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Minireview

Photosynthesis genes and their expression in *Rhodobacter sphaeroides* 2.4.1: a tribute to my students and associates

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Abstract

This minireview traces the photosynthesis genes, their structure, function and expression in *Rhodobacter sphaeroides* 2.4.1, as applied to our understanding of the inducible photosynthetic intracytoplasmic membrane system or ICM. This focus has represented the research interests of this laboratory from the late 1960s to the present. This opportunity has been used to highlight the contributions of students and postdoctorals to this research effort. The work described here took place in a much greater and much broader context than what can be conveyed here. The 'timeline' begins with a clear acknowledgment of the work of June Lascelles and William Sistrom, whose foresight intuitively recognized the necessity of a 'genetic' approach to the study of photosynthesis in *R. sphaeroides*. The 'timeline' concludes with the completed genome sequence of *R. sphaeroides* 2.4.1. However, it is hoped the reader will recognize this event as not just a new beginning, but also as another hallmark describing this continuum.

Abbreviations: BChl – bacteriochlorophyll; Crt – carotenoid; DMSO – dimethylsulfoxide; ETC – electron transport chain; ICM – intracytoplasmic membranes; LH 1 – B875 spectral complex; LH II – B800-850 spectral complex; PS – photosynthesis; RC – reaction center; SE – spheroidene; SO – spheroidenone

Introduction

I have interpreted, rather narrowly, the charge given by Govindjee and Howard Gest, who have kindly invited me to write this article. I have restricted my discussions of photosynthesis genes to those directing and regulating the synthesis of the ICM (intracytoplasmic membranes; photosynthetic membranes only in *Rhodobacter sphaeroides*). Further, I have emphasized the contributions from our laboratory in an effort to acknowledge the many and formidable students and postdoctorals with whom I have had the honor to associate. Finally, I am a microbial chauvinist, for which I make no apologies, and therefore I have attempted to view the photosynthesis genes through the behavior of *Rhodobacter sphaeroides* as that might occur *in situ*. For a general review of the 'lifestyle' of photosynthetic prokaryotes, see Pfennig (1978). For general reviews which follow the work described herein, see Kaplan (1978, 1981, 1988a, b), Kaplan et al. (1979), Kaplan and Arntzen (1982), Donohue and Kaplan (1986), Kaplan and Lee (1992), Lee and Kaplan (1996), Zeilstra-Ryalls et al. (1998a, b), and Oh and Kaplan (2001). At the suggestion of Govindjee, a photograph of myself and my current research group is shown (Figures 1 and 2).

Figure 1. The author (Sam Kaplan) in his office in Texas.

Early efforts

The earliest efforts to define, in the broadest possible sense, the genomic structure and function as these relate to the ICM of *Rhodobacter sphaeroides* (originally *Rhodopseudomonas spheroides*) were conducted in the laboratories of Drs June Lascelles (Lascelles 1978) and William Sistrom (Sistrom 1978; Sistrom et al. 1986), both of whom employed a biochemical-genetic approach to these early studies (see figures 3 and 4 for photographs of Lascelles and Sistrom, respectively).

F.E. Nano and C.S. Fornari[1] characterized the plasmids of *R. sphaeroides* (Fornari et al. 1984; Nano and Kaplan 1984). These and other efforts were intended to develop methods to obtain, propagate, and move DNA molecules between strains of *R. sphaeroides* (Miller and Kaplan 1978; Fornari and Kaplan 1982; Nano et al. 1985; Tai et al. 1988a; Moore and Kaplan 1989; Varga and Kaplan 1989; Donohue and Kaplan 1991; Suwanto and Kaplan 1992; Nereng and Kaplan 1998). The first major breakthrough came with the isolation of a DNA fragment by J.C. Williams (Williams et al. 1983, 1984) in George Feher's laboratory together with the assistance of the M.I. Simon laboratory at University of California at San Diego (UCSD). These DNA fragments contained the reaction center (RC) *L* and *M* genes, subsequently designated *pufL*, M.

J. Davis[2] (Davis et al. 1988) created the first directed mutation of a photosynthesis (PS) gene in *R. sphaeroides*. From that point on, many laboratories began to define those DNA segments, genes, mutants, etc., which encompass the PS gene cluster and assorted regulatory elements (Lynn et al. 1979; Marrs et al. 1980; Fornari and Kaplan 1983; Meinhardt et al. 1985; Hallenbeck and Kaplan 1987, 1988; Dryden and Kaplan 1990, 1993; Yun et al. 1990; Sabaty and Kaplan 1995; Gomelsky and Kaplan 1996; Mouncey et al. 2000). It was determined that in *R. sphaeroides* both *cycA* (cytochrome c_2, extensively studied by the T.J. Donohue[2] laboratory – Donohue et al. 1988b; Bradner et al. 1988) and the *puc* operon (Wu et al. 1991) encoding the B800–850 (light harvesting complex II, LH II) complex were closely linked to the PS gene cluster unlike that originally observed by J.E. Hearst's laboratory (Youvan et al. 1984) for *Rhodobacter capsulatus* where the sequence of the entire PS gene cluster was a remarkable achievement. The PS gene cluster in strain NCIB 8253 was sequenced by C.N. Hunter's laboratory (Naylor et al. 1999). We sequenced the entire ~65 Kb-gene cluster from strain 2.4.1 (Donohue et al. 1986a, b; Kiley et al. 1987; Kiley and Kaplan 1987; McEwan et al. 1989; Choudhary and Kaplan 2000). See Figure 5 for the 65 Kb cluster from strain 2.4.1.

