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# Regulation of Chlorophyll Synthesis in Photosynthetic Bacteria

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*Abstract*

Energy-transducing membranes of the nonsulfur purple photosynthetic bacteria are known to contain several species of bacteriochlorophyll (BChl) complexes. The reaction-centre complex (rc-BChl) is the locus of the charge separation that provides the "poles" of the photochemical electron transport system, whereas the other complexes serve light-harvesting functions. This report summarizes an investigation of the general features of the control mechanisms governing synthesis of the several chlorophyll complexes in *Rhodopseudomonas capsulata*. The results obtained indicate a close biosynthetic association between rc-BChl and one of the light-harvesting chlorophylls (complex I). Regulation of synthesis of light-harvesting complex II (during anaerobic photosynthetic growth) appears to be relatively independent, and intimately related to the "energy state" of the cell. Chlorophyll synthesis in *R. capsulata* cells growing aerobically in darkness was also studied. The presence of functional photosynthetic units in dark-grown cells, of very low BChl content, was clearly evidenced by demonstration of: the potentiality for resumption of anaerobic photosynthetic growth, light-induced oxidation of cytochrome$_{552}$ *in vivo*, and high photophosphorylation capacity (relative to BChl) of membrane fragments from such cells. Synthesis of light-harvesting BChl complex II is particularly inhibited in cells growing in darkness with respiratory phosphorylation as the source of energy, and it is suggested that this complex is a primary "target" of the biosynthetic control devices activated by change of light intensity or presence of molecular oxygen during growth of nonsulfur purple bacteria.

The "photosynthetic unit" is currently understood as an assemblage of chlorophylls and other types of molecules in which the absorption of a single quantum of light promotes an electron transfer event that initiates chemical (biochemical) reactions. It has been suggested [1] that in certain nonsulfur purple photosynthetic bacteria the unit contains some 40 molecules of "light-harvesting" (LH) bacteriochlorophyll (BChl) associated with a "reaction centre" in which one molecule of a special form of BChl effects the actual charge separation. The chlorophylls of such organisms ordinarily show a major *in vivo* absorbancy peak somewhere in the region 860-890 nm, the exact position depending on the species and the nutritional conditions. Identification of the reaction-centre bacteriochlorophyll (rc-BChl) component in a particular species is usually complicated by the fact that it has an absorbancy maximum in the same position or region as that of the more abundant LH-BChl. The rc-BChl component, however, can be detected as a pigment species subject to (reversible) bleaching by light or chemical oxidants [1].

Loss of the rc-BChl through mutation would be expected to lead to loss of ability to grow photosynthetically, and a *Rhodopseudomonas spheroides* mutant of this character (which can grow aerobically in darkness) has been described [2]. We recently reported [3] on the properties of a mutant of another nonsulfur purple bacterium, *Rhodopseudomonas capsulata,* in which the relative quantities of LH- and rc-BChl species can be readily altered through change of nutritional conditions, and in this communication we describe further experiments with this and related bacterial strains which give insights into the mechanisms controlling synthesis of the energy-converting complexes.

Typically, cells of nonsulfur purple bacteria grown photosynthetically appear to contain two species of LH-BChl complexes with different spectral characteristics [1-4]. For *R. capsulata,* we designate these as LH-BChl I, with a single major absorbancy peak at approximately 880 nm; and LH-BChl II, with absorbancy maxima at 802 and 855 nm. Photobleaching experiments indicate that rc-BChl in this bacterium has an absorbancy maximum at about 890 nm [3]. The spectral similarities of LH-BChl I and rc-BChl suggest close chemical, and possibly physical, relationships between these two species. According to Aagaard and Sistrom [4], the rc-BChl of *R. spheroides* has an absorbancy maximum at 870 nm and occurs in the ratio 1 rc-BChl : 20-25 molecules of a LH-BChl species with an absorbancy peak at 875 nm.

