Temporal Changes in the Pattern of Intra-cytoplasmic Membrane Protein Synthesis during the Swarmer Cell Cycle of *Rhodomicrobium vannielii*

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(Received 1 April 1986; revised 10 June 1986)

Swarmer cells and multicellular arrays of *Rhodomicrobium vannielii* synthesized different types of intra-cytoplasmic membrane proteins, as evidenced by pulse-labelling patterns with [³⁵S]methionine. Analysis of protein synthesis during the swarmer cell cycle revealed temporal changes in the rates of synthesis of several proteins, including those of the light-harvesting complex I (B885)–reaction centre pigment–protein complex and a 34000 *M_r* protein identified as flagellin. The results are discussed in terms of differentiation and polar growth in this and related members of the *Rhodospirillaceae*.

INTRODUCTION

Rhodomicrobium vannielii is a member of the *Rhodospirillaceae* in which cell growth is obligately polar and cell division asymmetric (Whittenbury & Dow, 1977; Dow *et al.*, 1983; Kelly & Dow, 1984). Photoheterotrophic growth in batch culture is characterized by the production of multicellular arrays of cells, linked by prosthecae, from the tips of which ovoid, peritrichously flagellate swarmer cells are produced (Whittenbury & Dow, 1977). When separated from the other cell types by a simple glass wool column filtration method (Whittenbury & Dow, 1977) and kept anaerobically in the dark, swarmer cells remain motile for several hours. However, when re-illuminated they synchronously (>90%) initiate a reproductive differentiation process in which the major morphological landmark events are loss of motility by the active shedding of flagella, synthesis of a prostheca at a polar location on the cell surface and production of a new daughter cell at the distal end of the prostheca (Dow *et al.*, 1983).

It is clear that many of the morphological landmarks observed during swarmer cell differentiation can be viewed as cell-surface- or membrane-associated events. *R. vannielii* belongs to that group of photosynthetic bacteria in which the photosynthetic apparatus is housed in a system of lamellate intra-cytoplasmic membranes (ICM; Trentini & Starr, 1967). The photochemical reaction centre (RC), closely associated with a bound cytochrome and light-harvesting (LH) complex (B885 or LHI) in addition to an accessory LH complex (B800-865 or LHII), characterize the photosynthetic pigment–protein complexes in this microbe (Kelly & Dow, 1985). As one consequence of the polar growth process, the ICM complex must be formed *de novo* in the daughter cell during swarmer cell differentiation (Whittenbury & Dow, 1977; Kelly & Dow, 1984). This must require both temporal and spatial control of gene expression. In view of these features, we have investigated the changes in ICM protein synthesis which occur during differentiation, the differential synthesis of ICM proteins in the different cell types in batch culture and the synthesis of certain components of the photosynthetic apparatus.

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Abbreviations: Bchl, bacteriochlorophyll; ICM, intra-cytoplasmic membrane(s); LH, light-harvesting (complex); RC, reaction centre.

METHODS

Growth conditions. *Rhodomicrobium vannielii* strain Rm5 from the University of Warwick culture collection was used throughout and was grown in PM medium (Whittenbury & Dow, 1977) containing (g l^{-1}): NH_4Cl , 0.5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.4; NaCl , 0.4; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.05; sodium pyruvate, 1.5; sodium hydrogen malate, 1.5. The pH was adjusted to 6.8 before autoclaving (121°C , 15 min) and sterile $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ buffer added aseptically afterwards to 5 mM final concentration. Cultures were capped with Suba-Seals (William Freeman & Co.), gassed for 15 min with O_2 -free N_2 , inoculated (0.4%, v/v) and incubated with stirring at 30°C under a light intensity of approximately 1000 lx. Cells were also grown under microaerobic conditions in 2 litre volumes of PM contained in 2.5 litre conical flasks on a rotary shaker at 175 r.p.m. and 30°C .

