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Both light-dependent protochlorophyllide oxidoreductase A and protochlorophyllide oxidoreductase B are down-regulated in the *slender* mutant of barley

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Abstract

The gibberellin-insensitive overgrowth mutant of barley, *slender*, exhibits altered expression of a number of nuclear genes in comparison with the wild type. There is a particularly marked reduction in *slender* seedlings of transcript encoding protochlorophyllide oxidoreductase (POR), the enzyme which catalyses the penultimate and only light-requiring step in chlorophyll biosynthesis. The expression of the two barley genes encoding light-dependent POR, *PORA* and *PORB* was investigated. Expression of both genes was found to be reduced in *slender* seedlings relative to the wild type, in both etiolated and light-grown leaf tissue; this was most marked in the zone of rapid cell extension. Western blot analysis showed that POR protein was also less abundant in etiolated and in light-grown *slender* than in the equivalent wild-type leaf tissue, although the effect was less pronounced than at the transcript level. Protochlorophyllide content in etiolated *slender* seedlings was reduced in comparison with wild-type seedlings, though chlorophyll content in light-grown leaf blades was unaffected. The reduction in *POR* expression in *slender* barley may reflect a novel response to the constitutive activation of gibberellin signalling in this mutant. Despite the consequences of the mutation for *POR* gene expression, *slender* seedlings develop apparently normal chloroplasts in the light, and etioplasts with well-defined prolamellar bodies when grown in continuous darkness. This suggests that the POR content of wild-type barley seedlings is well in excess of the minimum required for normal plastid development.

Key words: Chlorophyll, chloroplast, etioplast, gibberellin, *Hordeum vulgare*, prolamellar body.

Introduction

The *slender* mutation of barley, which results from a recessive mutation at a single nuclear locus (Foster, 1977), confers a phenotype resembling that of wild-type barley supplied with exogenous gibberellin. However, endogenous levels of the biologically active gibberellins GA_1 and GA_3 are much lower in *slender* than in wild-type plants. Application of exogenous gibberellin does not alter the *slender* phenotype, and the GA biosynthesis inhibitor paclobutrazol, which stunts growth of wild-type cereals, has no effect on *slender* (Croker *et al*., 1990). Hence the mutation may be classified as a GA-insensitive overgrowth mutant.

In an earlier study on extension growth in barley, a number of genes was identified whose expression was significantly altered in the *slender* mutant compared with the wild type (Schünmann and Ougham, 1996). Of these, the gene whose level of message was most severely affected was that encoding light-dependent NADPH : protochlorophyllide oxidoreductase (POR). In wild-type barley seedlings, the *POR* message was expressed throughout the light-grown leaf, but most strongly in the region corresponding to the zone of rapid cell

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extension, with very little message in the fully-expanded leaf blade. In *slender* plants, the expression pattern was similar, but the message abundance was very greatly reduced, especially in the extension zone. The effect of the *slender* mutation on *POR* gene expression was unexpected. The nuclear-encoded but plastid-localized enzyme POR catalyses the NADPH-dependent coreduction of protochlorophyllide to chlorophyllide (Apel *et al*., 1980; reviewed in Griffiths, 1991); this is the penultimate, and only light-dependent, step in chlorophyll biosynthesis in angiosperms (reviewed in Beale, 1999). In etiolated angiosperm seedlings POR is located in the prolamellar body, and is the most abundant protein in this plastid inner membrane structure (reviewed in Ryberg and Sundqvist, 1991). Although the *slender* mutation affects many aspects of development, these are not known to include differentiation of the photosynthetic apparatus, and chlorophyll content expressed on a fresh weight basis is the same in leaf blade tissue of the mutant as in wild-type leaves (Schünmann and Ougham, 1996).

