The Influence of Hydrophobic Inhibitors on the Growth of 
*Rhodobacter vannielii*

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*R. vannielii* swarmer cells were more resistant to the effects of rifampicin than chain or budding cells. RNA polymerase purified from the different cell types showed no difference in susceptibility to rifampicin or in the major protein subunits as visualized by two-dimensional gel electrophoresis. The susceptibility of swarmer and budding cells to sodium deoxycholate gave a similar pattern to that found for rifampicin, with swarmer cells being more resistant than budding cells. This suggests that the changes in susceptibility to the inhibitors as swarmer cells differentiate into budding cells are due to changes in the permeability properties of the cell envelope that relate to changes in the role of cells during the cell cycle.

**INTRODUCTION**

The responses of *Rhodobacter vannielii* swarmer cells to environmental stimuli have been exploited to obtain information on morphogenesis and differentiation in prosthecate bacteria (Whittenbury & Dow, 1977; Dow et al., 1983). They have been designated growth precursor cells and represent a non-growing dispersal stage adapted to survive conditions such as prolonged periods of low light intensity, when growth would either not be possible or not beneficial to the survival of the organisms (Dow et al., 1983). In this inhibited state the cells do not synthesize DNA, but RNA and protein are both made, albeit in low amounts (Potts & Dow, 1979; Dow et al., 1983; Scott & Dow, 1986a). Environmental stimuli, such as shifting swarmer cells from low to high light conditions (Whittenbury & Dow, 1977), induce growth and trigger the obligate sequence of morphogenesis and differentiation (maturation) which results in the swarmer cell becoming a non-motile reproductive cell. During this period the rates of synthesis of RNA and protein increase. Protein synthesis also displays a sequence of qualitative changes (Potts & Dow 1979; Dow et al., 1983, 1985). Growth proceeds with the synthesis of a prosthecum, from the end of which a daughter cell develops; DNA replication is uniquely associated with this reproductive period (Potts & Dow, 1979). At this stage the cell formed depends upon the environment and on the cell cycle being expressed. *R. vannielii* has two vegetative cell cycles. In one case, the simple cycle, the daughter cell is a swarmer cell which divides from the mother by fission; alternatively the daughter cell may be a chain cell giving rise to the complex cycle. These events are summarized in Fig. 1, which shows the key stages of the *R. vannielii* cell cycles.

Little is known about the regulation of the switch from a non-growing motile swarmer cell to a non-motile reproductive cell. Probing the cell cycle with growth inhibitors revealed that during the obligate differentiation sequence of swarmer cells, changes occurred in susceptibility to the DNA-dependent RNA polymerase inhibitor rifampicin: maturation was inhibited by approximately 40 μg rifampicin ml⁻¹ and the switch to the reproductive phase by approximately

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*Abbreviations*: IEF, isoelectric focusing; TGED, Tris/glycine EDTA buffer.
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Fig. 1. The two vegetative cell cycles of R. vannielii. (a) Simple cycle, (b) complex cycle.

20 µg ml⁻¹ (Whittenbury & Dow, 1977). One possible explanation for these data was that the RNA polymerase was modified during differentiation. However, the methodology of Whittenbury & Dow (1977) did not allow for the complex growth kinetics of the developing swarmer cell or for the rate of inhibitor uptake influencing such endpoint analyses. The importance of RNA polymerase modification in the control of gene expression (Losick & Pero, 1981; Doi, 1982; Doi & Wang, 1986) made a proper understanding of the rifampicin effect a necessary part of our study of cell cycle regulation.

Direct measurement of the influence of rifampicin on RNA synthesis has proved difficult because it has not been possible to label RNA in swarmer cells (Potts & Dow, 1979). In the present study, cell volume analysis and protein synthesis were therefore used as indicators of the influence of rifampicin on cell growth. Data are presented which confirm previous rifampicin studies and indicate that specific differences exist between the susceptibility of swarmer cells to rifampicin and that of cells from other stages in the cell cycle. These observations have not been correlated with changes in RNA polymerase holoenzyme (Scott & Dow, 1986b). Further analyses suggest that the changes in susceptibility are due to alterations in the permeability properties of the cell resulting from the functional alterations in the cell envelope that occur during the switch from the non-growing to the growing cell type (Dow et al., 1985).

