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Minireview

Interaction of photosynthesis, respiration and nitrogen fixation in cyanobacteria

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Abbreviations: DBMIB – 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone, DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea, FNR – ferredoxin-$NADP^+$-reductase, GOGAT – glutamine oxoglutarate aminotransferase, PET – photosynthetic electron transport, PS – photosystem, RET – respiratory electron transport

I Introduction

Until recently, respiratory electron transport (RET) in cyanobacteria was a rather neglected subject in cyanobacteriology (e.g., between 1969 and 1980, approximately 1–2 papers were published per year dealing with cyanobacterial RET). Accordingly, in one of the most comprehensive treatises on cyanobacteria (Carr and Whitton 1982), a chapter on respiratory electron transport is not present. During the last years, however, a steadily growing number of publications appeared, reporting data relevant for RET (1981–1985: approximately 12 papers per year). It became obvious that RET and photosynthetic electron transport (PET) are intimately coupled and interact with nitrogen fixation, so found both in heterocysts and in vegetative cells of particular species. This review deals with the close relationship between three major bioenergetic processes in cyanobacteria, i.e. respiratory electron transport, photosynthetic electron transport and nitrogen fixation. Reviews have been published by Binder (1982), Houchins (1984) and Peschek (1984, 1987).

II Localization of respiratory electron transport

Cyanobacteria as prokaryotic algae lacking organelles such as chloroplasts and mitochondria are able to perform oxygen-evolving photosynthesis and respiration (Carr and Whitton 1982, Peschek 1984). There is still some controversy in the literature concerning the site of respiratory electron transport, whereas little doubt exists that photosynthetic electron transport is exclusively localized in the thylakoid membrane system. It has been suggested that the thylakoid membrane also is the site of respiratory electron transport, based both on the isolation of the cytoplasmic membranes (Lockau and Pfeffer 1982, 1983; Omata and Murata 1984) and experiments performed with intact cells (Bisalputra 1969, Scherer and Böger 1985, Scherer et al. 1984). On the other hand, sound data have been reported by Peschek and coworkers, mainly for *Anacystis nidulans* (= *Synechococcus*), demonstrating a localization of at least part of RET on the cytoplasmic membrane, while the other part is localized on the thylakoids (for review see Peschek 1984). A third model suggests that part of RET is with the thylakoid membrane (where oxidation of reduced pyridine nucleotides takes place) and part with the cytoplasmic membrane which is suggested to be the site of cytochrome oxidase, the electrons being shuttled by some unknown mediator (Matthijs et al. 1984b, 1985). It seems, however, possible that different cyanobacteria exhibit different organization concerning the localization of respiratory electron transport (compare Scherer and Böger 1984, Nitschmann and Peschek 1985). The same may be true for the complex patterns of light-induced proton fluxes across the cytoplasmic membrane (for review see Scherer et al. 1988b), but further experimental work is needed to clarify these questions.

The following discussion will be restricted to the interaction of RET and PET on the thylakoid membrane with respect to nitrogenase and hydrogenase activities in both vegetative cells and heterocysts, regardless of whether some part of RET is localized additionally on the cytoplasmic membrane.

III Respiration in the light

It is not clear whether respiration in photosynthetically active cells is inhibited in the light. As suggested earlier, respiratory oxygen uptake in *Anacystis* and *Anabaena* is inhibited in the light. This was reasoned from ^{18}O-experiments and deduced from light intensity dependence of oxygen evolution (Kok effect, compare Jones and Myers 1963; for references see

Scherer and Böger 1982). It was shown for several cyanobacteria by $^{14}CO_2$ liberation in the light with saturating amounts of $^{12}CO_2$ present (Scherer and Böger 1982) that respiratory carbohydrate catabolism is, in most cases, indeed inhibited in the light (an exception was found for *Anabaena* under nitrogen fixing conditions, see section VI.1.1). Furthermore, "respiratory" electron flow to PS I in cyanobacteria is now well established for intact cells (Hirano et al. 1980, Aoki and Katoh 1982, Scherer et al. 1982, Mullineaux and Allen 1986, Myers 1986) as well as for the cell-free system which will be discussed in the next section.

The mechanism of light inhibition, however, still has to be elucidated. Whether a simple competition between cytochrome oxidase and P-700 for electrons (see Fig. 1) is responsible, or the regulation is controlled by the redox level of plastoquinone (compare Mullineaux and Allen 1986), deserves further experimental efforts. In *Rhodopseudomonas* (*Rhodobacter*) *capsulata*, the light-induced membrane potential has been suggested to regulate respiratory electron transport in the light (Cotton et al. 1983), but this mechanism cannot be demonstrated in *Anabaena* (Scherer, unpublished results).

