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Regulation of glutamine synthetase genes in leaves of *Phaseolus vulgaris*

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Abstract

Glutamine synthetase (GS) activity increased over three-fold in developing primary leaves of *Phaseolus vulgaris* L. This increase was shown to be the result of differential expression of three members of the GS gene family: *gln-α* and *gln-β*, which encode cytosolic GS polypeptides, and *gln-δ*, which encodes the chloroplast-located GS. The *gln-δ* gene was the most highly expressed GS gene and was regulated in a complex manner with two different transcripts accumulating differentially during leaf development. This gene was expressed weakly in the dark and was induced strongly by light; this induction was shown not to be an indirect effect of photorespiration. In the long term, *gln-δ* showed increased expression in photorespiring compared with non-photorespiring leaves. However, in the short term, there was no induction of *gln-δ* following transfer of plants to photorespiratory conditions. These results suggest that regulation of *gln-δ* by photorespiration was the result of indirect, long-term effects on cellular metabolism. In general, in all these experiments, analysis of cytosolic versus chloroplastic GS polypeptides and of the GS isoenzyme profiles showed the same pattern of changes in abundance as that observed for the mRNAs suggesting that regulation of GS gene expression occurred primarily at the mRNA level. However, it was noteworthy that the δ isoenzyme remained at a high abundance in older leaves, grown in both light and dark, despite a decrease in abundance of *gln-δ* mRNA.

Introduction

Assimilation of nitrogen in higher plants occurs primarily via the enzyme glutamine synthetase (GS; EC 6.3.1.2) which, in combination with glutamate synthase (EC 1.4.1.14/EC 1.4.7.1), leads to the production of glutamine and glutamate. Nitrogen, in the form of ammonium, is supplied to these enzymes from primary nitrogen via nitrate reduction, ammonium uptake and, in legumes, fixation of atmospheric nitrogen and from other pathways such as photorespiration and amino acid catabolism [29]. Photorespiration has been shown to be the major source of ammonium in the leaves of C3 plants, where it has been estimated to produce a 10-fold higher flux of ammonium than primary nitrogen assimilation [23].

GS in *Phaseolus vulgaris* L. is an octameric enzyme of about 320 kDa which occurs as a num-

ber of isoenzymes the subunits of which are encoded by four transcriptionally active, nuclear genes *gln-α*, *gln-β*, *gln-γ* and *gln-δ* [19]. The *gln-δ* gene encodes the 42 kDa subunits of the plastid-located δ isoenzyme and has been shown to be highly expressed in leaves and stems [27]. The *gln-α*, *gln-β* and *gln-γ* genes of *P. vulgaris* encode cytosolic GS polypeptides (α, β and γ) of about 39 kDa. These polypeptides assemble, perhaps randomly, to form the cytosolic GS isoenzymes [3].

It has been known for some time that the leaves of most higher plants contain both chloroplastic and cytosolic GS isoenzymes [28]. More recent work has begun to uncover the specific roles of each isoenzyme in this organ. In barley, mutations leading to a loss of the plastid-located GS were lethal under photorespiratory conditions [5, 39]. This isoenzyme, therefore, appears to be essential, at least in barley, for the reassimilation of photorespiratory ammonium. A role in photorespiratory nitrogen recycling has also been assigned to the chloroplastic GS of pea since the gene encoding this enzyme appears to be regulated by the photorespiratory status of the plant [17]. This pea gene also shows light regulation, mediated via the chromophore phytochrome [35]. In addition, experiments in which pea GS gene promoters were fused to the reporter gene β-glucuronidase (GUS) and expressed in tobacco indicated that the two promoters from genes encoding the chloroplastic polypeptides and one of the cytosolic polypeptides possess non-overlapping, cell-specific patterns of expression [18]. The chloroplastic-GS promoter was active in photosynthetic cell types reflecting its proposed role in the assimilation of ammonium generated by photorespiration and possibly also by nitrate reduction. The phloem-specific expression pattern seen with the cytosolic-GS promoter, on the other hand, indicated that the corresponding isoenzyme possibly functions to generate glutamine for intracellular nitrogen transport.

Previous studies carried out in this and other laboratories have provided information about the regulation of the GS genes of *P. vulgaris* in a number of organs including nodules, roots and cotyledons [3, 4, 11, 13, 20, 22, 31, 32, 34]. The work presented here wes designed to examine how this small gene family is regulated in developing leaves and to examine the effects of light and photorespiration on GS gene expression in this organ.

