LIMITED GENOME EXPRESSION IN BACTERIOPHAGE T4-INFECTED *ESCHERICHIA COLI*. II. DEVELOPMENT AND EXAMINATION OF A MODEL^{1,2}

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IN the previous paper (SNUSTAD 1966), limited genome expression was demonstrated, and several possible trivial explanations of the effect were ruled out. In the present report, a model is presented which offers a reasonable explanation of the observed phenomenon. The fit of the model to extensive experimental data is examined for both the *rII*-K112(λ h8) and the amber-S/6/5 conditional lethal systems. Finally, gene-product activity is investigated in cells mixedly infected with unequal-inputs of wild type and various mutant genomes carrying defects in one or more genes controlling vital phage functions.

MATERIALS AND METHODS

The bacteriophage and bacterial strains are those described by SNUSTAD (1966), with the exception of the amber bacteriophage mutants *am* N122, *am* N130 and *am* N58, which have been characterized by EPSTEIN *et al.* (1963). Preparation of phage stocks, media, plating conditions and experimental procedures are as in SNUSTAD (1966).

In experiments in which progeny phage were measured, each growth-tube was shaken with a few drops of chloroform at 90 minutes after infection ($T = 90$ min). After allowing the chloroform to settle, a 0.1 ml aliquot was plated (on the permissive host for total progeny phage or on the restrictive host for wild-type progeny).

In equal-input burst size experiments, cells were mixedly infected with multiplicities of approximately $7\frac{1}{2}$ each of mutant and wild-type phage. The multiplicities of infection used in unequal-input experiments are given along with the respective results.

EXPERIMENTAL RESULTS

In the preceding paper (SNUSTAD 1966), it was shown that when *E. coli* K112-(λ h8) was infected with a constant multiplicity of approximately 0.5 T4D⁺ per cell and varying multiplicities (0 and about 5, 10, 15, and 20) of an *rII* mutant, the number of infective centers capable of plating on K112(λ h8) decreased abruptly with increasing multiplicity of the *r* mutant. The model which is developed below is offered as a plausible explanation of the observed phenomenon.

Development of the model: The two premises upon which the model is based

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are: (1) only a constant, fixed number of phage genomes can function heterocatalytically in a given infected bacterium; and (2) the genomes which do express are drawn at random from the pool of infecting phage genomes. For the sake of clarity in developing the model, it is assumed that each bacterium which receives one or more wild-type genomes receives one and only one wild-type genome. (This, of course, is not true; however, because the multiplicity of infection (m.o.i.) of T4D⁺ was low (about 0.4–0.5), the error introduced is not large. Later, an estimate of the maximum error is obtained, and it is shown that the error introduced by this oversimplification is indeed small.) Moreover, the parental mutant phages are assumed to be Poisson-distributed over the infected bacteria (DULBECCO 1949).

Given the above assumptions, and defining

X = the proportion of potential infective centers (wild-type infected bacteria) which actually produce infective centers,

M = the multiplicity of the mutant parent, and

R = the number of mutant phages adsorbed to any given bacterium such that

P_R = the proportion of cells receiving exactly R mutant genomes,

it follows that if only one genome can be expressed in any given bacterium, then

$$X = \sum_{R=0}^{\infty} \frac{1}{R+1} \frac{M^R}{R!} e^{-M} \quad (1)$$

That is, the probability of an infected bacterium forming an infective center would be equal to the probability that within the bacterium the one expressing phage genome is wild-type.

Or, if a fixed number of genomes, K , can express their heterocatalytic functions in each infected cell, the relationship becomes

$$X = \sum_{R=0}^{K-1} \frac{M^R}{R!} e^{-M} + \sum_{R=K}^{\infty} \frac{K}{R+1} \frac{M^R}{R!} e^{-M} \quad (2)$$

$$= \sum_{R=0}^{K-1} \frac{M^R}{R!} e^{-M} + \frac{K}{M} \sum_{R=K}^{\infty} \frac{M^{R+1}}{(R+1)!} e^{-M} \quad (3)$$

Thus,

$$X = P_R \leq (K-1) + \frac{K}{M} P_R \geq (K+1). \quad (4)$$

At high mutant multiplicities of infection, this relationship, which was first suggested by MILLARD SUSMAN (personal communication), approaches $X = K/Y$ where Y equals the m.o.i. of the mutant plus one because $P_R \leq (K-1) \rightarrow 0$ and $M \approx Y$. Thus, the model predicts that a plot of X vs. Y should approximate a straight line of slope K at high mutant multiplicities of infection.