IV Components of electron transport

IV.1 NADH dehydrogenase and ferredoxin-NADP$^+$ reductase (FNR)

The pattern of interaction described in the following sections is summarized in the tentative scheme of Fig. 1, to which the reader is referred throughout this review. Although NADPH has been claimed to be the natural donor to respiration in *Phormidium* (Biggins 1969), in all cell-free preparations both NADPH and NADH were found to donate electrons to RET (e.g. Peschek 1980, Binder et al. 1981, 1984, Stürzl et el. 1982, 1984) but generally NADPH supported higher rates. NADH oxidation is due to a membrane-bound dehydrogenase which has been purified to a high extent, is specific for NADH in *Anabaena variabilis* and can only be removed from the membrane by detergents (Alpes et al. 1986, Alpes et al. forthcoming). NADH and NADPH are not only donors for RET but also for PS I, provided DCMU is present (Binder et al. 1981, Sandmann and Malkin 1983, Stürzl et al. 1984, Gonzales de la Vara and Gomez-Lojero 1986). Interestingly, an NAD(P)H dehydrogenase feeding electrons into PS I has been demonstrated for the green alga *Chlamydomonas* (Ben-Amotz and Gibbs 1975, Godde and Trebst 1980, Maione and Gibbs 1986). It has been shown by Stürzl et al. (1984) that different affinities for light dependent and dark mediated NAD(P)H oxida-

