CONTROL OF ENZYME SYNTHESIS DURING ADAPTATION IN SYNCHRONOUSLY DIVIDING POPULATIONS OF RHODOPSEUDOMONAS SPHEROIDES*

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The formation of the photosynthetic apparatus of R. spheroides can be considered as a primitive instance of bacterial morphogenesis (Cost & Gray, 1967). This process can be controlled by variations in light intensity or oxygen tension and is accompanied by alterations in the synthesis of the photopigment bacteriochlorophyll (Cohen-Bazire et al., 1957). The present study is an attempt to analyze this morphogenetic process by examining the regulation of synthesis of certain enzymes involved in bacteriochlorophyll production in synchronously dividing populations.

Two proposals have been advanced to explain the control of enzyme synthesis in synchronous populations of micro-organisms. Halvorson et al. (1964) have proposed that discontinuous enzyme synthesis is a result of a sequential process of transcription and further, have shown that addition of inducer at any time during the division cycle of yeast does not change the time of induced enzyme synthesis. Kuempel et al. (1965), Pardee (1966), and Goodwin (1966) have suggested that discontinuous enzyme synthesis occurs as a result of oscillations in feedback repression circuits. According to this view enzyme synthesis may occur at any time during the division cycle, and Masters and Donachie (1966) have shown that in B. subtilis the timing of enzyme synthesis can be specifically altered by the addition and removal of repressor.

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The results of the present experiments are consistent with the view that discontinuous enzyme synthesis is, at least in part, due to variations in the extent of repression. In synchronized cultures of R. spheroides, repression of enzyme synthesis, followed by removal of conditions responsible for repression resulted in an immediate derepressed synthesis of enzymes. This derepressed synthesis of enzymes could occur at any time and was not restricted to a particular period of the division cycle.

R. spheroides (strain 2.4.1) was grown anaerobically in the light as previously described (Gray, 1967) and the cultures were synchronized by the stationary phase method as described by Cutler and Evans (1966). Experiments were begun by dividing a large culture into two identical portions; one to serve as a control and the other to be subjected to alterations in environmental conditions. At the time of synchronization, gassing was begun with 95% N_2 - 5% CO_2. Samples were withdrawn from the main culture bottle at half hour intervals for both cell counts and specific analyses.

The bacteriochlorophyll concentrations in whole cells were determined by the method of Cohen-Bazire et al. (1957).

Assays of α-aminolevulinicacid (ALA) synthetase and ALA dehydrase activities were performed according to the procedures described by Burnham and Lascelles (1963) employing the color reaction of Mauzerall and Granick (1956). Samples of cell free extracts, obtained by ultrasonic disruption, equivalent to 1-2 x 10^9 bacteria were employed in each of the enzyme assays.

RESULTS

In cultures of R. spheroides the synthesis of ALA dehydrase and ALA synthetase occurs in a discontinuous manner (Ferretti and Gray, in preparation) at regular intervals in the division cycle. Studies by Lascelles (1959, 1960) have shown that the synthesis of these two enzymes is particularly responsive to alterations in environmental conditions. In cultures grown anaerobically at low light intensities which are abruptly exposed to either aerobic conditions or high light intensities, a repression

