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Review article

Carbon Metabolism in Lichens

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Abstract

Metabolic pathways in lichens proceed, not only in different cellular compartments, but also in two closely associated symbiotic partners. Some of the same primary pathways must operate in both symbionts, but others probably function in an analagous fashion in both the mycobiont and the phycobiont. Carbon that is fixed photosynthetically is transferred from the photobiont to the mycobiont where much of it is transformed into acyclic polyols. Appreciable carbon may be leached from the thallus, as well as deployed in cell walls or mucilaginous intercellular matrices, and in most species it is also used for synthesis of secondary metabolites. Regulatory controls and details of interactions between the mycobiont and its autotrophic associate are poorly understood, but carbon chemistry may be fundamental to maintenance of an equilibrium between the two symbiotic partners.

Keywords: lichen, photosynthesis, respiration, storage, slow growth, alternate pathways

Contents

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1. Introduction

Primary metabolism in lichens has received relatively little attention in comparison to that in other organisms. For example, non-lichenized fungi, which are of more commercial importance, have been studied to a much greater extent. Though the earliest biochemical investigations involving lichens focussed largely on their unique secondary metabolism, interest in carbon assimilation, respiration, storage and growth has increased steadily. The purpose of this paper is to present an overview of carbon chemistry in lichens, including interactions between symbionts, as well as between whole lichens and environment.

That there are metabolic differences between the mycobiont and phycobiont in a lichen is not surprising, because their respective ancestral forms appear to have diverged from one another in the Precambrian period (Knoll, 1992; Berbee and Taylor, 1993). Contrasting features of the two symbionts that will be discussed in this paper are shown in Table 1. In addition to those attributes listed, DNA base sequences will certainly have diverged, and some differences have already been documented in the introns of SSU rDNA (DePriest and Been, 1992). The heterotrophic and autotrophic partners in a lichen

Table 1. Biochemical features which differ in mycobionts and photobionts of a lichen

Attribute	Mycobiont	Photobiont
Pigments	Anthraquinones, usnic acids, depsides/ depsidones, carotenoids, chromones	Chlorophylls, carotenoids and, in some, phycobiliproteins, phytochromes
Major hydrophobic extracellular products	Dibenzofurans, depsides/depsidones	Unable to synthesize
Membrane complexes for capturing light energy	None	Reaction centers I and II and associated molecular complexes
Photosynthetic carbon-fixation	None	C-3 pathway of photosynthesis, RuBisCO and other RPC enzymes
Polyols	High levels of mannitol (or arabitol)	No mannitol, but ribitol, erythritol or sorbitol may be produced and exported
Glucans with both (1-3) and (1-4) linkages	Lichenan, pustulan, and isolichenan	None
Polymers of N-acetyl-glucosamine	Chitin	None
Algal-binding substances	Glycoproteins	None
Urea metabolism	Synthesizes urea	Degrades urea

are also probably disparate in respect to some aspects of primary metabolism, with various key primary metabolites (see below) synthesized along different pathways.

For example, in green algae and cyanobacteria the amino acid lysine is formed from pyruvate and aspartate, while in higher fungi, including ascomycetes and basidiomycetes, it is generated from 2-oxoglutarate and acetyl-CoA (Vogel, 1965). Lysine, therefore, is probably synthesized in two different ways within a lichen thallus, presumably reflecting the long period of time during which ancestors of present-day fungal and photosynthetic symbionts evolved separately from one another. Another amino acid, arginine, is catabolized differently in fungi and animals than in many prokaryotes and unicellular eukaryotic algae. In fungi the enzyme arginase hydrolyzes arginine, to produce urea and ornithine (Jennings, 1989), and in microalgae including *Trebouxia* arginine is deaminated by the enzyme arginine deiminase (Laliberté and Hellebust, 1991). Therefore, arginine in a lichen could be degraded either way, or along both routes.

Pyrroles form an essential part of the structure of phycobiliproteins, chlorophylls, cytochromes and phytochromes, and two molecules of alpha-aminolevulinate (ALA) are required for the synthesis of each of the pyrroles in a tetrapyrrole ring. However, ALA can be synthesized in two alternative ways. In yeasts, it is made from succinate and glycine, and in plants, algae and cyanobacteria, from glutamate (Beale and Weinstein, 1991). Thus, ALA is another pivotal metabolite, that is probably produced along two distinctly different routes within a lichen.

Distinctive forms of the same enzyme, as well as differences in pathways, may be found in the photosynthesizing partner and the fungus. For example, mycobionts and phycobionts may have distinctive types of fatty acid synthetase (FAS). In most bacteria and in plant chloroplasts, FAS is a series of seven or eight distinct uni-functional enzymes referred to as Type II FAS (Hopwood and Khosla, 1992); the photobiont probably has the same arrangement. In vertebrates and fungi (yeasts and *Penicillium*) FAS is a high molecular weight multifunctional complex with binding sites for all the conversions (FAS Type I). Because free-living fungi have all FAS functions on two polypeptides (Wang et al., 1991), the mycobiont in a lichen presumably has the same.

Distinguishing symbiotic partners

Because many metabolic reactions occur in both the lichen fungus and its photosynthetic partner, their respective contributions often are not clearly

evident. Lichen products are sometimes assumed to be synthesized by the mycobiont simply because they occur in high quantities. Unique lichen polysaccharides have been attributed to the fungus for this reason (Barreto-Bergter, 1989). Another approach to evaluating separate contributions of the partners is to examine each of the individual symbionts in pure culture. The presence of characteristic lichen glycans in mycobiont isolates confirms that these polysaccharides are indeed produced by the fungal partner (Takahashi et al., 1979). However, inferences concerning respective roles of the two symbionts can be misleading if based solely on culture experiments, as the metabolism of isolated cells can be quite different from that in the lichenized state.

Isolated phycobionts secrete fewer carbohydrates, and the compounds are different from those released in a lichen (Richrdson, 1973). Changed immunological and staining properties of the algal cell wall (Bubrick, 1988) are other indications that cultured algae are metabolically distinct from those in the lichenized form. The *Nostoc* photobiont of *Nephroma laevigatum* reverts to its filamentous form when isolated and cultured (Kardish et al., 1989), and cell walls become both structurally and immunologically different. Hydrogenases of cyanobionts are less evident in pure culture than in the lichenized state (Rai et al., 1992).

Mycobionts also differ *in vitro*, and may take up sugars and polyols that are not absorbed by hyphae in a lichen thallus (Galun et al., 1976; Feige, 1978). Cultured cells in some species become darkly pigmented or enlarge and fill with lipids, and the texture of wall surfaces may be altered (Honegger and Bartnicki-Garcia, 1991). Differing abilities to bind lectin probes suggest that cell wall components may not be the same in cultured mycobionts as in hyphae freshly isolated from a thallus (Galun, 1988). Phenolics not found in lichens may also be produced by isolated mycobionts (Mosbach, 1967; Kon et al., 1993); in fact phenolics of cultured lichen fungi may often be different from those in the intact thallus (Yoshimura et al., 1993). In single spore mycobiont isolates of *Ramalina siliquosa* the array of lichen products is more complex than in an intact thallus (Culberson et al., 1992).

The extent to which lichen metabolism can be understood by study of isolated symbionts is presumably limited, as the culture medium usually contains substances that are not available in an intact lichen. Also, pathways could be altered in culture due to absence of metabolites that are normally present in a natural thallus. Lichen enzymes are synthesized in response to intermediates such as arginine or urea (Planelles and Legaz, 1987; Legaz and Vicente, 1989), and enzyme activity is influenced by atranorin (Perez-Urria and Vicente, 1989), usnic acid (Planelles and Legaz, 1987) or ammonia (whether NH_3 or NH_4^+ not specified) (Legaz et al., 1986). Because activity is also affected by factors such

as light (Brown et al., 1982; Vicente, 1991) and water content (Gonzalez et al., 1984; Vicente, 1990), it is not surprising if metabolism in culture is different.

Another complication associated with using cultured isolates to investigate lichen metabolism is that the molecular form of a thallus enzyme may not correspond to that of either cultured symbiont. In the case of invertase in *Parmelia caperata*, the thallus isozyme is not produced in cultures of either the alga or the fungus and may be composed of subunits originating from both (Martin, 1973a,b; Fahselt, 1985). Interaction between symbionts to account for the enzyme form in a lichen, could be facilitated by intrathalline transport of soluble constituents within the mycobiont wall (Honegger and Peter, 1994). Like other secreted enzymes, invertase may undergo post-translational changes in connection with transport across cell walls (Ivanova, 1985) and, in the lichen thallus, the enzyme may be subject to different modifications than in isolated symbionts. Invertase is a glycoprotein and, at least in yeast, its activity is retained in spite of being altered (Yurkevich, 1985).

An alternative means of assessing the metabolic contributions of each symbiont, and one that does not necessitate extrapolation from cultures to whole lichens, is thallus fractionation. For example, structures such as purely fungal rhizines can be separated from thallus parts that include photobionts (Fahselt and Hageman, 1994). A useful technique that involves cutting below the algal layer to separate thalli into two tissue zones (Smith et al., 1969) has been used to study carbohydrate movement. Enzymes such as glutamate dehydrogenases have been characterized in dissected thalli (Bernard and Goas, 1979) and DNA has been studied in manually-separated fractions of *Nephroma laevigatum* that were subjected to endonuclease digestion (Kardish et al., 1990). For comparative purposes symbionts can be freshly isolated from thalli (Vicente and Legaz, 1988); fractions obtained from disrupted thalli by sucrose density gradient centrifugation have been used to assess intrathalline distribution of cellulases (Yague and Estevez, 1989).

Interpretation of differences observed between fractions must be tempered, however, by the possibility of differential regulation of gene expression within a normal intact thallus. Fungal processes are not the same in all layers or structures. For example, in phycolichens, chitinous cell walls maybe restricted to hyphae in particular layers of a stratified thallus (Schlarmann et al., 1990). Some extracellular phenolics are localized in the medulla while others are found mainly in the upper cortex (e.g., Hale, 1993; White and James, 1985). Levels of secondary metabolites are lower in the purely fungal prothallus than in areoles containing both bionts (Fahselt, 1976). A few isozymes in *Umbilicaria vellea* and *U. mammulata* were found exclusively in rhizines (Fahselt and Hageman,

1994). Such localization suggests either that the mycobiont is affected by proximity to the photobiont and its metabolites or by physical/chemical factors. If passive transport of solutes in apoplastic space occurs commonly throughout the lichen thallus (Honegger, 1993), it is more probable that localized differences in mycobiont metabolism are attributable to abiotic factors that could vary within the thallus, such as temperature, relative humidity or CO_2 concentration. Some effects could persist after experimental fractionation.

Selective destruction of one symbiont is another technique which permits particular metabolites to be localized in a lichen thallus, for example, the intact thallus of a cyanolichen can be treated with inhibitors that discriminate between prokaryotes and eukaryotes (Chambers et al., 1983). Thus, in *Peltigera polydactyla*, digitonin specifically disrupts fungal membranes and, because thallus pools of aspartate and alanine are lost after treatment, it can be assumed that the free forms of these two amino acids are concentrated mostly in the mycobiont (Rai et al., 1983).

Recent investigations concerned with the respective metabolic contributions of symbionts and biochemical interactions between them could benefit by more frequent use of immunological and tracer techniques. For example, immunogold labelling of antibodies that recognize specific lichen proteins and subsequent electronmicroscopy to locate the label appears to be a productive approach (Bergman and Rai, 1989; Lines et al., 1989; Rai et al., 1992), and the use of radioactively-labelled precursors (Galun et al., 1976; Lines et al., 1989) or enzymes (Schlarmann et al., 1990) also has considerable unexplored potential.

