RNA Metabolism of *Rhodopseudomonas spheroides* during Preferential Photopigment Synthesis

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SUMMARY

During anaerobic growth of *Rhodopseudomonas spheroides* the specific RNA content ($\mu\text{g. RNA}/\mu\text{g. protein}$) of cultures was directly proportional to the light intensity in which they were grown. Cultures subjected to a step-up in light intensity preferentially synthesized RNA until their specific RNA content increased to the value characteristic of growth at the higher light intensity. Conversely following a step-down in light intensity the rate of RNA synthesis fell below the rate of protein synthesis, and cellular RNA was diluted out to the value characteristic of growth at the lower light intensity. Adjustment of the differential rate of RNA synthesis in response to a change in light intensity was thus opposite to that of the differential rate of photopigment synthesis. Although a decrease in light intensity drastically decreased RNA synthesis some RNA continued to be formed. The RNA synthesized, like that in cultures maintained at a constant light intensity, consisted of soluble and ribosomal RNA and unstable RNA with sedimentation behaviour like m-RNA. Aerobic cultures of *R. spheroides* subjected to a marked decrease in O_2 tension synthesized photopigments *de novo* without appreciably increasing their net content of RNA, but continued to synthesize small amounts of all the usual classes of RNA.

INTRODUCTION

Rhodopseudomonas spheroides when grown aerobically is devoid of bacteriochlorophyll and contains only traces of carotenoids, but it synthesizes large amounts of these photopigments when grown anaerobically in the light or when incubated semi-aerobically in the dark (Cohen-Bazire, Sistrom & Stanier, 1957). The extent of pigment formation is inversely proportional to light intensity or to the O_2 tension under semi-aerobic conditions. Formation of bacteriochlorophyll is strictly dependent upon protein synthesis (Sistrom, 1963; Bull & Lascelles, 1963). This is in part explained by the fact that certain enzymic activities associated with bacteriochlorophyll formation increase in parallel with the ability to form photopigments (Lascelles, 1959; Gibson, Neuberger & Tait, 1963). However, the obligatory requirement for protein synthesis in already pigmented cultures, which presumably contain all the enzymes necessary for bacteriochlorophyll synthesis, suggests that bacteriochlorophyll formation must proceed with joint synthesis of some protein component of the photosynthetic apparatus. Evidence that proliferation of intracellular membranes is concomitant with development of photopigments in the *Athiorhodoaceae*

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supports this view (Cohen-Bazire & Kunisawa, 1963). The integral relationship between photopigment synthesis and protein synthesis suggests that control of bacteriochlorophyll formation may be at the genetic level. The role of RNA in mediating synthesis of specific proteins is well known. To gain information about the relationship of protein synthesis to bacteriochlorophyll formation I examined the RNA metabolism of *R. spheroides* under conditions where the organisms were increasing their specific photopigment content.

METHODS

Organisms. The strains of *Rhodopseudomonas spheroides* used were strain Ga, and strain M-25 which requires uracil for growth. These bacteria and the methods used to grow them were described previously (Lessie, 1965).

Semi-aerobic incubation of bacteria. Cultures were grown aerobically to a population equivalent to about 100 μ g. protein/ml. The organisms were centrifuged and suspended to ten times their original concentration in fresh medium, and 60 ml. volumes of the suspension were shaken at 34° in L-tubes (see Lascelles, 1959).

Determination of cellular nucleic acids, protein, and bacteriochlorophyll. RNA and DNA were extracted from organisms with hot trichloroacetic acid (TCA). RNA was estimated colorimetrically by reaction with orcinol (Schneider, 1945). DNA was estimated by reaction with indole (Ceriotti, 1952). TCA-insoluble protein was digested in NaOH and estimated by the Folin phenol method (Lowry *et al.* 1953). The details of these procedures were given elsewhere (Lessie, 1965). Bacteriochlorophyll was extracted from bacteria with methanol, and estimated by measuring the extinction of the methanolic extracts at 775 m μ (see Cohen-Bazire *et al.* 1957).

Incorporation of ¹⁴C-uracil into bacteria. Uptake of ¹⁴C-uracil into cellular RNA was measured as follows. Culture samples were diluted 1/2 to 1/10 in cold trichloroacetic acid (TCA) to give a concentration of between 20 and 100 μ g. protein/ml. in 5% TCA. Part of the TCA-treated sample (containing about 100 μ g. protein) was filtered through Millipore membranes (0.65 μ pore size) and the organisms washed with cold 5% TCA. The membranes were dried, cemented to planchettes, and their radioactivities determined by using a thin window Geiger tube. At the concentration of bacteria used it was unnecessary to correct for self-absorption. In a few experiments ¹⁴C-labelled RNA was extracted from the bacteria with hot TCA. The results were identical to those obtained using the Millipore technique.

