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Review

Oxygen intervention in the regulation of gene expression: the photosynthetic bacterial paradigm

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Abstract. The means by which oxygen intervenes in gene expression has been examined in considerable detail in the metabolically versatile bacterium *Rhodobacter sphaeroides*. Three regulatory systems are now known in this organism, which are used singly and in combination to modulate genes in response to changing oxygen availability. The outcome of these regulatory events is that the molecular machinery is present for the cell to obtain energy by means that are best suited to prevailing conditions, while at the same time maintaining cellular redox balance. Here, we explore the dangers associated with molecular oxygen relative to the various metabolisms used by *R. sphaeroides*, and then present the most recent findings regarding the features and operation of each of the three regulatory systems which collectively mediate oxygen control in this organism.

Key words. Oxygen control; *Rhodobacter sphaeroides*; FnrL; redox regulation; Prr two-component signal transduction regulatory system; PpsR; AppA; blue light repression.

Introduction

As details emerge regarding the actual molecular mechanisms by which cells sense and respond to their environment, this represents a good time to evaluate the collective information pertaining to one model system, the anoxygenic photosynthetic bacteria. Here we will examine what has been learned with respect to one major environmental parameter, oxygen. We hope to evaluate the status of our current understanding so that we might be prepared to assess new insights as they develop. The goals of this review are as follows: (i) to assess the literature with respect to the question as to why it is biologically important that oxygen play a role in the regulation of gene expression, (ii) to describe what is known or suspected about how this intervention takes place, (iii) to pose a number of questions that point to future research directions and finally (iv) lest we think there is nothing left to do, examine what is known about the role of oxygen in gene expression in other bacteria that confound our current understanding.

In order to present what we know about oxygen intervention in gene regulation, we need to describe why such intervention is anticipated. Our focus is on the facultative anoxygenic photosynthetic bacterium, *Rhodobacter sphaeroides*, which provides a unique as well as generalized perspective for examining this question, for reasons that will become clear during the discussion. In order to state the nature of the problem, we must first begin with an overview of energy metabolisms in this organism, and how oxygen-mediated regulation is manifest. We then consider how and why the need for intervention most likely arose, and examine the possible evolution of this requirement. Subsequently, the known molecular compo-

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nents of oxygen intervention will be described, and we will also consider the differences and similarities of oxygen regulation in other photosynthetic organisms when compared with *R. sphaeroides*.

Energy metabolism in *R. sphaeroides*

Figure 1 provides a diagrammatic overview of energy metabolism in *R. sphaeroides* involving a highly branched electron transport system, but does not include fermentative catabolisms or other substrate-centered mechanisms for energy production. This cartoon, although not exhaustive in detail, is adequate to serve as a guide to the discussion presented here. Apparent from this schematic is that *R. sphaeroides* can obtain energy by processes that require oxygen as well as several processes that do not. Not only does this organism have the enzymatic capacity to support this metabolic versatility, but importantly it possesses the 'regulatory' means to select the right metabolic route for the right conditions, and for changing conditions. The organism can deploy specialized complexes that allow electron flow to be rerouted from one endpoint, in which oxygen is the recipient, to another, in which an alternate molecule becomes reduced. Additionally, when conditions are appropriate, *R. sphaeroides* undergoes an entire morphological makeover involving differential changes to the inner membrane, accompanied by the assembly of the photosynthetic apparatus as the end product of these changes. The sole environmental parameter determining the timing of these processes is molecular oxygen, which makes good intuitive sense, because there exists the particular danger that is unique to chlorophyll-mediated energy acquisition when oxygen is present, i.e.

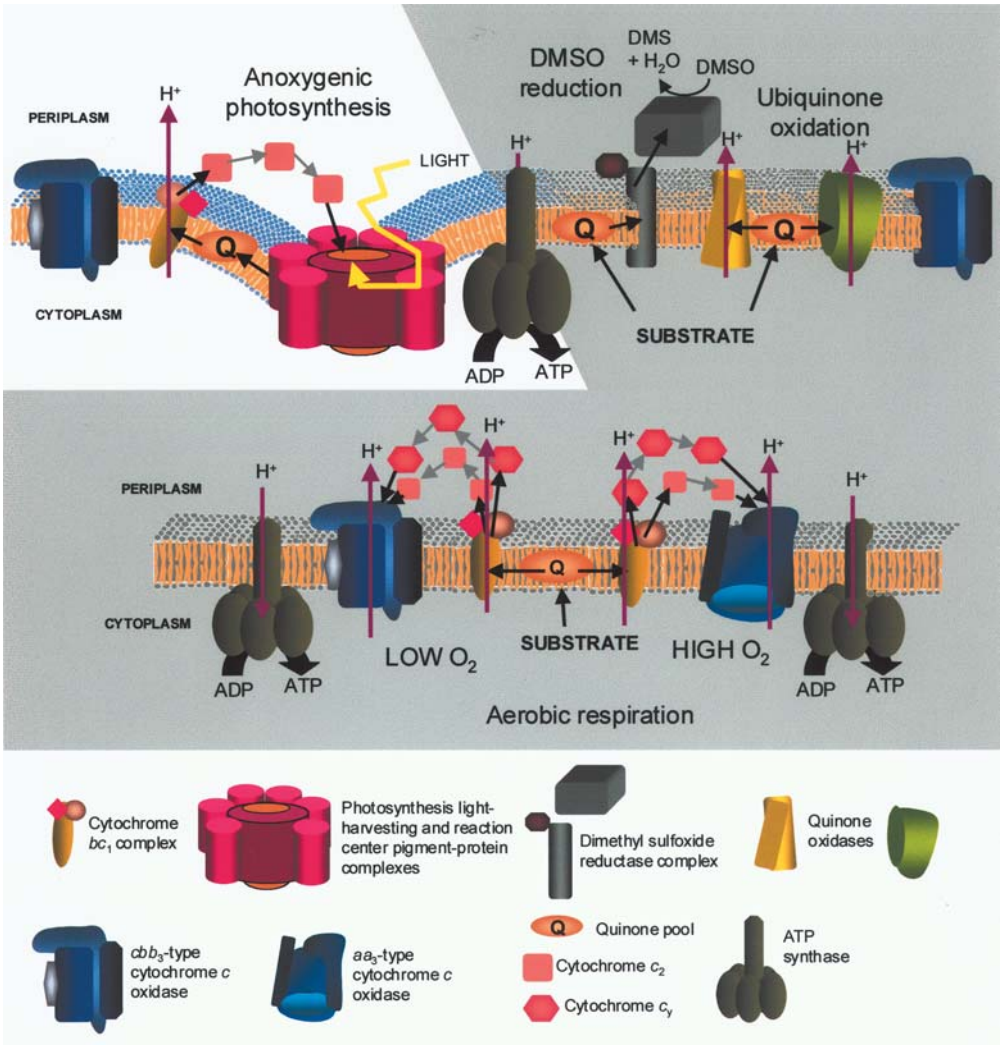

Figure 1. Cartoon diagram of catabolic metabolisms present in *R. sphaeroides*. Black arrows indicate electron flow, purple arrows indicate proton movement. 'Substrate' denotes electron source. Further information on the various enzyme complexes can be found in the following references: Photosynthesis light-harvesting and reaction center pigment-protein complexes [12–16]; dimethyl sulfoxide reductase complex [60, 61]; aa_3-type cytochrome *c* oxidase [62, 63]; cbb_3-type cytochrome *c* oxidase [58, 59, 125]; quinol oxidases [71].

the potential for the photosynthetic reaction itself to generate toxic oxygen species. This will be described in more detail presently. With this simple background, the likely importance of oxygen control of gene expression should be evident.

Oxygenic versus anoxygenic photosynthesis

The purpose of this limited description of photosynthesis is to orient the reader to the key distinctions between oxygenic versus anoxygenic photosynthesis. Many more detailed descriptions are available [1–4]. The major components of all photosynthetic organisms, defined here as those organisms that use chlorophyll (Chl) or bacteriochlorophyll (Bchl) to convert light energy into chemical free energy [5], consist of pigment molecules noncovalently combined with protein. Chls and Bchls are Mg-porphyrin-containing molecules that have a hydrophobic side chain that facilitates their association with proteins, as well as additional molecule-specific groups as part of the porphyrin ring. Chls are always present in oxygenic photosynthetic organisms, which include plants, algae and cyanobacteria, while anoxygenic photosynthesizers, which are exclusively bacterial, have Chl or Bchl. *R. sphaeroides* is an anoxygenic photosynthetic organism that uses the *a* type Bchl (see [1, 2] for further details).

There are two forms of pigment-protein complexes; those that harvest light energy and those which, in addition to harvesting light energy, also engage in the separation of charge, leading to the electron transport processes that will ultimately generate a proton motive force (PMF) for ATP formation and an electrochemical gradient. Within the light-harvesting complexes (LH), Chl or Bchl molecules are positioned, as a result of their interactions with the polypeptides, in a manner that allows them to optimally absorb light energy, and which also allows the 'energy' to be efficiently transferred to other similarly positioned Chls or Bchls. Energy can also be transferred to suitably positioned key Chls or Bchls that are present in a second form of complex, which leads to charge separation, and the initiation of electron transfer processes. This second type of complex is called the reaction center (RC).

