Lipid biosynthesis in synchronized cultures of the photosynthetic bacterium *Rhodopseudomonas sphaeroides*

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1. Lipid biosynthesis has been studied in photosynthetic cultures of *Rhodopseudomonas sphaeroides* that had been synchronized by stationary-phase cycling or by a centrifugation selection procedure. Synchrony index values in the range 0.70–0.80 were obtained for the first cell cycle with both synchronization methods. 2. The major membrane lipids phosphatidylethanolamine and phosphatidylglycerol were accumulated discontinuously during the cell cycle, their mass doubling immediately before cell division. This accumulation of lipid corresponded to peaks in incorporation of radioactivity from either [1-¹⁴C]acetate or [2-³H]glycerol into individual acyl lipids as measured in individual portions of bacteria. For phosphatidylglycerol an additional peak of incorporation of radioactivity from [2-³H]glycerol was found midway through the cell cycle. 3. In spite of their rather similar endogenous fatty acid compositions, the individual phosphoacylglycerols showed distinctive patterns of incorporation of radioactivity from [1-¹⁴C]acetate into their acyl moieties. 4. The discontinuous synthesis of acyl lipids observed in cultures of *Rhodopseudomonas sphaeroides* synchronized by either stationary-phase cycling or centrifugation selection procedures contrasted with the accumulation of chlorophyll–protein complexes whose amounts were found to increase throughout the cell cycle. 5. The implications of these findings for the control of lipid synthesis in bacterial photosynthetic membranes are discussed.

Photosynthetic bacteria that are grown under heterotrophic or phototrophic conditions differ not only in their content of photosynthetic pigments and proteins required for photosynthesis, but also in their lipid composition (Kenyon, 1978). One of the most widely studied species in this respect is *Rhodopseudomonas sphaeroides* (cf., e.g. Russell & Harwood, 1979). Kaplan and co-workers have extended the study of photosynthetic membrane biogenesis in strain 2.4.1 of this organism to include the use of synchronized cultures (Kaplan *et al.*, 1979; Cain *et al.*, 1981; Yen *et al.*, 1984). These experiments have demonstrated that the majority of the photosynthetic membrane components, i.e. reaction centres, light-harvesting complexes, carotenoids and cytochromes, are synthesized and integrated into the membrane continuously

through the cell cycle. In contrast, phospholipids are synthesized discontinuously. Kaplan and co-workers (Cain *et al.*, 1981) have interpreted their results in terms of a model in which phospholipids are synthesized in the cytoplasmic membrane between cell divisions and transferred to intracytoplasmic membranes at the onset of cell division, arguing that the cell-cycle-associated event is probably transfer rather than synthesis of phospholipids. However, the data are complex and the timing of phospholipid synthesis relative to cell division is variable [cf. Lueking *et al.* (1978) and Fraley *et al.* (1979a) with Cain *et al.* (1981)].

These previous studies have used stationary-phase cycling to synchronize cultures. This is an example of an induction-synchrony method (Mitchison, 1971). Induction-synchrony methods have the disadvantage that they rely on metabolic perturbation to induce synchrony; moreover, in stationary-phase cycling the reason for cessation of growth is unknown and the synchronized culture

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often has abnormally long cell-cycle times (Edwards, 1981; Lloyd *et al.*, 1982). Alternatively, cultures can be synchronized by using selection methods, which have the advantages that they generally give rise to fewer metabolic disturbances and it is easier to carry out suitable non-synchronized controls (Edwards, 1981). Thus we have used a selection method based on gentle centrifugation, in addition to stationary-phase cycling, to corroborate results obtained with the latter method.

