PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Interaction, Functional Relations and Evolution of Large and Small Subunits in Rubisco from Prokaryota and Eukaryota [and Discussion]

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Interaction, functional relations and evolution of large and small subunits in Rubisco from Prokaryota and Eukaryota

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In early biological evolution anoxygenic photosynthetic bacteria may have been established through the acquisition of ribulose bisphosphate carboxylase–oxygenase (Rubisco). The establishment of cyanobacteria may have followed and led to the production of atmospheric oxygen. It has been postulated that a unicellular cyanobacterium evolved to cyanelles which were evolutionary precursors of chloroplasts of both green and non-green algae. The latter probably diverged from ancestors of green algae as evidenced by the occurrence of large (L) and small (S) subunit genes for Rubisco in the chloroplast genome of the chromophytic algae *Olisthodiscus luteus*. In contrast, the gene for the S subunit was integrated into the nucleus in the evolution of green algae and higher plants. The evolutionary advantages of this integration are uncertain because the function of S subunits is unknown.

Recently, two forms of Rubisco (L_8 and L_8S_8) of almost equivalent carboxylase and oxygenase activity have been isolated from the photosynthetic bacterium *Chromatium vinosum*. This observation perpetuates the enigma of S subunit function. Current breakthroughs are imminent, however, in our understanding of the function of catalytic L subunits because of the application of deoxyoligonucleotide-directed mutagenesis. Especially interesting mutated Rubisco molecules may have either enhanced carboxylase activity or higher carboxylase:oxygenase ratios. Tests of expression, however, must await the insertion of modified genes into the nucleus and chloroplasts. Methodology to accomplish chloroplast transformation is as yet unavailable. Recently, we have obtained the first transformation of cyanobacteria by a colE1 plasmid. We regard this transformation as an appropriate model for chloroplast transformation.

The evolutionary context for Rubisco

Over a decade ago, the establishment of autotrophic CO_2 fixation during biological evolution was considered (McFadden 1973). Briefly, it was proposed that the acquisition of ribulose bisphosphate (RuBP) carboxylase and phosphoribulokinase by ancient heterotrophic bacteria would have conferred an enormous selective advantage at a time when hexoses (or intermediates of the Calvin cycle) had become limiting in the environment. We also postulated that phosphoribulokinase activity pre-existed in phosphofructokinase. It was then easy to imagine the evolution of a more efficient phosphoribulokinase as a result of duplication and mutation of the gene or genes encoding phosphofructokinase. Accordingly we hypothesized that the appearance of RuBP carboxylase in ancient photosynthetic bacteria may have triggered the establishment of autotrophic CO_2 fixation (McFadden & Tabita 1974). Aside from considerable interest in the evolution of RuBP carboxylase and autotrophs, there is also intense interest in other structural and functional properties of this protein, some of which are noted in the next section.

Prominent features of Rubisco

In 1972 it was established that the large (L) 55-kilodalton and small (S) 15-kilodalton subunits of tobacco RuBP carboxylase were encoded by the chloroplast and nuclear genomes respectively (Chan & Wildman 1972; Kawashima & Wildman 1972). Somewhat earlier, it had been noted that O_2 was a competitive inhibitor (with respect to CO_2) of soybean RuBP carboxylase (Ogren & Bowes 1971), an observation which led to the recognition that the carboxylase also catalysed the oxygenolysis of RuBP (Andrews *et al.* 1973; Lorimer *et al.* 1973). More recently, evidence has been summarized that ribulose bisphosphate-derived phosphoglycollate is the main precursor of glycollate, the substrate for photorespiration (Andrews & Lorimer 1978). The enigma, then, is why the bifunctional enzyme RuBP carboxylase–oxygenase (Rubisco) contributes to both photosynthesis and photorespiration (McFadden 1980).

A major curiosity in plant biology is the amount of Rubisco found in autotrophic species; indeed, this enzyme may be the most abundant protein in the world (Wildman 1979; Ellis 1979). It is even abundant in chemosynthetic and anoxygenic photosynthetic bacteria (McFadden *et al.* 1975), comprising more than 40% of the soluble protein in H_2-adapted *Rhodospirillum rubrum* (Schloss *et al.* 1979).

