

## Host Specificity of DNA produced by *Escherichia coli*

### V. The Role of Methionine in the Production of Host Specificity

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Bacteriophage  $\lambda$  grown in auxotrophic  $met^-$ ,  $pro^-$  or  $arg^-$  strains† of *Escherichia coli* K12 in the presence of the required amino acids show an efficiency of plating of approximately 1 on *E. coli* strains K12 and C. However, if  $met^-$  cells are deprived of methionine during a portion of the latent period, the efficiency of plating of the progeny phage is lower on the host K12 than on strain C. Similar results are obtained with  $met^-$  auxotrophs of strains B and K12(P1); deprivation of methionine during the latent period results in the production of phage with lower efficiency of plating on the host strain than on strain C. Such an effect is not observed following a similar starvation for proline or arginine. These results suggest that methionine is specifically required for the production of host specificity of DNA.

#### 1. Introduction

Host-controlled modification affects by non-mutational changes the host range of bacteriophage (Luria, 1953; Bertani & Weigle, 1953). For phage  $\lambda$ , growth is as a rule allowed on the host strain on which the phage had been produced before. Success of phage growth on other hosts depends on the genetic content of the infected bacterial strain. *E. coli* C appears to be a general acceptor for  $\lambda$ , regardless of the previous host, while *E. coli* K12 accepts the variant  $\lambda$ ·K (phage grown on K12) but restricts variants  $\lambda$ ·C or  $\lambda$ ·B (Arber & Dussoix, 1962). Infection of a host cell with a restricted  $\lambda$  variant leads to a rapid breakdown of the phage DNA (Dussoix & Arber, 1962). No DNA degradation occurs upon infection with nonrestricted  $\lambda$ , which differs from restricted  $\lambda$  only in having undergone host-controlled modification. The determinants provided by this modification have been called host specificity. They are carried on the DNA molecule (Arber & Dussoix, 1962; Dussoix & Arber, 1965), but their chemical nature remains unknown.

The experiments presented in this paper implicate the amino acid methionine in the production of host specificity. When phage  $\lambda$  is grown in appropriate amino acid-requiring host strains, starvation for methionine during a portion of the latent period leads to the production of progeny phage which partially lack the normally conferred host specificity. This effect is not observed upon starvation for other amino acids, suggesting that methionine is specifically required for the host-controlled modification process.

† Abbreviations used:  $met^-$ , methionine auxotroph;  $pro^-$ , proline auxotroph;  $arg^-$ , arginine auxotroph;  $B1^-$ , thiamin auxotroph;  $mal^+ - \lambda^s$ , maltose fermenting and sensitive to phage  $\lambda$ ;  $RC^{r*1}$ , relaxed control of RNA synthesis;  $RC^{str}$ , stringent control of RNA synthesis; e.o.p. = efficiency of plating.

## 2. Materials and Methods

### *Bacterial and bacteriophage strains*

(a) *Escherichia coli* K12 derivatives: strain 151 = Hfr C, *met*<sup>-</sup> *pro*<sup>-</sup> B1<sup>-</sup> RC<sup>re1</sup>, originally obtained from Dr S. E. Luria as W4032; strain 619 = a  $\lambda$ -lysogenic derivative of 151; strain 623 = a  $\lambda$ - and P1-lysogenic derivative of 151; strain 615 = *met*<sup>-</sup> B1<sup>-</sup> RC<sup>str</sup>, obtained in a cross of 151 with strain C600 (Appleyard, 1954); strain 620 = a  $\lambda$ -lysogenic derivative of 615.

(b) *E. coli* B derivatives: strain 251, a transduction hybrid with the *mal*<sup>+</sup>  $\lambda$ <sup>s</sup>-marker from K12 (Arber & Lataste-Dorolle, 1961); strain 628, a *met*<sup>-</sup> mutant of 251; and strain 629, a  $\lambda$ -lysogenic derivative of 628.

(c) *E. coli* C (Bertani & Weigle, 1953).

(d) The bacteriophage strains have been described by Arber & Dussoix (1962).

### *Media*

(a) Synthetic M9 medium: 0.7% Na<sub>2</sub>HPO<sub>4</sub>, 0.3% KH<sub>2</sub>PO<sub>4</sub>, 0.05% NaCl, 0.1% NH<sub>4</sub>Cl, 0.4% glucose, 10<sup>-4</sup> M-CaCl<sub>2</sub>, 10<sup>-3</sup> M-MgSO<sub>4</sub>.

