while the mechanism that produces the residual recombination is unaffected. The *rec*-1 mutation has an effect similar to that of *rec*-13.

The results suggest that the ultraviolet-stimulated recombination mechanism is identical with the primary mechanism that operates for nonirradiated phage. However, it is conceivable that the two mechanisms might not be completely identical, although it is clear that they must share at least one step in common, namely, the step blocked by the *rec*-1 and *rec*-13 mutations.

The primary mechanism for S13 recombination is not eliminated in all Rec$^-$ hosts. In the Rec$_1^-$ strain AB2470.5 the amount of phage recombination is approximately the same as that measured in the parental Rec$^+$ strain (Table 2). Thus the *rec*-21 mutation in AB2470.5, although it decreases bacterial recombination substantially (*3, 7*), has no significant effect on the primary mechanism of S13 recombination. The *rec*-21 mutation might possibly affect the secondary recombination mechanism, but this cannot be ascertained as long as the predominant primary mechanism is functioning.

IRWIN TESSMAN

Department of Biological Sciences, Purdue University, Lafayette, Indiana

References and Notes

1. I. Tessman, *Biochem. Biophys. Res. Commun.* **22**, 169 (1966).
2. A. J. Clark and A. D. Margulies, *Proc. Nat. Acad. Sci. U.S.* **53**, 451 (1965).
3. A. J. Clark, *J. Cell. Physiol.* **70**, Suppl. 1, 165 (1967).
4. P. van de Putte, H. Zwenk, A. Rörsch, *Mutation Res.* **3**, 381 (1966).
5. F. Jacob and E. L. Wollman, *Ann. Inst. Pasteur* **88**, 724 (1955).
6. P. Howard-Flanders and L. Theriot, *Genetics* **53**, 1137 (1966).
7. ———, J. B. Stedeford, *Biophys. J.* **6**, TC3 (Abstract) (1966); P. Howard-Flanders and and R. P. Boyce, *Radiation Res. Suppl.* **6**, 156 (1966).
8. A. J. Clark, M. Chamberlin, R. P. Boyce, P. Howard-Flanders, *J. Mol. Biol.* **19**, 442 (1966).
9. E. S. Tessman, *Virology* **25**, 303 (1965); R. Baker and I. Tessman, *Proc. Nat. Acad. Sci. U.S.* **58**, 1438 (1967).
10. I am grateful to Drs. R. P. Boyce, A. J. Clark, and P. Howard-Flanders for providing the bacterial mutants listed in Table 1, and to Dr. E. S. Tessman for criticism of the manuscript. Supported in part by NSF research grants GB-2748 and GB-7714X.

22 April 1968

Molecular Diversity of the Ribulose-1,5-Diphosphate Carboxylase from Photosynthetic Microorganisms

Abstract. *The ribulose-1,5-diphosphate carboxylases from green and blue-green algae and the purple sulfur photosynthetic bacterium* Chromatium *are proteins with high molecular weights and with sedimentation coefficients of 18 to 21 Svedberg units. The carboxylases of the Athiorhodaceae are smaller, that of* Rhodospirillum rubrum *being a 6.2*S *molecule, and those of the two species of* Rhodopseudomonas *are 12*S *and 14.5*S.

A large portion of the soluble protein of chloroplasts from higher green plants consists of a protein of high molecular weight with a sedimentation coefficient (s_{20}) of 18 to 21 Svedberg units (*S*), designated fraction I (*1, 2, 3*). Lyttleton and Ts'o (*2*) have reported that fraction I isolated by differential centrifugation contains very high concentrations of the central enzyme of the reductive pentose phosphate cycle, ribulose-1,5-diphosphate carboxylase [3-phospho-D-glycerate carboxylyase (dimerizing) E.C. 4.1.1.39]. This observation has been confirmed by Mendiola and Akazawa (*4*) with fraction I from rice leaves purified by starch-gel and Sephadex filtration. These authors found ribose-5-phosphate isomerase activity and ribulose-5-phosphate kinase activity, as well as that of ribulose-1,5-diphosphate carboxylase, associated with fraction I.

