Ribosome Synthesis in *Rhodopseudomonas capsulata*

Cells Growing In Continuous and Intermittent Light

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The ribosome content was markedly reduced when illumination (at 28 C) was repeatedly interrupted; an opposite trend was observed with continuous light as the temperature was decreased.

Intermittent illumination, at certain frequencies, of growing cultures of the photosynthetic bacterium *Rhodopseudomonas capsulata* causes a marked inhibition of growth rate and a substantial reduction in the total ribonucleic acid (RNA) content per unit dry weight (3, 4). In this communication, we report that intermittency [i.e., repeated interruption of in vivo adenosine triphosphate (ATP) regeneration] leads to a concomitant decrease in ribosome content. Data showing an increased ribosome level in cells growing in continuous light at suboptimal temperature are also presented.

*R. capsulata* (strain "St. Louis"; American Type Culture Collection no. 23782) was grown anaerobically in a synthetic medium containing 0.4% DL-malate and 0.1% ammonium sulfate (3), either in continuous light or with intermittent illumination, i.e., repeating light-dark cycles of 1-min duration, with light and dark periods of equal length (30 sec). The cultures, in Roux bottles, were incubated in a glass water bath, maintained at the temperatures indicated, and illuminated by an external bank of 60-w Lumiline lamps so that the incident light intensity was approximately 800 foot candles (ft-c) as measured with a Weston illumination meter model 756. This intensity is saturating for growth in continuous light (4). For experiments with intermittent illumination, the light source was alternately turned on and off at 30-sec intervals by a recycling cam timer (Industrial Timer Corp. model CM-2).

Cells were harvested during the logarithmic growth phase, after at least four mass doublings under each experimental condition. They were washed once with 0.01 M tris(hydroxymethyl)-aminomethane hydrochloride plus 10^{-4} M magnesium acetate buffer, pH 7.2, and were resuspended in buffer containing deoxyribonuclease (usually 4 μg/ml). Each suspension was disrupted by passage through a French pressure cell three times, and 0.4 ml of the resulting extract [equivalent to 214 μg (dry weight) of cells] was applied to 4.6 ml of a 4 to 20% sucrose gradient in the buffer noted. The gradients were centrifuged at 40,000 rev/min for 2 hr in a Spinco SW50L rotor and were analyzed with an Isco model D density gradient fractionator (with attached Texas Instrument Co. recorder).

Figure 1 illustrates typical ribosome (50S and 30S) profiles for cells grown at 28 C. Since equivalent dry weights of cells were used to prepare the extracts, it is evident that the ribosome content is greatly reduced in cells growing in intermittent illumination; in the particular comparison shown, the areas under the curves (between the vertical arrows) differ by a factor of 1.9. It should be noted that, with cells grown in intermittent light, the quantity of RNA associated with the rapidly sedimenting particulate fraction was found to be negligible. In the experiments of Fig. 1, the growth rates differed by about fourfold, i.e., 0.4 mass doubling/hr in continuous and 0.1 mass doubling/hr in intermittent light. Previous work (G. A. Sojka and H. Gest, Proc. Natl. Acad. Sci. U.S., *in press*; G. A. Sojka et al., Bacteriol. Proc., p. 47, 1968) has shown that, in respect to growth rate (and macromolecular composition), cells multiplying in the 1-min cycle with saturating light (>550 ft-c) behave as though they were growing in continuous dim light of an intensity of only about 50 to 60 ft-c. In other words, when saturating illumination is repeatedly interrupted in a 1-min cycle (with equal light and dark times), the net rate of conversion of light energy to chemical energy utilizable for biosynthesis appears to be disproporionately decreased.
The growth rate of *R. capsulata* in continuous saturating light was found to be little affected by change of temperature over the range 26 to 38°C. At 18°C, however, the growth rate was less than half that at 28°C. It has been suggested (2) that, in contrast to growth rate, the RNA content of bacterial cells growing in a given medium is independent of temperature over a considerable range; however, Tempest and Hunter (5) reported that cells of *Aerobacter aerogenes* growing at 25°C have a higher RNA content than when grown at 40°C, largely as a result of increased ribosome formation. A similar effect of temperature on the RNA content of *Candida utilis* was recently observed by Brown and Rose (1). Ribosome profiles, determined as described above, of *R. capsulata* cells grown at 18 and 38°C with continuous saturating illumination also indicate increased ribosome synthesis at the lower temperature (Fig. 2). Cells cultivated at 18°C contain, per unit of dry mass, approximately 35% more ribosomes than cells grown at 38°C. It is of interest that the areas under the ribosome profiles of cells grown under conditions of energy stress, that is, with intermittent illumination in the 1-min cycle, were virtually identical for growth temperatures of 18, 28 (lower curve, Fig. 1), and 38°C. With continuous energy supply, increased ribosome formation at lower temperatures may indicate the operation of a "homeostatic" control mechanism which tends to compensate for decreased ribosomal activity at such temperatures. Although a mechanism of this kind might be capable of maintaining a constant growth rate over some particular temperature range (5), it is evident that in *R. capsulata* the capacity for increased ribosome synthesis at 18°C is insufficient to compensate for the decrease in efficiency of ribosomal function at that temperature.

For particular purposes, the quantity of RNA (or other cell constituents) per cell or genome is the ratio of significance, but we consider the relationship to cell mass to be more meaningful for analysis of certain aspects of the regulation of biosynthetic metabolism. Total mass is a measure of the energy derived from light or other sources for biosynthesis, and the rate of mass increase reflects, in part, the energy conversion economy of the cell. Changes in the steady state chemical energy flux might be expected to cause alterations in the pattern of flow of intermediates into different biosynthetic pathways and, accordingly, alterations in cell composition. It seems likely that the effects of intermittent illumination in a 1-min cycle on *R. capsulata* result from a drastic reduction in the average ATP production rate and from related changes in concentrations of adenylate nucleotides which exercise control functions on various biosynthetic enzymes (4).

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**LITERATURE CITED**

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