Research Square

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Regulation of a novel DsGATA1 from Dunaliella salina on the synthesis of carotenoids under red light

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Abstract

Background

Dunaliella salina is a high-quality industrial effector for carotenoid production. Although the accumulation of carotenoids in *D. salina* increases under red light conditions, the content of carotenoids in the algal cell decreases. The mechanism by which red light regulates carotenoid synthesis is still unclear.

Results

In this study, a transcription factor of DsGATA1 with a distinct structure was discovered in *D. salina*. The recognition motif of DsGATA1 was comparable to that of plant and fungal GATA, despite its evolutionary proximity to animal-derived GATA. The expression of DsGATA1 in *D. salina* was still noticeably decreased when exposed to red light. Analysis of physiological and biochemical transcriptomic data from overexpressed, interfering and wild-type strains of DsGATA1 revealed that DsGATA1 acts as a global regulator of *D. salina* carotenoid synthesis. The upregulated genes in the CBP pathway by DsGATA1 were involved in its regulation of the synthesis of carotenoids. DsGATA1 also enhanced carotenoid accumulation under red light by affecting N metabolism. DsGATA1 was found to directly bind to the promoter of nitrate reductase to activate its expression, promoting *D. salina* nitrate uptake and accelerating biomass accumulation. DsGATA1 affected the expression of the genes encoding GOGAT, GDH and ammonia transporter proteins. Moreover, our study revealed that the regulation of N metabolism by DsGATA1 led to the production of NO molecules that inhibited carotenoid synthesis. However, DsGATA1 significantly enhanced carotenoid synthesis by NO scavenger removal of NO. The *D. salina* carotenoid accumulation under red light was elevated by 46% in the presence of overexpression of DsGATA1 and NO scavengers.

Conclusion

It was found that a transcription factor of DsGATA1 from *D. salina* has a distinct structure and recognition motif. The novel gene encoding DsGATA1 enhanced the production of carotenoids under red light and endowed *D. salina* with high algal biomass. The regulation of terpenoid metabolism by DsGATA1 is different from that reported for GATA factors. DsGATA1 yet enhanced the production of NO in *D. salina*. Nevertheless, our results indicated that DsGATA1 could be an important target for engineering carotenoid production.

1. Introduction

Microalgae have become a source of several natural metabolites due to their quick growth rate, high photosynthetic efficiency, cultivability in uncultivated areas [1], and excellent environmental tolerance. As a unicellular eukaryotic organism, *Dunaliella salina* has excellent potential for producing carotenoids with antioxidant properties [2].

The biomass of algal cells and the amount of carotenoids in the cells are the two key factors that determine the production of carotenoids in *D. salina* for industrial use [3]. Culture conditions suitable for cell growth are not usually conducive to the accumulation of secondary metabolites [4]. Carotenoid production in industry has been achieved by a two-step method, with *D. salina* cultivated in the best conditions for growth to produce more biomass [5], and then progressively switched to the best conditions for accumulating carotenoids with the highest carotenoid output. Red light is often employed during the growth period to boost biological yield since studies have shown that red light is more favorable to the growth of *D. salina* [6]. However, there is no clear report on the pattern and regulation of carotenoid synthesis in *D. salina* cells under red light.

GATA factors, a class of transcriptional regulators involved in the regulation of various physiological processes, are widely found in fungi, animals and plants and recognize the common sequence WGATAR (W = A/T, R = A/G), whose DNA binding domain is a $CX_2CX_{17-20}CX_2C$ class IV zinc finger pattern [7]. In animals, GATA transcription factors generally have two zinc fingers, of which only the C-terminal finger (C finger) is involved in DNA binding and the N-terminal zinc finger (N finger) is responsible for regulating the binding of the C finger to specific GATA sites [8]. In comparison, most GATA factors from fungi and plants contain only one zinc finger structural domain, which is generally located at the C-terminus. In animals, GATA factors have been shown to play a key role in the control of development, differentiation and cell proliferation [9], while fungal GATA factors are mainly involved in the regulation of various physiological functions, such as nitrogen control, iron carrier biosynthesis, light regulation and secondary metabolite accumulation [10]. In plants, GATA factors are also reported to have various physiological functions [11]. Although GATA motifs are prevalent in many promoter areas of plant light-responsive genes [12] and the GATA transcription factor has also been reported to be involved in the synthesis of terpenoids, no one has investigated whether GATA factors can regulate the accumulation of secondary metabolites by responding to light.

The DsGATA1 gene was chosen for investigation in this study. The effects of GATA factors on the carotene yield of *D. salina* were investigated by comparing physiological and biochemical data between transgenic and wild type plants under red light. *D. salina* overexpression and interference variants were created. Simultaneously, yeast one-hybrid and transcriptional activity analyses were used to investigate the binding motif of the DsGATA1 gene and the mechanism by which this transcription factor controls the accumulation of carotene in *D. salina*.

2 Materials And Methods

2.1 Acquisition and characterization of DsGATA1

Four GATA genes were discovered by our team while measuring the transcriptome of *D. salina* under red, white, and red–white light conversion circumstances. A unique DsGATA1 gene linked to red light was found after the analysis of expression variations under various light qualities. Its protein structure was examined by the CDD conserved domain from NCBI(https://www.ncbi.nlm.nih.gov/Structure/cdd), its

gene sequence was acquired by 3'RACE cloning, and its evolutionary tree was created using MEGA11 software. The pHis vector was modified to include the GATA and GATC motifs, and a yeast one-hybrid assay was run to investigate the binding motif of this transcription factor.

2.2 Construction and cultivation of DsGATA1 saltbush overexpression and interference strains

The CDS of the DsGATA1 gene was synthesized by PCR with the primers in Appendix 1, and the interfering gene fragment was designed on GPP Web Portal GPP Web Portal - Design hairpins (broadinstitute.org). The interfering fragment was obtained by chemical synthesis. These were constructed into the transgenic vector PGreen0029 by the method of Tang et al. [13], and the RNA of the overexpressed, wild-type, and interference strains was extracted for qRT—PCR to determine the expression of their DsGATA1 genes. The subsequently selected algal strain OE, WT, i1 was cultured under red light at 2000 Lux according to the method of Tang et al.