There is increasing evidence [5-8] that the regulatory effects of light intensity on total BChl synthesis are mediated through a chemical signal system in which the adenylate nucleotides (especially adenosine triphosphate; ATP) play a prominent role. It would be expected that normal turnover and the concentration profile of the nucleotides should be affected by inorganic arsenate ($As_i$), which can substitute for orthophosphate ($P_i$) leading to the formation of very unstable organic esters.

Synthesis of BChl and the photosynthetic growth rate of wild-type *R. capsulata* are, in fact, both progressively inhibited as the $As_i/P_i$ ratio in the growth medium is increased [8]; when the ratio is 1, almost complete inhibition of growth is observed [6]. Arsenate-resistant mutants of *R. capsulata* can be readily isolated and one of these, strain Z-1, has been studied in some detail [6]. Photosynthetic subculture of Z-1 in the presence of $As_i$ ($As_i/P_i = 1$) under certain conditions leads to a relative enrichment in LH-BChl I and rc-BChl [3]. This is illustrated in Fig. 1, which shows absorption spectra of membrane fragments* derived from wild-type and Z-1 cells grown photosynthetically under several sets of nutritional circumstances; for wild-type: 900 footcandle (fc) light intensity and 38°; for Z-1: 3,800 fc, $As_i/P_i = 1$, and either 38° or 22°.

The experimental difference spectrum between wild-type and "enriched" Z-1 fragments (from cells grown at 38°) reveals a substantial increment in enriched Z-1 of BChl species with absorbancy maxima close to 880 nm. We associate the latter with LH-BChl I + rc-BChl (separate light-induced bleaching experiments [3] have shown that an appreciable fraction of the 880-absorbing material is rc-BChl). The curve for Z-1 fragments from cells grown at 22° clearly indicates that the setting of the chemical signal system regulating BChl synthesis is profoundly affected by temperature. The growth rate at 22° is approximately half that at 38°, which speaks for a multitude of differences between the two situations. We assume that the lower growth rate at 22° reflects a greatly decreased flux of ATP regeneration (via photophosphorylation) and that this is a key factor in "resetting" of the regulatory signal system to a condition which favors the synthesis of LH-BChl II.

In intact cells, the ratio of absorbancy at 880 nm to that at 860 nm ($A_{880}/A_{860}$) can be taken as an index of enrichment of LH-BChl I + rc-BChl relative to LH-BChl II [3]. Figure 2 shows that the degree of enrichment in Z-1 cells (and in a derived streptomycin-resistant mutant, str-9) grown in the presence of $As_i$ is a function of temperature and light intensity conditions during growth, and that enrichment is somehow related to total BChl content in bacteria growing at a temperature of 30° or higher. Thus, cells growing at 38° and high light intensity produce less total BChl/mg cell protein and show higher values of $A_{880}/A_{860}$ than cells grown at 30° and lower intensity. Aagaard and Sistrom [4] have similarly observed, with *R. spheroides*, that with diminishing total BChl content, the ratio rc-BChl/LH-BChl increases. The data of Fig. 2 indicate that the quantitative aspects of synthesis of the several BChl species can differ significantly even in closely related strains grown under the same conditions, and that there is no clear-cut relation between the $A_{880}/A_{860}$ ratio achieved and the

* Entirely comparable results are observed when *in vivo* spectra of intact cells are examined.

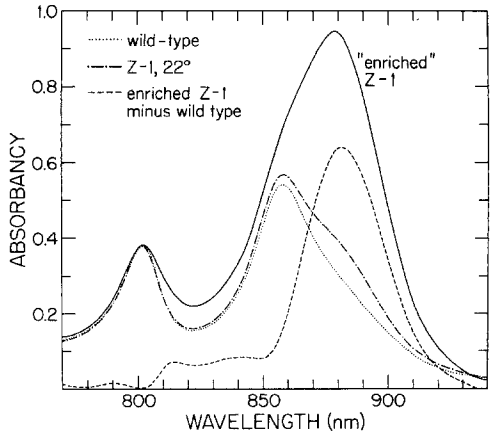


Figure 1. Bacteriochlorophyll absorption spectra (near-infrared region) of membrane fragments derived from *Rhodopseudomonas capsulata* wild-type and mutant Z-1 cells.

*R. capsulata*, wild-type (strain St. Louis; American Type Culture Collection No. 23782), was grown photosynthetically in a synthetic medium containing DL-malate and $(NH_4)_2SO_4$ as the respective carbon and nitrogen sources [3]. With mutant Z-1, the growth medium was supplemented with 10 mM sodium arsenate ($As_i/P_i = 1$). Membrane fragments ("particles") were isolated from crude extracts obtained by disruption of the bacterial suspensions in a French pressure cell [3, 20]. For spectral measurements (Cary 14 spectrophotometer operated in the IRI mode), membrane fragments were suspended in 25 mM glycylglycine buffer (pH 7.2) containing 0.1 mM sodium succinate. Particle concentrations were adjusted so as to give identical values of $A_{802nm}$-$A_{780nm}$ (where A designates absorbancy) and light scattering, measured as $A_{680}$, was equalized as necessary by addition of appropriate quantities of depigmented particles [wild-type fragments extracted with acetone : methanol (7 : 2, v/v)]. Solid curve, particles from "enriched" Z-1 cells (grown at 38° and ~3800 fc light intensity; fifth subculture); 7.8 μg BChl/mg protein. Dotted curve, particles from wild-type cells (38°, 900 fc; sixth subculture); 51 μg BChl/mg protein. Dash-dot curve, particles from Z-1 cells grown at 22° and ~3800 fc (fifth subculture); 9.7 μg BChl/mg protein. The dashed curve is an experimental difference spectrum for "enriched" Z-1 minus wild-type; suspensions of equal absorbancy at 802 nm were used, with the wild-type preparation serving as the "blank". Total BChl content of particles was determined by the acetone : methanol extraction procedure detailed in ref. 21, and protein content as described in ref. 3.

growth rate. Comparing cells with roughly the same total BChl content/mg protein from cultures grown at high light intensity, the great influence of low temperature (22°) on the pattern of BChl synthesis is strikingly apparent. It is clear that alteration of the light intensity and temperature conditions of photosynthetic growth can lead to pronounced changes in composition of the photochemical apparatus of *R. capsulata* Z-1 (and Z-1-str-9). Such changes presumably reflect the

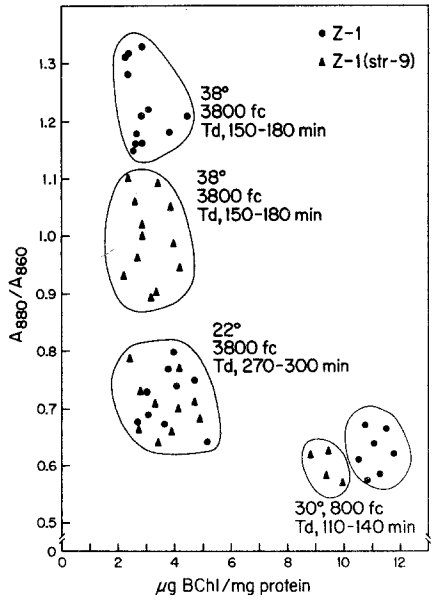


Figure 2. Influence of temperature and light intensity during growth of *R. capsulata* Z-1 and Z-1-str 9 (streptomycin-resistant mutant) on synthesis of BChl components with high absorbancy at 880 nm.

Intact cells were suspended in 25% bovine serum albumin [21], and the $A_{880}/A_{860}$ ratio determined using the Cary 14 spectrophotometer. The growth medium contained $As_i$ ($As_i/P_i = 1$) and measurements were made on bacteria harvested after a number of transfers under the conditions specified. To allow for maximal pigment synthesis, serial transfers were made every 72-84 h, except for cultures grown at 30° and 800 fc light intensity (24 h intervals). $T_d$, mass doubling times during the logarithmic phase of growth. Total BChl and protein were determined by the procedures used for the experiments of Fig. 1.

operation of control systems designed so as to enable the cell to achieve maximal biosynthetic economy over a wide range of environmental circumstances.

Ordinarily, nonsulfur purple bacteria also have the capacity to grow aerobically in darkness, in which case the energy for biosynthesis (ATP) is provided by oxidative, respiratory phosphorylation. During dark aerobic growth, the synthesis of BChl is greatly suppressed due to repression of synthesis of biosynthetic enzymes and other effects of molecular oxygen [9, 10]. This is illustrated in Fig. 3 for the wild-type and Z-1 strains of *R. capsulata*. The total BChl content/mg cell protein drops rapidly during the first few dark aerobic subcultures, and more slowly thereafter. With Z-1, the "final" BChl level is considerably lower

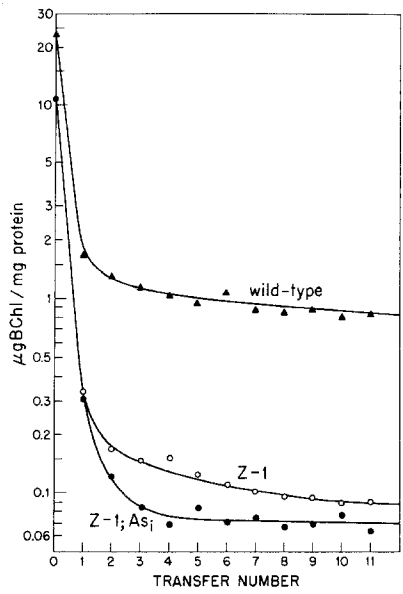


Figure 3. Effect of serial aerobic dark subculture of *R. capsulata* wild-type and mutant Z-1 on total cellular BChl content.

The bacteria were grown at 34° in the presence or absence of $As_i$ as noted. Cultures (in each case, 100 ml of inoculated medium in a 500 ml Erlenmeyer flask) were shaken on a rotary shaker operated at 180 revs/min and serial transfers (using 0.3 ml of previous culture) made at 21-24 h intervals. The "0" transfer points show the BChl content of cells (grown photosynthetically at 34° and 800 fc light intensity) used to inoculate the first dark cultures. Total BChl and protein were determined by the methods already noted.

than in the wild-type parental strain, and inclusion of $As_i$ in the growth medium leads to an even greater decrease in BChl content. Here again, growth rate does not seem to be an important factor in determining the total BChl level; thus, the mass doubling times for wild-type and Z-1 in these experiments were quite comparable (approximately 100 min). Despite the low total BChl content of aerobically grown *R. capsulata* cells, we have found that even after many subcultures they retain the capacity for anaerobic photosynthetic growth. According to Cohen-Bazire *et al.* [9] the nonsulfur purple bacteria *R. spheroides* and *Rhodospirillum rubrum* "eventually become physiologically incapable of photosynthesis" when grown in the presence of air, owing to total inhibition of BChl synthesis by oxygen. As far as we can tell, this is not the case with *R. capsulata.* During long-term aerobic dark growth of nonsulfur purple bacteria, nonphotosynthetic cell lines may arise through mutation or loss of genetic elements, and this deserves serious study.

The nature of the BChl species in *R. capsulata* cells transferred repeatedly under aerobic dark conditions is evidently of considerable interest in connexion with the problem of regulation of synthesis of the functional photosynthetic "unit". Subculture of wild-type photosynthetically grown *R. capsulata* cells under aerobic conditions in darkness markedly affects the *in vivo* BChl absorption spectrum (see Fig. 4). Cells

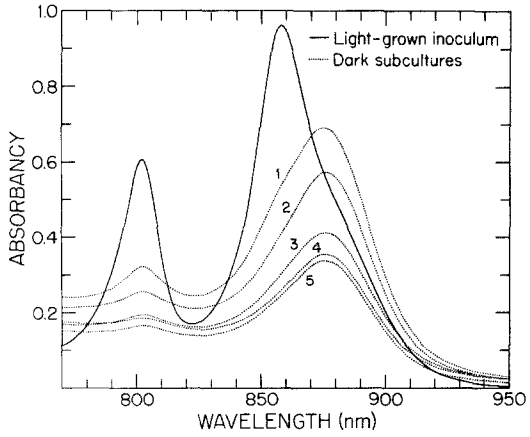


Figure 4. Alteration of the *in vivo* BChl absorption spectrum (near-infrared region) of *R. capsulata* wild-type during dark aerobic growth.

Serial transfers were made as described for Fig. 3. Harvested cells were suspended in 25% bovine serum albumin and the *in vivo* spectra determined using the Cary 14 spectrophotometer. Solid line curve, spectrum of the photosynthetically grown (30°, 800 fc) cells used to inoculate the first dark culture. Sample concentrations (mg dry weight of cells per ml)—photosynthetically grown cells, 0.7; dark-grown subcultures, in serial order (1-5): 5.2, 5.8, 5.6, 5.3, 5.5.

from the first transfer show a greatly diminished 802 nm peak and a pronounced shift in position of the major peak at longer wavelength. With continued subculture, these changes become more marked, and eventually the infrared spectrum closely resembles that seen in "enriched" (photosynthetically grown) Z-1 cells. The spectra of membrane fragments from both types of cells were also found to be very similar, differing in only two minor respects: the major peak of particles from dark-grown cells is at 875 nm (rather than 880 nm) and the ratio of absorbancy at this wavelength to that at 802 nm is slightly higher than $A_{880}/A_{802}$ in enriched Z-1 preparations.† Moreover, the membrane

† Klemme and Schlegel [11] have published spectra of particles from photosynthetically- and dark aerobically-grown cells of a different strain of *R. capsulata*, and a relative enrichment of the long wavelength-absorbing BChl species in the "aerobic particles" is apparent from their curves.

fragments from dark-grown cells resemble those from enriched Z-1 in that they also contain a BChl species subject to oxidative bleaching [in both cases, reduced (ascorbate) minus oxidized (ferricyanide) spectra show a peak at ~880 nm]. These observations indicate that although dark aerobically grown cells of *R. capsulata* have a very reduced total BChl content, they are enriched in respect to LH-BChl I + rcBChl. In other words, it appears that synthesis of the LH-BChl II component is particularly susceptible to inhibition by molecular oxygen.

As implied above, preservation of functional photosynthetic units in bacteria grown aerobically in darkness is indicated by the capacity of such cells to readapt to anaerobic photosynthetic growth. More direct evidence for the conservation of functional photosynthetic complexes in dark-grown cells is given by experiments on light-induced oxidation of cytochrome and photophosphorylation. Because of their very low pigmentation, Z-1 cells grown aerobically in darkness in the presence of $As_i$ provide an excellent experimental system for study of light-induced oxidation of endogenous cytochrome $c_{552}$ *in vivo*. This is evident from the results of Fig. 5, which clearly demonstrate the activity of the photochemical system in respect to interaction with cytochrome $c_{552}$. It is noteworthy that the extent of cytochrome $c_{552}$ photooxidation in such experiments corresponds to about 80% of the endogenous *c*-type cytochrome present.

The phosphorylation capacities of membranes from *R. capsulata* wild-type and mutant Z-1 cells, grown in light and dark, are shown by the data in Table I. It can be seen that the photophosphorylation activity (specific activity = $\mu$moles ATP synthesized/h/mg total BChl) of enriched Z-1 membranes is more than 10-fold higher than that of membranes from wild-type cells grown photosynthetically under "standard" conditions. The comparatively low photophosphorylation specific activity (referred to BChl) of the wild-type preparation reflects, in large measure, the presence of relatively large quantities of LH-BChl in such membranes. It is of interest to also compare the photophosphorylation rates per unit of protein since this gives some indication of energy-conversion rate in reference to the most abundant constituent of bacterial cells. On this basis, as well, enriched Z-1 cells apparently possess more effective machinery for utilizing light energy for biosynthesis than wild-type cells growing under "optimal" conditions.

Membrane fragments from dark-grown cells show high photophosphorylation specific activities, relative to BChl (Table I). These and similar observations reported by other investigators [11-13] confirm in an independent way the persistence of competent photosynthetic units in cells growing aerobically in darkness. Depending on the bacterial strain, composition of the growth medium, and the degree of aeration, the number of competent units per cell evidently may decrease to very low levels (as in Z-1 cultivated in the presence of $As_i$). The values in the

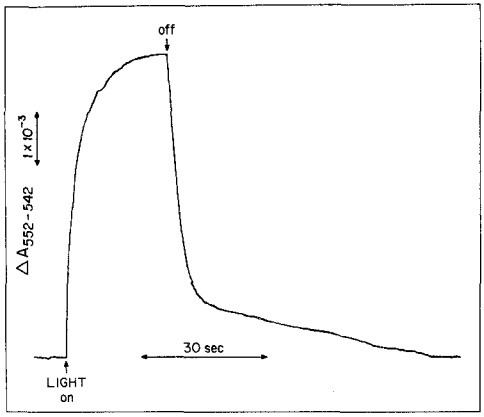


Figure 5. Light-induced oxidation of endogenous cytochrome-552 in intact, dark-grown cells of *R. capsulata* Z-1.

Cells grown aerobically in darkness in the presence of $As_i$ (10th transfer) were harvested, washed, and resuspended in "incomplete" medium (growth medium minus malate). The suspension was shaken aerobically (dark) for 1 h at 34° in order to deplete the bulk of the cellular endogenous reserves, and the cells then resuspended in "incomplete" medium minus $As_i$, at a density of 0.6 mg dry weight per ml; BChl content, 0.16 μg/mg cell protein. KCN (1.5 mM) was added to prevent oxidation of reduced cytochrome by atmospheric oxygen. The *in vivo* absorbancy changes were measured with a split beam/dual-wavelength spectrophotometer (designed by Dr. Bessel Kok), operated in the dual-wavelength mode using 552 nm light as measuring beam and 542 nm light as reference beam. After brief incubation of the suspension in darkness to permit reduction of cytochrome by residual endogenous substrates, the bacteria were irradiated (↑) with near infrared actinic light (wavelength > 760 nm) isolated from the radiations of a 600 watt tungsten-iodine lamp by interposition of a 1 in. water (cooling) filter and two Kodak Wratten filters (No. 88A). The photomultiplier was protected from stray light and scattered actinic light by a blue glass filter (Corning No. 4-96). Association of the observed absorbancy changes with cytochrome -552 was verified by scanning through the wavelength region 540-580 nm, which yielded a difference spectrum with a sharp peak at 552 nm.

last column of Table I give some quantitative notion of the relative development of the photophosphorylation and dark oxidative phosphorylation systems during growth under the circumstances specified. Clearly, the photophosphorylation system is much more highly developed in cells grown photosynthetically, whereas the oxidative phosphorylation mechanism predominates in membranes of low total BChl content from dark-grown cells. This is obviously in accord with expectations, and comparable findings were recently reported by Lampe and Drews [13]. The present results with *R. capsulata* strain Z-1 are particularly illustrative of the great plasticity of adaptive changes shown by nonsulfur purple bacteria in regard to energy metabolism.

TABLE I. Phosphorylation activities of membrane fragments derived from *R. capsulata* wild-type and Z-1 mutant cells

| Exp. | Cell type | Growth conditions | μg BChl/mg protein | Light-induced phosphorylation (LIP) μmoles ATP per hour per mg BChl | Light-induced phosphorylation (LIP) μmoles ATP per hour per mg protein | Oxidative phosphorylation (OP) per mg protein | LIP/OP (per mg protein) |
|---|---|---|---|---|---|---|---|
| 1 | Wild-type | Photosynthetic (800 fc) | 51 | 286 | 14.5 | 0.8 | 18.3 |
| 2 | Z-1 | Photosynthetic (3800 fc) + As | 7.8 | 3520 | 27.6 | 3.5 | 7.9 |
| 3 | Wild-type | Dark | 1.8 | 2360 | 4.2 | — | — |
| 4 | Z-1 | Dark | 2.8 | 1730 | 4.8 | 3.2 | 1.5 |
| 5 | Z-1 | Dark | 0.2 | 1702 | 0.3 | 1.5 | 0.2 |
| 6 | Z-1 | Dark + As | 0.1 | 1740 | 0.2 | 1.6 | 0.1 |

Dark-grown cells (exps. 3-6) were cultivated under conditions of vigorous aeration with air plus 1% carbon dioxide; in exp. 4, the aeration rate was decreased four-fold, and this accounts for the increased BChl content of the particles obtained (as compared with exp. 5). Membrane fragments from both photosynthetic and dark-grown cultures were prepared by the same procedure, as noted in the legend of Fig. 1. For measurement of phosphorylation, a hexokinase plus glucose "trap" was employed to convert [$^{32}$P] ATP, produced from $^{32}P_i$, to [$^{32}$P] glucose-6-phosphate; after deproteinization with trichloroacetic acid (TCA), organic $^{32}$P was separated and determined by the method of Avron [22]. The assay mixtures contained (μmoles/ml, or as noted): glycylglycine (pH 8), 70; adenosine diphosphate (ADP), 2; $P_i$, 5; $MgCl_2$, 7; glucose, 50; hexokinase, 5 units/ml; membrane fragments, as required; and sodium succinate (see below). For photophosphorylation, the mixtures were supplemented with 0.3 μmole sodium succinate per ml, and illuminated for 2 min with saturating white light; rates given are corrected for phosphorylation observed in appropriate dark controls. For oxidative phosphorylation assays, the mixtures were supplemented with 2.3 μmoles sodium succinate per ml, and incubated (2 min) in darkness; during incubation, the mixtures were constantly shaken and simultaneously aerated; corrections were applied for background $^{32}$P counts observed in "zerotime controls" in which TCA was added prior to addition of ADP, $^{32}P_i$, and $MgCl_2$.

*R. capsulata* is a typical nonsulfur purple bacterium in most respects; and it seems likely that the scheme for regulation of BChl synthesis in this organism includes basic features that are employed by other representatives of the group. From the information at hand we conclude that in *R. capsulata,* LH-BChl II is a primary "target" of the control devices activated by change of light intensity or presence of molecular oxygen. With this as a basis, we propose the following interpretive model. The syntheses of rc-BChl and LH-BChl I are regulated coordinately, whereas the "coarse" control of total BChl synthesis is based—as a first approximation—on relatively independent adjustment of the cellular content of LH-BChl II. The latter can oscillate over a wide range, the exact value under photosynthetic (anaerobic) conditions being dependent on the energy state of the cell; that is, on the rate of ATP regeneration by photophosphorylation and the adenylate "energy charge" [5, 6, 14, 15]. Under photosynthetic conditions, total BChl production is inversely related to light intensity [9, 16]. Accordingly, at low intensity cells grow slowly and manifest a high content of LH-BChl (especially II), which obscures the rc-BChl species in the infrared spectrophotometric profile. The compensatory synthesis of "excess" light-harvesting membrane at low light intensity is no doubt metabolically expensive to the cell, and this may be an important factor dictating slow growth rate under conditions of energy stress [17].