Multiple enzyme forms

The first multiple enzyme forms described in lichens were those of orcellinate depside hydrolase (Schultz and Mosbach, 1971). More recently, two enzyme forms that hydrolyze fructose-1,6-bisphosphate in *Peltigera rufescens* (Brown and Kershaw, 1986a) and at least three forms of arginase in *Evernia prunastri* (Planelles and Legaz, 1987) were characterized. In addition, multiple forms of several other enzymes have been demonstrated in intact lichens (e.g., Hageman and Fahselt, 1990), cultured lichen algae (Kilias et al., 1988) and isolated mycobionts (Fahselt, 1985). The similarity of electrophoretic banding patterns in cultured mycobionts or fungal structures and those in intact lichens (Fahselt, 1985; Fahselt and Hageman, 1994) suggests that enzyme forms in thallus extracts are mostly those of the mycobiont. This is not surprising as the mycobiont makes up 90–95% of the lichen biomass and some metabolic processes thus occur mainly in the fungus (Rai et al., 1983).

In lichenology the term, isozyme, has been used loosely to refer to any electromorph of a given enzyme, although genetic analyses have not been performed to determine the actual bases for inheritance of different enzyme forms. If only one allele per locus were present in a thallus, the term isozyme would be appropriate, even applying a strict definition. With lichens, however, the word must be used in a less restrictive sense because the possibility of genetic heterogeneity within a thallus (see below), and thus co-existence of two or more alleles, cannot be ruled out entirely.

Electrophoretic banding patterns of enzymes extracted from eukaryotes commonly include isozymes produced in different cellular compartments. In lichens, however, patterns sometimes appear to be particularly complex, perhaps because enzyme forms may be contributed by each of the symbionts. In addition, several genetic strains may be present in one thallus, a possibility that is suggested, at least in large umbilicate lichens, by a patchwork distribution of isozyme phenotypes in the same "individual" or thallus (Larson and Carey, 1986). This mosaicism strongly implies involvement of more than one mycobiont genotype in a single thallus and, because heterogeneity increases with age, it may be explained by incorporation of propagules or accumulation of somatic mutations over time (Larson and Carey, 1986). Photosynthetic productivity varies within a thallus (Larson and Carey, 1986), so photobionts may also contribute to intrathalline enzyme variation (Honegger, 1991).

In every lichen species examined so far, sexual or not, individual thalli in environmentally homogeneous stands differ from one another with respect to the electrophoretic patterns of at least some enzymes (e.g., Fahselt, 1994a; Hageman and Fahselt, 1990). Although enzymes reflect the underlying genetic character of a lichen, they are influenced by ecological factors that regulate genes or affect protein activity. Some enzyme patterns change from season to season (Hageman and Fahselt, 1986) or month to month during the summer (Skult et al., 1990). Care must thus be taken in the experimental design of physiological and biochemical investigations because, along with isozymes, many metabolic processes in lichens show pronounced seasonality.

2. Photosynthesis

The chloroplasts of green algae are believed to have been derived from a free-living procaryote via endosymbiosis (e.g., Margulis, 1984; Sherman et al., 1987) and, thus, while photosynthesis differs in some details (Table 2), it is basically the same in phycolichens and cyanolichens. Although rRNA base sequences are sometimes interpreted to indicate otherwise, evidence for a monophyletic origin of chloroplasts is strong (Cavalier-Smith, 1993). The

Table 2. Biochemical differences between cyanobionts and phycobionts in lichens

Feature	Cyanobiont	Phycobiont
Nitrogen-fixing enzymes	Yes	None
Export of ammonium to main thallus	Yes	No
Sheath around cell wall	Of irregularly branched glycans	None
Cell wall materials		
Murein	Yes	No
Cellulose	No	Yes
Sporopollenin	No	In some species
Susceptibility of wall to enzymatic degradation	Yes	Not if sporopollenin is present
Wall subject to hyphal intrusion	No	In simple crustose and some pyrenocarpous lichens, not if sporopollenin is present
Ability of some molecules (analine blue) to diffuse through walls	Impossible	Possible
Light energy capture		
Molecules	Phycobiliproteins	Chlorophyll *a* and *b* and carotenoids
Location	Phycobilisomes	Light harvesting complexes
Cyclic changes involving de-epoxidation of xanthophyll epoxides	No	Yes
α-Carotene & derivatives such as lutein	No	Yes
β-Carotene & derivatives such as echinenone & canthaxanthin	More than in phycobionts	Smaller amounts than in cyanobionts
Enzyme for synthesizing chlorophyll *b* from chlorophyll *a*	None	Present
Assimilation of CO_2 at low water potential	No	Yes
Location of non-solubilized RuBisCO	Polyhedral bodies	Pyrenoid
Transcription of RuBisCO	Genes for the two subunits transcribed together as a unit	Gene for the large subunit transcribed in the chloroplast, that for the small in the nucleus
Affinity of RuBisCO for CO_2	High affinity	Lower affinity
Sensitivity of carboxylation to O_2	No	Yes
Photosynthetic products exported	Glucose	Acyclic polyols, such as ribitol, erythritol and sorbitol
Storage		
Carbon	Glycogen, lipids	Starch, lipids
Nitrogen	Polyaspartic acid with some arginine residues	Proteins in pyrenoid?
Urease synthesis	Constitutive	Inducible

photosynthetic carbon-fixation pathway has not been demonstrated in lichens *per se*, but much of what is known about photosynthesis was discovered in free-living green algae. Molecular complexes concerned with electron capture and processing, photosystems I and II (PSI and PSII), are associated with thylakoid membranes and are the same in both chloroplasts and cyanobacterial cells. In lichens these may remain functional after storage at −25°C for as long as 9 years (Feige and Jensen, 1987).

In lichenized *Nostoc*, as is the case with other phycobilin-containing cyanobacteria, thylakoids are aligned separately (Fig. 1), but in chloroplasts of green algae (*Trebouxia*, Pleurastrophyceae) they are closely stacked (Fig. 2).

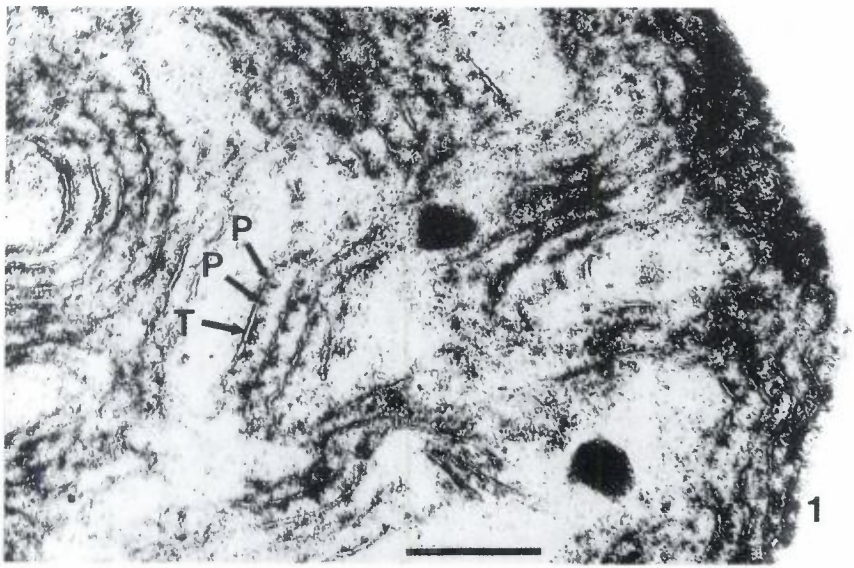

Figure 1. Transmission electron micrograph of a *Nostoc* (cyanobacterium) photobiont of *Peltigera canina*. T = thylakoids. P = Small protuberances on thylakoids are phycobilisomes, bar = 500nm (by courtesy of M.-C Boissière).

Photosystem reaction centers contain chlorophyll complexed with protein, carotenoids and many small polypeptides, and photons excite the chlorophyll in both PSI and PSII. Electrons from chlorophyll in PSII (P680) are quickly passed to phaeophytin and ultimately plastoquinone, leaving $P680^+$, a powerful oxidizing agent that extracts electrons from water and produces molecular oxygen and proteins.

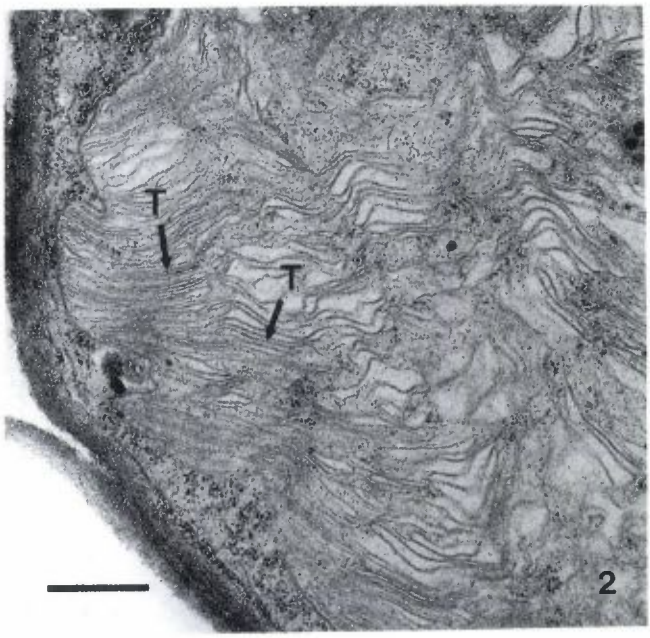

Figure 2. Transmission electron micrograph of a *Trebouxia* (green algae) photobiont. T = thylakoids. Thylakoids are closely packed, or appressed to one another, and have no visible protuberances on their surfaces, bar = 500 nm.

Antennae

In cyanobacteria antennae pigment complexes are closely associated with PSII and, in green algae, with PSI and PSII. Antennae complexes absorb light energy maximally in different parts of the visible spectrum than reaction center chlorophyll (Hopkins, 1995), and captured energy is transmitted to reaction centers.

The cyanobacterial antenna contains no chlorophyll or carotenoids (Hiller et al., 1991), and has instead water-soluble phycobiliprotein pigments with linear tetrapyrrole chromatophores closely associated with protein (Fig. 3). Phycobiliproteins, i.e., phycoerythrocyanin, phycoerythrin, phycocyanin and allophycocyanin, have been documented in various cyanolichens, including *Peltigera, Stereocaulon* and *Nephroma* (Czeczuga, 1988a). These pigments have absorbance maxima between the blue and red peaks of chlorophyll *a*, and are arranged within hemispherical phycobilisomes attached to the thylakoid membrane near PSII (Fig. 1). Most light energy used in photosynthesis is

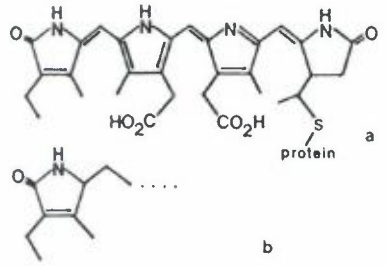

Figure 3. Linear tetrapyrrole structures making up the chromatophores in phycocyanins and phycoerythrocyanins, respectively. (a) phycocyanobilin and, (b) phycoerythrobilin. The point of attachment to the polypeptide chain is illustrated (modified from Leeper, 1991, used by permission of CRC Press, Boca Raton, FL).

captured by phycobilisomes and transferred to the reaction center along rod-shaped configurations of pigment molecules (Fig. 4) toward the reaction center. The proportions of different phycobiliproteins can be altered by the intensity

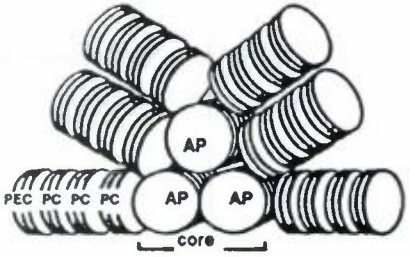

Figure 4. Model of a phycobilisome from a cyanobacterium. PEC = phycoerythrocyanin, PC = phycocyanin, AP = allophycocyanin. Energy is transferred down the PEC/PC rods towards AP in the core (modified from Wehrmeyer, 1990).

and quality of incident light, as well as by nutrient conditions (Wehrmeyer, 1990). For example, during exposure to low light intensities, such as in spring and fall, the proportion of phycocyanin to phycoerythrin in lichens increases (Czeczuga, 1988b). With the aging of a lichen thallus, phycobilisomes appear to be either degraded or disconnected, resulting in reduced photosynthetic productivity (Velly and LeClerc, 1987).

