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The Symbiontic Nature of Metabolic Evolution

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ABSTRACT

We discuss evolutionary aspects of metabolism, right from the beginning of life to the present day at various levels of organization, thereby including quantitative aspects on the basis of the Dynamic Energy Budget (DEB) theory. We propose a scheme for the evolution of the central metabolism with archaeal as well as eubacterial roots. After an extended initial phase of prokaryotic diversification, cycles of exchange of metabolites between partners in a symbiosis, integration of partners into new individuals and new specializations led to forms of symbiosis of various intensity ranging from loosely living together in species aggregates to several forms of endosymbiosis. While the prokaryotic metabolism evolved into a considerable chemical diversity, the eukaryotic metabolic design remained qualitatively the same but shows a large organizational diversity. Homeostasis of biomass evolved, introducing stoichiometric constraints on production and excretion of products that can be re-utilized; carbohydrates and inorganic nitrogen being the most important ones. This stimulates the formation of symbioses, since most are based on syntrophy, which is probably the basis of the huge biodiversity. A remarkable property of DEB theory for metabolic organization is that organisms of two species that exchange products, and thereby follow the DEB rules, can together follow a symbiogenic route such that the symbiosis behaves as a new organism that itself follows the DEB rules. This property of the reserve dynamics in the DEB theory also explains a possible evolutionary route to homeostasis. The reserve dynamics in DEB theory also plays a key role in linking the kinetics of metabolic pathways to needs of metabolites at the cellular level. Moreover, reserve kinetics, in combination with other DEB elements, explains how metabolic performance depends on body size and why such relationships work out differently within and between species. Apart from the key role of reserves, the dynamic interaction between surface areas and volumes is a basic feature of the DEB theory at all levels of organization (molecules, individuals, ecosystems). The explicit mass and energy balances of the DEB theory facilitates ecosystem modelling as it depends on nutrient exchange. The theoretical interest in this topic concerns the huge range in space-time scales that is involved in understanding the significance of the actions of life within the context of metabolic organization.

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7.1 INTRODUCTION

Underlying the metabolic organization in individuals is a long evolutionary history of acquisition and loss of new metabolic pathways, as well as a recombination of existing pathways. The boundaries of individuals are frequently crossed in symbioses that span the full range from loosely coupled populations, to a fully integrated individual that is hard to recognize as a consortium of individuals of different species. The metabolic requirements of life can be energetic ones, or they can concern particular nutrients, or both. A proper understanding of metabolic organization cannot be achieved without exploring its historic roots.

The metabolism of individuals has adapted over time to overcome the consequences of changing living conditions. The question here is how this might have happened in interaction with the environment. One possibility is through changing the system itself by mutation and selection. This is a very slow process, but essential for building up a basic diversity in metabolic performance between different species. This explains the slow start of evolution. Much faster is the exchange of plasmids that evolved among prokaryotes (Doolittle, 1999), which is further accelerated by the process of symbiogenesis, typical for eukaryotes. The latter also duplicate DNA and reshuffle parts of their genome, giving adaptive change even more acceleration. Mutation still continues, of course, but the reshuffling of metabolic modules occurs at rates several orders of magnitude higher. The response to changes in the environment is further accelerated by the development of food webs, and therefore of predation, which enhances selection. Owing to their advanced locomotory and sensory systems, animals play an important role in food webs, and so in the acceleration of evolutionary change.

Basic to these processes is the question of how pliable complex metabolic systems are, i.e. how much they can be dropped, added, or altered without harm? How much of the initial structures are kept right from the beginning or from stages developed soon afterwards? Or, should symbiogenesis be understood in terms, not of changeability of the systems but in principle from those of their rigidity?

Aim

The aim of the present paper is to integrate existing ideas on quantitative aspects of symbiotic interactions based on syntrophic relationships (Kooijman *et al*., 2004) with ideas on the chemical evolution of metabolism (Hengeveld and Fedonkin, 2004). The topics that we discuss are widely scattered in the specialized literature. By bringing them together into one framework, we hope to stimulate an important field of research that crosses the traditional boundaries between various specializations. We believe that barriers to

communication between molecular biology, physiology, microbiology, population biology, ecosystem ecology, and earth systems science hamper the development of a quantitative theory for metabolism at the various levels of organization.

Our view is that interactions among species can frequently be understood from their metabolic requirements, and that quantitative aspects of the metabolism of individuals can be understood from interactions between larger biochemical modules, in ways that are not too different from those between individuals. This also holds for systems of metabolically interacting species. Yet, basic differences exist between the various levels of organization. At the level of the individual, metabolic performance is studied as a dynamic system, *given* the concentrations of substrates, nutrients and/or food. At the ecosystem level such concentrations are not given but are part of the dynamic system that evolves interactively, which naturally leads to the study of nutrient cycles at this level.

When life first emerged, its quantitative impact on the environment cannot have been substantial. It need not have taken long, though, before considerable amounts of biomass built up to such levels that could have affected geochemical cycling. Precambrian cyanobacteria (stromatolites) in coastal areas testify to an increasing impact of life on its environmental conditions. Geochemical cycling cannot be studied without considering climate (temperature and water), substantially affecting (metabolic) rates, which makes it such that metabolism at larger spatial and temporal scales cannot be studied without involving climate and biogeochemical recycling in a holistic way (Kooijman, 2004).

We first present a brief introduction to the central metabolism of eukaryotes and then discuss its evolutionary history, starting with the first cells, the invention of phototrophy, diversification and interaction. So far, our discussion concerns prokaryotic evolution at the sub-organismic level that resulted in a substantial *chemical* diversity. After this, we consider the emergence of eukaryotes and their *organizational* diversity in the form of multicellularity, and the various direct and indirect syntrophic interactions at the supra-organismic level. Finally, we discuss quantitative aspects of metabolic organization.

Context of DEB theory

Every now and then, we will refer to the Dynamic Energy Budget (DEB) theory for quantitative aspects of the metabolic organization at the level of the individual (Kooijman, 2000, 2001; Nisbet *et al*., 2000). These references not only serve to point to opportunities of understanding particular aspects of evolution quantitatively, but also to demonstrate that the DEB theory has significance for understanding evolutionary processes. A property that sets this

theory apart from its (presently available) alternatives is the decomposition of biomass into reserves and structure and the special type of kinetics of the reserve, which quantify the metabolic memory of the system.

Perhaps contrary to what the term suggests, reserves are *not* characterized by "compounds set apart for later use"; their constituent compounds can have quite active metabolic functions. Each particular compound can belong to both reserve and structure. Most ribosomal RNA, for example, belong to the reserve, which implies that the rRNA content of the body increases with the growth rate (Elser, 2004), since abundant reserve comes with a large use of reserve (Vrede *et al.*, 2004). The dynamics of rRNA as part of the reserve is parsimonious given the role of rRNA in the elongation of peptides; if growth is low, there is little need for peptide elongation so less need for rRNA. The co-variation of reserve density and growth rate only holds for single-reserve systems and for the (most) limiting reserve in multiple-reserve systems. Non-limiting reserves typically show the opposite pattern of being more abundant for low growth rates.

7.2 THE CENTRAL METABOLIC PATHWAY

The idea that eukaryotes developed out of prokaryote assemblages, with or without a hypothetical "Urkaryote", is now widely accepted. Their evolutionary history implies that the metabolism of eukaryotic cells arose from several interacting prokaryotic modules. We can only hope to understand eukaryotic organization from that of prokaryotic ancestors, plus an appreciation of the interaction between the modules of the evolving eukaryotic cell. Therefore, let us focus on the organization of the central metabolic pathway first.

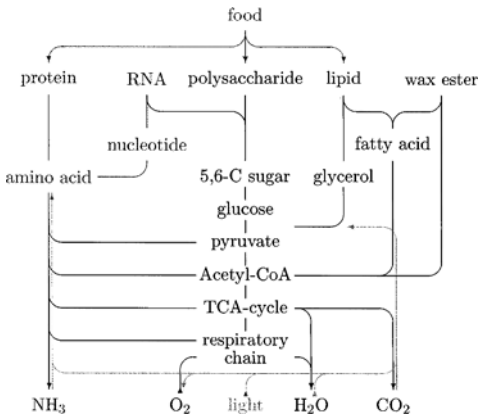

Figure 7.1. The very much simplified design of the central metabolism of eukaryotes and many prokaryotes, in which the Pentose Phosphate cycle, the glycolysis, the TriCarboxylic Acid cycle and the respiratory chain have a central position in the conversion of polymers. Heterotrophs use food (organic compounds) as a source for energy and building blocks; photo-autotrophs use light and nutrients for these purposes.

Four modules of central metabolism

The central metabolic pathway of many prokaryotes and almost all eukaryotes (Figure 7.1) consists of four main modules:

- The *Pentose Phosphate (PP) Cycle* comprises a series of extra-mitochondrial transformations by which glucose-6-phosphate is oxidized with the formation of carbon dioxide, reduced NADP and ribulose 5-phosphate. Some of this latter compound is subsequently transformed to sugar phosphates with 3 to 7 or 8 carbon atoms, whereby glucose-6-phosphate is regenerated. Some ribulose 5-phosphate is also used in the synthesis of nucleotides and amino acids. Higher plants can use the same enzymes also in reverse, thus running the reductive pentose phosphate cycle. The PP cycle is primarily used to interconvert sugars as a source of precursor metabolites and to produce reductive power. Theoretical combinatorial optimization analysis indicated that the number of steps in the PP cycle is evolutionarily minimized (Meléndez-Hevia and Isidoro, 1985; Meléndez-Hevia, 1990), which maximizes the flux capacity (Heinrich and Schuster, 1996; Waddell *et al*., 1997).
- The *Glycolytic Pathway* (aerobically) converts glucose-6-phosphate to pyruvate or (anaerobically) to lactate, ethanol or glycerol, with the formation of 2 ATP. The transformations occur extra-mitochondrially in the free cytoplasm. However, in kinetoplastids they are localized in an organelle, the glycosome, which is probably homologous to the peroxisome of other organisms (Bakker, 1998; Cavalier-Smith, 2002b). The flux through this pathway is under control by phospho fructokinase and by hormones. Heinrich and Schuster (1996) studied some design aspects of the glycolytic pathway. Most pyruvate is converted to acetyl and bound to coenzyme A.
- The *TriCarboxylic Acid (TCA) Cycle,* also known as the citric acid or the Krebs cycle, oxidises (without the use of dioxygen) the acetyl group of acetyl coenzyme A to two carbon dioxide molecules, under the reduction of 4 molecules NAD(P) to NAD(P)H. In eukaryotes that contain them, these transformations occur within their mitochondria. Some plants and micro-organisms have a variant of the TCA cycle, the glyoxylate cycle, which converts pyruvate to glyoxylate and to malate (hence a carbohydrate) with another pyruvate. Since pyruvate can also be obtained from fatty acids, this route is used for converting fatty acids originating from lipids into carbohydrates. Some plants possess the enzymes of the glyoxylate cycle in specialized organelles, the glyoxysomes.
- The *Respiratory Chain* oxidizes the reduced coenzyme NAD(P)H, and succinate with dioxygen, which leads to ATP formation through oxidative phosphorylation. Similarly to the TCA cycle it occurs inside mitochondria. Amitochondriate eukaryotes process pyruvate through pyruvate-ferredoxin oxidoreductase rather than through the pyruvate dehydrogenase complex. If

the species can live anaerobically, the respiratory chain can use fumarate, nitrate, or nitrite as electron acceptors in the absence of dioxygen (Tielens *et al*., 2002).