In addition to the Chls and Bchls, there are other classes of photopigments, and here we are concerned with the carotenoids (Crts). Crts play two critical roles in photosynthesis (for details see [6, 7]). First, they improve energy collection because they can absorb light at shorter wavelengths than the Chls and Bchls, and then transfer that energy to Chl and Bchl. Second, they protect photosynthetic cells because they can quench high energy states of Chl and Bchl, as well as the toxic singlet oxygen these 'energetic' Chls and Bchls have the potential to generate in the presence of oxygen. Before describing these aspects of photosynthesis in more detail, we need to first complete our description of the photosynthetic process relating to energy capture and then describe the differences and similarities between photosynthesis that involves the generation of oxygen and photosynthesis that does not lead to oxygen generation.

Electron flow stemming from charge separation in the reaction center involves the small molecule quinones, which transfer electrons between membrane-localized complexes that also act as proton pumps. The resulting proton motive force (PMF) is used by the cellular ATP synthase to form ATP. Therefore, with respect to ATP production, it is the mode of action involved in the initiation of electron transfer that distinguishes photosynthesis from respiration, i.e., light entrapment versus biological oxidation.

We can divide photosynthetic organisms into two general groups, based on the source of reducing power used to generate and sustain high-energy electron flow. A simple overview of electron flow in these two groups is provided in figure 2. One group extracts electrons from water, ultimately producing oxygen as an end product, hence the name oxygenic photosynthesis. These electrons are eventually transferred to $NAD(P)^+$, and thus electron flow in those organisms is a linear process. The oxygenic photosynthetic organisms employ two different types of RCs working in series to carry out these processes by generating sufficient energy to 'split' water. On the one hand, a RC of sufficiently (high) positive reducing potential is required to be able to accept electrons from water, which is then converted, through excitation energy transfer to an electron donor capable of transferring electrons to the quinone pool, and eventually to a second RC that in its excited state can transfer reductant to $NAD(P)^+$. The reduction potential that is spanned as the result of this two-step process involving the receipt of electrons from water and donating electrons to $NAD(P)^+$ is apparently too great to be accomplished by a single RC. The other group of photosynthetic organisms is incapable of extracting electrons from water, since the energy 'jump' is too great, and the electron transfer reactions are cyclic; the 'deenergized' electrons that were used to generate the PMF are ultimately returned to the photosynthetic RC where they will become reenergized. Because oxygen is not generated, since water is not an electron donor, this process is called anoxygenic photosynthesis. The RCs present in these organisms span a vastly different range in reduction potential in terms of their resting and excited states. This energy jump dictates how reducing power for those species that fix carbon is generated.

The purple bacteria, including *R. sphaeroides*, use the same Calvin cycle for fixing carbon that is present in many oxygenic photosynthetic organisms [8–10]. However, the NAD(P)H that is required for reducing carbon dioxide is not generated in the photosynthetic process, as occurs in oxygenic photosynthetic organisms, since the primary electron acceptor in purple bacteria, the quinone

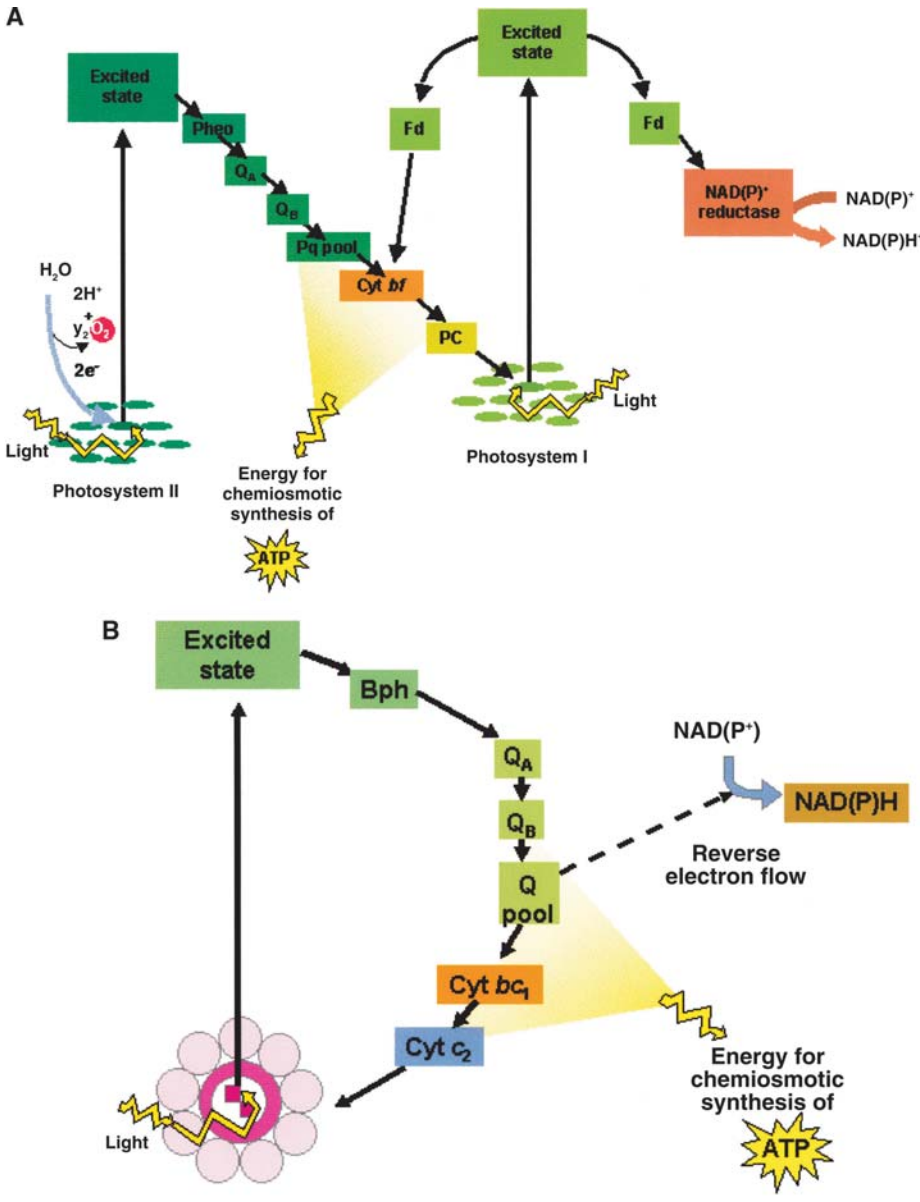

Figure 2. Comparative diagram of electron flow in oxygenic photosynthesis versus anoxygenic photosynthesis (in purple bacteria). Abbreviations in (*A*) are Pheo, pheophytin; Q_A and Q_B, plastoquinones bound to the reaction center; Pq pool, plastoquinone pool; Cyt *bf*, cytochrome *bf*; PC, plastocyanin; Fd, ferredoxin. Abbreviations in (*B*) are Bph, bacteriopheophytin; Q_A and Q_B, quinones bound to the reaction center; Q pool, quionone pool; Cyt bc_1, cytochrome bc_1; Cyt c_2, cytochrome c_2.

pool, is more electropositive than $NAD(P)^+$. Instead, these organisms use their PMF to drive low-energy electrons 'uphill' to a higher energy level, to achieve a reversed flow of electrons that are now capable of reducing $NAD(P)^+$. Because these electrons are removed from the system, i.e. carbon reduction, they need to be replenished in order for cyclic anoxygenic photosynthesis to proceed. It is interesting that the electrons used to replenish the pool of reducing power can be obtained from organic compounds, as well as inorganic compounds (see chapter 41 in [2] and [4]). Carbon dioxide fixation pathways other than the Calvin cycle are known to exist [11]. Details of those processes are beyond the scope of this review. To complete our description of the anoxygenic photosynthesizers, we also need to point out that some among these have RCs in which electrons are excited to sufficiently negative reduction potentials, i.e. highly energetic, that they can be transferred directly to $NAD(P)^+$. Others use primary electron acceptors of sufficient reducing potential that they can be employed directly in fixing carbon dioxide, and still others must deplete the PMF in the same manner as the purple bacteria to create sufficiently strong reducing potentials. Details of these events are described in [1, 2, 4]. But in no case is sufficient en-

ergy generated to use water as a source of reductant. Given this discussion, it is clear that one group of photosynthesizers developed the capacity to deal with the presence of oxygen early on, while the other group had no such need.