The above model would be correct if each cell which received one or more wild-type genomes received only one wild-type genome. In actuality, of course, some bacteria receive more than one wild-type genome even at the low multiplicities employed. Consider, for example, that of the cells which receive at least one wild-type genome, certain proportions C_1, C_2, C_3 , etc., receive one, two, three, etc., wild-type genomes, respectively. Then, if each bacterium is assumed to adsorb M mutant phages, a maximum estimate of the error introduced by the

oversimplification of exactly one wild-type genome per each wild-type infected cell can be calculated for any T4D⁺ m.o.i. Using these assumptions, the relationship becomes

$$X = \frac{C_1 K}{Y} + \frac{C_2 K(2Y+1-K)}{Y(Y+1)} + \frac{C_3 K(3Y^2+6Y-3YK+2-3K+K^2)}{Y(Y+1)(Y+2)} + \dots \quad (5)$$

For the special case of $K = 1$, this equals

$$X = \frac{C_1 K}{Y} + \frac{2C_2 K}{Y+1} + \frac{3C_3 K}{Y+2} + \dots \quad (6)$$

which at high multiplicities is approximately equal to

$$X = \frac{C_1 K}{Y} + \frac{2C_2 K}{Y} + \frac{3C_3 K}{Y} + \dots = \frac{K}{Y} (C_1 + 2C_2 + 3C_3 + \dots). \quad (7)$$

Comparisons of equations (5) and (7) with the oversimplification $X = K/Y$ show that equation (7) yields a maximum estimate of the error introduced by not considering the Poisson distribution of wild-type genomes among the infected cells, for all values of K . That is, the values of K obtained for given sets of values for C_1, C_2, C_3 , etc., X and Y from equation (7) deviate more from those obtained with the oversimplification ($K=XY$) than do those from equation (5), the true relationship.

Thus, by calculating the values of C_1, C_2, C_3 , etc., as the respective proportions of the total non-zero fraction of the Poisson distribution (DULBECCO 1949) and comparing the values of K obtained as described above, it can be shown that the maximum error introduced by assuming that each wild-type infected cell receives exactly one wild-type genome is 20% for a T4D⁺ m.o.i. of 0.5 (T4D⁺ m.o.i. being constant *within* each of the experiments). The values of K estimated on the basis of the oversimplification will thus be too large by something less than 20%.

Critical test of the model: The model presented above makes precise predictions regarding the results of the unequal-input experiments reported here. (1) There should be an interval in which only a negligible number of bacteria are infected with more phage genomes than can express, where no detectable loss of potential infective centers occurs. (2) There should be a region in which $Y > K$ where the plot of the proportion of the potential infective centers which actually produce infective centers, X , against the reciprocal of the average multiplicity of the mutant plus one, $1/Y$, approximates a straight line of slope K . (3) When the value of K estimated from the slope as described in 2 is used in equation (3) to calculate the predicted relationship between X and Y , the curve obtained should provide an accurate description of the data collected in the unequal-input experiments.

The preliminary results (SNUSTAD 1966) are consistent with the model. The data are far too limited, however, to test the model critically. Thus, the following experiments were undertaken to obtain data sufficient to test whether or not the model presented above provides an accurate description of the limited genome expression observed in this study. In order to accomplish this, it was necessary to infect with a wide range of phage multiplicities and to repeat each m.o.i.

FIGURE 1.—Fit of the model to r -K112(λ h8) interaction. The proportion of potential infective centers which plate, X , is plotted against the reciprocal of the average m.o.i. of the mutant plus one, Y , i.e., the approximate average total m.o.i. in the potential infective centers. The proportion of potential infective centers which plate was defined as equal to one in the control tubes singly infected with wild-type phage. The standard protocol was used. Infective centers were measured at 5 minutes after dilution to the growth-tube in all cases. ---- = extrapolation of the straight line approached at high multiplicities of infection where the model (equation 3) approximates $X=K/Y$ (see text). Slope = 4.3; $1/Y = 0.24$, $Y = 4.3$ at inflection. The three curved lines represent the specific predictions of the model (equation 3 of text) for $K=3$ (— · — · —), $K=4$ (.....), and $K=5$ (---). These were calculated from the following solutions of equation 3:

$$\text{for } K=3, X = \frac{3}{M} - e^{-M} \left(\frac{3}{M} + 2 + \frac{M}{2} \right);$$

$$\text{for } K=4, X = \frac{4}{M} - e^{-M} \left(\frac{4}{M} + 3 + \frac{M}{2} + \frac{M^2}{6} \right);$$

$$\text{for } K=5, X = \frac{5}{M} - e^{-M} \left(\frac{5}{M} + 4 + \frac{3M}{2} + \frac{M^2}{3} + \frac{M^3}{24} \right).$$

▲ = deletion mutant r H23, K112(λ h8) Sm^r34 + streptomycin sulfate at 1 mg/ml, T4D⁺ m.o.i. = 0.74; × = r ED220, K112(λ h8), T4D⁺ m.o.i. = 0.42; ● = r ED220, K112(λ h8), T4D⁺ m.o.i. = 0.49; ○ = r ED220, K112(λ h8), T4D⁺ m.o.i. = 0.48; □ = r ED220, K112(λ h8), T4D⁺ m.o.i. = 0.43.

several times to overcome the relatively large error inherent in individual measurements.