Quaternary structure of Rubisco

The dominant structure of Rubisco consists of eight large and eight small subunits; L_8S_8 species have been isolated from higher plants, green algae, cyanobacteria, anoxygenic photosynthetic bacteria and chemosynthetic bacteria (McFadden & Purohit 1978; McFadden 1978; McFadden 1980). However, forms lacking small subunits have been characterized from several prokaryotic sources and are included in table 1. Very recently we have separated L_8S_8 and

Table 1. Quaternary structures of Rubisco

origin[1]	structure	
higher plants	L_8S_8	—
green algae	L_8S_8	—
non-green algae	L_8S_8[2]	—
cyanobacteria	L_8S_8	—
anoxygenic photosynthetic bacteria:		
Chromatium vinosum[3]	L_8S_8	L_8
Ectothiorhodospira halophilum	L_8S_8	—
Thiocapsa roseopersicina	L_8S_8	—
Rhodopseudomonas sphaeroides	L_8S_8 (I)	L_{4-6} (II)
Rhodomicrobium vannielii	L_6S_6	—
Rhodospirillum rubrum	—	L_2
chemosynthetic bacteria:		
Pseudomonas oxalaticus	L_6S_6	—
Thiobacilli	L_8S_8[4]	L_8
Nitrobacter agilis	L_8S_8	—
Paracoccus denitrificans	L_8S_8	—
Alcaligenes eutrophus	L_8S_8	—

[1] For references to the primary literature except as supplemented below, see McFadden (1980).
[2] Cattolico, R. A., personal communication for the enzyme from *Olisthodiscus luteus*.
[3] Torres-Ruiz & McFadden (1985).
[4] Bowman & Chollet (1980).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

L_8 forms of Rubisco from *C. vinosum* (table 1) by centrifugation in the presence of 10% polyethylene glycol. Presumably the L_8S_8 form is stripped of small subunits by centrifugation at 175000 *g*, and a model has been proposed to account for its conversion to an L_8 structure. In any case, both forms are easily prepared under mild, non-denaturing conditions.

In table 2, the catalytic turnover numbers for the carboxylase activity of various well-characterized Rubisco molecules containing and lacking small subunits are compared; it is evident that they are quite similar. Discrepant reports of the isolation of highly active L_8 and L_8S_8 forms from *T. intermedius* (table 2) may be attributable to facile dissociation of the latter species as for the enzyme from *C. vinosum*.

TABLE 2. CATALYTIC TURNOVER NUMBERS OF RuBP CARBOXYLASES

source	form	turnover number mol CO_2 s^{-1} mol L subunit^{-1}
Chromatium vinosum	L_8S_8	3.7[1] (3.4, 3.2, 3.3)[2]
Chromatium vinosum	L_8	3.1[1] (1.4, 1.7, 1.7)[2]
Rhodospirillum rubrum	L_2	4.2[3]
Higher plants	L_8S_8	3.8–4.0[4]
Thiobacillus intermedius	L_8S_8	1.9[5]
Thiobacillus intermedius	L_8	2.8[6]

[1] Averages of six different isolates of enzyme; calculated from relative molecular masses of 550000 and 440000 for L_8S_8 and L_8 enzymes, respectively.
[2] The turnover numbers in parentheses are for three different batches of enzyme isolated (Torres-Ruiz & McFadden 1985) from a different strain of *C. vinosum*.
[3] Schloss *et al.* (1979).
[4] Berhow *et al.* (1982).
[5] Bowman & Chollet (1980).
[6] Purohit *et al.* (1976).