Some years earlier, J. Chory[1] (Chory and Kaplan 1982a) developed an *R. sphaeroides*-based *in vitro* transcription/translation system (Muller et al. 1985a, b; Chory et al. 1985). This system was used to produce, *in vitro*, the Form I carboxylase and other PS gene products.

Characteristic of the organization of most of the PS genes is a tight transcriptional coupling (Kiley and Kaplan 1988). Another organizational characteristic of both the *puf* and *puc* operons is the existence of differential gene expression of upstream versus downstream genes (Zhu et al. 1986; DeHoff et al. 1988; Donohue et al. 1988a; Lee et al. 1989a), and a short, translated open reading frame, *pufK* (Gong et al. 1994; Gong and Kaplan 1996), immediately upstream of the *pufBALMX* structural genes (Figure 5). Regulatory sequences, which direct the binding of the *R. sphaeroides* global regulator PrrA (Eraso and Kaplan 1994), are apparently present (Figure 5). Binding sequences for the repressor PpsR (TGT N_{12}ACA, Lee and Kaplan 1992a, 1995; Penfold and Pemberton 1994; Gomelsky and Kaplan 1995a, 1997) are positioned upstream of the *puc* structural genes, as well as some carotenoid (*crt*) and bacteriochlophyll (*bch*) genes (Figure 5). Uniquely positioned upstream of the *bchE* and *pucB* genes is a binding sequence for the *R. sphaeroides* FnrL protein (Zeilstra-Ryalls and Kaplan 1997; Oh et al. 2000) and finally, adding to the complexity of *puc* operon expression is an IHF binding sequence which overlaps the FnrL-binding sequence (Lee et al. 1993, Figure 5). We have shown that PrrA can interact with other regulatory proteins, e.g., FnrL,

Figure 2. Current members of the Kaplan laboratory. From left to right; Rebecca Cox, In-Jeong Ko, Agnes Puskas, Madu Choudhary, Jung Hyeob Roh, Jeong-Il Oh, Xiaohua Zeng, Raimo Pollanen, Bill Smith, Canna Ross, Joy Marshall and Chris Mackenzie.

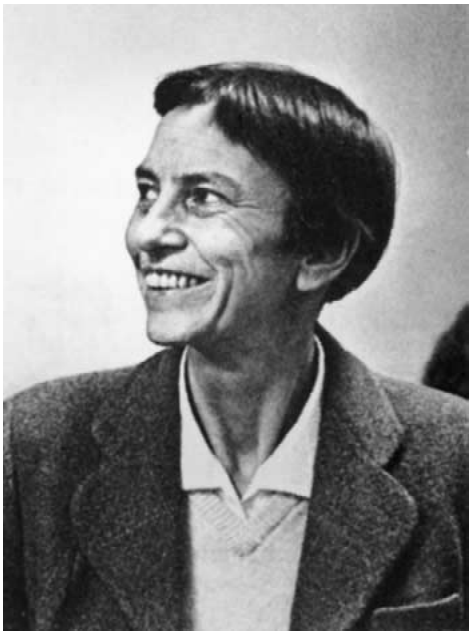

Figure 3. June Lascelles.

Figure 4. William Sistrom.

to jointly activate gene expression (Oh et al. 2000), which further embellishes the regulatory response.

Despite this emphasis on things 'DNA,' the ICM is composed of protein, lipid, pigments, metal, ions, quinones, etc. Pam Fraker[1] successfully developed methods for the large-scale purification of isolated ICM (Fraker and Kaplan 1971) or 'chromatophores,' as originally defined by Al Vatter and Ralph Wolfe (1958). Fraker was amongst the very first, if not the first, to demonstrate that bacteriochlorophyll (BChl)

was bound noncovalently to specific protein species within the ICM (Fraker and Kaplan 1972). These studies were followed by the studies of other students (Huang and Kaplan 1973a–c; Shepherd and Kaplan 1978a; Cohen and Kaplan 1981a, b; Hoger and Kaplan 1985; Hoger et al. 1986). V.D.-H. Ding[1], D. Baumgardner[3] and C.D. Deal[1], together with W.D. Shepherd[1] (Ding and Kaplan 1976; Baumgardner et al. 1980; Deal and Kaplan 1983a–c; Shepherd and Kaplan 1983), as well as the Robert Niederman laboratory (Niederman and Gibson 1978) characterized the cell envelope layers so that we could readily assess the purity, fractionation and interrelationships between the various membrane systems. Shepherd[1] (Shepherd et al. 1981) provided a qualitative and quantitative approach and addressed protein targeting and localization in bacteria.

