


Cloning of a novel endogenous promoter for foreign gene expression in *Phaeodactylum tricoratum*

Erdenedolgor Erdene-Ochir^{1,2} · Bok-Kyu Shin¹ ·
Md Nazmul Huda^{1,2} · Da Hye Kim¹ · Eun Ha Lee¹ ·
Dae-Geun Song¹ · Yoon-Mi Kim^{1,2} · Sang Min Kim^{1,2} ·
Cheol-Ho Pan^{1,2} 

Received: 20 October 2016 / Accepted: 11 November 2016 / Published online: 18 November 2016
© The Korean Society for Applied Biological Chemistry 2016

Abstract *Phaeodactylum tricoratum* is a model diatom, and its genomic sequence data and expressed sequence tag databases are available. This study was to discover a new endogenous promoter that drives strong constitutive expression of a protein of interest in *P. tricoratum*. To find promoter candidates, the intracellular proteins of *P. tricoratum* grown to stationary phase were extracted and identified by LC–MS/MS. Glutamine synthetase (GLNA) was one of the most abundantly expressed proteins during the stationary phase. Promoter is usually located on 5' upstream region of open reading frame of the gene. Thus, two fragments of 5' upstream region of the *GLNA* gene as putative promoters, 501 and 997 bp long, were amplified and cloned into enhanced Green Fluorescent Protein (eGFP) reporter systems. The constructed reporter systems were transformed into *P. tricoratum* and the eGFP expression levels were compared to those of reporter systems using the promoters of *fcpA* (fucoxanthin chlorophyll *a/c* binding protein A) and CIP1 (putative replication-associated proteins of a *Chaetoceros lorenzianus*-infecting DNA virus) as controls. The expression of eGFP driven by either *GLNA* promoter (501 and 997 bp) was linearly

related to cell density, and eGFP was expressed constitutively regardless of the cultivation phase. The eGFP expression level driven by the *GLNA* promoters was at least 4 times higher than the *fcpA*-driven eGFP expression level at the stationary phase. The 501 and 997 bp regions of the *GLNA* promoter had similar activity patterns for transcribing the downstream gene. These results indicate that at least 501-bp region of the *GLNA* promoter can be used as a strong constitutive promoter in genetic engineering of *P. tricoratum*.

Keywords Constitutive expression · Glutamine synthetase promoter · *Phaeodactylum tricoratum* · Transformation

Introduction

Alongside the gradual increase in the human population, the accompanying consumption of material goods is also increasing. The most important things include food, fuel, and pharmaceuticals. Nonrenewable natural resources will be exhausted in the near future, and we will need new systems that are able to create these useful products. Over the past few decades, researchers have made substantial progress in this field and are already developing these systems. Many of these new production systems involve the use of diatoms, which are eukaryotic phytoplankton which is able to fixate carbon and nitrogen sources while producing oxygen (Saade and Bowler 2009). Diatoms are increasingly used as cellular “factories” for commercial and industrial applications, including the production of biopharmaceuticals, recombinant proteins, and biofuels. Diatoms are preferred for these applications because they grow relatively rapidly and require only seawater, light, and relatively inexpensive inorganic nutrients such as

Electronic supplementary material The online version of this article (doi:10.1007/s13765-016-0235-y) contains supplementary material, which is available to authorized users.

✉ Cheol-Ho Pan
panc@kist.re.kr

¹ Systems Biotechnology Research Center, KIST Gangneung Institute of Natural Products, Gangneung 25451, Republic of Korea

² Department of Biological Chemistry, Korea University of Science and Technology (UST), Daejeon 34113, Republic of Korea

nitrogen, phosphorus, and carbon (Qin et al. 2012; Kadono et al. 2015).