2.3 Algal growth kinetics and N-source utilization kinetics

The growth curves of salt algae were plotted by taking 3 ml of algal solution every 1 day and measuring the absorbance value at a wavelength of 654 nm with a spectrophotometer.

Five milliliters of algal solution was collected every day from the first day of culture to the fifth day to measure the rate at which N sources were being utilized. The $NO3^-$ content in the culture medium of the brine algae was measured by high-performance liquid chromatography using a CIC-D160 ion chromatograph.

2.4 Determination of carotenoid content

The pigments were extracted by the acetone method, and the carotenoid content was calculated according to the following previously reported formula for photosynthetic pigments.

Ca = 12.21*A663-2.81*A647 Cb = 20.13*A647-5.03*A663

Car=(1000*A470-3.27*Ca-104*Cb)/229

Unitpigment content = Cx*V*dilution times/FW

Cx-pigment concentration (mg/L) V-extraction volume (mL)

FW-fresh weight of sample (g)

The pigment content of overexpressing strains, wild type strains, and interfering strains was measured daily to compare the pigment yield of transgenic algae from the second day of carotenoid accumulation during growth to the sixth day of a substantial yield difference.

The NO inhibitor cPTIO was added on the second day of incubation, and the change in pigment content was measured after two days to investigate the effect of NO.

2.5 Measurement of expression of carotenoid synthesis and N-metabolism pathway genes

RNA was extracted from the overexpressed, wild, and interference strains on the second day of carotenoid accumulation and the fourth day of significant difference using the method of Tang et al [13]. The expression levels of the important enzymes AMT, GDH, GOGAT, GS, NR, and NRT as well as the carotenoid synthesis genes GPPS, PDS, PSY, ZDS, and CRTISO were also measured by qRT—PCR using the primers in Appendix 1 in three different algae genotypes.

2.6 Identifying DsGATA1's impact on nitrate reductase NR

A yeast one-hybrid assay was performed using the Y187 yeast receptor cell-pHis system. The NR promoter was constructed onto the pHis vector, and DsGATA1 was attached to pGADT7-Rec2 to test whether DsGATA1 could directly activate the transcription of NR.

2.7 Determination of NO content

The NO content in the cells of three different genotypes of salt algae was measured on the fifth day when the difference in carotenoid production was relatively large according to the method of Zhu et al [14].

2.8 Statistical analysis

All experiments were performed with three biological replicates. The experimental data were graphed and plotted against the results using GraphPad Prism 8.0 software, and significance analysis was performed using SPSS Statistics software for t tests.

3. Results

3.1 Molecular characterization of DsGATA1

Transcriptome differential expression analysis revealed that the DsGATA1 gene (F01-cb1790-c8) behaved quite differently from several other GATAs under red light, and its transcriptional activity was significantly repressed; at the same time, its expression was significantly elevated when the incubation light quality was switched from red to white light (Fig. 1a). The CDS of the DsGATA1 gene was 1080 bp, and the protein consisted of 360 amino acids. A phylogenetic tree was constructed with 22 genes from animals, plants and fungi, and the different GATA factors were clustered according to their species, but DsGATA1, which comes from plants, was clustered into the category of animals. Conservative structural domain analysis also showed that its zinc finger structural domain located at the N-terminal end was more similar to the GATA structure in animals, while there were significant structural differences with some GATA molecules belonging to the same plant with zinc finger structural domains located at the C-terminal

end (Fig. 1b). The DsGATA1 gene in *D. salina* was able to stimulate the transcription of the GATA motif, according to yeast one-hybrid studies (Fig. 1c).

3.2 Construction and growth data of *D. salina* DsGATA1 overexpression and interference strains

DsGATA1 overexpression and interference strains were constructed to investigate the function of DsGATA1 with a unique structure. During the screening process, all wild-type algae died at a chloramphenicol concentration of 1 g/L because the vector carried a chloramphenicol resistance tag. The empty vector, interference strain GATAi, and overexpression strain OEGATA all survived, and the OE grew better (Fig. 2a). The expression of the GATA gene in the overexpression strain OE was six times higher than that of the wild type, that of the interference strain i was forty percent lower according to fluorescent quantitative PCR. For the subsequent experiments, the interference strain i and the overexpression strain OE were chosen (Fig. 2b). The growth curves of *D. salina* under red light showed that the growth rates of OE, WT and i did not differ much until the second day, with OE being slightly faster than the WT and the interfering strain significantly slower than the WT. After the second day, the growth rate of all three alga groups was significantly accelerated, and the slope showed that the slope of the overexpression strain was the largest and the growth rate was the fastest, while the slope of the interference strain was the smallest and the growth rate was the slowest, indicating that the DsGATA1 gene could significantly promote the growth of *D. salina*. (Fig. 2c)

3.3 DsGATA1 increases carotenoid production in *D. salina*

Red light was used to cultivate the interference strain, wild-type strain, and overexpression strain. On day two, the total carotenoid production, biomass and single-cell carotenoid production of the overexpressing, wild and interfering strains were not significantly different. The carotenoid concentration and biomass of *D. salina* started to differ significantly on the third day (Fig. 3a,b,c). The total carotenoid production of the overexpression strain was 25% higher than that of the wild type, and the biomass was 15% higher than that of the wild type; only 69% of the wild type's total carotenoid production and 74% of the wild type's biomass were produced by the interference strain. At this time, the difference in biomass caused a large difference in total carotenoid production (Fig. 3a).

The greatest difference in single-cell carotenoid production between the three strains was observed on day 4 (Fig. 3c), with the overexpression strain producing 1.2 times more carotenoids than the wild type and the interfering strain producing 87% more carotenoids per cell, while the total production of the overexpression strain increased by 40% and the total carotenoid production of the interfering strain decreased by 28%. From the fifth day, the single-cell carotenoid production of the three strains was basically unchanged, while the single production of the overexpression strain was 1.1 times higher than that of the wild-type strain and that of the interference strain was 90%(Fig. 3c). The biomass and carotenoid yield of the algae were measured during the incubation process, which showed that the overexpression of the DsGATA1 gene not only promoted the growth of the algae but also increased the

carotenoid content of the single cells of the algae and further increased the total *D. salina* carotenoid yield (Fig. 3a,b,c).

