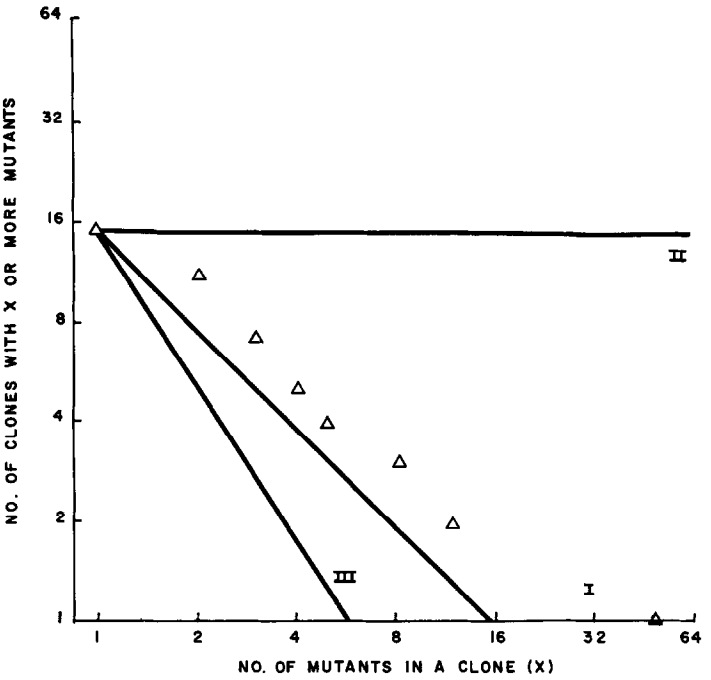

FIG. 2. The distribution of the 20 mutant (*r*) clones containing between 3000 and 10,000 PFU. The solid lines present the distributions to be expected for each of the three alternative mechanisms of replication of the viral genome (with 15 clones containing one or more mutants). Line I, geometric replication, Line II, "follow-the-leader" replication. Line III, "stamping machine" replication.

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Gene Order in Prophage P22[1]

Zinder (*1*), Subbaiah *et al.* (*2*), and more recently Young and Hartman (*3*) have mapped the phage P22 prophage near the proline locus of *Salmonella typhimurium* by means of colicine factor-mediated conjugation experiments. This report presents evidence confirming these findings. Some *S. typhimurium* strains with deletions in the proline region (*4*) are not able to form stable lysogens. In addition, bacterial mating experiments demonstrate a close linkage between the *pro A* cistron and prophage loci. These crosses also establish the order of three prophage genes in relation to the *pro A* locus. The order of these three genes in the prophage is a permutation of the vegetative phage map (*5*), similar to that reported for the λ prophage (*6–10*).

A number of F^- *pro*$^-$ strains were generously supplied by Dr. K. E. Sanderson from the collection of Dr. M. Demerec.

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These were tested for ability to form stable lysogens by infection in nutrient broth with wild-type phage P22 at a m.o.i. of about 15. After treatment with antiphage serum, the infected culture was diluted to approximately 10^3 cells/ml to prevent reinfection and then sampled at 30-minute intervals for the proportion of sensitive and phage-carrying cells. The culture was appropriately diluted to maintain the cell concentration at low levels.

Results typical of that previously noted for infection of wild-type cells (*11*) were obtained with infections of *pro A15* (a point mutation in the *pro A* cistron), *pro B31* (a point mutation in the *pro B* cistron), *pro B25* (a deletion in the *pro B* cistron), and *pro AB21* (a deletion of the entire *pro B* cistron and only part of the *pro A* locus) (*4*). Approximately 50% of the cells became stable lysogens, while the other 50% reacquired phage-sensitivity by segregation.

Infection of mutants *pro AB47*, *pro AB126*, and *pro A107* resulted in continued segregation of sensitive cells, while the number of phage carrying cells, after a few divisions, became constant. The phage was apparently incapable of replicating synchronously with the host. Mutants *pro AB47* and *pro AB126* are deletions of all of cistrons A and B and probably extend for a considerable distance to either side. However, *pro A107* deletes the point mutation *pro A15*, leaving the B cistron intact (*4*). A most reasonable interpretation is that these three mutants cannot be lysogenized because they are deleted in the region of prophage integration into the host genome. The prophage integration site thus appears to be to the *pro A side of the proline region.* Recombination studies support these interpretations.

Linkage of three phage markers, m_3, c_2, and h_{21} (*5*), in the prophage state, were examined by bacterial matings using the Hfr strain, SU576 *pur C7* (again generously supplied by Dr. Sanderson). The following two reciprocal crosses were performed:

Cross 1: SU576 *pur C7*(+++) × F⁻ *pro A15*($m_3c_1h_{21}$)

Cross 2: SU576 *pur C7*($m_3c_1h_{21}$) × *F⁻ pro A15*(+++)

Prototrophic recombinants from each cross were selected at random from minimal agar plates. These were purified by single-colony isolation on minimal agar plates spread with antiphage serum and then examined for the type of phage spontaneously liberated from the prophage state. The observed frequencies of occurrence of the eight possible genotypes for each cross are shown in Table 1. The two reciprocal crosses gave frequencies of recombinants which were essentially reciprocals of each other. The combined frequencies from the two crosses, referred to the phage classes observed in cross 2, are presented in column 5 of Table 1.