Phaeodactylum tricornerutum is a model diatom, and both its genome sequence and an expressed sequence tag (EST) database spanning 16 different growth conditions (Bowler et al. 2008) are available. In addition, molecular toolbox for genetic modification has been established (Apt et al. 1996; Siaux et al. 2007; De Riso et al. 2009). *P. tricornerutum* has been genetically engineered to produce various valuable products such as polyunsaturated fatty acids (Hamilton et al. 2014), glycerol, neutral lipid (Yao et al. 2014), and recombinant protein (Hempel et al. 2011). Generally, the strong endogenous *fc*p promoter, which was isolated and characterized from very closely related tandem genes that encode fucoxanthin chlorophyll *a/c* binding proteins (Bhaya and Grossman 1993), has been used in the genetic manipulation of *P. tricornerutum*. Lately, the heterologous promoter *CIP1* has been isolated from *Chaetoceros lorenzianus*-infecting DNA virus and applied in *P. tricornerutum* (Kadono et al. 2015). Unfortunately, *fc*p promoter is highly dependent on light and the circadian rhythms of diatom species (Falcioratore et al. 1999; Seo et al. 2015). *CIP1*, meanwhile, is suboptimal because heterologous promoters may be less efficient than endogenous promoters (Diaz-Santos et al. 2013). Thus, a strong, endogenous, constitutive promoter for foreign gene expression and metabolic engineering in *P. tricornerutum* is urgently needed.

This work identifies a novel endogenous promoter that can constitutively express recombinant proteins in *P. tricornerutum*. To do this, we first analyzed proteins expressed in *P. tricornerutum* and selected the glutamine synthetase (*GLNA*) promoter as a candidate. Second, the *GLNA* promoter was fused to an eGFP reporter system and the expression level of eGFP was measured. The *GLNA* promoter strongly and consistently expressed the reporter protein, eGFP, in *P. tricornerutum*.

Materials and methods

Culture condition

The *P. tricornerutum* Bohlin UTEX 646 strain was purchased from the UTEX Culture Collection of Algae (The University of Texas at Austin, USA). Cultures were grown in *f/2* media made with 50% artificial seawater (Guillard 1975). Mixed antibiotics, which consisted of ampicillin (50 µg/mL), kanamycin (10 µg/mL), and streptomycin (50 µg/mL), were added to the media to suppress bacterial contamination. Cultures were incubated at 20 °C under shaking at 200 rpm with constant aeration and continuous lighting from a cool white fluorescent lamp (1600 lx), if culture conditions were not described independently.

Preparation of the proteins expressed in *P. tricornerutum*

For the analysis of the proteins expressed in *P. tricornerutum*, cultures of *P. tricornerutum* were grown as described above. Cells were grown to 1×10^7 cells/mL, pelleted, and suspended in a lysis buffer, pH 8.0, containing 50 mM Tris-Cl, 150 mM NaCl, and 0.1% Triton X-100 (De Riso et al. 2009). They were then vortexed, sonicated, and the cell lysates were collected by the centrifugation at 17,000 rpm for 20 min at 4 °C. The protein concentrations were determined by the Bradford assay (Biorad, Hercules, CA, USA). Samples were prepared separately from three independent cultures.

SDS-PAGE and in-gel digestion

Protein samples (30 µg each) were resolved on 12% SDS-PAGE gel and visualized with Coomassie blue. The most abundant proteins were excised from the gels (Fig. 1A), and the gel slices were washed with deionized water. The gel slices were subjected to an in-gel digestion with slight modifications (Shevchenko et al. 2006).

LC-MS/MS analysis and protein identification through database searching

The peptide samples prepared by in-gel digestion were analyzed with a LTQ XL linear ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA) equipped with a nano-HPLC system (Eksigent, Dublin, CA, USA). Five microliters of each peptide sample was injected. Sorcerer-SEQUEST (Sage-N research, Milpitas, CA, USA) was used to analyze the MS/MS raw data, and Scaffold 4 (Proteome Software, Portland, OR, USA) was used to visualize and validate the MS/MS-based peptide and protein identifications. SEQUEST was set to search the UniProt *P. tricornerutum* protein database (15784 entries), with up to 2 missed trypsin cleavage sites (Kang et al. 2014).