The photosynthetic membrane of *R. sphaeroides*

Development of the specialized intracytoplasmic membrane (ICM) structure of cells that are competent for photosynthesis is induced when oxygen is limiting, and independent of the presence or absence of light. The ICM is the result of a differentiation process in which the cytoplasmic membrane invaginates, forming structures that contain the LH and RC pigment-protein complexes. The photosynthetic assembly consists of a RC complex that is surrounded by LHI, one of two types of light-harvesting pigment-protein complexes. The light collection capability of the LHI-RC system is augmented by arrays of the second type of light-harvesting complex, LHII, that are distributed around the LHI-RC core. A depiction of this arrangement is included in figure 1. Because the Bchls within the LH complexes are symmetrically arranged, they absorb light at discrete wavelengths that are characteristic for each LH type; for LHI this is 875 nm, while LHII has maxima at 800 and 850 nm, because the Bchl molecules are positioned in two arrangements. Thus, a common nomenclature for the LH complexes is B875 (B is Bchl) and B800–850, corresponding to LHI and LHII, respectively. In addition to the primary environmental signal that induces formation of the ICM, namely limiting oxygen concentrations, the number of invaginations and concentration of LHI-RC varies inversely with the incident light intensity, as does the ratio of LHII to LHI-RC complexes. A description of the differentiation process can be found in [12], and structural details are available for both the RC and LH pigment-protein complexes in [13–16]; for an on-line interactive source see http://www.life.uiuc.edu/crofts/bioph354/lect22.html

Genes and proteins of photosynthesis

The genes and proteins associated with photosynthesis in *R. sphaeroides* are listed in table 1. These are of three types: those coding for enzymes that catalyze the biosynthesis of Bchl, the enzymes for Crt biosynthesis, and the polypeptides of the LH and RC complexes. These genes are clustered at the photosynthesis region on the larger of the two chromosomes of the *R. sphaeroides* genome (note that not all of the genes in this region are of these three types, and so they are not included in table 1). The expression of the photosynthesis genes are regulated in a manner that ensures that pigments and proteins are produced at optimal rates, such that neither is present in vast excess of the other, nor are they produced under inappropriate (aerobic) conditions. While some genes coding for the regulatory proteins involved in this coordinated event are also located within the photosynthesis region, others are present at other loci within the genome.

Table 1. Photosynthesis genes and operons.

Gene or operon	Function
bchFNBHLM	Bchl biosynthesis
bchEJG	Bchl biosynthesis
bchCXYZ	Bchl biosynthesis
bchHDI	Bchl biosynthesis
pucBAC	LHII polypeptides (PucB and PucA) and assembly factor (PucC)
pufQ	LHI and LHII complex assembly factor
pufKBALMX	LH1 polypeptides (PufB and PufA), RC polypeptides (PufL and PufM), and PufB translation gating polypeptide (PufK)
puhA	RC polypeptide (PuhH)
crtA	Crt biosynthesis
crtIB	Crt biosynthesis
crtDC	Crt biosynthesis
crtEF	Crt biosynthesis
cycA	cytochrome c_2

It is perhaps useful to mention here, with respect to Bchl and Crt biosynthesis, only those genes whose products catalyze reactions that are specific to Bchl and Crt formation are present in the photosynthesis region, and are therefore regarded as photosynthesis genes. Thus, *bch* genes encode enzymes that catalyze Bchl biosynthesis reactions beginning with the insertion of magnesium into protoporphyrin IX (for more information, see [17]), and *crt* genes code for those enzymes required to produce the Crts spheroidene and spheroidenone from an isoprenoid pyrophosphate precursor (detailed in [6]). Also, the *cycA* gene, encoding the cytochrome c_2 protein, is within the photosynthesis region, and is often denoted as a photosynthesis gene, although it can participate in aerobic as well as photosynthetic electron transport. Finally, the DNA sequence of this region, as well as the remainder of the *R. sphaeroides* 2.4.1 genome, has been determined, and annotation information is available at http://www.rhodobacter.org

The yin and yang of oxygen

The timing of the evolution of photosynthesis relative to aerobic respiration is not firmly resolved, although several recent studies have suggested quite fascinating and novel hypotheses. It is widely accepted that evolution of oxygenic photosynthesis made possible aerobic metabo-

lisms, since prior to that event, free oxygen was not available. But this view has come into question [18–22]. A recent review and compilation of DNA sequences coding for the Rieske iron-sulfur protein-cytochrome *b*-cytochrome *c* enzyme, or cyt bc_1, which is a component of both respiratory and photosynthetic electron transfer chains (see fig. 1), presents evidence for its early, pre-photosynthesis evolution since it is represented in Archaeal species, which do not possess photosynthesis [23]. Together with cyt bc_1, identification of a cytochrome *c* oxidase in Archaea would establish the existence of an electron transport chain with oxygen as the final electron acceptor prior to the split into the Archaea and Bacteria evolutionary branches, i.e. before any form of photosynthesis had evolved. However, a consensus answer as to the presence of a cytochrome *c* oxidase before the split is currently lacking [24, 25]. Nevertheless, this work has provided the impetus for developing and exploring new ideas with respect to the evolution of the photosynthetic RCs. For example, recent investigations suggest that the RC protein may have been derived from cytochrome *b* or a similar molecule, with anoxygenic photosynthesis as it occurs in the purple bacteria, and using Bchl, evolving prior to oxygenic photosynthesis, which uses Chl (described collectively in [26–28]).

The hypothesis of an aerobic metabolism that predates the evolution of any photosynthetic process has bearing on this discussion, since if it is correct then one could assume that protection against toxic oxygen species generated in even the most primitive respiratory process might have evolved before photosynthesis, and been available for photoprotection as the oxygenic photosynthesis option evolved. This option would have given rise to higher concentration of oxygen, which would ultimately lead to oxygenic and anoxygenic photosynthesis.

Why is oxygen toxic to cells?

In vitro, molecular oxygen itself can apparently destroy iron-sulfur clusters, which can thereby inactivate enzymes requiring these structures (described in [29]). There is, in fact, good evidence that this destructive activity is central to a widely employed regulatory mechanism that prevents the needless synthesis of enzymes that are not functional or useful in aerobic metabolism (system I below). However, the level of actual toxicity associated with this direct role of oxygen in vivo is not yet known [29]. Known with greater certainty is the toxicity associated with other dangerous oxygen species that can be formed from molecular oxygen, either in response to external chemicals or by the cell itself, as it goes about its business of obtaining energy (reviewed in [29]): (i) flavins can be oxidized by oxygen, generating superoxide and hydrogen peroxide as products; (ii) in turn, enzymes that depend on iron-sulfur clusters and sulfhydryls are inactivated as these groups become oxidized by the action of the superoxide and hydrogen peroxide, both of which are more reactive than molecular oxygen; (iii) the oxidation of free iron by hydrogen peroxide forms hydroxyl radicals, which can interact with virtually any biomolecule, especially DNA.

Organisms that grow in the presence of oxygen, or use oxygen in respiration, have superoxide dismutases, peroxidases and catalases that can convert the toxic compounds that inevitably are generated to nontoxic chemicals. Obligate anaerobes, in addition to lacking oxygen radical detoxifying enzymes, also have dioxygen-sensitive enzymes that are not needed by aerobes but are used to maintain redox balance in the anaerobic organisms. For both of these reasons obligately anaerobic organisms cannot grow under aerobic conditions. But, it is the destruction of DNA mediated by hydroxyl radicals that probably kills them [29]. Recently, a previously unsuspected enzyme, superoxide reductase, was found to play a protective role in several obligate anaerobes (summarized in [29]). The importance of this discovery is that it identifies an entirely new means of detoxifying superoxide. A question that would be useful to address next will be whether or not there exist currently unknown enzymatic strategies to detoxify hydrogen peroxide, as well.

Antioxidant compounds are also present in both aerobes and anaerobes. Glutathione and thioredoxin are examples of antioxidants that can protect protein thiols via exchange reactions (see [30] for others present in prokaryotes). They can also detoxify toxic oxygen species directly, as can Crts and several other molecules (for a more extensive list see for example [31]); this function of Crts makes them highly useful in oxygen protection in both photosynthetic and nonphotosynthetic organisms. Therefore, we can visualize over time the development of two general strategies employed by photosynthetic organisms: (i) directly confront the presence of oxygen by coping with its dangerous downsides, especially in oxygenic photosynthesis, or (ii) develop strategies to employ the photosynthetic lifestyle only when oxygen is absent.

Special concerns with respect to photosynthesis

In photosynthetic organisms, oxygen toxicity stems from an additional source; namely, the photosynthetic process itself can generate toxic oxygen derivatives. This occurs when excited Bchls and Chls transfer the energy to oxygen instead of the RC, thereby generating highly reactive singlet oxygen. Note that the longevity of Bchl and Chl in the excited state is determined by how fast the downstream electron transfer reactions can occur, since charge separation is relatively instantaneous. Because of these differential rates, the level of oxygen toxicity in photosynthetic organisms is coupled to metabolic rates. Thus, carbon dioxide fixation and nitrogen fixation that con-

sume large amounts of ATP and reducing power actually become integral to the ability of photosynthetic organisms to tolerate high light flux. Therefore, it is not surprising that expression of genes associated with these processes is governed by some of the same mechanisms that regulate photosynthesis gene expression in *R. sphaeroides,* which possesses all of these metabolic capabilities, as well as the ability to utilize molecular oxygen. In higher photosynthetic organisms, this relationship is called sink regulation (reviewed in [32]).

Coping mechanisms

It is convenient to subdivide mechanisms to cope with oxygen in photosynthetic organisms into several categories. We include in one category those protective mechanisms that are not specific to photosynthesizers; the detoxifying actions of enzymes such as superoxide dismutase, peroxidase and catalase, and the antioxidant activity of compounds such as glutathione, thioredoxin and Crts, are in this category. Also placed in this category is simply metabolizing oxygen, thereby removing it as a toxic agent. A second category for coping encompasses those rapid changes in photosynthetic organisms that provide protection from dangers that arise through sudden changes in environmental conditions. Relatively slower changes that occur by modulating the presence of components that can generate toxic oxygen species are placed in a third category. A full consideration of all of these coping mechanisms is not feasible here, and the following descriptions pertaining to the first two categories have been selected for further discussion. We then provide a more in depth consideration of oxygen control of gene expression in *R. sphaeroides*, which belongs to the third category of coping mechanisms.

