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BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

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

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Abstract

Dunaliella salina is a high-quality industrial effector for carotenoid production. The mechanism by which red light regulates carotenoid synthesis is still unclear. 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 scavenger. Nevertheless, our results indicated that DsGATA1 could be an important target for engineering carotenoid production.

Key points

- *DsGATA1 with a distinct structure and recognition motif was found in D. salina*
- *DsGATA1 enhanced carotenoid production and biomass in D. salina under red light*
- *DsGATA1 is involved in the regulation of N metabolism and carotenoid synthesis*

Keywords *Dunaliella salina* · Carotenoid · GATA · Light regulation · N metabolism

Introduction

Microalgae have become a source of several natural metabolites due to their quick growth rate, high photosynthetic efficiency, cultivability in uncultivated areas (Saini et al. 2020), and excellent environmental tolerance. As a unicellular eukaryotic organism, *Dunaliella salina* has excellent potential for producing carotenoids with antioxidant properties (Rammuni et al. 2019).

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 (Raja et al. 2007). Culture conditions suitable for cell growth are not usually conducive to the accumulation of secondary metabolites (Tafreshi and Shariati 2009). 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 (Wichuk et al. 2014), 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

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have shown that red light is more favorable to the growth of *D. salina* (Pereira and Otero 2019). 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 $CX_2CX_{17\text{-}20}CX_2C$ class IV zinc finger pattern (Reyes et al. 2004). 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 (Trainor et al. 2000). 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 (Viger et al. 2008), 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 (Scazzocchio 2000). In plants, GATA factors are also reported to have various physiological functions (Schwechheimer et al. 2022). Although GATA motifs are prevalent in many promoter areas of plant light-responsive genes (Luo et al. 2010) and the GATA transcription factor has also been reported to be involved in the synthesis of terpenoids (Mihlan et al. 2003), 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*.

Materials and methods

Algal strains and culture conditions

D. salina strain FACHB 435 was provided by Freshwater Algae Culture Collection of the Institute of Hydrobiology, Wuhan, China. The culture medium contained 1.5 M NaCl, the red light intensity used for this study was 2000 Lux, and the growth conditions were as previously described (Wu et al. 2019). For the treatment of different NO content, the NO scavenger cPTIO (Carboxy-PTIO) from Sigma-Aldrich was added on the second day of incubation.

Acquisition and characterization of DsGATA1

Four GATA genes were discovered according to zinc finger structure by our team while measuring the transcriptome of *D. salina*. The expression of four genes under red, white, and red−white light conversion circumstances was detected by Q-PCR. 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 add the GATA and GATC motifs between *Eco*R I and *Sac* I; a yeast one-hybrid assay was run to investigate the binding motif of this transcription factor.

Construction and cultivation of DsGATA1 *D. salina* overexpression and interference strains

The CDS of the DsGATA1 gene was synthesized by PCR with the primers in Table S1, 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. The overexpressed and silenced sequences of DsGATA1 were digested with *Xho* I and *Kpn* I and inserted into the transgenic vector PGreen0029 (Figure S1 a) by the method of Lan (Lan et al. 2022); the sequences were in Table S2. And the RNA of the overexpressed, wild-type, and interference strains was extracted for qRT−PCR to determine the expression of their DsGATA1 genes.

Algal growth kinetics and N-source utilization kinetics

The growth curves of *D. salina* were plotted by taking 3 mL of algal solution every day and measuring the absorbance value at a wavelength of 654 nm with a spectrophotometer (Lan et al. 2022).

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 nitrate content in the culture medium was measured by high-performance liquid chromatography using a CIC-D160 ion chromatograph. The separation was performed on a Dionex IonPac AS 19 anion analytical column, eluting with 16 mM NaOH solution (Michalski and Kurzyca 2006).

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.

$$Ca = 12.21 * A663 - 2.81 * A647$$

$$Cb = 20.13 * A647 - 5.03 * A663$$

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

$$Ct = Ca + Cb$$

Ca is chlorophyll A, Cb is chlorophyll B, Car is carotenoids, and Ct is total chlorophyll.

$$Unitpigmentcontent = Cx * V * dilutiontimes/FW$$

Cx is pigment concentration (mg/L), V is extraction volume (mL), and FW is fresh weight of sample (g)

The pigment content of overexpressing strains, wild type strains, and interfering strains was measured on the fourth day to compare the pigment yield of transgenic *D. salina*. And the pigment content of OE, WT, and I was measured from the second day of carotenoid accumulation during growth to the sixth day of a substantial yield difference.

