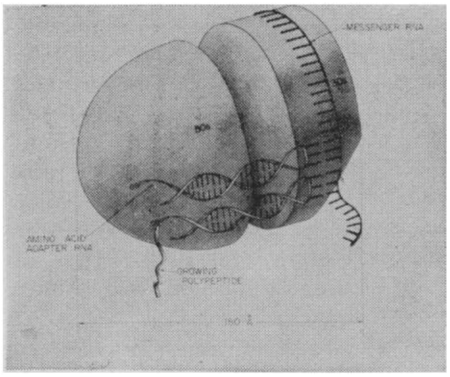

Fig. 4. Model of a 70*S* ribosome to demonstrate the role of the ribosome in protein synthesis. The ribosome provides a stabilizing surface for maintaining the messenger RNA in a configuration that would be thermodynamically unstable in solution and for maintaining the reacting adapter molecules in parallel register. Only part of the messenger is bound at one time. The dimensions of the ribosome were determined from electron microscopy.

there is something additional holding them together. (ii) Messenger RNA is dissociable from the ribosome, and ribosomes may associate with more than one messenger RNA with varying molecular weight (*11, 12*). (iii) The lifetime of the ribosome in bacteria is much greater than that of the messenger RNA (*22*). (iv) The 30*S* subunit and the 70*S* particle have been shown (*23*) to form a complex with synthetic messenger RNA under conditions where the 50*S* subunit does not. (v) The 30*S* subunit in *E. coli* contains a latent ribonuclease (*24*). (vi) In reticulocytes where the messenger RNA does not turn over, there is no evidence for a latent structural RNAse on the ribosome (*4*). (vii) Observations iii to vi support the idea that messenger RNA is bound to the 30*S* subunit.

The site of binding to the ribosome must allow the messenger RNA's of various size to be accommodated. In most cases, messenger RNA molecules would be too large for the entire molecule to be in direct contact with the ribosome simultaneously. Probably only part of the messenger is bound at one time, as is suggested in Fig. 4. Adjacently adsorbed adapters might, for greater stabilization, be adsorbed to the outer surface of the 50*S* particle, and the polypeptide synthesis would thus be more closely associated with the 50*S* ribosome. The function of the ribosome in this model is to provide a "stabilizing surface" for maintaining the messenger RNA in a configuration that would be thermodynamically unstable in solution and for maintaining the reacting adapter molecules in parallel register. The 50*S* and 30*S* subunits in the active particle would be more firmly held together than in the inactive 70*S* ribosome by virtue of the linked-adapter RNA which is connected to both the growing end of the polypeptide chain and the messenger RNA.

I have attempted to link structural and biochemical information into one coherent pattern, and to utilize the resulting knowledge in the production of a stereochemically sound model for the template mechanism in protein synthesis (*25*).

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8 May 1963

Photosynthetic Mutants Separate Electron Paramagnetic Resonance Signals of Scenedesmus

Abstract. *Current schemes for the mechanism of the photosynthesis reaction imply a two-part cycle; one part produces a strong photoreductant, the other functions to release molecular oxygen. Wild-type* Scenedesmus *exhibits the two light-induced electron-paramagnetic resonances typical of green algae and chloroplasts of higher plants. These resonances indicate the presence of unpaired electrons in at least two sites. By means of mutants which are blocked in one or the other part of the dual cycle, the narrow, rapidly decaying signal can be correlated with the photoreductant part, and the broad, slowly decaying signal can be correlated with the part releasing molecular oxygen.*

Most current schemes for the photosynthesis reaction imply the cooperation of two more or less separate photochemical reactions. One of these is believed to produce a strong photoreductant while the other is functional in the release of molecular oxygen (*1*). Since in such systems there is necessarily the transfer of electrons, the participation of free radicals in the overall process is to be expected. The occurrence of photoinduced unpaired electrons has been amply demonstrated (*2–4*) by means of electron paramagnetic resonance (EPR) spectroscopy. At least two separate light-induced signals may be observed in algal species and chloroplasts; these signals may be differentiated on the basis of their kinetics, form, and position in the electromagnetic spectrum. There has been, however, no rigorous demonstration of a relationship between these free radicals and photosynthesis as it occurs in natural systems. We now present evidence that the narrow, rapidly decaying (*R*) signal, with a *g*-value (*5*) of 2.0025, is correlated with the CO_2-fixing portion of the photosynthetic cycle, and that the broad, structured, slowly decaying (*S*) signal is visible evidence of the events leading to O_2 production.

