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Evolution of cytochromes and photosynthesis

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The following is an outline of the direction of research into the evolutionary origins of photosynthesis as revealed by the study of cytochromes *c*. Determination of the numbers of kinds of cytochromes, their structures, their functional roles, and their distribution are the principal kinds of data being collected and analyzed. A hypothesis on the origin of photosynthesis is presented.

Two recent advances have added a new dimension to study of the structure/function relationship and the evolution of cytochromes and photosynthesis. One is the solution of the crystal structures of photosynthetic reaction centers and the other is the genetic manipulation of cytochrome c_2. The crystal structures of reaction centers from *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* illustrate a fundamental difference in that there is an extra subunit in *Rps. viridis*, the 40 kDa tetraheme cytochrome *c* [1]. This subunit appears to be unnecessary because of its absence in *Rb. sphaeroides* [2]. On the other hand, the majority of purple bacterial species do appear to have this bound cytochrome [3]. It is likewise the case that most non-sulfur purple bacteria have a cytochrome c_2, which is able to interact with either the simple reaction center (second-order rate constants about 10^8 to 10^9 M^{-1} s^{-1}) [4,5] or with the bound cytochrome in the more complex reaction center (second-order rate constant about 10^6 M^{-1} s^{-1}) [6]. Electron transfer from the highest-potential heme 3 of the bound cytochrome, which is nearest to the reaction center special pair of *Rps. viridis*, is about $2 \cdot 10^6$ s^{-1} and from the next to highest potential heme 2, which is the third most distant, is about $3 \cdot 10^5$ s^{-1} [7,8]. Limiting first-order rate constants for reaction of cytochrome c_2 with the simple reaction centers are about 10^3–10^6 s^{-1} [4,5], which is comparable to the values for the bound cytochrome in *Rps. viridis*.

What is the function of the bound cytochrome in the reaction center and why is it not present in all species? One possibility is that it might facilitate reaction with soluble redox proteins other than cytochrome c_2 in those species in which the latter is absent. To address this question, we have examined the reaction of various redox proteins with the *Rps. viridis* reaction center. *Rps. viridis* cytochrome c_2 is the most reactive of a dozen proteins tested with ascorbate-reduced reaction center cytochrome. A negative charge on the reaction center cytochrome was determined by studying the effect of ionic strength on photooxidation of soluble proteins, consistent with reaction at the 300 mV heme 2 (which should have a net charge of about minus 3 near the site of reduction based on examination of the three-dimensional structure). Other soluble redox proteins react as much as three orders of magnitude more slowly with reaction centers and some show unfavorable electrostatic interactions. Under these conditions, the reaction center cytochrome does not appear to facilitate interaction with these proteins. However, other conditions should also be considered. We have not yet measured reaction with the 370 mV heme 3, which is adjacent to the reaction center chlorophyll species. This experiment should be performed without ascorbate and with reduced electron donors which have potentials higher than that of heme 2. The higher potential donors, such as the 405 mV *Rhodocyclus tenuis* cytochrome *c*-553 and the 370 mV *Rb. sphaeroides* cytochrome c_2 may then be more effective reductants for heme 3 than with the lower-potential heme 2, although heme 3 appears to be surrounded by positive charge (of magnitude about plus 3), which would repel the *Rc. tenuis* and *Rb. sphaeroides* cytochromes and result in a lower rate constant. The still lower potential (10 mV) heme 4, which is spatially located between hemes 2 and 3, may be the preferred site of interaction of very low potential donors such as *Ectothiorhodospira halophila* HiPIP (50

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mV) or cytochrome c-551 (58 mV). Heme 4 appears to be surrounded by a net charge of about plus 3, which would be favorable for interaction with these negatively charged proteins. We have not yet probed this site, which may require chemical substitution of a lower potential quinone for the measurement. The lowest-potential (−60 mV) heme 1 is also the most distant and least likely to mediate electron transfer, although it is the most exposed to solvent of all the four hemes. This heme is also surrounded by the largest net charge, which is about minus 5. Until these studies are completed, we tentatively conclude that the bound reaction center cytochrome does not facilitate electron transfer with soluble proteins. Alternatively, each reaction center species may be individually tailored to interact with specific donors. This possibility will be addressed in a future study with *Chromatium vinosum* or other reaction centers which do not have an accompanying cytochrome c_2.