In combination with nutrients (phosphates, sulphates, ammonia, iron oxides, etc), the first three pathways of the central metabolic pathway provide almost all the essential cellular building blocks, including proteins, lipids, and RNA. The universality of this central metabolic pathway is partly superficial or, if you like, the result of convergent evolution because the enzymes running it can differ substantially. This diversity in enzymes partly results from the modular make-up of the enzymes themselves. Some variation occurs in the intermediary metabolites as well.

The central role of carbohydrates

Obviously, glucose plays a pivotal role in the central metabolism. However, its accumulation as a monomer for providing a metabolism with a permanent source of substrate would give all sorts of problems, such as osmotic ones. This also applies to metabolic products. To solve these problems, cells typically store the supplies in polymeric form (polyglucose (i.e. glycogen), starch, polyhydroxyalkanoate, polyphosphate, sulphur, proteins, RNA), which are osmotically neutral. Their storage involves so-called inclusion bodies, the inherent solid/liquid interface of which controlling their utilization dynamics.

Quantitative aspects of metabolism

The understanding of the quantitative aspects of the central metabolism calls for kinetic modelling that is based on the availability of the interface between essential polymers and the cytosol, rather than of their amounts or concentrations. The concept of concentration hardly applies to polymers as a basis for kinetics. It is also very problematic for low concentrations of monomers given the complex spatial structure of a cell in which membrane-linked transformations dominate. Transporter proteins cause further deviations from the law of mass action on which classic enzyme kinetics is based. This is why DEB theory uses an alternative for classic enzyme kinetics as it is based on fluxes rather than concentrations. This alternative, synthesizing unit kinetics, is used to quantify simultaneous limitations and adaptations in assimilation, maintenance and growth (Kooijman, 1998, 2000; Kooijman *et al*., 2004; Brandt, 2002; Kuijper *et al*., 2003). It can deal with the dynamic interactions between surface areas and volume at all levels of organization. These interactions are a basic feature of the DEB theory

Almost all metabolites have a dual function as building blocks or as an energy source. It seems that reserves are required for modelling the regulation of these functions, where some of the enzyme molecules are part of the

reserve, whereas the growth rate depends on the amount of reserve (Kooijman and Segel, 2003). Pyruvate that is sent to mitochondria in eukaryotes, for instance, is partly used to generate ATP and reducing power, and partly for the synthesis of the intermediary metabolites of the TCA cycle, e.g. succinate and fumarate. The nine different enzymes of the TCA cycle are spatially organized in a super-macromolecule and the interaction between these enzymes controls the fate of intermediary metabolites. The problem is that the ratio of the cell's requirements for building blocks *versus* energy depends on the growth rate. So, the need for products and intermediary metabolites depends on the growth rate. If the growth rate varies, the amounts of enzymes vary in a very special way. As shown by the application of a model for pathway kinetics that is based on synthesizing units, this can have the effect that the varying metabolic needs at the cellular level are exactly matched. Without reserves, so without the possibility of varying enzyme concentrations, it will be difficult, if not impossible, to deal with these varying needs in a theoretically satisfactory way.

7.3 HOW DID METABOLIC SYSTEMS EVOLVE?

Since the central metabolic pathway involves the operation of a large number of enzymes, its evolution must have taken many steps. Dioxygen was rare, if not absent, during the time life emerged on earth which classifies the respiratory chain as an advanced feature. We doubt that glucose could have been that central during the remote evolutionary origins of life, since its synthesis and degradation typically involves dioxygen. Early life forms must probably be sought among the anaerobic chemolithoautotrophic bacteria (Wächtershäuser, 1988). Like all phototrophic eukaryotes, most of these bacteria fix inorganic carbon in the form of carbon dioxide through the Calvin cycle. At present, this cycle is part of the phototropic machinery, a rather advanced feature in metabolic evolution which is not found in any archaea (Schönheit and Schafer, 1995). It has glucose as its main product, which suggests that the central position of glucose and, therefore, of carbohydrates, evolved only after oxygenic phototrophy evolved. Like the Calvin cycle, eukaryotic and eubacterial glycolysis (the Embden-Meyerhof pathway) is not found in archaea either; hyperthermophilic archaea possess the Embden-Meyerhof pathway in modified form (Schönheit and Schafer, 1995; Selig *et al*., 1997), and generally do not use the same enzymes (Martin and Russell, 2003). This places the pyruvate processing TCA cycle at the origin of the central metabolism. However, if we leave out the glycolysis as a pyruvate-generating device, what process was generating pyruvate?

Early cells

Interestingly, the eubacteria *Hydrogenobacter thermophilus* and *Aquifex* use the TCA cycle in reverse, binding and transforming CO_2 into building blocks (lipids, cf. Lengeler *et al*., 1999), including pyruvate. Both species are *Knallgas* bacteria, extracting energy from the oxidation of dihydrogen. The green sulphur bacterium *Chlorobium,* as well as the archaea *Sulfolobus* and *Thermoproteus* (Madigan *et al*., 2000) also run the TCA cycle in reverse for generating building blocks. Hartman (1975), Wächtershäuser (1990) and Morowitz *et al*. (2000) hypothesized the reverse TCA cycle to be one of the first biochemical pathways.

The interest in hydrogen bacteria relates to the most likely energy source for the first cells on earth. *Hydrogenobacter* optimally thrives at 70-75ºC in Japanese hot springs. It is an aerobic bacterium, using ammonia and nitrate, but not nitrite and possesses organelles (mesosomes). Several enzymes of the PP cycle and the glycolytic pathway are present although their activities are low (Staley *et al*., 1989). The togobacterium *Aquifex* is even more interesting since its metabolism might still resemble that of an early cell. Although it is also aerobic, it tolerates only very low dioxygen concentrations, which may have been present when life emerged (Holland, 1994; Kasting, 2001; Anbar and Knoll, 2002). Growing optimally at 85ºC in marine thermal vents, it utilizes H_2, S^0 or $S_2O_3^-$ as electron donors and O_2 or NO_3^- as electron acceptors. With a genome size of only 1.55 Mbp, its genome amounts to only one third of that of *E. coli,* which is really small for a non-parasitic prokaryote. The archaeon *Nanoarchaeum equitans*, which lives symbiotically with the H_2-producing and sulphur-reducing archaeon *Ignicoccus,* has a genome size of 0.5 Mbp (Huber *et al*., 2002), one of the smallest known genomes for a non-parasitic bacterium. The phototrophic cyanobacterium *Prochlorococcus* has 1.7 Mbp (Fuhrman, 2003). These small genome sizes illustrate that autotrophy is metabolically not more complex than heterotrophy (see Discussion section).

The TCA cycle seems to be remarkably efficient, which explains its evolutionary stability. Moreover, it is reversible, which directly relates to its efficiency and the inherent small steps in chemical potential between subsequent metabolites. Yet, with its nine transformations, the TCA cycle is already rather complex and must have been preceded by simpler CO_2-binding pathways (Orgel, 1998, 2000) such as the (linear) acetyl-CoA pathway of homoacetogens: $2\ CO_2 + 4\ H_2 + CoASH \rightarrow CH_3COSCoA + 3\ H_2O$ (Hugenholtz and Ljungdahl, 1990; Ljungdahl, 1994). Apart from H_2, electron donors for acetogenesis include a variety of organic and C_1-compounds. Coenzyme A, which plays an important role in the TCA cycle, is a ribonucleotide and the main substrate for the synthesis of lipids, a remembrance of the early RNA world (Stryer, 1988). Several eubacteria and archaebacteria employ the acetyl-CoA pathway; they include autotrophic

homoacetogenic and sulphate-reducing bacteria, methanogens, *Closterium*, *Acetobacterium*, and others. The RNA-world is generally thought to predate the protein/DNA-world. RNA originally catalyzed all cellular transformations; protein evolved later to support RNA in this role. Many protein enzymes still have RNA-based cofactors (e.g. ribosomes and spliceozomes), while RNA still has catalytic functions. DNA evolved as a chemically more stable archive for RNA, probably in direct connection with the evolution of proteins. The step from the RNA to the protein/DNA world came with a need for the regulation of transcription.

The hyperthermophilic methanogens, such as *Methanococcus, Methanobacterium* or *Methanopyrus,* have also been proposed as contemporary models for early cells (Lindahl and Chang, 2001); they have the acetyl-CoA pathway, which they run in both the oxidative and the reductive direction (Simpson and Whitman, 1993). Like *Aquifex*, they are thermophilic and taxonomically close to the archaea/eubacteria fork (eukaryotes have some properties of both roots), have a small genome (*Methanococcus jannaschii* has 1.66 Mbp, coding for only 1700 genes), and they utilize H_2 as electron donor.

Intermezzo: Before the first cells

A possible exergonic process generating energy in the initial stages of life involves the formation of makinawite crusts at the interface of mildly oxydizing, iron-rich acidulous ocean water above basaltic floors from which alkaline seepages arose (e.g. Russell *et al.*, 1994). These crusts consist of FeS layers allowing free electron flow from the reducing environment beneath, generated by the activation of hydrothermal hydrogen. Thus, energy was constantly supplied which, moreover, could easily be tapped at the steep gradient formed by the crust. FeS can spontaneously form cell-like structures on a solid surface (Russell and Hall, 1997, 2002; Boyce *et al.*, 1983; Cairns-Smith *et al.*, 1992), and has a high affinity for the ATP ingredients organophosphates and formaldehyde (Rickard *et al*., 2001), which can form ribulose (see Bengtson, 1994: 81). The released energy could stimulate the formation of larger molecules at each inner surface, such as phosphorus or nitrogen compounds. The chemically labile energy-rich inorganic pyrophosphate compounds could have served as energy-transferring molecules (Baltscheffsky, 1996; Baltscheffsky *et al.,* 1999), whereas the nitrogen-containing molecules on the inner surface of the crust could have developed into nucleic acids or, later, into larger peptides. Of these, the peptides, in turn, could have combined with iron and sulphur complexes in the crust, thus initiating the formation of ferredoxins, or they could have nested themselves within the crust, thus forming the second step in the formation of membranes (Russell and Hall, 2002).