The discontinuous synthesis and/or transfer of phospholipids in intracytoplasmic membranes causes cyclical changes in the protein/phospholipid ratio and in the microviscosity of the photosynthetic membranes (Fraley *et al.*, 1979b). A large proportion of the phospholipid in photosynthetic membranes of *Rhps. sphaeroides* interacts with protein (Fraley *et al.*, 1978, 1979b; Birrell *et al.*, 1978), and it has been suggested that the transfer of additional phospholipid just before cell division might help facilitate partitioning of photosynthetic membranes between daughter cells or serve to regulate specific enzymes concerned with cell division (Wraight *et al.*, 1978; Fraley *et al.*, 1979a). The large changes in the lipid/protein ratio of intracytoplasmic membranes that would result from discontinuous synthesis or transfer of phospholipid might be expected to affect also the efficiency of energy transfer during photosynthesis. We have shown previously that the photosynthetic membranes of *Rhps. sphaeroides* are enriched with phosphatidylglycerol (Russell & Harwood, 1979), which may be associated with the pigment-protein complexes (Birrell *et al.*, 1978) and appears to play a functional role in photosynthesis (Harwood & Russell, 1984). Since phosphatidylglycerol turnover in *Escherichia coli* varies during the cell cycle (Ohki, 1972), it was of particular interest to determine the metabolism of this lipid and its relationship to photosynthetic pigments in synchronized cultures of *Rhps. sphaeroides*. The results of our preliminary studies using a double-labelling ($^{14}\text{C}/^3\text{H}$) technique with cultures synchronized by stationary-phase cycling have been published (Knacker *et al.*, 1982). By using two synchronization methods, and including a functional test of photosynthetic capacity, we now report an extension of these studies with strain N.C.I.B. 8253, which shows characteristics of lipid synthesis different from those of strain 2.4.1 (Donohue *et al.*, 1982).

Materials and methods

Materials

Sodium [$1\text{-}^{14}\text{C}$]acetate (1.9 GBq/mmol) and [$2\text{-}^3\text{H}$]glycerol (22 GBq/mmol) were purchased from

Amersham International, Amersham, Bucks., U.K. Lipid standards were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K., and fatty acid standards from Nu-Chek Prep., P.O. Box 172, Elysian, MN 56028, U.S.A.

Bacterial strain and cultural conditions

Rhodospseudomonas sphaeroides (N.C.I.B. 8253) was grown routinely in the Medium S described by Lascelles (1956) at 30°C with 2000 lux illumination (Russell & Harwood, 1979).

Synchronization procedures

Synchronization by the stationary-phase-cycling induction method was carried out essentially by the technique developed by Lueking *et al.* (1978). The starting A_{680} was 0.10–0.15, and cessation of exponential-phase growth took place at $A_{680} = 0.80\text{--}0.90$. After this point, a further 50–75% mass doubling was allowed ($A_{680} = 1.20\text{--}1.40$) and a portion of culture was removed to inoculate fresh prewarmed Medium S for a second growth cycle (the same protocol as above being used). Portions of culture from this second cycle were used to inoculate fresh, prewarmed Medium S for synchronous growth in an experiment. Such cultures gave a synchrony index (F) value of 0.70–0.80, which is regarded as being highly acceptable (Edwards, 1981).

Synchrony was also established by a selection method that employed differential centrifugation. Samples of culture were taken at the end of exponential-phase growth ($A_{680} = 0.80\text{--}0.90$) and centrifuged at $3500g_{\text{av}}$ for 12 min. The supernatant, which contained less than 5% of the cells, was decanted and discarded; the bacterial pellet was resuspended in the original volume of fresh prewarmed Medium S by gentle swirling. This suspension was centrifuged at $3500g_{\text{av}}$ for 8 min and the supernatant, containing about 10% of the cells, was used for synchronized-cell-growth experiments. The control (non-synchronized) sample for such experiments was subjected to the same centrifugation conditions, but the pellet and supernatant fractions at each step were recombined. Cultures synchronized by differential centrifugation gave a synchrony index (F) value of approx. 0.7 for the first division, but often considerably less for subsequent cell cycles.

Standard assay conditions

At the beginning of synchronous growth, the culture was divided into small samples (5–10 ml), placed in 10 ml ampoules, which were flushed with N_2 and sealed first with cottonwool and then with a rubber stopper. In some experiments a few crystals of pyrogallol (benzene-1,2,3-triol) dissolved in saturated K_2CO_3 were placed between

the cottonwool and rubber bung, but no differences in the results were observed. It was assumed, therefore, that flushing with N₂ and tightly sealing with a rubber stopper ensured that anaerobic conditions were preserved.