Working with *Rhodopseudomonas viridis*, Deisenhofer et al. (1985) crystallized the reaction center (RC). Shortly thereafter, George Fehers' and Jim Norris' laboratories (Allen et al. 1986; Schiffer and Norris 1993) crystallized the RC from *R. sphaeroides* for which a very robust gene exchange system had been developed (Nano et al. 1985; Davis et al. 1988; Suwanto and Kaplan 1992; Kaplan and Donohue 1993; Zeilstra-Ryalls et al. 1998b). C.N. Hunter and collaborators provided detailed spectroscopic analyses using a variety of cleverly constructed mutant strains of *R. sphaeroides* (Jones et al. 1992).

Recent efforts

Several curiosities do remain. There is a second *pucBA* ($pucBA_{II}$) gene cluster, including regulatory sequences (Lee et al. 1989b; X. Zeng and S. Kaplan, 2001) and which maps to chromosome I (CI) at position 1430 Kb clockwise from the PS gene cluster. Other elements which are involved either directly or indirectly or in a regulatory fashion in PS gene expression are the *ccoNOQP* operon (Zeilstra-Ryalls and Kaplan 1995a; O'Gara et al. 1998; Zeilstra-Ryalls et al. 1998a; Oh and Kaplan 1999), the *rdxBHIS* operon (O'Gara et al. 1998; Roh and Kaplan 2000, Figure 5A) and *cycY* (Zeilstra-Ryalls and Kaplan 1995b), first described for *R. capsulatus* (Myllykallio et al. 1999) and the *spb* gene (Shimada et al. 1996). The *ccoNOQP* operon encodes the cbb_3 terminal oxidase; the *rdxBHIS* encodes an assemblage of redox proteins which interact with the cbb_3 terminal oxidase. These are involved in the regulation of PS gene expression through the two-component Prr activation system (Eraso and Kaplan 1994, 1995; O'Gara et al. 1998). They also provide reducing equivalents, under anaerobic conditions, to a hypothetical 'OXO'-donor, which is used in the conversion of spheroidene (SE) to spheroidenone (SO) (Yeliseev et al. 1996; Yeliseev and Kaplan 1997; O'Gara and Kaplan 1997).

Cell biology

During the period of rapid PS gene discovery, our laboratory followed several independent lines of inquiry. J. Chory[1], P.J. Kiley[1], T.J. Donohue[2], A.R. Varga[2] and others (Chory and Kaplan 1982b; Chory et al. 1984; Jackson et al. 1987; Kiley et al. 1988) provided a detailed kinetic analysis of the development and function of the photosynthetic apparatus. Earlier, experiments initiated by M.H. Kozakowski[2] (Kosakowski and Kaplan 1974), and continued by R.T. Fraley[1] and D. Leuking[2], and then G. Yen[1], B.D. Cain[1] and T.J. Donohue[2] (Leuking et al. 1978; Wraight et al. 1978; Fraley et al. 1978a,b, 1979a,b; Cain et al. 1981, 1982; Yen et al. 1982, 1984; Kaplan et al. 1983; Snozzi and Crofts 1984; Hoger et al. 1987) shed important new information on the fate of the photosynthetic apparatus in steady-state photosynthetically growing *R. sphaeroides*.

Coming of age

Antonius Suwanto[1] (Suwanto and Kaplan 1989a,b) determined the genome architecture of *R. sphaeroides* and thereby enabled the placement of all of the relevant PS and regulatory genes throughout the genome. Suwanto revealed, for the first time, that prokaryotes could possess more than a single chromosome. Final confirmation of the genome architecture came with the recent genome sequence of *R. sphaeroides* 2.4.1 by the Joint Genome Institute and our laboratory (www.rhodobacter.org or www-mmg.med.uth.tmc.edu/sphaeroides/).

Collectively, studies initiated by Y.S. Zhu[1] and J.K. Lee[1] (Zhu et al. 1985, 1986; Lee et al. 1989a) yielded an analysis of PS mRNA species. In addition, the first definable regulatory mutants were isolated by J.K. Lee[1] (Lee et al. 1989a, 1993; Lee and Kaplan 1992a,b), who built upon the genetic and biochemical work of P.J. Kiley[1] (Kiley and Kaplan 1988; Kiley et al. 1988). Lee[1] also delineated the complexity of

