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Minireview

'Every dogma has its day'*: a personal look at carbon metabolism in photosynthetic bacteria

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Abstract

Dogmas are unscientific. What is perhaps the greatest biological dogma of all time, the 'unity of biochemistry' is, in the main, still having its day. According to present knowledge, the exceptions to this dogma are mere details when seen in relation to the biosystem as a whole. Nevertheless the exceptions are scientifically interesting and the understanding of them has led to a better comprehension of photosynthesis and ecology. Until the discovery of ^{14}C, photosynthetic CO_2 fixation was like a slightly opened black box. With ^{14}C in hand scientists mapped out the path of carbon in green plant photosynthesis in the course of a few years. The impressive reductive pentose phosphate cycle was almost immediately assumed to be universal in autotrophs, including anoxygenic phototrophs, in spite of the odd observation to the contrary. A new dogma was born and held the field for about two decades. Events began to turn when green sulfur bacteria were found to contain ferredoxin-coupled ketoacid-oxidoreductases. This led to the formulation of a novel CO_2-fixing pathway, the reductive citric acid cycle, but its general acceptance required much work by many investigators. However, the ice had now been broken and after some years a third mechanism of CO_2 fixation was discovered, this time in *Chloroflexus,* and then a fourth in the same genus. One consequence of these discoveries is that it has become apparent that oxygen is an important factor that determines the kind of CO_2-fixing mechanism an organism uses. With the prospect of the characterization of hordes of novel bacteria forecast by molecular ecologists we can expect further distinctive CO_2 fixation mechanisms to turn up.

Abbreviations: BChl – bacteriochlorophyll; FAc – monofluoroacetate; Fd – ferredoxin; FNR – ferredoxin-NAD(P)-reductase; RC – reaction center; RPPC – reductive pentose phosphate cycle; Rubisco – ribulose bisphosphate carboxylase/oxygenase

Introduction

The Oxford English Dictionary defines a dogma as 'That which is held as an opinion; a belief, principle, tenet.' It comes from a Greek word meaning to seem, or seem good. Scientific dogmas grow out of bodies of knowledge or thought that at the time seem reasonable to many scientists. Dogmas can stimulate researchers to do experiments to support or invalidate them but they can also have a daunting effect. Dogmas can also weaken scientific objectivity in the assessment of evidence. With these considerations in mind, it is useful to look back and see how research in a particular field has developed. Prior to the 1930s, it was thought that CO_2 fixation was limited to green plant

* I. Zangwill, speech, November 13, 1892. www.quotelady.com/authors/author-z.html (January 16, 2003).

and algal photosynthesis. Since then researchers have uncovered a number of versions of autotrophic CO_2 fixation in anoxygenic phototrophs. In this essay I will try to illustrate the influence of dogmas on some of the research that I have been interested in, and will concentrate mostly on CO_2 fixation pathways.

Dogmas

The reductive pentose phosphate cycle

Before World War II little productive research had been done on the biochemistry of carbon metabolism in autotrophs. The discovery of ^{14}C by Martin Kamen (Ruben and Kamen, 1941) was an event that transformed the situation. In the wake of this discovery, after the war, a team led by Calvin solved the complex pathway of CO_2 fixation in algae and plants within a relatively short time (Bassham et al. 1954). The adoption of paper chromatography, invented by Consden et al. (1940) was also instrumental in that success story.

Soon after, evidence for the reductive pentose phosphate cycle (RPPC) was obtained in purple bacteria (Stoppani et al. 1955) and chemoautotrophic bacteria (Trudinger 1955, 1956). This fitted in nicely with the dominant philosophical idea of the time, known as the 'unity of biochemistry.' A dogma developed that the RPPC was the default CO_2-fixing mechanism in autotrophs. It was called the 'autotrophic mechanism' by Sid Elsden (1962) and was to dominate thinking in the field of CO_2 fixation for years to come. My long time colleague Reidun Sirevåg recalls that at a meeting in Trondheim in 1980 she sat beside Melvin Calvin at dinner. He asked her what she was working on. 'I hesitate to say this,' she said, 'I am working with an autotrophic, photosynthetic bacterium which fixes CO_2 but not by the Calvin [RPPC] cycle.' 'I don't believe it,' Calvin replied with a grin.