Every 30 min during a 9 h growing period, sodium [1-¹⁴C]acetate and/or [2-³H]glycerol were added to individual samples in separate ampoules, together with non-radioactive acetate and/or glycerol, to give final concentrations of 37 kBq/ml (1 μ Ci/ml, 60 μ M) and 37 kBq/ml (1 μ Ci/ml, 6 μ M) respectively. Samples were re-flushed with N₂ and re-sealed. Portions (2 ml) were removed after 5 min and extracted by the method of Garbus *et al.* (1963). Lipids were separated by t.l.c. (Russell & Harwood, 1979) and individual lipid classes were removed for radioactivity counting in a scintillation cocktail of PCS (Amersham International)/xylene (2:1, v/v) with a LKB Rackbeta liquid-scintillation spectrometer. Counting efficiency was measured automatically by the external-standards-ratio method. For double-labelling experiments employing ¹⁴C and ³H, the amount of ¹⁴C spillover in the ³H channel was determined by using standards of known radioactivity, and the liquid-scintillation spectrometer programmed to calculate ¹⁴C and ³H channels separately, with automatic adjustment for any changes due to quenching.

Other measurements

Fatty acid methyl esters from individual acyl lipid classes were formed by transmethylation of lipid samples with H₂SO₄/dry redistilled methanol (1:39, v/v) at 70°C for 2 h. An internal methyl pentadecanoate standard was used for lipid quantification by g.l.c. analysis with 15% (w/w) EGSS-X columns (1.5 m $\times$ 4 mm internal diameter) on Chromosorb W AW (Supelco, Bellefonte, PA 16823, U.S.A.) at 185°C. For mass determinations a Perkin-Elmer F33 chromatograph fitted to a Varian CDS-111 integrator was used, and for radioactivity-plus-mass determinations a Pye GCD chromatograph fitted to a Panax Reigate-series gas-flow proportional counter was utilized.

Bacterial cell number was measured by colony counts of diluted samples grown on Medium S solidified with agar (2%, w/v) (Difco Laboratories, West Molesey, Surrey, U.K.). DNA was determined by using the diphenylamine assay (Burton, 1956) as modified by Lueking *et al.* (1978).

All spectrophotometric measurements were performed on whole cells suspended in 10 mM-Tris/HCl, pH 7.5, with 50% (v/v) glycerol to decrease problems that arose from light-scattering. Reaction-centre concentrations were determined using dual-wavelength spectrophotometry as described by Jones & Plewis (1974). Concentrations

of B875 and B800–850 antennae were calculated from absorbance spectra by using the approximations given by Crounse *et al.* (1963) and the absorption coefficient given by Clayton & Clayton (1981).

Results

In common with most induction-synchrony procedures, the stationary-phase-cycling technique adopted for synchronization of *Rhodospseudomonas sphaeroides* showed fluctuations in cell generation time. Unusual events during the first cell cycle in a synchronized culture may be caused by the synchronization procedure (Mitchison, 1971). Therefore the second and, if available, the successive cell cycles, represent more satisfactory conditions for analysing metabolism. The results of a representative synchrony experiment are shown in Fig. 1. The synchrony of the culture could be proved by viable-cell-number determination as well as by monitoring the discontinuous DNA accumulation. The relatively constant exponential increase in absorbance indicates that there was continuous production of cell mass during synchronous growth, although close inspection of the curve reveals some effect of cell division on A₆₈₀ values. The increase in amounts of phosphatidylethanolamine and phosphatidylglycerol (the major acyl lipids; cf. Russell & Harwood, 1979) followed a distinctly discontinuous pattern during the cell cycle, their increase occurring coincidentally with that of DNA and immediately before cell division (Fig. 1).

Before proceeding with studies on lipid metabolism during synchronous growth, the time course and extent of the labelling of lipid fractions in *Rhps. sphaeroides* from [1-¹⁴C]acetate and [2-³H]glycerol were examined. Since we wished to compare labelling rates of individual components, it was important to determine the period for which incorporation was linear. When using non-synchronized exponential-phase cultures, the labelling of all fractions from [1-¹⁴C]acetate was linear for at least 15 min; after 30 min the labelling rates of all fractions slowed, and for bacteriochlorophyll and diphosphatidylglycerol no increase in labelling rates was seen between 30 and 60 min incubation. The relative labelling of individual lipids was in the order phosphatidylethanolamine > bacteriochlorophyll > phosphatidylglycerol > phosphatidylcholine > diphosphatidylglycerol. Studies of the distribution of label in different parts of the phosphoacylglycerols (cf. Heinz & Harwood, 1977) showed that more than 96% radioactivity incorporated from [1-¹⁴C]acetate was present in acyl chains.