In green algae, light-harvesting pigments include both chlorophyll *a* and *b* as well as several carotenoids (Scheer, 1991); light-harvesting complexes are incorporated into the photosynthetic membrane, rather than projecting from its surface. Chlorophyll levels adjust to the available light energy,with

chlorophyll *b* increasing in the shade when the antenna system of PSII expands to intercept more light (Hopkins, 1995).

Dessication of lichens with green algal photobionts reduces transmission of energy from the light harvesting complex to PSII, but normal transfer can be resumed after exposure to humid air. Drying of cyanolichens prevents energy transfer between phycobilisomes and PSII, and restitution is possible only after thalli are wetted with liquid water (e.g., Bilger et al., 1989).

Protection

The ability to concentrate light energy must be highly adaptive, but superoptimal light intensities can be potentially damaging. Under high irradiance, energy transfer to the reaction center of the free-living cyanobacterium *Synechococcus* can be minimized by autofluorescence of phycoerythrin, thus reducing the risk of chlorophyll photo-oxidation (Wyman et al., 1985). In higher plants the enzyme superoxide dismutase (SOD, E.C. 1.15.1.1.) inactivates damaging free radicals of oxygen (O_2^-) that may be produced by reaction of excited chlorophyll with molecular oxygen. This enzyme in lichens, occurs both in fungal parts of the thallus and in fractions with photobionts and, thus, must scavenge radicals in addition to those produced through the interaction of light and chlorophyll. Photoprotection against singlet oxygen ($^1O_2$), and the excited triplet state of chlorophyll from which it forms, is provided by carotenoids bound in reaction centers (Frank and Cogdell, 1993), that is, while some carotenoids harvest light, others prevent destruction of photosystems by absorbing and dissipating excess excitation energy.

Xanthophyll intermediates that quench excess irradiation energy undergo rapid, reversible light-induced changes in concentration and facilitate rapid recovery of photosynthetic efficiency (Demmig-Adams et al., 1990a,b). Following high intensity exposures to excitation energy, phycolichens experience increased levels of zeaxanthin, which is produced in the xanthophyll cycle at the expense of the xanthophyll epoxide violaxanthin (Fig. 5; Hager, 1980). Antheraxanthin is an intermediate between violaxanthin and zeaxanthin, and interconversion among these three takes place in pigment complexes associated with reaction centres (Demmig-Adams et al., 1990b). Zeaxanthin was identified (Czeczuga, 1988b) in most of 143 lichen species surveyed, including some cyanolichens, but xanthophyll epoxides are produced only by green algal photobionts and not by cyanobionts (Demmig-Adams and Adams, 1993).

The xanthophyll cycle for rapid generation of zeaxanthin (Fig. 5) could play a role in determining ecological limits for a lichen. In response to continuous intense irradiation, cyanolichens produce relatively high levels of β-carotenes,

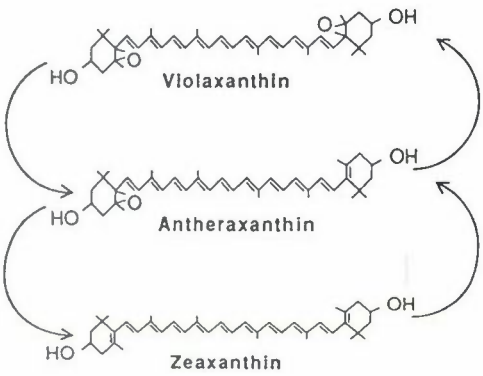

Figure 5. The xanthophyll cycle. ASC = ascorbic acid, GSSG = oxidized GSH,GSH = glutathione (modified from Hager, 1980, reprinted by permission of Chapman and Hall).

but only small quantities of zeaxanthin which do not originate through the xanthophyll cycle (Demmig-Adams et al., 1990a). As a result, cyanolichens are not afforded the same instantaneous protection from intense radiation energy as phycobionts, and they suffer more long-lasting photoinhibitory damage. Cyanolichens, thus, may not be as well adapted to widely fluctuating light conditions, and this may explain why species such as *Peltigera rufescens* and *Sticta fuliginosa* are restricted, respectively, to either open or continuously shaded habitats.

When the PSII reaction center suffers reversible irradiance damage, the fluorescence properties of chlorophyll are altered. This is reflected in a change in the ratio between induced fluorescence and maximum level of fluorescence (F_v/F_m), and the magnitude of decrease in this ratio indicates the extent of impairment (Manrique et al., 1993). The level of illumination that is damaging to PSII depends on the lichen, e.g., species growing naturally in brightly illuminated habitats are less subject to damage and those requiring a shorter time for a wet thallus to dry to constant weight are also less vulnerable to irradiation (Manrique et al., 1993).

Photosynthetic electron transport

After light energy is received in reaction-centers, electrons are rapidly displaced to a higher energy level, spontaneously moving through various electron carriers, until they leave the membrane system a few milliseconds later.

Cytochrome b_6-f catalyzes transfer of electrons from plastoquinone to plastocyanin, and then electrons are captured by the reaction center of PSI. They are next transferred to a site on the thylakoid membrane where redox molecules such as ferredoxin or flavodoxin are encountered. Ferredoxin, an extremely old protein (Rogers, 1987), has been isolated from two lichens, *Evernia prunastri* and *Lobaria pulmonaria* (Vicente and Legaz, 1988). Lichen ferredoxin, like that in other autotrophs, contains both iron and sulfur, but its absorbance properties are atypical of higher plant and some algal ferredoxins.

Ferredoxin-$NADP^+$ oxidoreductase (EC 1.18.1.2), which acts on reduced ferredoxin to catalyze electron transfer to $NADP^+$, is inhibited by high levels of NADPH, while $NADP^+$ produced in the xanthophyll cycle may stimulate the photosynthetic electron-to-oxygen transport system (Hager, 1980). Besides facilitating movement of electrons, the cytochrome b_6-f complex pumps protons into thylakoid space and creates a pH gradient across the membrane. Protons then flow out of thylakoid space through an enzyme assembly in the membrane that synthesizes ATP. Electrons from ferredoxin may also return to cytochrome b_6-f instead of going to $NADP^+$, in which case ATP is generated without formation of NADPH.

Carbon fixation

Over a period of time CO_2 is fixed in a series of reactions that mostly take place in the stroma of the chloroplast or in cytoplasmic inclusions of a cyanobacterial cell. Free-living cyanobacteria and algae reduce carbon dioxide to carbohydrates in a pathway variously known as the Calvin, C-3 or reductive pentose cycle (RPC); lichens seem also to use the C-3 pathway for photosynthesis (Hill, 1976; Brown and Kershaw, 1986b).

Higher than expected net rates of photosynthesis in cyanolichens and some phycolichens (Badger et al., 1993) are probably due to a CO_2 concentrating mechanism (CCM) that involves active uptake of inorganic carbon by the photobiont (Palmqvist, 1993). Carbonic anhydrase (CAN, EC 4.2.1.1), a reversible enzyme that catalyzes the dehydration of H_2CO_3 to CO_2, also enhances production potential under CO_2-limited conditions, and the littoral cyanolichen *Lichnia pygmaea* has a CCM with intracellular and, possibly, extracellular CAN (Raven et al., 1990). Thus, when thalli are submersed by tides, excreted CAN may facilitate acquisition of dissolved inorganic carbon from sea water. The presence of CAN in the cyanobacterial carboxysome (polyhedral body) together with enzymes that fix CO_2 is an efficient arrangement that facilitates assimilation of carbon (Badger and Price, 1992).

No CCM has yet been found in phycolichens with *Coccomyxa* or *Dictyochloris* as autotrophic partners, but it has been reported in *Trebouxia* lichens (Palmqvist, 1993). Less is understood about the operation of CCM in green algae (Badger and Price, 1992) and some phycolichens that lack a CCM appear to compensate with higher levels of internal CAN (Palmqvist, 1994). Carbonic anhydrase is known in several lichens, (e.g., Fahselt, 1988; Fahselt and Hageman, 1983; Skult et al., 1990), but isozymes found in rhizines of *Umbilicaria* spp. (Fahselt and Hageman, 1994), indicate that the enzyme is not restricted to photobionts.

Depending on the cortical anatomy of a lichen species, the thallus surface might become impervious when wet and, thus, prevent gases from entering the thallus (Lange, 1981; Kappen, 1988). In this case, CO_2 could be generated from internal sources, for example, by photorespiration or respiratory activity of the mycobiont; entry by CO_2 could also be gained from the lower side of an ecorticate thallus (Honegger, 1991).

Blanco et al. (1984) proposed that urea, from which lichens can produce carbon dioxide through the action of urease, may be another source of inorganic carbon for photosynthesis. Urease is inducible in phycolichens by exogenous sources of urea, but constitutive in cyanolichens (Vicente et al., 1984). It has been suggested that lichens growing on nitrogenous soils or trees might utilize urea both from the substrate (Perez-Urria et al., 1989) and endogenous sources. However, while urease is produced by several lichen species *in vitro* (Vicente and Legaz, 1988), the quantity of urea available under natural circumstances has not been related to photosynthetic requirements for CO_2.

The RPC requires NADPH and ATP to convert 3-phosphoglycerate into 3-phosphoglyceraldehyde, and ATP is required also to convert ribulose-5-phosphate into ribulose-1,5-bisphosphate. The one point in the cycle where carbon dioxide is taken up involves the intriguing bifunctional enzyme, ribulosebisphosphate carboxylase/oxygenase (RuBisCO) (EC 4.1.1.39) that carboxylates ribulose-1,5-bisphosphate in the light. Not surprisingly, RuBisCO occurs in lichens (Vicente and Legaz, 1988), as in many autotrophs. It is an enormous molecule, both in chloroplasts and cyanobacteria, consisting of eight large and eight small subunits (Tabita, 1987; Gregory, 1989). Small and large subunits are transcribed together in cyanobacteria (Tabita, 1987), but in green algae, because the gene for the large subunit is in the chloroplast and for the small in the cell nucleus, they must be transcribed separately. Large paracrystalline concentrations of RuBisCO are found in the carboxysomes of cyanobacteria and pyrenoids of green algae (Badger et al., 1993).

For its functioning, RuBisCO requires a high pH and supply of Mg^{2+} ions, conditions that are met as a result of reactions taking place in the light (Amesz,

1987; Gregory, 1989). Also, key phosphorylated metabolites, such as phosphogluconate and NADPH enhance carboxylase activity, and an "activase" enzyme to attach the carbon dioxide to RuBisCO is also necessary. However, unless CO_2 saturates RuBisCO, it will function predominantly as an oxygenase and the efficiency of carbon fixation will be lowered. Photosynthesis is more sensitive to molecular oxygen in a *Peltigera* phycolichen than in a related cyanolichen, suggesting that the affinity of RuBisCO for CO_2 is greater in a lichen with a cyanobiont (Palmqvist, 1993). *In vitro*, however, RuBisCO of a cyanobacterium has a lower affinity for CO_2 than that of a green alga (Badger et al., 1993), so the higher rate of net CO_2 assimilation in a cyanolichen may be related instead to its facility for concentrating CO_2 (Raven et al., 1990).

Cyanolichens differ from phycolichens in their ability to discriminate between C-isotopes, but the reason is not clear. From an atmospheric mixture of $^{12}CO_2$ and $^{13}CO_2$ RuBisCO selectively incorporates $^{12}CO_2$, but discrimination is somewhat less in cyanolichens. Lange and Zeigler (1986) suggested that the difference is related to a photosynthetic requirement for liquid water in cyanobionts, and the necessity for CO_2 to diffuse through water, rather than air, prior to assimilation. Raven et al. (1990) consider that C-isotope ratios are different due to the greater importance in cyanolichens of CCM membrane pumps for the uptake of dissolved inorganic carbon.

