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Essay

On the natural selection and evolution of the aerobic phototrophic bacteria

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Abstract

This contribution gives a brief survey of the short history since the discovery of the aerobic phototrophic bacteria to focus on a general evolutionary scenario. Most of the citations are of reviews that have covered the earlier literature and to which the reader is directed at appropriate places in the following text. The data summarized in these reviews are supplemented with information from recent or otherwise key primary publications in order to support a synthesis that addresses vexing questions about bacteria containing photosynthetic pigment–protein complexes, but which are incapable of growth with light as the sole, or even the major source of energy.

Abbreviations: APB – aerobic phototrophic bacteria; BChl – bacteriochlorophyll

Introduction

Purple photosynthetic bacteria conserve light energy in a cycle of electron transfer reactions dependent on bacteriochlorophyll (BChl) and membrane protein complexes, that translocate protons across the cytoplasmic membrane (Figure 1; Prince 1990; Okamura et al. 2000). Because of the cyclic nature of these electron transfer reactions, this type of photosynthetic energy transduction in principle does not require an external electron donor, in contrast to the cyanobacteria and eukaryotic chloroplasts, which oxidize water and evolve oxygen. Hence the terms 'anoxygenic' (as in the purple photosynthetic bacteria) and 'oxygenic' (cyanobacteria and chloroplasts) that are often used to designate these photosynthetic processes (Madigan et al. 2000). Purple photosynthetic bacteria have figured prominently in our understanding of fundamental aspects of photosynthesis, ranging from the first and subsequent high resolution structures of integral membrane proteins (Lancaster et al. 1995; Papiz et al. 1996), through the molecular biology of light- and oxygen-regulated gene expression (Bauer and Bird 1996; Oh and Kaplan 2001), to ecophysiological activities in natural environments (Van Gemerden and Mas 1995; Overmann et al. 1999).

For decades it was believed that purple bacterial photosynthesis is essentially limited to anaerobic environments because of the inhibitory effect of oxygen on the expression of photosynthesis genes in most purple photosynthetic bacteria and their typically anaerobic habitats (Cohen-Bazire et al. 1957; Pfennig 1967; Bauer and Bird 1996; Oh and Kaplan 2001). However, a large variety of obligately respiratory species that resemble purple photosynthetic bacteria has been discovered, starting in the late 1970s (Shiba et al. 1979) and contributing in subsequent years (Shimada 1995; Yurkov and Beatty 1998; Kolber et al. 2001). These organisms have puzzled microbiologists, and there has been a debate (and perhaps resultant confusion) about an appropriate common name to describe them as a group. For example, the appellations quasi-photosynthetic bacteria, erythrobacteria, photosynthetic rhizobia or aerobic anoxygenic phototrophic

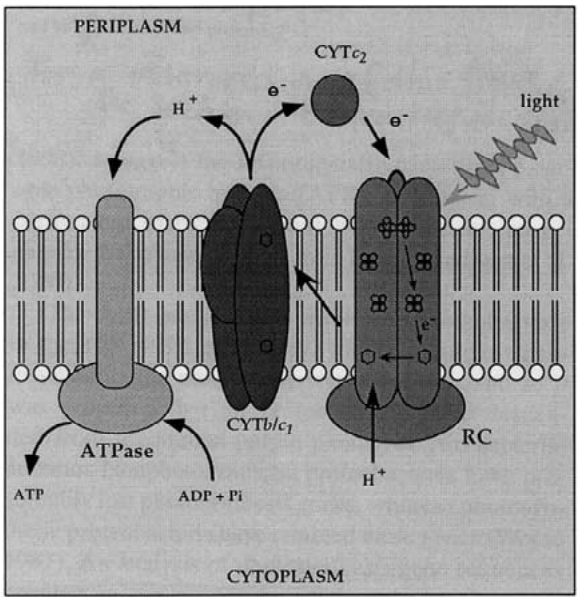

Figure 1. Representation of a membrane-imbedded, generic purple bacterial photosynthetic reaction center and related redox catalysts. Light-harvesting complexes are not shown. RC – the reaction center; CYTb/c_1 – the cytochrome b/c_1 complex; ATPase – the ATP phosphohydrolase complex; CYTc_2 – a mobile periplasmic cytochrome; e^- and H^+ – electrons and protons. For a color version of this figure, see section in the front of the issue.