Plasmid construction

The *fc*pA promoter was removed from a pPha-T1 expression vector which has zeocin-resistant gene as a selection marker (Zaslavskaja et al. 2000) by NdeI/EcoRI enzymes. The *CIP1* promoter (Kadono et al. 2015) was synthesized by Bioneer (Daejeon, Korea) with flanking NdeI/EcoRI enzyme sites. Then, the *CIP1* promoter and the potential promoter regions of *GLNA* (501 and 997 bp long), as amplified by PCR, were inserted at NdeI/EcoRI enzyme sites into the pPha-T1 vector. All of the primers used in this study are listed in Supplementary Table 1. Next, the reporter protein (eGFP) coding gene was PCR amplified

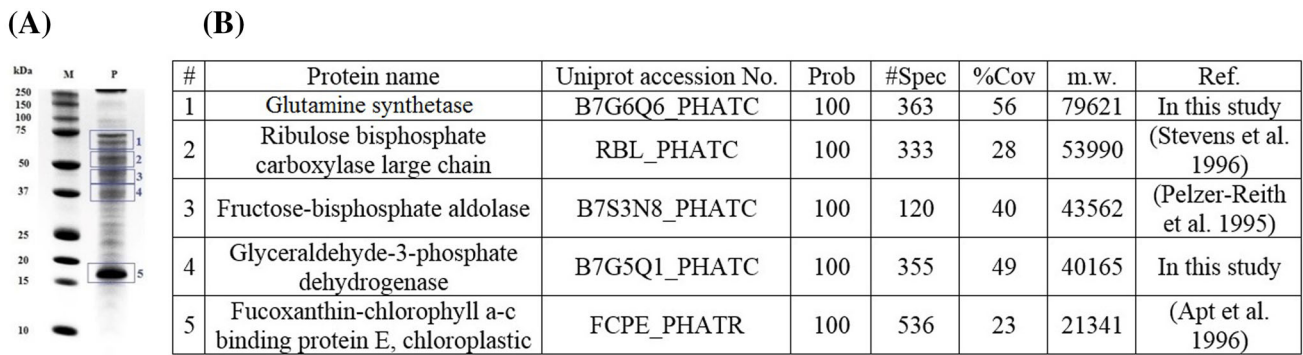


Fig. 1 Proteins of *P. tricornutum* resolved by SDS-PAGE (A) and proteins identified by LC-MS/MS (B). *M* size marker in kDa, *P* proteins from *P. tricornutum*, 1–5, sliced gel sections for LC-MS/

MS analysis, # gel slice number, *Prob* probability, #*Spec* total spectrum count, % *Cov* percentage of coverage, *m. w.* molecular weight by Dalton, *Ref.* references

from a pEGFP-C2 vector and inserted into the pPha-T1 vector at EcoRI/BamHI enzyme sites. Finally, a pPha-T1 vector containing the *fcpA* promoter but without the *egfp* gene was used as a mock construct. All sequencing was done by Macrogen (Seoul, Korea).

Transformation

The cells (3×10^8) were plated on the central third of the *f/2* agar plates, which also contained mixed antibiotics. The plates were incubated at 20 °C overnight. The next day, 3 mg of 0.6 μm gold microcarriers (9204298 RevD, Biorad) was coated with 15 μg of DNA for each of the vector constructs, according to the manufacturer's instruction for the Biolistic® PDS-1000/He Particle Delivery System (165-2257, Biorad). The coated microcarriers were used for four bombardments. Cell bombardment was carried out at 1350 psi. The distance between the target plate and the starting point of bombardment was 6 cm. Bombarded cells were allowed to recover at 20 °C for 2 days, and after that, cells were resuspended in *f/2* medium, and 10^8 cells were plated on *f/2* agar selection plate containing 100 μg/mL zeocin. After 4 weeks of selection, resistant colonies were further analyzed. During all experiments, resistant colonies were cultured in *f/2* medium with 100 μg/mL zeocin and mixed antibiotics under continuous lighting from a cool white fluorescent lamp (1600 lx).

Fluorescence measurement of eGFP by fluorometer

After measuring the cell density, 200 μL of cell culture was centrifuged at 3500 rpm for 5 min at 4 °C. The pelleted cells were lysed with lysis buffer, pH 8.0, containing 50 mM Tris-HCl, 150 mM NaCl, 1 mM PMSF, 1 mM EDTA, 1% nonidet, and 0.1% Tween-20. Lysates were incubated on ice for 20 min and then centrifuged at 3500 rpm for 15 min at 4 °C. The eGFP fluorescence of the supernatant was

measured by excitation at 485/20 nm and emission at 528/20 nm using a fluorometer (Multi-Detection Microplate Reader Synergy HT, Biotek, Winooski, VT, USA). All measurements were made on three independent replicates and autofluorescence value of the mock construct (containing *fcpA* promoter alone) was subtracted from the eGFP fluorescence value driven by *fcpA*, *CIP1*, *GLNA-501*, and *GLNA-997* promoter constructs.

Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was performed using Student's *t* test and one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparison. *p* value <0.05 was considered statistically significant.

Results

Protein identification

The gels resolved by SDS-PAGE were sliced into five bands (Fig. 1A), and 1836 proteins were totally identified from the bands by LC-MS/MS analysis. The most abundant protein identified from each band is listed as shown in Fig. 1B. The percent coverage of the proteins ranged from 23 to 56% by LC-MS/MS. Of these 5 proteins, ribulose biphosphate carboxylase (Stevens et al. 1996), fructose-bisphosphate aldolase (Pelzer-Reith et al. 1995), and fucoxanthin chlorophyll a/c binding protein E (Apt et al. 1996) have already had their promoters used to construct transformation vectors in diatom species. After considering the identified total spectrum counts and EST databases (Uma Maheswari et al. 2010) of the remaining two, glutamine synthetase (*GLNA*) and glyceraldehyde-3-phosphate dehydrogenase (*GapC1*), we selected the *GLNA*

promoter for further experimentation. The LC–MS/MS protein sequence coverage, spectrum, and fragmentation table of GLNA is available in Supplementary Fig. 1.

Plasmid construction and transformation

The pPha-T1 vector, which contains a zeocin resistance gene driven by the *fcpB* promoter (Zaslavskaja et al. 2000), was used as a backbone for all constructed vectors (Fig. 2A). The commonly used promoters for genetic manipulations of *P. tricornutum* are around 500 bp long. The potential promoter regions of *GLNA* (Uniprot Accession: B7G6Q6), which were 501 and 997 bp long, were extracted by the Biomart tool of Ensembl Protists (Kinsella et al. 2011) (Fig. 2B). The *fcpA* and *CIP1* promoters were used as endogenous and heterologous constitutive promoters that drive reporter protein expression. The pPha-T1 vector containing the *fcpA* promoter but without the *egfp* gene was used as a mock construct (Fig. 2C).

All prepared constructs were transformed into cells grown to stationary phase. Transformed cells were selected on an *f/2* agar selection plate containing 100 µg/mL zeocin for 4 weeks, and after that, all successfully transformed,

resistant colonies were transferred to a liquid *f/2* selection medium containing 100 µg/mL zeocin. Cells grown on liquid *f/2* media were further selected by genomic PCR analysis of whether foreign DNA was integrated (data not shown) and by eGFP fluorescence, indicating whether the reporter protein was expressed.

eGFP expression

A colony selected from each construct was cultured in *f/2* media, supplemented with 100 µg/mL zeocin and mixed antibiotics, until stationary phase. The cell density and eGFP expression of each cell culture were checked daily (Fig. 3). Cells were seeded with a concentration of 10^5 cells/mL at day 0 and grown to $\sim 10^7$ cells/mL at day 10. According to the cell growth curve, days 6 and 10 were the mid-log phase and stationary phase, respectively. The *fcpA* promoter-driven construct reached its highest eGFP expression level, which was 166.3 ± 6.0 relative eGFP fluorescence, at the log phase, and the expression decreased to 35.0 ± 2.5 relative eGFP fluorescence at the stationary phase (day 11) (Fig. 3A). The *CIP1*-driven construct did not show the predicted increase in expression between the lag and the stationary phase; the expression was 12.3 ± 0.6 relative eGFP fluorescence on day 6 and 16.0 ± 0.6 relative eGFP fluorescence on day 11 (Fig. 3B). Interestingly, the eGFP expression level of the *GLNA-501*-driven construct reached 44.3 ± 0.6 relative eGFP fluorescence during the early log phase and 156.0 ± 8.1 relative eGFP fluorescence during the stationary phase (day 11) (Fig. 3C). The *GLNA-997*-driven construct also showed constitutive eGFP expression, which was 72.7 ± 1.2 relative eGFP fluorescence during the middle log phase and 197.3 ± 1.7 relative eGFP fluorescence during the stationary phase (day 11) (Fig. 3D). Thus, the eGFP expression of the *GLNA-501* and *GLNA-997*-driven constructs was less than 50% compared to that of the *fcpA* promoter during mid-log phase (Fig. 3E). However, the *GLNA-501* and *GLNA-997*-driven constructs of the eGFP expression were 4 and 5 times higher, respectively, than that of the *fcpA* promoter during the stationary phase (Fig. 3F). We did not observe the predicted result from the *CIP1*-driven construct (Kadono et al. 2015) in these culture conditions. The eGFP expression level of the *CIP1*-driven construct was lower than that of all other promoters and increased slightly throughout stationary phase (Fig. 3B).