The membranes of membrane-bound vesicles are at the basis of transformations typical for life (Segré *et al*., 2001). Membranes need membranes (plus genes) for propagation; genes only are not enough (Cavalier-Smith, 2000). Strong arguments in favour of the hypothesis "cells before metabolism" include the abiotic abundance of amphiphilic compounds (even on arriving meteorites), the self-organization of these compounds into membranes and vesicles, and their catalytic properties (Deamer and Pashley, 1989). This argument only works if amphiphilic compounds tend to accumulate in very specific micro-environments; otherwise they will be too dilute. The modifications of substrates that are taken up from the environment to compounds that function in metabolism were initially probably small and gradually became substantial. Compartmentalization is essential for the accumulation of metabolites and for any significant metabolism. Norris and Raine (1998) suggest that the RNA world succeeded the lipid world, which is unlikely because the archaebacterial lipids consist of isoprenoid ethers, while eubacterial lipids consist of fatty acids (acyl esters) with completely different enzymes involved in their turnover (Kates, 1979; Kandler, 1998; Wächtershäuser, 1988). Lipids were probably synthesized first from pyruvate, the end product of the acetyl-CoA pathway and the reverse TCA cycle, before the extensive use of carbohydrates.

Koga *et al*. (1998) hypothesized that the eubacterial taxa made the transition from non-cellular ancestors to cellular forms independently from the archaebacteria (see also Martin and Russell, 2003). This seems unlikely, however, because they are similar in the organization of their genes (e.g. in operons) and genomes, and in their transcription and translation machinery (Olsen and Woese, 1996; Cavalier-Smith, 1998). Eubacteria do have a unique DNA replicase and replication initiator proteins however. These properties apply especially to cells, rather than to pre-cellularly existing forms, and are complex enough to make it very unlikely that they evolved twice. Woese (2002) hypothesized that lateral gene transfer could have been intense in proto-cells with a simple organization; diversification through Darwinian mutation and selection could only occur after a given stage in complexity had been reached, that is when lateral gene transfer could have been much less intense. The eubacteria, archaebacteria and eukaryotes would have crossed this stage independently. Since all eukaryotes once seem to have possessed mitochondria (Roger, 1999; Gupta, 1998; Keeling, 1998; Embley and Hirt, 1998), this origin is unlikely for them. Cavalier-Smith (2002a) argued that archaebacteria and eukaryotes evolved in parallel from eubacteria since about 850 Ma ago, and that eukaryotes have many properties in common with actinomycetes. However the differences in, for example, lipid metabolism and many other properties between eubacteria and archaebacteria are difficult to explain in this way. Moreover, carbon isotope differences between carbonates and organic matter of 2.8-2.2 Ga ago are attributed to archaean methanotrophs

(Knoll, 2003). Although so far the topic remains speculative, a separate existence of eubacteria and archaebacteria before the initiation of the lipid metabolism and before the origin of eukaryotes through symbiogenesis with mitochondria seems to be the least-problematic sequence explaining metabolic properties among these three taxa.

A hypothetical energy-generating scheme involving the consumption of dihydrogen and sulphur is based on the overall exergonic reaction $FeS + S \rightarrow FeS_2$ (Taylor *et al.*, 1979; Wächtershäuser, 1988; Madigan *et al*., 2000).

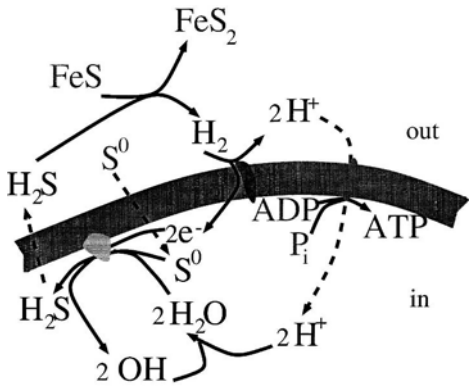

Figure 7.2. A possible early ATP generating transformation, based on pyrite formation, that requires a membrane and three types of enzyme: proto-hydrogenase, proto-ATP-ase and S^0-reductase; modified from (Madigan *et al*., 2000). Sulphur has to be imported in exchange for H_2S.

The scheme of Figure 7.2 may have applied to the initial cellular life forms because of the availability of the substrates in the deep ocean (van Dover, 2000), and few enzymes are required. Keefe *et al*. (1995) however, argue that the oxidation of FeS gives insufficient energy to fix carbon dioxide through the inverse TCA cycle. Yet, this fixation may have occurred along other pathways using accumulated ATP. Schoonen *et al*. (1999) demonstrated that the energy of this reaction diminishes sharply at higher temperatures. Contrary to pyrite, greigite ($Fe_5Ni_6S_8$) has structural moieties that are similar to the active centres of certain metallo-enzymes, as well as to electron transfer agents (see, for example, Russell and Hall, 2002), and catalizes the transformation $2\ CO_2 + CH_3SH + 8\ [H] \rightarrow CH_3COSCH_3 + 3\ H_2O$.

Concerning homeostatic membranes, transformations of substrates and products, occurring in the enclosed vesicle and catalyzed by membrane-bound enzymes, depend on the size of the vesicle, that is on the amount of enzyme proportional to the amount of membrane and therefore to the surface area of the cell. The transformation rate involves the ratio of surface area to volume,

which constitutes a measure of length. The change in this ratio naturally leads to the cell cycle, that is a cyclic pattern in the metabolism of the cell, and represents one of the cornerstones of the DEB theory. This theory implies that the turnover rate of reserve density, that is the ratio of the amounts of reserve and structure, is inversely proportional to a length measure in isomorphs, i.e. organisms that do not change in shape when they grow. The crucial parameter in reserve turnover, the energy conductance with the dimension of length per unit of time, testifies to the basic role of surface area-volume interactions in metabolic rate control.

A natural implication of the reversal of the TCA cycle is that the direction of glycolysis was initially reversed as well, and served to synthesize building blocks for e.g. carbohydrates. Comparing the carbohydrate metabolism among various bacterial taxa, Romano and Conway (1996) concluded that originally glycolysis must indeed have been reversed. Thus, the reversed glycolytic pathway probably developed as an extension of the reversed TCA cycle, and they both reversed to their present standard direction upon linking to the Calvin cycle, which produces glucose in a phototrophic process. So, what could have been the evolutionary history of photoptrophy?

Phototrophy

Phototrophy developed early in evolution; some workers even think that it has been present right at the origin of life (Woese, 1979; Cavalier-Smith, 1987b; Hartman, 1998; Blankenship and Hartman, 1992, 1998). In an anoxic atmosphere, and therefore without ozone, UV damage must have been an important problem for the early phototrophs though and protection and repair mechanisms against UV damage must have evolved in parallel with phototrophy (Dillon and Castenholz, 1999). The green non-sulphur bacterium *Chloroflexus* probably resembles the earliest phototrophs and is unique in lacking the Calvin cycle, as well as the reverse TCA cycle. In the hydroxypropionate pathway, it reduces two CO_2 to glyoxylate, using many enzymes also found in the thermophilic non-phototrophic archaeon *Acidianus*. Its photoreaction centre is similar to that of purple bacteria. The reductive dicarboxylic acid cycle of *Chloroflexus* is thought to have evolved into the reductive tricarboxylic acid cycle as found in *Chlorobium*, and further into the reductive pentose phosphate cycle, which is, in fact, the Calvin cycle (Hartman, 1998).

Like sulphur and iron-oxidizing chemolithotrophs, aerobic nitrifying bacteria use the Calvin cycle for fixing CO_2. The substrate of the first transformation of the monophosphate pathway for oxidizing C_1-compounds, such as methane, is very similar to the C_1-acceptor of the Calvin cycle, which suggests a common evolutionary root of these pathways (Madigan *et al*., 2000). The first enzyme in the Calvin cycle, RubisCO is present in most

chemolithotrophs and phototrophs and even in some hyperthermophilic archaea. It is the only enzyme of the Calvin cycle of which (some of) the code is found on the genome of chloroplasts. The enzymes that are involved in the Calvin cycle show a substantial diversity among organisms and each has its own rather complex evolutionary history (Martin and Schnarrenberger, 1997). This complicates the finding of its evolutionary roots (see Figure 7.3).

The thermophilic bacterium *Chlorobium tepidum* has a reverse TCA cycle and a RubisCO-like gene. In combination with the observations mentioned above, this suggests that the present central glucose-based metabolism evolved when the Calvin cycle became functional in CO_2 binding, and the glycolysis and the TCA cycle reversed to their present standard direction, operating as a glucose and pyruvate processing devices, respectively (see Figure 7.3).

Most phototrophs use the Calvin cycle for fixing CO_2 in their cytosol in combination with a pigment system in their membrane for capturing photons. Archaea use a low-efficient retinal-protein and are unable to sustain true autotrophic growth; five of the 11 eubacterial phyla have phototrophy. Bacterio-chlorophyll in green sulphur bacteria is located in chlorosomes, organelles bound by a non-unit membrane, attached to the cytoplasmic membrane.

Green non-sulphur and purple bacteria utilize photosystem (PS) II; green sulphur and Gram-positive bacteria utilize PS I, whereas cyanobacteria (including the prochlorophytes) utilize both PS I and II (Zubay, 2000). The cyanobacterium *Oscillatoria limnetica* can utilize their PS I and II in conjunction, thus being able to split water and to produce dioxygen. In the presence of H_2S as an electron donor, it uses only PS I, an ability pointing to the anoxic origin of photosynthesis. This anoxic origin appears to be ancient (Xiong *et al*., 2000). Oxygenic photosynthesis is a complex process that requires the co-ordinated translocation of four electrons. It evolved more than 2.7 Ga ago (Bjerrum and Canfield, 2002). Based on the observation that bicarbonate serves as an efficient alternative for water as an electron donor, Dismukes *et al*. (2001) suggested the following evolutionary sequence for oxygenic photosynthesis, starting from green non-sulphur bacterial phytosynthesis that uses organic substrates as electron donor:

Electron Donation	Pigment	Reaction Centre	Photo-synthesis
Oxalate $\rightarrow$ Oxalate$^+$	BChl-a		Anoxygenic
$Mn_2(HCO_3)_4 \rightarrow Mn_2(HCO_3)_4^+$	BChl-a		Anoxygenic
$2\ HCO_3^- \rightarrow O_2 + 2\ CO_2 + 2\ H^+$	BChl-g	$Mn_4O_x(HCO_3)_y$	Oxygenic
$2\ H_2O \rightarrow O_2 + 4\ H^+$	Chl-a	$CaMn_4O_x(HCO_3)_yY_z$	Oxygenic

The phototrophic machinery eventually allowed the evolution of the respiratory chain (the oxidative phosphorylation chain), which uses dioxygen that is formed as a waste product of photosynthesis, as well as the same enzymes in reversed order. If the respiratory chain initially used sulphate, for example, rather than dioxygen as electron acceptor, it could well have evolved simultaneously with the phototrophic system.