Other enzymes of the RPC are found in the cyanobacterial carboxysome along with RuBisCO (Beudeker and Kennen, 1981; Sainis and Harris, 1987) and these same enzymes are located in the chloroplast stroma of phycolichens (Foyer et al., 1987; Hopkins, 1995). In chloroplasts, four are stimulated by light: glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.13), fructose-1,6-bisphosphatase (EC 3.1.3.11), sedohepulose-1,7-bisphosphatase (EC 3.1.3.37) and ribulose-5-phosphate kinase (EC 2.7.1.19). Of these, photosynthetic fructose-1,6-bisphosphatase has been purified from lichens (Brown and Kershaw, 1986a); its activation energy and substrate affinity in *Peltigera rufescens* differ seasonally, a phenomenon that was attributed to conformational changes associated with temperature acclimation (Brown and Kershaw, 1986b).

Alternative pathways to assimilate CO_2 have not been identified in lichens, but some free-living autotrophic bacteria lack RuBisCO and fix carbon via a reductive citric acid cycle or the reductive non-cyclic acetyl-CoA pathway. In each case, key enzymes other than RuBisCO are required (Strauss and Fuchs, 1993). Another alternative mode of autotrophic assimilation has been recently discovered in prokaryotes, which uses 3-hydroxypropionate as an intermediate and acetyl-CoA carboxylase and propionyl-CoA carboxylase as CO_2-assimilating enzymes (Eisenreich et al., 1993).

In addition, a variety of pathways in plants, green algae and fungi may

fix CO_2 in the dark. Acetyl-CoA carboxylase (see below in relation to lipid synthesis) assimilates CO_2, as does pyruvate carboxylase, which has been found in yeast and *Aspergillus* (Webb, 1992). In a process referred to as β-carboxylation, phosphoenopyruvate carboxylase (PEP) of plants and green algae carboxylates phosphoenolpyruvate of the Embden-Meyerhof pathway to form a 4-carbon dicarboxylic acid that feeds into Kreb's cycle and replenishes intermediates drawn off by the synthesis of proteins and nucleic acids (Raven, 1974). Phosphoenolpyruvate carboxykinase and 'malate enzyme', both found in algae, also fix carbon (Raven, 1974; Johnstone and Raven, 1989), as do carbamoyl-phosphate synthases (Webb, 1992) that occur in basidiomycetes and bacteria. At present, it is unclear which of these transformations take place in lichens. Some lichen species have a capacity for dark fixation (Cowan et al., 1979), apparently by β-carboxylation, but in *Xanthoria calcicola* incorporation of labelled carbon into mannitol is abolished in darkness (Lines et al., 1989).

3. Photorespiration (The C-2 Pathway)

Carbon dioxide may be fixed by RuBisCO but, when oxygen levels are high and ribulose bisphosphate is oxygenated rather than carboxylated, CO_2 is generated instead. Initially 3-phosphoglycerate and phosphoglycolate are formed and a subsequent intermediate in the pathway, glycine, becomes decarboxylated. The carbon that is lost can be assimilated again, but only through expenditure of further energy. Photorespiration thus seems counterproductive, in that sugars produced in photosynthesis are re-oxidized for no apparent reason. RuBisCO, undoubtedly an ancient enzyme, must have originated in the Precambrian Period when there was much less oxygen in the atmosphere and little selective advantage to distinguishing between CO_2 and O_2. By the time oxygen levels increased, the oxygenase function was an integral part of the enzyme (Gregory, 1989).

Free-living cyanobacteria produce the C-2 pathway intermediate, glycolate, through the oxygenase activity of RuBisCO (Gross, 1993), and free-living green algae both synthesize and excrete it (Fiege, 1975; Geider and Osborne, 1992). Glycolate is generated when O_2 competitively inhibits RuBisCO carboxylase activity in *Ramalina maciformis*, but decarboxylation does not necessarily follow (Cowan et al., 1992). Although both glycolate and another intermediate, glyoxylate, have been reported in lichens (Culberson, 1970), the extent to which C-2 oxidation occurs is not known.

4. Carbon Transfer to the Mycobiont

The flow of nutrients from higher plant cells into fungal pathogens probably takes place by diffusion across the host cell membrane. The movement of solutes appears to be down a concentration gradient that is maintained both by high levels of photosynthate in the host and rapid conversion of the transferred molecules into fungal-specific metabolites in the pathogen (Smith et al., 1969; Hall et al., 1992). Evidence suggests a carrier-mediated active transport in lichens (Smith and Douglas, 1987). Temperature down to 5°C has little effect on rate of transport out of cyanobionts but, depending on the species, thallus hydration may influence the movement of fixed carbon (MacFarlane and Kershaw, 1982). The photobiont releases most assimilated carbon to the fungus (Smith and Douglas, 1987), a process confirmed by feeding experiments with ^{13}C (Lines et al., 1989).

In the case of cyanobionts such as *Nostoc*, carbon is released in the form of glucose, while phycobionts release acyclic alcohols, or polyols. *Trebouxia* and *Coccomyxa* export ribitol, *Trentepohlia* erythritol and some other phycobionts produce sorbitol (Smith et al., 1969; Richardson, 1985). A ^{13}C-NMR study (Lines et al., 1989) with *Xanthoria calcicola* indicated that most carbon is released largely as a single kind of molecule. On the other hand, MacFarlane and Kershaw (1985) suggested that the mixture of carbohydrates transferred from the photobiont to the fungus is complex, and Vicente (1991) indicated as well that several molecules are released. One repressor synthesized in the photobiont and exported to the fungus has been reported in *Evernia prunastri* (Perez-Urria et al., 1990), so other exported molecules may be regulatory as well.

It has been suggested that ammonia released from urea by urease (Blanco et al., 1984) increases permeability of algal cell membranes and promotes export of photosynthates (Vicente and Filho, 1979). However, there is apparently little evidence to support this. Lichens can absorb NH_4^+ from culture media (Rai, 1988), probably in a carrier-mediated fashion (Jennings, 1986), but it has not been shown that ecological concentrations enhance release of photosynthates to the mycobiont.

A wide array of organic compounds can be used as substrates by cultured lichens, including sucrose, sugar alcohols and glutamine (Yamamoto et al., 1993), as well as cellulose (Ahmadjian, 1987) and glucose (e.g., Feige, 1978). However, acquisition of carbon from higher plants by epiphytic lichens in natural settings has been little studied. Yague and Estévez (1988, 1989) have indicated that the necessary extracellular polysaccharidases for breaking down cellulose into simple sugars can be induced in epiphytic lichens, but there is

so far no evidence that natural substrates are actually degraded under field conditions (Vicente, 1990).

5. Respiration/Oxidation

In lichens, as in other organisms, high-energy phosphate molecules as well as reduced co-factors required for intermediary metabolism are presumably produced by respiratory sequences. The Embden-Meyerhof pathway (EM) and the pentose-phosphate pathway (PP) are common to non-lichenized fungi, green algae and cyanobacteria. The Krebs or tricarboxylic acid cycle (TCA) functions in free-living fungi and green algae, but is incomplete in cyanobacteria as the enzyme 2-oxoglutarate dehydrogenase is lacking (Lloyd, 1974; Raven, 1987). Reducing power can also be generated through the glyoxylate cycle. Besides energy currency, respiratory pathways of course provide precursors required for the synthesis of compounds such as fatty acids, amino acids and porphyrins.

Because photobionts and mycobionts are capable of independent growth in pure culture, all must have functional primary pathways, but most oxidative pathways have never been verified in lichens by following the course of labelled precursors or using inhibitors to produce accumulations of diagnostic intermediates. However, several respiratory enzymes (Table 3) have been identified in lichens, cultured *Trebouxia*, isolated cyanobionts or lichen fungi.

The Embden-Meyerhof pathway

The EM enzymes known to occur in crude extracts of lichen thalli are shown in Table 3. Hexokinase and fructose-1,6-bisphosphatase activities have been demonstrated in *Peltigera rufescens*, and D-phosphoglucose isomerase in *Cladonia stellaris* and in cultured *Trebouxia*. Glyceraldehyde-3-phosphate dehydrogenase (NAD^+) has been reported also in *Peltigera rufescens* and lactate dehydrogenase in extracts of *Parmelia omphalodes* and related species. Although these enzymes have been detected in only a few lichens, they undoubtedly occur much more widely.

The pentose phosphate pathway

The oxidative PP pathway interdigitates with the EM, and like the EM all reactions occur in the cytosol. Intermediates such as D-fructose-6-phosphate and D-glyceraldehyde-3-phosphate are common to both metabolic routes and, in free-living fungi, the balance between the two pathways may shift. For example, the PP, especially the non-oxidative part (Peleato et al., 1991), is

Table 3. Enzymes of primary metabolic pathways found in lichens

Enzyme & Pathway	EC+	Reaction	References
Embden-Meyerhof pathway			
Hexokinase	2.7.1.1	D-glucose → glucose-6-P	Brown et al., 1989
D-phosphoglucose isomerase	5.3.1.9	D-glucose-6-P → fructose-6-P	Kershaw et al., 1983, *Kilias et al., 1988
D-fructose bisphosphatase ($NADP^+$)	3.1.3.11	D-fructose-1,6-biP + H_2O → D-fructose-6-P + Pi	Brown et al., 1989
Glyceraldehyde-3-phosphate dehydrogenase (NAD^+)	1.2.1.12	Glyceraldehyde-3-P → 1,3-diphosphoglycerate	Brown & Kershaw, 1985
Lactate dehydrogenase (NAD^+)	1.1.1.27	pyruvate → lactate	Skult et al. 1986, 1990
Pentose phosphate pathway			
D-glucose-6-phosphate dehydrogenase	1.1.1.49	Glucose-6-P → 6-phospho-gluconolactone	**Fahselt, 1985; Hageman & Fahselt, 1984, 1990, Brown & Kershaw, 1985 Hamade et al., 1993
6-Phosphogluconate dehydrogenase	1.1.1.44	6-P-gluconate → ribulose-5-P	**Fahselt, 1985; Hageman & Fahselt, 1984, 1990; Skult et al., 1986, 1990
Tricarboxylic acid cycle			
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	Isocitrate → α-keto-glutarate	Hageman & Fahselt, 1984, 1990
Malate dehydrogenase (NAD^+)	1.1.1.37	Malate → oxaloacetate	Kershaw et al., 1983, *Kilias et al., 1988, Brown et al., 1989; Skult et al., 1990; Hamade et al., 1993
Amino acid synthesis			
Glutamate dehydrogenase ($NADP^+$)	1.4.1.4	Glutamate → α-keto-glutarate	Bernard & Goas, 1979, **Fahselt, 1985, Hageman & Fahselt, 1984, 1990; Skult et al., 1986, 1990, Hamade et al., 1993

+ International Union of Biochemistry Enzyme Code
* The isolated photobiont, *Trebouxia*
** The isolated mycobiont

more active during conidiogenesis than in post-sporulating mycelia (Ng et al., 1972; Turian and Bianchi, 1972). However, the possibility of an association between these pathways and reproductive *vs* vegetative growth has not been explored in lichens. The importance of the PP, in some cyanobacteria at least, may be determined by levels of nitrogenous intermediates such as amino acids that exert metabolic control over glucose-6-phosphate dehydrogenase, the enzyme that catalyzes the first step (Rowell and Simpson, 1990). Effects on green algal glucose-6-phosphate dehydrogenase activity depend on whether nitrogen is in the form of ammonium or nitrite; if NO_3^- is added to nitrogen-limited cells, an increase in activity is observed, but NH_4^+ produces no change (VanLerberghe et al., 1992).

Just as carbon can be admitted to the PP at several points, a variety of compounds can likewise be generated from PP intermediates; e.g., sugar phosphates are precursors for nucleotide synthesis and for shikimate-based products. In lichens and free-living fungi, starting materials are drawn from the PP for synthesis of storage products (Cochrane, 1976); fructose-6-phosphate is of particular significance to lichens as this is probably converted to polyols or sugar alcohols characteristic of the mycobiont (Vicente and Legaz, 1988).

All enzymes of the PP pathway have been reported in free-living fungi (Wood, 1985) and those generating NADPH have been found in cell-free thallus preparations of several lichen species (Table 3). Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase both occur in extracts of *Lasallia papulosa* and five species of *Umbilicaria*. The first of these has been found in *Cetraria islandica* and some species of *Parmelia*, and the second in *Peltigera rufescens*. All lichens must have PP enzymes, but so far most have not been tested.