The procedure adopted to increase the precision of the data was to prepare adjusted phage mixtures and adjusted log-phage bacterial cultures by the standard method and to repeat the steps of the standard protocol up to the measurement of infective centers several times in rapid succession using the same

preparations. Since the bacterial pellet was always resuspended in cold H-broth after refrigerated centrifugation, the adjusted log-phase cultures could be stored under refrigeration for 1 to 2 hours with no change in titer and no apparent change in capacity to undergo lytic infection. Since each repeat requires 20 minutes, it was possible to complete four to five repeats in 2 hours.

The data collected from extensive experiments with various r mutants and their restrictive hosts, K122(λ h8) and K112(λ h8) Sm^r 34, [a spontaneous streptomycin-resistant mutant of K112(λ h8)], are presented in Figure 1. On the basis of these results, it is concluded that the model offers a reasonable description of the limited r^+ expression observed in this study.

In order to facilitate discussion of the model in the following sections, the term "slots" shall be adopted in speaking of the factor(s) responsible for the limited expression. Furthermore, it will be assumed that one slot allows the expression of one genome. In terms of this jargon we can now ask what these slots are and consider their heterogeneity within populations and between different strains of bacteria.

The estimates of K from the data of Figure 1 indicate that about four phage genomes can express per cell. Moreover, they show that the heterogeneity in the

FIGURE 2.— Fit of model to *am* N130-S/6/5 interaction. See the legend to Figure 1 and the text for a description of the protocol and the plot.

— — — = predictions of the model for $K = 5$ (see Figure 1 legend).

- . - . - = predictions of the model for $K = 6$ where equation 3 yields

$$X = \frac{6}{M} - e^{-M} \left(\frac{6}{M} + 5 + 2M + \frac{M^2}{2} + \frac{M^3}{12} + \frac{M^4}{120} \right).$$

○ = Experiment 1, T4D+ m.o.i. = 0.39; ● = Experiment 2, T4D+ m.o.i. = 0.51.

0.9 = slots

number of slots per bacterium cannot be large in the cultures of K112(λ h8) used in these experiments.

Is limited heterocatalytic expression specific to the *r*-K112(λ h8) interaction or does it occur in other conditional lethal systems? The amber mutations were studied in an attempt to answer this question. Since the r^+ region has been shown to control an early function(s) (GAREN 1961; FERROLUZZI-AMES and AMES 1965), experiments were first undertaken with *am* N130, an early function mutant in gene 46 (EPSTEIN *et al.* 1963). The results of these experiments (Figure 2) show that a qualitatively similar loss of potential infective centers occurs in the amber-S/6/5 interaction. Quantitatively, however, the results differ from those obtained with the *r* mutants and K112(λ h8) in that S/6/5 appears to have more slots, i.e., five to six.

Is the phenomenon of limited expression a specific characteristic of the early functions, or is it a general phage-host interaction? Figure 3 shows the results obtained with *am* N58, a late function amber mutant in gene 34 (EPSTEIN *et al.* 1963), and S/6/5. The results clearly agree with those obtained with *am* N130.

Thus, both early functions and late functions seem to exhibit the same limited expression and, moreover, to the same degree. This leads one to ask whether the same genomes remain in their slots and express throughout the infectious cycle, or whether one set of *K* genomes express the early functions and then leave their slots to be replaced by a second set which express the late functions, or whether genomes are continuously passing in and out of the slots. If the latter occurs, however, the total number of genomes which occupy slots during the period of

FIGURE 3.—Fit of model to *am* N58-S/6/5 interaction. See the legends to Figures 1 and 2 and the text for a description of the protocol and the plot. — — — and — . — . — = predictions of the model (equation 3 of text) for $K = 5$ and $K = 6$, respectively. $\circ$ = Experiment 1, T4D⁺ m.o.i. = 0.34; $\bullet$ = Experiment 2, T4D⁺ m.o.i. = 0.44. Slope = 6.2.

either early function expression or late function expression is restricted to *K*, i.e., instead of four slots containing four genomes which express throughout, four genomes might occupy one slot in succession.

The availability of a multiple amber mutant, *am* X4E, containing amber defects in four different late function genes (EDGAR and LIELAUSIS 1965) facilitated discriminating between the first two possibilities. When a cell is mixedly infected with wild-type and a multiple mutant, recombination will cause a decrease in the proportion of wild-type genomes recovered in the progeny (DELBRÜCK and BAILEY 1946; HERSHEY 1946). Moreover, when the wild-type parent is very much in the minority in the input mixture (i.e., one T4D⁺ to ten *am* X4E) as in the present experiments, more recombinant than wild-type genomes will be recovered (DOERMANN and HILL 1953; VISCONTI and DELBRÜCK 1953). SATO and STENT (unpublished results cited in DELBRÜCK and STENT 1957) have shown that when cells are mixedly infected with an average of one r_1 r_7 m^+ phage and an average of ten r_1 r_7 m phage (r_1 , r_7 and m being distantly linked), the progeny contain "less than one half of a minority parental type per infected bacterium" as is to be expected according to the VISCONTI-DELBRÜCK theory.

