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Site- and Gene-specific Limited Heterocatalytic Expression in Bacteriophage T4-infected *Escherichia coli*

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Genetic evidence for site- and gene-specific variation in limited heterocatalytic expression in phage T4-infected *Escherichia coli* is reported, and the implications of such variation are discussed.

Edgar and Steinberg (2) demonstrated that as many as 30 T4 phage genomes can "participate" per cell when participation is evaluated in terms of marker survival. When evaluated in terms of heterocatalytic expression, however, participation is much more limited (4, 10, 11). The molecular basis (or bases) for this phenomenon remains obscure, although the recent results of Schachtele et al. (8, 9) suggest a possible mechanism. Snustad (11) suggested a mathematical interpretation of the results of genetic experiments which is consistent with the proposal of Schachtele et al. (8). Both of these interpretations, in their simplest form, predict host-specific limited heterocatalytic expression, but not gene- or site-specific limited expression.

Whereas Krieg (4) reported site-specific variation among *r*II mutants, Snustad (10; *unpublished data*) observed only host-specific variation with several *r*II deletion mutants and 10 *r*II point mutants. This difference may well have resulted from the fact that all of the point mutants studied by Snustad were of spontaneous origin and were thus probably small additions and deletions (3, 6). This possibility is supported by the observation that none of these point mutants complements any other mutant in the same cistron (D. P. Snustad, Ph.D. Thesis, Univ. of California, Davis, 1965), despite the occurrence of intragenic complementation in the *r*II region (S. P. Champe and S. Benzer, *personal communication*).

In analogous experiments with amber mutants of phage T4, no gene- or site-specific variation in limited heterocatalytic expression was observed (11). In this paper, evidence for rare gene- and site-specific variation is reported.

In all, 78 amber mutants in 61 different genes have been examined in unequal-input experiments of the type described in detail earlier (10). Of these mutants, 71 were described by Snustad (Table 1 of reference 12); the other 7 (H11, H32, B278, C137, H36, A489, and C208) were described by Sarabhai et al. (7). All of these mutants, except those in three genes, namely, genes 22, 23, and 24, yield responses of the type reported for the amber mutants studied earlier (11). However, mutants in genes 22, 23, and 24 yield quite different results. These results and their implications as to the basis of limited heterocatalytic expression are the subject of this note.

The basic experimental design employed was to mixedly infect a restrictive host of the amber mutants (*Escherichia coli* strain S/6/5 or B) with a constant, low multiplicity ($\simeq$ 0.5) of T4D wild-type and with various multiplicities (e.g., 0, 5, 10, 15, 20, etc.) of a given amber mutant. The number of bacteria yielding bursts was then determined by plating infective centers on the restrictive indicator. KCN was used in all experiments to prevent superinfection exclusion. The proportion of potential infective centers (wild-type infected cells) yielding bursts was then plotted against the reciprocal of the average total multiplicity of infection (MOI) to get an estimate of the average number of genophores which can "effectively" express themselves (for the particular function in question) per cell. If an unlimited number of genomes can express themselves per cell, then the proportion of infected bacteria which produces progeny phage should be independent of mutant MOI. If only a limited number can function per cell, then the proportion of infected cells which yields bursts should decrease as the mutant MOI increases. With a plot of the type mentioned above and equation 3 of Snustad (11), which describes the relationship between the

proportion of potential infective centers yielding bursts and the input multiplicities in terms of the average number of genomes that can function per cell, one can easily compare participation numbers for different mutants, different hosts, etc.

Data from experiments with *am*N90 (gene 21) are shown in Fig. 1; identical or very similar results were obtained with all of the other amber mutants studied (including three reported on earlier in reference 11), except those in genes 22, 23, and 24. With amber mutants in the latter three genes, the decrease in the proportion of infected bacteria producing progeny phage began at much lower mutant multiplicities, as is shown for *am*B272 (gene 23) in Fig. 1.

Previous studies with amber mutations located at different positions within the same cistron (e.g., ambers N91, A481, N52, and B280 in gene 37; see reference 1) failed to reveal any site-specific variation in the response observed (*unpublished data*). An investigation of the classic gene 23 colinearity mutants (7; generously provided by A. S. Sarabhai), however, yielded the only example of site-specific variation in limited heterocatalytic expression thus far observed with amber mutants. Eight of the nine mutants studied yielded responses indistinguishable from that shown for *am*H36 (Fig. 2). On the other hand, *am*C208 (Fig. 2) responded in almost the same way as mutants outside the gene 22 to 24 region.