Pulse-labelling batch cultures. A 1 litre culture of *R. vannielii* was grown to late-exponential phase (OD_{540} 1–2) and L-[^{35}S]methionine ($>800 \text{ Ci mmol}^{-1}$) added under anaerobiosis to a final activity of $1 \mu\text{Ci ml}^{-1}$ (37 kBq ml^{-1}). After 20 min, unlabelled L-methionine was added to 1 mM final concentration and the culture divided into three portions. A 50 ml sample was centrifuged immediately (20000 g, 4°C , 20 min) and the pellet was frozen in liquid N_2 . A further 50 ml sample was used to obtain multicellular arrays by three cycles of differential centrifugation (1000 g, 2 min, room temperature). After each cycle, the loose pellet was gently resuspended in 5 mM-phosphate buffer pH 6.8 and the supernatant, containing the swarmer cells, was discarded. The pellet was finally resuspended in 5 mM-phosphate buffer and frozen in liquid N_2 . The remaining 900 ml was used to obtain a homogeneous population of swarmer cells by filtration through glass wool (Whittenbury & Dow, 1977). The swarmer cells were centrifuged (20000 g, 4°C , 20 min), resuspended in phosphate buffer and frozen.

Pulse-labelling during the swarmer cell cycle. A 5 litre culture grown to the late-exponential phase was used to prepare a homogeneous swarmer cell population by glass wool filtration. The swarmer cells were collected in a foil-covered aspirator equipped with two N_2 -filled neoprene rubber bladders in equilibrium with the gas phase, so that successive samples could be taken without affecting the gas atmosphere. At time 0, the foil was removed and the culture incubated at 30°C under 1000 lx illumination. At hourly intervals, 100 ml samples were removed and pulse-labelled for 20 min with L-[^{35}S]methionine (final concentration $1 \mu\text{Ci ml}^{-1}$) under anaerobic conditions at the same light intensity. After labelling, L-methionine was added (final concentration 1 mM), and the cells were collected by centrifugation (20000 g, 4°C , 20 min), resuspended in 10 mM-Tris/HCl buffer pH 7.4 and stored frozen. At the same time intervals, 200 ml unlabelled samples were collected in the same way for bacteriochlorophyll (Bchl) and protein determinations.

Isolation of ICM. Cells were resuspended in 10 mM-Tris/HCl pH 7.4, washed once, resuspended again, and broken by sonication ($8 \times 15 \text{ s}$ bursts with 1 min cooling intervals in an MSE 12/76 Mk II sonicator). RNAse and DNase (each $25 \mu\text{g ml}^{-1}$) were added and debris and unbroken cells removed by centrifugation in a microfuge (11 600 g, 5 min, 4°C). The supernatant (300 μl) was layered onto a 25% (w/v) sucrose cushion (4.3 ml) in 10 mM-Tris/HCl pH 7.4 and centrifuged at 150000 g for 2.5 h at 4°C in the SW50.1 rotor of a Beckman ultracentrifuge. The membrane pellet was resuspended in 50–100 μl 10 mM-Tris/HCl pH 7.4 and stored at -20°C . For the detection of flagellin, ICM were also isolated on 20–60% (w/v) sucrose gradients [8 ml of 20, 30, 40 and 50% (w/v) sucrose + 2 ml 60% (w/v) sucrose] loaded with 3 ml cell-free extract and centrifuged at 100000 g for 16 h at 4°C in the SW28 rotor of a Beckman ultracentrifuge. The ICM, which banded at 36% (w/v) sucrose, were removed and stored at -20°C .

SDS-polyacrylamide gel electrophoresis (SDS-PAGE). This was done on 10–30% (w/v) exponential polyacrylamide gels (Hames & Rickwood, 1981). Samples were routinely solubilized at 75°C for 2 min in Laemmli sample buffer (Laemmli, 1970). Gels were run at 20 mA for 16 h at $4-10^\circ\text{C}$, fixed in 50% (v/v) methanol and either stained with Coomassie blue or silver stained (Wray *et al.*, 1981).