METHODS

Organisms and growth conditions. R. vannielii (strain RM5 complex cycle, Warwick), a simplified cell cycle derivative (Fig. 1) (Dow & France, 1980) and a spontaneous rifampicin resistant mutant (R4) were grown phototrophically in PM medium (Potts & Dow, 1979). Cultures were incubated, with agitation, under oxygen free N₂ at 30 °C with an incident light intensity of approximately 3 W m⁻² (tungsten light source). Growth (OD₅₇₀) was measured using a Pye Unicam SP500 spectrophotometer.

Swarmer cell isolation and growth. Populations of synchronized swarmer cells were obtained by the method of Whittenbury & Dow (1977). Swarmer cell growth was induced by re-illumination. Cell volume measurements were made using a ZBI Coulter counter and C1000 channelizer linked to a BBC model B computer system.

Measurement of [³⁵S]methionine incorporation. To measure the incorporation of [³⁵S]methionine (>800 Ci mmol⁻¹, >30 TBq mmol⁻¹, Amersham) samples were removed from the cultures and precipitated onto GFC filters (Whatman) by plunging into ice-cold TCA (7.5%, w/v). After washing twice with TCA and twice with ice-cold ethanol the filters were dried and the incorporated radioactivity was measured in Triton/toluene scintillation fluid using a Packard liquid scintillation counter.

Analysis of the effect of rifampicin on the growth of swarmer cells and chain cells (endpoint analysis). Swarmer cell populations of approximately 10⁷ cells ml⁻¹ were obtained by selective synchronization. They were either used immediately or allowed to grow for 17 h to produce populations of chain cells (clusters of four to eight cells, Fig. 1). The cells (10 ml samples) were subsequently incubated in the presence of rifampicin and 10 mM l-methionine (2 µCi, 74 kBq, [³⁵S]methionine ml⁻¹) for 18 h. The influence of rifampicin on growth was determined by phase
contrast microscopy, cell volume distribution analysis and measurement of $[^{35}S]$methionine incorporation (200 µl samples). The influence of rifampicin on the growth of complex and simple cell cycle exponential populations of *R. vannielii* was assessed in cultures grown to the mid (OD$_{540}$ = 0·5) or late (OD$_{540}$ > 1) exponential phase of growth which were then inoculated into medium, containing known concentrations of inhibitor, to give OD$_{540}$ = 0·1. Swarmer cells and budding cells (swarmer cells incubated in the light for 3 h) were incubated in PM, both in the light and in the dark with 40 µg rifampicin ml$^{-1}$ and $[^{35}S]$methionine (60 µCi, 2.22 MBq, in 10 ml). Cultures were sampled (50 µl) at intervals and the amount of radioactivity incorporated was measured.

Susceptibility of swarmer cells and budding cells to sodium deoxycholate. Swarmer cells and budding cells were incubated in PM containing 1 mg sodium deoxycholate ml$^{-1}$. The cells were kept in the dark to inhibit development. Samples were removed at intervals, washed with fresh medium and plated on PM agar to obtain viable counts. The plates were incubated under N$_2$ at 30 °C with illumination for 7 d. Samples were also taken for cell volume measurement.

Influence of rifampicin and chloramphenicol on the pulse labelling of proteins synthesized during swarmer cell differentiation. Inhibitors were added to synchronized swarmer cell cultures (S1) immediately prior to re-illumination. Samples (300 ml) were removed and pulsed with $[^{35}S]$methionine (0·5 µCi, 18·5 KBq, ml$^{-1}$); after 10 min, further labelling was prevented by the addition of 1 mM-L-methionine. After suspension in lysis buffer and sonication, the total soluble protein fractions were isolated (OFarrell, 1975) and stored at -20 °C until required. $[^{35}S]$methionine incorporation was measured as described above. The proteins synthesized during the pulse were analysed by PAGE.