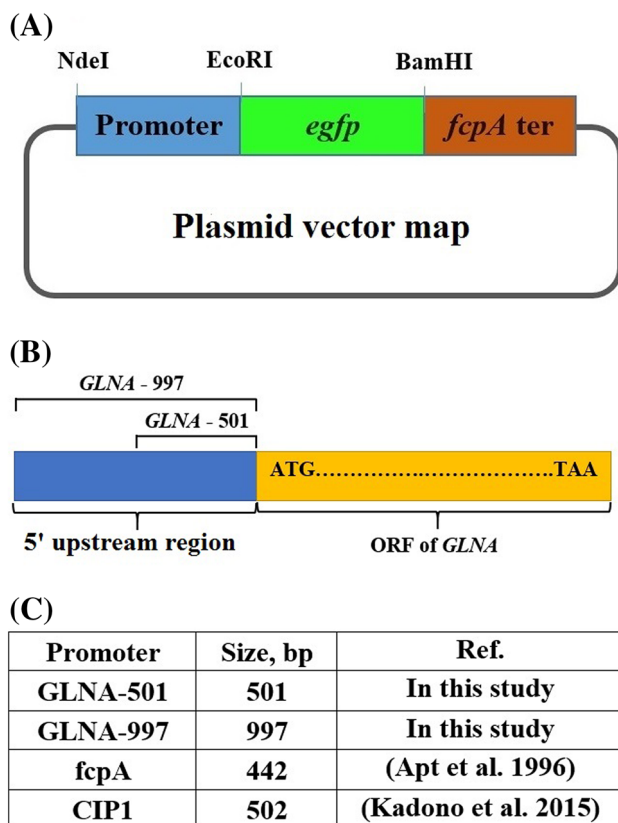


Fig. 2 Plasmid vector map (A), putative promoter regions of the *GLNA* gene (B), and promoters (C) used in this study. Promoter, promoters used in this study; *egfp*, enhanced green fluorescence protein coding gene; *fcpA* ter, *fcpA* terminator; Ref. references

Discussion

One of the most important genetic engineering tools for foreign gene expression and metabolic engineering is a strong constitutive promoter that is able to express large