The tricarboxylic acid cycle

Pyruvate generated by glycolysis is decarboxylated in the cytosol, bound to coenzyme A and transported in eukaryotes as acetyl-CoA into the mitochondrion where it may enter the TCA pathway. Most intermediates of this pathway have been reported in whole lichens (Culberson, 1970) and some enzymes detected in crude lichen extracts (Table 3). Isocitrate dehydrogenase is known in six species of the Umbilicariaceae in eastern Canada and in *Parmelia* collections from southern Finland, and malate dehydrogenase has been shown in four lichens, *Cladonia stellaris, Parmelia omphalodes, Cetraria islandica* and *Peltigera rufescens*, as well as in a cultured *Trebouxia* photobiont. At least in some lichens, enzymes of the TCA are less sensitive to dessication than those of photosynthetic C-fixation and at low humidities may produce intermediates

that are ready for use when metabolism is initiated by rehydration (Cowan et al., 1979a).

The glyoxylate cycle

This pathway, like the TCA, converts acetyl-CoA or lipids into carbohydrates and it involves a number of the same intermediates. However, it generates less reduced co-factor and no ATP or GTP. Isocitrate, which is at the branching point between these two pathways, may be metabolized either way, being diverted into the TCA when energy requirements are greater (Stryer, 1988). Although the glyoxylate cycle has been documented in free-living fungi (e.g., Ruch and Samuel, 1992), enzymes unique to the pathway, isocitritase and malate synthase, have not yet been found in lichens.

Oxidative phosphorylation

Electrons released during respiratory oxidation are transferred to pyridine nucleotide or flavin carriers and finally to O_2 through an electron transport chain that is coupled to ATP synthesis. The transport chain typically involves a series of molecular complexes that, in eukaryotes, are located in the inner membrane of the mitochondrion. In prokaryotes, electron carriers are probably situated in both thylakoid and plasma membranes (Peschek, 1987). Electrons emerging from the first complex may flow to O_2 either through two cytochrome-containing complexes, as in higher plants and some green algae, or along a completely different route, i.e., one that is not inhibited by cyanide (Weger and Dasgupta, 1993). The energy yield of the alternate pathway is considerably less than that of the cytochrome route, i.e., NADH yields three ATP along the phosphorylating cytochrome pathway and only one using the alternate. Lambers (1982) hypothesized that the alternate pathway is an energy overload device invoked when there is more reduced co-factor than can be accommodated in the cytochrome pathway and proposed that the less efficient route may be engaged when the supply of carbon exceeds the demand. It was suggested that the option of using an alternate route may be advantageous under fluctuating environmental conditions. Basal respiration in lichens is cyanide-resistant, so oxidation of co-factors normally appears to be by the alternate route (Farrar, 1988). However, the cytochrome pathway may be inducible, and could operate during the burst of respiration that occurs after dry thalli are wetted (Farrar, 1988).

6. Storage

Lichens presumably have metabolic resources that are sequestered as reserves and drawn upon in times of deprivation or active growth. Stores of N and P are closely integrated with C because N- and P-containing intermediates are ultimately taken from basic carbon cycles, and net assimilation of carbon depends upon adequate supplies of various nutrients, including N and P, for enzymes, and structural components of cells. Jennings (1989) has reviewed important aspects of the interdependency of C and N metabolism: both regulate the enzyme glutamate dehydrogenase that operates at a major branching point between C and N pathways, many fungi utilize protein as a source of C, the relative availability of C and N may be critical in determining cell wall composition, P anions maintain the cation/anion balance in cells while C is conserved for other purposes, and orthophosphate concentration interacts with pH to regulate glycolysis through the two glycolytic enzymes, hexokinase and phosphofructokinae. Phosphorus is of course pivotal to the energy currency used for assimilation and respiration.

Mycobiont storage products

Free amino acids

Both free and bound amino acids are found in lichens (Gorin et al., 1988). Though pools of some free amino acids appear to be associated with the mycobiont (Rai et al., 1983), the intrathalline location has not been determined for all. The metabolic role of these pools is essentially unknown although it may be related to storage, as amino acids can be transaminated or deaminated in lichens, even at relatively low humidities, and directed into the TCA cycle (Cowan et al., 1979a,b).

Polyols

There are a large number of different acyclic polyols in fungi (Pfyffer and Rast, 1980), but mannitol is especially prevalent in Ascomycetes (Rast and Pfyffer, 1989; Pfyffer et al., 1990). Of the many polyols in lichens (Vicente and Legaz, 1988), mannitol, produced only in the mycobiont (Feige, 1978), is usually reported to be the most abundant. It may be, however, that mannitol is most abundant in wet lichens, and more arabitol than mannitol is present when thalli are dry (Rees et al., 1989).

Various roles have been envisaged for polyols (Corina and Munday, 1971; Farrar, 1973, 1988; Richardson, 1985), but a clear consensus has not been reached. Sugar alcohols may be important to water relations of a fungus and

promote passive uptake of water (Smith et al., 1969; Farrar, 1988), and hydroxyl groups of polyols may also help to maintain membrane integrity during periods of dessication (Farrar, 1976), the latter a possibility that has been little explored. Polyols may constitute a reserve of carbon and reducing power (Hult and Gatenbeck, 1978; Hult et al., 1980; Farrar, 1988), with mannitol in particular considered as a form of low molecular weight storage (Sturgeon, 1985). However, mannitol may instead provide sustained protection against water loss, while arabitol, showing more pronounced variation on a day-to-day basis, is the more readily mobilizeable reserve (Armstrong and Smith, 1993). A phenomenon that suggests polyols might be somewhat unreliable as stores is the loss of considerable amounts by leaching from lichens during simulated rain (Dudley and Lechowicz, 1987) or from bryophytes during exposure to natural precipitation (Coxson et al., 1992).

Pathways of mannitol synthesis in lichens, based on those in other organisms, were proposed by Vicente and Legaz (1988). The suggested involvement of pentose phosphate pathway intermediates is supported by the time course investigations of Lines et al. (1989); ^{13}C-NMR spectra of *Xanthoria calcicola* indicated that the labelled carbon in $^{13}CO_2$ appears first in ribitol and only later in mannitol. Data are consistent with the production of mannitol via PP pathway, with D-xylulose-5-phosphate as an intermediate.

A mannitol cycle (Fig. 6) that generates D-mannitol from fructose-6-

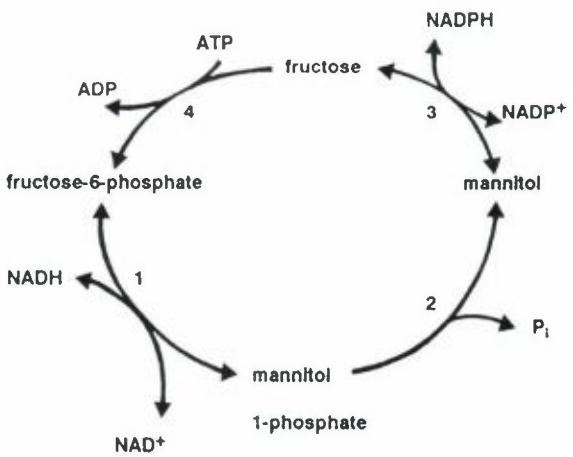

Figure 6. Reactions of the mannitol cycle. Enzymes: (1) mannitol-1-phosphate dehydrogenase, (2) mannitol-1-phosphatase, (3) mannitol dehydrogenase, (4) fructose kinase (from Hult et al., 1980).

phosphate is known in several free-living fungi (Hult and Gatenbeck, 1978, 1979; Hult et al., 1980), but only some of the enzymes needed to operate it have been identified in lichens. One of the enzymes, $NADP^+$-dependent D-mannitol dehydrogenase (EC 1.1.1.138), was detected in *Lasallia papulosa* and five species of *Umbilicaria* (Hageman and Fahselt, 1990), and Crittenden (1993, personal communication) has reported both NAD^+- and $NADP^+$-dependent forms of it. An NAD^+-dependent D-mannitol-1-phosphate dehydrogenase (EC 1.1.1.17), has been found in *Pseudevernia furfuracea* (Jensen et al., 1991). Since no mannitol occurs in the photobiont, these enzymes must be fungal. Pathways to mannitol could differ from one mycobiont to another, but the apparent lack of some enzymes must simply relate to difficulties of detection in the lichen species investigated. For example, in order for *P. furfuracea* to form mannitol, there must be a mannitol-1-phosphatase (EC 3.1.3.22) (Jensen et al., 1991), even though none has been found. It has been suggested that arabitol is formed from mannitol during dessication (Rees et al., 1989), but in *Parmelia conspersa* there is no correlation between levels of the two polyols, so at least in this species one is probably not derived from the other (Armstrong and Smith, 1993).

Lipids

Many phospholipids, neutral lipids and fatty acids have been documented in lichens by Dembitsky and co-workers (e.g., Dembitsky, 1992; Dembitsky et al., 1992a,b). The partitioning of constituents between the two symbionts in a thallus is not understood, but isolated mycobionts can be seen to produce lipid droplets (Honegger and Bartnicki-Garcia, 1991). Low temperature generally provokes changes in membrane lipid composition, and resistance to temperature stress probably depends, in part, on lipid rearrangements to restore normal membrane function (Mazliak, 1989). Water-stress in lichens also affects lipids, in that synthesis is depressed to a significant degree (Cowan et al., 1979a). Perhaps the marked variation in lipid content, with season and place of collection (Dembitsky, 1992), in part reflects the effects of differing environmental conditions. Because lipids can be degraded to acetyl-CoA and respired, they may be utilized as metabolic reserves.

Little is known of fatty acid synthesis in lichens, but the process has been investigated in free-living fungi. The first step is a two-stage carboxylation of acetyl-CoA that is catalyzed by the multifunctional enzyme, acetyl-CoA carboxylase (EC 6.4.1.2.), and produces malonyl-CoA. Fatty acid synthetase, in approximately seven steps, then adds two carbons from malonyl-CoA to acetyl-CoA to produce a longer coenzyme A thioester (Schweizer, 1984). Acetyl-CoA

carboxylase and fatty acid synthetases are both cytoplasmic, while desaturases that generate unsaturated fatty acids from corresponding saturated molecules are particulate enzymes localized in microsomes.

In lichens examined so far, the main fatty acids are 16 or 18 carbons long (Dembitsky, 1992) and to achieve chains of such lengths, the condensation process must be repeated; the synthetase acts on successively longer intermediates and increases the chain by two carbons each time. Fatty acid synthesis is generally promoted by high levels of acetyl CoA and low levels of TCA enzymes.

Lichen fatty acids may be polyunsaturated (Dembitsky et al., 1992b), in which case they are more susceptible to damage by peroxidation. Superoxide dismutase could protect fatty acids from oxygen toxicity in lichens as it does in yeasts (Bilinski et al., 1989). The lichen enzyme is produced in purely fungal structures such as rhizines (Fahselt and Hagemen, 1994) as well as in photobionts (Fahselt, 1985), and is commonly found in natural thalli.

Polymeric reserves

Polymeric stores are osmotically less active than free monomers and thus no energy is required to maintain high concentrations of carbon. In addition, polymers can be easily accessed by degradative enzymes. Many free-living fungi, including *Aspergillus* and *Penicillium* spp., have carbohydrate reserves in the form of amylose (Gorin and Barreto-Bergter, 1983), a linear homopolysaccharide based on (1-4) linked α-D-glucose units. However, amylose has not been confirmed in mycobionts. Another polysaccharide commonly produced by free-living fungi but not specifically identified in lichenized fungi is glycogen (Gorin et al., 1988), a polymer based on α-D-glucose, in which glucose units are connected into branched chains by (1-6) as well as (1-4) linkages.