3.4DsGATA1 regulates carotenoid synthesis pathway genes

To investigate the reason for the increase in carotenoid production in the overexpression strain, we extracted RNA from three genotypes of *D. salina*—wild, overexpressing, and interfering—at day 4 when the difference in single-cell carotenoid production was greatest (Fig. 3c) and performed qPCR assays on their carotenoid synthesis pathway genes. The expression levels of PSY, ZDS, and CRTISO were 1.4-fold, 1.3-fold, and 1.1-fold higher than those of the WT, respectively, while they were significantly lower in the interfering strain, 91%, 94%, and 84%, respectively (Fig. 3d). This indicates that the DsGATA1 gene can upregulate the key genes of carotenoid synthesis, PSY, ZDS, and CRTISO, to improve the carotenoid production of algae cells in *D. salina.*

3.5 DsGATA1 promotes N metabolism in *D. salina*

According to previous research, DsGATA1 can stimulate the development of *D. salina*, and growth and N metabolism are tightly connected. At the same time, it has been widely recognized that GATA transcription factors have a role in controlling biological N metabolism. As a result, the impact of DsGATA1 on N metabolism was examined in this research, and the concentration of nitrate in the medium was evaluated to ascertain the impact of DsGATA1 on the use of nitrogen sources in *D. salina*. On the first two days of growth, it was discovered that the overexpression strain consumed nitrate in the medium at a rate that was not significantly different from that of the wild type (Fig. 4a). The nitrogen source usage rate accelerated from the second to the third day. After three days, the overexpression strain's N source consumption curve had a slope that was noticeably higher than that of the wild-type strain. Just 70% of the wild-type form of nitrate was still present in the medium on the fifth day. Throughout the whole culture process, the interference strain's N source consumption rate was lower than that of the wild-type strain. On the fifth day, the medium still contained 1.2 times as much residual nitrate as the wild type (Fig. 4a).

The greatest difference in nitrate content between the three strains of algae in the medium was observed on day four. Extraction of RNA from the different genetic strains at that time and fluorescence quantitative PCR showed that the DsGATA1 gene could regulate the transcriptional activity of N metabolic pathway genes. First, the expression of nitrate reductase (NR), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH), the key enzymes of the nitrate conversion pathway, was increased by 30%, 27% and 48%, respectively, in the overexpression strain, and the expression of glutamine synthase (GS) and nitrate transporter protein (NRT) was also slightly increased. The expression levels of NR, GOGAT and GDH in the interference strain were 85%, 90%, and 90% of those in the wild type, respectively (Fig. 4C). DsGATA1 could promote the utilization of nitrate in *D. salina* by converting nitrate to ammonia and further participating in other metabolic pathways. We also found that ammonia transporter protein (AMT) expression was also increased by 36% in the overexpression strain, indicating

that DsGATA1 can also promote N metabolism in *D. salina* by facilitating the transport of ammonium ions.

3.6 DsGATA1 activates the transcription of NR, leading to NO accumulation

PlantCare was used to study the 2000 bp upstream of the NR gene in *D. salina*, and it was discovered that the promoter region of the NR gene had several binding sites for GATA transcription factors. A yeast one-hybrid assay with the Y187-pHis system showed that DsGATA1 binds directly to the promoter region of the nitrate reductase NR to activate its transcription (Fig. 4b). Nitrate reductase, a crucial enzyme in the N metabolic pathway, can catalyze the transformation of nitrate into nitrite and release a significant quantity of NO. On the fourth day, distinct transgenic algal cells were examined for their NO content. The NO content of the overexpression strain was three times that of the wild type, while the NO content of the interference strain was only forty percent of that of the wild type (Fig. 4d,e). This result suggested that the DsGATA1 gene could directly stimulate the transcriptional activity of the nitrate reductase NR, resulting in the accumulation of NO in *D. salina*.

3.7 NO inhibits carotenoid synthesis

NO is widely involved in the regulation of many metabolic processes as a signaling molecule [15]. On the second day, we added the NO inhibitor Cptiso to determine whether NO would alter carotene accumulation in *D. salina.* The NO content in the overexpression strain dropped by up to 27% when the inhibitor was added, while it dropped by 16% and 8% in the WT strain and interference strain, respectively (Fig. 5a). However, the growth of the overexpression, wild-type and interference strains was largely unaffected (Fig. 5b). At this time, carotenoid production was significantly higher in all the alga genotypes with the addition of inhibitors, and the total carotenoid production of the overexpression strain with the addition of inhibitors was 1.1 times higher than that of the normal overexpression strain and 1.46 times higher than the total production of the WT. The total yield was also increased by 11% and 12% for the wild and interfering strains, respectively, with the addition of the inhibitor (Fig. 5c). The single-cell carotenoid production of *D. salina* also changed significantly after the addition of the NO inhibitor, and the overexpression strain with the addition of inhibitor showed a significant increase in the single yield compared to that without the addition (Fig. 5d), indicating that NO inhibits carotenoid synthesis in *D. salina*.

Discussion

DsGATA1, with its unique structure, was involved in the regulation of carotenoid accumulation in *D. salina* under red light. GATA transcription factors are widely found in animals, plants, fungi and other microorganisms [16]. However, the structures of GATA in various organisms are significantly different. The unique zinc finger structural domain of GATA in plants and fungi is generally located at the C-terminus [17], whereas in animals, GATA usually consists of two distinct zinc finger structural domains located at the N-terminus and the C-terminus [18]. Our results showed that DsGATA1 was evolutionarily

closer to animal-derived GATA and was uncommon in plants and microorganisms. Although several transcription factors in the database were annotated as GATA from *Arabidopsis thaliana* and were anticipated to include a zinc finger domain at the N-terminus [7], it was yet unknown whether these transcripts are real or functional. There were also differences in the DNA motifs of the two types of GATA actions in animals and plants. The action motif of plant GATA factors is usually the GATA module [19], whereas in animals, the zinc finger structural domain of GATA factors located at the C-terminus activates transcription of the GATA module, and the structural domain located at the N-terminal end prefers to bind to the GATC module [20]. Despite its structural evolutionary similarity to animal GATA, the action motif of DsGATA1 is the GATA module and has no apparent binding ability to the GATC module. This unique structure of DsGATA1 could make an important contribution to its function. In *D. salina*, compared to other GATA factors that share the same zinc finger domain as those found in plants, the expression kinetics of DsGATA1 in the regulation of carotenoid accumulation by red light are clearly distinct.