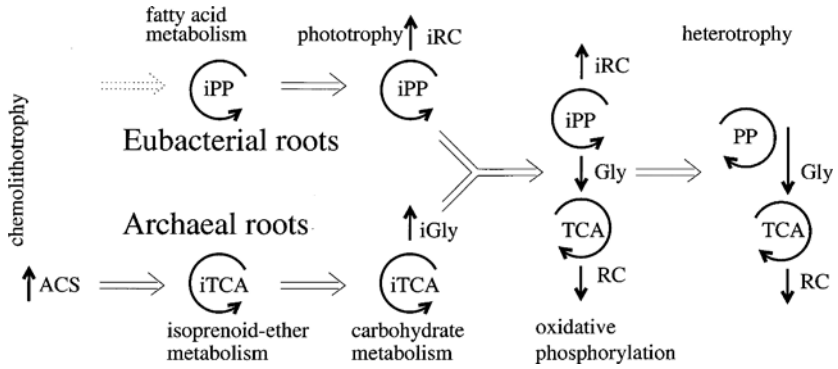

Figure 7.3. Evolution of the central metabolism among prokaryotes that formed the basis of eukaryotic organization of the central metabolism. ACS = acetyl-CoA Synthase pathway, iPP = inverse Pentose Phosphate cycle (= Calvin cycle), PP = Pentose Phosphate cycle, iTCA = inverse TriCarboxylic Acid cycle, TCA = TriCarboxylic Acid cycle (= Krebs cycle), iGly = inverse Glycolysis, Gly = Glycolysis, iRC = inverse Respiratory Chain, RC = Respiratory Chain. The arrows indicate the directions of synthesis to visualize where they reversed. All four main components of eukaryote's heterotrophic central metabolism originally ran in the reverse direction to store energy and to synthesize metabolites.

Figure 7.3 summarizes the broad pattern of the possible evolution of the central metabolism as it took place in prokaryotes and that formed the basis for the eukaryotes. It implies considerable conjugational exchange between the archaea and eubacteria, but given the long evolutionary history, such exchanges might have been very rare. The exchange must have been predated by a symbiontic coexistence of archaea and eubacteria to tune their very different metabolic systems. The production of dioxygen during phototrophy, which predates the oxidative phosphorylation, changed the earth (e.g. Dismukes *et al.*, 2001; Lane, 2002).

The availability of a large amount of energy and reducing power effectively removed energy limitations; primary production in terrestrial environments is mainly water-limited, that in aquatic environments nutrient-limited. This does not imply however, that the energetic aspects of metabolism could not be

quantified usefully; energy conservation also applies in situations where the energy supply is not rate-limiting.

Nutrients may have run short of supplies because of oxidation by dioxygen; this would have slowed down the rate of evolution (Anbar and Knoll, 2002). First, sulphur precipitated out, followed by iron and towards the end of the Precambrian by phosphate and, since the Cambrium revolution, by calcium as well. Also, under aerobic conditions, nitrogen fixation became difficult, which makes biologically required nitrogen unavailable, despite its continued great abundance of dinitrogen in the environment (see Bengtson, 1994: 41).

Since the Calvin cycle produces fructose 6-phosphate, those autotrophic prokaryotes possessing this cycle are likely to have a glucose-based metabolism. Indeed, the presence of glucose usually suppresses all autotrophic activity. Several obligate chemolithotrophic prokaryotes, such as sulphur-oxidizers, nitrifiers, cyanobacteria and prochlorophytes contain this cycle in specialized organelles, the carboxysomes, which are tightly packed with RubisCO. Facultative autotrophs, like purple anoxyphototrophs, use the Calvin cycle for fixing CO_2, although they lack the carboxysomes.

Diversification and interactions

The prokaryotes as a group evolved a wide variety of abilities for the processing of substrates, whilst remaining rather specialized as species (e.g. Amend and Shock, 2001). The nitrogen cycle in Figure 7.4 illustrates this variety, as well as the fact that the products of one group are the substrate of another.

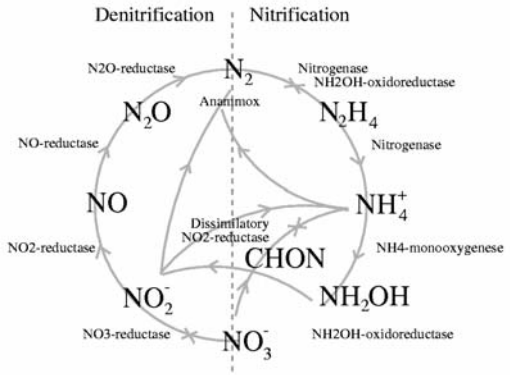

Figure 7.4. Conversions of inorganic nitrogen species by prokaryotes. The compound CHON stands for biomass. Modified from Schalk (2000).

Some of the conversions of inorganic nitrogen species can only be done by a few taxa. The recently discovered anaerobic oxidation of ammonia is only known from the planctobacterium *Brocadia anammoxidans* (Schalk, 2000) (nonetheless, it might be responsible for the removal of one-half to one-third

of the global nitrogen in the deep oceans (Dalsgaard *et al*., 2003)); the aerobic oxidation of ammonia to nitrite is only known from *Nitrosomonas*, the oxidation of nitrite into nitrate is only known from *Nitrobacter*; and the fixation of dinitrogen can only be done by a few taxa, such as some cyanobacteria, *Azotobacter*, *Azospirillum*, *Azorhizobium*, *Klebsiella, Rhizobium,* and some other ones (Sprent, 1987).

If the composition of structural mass, i.e. a combination of proteins, lipids, carbohydrates, etc., does not change too much, we have stoichiometric constraints on growth. These constraints are revealed when the nutrient concentrations in the environment change relative to each other. The DEB theory holds that growth happens at the expense of reserves rather than at that of nutrients in the environment. Also, nutrient uptake is a function of the nutrient concentration in the environment and the amount of structural mass only and is not a function of the amount of reserve. The consequence is that (some of) the utilized reserves that are not immediately used for maintenance or growth must be excreted in one form or another, which links homeostasis to excretion (see, for example, Smith and Underwood, 2000). The excretion of polysaccharides (carbohydrates) and other organic products by nutrient-limited photosynthesizers (such as cyanobacteria), stimulated heterotrophs to decompose these compounds through the anaerobically operating glycolytic pathway. Thus, other organisms came to use these excreted species-specific compounds as resources, and a huge biodiversity resulted.

Apart from the use of each other's products, prokaryotes, such as the proteobacteria *Bdellovibrio* and *Daptobacter,* invented predation on other prokaryotes. When the eukaryotes emerged, many more prokaryote species turned to predation, with transitions to parasitism causing diseases in their eukaryotic hosts. Predators typically have a fully functional metabolism, while parasites use building blocks from the host, reducing their genome with the codes for synthesizing these building blocks. The smallest genomes occur in viruses which probably evolved from their hosts and are not reduced organisms (Hendrix *et al.,* 1999; Sullivan *et al.,* 2003).

Prokaryotic mats on intertidal mud flats and at methane seeps illustrate that the exchange of metabolites between species in a community can be intense (van den Berg, 1998; Michaelis *et al*., 2002; Nisbet and Fowler, 1999). The occurrence of multi-species microbial flocks, such as in sewage treatment plants (Brandt and Kooijman, 2000; Brandt, 2002) further illustrates an exchange of metabolites among species. The partners in such syntrophic relationships sometimes live epibiotically, possibly to facilitate exchange. Internalization further enhances such exchange (Kooijman *et al*., 2003). The gradual transition of substitutable substrate to become complementary is basic to the formation of obligate syntrophic relationships. The mathematical framework for such a smooth transition is discussed in Brandt *et al.* (2003) and in Kooijman *et al.* (2003).

7.4 THE EMERGENCE OF THE EUKARYOTES

Symbiontic origins of mitochondria

Eukaryotes may have emerged from the internalization of a fermenting, facultative anaerobic H_2- and CO_2-producing eubacterium into an autotrophic, obligatory anaerobic H_2- and CO_2-consuming methanogenic archaebacterium (Martin and Mueller, 1998), the host possibly returning organic metabolites (see Figure 7.5). Once the H_2-production and consumption had been cut out of the metabolism, aerobic environments became available, where the respiratory chain of the symbiont kept the dioxygen concentration in the hosts' cytoplasm at very low levels. The internalization of (pro)mitochondria might be a response to counter the toxic effects of dioxygen. This hypothesis for the origin of eukaryotes explains why the DNA replication and repair proteins of eukaryotes resemble that of archaea, and not that of eubacteria. Notice that the eukaryotization, as schematized in Figure 7.5, just represents a recombination and compartmentation of existing modules of the central metabolism (cf. Figure 7.3).

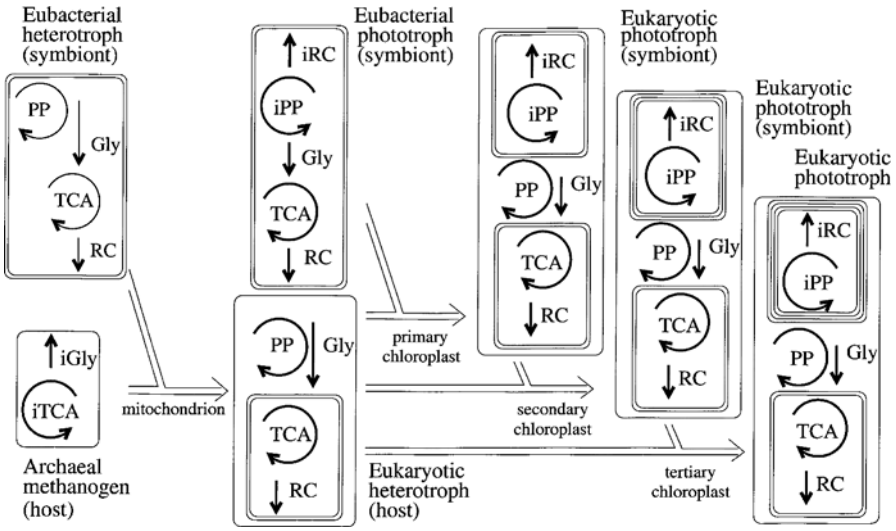

Figure 7.5. Scheme of symbiogenesis events; the first two primary inclusions of prokaryotes (to become mitochondria and chloroplasts respectively) were followed by secondary and tertiary inclusions of eukaryotes. Each inclusion comes with a transfer of metabolic functions to the host. The loss of endosymbionts is not illustrated. See Figure 7.3 for the meaning of the codes for the modules of the central metabolism and for the ancestors of the mitochondria and chloroplasts.