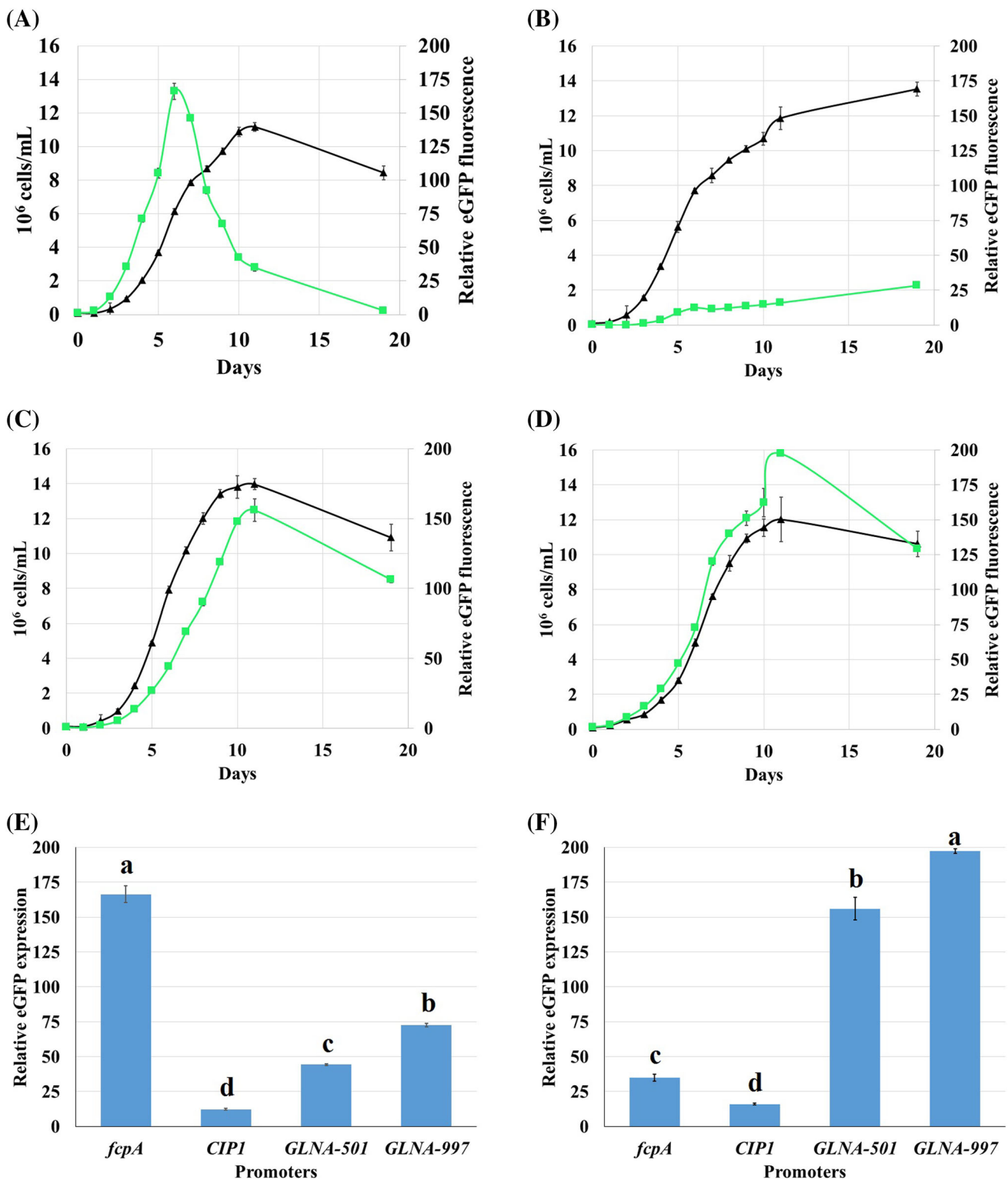


Fig. 3 Cell growth (filled triangle) and relative eGFP fluorescence (filled square) of *P. tricornutum* transformed with the construct containing *fcpA* (A), *CIP1* (B), *GLNA-501* (C), and *GLNA-997* promoter (D); statistical analysis on the eGFP expression level of each promoter at Day 6 (E) and Day 11 (F). Each transformed *P. tricornutum* was seeded with the concentration of 10⁵ cell/mL at the day 0 and cultivated. Cell density and eGFP expression were

evaluated daily. The values were expressed as mean ± SD of three independent experiments. Statistical analysis was performed using ANOVA followed by the Tukey’s test for multiple comparison at *p* < 0.05. *a*, *b*, *c*, and *d* indicate statistically significant differences on eGFP expression level of *fcpA*, *CIP1*, *GLNA-501*, and *GLNA-997*-driven constructs at Day 6 and Day 11

amounts of proteins inside or outside of (i.e., secreted from) the host organism. To maximize productivity, it is preferable to have the highest level of protein expression during the stationary phase of cell culture. The activity of the *fcpA* and *CIP1* promoters has also been evaluated during the stationary phase (Apt et al. 1996; Kadono et al. 2015). In this study, therefore, the search for novel candidate promoters focused on proteins that are highly expressed during the stationary phase. Thus, after evaluating the protein expression of *P. tricornutum* during the stationary phase, we examined the most highly expressed proteins (Fig. 1), such as GLNA and GapC1. Given its abundant protein expression and EST database information, GLNA was chosen as a candidate protein that is regulated by a strong constitutive promoter.