(b) Tryptone broth: 1% Difco Bactotryptone, 0.5% NaCl, pH 7.0; for solid medium completed with 1.5% agar.

Efficiencies of plating of  $\lambda$  were measured with pre-adsorption on indicator strains K12 = C600, K12(P1) = C600(P1), B = 251 and C, grown in Tryptone broth to about 5 × 10<sup>8</sup> cells/ml. and then starved in 0.01 M-MgSO<sub>4</sub> by aeration for 1 hr at 37°C. Table 1 gives the e.o.p. of different  $\lambda$  variants on the above hosts as previously determined under these standard conditions (Arber & Dussoix, 1962).

TABLE 1

*Efficiency of plating of phage  $\lambda$  variants on different host strains*

Phage variant	Efficiency of plating on host strains			
	K12	K12(P1)	B251	C
$\lambda$ -K	1	2 × 10 <sup>-5</sup>	10 <sup>-4</sup>	1
$\lambda$ -K(P1)	1	1	10 <sup>-4</sup>	1
$\lambda$ -B	4 × 10 <sup>-4</sup>	7 × 10 <sup>-7</sup>	1	1
$\lambda$ -C	4 × 10 <sup>-4</sup>	4 × 10 <sup>-7</sup>	2 × 10 <sup>-4</sup>	1

(From Arber & Dussoix, 1962)

## 3. General Experimental Plan

The general plan of the experiments to be discussed below is illustrated schematically in Fig. 1. Bacteria were grown in M9 synthetic medium supplemented with amino acids as required. While still in the exponential growth phase, the cells were either irradiated with an optimum induction dose of u.v. ( $\lambda$ -lysogenic strains) or infected with  $\lambda$  phage (non-lysogenic strains), and incubation was continued for a period of *t*<sub>1</sub> minutes. The cells were then washed by centrifugation, resuspended in fresh medium lacking the required amino acid to be tested, and incubated for *t*<sub>2</sub> minutes. Finally, the missing amino acid was added back for a period of *t*<sub>3</sub> minutes, in order to allow phage structural protein formation and maturation, after which the cells were prematurely lysed with chloroform. The liberated phage were assayed, (a) on *E. coli* C indicator, to determine total infective titre (see Discussion), and (b) on

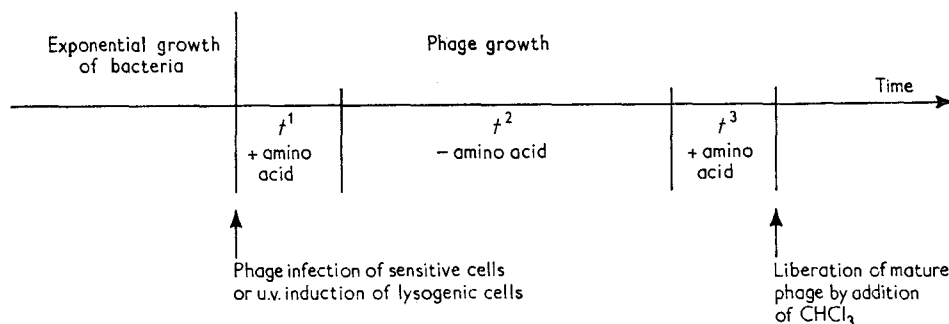


FIG. 1. Experimental plan.

the host strain. The ratio of the titre on C to that on the host strain was taken to indicate the extent of the host specificity conferred on the progeny phage.

#### 4. Results

A series of experiments was carried out with *E. coli* 619, a  $\lambda$ -lysogenic, doubly auxotrophic *met*<sup>-</sup>, *pro*<sup>-</sup>, *RC*<sup>res</sup> K12 strain. Phage production was induced by u.v. irradiation immediately before the beginning of the incubation period  $t_1$ . The influence of variations in  $t_1$ ,  $t_2$  (Table 2) and  $t_3$  (Table 3(a)) was investigated, as well as that of the omission of only methionine, only proline, or both, during  $t_2$  (Table 3(a)). The results can be summarized as follows.

(1) Experimental conditions were found such that methionine starvation during  $t_2$  resulted in lysates the e.o.p. of which on strain C was several hundred times higher than on the host strain K12.