It can be shown that such forms of syntrophy can easily lead to homeostatic assemblages, where the relative abundance of the partners become independent of variations of the primary resources in the environment (Kooijman *et al*., 2003). Moreover, it can also be shown that this merging of initially independently living populations, each following the rules of the DEB theory, can be such that the integrated assemblage again follows these rules (Kooijman *et al*., 2003). This remarkable property poses stringent constraints on reserve dynamics which the DEB model appears to satisfy. Given the common occurrence of symbiogenesis in evolutionary history, this property is required for any model that is not species-specific. Most (if any) alternative models will not have this property which makes them species-specific. Syntrophic associations between methanogens and hydrogenosomes are still abundant; ciliates can have methanogens as endosymbionts and interact in the exchange (Fenchel and Finlay, 1995).

Much discussion exists about which metabolites may have been exchanged between the pro-mitochondrial symbionts and their hosts; some workers believe that both were aerobic heterotrophs, although they do not give clues about the nature of the compounds being exchanged (e.g. Kurland and Andersson, 2000). Part of the problem is that mitochondria and hosts exchanged quite a few genes, and the genome of mitochondria reduced considerably, down to 1% of its original bacterial genome (Fenchel, 2002). The mitochondrial DNA in kinetoplasts, however, is amplified and can form a network of catenated circular molecules (Lee *et al.*, 2000).

Cavalier-Smith (1987a, 2002b) argued that eukaryotes descend from some actinobacterium that engulfed a phototrophic posibacterium (an α-proteobacterium) as mitochondrion, which later lost phototrophy, and used it as a slave to produce ATP. The ability to phagotise is central to his reasoning. Actomyosin mediates phagocytosis and actinobacteria have proteins somewhat related to myosin, although they do not phagotise. If he is right that the outer membrane of mitochondria is derived from the original posibacterium, and not from the host, there is little need for the existence of phagocytosis prior to the entry of a posibacterium to become a mitochondrion. At least one example exists of prokaryotic endosymbiosis (β-proteobacteria that harbour γ-proteobacteria, von Dohlen *et al*., 2001) in absence of phagocytosis. More examples exist of penetration through the membrane without killing the victim instantaneously (e.g. Guerrero, 1991). His present view, shared by others, is that it happened only once and the logical implication is just in a single individual. If phagocytosis would have been well established prior to the entry of a mitochondrion, it is hard to understand why it did not occur more frequently. It seems more likely that eukaryotic membrane transport (with applications in phagocytosis), the cytoskeleton (with applications in cilia) and the Endoplasmatic Reticulum (ER, including the nuclear envelope) became

operational somewhere between the entries of mitochondria and chloroplasts, which do have host-derived envelopes. The origin of eukaryotes is possibly some 1.5 Ga (Knoll, 2003) or 2.0 Ga (Raven and Yin, 1998) or 2.7 Ga (Brocks *et al*., 1999) ago. The rhodophytes were among the first eukaryotes having chloroplasts; their fossil record goes back to 1.2 Ga (Knoll, 2003) ago.

We agree with Cavalier-Smith on the need to understand the evolution of phagocytosis which is still enigmatic. A weak element in his reasoning is that phagocytotic entry was prior to enslavement to produce ATP for the host. The development of exchange systems for metabolites doubtlessly took many generations, while the endosymbiosis must have been operational right from the moment of penetration into the host cell for (more or less) co-ordinated cell growth and duplication. We cannot see how this is possible without a prior (epibiontic) existence of a syntrophic relationship between host and symbiont (Kooijman *et al.,* 2004). Moreover, the relationship between mitochondria and their host is much more complex than the delivery of ATP in exchange for pyruvate, ADP and P from the host. Kooijman and Segel (2003) argue that the delivery of intermediary metabolites by the mitochondria is at least as essential for the host.

No eukaryotes are known with plastids but are lacking mitochondria, which suggests that possessing mitochondria was compulsory for cyanobacteria to move in into the symbiotic relationship. Genes that moved from mitochondria to the genome of their host reveal that some eukaryotes (also) lost their mitochondria. As mentioned before, recent studies suggest that all eukaryotes once possessed mitochondria (Simpson and Roger, 2002; Stechmann and Cavalier-Smith, 2002), despite the many taxa that presently lack mitochondria.

The amitochondriate pelobiont *Pelomyxa palustris* has intracellular methanogenic bacteria that may have comparable functions. Other members of the α-group of purple bacteria (from which the mitochondria arose; Andersson *et al*., 1998) such as *Agrobacterium* and *Rhizobium,* can also live inside cells, and usually function in dinitrogen fixation; *Rickettias* became parasites, using their hosts' building blocks and reducing their own genome to viral proportions.

Symbiontic origins of chloroplasts

The process of internalization of a cyanobacterium of uncertain phylogenetic origin probably occurred only once in eukaryotic history (Delwiche, 1999; McFadden, 2001; Cavalier-Smith, 2002a), where the plastids of glaucophytes retained most of their genome and properties, whereas that of rhodophytes and chlorophytes became progressively reduced by transfer of thousands of genes to the nucleus (Martin *et al.*, 2002) and by gene loss. Secondary endosymbioses of red algae occurred in cryptophytes, haptophytes, heterokonts, dinoflagellates and apicomplexans and those of green algae

occurred in euglenoids and chlorarachniophytes. The presence of plastids in the parasitic Kinetoplastids and of cyanobacterial genes in the heterotrophic percolozoans (= Heterolobosea) suggests that secondary endosymbiosis did not take place in the euglenoids, but much earlier in the common ancestor of all excavates, where chloroplasts became lost in the percolozoans (Andersson and Roger, 2002). Alveolates (including dinoflagellates and ciliates) have a more dynamic association with plastids. Even weaker associations evolved between phototrophic dinoflagellates and chlorophytes on the one hand and heterotrophs on the other, such as fungi (lichens), foraminiferans, radiolarians, and animals (sponges, coelenterates, molluscs, platyhelmintes).

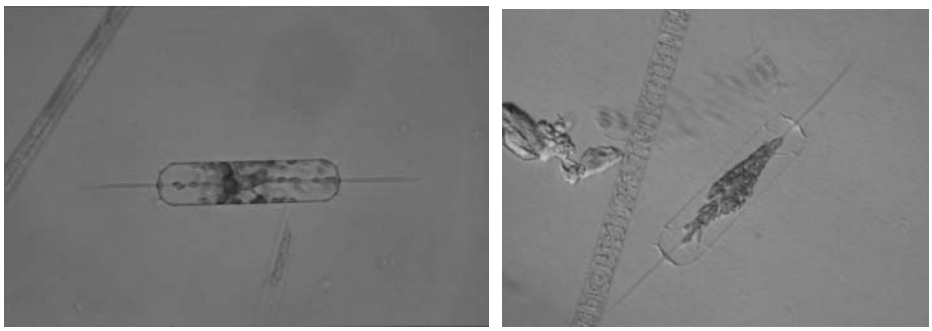

Figure 7.6. Chloroplasts of the marine diatom *Ditylum brightwellii* disperse at low light levels, and aggregate at high ones. They move in a co-ordinated way.

The intra-cellular dynamics of mitochondria and plastids is still poorly known (Osteryoung and Nunnari, 2003). Growth and division are usually only linked to the cell cycle. Mitochondria move actively through the cell and can easily fuse with each other (Kooijman *et al.,* 2003), in yeasts and chlorophytes even forming networks. Their numbers can range from a single one to many depending on species and conditions. In some algae, the single mitochondrion can cyclically divide into many small ones and fuse to a single one again. Moreover, the host cell can kill mitochondria and lysosomes can decompose the remains. Likewise chloroplasts can move through the cell sometimes in a co-ordinated way (see Figure 7.6). They can reversibly lose their chlorophyll and fulfil non-photosynthetic tasks, which are permanent in the kinetoplasts (e.g. the endoparasite *Tripanosoma*) and in heterotrophic plants (*Triurdaceae*, some *Orchidaceae, Burmanniaceae*, prothallium-stage of Lycopods and Ophioglossids), in parasitic plants (*Orobanchaceae, Rafflesiaceae, Balanophoraceae,* some *Convolvulaceae*), and in predatory plants (some *Lentibulariaceae*), for instance. (This list of exclusively heterotrophic plants suggests that heterotrophy might be more important among plants than is generally recognized.) Eukaryotes also had to master the control of

transmission of mitochondrial and chloroplast genomes. Most use a system in meiosis where these genomes come from a single parent; stochastic models for mitotic genome segregation seem to be most effective (Birky, 2001).

Features unique to eukaryotes

Eukaryotes have many properties not known from prokaryotes, which challenges the view that they are "simply" prokaryotic chimaeras. An example is the production of clathrin, a protein which plays a key role in the invagination of membranes such as during endocytosis. We are just beginning to understand the complex processes involved in membrane deformation (Bigay *et al.*, 2003). No prokaryote seems to be able to form vesicles, while membrane transport (including phagocytosis and pinocytosis, vesicle mediated transport) is basic in eukaryotes (de Duve, 1984; Gruenberg, 2001), and essential for endosymbiotic relationships. Today, only a single endosymbiotic relationship among prokaryotes is known (von Dohlen *et al.*, 2001), but the endosymbionts are probably not surrounded by a membrane of the hosts. Eukaryotes also have ATP-fuelled cytoplasmatic mobility driven by myosin and dynein.

Another example of a property unknown in prokaryotes is the vacuole (Leigh and Sanders, 1997), which is used for storing nutrients in ionic form and carbohydrates; sucrose, a precursor of many other soluble carbohydrates, typically occurs in vacuoles. This organelle probably evolved to solve osmotic problems that came with storing substrates. The storage of water in vacuoles allowed plants to invade the terrestrial environment; almost all other organisms depend on plants in this environment. The DEB theory predicts that the storage capacity of energy and building-blocks scales with volumetric length to the power of four; since eukaryotic cells are generally larger than prokaryotic ones, storage becomes more important to them. Diatoms typically have extremely large vacuoles, which occupy more than 95% of the cell volume, allowing for a very large surface area (the outer membrane, where the carriers for nutrient uptake are located), relative to their structural mass that requires maintenance. In some species, the large chloroplast wraps around the vacuole like a blanket. Since, according to the DEB theory, reserve does not require maintenance, the large ratio of surface area to structural volume explains why diatoms are ecologically so successful, and also why they are the first group of phytoplankton to appear each spring. Archaebacteria and posibacteria do have gas vacuoles but their function is totally different from that of eukaryotic vacuoles.

The Golgi apparatus, a special set of flat, staked vesicles, called dictyosomes, develops after cell division from the endoplasmatic reticulum. They appear and disappear repeatedly in the amitochondriate metamonad *Giardia*. The nuclear envelope can disappear in part of the cell cycle in some

eukaryotic taxa and it is also formed by the endoplasmatic reticulum. The amitochondriate parabasalid *Trichomonas* does not have a nuclear envelope while the planctobacterium *Gemmata oscuriglobus* has one. The possession of a nucleus itself is therefore not a basic requisite distinguishing between prokaryotes and eukaryotes. The situation is quite a bit more complex than molecular biology textbooks suggest; e.g. the macronuclei (sometimes more than one) in ciliates are involved in metabolism, while the micronuclei deal with sexual recombination.