Preparation and characterization of ribosomes and RNA. Bacteria were disrupted in a French press to obtain crude extracts containing ribosomes and soluble RNA. Ribosomal RNA was obtained by deproteinizing the crude extracts with sodium lauryl sulphate and phenol. The ribosomes and RNA were characterized by sucrose gradient centrifugation using an SW-39 rotor in a Spinco Model L ultracentrifuge. The detailed procedures followed to obtain and characterize *Rhodopseudomonas spheroides* ribosomes and RNA have already been reported (Lessie, 1965).

2-¹⁴C-uracil was obtained from the Radiochemical Centre Amersham, Buckinghamshire. Millipore filters were obtained from the Millipore Filter Co., Bedford, Mass., U.S.A.

RESULTS

Effect of light on the differential rate of RNA synthesis. Cultures of *Rhodospseudomonas spheroides* subjected to an increase in light intensity temporarily stopped forming photopigments and synthesized RNA preferentially. After the specific RNA content ($\mu\text{g. RNA}/\mu\text{g. protein}$) increased to the value value characteristic of growth at the higher light intensity increase of RNA proceeded in step with increase of protein. For example a culture of *R. spheroides* strain ga subjected to a step-up

Fig. 1. Increase of specific RNA content following an increase in light intensity. *Rhodospseudomonas spheroides* ga was grown in 400 ml. SG medium at a light intensity of 300 ft.c. The culture was bubbled with a mixture of 5% (v/v) CO_2 and 95% (v/v) N_2 . When the culture reached a concentration equivalent to about 50 $\mu\text{g. protein/ml.}$ it was divided into two portions. One portion was incubated as before in light at 300 ft.c.; the other portion was incubated in light at 1000 ft.c. At the indicated times duplicate samples were taken for determination of bacteriochlorophyll (+—+), protein (○—○), RNA (●—●), DNA (x—x).

in light intensity from 300 to 1000 foot-candles (ft.c.) synthesized RNA preferentially until its specific RNA content was increased about twofold (from 0.13 to 0.25, see Fig. 1). The amount of DNA/protein remained constant with a specific DNA content of about 0.1.

Conversely, a decrease in light intensity caused the rate of RNA synthesis to decrease below the rate of protein synthesis; as a consequence the cellular RNA was diluted. After the specific RNA content decreased to the value characteristic of growth at the lower light intensity, balanced synthesis of RNA was resumed. Accordingly, a culture subjected to a step-down in light intensity formed bacteriochlorophyll without significantly increasing its net content of RNA. For example,

following a decrease in light intensity from 1000 to 200 ft.c. the specific bacteriochlorophyll content of a culture of *Rhodospseudomonas spheroides* strain ga more than doubled while net increase of RNA was arrested (see Fig. 2). The specific DNA content increased slightly following the step-down in light intensity and then readjusted to its initial value.

Fig. 2. Decrease of specific RNA content following a decrease in light intensity. *Rhodospseudomonas spheroides* was grown in 400 ml. SG medium in light at an intensity of 1000 ft.c. The culture was bubbled with a mixture of 5 % (v/v) CO₂ and 95 % (v/v) N₂. When the culture reached a concentration equivalent to about 50 μg. protein/ml. it was divided into two portions. One portion was maintained in 1000 ft.c.; the other was placed in light at 200 ft.c. Duplicate samples were taken at specified times for determination of bacteriochlorophyll (+—+), protein (○—○), RNA (●—●), DNA (x—x).

RNA synthesis following a decrease in light intensity. RNA synthesis was not abolished by a decrease in light intensity. The bacteria incorporated radioactive uracil into their RNA at the same time as they preferentially synthesized bacteriochlorophyll. Figure 3 shows the kinetics of entry of ¹⁴C-uracil into the cold-TCA insoluble fraction of a culture of *Rhodospseudomonas spheroides* strain m-25 following a step-down in light intensity from 1200 to 120 ft.c. Incorporation which occurred after the initial rapid uptake amounted to about 10 % of that in cultures maintained at 1200 ft.c. Following a decrease in light intensity from 1200 to 400 ft.c. the rate of uracil incorporation was about 20 % the value at 1200 ft.c.