Lichen polysaccharides have been reviewed by Richardson (1985) and Gorin et al. (1988), and many of those occurring in lichen mycobionts and free-living fungi are characteristic water-soluble glucan homopolysaccharides not formed by other groups of organisms: lichenan, isolichenan and pustulans (Shibata et al., 1968). These polymers have not been identified in mycobiont cell walls, and apparently do not serve a structural purpose (Honegger and Bartnicki-Garcia, 1991), but their role is not understood. Isolichenan, a common polyglucan of α-D-glucose units containing both (1-3) and (1-4) linkages (Aspinall, 1983), has a variable proportion of these two linkage types, ranging in different species from 1:1 to 4:1 (Barreto-Bergter, 1989). Isolichenan usually occurs in low concentrations in lichen thalli and lichenan in higher (Common, 1991). Lichenan and pustulan are both based on β-D-glucose, linked in lichenan by (1-3) and (1-4)

connections and in pustulan by (1-6)'s. Pustulan is found in several species of umbilicate lichens with glucose units sometimes acetylated (Barreto-Bergter, 1989).

Galactomannans are quite variable; an unusual type that is extremely low in galactose residues has been reported by Baron et al. (1991) in the lichen, *Neuropogon aurantiaco.* Galactomannans are reported also in *Cladonia* spp. (Nishikawa et al., 1974), *Stereocaulon ramulosum* (Baron et al., 1989) and *Umbilicaria* spp. (Kjolberg and Kvernheim, 1989), in each case exhibiting a variety of branching patterns and linkages. The complexity of lichen glycans and glycopeptides as summarized by Gorin et al. (1988), is uncharacteristically high for storage molecules and indicates that they may not function as reserves. However, after incubation in the dark with tritiated water, thalli produce mannitol or pentitols labelled with ^{3}H, suggesting that at least some polysaccharides can be hydrolysed to generate polyols (Cowan et al., 1979b).

Polyphosphates in fungi could represent a source of inorganic phosphorus for synthesizing nucleotides and nucleic acids. However, they are conspicuous in walls and nucleii, as well as vacuoles, and may therefore play other roles, such as providing phosphorus anions to establish an appropriate charge balance within the cell for cation uptake (Jennings, 1989).

Arginine

Some lichens have large arginine pools (Planelles and Legaz, 1987), which can be degraded by the sequential action of arginase and urease to provide both N and C for synthetic activities. It has been suggested that arginine-derived urea could be mobilized under conditions of deprivation (Blanco et al., 1984).

Phycobiont storage

Lipids

To perpetuate chain extension in the synthesis of fatty acids in green algae, long-chain acyl-CoA derivatives that are products of fatty acid synthetase inhibit the enzyme and, thus, must be removed from the cycle by fatty acyl-CoA acceptors (Schweizer, 1984). Because electron micrographs show lipid bodies or pyrenoglobuli in the chloroplast pyrenoid of phycobionts, perhaps such acceptors are located in the pyrenoid and explain the concentration of lipids there. Although photomicrographs indicate that some photobionts have stored lipids, the nature of molecules that might act as metabolic reserves is not known.

Proteins

Polypeptides constitute another group of possible energy reserves, as well as a source of nitrogen. No specific proteins have been demonstrated to act as reserves in phycobionts, but high levels of RuBisCO occur in algal pyrenoids. In higher plants, RuBisCO is considered to be a storage material as well as an enzyme (Millard, 1988; Gregory, 1989).

Polysaccharides

Green algal food reserves are commonly in the form of starch, a homopolysaccharide that includes both amylose and amylopectin. Monomers of D-glucose are connected by (1-4) linkages, or (1-4)'s and (1-6)'s, in the chloroplast, and starch grains have been identified in the photobionts of many lichen species. However, in a lichen thallus, photobionts of *Trebouxia* (Fig. 7) may accumulate relatively little starch, compared to *Coccomyxa* (Honegger, personal communication).

Nevertheless, eight distinctive pyrenoid types have been identified in *Trebouxia*, each of which is stable in culture and associated with a particular group of species (Freidl, 1989). Pyrenoid morphology is the same whether a given alga is lichenized or grown in pure culture. In most cases, starch is relatively inconspicuous and deposited between pyrenoid thylakoids, but in some species, prominent starch grains surround the entire pyrenoid. All 26 species of *Trebouxia* studied to date have pyrenoglobuli, and in each species group these have a characteristic distributional pattern within the chloroplast.

Ultrastructural evidence of utilization

Utilization patterns obviously depend on the nature of available metabolic reserves in a particular lichen. Ultrastructural comparisons indicate that *Parmelia sulcata* has abundant starch grains and protein bodies in the chloroplast, but *P. laevigata* has little starch and no chloroplast protein bodies (Brown et al., 1988). A contrast can also be seen between species of *Umbilicaria*; little evidence of starch and lipid reserves can be seen in *U. americana*, while much more is found in *U. deusta* (Scott and Larson, 1986a).

Electronmicroscopy permits changes to be assessed in the chloroplast and other cytoplasmic structures and provides one indication that sequestered materials can actually be drawn upon when conditions are adverse. Fewer starch grains are present in the photobiont of *Umbilicaria deusta* in spring than in autumn, so this species presumably utilizes starch during the winter when buried under snow (Scott and Larson, 1986a). Starch and lipid also vary seasonally in

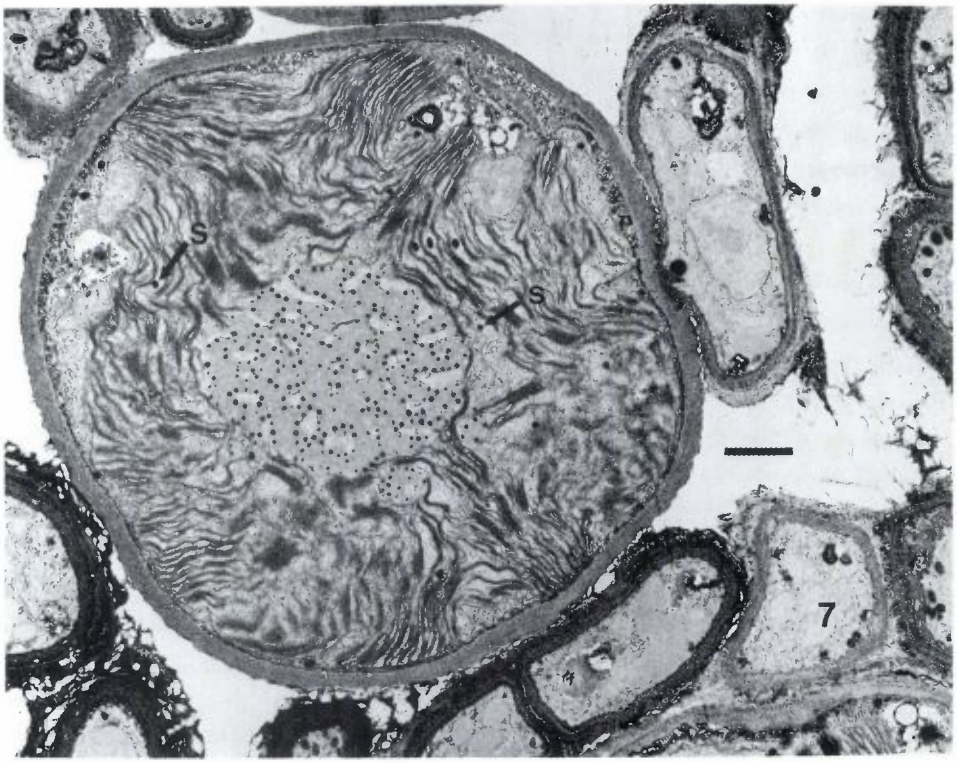

Figure 7. Transmission electronmicrograph of *Parmelia tiliacea* with cell of *Trebouxia* sp. photobiont showing a paucity of intergranal starch grains (S),bar = 1 μm (by courtesy of R. Honegger).

Hypogymnia physodes, being most abundant in the spring and early summer. The numbers of both starch grains and lipid bodies were markedly reduced in samples collected near the city of Zurich, compared to those taken from unpolluted rural areas, an observation that could mean either less assimilation of CO_2 and/or a greater demand on storage reserves close to an urban area (Fiechter and Honegger, 1988).

The pattern of mobilization following periods of deprivation may also depend upon species. In the dark, starch granules disappeared from *P. sulcata* and lipid-rich pyrenoglobuli in photobionts decreased (Brown et al., 1988), indicating that starch and lipids may be utilized as reserves when carbon is not being fixed through photosynthesis. Minimal starch was lost from *P. laevigata* in the dark and no change was observed in pyrenoglobuli, suggesting that this species places few demands on reserves (Brown et al., 1988). Under

dessication, the area of the pyrenoid occupied by pyrenoglobuli of *P. sulcata* remained unchanged (Brown et al., 1987), while dried thalli of *P. laevigata* showed a decreased number of pyrenoglobuli and perhaps also utilization of pyrenoid matrix proteins (Ascaso et al., 1988). Apparently lipids are mobilized differently in dry thalli of the two species.

After months of deep snow cover the conspicuous cytoplasmic lipid bodies in the photobiont of *U. deusta* undergo no visible change and thus may not be mobilizable for respiration. Instead, these may provide protection against freezing or possibly the periodic superoptimal moisture that this lichen encounters on horizontal rock surfaces (Scott and Larson, 1986a).

Cyanobiont storage

Dispersed among photosynthetic membranes of cyanobacteria (Allen, 1984) are inclusion bodies such as glycogen granules that contain polyglucosides as carbohydrate reserves, and several cyanobionts (Fig. 8a) contain glycogen bod-

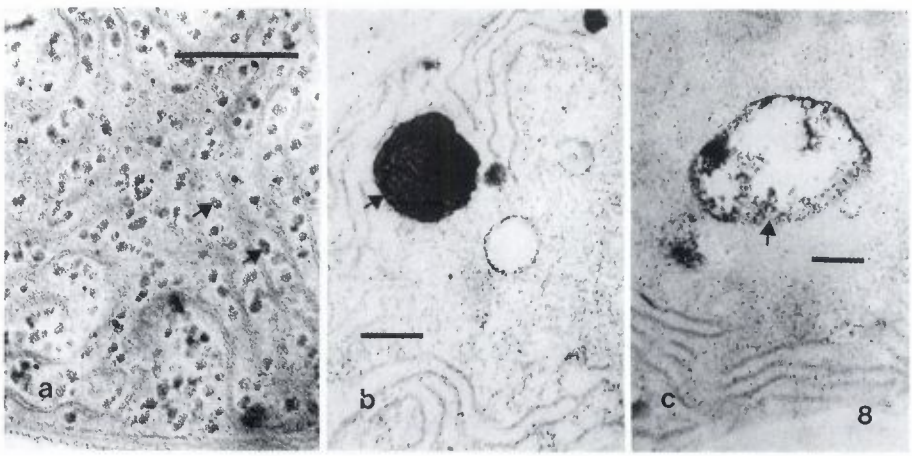

Figure 8. Transmission electronmicrographs of *Nostoc* symbiont, bar in each case = 400 nm, (a) glycogen or polyglucoside granules between thylakoids, (b) cyanophycin granule, (c) polyphosphate body (by courtesy of M.-C. Boissière).

ies. Stores in the *Nostoc* symbiont of *Peltigera canina*, are digestible with amylase, confirming their polysaccharide nature (Boissière, 1982). Glucose efflux from cultured *Nostoc* of *P. horizontalis* is accompanied by a decrease in cellular glucans, also suggesting utilization of glucans (Meindl and Loos, 1990).

Other osmiophilic inclusions called plastoglobules are found in lichenized *Nostoc*, where they are more plentiful than in free-living species of the genus (Boissière, 1982). These are considered to probably contain reserve products, but their precise nature is not known. Plastoglobules have also been found in cultured *Nostoc* cyanobionts (Boissière, 1982).

Cyanophycin, a branched polypeptide of polyaspartic acid with lateral arginine residues, has been viewed as a nitrogen reserve in cyanobacteria. It seems to be unique to cyanobacteria, though not present in all species. Cyanophycin granules have been reported in both cultured and lichenized cyanobionts (Fig. 8b) (Boissière, 1982) and could constitute storage material in a lichen. Another possible reserve of N in cyanobacteria is phycoerythrin. Accumulations of phycoerythrin that are not coupled to the photosynthetic apparatus may build up when N availability is high and provide a reservoir to be drawn upon when N supplies dwindle (Wyman et al., 1985).