Isolation of pigment-protein complexes. Portions (5 μl) of each membrane sample obtained from pulse-labelled cells during differentiation, as described above, were added to 45 μl 68 mM-Tris/HCl pH 7.4, 1.6% (w/v) SDS, 1.6% (w/v) Triton X-100, 5% (v/v) 2-mercaptoethanol, incubated at room temperature for 5 min and then microfuged for 2.5 min. Portions (2 μl) of the resulting supernatants were removed for scintillation counting before equal quantities of radioactivity (82000 c.p.m.) were directly applied to the stacker of a 10% (w/v) polyacrylamide gel (ratio of acrylamide to bisacrylamide 30:0.8) containing 0.05% SDS + 0.05% Triton throughout. The gel was run at $4-10^\circ\text{C}$ for 6 h, after which time the pigmented bands corresponding to the B885-RC complexes (Kelly & Dow, 1985) were excised as a strip and sealed to the stacking gel of a 10–30% (w/v) gradient gel (containing 0.1% w/v SDS) with hot 1% (w/v) agarose dissolved in Laemmli sample buffer. The gel was run at 20 mA overnight in the cold, fixed in methanol (50%, v/v) and fluorographed (Bonner & Laskey, 1974) using pre-flashed Kodak X-omat S film (Laskey & Mills, 1975).

Production of antisera. Antibodies were raised in New Zealand White, half-lop rabbits as follows. ICM protein (4 mg) was solubilized in 5% (w/v) Triton X-100 + 0.8% (w/v) SDS in 50 mM-Tris/HCl pH 7.4 for 1 h at room temperature. After microfuging 5 min, the supernatant was made up to 1 ml with 50 mM-Tris/HCl buffer and added to 1 ml complete Freund's adjuvant. After homogenization, the mixture was injected subcutaneously. A booster injection was given 1 month after the first, using Freund's incomplete adjuvant. Two weeks after this, rabbits were bled at regular intervals and the pooled serum was stored at -20°C .

Western immunoblotting. Proteins were transferred to nitrocellulose sheets (Burnette, 1981) in a Bio-Rad Trans-Blot cell at 50 V, 300 mA for 3.5 h. The sheets were incubated for 1 h with 8% (w/v) bovine serum albumin (BSA) in phosphate buffered saline (PBS) followed by the addition of 200 µl crude antiserum and incubation at room temperature overnight. The sheets were then washed six or seven times with PBS and incubated for 2 h with 8% (w/v) BSA in PBS containing 1×10^6 c.p.m. ^{125}I -labelled Protein A. After extensive washing with 1% (w/v) Triton X-100 in PBS, the sheets were air dried and autoradiographed at 4 °C for 1–3 d.

Preparation of flagella. The cells from a 5 l culture of *R. vannielii* were removed by centrifugation (20000 g, 4 °C, 30 min) and the supernatant was concentrated to a volume of 50 ml using an Amicon CH4 hollow-fibre concentrator (filter-type HIP-100). The concentrate was re-centrifuged (5000 g, room temperature, 30 min), and the supernatant was adjusted to 35% (w/v) CsCl, 15 mM Tris/HCl pH 7.5, 2% (w/v) Triton X-100 and centrifuged (90000 g, 4 °C, 50 h) in the 8×25 ml angle rotor of an MSE 65 ultracentrifuge. The flagella, which formed a visible, turbid band at a density of 1.336 g cm^{-3} , were removed and dialysed against 15 mM-Tris/HCl pH 7.5 overnight at 4 °C.

Analytical methods. Protein was determined by the Lowry method with the Folin phenol reagent. Bchl was determined by repeated extraction of whole cells with acetone/methanol (7:2, v/v) using a molar absorption coefficient of $75 \text{ mm}^{-1} \text{ cm}^{-1}$ at 772 nm (Clayton, 1963). Carotenoid was determined on the same samples and expressed as the absorbance at 472 nm per unit protein.

Cell volume distribution analysis was done using a Coulter counter ZBI and channelyzer C1000 linked to a BBC microcomputer and disc storage system.

Radioactivity in membrane samples was determined by scintillation counting (2–5 µl sample + 3 ml Beckman MP fluid) in an LKB Minibeta counter.

Chemicals. L-[^{35}S]Methionine was from Amersham, acrylamide from Eastman Kodak and bisacrylamide from Bio-Rad. Radioiodinated Protein A was a gift from Miss R. Lennox of this department. The Freund's adjuvants were obtained from Difco. M_r standards for SDS-PAGE were from Pharmacia.