Therefore, since all of the various recombinant types as well as the majority parent will contain amber defects in late function genes when *am* X4E is employed in the standard unequal-input experiment, the proportion of wild-type genomes present in the vegetative pool should decrease throughout the latent period (HERSHEY and CHASE 1951; DOERMANN 1953; LEVINTHAL and VISCONTI 1953; SUSMAN 1962).

The total distance in terms of the recombination values within the region covered by the four genes containing amber defects in *am* X4E is 85 units according to the map of EDGAR, DENHARDT and EPSTEIN (1964). If these intervals are calculated in terms of their elementary, additive intervals, the region involved in *am* X4E is over 400 map units (STAHL, EDGAR and STEINBERG, 1964). Since the maximum frequency of recombination in the T-even phages is 30% under conditions of normal lysis (HERSHEY and ROTMAN 1949; HERSHEY and CHASE 1951), the more distant defects in *am* X4E are clearly unlinked and even the closest defects are only loosely linked. Thus, the proportion of the minority wild-type genomes present in the vegetative pool should decrease rapidly throughout the latent period, and this decrease should become more exaggerated as the mutant input is increased.

The results of this experiment with *am* X4E should be very different depending upon whether (1) the same set of *K* infecting genomes remain in the slots throughout the entire latent period, or (2) a subsequent set (or sets) of genomes is drawn from the vegetative pool and allowed to express itself at any time after the onset of vegetative replication. In the former case, the results of this experiment should be identical to those obtained with the single mutants, *am* N130 and *am* N58. If the latter is correct, there should be a more drastic loss of potential infective centers since the proportion of wild-type genomes will have greatly decreased, and the probability of obtaining a wild-type genome in a slot correspondingly lowered.

FIGURE 4.—Fit of model to *am* X4E-S/6/5 interaction. Plot and protocol as described in the legends to Figures 1 and 2 and in the text. — — — and — . — . — = predictions of the model for $K=5$ and $K=6$, respectively. ● = Experiment 1, T4D⁺ m.o.i. = 0.52; ○ = Experiment 2, T4D⁺ m.o.i. = 0.45. Slope = 5.3.

FIGURE 5.—Summary of amber-S/6/5 interaction. See the text and the legends to Figures 1 and 2 for a description of the protocol and plot used. — — — and — . — . — = predictions of the model for $K=5$ and $K=6$, respectively. ● = *am* N130; ○ = *am* N58; □ = *am* X4E; each point is the mean of three observations. Slope = 5.9.

The data obtained with *am* X4E are shown in Figure 4. The similarity between the decrease in infective centers observed with *am* X4E and those observed with *am* N130 and *am* N58 is evident. Just how congruent the losses obtained with *am* N130, *am* N58 and *am* X4E are is emphasized in Figure 5 in which all of the means for the experiments with the three amber mutants are superimposed.

Burst size data and progeny ratios: Studies in which one or more gene functions are limiting indicate that burst size is a very sensitive indicator of the amount of phage-specific function present in phage-infected cells (CHAMPE and BENZER, personal communication from S. P. CHAMPE; BERNSTEIN, EDGAR and DENHARDT 1965). It was therefore of interest to determine burst sizes for cells mixedly infected with mutant and wild-type phage using the respective restrictive host. This was first done using equal inputs of wild-type and each of the 22 *rIIA* mutants shown in Figure 1 of FISHER and BERNSTEIN (1965). The results (SNUSTAD 1965) indicate that r^+ function is not limiting when K112(λ h8) is infected with equal inputs of r and r^+ phage. That is, r^+ appears completely dominant to r under these conditions.

Burst size measurements were also made in several unequal-input experiments. It seemed likely that although T4D⁺ might be completely dominant to the mutant under equal-input conditions, it might not be if the cell were "flooded" with mutant gene product. This would be expected to occur if the input were heavily biased in favor of the mutant phage. Thus, the burst sizes (in terms of progeny per yield) were examined in cells of the respective host infected with low multiplicities (about 0.5) of wild type and variable, high multiplicities of mutant. The results of these experiments (Figure 6) proved to be most interesting. The mutants studied fell into two distinct classes. Either (1) the burst size remained constant regardless of the mutant multiplicity—*am* N122 and the *rIIA* mutants, or (2) the burst size decreased sharply as the mutant input increased—*am* N58, *am* N130 and *am* X4E. Although several explanations are equally feasible (see DISCUSSION), the idea is favored at present that the difference may depend upon whether the product of the gene involved functions catalytically or stoichiometrically. More work is currently under way on this phenomenon.