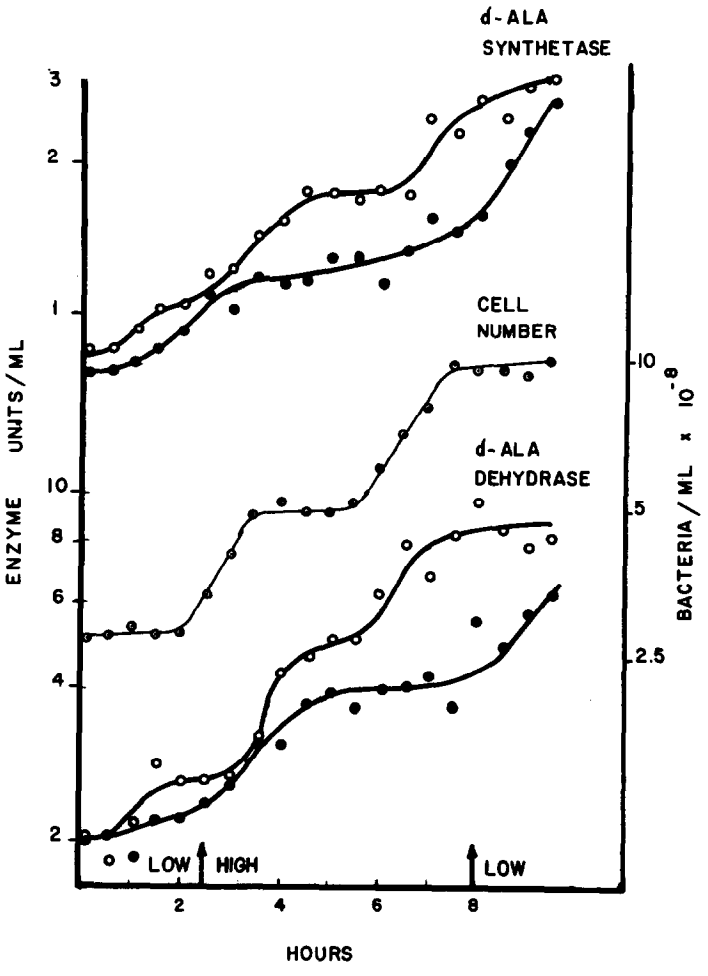

Figure 1. Effects of shifts in light intensity on enzyme synthesis. A culture grown at low light intensity (75 foot-candles) was divided at the time of synchronization into two portions. At the times indicated by the arrows, one of these (closed circles) was subjected to an increase in light intensity (1300 foot-candles) followed by a return to the low light intensity. The other culture (open circles) was maintained at the low light intensity. Samples were withdrawn at intervals and enzyme activity measured. Cell numbers were determined using a Petroff-Hausser counting chamber and were virtually identical in both cultures.

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of the synthesis of both enzymes is observed along with a concomitant inhibition of the synthesis of bacteriochlorophyll. Upon the return to the original pre-shift conditions, i.e., anaerobic conditions at a low light intensity, the synthesis of these two enzymes is immediately resumed and shortly thereafter the synthesis of bacteriochlorophyll begins again. If

similar results were to be obtained with synchronized cultures, it would be expected that following the repression of ALA synthetase and ALA dehydrase the synthesis of these two enzymes would be resumed immediately after the removal of repression, i.e., bursts of enzyme synthesis could take place at any time in the division cycle. On the other hand, if the genes specific for the synthesis of these enzymes were available for transcription only during a specific time in each division cycle, then enzyme synthesis would not resume again until the same time in a subsequent division cycle.

Two experiments involving alternating periods of repression and derepression were performed. The first experiment involved shifting the source of illumination incident on a synchronized culture from a low light intensity (75 foot-candles) to a high light intensity (1300 foot-candles) and is shown in Figure 1, along with a control culture which was maintained at a constant low light intensity. In the control culture ALA synthetase and ALA dehydrase activities increased discontinuously at regular intervals, while in the other culture enzyme synthesis stopped following exposure to an increased light intensity. The cessation of ALA synthetase synthesis was very rapid while ALA dehydrase activity continued to increase for a time before leveling off. Upon the return to a low light intensity of 75 foot-candles the synthesis of both enzymes resumed, however, at a different time in the division cycle than the time of corresponding enzyme increase in the control culture.

The synthesis of bacteriochlorophyll in this experiment is shown in Figure 2. Following the increase in light intensity bacteriochlorophyll synthesis is inhibited and does not resume until shortly after the return to the original light intensity. This resumption of synthesis always occurs after the increase in enzyme formation. Bacteriochlorophyll is synthesized continuously in the control culture.

The second experiment involves the introduction of air into a culture growing under anaerobic conditions. Assays of ALA synthetase activity and

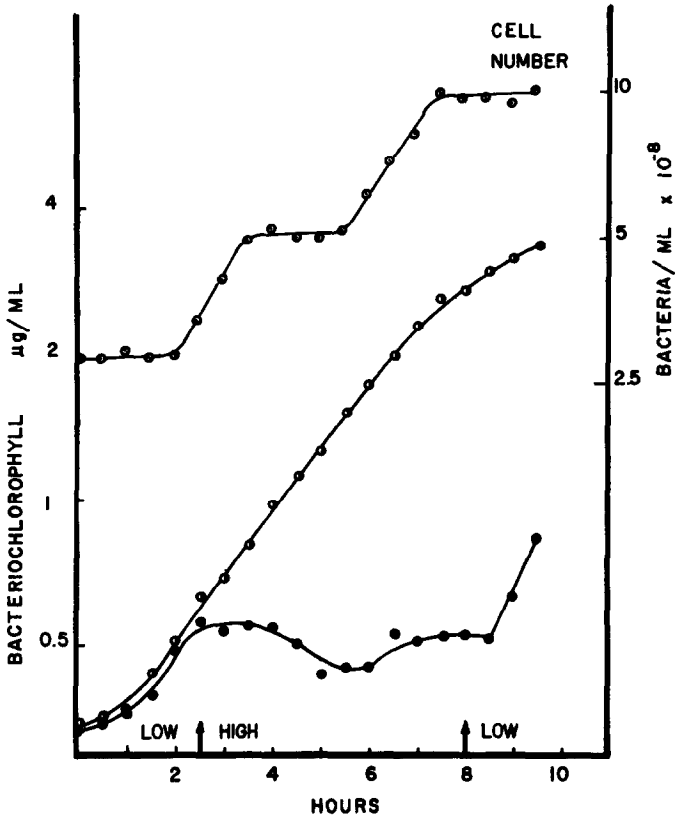

Figure 2. The effects of shifts in light intensity on bacteriochlorophyll synthesis. As in Figure 1 but pigments were extracted and measured. Open circles indicate culture at constant light intensity, closed circles refer to culture subjected to light shifts.