Nearly 30 years ago, Lumsden et al. [33] purified a superoxide dismutase from *R. sphaeroides*, but a clear picture of the physiological importance of this and other components comprising the generalized protection category is only beginning to emerge in *Rhodobacter* species. Recently, the superoxide dismutase coded for by the *sodB* gene of *R. capsulatus* has been shown to be essential for aerobic growth [34]. Interestingly, this enzyme appears to contain iron when isolated from photosynthetic cultures and manganese when isolated from aerobically grown cells [35]. The physiological significance of this property has not yet been described, but reconstitution studies of purified recombinant enzyme with one or the other metal reveal that they differ with respect to hydrogen peroxide sensitivity [35].

Hochman et al. [36] characterized a mutant strain of *R. capsulatus* lacking catalase-peroxidase activity, and observed differences in viability in aerobic stationary phase cultures relative to the wild type. However, growth of the mutant strain was not measurably different under either aerobic or photosynthetic conditions. Since *R. capsulatus* also has a second peroxidase, it was proposed that this second enzyme is more important for protection in growing cultures, while the catalase-peroxidase is required in stationary phase. To our knowledge, the role of the second peroxidase has not yet been described. Nevertheless, subsequent analysis of the *cpeA* gene, coding for the catalase peroxidase, shows expression is higher under aerobic conditions, and it is also peroxide inducible [37, 38].

The *trxA* thioredoxin gene of *R. sphaeroides* was identified by Pasternak et al. [39], and those workers also demonstrated that it is essential for aerobic and anaerobic respiration using dimethyl sulfoxide (DMSO) as terminal electron acceptor. More recently, Pasternak et al. [40] succeeded in constructing a mutant strain that is disabled in the chromosomal *trxA* gene, but whose growth is supported by the additional presence in trans of inducible *trxA* sequences on a plasmid, enabling for the first time an evaluation of the role of thioredoxin with respect to photosynthesis in this organism. This investigation revealed that TrxA, in reduced form, is required for normal Bchl content and messenger RNA (mRNA) levels of the photosynthesis proteins, suggesting that thioredoxin may have a regulatory role; whether this function is in addition to antioxidant activity is not yet known, and therefore we place it in the first category of protective mechanisms.

With respect to the second category of oxygen coping mechanisms, photosynthetic organisms can alter the abundance and/or efficiency of light-absorbing complexes such that excess radiant energy is diverted and/or dissipated. Knowledge of this process in plants has increased considerably in recent years, and reveals that posttranscriptional regulatory events are central. Preliminary observations suggest that some form of this regulation must also occur in *R. sphaeroides*. In plants, the process is called feedback deexcitation, and it occurs when the pH of the thylakoid lumen decreases, which would occur when the rate of generation of the PMF exceeds its rate of dissipation. This lower pH activates an enzyme present in the lumen which catalyzes the conversion of one species of Crt to another, and also causes the protonation of at least one of the subunits of plant photosystem II (fig. 2). The latter event precipitates a conformational change, and with the new Crt species, together these are able to deexcite the singlet Chls in the photosystem (reviewed in [7]). The following observations have been made with respect to *R. sphaeroides*: (i) there is evidence that the enzyme CrtA (table 1), which catalyzes the conversion of spheroidene (SE) to spheroidenone (SO), is posttranscriptionally regulated [41]; (ii) the B800-850 light-harvesting complex, composed of the *pucB* and *A* gene products (table 1) together with Bchl, shows a preference for binding SE, and thus the level of the B800–850 complex can be posttranscriptionally regulated in response to light intensity [42]; (iii) the DNA se-

quence of the *R. sphaeroides* genome confirms the existence of a second *pucBA*-like sequence which is not within the photosynthesis gene cluster (http://www.rhodobacter.org). Suitable experimentation will be required to determine the significance of these observations in relation to those found in green plants. However, it is noteworthy that *R. sphaeroides* is not the only anoxygenic photosynthetic bacterium having multiple copies of genes coding for photosynthesis polypeptides, and in certain species of anoxygenic photosynthetic bacteria the polypeptide composition of the LHII complexes is known to vary in response to changes in incident light intensity [43, 44].

In the next section, we consider a description of what is currently known or surmised for *R. sphaeroides* about coping strategies in the third category, which involve oxygen control of gene expression at the level of transcription.

The facultative photosynthetic bacteria: the price of metabolic vigor is the need for oxygen control of gene expression

The metabolic sophistication of facultative photosynthetic organisms suggests that in terms of gene regulation, they represent a 'one-stop shop' for the examination of mechanisms by which cells regulate genes to optimize energy production and redox flow, and at the same time to protect themselves as oxygen availability changes. At the transcription level, facultative anoxygenic photosynthetic cells have developed highly sophisticated mechanisms that enable them to seamlessly move between different growth modes.

Three regulatory systems relating to oxygen control of gene expression that have emerged are described below. We have limited this discussion to the most recent findings pertaining to these systems. Additional details, as well as other aspects of photosynthesis gene regulation, can be found in [45–47]. The nomenclature describing photosynthesis gene regulation and the associated regulatory proteins has become complex because homologues for certain protein components have been given different names in different organisms. To assist the interested reader, table 2 lists the names of several homologues as a key to that literature. Note that the description here will be dedicated to *R. sphaeroides*. Even though some protein components are very similar in other organisms, their regulatory role is not necessarily equivalent. Some well-defined examples of such differences are included.

System I: Regulation mediated by molecular oxygen

The FnrL protein of *R. sphaeroides* is considered to be a homologue of the *Escherichia coli* Fnr (fumarate and nitrate reduction regulatory protein), based on the presence in FnrL of amino acid homology to all the known functional domains that characterize the *E. coli* Fnr protein (reviewed in [48]). These include (i) a DNA binding domain towards the C-terminus having a predicted helix-turn-helix secondary structure, (ii) a more centrally localized dimerization domain and (iii) a characteristic cysteine motif consisting of a cluster of three cysteine residues near the N-terminus of the protein plus an additional cysteine that is near the dimerization domain. In *E. coli* Fnr, the cysteines have been shown to coordinate a 4Fe-4S cluster that is required for the protein to achieve a DNA binding-competent conformation [49]. This cluster is molecular oxygen labile, and Fnr is converted to a form that is inactive with respect to DNA binding when oxygen concentrations reach critical levels [50]. Figure 3 compares the predicted amino acid sequence of the *R. sphaeroides fnrL* gene product to that of *E. coli* Fnr, indicating important features of the proteins, together with a cartoon diagram of its action, extrapolated from thorough investigations of the Fnr protein of *E. coli* (for recent reviews, see [48, 51]).

Table 2. *R. sphaeroides* regulatory proteins and homologues.

Name of *R. sphaeroides* protein	Name of homologue(s): Organism	References[a]
FnrL	Fnr: *E. coli*	
	FnrL: *R. capsulatus*	[54]
PpsR	CrtJ: *R. capsulatus* [126, 127]	
AppA	none described	
PpaA	AerR: *R. capsulatus* [128]	
PrrA and B(also called RegA)	RegA and B: *R. capsulatus*,	[126, 127]
	RegA and B: *R. sulfidophilum*,	[90]
	RegA and B: *R. denitrificans*	[90]
PrrC	SenC: *R. capsulatus*,	
	SenC *Rhodovulvus sulfilobum,*	[90]
	SenC *Roseobacter denitrificans*	[90]
TspO (also called CrtK)	CrtK: *R. capsulatus*	[129]
	pk18: mammals	[110, 130]

[a] Cited references are review articles, if available.