EVOLUTION OF L AND S SUBUNITS

Over a decade ago, we emphasized that a quantitative comparison of L and S subunits by the $S\Delta Q$ method (McFadden & Tabita 1974) suggested that L subunits would prove to be homologous whereas S subunits would not. Indeed, L subunits have probably evolved at a rate far below the median rate for proteins (Shinozaki *et al.* 1983) whereas S subunits have evolved far more rapidly (Shinozaki & Sugiura 1983). Nevertheless, two limited regions inferred from deoxynucleotide sequences (Phe 12–Leu 21 and Tyr 54–Phe 63) have been completely conserved in S subunits from *A. nidulans* 6301 and from higher plants, which are otherwise 40% homologous (Shinozaki & Sugiura 1983). An analogous comparison of large subunits reveals 80% homology (Shinozaki *et al.* 1983). The limiting structures in terms of change in L subunit sequence are those from *R. rubrum* and higher plants, in which there is 31% homology if corresponding regions are aligned as established by Hartman *et al.* (1984). These authors have stressed that striking homology exists between L subunits around those residues in the catalytic–activation domain. Specifically, conservation occurs around spinach Lys 201, the residue that binds activator CO_2, and around Lys 175, Lys 334 (Hartman *et al.* 1984) and His 298 (Igarashi *et al.* 1985; Saluja & McFadden 1982) in the active site domain, when these residues are compared with their counterparts in the *R. rubrum* enzyme. Similarly conserved regions are evident in a comparison of L subunits from Rubisco of *A. nidulans* 6301 and higher plants (Shinozaki *et al.* 1983). Of significance is the placement of Lys 175 near His 298 in the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

spinach active-site domain (Bhagwat & McFadden 1983; Igarashi *et al.* 1985). An essential arginine residue in the higher plant (Lawlis & McFadden 1978; Schloss *et al.* 1978), *R. rubrum* (Schloss *et al.* 1978), *P. oxalaticus* (Lawlis & McFadden 1978) and *Thiocapsa roseopersicina* (Purohit *et al.* 1979) enzymes is as yet unplaced.

Recently Hartman and colleagues have found good alignment between the hydrophobic regions of the *R. rubrum* enzyme and L subunits of tobacco Rubisco (Janson *et al.* 1984). Thus it seems highly likely that these polypeptides are similarly folded. Nevertheless, we have stressed (McFadden & Majumdar 1984) that appreciable differences exist between these polypeptides in terms of binding in the active site domain of ligands such as 6-phosphogluconate (Tabita & McFadden 1972), 2-carboxy-D-arabinitol 1,6-bisphosphate (Roach *et al.* 1983) and [RuBP–Mg]$^{2-}$ (Roach & McFadden 1983).

Given the simplicity of the L_2 structure of Rubisco from *R. rubrum* and the fact that it lacks S subunits, we have proposed that it reflects a primitive molecular form of Rubisco and that S subunits were added later in evolution (McFadden & Tabita 1974). Indeed *R. rubrum* may have descended from ancient photosynthetic bacteria, a hypothesis that fits fossil records and the capacity of *R. rubrum* to grow photoheterotrophically (McFadden & Tabita 1974). The finding of Rubisco containing S subunits in other anoxygenic photosynthetic bacteria (table 1) suggests that this molecular form arose later, before the appearance of O_2. In this context, it may be relevant that *Rhodopseudomonas sphaeroides* contains both types of Rubisco, an L form (II) of unknown aggregation state, and an L_8S_8 (I) form (table 1). Because the form II gene hybridizes strongly with the Rubisco gene from *R. rubrum* and a second chromosomal region hybridizes at lower stringency, forms II and I are probably products of two distinct structural genes (Quivey & Tabita 1984). Thus at least part of the genome of *R. sphaeroides*, which can be grown photoheterotrophically, may have descended from ancient photosynthetic bacteria.

Fossil records and other considerations suggest that cyanobacteria arose in the early Proterozoic era, about 2500 Ma ago and that photosynthetic O_2 production may have been rapid (Schopf & Walter 1982). If the origin of obligate aerobes occurred about 1700 Ma ago (Walker *et al.* 1983), the chemosynthetic bacteria, all of which can be cultured aerobically, (McFadden & Tabita 1974) and contain predominantly L_8S_8 Rubisco molecules (table 1), arose at or about that time. A very tentative evolutionary tree is shown in figure 1.