Van Niel's hypothesis

Another dogma that has influenced the field of carbon metabolism in photosynthetic bacteria has to do with how light is transformed into chemical energy. We know a lot about photosythetic electron transfer systems today, enough to be able to see how they can influence the kind of carbon assimilation mechanisms an organism uses. Fifty years ago we had less well defined ideas, and C.B. van Niel's (1935) hypothesis was still dominant – photosynthesis began with a photolysis of water to give a reduced product H and an oxidized product OH. The H was used solely for CO_2 fixation while the OH could either be converted to oxygen and water or be reduced by an accessory electron donor, commonly H_2S. Van Niel's hypothesis allowed one to see the analogy between oxygenic and anoxygenic photosynthesis, and as such it was very useful in a period when the understanding of photosynthesis was far poorer than it is today.

Van Niel assumed that organic substrates in purple bacterial photosynthesis were completely degraded to CO_2 and H, and at the same time a CO_2 fixation was thought to occur that was proportional to the amount of H made available in the degradation. This is a roundabout and wasteful mechanism and quite early on, a simpler explanation was provided independently by H. Gaffron (1933, 1935). Using Warburg manometry Gaffron examined fatty acid photometabolism in purple bacteria and found that it was accompanied by only a small amount of CO_2 fixation, proportional to the chain length of the fatty acid. He proposed that the fatty acids were photoassimilated directly, with any excess H formed being used for CO_2 fixation. However, there is a weakness here – the manometric method shows only gross pressure changes, so there could have been some fixation that was hidden by a CO_2 production. Gaffron's data did not give sufficient grounds for distinguishing between his own interpretation and van Niel's.

Fluoroacetate experiments on *Rhodospirillum*

In the early 1950s, I was working on my PhD at Sheffield University. In those days, Sheffield, although beautifully cradled in the Pennine hills, was badly polluted by industrial smoke. Thick fog in winter was also visible indoors because of the low temperature, and I can still see in my mind's eye the lady who worked at the bench adjacent to mine, Margot Kogut, standing there in white lab coat and scarf huddled over a cup of hot tea.

My supervisor, Sidney Elsden (see Figure 1), had done some preliminary experiments with *Rhodospirillum rubrum* using fluoroacetate (FAc), which in cells possessing the citric acid cycle is converted into fluorocitrate which blocks aconitase (Peters et al. 1953). The results did not support van Niel's hypothesis because they indicated that substrate metabolism anaerobically in the light involved direct assim-

Figure 1. Members of the Microbiology Unit at Sheffield University, about 1953. Sid Elsden is fourth from right, back row and Jane Gibson second from right, front row. John Ormerod is at the far left, back row.

ilation of substrates rather than complete breakdown to CO_2 accompanied by CO_2 fixation.

My first task was to repeat and extend these experiments (Elsden and Ormerod, 1956). I used Warburg manometry and analysed for citrate accumulation. Because manometry showed only gross pressure changes we also measured $^{14}CO_2$ fixation during substrate assimilation (Ormerod, 1956). Surprisingly, there was considerable endogenous $^{14}CO_2$ fixation that did not register manometrically, which was attributed to exchange reactions. When this was corrected for, the only substrate that gave agreement between the manometric and the $^{14}CO_2$ fixation data was butyrate. In other words, no CO_2 was produced during butyrate assimilation, thus vindicating completely Gaffron's 20-year-old interpretation. With other substrates CO_2 fixation was cryptic and could be exposed only by using $^{14}CO_2$.

We assumed that the RPPC was the CO_2 fixation mechanism accompanying substrate assimilation. This was confirmed more recently by Tabita and coworkers (e.g., Tichi and Tabita 2000) who inactivated the Rubisco gene(s) in purple bacteria.

An unexpected observation in my experiments was that FAc inhibited the autotrophic photoreduction of CO_2 by hydrogen in *R. rubrum* (Elsden and Ormerod, 1956). We commented: '. . . it is not easy to understand how the (tricarboxylic acid) cycle participates in this reaction, the more so since Glover et al. (1952) have shown that CO_2 fixation in *Rsp. rubrum* involves phosphoglyceric acid just as in green plant photosynthesis.'

FAc was also tested with the obligate autotroph, *Chlorobium thiosulfatophilum,* isolated earlier by Helge Larsen (Larsen 1952). Larsen visited the department in 1954. (Incidentally, during his visit he asked me if I would consider spending a year in his department in Norway when I was through at Sheffield, and I said yes. I am still in Norway.) The results of the joint experiment that we performed with *Chlorobium* were unequivocal – CO_2 fixation with thiosulfate was inhibited about 80% by 1 mm FAc. In my thesis discussion (Ormerod 1957), the improbable explanation was offered that it was thiosulfate metabolism that was being inhibited rather than CO_2 fixation. This was not pursued any further at the time.

