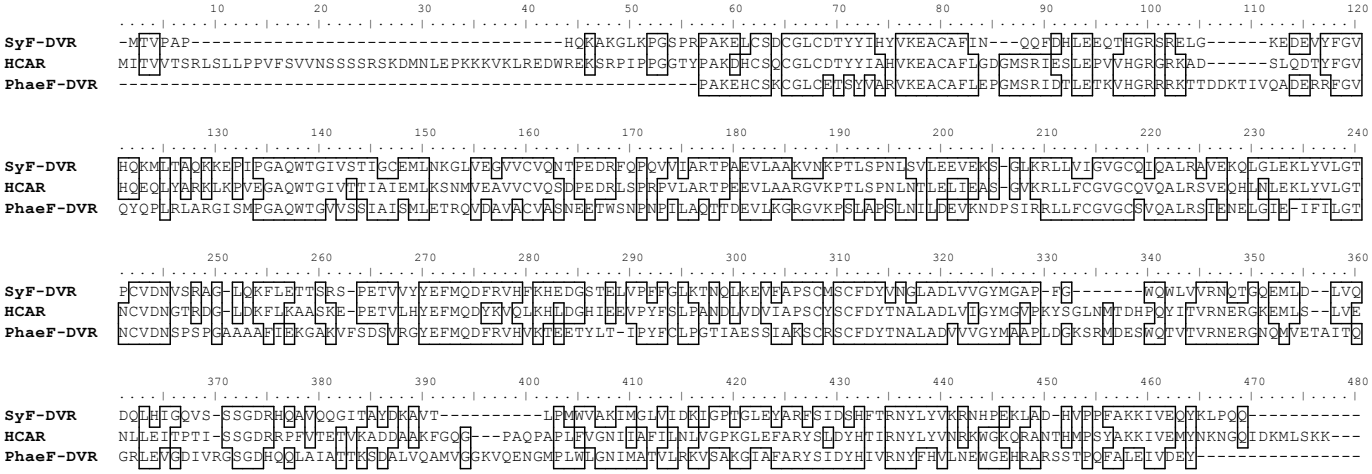
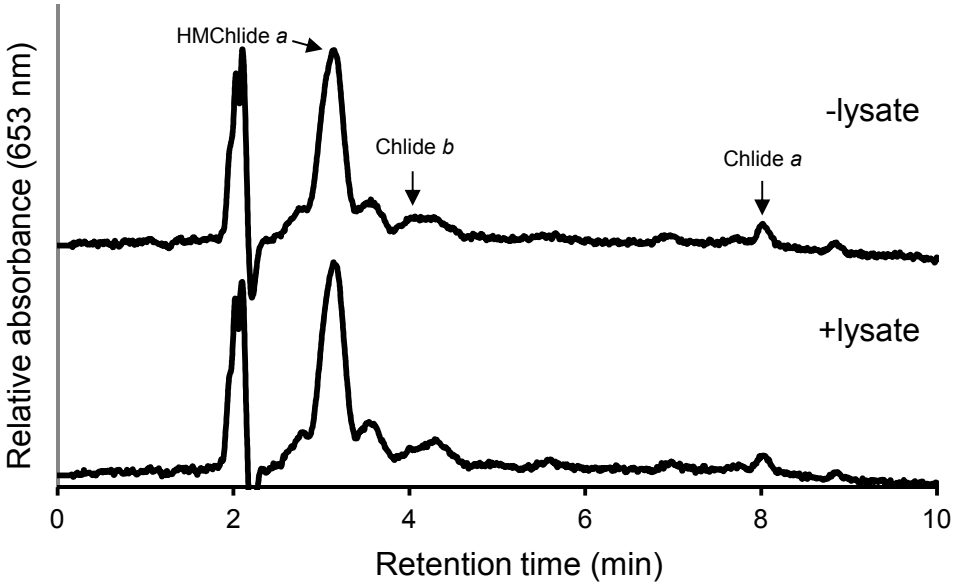
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Title	Evolution of a New Chlorophyll Metabolic Pathway Driven by the Dynamic Changes in Enzyme Promiscuous Activity
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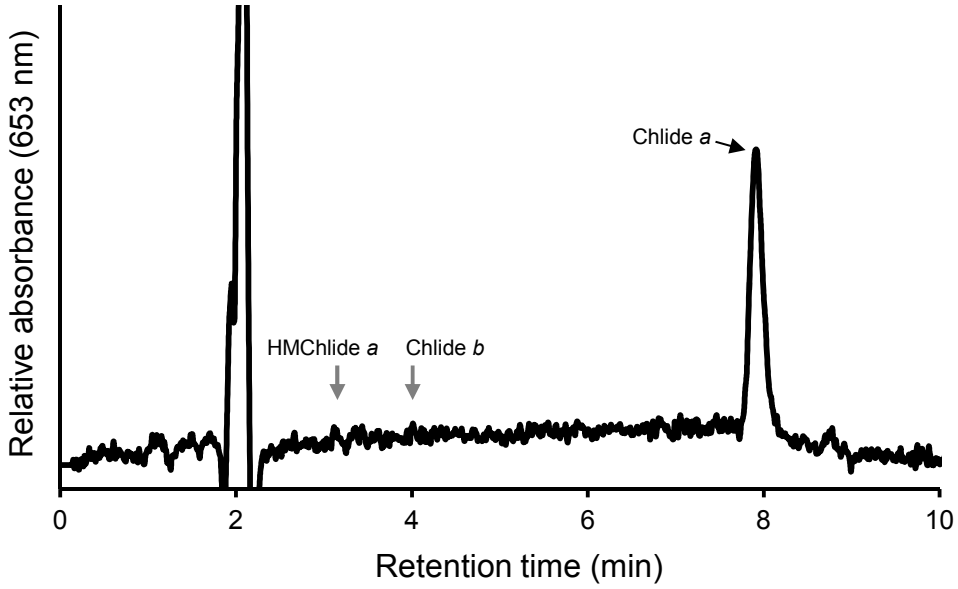
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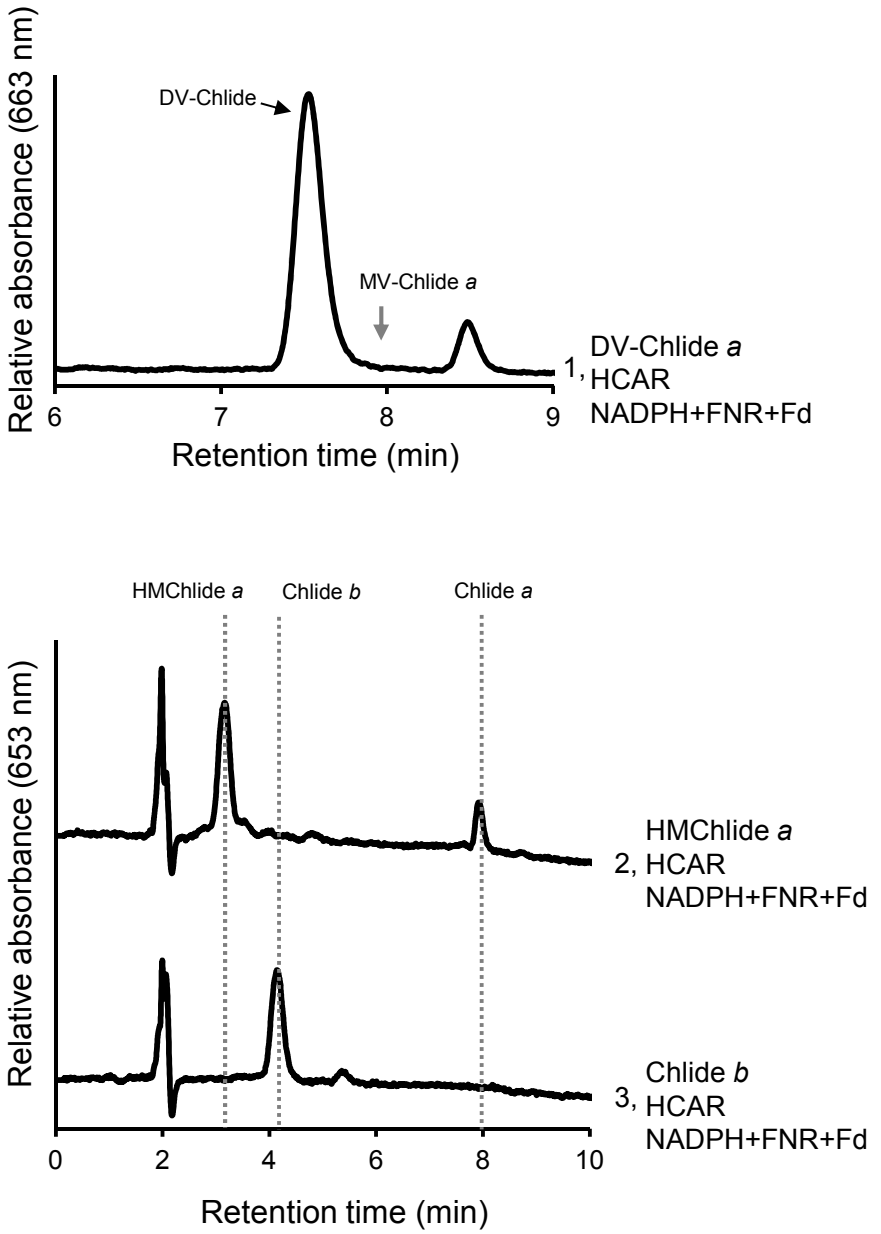