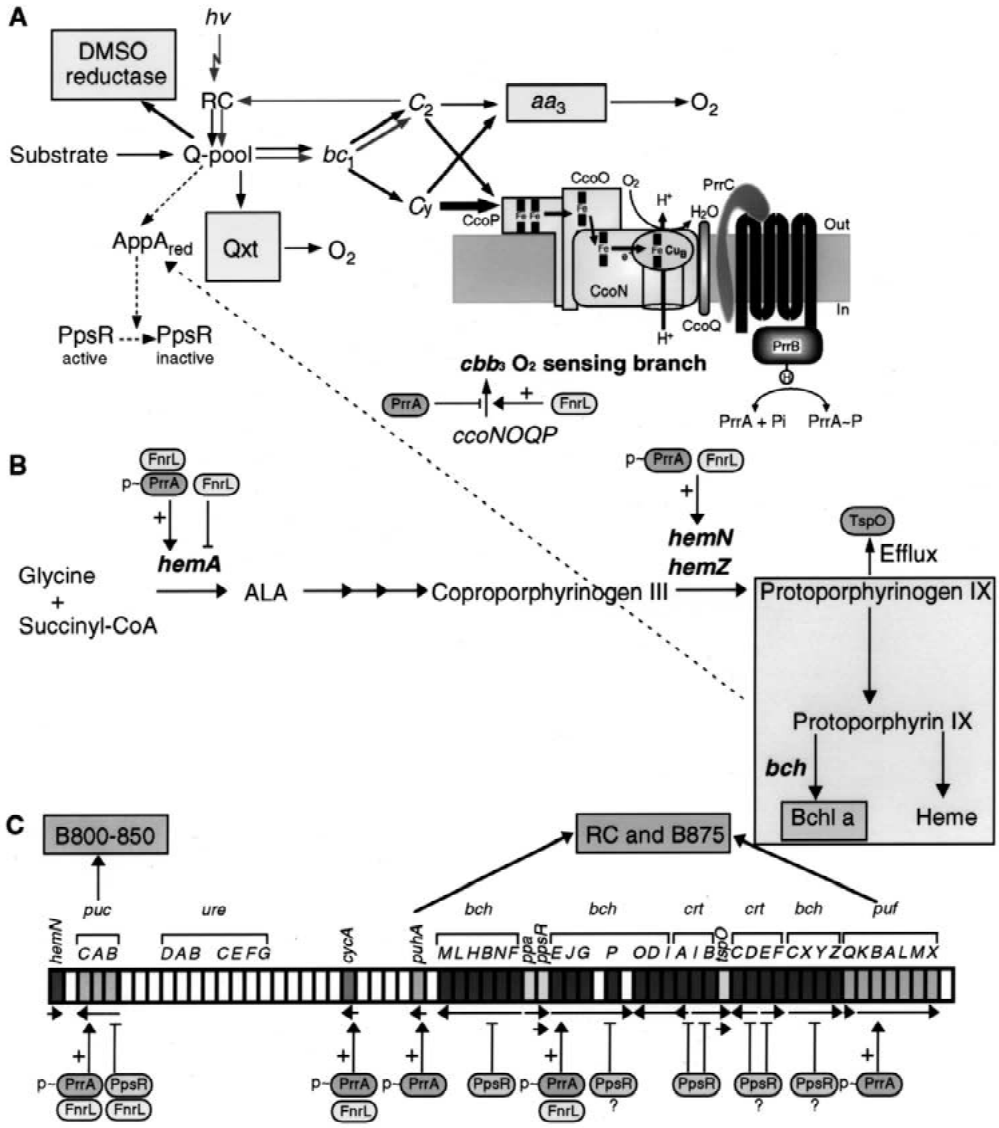

Figure 5. Model for the regulation of PS gene expression in *R. sphaeroides*. (A) Respiratory and photosynthetic electron transport pathways and coupled signal tranduction pathways. Red arrows indicate phototsynthetic cyclic electron flow. The thickness of the arrows is a measure of the relative contribution of cytochromes c_2 and c_y to channel electrons from the bc_1 complex to the cbb_3 oxidase. The cbb_3 oxidase is composed of CcoN, O, Q, and P subunits; the redox centers and intramolecular electron transfer within the cbb_3 oxidase are depicted. PrrC is part of the signaling pathway and crosses membrane. The histidine kinase, PrrB, is a membrane-bound protein and presumably exists as a dimmer. (B and C) Tetrapyrrole biosynthetic pathway and PS gene cluster of *R. sphaeroides* 2.4.1. The regulation of PS genes, which is either established or predicted on the basis of sequence analyses, is depicted by using the regulatory symbols (PrrA: PrrBA two-component system, PpsR: AppA/PpsR-antirepressor/repressor system, and FnrL). The question mark below a regulatory symbol indicates that regulation by the cognate regulator is inferred from sequence analyses only. The arrows with a '+' sign and blunt arrows represent induction under oxygen-limiting conditions (semiaerobic and anaerobic) and repression under high-oxygen conditions, respectively. TspO affects the expression of PpsR target genes by controlling the efflux of (a) porphyrin intermediate(s), probably protoporphyrinogen IX or beyond, of the tetrapyrrole biosynthetic pathway enclosed in the grey box. The arrows below the PS genes represent transcriptional organization and direction. The *puc*, *puf*, and *puhA* encode the structural polypeptides and assembly factors of the photochemical reaction center and light harvesting complexes (B800–850 and B875). Abbreviations: DMSO, dimethyl sulfoxide; RC, photochemical reaction center; Q-pool, quinone pool; bc_1, bc_1 complex; c_2 and c_y, cytochromes c_2 and c_y; aa_3, aa_3 cytochrome *c* oxidase; Qxt, quinol oxidase; *hv*, light; red, reduced; ALA, 5-aminolevulinic acid; Bchl *a*, bacteriochlorophyll *a*. This figure and legend are reprinted with the kind permission of Blackwell Sciences, Blackwell Publishers, Polity Press. It first appeared in 'Generalized approach to the regulation and integration of gene expression,' Mol. Microbiol., Vol 39, No. 5, pp. 1116–1123 (2001). For a color version of this figure, see section in the front of the issue.