Fig. 1. Changes in absorbance, cell number, DNA and the major acyl lipids during the growth of *Rhodospseudomonas sphaeroides* cultures synchronized by the stationary-phase-cycling technique

Synchronization and measurements were done as described in the Materials and methods section. Points represent averages of two to four determinations. ▼, A_{680} ; △, no. of viable cells; ($\times 10^{-9}/\text{ml}$) ▲, DNA ($\mu\text{g}/\text{ml}$); ●, phosphatidylethanolamine; (nmol/ml) ○, phosphatidylglycerol, (nmol/ml)

In preliminary experiments with $[1-^{14}\text{C}]$ acetate or $[2-^3\text{H}]$ glycerol, the relative incorporations of radioactivity from these two precursors into *Rhps. sphaeroides* lipids were calculated. We were thus able to carry out subsequent double-labelling

experiments where total incorporation of ^{14}C and ^3H radioactivity into lipids was comparable. This allowed more accurate scintillation counting to be carried out (see the Materials and methods section). The $2-^3\text{H}$ isotopic isomer of glycerol was used because this is a relatively specific label for the non-acyl moiety of phospholipids owing to the loss of ^3H radioactivity from glycerol 1-phosphate when it is converted into dihydroxyacetone phosphate, which eventually gives rise to acetyl-CoA, the precursor of fatty acids (see, e.g., Slack *et al.*, 1979).

Rhps. sphaeroides was synchronized by the stationary-phase-cycling technique, and every 0.5 h over a 10 h period samples were double-labelled with $[^3\text{H}]$ glycerol and $[^{14}\text{C}]$ acetate. Labelling of total lipids from $[^3\text{H}]$ glycerol was essentially linear for 15 min, whereas that from $[^{14}\text{C}]$ acetate tended to decrease after 8 min. On the basis of these results it was decided to use 5 min as the routine incubation time for double-labelling experiments with synchronous culture. The linearity of the incorporation of radioactivity was independent of the cell-cycle stage; thus it was possible to derive rates from single 5 min samplings, which enabled fairly large numbers of samples to be taken at relatively frequent times during two or three cell cycles.

The biosynthesis of neutral lipids (including pigments), phosphatidylethanolamine and phosphatidylglycerol over three successive cell cycles is depicted in Fig. 2. In order to prevent changes in lipid composition, which can be caused by drastically altered growth conditions (Onishi & Niedermann, 1982), the concentrations of the precursors were less than 1% of those required to give saturating conditions for uptake/incorporation.

The radioactivity from each precursor was incorporated discontinuously during synchronous growth. Each cell division was preceded by an increase in the rates of lipid synthesis for total lipids and for individual lipid classes, although this increase was often rather small at the end of the first cycle. Particularly high rates of incorporation from both precursors into the neutral lipid fraction occurred at the end of the second and third cell cycles. The labelling profiles of phosphatidylethanolamine and phosphatidylglycerol from $[1-^{14}\text{C}]$ acetate were similar to the quantitative changes in the contents of these phospholipids (cf. Figs. 1 and 2). Since the fatty acyl chains represent approximately two-thirds of the mass of these phosphoacylglycerols, and because radioactivity from $[^{14}\text{C}]$ acetate labels such groups virtually exclusively (see above), this result was perhaps not surprising. However, the time courses of incorporation from $[^3\text{H}]$ glycerol were rather different. Whereas the incorporation of radioactivity into

Fig. 2. Rates of labelling of acyl lipids at different stages of the cell cycle of *Rhodospseudomonas sphaeroides* synchronized by the stationary-phase-cycling technique

Synchronization, labelling with [^3H]glycerol and [^{14}C]acetate, the extraction of lipids and their purification are detailed in the Materials and methods section. Incubations were for 5 min. The plots on the left side represent incorporation of ^3H label (open symbols) and those on the right, incorporation of ^{14}C label (filled symbols). Δ , $\blacktriangle$, Viable cells; $\square$, $\blacksquare$, total lipid; $\diamond$, $\blacklozenge$, neutral lipids (including pigments); $\circ$, $\bullet$, phosphatidylethanolamine; ∇ , $\blacktriangledown$, phosphatidylglycerol.

phosphatidylethanolamine reached maxima at the end of the second and third cell cycles, a different pattern of incorporation into phosphatidylglycerol was seen, consisting of a series of maxima over three successive cell cycles (Fig. 2); although these maxima represent only single samplings, the same pattern was obtained consistently from one experiment to another.