PAGE. One-dimensional PAGE used in this study was based on that of Laemmli (1970); 10-30% (w/v) acrylamide exponential gradient gels were loaded with 50000 c.p.m. per track and run at 10 mA constant current for 24 h. The gels were fluorographed as described by Bonner & Laskey (1974). Two-dimensional PAGE-IEF was as described by O'Farrell (1975). The second-dimension gel was a 10-30% (w/v) acrylamide exponential gradient gel containing 0·1% (w/v) SDS. The two-dimensional (non-denaturing, denaturing) PAGE system was as described by Scott & Dow (1986b). The gels were stained with silver nitrate (Wray et al., 1981). Gels were loaded with 10 µg protein extract.

Rapid partial purification of DNA-dependent RNA polymerase from *R. vannielii*. The method used was based on that of Gross et al. (1976) with the modifications described below. Cells (0·25 g wet wt) suspended in 10 ml 0·01 M-Tris/HCl, 0·1 mM-EDTA, 5% (w/v) glycerol, 0·1 mM-dithiothreitol, pH 7·9, buffer (TGED) + 0·1 mM-NaCl (Burgess & Jendrisak, 1975) were lysed by three passages through a French pressure cell (137 MPa). Cell thymus DNA (10 mg, washed in 2 mM-NaCl, precipitated in ethanol -20 °C) in 2 ml TGED + 0·1 mM-NaCl was added to the suspension, which was incubated at 4 °C for 15 min. DNA was precipitated by the addition of an equal volume of 20% (w/v) PEG 6000/0·5 mM-NaCl solution and incubated at 4 °C for 10 min. This suspension was centrifuged for 10 min at 5000 g. The pellet was suspended in 1 ml buffer E (Gross et al., 1976) and 12·3 ml TGED and passed through a washed (TGED + 1 mM-NaCl) and pre-equilibrated (TGED + 0·15 mM-NaCl) DNA cellulose column (1 x 2 cm). The column was washed with 10 ml TGED + 0·15 mM-NaCl then eluted with 2 ml TGED + 0·75 mM-NaCl. The eluate was diluted with 12 ml TGED and passed through a pre-equilibrated (TGED + 0·11 mM-NaCl) DEAE-Sephadex column (1 x 2 cm). This column was washed with 10 ml TGED + 0·11 mM-NaCl and the proteins were eluted with 1·5 ml TGED + 0·5 mM-NaCl. The RNA polymerase extract was dialysed into TGED storage buffer (Burgess & Jendrisak, 1975) and stored at -20 °C until required. RNA polymerase activity was assayed using the method of Jaehning et al. (1979).

RESULTS

Rifampicin susceptibility

Fig. 2 (a) shows the results of an endpoint analysis of swarmer cell growth. Examination of the cells by microscopy indicated that the response of swarmer cells to rifampicin was similar to that reported by Whittenbury & Dow (1977); the stages of growth reached by the swarmer cells are shown. The incorporation of $[^{35}S]$methionine was also used as an indicator of growth as it allowed comparison to be made between the influence of rifampicin on swarmer cell and chain cell growth, and because direct measurement of RNA synthesis in swarmer cells is difficult (Potts & Dow, 1979). Analysis of $[^{35}S]$methionine incorporation revealed that swarmer cell growth was considerably more resistant to rifampicin than that of chain cells. This observation was confirmed by a second approach which also showed that the effect was not an artefact of the synchronization. Fig. 2 (b) shows the growth kinetics of *R. vannielii*, monitored by OD$_{540}$, as a simplified vegetative cell cycle and as a complex vegetative cycle (RMS5) in media containing various concentrations of rifampicin. The simplified cell cycle (mainly swarmer cells and pairs)
Fig. 2. Influence of rifampicin on the growth of *R. vannielii*. (a) $[^{35}S]M$ethionine incorporation into swarmer cells (■) and chain cells (▲) during an 18 h incubation; the stage of growth reached by the swarmer cell is indicated. (b) The growth of cells of a simple cycle (■) and a complex cell cycle (RMS) (□), monitored by measurement of OD$_{540}$ in PM media containing various concentrations of rifampicin. The numbers in parentheses indicate the concentrations of rifampicin used (µg ml$^{-1}$).

was more resistant to rifampicin than the complex cell cycle (RMS) (mainly chain cells). The age of the inoculum or the cell density, both of which were related to the available light intensity, had no influence on the final result (data not shown).