In chlorophytic plants, L and S subunits of Rubisco are encoded by the chloroplast and nuclear genomes, respectively, as mentioned previously. Of considerable interest is the evolutionary relationship envisaged between cyanelles and chloroplasts (figure 1). In certain photosynthetic biflagellated protists, such as *Cyanophora paradoxa*, intracellular organelles termed cyanelles are found. Each cell contains 2–4 cyanelles, which have a fine structure closely resembling that of unicellular cyanobacteria, except that a typical cell wall is not evident in electron micrographs. Cyanelles are, however, surrounded by a thin peptidoglycan layer that is probably homologous to the innermost layer of the cell wall of cyanobacteria and other Gram-native bacteria. These findings support the hypothesis that cyanelles descended from endosymbiotic cyanobacteria that lost the capacity to synthesize the outer lipopolysaccharide cell wall layer characteristic of all free-living cyanobacteria (Herdman & Stanier 1977). When DNA base compositions and genome sizes of chloroplasts from a wide range of organisms were compared with those of the cyanelle and of unicellular cyanobacteria, it was concluded that the cyanelle is a photosynthetic organelle rather than an endosymbiotic cyanobacterium. However, enclosure of the cyanelle by a peptidoglycan layer was construed as powerful

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

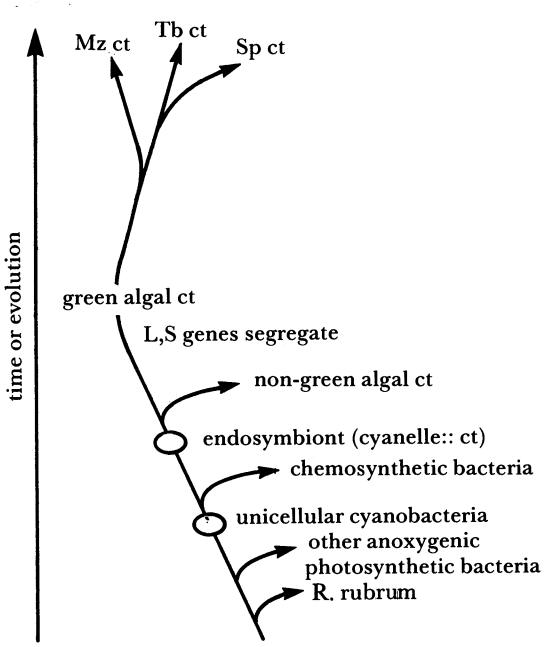

FIGURE 1. Evolutionary tree for autotrophic CO_2 fixation, partly based on structure, function, and gene dispersion for Rubisco. Abbreviations: ct, chloroplasts; Mz, maize; Tb, tobacco; Sp, spinach.

evidence for its evolutionary derivation from an endosymbiotic cyanobacterium (Herdman & Stanier 1977). Supporting this evolutionary origin has been more recent evidence placing the genes for the L and S subunits of Rubisco in close proximity on the cyanelle chromosome (Heinhorst & Shively 1983), a situation similar to that for *Anacystis nidulans* 6301 (Shinozaki & Sugiura 1983).

The implication that present-day chloroplast genomes from some species may contain genes for both L and S subunits remained untested until quite recently. Reith & Cattolico (1986) have discovered that the genes for both subunits are indeed present and close to one another in the chloroplast DNA of the non-green chromophytic alga *Olisthodiscus luteus* (figure 1). Moreover, Cattolico (1985) has recently reported that antibodies to the S subunit of Rubisco in this organism cross-react with S subunits isolated from the rhodophyte *Griffithsia pacifica*. It will be crucial to examine the L and S subunit gene distribution in this and other red algae. This is of special significance because the double-membrane limited chloroplasts of red algae may have evolved from symbiotic cyanobacteria (Gibbs 1981). In contrast, chromophytes such as *O. luteus* contain chloroplasts that are limited by four membranes (Gibbs 1981). Chromophytic chloroplasts, like those from cryptomonads, may have arisen from a red algal-like cell that took in a prokaryote and was eventually acquired by an ancestral micro-organism (Wilcox & Wedemayer 1985). Cattolico (1985) has stressed the power of using diverse chloroplast DNA probes to examine the relatedness of Chlorophyta, Chromophyta and Rhodophyta and other interesting autotrophic protists such as cryptomonads and dinoflagellates.