Supplementary Figure S1. Multiple amino acid sequence alignment of *Synechocystis* F-DVR, *Arabidopsis* HCAR and predicted F-DVR of *Phaeodactylum*. *Synechocystis* F-DVR (SyF-DVR) (accession No. NP_441896), *Arabidopsis* HCAR (accession No. XP_002285592) and predicted F-DVR of *Phaeodactylum* (PhaeF-DVR) (accession No. XP_002186085) sequences were aligned using ClustalW, as implemented in BioEdit. The conserved residues are outlined.



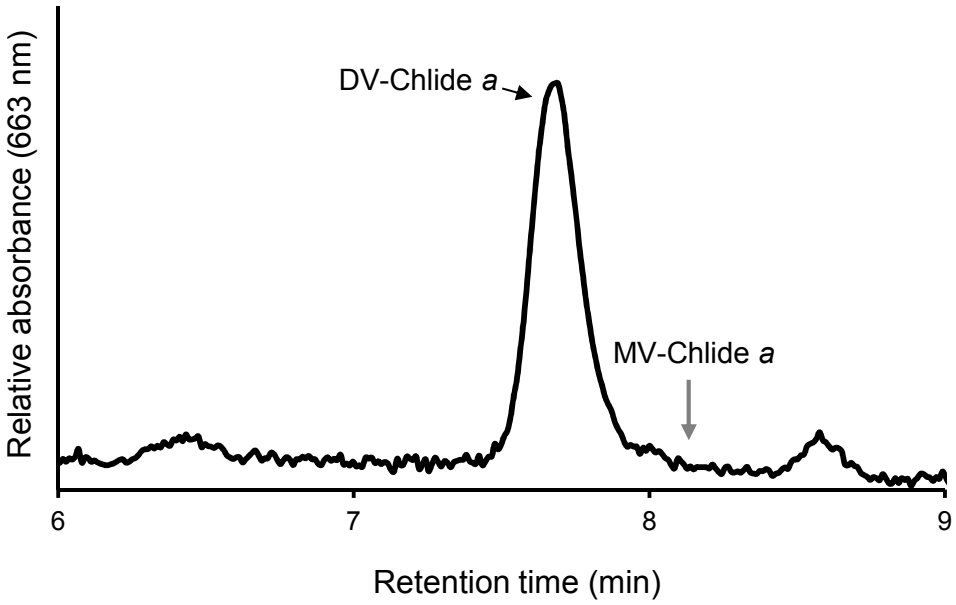
Supplementary Figure S2. The effects of *E. coli* lysate on the *Synechocystis* F-DVR activity. HMChlide *a* and purified *Synechocystis* F-DVR were incubated with or without a culture lysate of *E. coli* containing the empty pET vector. NADPH, FNR and Fd were included in the reaction mixture. The pigment composition after incubation were analyzed by HPLC.