Our current hypothesis is that the purpose of the bound cytochrome is to rapidly reduce the photooxidized special pair chlorophyll before back reaction from quinone can occur, thus allowing the less efficient soluble donors such as HiPIP, cytochrome *c*-553, or copper proteins to mediate between the cytochrome bc_1 complex and reaction center. Acquisition of the highly reactive cytochrome c_2 appears to have been recent and to have made the bound cytochrome superfluous and subject to deletion. This may be why there are relatively few species which have both cytochrome c_2 and bound reaction-center cytochrome. One may ask why the *Rps. viridis* cytochrome c_2 has not replaced the bound cytochrome, such as presumably happened in *Rb. sphaeroides*. We have also studied kinetics of reaction of soluble proteins with the *Rb. sphaeroides* reaction center and find that horse and *Rb. sphaeroides* cytochromes c_2 are about two orders of magnitude or more reactive than other proteins tested, including *Rps. viridis* cytochrome c_2. This work is not yet complete, but suggests that each donor/acceptor pair has evolved to interact most effectively in each species.

Genetic studies have contributed to our knowledge of the role of cytochrome c_2 in photosynthetic electron transfer. Cytochrome c_2 appears to be unnecessary in at least one species which has a simple reaction center in that its gene has been deleted in *Rhodobacter capsulatus* without deleterious effects on photosynthesis [9]. The analogous experiment performed with *Rb. sphaeroides* resulted in loss of photosynthetic capability, although spontaneous suppressor mutants regained the capacity to grow in the light [10]. This is apparently due to expression of an isozyme of cytochrome c_2 [11], which is as different as any species of cytochrome c_2. Is there a constitutive membrane-bound cytochrome c_2 isozyme in *Rb. capsulatus*, which is too divergent to recognize by gene hybridization? How many of the apparently cytochrome c_2 minus species have a cytochrome c_2 gene which is not normally expressed (such as the *Rb. sphaeroides* isozyme) or which is normally membrane bound? We expect that a few more species will be found to have at least one cytochrome c_2 gene, but that the majority of species probably do not have a cytochrome c_2 at all. The high potential cytochrome reported in *C. vinosum* [12,13] is probably not a cytochrome c_2, but is more likely related to one of the *Pseudomonas* cytochromes as discussed below.

Determination of not only the numbers of cytochrome c_2 isozymes in each species but also which species have cytochromes c_2 appears simple in comparison with establishing how many types of totally unrelated cytochromes there are, and how they might be involved in photosynthesis. Prior to crystal structure analysis of the *Rps. viridis* reaction center, there were only three known kinds of unrelated *c*-type cytochromes (class 1 = cytochrome c_2, mitochondrial cytochrome *c*, and smaller bacterial relatives; class 2 = cytochrome c' and low spin isozymes; and class 3 = *Desulfovibrio* cytochrome c_3,) [14]. The tetraheme reaction center cytochrome subunit is now representative of a fourth unrelated structure which has covalently bound heme and appears to have arisen by two successive gene doublings of a 10 kDa monomer. There are other multiheme cytochromes, which appear to have independently arisen by gene doubling, such as the diheme cytochrome c_4 [15] and flavocytochrome *c* [16] (which are class 1 cytochromes). Cytochrome c_3, which has four hemes, was doubled twice, yet the resulting protein is only 14 kDa [17]. A low-potential cytochrome *c* from *Rb. sphaeroides*, which has two hemes at opposite ends of a 16 kDa protein, was apparently not doubled [16]. *Pseudomonas stutzerii* cytochrome *c*-552 is a 26 kDa diheme protein [16,18], which shows no evidence for gene doubling and there is no obvious similarity to the *Rb. sphaeroides* cytochrome. *Pseudomonas aeruginosa* cytochrome *c* peroxidase is a 34 kDa diheme protein [19], which does not appear to be related to either the *Rb. sphaeroides* or the *P. stutzerii* cytochromes. Until the three-dimensional structures are determined, we cannot say whether these three diheme cytochromes are truly unique or distantly related to one another or whether they are related to the reaction-center cytochrome. We have sequence evidence for the presence of the above two *Pseudomonas* proteins in the widely divergent purple bacteria *Rc. tenuis* and *Rb. capsulatus* [16], but have no functional role for them.

