ciently in ice at 90°K (*11*), and OH should preferentially be lost downward to the planet's surface. If Saturn's rings are ice, an adequate description of their kinetics must certainly be very complex.

HALSTEAD HARRISON
R. I. SCHOEN
Boeing Scientific Research Laboratories, Seattle, Washington

References and Notes

1. G. P. Kuiper, *The Atmospheres of the Earth and Planets* (University of Chicago Press, Chicago, ed. 2, 1952), p. 365.
2. T. Owen, *Science* **149**, 974 (1965).
3. D. H. Robey, *J. Brit. Interplanet. Soc.* **17**, 205 (1959).
4. K. Dressler and O. Schnepp, *J. Chem. Phys.* **33**, 270 (1960).
5. P. J. Nawrocki and R. Papa, *Atmospheric Processes* (Prentice-Hall, New York, 1961), pp. 2–8.
6. N. Ockman, *thesis*, University of Michigan, 1957.
7. F. S. Johnson, *J. Meteorol.* **11**, 431 (1954); L. Goldberg and A. K. Pierce, *The Photosphere of the Sun*, (Springer-Verlag, New York, 1959), p. 17.
8. G. Herzberg, *Molecular Spectra and Molecular Structure. II. Infrared and Roman Spectra of Polyatomic Molecules* (Van Nostrand, Princeton, N.J., 1945), p. 207.
9. M. Neugebauer and C. W. Snyder, *J. Geophys. Res.* **71**, 4469 (1966).
10. Close to an upper limit: see M. Kaminsky, *Atomic and Ionic Impact on Metal Surfaces* (Academic Press, New York, 1965), p. 151.
11. R. T. Brackmann and W. L. Fite, *J. Chem. Phys.* **34**, 1572 (1961).

26 June 1967

Orientation of Nonsense Codons on the Genetic Map of the Lac Operon

Abstract. *Intracodon recombination is used to orient the nonsense codons UGA, UAG, and UAA on the genetic map of the (lactose) lac operon of* Escherichia coli. *The 5′-end of these triplets is toward the operator end of the operon. A hypothesis is presented to explain the fact that the frequency of recombination between adjacent nucleotides is many times lower than expected.*

Two genetic markers must be at different sites on the chromosome in order that we may observe recombination between them. The closest possible pair of markers from which recombination is to be expected are neighboring nucleotides. Recombination between adjacent nucleotides was first studied by Guest and Yanofsky (*1*) who determined the base sequence of mutant codons from the amino acid substitutions that these mutations produced in tryptophan synthetase. Recombination frequencies of the order of 10^{-3} percent were found between markers in adjacent nucleotides as compared to 4 percent for the whole tryptophan synthetase *A* gene which has a length of 280 amino acids. By measuring the frequency of unselected outside markers in these crosses, Guest and Yanofsky were able to orient several codons on the genetic map showing that the 5′-end of the "sense" strand triplet was toward the NH_2-terminal end of the protein. The sense-strand of DNA is the one with the same base sequence as mRNA (*2*). Thus, for example, if the mRNA codon is UGA, the sense-strand DNA codon will be TGA. Here we only use U and not T, and the 5′ end is always on the left.

In the case of the three nonsense codons UGA, UAG, and UAA, it is possible to determine the identity of mutant codons without any analysis of the protein involved. This is accomplished by using a series of outside suppressors that suppress nonsense mutations with sufficient specificity to determine which of the three possible nonsense triplets cause the defect. Examination of the base sequence of the three nonsense triplets indicates that recombinants should be obtained from crosses between UGA and UAG but not between UGA and UAA or between UAG and UAA when these triplets occur in the same codon. This expectation was shown to be true in crosses between a series of nonsense mutants in the same codon of the *r*II gene of T4 bacteriophage (*3*).

In crosses between UGA and UAG in the same codon, two recombinant triplets are possible, that is, UAA and UGG. The UAA is also nonsense and thus cannot generally be selected from the parental mutations. The codon for typtophan (UGG) will give an active protein only if tryptophan is an acceptable amino acid in the particular case being studied. Tryptophan will always be acceptable if the UGA and UAG mutations used in a cross are derived from the same UGG wild-type codon by single-step mutational events. A pair of UGA and UAG mutants that fit these criteria have been identified (*4*).