bacteriochlorophyll levels are shown in Figure 3. It is evident that air causes a cessation of the synthesis of bacteriochlorophyll and ALA synthetase. The synthesis of ALA synthetase is repressed for an entire division cycle, and subsequent derepression by the restoration of anaerobic conditions results in an immediate synthesis of the enzyme but at a later time in the division cycle than the normal burst of synthesis in the control culture. The level of enzyme activity following derepression is comparable to that of the control and remains constant until the usual time of enzyme formation.

These experiments suggest that one of the primary factors responsible for discontinuous enzyme synthesis in synchronous cultures is the periodic

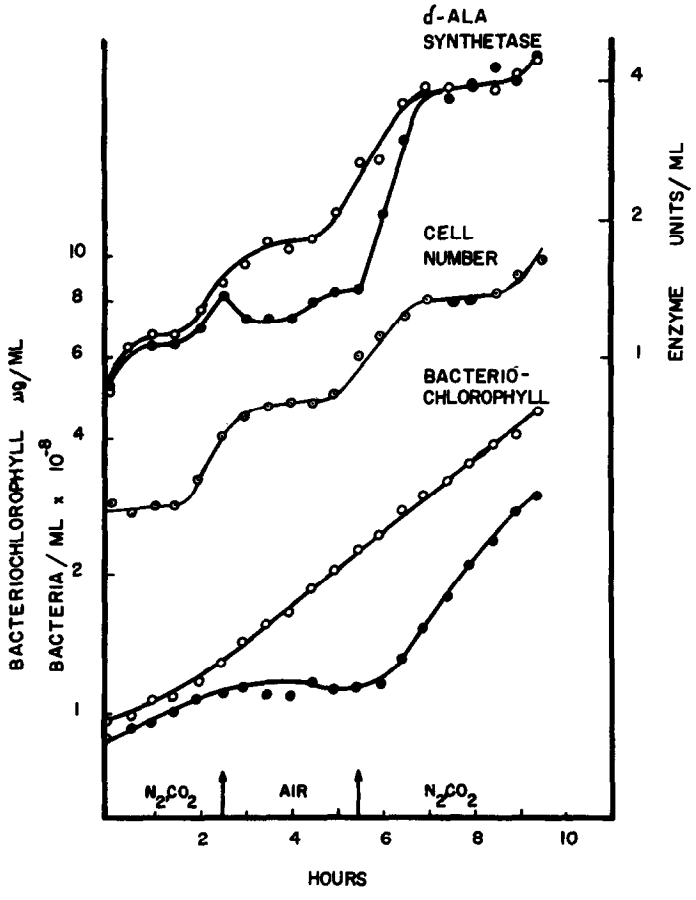

Figure 3. The effect of air on synthesis of ALA synthetase and bacteriochlorophyll. At the times indicated by the arrows, one portion of a culture divided in half at the time of synchronization was gassed with air and then returned to 95% N_2 - 5% CO_2 gassing (closed circles). The other half (open circles) was gassed continuously with N_2 - CO_2. Light intensity was maintained at a constant level. The cell numbers were the same in both cultures.

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fluctuations in repression which presumably oscillate in opposition to enzyme synthesis. It has been proposed that repression may become periodic as a result of the sequential production of end products which are generated initially by the sequential transcription of the genome. Goodwin (1966) has proposed that discontinuous enzyme synthesis may be entrained to the period of gene replication by the synthesis of a small amount of m-RNA, and the genome to be available for transcription at any time. Cutler and Evans (1967)

have found this latter to be the case in E. coli and indirect evidence from several studies on R. spheroides also support this hypothesis. Cost and Gray (1967) have demonstrated that the synthesis of rapidly labeled RNA is increased when asynchronous cultures are subjected to periods of altered environmental conditions. Further, Gray (1967) has shown that the amount of rapidly labeled RNA which hybridizes with DNA is increased following exposure of asynchronous cultures to altered environmental conditions. These experiments, along with the fact that there is little difference between the rates of derepressed synthesis of ALA synthetase or ALA dehydrase as measured in the present studies with synchronized cultures, and in asynchronous cultures (Lascelles, 1960) may indicate that the RNA formed during adaptation is a result of differential gene transcription. Results such as these are consistent with the hypothesis of Jacob and Monod (1961) that induction or derepression stimulate the formation of specific messenger RNA.

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