In barley, POR is encoded by two related genes, whose expression is differentially regulated developmentally and by light (Holtorf *et al*., 1995; Holtorf and Apel, 1996). *PORA* is expressed predominantly in dark-grown (etiolated) tissue of young seedlings, and its mRNA is readily detectable only at the beginning of greening. In contrast, *PORB* mRNA is found constitutively during the greening of etiolated seedlings, and is also present in light-grown leaf tissue. Although distinct biochemical functions for barley PORA and PORB in photo-oxidative protection, prolamellar body formation, light-harvesting, and pigment-binding have been postulated in recent years (reviewed in Reinbothe *et al*., 1996, 1999), most of the available evidence argues against these hypotheses (Klement *et al*., 1999; Scheumann *et al*., 1999; reviewed in Armstrong *et al*., 2000). In the previous characterization of genes differentially expressed in the *slender* mutant versus wild-type barley, it was a *PORA* cDNA clone that was isolated (Schünmann and Ougham, 1996). The current study was designed to answer the questions: does the mutation affect expression of one *POR* gene alone, or of both genes; and does its effect on *POR* expression cause any alterations in chloroplast and etioplast morphology?

Materials and methods

Plant material and growth conditions

The *slender* mutation is recessive, and plants homozygous for the mutant allele are sterile. It is therefore impossible to obtain a pure seed lot homozygous for the *slender* allele. Two seed stocks were used, both having the genetic background *Hordeum vulgare* cv. Herta Cb3014: homozygous for the wild-type allele; and segregating 1 : 3 *slender* : wild type. Seeds were sown in moist vermiculite in a controlled environment chamber at 20 °C, in continuous darkness or with a 16 h light-period (photon flux density *c*. 350 μmol m^{-2} s^{-1}). They were harvested in the mid-light period, 5 d after sowing, at which stage the primary leaf was 6–7 cm in length for wild-type barley, 10.5–12.5 cm for *slender*. Tissue for RNA and protein extraction was taken from three zones of the leaf. Zone A (leaf base; region of cell division) consisted of the first 5 mm of the leaf for the wild type and the first 7.5 mm for *slender*. Zone B (region of maximal cell extension rate) was the section 10–15 mm from the leaf base for the wild type and 15–22.5 mm from the base in *slender*. Zone C (leaf blade) was a 10 mm section taken 10 mm below the tip of the leaf in each case. Leaf segments from 10 plants were pooled for protein or RNA extraction. For immunolocalization studies, only zone C was used. To facilitate a direct comparison with the results reported earlier (Schünmann and Ougham, 1996), zone A is equivalent to sections 1 + 2 for the wild type in the latter paper, and to sections 1 + 2 + the basal half of section 3 for *slender*; zone B represents section 4 for the wild type and section 5 + the basal half of section 6 for *slender*; and zone C is equivalent to section L in both cases. The sections were chosen to represent equivalent developmental stages; Schünmann *et al*. used cell-free translation of mRNA isolated from leaf segments to determine which zones of wild-type and *slender* barley were comparable with respect to translatable mRNA profile and hence developmentally equivalent in at least this regard (Schünmann *et al*., 1994).

RNA extraction and Northern blot analysis

Total RNA was extracted from leaf tissue using a Fast-RNA Green kit (Bio 101). It was subjected to denaturing gel electrophoresis on 1.5% agarose/formaldehyde gels, using 10 μg total RNA per track, stained with ethidium bromide and transferred to nylon membrane (Boehringer Mannheim) as described previously (Schünmann and Ougham, 1996). Photography of the ethidium bromide-stained gels (not shown) was used to confirm uniformity of gel loading before blot transfer. Duplicate blots were hybridized with RNA probes specific to the 3′ untranslated regions of the barley *PORA* and *PORB* genes (Holtorf *et al*., 1995). The *PORA* and *PORB* sequences were, respectively, 94 and 96 bases in length. A non-radioactive protocol was used in which the RNA probes were labelled with digoxigenin and detected by chemiluminescence as recommended by the manufacturers of the DIG Northern kit (Boehringer Mannheim).