The labelling patterns of the fatty acids of individual phosphoacylglycerols from [^{14}C]acetate were also examined. Interestingly, despite the facts that the endogenous fatty acid patterns of the principal phosphoacylglycerols were essentially identical and that this bacterium uses fatty acid synthetase for the production of both saturated and unsaturated fatty acids, the relative incorporation of radioactivity into different fatty acids varied

between lipids (Table 1). This difference was particularly marked for palmitic acid, which was labelled rather poorly in phosphatidylethanolamine and relatively well in phosphatidylcholine, compared with the percentage proportions by mass. In the latter phospholipid, *cis*-vacenate was labelled less, and stearate more, than in phosphatidylethanolamine and phosphatidylglycerol. Palmitoleate was labelled rather poorly in all phospholipids.

We also examined the accumulation of the pigment-protein complexes in our synchronized cultures for comparison with that of phospholipids, in view of the proposed roles of some lipids in photosynthesis (see the introduction). Fig. 3 shows that, in contrast with the discontinuous accumulation of phospholipid, the accumulation of the pigment-protein complexes rose continuously and smoothly after an initial lag of 3–4 h. The rate of accumulation of the B800–850 antennae rose faster than that of the B875 antennae, as increasing culture turbidity gave self-shading effects during the course of the experiment (Fig. 3). There was an approx. 4-fold increase in the amount of bacteriochlorophyll per ml of culture during the experiment (results not shown). The results in Fig. 3 also show that the B875 antenna/reaction-centre ratio remained constant during the experiment.

An alternative method of synchronizing cultures is by differential centrifugation, and a number of such experiments were carried out. A representative experiment is shown in Fig. 4, and it can be seen that good synchrony was achieved for only one division. The increase in viable cell numbers for the second division was only about 50%. Furthermore, the times between divisions were erratic: the first cycle usually taking approx. 4 h, but the second cycle only 2–2.5 h. With the stationary-phase-cycling method a doubling time of 4.5 h was obtained consistently for two successive divisions.

Double-labelling experiments were carried out with cultures synchronized by differential centrifugation, and the results are shown in Fig. 4. Most lipid fractions displayed similar patterns of labelling from either [^{14}C]acetate or [^3H]glycerol: characteristically there was a large increase in the rate of labelling just preceding the first cell division. This labelling peaked at the beginning of the second cell cycle (4.5 h). Although control (non-synchronized) cultures did not show the same obvious peak in labelling rates, several lipids were also labelled at increased rates around 4.5 h (Fig. 4). The labelling of all lipid fractions in synchronized cultures declined markedly during the second cell cycle and showed only a small increase at the time of the second division (approx. 6.5 h). In contrast, the labelling rates of non-synchronized

Table 1. Labelling of individual fatty acids of phosphoacylglycerols from [$1\text{-}^{14}\text{C}$]acetate in *Rhodopseudomonas sphaeroides* growing non-synchronously

Bacteria were grown non-synchronously and samples were taken during exponential-phase growth and incubated with [$1\text{-}^{14}\text{C}$]acetate under phototrophic conditions (see the Materials and methods section). Samples were removed at 1, 2, 4, 8, 15, 30, 45 and 60 min, the lipids extracted and separated, and fatty acid compositions determined as described in the Materials and methods section. Results are shown for radiolabelling at 8, 15 and 60 min, but other time intervals gave essentially the same results. The fatty acyl mass compositions are shown for each phosphoacylglycerol for comparison with the radioactive labelling; the mass pattern did not alter during the experiment. Data for radiolabelling are the averages of duplicates, whereas the mass patterns are means $\pm$ S.D. ($n = 8$). Abbreviations used: $\text{C}_{16:0}$, palmitic acid; $\text{C}_{16:1}$, palmitoleic acid; $\text{C}_{18:0}$, stearic acid; $\text{C}_{18:1}$, *cis*-vaccenic acid (cf. Russell & Harwood, 1979); PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PC, phosphatidylcholine.