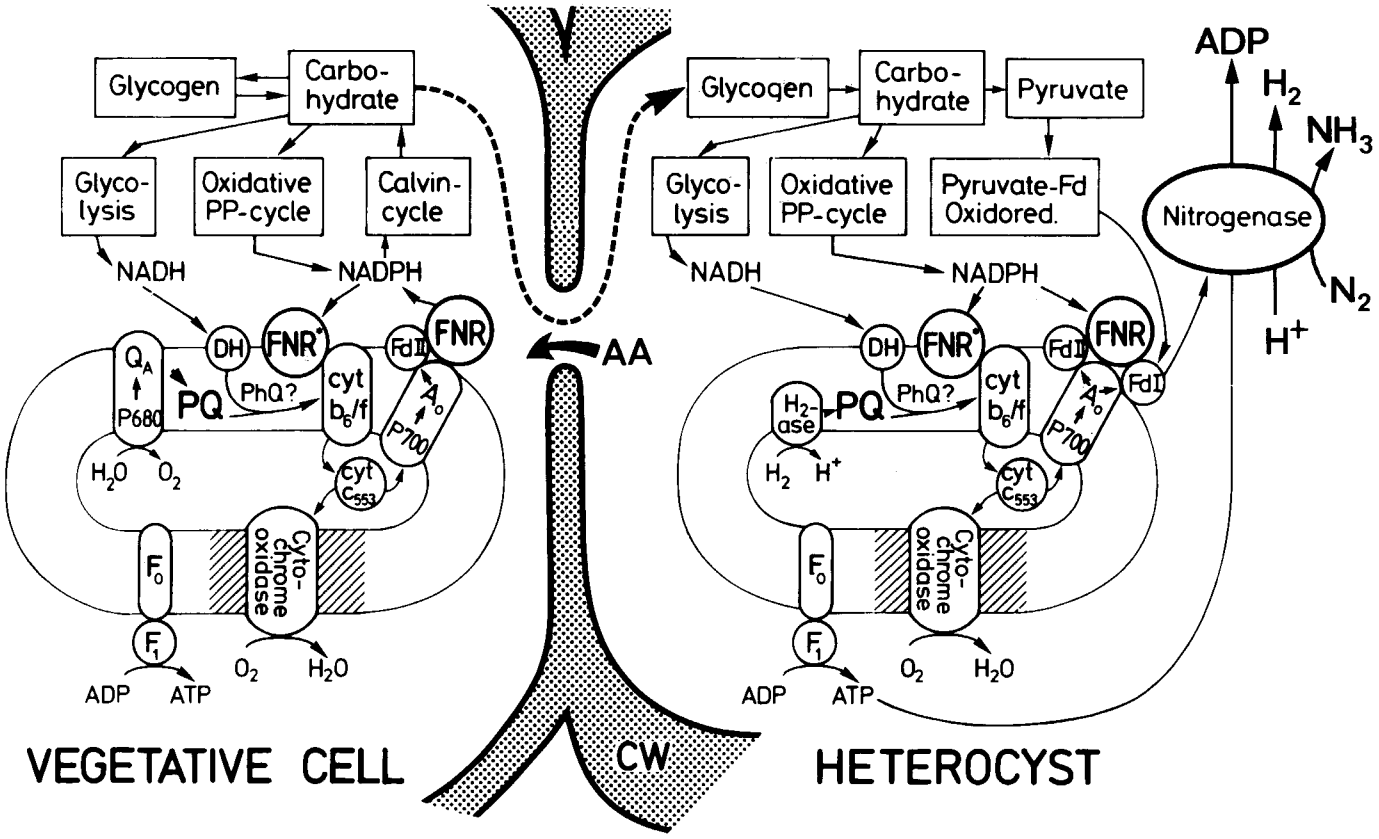

Fig. 1. Tentative scheme for the functional interaction of photosynthesis, respiration and nitrogen fixation in a heterocystous cyanobacterium. The interaction of photosynthesis and respiration shown for the vegetative cell reflects also the situation in non-heterocystous species. The thick arrow indicates transport of fixed nitrogen (presumably glutamine; compare Wolk et al. (1976)) into vegetative cells. The broken arrow stands for transport of reductant into heterocysts. It is not known whether all imported reductants are first converted to glycogen or, at least in part, directly catabolized yielding reduced pyridine nucleotides or possibly reduced ferredoxin. See text for further comments. AA, amino acids; cyt b_6/f, cytochrome b/f complex; Cyt c_{553}, soluble cytochrome c, may be replaced by plastocyanin in some species; CW, cell wall; DH, NADH dehydrogenase; Fd I, ferredoxin I, only in heterocysts; Fd II, ferredoxin II; FNR, ferredoxin-NADP$^+$ reductase, active in the light; FNR*, FNR active in the dark as NADPH dehydrogenase; F_0F_1, ATP synthetase; H_2ase, membrane-bound uptake hydrogenase; PP, pentose phosphate; P 680 → Q_A, photosystem II; P

tions exist in *Anabaena*. Whether the enzymes are light-modulated is not yet clear. In heterocysts, NADH and NADPH donate electrons to PS I and subsequently to nitrogenase. NADPH has been suggested to be oxidized by FNR in heterocyst preparations producing reduced ferredoxin (Houchins and Hind 1982, Schrautemeier et al. 1985). Interestingly, NADPH oxidation activity of a membrane preparation of vegetative cells of *Anabaena* can be destroyed by washing membranes without any detergent while NADH oxidation remains intact (Table 1); NADPH oxidation is inhibited by $NADP^+$, which is a feature characteristic for the FNR (Alpes et al. 1985). The same was found for oxidative phosphorylation, driven by either NADPH or NADH (Scherer et al., in preparation). Kinetics and immunological characterization of partially purified NADPH oxidizing protein from *Anabaena* membranes provided strong evidence that FNR acts as a NADPH dehydrogenase in the dark, donating electrons to RET (Scherer et al., in preparation). The apparent association of FNR with the cytochrome b–f complex (Clark et al. 1984, Coughlan et al. 1985), although demonstrated in chloroplasts, may also be significnt with respect to our model. It has been shown that FNR is light-regulated (e.g. Carrillo and Vallejos 1983), and we assume that it is active as a "NADPH dehydrogenase" in the dark only, indicated in Fig. 1 as FNR*. Reconstitution of RET with an FNR-containing fraction (Gonzales dela Vara and Gomez-Lojero 1986, as well as with purified FNR (Scherer et al., in preparation), has proven to be difficult and yields only marginal rates of cyanide-sensitive, NADPH-dependent oxygen uptake. Although NAD(P)H oxidation by PS I is common in

Table 1. NAD(P)H-oxidizing activities in the dark. Extracts of *Anabaena variabilis* cells were treated by washing steps and assayed for different electron-transport activities. Values are expressed in % of total activity. The values in parentheses (col. 4) indicate the data obtained after ultracentrifugation. By comparing cols. 1–3 with col. 4 it is evident that NADPH-oxidizing activities are soluble and due to FNR whereas NADH oxidation is membrane-bound. (Data from Alpes et al. 1985.)

Washing step	Electron donor/acceptor systems			
	(1) NADPH → thio-NADP	(2) Hydrogenase, ferredoxin → NADP	(3) NADPH → menadione	(4) NADH → menadione
1st Supernatant	77	78	83	28 (0)
1st Pellet	22	15	16	27 (91)
2nd Supernatant	11	6	10	3
2nd Pellet	15	8	7	57
3rd Supernatant	5	4	4	0
3rd Pellet	5	2	5	52

vegetative cells, it seems to be of main physiological significance in heterocysts, e.g. providing reducing power for nitrogen fixation (compare section VI.1).