Protein extraction and Western blot analysis

Plant tissue was ground in liquid nitrogen and the protein extracted as described earlier (Mae *et al*., 1993). The extract was treated at 95 °C for 45 s immediately before loading onto a 12.5% polyacrylamide-SDS gel. Proteins were separated by electrophoresis using a Mini-Protean II apparatus (BioRad) for 45 min at 90 V, and transferred onto nitrocellulose membrane (Schleicher & Schuell) by electroblotting. They were subjected to Western blot analysis using a primary antibody raised against oat POR, the kind gift of WT Griffiths. Preimmune serum was used as a control. Detection was performed using a Chemiluminescent Western Blot Kit as recommended by the manufacturers (Boehringer Mannheim).

Protochlorophyllide determination

Protochlorophyllide content of etiolated leaf segments was determined by room-temperature fluorescence spectroscopy of 80% (v/v) acetone extracts. Each individual leaf segment was

dissected in darkness and extracted in 1 ml of 80% acetone, and fluorescence measurements made using an excitation wavelength of 433 nm. Protochlorophyllide was determined using the formula of Sperling (Sperling, 1998):

$$Protochlorophyllide = (F_{628} - 0.09 \times F_{668} + 0.006 \times F_{650}) \times 2.2$$

The resulting value indicates the protochlorophyllide content in ng ml^{-1} when the excitation and emission beam slit widths are both set to 4 nm. Fresh weights of an equivalent set of leaf segments were determined in order to express the pigment content in μg g^{-1} FW.

Immunolocalization and electron microscopy

Tissue pieces (approximately 1.5 mm^2) were dissected under, and infiltrated, in fixative (67 mM sodium cacodylate buffer pH 7.4 containing, 0.25% (w/v) glutaraldehyde, 4% (w/v) paraformaldehyde). Infiltration was carried out using mild vacuum for 15 min. Samples were washed, dehydrated in a graded ethanol series whilst lowering the temperature to −30 °C, and infiltrated with resin (Unicryl™; Scala *et al*., 1992; British BioCell International, UK) at −30 °C. The samples were transferred to BEEM™ capsules in fresh resin and the resin polymerized by UV (from a 15 W 360 nm light tube) at −20 °C for 48 h.

Ultrathin sections (silver/gold) for EM were collected on gold grids with a support film of formvar and carbon, which were glow discharged to assist section recovery and adhesion. After immunocytochemical labelling, sections were counter-stained with 2% (w/v) uranyl acetate for 15 min followed by lead citrate solution (Reynolds, 1963) for 3 min, and examined in a JEOL JEM 100S or JEM1010 electron microscope (JEOL, Tokyo) at 80 kV. Images were recorded on Kodak electron microscope film (type 4489, Agar Scientific, UK).

Immunogold labelling was performed as described earlier (Gordon *et al*., 1992), but with dilutions of primary antibody and colloidal-gold labelled secondary antibodies made in CTM buffer (154 mM NaCl, 10 mM Tris/HCl pH 7.4, 0.5% casein Hammarsten, and 0.02% thiomersal; Kenna *et al*., 1985) only modified to include 0.015% Tween-20 (CTM-T). Sections were counter-stained and examined. Controls were used to ensure the specificity of the primary antibody gave no or only background labelling. These were (1) the primary antibody was replaced with pre-immune serum or CTM-T buffer only, or (2) the second, detecting antibody was replaced with either colloidal gold-labelled BSA or colloidal gold-labelled goat anti-rat IgG.