Lipid	Incubation time (min)	^{14}C -labelled fatty acid . . .	Proportion (% of total)			
			$\text{C}_{16:0}$	$\text{C}_{16:1}$	$\text{C}_{18:0}$	$\text{C}_{18:1}$
PE	8		8.4	2.8	18.2	71.9
	15		7.7	3.1	15.9	73.2
	60		5.5	1.3	17.1	76.1
	Average (all times $n = 8$) . . .		6.8 ± 1.6	2.2 ± 1.0	17.9 ± 1.4	73.6 ± 2.1
	Mass distribution . . .		14.8 ± 3.9	4.4 ± 2.4	16.8 ± 2.4	64.9 ± 7.1
PG	8		7.7	2.1	20.9	70.3
	15		8.0	1.4	26.7	64.6
	60		13.1	2.3	14.8	69.8
	Average (all times $n = 8$) . . .		8.8 ± 2.5	2.0 ± 0.5	21.5 ± 4.7	68.5 ± 3.4
	Mass distribution . . .		14.2 ± 3.9	5.5 ± 3.5	19.0 ± 4.0	61.0 ± 10.3
PC	8		12.5	3.8	35.0	50.6
	15		25.7	4.0	30.8	36.9
	60		19.6	1.5	36.3	42.6
	Average (all times $n = 8$) . . .		20.3 ± 5.6	3.2 ± 1.3	32.6 ± 3.5	44.7 ± 6.5
	Mass distribution . . .		14.5 ± 3.7	3.8 ± 1.9	18.9 ± 3.4	63.3 ± 6.5

cultures increased throughout this part of the growth period, with a small peak at 6.5 h.

Discussion

An important consideration for experiments with synchronized cultures is to ensure that the experimental observations are normal cell-cycle-associated events and are not induced by the manipulations necessary to achieve synchrony. Thus, even though results might be reproducible and the experiments repeated in different ways, it is desirable to verify the data by using synchronization procedures based on different principles. We have sought, therefore, to verify published information on phospholipid synthesis in synchronous cultures of *Rhodopseudomonas sphaeroides* by using two methods to induce synchrony. In addition, we have used a different method utilizing double-labelling to monitor both head-group and fatty-acyl-chain metabolism, and included a test of photosynthetic capacity to correlate with phospholipid changes. The following discussion will show that, by the use of different methods, we have confirmed many of the findings of Kaplan's group (Kaplan *et al.*, 1979; Cain *et al.*, 1981; Yen *et al.*,

1984), although there are significant differences, as well as extending their observations.

Good incorporation rates were obtained by using non-synchronous cultures incubated with [^{14}C]acetate, and synchronous cultures incubated with [^{14}C]acetate and [^3H]glycerol. The faster rates of labelling of phosphatidylglycerol compared with diphosphatidylglycerol (cardiolipin), and of phosphatidylethanolamine compared with phosphatidylcholine, from both radioactive precursors, probably reflects the precursor-product relationship of these pairs of phospholipids in bacteria (Harwood & Russell, 1984); a similar observation was made by Cain *et al.* (1981) for phosphatidylethanolamine and phosphatidylcholine. In the present study, in which strain N.C.I.B. 8253 was used, good incorporation rates were obtained for all phospholipids. In comparison, Cain *et al.* (1981) found in strain 2.4.1 that phosphatidylcholine was labelled at a very low rate (usually less than 5% of the total radioactivity). These workers cite the data of Lascelles & Szilagyi (1965), who also used strain N.C.I.B. 8253, as being in support of their results in strain 2.4.1. In fact, Lascelles & Szilagyi (1965) showed that the initial rate of phosphatidylcholine labelling was

Fig. 3. Accumulation of lipid and pigment-protein complexes during synchronous growth of *Rhodopseudomonas sphaeroides*

Techniques for synchronization by stationary-phase cycling and the determination of acyl lipids and pigment-proteins are described in the Materials and methods section. ▲, Total fatty acids; △, viable cell counts; ■, reaction centres; ●, B800-B850 antennae; ○, B875 antennae.