We were interested to know why swarmer cells reached different stages during maturation in the various rifampicin concentrations. There are a number of possible explanations: growth rate may be inversely proportional to rifampicin concentration; alternatively the time of inhibitor action could either by related to the time required for an inhibitory concentration to enter the cell or be a consequence of a change in cellular properties relating to inhibitor function. To investigate these possibilities the growth of synchronized swarmer cells was monitored by Coulter counter analysis. The growth of synchronized swarmer cells (no added rifampicin) reintroduced into the light was biphasic. During maturation there was a small but measurable increase in median cell volume. Once the cells entered the reproductive phase there was a rapid acceleration of the cell volume increase. Rifampicin concentrations greater than 40 µg ml$^{-1}$ inhibited growth within maturation. The cells did not differentiate and remained motile for up to 9 h. Concentrations between 1 and 40 µg rifampicin ml$^{-1}$ reduced the rate of volume increase once the cells entered the reproductive phase (4 h); in no case was growth completely inhibited.

Analysis of protein synthesis (Fig. 3) revealed further information on the timing of rifampicin action. Pulse labelling carried out through maturation and reproduction for the analysis of protein synthesis by PAGE allowed us to compare the incorporation of $[^{35}S]$methionine in a control swarmer cell culture and one containing 40 µg rifampicin ml$^{-1}$. Both cultures showed an increase in label incorporation initially; however 40 µg rifampicin ml$^{-1}$ reduced the rate of protein synthesis early in the maturation phase (within the first 2 h) (data not shown). This was earlier than expected from the cell volume data discussed above.

Fig. 3 (a) shows a PAGE analysis of the sequential pattern of protein synthesis during maturation and reproduction of synchronized swarmer cells (no added rifampicin). In this experiment the swarmer cells had lost motility and developed stalks by the second hour. In this
Fig. 3. PAGE analysis of proteins synthesized during swarmer cell growth (a) and swarmer cell growth in the presence of 40 µg rifampicin ml⁻¹ (b). The gels used for this study were 10 to 30% (w/v) acrylamide gradient gels. After electrophoresis the gels were fluorographed to show the proteins which were synthesized during the pulse labelling period. A–L, swarmer cell proteins.
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Fig. 4. Influence of rifampicin (40 μg ml⁻¹) on incorporation of [³⁵S]methionine into swarmer cells (a) and budding cells (b). The graph shows the incorporation ratio (cells treated with 40 μg rifampicin ml⁻¹/untreated cells) for swarmer cells incubated in the light (■), swarmer cells incubated in the dark (○), budding cells incubated in the light (▲) and budding cells incubated in the dark (▼).

period, particularly within the first hour, there was an increase in the amount of proteins B, C, D, E and F found on the gel; their apparent rates of synthesis fell as differentiation proceeded. During the 2–6 hour period, stalk synthesis and daughter cell formation, proteins A, G, H and J were found in increased amounts. At 21 h proteins K and L were found in increased amounts.

The presence of 40 μg rifampicin ml⁻¹ in the culture delayed the loss of motility and stalk formation for the majority of the swarmer cell population (Fig. 3b). The general reduction in [³⁵S]methionine incorporation was not reflected by a general delay in the sequence of protein synthesis. While proteins K and L were not made in increased amounts in the 1260 min sample – this was expected as the cells did not reach the chain stage – most of the other proteins were unaffected. However, two of the early proteins were influenced: protein B retained a high relative rate of synthesis and protein E was switched on much later. Also the amount of protein G on the gel was found to have increased. These observations raise a number of important questions that cannot be answered yet. The simplest explanation for our observations is that some of the alterations in protein synthesis relate to an early trigger (not influenced by rifampicin) and one not directly related to morphology or the absolute rate of protein synthesis.

The synthesis of other proteins does relate to morphology and cell type, for instance L is not synthesized in the presence of rifampicin as it is chain cell specific. Within this interpretation there is room to suggest that they may also be subtle changes in the transcription properties of the enzyme RNA polymerase. It is possible that the changes in protein synthesis observed are due to there being different forms of the enzyme with different sensitivities to rifampicin.