Results

RNA gel blot analysis of PORA *and* PORB *expression*

To examine the expression of *PORA* and *PORB* genes in *slender* compared with wild-type barley, gene-specific riboprobes were used (Holtorf *et al*., 1995). Expression of both *PORA* and *PORB* could be detected in *slender* as well as in wild-type barley, but the mutant plants had reduced levels of both transcripts (Fig. 1). *PORA* was strongly expressed in dark-grown tissue, particularly in the zone of most rapid cell extension (tracks B). This zone is 10 mm from the leaf base in the wild type and 15 mm from the base in *slender* (Schünmann *et al*., 1994). It was in this region that the difference in *PORA* transcript abundance was most marked between wild-type and *slender* seedlings. In light-grown leaves, a small amount of *PORA* transcript could be detected in wild-type leaf bases. By heavily overexposing the luminogram, a trace could also be detected in *slender* leaf bases (data not shown). The upper regions of light-grown leaves had no measurable *PORA* transcript. *PORB* transcript, in contrast, was readily detected in both light- and dark-grown seedlings of both genotypes. In the light, it was present in the leaf base and extension zone, and was much more abundant in wild-type than *slender* seedlings. In etiolated seedlings, maximum *PORB* expression was, as for *PORA*, in the cell extension zone and again a large difference in expression between wild-type and *slender* was seen in this region, *slender* seedlings containing less of the transcript than the wild type. The light-grown leaf blade contained little *PORB* transcript, but overexposure of the luminogram (data not shown) revealed a trace even in *slender* leaf blades. Total POR transcript accumulation in dark-grown barley leaf sections obtained from an independent experiment (data not shown) and detected using a full-length barley PORA cDNA probe (Schulz *et al*., 1989) displayed a pattern similar to that observed with the PORA-specific riboprobe (Fig. 1). This correspondence is presumably due to the relative expression levels of PORA and PORB in etiolated barley seedlings (Holtorf *et al*., 1995).

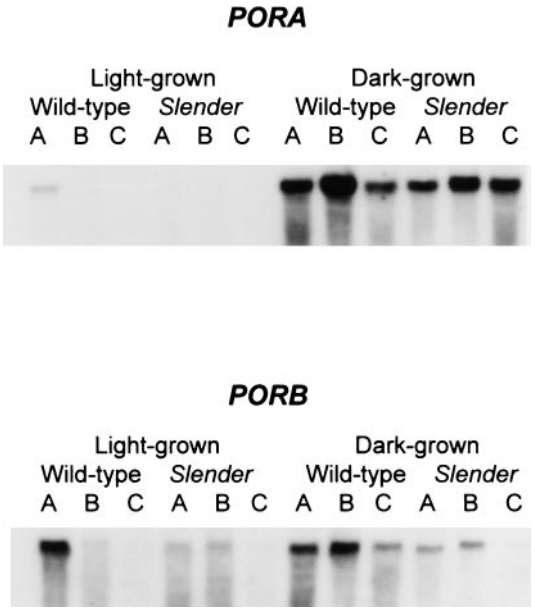

Fig. 1. RNA gel blot analysis of *PORA* and *PORB* transcripts in different leaf segments of 5-d-old seedlings of wild-type and *slender* barley grown under a 16/8 h light/dark regime, or in continuous darkness. Total RNA was extracted from leaf tissue and 10 μg was loaded per track. Duplicate blots were hybridized with gene-specific probes corresponding to the 3′-untranslated regions of the barley *PORA* and *PORB* genes (upper and lower panels, respectively). (A) Leaf base (cell division zone). (B) Zone of maximum extension rate. (C) Leaf blade.

The results shown in Fig. 1 were obtained using riboprobes containing *PORA* and *PORB* gene-specific sequences derived from their respective 3′ untranslated regions. For both riboprobes, it was necessary to heat-denature the product of the *in vitro* transcription reaction before adding it to the hybridization solution in order to obtain a signal, due to an unusually high proportion of predicted secondary structure.