bacteria have been used (Gest 1993; Shimada 1995; Fleischman and Kramer 1998; Yurkov and Beatty 1998). I suggest the taxonomically trivial name aerobic phototrophic bacteria (APB), in keeping with a current use of the term phototroph, namely 'an organism that obtains energy from light' (Madigan et al. 2000).

The APB are grouped within the proteobacteria in the 16S rRNA phylogeny, which intermixes photosynthetic and nonphotosynthetic species, and so it was proposed that all proteobacteria have descended from a common purple photosynthetic bacterial ancestor Nonphotosynthetic proteobacteria have presumably lost photosynthesis genes, whereas photosynthetic proteobacteria have retained these genes (Woese 1987). An analysis of photosynthesis gene sequences resulted in the proposal that a precursor of the purple photosynthetic BChl *a* biosynthetic pathway is ancestral to the homologous pathways of all extant photosynthetic organisms (Xiong et al. 2000).

The large number and variety of APB isolates from throughout the world indicate that they are not a rare oddity. Instead, the APB are a class of bacteria with metabolic properties that enable them to thrive in competition with their physiologically disparate proteobacterial relatives.

Discussion

The APB and purple photosynthetic bacteria share a highly conserved photosynthetic apparatus (Figure 1); this conservation exists not only in pigment–protein complex composition and amino acid sequences of proteins, but also in the chromosomal organization of key photosynthesis genes (Nishimura et al. 1996; Yurkov and Beatty 1998). However, in contrast to the related purple photosynthetic bacteria that grow robustly by photosynthesis in the absence of oxygen (with cell carbon provided either by assimilation of organic compounds or reduction of carbon dioxide with inorganic electron donors), the APB are unable to use light as the primary source of energy for anaerobic growth. Instead, the APB require oxygen or other terminal electron acceptors to respire organic compounds, which are essential for growth. Short-term exposure of dark-grown, aerobic APB cultures to light resulted in increases in cell numbers (from ~10% to almost a factor of two), indicating that these organisms are capable of using light energy to supplement an otherwise heterotrophic metabolism (Shimada 1995; Yurkov and Beatty 1998). Biochemical experiments on preparations from APB cells were interpreted as showing that the redox state of electron carriers (such as quinones and cytochromes) that participate in both photosynthetic and respiratory redox reactions affects the efficiency of light-dependent electron and proton transfers (Yurkov and Beatty 1998; Schwarze et al. 2000; Candela et al. 2001). That is, when the quinone and cytochrome pools that are shared between these two bioenergetic processes are electrochemically reduced (as would be the case *in vivo* if the rates of oxidation of organic carbon electron donors exceeded rates of reduction of terminal electron acceptors) light energy may excite BChl molecules, but cyclic electron transfer (Figure 1) would encounter a bottleneck because of the absence of sufficient numbers of oxidized quinones and/or cytochromes. Other experiments on APB laboratory cultures and natural samples from ocean surface waters indicated a lower content of photosynthetic complexes when cells have access to high concentrations of organic substrates (Shimada 1995; Kolber et al. 2001; Suyama et al. 2002). Thus, it seems that APB are adapted to oligotrophic environments in which the preferred mode of energy conservation

is respiration of organic compounds, although these bacteria are capable of using photosynthetic redox reactions to supplement their heterotrophic metabolism.

The properties of the APB described above were puzzling because of differences from the related purple photosynthetic bacteria, which gave rise to the question: why would the photosynthesis genes be retained in the APB when the major fraction of energy is obtained by respiration of organic compounds, with only an estimated 10–50% of the cellular energy budget provided by photosynthetic electron transfer and associated proton translocation? Recent publications provide a potential answer to this question, as discussed below.

