ASSOCIATION OF TEMPERATE PHAGE P2 WITH THE PRODUCTION OF HISTIDINE NEGATIVE SEGREGANTS BY ESCHERICHIA COLI.

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Prototrophic Escherichia coli K strains carrying P2 prophage in location H, very closely linked with the histidine locus, have been found to spontaneously segregate his^- cells, most of which are non-lysogenic. No his^- segregants have been found in non-lysogenic E. coli K or C strains, or in E. coli C strains carrying P2 prophage in a variety of locations. His^- isolates are also obtained following P2 infection of K strains but not of C strains.

Phage P2, unlike most temperate phages, can establish itself as prophage at a number of different chromosomal sites in its host, E. coli (Bertani and Six, 1958). In E. coli K, one of these sites (location H) is very closely linked with the histidine operon, showing a co-transduction frequency of approximately 40% in P1 transductions (Kelly, 1963; Sunshine and Kelly, 1967). One of the sites occupied by P2 prophage in E. coli C, location I, is more distantly linked with histidine. P1 transductions from non-lysogenic E. coli C strains to strains carrying P2 prophage in location I show a co-transduction frequency for non-lysogenicity of 5-6%, but co-transduction of P2 prophage with histidine selection has not been found to occur (Sunshine and Kelly, 1967).

Results and Experimental

The properties of phage P2 and of its mutants, and the media used have been described by G. Bertani (1951, 1954) and by L. E. Bertani (1959). Davis glucose agar was routinely supplemented with B1 to satisfy the growth requirement of K-40 and its derivatives.

When single colony isolates of *his*$^+$ *E. coli K* strains carrying *P2* prophage in location H were transferred to nutrient broth (NB), incubated overnight, diluted approximately 10^{-5}, and spread on Davis glucose agar containing 1 to 2 ug/ml histidine (low histidine agar), some of the resulting colonies were very small and translucent. Such colonies were transferred to NB, incubated overnight, diluted, and spotted on Davis glucose agar without histidine and with 20 ug/ml histidine.

As shown in table 1, *E. coli K* strains carrying *P2* prophage in location H yielded about 0.3% *his*$^-$ colonies. *E. coli K* strains carrying *P2* prophage in other locations, *E. coli C* strains carrying *P2* prophage in various locations and non-lysogenic *E. coli K* and *C* strains did not produce *his*$^-$ colonies under these conditions.

In addition to the strains listed in table 1, nine substrains of *K-40*, newly lysogenized with *P2 rd 1 c* by Miss Sandra Reid, were tested for segregation of *his*$^-$ colonies, and also tested for prophage location by bacterial crosses. Five of these strains carry the prophage in a location closely linked with the histidine locus, presumably location H. All of these five strains segregate *his*$^-$ cells. No *his*$^-$ segregants were obtained from the other strains which carry the prophage in other locations.

To test whether infection of *E. coli K* strains with *P2* might similarly lead to the production of *his*$^-$ cells, wild type *P2* was plated on *E. coli K-12* and *K-40*. Ten plaques from each culture were picked separately to 1 ml NB, incubated overnight and diluted. 0.1 ml amounts were plated on low histidine agar, producing, after incubation, 5-600 colonies per plate. *His*$^-$ colonies were found on 8 out of 10 of the plates of *K-12* or *K-40* derived in this way from *P2* plaques. Controls, in which 10 randomly chosen spots on uninfected lawns of *K-12* or *K-40* were similarly tested, showed no *his*$^-$ colonies. Similar experiments with *P2 xt* and with *P2 hy dis* also led to the production of *his*$^-$ colonies. No *his*$^-$ colonies were found when *C-2* was infected with wild type *P2*.

Table 1

Occurrence of histidine negative colonies on low histidine agar in E. coli strains lysogenic for P2.

Strain	Pertinent genetic structure	Approximate number of colonies plated	Frequency of his⁻ segregants
K-40	Nonlysogenic for P2	3,600	0
LG-102	$(P2)_{II}$	6,000	0
LG-103	$(P2)_{II}$	6,000	0
LG-106	$(P2)_{H}$	13,400	0.3%
K-12	Nonlysogenic for P2	2,500	0
LG-4	$(P2)_{H}$	9,000	0.38%
LG-5	$(P2)_{H}$	7,400	0.31%
C-2	Nonlysogenic for P2	3,000	0
C-27	$(P2\ rd\ 1\ c)_{I}$	3,000	0
C-67	$(P2\ c)_{II}$	3,000	0
C-77	$(P2)_{III}$	3,000	0

$(P2)_{H}$ indicates a strain lysogenic for P2 in location H. LG-102, 103, and 106 are P2 lysogenic isolates numbers 2, 3, and 6 of E. coli K-40; LG-4 and LG-5 are P2 lysogenic isolates numbers 4 and 5 of E. coli K-12 (Kelly, 1963).

The his⁻ isolates from lysogenic or infected K strains were tested for the presence of phage by spotting on appropriate indicator bacteria, and tested for immunity to P2 or P2 hy dis by cross-streaking against a phage stock containing approximately 2.5×10^8 p.f.u./ml. Of 206 his⁻ isolates from lysogenic strains carrying P2 prophage in location H (LG-106, LG-4 and LG-5), 201 are non-lysogenic and sensitive to P2. Of 92 his⁻ isolates obtained following infection of K strains with P2, P2 xt, or P2 hy dis, 79 are non-lysogenic and sensitive.