As it has been suggested that the energetic state of a bacterial cytoplasmic membrane can influence the uptake of hydrophobic compounds (Hancock, 1984), rifampicin (40 μg ml⁻¹) was tested on light incubated and dark incubated swarmer cells and budding cells. In this experiment effects were measured by analysing uptake of [³⁵S]methionine. No carrier methionine was included, to ensure sufficient incorporation during the initial stages of incubation. This meant that the absolute amount of label incorporated into the different cell types was more susceptible to influence by differences in rate of uptake and methionine pool size within the cells. Allowing for these considerations the ratios of incorporation clearly show that budding cells were far more susceptible to rifampicin than swarmer cells (Fig. 4). It is interesting that dark incubated swarmer cells did not initiate differentiation and budding cells did not continue to grow (increase in volume) yet they incorporated label; further, protein synthesis in the budding cells that had been switched from light to dark growth conditions was more resistant to rifampicin than that in the budding cells maintained in the light. It was not possible to detect any difference between the incorporation into light incubated and dark incubated swarmer cells at the rifampicin concentrations tested. While the data presented above indicate that light may play some small part in the rifampicin phenomenon it is not the most important feature. Rather, the influence of rifampicin on cell volume, protein synthesis and [³⁵S]methionine incorporation points to there being a basic difference between the properties of swarmer cells and budding cells.
Hydrophobic inhibitors and R. vannielii

One explanation for the phenomena discussed above was that DNA-dependent RNA polymerase was modified during maturation. Indeed, while the data of Whittenbury & Dow (1977) were inconclusive, the data presented in Fig. 4 would suggest a major alteration in the properties of the enzyme. The response of RNA polymerase to rifampicin was assayed by the method of Jaehning et al. (1979) with enzyme isolated from different stages of the cell cycle, i.e. swarmer cells, budding cells and chain cells. It was not possible to resolve any change in susceptibility; the enzyme extracts always showed 50% inhibition with 5 ng rifampicin ml\(^{-1}\). These assays were done using various DNA templates (calf thymus; T7D111; R. vannielii) and with either partially purified enzyme (ammonium sulphate stage, Burgess, 1969), enzyme purified by a range of column procedures – heparin (Sternbach et al., 1975), DNA-cellulose, DEAE-Sephadex (Scott & Dow, 1986a,b) – or with enzyme isolated by the rapid purification system described in this paper. Only with rapidly purified enzyme isolated from a rifampicin resistant mutant R4 (resistant to 100 μg rifampicin ml\(^{-1}\) \textit{in vivo}) was a different inhibitory concentration of 5 μg ml\(^{-1}\) (for 50% inhibition) found.

Although these data suggest some other explanation for the \textit{in vivo} changes in rifampicin susceptibility, other than RNA polymerase modification, they do not rule out the possibility that the enzyme assay was not sensitive enough to detect any minor changes in sensitivity produced by differences in subunit composition of RNA polymerase. To explore this possibility a rapid partial purification scheme, based upon that of Gross et al. (1976), was utilized in association with two-dimensional PAGE to identify RNA polymerase subunits in differentiating swarmer cells. The detergent lysis procedure used by Gross et al. (1976) was found to be inefficient with \textit{R. vannielii} swarmer cells. To obtain sufficient lysis with all cell types a French pressure cell was used; this, however, made the addition of carrier DNA to the cell lysate necessary for reproducible PEG 6000 precipitation of RNA polymerase. The addition of DNA did not influence the proteins isolated from the extracts. PAGE analysis of purified enzyme preparations from \textit{R. vannielii} and \textit{Escherichia coli} indicated that they did not contain any proteins of comparable \(M_r\).