Prophage from the donor was transferred intact to the recipient with a frequency of

TABLE 1

FREQUENCIES OF GENOTYPES OBTAINED FROM CROSSES 1 AND 2

Genotypes	Cross 1[a]		Cross 2		Cross 1 and 2, combined observed frequencies	Formulas used to compute expected frequencies	Expected frequencies
	N	*F*	*N*	*F*			
m_3 c_1 h_{21}	71	0.104	374	0.637	0.643	$(1 - r_1)(1 - r_2)(1 - r_3)$	0.638
+ + +	443	0.649	64	0.109	0.107	$r_1(1 - r_2)(1 - r_3)$	0.105
+ c_1 h_{21}	19	0.028	107	0.182	0.162	$(1 - r_1)(1 - r_2) r_3$	0.163
m_3 + +	98	0.143	8	0.013	0.021	$r_1(1 - r_2) r_3$	0.027
m_3 c_1 +	5	0.007	3	0.005	0.014	$(1 - r_1) r_2 r_3$	0.012
+ + h_{21}	16	0.023	3	0.005	0.006	$r_1 r_2 r_3$	0.002
m_3 + h_{21}	29	0.042	7	0.012	0.007	$r_1 r_2 (1 - r_3)$	0.007
+ c_1 +	2	0.003	22	0.037	0.040	$(1 - r_1) r_2 (1 - r_3)$	0.046
	683	0.999	588	1.000	1.000		1.000

[a] *N*, number; *F*, frequency.

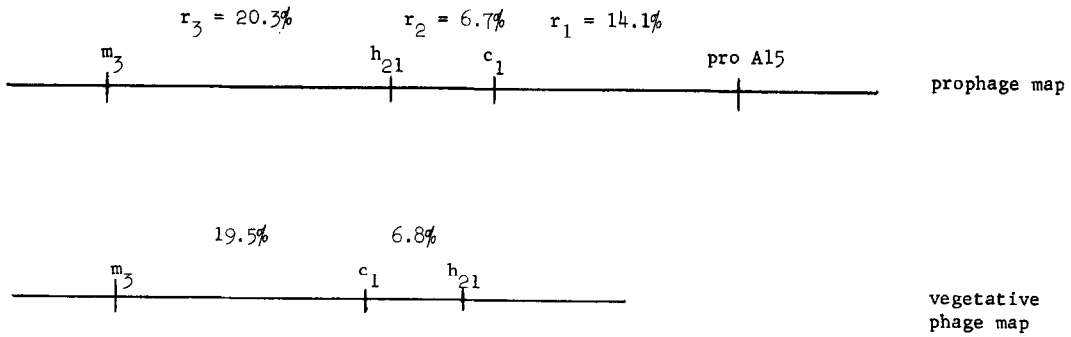

FIG. 1. Comparison of the prophage and vegetative maps of phage P22.

64 %, indicating close linkage of the prophage to *pro A15*. Recipient cell prophage remained intact 10.7 % of the time. The remaining 25 % of the prophages were recombinant for phage markers. The only order of markers for the prophage which is compatible with the observed frequencies of recombination is $m_3-h_{21}-c_1$. For example, with this order of genes, a triple crossover such as $++h_{21}$ would be expected to occur with low frequency in comparison to a single crossover such as $+c_1+$. The observed frequencies of 4 % for $+c_1+$ and 0.6 % for $++h_{21}$ agree with this expectation. However, if the vegetative order of genes $m_3-c_1-h_{21}$ were preserved, then $+c_1+$ would be a triple crossover event and $++h_{21}$ a single crossover, clearly contradicting the data.

Accepting this order, $m_3-h_{21}-c_1$, calculation of percentage recombination between pairs of markers gave 14.1 % for *pro A15*$-c_1$, 6.7 % for $h_{21}-c_1$, and 20.3 % for m_3-h_{21} (Fig. 1). The "expected" frequency of each genotype was computed by using these map distances. Observed and expected frequencies show close agreement (Table 1).

It appears that phage P22 undergoes a permutation of its vegetative map upon becoming prophage. These findings are completely analogous to the situation in phage λ and support the integration model of Campbell (*12*).

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Disappearance of Tumorigenic Activity from Human Adenovirus Type 12

Adenovirus type 12 has been found to induce tumors in Syrian hamsters (*1*, *2*). Recently we have found an improved assay method for adenovirus type 12 prototype strain, by using a clonal line of porcine kidney stable cells, PS(Y-15) cells, cultivated at 41° (to be published). The present