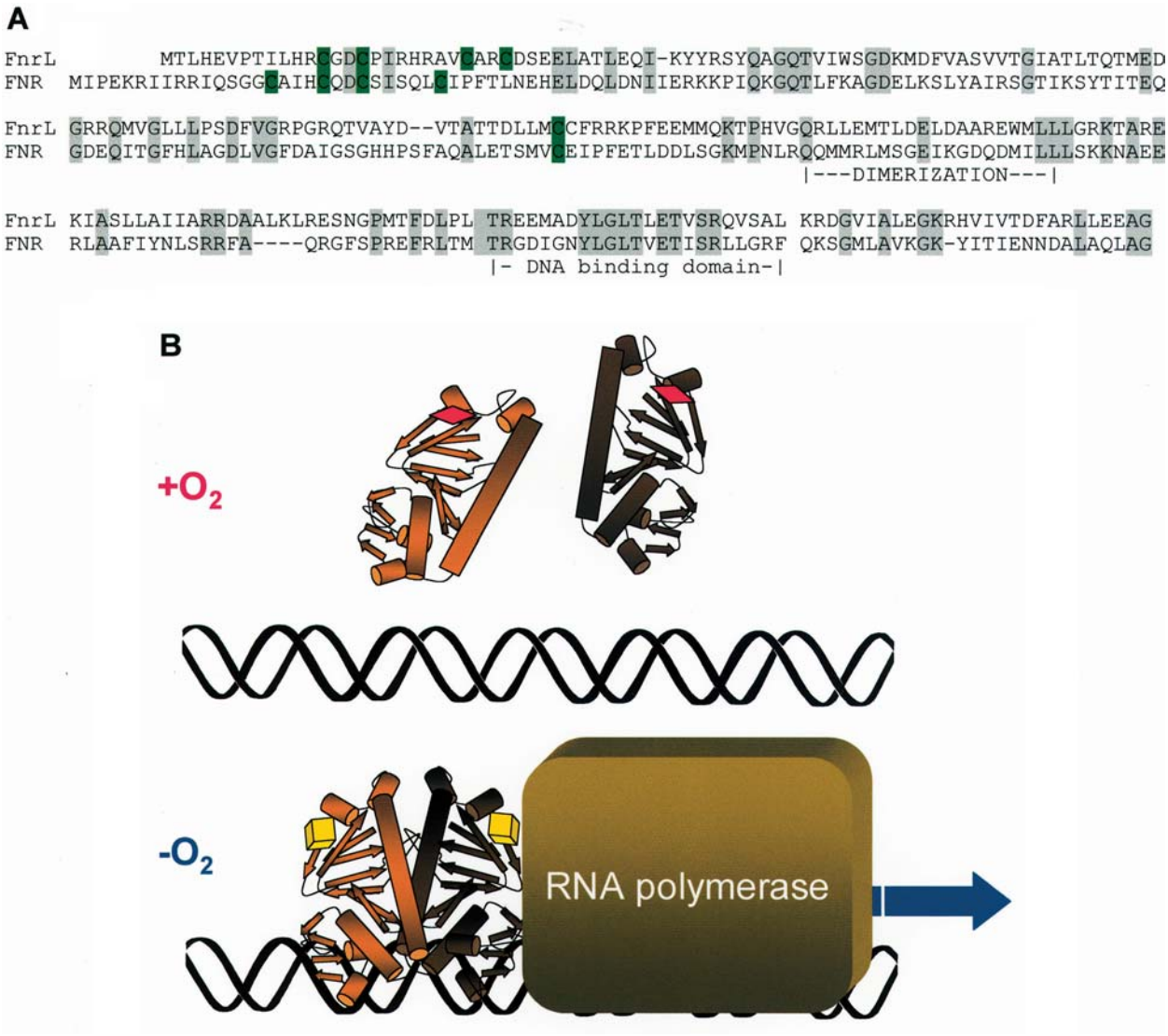

Figure 3. Alignment of *R. sphaeroides* FnrL and *E. coli* Fnr proteins, and cartoon diagram of FnrL activity. Indicated in (*A*) are functional domains of FnrL protein, predicted from domains of *E. coli* Fnr protein. The diagram of FnrL-mediated regulation shown in (*B*) is based on the activity of *E. coli* Fnr (reviewed in [48, 51]). In the absence of oxygen, FnrL activates transcription by binding DNA as a dimer; each subunit has a [4Fe-4S] center. When oxygen is present, the [4Fe-4S] center is converted to a [2Fe-2S] center, which induces a conformational change in the protein, and it is no longer capable of binding to DNA.

The *E. coli* Fnr regulon includes more than 100 genes [52]. Apparently, the theme uniting genes belonging to this expansive regulon is that they are associated with adapting the expression of genes involved in energy metabolism in the cell from that which is suited to an oxygen-rich environment to that which is effective in an oxygen-poor environment. Therefore, a predictor one might use to identify gene members of the FnrL regulon of *R. sphaeroides* would be that they encode products required to support growth when oxygen becomes limiting. This was substantiated in the first instance by virtue of the fact that FnrL$^-$ mutant strains are incapable of anaerobic dark (with DMSO) or light (photosynthetic) growth [53]. Additional evidence came from inspecting the upstream sequences of genes, which are involved in these growth modes, for the presence of DNA sequence corresponding to the Fnr binding site, TTGAT-N_4-ATCAA [54]. Several genes whose upstream sequences include this motif have now been individually evaluated for the contribution of FnrL to their expression, and in all cases, findings were consistent with a role for FnrL. Some notable examples, which serve to further delineate the character of the FnrL regulon, include the *puc* operon [55] and *bchE* [56], genes that are associated with or essential for photosynthesis (table 1); the *ccoNOQP* operon [57], which codes for the high-oxygen-affinity cbb_3-type cytochrome *c* oxidase that is suited for aerobic respiration in oxygen-restricted environments [58, 59], and *dorSR* [60], whose

role is to activate *dorBAC* expression under anaerobic conditions when DMSO respiration is appropriate [60, 61].

If FnrL really determines the cell's metabolic profile, then turning on genes required for oxygen-poor metabolic processes should be accompanied by a turn-off of genes that would operate in an oxygen-rich-requiring metabolism. This hypothesis finds support in that the upstream sequences of genes coding for the *R. sphaeroides* low-oxygen-affinity aa_3-type cytochrome *c* oxidase, contain Fnr consensus-like sequences [62, 63], believed to serve as targets for FnrL-mediated repression of these genes. This terminal oxidase has a low affinity for oxygen, and is used for aerobic respiration when oxygen concentrations are high. The predicted regulation of these genes by FnrL at first appears to be unsubstantiated by transcription analyses [64]. However, there are two known cases in which genes can be both directly and indirectly regulated by FnrL [55, 65], and the aa_3-type cytochrome *c* oxidase genes might represent additional examples. Another possibility suggested by Flory and Donohue [64] is that FnrL, in combination with another transcription factor that was not present in their assays, mediates the repression event. Further studies will be required to reach a final conclusion as to whether or not FnrL regulates these genes.

There is also a major distinction between the role of FnrL among different species of *Rhodobacter* as revealed by comparing growth characteristics of FnrL$^-$ mutant strains of *R. sphaeroides* and *R. capsulatus*. Whereas an intact *fnrL* gene is required for both anaerobic dark growth with DMSO and photosynthetic growth of *R. sphaeroides* [53], photosynthetic growth of *R. capsulatus* does not require an intact *fnrL* gene, although anaerobic dark growth with DMSO does [54]. It had been noted that while an FNR binding motif could be discerned in the upstream sequences of the *bchE* gene in *R. sphaeroides*, no motif could be identified in the upstream sequences of the *R. capsulatus bchE* gene [54]. FnrL-mediated regulation of the *bchE* gene in *R. sphaeroides* has now been confirmed [56], and since Bchl is essential for photosynthesis, the requirement for FnrL to activate transcription of this gene is already sufficient to account for the growth differences between the two FnrL$^-$ mutant species.

FnrL also regulates Bchl formation by modulating expression of genes whose products catalyze reactions in the biosynthesis of the tetrapyrrole nucleus of Bchl, protoporphyrin IX. Protoporphyin IX is the precursor of heme, as well, which is required for both aerobic and anaerobic metabolisms, including photosynthesis (reviewed in [66, 67]). Thus, *R. sphaeroides* is confronted with the challenge of ensuring that Bchl formation is strictly off in the presence of oxygen, while heme formation must continue, but also be responsive to changes in oxygen availability, since the need for heme is higher for photosynthesis than it is for aerobic respiration. The known gene targets of FnrL regulation in the protoporphyrin IX biosynthetic pathway are *hemA*, one of two genes [68, 69] that code for enzymes which catalyze the first step in the pathway, and *hemN* and *hemZ*, which encode isoenzymes [53, 70] that catalyze the penultimate step in the pathway. While *hemZ* transcription is absent without FnrL [56], both *hemA* and *hemN* are regulated in a more complex manner, and even in the absence of FnrL they are transcribed at basal levels [53, 56]. On the other hand, both *hemA* and *hemN* induction in response to lowering oxygen tensions absolutely requires an intact *fnrL* gene [53, 56, 65]. Thus, the added requirement for tetrapyrrole as oxygen tensions decrease stimulates all FnrL-controlled genes involved in tetrapyrrole formation. This complex pattern of tetrapyrrole biosynthesis gene expression helps to ensure that the necessary levels of heme precursors are formed for both aerobic and anaerobic growth, while also accommodating the increased need for these same molecules in Bchl production as the cell prepares for photosynthesis. However, this is by no means a complete description of the regulation of tetrapyrrole biosynthesis in this metabolically complex organism, and some additional aspects will be described below.

It is difficult to imagine that FnrL regulation alone would allow an organism with so many anaerobic metabolic options to be able to determine which amongst them should prevail. This appears to be correct since (i) the upstream sequences of operons coding for two quinone oxidases in *R. sphaeroides* have no discernable FNR consensus-like sequence [71], (ii) FnrL participates in a multilayered regulatory mechanism that, in the presence of DMSO and in the absence of oxygen, leads to activation of DMSO reductase genes [61]; (iii) FnrL has no role in the expression of genes associated with the denitrification metabolism which exists among some strains of *R. sphaeroides* [72, 73] and (iv) it is clear that with respect to photosynthesis genes, additional regulatory factors are involved (see below).

System II: regulation mediated by electron flow

The curtailed growth capabilities of FnrL$^-$ mutant strains of *R. sphaeroides* leave little doubt that the *fnrL* gene is indispensable for activating genes that are central to obtaining energy when oxygen is in short supply. But, even when energy needs can be met, without the ability to maintain redox balance, i.e. reoxidize NAD(P)H, growth would not be possible. In other words, besides deploying the correct assemblages of cellular components to be able to generate cellular energy according to the resources which are available, cells must adapt their metabolic capability such that energy production keeps pace with energy consumption – and vice versa.