Western blot analysis of POR protein

Figure 2 shows a Western blot analysis of POR protein in the same leaf tissues. The polyclonal antibody used does not distinguish between the PORA and PORB isoforms. For the results illustrated, the blots were challenged together with the same dilution of primary anti-POR antiserum, and detection was carried out on them in parallel; the resulting luminogram was exposed for the same time (5 min). The relative intensity of signal between the two blots may thus be considered a reasonable reflection of the relative protein abundance in light- and dark-grown tissue. The antiserum detected several distinct bands at approximately 40 kDa; these are typical of monocot leaf extracts (Griffiths *et al*., 1985; Dehesh *et al*., 1986; Davies *et al*., 1989; Holtorf *et al*., 1995; Holtorf and Apel, 1996; Klement *et al*., 1999). In barley seedlings PORA has been suggested to display a slightly smaller apparent molecular weight than PORB, on the basis of correlations with the accumulation patterns of the corresponding messages (Holtorf *et al*., 1995). These assignments of immunoreactive species are also consistent with the patterns of *POR* transcript and POR protein accumulation seen in different light-grown leaf sections of *slender* and wild-type barley (Fig. 2). However, in the absence of monoclonal anti-POR antibodies or N-terminal amino acid sequence information, it is not possible conclusively to assign the different immunoreactive signals to specific POR isoforms.

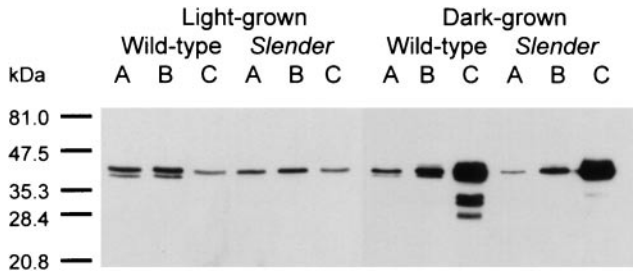

Fig. 2. Western blot analysis of POR protein in different leaf segments of 5-d-old seedlings of wild-type and *slender* barley grown under a 16/8 h light/dark regime, or in continuous darkness. Each track was loaded with protein extracted from an equal fresh weight of leaf tissue. (A) Leaf base (cell division zone). (B) Zone of maximum extension rate. (C) Leaf blade. The blot was challenged using a polyclonal antibody which recognises both PORA and PORB. Molecular weight markers are indicated on the left.

In Fig. 2 it appears that POR from light-grown wild-type barley (leaf base and extension zone) runs as a doublet between 37 and 41 kDa, whereas light-grown *slender* shows only a single band. In fact, longer exposure of the luminogram reveals the doublet in the case of the *slender* protein extracts as well (data not shown). While both components of the doublet are affected in *slender*, it is the lower band which shows the greater reduction. In dark-grown seedlings, POR is most abundant in the upper part of the leaf, with three additional, lower molecular weight bands between 35 and 28 kDa visible in this region (again, overexposure reveals them in the case of *slender* too). These signals may correspond to minor POR-derived degradation products. Small amounts of such products have been observed by other groups in protein samples from etiolated barley and wheat seedlings (Dehesh *et al*., 1986, Fig. 6; unpublished data cited in Rowe and Griffiths, 1995). In the leaf base and extension zone, the doublet is again observed for both genotypes, with the lower band more strongly affected than the upper band by the *slender* mutation. To confirm the reproducibility of these results, the Western blot analyses were repeated with the same antiserum using independently-isolated samples from a duplicate experiment. Qualitatively similar results (data not shown) were also obtained using another set of independent samples tested with antisera raised against purified barley POR (Apel, 1981) and against a barley PORA-LacZ fusion protein expressed in *E. coli* (Schulz *et al*., 1989).

It had previously been observed that in light-grown tissue the abundance of POR protein was reduced in *slender*, though not to the same extent as *POR* message (Schünmann and Ougham, 1996). The current results show that this also applies to dark-grown tissue. It was noted that the gradient of POR accumulation observed in different sections of etiolated *slender* and wild-type leaves closely parallels earlier measurements of the enzyme activity in leaf sections from the wild type (Dehesh *et al*., 1983).