DsGATA1 acted as a global regulator involved in the regulation of carotenoid synthesis in *D. salina* under red light. DsGATA1 can directly affect the expression of functional genes in the CBP pathway, and our study showed that overexpression of DsGATA1 greatly upregulated several functional genes, including PSY, ZDS, and CRTISO, which are essential genes for carotenoid synthesis, and increased the amount of carotenoid synthesis in *D. salina.* These effects of DsGATA1 were clearly different from the reported mode of action of GATA in other species. Previous reports have identified a GATA transcriptional regulator, AreA, in *Ganoderma lucidum* that is involved in the synthesis of the terpenoid ganoderic acid [14]. However, AreA targets the synthesis genes of the ganoderic acid synthesis precursor FPP rather than having a direct effect on the functional genes of the ganoderic acid synthesis pathway. DsGATA1 also regulated the accumulation of carotenoids under red light in *D. salina* by affecting N metabolism. Our results showed that DsGATA1 significantly increased the expression of the gene encoding the nitrate reductase in N metabolism, which promotes nitrate uptake and the rapid growth of the algae under red light, thereby increasing carotenoid accumulation. Increased NR expression could lead to an increase in $NH4^+$ in vivo, and to balance the homeostasis of $NH4^+$ in vivo and reduce its toxicity to algal cells [21], DsGATA1 upregulated the expression of the gene encoding GOGAT, which can convert $NH4^+$ to Glu and reduce the amount of $NH4^+$. GOGAT has a stronger affinity for $NH4^+$ [22]and can keep the concentrations of $NH4^+$ very low. When the $NH4^+$ concentration was too high, DsGATA1 upregulated the expression of GDH, which can play a role in high concentrations of $NH4^+$ [23]and convert $NH4^+$ into Glu. More intriguingly, DsGATA1 also upregulated AMT activity, implying the possibility of using $NH4^+$ as a nitrogen source in *D. salina*. Although we did not examine the efficiency of the transgenic algae to utilize $NH4^+$ in this paper, the role of DsGATA1 in balancing $NH4^+$ provides insights for the culture of *D. salina* by using ammonium nitrogen.

DsGATA1 improved the ability of *D. salina* to utilize $NO3^-$ but resulted in the production of more NO molecules. We eliminated NO produced by different genotypes of algae in culture and found a significant elevation in the amount of carotenoids, indicating that the effect of NO molecules on carotenoid synthesis is negative. This was also found in *Ganoderma lucidum*. In that study, AreA produced a more pronounced inhibitory effect on the synthesis of ganoderic acid. By comparison, in this study, DsGATA1

from *D. salina* promoted carotenoid synthesis. A 28% increase in carotenoid amounts was observed in the overexpression strain compared to the WT. The possible reason for this is that DsGATA1 can directly upregulate CBP pathway genes to eliminate the inhibitory effect of NO. It was also a possible consequence of the unique structure of DsGATA1. When we used transgenic DsGATA1 for carotenoid synthesis, the further addition of NO inhibitor during the culture of the overexpression strain resulted in a 46% increase in carotenoid production compared to the WT. This could be a valuable approach for the production of carotenoids using *D. salina* under red light.

Conclusion

It was found that a transcription factor of DsGATA1 from *D. salina* has a distinct structure and recognition motif. The novel gene encoding DsGATA1 enhanced the production of carotenoids under red light and endowed *D. salina* with high algal biomass. The upregulated genes in the CBP pathway and N metabolism by DsGATA1 were involved in its regulation of the synthesis of carotenoids. However, DsGATA1 enhanced the production of NO in *D. salina*, which is an inhibitor of carotenoid synthesis. A more valuable approach to the efficient production of carotenoids is thus that transgenic *D. salina* overexpressing the DsGATA1 gene could be used in a culture medium with NO inhibitor.

Abbreviations

α-oxo	Alpha ketoglutaric acid
AMT	Ammonium transporter
CRTISO	Carotenoid isomerase
DMAPP	Dimethylallyl pyrophosphate
FPP	Farnesyl diphosphate
FPPS	Farnesyl diphosphate synthase
GDH	Glutamate dehydrogenase
Glu	Glutamate
Gln	Glutamine
GOGAT	Glutamate synthase

GGPP	Geranylgeranyl diphosphate
GGPPS	Geranylgeranyl diphosphate synthase
GS	Glutamine synthetase
IPP	Isopentenyl pyrophosphate
NR	Nitrate reductase
NIR	Nitrite reductase
NRT	Nitrate transporter
PDS	Phytoene desaturase
PSY	Phytoene synthase
ZDS	Zeta-carotene desaturase

Declarations

Authors' contributions

YS and HX designed the study, performed the experiments, analysed the results and drafted the manuscript. HX and YC as the corresponding author, developed the concept of this research, supervised the progress of this research, and commented on the manuscript. YHL and KL assisted in the experiments. DRQ participated in the design of the study and commented on the manuscript. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors approved the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figures