Materials and methods

Plant material

The leaf material analysed in Figs. 1 to 4 was harvested from non-nodulated plants of *P. vulgaris* cv. Tendergreen grown from seeds imbibed overnight in tap-water and grown under a 12 h light/12 h dark cycle in vermiculite under the growth room conditions previously described [27]. For all the other leaf tissue samples, non-nodulated *P. vulgaris* cv. Tendergreen plants were grown in vermiculite in growth cabinets at 25 °C and between 80 and 100% RH. Etiolated plants were obtained by imbibing and germinating seeds in vermiculite in the dark in a growth cabinet within a light-proof room. Leaves were harvested in complete darkness. Light-grown plants were exposed to continuous white light illumination of 100 μmol m^{-2} s^{-1}. The effect of photorespiration was investigated by comparing plants grown in a growth cabinet in air (0.02% CO_2) with plants grown in a growth cabinet into which 100% CO_2 was injected as a slow stream in order to maintain a CO_2 concentration in the cabinet of between 2 and 4%. The CO_2 concentration within the cabinet was monitored using a CO_2 analyser; the light intensity, temperature and humidity were identical to those of the air-filled cabinets. All leaf samples were from plants which were watered with a nutrient solution containing 1 mM Ca $(NO_3)_2$[9]. Total root RNA was prepared from roots of plants grown for 10 days in perlite and watered with a nutrient solution lacking a nitrogen source [9]. Total nodule RNA was prepared from nodules harvested 13 days after inoculation of plants grown as described by Chen and Cullimore [10] with the wild-type *Rhizobium leguminosarum* bv. *phaseoli* strain CE3 [30]. In all cases, tissue was harvested, frozen in liquid nitrogen and stored at −80 °C.

RNase protection assay of mRNA abundance

An RNase protection method [24] was used to assay for specific mRNAs. Isolation of total RNA and synthesis of probes specific to *gln-δ*, *gln-α* and *gln-β* have been described previously [4]. GS mRNA was quantified by comparison with RNase protection of calibration curves of (m)RNA synthesised *in vitro* from GS cDNA clones [4]. A cDNA library derived from mRNA of *P. vulgaris* cv. Tendergreen primary leaves [27] was screened with a pea CAB probe and the most abundant hybridising species, as determined by restriction mapping, was selected for use as a CAB probe. A 500 bp *Hin*d III fragment from this CAB cDNA was subcloned into pGEM-4Z and digested with *Eco* RI so that the transcribed probe was of about 400 nucleotides. A 160 bp *Sac* I/*Bam* HI fragment of a *P. vulgaris* EF-1α cDNA [2] was cloned into pGEM-4Z which was then linearised with *Eco* RI and used as the template for the EF-1α probe. Probe corresponding to mRNA of the phenylalanine ammonia-lyase gene PAL1 was transcribed from pGEM-3Z containing the 1427 bp *Sac* I/*Hin*d III fragment of pPAL5 [16] linearised with *Hin*c II. Ten nucleotides of this 311 nucleotide probe are derived from the vector. The probe used to analyse the 5′ end of *gln-δ* mRNA in Fig. 4 was derived from the cloned *gln-δ* gene (J.M. Cock and J.V. Cullimore, unpublished data). A 2665 bp *Pst* I fragment was subcloned into pGEM-3Z and linearised with *Bgl* II so that a 751 nucleotide antisense transcript produced from the T7 promoter starts from within an intron and runs upstream through a 5′ untranslated exon (as defined by comparison with a *gln-δ* cDNA sequence [27]. (Note that several of the probes used produced a number of protected fragments of approximately the same size rather than the expected single fragment. This was assumed to be due to 'breathing' at the ends of the hybridising region and RNase digestion of the exposed RNA strands. However, as the multiple fragments were always present in the same ratio and as the same pattern occurred in the standard curves, this did not interfere with quantitation of the mRNAs.)

Analytical procedures

Western blots were carried out as described by Bennett and Cullimore [3] with either constant amounts of 200 nmol/min GS_t activity (Fig. 3A) or constant amounts of 100 μg soluble protein (Fig. 6A) loaded in each track. Ion-exchange high-performance liquid chromatography (IE-HPLC) was carried out as described in Bennett and Cullimore [3]. GS transferase (GS_t) activity in cell-free extracts was assayed as described by Cullimore and Sims [12]. Protein was determined by the method of Bradford [8] and specific enzyme activities were then expressed as μmol/min per mg protein.