In order to ascertain that under the above conditions uracil was incorporated into RNA and to determine which classes of RNA were being synthesized the following experiments were performed. Cultures of *Rhodospseudomonas spheroides* m-25 were subjected to a step-down in light intensity from 1200 to 120 ft.c., and then given a

2 min. pulse of ^{14}C -uracil which was 'chased' for different times with excess ^{12}C -uracil. Phenol purified RNA was prepared from the bacteria, and characterized by sucrose gradient centrifugation. Figure 4 shows the sucrose gradient profiles of RNA from bacteria which were given a 2 min. pulse of ^{14}C -uracil, and then incubated for 30 sec. or 2, 10, or 20 min. with an excess of unlabelled uracil. The sedimentation patterns were similar when the bacteria were subjected to a step-down in light

Fig. 3. Uracil incorporation into RNA during preferential bacteriochlorophyll formation under anaerobic conditions. *Rhodospseudomonas spheroides* M-25 was grown in 1200 ft.c. in 200 ml. SG medium containing 5 μg . uracil/ml. The culture was bubbled with a mixture of 5 % (v/v) CO_2 and 95 % (v/v) N_2 . When the cells reached a concentration equivalent to about 100 μg . protein/ml. they were centrifuged and suspended in 200 ml. SG medium containing 5 μg . uracil/ml. and a total of 20 microcuries 2- ^{14}C -uracil (3730 counts/100 sec./ μg . uracil). The suspension was incubated in light at an intensity of 120 ft.c., and gassed as before. At the indicated times samples were removed for determination of bacteriochlorophyll (+—+), RNA (●—●), protein (○—○), radioactive uracil in cold-TCA washed cells from 1 ml. culture (×—×).

intensity from 1200 to 400 ft.c. or maintained at 1200 ft.c. It should be noted that *R. spheroides* is devoid of the usual 23S ribosomal RNA (Lessie, 1965). The first RNA to be labelled was unstable and sedimented differently from the bulk ribosomal and soluble RNAs. The sedimentation behaviour of this heterogeneous fraction of RNA was similar to that of m-RNA. Within 10 min. most of the label passed into the stable ribosomal and soluble RNA fractions.

As expected from the kinetics of labelling of phenol-purified RNA, label was found to enter rapidly into the ribonucleoprotein particles of bacteria subjected to a

decrease in light intensity. Figure 5 shows the kinetics of appearance of ^{14} -uracil in the 30S and 50S ribosomes of bacteria which were subjected to a step-down in light intensity from 1200 to 120 ft. c. Similar incorporation of label into ribosomes were obtained with bacteria transferred from 1200 to 400 ft.c. or bacteria maintained at 1200 ft.c. In all three instances the 30S ribosomes were labelled more rapidly than were the 50S ribosomes.

RNA synthesis in semi-aerobic cultures. Suspensions of aerobically grown *Rhodospseudomonas spheroides* subjected to a marked decrease in O_2 tension formed photopigments without significantly increasing their net content of RNA. The bacteria did, however, continue to incorporate ^{14}C -labelled uracil into cellular RNA (see

Fig. 4. RNA synthesized following a decrease in light intensity. *Rhodospseudomonas spheroides* was grown in 1200 ft.c. and transferred to 120 ft.c. as described in Fig. 3, except that transfer was to medium containing one $\mu g.$ uracil/ml. and no ^{14}C -uracil. After 3 min. 20 $\mu c.$ 2- ^{14}C -uracil were added in one ml. (zero time). Two min. later 3.6 ml. 10^{-2} M- ^{14}C -uracil were added (final uracil conc. 20 $\mu g./ml.$). At 30 sec. and at 20 min. 100 ml. volumes of culture were poured onto 50 g. crushed ice containing 0.75 ml. 2×10^{-1} M- NaN_3 . The bacteria were centrifuged, washed twice with cold SG medium containing 10^{-3} M- NaN_3 , and then frozen. An identical culture was given a two min. pulse of ^{14}C -uracil, but the bacteria were sampled, washed, and frozen after 2 min. and after 10 min. chase periods in the presence of excess ^{14}C -uracil. Crude ribosome extracts and phenol-purified RNA in 5×10^{-3} M-tris buffer (pH 7.4) and 10^{-4} M- $MgCl_2$ were prepared from thawed pulse-labelled bacteria combined with unlabelled bacteria from a 300 ml. culture (100 $\mu g.$ protein/ml.) grown in 1200 ft.c. 0.2 ml. portions of the phenol-purified RNA preparations were centrifuged at 38,000 rev./min. for 5 hr. through 5 ml. 5–15% sucrose gradients containing 5×10^{-3} M-tris buffer (pH 7.4) and 10^{-4} M- $MgCl_2$. The bottoms of the tubes were punctured, and 5 drop fractions were collected. Extinction at 260 mμ (●—●) and radioactivities (×---×) of each sample were determined.