Trown (*5*) and Thornber, Ridley, and Baily (*6*) have isolated ribulose-1,5-diphosphate carboxylase, which is physically similar to fraction I, free of the isomerase and kinase activity. Whether or not these three enzymes are associated in vivo, there is no doubt that fraction I is associated with the photosynthetic apparatus in higher green plants.

The purpose of the present experiments was to ascertain whether fraction I or some other protein component with carboxylase activity could be observed in extracts of the green alga *Euglena gracilis*, an organism that contains chloroplasts, and in extracts of the procaryotic blue-green alga *Anacystis nidulans*. In addition, we compared the properties of the carboxylases of the photosynthetic bacteria *Rhodospirillum rubrum, Rhodopseudomonas spheroides, Rhodopseudomonas palustris*, and *Chromatium*, organisms that contain ribulose-1,5-diphosphate carboxylase but do not have the eucaryotic cell structure.

Euglena gracilis strain Z was grown under photoautotrophic conditions in a mixture of CO_2 (5 percent) and air on the medium of Hutner, Bach, and Ross (*7*) and was also grown in the dark on *Euglena* broth (Difco). *Anacystis nidulans* was grown under photoautotrophic conditions, in an atmosphere of 5 percent CO_2 in air, on medium D of Kratz and Myers (*8*). *Chromatium* strain D, a purple sulfur photosynthetic bacterium, was cultured on $NaHCO_3$, $(NH_4)_2SO_4$, and $Na_2S_2O_3$ (*9*). The nonsulfur photosynthetic bacterium *Rhodospirillum rubrum*, strain S-1, was grown under completely autotrophic conditions, with continuous gassing with 1 percent CO_2 and 99 percent H_2, on a basal salts medium (*9*) containing 2.5 m*M* $(NH_4)_2SO_4$. *Rhodopseudomonas spheroides* was grown similarly, with 10 m*M* $(NH_4)_2SO_4$ as the nitrogen source and with 1 mg of nicotinic acid and of thiamine per liter; and *Rhodopseudomonas palustris*, with 5 mg of *p*-aminobenzoic acid per liter added. Concentrations of CO_2 were selected to give optimum growth. No attempt was made to determine optimum CO_2 concentration for formation of ribulose-1,5-diphosphate carboxylase in the organisms we studied.

Extracts were prepared as previously described (*10*) and centrifuged for 2 hours at 100,000*g* to remove cellular particulates prior to analytical ultracentrifugation and for 15 minutes at 270,000*g* prior to sucrose density-gradient analysis. *Rhodospirillum rubrum* ribulose-1,5-diphosphate carboxylase was further purified through the ammonium sulfate–precipitation step prior to sucrose density-gradient analysis (*11*); the same relative sedimentation value was obtained with a crude extract. The relative sedimentation values of carboxylases from all sources were determined by the density-gradient method of Martin and Ames (*12*). The gradients were 10 m*M* in potassium phosphate at *p*H 7.9, 10 m*M* in $MgCl_2$, 100 μM in dithioerythritol, and linear, between 5 and 20 percent, in sucrose. An SW 39 or SW 65 rotor in a Beckman model L preparative ultracentrifuge was used for the analysis. For low-speed runs (22,000 to 50,000 rev/min) 100 to 200 μl of extract was layered onto the gradient, and for high-speed runs (65,000 rev/min) up to 300 μl of extract was used. Gradients were centrifuged at 4°C for a period of time sufficient to move the marker and the carboxylase a distance

Table 1. Relative sedimentation values for ribulose-1,5-diphosphate carboxylases from various photosynthetic organisms. Values, obtained as described in the text, are the average of at least two determinations with extracts from different cultures of cells.