Results

Developmental regulation of GS in leaves of P. vulgaris

Seeds of *P. vulgaris* were imbibed and grown for 13 days under 12 h light/12 h dark illumination and samples of plumules/primary leaves were harvested every 24 h. The fresh weight of the leaves increased more than 160-fold during the course of the experiment. Figure 1 shows that GS activity was detectable in extracts of plumules from dry seeds and increased by more than 3-fold

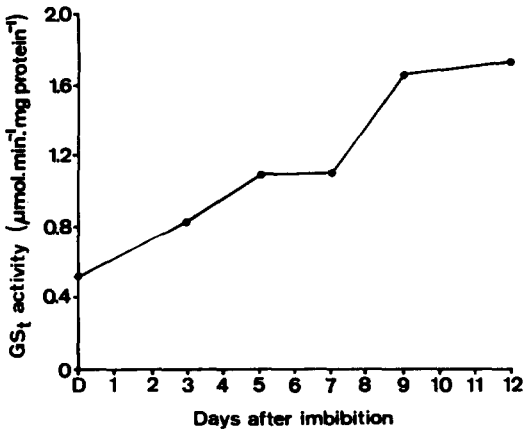

Fig. 1. Changes in the specific activity of glutamine synthetase during leaf development. D indicates plumules from dry seeds.

during the developmental period. The curve of the graph, however, suggests that the kinetics of accumulation of GS activity were complex. The aim of the following experiments was to analyse the kinetics of this accumulation at the level of the mRNA, polypeptide and isoenzyme for each of the GS genes.

An RNase protection technique was used to measure the abundances of gene specific mRNAs in developing leaves (Fig. 2). Three GS genes were shown to be differentially expressed resulting in transient peaks of accumulation of *gln-α* mRNA, followed by *gln-β* mRNA and finally of *gln-δ* mRNA. The *gln-δ* gene was the most highly expressed GS gene (Fig. 2) and accumulation of its mRNA corresponded approximately with greening of the leaves (data not shown). The

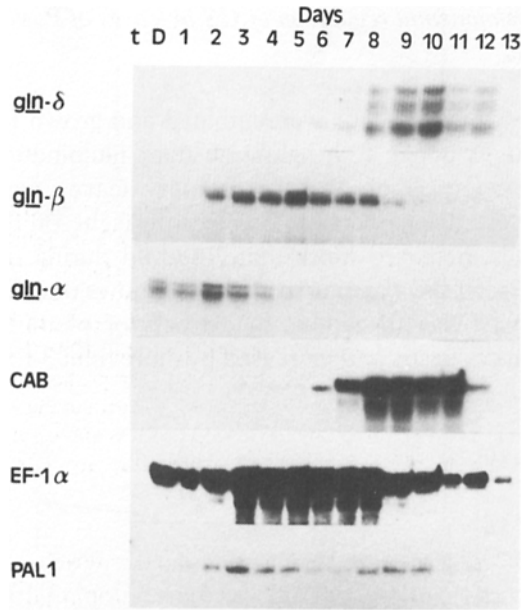

Fig. 2. Abundances of mRNAs corresponding to three GS (*gln*) genes and of mRNAs encoding CAB, EF-1α and PAL1 in developing leaves. The mRNA abundances were measured using an RNase protection technique in total RNA from plumules/leaves from dry seeds (D) and from seedlings harvested 1 to 13 days after imbibition and, as a control, in yeast tRNA (t). Maximum abundances of GS mRNAs were calculated (by comparison with standard curves of (m)RNAs synthesized *in vitro*) as 30 pg/μg total RNA for *gln-α* at day 2, 60 pg/μg total RNA for *gln-β* at day 5 and 160 pg/μg total RNA for *gln-δ* at day 10.

mRNAs corresponding to a chlorophyll *a*/*b*-binding (CAB) protein gene, a translation elongation factor (EF-1α) gene and the phenylalanine ammonia-lyase PAL1 gene were also assayed for comparison (Fig. 2). The CAB and the EF-1α mRNAs showed similar changes in abundance to *gln-δ* and *gln-β* respectively whilst the PAL1 mRNA showed a different, complex pattern of expression with peaks of abundance at 3 and 9 days.

Western immunodetection of GS polypeptides in extracts of leaves from the developmental series (Fig. 3A) showed that the cytosolic subunits (α and/or β; these are indistinguishable on a one-dimensional gel) were most abundant in plumules and leaves of young seedlings whereas the chloroplast-located subunit, δ, was first detected at day 5 and thereafter increased to account for over 90% of the GS protein in mature leaves. GS isoenzymes in these same extracts were separated by IE-HPLC and detected in the column fractions by enzyme activity (Fig. 3B). In general changes in abundance of the three GS isoenzymes closely matched changes in abundance of their corresponding GS mRNA and polypeptide. It was noted, however, that δ isoenzyme activity continued to increase between day 9 and day 12 despite a decrease in *gln-δ* mRNA abundance occurring after day 10. Note that no polypeptide or isoenzyme corresponding to the fourth expressed GS gene in *P. vulgaris*, *gln-γ*, was detected in leaves (Fig. 3); this confirms a previous observation that *gln-γ* mRNA is not detectable in this organ [4].