There is no obvious sequence similarity between cytochromes c_1 and *f*, although they are components of related cytochrome bc_1 and b_6f complexes [20–22], which are integral components of photosynthetic pathways and each contain a Rieske iron-sulphur centre. Cytochromes c_1 and *f* have about the same molecular masses (approx. 30 kDa) and have only one heme,

located near the N-terminus. Their sixth ligands are apparently located in different parts of the sequences. At present they appear to be representative of at least one more unrelated structure. Based on the above evidence, it is likely that the number of kinds of unrelated *c*-type cytochromes is likely to double in the future.

How might the information discussed up to this point be used to reconstruct evolution in photosynthetic bacteria and what other types of data are being used? We had previously shown that evolutionary trees for cytochrome c_2 and for r-RNA based on matrices of sequence identities were generally invalid when applied to a wide range of species because of convergence [23]. Insertions and deletions, identified through comparison of three-dimensional structures, are less susceptible to convergence and give a better representation of relationships. This is especially true for the cytochromes c_2. However, multiple genes and gene transfer are additional complicating factors, which have been difficult to assess. As noted above, we are just now beginning to learn about multiple cytochrome c_2 genes in purple bacteria. The numbers of kinds of cytochromes, their functions, and how they are distributed among the species may be as useful or more so in understanding relationships than quantitative sequence data for the above reasons.

Using these kinds of data, we can speculate on the evolutionary relationships among photosynthetic bacteria. We know that purple bacterial reaction centers are homologous to plant Photosystem II [24] and green bacterial reaction centers are expected to be homologous to plant Photosystem I because they have similar characteristics [25]. It is possible that cyanobacteria evolved by acquisition of early green and purple bacterial reaction centers [25] just as *Chloroflexus* is likely to be a chimera composed of purple bacterial reaction centers and green bacterial light-harvesting complexes. Evidence for common origin of all three major kinds of phototrophic bacteria may be seen in the homology among green bacterial cytochrome *c*-555 [26], cyanobacterial and algal cytochrome *c*-553 [27,26], and cytochrome *c*-551 from the purple bacterium, *E. halophila* [29]. It has not yet been proven, but all three of the above cytochromes may have a similar role as electron donor to reaction centers. Still more interesting is that these cytochromes are most similar to *Pseudomonas mendocina* cytochrome c_5 [30]. This may be coincidental. However, other *Pseudomonas* redox proteins are found in photosynthetic bacteria besides cytochrome c_5 homologs, which strengthens this analogy. *Chloroflexus* contains azurin homologs called auracyanin (Ref. 31; Van Beeumen et al., unpublished data). The chloroplast and cyanobacterial copper protein plastocyanin is homologous to azurin [32]. *C. vinosum* has a cytochrome c_4 [16] and spectral evidence suggests its presence in *Rc. gelatinosus, Rc. tenuis*, and *Thiocapsa pfennigii*. *Rc. gelatinosus* and *Rc tenuis* have a cytochrome *c*-551 homologous to *Pseudomonas* cytochrome *c*-551 [33]. *Rc. tenuis* has a (*P. stutzerii* type) cytochrome *c*-552 [16], and *Rb. cansulatus* has a cytochrome *c*-556 [16] related to *Pseudomonas* cytochrome *c* peroxidase. The *Azotobacter / Pseudomonas* type of (7Fe, 8S) ferredoxin [34] appears to be widespread in the purple bacteria (Refs. 35, 36; Van Beeumen et al, unpublished data). Rather than acquisition through gene transfer, the distribution and relationships among the above proteins suggest that *Pseudomonas* predated modern photosynthetic organisms. We suggest that photosynthesis may have originated with *Pseudomonas* and many of the *Pseudomonas* proteins may be vestigial in the purple bacteria. Cytochrome c_5 and homologs may provide a unique record of photosynthetic development not available with any other gene which has the same function in all photosynthetic bacterial families. However, we need sequences, three-dimensional structures and genetic analyses of additional proteins from photosynthetic bacteria and *Pseudomonas* such as cytochrome bc_1 complexes and reaction centers to develop this hypothesis.