From the emerging evidence, then, it seems likely that an evolutionary branch to the algae occurred and that one evolutionary sequence established chlorophytic chloroplasts whereas the other sequence established chloroplasts in other algal species (figure 1).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Function of the small subunit

Conjecture about the evolution of Rubisco is seriously limited by the lack of information about S subunit function. Thus the evolutionary advantages accruing to autotrophic eukaryotic microbes in segregating the genes for S and L subunits into nuclear and chloroplast genomes are not evident. In fact, this segregation has required the fusion of a 4–6 kDa transit sequence at the N-terminus of S subunits that enables transport of the cytoplasmically synthesized precursor into chloroplasts (for a review, see Freyssinet & Buetow 1984). An analogous transit peptide is probably lacking in the genomic region encoding cyanobacterial S subunit (Shinozaki & Sugiura 1983; Nierzwicki-Bauer *et al.* 1984).

The evolutionary transfer of S subunit genes for Rubisco from the chloroplast to nucleus may not have been unique, however, in that an analogous transfer of genes for ferredoxin and light-harvesting chlorophyll protein may have occurred. Both polypeptides are cytoplasmically synthesized as precursors in chlorophytes (Freyssinet & Buetow 1984; Smeekens *et al.* 1985). The deduced sequence of the transit peptide for ferredoxin, in fact, shows a stretch of three amino acids near the processing site that is identical with an analogous sequence in the S subunit precursor, suggesting, perhaps, a common mechanism of processing (Smeekens *et al.* 1985). It will be important to characterize the locus of these genes and gene products in non-green algae and, especially, to probe for the existence of transit peptides. A further complication in understanding the evolution of Rubisco is the observation that spinach L subunits are synthesized in two forms *in vitro*, one of which is 1–2 kDa larger than the other (Langridge 1981) and that a 14-amino acid N-terminal peptide may correspond to the sequence that is removed (Zurawski *et al.* 1981). Although the function of the implied chloroplastic processing of the precursor of L subunits is obscure, it is significant that the presence of a similar N-terminal peptide has been proposed on the basis of the deoxynucleotide sequence of the cyanobacterial L subunit gene (Shinozaki *et al.* 1983).

Returning to the puzzle of the function of S subunits in Rubisco, the following observations are relevant. Ribulose bisphosphate oxygenase activity exists in the L_2 dimer from *R. rubrum* (McFadden 1974) thus ruling out a requirement of S subunits for this activity. In recent years, a substrate specificity factor $(V_c/K_o)/(V_o/K_c)$ (Laing *et al.* 1974) has been used to measure the specificity of Rubisco from various sources. Of interest was the finding that the specificity factors for Rubisco from *R. rubrum* and *Rps. sphaeroides* (form II), both of which lack small subunits, were 15 and 9 respectively. This factor increased to 47–48, 54–63, and 78–82 for the L_8S_8 enzymes from cyanobacteria, green algae, and C_4 or C_3 plants, respectively. The authors (Jordan & Ogren 1981) suggested that evolutionary modifications which increased carboxylation efficiency and the affinity for CO_2 have occurred during the course of natural history. The trends noted are in accord with the evolutionary sequence suggested in figure 1. Recently, Andrews and colleagues have succeeded in dissociating Rubisco from a marine cyanobacterium, *Synechococcus* (strain RRIMP N1), by precipitating L subunits under mildly acid conditions (pH 5.2–5.3). S subunits could then be added back to the L_8 core containing almost no S (Andrews & Abel 1981) to reconstitute the carboxylase activity. In addition, a K_d value for $LS \rightleftharpoons L + S$ of ca. 10^{-9} M was found for the *Synechococcus* enzyme when albumin was used to suppress surface binding (Andrews & Ballment 1983). Quite recently, hybridization of S subunits from *Prochloron* (Andrews *et al.* 1984) and spinach (Andrews & Lorimer 1985) with acid-precipitated Rubisco core from the marine *Synechococcus* sp. has revealed some