Organism	s_{rel}	Approximate molecular weight
Rhodospirillum rubrum	6.2	120,000*
Rhodopseudomonas palustris	12	3 × 120,000†
Rhodospeudomonas spheroides	14.5	
Chromatium, strain D	18	5.5 × 120,000†
Anacystis nidulans	19.5	
Euglena gracilis	19	

* Based on s_{rel} value and Stokes radius determination by Sephadex-gel filtration. Calculated by using an assumed partial specific volume according to Siegel and Monty (*20*). † Approximated from $s_1/s_2 = (mw_1/mw_2)^{\frac{2}{3}}$, using the s_{rel} and molecular weight values for the *R. rubrum* enzyme. This approximation assumes spherical molecules which, of course, may not be the case. The molecular weight of the higher plant carboxylase is 557,000 (*18*).

not more than 3/4 nor less than 1/4 the length of the gradient. Fractions (0.1 ml) were collected and assayed for activity. Peak fractions contained enough activity to form at least 5 nmole of 3-phosphoglyceric acid per minute in the ribulose-1,5-diphosphate carboxylase assay. Catalase, 11.3*S* (*12*), was used as a marker except that glyceraldehyde-3-phosphate dehydrogenase, 7.7*S* (*13*), was used with the enzyme from *R. rubrum*. Ribulose-1,5-diphosphate carboxylase was assayed by means of the spectrophotometric assay of Racker (*14*), glyceraldehyde-3-phosphate dehydrogenase by the method of Wu and Racker (*15*), and catalase as described by Martin and Ames (*12*). Protein was estimated by the method of Warburg and Christian (see *16*).

Inspection of the schlieren patterns obtained during centrifugation of extracts from *Euglena* grown under photosynthetic conditions reveals the presence of an apparent 18*S* peak, but such a peak is not observed in extracts from the *Euglena* grown in the dark (Fig. 1, a and b). This peak appears to be similar, if not identical, to the 18*S* peak observed in extracts of higher green plants designated chloroplast fraction I. The relative sedimentation value for ribulose-1,5-diphosphate carboxylase from *Euglena* was 19*S* (Table 1), in agreement with the value of the fraction I peak. Therefore, similar to fraction I of higher plants, fraction I of the alga *Euglena* has ribulose-1,5-diphosphate carboxylase activity.

Like *Euglena* and higher plants, the blue-green alga *Anacystis nidulans* has a 19.5*S* ribulose-1,5-diphosphate carboxylase (Table 1). These procaryotic algae do not contain chloroplasts; and thus fraction I (or 18*S* to 21*S* ribulose-1,5-diphosphate carboxylase) is not uniquely associated with the chloroplast structure.

Schlieren diagrams of extracts of the purple bacterium *Chromatium* also show a 19.7*S* peak that corresponds to chloroplast fraction I (Fig. 1c). The relative sedimentation value of the ribulose-1,5-diphosphate carboxylase from this bacterium is also 18*S*. This, apparently, is a "bacterial fraction I" with ribulose-1,5-diphosphate carboxylase activity. Thus fraction I is characteristic not only of higher plants and of procaryotic and eucaryotic algae but also of a photosynthetic sulfur bacterium.

In sharp contrast, two distinctly different ribulose-1,5-diphosphate carboxylases are found in a third, and possibly more primitive, group of photosynthetic bacteria, the Athiorhodaceae. The carboxylases from *R. palustris* and *R. spheroides* are 12*S* and 14.5*S* molecules (Table 1). It is interesting that the dimers of 12*S* and 14.5*S* proteins are 17*S* and 20*S* molecules. One explanation of these data could be that the 18*S* to 21*S* enzyme found in higher plants, algae, and *Chromatium* is a stable dimer of the carboxylase found in the rhodopseudomonads. It is apparent, in any case, that fraction I (or 18*S* to 21*S* ribulose-1,5-diphosphate carboxylase) is not characteristic of all photosynthetic organisms.