One way in which *R. sphaeroides* regulates gene expression to achieve this apparent homeostasis is to monitor the rate of electron flow, then to adjust the availability of proteins required for both energy generation and consumption, accordingly. This monitor is proposed to be the cbb_3-type cytochrome *c* oxidase in *R. sphaeroides*, and recent findings that will be presented below have provided a plausible argument for the actual mechanics of the process by which monitoring takes place. There is now considerable evidence to suggest that the adjustment process, i.e. altered transcription of genes, is mediated by the Prr (photosynthesis response regulator) proteins, and the current understanding of this mechanism will also be described.

The genes coding for the PrrB, C and A proteins are arranged as shown in figure 4 [74], and PrrB and PrrA comprise a two-component signal-transduction system. Investigations performed in several laboratories have collectively provided important details about these two proteins. The PrrB protein consists of a membrane-spanning region, constituting the signal-sensing domain of the protein, and a cytoplasmically localized region corresponding to the transmitter domain [75–77]. PrrB activities include all of those typically associated with the histidine kinase proteins of two-component regulators. These include PrrB autophosphorylation, and both kinase and phosphatase activity towards PrrA, its partner DNA-binding response regulatory protein [76–78]. In the absence of an incoming signal, the PrrB kinase activity predominates, and the levels of phosphorylated PrrA (PrrA~P) are high [76]. Thus, the kinase-dominant mode constitutes the default state of PrrB. In vitro transcription assays using purified protein components and DNA template corresponding to regulatory sequences of the *cycA* gene coding for cytochrome c_2 (see table 1), which is known to be regulated by Prr [79], demonstrated that the DNA binding affinity of PrrA ~ P is approximately 15-fold higher than for PrrA [77]. Nonetheless, PrrA is also capable of binding DNA [77], and it has been suggested that the binding activity of unphosphorylated PrrA could explain feedback regulation of the PrrBA system at the level of transcription [76, 77].

While the arrangement of the *prr* genes had long suggested that all three Prr proteins belong to the same regulatory locus, the role of PrrC had been difficult to ascertain. Once it became clear that *prrA* is cotranscribed with *prrC*, suitable PrrC$^-$ mutant strains that avoided problems of polarity effects on the expression of *prrA* could be constructed [74]. Analysis of these mutants revealed that disabling *prrC* results in the inability to keep transcription of the *puc* and *puf* operons (containing genes which encode polypeptides of the LH and RC complexes; see table 1) muted in the presence of oxygen, thereby firmly establishing the PrrC protein as an additional member of the PrrBA regulatory system [74]. Another study reports PrrC can bind copper, and there is evidence to suggest it possesses oxidoreductase activity [80]. The significance of these functions is not yet understood, but they may be involved in transmitting the regulatory signal to PrrB generated from the upstream monitor of electron flow, the cbb_3-type cytochrome *c* terminal oxidase.

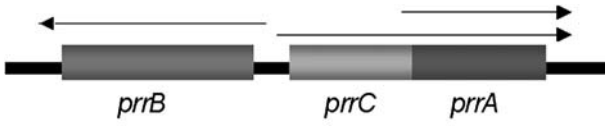

Figure 4. Relative arrangement of the *prr* genes of *R. sphaeroides*. Arrows denote direction of transcription; the two arrows above *prrC* and *prrA* refer to hybridization analyses in which transcripts corresponding to *prrA* and *prrCA* were detected [74].

The polypeptides of the cbb_3-type cytochrome *c* oxidase are coded for by four genes comprising the *cco* operon. FnrL activates expression of the operon in response to lowering oxygen tensions [57], while simultaneously expression of the aa_3-type cytochrome *c* oxidase expression decreases [64], so that while the overall level of electron flow directed through the cbb_3-type cytochrome *c* oxidase increases with decreasing oxygen tension, the flow through individual cbb_3 oxidase complexes is nevertheless diluted by the increase in concentration of the enzyme. It has been known for several years that photosynthesis gene expression is elevated in the presence of oxygen in cells lacking a functional cbb_3-type cytochrome c oxidase [81]. The working hypothesis to describe this observation suggested that the cbb_3-type cytochrome *c* oxidase generates a signal that increases in magnitude with increasing electron flow through this enzyme [46, 47]. This signal, in turn, results in the absence of the phosphorylated form of PrrA, namely PrrA ~ P, because the PrrB phosphatase activity is stimulated [76]. Therefore, gene targets of PrrA~P activation are quiescent and, hence, with respect to expression of PrrA ~ P-activated genes, the signal is inhibitory in nature.

Considerable effort has been directed towards determining the identity of the postulated 'inhibitory' signal, and how it is communicated to PrrB. This is no easy task, since phenotypes associated with the regulatory event are only apparent in the absence of the signal. Nevertheless, progress has been made, and the key findings are reported here. First, it is now known that the signal does not emanate from an alteration in the PMF [46]. Second, it has been determined that the signal is absent when electron flow is altered such that electrons do not arrive to the cbb_3-type cytochrome *c* oxidase or when the oxidase itself is absent [46]. The need for a functional cbb_3-type cytochrome *c* oxidase has been confirmed by recent studies that have clarified the role of the CcoQ protein. A cat-

alytically active cbb_3 enzyme contains the CcoN, CcoP and CcoO polypeptides, and it is now known that the fourth polypeptide, CcoQ, is required to stabilize the enzyme in the presence of oxygen [82]. Therefore, all available evidence is consistent with the hypothesis that electron flow through catalytically active cbb_3 oxidase protein generates the inhibitory signal. Finally, PrrC is now known to be a participant in the communication process between the signal generator, the cbb_3-type cytochrome *c* oxidase, and the signal receiver, PrrB [74], and learning more about this protein should provide additional insights into the mechanism of this regulatory system.

Since maintaining redox balance requires coupling energy production rates to energy utilizing rates, it makes good sense to couple the regulation of genes required for these processes. That such coupling exists in *R. sphaeroides* is supported by evidence that, in addition to photosynthesis genes, Prr also regulates expression of genes required for carbon dioxide fixation [83]. *R. sphaeroides* possesses two operons which each contain genes coding for Calvin cycle enzymes [8, 9]. The two ribulose-bisphosphate carboxylase/oxygenase (RuBisCO) enzymes have different affinities for carbon dioxide [10]. In addition to carbon dioxide fixation-specific transcription factors [84, 85], the two operons are differentially regulated by Prr [85, 86]. This provides the cell with considerable flexibility to adjust energy- and electron-consuming metabolism in a manner that compensates for changes in energy production rates.

Additional studies have demonstrated the presence of remarkable redox-balancing capability in *R. sphaeroides* that includes other processes. When the ability to fix carbon is abolished by disabling the genes coding for Calvin cycle enzymes, cells cannot grow anaerobically in the light, even when organic carbon is available. However, the addition of DMSO restores this capability because the cells have been provided with an alternate electron acceptor [10]. This early observation suggested that DMSO reduction might also be regulated by the by the cbb_3 oxidase-Prr system. Consistent with this idea, Mouncey and Kaplan have determined that expression of the *dorCBA* operon, which contains the genes coding for the structural components of DMSO reductase, is affected by the presence or absence of the cbb_3-type cytochrome *c* oxidase [61]. Eraso and Kaplan demonstrated that the DorA protein (see fig. 1) could be detected in extracts of aerobically grown mutant cells lacking PrrC [74], a result that is consistent with the elevated levels of photosynthesis gene products present in the $PrrC^-$ mutant strain, and which again indicates a reduction in the repression of many genes in the presence of oxygen.

Another display of exquisite redox balancing in this same organism was recorded by Joshi and Tabita [87]. Beginning with mutant strains of *R. sphaeroides* lacking Calvin cycle enzymes, they selected for cells that had recovered the ability to grow photosynthetically in the absence of an additional electron acceptor, such as DMSO. Analysis of a photosynthesis-competent revertant revealed that the compensatory mutation had resulted in derepressed synthesis of nitrogen fixation enzymes, thereby providing the cells with an alternative outlet for the excess reducing power. When the *prrB* gene is disabled in the revertant strain, photosynthetic growth is again abolished, thus demonstrating that Prr is directly involved in this event [87]. Therefore, the true metabolic scope of this important regulatory system extends considerably beyond photosynthesis, and includes both carbon and nitrogen fixation.

Recently, conservation of not only sequence but also function among PrrB and PrrA homologues has been shown, extending to several nonphotosynthetic α-Proteobacteria [88], and recently a γ-Proteobacterium [89]. Interestingly, it is only in photosynthetic species that a gene coding for PrrC is also present; thus a *prr* gene arrangement is present in *Rhodovulum sulfidophilum, Roseobacter denitrificans*, and *Rhodobacter capsulatus* [90, 91], but there are no identifiable sequences that code for PrrC homologues in *Bradyrhizobium japonicum* or *Sinorhizobium meliloti*. Perhaps PrrC is a necessary additional component of the PrrBA-mediated regulatory system that ensures photosynthesis genes are also tuned to respond to changes in electron flow as oxygen availability is altered.

System III: regulation mediated by an oxygen-reactive center

Whatever means the cell uses to achieve redox balance, suitable regulation must also be present to ensure that the cell protects itself from conditions that would lead to the particularly dangerous situation of having photosynthetic reactions occurring in the presence of oxygen. The AppA-PpsR regulatory system appears to be critical for preventing this from happening.

