# Selective Stimulation of One of the Mechanisms for Genetic Recombination of Bacteriophage S13

Abstract. *In certain recombination-deficient (Rec⁻) bacterial strains genetic recombination of bacteriophage S13 is reduced, but the existence of some residual recombination has suggested that there is a secondary mechanism of phage recombination that is still functioning. In these Rec⁻ strains it is found that there is no stimulation of recombination by irradiation of the parental phage with ultraviolet light, in contrast to the large increase found when irradiated phage particles infect a Rec⁺ host. This selective stimulation of phage recombination in the Rec⁺ but not in the Rec⁻ strains supports the view that the phage uses at least two mechanisms of genetic recombination.*

At least two mechanisms for genetic recombination of bacteriophage S13 have been detected by crossing the phage in a recombination-deficient (Rec⁻) strain of *Escherichia coli* (*1*). Recombination frequencies were found to be 4 to 40 times lower in the Rec⁻ strain JC1553 [which contains the *rec*-1 allele (*2, 3*)] than in the parent Rec⁺ strain JC411. This result showed that the primary mechanism of S13 recombination is blocked in the Rec⁻ strain and also that phage and host share a common step in their recombination processes.

This Rec⁻ mutation produced a more severe reduction of bacterial than of phage recombination; with certain genetic markers and donor cells bacterial recombination in the Rec⁻ strain was 1000 times lower than in the parental Rec⁺ strain, and even the small amount of bacterial recombination left might have been due to the introduction of a *rec*⁺ allele by the donor cell (*2–4*). Since the proportion of phage recombination remaining in this Rec⁻ strain was much greater than the proportion of bacterial recombination, it appeared likely that the remaining phage recombination was due to something other than residual activity of the mutant *rec* gene. It was therefore concluded that a secondary mechanism for phage recombination must still be functioning in the Rec⁻ strain (*1*).

It will be shown that at least two phage recombination mechanisms can be distinguished by virtue of their being affected differently by irradiation of the phage with ultraviolet light. An increase in recombination frequency by ultraviolet irradiation [a phenomenon originally shown for phage λ (*5*)] is observed for S13 crosses in a Rec⁺ host. However, in a Rec⁻ host irradiation causes no significant increase in frequency above the residual level.

Previously (*1*) only the recombination-deficient mutant JC1553, which was derived from the Rec⁺ strain JC411 (*2*), was used. Two additional Rec⁻ mutants have now been studied, AB2463 and AB2470, both derived from the Rec⁺ strain AB1157 (*6*) (Table 1). Because these mutants do not adsorb S13 they were mutagenized further with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and S13-sensitive derivatives, AB2463.3, AB2470.5, and AB1157.6, were selected by a replica-plating method (*1*). Mutant AB2463 is similar in its Rec⁻ properties to JC1553 [Table 1 and (*3*)].

Table 1. Recombination-deficient (Rec⁻) mutants used as hosts for S13 crosses. All strains are F⁻.

| Mutants | *rec* Allele | Phenotypic group* | Radiation-induced DNA breakdown † |
|---|---|---|---|
| Derivatives of AB1157 (Rec⁺) | | | |
| AB2463 | *rec*-13 | $Rec_2^-$ | "Reckless" |
| AB2470 | *rec*-21 | $Rec_1^-$ | "Cautious" |
| Derivative of JC411 (Rec⁺) | | | |
| JC1553 | *rec*-1 | $Rec_2^-$ | "Reckless" |

* Complementation tests (*3*) suggest that the two phenotypic groups represent defects in different genes. † The "reckless" strains are abnormally high in radiation-induced breakdown of the cell DNA; the "cautious" strain is abnormally low (*7*). The "reckless" property of JC1553 is inferred from that of JC1569, which is a Mal⁺, $\lambda^s$ derivative of JC1553 (*8*).

Crosses were performed as previously described (*1*), with temperature-sensitive (*t*) and suppressible (*su*) mutants under conditions that permit growth. Only the wild-type recombinant was scored. In the irradiation experiments the free phage particles were irradiated with a 15-watt germicidal lamp to a survival of 20 to 40 percent and the cells were infected with an average of five to ten phage particles of each type, including both live and dead particles. The burst sizes ranged from approximately 10 to 30 when the phage particles were irradiated and 50 to 100 when unirradiated. Phage recombination frequencies appear to be lower in strains AB1157 and AB2463 than in JC411 and JC1553 when the same crosses are compared in either the Rec⁺ or the Rec⁻ hosts (see *1*).

At the ultraviolet dose used the recombination frequency is increased approximately 20 to 30 times in the Rec⁺ strain AB1157.6, but is not significantly increased in the Rec⁻ strain AB2463.3 (Table 2). Thus the gene in which the *rec*-13 mutation occurs controls a recombination mechanism that is stimulated by ultraviolet light,

Table 2. Recombination of S13 mutants in Rec⁺ and Rec⁻ hosts with and without ultraviolet (UV) irradiation of the phage. All crosses were performed at 37°C.

| Mutants crossed* | Host | Frequency of wild-type recombinants (in units of $10^{-5}$) | | Ratio + UV / − UV |
|---|---|---|---|---|
| | | − UV | + UV | |
| *t*H39(I) × *t*H11(IIIb) | AB1157.6 (*rec*⁺) | 5.7 | 120 | 21 |
| | AB2463.3 (*rec*-13) | 0.55 | 0.59 | 1.1 |
| *t*H76(II) × *t*H266(IIIb) | AB1157.6 (*rec*⁺) | 13.3 | 380 | 29 |
| | AB2463.3 (*rec*-13) | 1.8 | 3.6 | 2.0 |
| *su*HS149(II) × *su*HS16(IV) | AB1157.6 (*rec*⁺) | 58 | 1600 | 28 |
| | AB2463.3 (*rec*-13) | 0.90 | 1.2 | 1.4 |
| | AB2470.5 (*rec*-21) | 33 | 1500 | 44 |
| *t*H76(II) × *t*H11(IIIb) | JC411.5 (*rec*⁺) | 15 | 63 | 4.3 |
| | JC1553.1 (*rec*-1) | 2.0 | 1.1 | 0.6 |
| *su*HS149(II) × *su*HS66(IIIb) | AB1157.6 (*rec*⁺) | 30 | | |
| | AB2470.5 (*rec*-21) | 43 | | |
| *su*HS149(II) × *su*HS16(IV) | AB1157.6 (*rec*⁺) | 55 | | |
| | AB2463.3 (*rec*-13) | 0.50 | | |
| | AB2470.5 (*rec*-21) | 70 | | |

* In parenthesis is the complementation group for each mutant (*9*).

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.

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### 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) (196[illegible] P. Howard-Flanders 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).
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## 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 $\mu M$ 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 $\mu$l of extract was layered onto the gradient, and for high-speed runs (65,000 rev/min) up to 300 $\mu$l 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