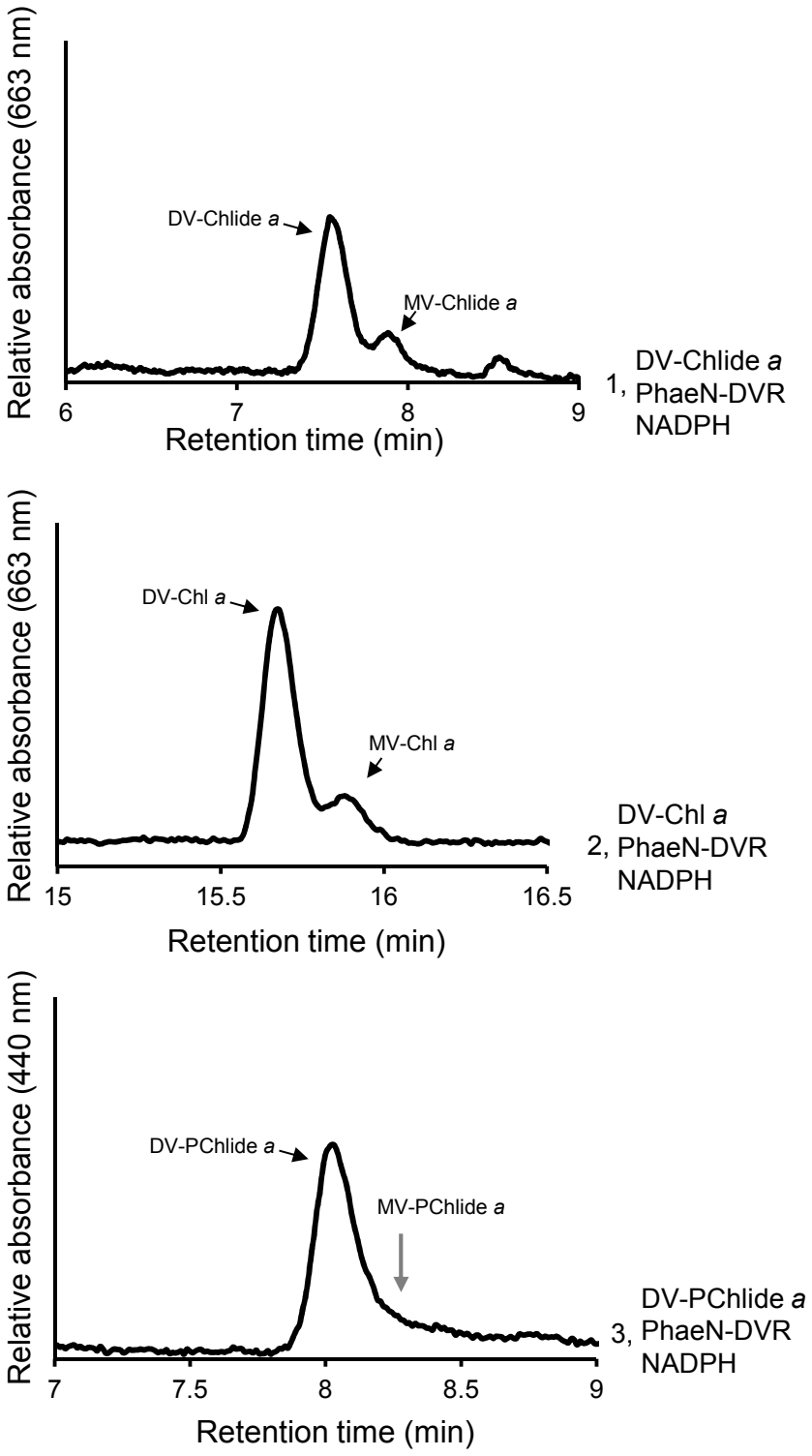
Supplementary Figure S3. *Synechocystis* F-DVR activity on Chlide *a*. Chlide *a* was incubated with a culture lysate of *E.* coli containing *Synechocystis* F-DVR under the same conditions of Fig. 4C. NADPH, FNR and Fd were included in the reaction mixture. The pigment composition after incubation were analyzed by HPLC. Gray arrows indicate elution positions of HMChlide *a* and Chlide *b*.