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References

1 Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1965) Nature 318, 618–624.
2 Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1987) Proc. Natl. Acad. Sci. USA 84, 6162–6166.
3 Bartsch, R.G. (1991) Biochim. Biophys. Acta 1058, 28–30.
4 Rickle, G.K. and Cusanovich, M.A. (1979) Arch. Biochem. Biophys. 197, 589–598.
5 Overfield, R.E., Wraight, C.A. and Devault, D. (1979) FEBS Letts. 105, 137–142.
6 Shill, D.A. and Wood, P.M. (1984) Biochim. Biophys. Acta 764, 1–7.
7 Dracheva, S.M., Drachev, L.A., Zaberezhnaya, S.M., Konstantinov, A.A., Semenov, A.Y. and Skulachev, V.P. (1986) FEBS Lett. 205, 41–46.
8 Fritzsch, G., Buchanan, S. and Michel, H. (1989) Biochim. Biophys. Acta 977, 157–162.
9 Daldal, F., Cheng, S., Applebaum, J., Davidson, E. and Prince, R.C. (1986) Proc. Natl. Acad. Sci. USA 83, 2012–2016.
10 Donohue, T.J., McEwan, A.G., Van Doren, S., Crofts, A.R. and Kaplan, S. (1988) Biochemistry 27, 1918–1925.
11 Fitch, J., Cannac, V., Meyer, T.E., Cusanovich, M.A., Tollin, G., Van Beeumen, J., Rott, M.A. and Donohue, T.J. (1989) Arch. Biochem. Biophys. 271, 502–507.
12 Gray, G.O., Gaul, D.F. and Knaff, D.B. (1983) Arch. Biochem. Biophys. 222, 78–86.
13 Tomiyama, Y., Doi, M., Takamiya, K. and Nishimura, M. (1983) Plant Cell Physiol. 24, 11–16.

14 Ambler, R.P. (1982) in From Cyclotrons to Cytochromes (Kaplan, N.O. and Robinson, A.B., eds.), pp. 263–280, Academic Press, New York.
15 Ambler, R.P., Daniel, M., Melis, K. and Stout, C.D. (1984) Biochem. J. 222, 217–227.
16 Van Beeumen, J. (1991) Biochim. Biophys. Acta 1058, 56–60.
17 Ambler, R.P. (1973) System. Zool. 22, 554–565.
18 Denariaz, C.M., Liu, M.Y., Payne, W.J., LeGall, J., Marquez, L., Dunford, H.B. and Van Beeumen, J. (1989) Arch. Biochem. Biophys. 270, 114–125.
19 Ronnberg, M., Kalkkinen, N. and Ellfolk, N. (1989) FEBS Letts. 250, 175–178.
20 Trumpower, B.L. (1990) Microbiol. Rev. 54, 101–129.
21 Hauska, G., Hurt, E., Gabellini, N. and Lockau, W. (1983) Biochim. Biophys. Acta 726, 97–133.
22 Kallas, T., Spiller, S. and Malkin, R. (1988) Proc. Natl. Acad. Sci. USA 85, 5794–5798.
23 Meyer, T.E., Cusanovich, M.A. and Kamen, M.D. (1986) Proc. Natl. Acad. Sci. USA 83, 217–220.
24 Komiya, H., Yeates, T.O., Rees, D.C., Allen, J.P. and Feher, G. (1988) Proc. Natl. Acad. Sci. USA 85, 9012–9016.
25 Mathis, P. (1990) Biochim. Biophys. Acta 1018, 163–167.
26 Van Beeumen, J., Ambler, R.P., Meyer, T.E., Kamen, M.D., Olson, J.M. and Shaw, E.K. (1976) Biochem. J. 159, 757–774.
27 Ambler, R.P. and Bartsch, R.G. (1975) Nature 253, 285–288.
28 Cohn, C.L., Hermodson, M.A. and Krogmann, D.W. (1989) Arch. Biochem. Biophys. 270, 219–226.
29 Ambler, R.P. and Meyer, T.E. (1991) Biochem. J., in press.
30 Ambler, R.P. and Taylor, E. (1973) Biochem. Soc. Trans. 1, 166–168.
31 Trost, J.T., McManus,, J.D., Freeman, J.C., Ramakrishna, B.L. and Blankenship, R.E. (1988) Biochemistry 27, 7858–7863.
32 Norris, G.E., Anderson, B.F. and Baker, E.N. (1983) J. Mol. Biol. 165, 501–521.
33 Ambler, R.P., Meyer, T.E. and Kamen, M.D. (1979) Nature 278, 661–662.
34 Stout, C.D. (1989) J. Mol. Biol. 205, 545–555.
35 Saeki, K., Suetsugu, Y., Yao, Y., Horio, T., Marrs, B.L. and Matsubara, H. (1990) J. Biochem. 108, 475–482.
36 Duport, C., Jouanneau, Y. and Vignais, P.M. (1990) Nucleic Acids Res. 18, 4618.