Fig. 6). Pulse experiments with ^{14}C -uracil indicated that, during preferential photopigment synthesis and during the period of adaptation to form photopigments, label was incorporated into ribosomal and soluble RNAs after flowing through unstable RNA like that in Fig. 4.

Fig. 5. Synthesis of ribosomes following a decrease in light intensity. 0.2 ml. samples of the crude ribosome extracts obtained from the samples described in Fig. 4 were centrifuged at 38,000 rev./min. for 2.5 hr through 5–10 % sucrose gradients containing 5×10^{-3} M-tris buffer (pH 7.4) and 10^{-4} M-MgCl₂. Samples were collected and their radio-activities (x---x) and 260 mμ extinctions (O—O) were determined.

Uracil requirement for bacteriochlorophyll synthesis. Aerobically grown *Rhodospseudomonas spheroides* incubated semi-aerobically without uracil formed no photopigments, nor did anaerobically grown bacteria deprived of uracil and subjected to a stepdown in light intensity.

DISCUSSION

The present results indicate that while preferentially synthesizing photopigment *Rhodospseudomonas spheroides* synthesizes all the usual classes of RNA, although more slowly than during balanced growth. There appears to be no striking change in nucleic acid metabolism (such as a greatly increased output of m-RNA) signalling a preferential rise in bacteriochlorophyll formation. However, the observation that *R. spheroides* strain m-25 did not form bacteriochlorophyll when its RNA synthesis was restricted by deprivation of uracil suggests that RNA synthesis is necessary for bacteriochlorophyll formation. These data are consistent with a role of RNA

synthesis in bacteriochlorophyll formation as already implied by the results of Sistrom (1963) and Bull & Lascelles (1963) which established that bacteriochlorophyll formation is coupled to protein synthesis. It is possible that the primary control of photopigment synthesis operates at the genetic level by regulating synthesis of specific informational RNA required to promote synthesis of some protein integrally involved in bacteriochlorophyll synthesis. Our results indicate that if such a control exists detection of the informational RNA involved would depend upon more sensitive technique than sucrose gradient resolution of the entire bacterial complement of RNA.

It has been shown that *Rhodopseudomonas spheroides* is devoid of the usual 23S ribosomal RNA (Lessie, 1965). The experiments reported here show that *R. spheroides* contains unstable RNA which sediments more rapidly than does 16S

Fig. 6. Uracil incorporation into RNA during semi-aerobic incubation. *Rhodopseudomonas spheroides* was grown aerobically to a concentration equivalent to about 100 $\mu\text{g.}$ protein/ml., centrifuged, and suspended to about 1 mg. protein/ml. in SG medium containing 20 $\mu\text{g.}$ uracil/ml. Three 65 ml. volumes of the suspension were distributed into separate 1-l. tubes. 20 microcuries $2\text{-}^{14}\text{C}$ -uracil in one ml. were added to one of the tubes (2210 counts/100 sec./ $\mu\text{g.}$ uracil). All three tubes were shaken gently at 34° C. At 15 min. intervals 3 ml. samples for bacteriochlorophyll determination were taken alternatively from the two tubes without ^{14}C -uracil. At the indicated times 0.5 ml. samples of the culture with ^{14}C -uracil were pipetted into 4.5 ml. cold 5% TCA. 4 ml. of the TCA-treated sample was assayed for RNA and for protein; the remainder was used to determine radioactivity in the cold-TCA insoluble cell fraction. ●—●, RNA; ○—○, protein; +—+, bacteriochlorophyll; ×—×, radioactive uracil in cold TCA-washed bacteria from one ml. culture.

ribosomal RNA. Two observations make it unlikely that this RNA represents a small pool of 23S ribosomal RNA. First, examination of the kinetics of incorporation of labelled uracil into RNA indicated that the RNA under discussion was labelled more rapidly than was 16S RNA. Secondly, under the same conditions 30S ribosomes were labelled more rapidly than 50S ribosomes. Since 16S RNA is presumed to comprise the RNA of 30S ribosomes, it is unlikely that 16S RNA would be labelled more slowly than RNA of 50S ribosomes. I presume that the RNA in question was not derived from 50S ribosomes.

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