The *fcpA* promoter-harboring construct showed the expected pattern of eGFP expression. Its eGFP expression level increased from the lag to the log phase and decreased from the log to the stationary phase, resulting in a similar expression level to the *CIP1*-promoter-harboring construct during the early stationary phase (Fig. 3A, B). However, in its original description, the *CIP1*-promoter-harboring construct expressed about three times more reporter protein than the *fcpA* promoter-harboring construct during the stationary phase (Kadono et al. 2015). We suspect that different experimental conditions, especially light intensity, could be the main factor for the reduction of the *CIP1* promoter activity, so we changed the light intensity from 1600 lx to 3000 lx during cultivation. At the result, the ratio of the expression of eGFP from the two promoters (3:1, *CIP1*:*fcpA*) was similar to the reference (Kadono et al. 2015), which implies that *CIP1* could be a light-inducible promoter (Supplementary Fig. 2). The eGFP expression driven by the GLNA promoters was linearly related to cell density, and the reporter protein was expressed constitutively during all cell cultivation phases. The eGFP expression driven by the GLNA promoter regions was at least four times greater than that driven by the *fcpA* promoter during the stationary phase (day 11) (Fig. 3F). This is higher still than the eGFP expression driven by *CIP1* under ideal conditions, which was only three times greater than that of the *fcpA* promoter (Kadono et al. 2015). Although the eGFP expression driven by the GLNA-997 promoter region was around 30% greater than that driven by the GLNA-501 promoter region, GLNA promoter region of at least 501 bp was able to express eGFP consistently in these culture conditions.

In this study, the GLNA promoter caused strong constitutive expression of the reporter protein, regardless of the cell cultivation phase. GLNA promoter region over 501 bp could be used as genetic manipulation tool for foreign gene expression or metabolic engineering of *P. tricornutum*. Further study is needed to better understand the function of

the GLNA promoter and to express valuable recombinant proteins in *P. tricornutum* using the GLNA promoter.

Acknowledgements This research was performed as part of the project titled ‘Development and industrialization of high value cosmetic raw materials from marine microalgae,’ funded by the Ministry of Oceans and Fisheries, Korea, and was supported by an intramural Grant (2Z04690) from KIST Gangneung Institute of Natural Products.

References

- Apt KE, Kroth-Pancic PG, Grossman AR (1996) Stable nuclear transformation of the diatom *Phaeodactylum tricornutum*. *Mol Gen Genet* 252:572–579
- Bhaya D, Grossman AR (1993) Characterization of gene clusters encoding the fucoxanthin chlorophyll proteins of the diatom *Phaeodactylum tricornutum*. *Nucleic Acids Res* 21:4458–4466
- Bowler C, Allen AE, Badger JH, Grimwood J, Jabbari K, Kuo A, Maheswari U, Martens C, Maumus F, Otillar RP, Rayko E, Salamov A, Vandepoele K, Beszteri B, Gruber A, Heijde M, Katinka M, Mock T, Valentin K, Verret F, Berges JA, Brownlee C, Cadoret JP, Chiovitti A, Choi CJ, Coesel S, De Martino A, Detter JC, Durkin C, Falciatore A, Fournet J, Haruta M, Huysman MJ, Jenkins BD, Jiroutova K, Jorgensen RE, Joubert Y, Kaplan A, Kroger N, Kroth PG, La Roche J, Lindquist E, Lommer M, Martin-Jezequel V, Lopez PJ, Lucas S, Mangogna M, McGinnis K, Medlin LK, Montsant A, Oudot-Le Secq MP, Napoli C, Obornik M, Parker MS, Petit JL, Porcel BM, Poulsen N, Robison M, Rychlewski L, Rynearson TA, Schmutz J, Shapiro H, Saut M, Stanley M, Sussman MR, Taylor AR, Vardi A, von Dassow P, Vyverman W, Willis A, Wyrwicz LS, Rokhsar DS, Weissenbach J, Armbrust EV, Green BR, Van de Peer Y, Grigoriev IV (2008) The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature* 456:239–244
- De Riso V, Raniello R, Maumus F, Rogato A, Bowler C, Falciatore A (2009) Gene silencing in the marine diatom *Phaeodactylum tricornutum*. *Nucleic Acids Res* 37:e96
- Diaz-Santos E, de la Vega M, Vila M, Vigarra J, Leon R (2013) Efficiency of different heterologous promoters in the unicellular microalga *Chlamydomonas reinhardtii*. *Biotechnol Prog* 29:319–328
- Falciatore A, Casotti R, Leblanc C, Abrescia C, Bowler C (1999) Transformation of nonselectable reporter genes in marine diatoms. *Mar Biotechnol* 1:239–251
- Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith W, Chanley M (eds) Culture of marine invertebrate animals, 1st edn. Springer, New York, pp 29–60
- Hamilton ML, Haslam RP, Napier JA, Sayanova O (2014) Metabolic engineering of *Phaeodactylum tricornutum* for the enhanced accumulation of omega-3 long chain polyunsaturated fatty acids. *Metab Eng* 22:3–9
- Hempel F, Lau J, Klingl A, Maier UG (2011) Algae as protein factories: expression of a human antibody and the respective antigen in the diatom *Phaeodactylum tricornutum*. *PLoS One* 6:e28424
- Kadono T, Miyagawa-Yamaguchi A, Kira N, Tomaru Y, Okami T, Yoshimatsu T, Hou L, Ohama T, Fukunaga K, Okauchi M, Yamaguchi H, Ohnishi K, Falciatore A, Adachi M (2015) Characterization of marine diatom-infecting virus promoters in the model diatom *Phaeodactylum tricornutum*. *Sci Rep* 5:18708
- Kang K, Song DG, Lee EH, Lee KM, Park YG, Jung SH, Pan CH, Nho CW (2014) Secretome profiling reveals the signaling molecules of apoptotic HCT116 cells induced by the dietary