Neither an 18*S* to 21*S* fraction nor a 12*S* to 14.5*S* fraction was found in extracts of *Rhodospirillum rubrum* (Fig. 1d). In contrast, the ribulose-1,5-diphosphate carboxylase from *R. rubrum* was 6.2*S* (Table 1). This carboxylase is thus physically distinct from both fraction I and from the carboxylase of *Rhodopseudomonas spheroides* and *R. palustris*.

Like the plant enzyme, the carboxylase from *R. rubrum* has a low affinity for CO_2 (K_m, 12.5 m*M*) and a higher affinity for ribulose-1,5-diphosphate (K_m, 8×10^{-2} m*M*) (*17*). The respective K_m's for the plant enzyme are 22 and 1.2×10^{-1} m*M* (*18*). Unlike the plant enzyme, however, the enzyme from *R. rubrum* does not require the addition of a divalent cation for activity. Per mole of enzyme, the turnover number for the *R. rubrum* enzyme is roughly half that for the plant enzyme (*17*). The *R. rubrum* enzyme is thus more efficient in terms of size than the carboxylase found in higher plants, but half as efficient per mole of enzyme.

It is not clear whether the purple, nonsulfur, photosynthetic bacterium *R. rubrum* contains a carboxylase that is an evolutionary precursor of the 14*S* or 18*S* carboxylase, or one that is a different enzyme.

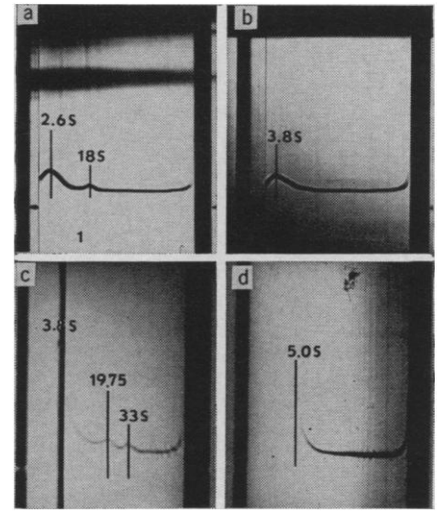

Fig. 1. Ultracentrifuge diagrams of extracts from microorganisms. (a) Autotrophically cultured *Euglena*. The single sector cell contained 8.5 mg of protein per milliliter in 0.1*M* tris-HCl, *p*H 8.5. An AnD rotor was used at 50,740 rev/min. Temperature during the run was 20°C. The faster moving component is 18*S*, the slower moving component 2.6*S*. The 18*S* peak corresponds to the 18*S* peak observed in higher plant extracts, which has been designated fraction I. Photograph was taken approximately 17 minutes after reaching speed and at a bar angle of 70 deg. (b) Dark, heterotrophically cultured *Euglena*; the cell contained 12.5 mg of protein per milliliter in 0.1*M* tris-HCl buffer; 19°C; peak is 3.8*S*. Photograph was taken 19 minutes after reaching speed. There is no peak corresponding to fraction I. Ribulose-1,5-diphosphate carboxylase activity is nil in dark-gorwn *Euglena* (*21*). (c) *Chromatium*. The cell contained 33 mg of protein per milliliter in 0.1*M* tris-HCl. Temperature during the run was 20°C. The two faster moving components were 19.7*S* (corresponding to fraction I) and 33*S*, respectively; the main component was 3.8*S*. Photograph was taken approximately 34 minutes after reaching speed; 38 μmole of phosphoglyceric acid per gram of protein per minute were produced in the assay for ribulose-1,5-diphosphate carboxylase. (d) *Rhodospirillum rubrum*. The cell contained 10.2 mg of protein per milliliter in 0.1*M* tris-HCl, *p*H 8.5; temperature, 16°C; the peak is 5.0*S*. Clearly there is no 18*S* peak corresponding to fraction I. Photograph was taken approximately 34 minutes after reaching speed and at a bar angle of 70 deg. The extract produced 499 μmole of phosphoglyceric acid per gram of protein per minute in the assay for ribulose-1,5-diphosphate carboxylase.