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

activity of hybrids. K_d values were 9- to 25-fold higher for heterologously associated enzymes, and the K_m for CO_2 of the *Synechococcus*–spinach hybrid was about twice as high as that for the homologously reassociated hybrid. However, the specificity factor was not altered by heterologous association (Andrews & Lorimer 1985) suggesting that catalytic partitioning between the carboxylation and oxygenation pathways may be specified entirely by the large subunit. Of considerable interest was the observation by Andrews & Ballment (1984) that the binding of CO_2 and the transition state analogue 2-carboxyarabinitol 1,5-bisphosphate (CABP) by the *Synechococcus* enzyme was unaltered by the depletion of S subunits through acid precipitation. These authors concluded that the necessity for S subunits in catalysis must stem from a more subtle involvement in the catalytic mechanism itself.

Other studies have suggested that S subunits can be removed from Rubisco from the super-halophytic cyanobacterium *Aphanotheca halophytica* by low-salt treatment and that the carboxylase activity can be restored by addition of S subunits to the core (Asami *et al.* 1983). More recently, Jordan & Chollet (1985) have removed S subunits from the partially purified enzyme from *C. vinosum* at pH 9.6 and have reported reconstitution of activity by addition of S subunits to the L core. With enzyme from either of these sources, the S-depleted L-subunit core can also bind CABP and Mg^{2+} and CO_2 (Takabe *et al.* 1984; Jordan & Chollet 1985).

To recapitulate, the studies of S-depleted Rubisco from two different cyanobacteria indeed suggest a subtle role of S subunits in catalysis. Nevertheless, the possibility that S subunits are only required to renature L subunits cannot be excluded. This may be relevant in reconciling the results obtained for the *C. vinosum* enzyme at pH 9.6 (Jordan & Chollet 1985) with those from our laboratory. It is noteworthy that we have isolated two forms (L_8 and L_8S_8) of Rubisco from the same organism under very mild conditions and that they have comparable carboxylase and oxygenase activities (table 2; see also Torres-Ruiz & McFadden 1985). Whether these forms differ in the specificity factor (Jordan & Ogren 1981) reflecting carboxylation: oxygenation remains to be determined.

The precise function of the S subunits of Rubisco is still a major enigma in plant biology. Perhaps the approach of oligonucleotide-directed mutagenesis will elucidate the function of these subunits. If so, our understanding of the molecular evolution of Rubisco will surely be enhanced.

New research initiatives

Quite recently, deoxyoligonucleotide-directed mutagenesis of the L subunit gene from *R. rubrum* has been used to substitute Glu for either Asp 188 or Lys 191 (Gutteridge *et al.* 1984; Estelle *et al.* 1985). The former substitution (originally identified at Asp 198) resulted in slight alterations of the metal ion environment and catalysis. In contrast, the latter substitution at the lysyl residue that binds activator CO_2 abolished catalysis and formation of the highly stable quaternary complex between enzyme, CO_2, Mg^{2+} and CABP. The L gene used in these cases was actually a recombinant gene that encodes a 25-residue segment of β-galactosidase that is fused to the N-terminus of the L subunit.

The continued use of deoxyoligonucleotide-directed mutagenesis promises to deepen our understanding of structure–function correlates for Rubisco. This approach will be of particular interest in examining the L_8S_8 cyanobacterial enzyme, the genes for which have recently been cloned in *E. coli* through the use of high expression vectors, pSV55 (Gatenby *et al.* 1985) and

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

pCS75 (Tabita & Small 1985). In these clones, deoxyoligonucleotide-directed assembly defects will be readily apparent in the Rubisco(minus) background of *E. coli*. To date, cloning of the gene for the maize L subunit in *E. coli* has yielded insoluble L subunits with no detectable enzyme activity and no capacity to bind CABP (Gatenby 1984). Ideally, higher plant L and S subunit genes should be cloned and expressed together to study correlations of structure and function but this approach awaits the construction of suitable high-level expression vectors.