Polyphosphate bodies (Fig. 8c) include a high molecular weight polymer of orthophosphate, which may serve as a store of inorganic phosphorus for syntheses. These bodies were found, for example, in two species of *Collema* (Chilvers et al., 1978), as well as in the photobionts of *Peltigera canina* and *Collema tenax* (Boissière, 1982).

Carbon reserves and species ecology

A direct relationship between photosynthesis and growth has been seen in *Parmelia caperata*; lobes with more total chlorophyll and higher net carbon assimilation grew faster than parts of the thallus with less chlorophyll and lower rates of CO_2 assimilation (Tretiach and Carpanelli, 1992). Although the rate of carbon-fixation is obviously critical, as shown by these authors, it is presumably the overall balance among photosynthesis, respiration, sequestering and loss from the cytoplasm or thallus that affects the availability of carbon for growth.

Lichen species differ in regard to utilization of metabolic resources. *Umbilicaria americana*, on vertical rock faces, has a relatively low rate of CO_2 exchange, but it can photosynthesize successfully under a wide range of thallus moisture levels and temperatures (Scott and Larson, 1985). In the winter, carbon fixation is possible at approximately 0°C, but based on ultrastructure evidence, this species apparently sets aside few energy reserves. Its large thalli suggest that *U. americana* channels most of its energies into growth. In contrast, *U. deusta* has a higher photosynthetic rate and, before the beginning of winter, sequesters large amounts of carbon that are gradually depleted during

burial by snow on the horizontal rock surfaces where it grows (Scott and Larson, 1986b). In the small thalli of *U. deusta*, energy is seemingly partitioned more in the direction of storage products and less toward growth.

7. Growth

Proteins required during growth originate from basic respiratory pathways; at least 12 amino acids are derived from 2-oxoglutarate (in the TCA pathway), and others are synthesized from intermediates such as oxaloacetate, pyruvate and phosphoenolypyruvate (EM pathway). Glutamate dehydrogenase, an important enzyme that directs carbon towards amino acid synthesis, has been found in various lichens (e.g., Hageman and Fahselt, 1990; Skult et al., 1990). It was purified by Bernard and Goas (1979) who found that the enzyme in *Lobaria laetevirens* was probably more active in glutamate biosynthesis than in the reverse reaction and that most thallus activity was in the mycobiont. Bases are also derived from reactants of the TCA, purines indirectly from 2-oxoglutarate and pyrimidines from oxaloacetate. In addition to being needed for metabolic intermediates, carbon is required for structural elements of cells.

Cell walls

Cell walls of free-living fungi are biochemically relatively uniform within major taxonomic groups, and mycobiont walls in ascolichens seem to resemble those of other ascomycetes (Boissière, 1987). In regard to polysaccharides, however, there is a wide range of polysaccharide branching patterns and proportions of monomer linkage types in different species. The role of insoluble polysaccharides in lichens has been under-investigated (Richardson, 1985), but in addition to playing a structural role, the mycobiont cell wall/matrix system is implicated in water management of the thallus (Honegger, 1993).

Chitin and glucans are important structural elements of many fungal walls (Richmond, 1991). Two of the enzymes required for synthesis of chitin, a stable polymer of (1-4) linked β-N-acetylglucosamine units, have been isolated from *Evernia prunastri*: glucosamine 6-phosphate isomerase (EC 5.3.1.19) (Rapsch and Cifuentes, 1983) and N-acetylglucosamine 6-phosphate synthetase (EC 2.3.1.4) (Cifuentes and Rapsch, 1983). The first of these is inducible by exogenously supplied glucose and ammonia, and synthesized in much larger quantities at the end of summer than in winter-time. The tritiated form of N-acetylglucosamine, which is a chitin precursor, can be traced by means of a label to its site of incorporation in hyphal walls (Galun et al., 1976).

The extent to which chitin occurs in mycobiont walls seems to be related to the type of photobiont in the thallus, with chitin microfibrils being present in all cyanolichens (Schlarmann et al., 1990). In a few phycolichens, chitin is distributed throughout the thallus, but staining reactions suggest that it occurs in relatively low amounts. The differences in walls between phycolichens and cyanolichens may be related to the relative abundance of available C and N; the C/N ratio is reflected in fungal cell wall composition, with the wall acting as a C sink if the ratio is high and as a N sink when it is lower (Jennings, 1989). In lichens with green algal partners, chitin may be localized in the lateral walls of hyphae in the algal layer and the inner wall layer of cortical hyphae but, even if levels are low in other parts of the thallus, chitin can be found in hyphal walls of growing tips (Schlarmann et al., 1990).

The presence of chitin has been confirmed in purified mycobiont walls of *Cladonia macrophylla, C. caespiticia* and *Physcia stellaria* through the use of X-ray diffractometry, but in these lichens the major polysaccharide is a branched β-(1-3) (1-6) glucan that is found in other ascomycetes (Honegger and Bartnicki-Garcia, 1991). In *Lasallia pustulata* and *Peltigera canina*, a structural skeleton composed of chitin is closely associated with β-(1-3) and (1-6) glucans, as matrix materials (Boissière, 1987).

Mucilages, which are complex heteropolysaccharides, proteins and lipids, cover the outer surface of the mycobiont wall (Honegger, 1988) and render it hydrophobic. Crystals of extracellular secondary metabolites, seen by scanning electronmicroscopy, are deposited on top and contribute to this effect (Honegger, 1986). Cryo-electronmicroscopy (Honegger and Peter, 1994) and experiments using hand-sectioned thalli and water soluble dyes (Armaleo, 1993) support the idea that interhyphal space in a thallus does not fill with liquid water. The outer wall layer may function like a cuticle, transforming apoplastic space, in the wall, into a conduit within which water and solutes can be transported, and maintaining hyphal cell wall surfaces unimpeded by free water (Honegger, 1990). In lichens with cyanobionts, the outer wall of mycobiont hyphae is seen through freeze fracture techniques to consist of tightly packed "rodlets", while in phycolichens, each bundle of rodlets is obscured by a cover of amorphous material, probably of lipid and phenols (Honegger, 1991). The lichenan-like polysaccharides and mucilaginous materials on the outer wall of hyphae may vary in different parts of the same thallus (Boissière, 1987; Schlarmann, 1987).

In non-lichenized algae, cell walls are diverse and microfibrillar composition may differ from one cell to another (Richmond, 1991); several types have been described in chlorophyceans. Walls of lichen photobionts are also diverse, and

such variability may be relevant to the process of recognition, or partner selection, which precedes lichenization (König and Peveling, 1984). Green algal cell walls may include "cellulosic polysaccharides" that, when hydrolysed, yield mostly glucose, or glucose with small amounts of mannose. Thus, mannans and/or a variety of other heteropolysaccharides must be present along with cellulose. Another cell wall fraction yields mixtures of monoses such as rhamnose, xylose, galactose and glucose, some of which are species-specific (König and Peveling, 1984). The β-1,4-glucanase of lichens is mainly an algal enzyme, and perhaps enables the alga to self-digest or degrade cellulose, a β-(1-4)-D-glucan, in its own wall. This could facilitate cell expansion (Yague and Estevez, 1989) and contribute to the increase in photobiont cell size that occurs with age (Hill, 1989).

One type of phycobiont wall, as described by Honegger (1990) is that of *Coccomyxa* and *Elliptochloris*. It has an external layer, called the trilaminar sheath, which is essentially an extended sandwich of protein filled with a poorly understood combination of polymers called sporopollenin. In lichens, this non-reactive polymer is probably synthesized largely from precursors such as fatty acids (Guilford et al., 1988), rather than from carotenoids like the sporopollenin in *Lycopodium*. Only relatively small molecules can diffuse through the trilaminar sheath and haustoria seldom penetrate walls that include this layer, presumably because of sporopollenin (Honegger, 1986). In phycolichens so far investigated, algal cells are enveloped by a hydrophobic layer, including lipids, proteins, carbohydrates and phenolics, that originates from the fungal partner (Honegger, 1990). Photobiont surfaces would thus be devoid of free water and open to CO_2 that might be concentrated for photosynthesis (Palmqvist, 1993). Solutes within the hyphal apoplast may include HCO_3^- that could also be assimilated.

The cyanobacterial cell boundary seems to have a thin inner layer which is thought to be composed of peptidoglucan, or murein, in addition to an outer layer and a prominent sheath (Büdel and Rhiel, 1985, 1987). Cyanobacterial walls probably do not contain cellulose and, in lichenized *Nostoc*, for example, the outer envelope is lipopolysaccharide (Painter, 1983) or mucopolysaccharide (Boissière, 1982).

Unusual structural features, giving the appearance of limp ribbons overlapping one another, have been reported by Büdel and Rhiel (1985) on the cell surface of the cyanobiont *Chroococcidiopsis* from the lichen *Psorotichia columnaris*. However, the chemistry and significance of these structures is not yet known.

Cyclic nucleotides and phytochrome

Cyclic nucleotides are implicated in a multitude of metabolic and growth responses in free-living fungi and have also been found in lichens. Cyclic AMP (cAMP), with a phosphate group linked to ribose in a 6-membered ring, is synthesized from ATP by the enzyme adenylate cyclase and functions in cellular signal transduction. Fungi seem to possess most of the main components of the transduction model proposed for higher eukaryotes (Pitt and Kaile, 1990), with cAMP or cGMP being formed in response to "first messengers" or external stimuli received by a cell and, possibly through phosphorylation of proteins, initiating physiological responses (Hardie, 1991).

Cyclic AMP occurs in *Evernia prunastri* (Avalos and Vicente, 1987) and *Himantormia lugubris* (Mateos et al., 1991) and cGMP in *Usnea aurantiaco-atra* (Pedrosa et al., 1992). In lichens, cyclic nucleotides appear to facilitate uptake of exogenously supplied metabolites (Avalos and Vicente, 1989) as well as induce several enzymes, including to date D-usnic acid dehydrogenase (Avalos and Vicente, 1987), D-usnic acid oxidoreductase (Avalos and Vicente, 1989; Vicente an Avalos, 1990) and nitrate reductase (Avalos and Vicente, 1985). Cyclic AMP is critical in the free-living fungus *Neurospora* for establishing a normal pattern of hyphal dominance within a mycelial mat (Pall and Robertson, 1986). If hyphal organization in lichens is determined in a similar way, cAMP may also influence hyphal growth and metabolism in a lichen thallus.

Phytochrome in plants behaves as a first messenger by attaching to the cell membrane and initiating intercellular communication (Hardie, 1991). It is comprised of two identical polypeptides, each with a tetrapyrrole chromophore, but two forms are interconvertable by red (667 nm) and far-red (720 nm) light. Cyclic AMP in *Evernia prunastri* is produced only under high red light, a phenomenon consistent with phytochrome involvement in its synthesis (Vicente and Avalos, 1990). Because the proportion of red to far-red in natural radiation differs with the time of day and the season of the year, cAMP may be involved in governing diurnal and seasonal differences in lichen metabolism.

Biochemistry of low growth rates

Several factors may contribute to the extremely slow growth that characterizes many lichen species. Rates of photosynthetic C-fixation in lichens were not considered by Fox and Mosbach (1967) to be particularly limiting, and Farrar (1988) similarly suggested that the rate of CO_2 incorporation is not unlike that of any autotrophic eukaryote. However, Werner (1992) indicated that, in comparison to free-living unicellular green algae and cyanobacteria, photosynthetic activity in lichens on a dry weight basis is low, even taking

into account that only a small part of the thallus biomass is attributable to the photobiont.

Also relevant to growth of lichens is respiration rate; at least the respiration involved in growth, as opposed to that related to maintenance, must be lower than in other organisms. However, respiration activated by dew-moistening during the night could draw significantly on carbohydrate reserves, as could short-term increases in respiration produced by immersion of dry thalli in water at any time of day (Smith and Douglas, 1987). Nevertheless, rehydration respiration cannot always explain slow growth because thallus expansion rates are limited even in environments where dry thalli are seldom wetted.