Kolber et al. (2000) discovered the global distribution of APB in ocean surface waters, and followed up on this report with a direct enumeration of APB in this oligotrophic environment (Kolber et al. 2001). Approximately 10% of *all* (phototrophic and heterotrophic) microbial cells at a site in the North Pacific Ocean were found to contain BChl, and indirect evidence indicated the likelihood of much higher numbers at other ocean surface locations. These presumed APB were photosynthetically active on the basis of a BChl-specific infrared fluorescence kinetics measurement technique (Kolber et al. 1998). Other experiments on natural samples and laboratory cultures indicated an inverse correlation between organic carbon availability and photosynthetic competence (Shimada 1995; Kolber et al. 2001).

Collectively, these results confirm that the APB are aerobic heterotrophs and differ from their proteobacterial relatives that have either completely lost photosynthetic capability or are able to grow with light as the sole source of energy. Instead, the APB appear to have retained a residual photosynthetic competence that is of greatest significance when they are starved for organic carbon electron donors, and which provides a selective advantage in aerobic, transiently illuminated oligotrophic ecosystems such as ocean surface waters. This advantage allows the APB to persist as a globally huge population when numbers are integrated over the $\sim$75% of the earth's surface covered by water. The restriction of maximal BChl synthesis to APB cells that are organic-carbon-starved limits the greatest production of the potentially toxic BChl (the combination of BChl, light and oxygen produces chemically reactive singlet oxygen that damages cell components; Cogdell and Frank 1987) to oligotrophic situations in which photosynthesis provides an advantage in competition with strictly heterotrophic organisms.

Other curious attributes that are characteristic of APB are a high carotenoid content, and the fact that even low intensities of continuous illumination greatly inhibit BChl synthesis and the formation of the photosynthetic apparatus (Shimada 1995; Yurkov and Beatty 1998). This pronounced light inhibition of photocomplex synthesis seemed to be counter-intuitive and gave rise to a second question: why turn off the production of the light energy transduction apparatus in response to the availability of light? This thorny question is addressed as follows.

The production of the photosynthetic apparatus in purple photosynthetic bacteria is partially repressed by high light intensities; as in the APB, this repression is controlled at least in part at the level of transcription (Bauer and Bird 1996; Nishimura et al. 1996; Oh and Kaplan 2001). Thus, this light response appears to have evolved in a photosynthetic ancestor in common to all proteobacteria, and so could be manifested in the APB as an extreme modification of a pre-existing genetic regulatory mechanism. Like the organic carbon control (see above), this evolutionary development is consistent with the aerobic lifestyle of the APB, in which the synthesis of BChl in the presence of oxygen and high light intensity would be dangerous and may be lethal. Perhaps the APB have solved the problem of survival in aerobic, illuminated oligotrophic environments by balancing on a knife-edge between BChl synthesis (to enhance survival during organic carbon starvation) and cessation of BChl production (to minimize toxicity during solar irradiation), by exaggerating a previously evolved light regulatory mechanism, such that BChl synthesis is induced during darkness and completely repressed during illumination. In other words, the photosynthetic complexes produced at night (in greatest amounts during nutrient deprivation) function to supplement APB respiratory electron transport during the day. Sunlight strikes a site on the earth's surface only 50% of the time on average, and so there is sufficient time to produce BChl during the night for subsequent use during the day. This is because, once formed and bound in photosynthetic protein complexes, BChl is not degraded (Yurkov and Beatty 1998), and microbial growth rates in oligotrophic aquatic environments may be one cell division or less per day (Furnas 1990; Overmann et al. 1991). With such slow growth rates, the BChl content per APB cell would not be significantly decreased during illuminated cell growth and division. Caroten-

oids provide a light-harvesting function in a variety of photosynthetic organisms that is most important at aquatic depths penetrated by light wavelengths of only about 450–550 nm (Pfennig 1967; Cogdell and Frank 1987; Overmann et al. 1991). However, in APB that contain large amounts of carotenoids, most of these carotenoids do not transfer light energy to the reaction center (Yurkov and Beatty 1998). Perhaps this high content of carotenoids is an adaptation to aerobic environments to provide protection from singlet oxygen (Cogdell and Frank 1987).