RESULTS

Cell-type-specific ICM proteins

Light microscopy and representative cell volume distribution profiles showed that swarmer cells and multicellular arrays obtained from pulse-labelled batch cultures by column filtration and differential centrifugation respectively were homogeneous with respect to cell type.

In order to examine the ICM proteins synthesized during the pulse, we equalized both protein and radioactivity on denaturing polyacrylamide gels (Fig. 1). Coomassie blue staining revealed one major band of M_r 34000 which stained prominently only in the swarmer cell ICM tracks. Few other proteins could be ascribed to a particular cell type from an analysis of such stained gels. However, fluorography showed the 34000 M_r protein to be strongly labelled in the swarmer cell ICM, with some labelling in ICM from multicellular arrays. Several other proteins (M_r 28000, 38000, 65000, 68000) were also strongly labelled in swarmer cell ICM during the pulse, as were proteins (M_r 45000, 70000, 77000, 85000) specific to the ICM of the multicellular arrays.

The identity of the majority of these differentially synthesized proteins is unknown. However, we noted that the flagellin monomer from purified *R. vannielii* flagella had an apparent M_r of 34000 (J. Macdonald & C. S. Dow, unpublished) and this raised the possibility that a membrane-bound form of flagellin was present. This was confirmed by using antisera raised against detergent-solubilized *R. vannielii* ICM as a probe in Western immunoblotting experiments (Fig. 2). Purified flagella from CsCl gradients produced one band of M_r 34000 which reacted strongly with this probe. Equivalent bands of the same M_r were also observed in ICM from cells grown either photosynthetically or chemoheterotrophically under microaerobic conditions in the dark. The 34000 M_r polypeptide was present in ICM isolated either on sucrose cushions or on sucrose gradients, indicating that it is not a co-sedimenting contaminant.

Synthesis of ICM proteins during swarmer cell differentiation

In order to gain information regarding the types of ICM proteins synthesized during swarmer cell differentiation, a homogeneous population of synchronized swarmer cells was pulse-labelled for 20 min periods every hour under phototrophic conditions and the morphological changes were followed microscopically and by cell volume distribution analysis. The changes in peak cell

Fig. 1. ICM synthesis during pulse-labelling of *R. vanielii* batch cultures. A photoheterotrophically grown batch culture was pulse-labelled with $L\text{-}^3\text{S}$ methionine ($1 \mu\text{Ci ml}^{-1}$) and then separated into its constituent cell types (see Methods). ICM were prepared from the whole culture, containing all cell types (tracks 2 and 5), from the fraction containing multicellular arrays (tracks 3 and 6) and from the swarmer cell fraction (tracks 4 and 7). After solubilization (75°C for 2 min) the samples were loaded equally on the gel (10–30% w/v, polyacrylamide gradient) with respect to protein (120 μg ; tracks 2–4) or radioactivity (60000 c.p.m.; tracks 5–7). Tracks 1 and 8 contained M_r markers. (a) Coomassie-blue-stained gel; (b) fluorogram of the same gel after 2 weeks exposure.

Fig. 2. Detection of flagellin in the ICM of *R. vannielii* by immunoblotting. Track 1, purified flagella (20 µg protein); track 2, ICM from photoheterotrophically grown cells prepared on 25% (w/v) sucrose cushions; track 3, ICM from photoheterotrophically grown cells prepared on 20–60% (w/v) sucrose gradients (40 µg protein); track 4, ICM from chemoheterotrophically grown cells prepared on 20–60% (w/v) sucrose gradients (40 µg protein). The samples were denatured and run on a 10–30% (w/v) polyacrylamide gel, the proteins were blotted onto microcellulose (see Methods) and the blot was probed with antibodies raised to detergent-solubilized ICM from photoheterotrophically grown cells. The figure shows the resulting autoradiograph (3 d exposure) after incubation with ¹²⁵I-labelled protein A.