An almost complete separation of the two signals, heretofore observed only in combination in intact systems, has been achieved by use of two classes of photosynthetic mutants of the green alga *Scenedesmus obliquus*. The photochemical characteristics of one of these

Table 1. Rates of photosynthesis, photoreduction, and the Hill reaction of heterotrophically grown cultures of normal and mutated *Scenedesmus* (ScD_3). Photosynthesis and photoreduction were measured manometrically at 25°C in 0.05*M* phosphate buffer, *p*H 6.5. The gas phase for photosynthesis was air containing 4 percent CO_2, for photoreduction H_2 containing 4 percent CO_2. A suspension of 25 μl cells in 3 ml was used in all experiments. The light was white (saturating). Maximum photoreduction rates for normal algae were determined in the presence of 2×10^{-6} *M* DCMU. The Hill reaction was determined in N_2 containing 4 percent CO_2 with saturating red light. One milligram of *p*-benzoquinone was added in final volume of 3 ml. Each value represents an average of three or more determinations. Chl, chlorophyll.

Algal strain ScD_3	Oxygen (μl hr^{-1})				Carbon dioxide (μl hr^{-1})	
	Photosynthesis		Hill reaction		Photoreduction	
	per μl cells	per mg Chl	per μl cells	per mg Chl	per μl cells	per mg Chl
Control	7.8	444.4	6.7	406	2.4	143.0
Mutant No. 8	0.6	15.3	2.0	200	.1	8.1
Mutant No. 40	0.2	16.3	.6	44	2.4	167.1

has been reported (*6*). These mutants have no pigment defect; they possess a normal concentration of chlorophylls, carotenes, and carotenoids. They have been characterized further as either "CO_2" mutants—strains unable to assimilate carbon dioxide in normal photosynthesis or photoreduction, but able to evolve oxygen in the quinone-Hill reaction—or "O_2" mutants which participate in neither the Hill reaction nor photosynthesis but assimilate carbon dioxide in a normal fashion during photoreduction. The photochemical characteristics of an "oxygen" and a "carbon dioxide" mutant are compared to those of the parent strain in Table 1.

Electron paramagnetic resonance studies on suspensions of intact cells of these two classes of mutants have revealed a striking distinction between them. The oxygen mutant shows characteristically only the narrow, rapid (*R*) signal, and lacks all but a trace of the broad, slow (*S*) signal. A typical set of traces made without and with illumination is shown in Fig. 1 (*a* and *b*) in comparison with a suspension of wild-type cells of equal cell concentration handled in precisely the same manner (Fig. 1, *c* and *d*). When the magnetic field is fixed at the point of maximum recorder deflection, and the light is turned on and off, the rise time of this signal to its maximum value is practically instantaneous; it has a very small slow component, in contrast to the behavior of the wild type (Fig. 2).

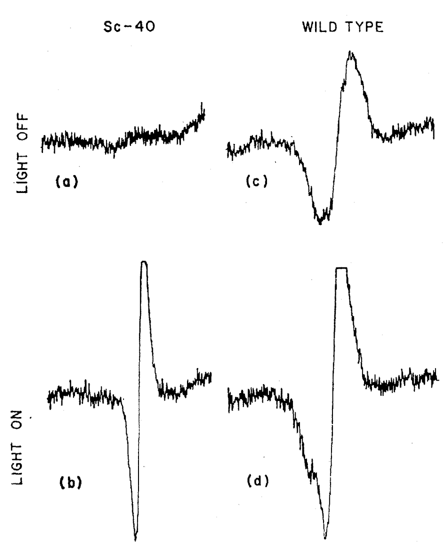

Fig. 1. (*a*) Electron paramagnetic resonance spectrum from a suspension of an "O_2" mutant of *Scenedesmus* (No. 40) in the absence of illumination, illustrating the absence of the prominent *S* signal present in the wild type (*c*). (*b*) The narrow *R* signal with illumination (700 mμ) also observable in wild type (*d*) but superimposed on the persistant *S* signal. All tracings made with suspensions containing 6.6 $\times 10^8$ cells per milliliter and with identical instrumental parameters.