IV.2 Membrane-bound hydrogenase

In heterocystous cyanobacteria, a membrane-bound hydrogenase, claimed to be localized only in the heterocysts (Peterson and Wolk 1978), takes up hydrogen produced by nitrogenase. H_2 has been shown to be an electron donor for RET (Eisbrenner and Bothe 1979, Houchins and Hind 1982) and to support oxygen-dependent ATP synthesis as well (Peterson and Burris 1978, Ernst et al. 1983). In addition, H_2 is the effective electron donor for nitrogen fixation in isolated heterocysts. Electrons from hydrogen, however, have to pass PS I before reducing ferredoxin which is the ultimate electron donor to nitrogenase (Houchins and Hind 1982, Ernst et al. 1983, Schrautemeier et al. 1984. The apparent K_m for hydrogen with nitrogen as electron acceptor in the light is very low (1.3 μM, see Schrautemeier et al. 1984). Comparison of H_2-dependent oxygen uptake and nitrogen fixation provided early evidence for the interaction of RET and PET in cyanobacteria (Fig. 1). Whether expression of membrane-bound uptake hydrogenase activity is advantageous with respect to the energy budget of nitrogen-fixing cyanobacteria will be discussed later.

Interestingly, membrane-bound hydrogenase activity has also been measured in the unicellular cyanobacterium *Anacystis*, with hydrogen serving as electron donor for oxygen reduction and ATP synthesis (Peschek 1980a, 1982b).

IV.3 Plastoquinone and phylloquinone

Plastoquinone is active in PET of both chloroplasts and cyanobacteria. In the latter, other quinones have been demonstrated, such as phylloquinone (vitamin K_1) and, in very low concentrations, "polar naphthoquinone" (Peschek 1980b). Plastoquinone is present in a 10-to-20 fold higher concentration compared to phylloquinone. Neither ubiquinone nor menaquinone, active in RET of mitochondria and photosynthetic bacteria, respectively, have been detected in cyanobacteria.

The first suggestion on a dual function of plastoquinone in both RET and PET (Fig. 1) has been published by Eisbrenner and Bothe (1979) who demonstrated that DBMIB inhibited H_2 dependent oxygen uptake in the dark. Peschek and Schmetterer (1982) showed by plastoquinone reconstitution experiments that reduction of cytochrome f in the dark (NADPH

as electron donor) was dependent on plastoquinone. Phylloquinone has not been assayed in these experiments (see below). The quinone pool of intact *Synechococcus* sp. is reduced by respiratory substrate such as glucose and fructose, which has been inferred by the analysis of the early fluorescence induction (Aoki and Katoh 1982). The size of the quinone pool active in respiration and photosynthesis was estimated to be the same (Aoki and Katoh 1983). Although these experiments do not allow for the conclusion that only plastoquinone is active in respiration, the data show that reducing equivalents from carbohydrate breakdown can enter PET at the plastoquinone level.

Following the flash-induced oxidation and subsequent reduction of cytochrome c-553 in intact *Synechococcus* cells, Hirano (1980) demonstrated a dual function of cytochrome c-553 in RET and PET (compare section IV.4, but the results do not allow any conclusions on the nature of the quinone involved.

Extraction of quinone from thylakoids of *Anacystis nidulans* and subsequent reconstitution with phylloquinone and plastoquinone has shown that phylloquinone is active preferentially in RET whereas plastoquinone is in PET (Peschek 1980b). These experiments have been extended to *Anabaena variabilis* and *Nostoc* sp. (Peschek and Kuntner 1987, Table 2), yielding essentially similar results. The authors raise the interesting hypothesis that their data may "reflect different affinities of the quinones toward the donor side of PS II and the respiratory dehydrogenase(s) . . ." Although a specific function of phylloquinone as acceptor of electrons from respiratory dehydrogenases is an attractive hypothesis in terms of regulation of RET in the light, a final conclusion on the nature of the respiratory quinone is not yet possible.

Table 2. Effect of quinone extraction and reconstitution on photosynthetic and respiratory electron transfer reactions in isolated membranes of *Anabaena variabilis*. DPC, diphenylcarbazide; DPCIP; dichlorophenol indophenol; PhQ, phylloquinone; PQ-9, plastoquinone. The arrows stand for donor → acceptor systems. Rates are given as nmol acceptor reduced per min and mg protein. Data are taken from Peschek and Kuntner (1987), for experimental details the reader is referred to this publication.