volume, culture OD₅₄₀, protein and pigment content are shown for a representative experiment in Fig. 3. The first observable morphological event was the loss of motility due to shedding of flagella, and this occupied the period from 1 to 2 h. However, increases in OD₅₄₀ and whole-cell protein levels occurred from time 0. Prostheca synthesis occupied the period from 2 to 3.5 h and was associated with a plateau on the OD₅₄₀ but not the protein curve, indicating a change in the light-scattering properties of the cells. Daughter cell synthesis commenced at 3–3.5 h, followed by rapid growth and enlargement, which was reflected in the increase in the peak cell volume from this time to the end of the experiment. No change in the culture Bchl or carotenoid concentration was detected under these conditions but the continuously increasing protein content meant that a gradual decrease in the cellular specific pigment content was observed: over the 6 h of the experiment the cellular specific Bchl content decreased from an initial value of 34 nmol (mg protein)⁻¹ to about 15 nmol (mg protein)⁻¹. The change in carotenoid content paralleled that of Bchl.

In Fig. 4, the changes associated with the ICM system during differentiation are shown for a different synchronized swarmer cell population, but the timing of the morphological events of the cell cycle was quite reproducible from one experiment to another (compare Fig. 3 and Fig. 4). The ICM specific pigment content showed a decline up to about 3 h after the initiation of differentiation but thereafter an increase was observed, over the period of daughter cell formation. Measurements of the amount of [³⁵S]methionine incorporated per unit ICM protein showed that this was low in swarmer cells but increased in successively labelled samples during differentiation.

The proteins synthesized during pulse labelling were analysed on polyacrylamide gels equalized with respect to both protein and radioactivity. Those loaded with equal protein were silver stained. The relative abundance of the majority of the 60–70 detected polypeptides

Fig. 3. Changes in cell volume, OD₅₄₀, and cellular pigment and protein contents in differentiating *R. vannielii* swarmer cells. A 5 litre batch culture was synchronized and the swarmer cells were incubated under photoheterotrophic conditions. Differentiation of 200 ml samples removed at 0.5 h intervals was monitored by microscopy, cell volume distribution analysis (●) and the change in OD₅₄₀ (■). The culture protein concentration (○), Bchl concentration (nmol ml⁻¹, ◆) and carotenoid concentration (A₄₇₂ units ml⁻¹ ◇) were also determined and used to calculate the cellular specific Bchl (▲) and carotenoid (△) contents.

Fig. 5. Synthesis of ICM proteins in differentiating swarmer cell populations. Samples (100 ml) of a synchronized swarmer cell culture incubated under phototrophic conditions were pulse-labelled at 1 h intervals (tracks 0–8) for 20 min with L-[³⁵S]methionine (1 μ Ci ml⁻¹). ICM proteins were prepared on 25% (w/v) sucrose cushions and equal quantities of radioactivity (40000 c.p.m.) applied to a 10–30% (w/v) gradient gel. The figure shows the resulting fluorogram (2 weeks exposure). The M_r values of polypeptides showing a prominent decrease (left-hand side) or increase (right-hand side) in rate of synthesis during differentiation are indicated.

showed no variation during the course of differentiation. The polypeptides of the photochemical RC (M_r 26000, 28000 and 31000) a *c*-type cytochrome (M_r 38000) and those of the LH apparatus (LHI/B885 12000 and 14000 LHII/B800–865 11000 and 13000) identified in a separate study (Kelly & Dow, 1985) were present in all cell types. However, analysis of such stained gels gave no information about the period or rate of synthesis of the individual protein species. Therefore, fluorographs of gels loaded with equal radioactivity were prepared, on which the band intensity is proportional to the net rate of synthesis of the protein (Fig. 5). Such fluorographs revealed several protein species whose rate of synthesis was modulated during swarmer cell differentiation. For example the M_r 34000 flagellin monomer was only synthesized in motile swarmer cells (0–1 h) and upon loss of motility, there was no further incorporation of radioactivity into this polypeptide. A polypeptide of M_r 14000 and a group of proteins of M_r 70000–80000, possibly corresponding to those synthesized in multicellular arrays (Fig. 1), were particularly strongly synthesized over the period of daughter cell formation 3–8 h.