- polyacetylene gymnasterkoreayne B. J Agric Food Chem 62:2353–2363
- Kinsella RJ, Kahari A, Haider S, Zamora J, Proctor G, Spudich G, Almeida-King J, Staines D, Derwent P, Kerhornou A, Kersey P, Flicek P (2011) Ensembl BioMarts: a hub for data retrieval across taxonomic space. Database (Oxford) 2011 p bar030
- Maheswari U, Jabbari K, Petit J-L, Porcel BM, Allen AE, Cadoret J-P, Martino AD, Heijde M, Kaas R, Roche JL, Lopez PJ, Martin-Jézéquel V, Meichenin A, Mock T, Parker MS, Vardi A, Armbrust EV, Weissenbach J, Katinka M, Bowler C (2010) Digital expression profiling of novel diatom transcripts provides insight into their biological functions. Genom Biol 11:R85
- Pelzer-Reith B, Freund S, Schnarrenberger C, Yatsuki H, Hori K (1995) The plastid aldolase gene from *Chlamydomonas reinhardtii*: Intron/exon organization, evolution, and promoter structure. Mol Gen Genet 248:481–486
- Qin S, Lin H, Jiang P (2012) Advances in genetic engineering of marine algae. Biotechnol Adv 30:1602–1613
- Saade A, Bowler C (2009) Molecular tools for discovering the secrets of diatoms. Bioscience 59:757–765
- Seo S, Jeon H, Hwang S, Jin E, Chang KS (2015) Development of a new constitutive expression system for the transformation of the diatom *Phaeodactylum tricorutum*. Algal Res 11:50–54
- Shevchenko A, Tomas H, Havlis J, Olsen JV, Mann M (2006) In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat Protoc 1:2856–2860
- Siaut M, Heijde M, Mangogna M, Montsant A, Coesel S, Allen A, Manfredonia A, Falciatore A, Bowler C (2007) Molecular toolbox for studying diatom biology in *Phaeodactylum tricorutum*. Gene 406:23–35
- Stevens DR, Roehaix J-D, Purton S (1996) The bacterial phleomycin resistance gene *ble* as a dominant selectable marker in *Chlamydomonas*. Mol Gen Genet 251:23–30
- Yao Y, Lu Y, Peng K-T, Huang T, Niu Y-F, Xie W-H, Yang W-D, Liu J-S, Li H-Y (2014) Glycerol and neutral lipid production in the oleaginous marine diatom *Phaeodactylum tricorutum* promoted by overexpression of glycerol-3-phosphate dehydrogenase. Biotechnol Biofuels 7:1–9
- Zaslavskaja LA, Martek JCL, Kroth PG, Grossman AR, Apt KE (2000) Transformation of the diatom *Phaeodactylum tricorutum* (Bacillariophyceae) with a variety of selectable marker and reporter genes. J Phycol 36:379–386