In order to consider whether there is any relationship between gene expression and gene replication, progeny ratios were determined for cells mixedly infected with unequal inputs of mutant and wild-type phage. This has been done for *r*62, *am* N122, *am* N130 and *am* X4E. Figure 7 shows the results of several experiments in which progeny ratios were determined. These results suggest a difference between the amber-S/6/5 interaction and the *r*-K112(λ h8) interaction. With the amber-S/6/5 system, the progeny ratios either follow or approach the input ratios. With the *r*-K112(λ h8) system, the progeny contain a higher proportion of wild-type than the input. The *r*/wild-type progeny ratios remain approximately equal to the input ratios at r m.o.i.'s below four. Above r multiplicities of about four, the *r*/wild-type progeny ratios equal about one-half the *r*/wild-type input ratios. The loss of r genomes in the progeny is not large enough, however, to account for the decrease in infective centers by an exclusion mechanism (see *Superinfection breakdown and/or exclusion*, SNUSTAD 1966). One possibility is

FIGURE 6.—Burst sizes of cells mixedly infected with unequal inputs of mutant and wild-type phage. In all cases the restrictive host of the respective mutant was infected with a low (about 0.5) m.o.i. of wild type and varying m.o.i.'s of mutant. In the case of the *r* mutants and *am* N122 where the burst size (progeny/infected bacteria) remains essentially constant regardless of the mutant/wild type input ratio, unadsorbed phage and leakiness were insignificant. With the other amber mutants, however, it was necessary to eliminate unadsorbed phage with T4-antiserum. Multiple amber X4E was found to be nonleaky as expected. Leakiness was corrected for in the *am* N130 experiments using a phage per nonyielder (infected bacterium not producing wild-type progeny phage) factor—this was required because of the multiplicity-dependent variation in the number of infective centers formed. The data for *am* N58 is not corrected for leakiness or unabsorbed phage; the results at higher phage multiplicities were therefore obscured by these factors. □ = *am* N122, T4D⁺ m.o.i. = 0.53; ■ = *am* N130, averages of two repeats, T4D⁺ m.o.i. = 0.39; ▲ = *am* N58, T4D⁺ m.o.i. = 0.34; ○ = *am* X4E, Experiment 1, averages of two repeats, T4D⁺ m.o.i. = 0.43; ● = *am* X4E, Experiment 2, averages of two repeats, T4D⁺ m.o.i. = 0.43; × = *r*ED220 and *r*62, T4D⁺ m.o.i.'s = 0.40–0.60, → numerous controls in *r* experiments have burst sizes in this range; space does not permit presenting the data, however. * based on less than 60 plaque counts.

that “expressing” and “nonexpressing” genomes may replicate with different efficiencies.

DISCUSSION

The model: Of the two premises upon which the model is based, the first (that only a constant, fixed number of phage genomes can function heterocatalytically

FIGURE 7.—Progeny ratios for various mutant input multiplicities. Unadsorbed phage were inactivated with T4-antiserum in all the *am* X4E and *am* N130 experiments. With *am* N122 and *r62*, burst sizes remain large (about 300); therefore, unadsorbed phage were insignificant and comparable results were obtained both with and without antiserum. ○ = *am* X4E, Experiment 1, averages of two repeats, T4D⁺ m.o.i. = 0.43; ● = *am* X4E, Experiment 2, averages of two repeats, T4D⁺ m.o.i. = 0.43; ▲ = *am* N130, averages of two repeats, T4D⁺ m.o.i. = 0.39; ■ = *am* N122, T4D⁺ m.o.i. = 0.53; × = *r62*, T4D⁺ m.o.i.'s = 0.43–0.56; * at least one measurement is based on less than 60 plaque counts.

in a given infected bacterium) is of major concern. Although the second, that the genomes which do express are drawn at random from the pool of infecting phage genomes, is essential to the model, there would not seem to be much doubt of its validity. It appears very unlikely that the mechanism controlling gene expression would be able to distinguish between wild-type genomes and a host of different *r* and amber mutant genomes which probably only differ from wild type by single base-pair alterations.

The results obtained with the amber mutants (Figures 2–5) support the simplest form of the model, namely, that the same *K* genomes express throughout the latent period. This is not consistent with the possibility that the early functions are expressed only by the infecting DNA while the late functions are expressed only by newly synthesized DNA (LURIA 1962). Other recent reports are likewise inconsistent with this concept of regulation of the early and late functions. EDLIN (1965), studying 5-fluorouracil-induced phenotypic reversion of amber mutants of T4 which affect phage DNA synthesis, has concluded “that the late genes can be transcribed from the injected DNA without replication.” This, of course, would be the case if the model presented here were correct.

An alternate explanation of the limited genome expression observed with *am* N58 and *am* X4E is suggested by the results of WOMACK and BARRICELLI (1965). On the basis of irradiation studies, WOMACK and BARRICELLI suggest that the gene 34 function is of a type which they call a “macrotarget” function. They propose that such functions survive only if expression occurs from one of the two injected parental strands early in the infectious process (prior to replication) and subse-

quently from copies of the complementary strand (after replication). If, indeed, the gene 34 function is required before replication from expression of one of the infecting parental strands, then the loss of potential infective centers observed with the late function mutants in gene 34 might be caused only by the lack of early or prereplication expression. If this is correct, the results obtained in this study do not necessarily have any implications as to the number of genomes which can express late functions.