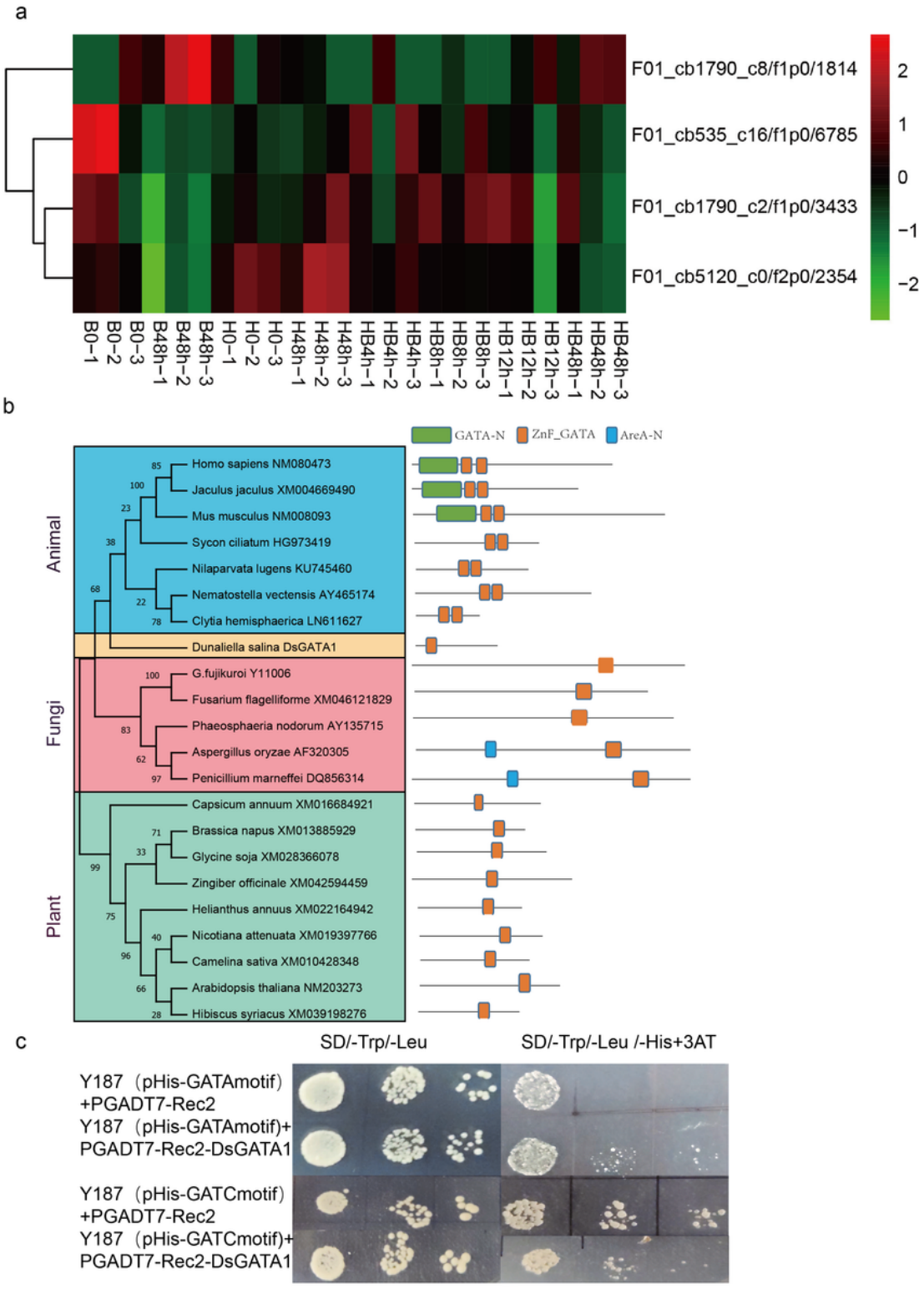


Figure 1

Molecular characterization of DsGATA1 (a) Heatmap of GATA gene expression in *D. salina* under different light qualities, H-red light, B-white light, HB-red light to white light (b) Phylogenetic tree and conserved structural domain analysis of GATA molecules in animals, plants and fungi (c) DsGATA1 specifically binds to the GATA module and activates its transcription

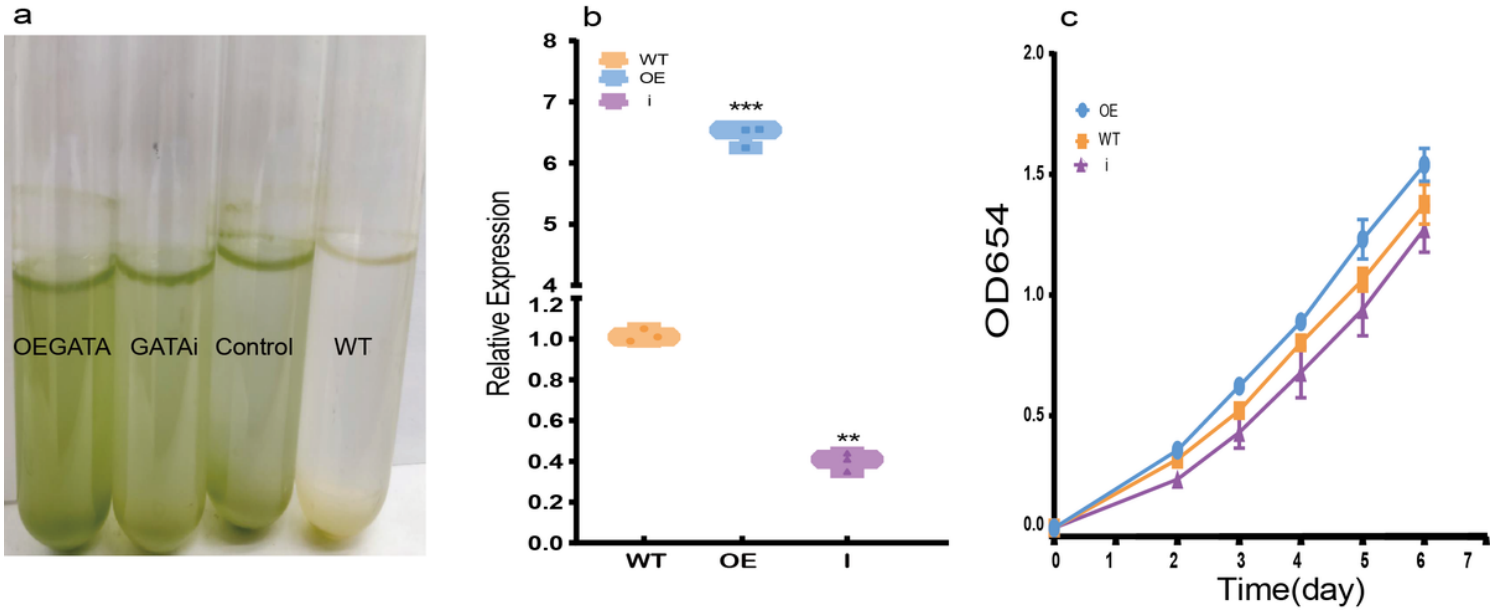


Figure 2

Screening and growth comparison of DsGATA1 overexpression strain and interference strain (a) Growth status of DsGATA1 overexpression strain OE, interference strain i, algal cells transferred into empty vector Control and wild-type WT under 1 g/Lchloramphenicol screening conditions (b) Transcript levels of DsGATA1 in WT, OE, i cells. Error bars: SD from three replicates. Asterisks indicatesignificant differences compared with the controls according to Student's t test: (**P < 0.01, ***P < 0.001). (c) Growth of overexpression strain OE, interfering strain i and wild-type WT during culture.