Identification of two different gln-δ *mRNA transcripts*

A probe derived from the 5′ region of the cloned *gln-δ* gene was found to detect the presence of two *gln-δ* mRNA transcripts differing in the length of their 5′ untranslated region (Cock, Hémon and Cullimore, in preparation). RNase protection was used to measure the abundance of these two transcripts during leaf development (Fig. 4A). The two *gln-δ* transcripts showed different kinetics of

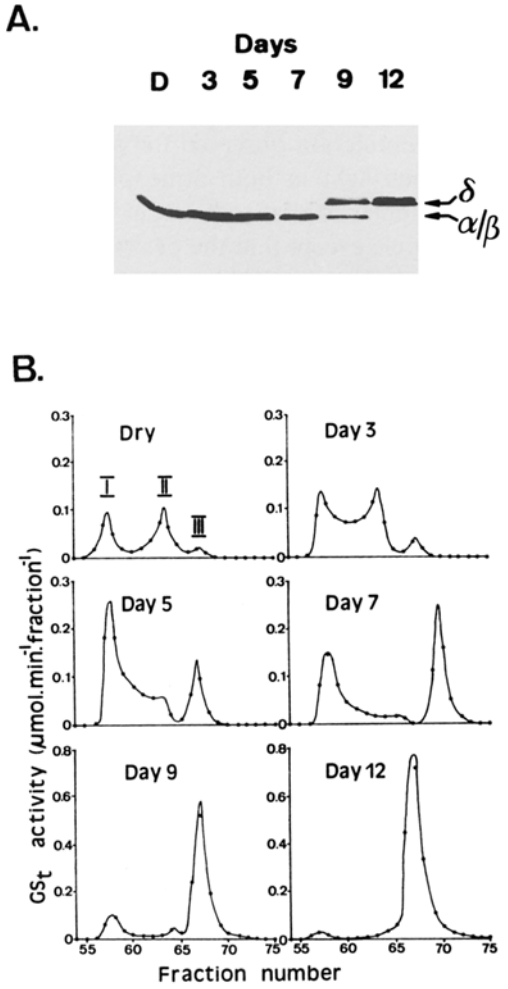

Fig. 3. Changes in abundances of GS polypeptides and GS isoenzymes in plumules/leaves from dry seeds (D) and from seedlings harvested up to 12 days after imbibition. A. Western blot of GS polypeptides. Note that the α and β polypeptides are not separated on a one dimensional gel. B. IE-HPLC of GS isoenzymes in leaf extracts. The three GS peaks, marked I, II and III, have been shown previously [3] to contain predominantly β, α and δ subunits respectively. Activities eluting between peaks I and II have been shown to be composed of isoenzymes containing mixtures of α and β polypeptides [3]. GS activity is plotted as μmol/min per fraction corrected for a column loading of 1 mg soluble protein.

accumulation; one transcript was first detected at day 4, accumulated to a peak at day 9 and then decreased in abundance, whilst the second transcript species only appeared after day 8 but then accumulated to a greater molar abundance by day 10 (Fig. 4A).

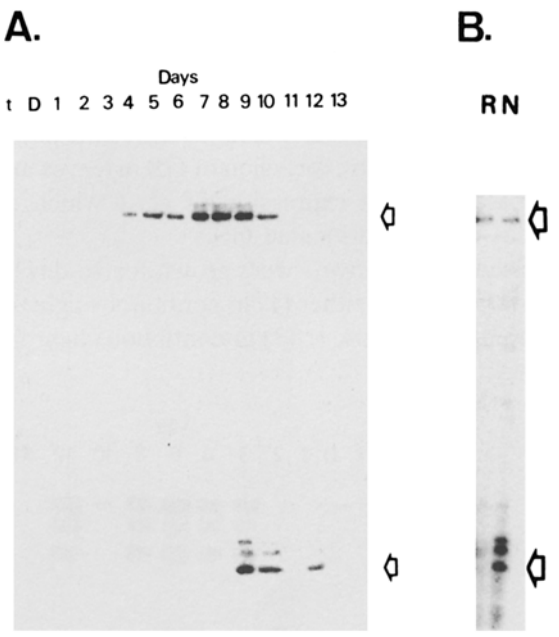

Fig. 4. Identification of two differentially regulated transcripts of *gln-δ*. Transcripts of *gln-δ* were detected by RNase protection using a probe from the 5′ end of the *gln-δ* gene. A. Differential accumulation of *gln-δ* transcripts during leaf development. The RNA samples were exactly as described for Fig. 2. B. Detection of *gln-δ* transcripts in total RNA from roots (R) and nodules (N). This autoradiograph has been exposed for longer than the autoradiograph shown in Fig. 4A in order to detect the *gln-δ* mRNA which is less abundant in these organs [4, 11]. The arrows indicate the major transcripts.