These strains were also tested for the ability to grow on 150 ug/ml L-histidinol. Only one strain able to grow on L-histidinol was isolated. This was obtained from K-12 infected with P2 hy dis, and is one of the minority of strains which is lysogenic for the infecting phage. Upon primary isolation and spotting

on histidinol this isolate produced very thin, translucent background growth with opaque papillae. The two types of colonies were isolated and retested on histidinol. The opaque colonies grew well on histidinol. The translucent growth did not produce individual colonies on histidinol, but when spotted, again produced translucent background growth and opaque papillae. Both types of colonies remained his$^-$.

UV induced his$^-$ strains in our collection, when spotted on Davis glucose agar supplemented with 20 ug/ml histidine, appear to grow as well as his$^+$strains This was not true of the his$^-$ segregants isolated. These strains uniformly grew more slowly, and the final growth obtained was less opaque.

Two his$^-$ segregants from LG-106, two from LG-4, and two from LG-5 were tested for reversion to his$^+$ by plating approximately 6 x 10^9, 6 x 10^8 and 6 x 10^7 washed cells per plate in soft agar layers on Davis glucose agar supplemented with B1. No revertants were found after 4 days. A UV induced his$^-$ mutant of LG-106 produced 7 revertant colonies under the same conditions. In another experiment, 5 independent his$^-$ segregants from LG-106 were tested for spontaneous reversions and for induction of reversions by nitrosoguanidine (NTG) and diethylsulfate (DES), using the method of Eggertsson and Adelberg (1965). A series of six Davis glucose agar plates supplemented with 0.01% NB and B1 were inoculated with approximately 2.5 x 10^9 cells in a soft agar layer. On two plates of each series a filter paper disc impregnated with 0.025 ml of 2 mg/ml solution of NTG was placed; on two plates a filter paper disc impregnated with 0.025 ml of liquid DES was placed; on two control plates, the filter paper discs were moistened with sterile distilled water. Similar sets of plates were prepared with a 10 fold dilution of cells. No revertants were found after 72 hours. Similar plates inoculated with a UV induced his$^-$ mutant of LG-106 showed a few spontaneous revertants and a circle of revertant colonies around the discs impregnated with NTG and DES.

In order to determine whether other types of auxotrophs are produced as a result of interaction of P2 and host chromosome, some of the strains pre-

viously tested on low histidine agar were examined for the presence of small translucent colonies when grown on Davis glucose agar supplemented with 0.05% or 0.01% NB (low NB agar). Presumptive auxotrophs occurring on these plates were re-tested, and their growth requirements determined by spotting on appropriately supplemented Davis agar, or by incorporating the bacteria in a soft agar layer on Davis minimal medium and spotting with a variety of growth factors. The results are summarized in table 2. His⁻ auxotrophs were found among colonies of LG-106 and among the colonies derived from plaques of P2 on K-40. A few other auxotrophs of a variety of types were found but there was no obvious pattern to their occurrence.

Discussion

The production of his⁻ cells from E. coli strains lysogenic for P2 appears to be related to the presence of P2 prophage in location H, a location very close to the histidine locus. Since the great majority of his⁻ isolates are non-lysogenic, and since the few which have been studied appear not to revert to histidine independence, it seems probable that a chromosomal deletion has occurred concomitantly with the loss of prophage, probably by an aberrant cross-over of the type which is postulated to occur in the formation of λdg (Campbell, 1962). This interpretation is consistent with the observed slow growth of the his⁻ segregants, compared with the reverting his⁻ mutants obtained following exposure to UV.

Whether there is some specific anomaly in the histidine region of E. coli K which causes this phenomenon to occur, or whether a similar loss of prophage and production of chromosomal deletions occurs at any location where P2 is integrated is not known. An attempt to find other auxotrophs produced in this way by plating various strains on Davis glucose agar supplemented with small amounts of NB did not reveal any pattern to the occurrence of the few auxotrophs found. However, the chromosomal regions adjacent to other P2 locations may control functions not amenable to testing in this way.

Since the production of his⁻ isolates following infection with P2 appears

to be limited to K strains in which location H is available for prophage integration, it seems possible that this effect depends upon the initiation of lysogenization.

Table 2

Occurrence of auxotrophs on low NB agar in E. coli strains lysogenic for P2 or infected with P2.

Strain	Approximate number of colonies examined	Number of auxotrophs	Phenotype of auxotrophs
LG-106	5,000	17	his$^-$
LG-102	5,000	1	Shows the same slight growth on Davis glucose agar unsupplemented or supplemented with histidine or NB.
K-40	4,500	1	leu$^-$
C-2	5,000	1	Unidentified. Very high reversion rate.
C-27	5,000	0	
C-67	7,000	0	
C-77	5,000	0	
K-40 + P2$^+$	4,000	17	his$^-$
		2	pro$^-$
		1	cys$^-$/ met$^-$ joint requirement
C-2 + P2$^+$	4,000	1	lys$^-$
		1	Unidentified. Very high reversion rate.

Symbols used: his$^-$, leu$^-$, pro$^-$, lys$^-$, requires respectively histidine, leucine, proline or lysine. cys/met$^-$, requires both cysteine and methionine.

The genetic structure of the his$^-$ isolates remains to be investigated. If these represent deletions of a region adjacent to the histidine operon, they may extend into that operon for varying distances. The isolation of a mutant from one of the his$^-$ segregants which responds to histidinol is con-

sistent with this interpretation. This isolate may resemble the secondary mutants of Salmonella typhimurium which respond to histidinol reported by Ames et al (1963). Some of these have been shown to be extensions of an original deletion.

The phenomenon reported here appears to be essentially different from that reported by Taylor (1963) with phage Mμ 1, in which the production of auxotrophs appeared to be related to the establishment of the lysogenic state

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