A few years later, Sadler and Stanier (1960) showed that acetate could be utilized by *Chlorobium* provided that CO_2 and an electron donor were present in substrate quantities. They suggested that these were required because *Chlorobium* could not itself oxidize acetate to CO_2 and reducing power. The added acetate almost doubled the growth rate and yield and furthermore the distribution of assimilated acetate carbon was

the same as that of CO_2 carbon. These authors did not discuss the bearing of their results on the question of the autotrophic CO_2 fixation mechanism in *Chlorobium*. It seems as though they believed acetate utilization and autotrophic CO_2 fixation had nothing in common metabolically. Shortly after, Smillie et al. (1962) reported that all the enzymes of the RPPC were present in cell free extracts of *Chlorobium*. The cycle had again won the day.

New ways in *Chlorobium*: the reductive citric acid cycle

It looked as though the RPPC was present in all anoxygenic phototrophs as well as the oxygenic ones and chemoautotrophs. A second investigation of acetate assimilation in *Chlorobium* (Hoare and Gibson 1964), carried out in the Sheffield department, was concerned mostly with pathways of amino acid synthesis from labeled acetate and CO_2. The labeling indicated that acetate carbon was incorporated into most of the amino acids, that acetate was carboxylated to give pyruvate and that C1 of glutamate was derived from CO_2. Explaining their results the authors wrote that '. . . the carbon atom skeletons of the amino acids are built up mainly from bicarbonate carbon atoms by autotrophic assimilation and acetate carbon atoms simply supplement this' presumably implying that autotrophic assimilation was by the RPPC.

However, two years later a radical publication appeared like a bolt from the blue: Evans, Buchanan and Arnon (Evans et al. 1966) proposed the operation of a CO_2-fixing, reversed or reductive citric acid cycle in *Chlorobium*. The idea was attractive because it accounted for two ferredoxin (Fd)-coupled, CO_2-fixing enzymes which the Berkeley group had discovered in *Chlorobium*: pyruvate-Fd oxidoreductase (pyruvate synthase) and α-ketoglutarate-Fd oxidoreductase (α-ketoglutarate synthase). These reactions are reversible, in contrast to the corresponding dehydrogenases. Citrate synthase was replaced by citrate lyase. It all looked very plausible and offered an explanation for Hoare and Gibson's (1964) amino acid data. The reductive citric acid cycle was indeed a brilliant idea and an exciting one.

In the fall of 1968, Reidun Sirevåg (see Figure 2) started working with me on the possible relationship between the earlier observed FAc inhibition and a reductive citric acid cycle in *Chlorobium.* There was no accumulation of isocitrate or citrate but we did detect keto acids in the FAc-inhibited washed suspensions, mostly α-ketoglutarate (Sirevåg and Ormerod 1970a, b). However, the control also contained keto acids, but this was a mixture, mostly α-keto-β-methyl valerate, with less α-ketoglutarate and other keto acids. About half the CO_2 fixed in the control was in polyglucose but this was greatly reduced in the presence of FAc. Although the site of FAc inhibition is still not known, the conversion of α-ketoglutarate to other ketoacids and polyglucose was evidently being affected. Evans et al. (1966) had proposed two CO_2-fixing mechanisms in *Chlorobium*, the RPPC for carbohydrate synthesis and the reductive citric acid cycle for amino acids. But the RPPC is not inhibited by FAc (Kelly 1968). Thus although carbohydrate was a major product in resting *Chlorobium* cells, the sensitivity to FAc indicated that it could not have been formed by the RPPC. The fact that ad-

Figure 2. Reidun Sirevåg and John Ormerod in 1969 (Madrid, FEBS meeting).

ded acetate gave the same products as CO_2 and that added succinate caused increased accumulation of α-ketoglutarate was further evidence for operation of the reductive citric acid cycle (Sirevåg and Ormerod 1970b).