Although we do not know the complete details of the mechanism of the enzyme-catalyzed reaction of CO_2 with ribulose-1,5-diphosphate yielding two moles of 3-phosphoglyceric acid (*19*), we can assume that the overall reaction is similar, if not identical, in the many organisms that carry out photosynthesis. We are, therefore, faced with a situation in which the same or a very similar reaction is catalyzed by an enzyme in at least three quite different molecular forms. Additional experiments are required to determine whether the three carboxylases (6*S*, 13*S*, and 18*S*) are indeed chemically similar or chemically unrelated proteins.

LOUISE E. ANDERSON
G. B. PRICE
R. C. FULLER

Biology Division, Oak Ridge National Laboratory, and *University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge, Tennessee 37830*

References and Notes

1. S. J. Singer, L. Eggman, J. M. Campbell, S. G. Wildman, *J. Biol. Chem.* **197**, 233 (1952).
2. J. W. Lyttleton and P. O. P. Ts'o, *Arch. Biochem. Biophys.* **73**, 120 (1958).
3. N. G. Pon, *ibid.* **119**, 179 (1967).
4. L. Mendiola and T. Akazawa, *Biochemistry* **3**, 174 (1964).
5. P. W. Trown, *ibid.* **4**, 908 (1965).
6. J. P. Thornber, S. M. Ridley, J. L. Bailey, in *Biochemistry of Chloroplasts*, T. W. Goodwin, Ed. (Academic Press, London, 1966), vol. 1, pp. 275–284.
7. S. H. Hutner, M. K. Bach, G. I. M. Ross, *J. Protozool.* **3**, 101 (1956).
8. W. A. Kratz and J. Meyers, *Amer. J. Bot.* **42**, 282 (1955).
9. S. K. Bose, in *Bacterial Photosynthesis*, H. Gest *et al.*, Eds. (Antioch Press, Yellow Springs, Ohio, 1963), pp. 501–510.
10. L. Anderson and R. C. Fuller, *Plant Physiol.* **42**, 497 (1967).
11. L. Anderson, L. E. Worthen, R. C. Fuller, *Federation Proc.* **26**, 725 (1967).
12. R. G. Martin and B. N. Ames, *J. Biol. Chem.* **236**, 1372 (1961).
13. J. B. Fox, Jr., and W. B. Dandliker, *ibid.* **218**, 53 (1956).
14. E. Racker, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1962), vol. 5, pp. 266–270.
15. R. Wu and E. Racker, *J. Biol. Chem.* **234**, 1029 (1959).
16. E. Layne, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1957), vol. 3, pp. 447–454.
17. L. Anderson, unpublished observations.
18. J. M. Paulsen and M. D. Lane, *Biochemistry* **5**, 2350 (1966).
19. Some of the details of the mechanism of the reaction catalyzed by the plant enzyme have been elucidated by G. Müllhofer and I. A. Rose [*J. Biol. Chem.* **240**, 1341 (1965)] and by F. Fiedler, G. Müllhofer, A. Trebst, I. A. Rose [*European J. Biochem.* **1**, 395 (1967)].
20. L. M. Siegel and K. J. Monty, *Biochim. Biophys. Acta* **112**, 346 (1966).
21. R. C. Fuller and M. Gibbs, *Plant Physiol.* **34**, 324 (1959).
22. We thank P. von Hippel for encouragement and advice, K. Y. Wong for aid in the use of analytical ultracentrifuge, and L. Worthen and N. Rigopoulos for technical assistance. A portion of this work was done at Dartmouth Medical School supported by contract AT (30-1) 2801 from AEC and grant GM 10705 from PHS; and at Oak Ridge National Laboratory supported by AEC under contract with the Union Carbide Corporation.