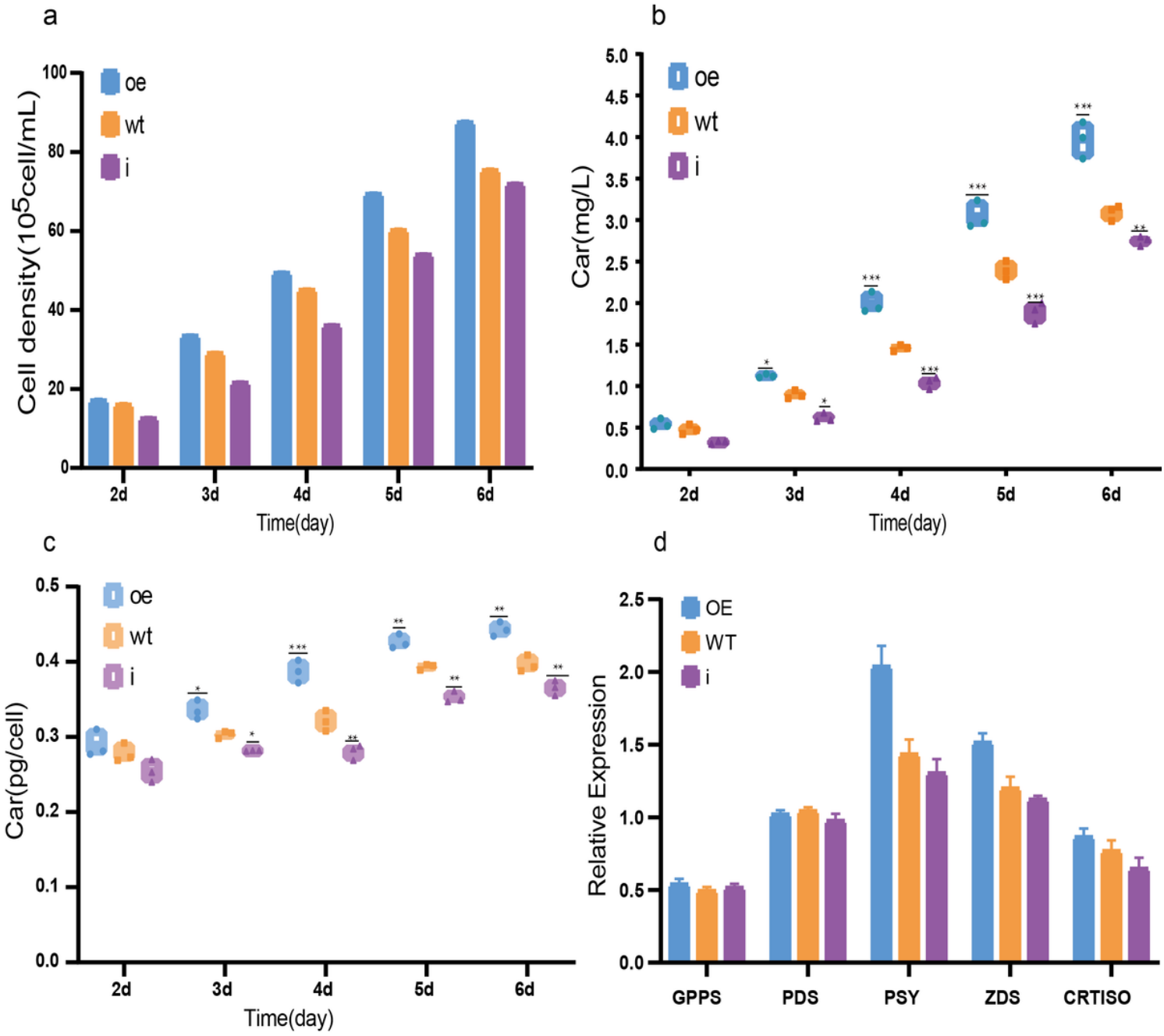


Figure 3

Carotenoid accumulation in different genotypes of *D. salina* (a) Biomass of OE, i, and WT at different time periods of culture (b) Total carotenoid production (c) Carotenoid production in single cells (d) Expression levels of key genes of the carotenoid synthesis pathway in OE, i, and WT cells on the fourth day of maximum difference in single cell production. Error bars: SD from three replicates. Asterisks indicate significant differences compared with the controls according to Student's t test: (*P<0.05, **P < 0.01, ***P < 0.001)