factors involved in the control and expression of the *puc* operon (Lee and Kaplan 1992a, b; Lee et al. 1993). J.M. Eraso[2] (Eraso and Kaplan 1994, 1995, 1996) extended the work of Lee by defining the Prr two-component activation system from those mutants isolated by Lee (see Figure 5A). Eraso[2] (Eraso and Kaplan 2000) also revealed the global nature of the response regulator PrrA control over PS gene expression. F.R. Tabita's laboratory demonstrated the importance of PrrA control of genes involved in both CO_2 and N_2 fixation (Joshi and Tabita 1996). S. Ouchane[2] (Ouchane and Kaplan 1999) formally demonstrated the membrane topology of the histidine kinase PrrB.

M. Gomelsky[2] went on to characterize the PpsR repressor/AppA antirepressor system (Gomelsky and Kaplan 1995a, b, 1997, 1998; Gomelsky et al. 2000), following the very first demonstration by Penfold and Pemberton (1994) of the *ppsR* gene, mapping to the PS gene cluster (Figure 5) PpsR encodes a repressor of PS genes involved in pigment biosynthesis and the *puc* operon. Gomelsky[2] (Gomelsky and Kaplan 1995a–c) discovered the existence of *appA*, encoding a redox active protein which appears to act as an antirepressor through its presumed direct interaction with the PpsR repressor (Figure 5A). The AppA protein was shown to bind a flavin at the amino terminal end, as well as other ligands. Gomelsky[2] (Gomelsky and Kaplan 1997, 1998) also showed that the PpsR/AppA pair plays a significant role in light regulation of PS gene expression.

J.H. Zeilstra-Ryalls[2] (Zeilstra-Ryalls and Kaplan 1995a, b, 1996) continued the work of E. Neidle[2] (Neidle and Kaplan 1993a, b) who defined the expression of the *hemA* and *hemT* genes, originally discovered by T.-N. Tai[1] (Tai et al. 1988b) encoding isoenzymic forms of 5-aminolevulinic acid synthase (Figure 5B). Zeilstra-Ryalls[2] demonstrated the existence of an *E. coli* Fnr homologue (designated FnrL, L in honor of June Lascelles) in *R. sphaeroides*, as well as the *ccoNOQP* operon encoding the cbb_3 terminal oxidase (Figures 5A, B). Immediately downstream of the *cco* operon, she discovered the *rdxB* gene and operon (see below) a homolog of the *rdxA* gene, which had been discovered by Neidle[2] (Neidle and Kaplan 1992). Zeilstra-Ryalls[2] revealed that in *R. sphaeroides*, FnrL is involved in the regulation of a highly selective set of PS genes (Zeilstra-Ryalls and Kaplan 1997), which are critical in porphyrin biosynthesis (Figure 5B). She also showed, together with the R.G. Kranz laboratory (Zeilstra-Ryalls et al. 1997), that FnrL was essential to the expression of the dimethylsulfoxide (DMSO) reductase system of *R. sphaeroides* and *R. capsulatus*.

N.J. Mouncey[2] continued the studies of the DMSO reductase (Mouncey et al. 1997; Mouncey and Kaplan 1998a–c, *dor* operon) of *R. sphaeroides*, which is also under redox control by the cbb_3/PrrBA system. Mouncey[2] (Mouncey and Kaplan 1998a) also revealed that FnrL was critical to *cco NOQP* operon expression and that under anaerobic conditions, *cco* expression was twice that under aerobic growth. Importantly, low O_2 conditions showed the highest levels of *cco* expression dependent upon FnrL.