It has recently been shown that there is an accumulation of *gln-δ* mRNA in developing nodules [4]. It was therefore of interest to examine whether this accumulation also involved differential regulation of the two transcripts detected in leaves. The results (Fig. 4B) show that total RNA from roots contained only one of the two transcripts but that both transcripts were present in total RNA from nodules. Moreover, the accumulation of *gln-δ* mRNA in nodules compared with roots appeared to be due entirely to the accumulation of this second transcript.

Effect of light and photorespiration on the expression of gln-δ *in leaves*

The experiments described in this section were designed to study the effects of light and photorespiration on the expression of GS in leaves and, in particular, on expression of *gln-δ* which encodes the plastid-located GS.

Seeds of *P. vulgaris* were grown for 16 days in growth cabinets either (1) in continuous light, (2) in continuous dark or (3) in continuous light in a high CO_2 atmosphere (in order to suppress photorespiration). The abundances of the *gln-δ*, *gln-β*, CAB and EF-1α mRNAs were then analysed by RNase protection (Fig. 5A). The pattern of mRNA accumulation observed for plants grown in continuous light in both atmospheric conditions was similar to that seen under a 12 h light/12 h dark cycle except that the peaks of accumulation of all four mRNAs occurred earlier (Fig. 5A; cf. Fig. 2). This difference in kinetics of accumulation may be due to the fact that the