The NO scavenger 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.

Absolute content of different carotenoid fractions xanthophyll, β-carotene, neoxanthin, violaxanthin, zeaxanthin, and α-carotene in overexpressing, wild, and interfering strains on the fourth day of cultivation was determined using HPLC external standard targeted assay (Irakli et al. 2011; Melendez-Martinez et al. 2010; Rasmussen et al. 2012).

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 (Tang et al. 2020). The expression levels of the important enzymes ammonium transporter (AMT), glutamate dehydrogenase (GDH), glutamate synthase (GOGAT), glutamine synthetase (GS), nitrate reductase (NR), and nitrate transporter (NRT) as well as the carotenoid synthesis genes geranylgeranyl diphosphate synthase (GPPS), phytoene desaturase (PDS), phytoene synthase (PSY), zeta-carotene desaturase (ZDS), and carotenoid isomerase (CRTISO) were also measured by qRT−PCR using the primers in Table S1 in three different genotypes of *D. salina*.

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 digested with *Eco*R I and *Mlu* I and inserted into the pHis vector. DsGATA1 was digested with *Xba* I and *Xho* I and attached to pGADT7-Rec2 vector to test whether DsGATA1 could directly activate the transcription of NR, and vector maps are in Figure S1.

Determination of NO content

The NO content in the cells of three different genotypes was measured on the fifth day when the difference in carotenoid production was relatively large according to the method of Zhu (Zhu et al. 2019).

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. Statistical analyses were performed using the SPSS software for Windows (Windows version 26.0), and Duncan's multiple range test was used for the determination of significance.

Results

Molecular characterization of DsGATA1

Fluorescent quantitative PCR results 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).