Membranes	Light reaction		Dark reaction	
	$H_2 \rightarrow NADP^+$	DPC → DCPIP	$H_2 \rightarrow O_2$	$NADPH \rightarrow O_2$
Native	69.8	27.5	42.5	20.7
Depleted	5.2	7.3	3.8	2.0
Reconstituted				
with PQ-9	43.0	36.8	11.7	6.9
with PhQ	16.4	20.9	29.9	13.6

IV.4 Cytochrome b_6f complex

The cytochrome b_6f complex of cyanobacterial PET is remarkably similar to the corresponding complex in higher plants (Hauska et al. 1983). During the preparation of this complex from *Anabaena variabilis*, no evidence has been found for a respiratory cytochrome b/c complex, which has been taken as evidence for its dual function in both PET and RET (Krinner et al. 1982, Fig. 1).

Cytochrome f and cytochrome b-563 of *Anacystis nidulans* could be reduced by NADPH (Peschek 1982a); additionally, cytochrome f was oxidized in a cyanide-sensitive reaction by oxygen in the dark (Peschek and Schmetterer 1982). The latter finding was corroborated for *Aphanocapsa* (Sandmann and Malkin 1984). In heterocysts of *Anabaena variabilis*, cytochrome b-563 and cytochrome f were oxidized by oxygen in the dark (Böhme and Almon 1983). Similar results were obtained by flash-spectroscopy investigations of PET in isolated heterocysts of *Anabaena* sp. (Houchins and Hind 1983), thus providing evidence for a dual function of the cytochrome b_6/f complex in RET and PET of heterocysts as well as of vegetative cells. The influence of inhibitors, specific for the cytochrome b_6/f complex, on RET was taken as additional evidence for its dual function (Binder et al. 1984, Matthijs et al. 1984a, Schrautemeier et al. 1984, Scherer et al. 1987).

IV.5 Cytochrome c-553 and plastocyanin

The direct electron donor to PS I in higher plants is plastocyanin, which is also found in several cyanobacteria. In the latter (as well as in several algae), however, a soluble cytochrome c is also frequently found and is able to functionally replace plastocyanin under conditions of copper deficiency (Sandmann 1986). A soluble cytochrome, which could be a specific electron donor for cytochrome oxidase in cyanobacteria has not been isolated so far. It has been shown by Lockau (1981) for the first time that both cytochrome c-553 and plastocyanin from *Anabaena variabilis* donate electrons to PS I and cytochrome oxidase (Fig. 1), although the donor specificity is different for photo-oxidation and dark oxidation. A stimulation of PS I and cytochrome oxidase by soluble cytochrome c-553 has also been demonstrated for *Mastigocladus laminosus* (Binder et al. 1984) and *Spirulina maxima* (Gonzales de la Vara and Gomez-Lojero 1986). Other cytochromes, for instance from horse heart or from *Rhodopseudomonas* (*Rhodobacter*), are also active as electron donors to cytochrome oxidase (Lockau 1981, Kienzl and Peschek 1982). Therefore, the dual role of cytochrome c and/or plastocyanin cannot safely be deduced from such type of experiments. Using a membrane

preparation from *Nostoc muscorum*, Stürzl et al. (1982) could restore electron flow from water to NADP in the light and from NAD(P)H to oxygen in the dark by adding *Nostoc* cytochrome c-553, which, accordingly, not only donated electrons to the oxidase and P-700 but also accepted electrons from the cytochrome b_6/f complex. Only the *Nostoc* cytochrome was active in this membrane preparation, not even cytochromes isolated from other cyanobacteria were found active. Together with the finding that both PET and RET were inhibited by an antibody against cytochrome c-553 of *Nostoc* (Alpes et al. 1984), the data convincingly document that cytochrome c-553 is the native donor for cytochrome oxidase at least in *Nostoc*.

With *Anacystis* other results have been obtained. The basic horse heart cytochrome has been found the best donor for cytochrome oxidase (Kienzl and Peschek 1982) but did not stimulate PS I activity. The native cytochrome c-553 from *Anacystis*, which is an acidic protein, stimulated PS I but not cytochrome oxidase activity, which has been taken as evidence that in *Anacystis* the soluble cytochrome may not be shared by RET and PET (Peschek and Schmetterer 1982). If this hypothesis will be corroborated, a substantial difference will exist between filamentous and coccoid cyanobacteria. A detailed study of whether the affinities of PS I and cytochrome oxidase towards their electron donors are different or subject to light regulation is still lacking (see section III).

