Expression and organisation of antenna proteins in the light- and temperature-sensitive barley mutant *chlorina*<sup>-104</sup>

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Abstract. The nuclear gene mutant *chlorina*<sup>-104</sup> of barley (*Hordeum vulgare* L.) is chlorophyll-deficient when grown under high irradiance, particularly at low temperatures. *Chlorina*<sup>-104</sup> chloroplasts had fewer thylakoids than the wild type, and fewer appressed lamellae relative to non-appressed lamellae. The freeze-fracture ultrastructure showed a loss of particles from the protoplasmic fracture face of the stacked thylakoid region (PFs), consistent with the loss of most of the light-harvesting complex (LHC) II, and a loss of some of the large particles from the same face of the unstacked thylakoid region (PFu), indicating a loss of photosystem-I particles. The mutant is remarkable for the high density of particles on the exoplasmic fracture face of the unstacked thylakoid region (EFu), levels of which fell to normal after transfer to low light. The chlorophyll deficiency was shown to be primarily caused by the loss of LHCII and LHCI–680, with the consequent loss of much of the chlorophyll (Chl) b and the xanthophylls neoxanthin and lutein. The use of a monoclonal antibody which recognises the 23-kDa polypeptide of LHCI–680, confirmed that it was severely depleted in chloroplasts from *chlorina*<sup>-104</sup> grown under restrictive conditions. The 77 K fluorescence emission spectrum was characterised by a pronounced shoulder at 720 nm, arising from the photosystem-I reaction centre (CPI). Since fluorescence from CPI is normally quenched by LHCI–730, this indicates that LHCI–680 mediates excitation energy transfer between LHCI–730 and the reaction centre. After moving seedlings to permissive conditions, LHCII and LHCI–680 began to accumulate in the chlorotic leaves and the fluorescence emission spectrum resembled that of wild-type leaves. Measurement of the steady-state mRNA levels with specific *Cab* probes, showed no difference between wild type and mutant, indicating that control of LHCII and LHCI–680 accumulation was at a post-transcriptional level.

Key words: *Cab*-gene expression – Excitation energy transfer – *Hordeum* – Light harvesting complex I (monoclonal antibody) – Mutant (barley) – Thylakoid (freeze-fracture)

Introduction

The major light-harvesting antennae of green plants are functionally specialised chlorophyll (Chl) a/b-binding complexes. In PSII there are at least four distinct Chl b-containing antenna complexes. In addition to light-harvesting complex (LHC) II, which binds 40–60% of the total Chl of the thylakoid membrane, three other Chl a/b-proteins (CP29, CP26 and CP24) transfer energy to the PSII reaction centre (Dunahay and Staehelin 1986; Bassi et al. 1987). In PSI, two separate antenna complexes, named LHCI–730 and LHCI–680 can be isolated (Lam et al. 1984; Bassi and Simpson 1987). This antenna diversity reflects the expression of a large family of different nuclear genes. In tomato, where the gene family coding for light-harvesting Chl a/b-proteins has been studied most extensively, about 18 *Cab* genes coding for Chl a/b-proteins have been identified (Pichersky et al. 1985, 1987a, b, 1988, 1989; Hoffman et al. 1987; Pichersky and Green 1990; Schwartz and Pichersky 1990). The coordination of pigment-protein synthesis and Chl and carotenoid biosynthesis involves both nuclear and plastidic genes during chloroplast development or during adaptations which result in significant stoichiometric changes in the antenna components. The use of mutants...
to study mechanisms of regulatory communication between the two genomes is well established (Somerville 1986; Oelmüller 1989). The conditional barley mutant chlorina-104 from the Copenhagen mutant collection, identified by its pigment-deficient phenotype and chlorophyll fluorescence properties as originally described by Simpson et al. (1985), appeared to be a promising candidate for investigation. The mutant is Chl b-deficient when grown under the restrictive conditions of low temperature (15°C) and moderate light (250 μmol photons m⁻² s⁻¹) and shows an unusually high F₆₀/F₅₀ ratio when examined by room-temperature fluorescence induction kinetics. Chlorina-10₄ returns to a wild-type phenotype when shifted to permissive conditions of higher temperature (22°C) and lower irradiance (75 μmol photons m⁻² s⁻¹). It is the first barley mutant in which mutant leaf tissue returns to normal as the result of being transferred from restrictive conditions (Knoetzel and Simpson 1990).

In the present paper we describe the alterations which accompany Chl b-deficiency in chlorina-104 using 77 K fluorescence emission spectroscopy, reversed-phase high-performance liquid chromatography (RP-HPLC) pigment analysis, non-denaturating green gel electrophoresis and Western blot analysis. The biochemical characterisation is extended by ultrastructural studies indicating that the lack of antennae leads to changes in the degree of thylakoid stacking and the density of freeze-fracture particles. Northern blot analysis using barley mRNA and tomato Cab genes coding for two PSII-antenna proteins, Cab 3C (PSII, Type I) and Cab 9 (PSII, CP29), and three PSI antennae, Cab 6A (PSI, Type I), Cab 7 (PSI, Type II) and Cab 8 (PSI, Type III) revealed that expression of these Cab genes is under post-transcriptional control in chlorina-104.

Materials and methods

Plant material. Seeds of wild-type barley (Hordeum vulgare L. cv. Svalöf’s Bonus) and the nuclear gene mutant chlorina-104 were germinated in tap-water moistened vermiculite and grown under different light and temperature conditions as indicated in the text.

Low-temperature fluorescence emission spectra. Spectra were recorded from intact leaves and by solid dilution at 77 K as described by Simpson and von Wettstein (1980) and Simpson et al. (1985). Spectral deconvolution into component Gaussian curves was performed with the programme RESOL (Simpson 1988).