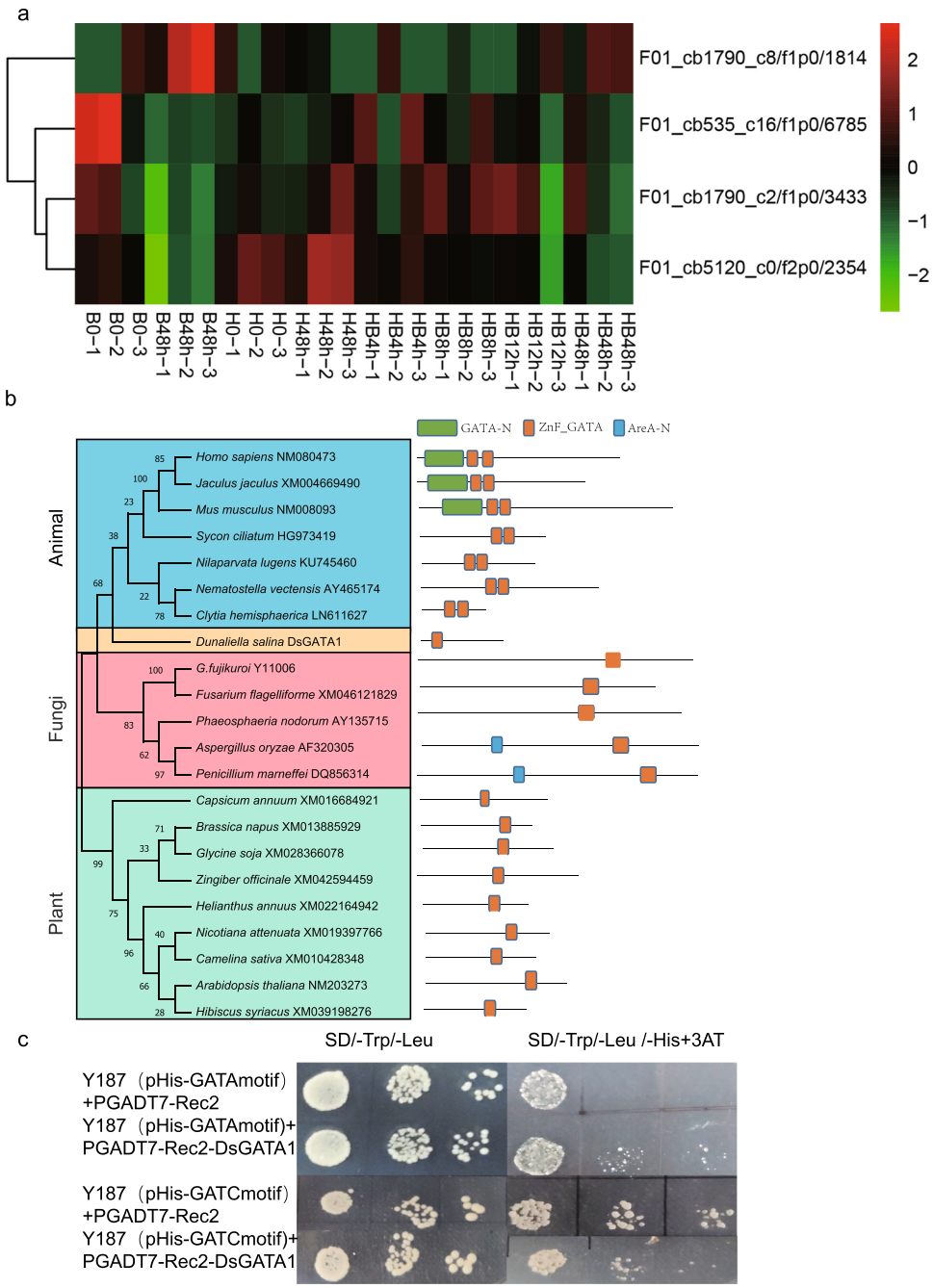

Fig. 1 Molecular characterization of DsGATA1. **a** Heatmap of GATA gene expression in *D. salina* under different light qualities, H-red light, B-white light, and HB-red light to white light. **b** Phylogenetic tree and conserved structural domain analysis of GATA molecules in animals, plants, and fungi. **c** DsGATA1 specifically binds to the GATA module and activates its transcription

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

Three overexpression strains, three interfering strains, and a no-load as a control were constructed to investigate the function of the DsGATA1. The expression of the DsGATA1 in overexpression strains OE1, OE2, and OE3 was 6.3, 2.3, and 3.6 times greater than that of the wild type, respectively, according to the results of Q-pcr analysis. The expression of the interfering strains I1, I2, and I3 was 86%, 61%, and 35% of that of the wild type, respectively (Fig. 2a). In all the transgenic strains, the resistance gene Cmr (520 bp) on transgenic vector was found, confirming the correct insertion of DsGATA1 in the genome of *D. salina* (Figure S2). After being incubated, it was discovered that the vector had no effect on the growth of because there was no discernible difference in the carotenoid accumulation or growth rate between the wild type and no-load under various lighting conditions. The growth curves of the *D. salina* under red and white light revealed that there was no significant difference in the growth rate of different genotypes of *D. salina* on the first day of growth. After the first day of growth, the slope of