Although some prokaryotic cells, such as the planctobacteria, are packed with membranes, eukaryotic cells are generally more compartmentalized, both morphologically and functionally. Compounds can be essential in one compartment, and toxic in another (Martin and Schnarrenberger, 1997). Eukaryotic cilia differ in structure from the prokaryotic flagella, and are therefore called undulipodia to underline the difference (Margulis, 1970). The microtubular cytoskeleton of eukaryotes is possibly derived from protein constricting the prokaryotic cell membrane during fission, as both use the protein tubulin (van den Ent *et al*., 2001).

Another feature particular to eukaryotes concerns the organization of their genome into chromosomes (Chela-Flores, 1998), with a spindle machinery for genome allocation to daughter cells and telomerase guide RNA. Chromosomes are linked to the evolution of reproduction, which includes cell-to-cell recognition, sexuality and mating systems. Moreover, many eukaryotes have haploid as well as diploid life stages and two or more (fungi, rhodophytes) sexes (Kirkpatrick, 1993). Although reproduction may seem to have little relevance to metabolism at the level of the individual, metabolic rates at the population level depend on the amount of biomass and, hence, on rates of propagation. Eukaryotes also have a unique DNA topoisomerase I, which is not related to type II topoisomerase of the archaea (Forterre *et al.*, 1996) which further questions their origins.

Despite all their properties, the eukaryotic genome size can be small; the genome size of the acidophilic rhodophyte *Cyanidoschyzon* is 8 Mbp, only double the genome size of *E. coli* (Chela-Flores, 1998); the chlorophyte *Ostreococcus tauri* has a genome of only 10 Mbp, and the yeast *Saccaromyces cerivisiae* of 12 Mbp (Derelle *et al*., 2002).

This list of metabolic differences between prokaryotes and eukaryotes largely concerns biochemical and morphological ones. The following sections will focus on their organizational differences: multicellularity and syntrophy.

7.5 MULTICELLULARITY AND BODY SIZE

Multicellularity evolved many times in evolutionary history, even among the prokaryotes but particularly among the eukaryotes. It allows a

specialization of cells to particular functions, and the exchange of products is inherently linked to specialization. Think, for instance, of filamental chains of cells in cyanobacteria where heterocysts specialize in N_2 fixation. To this end, specialization requires adaptations for the exclusion of dioxygen and the production of nitrogenase. The existence of dinitrogen-fixation unicellular cyanobacteria shows that all metabolic functions can be combined within a single cell, which is remarkable as its photosynthesis produces dioxygen, inhibiting dinitrogen fixation. A temporal separation of the processes solves the problem, but restricts dinitrogen fixation during darkness; specialization can be more efficient under certain conditions. The mixobacterium *Chondromyces* and the proteobacteria *Stigmatella* and *Mixococcus* have life cycles that remind us of those of cellular slime moulds, involving a multicellular stage, whereas acetinobacteria, such as *Streptomyces* resemble fungal mycelia (e.g. Dworkin, 1985).

Pathogens, such as viruses can kill individual cells without killing the whole organism, which is an important feature of multicellularity, and is basic to the evolution of defence systems.

Cell differentiation is minor in poriferans, reversible in coelenterates and plants, and irreversible in vertebrates. The number of cells of one organism very much depends on the species, and can be up to 10^{17} in whales (Rizzotti, 2000), which requires advanced communication. Many larger organisms, including opisthokonts (fungi plus animals), tracheophytes, rhodophytes and phaeophytes, evolved elaborate transport systems to facilitate exchange of metabolites among the cells and with the environment. Animals evolved advanced locomotory abilities, which require accurate co-ordination by a nervous system. This latter system not only took tasks in information exchange and processing but also in metabolic regulation. Animals also evolved an immune system which supplements chemical defences to fight pathogens.

Differentiation and cellular communication

Multicellularity has many implications. Cells can be organized into tissues and organs, which gives metabolic differentiation once more an extra dimension. It comes with a need for regulation of the processes of growth and apoptosis of cells in tissues (Rothenberg and Jan, 2003), in which communication between cells plays an important role. Animals (from cnidarians to chordates) use gap junctions between cells of the same tissue, where a family of proteins called connexins form tissue-specific communication channels. They appear early in embryonic development (in the eight-cell-stage in mammals) and are used for nutrient exchange, cell regulation, conduction of electrical impulses, development and differentiation. Together with the nervous and endocrine systems, gap junctions serve to synchronize and integrate activities. When cell-to-cell communication systems

fail, tumours can develop; only a small fraction of tumours result from DNA damage (van Leeuwen and Zonneveld, 2001). Plants use plasmodesmata to interconnect cells, which are tubular extensions of the plasma membrane of 40-50 nm in diameter, that traverse the cell wall and interconnect the cytoplasm of adjacent cells into a symplast. Higher fungi form threads of multi-nucleated syncytia, known as mycelia; sometimes septa are present in the hyphae, but they have large pores. Otherwise the cells of fungi only communicate via the extracellular matrix (Moore, 1998). Rhodophytes have elaborate pit connections between the cells (Dixon, 1973), which have a diameter in the range 0.2-40 μm, filled with a plug that projects in the cytoplasm on either side. Ascomycetes and Basidiomycetes have similar pit connections, but lack the plug structure and the cytoplasm is directly connected, unlike the situation in rhodophytes.

The cell-individual-population continuum

The boundaries between cells, individuals, colonies, societies and populations are not sharp at all. Fungal mycelia can cover up to 15 hectares as in the basiodiomycete *Armillaria bulbosa*, but they can also fragment easily. Cellular slime moulds (dictyostelids) have a single-celled free-living amoeboid stage, as well as a multicellular one; the cell boundaries dissolve in the multicellular stage of acellular slime moulds (eumycetozoa), which can now creep as a multi-nucleated plasmodium over the soil surface. The mycetozoans are not the only amoebas with multi-nuclear stages; *Mastigamoeba* (a pelobiont) is another example (Bernard *et al.,* 2000). Many other taxa also evolved multi-nucleated cells, plasmodia or stages, e.g. ciliates, Xenophyophores, Actinophryids, Biomyxa, Loukozoans, Diplomonads, Gymnosphaerida, Haplosporids, Microsporidia, Nephridiophagids, Nucleariidae, Plasmodiophorids, Pseudospora, Xanthophyta (e.g. Vaucheria), most classes of Chlorophyta (Chlorophyceae, Ulvophyceae, Charophyceae (in mature cells) and all Cladophoryceae, Bryopsidophyceae and Dasycladophyceae)) (Patterson, 1999; van den Hoek *et al.,* 1995); the Paramyxea have cells inside cells. Certain plants, such as grasses and sedges, can form runners that give off many sprouts and cover substantial surface areas; sometimes, these runners remain functional in transporting and storing resources such as tubers, whereas in other cases they soon disintegrate. A similar situation can be found in, for example, corals and bryozoans where the tiny polyps can exchange resources through stolons. Behavioural differentiation between individuals, such as between those in syphonophorans, invites one to consider the whole colony an integrated individual, whereas the differentiation in colonial insects and mammals is still that loose that it is recognized as a group of co-ordinated individuals.

These examples illustrate the vague boundaries of multicellularity and even those of individuality. A sharpening of definitions or concepts may reduce the number of transition cases to some extent, but this cannot hide the fact that we are dealing here with a continuum of metabolic integration in the twilight-zone between individuals and populations. This illustrates that organisms, and especially eukaryotes, need each other metabolically.

The implications of body size and shape

Although some individual cells can become quite large, with inherent consequences for physiological design and metabolic performance (Hope and Walker, 1975; Raven and Brownlee, 2001), multicellularity can also lead to really large body sizes. According to the DEB theory, the ultimate body size is determined by the ratio of the assimilation flux, coupled to its surface area, and the maintenance requirements, which are coupled to body volume. Surface area is proportional to volume$^{2/3}$ in isomorphs (organisms that do not change in shape during growth), which explains why maximum body size has an upper boundary, even in the presence of abundant food; an insight that goes back to A. R. Wallace in 1865 and results in a von Bertalanffy growth curve at constant food density. This does not hold for e.g. V1-morphs, where surface area is proportional to volume^{1}; they can really grow to large sizes, as shown by some fungal mycelia, which can cover some 15 hectares (Smith *et al*., 1992). Their growth curve is typically exponential at constant food density. Growing crusts, such as lichens on a rocky surface, can be conceived as a dynamic mixture between a V1-morph (the outer annulus) and a V0-morph (the centre), where the surface area is proportional to volume^{0} (i.e. constant). The implication being that the diameter of a crust grows linearly in time at constant food density. These seemingly different growth patterns demonstrate their common feature: the significance of surface area-volume relationships at the individual level; we already discussed these relationships at the molecular level.

The DEB theory implies that the inter-specific storage capacity for reserves of organisms is proportional to the (maximum) structure's volumetric length to the power of four (Kooijman, 1986, 2000). Thus, the physiological condition of large organisms, therefore, follows environmental changes only slowly; maximum starvation times increase with body length. Body size has a bearing on the transport of material (feeding, respiration, excretion); moreover, body size and capacity for metabolic memory are interdependent. Many physiological properties are linked to transport rates and storage capacity. A classic topic of this concerns the respiration rate, also known as the metabolic rate, which is less than proportional to the weight of an organism. According to the DEB theory, this scaling is because reserves do not require maintenance costs and body weight has contributions of structural mass and of reserve; the

latter contributions are relatively more important for large-bodied species. Freshly-produced eggs or seeds beautifully illustrate that reserves do not require maintenance; they consist entirely of reserve and hardly respire. Structure is growing at the expense of reserve, which explains why total embryonic mass decreases, while its respiration rate increases. Once the feeding process is initiated at birth, we see the reversed pattern: respiration rate is increasing with body mass.

The basic difference between reserve and structure in the context of the DEB theory is in the turnover of their compounds. All compounds in the reserve have the same turnover rate, which is inversely proportional to body length in isomorphs, so the turnover rate decreases during growth. Compounds in the structure can have compound-specific turnover rates, independent of the size of the organism, due to the somatic maintenance efforts. Since large-bodied species have relatively more reserves, more of the compounds in their body have the same (low) turnover time.

Physical arguments indicate that energy costs for movement (walking, swimming, flying) scale with surface area, while energy investment scales with volume (mass). Mean travelling distance, therefore, scales with length. This implies that the diameter of the home range scales with length. The fact that maximum starvation times scale with length and feeding rate with surface area all match beautifully. In an ecosystem with many organisms of widely different body sizes, the smaller organisms live at other space-time scales than the larger ones, which has profound consequences for ecosystem dynamics as well as the stability of species diversity. Small organisms can live in locally homogeneous environments whereas large ones cannot. As a result, for instance, we have to quantify resource availability for bacteria and micro plankters in terms of concentrations (amounts per volume), but for large herbivores (such as cows) in terms of amounts per surface area. This has profound consequences for ecosystem modelling.