Protochlorophyllide content of etiolated leaves

Room-temperature fluorescence spectroscopy measurements were used to measure protochlorophyllide content in dark-grown wild-type and *slender* barley seedling leaves. The results are shown in Table 1. No protochlorophyllide was detectable in the leaf base (cell division zone) of either genotype, nor in the zone of maximum cell extension for *slender*. In contrast, the wild-type seedlings had an appreciable amount of protochlorophyllide in the cell extension zone. In the zone just below the leaf tip, wild-type seedlings had more than four times the amount present in *slender*, expressed on a fresh weight basis. Thus, in etiolated seedlings of the same age, the appearance of protochlorophyllide is delayed in *slender*,

and its abundance in the leaf tip zone is much reduced, in comparison with wild-type barley.

Immunolocalization of POR in plastids

Immunolocalization of POR protein in leaf tissue (Fig. 3), using the same antibody as for the Western blot, shows that in the blade section of etiolated seedlings, the immunoreactive protein is located mainly in the prolamellar body of the etioplasts (Fig. 3A, B). The chloroplasts of light-grown seedling leaf blades also contain POR (Fig. 3C, D), but very much less than in the etioplasts. There is no consistent difference in chloroplast or etioplast morphology or numbers between *slender* and wild-type barley, and the amount of immunoreactive protein is also similar in etioplasts, and in chloroplasts, from each genotype. A pre-immune serum used as a control gave no significant signal in any of the sections examined (example shown in Fig. 3E).

Table 1. *Protochlorophyllide content in different leaf segments of 5-d-old seedlings of wild-type and* slender *barley grown in continuous darkness*

Protochlorophyllide was measured by fluorescence of acetone extracts. Segment A: leaf base (cell division zone). B: Zone of maximum extension rate. C: Upper part of leaf. Mean values (μg g^{-1} fresh weight) ± SD of three independent replicates. No protochlorophyllide could be detected in light-grown seedlings of either genotype.

	Wild type	*slender*
Zone A	0	0
Zone B	1.71 ± 0.4	0
Zone C	12.3 ± 0.4	2.85 ± 0.8

Discussion

The results of RNA blot analysis show that while *slender* barley expresses both *PORA* and *PORB*, the presence of the mutation causes a reduction in abundance of both transcripts in most regions of the leaf. It does not, however, qualitatively affect the transcript distribution throughout the leaf, or the light-dependence of gene expression.

The large difference in *POR* transcript abundance between wild-type and *slender* light-grown tissue makes it easy to understand why a *POR* cDNA was originally cloned by differential screening of cDNA libraries which