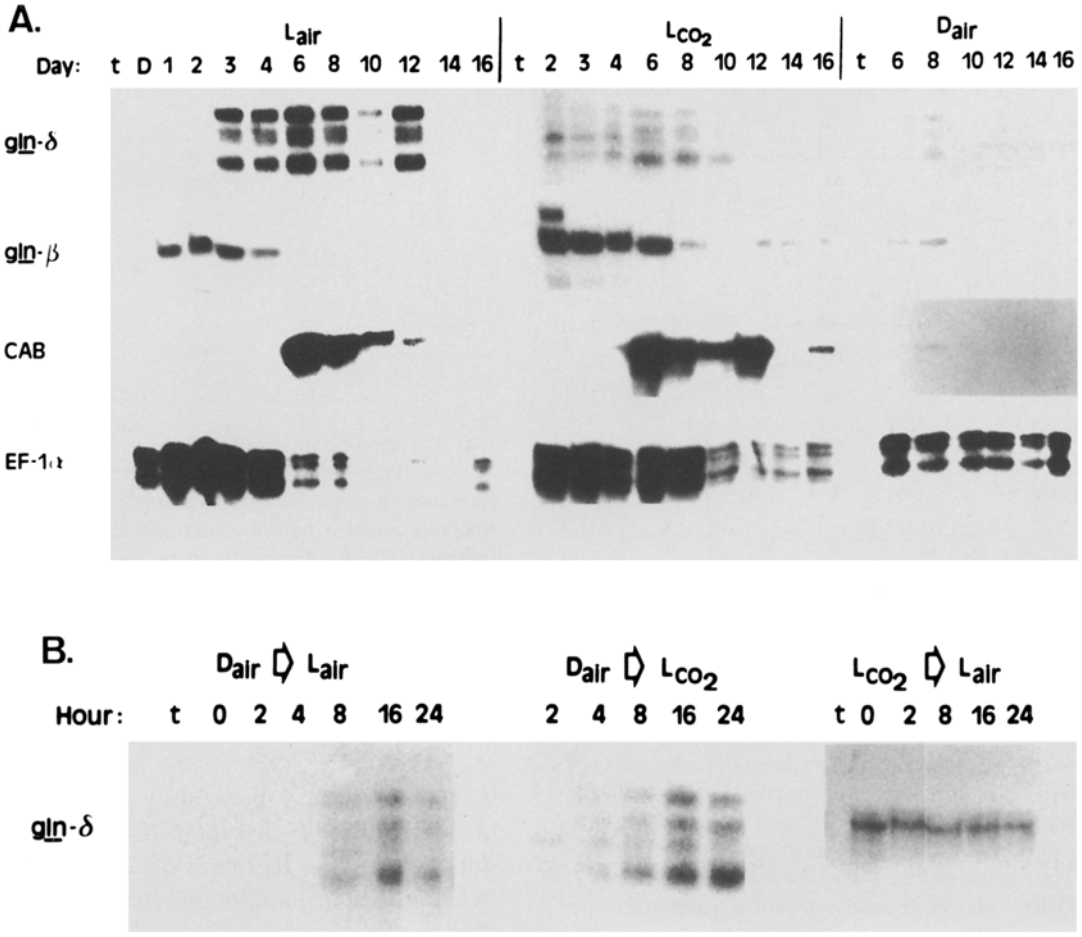

Fig. 5. Effect of light and photorespiration on the abundance of *gln-δ* mRNA in leaves. A. Long-term experiment. Abundances of mRNAs corresponding to *gln-δ*, *gln-β*, CAB and EF-1α were measured by RNase protection in total RNA from plumules/leaves of dry seeds (D) and of seedlings harvested between 1 and 16 days after imbibition and in yeast tRNA (t). Seedlings were grown in continuous light under air (L_{air}), in continuous light under high CO_2(L_{CO_2}) or in continuous dark under air (D_{air}). B. Short-term experiment. Abundance of *gln-δ* mRNA measured by RNase protection in total RNA of leaves. Seedlings were transferred 14 days after imbibition either from the dark under air to the light under air ($D_{air}\rightarrow L_{air}$), from the dark under air to the light under high CO_2 ($D_{air}\rightarrow L_{CO_2}$) or from the light under high CO_2 to the light under air ($L_{CO_2}\rightarrow L_{air}$). The *gln-δ* mRNA was assayed in leaves harvested between 0 and 24 hours after transfer, or in a yeast tRNA control (t).

plants developed more quickly under the continuous light conditions. Plants grown under a high CO_2 atmosphere in the light accumulated *gln-δ* mRNA to, approximately, a 5-fold lower abundance than control plants grown under air in the light. CAB, *gln-β* and EF-1α mRNAs, however, accumulated to at least the same abundances under high CO_2 compared with air and, in fact, even appeared to persist for longer under the high CO_2 atmosphere (Fig. 5A). The GS polypeptides and isoenzymes were analysed at day 14 and it was found that the high CO_2 atmosphere resulted in a slightly reduced accumulation of the δ polypeptide (Fig. 6A) and in a reduced specific activity of the δ isoenzyme (Fig. 6B) when compared to plants grown in air. Note that at this time the δ isoenzyme was present at high levels despite the *gln-δ* mRNA having decreased in abundance.

Plants grown in the dark also accumulated *gln-δ* mRNA but more transiently and to a much lower abundance than plants grown in the light (Fig. 5A). This result was confirmed in a duplicate experiment. Western immunodetection and IE-HPLC analysis showed that δ polypeptide was present in 6-day dark-grown leaves and that it was assembled into an active isoenzyme (Fig. 6). Surprisingly, the abundance of the δ polypeptide and the specific activity of the δ isoenzyme were slightly higher by day 14 in the dark (Fig. 6B) despite the fact that *gln-δ* mRNA was barely detectable after day 8 (Fig. 5A).

Abundance of the CAB mRNA was also decreased in dark compared with light-grown leaves but nonetheless showed a transient peak around day 8. The abundance of EF-1α mRNA appeared to be higher in dark compared with light grown leaves at the later stages of development, although this may simply be a result of a lower abundance of other RNA species.

Experiments were also conducted to test the short-term effect of altered light and CO_2 environments on *gln-δ* expression in leaves of 14-day old plants (Figs. 5B, 6). These experiments tested the effect of light in the absence of photorespiration (transfer of plants grown in the dark under air to light under a high CO_2 environment), the effect of photorespiration (transfer from high CO_2