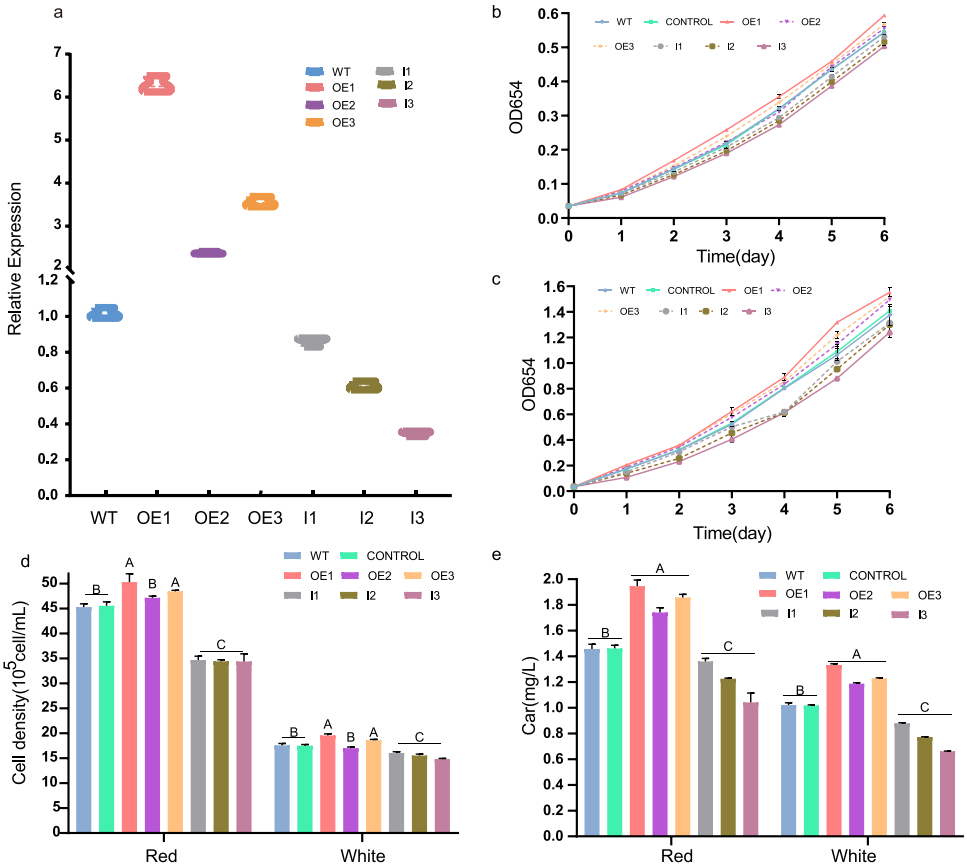

Fig. 2 Screening and growth comparison of DsGATA1 overexpression strain and interference strain. **a** Transcript levels of DsGATA1 in WT, OE1, OE2, OE3, I1, I2, and I3 cells. Error bars: SD from three replicates. **b** Growth of different genotypes of *D. salina* during red culture. **c** Growth of different genotypes of *D. salina* during white culture. **d** Biomass in different genotypes of *D. salina* under red or white light on the fourth day. **e** Total carotenoid production in different genotypes of *D. salina* under red or white light on the fourth day. Error bars: SD from three replicates. Different letters indicate significant differences between the treatments according to Duncan's multiple range test ($P < 0.01$)

the overexpression strain was significantly faster, the growth rate was significantly higher than that of the wild type, and the growth of the interfering strain was significantly inhibited. From the growth curves under different lights, it can be seen that the growth rates of wild type, overexpression strain, and interference strain in red light culture were significantly faster than those in white light. The biomass was determined on the fourth day of incubation under different light conditions, the biomass of wild type under red light increased by 2.8×10^6 cell /mL compared with that under white light, and overexpressed strain O1 and interfered strain I3 increased by 3.0×10^6 cell /mL and 2.0×10^6 cell /mL, respectively (Fig. 2b–d). Under red light, the biomass of overexpressed strains increased more than that of wild type. At the same time, red light can also improve the chlorophyll content in *Dunaliella*.

Compared with white light, the content of wild type chlorophyll was increased by 33% under red light, and the content of chlorophyll in overexpressed strain O1 was significantly increased by 53%. Interfering strains increased less, and I1 and I3 increased 26% and 7%, respectively (Figure S3). Red light can improve the chlorophyll content especially in overexpressed strains. This phenomenon demonstrates that both DsGATA1 gene and red light could significantly increase the biomass and chlorophyll content of *D. salina*.

Carotenoids were extracted from samples of various genotypes under various lights on the fourth day. The carotenoid content of the overexpression strain was significantly higher than that of the wild type under the same light, and the lowest yielding OE2 in the overexpression strain increased by 19.4% compared to both the wild type under red light and by 18.6% under white light. And the interfering strain's carotenoid synthesis was much reduced, with I3 producing just 71.5% as much carotenoid as the wild type under red light and a 34% less amount under white light. In contrast to white light, carotenoid yield was significantly improved in red light by 46% for OE1, 45% for the wild type, and 37% for the interfering strain I3 (Fig. 2e). The overexpression strain OE1 and the interfering strain I3 possessed the finest genetic features, and there was no significant difference between wild type and no-load. We selected OE1 as the overexpression strain OE, I3 as the interfering strain I, and wild type as the control for the following assays. All next investigations used red light culture because *D. salina* grew more quickly and accumulated more carotenoids when exposed to it.