Suspensions of mutant 8, a CO_2 mutant, do not show the *R* signal at all and yield only the broad, slow signal, even under intense white-light illumination. This signal has the same characteristics as that of the normal alga in the absence of illumination but with an amplitude about one-half that of the *S* signal of wild type at equal cell concentrations. This is not inconsistent with the data in Table 1, which reveal a Hill reactivity, calculated per cell, in the wild type about three times that of mutant 8. This total lack of the *R* signal in an organism that has quantities of chlorophyll, both *a* and *b*, as well as the other wild-type pigments, is particularly striking since published observations have been consistent with a 1:1 correlation of an *R* signal (or one very similar to it) and chlorophyll, whether or not the chlorophyll was in an intact cell, in a chloroplast, in solution, dried, lyophilized, at room temperature or higher, or at liquid-nitrogen temperatures (*2–4, 7*). Our observation on mutant 8 has been repeated numerous times under conditions of high instrumental sensitivity. Among three similar "CO_2" mutants, only No. 8 completely lacks the *R* signal. A second one, No. 18, has a very slight *R* signal with illumination, and a third, No. 17, has an *R* signal of the same order of magnitude as wild type, despite its inability to reduce CO_2. Therefore, loss of *R* signal may indicate loss of ability to reduce CO_2 but the converse is not true (Fig. 3).

Our evidence, derived from three independently isolated "O_2" mutants, strongly supports the conclusion that the *S* signal can be correlated with the oxygen-evolving ability of the cell. Strains of *Chlamydomonas* with impaired Hill activity have also displayed a reduced *S* signal (*8*). Other observations are in harmony with this concept; the earliest was that photosynthetic bacteria, which are unable to evolve oxygen, have an apparently typical *R* signal but no *S* signal (*9*). Manganese-deficient cultures, although retaining all pigments, are unable to evolve oxygen and lack the *S* signal but display a prominent *R* signal (*10*); they strikingly resemble the "O_2" mutants in their

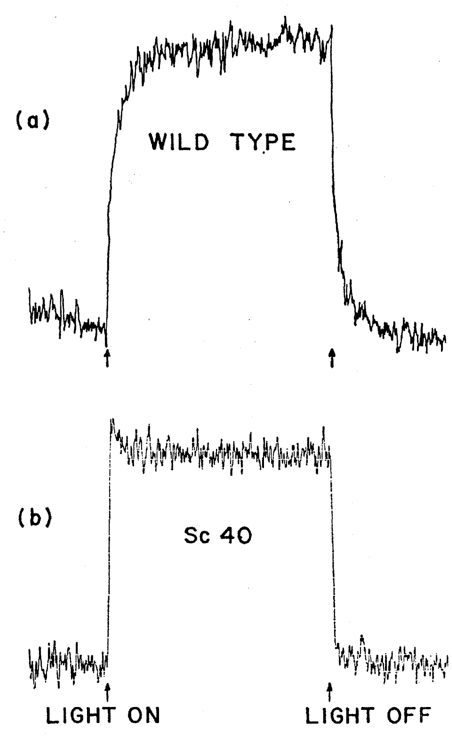

Fig. 2. (*a*) Rise and decay curves for wild-type *Scenedesmus*. These were made by fixing the magnetic field at the point of maximum deflection and turning the light (700 mμ) on and off. Both rise and decay have fast and slow components. (*b*) Similar trace made with an "O_2" mutant, Sc 40. Note absence of the slow component. These comparisons are qualitative, and were made with similar but not identical concentrations of cells.

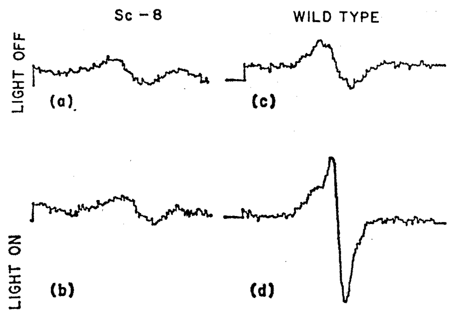

Fig. 3. (*a*) Electron paramagnetic resonance spectrum from a suspension of a "CO_2" mutant (No. 8) in the absence of illumination. (*b*) Spectrum from the same suspension under white light illumination, illustrating lack of *R* signal present in a wild-type suspension under illumination (*d*). (*c*) Spectrum of *S* signal from wild-type cells; the larger amplitude reflects the greater Hill activity of wild type (see Table 1). All tracings are made with suspensions containing 5×10^8 cells per milliliter and with identical instrument parameters.