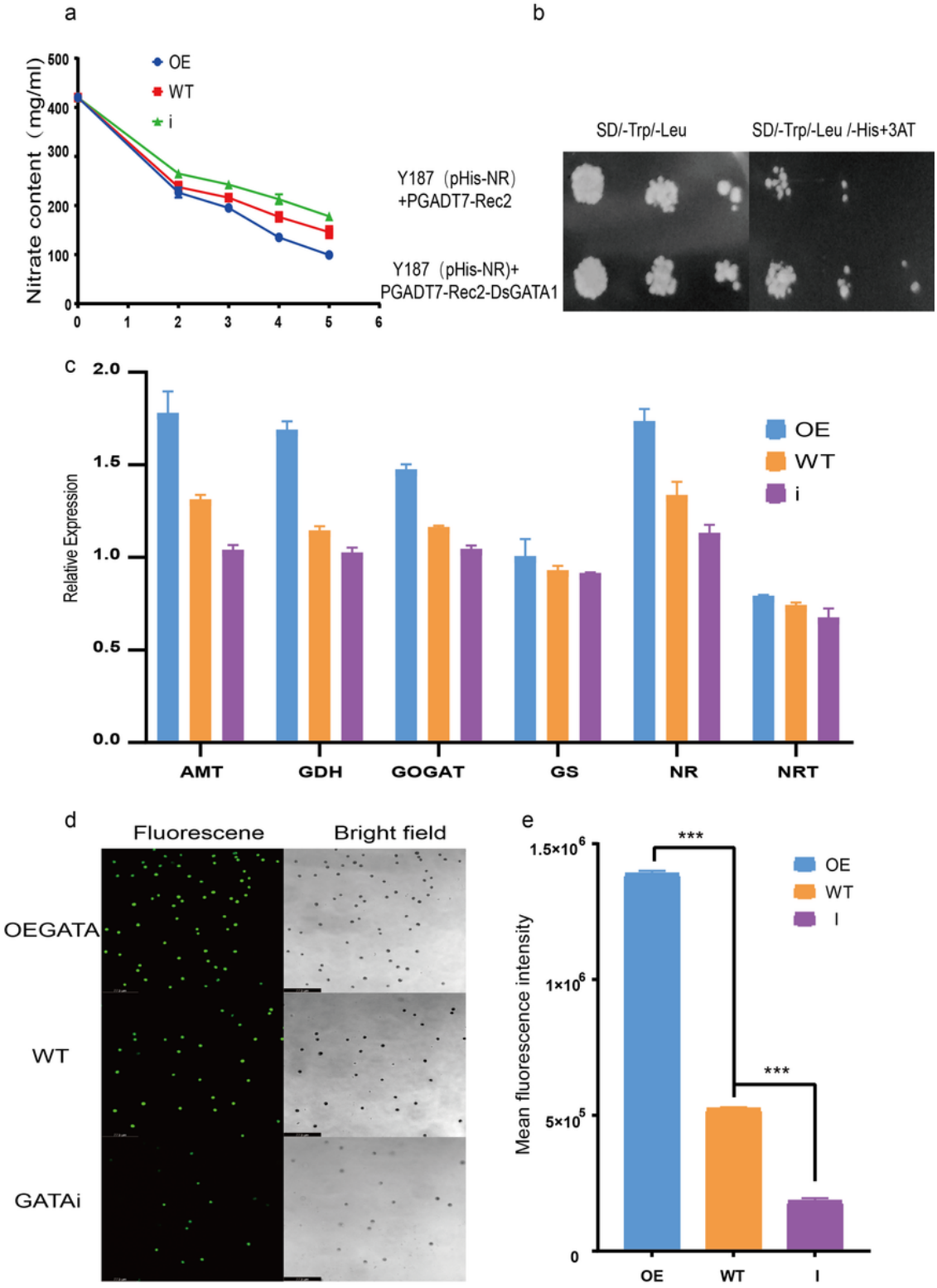


Figure 4

Regulation of N metabolism by DsGATA1 in *D. salina*. (a) Nitrate content in the medium during OE, WT, and i culture. (b) DsGATA1 binds to the promoter region of nitrate reductase (NR) and activates the transcription of NR.(c) Expression levels of key genes of the N metabolic pathway in OE, WT, and i cells at day 4. (d) NO content in OE, WT, and i cells loaded with a DAF-AM probe and detected by fluorescence microscopy.(e) Relative fluorescence ratio in OE, WT, and i cells. Error bars: SD from three replicates.

Asterisks indicatesignificant differences compared with the controls according to Student's t test: (***P < 0.001)