Isolation of thylakoid membranes and pigment-protein complexes. Barley leaves were homogenized in 50 mM N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine (Tricine)-NaOH (pH 7.8), 10 mM MgCl₂, 0.4 M sucrose. The pellet membranes were washed twice in 10 mM 4-(2-hydroxyethyl)-1-piperazineneethanesulphonic acid (Hepes)-NaOH (pH 7.5), 1 mM EDTA, adjusted to 2 mg Chl·ml⁻¹, and stored at -80°C in 50% glycerol. Membranes were solubilised by adding an equal volume of 4 mM Tris-maleate (pH 7.0), 68% glycerol, 4% octylglucoside to give a final detergent: Chl ratio of 20:1. Non-denaturating gel electrophoresis was carried out with 0.05 M Tris, 0.49 M glycine, 0.1% sodium dodecyl sulphate (SDS) as running buffer and a constant current of 0.5–0.8 mA per tube for 3 h at 4°C using a Bio-Rad tube gel electrophoresis cell model 155 with tubes of 12.5 cm length and 0.5 cm internal diameter (Bio-Rad, Richmond, Cal., USA). The separating gel contained 11% acrylamide (11% T, 2.7% C), 0.42 M Tris-HCl (pH 8.8), 13% (v/v) ammonium persulphate and 0.0625% (v/v) N,N,N',N'-tetrathiomethyleneamidine (TEMED). The gel was overlayed by a 3-mm stacking gel consisting of 4% acrylamide (4% T, 2.7% C), 0.25 M Tris-HCl (pH 8.45), 13% (v/v) glycerol, 0.08% (w/v) ammonium persulphate and 0.08% (v/v) TEMED. The use of this stacking gel buffer, introduced by Schägger and von Jagow (1987), led to a preferential stacking of the faster-migrating pigment-protein complexes such as CP24, CP26, and CP29 (while CPI* and CPI are destacked), which results in an optimal resolution of all complexes. In order to isolate the PSI antenna LHCI-730 and LHCl-680 PSI-200 was prepared following the protocols of Mullet et al. (1980) and Bassi and Simpson (1987), subjected to green gel electrophoresis or, after dodecyl maltoside solubilisation, separated by sucrose gradient ultracentrifugation (Bassi and Simpson 1987). Photosystem II membranes (BBY particles) were obtained according to Berthold et al. (1981), omitting the second detergent treatment (Dunahay et al. 1984).

Pigment analysis. Reversed-phase high-performance liquid chromatography (RP-HPLC) of pigment extracts from thylakoid membranes and CPI* was carried out as described previously (Knoetzel et al. 1988). Chlorophyll concentrations were determined according to Arnon (1949).

Denaturating sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot assay. Polypeptides were separated on 8–25% gradient gels with the denaturing SDS-PAGE system of Fling and Gregerson (1986) using a Mini Protein II electrophoresis cell (Bio-Rad). Immediately after electrophoresis, the polypeptides were blotted onto nitrocellulose filters (HAHY, pore size 0.45 μm; Millipore, Bedford, MA, USA) using a semi-dry electroblotter. The Carlsberg monoclonal antibodies CMpLHCI-2 and CMpChla/b-Pl:1 (henceforward referred to as α-LHCI and α-CP29, respectively) originally described by Höyer-Hansen et al. (1988) were used in the immunoblot assays. The assays and detection of the immobilized antenna proteins were done according to Höyer-Hansen et al. (1985) except that 1% (w/v) skim milk powder in phosphate-buffered saline (pH 7.2) was used instead of 10% newborn calf serum.

Messenger-RNA isolation and Northern blot hybridisation. For mRNA isolation, 20 g of frozen leaves were rapidly pulverized in a coffee mill and the SDS-proteinase K-method of Klopsteg and Schweiger (1976) was followed. Polyadenylated [Poly (A)⁺] RNA was obtained by one cycle of oligo (dT)-cellulose chromatography as described by Sambrook et al. (1989) using 0.4 M LiCl in the first column-loading buffer. The poly(A)⁺ RNA was fractionated by electrophoresis in 1.2% agarose gels containing formaldehyde and capillary-transferred to a nylon filter (Hybond N; Amersham International, Buckinghamshire, UK) essentially as described in Sambrook et al. (1989), or denatured RNA was dotted directly onto Hybond N according to the manufacturers protocol using the Mini-1 dot blotter (Schleicher and Schuell, Dassel, FRG). The bound RNA was immobilized on the membrane by UV irradiation for 5 min.

The membranes were hybridized with α-[³²P]CTP-labelled randomly primed fragments (Feinberg and Vogelstein 1983) with specific activities of 2·10⁶ cpm per μg DNA. The following probes were used: Cab 3C (Hind III/Hind III fragment about 500 basepairs (bp) long; Pichersky et al. 1985), Cab 6A (Sal I/Xba I fragment about 1000 bp long, Eco RI/Nco I fragment about 450 bp long, Nco I/Nco I fragment about 300 bp long; Pichersky et al. 1987), Cab 7 (Hind III/Eco RI fragment about 900 bp long; Pichersky et al. 1988), Cab 8 (Sal I/Xba I fragment about 1100 bp long, Hind III/Hind III fragment about 500 bp long; Pichersky et al. 1989) and Cab 9 (Sal I/Sal I fragment about 1300 bp long; Pichersky and Green 1990). The plasmids containing the tomato nuclear DNA fragments were obtained from Dr. E. Pichersky of the University of Michigan, Ann Arbor, Mich., USA. Plasmids were transferred.