(2) This high e.o.p. ratio was observed only after methionine starvation, but not after proline starvation. Starvation for both methionine and proline also gave phage with a higher e.o.p. on C than on K12.

(3) Starvation for either amino acid during the latent period sharply reduced the titre of progeny phage; this reduction is partially explained also by the premature lysis of the productive bacteria.

(4) The u.v.-irradiated bacteria had to be pre-incubated with all required amino acids for 10 to 20 minutes ( $t_1$ ) previous to the starvation in order to produce a titre exceeding the low level of phage particles (about  $10^4$  phage/ml.) which were liberated by the small fraction of spontaneously induced cells and which, at the moment of deprivation of the amino acid, were already matured intracellularly.

(5) Prolongation of  $t_2$  in the absence of methionine from 50 to 100 minutes resulted in a slow decrease of the final plaque titre; this decrease was more pronounced on K12 indicator than on C. Hence the effect of methionine deprivation was more striking for prolonged starvation.

(6) A minimum time  $t_3$  of about 10 minutes after re-addition of the amino acids was required before the number of mature phages with plaque-forming ability on C increased significantly over the background of spontaneously produced phages, but then this number rose rapidly as a function of time to reach after 35 minutes a value corresponding to an average burst size of one phage per induced cell. Highest C/K12 ratios were obtained for  $t_3$  of about 20 minutes. Upon longer incubation, DNA gradually recovered its host specificity.

TABLE 2

*The effect of methionine starvation during the latent period on the production of  $\lambda$  phage in u.v.-induced K12( $\lambda$ ) bacteria*

<i>t</i> 1	<i>t</i> 2	<i>t</i> 3	Lysates, obtained from $1.4 \times 10^8$ induced bacteria/ml.			
			assayed on C (plaques/ml.)	assayed on K12 (plaques/ml.)	ratio C/K12	assayed on B (plaques/ml.)
Duration of incubation with 10 $\mu$ g/ml. methionine (min)	Duration of methionine starvation (min)	Duration of incubation after re-addition of 10 $\mu$ g/ml. (methionine) (min)				
0	50	20	$2.2 \times 10^4$	$1.6 \times 10^4$	1	$< 10^1$
	75		$2.7 \times 10^4$	$2 \times 10^4$	1	$< 10^1$
	100		$1.6 \times 10^5$	$6 \times 10^3$	27	$< 10^1$
10	50	20	$5.2 \times 10^4$	$6 \times 10^3$	9	$< 10^1$
	75		$5.5 \times 10^5$	$3.6 \times 10^4$	15	$4 \times 10^1$
	100		$3.4 \times 10^6$	$2.9 \times 10^4$	117	$2.5 \times 10^2$
20	50	20	$1.5 \times 10^7$	$1.4 \times 10^5$	107	$1.2 \times 10^3$
	75		$1.8 \times 10^7$	$5.2 \times 10^4$	346	$9 \times 10^2$
	100		$8.4 \times 10^6$	$2.1 \times 10^4$	400	$6.3 \times 10^2$
30	50	20	$5.7 \times 10^7$	$1.7 \times 10^6$	33	$4.2 \times 10^3$
	75		$2.3 \times 10^7$	$2.5 \times 10^5$	92	$2.7 \times 10^3$
	100		$1.2 \times 10^7$	$5.1 \times 10^4$	235	$8.8 \times 10^2$
120	0	0	$3.5 \times 10^9$	$3.1 \times 10^9$	1	$2.4 \times 10^5$

Bacteria of strain 619 (K12( $\lambda$ )<sup>+</sup> *met*<sup>-</sup> *pro*<sup>-</sup> *B1*<sup>-</sup> *RC*<sup>res1</sup>) were grown in M9 medium, supplemented with vitamin B1 (1  $\mu$ g/ml.) and methionine and proline (25  $\mu$ g/ml. each), to a concentration of  $7 \times 10^8$  cells/ml., washed by centrifugation, resuspended at the same concentration in M9 + B1 (1  $\mu$ g/ml.) + methionine and proline (10  $\mu$ g/ml. each), irradiated for 90 sec with u.v. (0.45% survival) and incubated for *t*1 min with aeration. The cultures were then chilled, washed once by centrifugation in the cold, resuspended in an equal vol. of M9 + B1 (1  $\mu$ g/ml.) + proline (10  $\mu$ g/ml.), incubated for *t*2 minutes, diluted fivefold into fresh M9 with final concentrations of 1  $\mu$ g/ml. B1, 10  $\mu$ g/ml. methionine and 10  $\mu$ g/ml. proline, incubated for another *t*3 minutes and then chloroformed. The active phage titres were assayed on C, K12 and B indicators. In one instance (tube of *t*1 = 20 min) the free phages were assayed on K12 indicator 10 min after resuspension in the medium without methionine: the titre, adjusted to the number of bacteria producing the final lysate, was  $1.5 \times 10^4$  phages/ml.