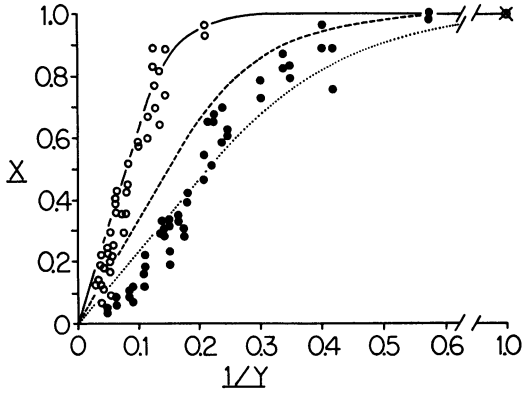

FIG. 1. *Limited heterocatalytic expression in E. coli strain S/6/5 cells mixedly infected with wild-type T4D and either amN90 (gene 21), ○, or amB272 (gene 23), ●. The proportion of potential infective centers which yields progeny phage, X, is plotted against the reciprocal of the average total multiplicity of infection of the potential infective centers, 1/Y. The lines indicate the relationships expected, assuming that the genomes which express themselves are a random sample of the intracellular pool, if only two (dotted line), three (dashed line), or six (solid line) genomes can express themselves per cell.*

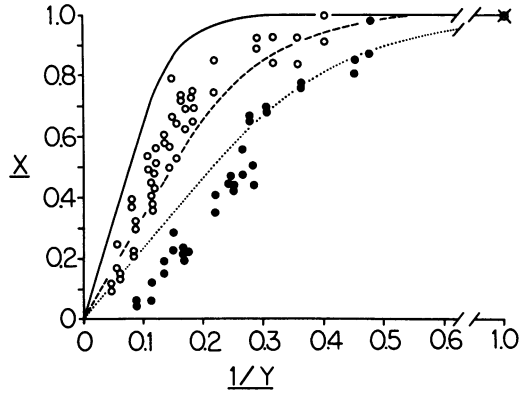

FIG. 2. *Limited heterocatalytic expression in E. coli strain S/6/5 cells mixedly infected with wild-type T4D and either amC208 (○) or amH36 (●), both in gene 23. The plot is as described in the legend to Fig. 1.*

The possibility that the amber stocks employed in these experiments might have contained "killer" particles, which could participate in killing sensitive cells by lysis-from-without although unable to give rise to bursts of progeny phage, was eliminated by directly comparing the infective center titer with the killing titer of the stocks used. All of the gene 23 mutant stocks were tested, and the killing titer (input bacteria minus viable cells) was found to equal the infective center titer. The gene 23 location of the amber defect in the C208 stock used was also verified by complementation tests.

Snustad (11) initially attempted to explain limited heterocatalytic expression by proposing that only a limited number of phage genomes could express themselves per cell. It was suggested that the infecting phage genome had to interact with some cellular component (e.g., membrane attachment site) which was present in limited quantity as a prerequisite to transcription. Subsequently, Schachtele et al. (8, 9), in studies on the mechanism of canavanine death and the inability of T4 to reproduce in canavanine-killed cells, proposed that "there are a limited number of specialized membrane sites in the bacterium at which DNA replication is organized, and that detachment or jamming of the genome at these sites stops transcription."

However, neither of these models can explain gene- or site-specific variations, or both, in limited heterocatalytic expression, which suggests that either (i) such mechanisms are not the basis for limited heterocatalytic expression or (ii) limited heterocatalytic expression has at least two components. The more restricted "effective" (progeny phage production) heterocatalytic ex-

pression observed with amber mutants in genes 22, 23, and 24 may result from dominance interactions at the polypeptide level (1, 12). Gene 23 codes for the major structural component of the phage head (7), whereas genes 22 and 24 appear to code for minor structural components (5). These three genes thus account for over one-half of the protein synthesized during the latter part of the latent period (7). Note that *am*C208 makes the longest polypeptide fragment of all the gene 23 mutants (7). Perhaps the *am*C208 fragments are sufficiently complete so that when mixed with wild-type gene 23 polypeptides they will yield infective progeny phage, whereas the shorter fragments produced by the other gene 23 amber mutants will not. For mutants such as these, in which the burst size becomes small in mixed infections with wild-type (12), this could explain the difference between *am*C208 and the other gene 23 amber mutants. If mixed infections of the restrictive host by *am*C208 and am^+ yield progeny having heads composed of mixed *am* and am^+ subunits, these phage might exhibit altered physical properties. However, attempts to demonstrate a difference in thermal stability between such progeny and am^+ phage have not been successful.

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