Enzyme partially purified by the micro-purification system was analysed by two-dimensional non-denaturing-denaturing PAGE (Scott & Dow, 1986b). Silver nitrate stained second-dimension gels of partially purified enzyme from swarmer cells, budding cells and pairs are shown in Fig. 5. In all of these cases the major enzyme components were as reported previously.
(\(\beta\'), \(M_r\), 155,000; \(\alpha\), \(M_r\), 38,000; and \(\sigma\), \(M_r\), 98,000) for enzyme isolated from swarmer cells (Scott & Dow, 1986a) and from a heterogeneous culture (Scott & Dow, 1986b). With this gel system several other proteins remained associated with the core; four of these proteins are indicated in Fig. 5 (\(M_r\), 88,000, 80,000, 78,000 and 50,000). Proteins of the same \(M_r\) have been detected in RNA polymerase extracts from heterogeneous cultures (simplified cell cycle and complex cell cycle) and simplified cultures prepared by the heparin column method of Sternbach et al. (1975).

Visual observation of one-dimensional and two-dimensional polyacrylamide gels of RNA polymerase extracted by both large scale (Scott & Dow, 1986b) and small scale procedures suggested that the amounts of minor proteins, relative to core subunits, changed during maturation: \(M_r\), 80,000, \(M_r\), 78,000 and \(M_r\), 50,000 increased in amount and \(M_r\), 88,000 decreased.

It has been previously reported that \(\alpha\) and \(\sigma^{98,000}\) have multiple forms with different isoelectric pH values (Scott & Dow, 1986b). These observations were tested with RNA polymerase isolated from different stages of the cell cycle using the two-dimensional IEF procedure of O'Farrell (1975). In this case the second-dimension gels were silver stained (Wray et al., 1981). No differences were detectable between swarmer cells and budding cells; both had multiple forms of \(\sigma^{98,000}\) and \(\alpha\), qualitatively and quantitatively similar to those reported for enzyme isolated from heterogeneous cultures (data not shown).

Susceptibility of swarmer cells to sodium deoxycholate and chloramphenicol

The inability to show a link between RNA polymerase modification and the changes in the rifampicin susceptibility led us to test whether an alteration in envelope structure during maturation may have brought about a change in the permeability properties of the cell, with respect to hydrophobic compounds (Nikaido, 1976; Nikaido & Vaara, 1985). It was not possible to measure rifampicin uptake directly, but to test whether we were dealing with a general permeability phenomenon two other hydrophobic inhibitors, sodium deoxycholate and chloramphenicol, were used to probe swarmer cell growth. These were chosen because it was possible to assay their effects. Empirical observations of the efficiency of detergent lysis procedures (Stonington & Pettijohn, 1971; Gross et al., 1976) indicated that swarmer cells were more resistant to lysis than reproducing cells. The viabilities of the two cell types (swarmer cell, budding cell) in 1 mg sodium deoxycholate ml\(^{-1}\) were ascertained (Fig. 6). The viability of the motile swarmer cells was unaffected by incubation in the dark or by the detergent, and no cell lysis was detected. Although the viability of the budding cells reintroduced to dark conditions fell to 70\% of the initial value, no cell lysis was observed in the control. The budding cells were very sensitive to the detergent, viability falling to 20\% of the original value within 4 h. Cell lysis was detected by Coulter counter analysis and phase contrast microscopy.

Analysis of swarmer cell susceptibility to chloramphenicol indicated that the time of inhibitor action related directly to concentration. There was no sudden change in the susceptibility of the
cells as they entered the reproductive phase. PAGE analysis of protein synthesis supported these observations. There was no evidence for any differential effects of chloramphenicol on protein synthesis; however progress through the specific changes in protein synthesis associated with maturation and reproduction was delayed in accord with the reduction in label incorporation (data not shown).

**DISCUSSION**

Probing the *R. vannielii* cell cycle with growth inhibitors clearly indicated that cells from different stages reacted in fundamentally different ways; protein synthesis in swarmer cells was more resistant to rifampicin than that in cells from later in differentiation or in chain cells. These differences, we believe, are due to changes in the amount of transcription, but it is possible that the net amounts of protein synthesis are being influenced by the activity of proteases (Russell & Mann, 1984; Turner et al., 1985) or the presence of long lived mRNA or RNA species synthesized in the mother cell prior to separation, as is the case for flagellin mRNA in *Caulobacter crescentus* (Milhausen & Agabian, 1983). Preliminary observations of *R. vannielii* suggest that pre-division synthesized RNA is not involved in flagellin synthesis in *R. vannielii*. Light influenced, to a small extent, the response of cells to rifampicin. One explanation for this was that the absence of light triggered the translation of pre-existing long lived mRNA that coded for components of the photosynthetic apparatus. Such mRNA has been shown to exist in *Rhodopseudomonas capsulata* (Dierstein, 1984).

