluc(1x) background, defective in RNAi²⁸. Values, normalized to those in the wild type strains, are means \pm SD of three independent experiments. (B) Diagram of the *Cre12.g552950* gene (from Phytozome v11, coding region shown in orange), indicating the binding sites for miR_t70 and two putative, perfectly complementary, siRNAs.



Figure S6. Relationship between miRNA expression level and number of predicted targets in *C. reinhardtii.* The expression level and the number of predicted targets (blue diamonds for cleavage targets and orange squares for translational repression targets) are plotted for each miRNA identified in the AGO3-pulldown libraries from *C. reinhardtii.*