We conclude that phage DNA produced during methionine starvation lacks, at least partially, the determinants for host specificity required for successful infection of K12, whereas DNA synthesized in cells deprived of proline carries the normal host specificity.

The results of similar experiments (not presented) carried out with independently derived singly auxotrophic K12( $\lambda$ ) *met*<sup>-</sup> and K12( $\lambda$ ) *arg*<sup>-</sup> strains agreed with the above findings. Starvation for methionine following u.v. induction led to the formation of phage that was restricted on the host strain K12, whereas arginine deprivation did not influence the relative efficiency of plating of the progeny on K12 and C.

Although the methionine effect described above is more striking on *RC* relaxed strains (Stent & Brenner, 1961), it can also be observed with *RC* stringent strains (Table 3(b)). In the *RC* stringent host, mature intracellular phage appeared after the same short incubation period *t*3 following re-addition of methionine as was observed in the *RC* relaxed strain, suggesting that some phage DNA was produced during *t*2

TABLE 3

*Phage liberated by premature lysis of u.v. induced K12( $\lambda$ ) met<sup>-</sup> pro<sup>-</sup>,  
after ninety minutes of starvation for amino acids*

Amino acid omitted during $t_2 = 90$ min	$t_3$ Duration of incubation after re-addition of amino acids (min)	Lysates			
		assayed on C (plaques/ml.)	assayed on K12 (plaques/ml.)	ratio C/K12	assayed on B (plaques/ml.)
(a) <i>Strain 619:</i> K12( $\lambda$ ) <sup>+</sup> met <sup>-</sup> pro <sup>-</sup> B1 <sup>-</sup> RC <sup>res1</sup> ( $7.6 \times 10^7$ infective centres/ml.)					
Proline	5	$2.7 \times 10^4$	$2.7 \times 10^4$	1	—
	14	$4.6 \times 10^4$	$4.2 \times 10^4$	1	$2 \times 10^1$
	18	$5.5 \times 10^5$	$4.2 \times 10^5$	1	$5 \times 10^1$
	22	$2.6 \times 10^6$	$2.4 \times 10^6$	1	$3.3 \times 10^2$
	26	$8.7 \times 10^6$	$7.1 \times 10^6$	1	$9.8 \times 10^2$
	35	$3.5 \times 10^7$	$3.9 \times 10^7$	1	$3.5 \times 10^3$
Methionine	5	$4.6 \times 10^4$	$2.6 \times 10^3$	18	—
	14	$4.5 \times 10^5$	$2.6 \times 10^3$	173	$5 \times 10^1$
	18	$3.2 \times 10^6$	$1.4 \times 10^4$	229	$4.3 \times 10^2$
	22	$9.3 \times 10^6$	$4.3 \times 10^4$	216	$1.4 \times 10^3$
	26	$1.9 \times 10^7$	$1.3 \times 10^5$	146	$2.3 \times 10^3$
	35	$3.9 \times 10^7$	$8.3 \times 10^5$	47	$2.8 \times 10^3$
Proline and methionine	22	$6.3 \times 10^6$	$1.5 \times 10^5$	42	$7.2 \times 10^2$
None	10	$6.9 \times 10^8$	$6.0 \times 10^8$	1	$8.6 \times 10^4$
(b) <i>Strain 620:</i> K12 ( $\lambda$ ) <sup>+</sup> met <sup>-</sup> B1 <sup>-</sup> RC <sup>str</sup> ( $5.6 \times 10^7$ infective centres/ml.)					
Methionine	5	$5 \times 10^4$	$1.1 \times 10^4$	5	—
	14	$3.3 \times 10^6$	$2.8 \times 10^5$	12	$3.7 \times 10^2$
	18	$1.4 \times 10^7$	$1.1 \times 10^6$	13	$2.5 \times 10^3$
	22	$4.6 \times 10^7$	$4 \times 10^6$	11	$4.2 \times 10^3$
	26	$7 \times 10^7$	$8.5 \times 10^6$	8	$7.2 \times 10^3$
	35	$8.6 \times 10^7$	$1.1 \times 10^7$	8	$8 \times 10^3$
None	10	$2.9 \times 10^9$	$2.5 \times 10^9$	1	$3.9 \times 10^5$

This is essentially the same procedure as described in Table 2. The bacteria were grown to about  $5 \times 10^8$  cells/ml. before the induction. The fraction of cells surviving the u.v. irradiation was 2.5% for (a) and 5% for (b).  $t_1$  and  $t_2$  were kept constant at 20 and 90 min, respectively.

in the absence of methionine. This may be in agreement with the observation of Kellenberger, Lark & Bolle (1962) that some residual DNA synthesis occurs in T2-infected, stringent strains.

Another set of experiments was carried out by *infection* of non-lysogenic K12 *met*<sup>-</sup> *pro*<sup>-</sup> strains with phage  $\lambda$  rather than by induction of phage production in lysogenic strains. Here any pre-incubation with amino acids (*t*<sub>1</sub>) prior to starvation for either methionine or proline resulted in a high yield of progeny phage which plated on K12 and C indicators with equal efficiency. However, an effect of methionine starvation was observed if the procedure was modified to omit the pre-incubation *t*<sub>1</sub>. In the experiment shown in Table 4, amino acid-starved K12 *met*<sup>-</sup> *pro*<sup>-</sup> bacteria were infected with  $\lambda$ -K, incubated in the absence either of methionine or proline for the

TABLE 4  
*The effect of amino acid starvation during the latent period on the production of  $\lambda$  in phage-infected K12 bacteria*

Amino acid omitted during <i>t</i> <sub>2</sub>	<i>t</i> <sub>2</sub> Duration of amino acid starvation (min)	<i>t</i> <sub>3</sub> Duration of incubation after re-addition of missing amino acids (min)	Lysates		
			assayed on C (plaques/ml.)	assayed on K12 (plaques/ml.)	ratio C/K12
(a) <i>Strain 151: K12 met</i> <sup>-</sup> <i>pro</i> <sup>-</sup> <i>B1</i> <sup>-</sup> <i>RC</i> <sup>rel</sup> ( $6 \times 10^7$ infected cells/ml.)					
Proline	55	18	$9 \times 10^5$	$1.2 \times 10^6$	0.8
Methionine	30	18	$1.7 \times 10^7$	$3.6 \times 10^6$	4.7
Methionine	55	18	$1.3 \times 10^7$	$1.5 \times 10^6$	8.7
Proline and methionine	55	18	$4.3 \times 10^6$	$7.5 \times 10^5$	5.7
None	0	60	$3.7 \times 10^9$	$4.0 \times 10^9$	0.9
(b) <i>Strain 615: K12 met</i> <sup>-</sup> <i>B1</i> <sup>-</sup> <i>RC</i> <sup>str</sup> ( $6 \times 10^7$ infected cells/ml.)					
Methionine	30	18	$2.9 \times 10^8$	$8.2 \times 10^7$	3.5
Methionine	55	18	$3.5 \times 10^8$	$6.0 \times 10^7$	5.8
None	0	60	$5.3 \times 10^9$	$4.8 \times 10^9$	1.1

Complete medium for strain 151: M9+B1 (1  $\mu$ g/ml.) + methionine (10  $\mu$ g/ml.) + proline (10  $\mu$ g/ml.) and for strain 615: M9 + B1 (1  $\mu$ g/ml.) + methionine (10  $\mu$ g/ml.). Bacteria were grown to a concentration of  $8 \times 10^8$  cells/ml., re-suspended and incubated for 30 min in equal vol. of M9 nonsupplemented with the required amino acids, centrifuged again, resuspended in 0.01 M-MgSO<sub>4</sub> at a concentration of  $2 \times 10^9$  cells/ml. and incubated for an additional 30 min. Then the bacteria were infected at a multiplicity of 0.6 phage  $\lambda$ *cb*<sub>2</sub>-K per cell. After 15 min adsorption at 37°C without aeration, the complexes were diluted fivefold into fresh M9 + B1 medium and aerated for 5 min. Then the bacteria were washed twice by centrifugation in the cold and resuspended at a concentration of  $6 \times 10^8$  cells/ml. in M9 medium completed under omission of the indicated amino acids. (Pre-incubation in the complete medium was omitted, i.e. *t*<sub>1</sub> = 0 min). After the incubation time *t*<sub>2</sub>, cells were diluted fivefold into complete medium and incubated for an additional *t*<sub>3</sub> minutes, before intracellular phage particles were liberated by addition of chloroform.

period indicated ( $t_2$ ), and then supplemented with the missing amino acid for 18 minutes ( $t_3$ ) prior to premature lysis. As before, proline starvation had no effect on the host specificity of the progeny, whereas methionine starvation produced a decrease in the relative e.o.p. of the liberated phage on K12. The effect was less pronounced than that observed in u.v.-induced lysogenic strains, and its magnitude was not increased by irradiating the cells before infection with a dose of u.v. corresponding to the induction dose for lysogenic strains.

In one such experiment, the infecting phage had been density-labelled with deuterium. The one-cycle lysate, grown in nondeuterated, methionine-starved K12 *met<sup>-</sup>* bacteria (multiplicity of infection = 1,  $t_1 = 0$  min,  $t_2 = 90$  min,  $t_3 = 25$  min) was centrifuged to density equilibrium in CsCl. Fractions were collected dropwise from the bottom of the centrifuge tube and assayed on indicators K12 and C. All phages carrying semiconserved parental DNA plated with the same efficiency on K12 and on C, whereas phages with only new DNA gave 12 times more plaques on C than on K12. We conclude that methionine deprivation did not lead to destruction of the host specificity of the parental DNA, and that K12 directed host specificity had been conferred to some extent on the newly replicated DNA, presumably between the time of re-addition of the methionine and phage maturation. It is not known how to explain incomplete modification of a phage population: whether by the existence of single DNA molecules provided at intermediate degrees with host specificity or by a mixture of phages with pure unmodified and with pure modified DNA.

To test the generality of the methionine effect on host specificity, experiments were carried out with methionine auxotrophs on *E. coli* strains B (Table 5) and K12(P1) (Table 6). Methionine deprivation of u.v.-induced bacteria during the latent period of phage development resulted in both cases in incomplete production of host specificity.

TABLE 5

*The effect of methionine starvation during the latent period on the production of  $\lambda$  in u.v.-induced B( $\lambda$ ) bacteria*

Duration of incubation with 10 $\mu$ g/ml. methionine (min)	$t_2$ Duration of methionine starvation (min)	$t_3$ Duration of incubation after re-addition of 10 $\mu$ g/ml. methionine (min)	Lysate			
			assayed on C (plaques/ml.)	assayed on B (plaques/ml.)	ratio C/B	assayed on K12 (plaques/ml.)
25	100	20	$2.3 \times 10^7$	$3.0 \times 10^6$	7.7	$1.1 \times 10^4$
120	0	0	$3.8 \times 10^9$	$3.7 \times 10^9$	1	$1.6 \times 10^6$

Bacteria of strain 629 (*Bc mal<sup>+</sup> - $\lambda^s$ ( $\lambda$ ) met<sup>-</sup> RC<sup>str</sup>*) were grown in M9 medium supplemented with methionine (25  $\mu$ g/ml.) to a concentration of  $3 \times 10^8$  cells/ml., washed by centrifugation, resuspended at the same concentration in M9 + methionine (10  $\mu$ g/ml.), irradiated for 50 sec with u.v. ( $6 \times 10^{-5}$  survival) and incubated for  $t_1$  min with aeration. The culture was then chilled, washed once by centrifugation in the cold, resuspended in an equal vol. M9, incubated for  $t_2$  min, diluted fivefold into M9 + methionine (10  $\mu$ g/ml.), incubated for another  $t_3$  min and then chloroformed. The active phage titres were assayed on C, B and K12 indicators.

TABLE 6

*The effect of amino acid starvation during the latent period on the production of  $\lambda$  in u.v.-induced K12(P1)( $\lambda$ ) bacteria*

t1 (min)	t2 Amino acid omitted	t3 (min)	Lysate						
			assayed on C (plaques/ml.)	assayed on C(P1) (plaques/ml.)	ratio C/C(P1)	assayed on K12 (plaques/ml.)	ratio C/K12	assayed on K12(P1) (plaques/ml.)	
20	Methionine	90	20	$10^5$	$2.4 \times 10^4$	4	$7.9 \times 10^3$	13	$7.8 \times 10^3$
20	Proline	90	20	$1.6 \times 10^6$	$1.1 \times 10^6$	1.5	$1.1 \times 10^6$	1.5	$1.2 \times 10^6$

The experiment was carried out as in Table 2, using strain 623: K12(P1)( $\lambda$ ) *met<sup>-</sup> pro<sup>-</sup> B1<sup>-</sup> RC<sup>res</sup>*; u.v. induction dose : 80 sec (0.3% survival).

However, the effects appeared to be less pronounced than in K12; in B this may be related to the fact that the strain was *RC* stringent, while in K12(P1) the yields of progeny phage were consistently low, perhaps due to the presence of the P1 genome. By plating the progeny lysate from u.v.-induced K12(P1)( $\lambda$ ) *met<sup>-</sup> pro<sup>-</sup>* on K12 and C(P1) indicators as well as on C, it was possible to estimate independently the effects of amino acid starvation on the host specificities conferred by the K12 host and by the P1 prophage. As shown in Table 6, methionine starvation decreased both K12-specific and P1-specific modification, whereas proline starvation affected neither.

Many of the lysates obtained in the experiments described above were assayed not only on bacteria of the donor strains and on C, but also on the other available hosts of  $\lambda$ , in particular on those that restrict the acceptance of phage grown under normal conditions. The results of these assays are included in Tables 2, 3 and 5. It was found as a rule that the titres on the restricting indicator strain paralleled those obtained on indicator C, being lower by a factor typical for the strains involved and equal to the factor of restriction measured for phage grown under normal conditions. It is seen in Table 2 for example that the phage titres obtained upon assay on B were about  $10^4$  times lower than those determined on C, independently of whether the titre of phages active on K12 was equal to, or much lower than, that on C. This observation might suggest that a low proportion of  $10^{-4}$  cells of strain B do accept restricted phage  $\lambda$  independently of whether the phage DNA contains K12-directed host specificity or none. The following experiment is consistent with these ideas. Lysates of phage  $\lambda$  were made in methionine-starved *met<sup>-</sup> C* bacteria under conditions identical to those applied above. These lysates showed the same ratios of restriction on K12 and B indicators as control lysates obtained in cells supplemented with methionine.

The experiments presented in Table 7 were carried out in order to test the effects of substituting the analogues ethionine and norleucine for methionine, during either period t2, t3 or both. Comparing line 1 with lines 2 to 5, it is evident that neither analogue fulfils the methionine requirement for conferring host specificity during period t2. From lines 6 to 8, it can be seen that, following starvation for methionine (t2), incubation with ethionine rather than methionine does not significantly decrease the yield of progeny phage, whereas substitution of norleucine gives an appreciably lower titre.

TABLE 7  
*Substitution of methionine by the methionine analogues  
 ethionine or norleucine*

Amino acid present during			Lysates, obtained from $5.4 \times 10^7$ induced bacteria/ml.			
$t_1 = 20$ min	$t_2 = 90$ min	$t_3 = 20$ min	assayed on C (plaques/ml.)	assayed on K12 (plaques/ml.)	ratio C/K12	assayed on B (plaques/ml.)
(1) Methionine	Met	Met	$9 \times 10^8$	$1.2 \times 10^9$	1	$7.4 \times 10^4$
(2) Methionine	Eth	Eth	$4.2 \times 10^5$	$2 \times 10^4$	21	$1.3 \times 10^2$
(3) Methionine	Eth	Eth + Met	$2.7 \times 10^7$	$3.1 \times 10^8$	9	$2.3 \times 10^3$
(4) Methionine	Norl	Norl	$3.3 \times 10^4$	$8.3 \times 10^3$	4†	$< 10^1$
(5) Methionine	Norl	Norl + Met	$3.4 \times 10^7$	$3.2 \times 10^8$	11	$3.7 \times 10^3$
(6) Methionine	None	Met	$6.7 \times 10^6$	$1.2 \times 10^5$	56	$1.1 \times 10^3$
(7) Methionine	None	Eth	$3.9 \times 10^6$	$7.7 \times 10^4$	51	$10^3$
(8) Methionine	None	Norl	$2.2 \times 10^5$	$2.0 \times 10^3$	110	$2 \times 10^1$

† Not significant because of low phage yield.

The experiment was carried out with strain 619 as described in Table 2, except that the amino acids present during the various periods were as shown (L-methionine 10  $\mu\text{g/ml.}$ , DL-ethionine 20  $\mu\text{g/ml.}$ , DL-norleucine 20  $\mu\text{g/ml.}$ ). In addition, L-proline (10  $\mu\text{g/ml.}$ ) was present during the whole experiment.

## 5. Discussion

The interpretation of the experiments presented here is based on the assumption that *E. coli* C will not exert a restriction on DNA devoid of host specificity determinants. Unfortunately, the phage titres in these experiments were too low to allow direct verification of this point by particle count in the electron microscope. However, the following observations support such an assumption: strain C accepts phage  $\lambda$  grown on all other known hosts for  $\lambda$ , including modification-less mutants of K12 and B (Wood, 1965). Restriction-less mutants of K12 and B (Wood, 1965) plate all  $\lambda$  variants tested with the same efficiency as does strain C. Furthermore, in the lysates obtained following methionine deprivation, the phage titres as measured on a restricting host strikingly paralleled the titres obtained in C (but not those measured on the host itself), being lower by a factor of restriction characteristic for phage grown under normal conditions.

The finding that phage lysates produced during methionine starvation plate with higher efficiency on indicator C than on the host strain is taken as evidence that the phage DNA is not, or is only incompletely, supplied with host specificity in the absence of methionine.† A methionine effect is obtained for all the host specificities investigated: those of K12, of B and of prophage P1. The effect appears to be specific for

† The DNA of such phage is probably degraded upon re-infection of the host strain. The question then arises, why the DNA produced under methionine starvation and deficient in host specificity is not degraded by the host cells immediately following its synthesis. It is possible that some of the newly synthesized DNA molecules are broken down; indeed, the phage yield was low following methionine starvation and decreased as period  $t_2$  was prolonged. On the other hand, preliminary experiments (Wood, personal communication) have suggested that restriction also is specifically depressed by methionine starvation. An alternative explanation may be that the restricting functions are exerted more efficiently on the cell surface than in the cytoplasm and thus act specifically on DNA entering the cell.

methionine, since phage grown in cells starved for either proline or arginine show normal host specificity. It thus seems improbable that the deficiency of host specificity is a consequence of the non-production of a specific protein, such as an enzyme involved in conferring host specificity. A more likely interpretation might be that methionine is directly involved in the production of host specificity. One attractive possibility which would not contradict any of the experimental observations so far available (Dussoix & Arber, 1965) is that host specificity is conferred by the alkylation of specific sites on the phage DNA. This could occur in enzymic reactions such as those described by Borek, Mandel & Fleissner (1962) and Gold & Hurwitz (1963), in which methionine is known to serve as a methyl donor. Experiments designed to test this hypothesis are now in progress.

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#### REFERENCES

- Appleyard, R. K. (1954). *Genetics*, **39**, 440.  
Arber, W. & Dussoix, D. (1962). *J. Mol. Biol.* **5**, 18.  
Arber, W. & Lataste-Dorolle, C. (1961). *Path. Microbiol.* **24**, 1012.  
Bertani, G. & Weigle, J. J. (1953). *J. Bact.* **65**, 113.  
Borek, E., Mandel, L. R. & Fleissner, E. (1962). *Fed. Proc.* **21**, 379.  
Dussoix, D. & Arber, W. (1962). *J. Mol. Biol.* **5**, 37.  
Dussoix, D. & Arber, W. (1965). *J. Mol. Biol.* **11**, 238.  
Gold, M. & Hurwitz, J. (1963). *Cold Spr. Harb. Symp. Quant. Biol.* **28**, 149.  
Kellenberger, E., Lark, K. G. & Bolle, A. (1962). *Proc. Nat. Acad. Sci., Wash.* **48**, 1860.  
Luria, S. E. (1953). *Cold Spr. Harb. Symp. Quant. Biol.* **18**, 237.  
Stent, G. S. & Brenner, S. (1961). *Proc. Nat. Acad. Sci., Wash.* **47**, 2005.  
Wood, W. B. (1965). *Path. Microbiol.* **28**, 73.