I now report the results of crosses between these UGA and UAG mutations in the same codon of the β-galactosidase (*Z*) gene of *E. coli*. When I used outside Z^- markers and selected for lac+ recombinants, it was possible to orient these triplets on the map of the lac operon and to show that the 5′-end of the codon is toward the operator end of the operon. No recombination was found in crosses between UGA or UAG and a UAA mutant in the same codon. This expected result serves as added confirmation that the UAG and UGA mutants studied were really in the same codon. The recombination frequency between adjacent nucleotides was measured and was found to be an order of magnitude lower than expected from the size of the *Z* gene given by data on genetic and protein structure. An hypothesis to explain this positive interference in recombination between adjacent bases follows.

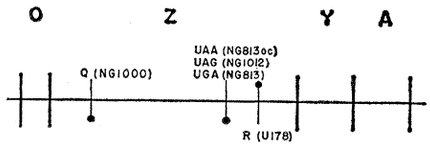

Fig. 1. Map of the lac operon of *Escherichia coli*. *O*, operator; *Z*, β-galactosidase; *Y*, permease; and *A*, thiogalactoside-transacetylase. The position of the markers in these experiments is roughly to scale. Since UGA, UAA, and UAG are in the same codon, they are shown to map at a single point.

Preliminary tests indicated that the recombination frequency between adjacent nucleotides was so low that recombinants could not be observed above the background of spontaneous lac+ revertants. To overcome this problem and to provide information for ordering the adjacent markers, double lac− mutants were constructed. To insure very high transfer of the lac region to the F− strains, F′ lac homozygotes were used as the male donors.

Table 1. Results of recombination between adjacent nucleotides. Q and R refer to NG1000 and U178 Z^- mutants, respectively. The UAG mutant is NG1012, the UGA is NG813, and the UAA is NG813oc. The NG813oc was derived from NG813 by the use of suppressors as described previously (*3*). The total number of recombinants refers to the number of lac+ colonies produced by a total of 3.2 ml of mating mixture on eight plates in 48 hours.

Parents		Total recombinants
F′ Male	F⁻ Female	
	Single crossover	
Q . . . UAG	UGA . . . . R	35
	Triple crossover	
Q . . . UGA	UAG . . . . R	7
	Nonrecombinant controls	
Q . . . UAA	UAG . . . . R	1
Q . . . UAA	UGA . . . . R	2
Q . . . UGA	UGA . . . . R	1
Q . . . UAG	UAG . . . . R	4
	Nearest outside marker	
UAG	R	102,000

All crosses were performed by mixing equal volumes of fresh Penassay broth cultures (of the two parental strains) that have about 5×10^8 bacteria per milliliter. The mixtures were allowed to stand for 30 minutes at 37°C and then were plated on minimum lactose plates. A total of 3.2 ml of mating mixture was plated for each cross. The plates were incubated at 37°C for 48 hours, and the lac+ colonies were counted. This method of crossing F− with F′ lac bacteria gives about ten times more recombinants per plate than were obtainable with crosses between Hfr and F− strains. This is critical in these experiments because of the very low recombination rates to be measured. The origin of the lac− mutants and the construction of the double mutants have been previously described (*4*, *5*). A map of all the markers used in these experiments is shown in Fig. 1.

To establish the orientation of the nonsense triplets, seven different crosses were performed (Table 1). "Nonrecombinant controls" are those crosses that would be expected to give no recombination. The lac+ colonies on these controls arise from a mixture of reversion and recombination events, that is, the reversion of one of the two lac− markers in a double mutant preceded or followed by a recombination which separates it from the other lac− marker. The maximum number of lac+ colonies in a control was four, as compared to 35 where recombination takes place. It is also clear (Table 1) that the crosses between UGA and UAA and between UAG and UAA fall into the nonrecombinant class, as expected. The UAA mutant was derived in a single step from the UGA mutant (*4*). The single crossover class exceeds the triple crossover class by fivefold, thus clearly establishing the orientation of the codons as operator ... UAG (or UGA). This is the order generally assumed and is consistent with the tryptophan synthetase results above.