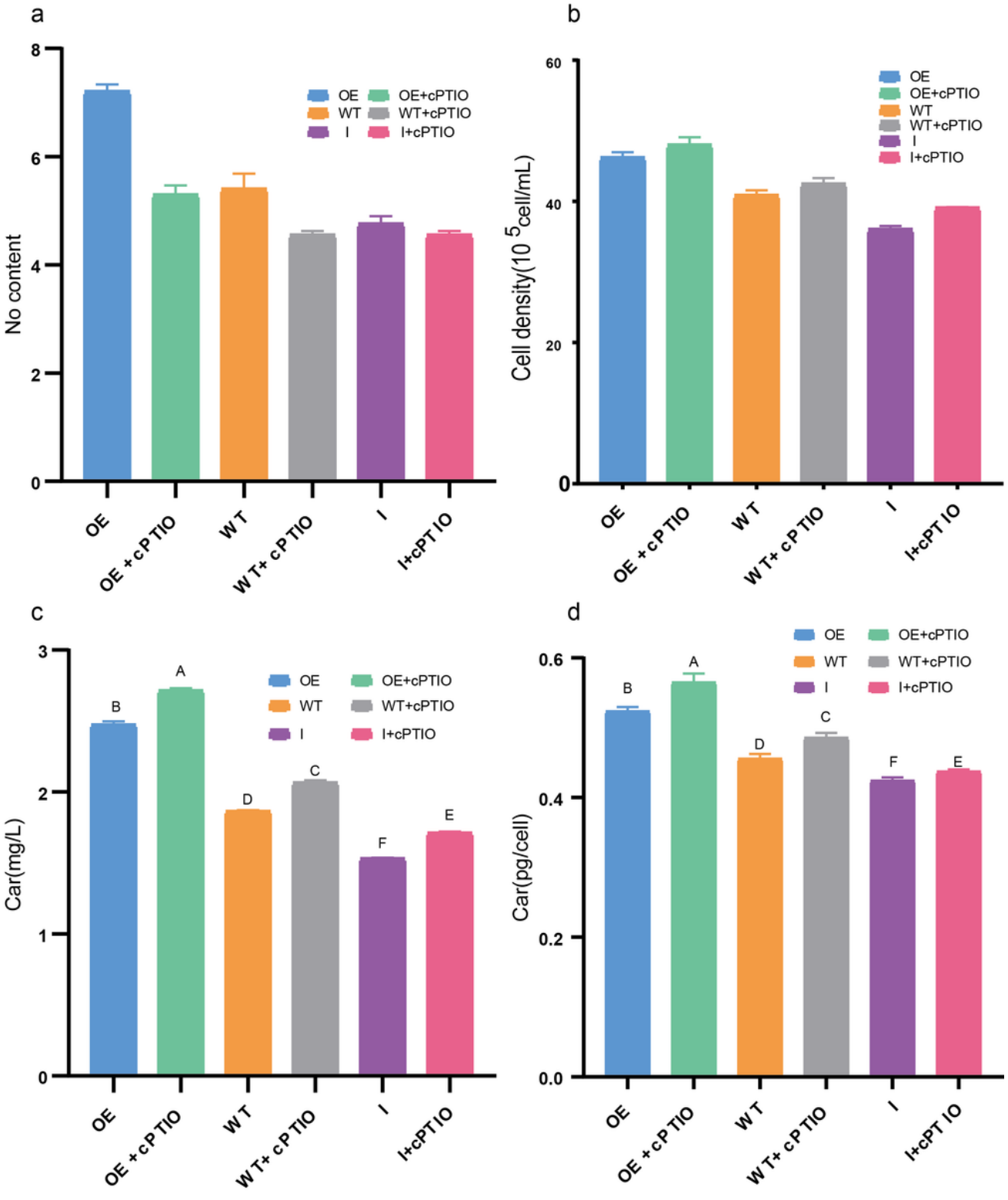


Figure 5

Effect of NO inhibitor on different genotypes of *D. salina* (a) NO content in OE, WT, i cells after addition of NO inhibitor (b) Biomass (c) Total carotenoid production (d) carotenoid production in single cells. Error

bars: SD from three replicates. Different letters indicate significant differences between the treatments according to Duncan's multiple range test ($P < 0.01$)

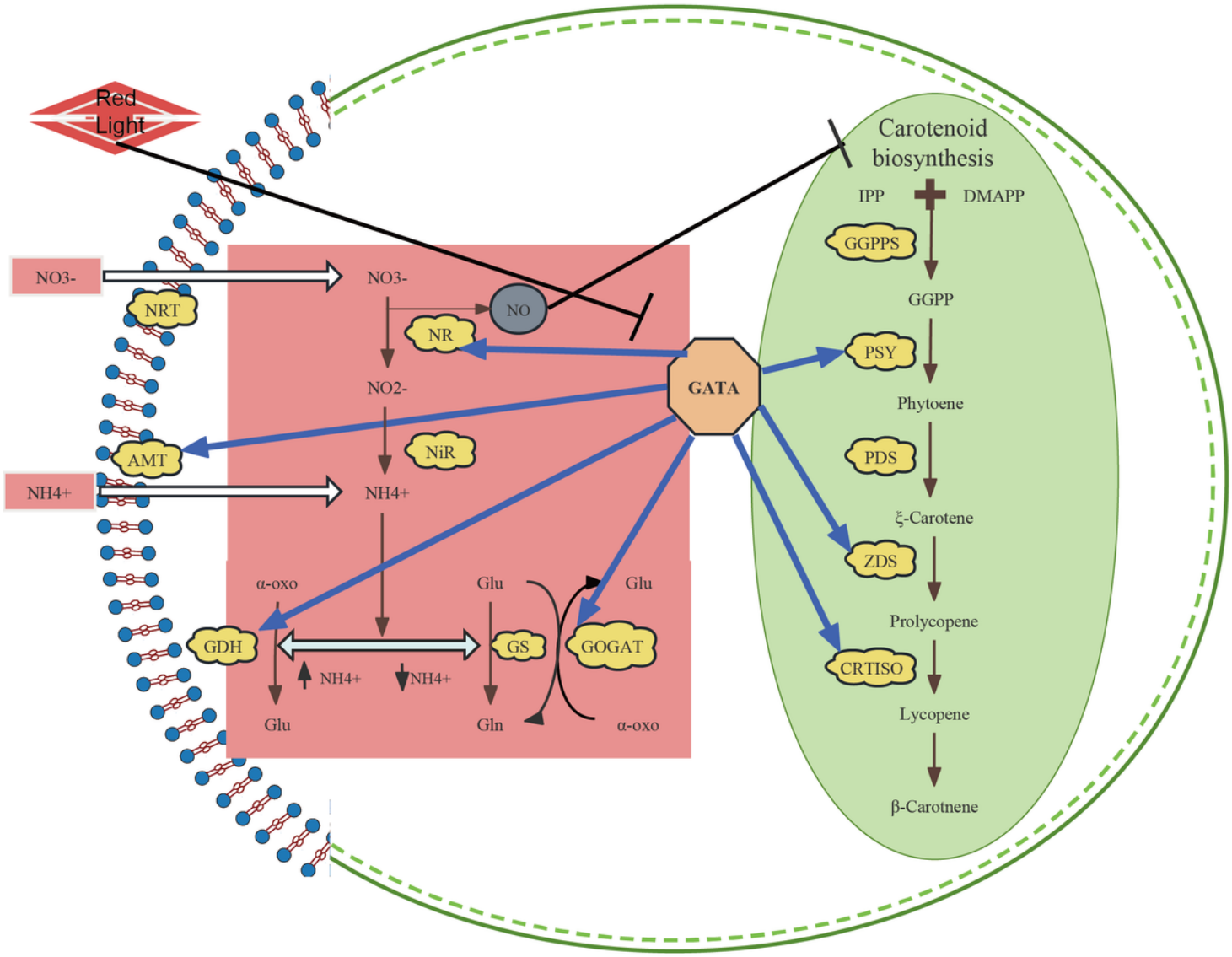


Figure 6

Proposed working model of DsGATA1 in regulating carotenoid synthesis in *D. salina*under red light. DsGATA1 acts as a global regulator to upregulate carotenoid synthesis in *D. salina*under red light. DsGATA1 upregulatesthe expression of several functional genes in the CBP pathway for carotenoid synthesis, including PSY, ZDS, and CRTISO; on the other hand, DsGATA1 also regulates carotenoid accumulation by affecting N metabolism. DsGATA1 directly binds to the nitrate reductase promoter to activate its expression, which promotes nitrate uptake in *D. salina* but produces NO molecules that inhibit carotenoid synthesis in the process. It also regulates the expression of the N-metabolism pathway genes GOGAT, GDH and ammonia transporter proteins to accelerate biomass accumulation and increase carotenoid content in *D. salina*. Blue line indicates activation, black termination line indicates inhibition

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx