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Genetic Mutations Affecting the Respiratory Electron-Transport System of the Photosynthetic Bacterium *Rhodopseudomonas capsulata*

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Alternative energy-converting systems permit the nonsulfur purple photosynthetic bacterium *Rhodopseudomonas capsulata* to grow either with light or (dark) respiration as the source of energy. Respiratory mutants, unable to grow aerobically in darkness, can be readily isolated and the defective step(s) in their respiratory mechanisms can be identified by study of biochemical activities in membrane fragments derived from photosynthetically grown cells. Such analysis of appropriate mutants and revertants permits construction of a model for the respiratory electron-transport system of the wild type. The results obtained indicate differential channeling of electrons derived from succinate and reduced nicotinamide adenine dinucleotide, and are interpreted in terms of a branched electron-transport scheme. The scheme provides a guide for further, more refined analysis of the respiratory mechanisms through biochemical genetic approaches, and several of the mutants isolated can be exploited for investigation of unsolved problems relating to interactions between respiratory and photosynthetic electron transport and the mechanism of inhibition of bacteriochlorophyll synthesis by molecular oxygen.

Typical nonsulfur purple bacteria can obtain energy for growth either by anaerobic photophosphorylation or dark aerobic (respiratory) phosphorylation. Both energy-conversion systems appear to be localized in a common membranous framework, but, although they are known to interact in various ways, the molecular details are still unclear. One approach to elucidation of the alternative energy-yielding schemes and their relationships is through the study of appropriate mutants. We chose to examine mutants (of *Rhodopseudomonas capsulata*) blocked in respiration, which can still grow photosynthetically. In mutants of this kind, the residual components of the respiratory electron-transport system (ETS) present in photosynthetically grown cells can be characterized, and this facilitates definition of the normal respiratory scheme. A previous report (9) from this laboratory described a mutant strain of *R. capsulata*, respiratory-deficient by virtue of a lesion in the reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase complex, and in this paper we describe other types of electron-transport mutants. These include strains with defects in the terminal portion of the ETS, detected through use of the "Nadi reaction" which has been employed in studies of mechanisms of biological respiration since 1884 (5): α-naphthol + dimethyl-*p*-phenylenediamine + O_2 $\rightarrow$ indophenol blue + H_2O. The mixed organic reactants (termed "Nadi" reagent, an acronym composed of the first two letters of each chemical name) are oxidatively converted to the blue dye through the agency of cytochrome *c* plus cytochrome oxidase. Analysis of the various mutants isolated indicates that the ETS of *R. capsulata* is branched and that electrons tend to be preferentially "channeled" through alternative routes, depending on their metabolic origins. Related studies, presented in an accompanying report (8), suggest that a flow of electrons from an intermediate catalyst of the ETS is essential for bacteriochlorophyll synthesis in the presence of molecular oxygen.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Unless otherwise noted, the growth medium used was the so-called RCV synthetic medium (9; initial pH, 6.8),

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containing 0.4% DL-malic acid, 0.1% $(NH_4)_2SO_4$, 0.0001% thiamine hydrochloride, 0.01 M KPO_4, and additional inorganic salts as specified by Ormerod et al. (12). Illumination for photosynthetic growth was provided by Lumiline lamps (Sylvania) at an illuminance of approximately 500 foot-candles (5.38×10^3 lux). Such cultures were grown in vessels filled to capacity with medium, or on agar plates (see below).

Strain Z-1 is an arsenate-resistant spontaneous mutant (16) of *R. capsulata* strain St. Louis (ATCC 23782). Strain M1 (previously designated as Z-1 [*aer-13*]; reference 9) is a respiratory-deficient derivative of Z-1 which lacks NADH dehydrogenase activity and, consequently, is unable to grow aerobically in darkness (i.e., with oxidative phosphorylation as the energy supply). Strain M2 lacks both NADH and succinate dehydrogenase activities and was isolated as follows. M1 was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and survivors were recovered as previously described (9). Mutagenized cells were washed with 0.01 M KPO_4 buffer (pH 6.8), containing 0.1% $(NH_4)_2SO_4$, and resuspended in a succinate-minimal medium (RCV-modified to contain 30 mM succinate instead of malic acid and only one-third as much $CaCl_2$). The culture was incubated anaerobically (argon atmosphere) in the light until its turbidity had increased threefold, at which time 200 U of penicillin G/ml were added. Photosynthetic conditions were maintained for 34 h, and surviving cells were then washed free of penicillin. The washed survivors were spread on the surfaces of peptone plus yeast extract (0.3% each) agar plates, which were incubated in an illuminated GasPack anaerobic system (BioQuest). Colonies of mutants unable to utilize succinate were subsequently identified by replica plating to agar plates containing either succinate or malate minimal medium. Strain M3 was isolated from M2 as a spontaneous revertant which had regained the ability to grow aerobically in darkness on malate minimal medium (but not on succinate minimal).

Strain Z-1 was used as the parental type for isolation of mutants with defects in the terminal oxidase region of the respiratory system. Such mutants were identified by staining colonies on agar plates with the classical Nadi reagent (5) as follows. Plates are flooded for approximately 15 s with a freshly prepared 1:1 mixture of 1% α-naphthol in 95% ethanol and 1% *N*,*N*-dimethyl-*p*-phenylenediamine monohydrochloride (DMPD) in water, then drained, and exposed to air. Colonies (grown either under anaerobic, photosynthetic or aerobic, dark conditions) composed of cells containing functional terminal oxidases stain a deep-blue color within 1 to 2 min. Lesions in terminal oxidase activities result in either a slow or virtual absence of staining. Survivors may be recovered from colonies on stained plates if the colony is sufficiently large (>2 mm diameter) and if the cells are restreaked on a fresh plate within a few minutes of application of the stain. The Nadi-negative strains M4 and M5 were identified as unstained colonies formed by cells from cultures which had undergone penicillin selection against aerobic growth as described earlier (9; we also isolated mutants similar to M4 [see below] by staining colonies produced by mutagenized cells without prior selection).

Strains M6 and M7 are spontaneous revertants of respiratory-deficient strain M5; the revertants regained the capacity to grow aerobically in darkness.

Preparation of cell extracts and membrane fragments. Insofar as possible, membrane fragments (particles) were prepared from cells which had experienced very similar culture conditions for many generations prior to disruption and extraction (with strains unable to grow aerobically, however, the medium was supplemented with 0.5 mg of L-ascorbate per ml [9]; controls showed that such addition had little or no effect on the activities measured). A "preculture" was inoculated with cells from frozen storage stocks (photosynthetically grown in RCV medium; glycerol was added to give a 10% concentration just prior to storage at −70 C). The preculture was incubated under anaerobic, photosynthetic conditions until early stationary phase was achieved (about 18 h at 30 C). A 5-ml amount of preculture was used as inoculum for the "final" culture (180 ml), grown in the same fashion. Cells were harvested in early stationary phase and disrupted in a French pressure cell, and pigmented membrane fragments were isolated and stored essentially as described earlier (9). In the current series of experiments, the supernatant fluid of centrifuged extracts was decanted and the "fluffy" layer of relatively unpigmented material, just above the deeply pigmented membrane sediment, was removed by vigorous "flick-of-the-wrist" snapping of the centrifuge tube. The membrane fragments were suspended in 100 mM glycylglycine (pH 7.2) containing 4 mM $MgCl_2$, and an equal volume of glycerol was added.

Analytical methods. Rates of O_2 consumption by membrane fragments suspended in 0.05 M KPO_4 (pH 7.5; 1 mg of protein/ml) were measured polarographically with a Yellow Springs Instrument Co. oxygen meter, model 51, at 25 C; substrate concentrations were: 4 mM NADH, 40 mM succinate, 0.2 mM *N*,*N*,*N'*,*N'*-tetramethyl-*p*-phenylenediamine (TMPD) plus 21 mM L-ascorbate, or 0.027 mM equine cytochrome *c* plus 21 mM L-ascorbate. NADH oxidase activity was also determined by spectrophotometric measurement of NADH oxidation rate in aerobic reaction mixtures as described earlier (9). Spectrophotometric determination of oxidation rate of reduced cytochrome *c* was performed as follows. A 1 mM solution of oxidized cytochrome *c* (equine, fraction III, Sigma Chemical Co.) was stoichiometrically reduced (as determined by absorbancy measurements) by titration with 1 mM L-ascorbate. The reduced cytochrome *c* was added to reaction mixtures [in 0.02 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 8.5; ca. 0.2 mg of membrane protein per ml] to give an initial concentration of 0.04 mM. Rate of absorbancy change at 550 nm was measured with a Cary 14 recording spectrophotometer, and a reduced-minus-oxidized molar extinction coefficient of 2.1×10^4 cm^{-1} was assumed (15).

Cytochrome *c* reductase activities were assayed spectrophotometrically as previously described (9).

Oxidative phosphorylation was assayed by meas-

urement of esterification of ^{32}P-labeled orthophosphate as detailed by Melandri et al. (10).

Protein content of membrane fragments was measured by the method of Lowry et al. (7), as in an earlier study (9).

RESULTS

Nadi reaction and growth characteristics of Z-1 and derived strains. Table 1 lists the *R. capsulata* strains considered in this investigation and shows their facultative capacities for dark, aerobic growth, as well as response to the Nadi reagent. Since use of the complete strain descriptions in discussions tends to be cumbersome, each isolate has been assigned a short designation.

R. capsulata Z-1 is a typical nonsulfur purple bacterium in respect to ability to grow either photosynthetically (anaerobically) or aerobically in darkness. Strain M1 shows the normal Nadi reaction, but is unable to grow as an aerobe in the dark because of a severe block in an early step of the ETS, viz., the NADH dehydrogenase reaction (9). Strain M2, derived from M1, is additionally blocked in the succinate dehydrogenase reaction (see Table 3); this added alteration leads to inability to grow photosynthetically with succinate as the source of carbon and reducing power. M2 was constructed to obtain a cell type, blocked in both of the major pathways through which electrons enter the ETS, to study the mechanism by which O_2 regulates bacteriochlorophyll synthesis (see reference 8). Strain M3 is a revertant obtained from M2, which has regained the ability to grow aerobically in darkness with malate as the substrate. Since the succinate dehydrogenase lesion persists in M3, succinate cannot support growth of this strain, either photosynthetically or aerobically in darkness. It is apparent that the succinate dehydrogenase lesion alone does not confer the respiratory-deficient phenotype. The latter, however, can result from a severe block in the NADH dehydrogenase step, since the bulk of the electrons used for dark oxidative phosphorylation are derived from NADH.

Mutants with impaired ability to catalyze the Nadi reaction can be readily obtained. Strains M4 and M5 were selected for further study because they showed particularly low Nadi reaction activities. Although M4 is Nadi-negative, it grows readily under aerobic conditions in darkness. To our initial surprise, we observed that a large fraction of Nadi-negative strains behaved in this way. M5, however, is Nadi-negative and unable to grow aerobically (some similar isolates were found to have diminished NADH dehydrogenase activity, and such presumed double mutants are not considered in this communication). Under aerobic dark conditions in the liquid malate medium (RCV), revertants M6 and M7—derived from M5—show the same growth rate and cell yield as the parent Z-1 strain (also true for M4).

From Table 1, it is evident that anaerobic photosynthetic growth is independent of the Nadi characteristic, as expected.

Oxidase activities in membrane fragments. To more precisely determine the loci of defects in the Nadi-negative strains, membrane fragments were analyzed for their oxidase activ-

TABLE 1. *Phenotypes of R. capsulata Z-1 and derivative strains*

Strain designation	Mutations[a]	Nadi reaction	Aerobic dark growth[b]		Photosynthetic growth[b]	
			Malate	Succinate	Malate	Succinate
Z-1	*asr-1*	+	+	+	+	+
M1	*asr-1, aer-13*	+	−	−	+	+
M2	*asr-1, aer-13, sdh-1*	+	−	−	+	−
M3	*asr-1, aer-13r4, sdh-1*	+	+	−	+	−
M4	*asr-1, aer-414*	−	+	+	+	+
M5	*asr-1, aer-412-512*	−	−	−	+	+
M6	*asr-1, aer-412r20-512*	+	+	+	+	+
M7	*asr-1, aer-412-512r34*	−	+	+	+	+

[a] *asr*, genes determining sensitivity to inorganic arsenate; mutation *asr-1* permits growth in media containing equimolar concentrations of phosphate and arsenate (16). The designations *13r4*, *412r20*, and *512r34* are tentatively used to indicate genotypes of revertants, and reflect our present inability to distinguish among true reversions, second-site reversions, and suppressor mutations.

[b] Growth on malate or succinate minimal agar plates (except for tests with M4-M7 on succinate). Symbols: +, colonies visible after 1 day of incubation (usually several millimeters in diameter by 3 days); −, no visible colony formation after 1 week of incubation at 30 C. Tests for photosynthetic (anaerobic) and aerobic dark growth of M4, M5, M6, and M7 on succinate were conducted by using liquid medium; as indicated, growth occurred in all instances except with M5 under aerobic, dark conditions.

ities with several substrates (Table 2). As anticipated, the TMPD oxidase activities of the particles correlated exactly with the in vivo (colony) DMPD staining reactions, and we conclude that the Nadi-negative strains described are blocked in the ability to transport electrons from the substituted phenylenediamine dyes to O_2. With exogenous, reduced cytochrome *c* (and reduced dichlorophenolindophenol, data not shown), the same pattern of activities was observed, indicating that TMPD and reduced cytochrome *c* are both oxidized via electron-transfer pathways with common elements. (Reduced cytochrome *c* oxidase activities measured spectrophotometrically corresponded closely to those determined by the O_2 consumption method.)

Antimycin A (20 μM) was found to have no effect on the rate of TMPD oxidation by *R. capsulata* particles, and this is consistent with the generally accepted conclusion (4, 5) that endogenous cytochrome *c* is the point of entry for electrons from donors such as TMPD (and exogenous reduced cytochrome *c*) in various systems. Attention is drawn to the fact that certain mutants (viz., M4 and M7), blocked in the ability to transport electrons from exogenous reduced cytochrome *c* to O_2, retain respiratory activity with NADH and succinate (see below).

Table 3 shows that the cytochrome *c* reductase activities of Nadi-negative strains are as high as those observed in the parental Z-1 strain, and this provides additional evidence that the mutational blocks in M4, M5, and M7 must affect the oxidation of reduced, endogenous cytochrome *c*. As expected, antimycin A was found to inhibit NADH-cytochrome *c* reductase activity (90% inhibition with 20 μM antibiotic) as well as NADH oxidase activity (70% inhibition; cf. reference 6).

TABLE 2. *Oxidase activities of membrane preparations from Z-1 and derivative strains*

Strain	Oxidase activities[a] (μmol of O_2 consumed or NADH oxidized per h per mg of protein)				
	NADH[b]	NADH	Succinate	Reduced cytochrome *c*	TMPD
Z-1	3.3	1.3	0.9	2.5	5.0
M2	0.37	0.09	0.03	2.6	5.1
M4	4.5	1.5	0.9	0.04	0.05
M5	0.26	0.06	0.07	0.00	0.00
M6	1.6	0.7	0.7	2.5	4.6
M7	4.3	1.6	0.8	0.03	0.08

[a] Except as noted, the values given were determined by measuring O_2 uptake; corrections were applied for endogenous O_2 consumption and autooxidation of substrates. Each value represents the average of at least two determinations, except values for Z-1 which are the averages of about five determinations. Activities may vary as much as twofold; however, in general, relative values obtained with particles prepared and assayed in parallel do not show appreciable variability.

[b] NADH $\rightarrow$ NAD^+ determined spectrophotometrically, in the presence of air.

TABLE 3. *Cytochrome c reductase activities of membrane fragments*

Strain	Reductase activities (μmol of equine cytochrome *c* reduced per h per mg of membrane protein)	
	NADH	Succinate
Z-1	12.2	4.5
M2	0.7	0.2
M3	14.7	0.1
M4	14.5	4.2
M5	16.1	4.7
M6	20.6	6.0
M7	23.7	4.9

Oxidative phosphorylation by membrane fragments. The data in Table 4 indicate that membranes from mutants M2 and M5, which have little or no ability to oxidize NADH and succinate with O_2, show correspondingly low capacity to catalyze oxidative phosphorylation (in vitro) with these substrates. On the other hand, membranes of strains M4 and M7, though blocked in transport of electrons from reduced cytochrome *c* to O_2, show appreciable oxidative phosphorylation activities, especially with NADH.

Channeling of electron flow from NADH and succinate. The data obtained with strains M6 and M7 suggested that different mutational changes in the terminal portion of the ETS may affect the flow of electrons from NADH and succinate differentially. Although these strains show very similar cytochrome *c* reductase activities (Table 3), their NADH and succinate oxidase activities differ relative to Z-1. Thus, in M6 the NADH oxidase activity is relatively more depressed than that of succinate oxidase (Table 2); strain M7 shows a slight effect in the opposite direction. Separate tests of independently arising revertants of M5 (capable of aerobic dark growth and showing the Nadi-positive phenotype) disclosed that all four isolates manifested comparable, depressed NADH oxidase activities, whereas their succinate oxidase activities were at the level observed in the parental strain (Z-1). Differential electron-flow rates can also be inferred from the oxidative

TABLE 4. *Oxidative phosphorylation activities of membrane fragments*

Strain	Phosphorylation activities (μmol of ATP synthesized per h per mg of membrane protein)		
	Endogenous[a]	NADH[b]	Succinate[b]
Z-1	0.10	0.26	0.61
M2	0.11	0.05	0.04
M4	0.12	0.18	0.08
M5	0.12	0.02	0.02
M6	0.19	0.25	0.85
M7	0.20	0.18	0.12

[a] Rate without added substrate.

[b] Rate with substrate added, corrected for endogenous rate.

phosphorylation data of Table 4. Observations suggesting channeling of electrons from the two substrates are summarized in Table 5. It is clear that electrons originating from NADH and succinate do not necessarily share a common transport pathway to oxygen, since different lesions in the terminal portion of the ETS can inhibit electron flow from these sources differentially.

The channeling possibility was further explored by spectrophotometric experiments as follows. A suspension of membrane fragments from strain Z-1 (2 mg of protein/ml in 0.05 M KPO_4 buffer, pH 7.5) was supplemented with a saturating concentration of NADH (10 mM) and separated into two portions. These were placed in the reference and sample cuvettes, respectively, of a Cary 14 recording spectrophotometer, and, as expected, no absorbancy difference was observed in the region between 530 and 580 nm. A precisely measured volume of a succinate solution was then added to the sample cuvette (final succinate concentration, 100 mM), and an equal volume of buffer was added to the reference cell. This resulted in a clear-cut difference spectrum with a peak at 560 nm accounting for some 20% of the maximal 560-nm absorbancy observable in a dithionite-minus-ferricyanide difference spectrum, in accord with the hypothesis that there are differences in accessibility of cytochrome *b* molecules within the membrane to electrons derived from succinate as opposed to NADH.

TABLE 5. *Evidence for channeling of oxidative electron flow in membrane fragments*

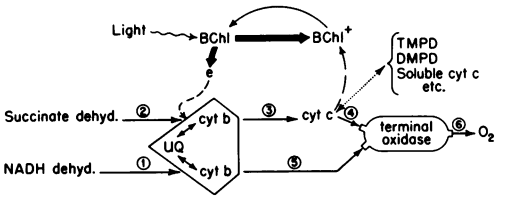
Strain	Activity ratio[a]	
	O_2 uptake	ATP synthesis
M6	1.0	0.29
Z-1	1.4	0.43
M7	2.0	1.5

[a] NADH-dependent/succinate-dependent; calculated from data of Tables 2 and 4.

DISCUSSION

The data obtained with the various mutants can be rationalized by the (minimal) scheme proposed in Fig. 1. In brief, the dark, aerobic ETS is envisaged as consisting of two "parallel" sequences which can be cross-linked at the ubiquinone-cytochrome *b* stage, and which share a common terminal oxidase. It is suggested that electrons from succinate preferentially (but not exclusively) follow the channel represented by the reaction sequence: 2, 3, 4, 6; and those from NADH, the alternative route: 1, 5, 6. Crossover between the sequences presumably can occur through the agency of ubiquinone or by electron transfer between the cytochrome *b* components.

Several aspects of the reasoning leading to the scheme warrant more detailed comment. The branched model accounts for the fact that



strain	missing step(s)
M1	1
M2	1,2
M3	2
M4	4
M5	4,5
M6	5
M7	4

FIG. 1. *Electron transport pathways responsible for energy conversion in R. capsulata. Numbered arrows represent steps in respiratory electron-transport pathways; some steps may involve the function of more than one electron carrier. BChl, bacteriochlorophyll; cyt, cytochrome; UQ, ubiquinone; e, electron. The strains listed carry genetic defects which render the indicated steps inactive. The arrangement of cytochromes b and UQ in the diagram is intended to suggest the mechanism of electron channeling; see text for further details. TMPD, DMPD, etc. are thought to interact with the ETS via membrane-bound cytochrome c. The upper part of the diagram depicts, in simplified form, the light-driven cyclic electron-flow system responsible for anaerobic photophosphorylation: heavy arrows indicate primary events consequent on absorption of light by BChl; cytochromes b and c are represented as being shared by the photosynthetic and dark aerobic systems—sharing of certain catalysts could account for inhibition of respiration by illumination and various other interactions between the alternative energy-transducing mechanisms (see 2, 9, 14).*

certain mutant strains (M4 and M7) blocked in the transport of electrons from exogenous donors such as TMPD, etc. to O_2 retain the capacity for dark aerobic growth. The possibility that absence of oxidase activity with the exogenous electron donors noted could be due simply to inaccessibility of such donors to the endogenous cytochrome *c* of the particles is considered unlikely on two grounds, namely (i) reductase activity with exogenous cytochrome *c* serving as acceptor is unaffected by the Nadi-negative type mutational blocks, and (ii) separate tests showed that treatment of M4 membrane fragments with a variety of detergents did not elicit oxidase activity with TMPD as substrate.

Strain M5, which is devoid of oxidase activities, is represented as a double mutant blocked in both branches (steps 4 and 5). In accord with this interpretation is the observation that such mutants were found to occur with lower frequency than mutants of the M4 type. Moreover, the model predicts, as observed, that M5 should give rise to two distinct classes of revertants capable of dark aerobic growth, namely, a Nadi-positive class which has regained the ability to oxidize TMPD but still shows depressed oxidase activity with NADH (M6), and a Nadi-negative class (M7) with properties similar to those of M4.

The branched scheme also lends itself to explanation of the observations indicating differential channeling of electrons derived from NADH as opposed to those from succinate. Current discussions (1, 3) on the fine structure of energy-converting membrane systems emphasize the accumulating evidence for a precise topological orientation of electron transfer catalysts, and on this basis a functional distinction among cytochrome *b* molecules within the same membrane matrix can be entertained. This is, in fact, suggested by the spectrophotometric observations described which indicate that a substantial fraction of the total membrane-bound cytochrome *b* is reducible by succinate, but not by NADH. Similar findings for *Rhodospirillum rubrum* membranes have been reported by Horio et al. (4).

The nature of the lesions which block electron flow at steps 4 and 5 in the various mutant strains isolated is still unknown. These blocks could be associated with still unidentified electron carriers or with the terminal oxidase itself. The chemical identity of the latter in *R. capsulata* also remains unclear; an exhaustive study by Klemme and Schlegel (6), however, appears to rule out *a*-type cytochromes and catalysts of the cytochromoid *c* variety. We suggest that the branches of the ETS rejoin at a common terminal oxidase because the NADH oxidase activities of membranes from strains Z-1, M4, and M6 were found to show identical sensitivity to KCN (90% inhibition with 1 mM KCN). The possible existence of two terminal oxidases with similar sensitivities to KCN, however, cannot be excluded at present. A number of (nonphotosynthetic) bacterial species, in fact, have branched ETSs and two kinds of terminal oxidases (13). Even if the NADH and succinate oxidase activities of *R. capsulata* are mediated by (two) specific terminal oxidases, interpretation of the data presented would not be substantially altered. Thus, crossover between the two sequences prior to the oxidases would still have to be assumed, since (i) exogenous cytochrome *c* is reduced with both NADH and succinate as substrates and (ii) succinate can be oxidized in the absence of electron flow between cytochrome *c* and the terminal oxidase, as seen in strains M4 and M7 (Table 2; compare results with succinate, reduced exogenous cytochrome *c*, and TMPD).

Klemme and Schlegel (6) have compared the oxidase activities of aerobic, dark and photosynthetically grown wild-type *R. capsulata* and found no qualitative differences between the systems from cells grown in these two modes (see also reference 11, for a summary of literature on this and related questions). In view of this and the internal consistency of our own results (especially with M5 and its revertants), we regard the study of membranes from photosynthetically grown cells of respiratory-deficient mutants as a valid approach to analysis of the oxidase components of the ETS.

It can be anticipated that the mutants described will prove to be useful for study of a variety of problems relating to the energy metabolism of purple bacteria. The model advanced for the ETS of *R. capsulata* provides a basis for further studies by the biochemical genetics approach, especially in that it suggests the types of mutants which should be sought to clarify unresolved questions (e.g., whether or not there is more than one terminal oxidase). Certain of the mutants have been particularly helpful for study of the mechanism by which O_2 inhibits bacteriochlorophyll synthesis (see reference 8), and it is likely that other mutants will permit an incisive attack on the refractory problem of whether or not certain catalysts are shared in the photosynthetic and aerobic electron-transport pathways.

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ADDENDUM IN PROOF

An investigation by Baccarini, Melandri, Zannoni, and Melandri (personal communication) on the electron transport system of aerobically grown *R. capsulata* indicates the presence of two terminal oxidases, and they propose a branched scheme very similar to that suggested by our study of electron transport mutants.

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