Fig. 4. Changes associated with the ICM system during the differentiation of *R. vannielii* swarmer cells. ●, Cell volume; ■, culture OD₅₄₀. At 1 h intervals, 100 ml samples were removed and pulse-labelled with L-[³⁵S]methionine, the ICM were isolated on 25% (w/v) sucrose cushions and the amount of radiolabel incorporated during the pulse was measured (○). The ICM specific Bchl (▲) and carotenoid (△) contents were determined after extraction into acetone/methanol (7:2, v/v).

Fig. 6. Synthesis of the B885-RC pigment-protein complex during swarmer cell differentiation. Synchronized swarmer cells were pulse-labelled as described in the legend to Fig. 5 and ICM prepared from them were used in the preparation of the B885-RC complex as described in Methods. The figure shows the fluorogram (5 weeks exposure) resulting after re-electrophoresis of the radiolabelled B885-RC complex on a 10–30% (w/v) polyacrylamide gradient gel. Tracks 0–6 represent 20 min pulse-labelling periods carried out hourly from 0–6 h after transfer of swarmer cells to phototrophic conditions.

Synthesis of the photosynthetic apparatus

The periodicity in the synthesis of the M_r 14000 ICM polypeptide (see Fig. 5) was thought to be indicative of a change in the expression of the RC-associated LH complex I during differentiation. We confirmed this by subjecting the native RC-HLI-cytochrome complex obtained from pulse-labelled membrane samples on SDS-Triton gels to SDS denaturing PAGE and fluorography (Fig. 6). The M_r 14000 B885 (LHI) polypeptide showed the same pattern of synthesis as the M_r 14000 polypeptide observed in Fig. 5. Under these conditions (Fig. 6) the native holocomplex was not fully denatured and this allowed correlation of the synthesis of its constituent polypeptides with that of the native complex itself. The period of prostheda elongation and daughter cell synthesis was characterized by an increased rate of synthesis of the RC polypeptides and the 38000 M_r cytochrome c_{553} in addition to that of 14000 M_r B885 protein. Some label was also incorporated into a polypeptide (M_r 61 000) which appeared to be a contaminant, or loosely associated with the B885-RC complex. However, this protein was synthesized throughout the cell cycle. Little [^{35}S]methionine was incorporated into the 12000 M_r B885 polypeptide or into the B800-865 polypeptides during differentiation.

DISCUSSION

Pulse-labelling during the swarmer cell cycle clearly indicated the existence of a temporal programme of ICM protein synthesis. Previous studies with *R. vannielii* (Dow *et al.*, 1983) and the non-photosynthetic prosthecae bacterium *Caulobacter crescentus* (Cheung & Newton, 1977; Iba *et al.*, 1978; Milhausen & Agabian, 1981) have shown variations in the rates of synthesis of a number of soluble proteins during the swarmer cell cycle. In the study of Milhausen & Agabian (1981), 37 developmentally regulated polypeptides were identified using two-dimensional gels, of which 7 were identified as membrane proteins. Even with the use of one-dimensional gels, as here, the synthesis of a relatively large number of ICM polypeptide species appeared to be under regulation.

The present study also showed that marked differences exist in the types of ICM proteins synthesized by swarmer cells and multicellular arrays in batch culture. The generation time of *R. vannielii* (approx. 6 h; Whittenbury & Dow, 1977) is relatively long compared with the labelling period used (20 min) thus assuring that the observed patterns were representative of the proteins produced by each cell population. Considerable differences were observed between the staining intensities and fluorograph band intensities of some of the proteins, which may be partially attributable to their different methionine contents. Only in some cases, for example the 34000 M_r flagellin monomer, was the relative abundance indicated by staining clearly comparable to the labelling pattern. In other cases, as with the group of proteins of 70000–80000 M_r synthesized only in the multicellular arrays, these proteins could nevertheless be detected in swarmer cells by staining. This could be an indication that they are segregated to the swarmer cells post-translationally and/or have a low rate of turnover. Post-translational segregation of membrane proteins has been documented during the *Caulobacter* cell cycle (Agabian *et al.*, 1979).