22% of that for phosphatidylglycerol, rising to 39% after 3 h incubation; in photosynthetic cultures the specific radioactivity of phosphatidylcholine was 41% of that for phosphatidylglycerol. In agreement with Lascelles & Szilagyi (1965), we also found that

the rate of phosphatidylcholine labelling was significant, but less (43–73%) than that of phosphatidylglycerol and phosphatidylethanolamine. The difference in phosphatidylcholine labelling between strains 2.4.1 and N.C.I.B. 8253 probably reflects true differences in lipid metabolism, because only strain 2.4.1 accumulates large amounts of *N*-acylphosphatidylserine during growth in Tris buffer (Donohue *et al.*, 1982). Thus we might anticipate other differences in phospholipid metabolism between the two strains.

There were specific differences in the pattern of radioactive labelling of the three major phospholipids, and a general observation is that the saturated fatty acids, palmitate and stearate, contained proportionately less radioactivity than indicated by their mass-distribution values, whereas the opposite was true of the unsaturated fatty acids (palmitoleate more so than oleate; Table 1). Assuming that the anaerobic pathway for fatty acid biosynthesis in *Rhps. sphaeroides* operates in essentially the same way as that in *E. coli* (Harwood & Russell, 1984), it would be anticipated that there might be regulation of the proportion of saturated and unsaturated fatty acids at the level of the dehydrase(s) or the β -oxoacyl-acyl-carrier-protein synthetase(s) (de Mendoza & Cronan, 1983). Regulation in *Rhps. sphaeroides* could be by light or O_2 partial pressure, which might be different enough under the small-scale short-term radioactive incubation conditions compared with those of bulk growth of larger-scale cultures used for lipid analyses to give differences in radioactivity distribution compared with mass distribution. Alternatively, there may be differential turnover of acyl moieties of individual phosphoacylglycerols such as occurs in other photosynthetic tissues (cf. Wintermans & Kuiper, 1982) and in *E. coli* (Kanemasa *et al.*, 1967; Kito *et al.*, 1975). Regulation of fatty acid synthetase by light has not been observed in bacteria, and this is a point worth further investigation.

We used [^{14}C]acetate and [3H]glycerol in our experiments, compared with [^{32}P]phosphate used by Kaplan and co-workers (Kaplan *et al.*, 1979; Cain *et al.*, 1981; Yen *et al.*, 1984), and by Lascelles & Szilagyi (1965). Glycerol and phosphate label the head group, and acetate labels the fatty acyl chains. Therefore our labelling patterns almost certainly reflect synthesis of the whole phospholipid molecule. Cain *et al.* (1981) point out that a disadvantage of using [^{32}P]phosphate is the extremely high intracellular pools of organophosphates during photosynthetic growth (Fuller, 1978), a problem that can be avoided by using acetate and glycerol as the radioactive precursors.

Despite the strain-dependent differences in phospholipid metabolism noted above, we could

Fig. 4. Labelling of individual acyl lipids at different stages of the cell cycle of *Rhodospseudomonas sphaeroides* synchronized by differential centrifugation

Cultures were synchronized by differential centrifugation as detailed in the Materials and methods section. Portions of culture were incubated for 5 min with [^{14}C]acetate or [^3H]glycerol, and labelled lipids were extracted and purified as described in the Materials and methods section. For simplicity only four individual lipids are shown, but other lipids showed similar labelling patterns. ●, ^{14}C -labelled phosphatidylethanolamine ([^{14}C]PE); ○, ^{14}C -labelled phosphatidylglycerol ([^{14}C]PG); ▲, ^{14}C -labelled phosphatidylcholine ([^{14}C]PC); △, ^3H -labelled phosphatidylcholine ([^3H]PC); ■, viable cells.

confirm the observation by Lueking *et al.* (1978) that discontinuous accumulation of phospholipid occurs in cultures of *Rhps. sphaeroides* growing synchronously. Lueking *et al.* (1978) did not measure amounts of individual phospholipids, but showed that the increase in total phospholipid was coincident with cell division. In our experiments there was a sharp increase in the amount of the major phospholipids, phosphatidylethanolamine and phosphatidylglycerol, just before cell division,

and there was little change in their amounts during interphase (Fig. 1). The slight difference in the timing of phospholipid accumulation may reflect our use of colony counts to measure viable-cell counts, compared with direct cell counting to determine total cell number as used by Kaplan and co-workers (Kaplan *et al.*, 1979; Cain *et al.*, 1981; Yen *et al.*, 1984). Viable-cell counting, unlike direct cell counting, depends on complete separation of cells before they are recorded as having

doubled their number, which would delay apparent cell division and, therefore, place the burst in lipid synthesis slightly earlier.