21 May 1968

Sensors Monitor Tensions in Transpiration Streams of Trees

Abstract. *Resistance of implanted sensors fluctuated predictably with uptakes and loss of water by plants, and correlated positively with coincidental measurements of leaf-water potential when transpiration was minimized. Preliminary results indicate that transmission of change in tension may be almost instantaneous, regardless of transpirational flow rates. Rates of relaxation of tension were very rapid after precipitation, but only after water had percolated to root surfaces.*

According to the transpiration-cohesion theory of the ascent of (xylem) sap, the sap stream should be subjected to varying tension that depends on relative rates of water absorption and transpiration. I now describe sensors that permit continuous monitoring of stream tension in woody plants.

Each sensor consisted of a pair of stainless steel electrodes, spaced by fiberglass wrapping around one of them, embedded in a gypsum (plaster-of-Paris) cylinder (Fig. 1). Both ends of each sensor were "sealed" and strengthened with epoxy resin, and short leads were soldered to the electrodes before implantation of the sensor.

The amount of water in such a sensor depends, of course, on the relative water potential (ψ) of moisture in its environment; thus sensor resistance is determined by this volume of water and the concentration of electrolytes in solution. Since the concentration of sap solutes does not fluctuate greatly—over short periods, at least—and since the $CaSO_4$ component would quickly saturate any water held in the sensor matrix, changes in sensor resistance are thought to reflect primarily changes in the volume of water held. The xylem matric ψ should be relatively constant, so that changes in external ψ must be due to changes in tension. Thus change in sensor resistance is a result of change in sap tension.

Sites for implantation of sensors were selected on stems, branches, and shallow roots that were at least 2 cm in diameter and not exposed to direct sunlight. The bark was removed from a small area, and a hole slightly larger in diameter than the dry sensor was bored in the wood to sufficient depth to accommodate the entire length of the gypsum cylinder. Sensors were then gently inserted, and the outer tissue that had been removed was replaced with putty; only the sensor leads protruded from the plant. Sensor resistance was then measured with either portable a-c resistance bridges or (for the most part) a Bristole Dynomatic high-resistance recorder; the latter permitted automatic monitoring of from 1 to 24 sensor points, with intervals of 10 to 15 seconds between consecutive readings.

Implantation obviously disrupted the transpiration stream in the area of the wound. After implantation, however, the gypsum swelled markedly when it absorbed moisture from the wood. The ensuing high degree of contact between sensor and tissue resulted in formation of a continuous liquid-water phase between the sensor and adjacent intact portions of the stream. Such moisture "bridges" probably account for the great sensitivity of the sensors, because of the exchange of water by bulk flow (*1*).

The amount of contact between sensor and stream, however, differed between implants, so that any preinsertion standardization or calibration of readings was precluded. This problem was overcome by correlation of sensor readings with pressure-bomb estimates of leaf ψ (*2*) during relatively static transpirational conditions. Tensions under such conditions should be equivalent to the vector sum of forces in any one direction.

I produced static conditions in saplings growing in pots by placing them in darkness long enough (usually overnight) to minimize transpiration. Tensions were varied in these trees by drying or wetting of the soil to different degrees. Satisfactory localized "static" conditions were also produced by enclosure, in opaque black plastic bags for a sufficient length of time, of only the foliage to be sampled (*3*). Saplings used included sour cherry (*Prunus cerasus* L. var. Montmorency) and two species of *Eucalyptus*.

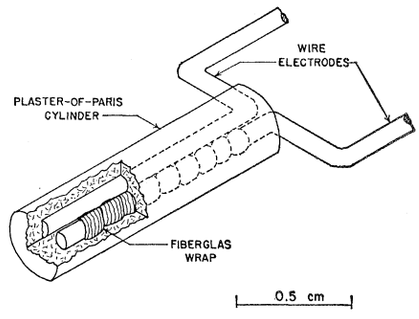

Fig. 1. Diagram of the moisture-tension sensor for plants.