The PpsR protein contains (i) a DNA binding domain of the helix-turn-helix type, (ii) two critical cysteine residues and (iii) two PAS domains that are involved in oxidation/reduction reactions [92–94]. This protein binds to the DNA sequence motif TGT-N_{12}-ACA [94–96]. Known targets of aerobic repression by PpsR protein are genes and operons coding for Bchl biosynthetic enzymes [93, 95, 96], Crt biosynthesis enzymes [95], and the *puc* operon [96], coding for polypeptides of the B800-850 light harvesting complex (see table 1). The CrtJ protein of *R. capsulatus* is 53% identical to PpsR at the amino acid sequence level [95] and binds to the same motif [97, 98]. While *bch*, *crt* and *puc* genes are also regulated by CrtJ (reviewed in [99]), the specific genes encompassing the CrtJ regulon are not the same as those of the PpsR regulon. The significance of this observation is not yet understood, although as already mentioned else-

where, it is also known that other homologous regulatory proteins, such as FnrL, in the two *Rhodobacter* species have different cohort genes as well. Additional clarification may be forthcoming in light of the fact that *R. capsulatus* apparently lacks any homologue of AppA.

AppA is encoded by the *appA* gene, which was identified through a screen of a *R. sphaeroides* 2.4.1 cosmid library to identify DNA sequences that suppress defects associated with the absence of PrrB and PrrA activity [100]. Thus, the nature of the screen, and additional analyses [101], established AppA-mediated regulatory phenomena as Prr independent. Understanding how AppA regulates gene expression proved to be a difficult, but ultimately incredibly rewarding task in that, for the first time, light and redox regulation could be coupled. A few of the major milestones along this arduous path are presented.

Milestone 1: PpsR and AppA are part of the same regulatory pathway [101]

$PpsR^-$ mutant and $AppA^-$ $PpsR^-$ double-mutant strains were found to have similar phenotypes. This observation suggested to Gomelsky and Kaplan [101] that PpsR is active upstream of AppA in the same regulatory pathway, which was confirmed by isolation of $AppA^-$-derived mutant strains that had regained the ability to grow photosynthetically. These suppressor mutants were shown to have mutations in *ppsR* that abolish PpsR-mediated repression. As a final demonstration of the participation of PpsR and AppA in the same pathway, an expression study was performed in a heterologous host, *Paracoccus denitrificans*, which is closely related to *R. sphaeroides* but incapable of photosynthesis. It was predicted that *P. denitrificans* would not possess *ppsR* or *appA* genes, which appeared to be exclusively involved in regulation of genes associated with photosynthesis. This study showed that repression of transcription from sequences containing the PpsR-binding consensus sequence occurred only in the presence of *ppsR*. When *appA* was added, the predicted antirepression activity was observed in the *P. denitrificans* background.

Milestone 2: AppA is a flavoprotein [102]

To understand the mechanism of antirepression, Gomelsky and Kaplan [102] attempted to purify AppA but were unsuccessful, as the overexpression system generated protein in recalcitrantly insoluble form. However, determining the cause of the yellow coloration associated with the insoluble inclusions revealed an important property of the protein; namely, AppA binds FAD in a 1:1 stoichiometry.

Milestone 3: the behavior of *R. capsulatus* versus *R. sphaeroides* towards blue light can be explained by the absence of AppA in *R. capsulatus* [103]

The *puc* and *puf* operons, containing genes encoding the polypeptides of the LH and RC complexes (see table 1), are repressed by light in semi-aerobically grown *R. sphaeroides,* but this repression does not occur in *R. capsulatus* [103, 104]. This phenomenon was shown to be due to AppA [103], which is present in *R. sphaeroides* but absent in *R. capsulatus* [94, 103]. The interaction of AppA with PpsR demonstrated previously [101] was consistent with these newer findings, i.e. AppA is required to release the repressor effect of PpsR, and this occurs in two modes. One mode responds to changes in oxygen availability. When oxygen is plentiful, conditions in which aerobic respiration is used to obtain energy, PpsR binds to DNA, and under these conditions AppA is incapable of interacting with PpsR, keeping photosynthesis genes off. As oxygen becomes limiting, the cell resorts to other catabolic options and, appropriately, AppA can interact with PpsR because its conformation has been altered. This interaction releases PpsR from the DNA, and thus the photosynthesis genes under PpsR control are derepressed. The other mode operates in the presence of (blue) light when oxygen, although limiting, is still present; under these conditions, photosynthesis needs to be tightly controlled since it is precisely this situation that can lead to the production of toxic oxygen species via energized Bchl, as described previously. Under these semi-aerobic conditions, interaction between AppA and PpsR is not possible because AppA, in response to blue light, has undergone yet another conformational change that also prevents it from interacting with PpsR.

Precisely parallel with these last in vivo studies, an analysis of the biochemical properties of both PpsR and AppA was conducted [94]. Three results from this analysis added additional insights into the regulatory process. First, it was demonstrated that PpsR contains a disulfide bond in the presence of oxygen. Second, the PpsR disulfide bond was shown to be reduced by the action of AppA. Third, the flavin-bound AppA displays a spectral shift indicative of an excitation state in response to blue light. A meld of these results with those of Braatsch et al. [103] suggests the following: AppA and PpsR are both oxidized under aerobic conditions, and when oxygen tensions are reduced, AppA is reduced and interacts with PpsR, reducing its disulfide bond, and thereby releasing the repressor from the DNA.

At this point, the model of AppA-PpsR regulation presented by Masuda and Bauer [94] differs from that of Braatsch et al. [103]. Whereas the presence of some oxygen is indispensable for blue light repression in the model of Braatsch et al. [103], it would seem that oxygen is not a strict requirement for the same event in the model presented by Masuda and Bauer [94]. In turn, this suggests that the excited conformation of the AppA protein is compatible with an interaction with PpsR. But it is not clear how PpsR, which would be reduced, reacquires the ability to bind to DNA. One possibility is that the PAS do-

mains are involved in the oligomerization of PpsR when the disulfides become reoxidized. On the other hand, if oxygen is indeed absolutely required for blue light repression, it suggests the following: the flavin of AppA catalyzes the formation of superoxide, and the superoxide can reoxidize PpsR, which is again capable of binding DNA. Under strictly anaerobic conditions, this oxidation is not possible because superoxide formation cannot occur. This scenario would be consistent with the observation that photosynthesis genes are not repressed regardless of the presence or absence of blue light when cells are respiring DMSO and are therefore strictly anaerobic [103]. Thus, AppA is cast in the role of the canary in a coal mine, as an exquisitely sensitive monitor of conditions that are most dangerous for photosynthetic cells: the combined presence of light and oxygen. If the AppA flavin can monitor quinone pool status, this would also couple both light and oxygen sensing. While details of the AppA photo-excitation process emerge [105], other features of this complex protein have yet to be examined, including a potential heme binding domain and an iron-sulfur center [47]. A possible role for the heme binding domain will be presented later.

Regulatory networking

Tetrapyrrole formation is governed by all three of the above regulatory systems. This biosynthetic pathway has several branches leading to end products that are required in different amounts, according to the metabolic state of the cell. These diverse regulatory systems work in concert, in order to provide the degree of responsiveness necessary to produce the right levels of the various end products, as environmental conditions dictate. Here, we present a summary of what is known about how these three systems regulate tetrapyrrole formation. As already described, the *hemA*, *hemN*, *hemZ* and *bchE* genes are all regulated by FnrL. In *R. sphaeroides*, the *cco* operon, which codes for the cbb_3-type cytochrome *c* oxidase, was identified by virtue of the fact that a Cco^- mutant strain has elevated transcription from *hemA* sequences [81], establishing redox regulation as an additional modulator of expression of this gene. An FnrL-independent increase in *hemN* transcription has also been demonstrated in Cco^- mutant bacteria [56]. Expression of the *bchEJG* operon is subject to this dual regulation as well [56]. Finally, the *bchEJG*, *bchCXYZ* and *bchFNBHLM* operons all have PpsR DNA binding motifs in their upstream sequences, and PpsR regulation of the latter operon has been confirmed [96]. One can interpret this information to suggest that regulation of *bch* genes by all three of these regulatory systems is required to ensure that Bchl formation is the most carefully guarded event in *R. sphaeroides*. Additionally, it indicates that since genes whose products catalyze tetrapyrrole biosynthesis steps that are common to the formation of Bchl and other end products are thus not regulated by PpsR, with the combination of FnrL and redox regulation being sufficient to provide adequate flexibility to the cell. However, as detailed below, additional studies caution against concluding that we now have a complete understanding of the regulation of tetrapyrrole formation, and also indicate that tetrapyrrole molecules may participate in transcriptional regulation themselves in *Rhodobacter* species. A description of their known involvement in posttranscriptional regulatory processes is beyond the scope of this review.