A major objection to the model presented here is that, at least in its simplest form, it predicts that recombinants arising intracellularly will not subsequently express prior to maturation. SÉCHAUD and STREISINGER (1962) have presented evidence which indicates that recombinant *h* genomes do express themselves in the same latent period during which they were formed. Decisive evidence on the efficiency of expression of recombinants, however, is needed. It should be obtainable in the form of "pseudocomplementation" between amber mutants located at the extremes of gene 34 which is several recombination units long. If WOMACK and BARRICELLI's proposal is correct, that certain functions require expression both from one of the infecting strands and from copies of its complement, then the model presented here is not necessarily inconsistent with the expression of recombinants since it might apply only to "early" expression.

The model provides a simple and accurate description of the phenomenon. Its molecular counterpart, however, remains obscure although the studies of EPSTEIN (1960), KOZINSKI and LIN (1965a,b) and others, suggest plausible molecular mechanisms. The fit of the model to experimental data is convincing—the only significant deviation being at the highest multiplicities used. At these high multiplicities, fewer infective centers are recovered than the model predicts. This was expected, however, since at multiplicities of 20 and above, some potential infective centers are lost owing to lysis-from-without. At an m.o.i. of 30, for example, about one half of the infected bacteria are lost under the conditions used in these experiments because of lysis-from-without (SNUSTAD 1966, see Figure 4). A comparable loss is therefore expected in the unequal-input experiments.

It should be emphasized that the data reported here can be explained by any type of a mutant-multiplicity-dependent abortion of the infectious cycle. The observations of EPSTEIN *et al.* (1963) with the amber system, however, argue against any such mutant-induced abortion since infection of the restrictive host with any of a large number of late-function amber mutants results in a normal lytic cycle except for the absence of the component(s) specified by the defective gene(s).

Burst sizes in unequal-input experiments: The results of burst size measurements for cells infected with increasing mutant/wild-type phage input ratios (Figure 6) show that the mutants studied fall into one of two distinct classes. Either the burst size is unaffected by the mutant multiplicity (*am* N122 and the *r* mutants) or the burst size decreases rapidly with increasing mutant multiplicity (*am* N130, *am* N58 and *am* X4E). This indicates that by "flooding" the cell with mutant genomes and, therefore, probably also with mutant protein, one has restricted gene-product activity in the case of *am* N130, *am* N58 and *am* X4E but not in the case of *am* N122 and the *r* mutants.

At present, several explanations are equally feasible. One possibility is that activity is lost at the gene-product level owing to aggregation between mutant and wild-type polypeptides with the mutant being either dominant or codominant. Such an interaction would probably be mutant-specific as is the case with intragenic complementation.

A second possibility is that the genes involved are simply under two different types of regulation. In *am* N122 and *r*-infected cells, for example, synthesis of gene product may be extended over a longer period of time until enough gene function is present to yield a normal burst. With *am* N130, *am* N58 and *am* X4E, there may be no such compensatory mechanism. It should be noted, however, that the only regulatory mechanism now known in phage T4-infected cells is an "alternate on-off" regulation of the expression of the early and late functions. And, since both *am* N130 (decreasing burst size) and *am* N122 (constant burst size) are mutants in genes controlling early functions, the alternate responses cannot be due to any general difference between the expression of early functions and the expression of late functions.

A third plausible explanation is that *am* N122 and the *r* mutants are in genes whose products are catalytically active while the *am* N130, *am* N58 and *am* X4E defects are in genes which control stoichiometric functions. In the former case, the decrease in the amount of wild-type gene product might be compensated for by an extended period of activity. Such compensation would not be possible in the case of a stoichiometric function. If this explanation or any explanation based upon regulation were correct, one would predict that the response would be gene-specific rather than mutant or site-specific. Experiments are currently underway to determine which type of response occurs and to extend the analysis to amber defects in several other genes.

Regardless of the correct explanation, the decreasing burst size with increasing mutant m.o.i. observed with *am* N130, *am* N58 and *am* X4E may be of considerable value in determining the validity of the model presented above. If only a fixed number of genomes can express heterocatalytically, then the mutant/wild-type gene-product ratio should reach a maximum when the mutant input is K minus one. Therefore, the burst size should not decrease further as one increases the mutant multiplicity above that value. This would be strictly true, however, only if every bacterium was infected with exactly the average m.o.i. Actually, at average total m.o.i.'s slightly higher than K , certain proportions of the infected population of cells adsorb less than K phage, and since the burst sizes are decreasing so sharply with increasing mutant multiplicity in this range (Figure 6), these cells may contribute a major proportion of the progeny phage. One would still expect a decrease in burst size, therefore, as the mutant input multiplicity increases above K minus one to the point where essentially no cells are infected with less than K minus one mutant phage. Nevertheless, the model predicts a plateau in the decrease in burst size at the higher mutant multiplicities where essentially all of the input bacteria are infected by K or more phage.