DsGATA1 increased carotenoid production in *D. salina*

On day two, the total carotenoid production, biomass, and single-cell carotenoid production of the overexpressing, wild, and interfering strains were not significantly different under red light. The carotenoid concentration and biomass of *D. salina* started to differ significantly on the third day (Fig. 3a–c). The total carotenoid production of the overexpression strain was 1.12 mg/mL which 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%. At the same time, the overexpression strain showed a 9% increase in xanthophyll content and a 25% increase in β-carotene content, while the interference strain showed a 4% decrease in xanthophyll content and a 9% decrease in β-carotene, and there was no significant difference in the content of neoxanthin, violaxanthin, zeaxanthin, and α-carotene (Fig. 3e). 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 was 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 especially xanthophyll and β-carotene (Fig. 3a, b, c, e).

DsGATA1 regulated 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,

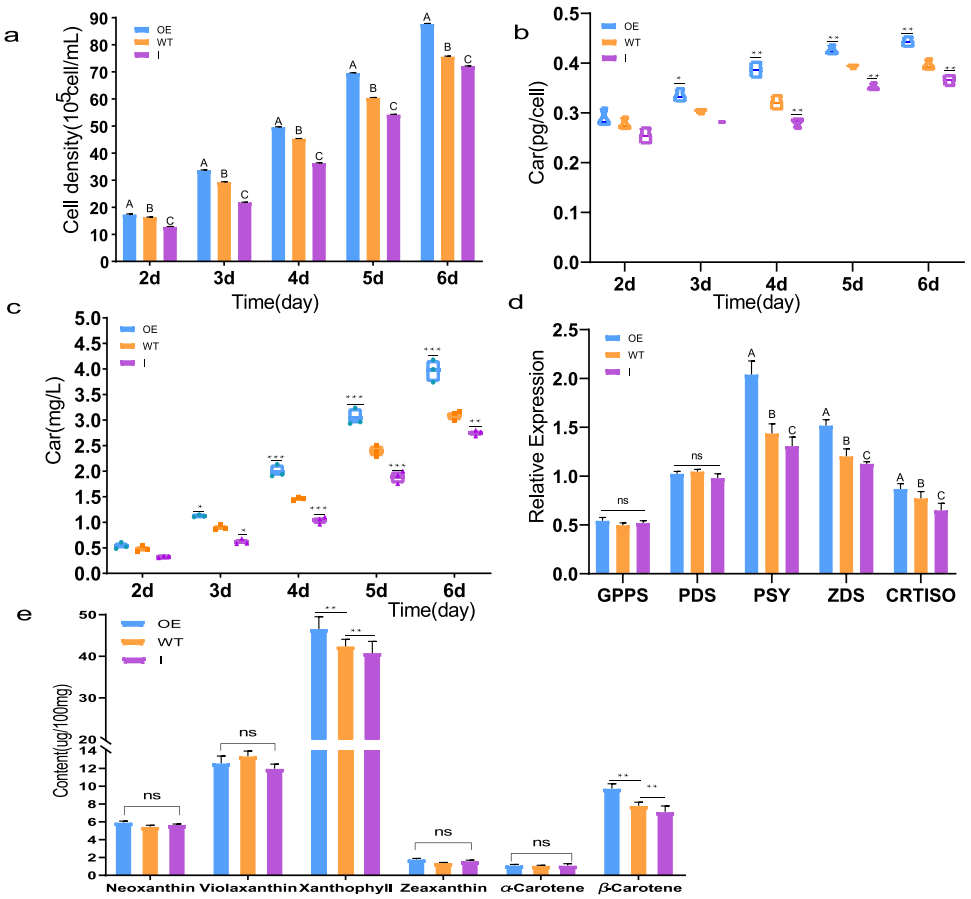

Fig. 3 Carotenoid accumulation in different genotypes of *D. salina*. **a** Biomass of OE, I, and WT at different time periods of culture. **b** Total carotenoid production. **c** Carotenoid production in single cells. **d** Expression levels of key genes of the carotenoid synthesis pathway in OE, I, and WT cells on the fourth day of maximum difference in single cell production. **e** Different carotenoid yields on the fourth day. Error bars: SD from three replicates. Asterisks indicate significant differences compared with the controls according to Student's t test: (*$P<0.05$, **$P<0.01$, ***$P<0.001$)

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*.

DsGATA1 promoted N metabolism in *D. salina*

It was mentioned earlier that 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.

DsGATA1 activated 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*.