V Phosphorylation

Photophosphorylation proceeds in cyanobacteria with an approximately identical efficiency as is known for chloroplasts (e.g. Spiller 1980, Wax and Lockau 1980) yielding P/2e ratios of about 1 in the cell-free system. Rates for ATP synthesis were found between 100–300 (linear photophosphorylation), and 300–1800 (cyclic photophosphorylation) μmol ATP per mg chlorophyll per hour. Oxidative phosphorylation in intact cells was described with comparatively high P/O ratios (up to 3) for a variety of species (Nitschmann and Peschek 1982, Scherer et al 1984, 1988a) with rates ranging between 20 and 100 μmol ATP per mg chlorophyll and hour, which is, as was expected from electron transport rates, only about 10–20% of the rates in the light. Oxidative phosphorylation in cell-free systems has been demonstrated only with limited success (Frei et al. 1984, Matthijs et al. 1984), yielding low rates and low P/O ratios of 0.8 at maximum (Scherer et al. 1987).

It is questionable whether cyanide-insensitive respiration, coupled or not with ATP synthesis, exists in cyanobacteria (Scherer et al. 1988a). No

evidence is available pointing to different ATP-synthetases in the light and in the dark. Indeed, with respect to the spatial organization of both processes (Fig. 1), only *one* enzyme seems reasonable, which has been partially isolated and characterized (e.g. Lubberding et al. 1983) without evidence for a second enzyme.

VI. Nitrogen fixation

All heterocystous and many nonheterocystous cyanobacteria are capable of nitrogen fixation, i.e. reduction of molecular nitrogen to ammonia (Rippka et al. 1979). This process is catalyzed by nitrogenase, an enzyme extremely sensitive to oxygen. Since photosynthetic oxygen evolution and oxygen-labile nitrogenase activity are incompatible processes, several strategies have been realized to allow for both metabolic pathways within the organism. Heterocystous species perform a spatial separation with nitrogenase being located in specialized cells, called heterocysts (Wolk 1982), while oxygen-evolving photosynthesis is restricted to the vegetative cells. In nonheterocystous cyanobacteria, apparently a temporal separation of both processes has to be accomplished.

VI.1. Heterocystous species

Figure 1 represent a schematic view of the complicated interactions of photosynthesis, respiration and nitrogen fixation in a filamentous heterocystous cyanobacterium. Nitrogenase is localized in the heterocyst, where a microaerobic environment is maintained by a modified photosystem consisting of an active photosystem I only (Tel-Or and Stewart 1977, Almon and Böhme 1980), a thick cell wall reducing gas diffusion (Walsby 1985) and high respiratory activity removing oxygen entering the heterocyst. There is evidence that even under microaerobic conditions nitrogenase activity is *not* induced in vegetative cells (Murry et al. 1984). Hydrogen metabolism is closely connected to nitrogen fixation, since H^+-reduction resulting in H_2-formation is an obligatory side reaction of N_2-reduction (Eady 1986). Hydrogen produced by nitrogenase can be taken up by hydrogenase, a membrane-bound enzyme located exclusively within heterocysts (Peterson and Wolk 1978).

VI.1.1. ATP supply and electron donation to nitrogenase

Nitrogen fixation is an ATP-requiring process and per molecule N_2 reduced at least 16 molecules ATP have to be provided. This is the minimum theoretical energy requirement assuming a ratio of 1 molecule H_2 formed per

molecule N_2 reduced. Since in vivo this ratio is shifted in favor of hydrogen evolution, a higher ATP requirement for N_2-fixation has to be assumed. Heterocysts containing a functional photosystem I are capable of producing ATP by cyclic photophosphorylation (Almon and Böhme 1982). In the dark however, ATP can only be provided by respiratory breakdown of stored carbohydrates, a process generating less ATP per unit of time than photophosphorylation and which is dependent on the amount of storage material present in the cyanobacteria. Even in case of a maximum amount of storage compounds available, rates of nitrogenase activity in the dark hardly exceed 50% of the rate measured in the light (Ernst et al. 1984). Apparently, nitrogenase activity in the dark is ATP-limited whereas in the light electron donation to nitrogenase is rate-limiting.