Unfortunately, the results of experiments designed to test this prediction are somewhat ambiguous (Figure 6). With *am* N130, no plateau is evident. The results obtained in two experiments with *am* X4E, on the other hand, do show

a plateau at the higher multiplicities. Moreover, the latter results must be considered more reliable because of the nonleaky nature of *am* X4E. With *am* N130, it was necessary to correct for leakiness by means of a progeny phage per non-yielder factor (see the legend to Figure 6). Thus in the case of *am* N130, burst size calculations depend upon experimental measurements of infective centers, progeny and leakiness, and, as a result, the error may be considerable. Moreover, at the higher multiplicities, the minimum dilution to the growth-tube which will eliminate the effect of the KCN and antiserum (about 10^4) results in a very low titer of yielders so that even direct platings yield few counts; this further limits the accuracy of these results.

At present, it can only be concluded that the results available are not sufficient to either verify or discredit this prediction of the model. More work must be done on this aspect of the problem.

The effect of small burst size on the recovery of infective centers: Earlier in this discussion, the slight deviation of the data obtained at high mutant input multiplicities from the prediction of the model was mentioned, and it was pointed out that such a deviation was expected because of lysis-from-without which occurs at these high m.o.i.'s. In the case of those mutants which exhibit a decreasing burst size with increasing mutant input, *am* N130, *am* N58 and *am* X4E, however, a similar deviation would be expected on a different basis. The burst sizes of cells infected with these mutants decrease to about ten progeny phage at mutant multiplicities of 15 and higher. If maturation of genomes is at random with respect to genotype as studies on phenotypic mixing indicate (STREISINGER 1956), then one would expect a certain proportion of the potential infective centers not to plate on S/6/5 even though they contain a wild-type genome which has expressed heterocatalytically simply because no wild-type genomes were "wrapped up" during the maturation process. At the higher m.o.i.'s with burst sizes of ten and mutant/wild type input ratios of 20–30/1, a major proportion of the potential infective centers should be lost for this reason.

The effect of small burst size on the recovery of infective centers which plate on the respective restrictive host, i.e., contain at least one genotypically wild-type progeny phage, should be even more extreme in *am* X4E-infected cells since the proportion of wild-type genomes will be reduced by recombination. The work of SATO and STENT (unpublished results cited in DELBRÜCK and STENT 1957—see RESULTS) indicates that even in "normal" bursts, as few as one or less wild-type phage per cell was recovered when a multiple mutant containing three "distinctly linked" markers was employed in an unequal-input experiment of this type. Thus, one would expect a large proportion of the potential infective centers not to yield a genotypically wild-type progeny phage in the present experiments with *am* X4E in which the average burst size decreases to about ten phage per cell. As Figure 5 shows, however, the deviation from the model observed with *am* X4E is not any larger than that observed with *am* N130 or *am* N58. The present experiments differ from those of SATO and STENT in one very important aspect. In their experiments, the permissive host of the *r* mutants, *E. coli* B, was infected instead of the restrictive host. All of the cells would, therefore, produce normal bursts irrespective of whether the minority parent expressed itself. On the other hand, in the

present experiments only those cells in which the minority wild-type genomes express themselves heterocatalytically produce infectious progeny phage.

What the present results seem to suggest is that the expressing genomes are protected from recombination, and, moreover, that they are matured preferentially. Both possibilities seem reasonable. The former might be expected if, for example, genomes are in a unique state during transcription. Likewise, the latter might result simply because of a localization of gene-products around the expressing genomes.

Studies on the transfer of parental DNA to progeny phage indicate that the parental DNA of phage is rather widely dispersed among the progeny (KOZINSKI 1961; ROLLER 1964; KONRAD and STENT 1965). If the expressing genomes are protected from recombination, a few conserved molecules should be observed. However, since these would only represent about 1% of the progeny genomes, they might well go undetected. It is interesting to note that KONRAD and STENT (1965) recovered a "minor peak of transferred ^{32}P atoms . . . at the density of the fully-heavy parental DNA, possibly representing the DNA of progeny phage which had received their entire DNA complement from the parent." Their preferred explanation of this peak is as follows: "More likely, however, this fully-heavy DNA represents ^{32}P from a fraction of the phages of the parental population that were dead or that had not properly infected the host cells and were somehow carried over into the lysate and reisolated along with the progeny."

It should be emphasized that the experiments reported in the present paper were not designed to establish whether or not a relationship(s) exists between heterocatalytic expression, replication and recombination, but to determine whether there is a limit to the number of genomes which can express per bacterium. Additional control experiments and more extensive data are necessary before any conclusion(s) can be made about the above-mentioned relationships.