It appears, then, that a variety of known point mutations in L and S subunit genes will soon become available. Especially interesting modifications of each Rubisco gene will lead to isolable gene products with enhanced carboxylase activity or an increase in the ratio of carboxylase:oxygenase. Either type may increase plant productivity if the genes can be reinserted into the nuclear or chloroplast genome. As a model system for the transformation of chloroplasts, we have been studying the transformation of *A. nidulans* 6301 by the 4.3 kilobase colE1 plasmid, pBR322. Transformation to ampicillin resistance has been observed if contact between plasmid and cells is suitably long. Transformation is more effective in the light than in the dark (figure 2). Moreover, our recent studies (Daniell *et al.* 1986) have shown that transformation efficiency in the light is enhanced about 50-fold when cells are first subjected to lysozyme–EDTA treatment to prepare permeaplasts. β-lactamase is present in transformants as evidenced by hydrolysis of the β-lactam ring of nitrocefin by extracts. β-lactamase can also be immunoprecipitated by anti β-lactamase from extracts of ^{35}S-Met labelled transformants and co-precipitates with labelled Rubisco. The latter was confirmed by immunoprecipitation

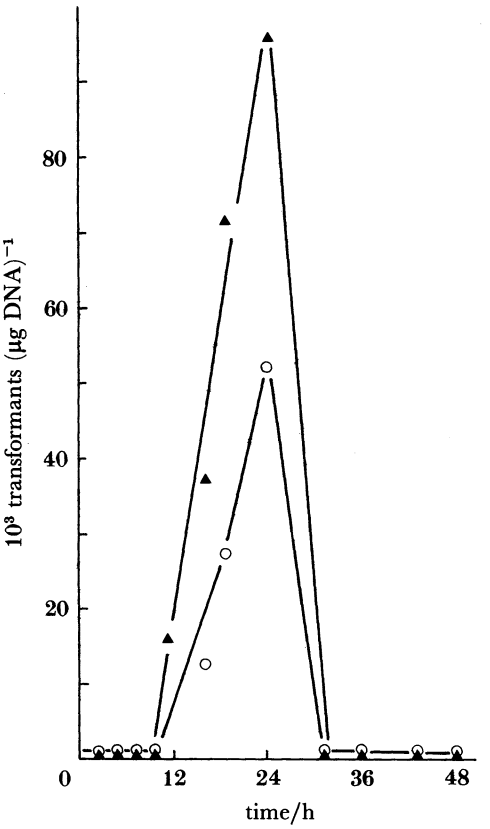

FIGURE 2. Effect of continuous, cool white fluorescent light (▲) and darkness (○) on transformation at 32 °C of *A. nidulans* 6301 (Daniell *et al.* 1986) to ampicillin (0.5 μg ml^{-1}) resistance by pBR322.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

with anti-spinach Rubisco (which, curiously, precipitated β-lactamase). Expression of Rubisco L and S subunits was greatly amplified in transformants and was even evident in Coomassie-blue-stained SDS–polyacrylamide gels. Chromosomal DNA content per cell was increased sixfold in pBR322-transformed *A. nidulans*, and 4.3 kilobase plasmids could be reisolated from pBR322 transformants in addition to the endogenous plasmids pUH24 and pUH25. These results reflect the first transformation of cyanobacteria by an *E. coli* plasmid and may open the way to genetic engineering of photosynthesis and N_2 fixation in these important organisms. The mechanism of the observed transformation is considered elsewhere (Daniell *et al.* 1986).

Quite recently we have established that the specific activity of Rubisco in pBR322 transformants is about six times as high as in untransformed (WT) *A. nidulans* (figure 3), reflecting, perhaps, the chromosomal amplification mentioned previously. It is noteworthy that transformed cells grow at about the same rate as untransformed cells in spite of the sixfold higher Rubisco content. We are presently investigating the mechanism of transformation, the dispersion of L and S subunit genes in the genome of transformed *A. nidulans*, and the correlation of growth rate with Rubisco content.