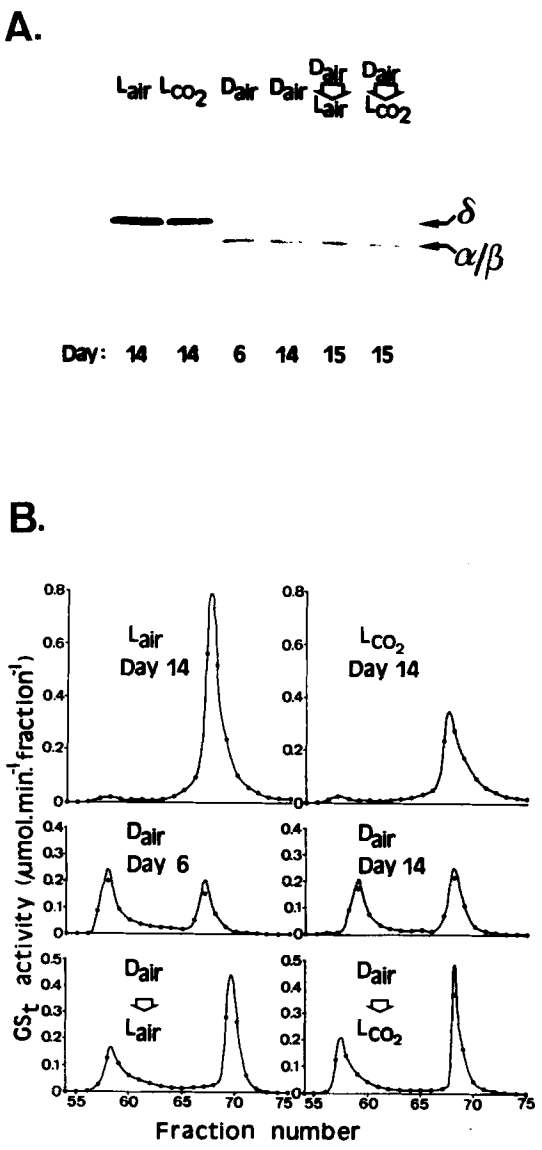

Fig. 6. Effect of light and photorespiration on the abundance, in leaves, of GS polypeptides and GS isoenzymes. Leaf samples were from seedlings grown in constant light for 14 days under either air (L_{air}) or high CO_2 (L_{CO_2}), from seedlings grown in the dark (D_{air}) for 6 or 14 days or from seedlings grown in the dark under air for 14 days and then transferred to the light under either air ($D_{air} \rightarrow L_{air}$) or high CO_2 ($D_{air} \rightarrow L_{CO_2}$) for 24 hours. A. Western blot of GS polypeptides. Note that the α and β polypeptides are not separated on a one-dimensional gel. B. IE-HPLC of GS isoenzymes. GS_t activity is plotted as μmol/min per fraction corrected for a column loading of 1 mg soluble protein.

to air in constant light) and the effect of both light and photorespiration (transfer from dark to light in an air environment). Transfer to the light, both in the presence and absence of photorespiration, resulted in a more than 10-fold increase in *gln-δ* mRNA abundance over 24 h (Fig. 5B). There were also increases, in both conditions, in δ isoenzyme abundance by 24 h (Fig. 6B). Photorespiration did not appear to influence the induction of *gln-δ* expression by light; *gln-δ* mRNA and isoenzyme accumulated to the same extent under both air and high CO_2 atmospheres (Figs. 5 and 6). In addition, transfer of illuminated plants from high CO_2 to air, which promotes photorespiration, did not lead to any increase in the abundance of the *gln-δ* mRNA (Fig. 5B).

Discussion

Differential expression of the GS gene family in developing leaves

The changes in GS specific activity which occur during primary leaf development in *P. vulgaris* (Fig. 1) were shown to be the result of a complex pattern of expression of three GS genes (Figs. 2 and 3). Initially, in plumules of dry and germinating seeds, the most highly expressed GS gene was *gln-α*, followed by *gln-β* in young leaves up to about 6 days after germination and finally *gln-δ* in maturing, green leaves. We suggest that the three GS isoenzymes fulfil different metabolic roles as the leaf develops from the plumule stage, which represents a sink for carbon and nitrogen supplied by the cotyledons, into a mature, photosynthesising leaf. In the germinating seed, mobilisation of the cotyledonary storage proteins leads to the release of ammonium, firstly in the cotyledons and later in the developing embryo, which must then be reassimilated via GS [25]. The *gln-α* gene is the predominantly expressed GS gene both in plumules at this early stage (Figs. 2 and 3) and, interestingly, also in radicles [31] and cotyledons [34]. Following initiation of imbibition there is an exponential increase in leaf fresh weight, dry weight and blade area up to day 8 [15]. This increase is a result of cell expansion concomitant with imbibition up to day 2 followed by periods of cell division and cell expansion up to day 8 [15, 38, 40]. Maximal expression of *gln-β* appears to correspond with this period of exponential leaf growth. Following the cessation of cell division, the chloroplasts develop [26] leading to greening of the leaves at approximately day 6 [15]. There is then a continued growth of the leaf resulting finally in an approximately 800-fold increase in leaf blade area [38]. Accumulation of both *gln-δ* and the CAB mRNA corresponded with chloroplast development and the concomitant onset of photosynthesis and photorespiration.

The functional significance of the two transcripts of the *gln-δ* gene (Fig. 4), which showed different patterns of regulation during leaf development, is not known. The induction of one of the two transcripts corresponded with greening of the leaves but its appearance is not dependent on chloroplast development as this transcript also accounts for the increase in *gln-δ* mRNA that occurs during nodule development (Fig. 4).