Certain observations imply that light intensity and molecular oxygen may control BChl synthesis by way of a common mechanism [9], but there is also ample reason to believe that this is only a superficial interpretation. LH-BChl II is clearly dispensable for dark aerobic growth and it is not surprising that this component rapidly disappears from cells growing with respiratory phosphorylation as the source of energy. It has been suggested [9, 18] that oxygen regulates BChl formation through an influence on the redox state of an (unspecified) electron transfer carrier of the respiratory chain, but recent experiments ([19]; B. Marrs and H. Gest, *J. Bacteriol.,* June 1973) with respiratory-deficient mutants of *R. capsulata* Z-1 indicate a complex control mechanism in which direct effects of oxygen on the BChl synthesis machinery play a primary role.

Little is known of the comparative molecular properties of the several chlorophyll complexes produced by a single type of photosynthetic cell, and it is probable that they differ primarily in respect to associated protein components. Accordingly, the regulatory phenomena described may eventually be referable to controls exerted on the synthesis of specific membrane proteins. Because of their physiological versatility and other properties, the nonsulfur purple photosynthetic bacteria may well provide unusually valuable experimental systems for further study of this important area of metabolic regulation.

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*References*

1. R. K. Clayton, *Molecular Physics in Photosynthesis,* Blaisdell Publishing Co., 1965.
2. W. R. Sistrom and R. K. Clayton, *Biochim. Biophys. Acta,* 88 (1964) 61.
3. S. Lien, A. San Pietro and H. Gest, *Proc. Nat. Acad. Sci., U.S.A.,* 68 (1971) 1912.
4. J. Aagaard and W. R. Sistrom, *Photochem. Photobiol.,* 15 (1972) 209.
5. G. A. Sojka and H. Gest, *Proc. Nat. Acad. Sci., U.S.A.,* 61 (1968) 1486.
6. J. W. Zilinsky, G. A. Sojka and H. Gest, *Biochem. Biophys. Res. Commun.,* 42 (1971) 955.
7. M. Fanica-Gaignier, J. Clement-Metral and M. D. Kamen, *Biochim. Biophys. Acta,* 226 (1971) 135.
8. G. Forti, M. Avron and A. Melandri (eds.), *Proc. Second Internat. Cong. Photosynthesis Res.,* Dr. W. Junk, N. V. Publishers, The Hague, vol. 3 (1972) 2701.
9. G. Cohen-Bazire, W. R. Sistrom and R. Y. Stanier, *J. Cell. Comp. Physiol.,* 49 (1957) 25.
10. J. Lascelles and D. Wertlieb, *Biochim. Biophys. Acta,* 226 (1971) 328.
11. J.-H. Klemme and H. G. Schlegel, *Arch. Mikrobiol.,* 68 (1969) 326.
12. G. Drews, H. H. Lampe and R. Ladwig, *Arch. Mikrobiol.,* 65 (1969) 12.
13. H. H. Lampe and G. Drews, *Arch. Mikrobiol.,* 84 (1972) 1.
14. H. Gest, in: *Advances in Microbial Physiology,* A. H. Rose and D. W. Tempest (eds.), Academic Press, London, 7 (1972) 243.
15. Z. Vanek, Z. Hostalek and J. Cudlin (eds.), *Genetics of Industrial Microorganisms,* Academia (Publ. House of Czechoslovak Acad. Sci.), Prague (1973) 131.
16. W. R. Sistrom, *J. Gen. Microbiol.,* 28 (1962) 607.
17. G. A. Sojka, A. Baccarini and H. Gest, *Science,* 166 (1969) 113.
18. T. Wittenberg and W. R. Sistrom, *J. Bacteriol.,* 106 (1971) 732.
19. B. Marrs, C. L. Stahl, S. Lien and H. Gest, *Proc. Nat. Acad. Sci., U.S.A.,* 69 (1972) 916.
20. A. Baccarini-Melandri, H. Gest and A. San Pietro, *J. Biol. Chem.,* 245 (1970) 1224.
21. G. A. Sojka, H. H. Freeze and H. Gest, *Arch. Biochem. Biophys.,* 136, (1970) 578.
22. M. Avron, *Biochim. Biophys. Acta,* 40 (1960) 257.