Gaining perspective

J.P. O'Gara[2] created a more rigorously defined mutation in *ccoP* (O'Gara and Kaplan 1997) as well as in the downstream *rdxB* gene of the *rdxBHIS* operon. He demonstrated that all such mutations led to the aerobic expression of the PS genes. In a collaborative effort with J.M. Eraso[2] (O'Gara et al. 1998), he showed that expression of the PS genes in Cco and Rdx mutants was dependent upon an intact Prr two-component activation system, and therefore concluded that a signal transduction pathway existed between the cbb_3 terminal oxidase and the Prr system (Figure 5A). Eraso[2] (Eraso and Kaplan 2000) showed that the PrrC protein is part of this pathway. These findings, and those to follow, formally established innovative hypothesis advanced by G. Cohen-Bazire et al. (1956). A similar interpretation was also reached by P.L. Hallenbeck[1] studying the control of genes involved in CO_2 fixation (Hallenbeck et al. 1990a, b).

J.P. O'Gara[2] et al. observed that mutants of cbb_3 and *RdxB* also gave rise to cells which, when grown photosynthetically, contained almost exclusively spheroidenone (SO, pink) as the major carotenoid instead of spheroidene (SE, orange). These findings coincided with studies of A. Yeliseev[2] (Yeliseev et al. 1996), who showed that the ratio of SE/SO was determined by the redox state of the growing cells, with SO predominating under more oxidized conditions. Yeliseev[2] also revealed that assembly of the B800–850 complex preferentially incorporated SE relative to SO, but the RC and B875 complexes showed no apparent Crt preferences (Yeliseev et al. 1996). These studies also raised an interesting paradox, namely, what is the source of the 2-OXO group of SO under anaerobic conditions? Yeliseev[2] showed

that water is not the source, leaving open an important question (Yeliseev and Kaplan 1997).

A. Yeliseev demonstrated that *crtK* of *R. sphaeroides* (Figure 5C) actually encodes an outer membrane protein and not an enzyme in Crt biosynthesis (designated TspO, Yeliseev and Kaplan 1995, 1999, 2000), whose absence leads to an acceleration in the transcription of the *puc* operon and genes of the BChl and Crt biosynthetic pathways during the transition of cells from aerobic to anaerobic growth (Figure 5B). In a collaborative study with Karl Krueger at George Washington University, Yeliseev[2] (Yeliseev et al. 1997) revealed that the rat PrK18 homologue (encoding the mammalian peripheral benzodiazepine receptor) of the *R. sphaeroides* TspO could substitute for the bacterial TspO (CrtK renamed) in *R. sphaeroides* strains mutant for TspO. These and many other observations strengthen the likely relationship between the non-sulfur purple bacteria and the 'early' mitochondrial endosymbiont (Yang et al. 1985). Yeliseev[2] suggested that the mode of TspO action is to 'regulate' the efflux of intermediates in porphyrin biosynthesis. Yeliseev[2] (Yeliseev and Kaplan 2000) suggested that a critical porphyrin intermediate(s) acts as a coactivator of the antirepressor, AppA, which in turn regulates the functional state of the PpsR repressor. Recently, X. Zeng[2] (Zeng and Kaplan 2001) has shown that TspO regulates PS gene expression through the PpsR/AppA regulon (Figure 5B).

J.P. O'Gara[2] and J.M. Eraso[2] and later J.-L. Oh[2] (Oh and Kaplan 1999, 2000, 2001) concluded that the cbb_3-generated inhibitory signal continues to act as a 'brake' on PS gene expression under photosynthetic conditions, i.e., there is electron flow through the cbb_3 terminal oxidase, anaerobically. This fits nicely with the observation of N.J. Mouncey[2] that FnrL is active under conditions of low O_2 as well as anaerobically, in stimulating *cco* operon transcription (Mouncey and Kaplan 1998a–c).

J.-L. Oh[2] (Oh and Kaplan 2001) made a series of mutant strains whereby different segments of the branched aerobic electron transport chain (ETC) could be isolated and studied. These studies, together with the use of specific electron transport chain (ETC) inhibitors in wild-type, enabled Oh to conclude that it is the volume of electron flow through the cbb_3 terminal oxidase which generates an inhibitory signal dampening the kinase activity of PrrB (Figure 5A). Oh et al. (2001) further demonstrated that the default state for the PrrB histidine kinase is in the kinase positive mode.

J.-L. Oh showed that removal of His^{407}, which is involved in binding the low spin heme of the catalytic N subunit gave rise to an altered cbb_3 resulting in the 'turn on' of PS gene expression in the presence of high oxygen (Figure 5A). Unlike the other His substitutions within the cbb_3 oxidase, which also 'turn on' PS gene expression, this strain had substantial residual oxidase activity and normal levels of subunit proteins in the membrane, as well as a normal carotenoid profile. This phenotype resembles the phenotype of an in frame deletion of the *Q* gene of the *ccoNOQP* operon (Oh and Kaplan 1999, 2001).

A study by J.-H. Roh[2] (Roh and Kaplan 2000) suggested that the RdxB polypeptide is involved in shunting electrons from cbb_3 to a hypothetical (organic) electron acceptor, which is involved in SE/SO synthesis (see below). We also concluded, as suggested for *R. capsulatus* and *Sinorhizobium*, that the corresponding *fix* genes (Preisig et al. 1996, *fixHIS*) are involved in assembly of the cbb_3 oxidase (Koch et al. 1998), i.e., *ccoHIS*.