NO inhibited carotenoid synthesis

NO is widely involved in the regulation of many metabolic processes as a signaling molecule (Gupta et al. 2022). On the second day, we added the NO scavenger cPTIO to determine whether NO would alter carotenoid accumulation in *D. salina*. The NO content in the overexpression strain dropped by up to 27% when the scavenger 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 scavenger, and the total carotenoid production of the overexpression strain with the addition of scavenger 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 scavenger (Fig. 5c). The single-cell carotenoid production of *D. salina* also changed significantly after the addition of the NO scavenger, and the overexpression strain with the addition of scavenger 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*.

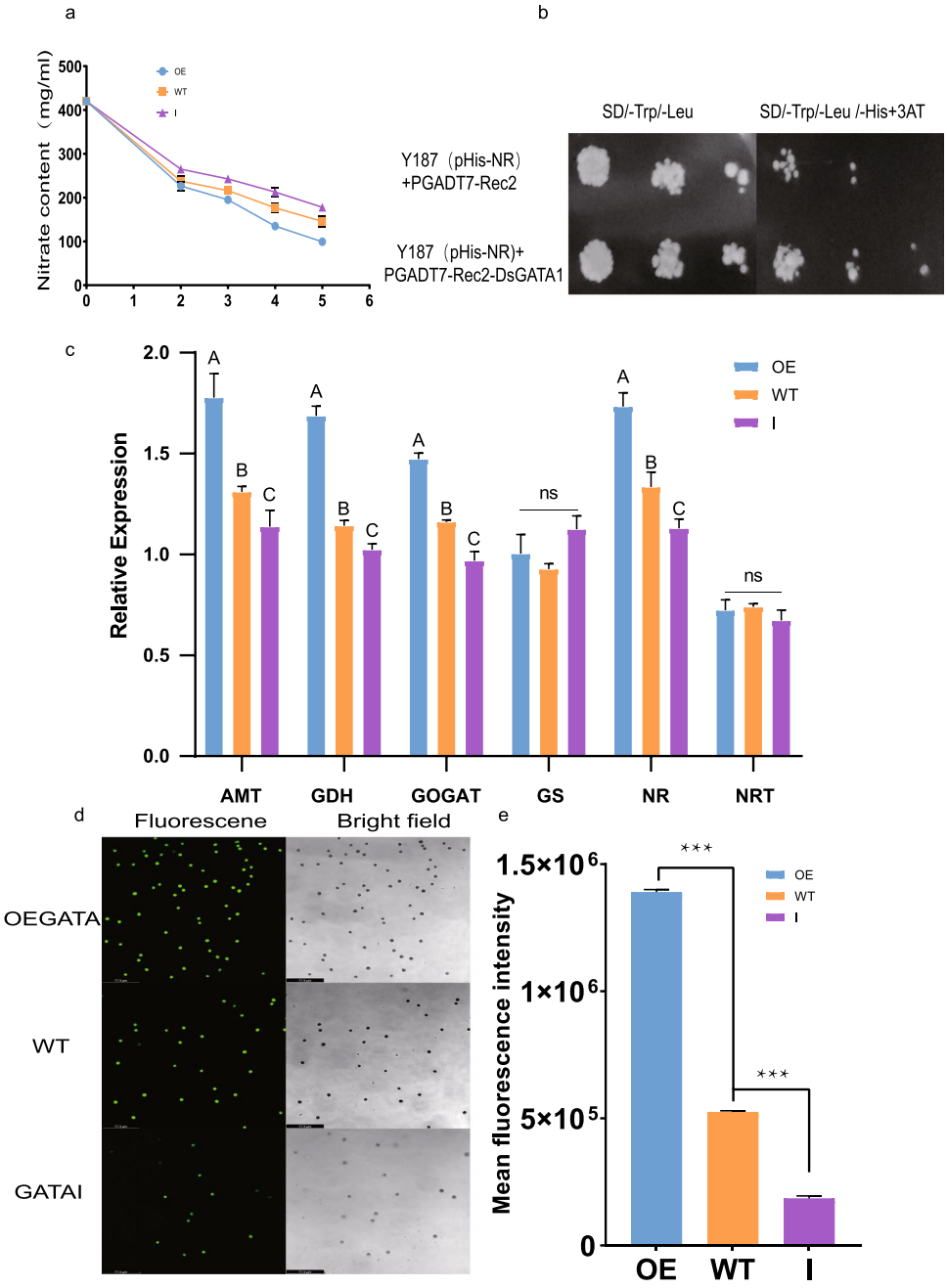

Fig. 4 Regulation of N metabolism by DsGATA1 in *D. salina*. **a** Nitrate content in the medium during OE, WT, and I culture. **b** DsGATA1 binds to the promoter region of nitrate reductase (NR) and activates the transcription of NR. **c** Expression levels of key genes of the N metabolic pathway in OE, WT, and I at day 4. **d** NO content in OE, WT, and I loaded with a DAF-AM probe and detected by fluorescence microscopy. **e** Relative fluorescence ratio in OE, WT, and I. Error bars: SD from three replicates. Asterisks indicate significant differences compared with the controls according to Student's t test: (***$P < 0.001$)