This type of mechanism may also be important in the assembly of particular cell-surface structures such as the flagellum. The identification of flagellin in the ICM fraction of *R. vannielii* provides evidence for the involvement of these subunits in the assembly of the flagellar organelle, a conclusion supported by the cell-cycle-dependent correlation observed between shedding of flagella and the cessation of flagellin synthesis. In *C. crescentus* (Huguenel & Newton, 1984) flagellins A and B are also temporally regulated during swarmer cell differentiation and assembled through the intermediary of a membrane-bound 'pool' of subunits.

During growth in batch culture, swarmer cells become light-limited due to self-shading and this prevents them from initiating differentiation (Dow *et al.*, 1983). Removal of the multicellular arrays during synchronization reduces the optical density of the culture about 10-fold, so relieving light-limitation when the cells are re-illuminated. This accounts for the drop in the specific photopigment content observed during the maturation phase of the cell cycle as a growth-dilution effect. When expressed on a whole-cell protein basis, a gradual decrease in Bchl content was observed throughout the cell cycle but when expressed in terms of ICM protein, the specific Bchl content increased over the reproductive phase of the cell cycle. This is consistent with only limited ICM development in the daughter cell due to the high light availability per cell in these synchronized cultures. The increase in ICM photopigment content over the period of daughter cell formation appeared to be due largely to the synthesis of the B885-RC pigment-protein complex. Conclusions about the pattern of synthesis of the B800-865 (LHII) complex and the M_r 12000 B885 polypeptide were precluded due to their lack of methionine incorporation. Low specific labelling rates of LH proteins and a lack of proportionality between methionine content and [^{35}S]methionine incorporation were also found by Peters *et al.* (1983) in *Rhodopseudomonas capsulata*.

Taken together, the pigment and protein data are consistent with the incorporation of new photosynthetic units into the daughter cell membrane during differentiation – a process which must accompany *de novo* membrane synthesis arising from polar growth (Whittenbury & Dow, 1977; Dow *et al.*, 1983; Kelly & Dow, 1984). This type of synthetic pattern is rather different from that observed in induction-synchronized cultures of *Rhodopseudomonas sphaeroides* (Fraley *et al.*, 1978; Wraight *et al.*, 1978). Although such cultures have been used in cell cycle studies (Leuking *et al.*, 1981; Kaplan *et al.*, 1983) the use of selection synchronization methods with the

photosynthetic prosthecate and budding bacteria would appear to offer a system in which *de novo* membrane assembly and reproduction by polar growth are obligately coupled during the cell cycle.

Our data indicates that the morphologically different cell types characteristic of cultures of photosynthetic prosthecate and budding bacteria are distinct with respect to several aspects of membrane physiology. Studies of asynchronous batch cultures of such organisms, for example *Rhodopseudomonas palustris* (Firsow & Drews, 1977; Varga & Staehelin, 1983) will only give averaged values for the whole population. It is therefore essential that cell-type heterogeneity be taken into account in studies on photosynthetic bacteria.

D. J. Kelly was the recipient of a Science and Engineering Research Council studentship.

We thank Miss J. Macdonald for the preparation of flagella and for valuable discussion.