Some relevant papers appeared during the succeeding years. Buchanan et al. (1972) could not detect Rubisco in the *Chlorobium* strain (Tassajara) used by Evans et al. (1966). Sirevåg (1974) found neither Rubisco nor phosphoribulokinase in strain 8327, and showed that aconitase and isocitrate dehydrogenase activities were adequate for the growth rate and were unaffected by acetate in the medium. Acetate added to a culture growing on thiosulfate plus CO_2 caused the growth rate to increase immediately to the new rate (Ormerod 1983), so obviously the enzymes were constitutively expressed at the maximum rate. Sirevåg (1975) incubated *Chlorobium* with labelled acetate and degraded by fermentation with yeast and *Zymomonas* the polyglucose produced. The results showed that glucose had been formed via pyruvate synthase and reversed glycolysis.

Three different green sulfur bacteria all reacted similarly to FAc (Sirevåg and Ormerod 1970a), indicating metabolic uniformity within the family. One of these was a fresh isolate from nature. The reader might be interested to learn how we took a sample from 6 m depth in the middle of a lake without using a boat. The meromictic lake just south of Oslo has a layer of green sulfur bacteria at a depth of 6 m; below this is H_2S-rich relict sea water. On a warm sunny day we performed this bizarre but enjoyable exercise by swimming out lying face down on inflatable mattresses with gear in tow, consisting of a sampler on a 6-m-long string. Back in the laboratory we isolated the green organism [probably *Pelodictyon luteolum* (Pfennig and Trüper 1971)] and examined its metabolism.

At one time it seemed as though succinate could be a direct carboxylation product (Sirevåg 1974). The obvious possibility was the propionate carboxylation reaction discovered in *Chlorobium* by Larsen (1951). This was tested for with N_2O, which inhibits vitamin B_{12} enzymes (Deacon et al. 1978). N_2O had no effect on either growth rate or CO_2 fixation rate, yet it eliminated the increase in growth rate and yield normally caused by added propionate (Ormerod, unpublished). Therefore, propionate carboxylation could not be part of the autotrophic CO_2 fixing mechanism in *Chlorobium*. However, later work in our lab was to implicate this reaction in CO_2 fixation in *Chloroflexus* (Holo 1989).

The N_2O experiments led to a serendipitous discovery that changed the focus of my own research for many years. The *Chlorobium* cultures containing N_2O grew at the normal rate but they were greyish yellow rather than green. N_2O was inhibiting BChl *d* synthesis, which requires B_{12} (Fuhrmann et al. 1993; Gough et al. 2000). Other anesthetic gases also inhibit BChl *d* synthesis (presumably for other reasons) and some of this work is described in Ormerod et al. (1990).

A short lived regression

As summarized above, the evidence in favor of the reductive citric acid cycle was mounting as we entered the 1970s. In the meantime, Tabita et al. (1974) reported the purification of Rubisco from *Chlorobium.* The activity was low, and the choice of experimental material could have been better – a cell paste, airmailed from Germany to the USA at ambient temperature. Buchanan and Sirevåg (1976) did a similar purification experiment with the same strain (Tassajara) with similar results, but found no labeled phosphoglycerate. Use of a fresh cell paste gave even lower activity. Takabe and Akazawa (1977) could not detect glycollate formation by *Chlorobium* in the presence of oxygen, nor was CO_2 fixation inhibited by cyanide. Possible differences in isotope discrimination due to different carboxylating enzymes were investigated by several groups, and all their results argued against Rubisco (Bondar et al. 1976; Quandt et al. 1977; Sirevåg et al. 1977).

An inhibitor study in Gerhardt Gottschalk's laboratory (Quandt et al. 1978) showed that glyoxylate inhibits CO_2 fixation in *Chlorobium*. Glyoxylate inhibits pyruvate synthase (Thauer et al. 1970). Surmising that acetate might be accumulating, I repeated some of Quandt et al.'s experiments and, using a gas chromatograph, found considerable acetic acid accumulation in the inhibited cultures, enough to account roughly for the drop in cell yield. (Ormerod 1980). My student Geir-Olav Fjeldheim and I also showed that *Chlorobium* growing in a thiosulfate/CO_2 medium without inhibitor secretes acetate to give just under 1 mm concentration (Fjeldheim 1982; Ormerod 1983).

Fuchs et al. (1980a) published experiments with *Chlorobium* cultures to which they added labeled propionate and degraded the protein amino acids to show unequivocally that all of the reactions of the reductive citric acid cycle must have been involved. With labeled

pyruvate (Fuchs et al. 1980b) the specific radioactivity of alanine and of glucose (from polyglucose) showed beyond doubt that hexosephosphate synthesis from CO_2 had proceeded via the pyruvate pool, not the RPPC. We seemed to be at the end of the road at last. There is, however, one more interesting turn to the story: two decades later a Rubisco-like gene was detected in the genome sequence of *Chlorobium tepidum* (Eisen et al. 2002).