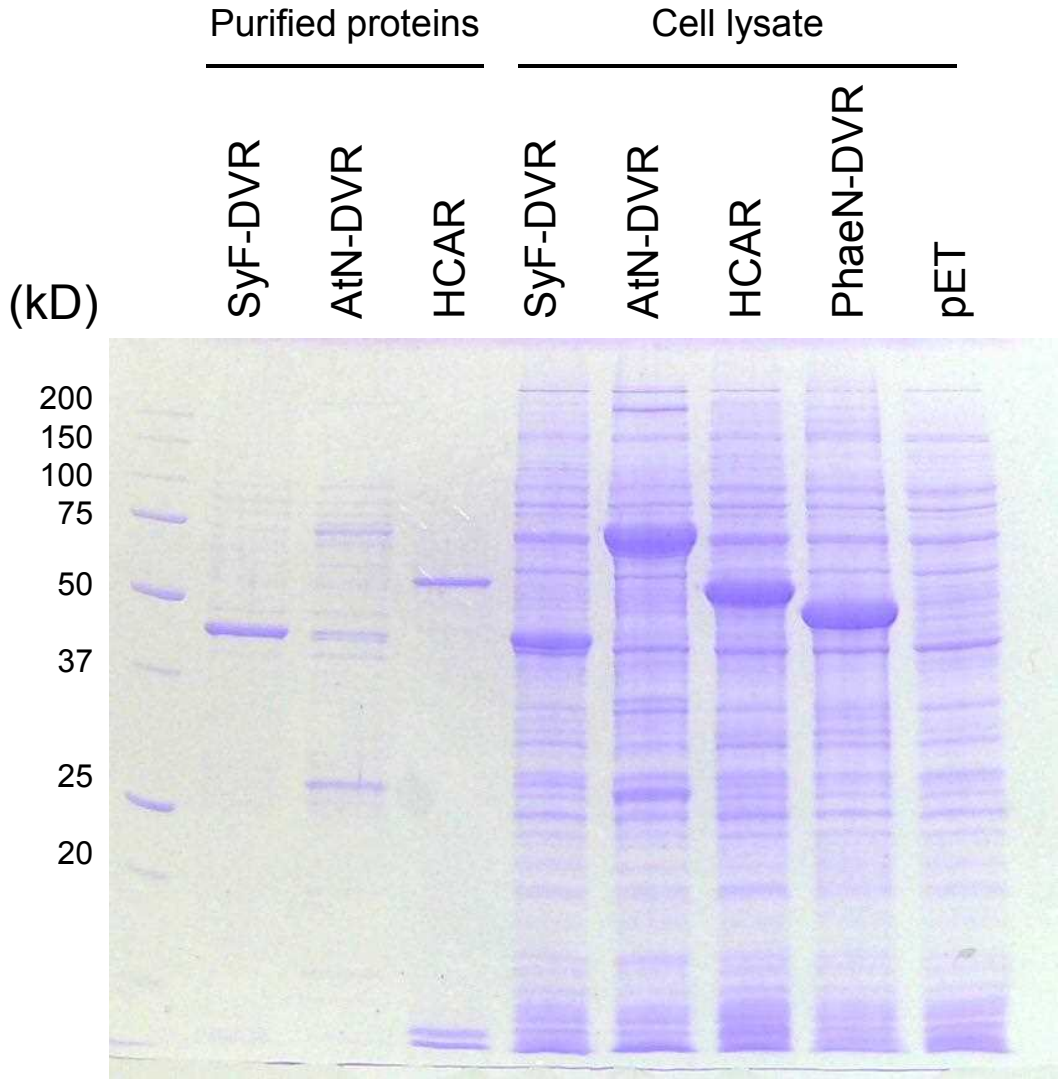
Supplementary Figure S4. HCAR activity toward DV-Chlide *a*, HMChlide *a* and Chlide *b*. DV-Chlide *a*, HMChlide *a* and Chlide *b* were incubated with purified recombinant *Arabidopsis* HCAR. All reaction mixtures contained NADPH, FNR and Fd. The pigment compositions were analyzed by HPLC. 1, DV-Chlide *a* incubated with HCAR. 2, HMChlide *a* incubated with HCAR. 3, Chlide *b* incubated with HCAR. Gray arrow indicates elution position of MV-Chlide *a*.



Supplementary Figure S5. NADH effect on DV-Chlide *a* reduction. DV-Chlide *a* was incubated with purified *Synechocystis* F-DVR and NADH. The pigment compositions were analyzed by HPLC. Gray arrow indicates elution position of MV-Chlide *a*.



Supplementary Figure S6. Substrate specificity of *Phaeodactylum* N-DVR. The *Phaeodactylum* gene homologous to *Arabidopsis* N-DVR (PhaeN-DVR) was expressed in *E. coli*, and the lysate was used for the analysis of DVR activity. After incubation of the pigments with the *E. coli* lysate, the pigment compositions were analyzed by HPLC. 1, DV-Chlide *a* incubated with PhaeN-DVR. 2, DV-Chl *a* incubated with PhaeN-DVR. 3, DV-PChlide *a* incubated with PhaeN-DVR. Gray arrow indicates elution position of MV-PChlide *a*.