DEB theory makes a fundamental distinction between intra- and inter-species scaling relationships. When a young (small) organism is compared with a large (fully grown) con-specific, its specific respiration rate will be higher, just as expected for inter-species comparisons, but for a totally different reason: Overhead costs of growth make the difference. Fully grown mice and elephants, to the contrary, both do not grow.

Temperature

Temperature directly affects metabolic rates, expressed quantitatively by the Arrhenius relationship. In a variety of species, large body sizes have also led to the control of body temperature for accelerating metabolic functions, which is impossible for unicellular organisms. Some *Arum* species can elevate the temperature of their flowers metabolically, which helps volatile smells to

escape and thus to attract insects and to ripen the fruit. Also, in preparation for flying, many insects warm up their body to allow them to generate enough energy (Heinrich, 1993); they generate heat metabolically, as well as by their movements. Similarly, tuna fish can increase their body temperature up to ten degrees above that of seawater; whereas birds and mammals are well known for regulating their body temperature, often within narrowly defined boundaries. Behavioural mechanisms for controlling body temperature are known in many animals and some plants (such as in the mountain avens *Dryas*). The regulation of body temperature is just another example of achievements made possible by multicellularity.

From supply to demand systems

By feeding on organisms, which body composition varies to a limited extent only, animals achieved a relatively high level of homeostasis; the dynamics of their metabolism is well described by the DEB theory using a single reserve (consisting of a generalized compound, which is a mixture of many compounds). A small group of animals, mainly birds and mammals, pushed the condition of a (relatively) constant internal environment one step further by using extensively neuronal and hormonal regulation systems that allowed them to become more independent of their environment. These systems are probably intimately linked to their high degree of endothermy. Organisms can be ranked on a scale from supply to demand systems, where supply systems are characterized by "eating what is available" (constrained by the food processing capacity, of course), and demand systems by "eating according to the needs" (where the needs are more or less "pre-programmed"). Organisms excluding animals and sea anemonies are examples at the supply end of the spectrum and birds and mammals at the demand end; other animals taking an intermediate position. Supply systems typically have a much larger ratio between the maximum feeding rate and the minimum one to stay alive; they can easily cease growth in response to food shortage without adverse effects on their health. Demand systems typically suffer from serious health problems if food intake no longer covers growth needs. The maximum feeding rate of demand systems can be regulated to temporary needs; up-regulation occurs prior to migration, and during egg synthesis or pregnancy and lactation. (Down-regulation, such as during hibernation and even deeper forms of turpor, follows more complex patterns in the supply-demand spectrum.) Demand systems also tune their diet more finely to their needs than supply systems. While the adult chicken does well on seed, for instance, the young and fast-growing chicken favours protein-rich insects for food.

The reserve dynamics of the DEB theory is special in its capacity to reduce the number of reserves from many (the native state of supply systems) down to one (the evolutionary advanced state of demand systems) in a smooth way,

using incremental changes of parameter values. The mechanism is the same as for the integration of a syntrophic symbiosis to a single system as discussed earlier. The stepwise integration of a metabolic system is of a much wider significance than for symbiogenesis only.

Respiration and feeding

The processes of aging develop gradually in multicellular organisms, rather than binary for individual cells (Kooijman, 2000). Free radicals, which play an important role in aging, might be used to accelerate changes in the genome across generations (Kooijman, 2000). Since free radical generation is linked to respiration, and that to food intake, the processes of aging and tumour induction in organisms with irreversible cell differentiation have intimate links with energetics, and so with body size (van Leeuwen and Zonneveld, 2001; van Leeuwen *et al*., 2002). High food intake levels generally reduce life spans, although the patterns are complex in detail. An important function of sleeping in animals seems to be in the repair of neuronal damage by free radicals (Siegel, 2003). The time allocated to sleeping among animal species of different body size does follow the pattern of metabolic rate per body mass, which means that small-bodied species take more sleeping time. This observation links sleeping to energetics and aging.

The DEB theory uses conservation of time to quantify feeding rate as a function of food density in the environment. In its simplest formulation the maximum feeding rate follows from the time required to process food items; this not only includes the mechanical handling but also digestion and further metabolic transformation to reserve(s). The switching between searching and handling causes food intake to depend hyperbolically on the rate of encounters with food items under a wide range of "details" for the various sub-processes. From a more abstract point of view the feeding process has a lot in common with the mechanism of enzyme kinetics. The conservation of time argument can be used to account for behavioural modification of this feeding process, where time allocation to social interaction (such as territorial defence or sexual behaviour) and sleeping reduce the time allocated to searching for food. Since time allocated to sleeping relates to food intake, as discussed, rather complex patterns in feeding rates can emerge. The details of the feeding process are further complicated by diurnal cycles and the synchronization of these cycles between predator prey species.

7.6 SYNTROPHY

Product formation and excretion are basic to metabolism. The dynamic difference in the context of the DEB theory between product formation and the excretion of unusable reserves is that product formation is linked with fixed

weighting coefficients to the basic fluxes of assimilation, maintenance, and growth, whereas the excretion of unusable reserves depends on the amounts of reserves, relative to structure, which can vary much more dynamically over time. The reason for reserves becoming unusable so that they must be excreted, is to be found in homeostatic relationships; excretion must occur when the product has a fixed composition and requires substrates in fixed proportions, whereas the proportions in arriving substrates actually vary. The DEB theory does not distinguish between waste products (e.g. faeces production which is associated with assimilation only and urine production which also has contributions from growth and maintenance) and other products (such as penicillin production by some fungi and secondary metabolite production by plants), since waste products also can have vital functions for the organism, while the functions of some products are not always clear.

In syntrophic relationships one organism lives off the products and/or excretions of another one. However, excretion can also be toxic to some other organism, such as the nitrogen-containing domoic acid excreted by the diatom *Pseudonitzschia*, which is neurotoxic to most fish. Such an excretion particularly occurs when the silicon reserve of *Pseudonitzschias* becomes depleted, causing the diatom to get rid of its nitrogen reserve, as quantified by the DEB theory. Another type of toxic interactions occurs in bacteria, which first transform readily degradable organic compounds into acetate, resulting in a lowering of the pH which, in turn, has a negative effect on competing species. When only acetates are left they use these as a substrate.

In the next two sections, we first discuss syntrophic interactions between autotrophs and heterotrophs, which both evolved from specialization of mixotrophs, after which we concentrate on aspects of food and nutrient recycling.

Direct symbiotic syntrophy

The demand of nutrients and energy in the form of carbohydrates has led to many syntrophic relationships between carbohydrate-supplying photo-autotrophs and nutrient-supplying heterotrophs. Pure photoautotrophs are probably rare, if they exist at all; either they have mixotrophic capabilities or they form associations with heterotrophs. Being able to move independently and over considerable distances, jellyfish, for example, are able to commute between anaerobic conditions at lower water strata for nitrogen intake and higher ones for photosynthesis by their dinozoan endosymbionts supplying them with energy stored in carbohydrates. Dinozoans are engaged in similar relationships with hydropolyps (corals) and molluscs; extensive reefs testify of the evolutionary success of this association.

A close relationship between chlorophytes (or cyanobacteria) and fungi (mainly ascomycetes) evolved relatively recently, i.e. only ca. 450 million years ago, in the form of lichens and *Geosiphon* (Schüßler, 2002). The fungal partner specialized in decomposing organic matter, which releases nutrients for the algae in exchange for carbohydrates not unlike the situation in corals. Similarly, mycorrhizas exchange nutrients against carbohydrates with plants which arose in the same geological period. The endomycorrhizas (presently recognized as a new fungal phylum, the glomeromycetes) evolved right from the beginning of the land plants; the ectomycorrhizas (ascomycetes and basidiomycetes) evolved only during the Cretaceous. These symbioses seemed to have been essential for the invasion of the terrestrial environment (Selosse and Le Tacon, 1998). Some plants can also fix dinitrogen with the help of bacteria, encapsulated in specialized tissues. A single receptor seems to be involved in endosymbiontic associations between plants on the one hand and bacteria and fungi on the other (Stracke *et al*., 2002), but the recognition process is probably quite complex (Parniske and Downie, 2003) and not yet fully understood. Associations between the dinitrogen-fixation cyanobacterium *Nostoc* and the fern *Azolla* have been known for some time, but the association with the bryophyte *Pleurozium schreberi* has only recently been discovered (DeLuca *et al*., 2002); this extremely abundant moss covers most soil in boreal forests and in the taiga. The cyanobacteria are localized in extra-cellular pockets in these examples, but in some diatoms they live intracellularly. See Rai *et al.* (2000) for a review of symbioses between cyanobacteria and plants.

Heterotrophs not only have syntrophic relationships with photoautotrophs, but also with chemolithoautotrophs. A nice example concerns the gutless tubificid oligochaete *Olavius algarvensis*, with its sulphate-reducing and sulphide-oxidizing endosymbiontic bacteria (Dubilier *et al*., 2001). These symbionts exchange reduced and oxidized sulphur; the fermentation products of the anaerobic metabolism of the host provide the energy for the sulphate reducers, whereas the organic compounds produced by the sulphide oxidizers fuel the (heterotrophic) metabolism of the host. Taxonomic relationships among hosts can match that among symbionts (van Dover, 2000), which suggest considerable co-evolution in syntrophic relationships.

When tree leaves fall on the forest floor, fungi release nutrients locked in them by decomposition; the soil fauna accelerates this degradation considerably (van Wensum, 1992). Without this activity by fungi and the soil fauna, trees soon deplete the soil from nutrients, as most leaves last for only one year, even in evergreen species. As mentioned trees, and plants in general, also need mycorrhizas to release nutrients from their organic matrix. Moreover, most of them also need insects, birds or bats and other animals to be pollinated (e.g. Proctor and Yeo, 1973; Barth, 1991), and yet other animals for seed dispersal. Thus, berries, for example of *Caprifoliaceae*, *Solanaceae*

and *Rosaceae*, are "meant" to be eaten (Snow and Snow, 1988); some seeds have edible appendices (e.g. *Viola*) to promote dispersal, but others have no edible parts in addition to the seed, such as *Adoxa* and *Veronica*, and germinate better after being eaten by snails or birds and ants, respectively. Still other seeds stick to animals (e.g. *Boraginaceae, Arctium*) for dispersal. Fungi, such as the stinkhorn *Phallus* and the truffle *Tuber,* also interact with animals for their dispersal. By shading and evaporation, trees substantially affect their microclimate and thereby allow other organisms to live there as well. This too can be seen as an aspect of metabolism.