With respect to supply of reductant, nitrogenase depends on carbohydrates which are photosynthetically produced in vegetative cells and transported into heterocysts as sugars (Wolk 1982) or even amino acids (Jüttner 1983). Within heterocysts, carbohydrates are transiently stored as glycogen or become degraded to pyridine nucleotides or possibly pyruvate. These compounds eventually reduce ferredoxin, which is the ultimate electron donor to nitrogenase. Interestingly, the ferredoxin involved in reduction of nitrogenase seems to be different from the ferredoxin operative in photosystem-I electron transport (Böhme and Schrautemeier 1987). Degradation of carbohydrates via glycolysis results in formation of NADH, which is taken up by a membrane-bound dehydrogenase (see IV.1). Electrons from NADH are then fed into photosystem I, and reduced ferredoxin is formed in a light-dependent reaction. The laboratory of Bothe favors a pathway in which ferredoxin is reduced by pyruvate:ferredoxin oxidoreductase (Neuer and Bothe 1985). This hypothesis is supported by experiments documenting the presence of this enzyme in heterocysts. The reported rates of pyruvate-dependent nitrogenase activity, however, are comparatively low. In our opinion, carbohydrate breakdown via the oxidative pentose phosphate cycle with concomitant generation of NADPH is more likely to be operative in heterocysts, because high enzyme activities have been measured and NADPH was shown to support high rates of nitrogenase activity in cell-free extracts from heterocysts (Schrautemeier and Böhme 1984). Two pathways for reduction of ferredoxin by NADPH have been assumed. In the dark ferredoxin is reduced via NADPH:ferredoxin oxidoreductase; in the light, however, this pathway appears to be inoperative, and electrons from NADPH must pass through photosystem I with ferredoxin being reduced in a light-dependent reaction.

VI.1.2. Hydrogen metabolism

Hydrogen evolution is an energy-wasting process; an active uptake hyd-

rogenase should therefore be advantageous for N_2-fixing organisms. Nevertheless, in a series of experiments with nickel-depleted cultures of *Anabaena variabilis*, containing low levels of hydrogenase activity, we found no significant differences with respect to growth and nitrogenase activity in comparison to nickel-grown cyanobacteria containing high levels of hydrogenase (Almon and Böger 1984, Almon and Böger 1987). These findings are supported by similar investigations published by Daday et al. (1985). In addition, studies with hydrogenase-containing (Hup^+) and hydrogenase-deficient (Hup^-) strains of *Rhizobium* and their corresponding host plants are contradictory with respect to better growth of host plants containing Hup^+ *Rhizobium* symbionts (Stam et al. 1987). It is questionable, whether uptake hydrogenase plays a major role in the energy balance of nitrogen-fixing organisms, but rather may lower O_2 partial pressure.

An alternative pathway of hydrogen consumption involving nitrogenase itself has recently been proposed by our laboratory. Based on the observation that hydrogen was taken up by *Nostoc muscorum* during culture phases in which hydrogenase activity was nearly zero, hydrogen uptake activity of nitrogenase was postulated (Weisshaar and Böger 1985). Additional studies supported this hypothesis, since in experiments with other cyanobacterial strains lacking hydrogenase activity, again significant rates of hydrogen uptake were measured and correlation with nitrogenase activity was found (Chen et al. 1986). These observations provide some physiological relevance for measurements performed with the isolated Mo-Fe subunit of bacterial nitrogenase, demonstrating hydrogen uptake by this component (Wang and Watt 1984).

The role of (soluble) reversible hydrogenase, which is present in low amounts in heterocysts and vegetative cells as well, remains unclear at present (compare Houchins 1984). No connection to nitrogen fixation is obvious, as is the case for two hydrogenases postulated to be present in the non-nitrogen-fixing unicellular cyanobacterium *Anacystis nidulans* (Peschek 1979).

VI.2. Nonheterocystous species

The capacity for nitrogen fixation is not restricted to heterocystous cyanobacteria; many nonheterocystous species (filamentous and unicellular forms) also fix dinitrogen, although only under microaerobic conditions (Rippka et al. 1979). These cyanobacteria must *temporarily* separate oxygen-evolving photosynthesis and oxygen-labile nitrogenase activity either during the naturally occurring light/dark cycle (Stal and Krumbein 1985a) or under conditions of continuous illumination during different phases of

the cell cycle (Mitsui 1986). Even *Gloeothece*, a unicellular cyanobacterium fixing N_2 aerobically in the light, depends on respiratory processes to support nitrogenase activity (Maryan et al. 1986). A *spatial* separation of nitrogen fixation and photosynthesis, however, has also been described (Carpenter and Price 1976). According to the observation of these authors, nitrogen fixation takes place in the center of marine *Oscillatoria* colonies, where a microaerobic environment is maintained and photosynthesis is not operative.

VI.2.1. Supply of ATP and reductant

During phases of nitrogen fixation, nonheterocystous cyanobacteria totally depend on respiratory processes which have to support energy-demanding enzyme activity. Carbohydrate storage compounds must therefore be produced and stored during phases of photosynthetic activity. Generation of ATP and reduced ferredoxin by (respiratory) breakdown of storage compounds requires oxygen, although in net concentrations which are not inhibitory to nitrogenase. Under these conditions respiration is expected to be oxygen-limited and to proceed at suboptimal rates.