Interestingly, the *gln-γ* gene has also been shown to contain multiple transcription start sites [20] as have the chloroplast GS genes of both pea [17] and barley [21] but it is not known whether the multiple transcripts of these genes are differentially regulated. However, other plant genes, including the phytochrome gene of pea [33, 36] and a chalcone isomerase gene of petunia [37], have been shown to be differentially regulated at more than one promoter site.

The effect of light on expression of the gln-δ *gene*

The *gln-δ* mRNA accumulated in etiolated leaves but transiently and to a much lower abundance than in light grown leaves (Fig. 5A). The δ isoenzyme also accumulated in the dark and to a specific activity only 3- to 4-fold lower than in light grown leaves (Fig. 6B) which could suggest that the δ isoenzyme has a role in dark-grown tissue as has been suggested for the chloroplastic GS of pea [17]. However, it should be noted that the CAB mRNA was also shown to accumulate, al-

though to a very low level, in etiolated leaves (Fig. 5A) presumably in response to a developmental signal as these leaves would not have contained functional chloroplasts (CAB mRNA has previously been shown to accumulate in dark-grown leaves of a number of other plant species [1, 14]). The timing of the transient accumulation of the *gln-δ* and CAB mRNAs may correlate with the invagination of the inner membrane of proplastids which later forms the prolamellar body of mature etioplasts [6]. During this time, leaves of *P. vulgaris* accumulate all of the enzymes of the photosynthetic dark cycle [7]. The expression of both *gln-δ* and CAB in etiolated leaves may, therefore, come under the control of signal(s) regulating the developmental expression of a wide range of photosynthesis related genes in the dark irrespective of the need for the functioning of their protein products.

Despite its expression in the dark, light is a major factor regulating the expression of the *gln-δ* gene in leaves. Its mRNA accumulated over 10-fold in response to illumination of dark-grown plants (Fig. 5A). The kinetics of this accumulation were similar to the pattern observed for the mRNA of the chloroplast located GS of pea [17]. Accumulation of *gln-δ* mRNA has been shown to be light dependent in cotyledons of *P. vulgaris*, which are green for a short time before they are abscised [34].

The role of photorespiration in the regulation of the gln-δ *gene*

In leaves grown in continuous light, *gln-δ* mRNA, polypeptide and isoenzyme were all shown to accumulate to higher abundances in air than under a high CO_2 atmosphere (Figs. 5 and 6). This effect was specific to *gln-δ* in so far as neither the *gln-β*, the CAB nor the EF-1α mRNA showed the same difference in abundance under the two conditions (Fig. 5B). The reduced expression of *gln-δ* under high CO_2 could suggest, as proposed by Edwards *et al.* [17] for the pea chloroplast GS, that ammonium produced by photorespiration induced expression of the *gln-δ* gene. It was therefore surprising that no accumulation of *gln-δ* mRNA was observed following transfer of light grown leaves from a high CO_2 atmosphere to air (Fig. 5B), conditions which should immediately switch on photorespiration. In addition, the accumulation of *gln-δ* mRNA, polypeptide and isoenzyme following the transfer of etiolated leaves to the light was essentially the same whether the plants were transferred to an air or a high CO_2 atmosphere (Figs. 5 and 6). These experiments suggest, that the abundance of *gln-δ* mRNA is not directly regulated by the flux of photorespiratory ammonium through the photorespiratory pathway and, moreover, have eliminated the possibility that the light regulation of *gln-δ* is mediated by a photorespiration control. In conclusion, the role of photorespiration in the regulation of *gln-δ* remains unclear; although *gln-δ* expression was shown to be influenced in the long term by the photorespiratory status of the leaf, it is unlikely that this effect is directly mediated by the ammonium produced by photorespiration and may result from other effects such as differential growth rates or differences in the cellular pH.

Relationship between the abundances of the GS mRNAs and their protein products

During initial stages of leaf development, both in the light and in the dark, it was noticeable that changes in abundance of the three GS isoenzymes and polypeptides closely matched changes in abundance of their corresponding GS mRNAs (Figs. 2, 3, 5 and 6). These results indicate that the level of GS activity in leaves at these times is determined largely, if not solely, by the abundances of the GS mRNAs. A similar observation has been made with regard to the increase in GS activity that occurs during nodulation [3, 4]. In older leaves, however, grown both in the light and in the dark, the *gln-δ* mRNA diminished to barely detectable levels yet there was no corresponding decline in abundance of its polypeptide or isoenzyme (Figs. 2, 3, 5 and 6). The most likely explanation for this observation is that the polypeptide/isoenzyme is more stable than the mRNA

although we cannot rule out the possibility that, in mature leaves, a lower abundance of *gln-δ* mRNA may support a greater translational activity.

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