Other reasons for slow growth could be the relatively short periods of time in a day, or in a growing season, when thallus moisture content is optimal for photosynthesis (Ahmadjian, 1987). Nutrients such as N may also be limiting, particularly in phycolichens (Fox and Mosbach, 1967).

To test whether enzymes of primary metabolism in lichens differed kinetically from those in other organisms, three of *Cetraria islandica* were extracted and characterized (Yamamoto et al., 1994). The specific activity of glucose-6-phosphate dehydrogenase was low compared to that in plants and other microorganisms, but all remaining parameters that were studied were similar. Malate dehydrogenase and glutamate dehydrogenase of lichens resembled those of other organisms with respect to all criteria tested, such as K_m, optimum temperature and thermal stability. Thus, it was concluded that the slow growth of lichens could probably not be attributed to limitations imposed by the enzymes examined. Synthesis of enzymes necessary for growth might be prevented, however, if extraneous nucleotide sequences (introns) were inserted into critical pieces of DNA (DePriest, 1993). For example, if introns are not excised from regions that code for ribosomal RNA subunits, ribosomes would be non-functional and growth impaired.

Loss of energy-rich materials from the cytosol may also curb growth. Although actual quantities have not been estimated, considerable resources must be used to produce thick mycobiont walls and intercellular matrices (Fig. 9) and might represent a significant drain on resources (Ahmadjian and Jacobs, 1983). Also, appreciable amounts of various compounds may be released due to dessication-induced changes in membranes and lost from the thallus by leaching (Cooper and Carroll, 1978; Dudley and Lechowicz, 1987; Smith and Douglas, 1987; Coxson et al., 1992).

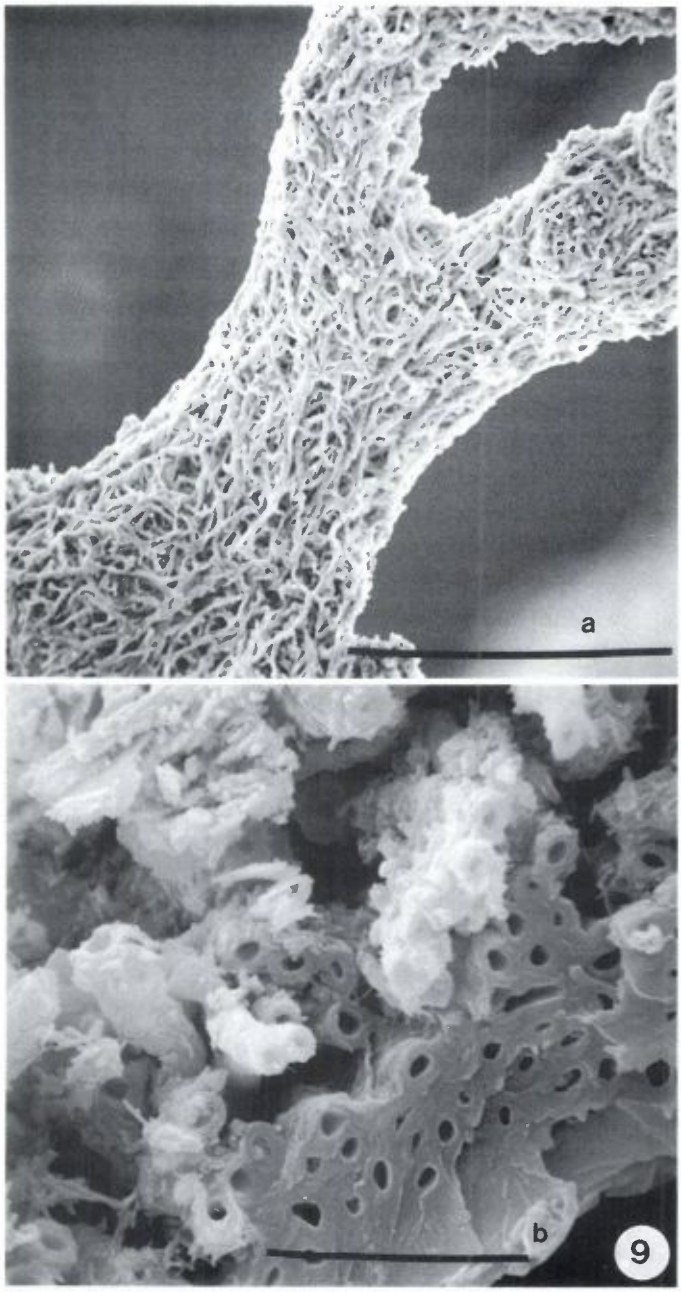

Figure 9. *Cladina rangiferina*. (a) ecorticate podetial surface. Bar = 100 μm. (b) transverse cut through podetium. Cell lumen is small and wall is thick, with matrix filling intercellular space. Bar = 10 μm.

Growth substances

The course of development and pattern of carbon utilization may be influenced by growth substances. The occurrence of hormones in lichens is not totally unexpected since they are well known in both free-living algae and fungi. Therefore, either symbiotic partner could generate substances that regulate growth of the other. Mere presence of phytohormones, however, does not constitute a demonstration of their role in the thallus (Jameson, 1993). Also, growth hormones usually exhibit interactive effects, so that trials involving individual substances in isolation from others may not be very instructive.

Mycobionts in culture are capable of synthesizing indole acetic acid (IAA) from tryptophan (Fortin and Thibault, 1972), and IAA has also been identified in *Ramalina duriaei* (Epstein et al., 1986). Treatment with auxins and cytokinins produces in resynthesized *Cladonia cristatella* a structural similarity to natural thalli that is otherwise lacking, a phenomenon that suggests growth substances are normally present in this lichen (Remmer et al., 1986).

Higher plants increase levels of growth substances, e.g., auxins and cytokinins, after infection by fungal or bacterial pathogens (Spanu and Boller, 1989), so a photobiont might respond in the same way. Hormonal involvement has previously been postulated in connection with the onset of lichenization, as well as with maintenance of a balance between the two symbiotic partners (Remmer et al., 1986).

Ethylene is found in all plants tested (Jameson, 1993), and in bacteria, free-living fungi and lichens (Lieberman, 1979; Epstein et al., 1986; Ott and Zwoch, 1992). Its role in lichens has not been determined specifically, but in other organisms it affects transcription. In plants, ethylene induces enzymes such as β-1,3-glucanase and chitinase that degrade fungal walls (Boller, 1988) and cause abnormal hyphal growth (Selintrennikoff et al., 1992), and ethylene has been viewed as a signal causing plants to activate protective measures against pathogenic fungi (Salisbury and Ross, 1992). Since it is volatile, ethylene can coordinate processes within the cells where it is produced or tissues nearby (Salisbury and Ross, 1992), but whether it diffuses from the photobiont and affects the mycobiont in a lichen is unknown. Lichens are similar to higher plants in that increased amounts of ethylene are synthesized under conditions that could be termed "stressful" (Lieberman, 1979; Epstein et al., 1986; Lurie and Garty, 1991).

In higher plants, ethylene is derived from the amino acid, methionine, via a pathway that requires molecular oxygen: 1-amino-cyclopropane-1-carboxylic acid (ACC) is the immediate precursor and ACC is made from S-adenosylmethionine (SAM). This earlier intermediate is partly salvaged and

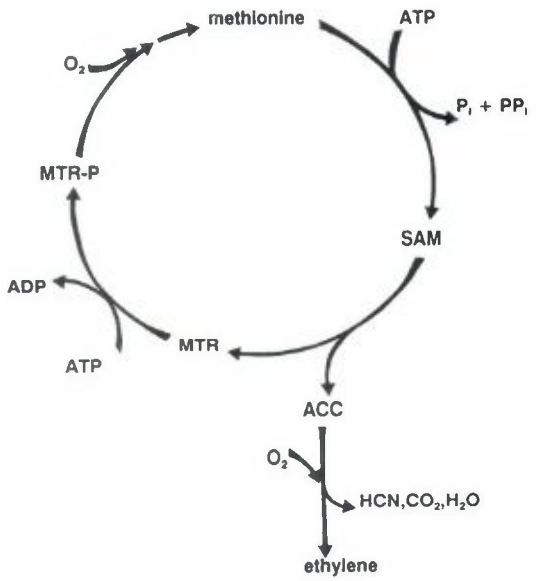

Figure 10. Production of ethylene by the ACC pathway in higher plants (modified from Salisbury and Ross, 1992, p. 394). SAM = S-adenosyl methionine, ACC = 1-amino-cyclopropane-1-carboxylic acid, MTR = methyl thioribose, MTR-P = methylthioribose-1-phosphate.

reconstituted as methionine (Fig. 10). Lurie and Garty (1991) reported that ethylene is formed independently of ACC in *Ramalina duriaei*, but Ott and Zwoch (1992), using more replicates, have indicated that ACC is involved in the production of ethylene in each of eight species: seven phycolichens and one cyanolichen. Bacteria do not use methionine as a precursor or utilize the ACC pathway to make ethylene (Jameson, 1993), and cyanobionts probably would not either. Thus, ethylene production, at least in one cyanolichen, may be attributed largely to the mycobiont. In any case, neither ethylene nor any other growth substance in lichens has so far been shown to place limits on the rate of thallus expansion.

Alternate metabolism

There is no evidence suggesting that the less-efficient glyoxylate pathway is utilized in lichens as an alternative to the TCA pathway, so it cannot be speculated that this accounts for low growth rates. However, a cyanide-resistant, less productive, respiratory electron to oxygen transport route (Weger and Dasgupta, 1993) does operate (Farrar, 1988), and may restrict growth. Also,

lichens certainly direct carbon out of primary metabolic sequences toward alternative secondary pathways, with the mycobiont generating characteristic secondary metabolites such as depsides, depsidones and dibenzofurans.

The level of carbon may be a factor affecting the onset of secondary metabolism in fungi. In *Gibberella fujikuroi*, low levels of glucose in a growth medium restrict the synthesis of overflow lipids and phenols (Borrow et al., 1961). Increased utilization of urea, or more CO_2 produced by its enzymatic hydrolysis, increases the ratio of polyols to glucose in lichen thalli (Vicente et al., 1984). Urea labelled with ^{14}C is more frequently incorporated into secondary phenolics in the light than in the dark, suggesting that the availability of assimilated carbon could promote polyketide synthesis (Blanco et al., 1984). Increased light, that could increase photosynthetic C-fixation, enhances production of certain depsides (Fahselt, 1981; Culberson et al., 1983; Blanco et al., 1984).

Citrate accumulations activate the enzyme acetyl-CoA carboxylase and cause increased levels of malonyl-CoA (Rasmussen and Kleink, 1974), a necessary intermediate for production of polyketides (Haslam, 1985). High levels of citrate, thus, promote synthesis of fatty acids, phenolics or both (Ward and Packter, 1974). Starting from malonyl-CoA, less reducing power is required to synthesize polyphenolics than fatty acids, so the supply of NADPH may regulate partitioning between these two alternatives (Mosbach, 1973) and determine the extent to which characteristic lichen products are formed.

It has been shown that intermediates of primary pathways in some cases depress accumulation of secondary products (Herrero-Yudego et al., 1989). A high glycolytic flux produced by glucose, or possibly another carbon source, may in some cases be inhibitory (the glucose or Crabtree effect, or catabolite repression). Catabolite repression in bacteria is mediated by cAMP and a protein called catabolite gene activator protein (CAP). Before transcription can take place, CAP, in the presence of cAMP, must bind DNA near the promoter region, but when glucose is present cAMP concentration is decreased and CAP is prevented from binding near the promoter (Lehninger et al., 1993). If high levels of glucose interact with DNA of lichens in the same way, synthesis of secondary enzymes could be prevented and lower quantities of certain phenolics would result (Vicente, 1991).

Secondary substances frequently occur as extracellular deposits, and thus, like polyols removed by leaching (Dudley and Lechowicz, 1987) constitute energy-rich metabolites removed from the cytosol. Although secondary products are probably highly adaptive (Fahselt, 1994b), they do not directly contribute to cell enlargement and proliferation or to thallus expansion. Such

carbon deployment thus may be a factor that limits growth possibilities for lichen thalli.

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