We assume, and the data of A. Yeliseev[2] (Yeliseev and Kaplan 1999, 2000) support the idea, that an 'organic' donor is the source of the 2-OXO group during the conversion of SE to SO by the CrtA protein. Therefore, electron flow and factors that influence this flow through the cbb_3 under anaerobic conditions will reduce the 2-OXO donor (X=O) to a hypothetical hydroxyl form [X-OH]. We speculate that the RdxB protein is involved in this reaction by taking electrons from the cbb_3 to the CrtA-catalyzed reaction sequence. Continued electron flow occurring through the cbb_3 terminal oxidase, under anaerobic conditions, implies that aerobic respiration immediately becomes the dominant energy mode when cells are returned to air, even in the presence of a fully functional ICM because electrons are 'drained' from the bc_1 complex to the cbb_3 terminal oxidase to O_2.

Synthesis

We have devised a general model (Oh et al. 2000; Oh and Kaplan 2000, 2001) whereby the two major regulatory pathways, repressor/antirepressor, and two component activation systems are sensitive to redox control by monitoring the different ends of the ETC (Figure 5). PpsR/AppA senses the redox state of the quinone pool through, we suggest, AppA, which is both sensitive to oxygen levels and, when anaerobic, to light intensity. This also explains the dominance

of oxygen control to light regulation. The Prr system is sensitive to the flow of reductant through the cbb_3 terminal oxidase, which determines the relative activity of PrrB and ultimately the activation of PrrA. However, PrrA is also likely to be phosphorylated by other heterologous histidine kinases (Gomelsky and Kaplan 1995c) such that the photosynthetic 'lifestyle' is fully integrated into the totality of cellular activity. Whereas PpsR is primarily involved in regulating pigment and *puc* genes, PrrA is involved in regulating virtually all PS genes. FnrL regulates *hemA* (the first gene in tetrapyrrole synthesis), *hemN*, and *hemZ* (encoding isoenzymic forms of coproporphyrinogen III oxidase) and *bchE* (and presumably the entire operon of which this is the first gene). In the absence of FnrL, the BChl-biosynthetic pathway is effectively off, although the apoproteins of the spectral complexes can be made at low levels. These nascent apoproteins find their way into the cell membrane, but as A.R. Varga[2] (Varga and Kaplan 1993, 1995) has shown, they are rapidly broken down. However, when a *cco* mutation is combined with an *fnrL* mutation, cells regain the ability to grow photosynthetically, i.e., the cbb_3 defect suppresses the loss of FnrL. The question is: how? We concluded that PrrA and FnrL must act together at those sites where FnrL is involved in activating transcription and the presence of unusually high levels of activated PrrA (due to the absence of cbb_3) must permit optimal binding of RNA polymerase in the absence of FnrL (Oh et al. 2000). Mechanistically, these data suggests interactions between RNA polymerase, FnrL, and activated PrrA.

FnrL regulates the expression of the *ccoNOQP* and *rdxBHIS* operons and thereby controls the strength of the inhibitory signal originating from cbb_3. In addition to this autoregulatory cycle involving PrrA and FnrL, PrrA also regulates the expression of PrrB, which in turn activates PrrA (Oh et al. 2002). Thus, the system is dynamically tuned to respond to any and all signals, which are ultimately translatable into active redox flow.

By controlling the intracellular levels of (a) critical porphyrin(s), TspO partially regulates AppA activity, which senses the redox state of the quinone pool, as described above. As the repressor loses activity (AppA becomes more active) as the result of anaerobiosis and decreasing light intensity, pigment synthesis accelerates, but the presence of TspO insures the continued efflux of porphyrin intermediates, which modulates or fine-tunes the functional state of AppA; i.e., AppA becomes less functional, resulting in increased repressor activity, keeping pigment production in check. Again, the regulatory system becomes infinitely responsive to changing growth conditions by being able to assess redox state of the quinone pool, as well as the extent of porphyrin accumulation.