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 (Manzoor et al. 2021). 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 (Niu et al. 2020), whereas in animals, GATA usually consists of two distinct zinc finger structural domains located at the N-terminus and the C-terminus (Patient and McGhee 2002). 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 (Reyes et al. 2004), 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 (Teakle et al. 2002), whereas in animals, the zinc finger structural domain of GATA factors located

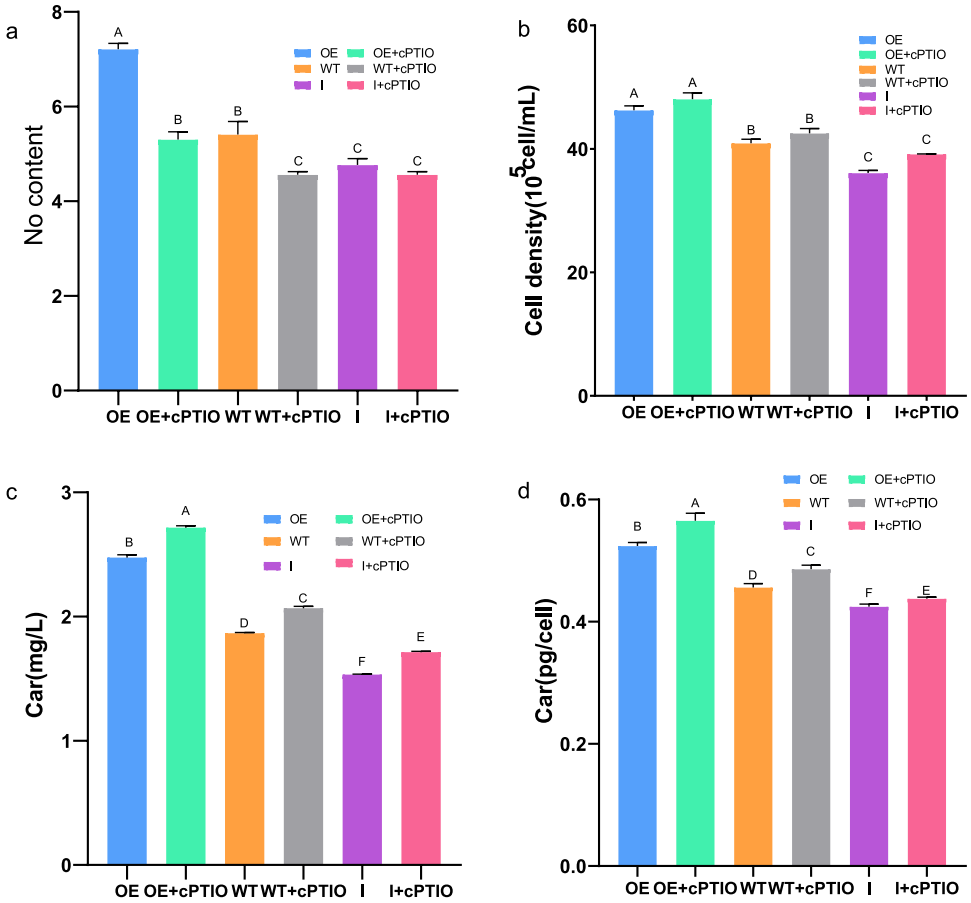

Fig. 5 Effect of NO inhibitor on different genotypes of *D. salina*. **a** NO content in OE, WT, and I after addition of NO inhibitor. **b** Biomass. **c** Total carotenoid production and **d** carotenoid production in single cells. Error bars: SD from three replicates. Different letters indicate significant differences between the treatments according to Duncan's multiple range test ($P < 0.01$)

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 (Newton et al. 2001). 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 is clearly distinct.