EPR behavior. Washed chloroplasts show a decreased *S* signal (*2, 4, 11*), and this may be correlated with the decline in Hill activity brought about by such treatment. However, hydrogen-adapted wild-type *Scenedesmus,* stabilized (*6*) with 3(3,4-dichlorophenyl)-1-1-dimethylurea (DCMU) possess a prominent *S* signal despite the fact that they are evolving no oxygen (*12*). Thus, the block imposed by DCMU occurs nearer the point of oxygen release than that brought about by manganese deficiency or by any of the mutants yet examined, and agrees with an earlier conclusion of Bishop (*13*).

The semiquinone of plastoquinone has been suggested as the organic free radical responsible for the slow signal (*9, 14*). Mutant 11 contains plastoquinone in normal amounts, and manganese deficiency does not alter the content of plastoquinone in wild-type or mutant cells. It is possible that manganese deficiency, thorough washing of chloroplasts, or whatever change it is that produces the "O_2" mutants, while not removing or preventing the formation of quinone, interferes with its oxidation or reduction or both, and therefore prevents the formation of a steady-state semiquinone, which is the free radical observable by EPR spectroscopy (*15*). Perhaps some water-soluble compound, dependent on manganese for its synthesis, is a necessary link in the electron pathway. The structural integrity of the intact system may also have been altered, since treatment of *Chlorella* with ultrasound abolishes the *S* signal, although the cell material was not fractionated (*16*).

The *R* signal has been assumed to be a free radical form of chlorophyll or of some component intimately and invariably associated with the primary light acceptor. Its absence in cells that have large amounts of the known photosynthetic pigments implies that an electron acceptor or donor which may not yet be identified is required for the production of the *R* signal. The *R* signal may be due to the photooxidized form of a pigment absorbing at about 700 mμ (P700) which, although it has not been isolated or further characterized, is believed to act as the terminal energy collector in photosynthesis by accepting energy from an excited state of chlorophyll (*17*). One possible explanation for the lack of *R* signal in mutant 8 is that it lacks P700. Another possibility is that the structure of the chloroplast is faulty, and that the electron evident as the *R* signal is not in this mutant, separated from the chlorophyll molecule.

We have reported the separation of the two typical photoinduced EPR signals in algae by the use of mutant strains blocked in different portions of the photosynthetic cycle, and made some guesses on the possible explanation for the observations. When the explanations can be offered on a solid basis, they should contribute to the understanding of the overall process (*18*).

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Organized Element: Possible Indentification in Orgueil Meteorite

Abstract. *Ragweed pollen stained by the Gridley method becomes distorted so that it resembles Claus and Nagy's Type 5 organized element, a particle found in a Gridley-stained preparation of the Orgueil carbonaceous chondrite.*

One of the most striking apparently biogenic particles reported from carbonaceous chondrites is the "Type-5 organized element" (*1*). It is hexagonal, with tubular protrusions extending from alternate walls, and is surrounded by a clear halo. It seems to be quite rare. Claus and Nagy (*2*) found only two such particles (along with several fragments) in Orgueil, and four in Tonk. One of these particles has been illustrated in the literature (*1–3*); this is the only particle which we were able to examine personally and to photograph in Dr. Claus' laboratory (Fig. 1). It appears on a slide stained with the Gridley method, and is orange-brown in color.

Claus and Nagy pointed out that this particle "is entirely dissimilar in its morphology to known terrestrial form[s]" (*1*), and suggested that it be recognized as an extraterrestrial genus, *Daidaphore berzelii* (*2*). The tubular projections were stated to contain up to 50 filaments (*2*). Mueller, on the other hand, has suggested that this particle is a limonite pseudomorph after troilite (*4*). Orange, hexagonal mineral grains do occur in Type 1 carbonaceous