The real world

When cells undergo a transition from aerobic to anaerobic conditions, the flow of reductant through the aa_3 and cbb_3 terminal oxidases decreases, the strength of the inhibitory signal affecting PrrB, as generated via electron flow through the cbb_3, diminishes, and PrrB becomes more active in activating PrrA, which is now able to activate PS gene expression. FnrL becomes active as oxygen tensions decline and together with a gradual decline in PpsR strength, due to the activation of AppA, as the result of the quinone pool becoming more reduced, pigment synthesis is gradually turned on. However, under limiting levels of pigment, the spectral complex assembly systems reflect the hierarchy of BChl insertion into the RC and B875 complexes followed by the B800–850 (Sockett et al. 1989; Gong et al. 1994; Gong and Kaplan 1996) and because of the continuing presence of oxygen, significantly more SO relative to SE is produced. The net effect is to post-transcriptionally dampen the assembly of the B800–850 relative to the RC and B875, thereby diverting BChl into the RC and B875 complex. Since the *puf* and *puhA* operon expression is relatively independent of PpsR/AppA, activated PrrA can result in strongly increased levels of the apoproteins comprising the RC and B875. Now, the system is poised for full-scale ICM development, once oxygen disappears. Counter-balancing these trends is the increased activity of FnrL in derepressing transcription of the *ccoNOQP* operon, giving rise to increased levels of cbb_3. This, in turn, because of its very high affinity for oxygen, can accelerate electron flow through the cbb_3, thereby countering the increased kinase activity of PrrB by increasing the strength of the inhibitory signal. The net effect is to dampen PrrA PS gene activation and only gradually turn on PS gene expression, especially the apoproteins for the B875 and RC. Likewise, the presence of TspO (increased synthesis as oxygen declines) serves to lower the intracellular concentration of the porphyrin intermediate(s) which serves as a coactivator of AppA, resulting in an increase in PpsR strength, thereby slowing the rate of pigment production. The continued presence of O_2

mitigates full FnrL activation and the *bchE* operon is not fully derepressed. Thus, the turn on of PS genes at low oxygen tensions is held in check by regulatory elements and effector molecules such that 'turn on' is slowed. If high levels of oxygen are reintroduced, the cell is well able to reverse direction.

If the levels of oxygen continue to fall to zero, the 'brakes' on the PpsR/AppA and Prr systems are eliminated, and the system is ready to develop optimally and robustly. The Prr system remains responsive to minimal levels of electron flow through the cbb_3. Light intensity, by determining the redox state of the quinone pool, is reflected in the activity of the PpsR/AppA system, which is now the dominant controlling element. In the absence of oxygen, the role of FnrL is optimized and now pigment gene expression and *puc* operon expression are more responsive to changes in the strength of the PpsR/AppA repressor/antirepressor system, which is under light control. As light intensity drops, the quinone pool becomes more reduced and AppA is more active, resulting in diminished PpsR activity and increased *puc* and pigment gene expression. Likewise, an increase in the reduced state of the quinone pool increases electron flow to the cbb_3, although minimal relative to aerobic growth. It nevertheless serves to maintain the presumptive 2-OXO donor (X=O) in the reduced state (X-OH), which increases the relative levels of SE to SO, insuring assembly of the B800–850 complex and an increase in the size of the variable photosynthetic unit (VPU). Extensive data indicate that the apoproteins can be and are synthesized in great excess relative to the levels of pigment ultimately available for spectral complex formation (Varga and Kaplan 1995). This means that there is likely to be no free pigment, which could result in irreparable harm to the cell. It also means that, under anaerobic conditions, it is the levels of pigments, regulated by light intensity, which ultimately set the final cellular levels of the spectral complexes. At higher light intensities, the quinone pool is relatively more oxidized, resulting in diminished AppA activity and increased PpsR activity, and diminished pigment and *puc* operon expression. Likewise, at high light, there is lowered electron flow to the cbb_3 and increased SO synthesis relative to SE, together resulting in a decrease in the size of the VPU (variable photosynthetic unit). However, by default, the decreased expression of the *puc* operon leads to relatively increased expression of *puf* and *puhA*, further increasing the size of the fixed photosynthetic unit (FPU). Such a scenario also describes why the action spectrum for PS gene expression follows the absorbance profile of the spectral complexes, since these describe the redox state of the quinone pool except when the redox state of the quinone pool is affected by other factors, e.g. DMSO reductase activity.

Upon re-introduction of oxygen and despite the presence of a fully functional ICM, the presence of a pre-existing, functional cbb_3 terminal oxidase insures that aerobic electron flow will prevail and cyclic electron flow will cease. FnrL will become nonfunctional turning down the pathway of porphyrin synthesis, as well as the *bchE* operon and *ccoNOQP* transcription. The quinone pool becomes highly oxidized, making its redox state independent of light intensity, since the cbb_3 is the major conduit for electron flow until significant levels of the aa_3 accumulate. As a result, AppA becomes non-functional and PpsR is restored to full functionality, just as the Prr system becomes nonfunctional, the PS genes are turned off. This shut down is virtually immediate; i.e., there is no 'brake' which restricts this shutdown. The synthesis of the aa_3 oxidase resumes and with more membrane surface area per cell (old ICM), we suggest that the aa_3 rapidly becomes the predominant terminal oxidase.

It ain't over 'till

Where do we go from here? Together with the ongoing protein, physiological, genetic and molecular analyses, we can now add the complete genome sequence of *R. sphaeroides* 2.4.1. There is obviously much that is still missing, as well as alterations which will be introduced to the overall model, but researchers now stand poised with a fuller armament of ideas and methodologies in order to fully understand photosynthesis and photosynthesis gene expression within the context of an entirely free-living organism, and how these contribute to the lifestyle of *R. sphaeroides*.

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Notes

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