As mentioned, non-photosynthesizing plastids are still functional in plants; such plants can still have arbuscular mycorrhizas as are found in the orchid *Arachnitis uniflora* (Hibbett, 2002). Although the plant cannot transport photosynthetically produced carbohydrate to their fungal partner *Glomus*, it is obviously quite well possible that other metabolites are involved in the exchange. The complex role of plastids shows that the plant is not necessarily parasitizing the fungus.

Like plants, animals need other organisms (e.g. for food). The processing of food requires symbiosis too. We briefly discuss some aspects.

Many animals feed on cellulose-containing phototrophs but no animal can itself digest cellulose. Most animals have associations with prokaryotes, amoebas and flagellates to digest plant-derived compounds (Smith and Douglas, 1987). These micro-organisms transform cellulose to lipids in the anaerobic intestines of their host animal; the lipids are transported to the aerobic environment of the tissues of the animal for further processing. Attine ants even culture fungi to extract cellulases (Martin, 1987). Many symbioses are still poorly understood, such as the *Trichomycetes*, which live in the guts of a wide variety of arthropods in all habitats (Misra and Lichtwardt, 2000); the role of smut fungi (*Ustilaginales*) in their symbioses with plants also seems more complex than just a parasitic relationship (Vánky, 1987).

Faeces, especially that of herbivores, represent nutritious food for other organisms. This is because proteins often limit food uptake, implying that other compounds must be excreted; protein supplements to the grass diet of cows can greatly reduce the amount of grass they need. Organisms specialized on the use of faeces as a resource are known as coprophages. Examples are the bryophyte *Splachnum*, which lives off faeces of herbivores (*S. luteum* actually lives off that of the moose *Alces alces*); the fly *Sarcophaga* which lives off cattle dung; the fungus *Coprinus* which lives off mammalian faeces, similar to beetles of the dung beetle family *Scarabaeidae*.

Dead animals are processed by a variety of other animals; burrowing beetles of the family *Silphidae* specialize in this activity, for instance. Almost all animal taxa engage in carrion feeding, since the chemical make up of organisms does not differ that much; because of their great capacity of moving around, animals are often the first to arrive at the feast. Many examples

illustrate that it is just a small step from feeding off dead corpses to that of living off live ones. Predation, a specialization of most animals, has many consequences and some can actually be "beneficial" for the prey: nutrient recycling, selection of healthy individuals, reduction of competition by weak individuals, reduction of transmission of diseases and enhancing the co-existence of prey species are all implications of predation (Kooi and Kooijman, 2000; Kooi *et al.*, 2004; Kooijman *et al*., 2004).

Intricate relationships between organisms evolved, especially in prey-predator interactions, such as those between insects and plants (e.g. Schoonhoven *et al*., 1998). A low predation pressure on symbiotic partners enhances their stable co-existence (Kooi *et al*., 2004), whereas co-existence becomes unstable at a high pressure and easily leads to the extinction of both prey and predator. This points to a co-evolution of parameter values quantifying the dynamics in prey-predator systems. The time scale of the effects on fitness is essential; short-term positive effects can go together with long-term negative effects of behavioural traits on fitness. Time scales and indirect side effects that operate through changes in food availability are important aspects that are usually not included in the literature on evolutionary aspects of life history strategies.

Indirect symbiontic syntrophy

In this section, we only give some examples of the many indirect trophic relationships that exist between species.

Phytoplankters bind nutrients in the photic zone of the oceans, sink below it, die and are degraded by bacteria. Subsequently, a temporary increase in wind speed brings some of the released nutrients back to the photic zone by mixing and enables photosynthesis to continue. The sinking of organic matter is accelerated by grazing zooplankters. The result of this process is that, over time, phytoplankters build up a nutrient gradient in the water column, that CO_2 from the atmosphere becomes buried below the photic zone, and that organic resources are generated for the biota living in the dark waters below this zone and on the ocean floor. Mixing by wind makes phytoplankters commute between the surface, where they can build up and store carbohydrates by photosynthesis, and the bottom of the mixing zone, where they store nutrients. Reserves are essential here for growth, because no single stratum in the water column is favourable for growth; their reserve capacity must be large enough to cover a commuting cycle, which depends on wind speed. Although nutrient availability controls primary production ultimately, wind is doing so proximately.

The rain of dead or dying phytoplankters fuels the dark ocean communities, not unlike the rain of plant leaves fuelling soil communities, but then on a vastly larger spatial scale. Little is known about the deep ocean food web;

recent studies indicate that cnidarians (jellyfish) form a major component (Dennis, 2003).

When part of this organic rain reaches the anoxic ocean floor, the organic matter is decomposed by fermenting bacteria (many species can do this); the produced hydrogen serves as substrate for methanogens (i.e. archaeans), which convert carbon dioxide into methane. This methane can accumulate in huge deposits of methane hydrates, which serve as substrate for symbioses between bacteria and a variety of animals, such as the ice worm *Hesiocoeca*, a polychaete. The total amount of carbon in methane hydrates in ocean sediments is more than twice the amount to be found in all known fossil fuels on Earth. If the temperature rises in the deep oceans, the hydrates become unstable and result in a sudden massive methane injection into the atmosphere. This happened e.g. 55 Ma years ago (e.g. Zachos *et al*., 2003), the Paleocene-Eocene Thermal Maximum (PETM) event, which induced massive extinctions globally (Kroon *et al.*, 2001). Methanogens are involved in similar synthrophic relationships with chemolithotrophic bacteria in the deep underground (>1 km), that release hydrogen in the transformation $FeO + H_2O \rightarrow H_2 + FeO_2$ (Madigan *et al*., 2000). We are just beginning to understand the significance of these communities on ocean floors and deep underground.

The colonization of the terrestrial environment by plants may in fact have allowed reefs of brachiopods, bryozoans and molluscs (all filter feeders) to flourish in the Silurian and the Devonian; the reefs in these periods were exceptionally rich (Wood, 1999). With the help of their bacterial symbionts, the plants stimulated the conversion from rock to soil, which released nutrients that found their way to the coastal waters, stimulated algal growth, and, hence, the growth of zooplankton, which the reef animals, in turn, filtered out of the water column. The reefs degraded gradually during the time Pangea was formed towards the end of the Permian, which reduced the length of the coastline considerably and thereby the nutrient flux from the continents to the ocean. Moreover, large continents come with long rivers, and more opportunities for water to evaporate rather than to drain down to the sea; large continents typically have salt deposits. When Pangea broke up, new coastlines appeared. Moreover, this coincided with a warming of the globe, which brought more rain, more erosion, and high sea levels, which caused covering of large parts of continents by shallow seas. This combination of factors caused planktontic communities to flourish again in the Cretaceous, and completely new taxa evolved, such as the coccolithophorans and the diatoms. This hypothesis directly links the activities of terrestrial plants to the coastal reef formation through nutrient availability. Although plants reduce erosion on a time scale of thousands of years, they promote erosion on a multi-million years time scale in combination with extreme but very rare physical forces that remove both vegetation and soil (Kooijman, 2004).

The geological record of the Walvis Ridge suggests that the mechanism of physical-chemical forces that remove the vegetation, followed by erosion and nutrient enrichment of coastal waters in association with recolonization of the rocky environment by plants might also have been operative in e.g. the 0.1 Ma recovery period following the PETM event (Kroon, personal communication).

A direct quantitative relationship exists between the fossil carbohydrates (methane hydrates, coal, oil, gas, all of biotic origin) and dioxygen in the atmosphere. Although dioxygen, a by-product of oxygenic photosynthesis, was doubtlessly very toxic for most organisms when it first occurred freely in the atmosphere; today most life is dependent upon it, both directly, as well as indirectly, such as the ozon shield against UV radiation. So phototrophs generate dioxygen that is used by heterotrophs; again a form of syntrophy.

A discussion of the interactions between biota and climate is beyond the scope of this paper, see e.g. Kooijman (2004) for further discussion. The example illustrates the dynamic interaction between surface areas (where erosion takes place) and volumes (in which nutrients are diluted) at the ecosystem level. We have already discussed the importance of these interactions at the individual and molecular levels.

7.7 DISCUSSION

The red thread through our presentation is that the evolutionary invention of homeostasis comes with stoichiometric constraints on production and with the excretion of metabolic products, which promote syntrophy and the formation of symbioses that are based on syntrophy. Organisms became increasingly connected metabolically; loose forms of symbiosis can evolve into tight forms and even into a full integration, processes that happened frequently and repeatedly throughout the evolutionary history of prokaryotes and especially of eukaryotes. Syntrophy is the basis of biodiversity and supplements Darwin's notion of survival of the fittest, which is based on competitive exclusion (Ryan, 2003).

We do realize that our account of metabolic evolution is sketchy at best, and controversial in places. Contrary to our present evaluation, for instance, fermentation is still widely seen as the origin of metabolism (Alberts *et al*., 2002; Heinrich and Schuster, 1996; Fenchel, 2002). The main motivation is probably that few steps seem to be required to convert organic compounds into "biomass". As genome size already suggests, the extracellular extraction of energy from organic matter is not necessarily simpler than from inorganic compounds, though; energy supply by chemolithoautotrophy still allows the uptake of organic building blocks. Each heterotrophic bacterial species can handle only a very limited number of organic substrates. Moreover, contemporary fermenting prokaryotes have a glucose-based metabolism,

which must have been an advanced feature. The ionic strength of cytoplasm equals that of seawater, which suggests that life arose in the sea. It seems unlikely that pre-biotic organic compounds could accumulate in concentrations that allowed the emergence of life in ocean water without separation and containment. Forterre and Philippe (1999) argue that the eukaryotes are at the root of life, from which prokaryotes developed by simplification. Although cladistic analysis of the properties of archaea, eubacteria and eukaryotes still allow multiple interpretations for the roots of life, given the metabolic uniformity of eukaryotes and the advanced nature of their heterotrophic metabolism, we find it hard to accept that life would originate with eukaryotes in a geochemical context.

A proper qualitative understanding of metabolism at the molecular level involves ecological, evolutionary and geochemical aspects. While theory on competition and predation dominates population ecology, our aim has been to reveal the increasing importance in evolution of metabolic interdependence of the various forms of life based on an exchange of nutrients and metabolites. Organisms did not only become increasingly dependent on each other, but the interaction with geochemical cycling of macro nutrients, and with the climate system also became stronger (Kooijman, 2004).

A proper quantitative understanding of metabolic organization involves a holistic setting. It is essential, though, to delineate proper modules that represent entities with similar time scales, and to nest modules for keeping the models relatively simple, and thereby useful for developing a better understanding of life at both the organismic as well as the supra-organismic level. The DEB theory is useful in linking the various levels of organization, where surface area-to-volume interactions are operative at all levels, and reserves are basic in the understanding of metabolic organization.

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