Summary

The foregoing gives a view of the evolution and global dispersion of the APB, driven by natural selection. These suggestions are consistent with current thoughts on the evolution of photosynthesis (Woese 1987; Xiong et al. 2000) and the fundamental principle that organisms evolve by modification of preexisting properties, to cope with selective pressures that arise from environmental changes (Presti and Delbrück 1978). This view is summarized as a speculative model in the following paragraphs.

The APB diverged from a purple photosynthetic-like bacterial ancestor by becoming dependent on respiration of organic compounds for survival, losing (or not developing) oxygen repression of the photosynthetic apparatus, and producing large amounts of carotenoids. These changes arose after the accumulation of oxygen in the earth's biosphere, which allowed heterotrophic respiration with oxygen as the terminal electron acceptor to become a primary mechanism for energy generation. The retention of photosynthesis in the APB was selected for in oligotrophic, illuminated aquatic environments, where it provided a way to survive organic carbon starvation. Although the APB produce a photosynthetic apparatus under aerobic conditions, the simultaneous presence of high light intensity would create a 'love–hate' relationship in which absorption of light by BChl would provide not only energy from photosynthesis, but also the toxic singlet oxygen. (This may be the major selective advantage afforded by a relatively high carotenoid content in such species.) The APB adapted to these selective pressures by induction of BChl synthesis during the night (when they were safe from light) and repression of BChl synthesis during the day, when they used BChl dangerously to harvest light energy. Therefore, I see the APB as existing on a cusp between

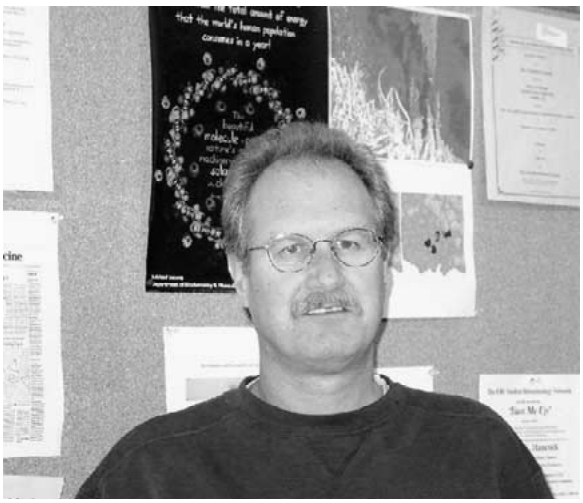

Figure 2. The author (J. Thomas Beatty).

aerobic photosynthetic and heterotrophic energy transduction, the relative contributions of which are shifted by the availability of organic carbon substrates.

Evolution has produced the purple photosynthetic bacteria, the non-photosynthetic proteobacteria and the APB, all of which appear to have diverged from a single progenitor. Natural selection has given rise to each of these evolutionary products as a result of alternative adaptations to advantages and problems presented by various environments. It is possible that the reason why the APB are so widespread is because these bacteria multiplied to reach globally massive numbers in the open ocean environment after the accumulation of molecular oxygen on Earth (i.e., recently relative to the origin of photosynthesis; Des Marais 2000), and then spread to other ecosystems (Kolber et al. 2001).