DsGATA1 acted as a global regulator involved in the regulation of carotenoid synthesis in *D. salina* under red light (Fig. 6). 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 (Zhu et al. 2019). 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 NH_4^+ in vivo and to balance the homeostasis of NH_4^+ in vivo and reduce its toxicity to algal cells (Kronzucker et al. 2001), DsGATA1 upregulated the expression of the gene encoding GOGAT, which can convert NH_4^+ to Glu and reduce the amount of NH_4^+. GOGAT has a stronger affinity for NH_4^+ (Gunka and Commichau 2012) and can keep the concentrations of NH_4^+ very low. When the NH_4^+ concentration was too high, DsGATA1 upregulated the expression of GDH, which can play a role in high concentrations of NH_4^+ (Reitzer 2003) and convert NH_4^+ into Glu. More intriguingly, DsGATA1 also upregulated AMT activity, implying the possibility of using NH_4^+ as a nitrogen source in *D. salina*. Although we did not examine

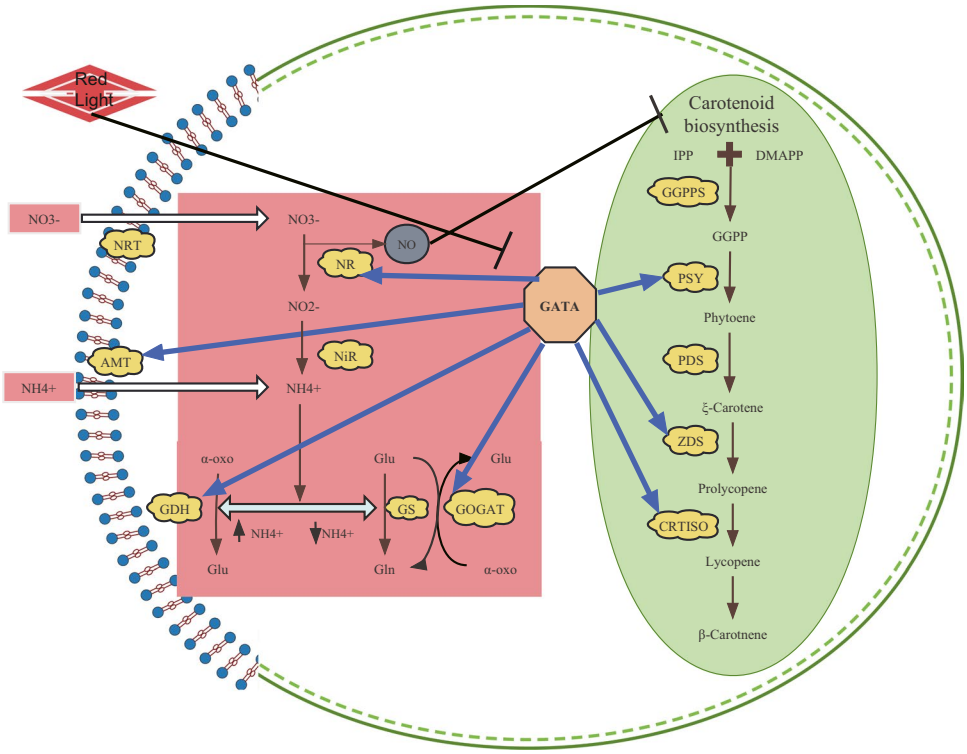

Fig. 6 Proposed working model of DsGATA1 in regulating carotenoid synthesis in *D. salina* under red light. DsGATA1 acts as a global regulator to upregulate carotenoid synthesis in *D. salina* under red light. DsGATA1 upregulates the expression of several functional genes in the CBP pathway for carotenoid 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, and black termination line indicates inhibition. NIR, nitrite reductase; α-oxo, alpha-ketoglutaric acid; Glu, glutamate; Gln, glutamine; IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate; GGPP, geranylgeranyl diphosphate

the efficiency of the transgenic algae to utilize NH_4^+ in this paper, the role of DsGATA1 in balancing NH_4^+ provides insights for the culture of *D. salina* by using ammonium nitrogen.

DsGATA1 improved the ability of *D. salina* to utilize nitrate but resulted in the production of more NO molecules. We eliminated NO produced by different genotypes of *D. salina* 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 scavenger 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.

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Author contribution YS and HX designed the study, performed the experiments, analyzed the results, and drafted the manuscript. HX and YC are the corresponding authors who 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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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

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Competing interests The authors declare no competing interests.

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