Under laboratory conditions, nitrogenase activity in nonheterocystous cyanobacteria can be induced during continuous illumination either at low light intensity or at high light intensity in the presence of DCMU, which partially inhibits photosynthetic oxygen production (Weisshaar and Böger 1983). In experiments performed at higher light intensities, rates of nitrogenase activity are usually higher, because of additional ATP production by cyclic photophosphorylation, which is still possible in the presence of PS-II inhibitor DCMU.

VI.2.2. Hydrogen metabolism

In contrast to heterocystous cyanobacteria, no uptake hydrogenase activity has been measured in nonheterocystous N_2-fixing strains (Weisshaar aned Böger 1983, Mitsui et al. 1985, compare also Houchins 1984). This may be explained according to Stam et al. (1987), who argued that under oxygen-limiting conditions respiratory hydrogen consumption is energetically unfavorable in comparison to respiratory carbohydrate consumption. Since nonheterocystous cyanobacteria obviously are oxygen-limited (and energy limited) during periods of nitrogen fixation, expression of uptake hydrogenase activity would be of greater disadvantage than lack of hydrogen recycling. Concomitant with lack of uptake hydrogenase activity, net hydrogen evolution by nitrogen-fixing nonheterocystous species is significantly higher than hydrogen evolution by heterocystous strains (Weisshaar and Böger 1983, Mitsui et al. 1985).

VI.3. Regulation of nitrogenase activity

Nitrogenase is an enzyme requiring large amounts of energy; nitrogenase activity is therefore only induced upon limitation of combined nitrogen (NO_3^-, NH_4^+) in the growth medium of the cyanobacteria. In contrast, nitrogenase activity is turned off, when combined nitrogen becomes available again. Until recently, this regulatory mechanism was thought to be operative on the level of nitrogenase synthesis only i.e., a comparatively slow response of cyanobacterial metabolism to changes in the nutrient composition of the growth medium has been assumed. Several strains of N_2-fixing phototrophic bacteria, however, have been shown to very rapidly "switch on" or "switch off" nitrogenase activity as a consequence of ammonium being added to their growth medium (for review see Vignais et al. 1985). A similar reaction of cyanobacterial nitrogenase could also be demonstrated, if ammonia was allowed to rapidly enter cyanobacterial cells (Reich et al. 1986). Ammonia itself, however, is not directly involved in regulation of nitrogenase activity, since at least its conversion to glutamine via glutamine synthetase is required to observe inhibition of nitrogenase activity. Whether other enzymes of the ammonia-assimilating system (e.g., GOGAT or transaminases) also participate in nitrogenase regulation is under investigation (Reich et al., this laboratory, unpublished). Besides the ammonia-dependent switch-off mechanism, a reversible oxygen-dependent switch-off/switch-on reaction of cyanobacterial nitrogenase has also been described (Stal and Krumbein 1985b) and a modification of the Fe-protein subunit of cyanobacterial nitrogenase after exposure to oxygen was recently demonstrated by Smith et al. (1987).

VII. Concluding remarks

In the cyanobacterial cell, a most intimate functional interaction between major bioenergetic processes is evident. According to most data known at present, respiratory electron transport, except for cytochrome oxidase, is included in photosynthetic electron transport as far as the identity (not the arrangement) of the components is concerned. In vegetative cells of non-heterocystous cyanobacteria and in heterocysts these electron transport chains are coupled to hydrogen and nitrogen metabolism, the separation of oxygen-evolving photosynthesis and nitrogen fixation being realized in different ways. After several years of rather intensive research with hundreds of papers being published, however, the regulatory features as well as the topography of these metabolic processes in or at the thylakoid membrane

are far from being resolved. Therefore, the summary given in Fig. 1 reflects functional relationships which can be measured under more or less artificial conditions rather than the real spatial organization of the components under discussion. Whether the spatial arrangement of the components implicated in Fig. 1 reflects the situation within the intact cell still remains to be shown.

Note added in proof:

Recently, N. Murata (Japan) and G.A. Peschek (Austria) reported (EMBO workshop on Oxygenic and Anoxygenic Electron Transport Systems in Cyanobacteria, September 1987, Cape Sounion, Greece) that in *Anacystis* cells of the linear growth phase a cytochrome oxidase is found on the cytoplasmic membrane, whereas in logarithmically growing cells cytochrome oxidase seemed to be restricted to the thylakoid membrane. This finding may explain the strikingly different results obtained in these two groups, as described in section II of this review.

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