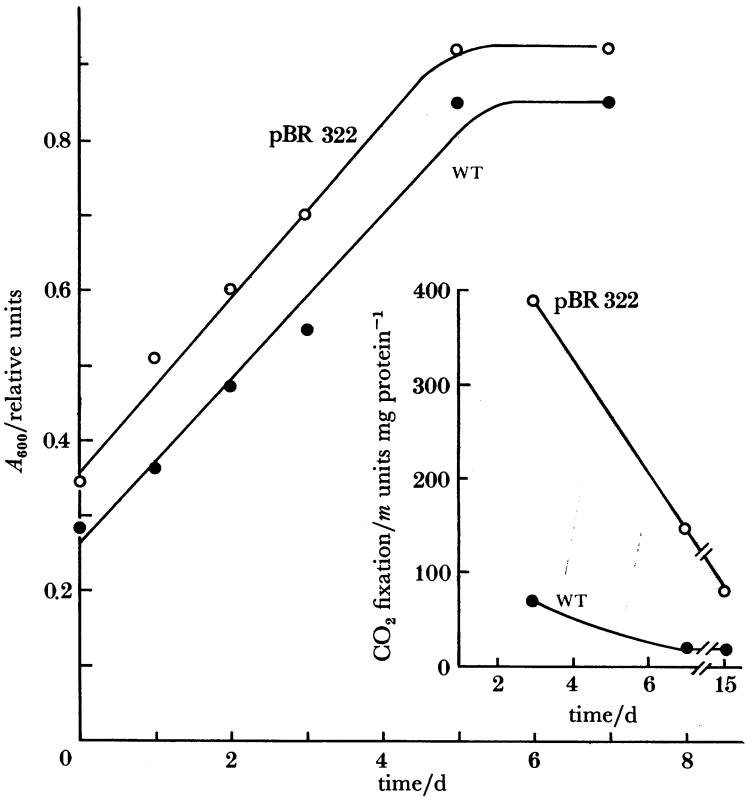

FIGURE 3. Growth at 32 °C (expressed as the absorption at 600 nm, A_{600}) and Rubisco content of pBR322-transformed (Daniell *et al.* 1986) and wild type (WT), untransformed *A. nidulans* 6301 in the presence (1.0 μg ml^{-1}) and absence of ampicillin, respectively. (One *m* unit equals one nmol RuBP-dependent CO_2 fixation min^{-1} at 30 °C (Torres-Ruiz & McFadden 1985).)

To recapitulate, our recent results establish that cyanobacteria can be transformed by colE1 plasmids lacking a cyanobacterial replicon. Permeability of recipients to DNA has been enhanced by the use of permeaplasts (Daniell *et al.* 1986). Certainly the results raise our expectations of introducing foreign DNA into chloroplasts.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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Discussion

G. Voordouw (*Department of Biochemistry, Agricultural University, Wageningen, The Netherlands*). You referred in your talk to the form II Rubisco from *Rhodopseudomonas sphaeroides* as an L_4–L_6 enzyme. Since an active dimer, similar to the L_2 enzyme of *Rhodospirillum rubrum*, has also been described for *Rh. sphaeroides* (Chory *et al.* 1985), I propose that we refer to this enzyme as a L_2 Rubisco with self-assembly properties. This underlines the homology between these two enzymes.

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Chory, J. D., Muller, E. D. & Kaplan, S. 1985 DNA-directed *in vitro* synthesis and assembly of the form II D-ribulose-1,5-bisphosphate carboxylase/oxygenase from *Rhodopseudomonas sphaeroides*. *J. Bact.* **161**, 307–313.

B. A. McFadden. I agree that incisive and clear nomenclature is required to emphasize the fact that Rubisco molecules that lack small subunits (form II) may differ in aggregation state. Such seems to be the case for the rhodopseudomonad enzymes in comparison with the L_2 form from *Rhodospirillum rubrum*. Dr S. Kaplan (Department of Microbiology, University of Illinois, Urbana, IL 61803) invites suggestions as to how to differentiate between the bacterial operon for Rubisco containing large and small subunit genes (presumably the L_8S_8 enzyme is the *normal* gene product) and the bacterial structural gene unit that encodes the large subunit only (presumably the aggregation state of L varies). I prefer the genotypic designations rbc-ls and rbc-l to distinguish between these limiting cases with the understanding that aggregation states of isolated enzymes and active enzymes may differ even within the category of rbc-ls. The latter designation and the designation rbc-l would be synonymous with the higher plant-type and *R. rubrum*-type enzymes, respectively.

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