The discontinuous accumulation of phospholipids just before cell division is mirrored by a burst of synthesis at the same time (cf. Figs. 1 and 2). By using the same kind of experimental protocol (i.e. pulse-labelling samples of culture at intervals during the cell cycle), a similar result for cell total phospholipid was obtained by Fraley *et al.* (1979a) and Kaplan *et al.* (1979). By complete contrast, in a later publication from the same group (Cain *et al.* 1981), it was shown that cell total phospholipid was constant during cell division but increased during interphase. No explanation of the differences in these published data were offered, but in the later publication it was shown that, compared with whole-cell phospholipids, the total and individual phospholipids of chromatophores showed a quite different pattern of radioactive labelling, with maxima at the time of cell division (Cain *et al.*, 1981). In order to explain the difference in whole-cell and chromatophore labelling patterns it was argued that the cell-cycle-associated event is transfer of phospholipid into the intracytoplasmic membrane, rather than synthesis *de novo*. Data from pulse-chase experiments were presented in support of this model, but, notwithstanding the reasonable deductions and careful allowance for errors in their assumptions, no direct experiments *in vitro* have been done with isolated chromatophores, and one has to measure radioactivity in chromatophores prepared from intact bacteria after incubation with radioactive precursor. Thus it is impossible to state categorically that intracytoplasmic membranes do not synthesize phospholipids.

Cain *et al.* (1981) give values of 30–35% for the proportion of phospholipid in intracytoplasmic membrane relative to whole-cell phospholipid, in comparison with the 50–70% quoted by Kaplan *et al.* (1979). We would agree with a value of *at least* 70% in photosynthetically growing bacteria, and suggest that our data on whole-cell phospholipid largely reflect the synthesis of intracytoplasmic membrane phospholipid. Of course, this does not preclude the interpretation that the event which is controlled by the cell cycle is phospholipid transfer from the cytoplasmic (plasma) membrane to the intracytoplasmic membranes, because transfer could well regulate synthesis; phospholipids synthesized in the cytoplasmic membrane just before cell division might be transferred immediately to the intracytoplasmic membrane. Furthermore, as noted above, one cannot rule out direct phospholipid synthesis by the intracytoplasmic membranes.

We were particularly interested in whether there was some special feature of phosphatidylglycerol

synthesis, in view of its possible functional association with photosynthesis (see the introduction). The pattern of phosphatidylglycerol synthesis from [^{14}C]acetate was the same as that of phosphatidylethanolamine (Fig. 2), probably reflecting the use of a common pool of fatty acid precursors. In contrast, the rate of synthesis of phosphatidylglycerol from [^3H]glycerol was multiphasic, displaying maxima of incorporation in the interphases as well as just before cell division (Fig. 2). We suggest that this is caused by differential synthesis (or turnover) rates of the glycerols forming the backbone and hydrophilic end of phosphatidylglycerol. The glycerols of phosphatidylglycerol in some other bacteria are metabolized independently (White & Tucker, 1969; Short & White, 1972; Ballesta *et al.*, 1973).

A direct test of the relationship between photosynthetic-pigment synthesis and photosynthetic activity is by measurement of antenna complexes and reaction centres. In contrast with phospholipids, there was a smooth rise in the amount of antenna complexes and reaction centres during two successive cell cycles (Fig. 3). Our observations of reaction centres are similar, both qualitatively and quantitatively, to those of Wraight *et al.* (1978). We were able to distinguish between B800–850 and B875 antennae; the faster rise in amounts of B800–850 antennae probably reflects self-shading, which becomes more significant as cell numbers increase during growth. The constant ratio between antennae and reaction centres of B875 agrees with observations of other workers (Aagaard & Sistrom, 1972).

Only a single, induction-synchrony, method had been used previously in experiments with *Rhps. sphaeroides*. For the reasons given above, we considered that it was important to verify this information with experiments employing a selection-synchronization method. Accordingly, we have been able to confirm the cell-cycle-dependent synthesis of phospholipids by using a centrifugation-selection method (Fig. 4). This was despite the fact that an acceptably high synchrony index could generally be obtained for only a single cell cycle, and the observation that centrifugation alone introduced a small degree of synchrony. However, the two methods give essentially the same results, yet use quite different means of introducing synchrony.

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