Future prospects

Analyses of APB genome sequences will undoubtedly shed light on their evolutionary history, including complexities arising from species-specific properties, and carefully controlled experiments would help to evaluate the validity of other ideas put forth in this paper. For example, the supposed *in vivo* electrochemical reduction of shared photosynthetic and respiratory electron and proton carriers, when APB cells encounter high concentrations of readily oxidized organic substrates, implies that the rate of entry of electrons into the respiratory chain (catalyzed mainly by NADH dehydrogenase and succinate de-

hydrogenase) greatly exceeds the catalytic activity of the terminal oxidase(s). Biochemical comparisons of such activities in membranes from purple photosynthetic *Rhodobacter* species (Hochkoeppler et al. 1995; Daldal et al. 2001) and the APB *Roseobacter denitrificans* (Candela et al. 2001) indicate differences between these species that are consistent with this notion (D. Zannoni, personal communication).

The lower amounts of photosynthetic complexes in APB cells grown in media containing high concentrations of one or more organic compounds, relative to the amounts in cells cultivated in media containing low concentrations, indicates that genetic regulatory mechanisms repress photosynthesis gene expression in response to the redox state of respiratory components. In this connection, recent work on *Rhodobacter sphaeroides* implicates components of the respiratory chains in this purple photosynthetic bacterium in such a regulation (Daldal et al. 2001; Oh and Kaplan 2001).

Finally, a better understanding of how light intensity is transduced in this genetic regulatory process may arise from comparative studies of APB and purple photosynthetic bacteria. I note that light absorption by photosynthetic complexes and organic carbon oxidation in respiration both lead to redox reduction of shared electron and proton carriers, and so a related mechanism may be operative in these two regulatory processes. A recent report on the involvement of a bacteriophytochrome in the regulation of photosynthetic complexes (Giraud et al. 2002) raises additional interesting questions.

As requested by the Editors, Figure 2 shows a photograph of the author.

Acknowledgments

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References

Bauer CE and Bird TH(1996) Regulatory circuits controlling photosynthesis gene expression. Cell 85: 5–8

Candela M, Zaccherini E and Zannoni D (2001) Respiratory electron transport and light-induced energy transduction in membranes from the aerobic photosynthetic bacterium *Roseobacter denitrificans*. Arch Microbiol 175: 169–177

Cogdell RJ and Frank HA (1987) How carotenoids function in photosynthetic bacteria. Biochim Biophys Acta 895: 63–79

Cohen-Bazire G, Sistrom S and Stanier RY (1957) Kinetic studies of pigment synthesis by non-sulphur purple bacteria. J Cell Comp Physiol 49: 25–68

Daldal F, Mandaci S, Winterstein C, Myllykallio H, Duyck K and Zannoni D (2001) Mobile cytochrome c_2 and membrane-anchored cytochrome c_y are both efficient electron donors to the cbb_3- and aa_3-type cytochrome *c* oxidases during respiratory growth of *Rhodobacter sphaeroides*. J Bacteriol 183: 2013–2024

Des Marais DJ (2000) Evolution: when did photosynthesis emerge on Earth? Science 289: 1703–1705

Fleischman D and Kramer D (1998) Photosynthetic rhizobia. Biochim Biophys Acta 1364: 17–36

Furnas MJ (1990) *In situ* growth rates of phytoplankton: approaches to measurement, community and species growth rates. J Plankton Res 12: 1117–1151

Gest H (1993) Photosynthetic and quasi-photosynthetic bacteria. FEMS Microbiol Lett 112: 1–6

Giraud E, Fardoux J, Fourrier N, Hannibal L, Genty B, Bouyer P, Dreyfus B and Verméglio A (2002) Bacteriophytochrome controls photosystem synthesis in anoxygenic bacteria. Nature (London) 417: 202–205

Hochkoeppler A, Jenney FE, Lang SE, Zannoni D and Daldal F (1995) Membrane-associated cytochrome c_y of *Rhodobacter capsulatus* is an electron carrier from the cytochrome bc_1 complex to the cytochrome *c* oxidase during respiration. J Bacteriol 177: 608–613

Kolber ZS, Prasil O and Falkowski PG (1998) Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. Biochim Biophys Acta 1367: 88–106

Kolber ZS, Vandover CL, Niederman RA and Falkowski PG (2000) Bacterial photosynthesis in surface waters of the open ocean. Nature (London) 407: 177–179