The last line of Table 1 shows the number of colonies observed when the UGA mutant and the nearest outside marker are crossed under identical conditions to the other crosses. The distance between these markers was previously shown to be less than 0.1 percent (*6*). From these data, the recombination frequency for a single base pair can be calculated to be less than 3.5×10^{-5} percent. The length of the whole β-galactosidase gene is about 3.3×10^3 bases, as calculated from the polypeptide molecular weight (*7*). Thus, the expected recombination length of the whole *Z* gene obtained by multiplying the single base-pair recombination frequency by the number of base pairs is about 0.1 percent. The observed value of the length of the *Z* gene, as measured by ordinary recombination frequency, is about 1 percent; this is ten times larger than expected from the single base-pair recombination frequency.

Recombination between the three nonsense triplets (UGA, UAG, and UAA) mapping in the same codon qualitatively follows the ordinary rules of genetics: recombination is only observed when there are two noncomplementary base pairs in the same codon; that is, the cross between UGA and UAG gives recombination, but the crosses between UGA or UAG and UAA do not. Multiple recombination events are much less frequent than single ones, allowing the ordering of adjacent nucleotide markers.

When the quantitative aspects of adjacent nucleotide recombination are studied, however, the results are more complex. The frequency of recombination between adjacent bases is an order of magnitude too low to account for the total observed recombination length of the β-galactosidase gene. This result is just the opposite of what would be expected from studies of recombination between close markers. As markers get closer together, an increase in the ratio of recombination frequency to distance is observed. This is hypothesized to be due to enzyme systems which excise regions of hybrid DNA in which there are nucleotide mispairs (*8*). Such excision and then repair would lead to a much higher frequency of recombination between close markers than expected if an actual break-and-join event had to take place between them.

Our explanation of the low recombination frequency between adjacent nucleotides is that neighboring mispaired nucleotides tend to be excised together from hybrid DNA duplexes. If both markers are removed together, the excision mechanism would not contribute to the recombination frequency. Thus, the recombination frequency would be much lower than expected from studies of markers in which the excision mechanisms make a major contribution to recombination.

DAVID ZIPSER
Department of Biological Sciences, Columbia University, New York 10027

References

1. J. R. Guest and C. Yanofsky, *Nature* **210**, 799 (1966).
2. Abbreviations: mRNA, messenger RNA; U, uracil; A. adenine; G. guanine; T. thymine: UGA, TGA, UAG, UAA, and UGG, nucleotides.
3. S. Brenner, L. Barnet, E. R. Katz, F. H. C. Crick, *Nature* **213**, 449 (1967).
4. D. Zipser, *J. Mol. Biol.*, in press.
5. ——— and A. W. Newton, *ibid.* **25**, 567 (1967).
6. A. W. Newton, J. Beckwith, D. Zipser, S. Brenner, *ibid.* **14**, 290 (1965).
7. J. L. Brown, S. Koorajian, J. Katze, I. Zabin, *J. Biol. Chem.* **241**, 2826 (1966).
8. R. Holliday, *Genet. Res.* **5**, 282 (1964).
9. Supported in part by NSF grant GB3231 and NIH grant GM14676-01.

19 July 1967

Hydrous Sodium Silicates from Lake Magadi, Kenya: Precursors of Bedded Chert

Abstract. *Two new hydrous sodium silicates, $NaSi_7O_{13}(OH)_3 \cdot 3H_2O$ (magadiite) and $NaSi_{11}O_{20.5}(OH)_1 \cdot 3H_2O$ (kenyaite), were found in lake beds at Lake Magadi, Kenya. Both are well-crystallized layered silicates with large basal spacings. Concretions within the magadiite bed consist of kenyaite or quartz (chert) in the center, surrounded by kenyaite. In dilute acids magadiite and kenyaite are converted to $6SiO_2 \cdot H_2O$ (SH), the first known crystalline hydrate of silica. The magadiite bed probably represents a chemical precipitate from alkaline brines. Percolating waters convert magadiite to kenyaite and eventually to chert. Thus a mechanism has been outlined for the formation of bedded chert deposits through inorganic precipitation. Alternations between silica-rich and iron-rich bands of iron formations may be due to concentration cycles in alkaline lakes.*

The Eastern, or Gregory Rift Valley, in East Africa contains a number of sodium carbonate-rich, alkaline lakes (*1*); the most saline of these is Lake Magadi in southern Kenya (*2*). It lies in a closed basin at 1978 feet (603 m) above sea level in the heavily block-faulted floor of the valley, 2° south of the equator. It is intermittently dry, except for brine pools ("lagoons") near the margins. It contains a vast deposit of trona ($Na_2CO_3 \cdot NaHCO_3 \cdot$