Supplementary Figure S7. SDS-PAGE analysis monitoring the expression and purification of recombinant proteins. Purified recombinant proteins (1 µg) and *E. coli* lysate (0.5 µL) were loaded onto a 12% acrylamide gel, separated with SDS-PAGE and Coomassie Brilliant Blue stained. For enzyme assay, 10 µg of purified proteins or 10 µL of lysate were used. *Synechocystis* F-DVR (SyF-DVR), *Arabidopsis* N-DVR (AtN-DVR) and *Arabidopsis* HCAR were purified using a nickel column. AtN-DVR was further purified using an ion exchange column. PhaeN-DVR; *Phaeodactylum* N-DVR.

Supplementary Table S1. Primers used in the construction

Gene	Forward primer	Reverse primer
Synechocystis F-DVR	5'-CATATGACCGTTCCTGCCCCCCA-3'	5'-CTCGAGTTGCTGGGGAAGTTTATA-3'
Arabidopsis N-DVR	5'-CATATGTCACTTTGCTCTTCCTT-3'	5'-CTCGAGGAAGAACTGTTCACCGAGTT-3'
Arabidopsis HCAR	5'-GGATCCTCCGTCGTTAACTCTTCTTC-3'	5'-CTCGAGTTTCTTGGAGAGCATTTTAT-3'
Phaeodactylum N-DVR	5'-GGATCCATGAGTGTGCGTCGGTTCCG-3'	5'-CTCGAGCTTGCCGGTCACGGGTTCGG-3'