Progeny ratios: Progeny ratios have been determined at various input multiplicities for $r62$ -infected K112($\lambda h8$) and *am* N122, *am* N130, and *am* X4E-infected S/6/5 (Figure 7). Although the results obtained with amber-infected S/6/5 are limited, they indicate that the progeny ratios are approximately equal to the input ratios, possibly being slightly skewed towards a higher proportion of wild type. With $r62$ -infected K112($\lambda h8$), the progeny ratios equal the input ratios at average total m.o.i.'s below K . At total multiplicities above K , however, the mutant/wild-type progeny ratios are considerably lower than the mutant/wild-type input ratios, indicating a preferential recovery of wild-type genomes. This could be explained by a differential rate of replication of expressing and nonexpressing genomes since one is looking at a subpopulation of cells in all of which at least one wild-type genome is expressing itself. A preferential maturation of the expressing genomes would also bias the progeny ratios in this direction.

On the nature of the "slots": On the basis of the results presented above, it has been concluded that only a constant, fixed number of phage genomes can express heterocatalytically in a given bacterium. These results suggest that about four genomes can be expressed per cell in the case of *E. coli* strain K112($\lambda h8$) and that about six can be expressed in *E. coli* strain S/6/5.

These estimates of the number of genomes which can express themselves per

cell are in good agreement with the number of "phage-synthesizing centers" revealed by irradiation of cells either before or after phage infection (EPSTEIN 1960). If these slots were the active sites of mRNA synthesis, they would be expected to consist of "primarily nucleic acid, as can be concluded from the results of a rough action spectrum for inactivation of phage production by infected cells" (EPSTEIN 1960). One is tempted to propose, therefore, that the slots represent sites in the cell where transcription occurs, i.e., some structural component of the cell essential to this process. The results presented above, however, only justify proposing that some cellular component, molecule, structure, environment, etc., which is required for the heterocatalytic expression of phage genomes is present in the bacterium in limited quantity—about four per cell of K112(λ h8) and six per cell of S/6/5.

It seems probable to this author that the slot might represent a regulatory molecule with which the injected genome must interact in order to initiate expression. Such a controlling mechanism is appealing in that it could represent "on" switch for the expression of early functions. A mechanism of this type might have a selective advantage in that it could assure that transcription begins at the correct position on the genome and only when the genome is in the cellular microenvironment most favorable for transcription and/or translation.

KOZINSKI and LIN (1965a,b) have done experiments with T4-infected *E. coli* B which indicate that the nascent or "replicative" T4 DNA, like that of *B. subtilis* (GANESAN and LEDERBERG 1965), enters a proteinaceous complex which floats on the surface of a CsCl gradient. KOZINSKI and LIN (1965b) have used chloramphenicol to demonstrate that the protein(s) involved is present in the host cell prior to infection. Their results suggest that replicating T4 genomes may be attached to the bacterial membrane or cell wall. As they point out, "the possible regulatory role of complex formation in DNA replication should be emphasized." This could likewise apply to regulation of heterocatalytic expression.

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SUMMARY

In this and a companion study (SNUSTAD 1966), it is demonstrated that when cells of K112(λ h8) are infected with a constant, low multiplicity of wild type T4D and varying multiplicities of an *rII* mutant, the proportion of cells infected with wild type which produce progeny phage decreases sharply as the mutant multiplicity increases. A similar response is obtained when S/6/5 is infected with a low multiplicity of wild type and varying multiplicities of an amber mutant, regardless of whether the amber mutant is located in a gene controlling an early function or a late function. Moreover, an identical response occurs when the amber mutant used is a multiple mutant containing defects located in four different late-function genes.—A model which offers a reasonable explanation of these phenomena is developed on the basis of the following two premises: (1)

that only a constant, fixed number of phage genomes can function heterocatalytically in a given bacterium, and (2) that the genomes which do express themselves are drawn at random from the pool of infecting phage genomes. It is estimated that about four phage genomes can be expressed per cell of K112(λ h8) and about six per cell of S/6/5.—On the basis of burst sizes in unequal-input experiments, the mutants studied can be divided into two classes. In cells of the restrictive host infected with one class including *am* N122 and the *r* mutants, the burst size remains large (approximately 300) regardless of the mutant multiplicity. With the other class, *am* N130, *am* N58 and *am* X4E, burst size decreases sharply with increasing mutant input.—Evidence from progeny ratios and from the recovery of infective centers with low burst sizes is discussed which suggests that expressing and nonexpressing genomes may replicate at different rates and mature with different efficiencies. Finally, possible molecular bases for the limited genome participation are discussed.—It is suggested that the limited genome expression observed may be the genetic counterpart to the restricted number of targets observed by EPSTEIN (1960) in irradiation studies. His estimates of the number of “phage-synthesizing centers” agree well with estimates of the number of genomes which can be expressed per bacterium in the present study.

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