The tryptophan-rich outer membrane-localized TspO protein is the *R. sphaeroides* homologue of the mammalian peripheral benzodiazepine receptor, and is involved in the transport or efflux of tetrapyrrole intermediates of the heme/bacteriochlorophyll biosynthetic pathway [106, 107]. While the role of this activity in *R. sphaeroides* physiology is not yet understood, investigating its function has provided new insights into the regulation of tetrapyrrole, and photosynthesis gene expression. Disabling the *tspO* gene results in elevated aerobic expression of certain photosynthesis genes, and a similar effect is observed in wild-type cells with multiple copies of *hemN*, in trans [108, 109]. Thus, both of these alterations lead to intracellular accumulation of tetrapyrrole intermediate(s). Since (i) only photosynthesis genes that also belong to the AppA-PpsR regulon are affected [110], (ii) the effect is only observed in cells having an intact *appA* gene [109] and (iii) AppA protein possesses a heme-binding motif [47], it has been proposed that heme, or certainly a tetrapyrrole intermediate derived from protoporphyrinogen IX whose formation is catalyzed by the *hemN* gene product, is a coeffector with the AppA protein. In light of the recent findings pertaining to AppA-PpsR activity (described above), it would appear that in the absence of other stimuli (blue light), AppA + a coeffector work antagonistically towards PpsR, resulting in derepression of PpsR-regulated genes: presumably, only low levels of this coeffector would normally be present under aerobic conditions, and so PpsR is competent for binding to DNA. Then, as tetrapyrrole biosynthesis increases in response to lowering oxygen availability (mediated in part through transcriptional activation of tetrapyrrole biosynthesis genes by FnrL), AppA + a coeffector complex forms, and acts on PpsR, resulting in derepression of the target genes.

Another tetrapyrrole-mediated regulatory event was reported by Rodig et al. [111] who found that *R. capsulatus* mutant strains disabled in *bchl* synthesis genes have altered levels of transcription of genes coding for the LH and RC polypeptides. Additional studies indicate that the *R. capsulatus* Prr homologues are required for this regulation [112]. In light of the several regulatory differences between the two species of *Rhodobacter*, some of which have been described here, whether a similar event occurs

in *R. sphaeroides* cannot be predicted. However, an investigation into the presence or absence of this Bchl (or Bchl precursor)-mediated regulation in *R. sphaeroides* does seem warranted.

Finally, a corrinoid has also been proposed to participate in the regulation of photosynthesis genes. This proposal stems from investigations of the *ppaA* gene product. The *ppaA* gene codes for a protein that affects expression of photosynthesis genes through a mechanism that is distinct from the FnrL, Prr and AppA-PpsR regulation. However, the *ppaA* gene itself is repressed by PpsR in the presence of oxygen, which suggests that PpaA-mediated regulatory events are primarily associated with low oxygen or anaerobic conditions. A conserved amino acid sequence found in several vitamin B_{12}-binding enzymes has also been identified in the PpaA protein, which suggests that PpaA activity requires a corrinoid cofactor. Since the *ppaA* gene is regulated by PpsR, and vitamin B_{12} is needed for both Bchl and Crt formation, it is proposed that PpaA activity couples vitamin B_{12} availability with photosynthesis gene expression. For more details of this study, see [113].

Why not just avoid the problem of oxygen altogether?

Motile bacteria can move towards attractants and away from repellants, and this includes substances such as terminal oxidants. In *Escherichia coli*, for example, aerotaxis is now a fairly well described phenomenon, in which the bacteria migrate towards optimal concentrations of oxygen [114, 115]. Since *R. sphaeroides* is motile, the presence of a taxis response is not surprising. But, with so many metabolic options available to this organism, it is also not surprising that the final direction of movement is the outcome of a large and very complex set of chemosensory genes (described in [116]). Based on motility observations, microaerophilic conditions are optimal for *R. sphaeroides*, regardless of the presence or absence of light (described in [117]), which has been interpreted to mean that under these conditions, the rate of electron transport is maximal within the constraints of other metabolic considerations. Recent studies have demonstrated that the Prr-mediated redox-responsive regulatory system participates in motility decisions [117], and thus taxis is conveniently coupled to expression of genes associated with energy, carbon and nitrogen metabolisms.

The paradoxical situation of the oxygen-requiring anoxygenic photosynthetic bacteria

This discussion has described at length how vigilant *R. sphaeroides* is in avoiding the quintessential dangerous situation for this organism of having Bchl in the presence of both oxygen and light. Yet, nature has proven that variety is the spice of life as well as science, and reset the baseline of our overall understanding of how this works, as there exist both bacteria that synthesize photosynthetic complexes under highly aerobic conditions [118–120], as well as obligately aerobic, but anoxygenic, photosynthetic bacteria (reviewed in [121]). Here, we explore what is currently known about the role oxygen plays in their lives.

Prr homologues have been identified in *Rhodovulum sulfidophilum*, which synthesizes photosynthesis complexes under highly aerobic conditions [90], and the PrrA and PrrB homologues are capable of complementing defects in the corresponding genes in *R. capsulatus* [90]. Also, mutant strains of *R. sulfidophilum*, in which the genes coding for PrrA or PrrB homologues have been disabled, have slower growth rates under anaerobic conditions, and pigment-protein complex levels are reduced under both aerobic and anaerobic conditions, relative to those present in wild-type cells under the same conditions [90]. These observations correlate with phenotypes of the corresponding mutant strains of *R. sphaeroides*, and indicate that these proteins participate in similar regulatory events in all these organisms. However, analysis of Bchl content in *R. sulfidophilum* wild-type strains grown under different conditions reveals a striking difference in oxygen control of Bchl biosynthesis, relative to that which exists in species of *Rhodobacter*: when examined in parallel under the same illumination conditions, Bchl content in *R. sulfidophilum* cells grown aerobically was found to be 51% of that present in anaerobically grown cells, while the content present in aerobically grown *R. capsulatus* cells was found to be less than 1% of anaerobic levels [122]. Understanding how *R. sulfidophilum* tolerates the presence of Bchl under aerobic conditions will almost certainly reveal a profound difference in the oxygen-coping strategies that are present in these oxygen-tolerant anoxygenic photosynthetic organisms, but are absent in *Rhodobacter*.

The existence of the second group of organisms, the obligately aerobic, but anoxygenic, photosynthetic bacteria, raises obvious questions like two sides of a coin: Why are they obligate aerobes, or why are they photosynthetic? Apparently photosynthesis activity is not obligatory in these organisms, but provides additional energy, perhaps offsetting respiratory rate changes that could occur as temperatures fluctuate, as an example [121]. These organisms are not capable of fixing carbon dioxide, and therefore the role of photosynthesis appears to be strictly energetic in an ancillary sense [121, 123]. Clear evidence of this specialized role of photosynthesis in these organisms stems from an analysis of Bchl production, which reveals that light abolishes Bchl formation, and so photosynthesis is only as long-lived as is the limited availability of Bchl produced before illumination; Bchl levels will

inevitably fall below a minimum amount required for photosynthesis as the cells grow and divide. Intriguingly, Prr homologues have also been identified in a member of this group of bacteria [90], and similarity of function was demonstrated by complementation studies in *R. capsulatus* mutant strains [90]. Perhaps, as alluded to by Candela et al. [123], the oxygen requirement of these anoxygenic photosynthetic organisms is due to the absence of a suitable outlet for photosynthetically generated reducing power under anaerobic conditions. This hypothesis predicts that introducing genes into these organisms that would confer the ability to carry out carbon dioxide fixation as a suitable electron sink would then allow them to grow in the absence of oxygen. Such an outcome would be consistent with the reverse observation that has already been made for *R. sphaeroides*; namely, abolishing carbon dioxide fixation confers an inability to grow by anoxygenic photosynthesis [10, 87, 124].

Conclusions so far: a work in progress

Protection mechanisms in oxygenic photosynthetic organisms must have evolved to a fine art to accommodate generating oxygen in the same organism as where the dangers inherent to its presence in photosynthesis reside. The evolution of the anoxygenic photosynthetic process before oxygenic photosynthesis is not in dispute, and so it is clear that protective mechanisms were either already present or evolved in some manner, which ultimately enabled the development of the water-splitting reaction center to be tolerated. There are many other evolutionary questions that remain unanswered at this point, but if one assumes that aerobic respiration predated photosynthesis, then some protective mechanisms must have already existed, and could have been available for 'recruitment' for protection during oxygenic photosynthesis. Conversely, if photosynthesis predated the evolution of aerobic respiration, then protective mechanisms would have had a strong selective advantage once oxygenic photosynthesis evolved. It is perhaps premature to speculate about the evolutionary history of these events until the timing of aerobic respiratory and photosynthetic metabolisms is unequivocally established.

Regardless of how they evolved, three systems that regulate genes through the intervention of oxygen in *R. sphaeroides* are now recognized in this organism. Although in no case is the entire regulatory cascade fully understood, we can conclude the following: all three of these regulatory systems modulate gene transcription in response to changes in oxygen, but in different ways; perhaps each evolved as oxygen tensions in the atmosphere changed, but this has not been studied. The first of these systems responds directly to the presence of molecular oxygen. The second system is tuned to respond to changes in cellular redox that are a consequence of changes in oxygen availability. The third system also perceives changes in cellular redox, but since it also senses changes in light intensity, its regulatory output is an integrated response to both parameters. Apparently, all three are needed in *R. sphaeroides* in order to provide this organism with sufficient plasticity in gene expression, but also security checks for the appropriate and safe deployment of the metabolism best suited to environmental conditions. It may be that this degree of regulatory sophistication is unnecessary in less metabolically versatile organisms.

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