REFERENCES

- AGABIAN, N., EVINGER, M. & PARKER, G. (1979). Generation of asymmetry during development: segregation of type-specific proteins in *Caulobacter*. *Journal of Cell Biology* **81**, 123-136.
- BONNER, W. M. & LASKEY, R. A. (1974). A film detection method for tritium labelled proteins and nucleic acids in polyacrylamide gels. *European Journal of Biochemistry* **46**, 83-88.
- BURNETTE, W. N. (1981). 'Western blotting': electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radiiodinated protein A. *Analytical Biochemistry* **112**, 195-203.
- CHEUNG, K. K. & NEWTON, A. (1977). Patterns of protein synthesis during development in *Caulobacter crescentus*. *Developmental Biology* **56**, 417-425.
- CLAYTON, R. K. (1963). Toward the isolation of a photochemical reaction centre in *Rhodopseudomonas sphaeroides*. *Biochimica et biophysica acta* **75**, 312-323.
- DOW, C. S., WHITTENBURY, R. & CARR, N. G. (1983). The 'shut down' or 'growth precursor' cell - an adaptation for survival in a potentially hostile environment. *Symposia of the Society of General Microbiology* **34**, 187-247.
- FIRSOW, N. N. & DREWS, G. (1977). Differentiation of the intra-cytoplasmic membrane of *Rhodopseudomonas palustris* induced by variations of oxygen partial pressure or light intensity. *Archives of Microbiology* **115**, 299-306.
- FRALEY, R. T., LUEKING, D. R. & KAPLAN, S. (1978). Intra-cytoplasmic membrane synthesis in synchronous cell populations of *Rhodopseudomonas sphaeroides*. Polypeptide insertion into growing membrane. *Journal of Biological Chemistry* **283**, 458-464.
- HAMES, B. D. & RICKWOOD, D. (1981). *Gel Electrophoresis of Proteins: a Practical Approach*. London: IRL Press.
- HUGUENEL, E. D. & NEWTON, A. (1984). Evidence that subcellular flagellin pools in *Caulobacter crescentus* are precursors in flagellum assembly. *Journal of Bacteriology* **157**, 727-732.
- IBA, H., FUKUDA, A. & OKADA, Y. (1978). Rate of major protein synthesis during the cell cycle of *Caulobacter crescentus*. *Journal of Bacteriology* **135**, 647-655.
- KAPLAN, S., CAIN, B., DONOHUE, T. J., SHEPHERD, W. D. & YEN, G. S. L. (1983). Biosynthesis of the photosynthetic membranes of *Rhodopseudomonas sphaeroides*. *Journal of Cellular Biochemistry* **22**, 15-29.
- KELLY, D. J. & DOW, C. S. (1984). Microbial differentiation: the role of cellular asymmetry. *Microbiological Sciences* **1**, 214-219.
- KELLY, D. J. & DOW, C. S. (1985). Isolation, characterization and topographical relationships of pigment-protein complexes from membranes of *Rhodomicrobium vannielii*. *Journal of General Microbiology* **131**, 2941-2952.
- LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* **227**, 680-685.
- LASKEY, R. A. & MILLS, A. D. (1975). Quantitative film detection of ^3H and ^{14}C in polyacrylamide gels by fluorography. *European Journal of Biochemistry* **56**, 335-341.
- LUEKING, D. R., CAMPBELL, T. B. & BURGHARDT, R. (1981). Light-induced division and genomic synchrony in phototrophically growing cultures of *Rhodopseudomonas sphaeroides*. *Journal of Bacteriology* **146**, 790-797.
- MILHAUSEN, M. & AGABIAN, N. (1981). Regulation of polypeptide synthesis during *Caulobacter* development: two-dimensional gel analysis. *Journal of Bacteriology* **148**, 163-173.
- PETERS, J., TAKEMOTO, J. & DREWS, G. (1983). Spatial relationships between the photochemical reaction centre and the light-harvesting complexes in the membranes of *Rhodopseudomonas capsulata*. *Biochemistry* **22**, 5660-5667.
- TRENTINI, W. C. & STARR, M. P. (1967). Growth and ultrastructure of *Rhodomicrobium vannielii* as a function of light intensity. *Journal of Bacteriology* **93**, 1699-1784.
- VARGA, A. & STAEHELIN, L. A. (1983). Spatial differentiation in photosynthetic and non-photosynthetic membranes of *Rhodopseudomonas palustris*. *Journal of Bacteriology* **154**, 1414-1430.
- WHITTENBURY, R. & DOW, C. S. (1977). Morphogenesis and differentiation in *Rhodomicrobium vannielii* and other budding and prosthecate bacteria. *Bacteriological Reviews* **41**, 754-808.
- WRAIGHT, C. A., LUEKING, D. R., FRALEY, R. T. & KAPLAN, S. (1978). Synthesis of photopigments and electron transport components in synchronous phototrophic cultures of *Rhodopseudomonas sphaeroides*. *Journal of Biological Chemistry* **253**, 465-471.
- WRAY, W., DOULIKAS, T., WRAY, U. P. & HANCOCK, R. (1981). Silver staining of proteins in polyacrylamide gels. *Analytical Biochemistry* **118**, 197-203.