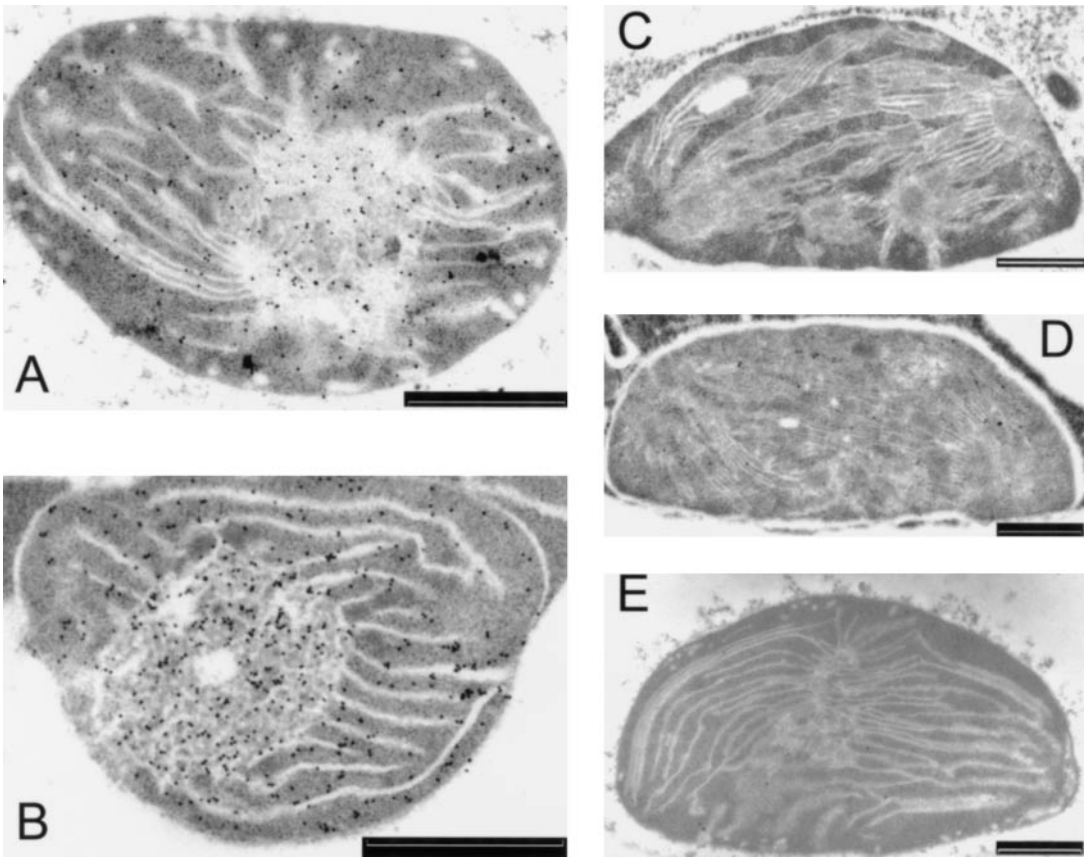

Fig. 3. Immunogold localization of POR protein in etioplasts (A, B) and chloroplasts (C, D) of transverse sections of wild-type (A, C) and *slender* (B, D) barley seedling leaf blades. A pre-immune serum was used as a control; example shown here for *slender* etioplasts (E). Scale bar represents 1 μm in each case.

were prepared from extension zone tissue of light-grown seedlings (Schünmann and Ougham, 1996). More surprising is the fact that it was *PORA*, not *PORB*, which was isolated in this way, since *PORB* message predominates in the light. However, the high degree of homology between the barley *POR* genes (Holtorf *et al*., 1995) would mean that the differential screen would have identified *PORA* and *PORB* clones indiscriminately, and it is possible that the clone selected for further study represented one of the minority *PORA* sequences in this population, rather than one of the *PORB* sequences which would have been statistically most likely to be chosen.

Western blot analysis showed that in light-grown tissue, *slender*, like wild-type barley, has the POR doublet characteristic of monocots in the lower part of the leaf, whereas both genotypes have only the higher molecular weight (40–41 kDa) component in the leaf blade. This must correspond to the protein detected in chloroplasts by immunogold localization, and it is reasonable, based on the literature, to assume that it is PORB. Previous work on expanding leaves of the grass *Lolium temulentum* had similarly shown a POR doublet in proteins from the lower part of the light-grown leaf, with a single band in the leaf blade (Ougham *et al*., 1987). The lower molecular weight band (37–38 kDa) seen in barley and *Lolium* may represent PORA. Holtorf and Apel found that in young barley seedlings grown under a 12/12 h light/dark cycle, the putative PORA polypeptide reaccumulated towards the end of the dark period, though this no longer occurred by the time the seedlings were 6-d-old (Holtorf and Apel, 1996). If this band is indeed PORA, the polypeptide proved more light-stable in the present study than in that of Holtorf and Apel (Holtorf and Apel, 1996), since the leaf tissue in this study was harvested half-way through the 16 h light period. However, Holtorf *et al*. found that when 5-d-old etiolated barley seedlings were shifted to white light, the putative PORA and PORB polypeptides were of roughly equal abundance after 8 h, and the putative PORA signal was still apparent even after 12 h (Holtorf *et al*., 1995). Furthermore, the putative PORA band was observed in 5-d-old etiolated seedlings which had received an additional 16 h of white light (Holtorf, 1995). In summary, some evidence argues that the putative PORA protein can persist for an extended period in illuminated barley seedlings. Alternatively, the assumed correspondence between POR proteins and bands detected in the Western blots is incorrect.