Chloroflexus: rara avis

Richard Castenholz, the co-discoverer of *Chloroflexus* (Pierson and Castenholz 1974), spent a sabbatical year in our lab in 1977–1978 and worked with Sirevåg on C-metabolism in *C. aurantiacus* strain OK-70fl. Evidence was obtained for the involvement of the (oxidative) citric acid cycle in heterotrophic metabolism (Sirevåg and Castenholz 1979). It was important to find a good method of cultivating *Chloroflexus* autotrophically and Sirevåg established such a culture on H_2/CO_2 in a chemostat. She and her PhD student Helge Holo utilized this to investigate the CO_2-fixing mechanism (Holo and Sirevåg 1986). *Chloroflexus* is of ancient lineage (Fox et al. 1980), and is rather special because it has chlorosomes like *Chlorobium* yet its RC and physiology are like those of a purple bacterium. Tests for Rubisco and phosphoribulokinase were negative. Discrimination experiments with $^{13}CO_2$ also indicated the absence of the RPPC, while labeling with $^{14}CO_2$ gave amino acids as early products. Although FAc inhibited growth and caused citrate accumulation, showing that aconitase was being inhibited, CO_2 fixation was not inhibited since polyglucose synthesis took off at a very high rate. Thus, aconitase could not be involved in the CO_2 fixation. Some labeled compounds added in the presence of FAc contributed to polyglucose synthesis. Acetate was the best. Neither formate nor CO carbon was incorporated, thus eliminating the acetyl-CoA (C_1) mechanism (Wood et al. 1986). A tentative pathway was suggested, in which acetyl-CoA, formed in some unknown way, and pyruvate, occupied central positions (Holo and Sirevåg 1986).

At this stage, Sirevåg left on sabbatical leave. Since labeled alanine was an early product of $^{14}CO_2$ fixation (Holo and Sirevåg 1986), Holo and Grace (1987) used [3-^{13}C]-alanine in the presence of FAc to label polyglucose, which was analysed by NMR. Labeling was symmetrical indicating reverse glycolysis in the conversion of pyruvate to polyglucose. However, with labeled [^{13}C]-acetate the results were very different, indeed astounding – the acetate carboxyl C ended up mostly in C1 and C6 of glucose, while the acetate methyl carbon was mostly in C2 and C5 of glucose. Only about one-third of the polyglucose carbon was derived from acetate, the rest from CO_2. C1-labeled acetate was being formed from C2-labeled and *vice versa*. This indicated that a novel mechanism was operating. Eventually, Holo (1989) identified 3-hydroxypropionate in FAc treated cultures. The ^{13}C labeling of this intermediate led Holo to conclude that it was probably formed from acetyl-CoA via malonyl-CoA and malonaldehyde. Reduction to propionate and carboxylation would give methylmalonyl-CoA which was converted into two molecules of acetyl-CoA by a then unknown mechanism. Thus, Holo had laid the very substantial basis for a completely novel cycle involving reactions better known from fatty acid metabolism. It is interesting to follow Holo's reasoning concerning the switching of the acetate carbons during cycling. Switching occurs because it is the activated malonyl carboxyl-CoA (derived from acetyl carboxyl) that becomes reduced to a methyl group, which eventually forms the acetate methyl moiety.

Holo's PhD work was now finished. He relinquished the field leaving the cycle dangling like an almost ripe plum for any investigator with sufficient microbiological expertise to grow *Chloroflexus* autotrophically to follow up. This was done in immaculate fashion by Georg Fuchs and co-workers. Fuchs was Holo's external PhD thesis examiner. He and his group have in a series of papers verified most of Holo's work and added to it (Strauss et al. 1992; Eisenreich et al. 1993; Strauss and Fuchs 1993; Herter et al. 2001). Two novel, very interesting key enzymes have been characterized: malonyl-CoA reductase (Hugler et al. 2002) and propionyl-CoA synthetase (Alber and Fuchs 2002). There is also evidence that acetyl-CoA and glyoxylate are formed in the final step of the cycle (Herter et al. 2002). It was shown earlier (Løken and Sirevåg 1982) that *Chloroflexus* has the enzymes of the glyoxylate cycle. The hydroxypropionate cycle is also believed to be present in a number of archaea (Burton et al. 1999; Ishii et al. 1997; Ménendez et al. 1999). The cycle is apparently not present in all *Chloroflexus* strains. Russian scientists (Ivanovsky et al. 1993; Ugolkova and Ivanovsky 2000) have presented evidence for a different mechanism in strain B-3, involving pyruvate synthase. The pyruvate formed is converted into malate which then is

split to give acetyl-CoA and glyoxylate. This is then converted to phosphoglycerate by the serine pathway. A closely related organism, *Oscillochloris*, uses the RPPC (Ivanovsky 1999). Thus, the Chloroflexaceae show considerably more metabolic variation than the Chlorobiaceae.