It has not been possible to relate the *in vivo* changes of rifampicin susceptibility with changes in the RNA polymerase holoenzyme. PAGE analysis has pointed to a number of minor proteins that remain physically associated with the core. Further analysis is required to establish the physiological basis of these associations and to discover whether the amounts of enzyme subunits recovered reflect true *in vivo* amounts. Nevertheless, this is an interesting observation as it may indicate the existence of a number of different forms of the enzyme as is the case in *Bacillus subtilis*. In this organism five holoenzyme complexes have been detected: one major form and four minor forms that may have some role in sporulation (Losick & Pero, 1981; Doi, 1982; Price & Doi, 1985; Doi & Wang, 1986). Recent analysis of *E. coli* has also indicated the presence of two additional sigma factors (Neidhardt et al., 1984; Grossman et al., 1984; Hirschman et al., 1985) and a number of core associated proteins that influence enzyme function (Ishihama et al., 1983).

The similarity in response of swarmer cells and budding cells to rifampicin and sodium deoxycholate suggest the possibility of a single, simple explanation in that there may be a change in membrane permeability properties during differentiation. Other observations support this notion, for instance *in vivo* labelling of RNA with radioactive precursors has proved more difficult in swarmer cells than in budding cells (Potts & Dow, 1979; Dow et al., 1983), although it has been possible to show RNA synthesis in both, once the cell membranes have been permeabilized by toluene treatment (Scott & Dow, 1986a). Also a study with DNA inhibitors (Whittenbury & Dow, 1977) and one using [3H]adenosine incorporation as an indicator of DNA synthesis (Potts & Dow, 1979) provide conflicting evidence as to when DNA synthesis is actually initiated. This anomaly could be explained if there was a change in the permeability of the swarmer cells during maturation to either the inhibitor or to the labelled precursor.

In Gram-negative bacteria the outer membrane acts as a permeability barrier to antibiotics and inhibitory compounds (Hancock, 1984; Nikaido & Vaara, 1985). Hydrophobic compounds cross this membrane though the lipid bilayer and not through pores, the rate of entry being restricted by the lipopolysaccharide (LPS) and protein content of the outer membrane bilayer (Nikaido & Vaara, 1985). Alterations in the protein content of this membrane have been shown to change the susceptibility of *E. coli* to rifampicin (Grundstrom et al., 1980) and to deoxycholate (Scott, 1982). It is not known how hydrophobic compounds cross the *R. vannielii* outer membrane or whether there are changes in the outer membrane proteins or LPS content during maturation. Such changes are likely, however, as the cell loses its flagella at this time and there is localized disruption of the cell envelope at the growing point during prosthecum and bud
formation. It is not yet known what causes the major differences in susceptibilities shown by swarmer cells and chain cells. Other factors that may influence permeability include the presence of aminoacyl hopanoids in the *R. vannielii* membrane (Neunlist *et al.*, 1985) and the presence of the prosthecum, which is important for the oligotrophic way of life, as the increased surface area to volume ratio it provides allows for an increase in the rate of nutrient uptake (Scott, 1982; Tam & Pate, 1985).

We must be cautious not to imply that any one single event or change in structure was responsible for all the phenomena observed; indeed chloramphenicol susceptibility did not appear to be influenced by differentiation, possibly because chloramphenicol may enter the cell through hydrophilic pores as it does in other organisms (Nikaido, 1976). Rather, there may be a number of subtle changes in the cell envelope (structural or functional) that relate to the change in cell function. The interface provided by the outer membrane of a growing reproductive cell may be different to that of a non-growing swarmer cell as a consequence of the external environment changing from one that is hostile to cell growth to one that would support growth. Such a change in envelope structure may be required to enable growing cells to take up enough nutrients to support growth whereas swarmer cells need only take enough, if any, to support basic survival functions.

**REFERENCES**


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