Chlorophyll *a* and *b* levels in fully-expanded leaf blade tissue of light-grown seedlings are almost identical (Schünmann and Ougham, 1996); this observation was confirmed for the present study (data not shown). In contrast, the *slender* mutation results in a marked reduction in the protochlorophyllide content of etiolated leaf tissue, and a delay in its appearance, since it is detectable in the zone of maximum cell extension in wild-type but not in *slender* barley. Thus the mutation affects the abundance of the substrate for POR, as well as the *PORA* and *PORB* transcripts and POR protein.

In etioplasts, POR is the major protein of the prolamellar body, where it is associated with protochlorophyllide and NADPH in a dark-stable complex (Griffiths, 1991; Ryberg and Sundqvist, 1991). Upon illumination, POR catalyses the very rapid reduction of protochlorophyllide to chlorophyllide, and following this conversion the POR protein is rapidly degraded by a light-induced protease (Reinbothe *et al*., 1995). Shaw *et al*. used immunogold localization to demonstrate that in wild-type barley etioplasts POR is associated with the prolamellar body (Shaw *et al*., 1985). Results in this study obtained with the same antiserum show that this is also the case in *slender* barley, which has etioplasts of qualitatively normal appearance. Thus although the abundance of *POR* message and, to a lesser extent, POR protein is reduced in *slender* barley compared with the wild type, as is the protochlorophyllide content, the levels of the protein and protochlorophyllide are apparently still sufficient to permit the development of normal etioplasts. When etiolated *slender* barley seedlings are exposed to light, they green over a similar time period as that required by wild-type seedlings (HJ Ougham and PHD Schünmann, unpublished data), showing that the POR : protochlorophyllide : NADPH complex is functional and that *slender* seedlings do not appear to be more susceptible to photo-oxidative damage than wild-type seedlings.

Chloroplasts in the blades of wild-type and *slender* barley seedlings are also of similar appearance, and immunolocalization revealed small amounts of POR associated with the thylakoid membranes in both cases. As mentioned above, this must represent the 40–41 kDa protein (presumably PORB) which is the upper band in the doublet seen on Western blots, since only this band is detected by Western blotting in proteins from this zone of the light-grown leaf.

It was concluded that the *slender* mutation results in a down-regulation of both *PORA* and *PORB* at the transcript level and, to a lesser extent, a reduction in POR protein content, together with a reduced protochlorophyllide content in dark-grown seedlings. However, these effects are not sufficient to impair detectably the development of normal etioplasts or chloroplasts in mutant seedlings, suggesting that *POR* gene expression in barley is normally in excess of the minimum required.

The basis for the effect of *slender* on *POR* expression in barley is unknown, but is likely to be due to the altered hormonal status of mutant plants. Although *slender* barley has lower levels of biologically-active gibberellins than wild-type, processes such as leaf extension rate and amylase secretion from the aleurone layer, which are normally gibberellin-regulated, are

gibberellin-independent in *slender*. In a few other angiosperms gibberellins have been shown to influence *POR* expression and plastid development. Exogenous gibberellin GA_3 enhances *POR* transcript accumulation in excised cucumber cotyledons (Kuroda *et al*., 1996). Younis *et al*. found that the gibberellin GA_3 enhanced etioplast development in dark-grown wheat seedlings, increasing protochlorophyllide content and prolamellar body development (Younis *et al*., 1995). *POR* message level was not determined in their study. It has been observed that treating wild-type barley with gibberellin reduces the amount of total *POR* transcript in leaf RNA to a level similar to that in *slender* (data not shown), and there is no obvious difference in etioplast development between *slender* and wild-type barley. The apparent inconsistency between the wheat and barley results may simply be due to a species difference, or it may be that *POR* transcript abundance is not the limiting factor in regulating etioplast development.

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