The heliobacteria have so far not been grown autotrophically. Pickett et al (1994) detected pyruvate synthase and α-ketoglutarate synthase in cell free extracts, but not Rubisco, citrate lyase, aconitase or citrate synthase. Heliobacteria are strict anaerobes and have a PS I type RC (Madigan and Ormerod 1995).

General consideration of CO_2-fixing mechanisms at the start of the genome era

Microbiological knowledge has advanced so much that we can begin to explain why one or another CO_2-fixing mechanism is present in a particular organism. For *Chlorobium*, whose chief métier is growth at very low light intensities, the reductive citric acid cycle provides an economical way of exploiting the product (Fd_{red}) of the PS I type RC. The cycle supplies the most important, central biosynthetic metabolites directly, but it is dependent on a considerable pool of Fd_{red}. Therefore, it seems very likely that the oxidative version of the cycle evolved from the reductive when oxygen reared its ugly head in the biosphere.

For many years, I was intrigued by the fact that the RPPC is not found in strictly anaerobic bacteria while it occurs ubiquitously in oxygenic phototrophs. I discussed this some years ago with a polymath friend, Olav A. Christophersen, who has an interest in photosynthesis. Although Rubisco's oxygenase activity has long been assumed to have a protective function (Tolbert 1980), the real nature of this has not been clear. Christophersen (personal communication) has suggested that an essential function of the RPPC (linked to FNR) in oxygenic phototrophs is to act as a sink for Fd_{red} produced by the PS I RC. If this one-electron carrier accumulated it would react rapidly with oxygen to form damaging free radicals. Equally important is the fact that Fd_{red} removal continues by the agency of photorespiration even when the concentration of CO_2 is low. Otherwise the cells would risk damage each time they run out of CO_2. For this reason the RPPC is a *sine qua non* for oxygenic phototrophs. Indeed the Rubisco in oxygenic phototrophs with its double functions seems to be tailor-made for the purpose of fixing CO_2 in the presence of oxygen and Fd-reducing RCs. As a component of a CO_2-fixing mechanism it is not particularly efficient and would only have superseded other mechanisms when oxygen became a problem.

A glance at the literature shows that Rubisco has had a long and extremely complex evolution (see for example Watson and Tabita 1977) and we must assume that the present forms of the enzyme have evolved to cope with the conditions of the particular ecological niche of each organism. Therefore, attempts to genetically modify the enzyme's affinity for oxygen so as to enhance CO_2 fixation for environmental or agricultural purposes would at the present time seem fairly hopeless.

Purple bacteria probably use the RPPC because they move about in situations where oxygen can occur. During phototrophic growth under conditions that require Fd-coupled reactions, e.g., nitrogen fixation, the purple bacteria are strictly anaerobic. We must remember, however, that only a handful of purple bacteria have been examined in any detail. The situation with the Chloroflexaceae is also complex because so far three different mechanisms of autotrophic CO_2 fixation have been found there.

In view of the earlier Rubisco/*Chlorobium* controversy (see Buchanan and Arnon 1990), the finding of a Rubisco-like gene in *Chlorobium tepidum* (Eisen et al. 2002) was surprising, although similar genes have been found in anaerobic Archea, e.g., *Archaeoglobus* (Klenk et al. 1998). The fact that *Chlorobium* has this gene does not necessarily mean that it represents a primitive form of Rubisco. The gene was cloned and expressed in *E. coli* but the purified protein had no Rubisco activity (Hanson and Tabita 2001). The authors also described the inevitable, very interesting experiment of interrupting the gene and transferring it back by the natural transformation system present in *Chlorobium*. Such 'knockout' cells grew more slowly, accumulated more S^0 than the control, and contained less BChl *c*. Hanson and Tabita (2001) suggested that the Rubisco-like protein is involved in oxidative stress responses and/or sulfur metabolism.

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