Kolber ZS, Plumley FG, Lang AS, Beatty JT, Blankenship RE, Vandover CL, Vetriani C, Koblizek M, Rathgeber C and Falkowski PG (2001) Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. Science 292: 2492–2495

Lancaster CRD, Ermler U and Michel H (1995) The structures of photosynthetic reaction centers from purple bacteria as revealed by X-ray crystallography. In: Blankenship RE, Madigan MT and Bauer CE (eds) Anoxygenic Photosynthetic Bacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands

Madigan MT, Martinko JM and Parker J (2000) Brock Biology of Microorganisms. Prentice Hall, Upper Saddle River, New Jersey

Nishimura K, Shimada H, Ohta H, Masuda T, Shioi Y and Takamiya K-I (1996) Expression of the *puf* operon in an aerobic photosynthetic bacterium, *Roseobacter denitrificans*. Plant Cell Physiol 37: 153–159

Oh J-I and Kaplan S (2001) Generalized approach to the regulation and integration of gene expression. Mol Microbiol 39: 1116–1123

Okamura MY, Paddock ML, Graige MS and Feher G (2000) Proton and electron transfer in bacterial reaction centers. Biochim Biophys Acta 1458: 148–163

Overmann J, Beatty JT, Hall KJ and Pfennig N (1991) Characterization of a dense, purple sulfur bacterial layer in a meromictic salt lake. Limnol Oceanogr 36: 846–859

Overmann J, Hall KJ, Northcote TG and Beatty JT (1999) Grazing of the copepod *Diaptomous connexus* on purple sulphur bacteria in a meromictic salt lake. Environ Microbiol 1: 213–221

Papiz MZ, Prince SM, Hawthornthwaite-Lawless AM, McDermott G, Freer AA, Isaacs NW and Cogdell RJ (1996) A model for the

photosynthetic apparatus of purple bacteria. Trends Plant Sci 1: 198–206

Pfennig N (1967) Photosynthetic bacteria. Annu Rev Microbiol 21: 285–384

Presti D and Delbrück M (1978) Photoreceptors for biosynthesis, energy storage and vision. Plant Cell Environ 1: 81–100

Prince RC (1990) Bacterial photosynthesis: from photons to Δp. Bacteria 12: 111–149

Schwarze C, Carluccio AV, Venturoli G and Labahn A (2000) Photo-induced cyclic electron transfer involving cytochrome bc_1 complex and reaction center in the obligate aerobic phototroph *Roseobacter denitrificans*. Eur J Biochem 267: 422–433

Shiba T, Simidu U and Taga N (1979) Distribution of aerobic bacteria which contain bacteriochlorophyll *a*. Appl Environ Microbiol 38: 43–45

Shimada K (1995) Aerobic anoxygenic phototrophs. In: Blankenship RE, Madigan MT and Bauer CE (eds) Anoxygenic Photosynthetic Bacteria, pp 105–122. Kluwer Academic Publishers, Dordrecht, The Netherlands

Suyama T, Shigematsu T, Suzuki T, Tokiwa Y, Kanagawa T, Nagashima KVP and Hanada S (2002) Photosynthetic apparatus in *Roseateles depolymerans* 61A is transcriptionally induced by carbon limitation. Appl Environ Microbiol 68: 1665–1673

Van Gemerden H and Mas J (1995) Ecology of phototrophic sulfur bacteria. In: Blankenship RE, Madigan MT and Bauer CE (eds) Anoxygenic Photosynthetic Bacteria, pp 105–122. Kluwer Academic Publishers, Dordrecht, The Netherlands

Woese CR (1987) Bacterial evolution. Microbiol Rev 51: 221–271

Xiong J, Fischer WM, Inoue K, Nakahara M and Bauer CE (2000) Molecular evidence for the early evolution of photosynthesis. Science 289: 1724–1730

Yurkov V and Beatty JT (1998) Aerobic anoxygenic phototrophic bacteria. Microbiol Mol Biol Rev 62: 695–724.