General transducing phages like *Salmonella* phage P22 isolated using a smooth strain of *Escherichia coli* as host

Tarlochan S. Dhillon a, Alice P.W. Poon b, Dorothy Chan c, Alvin J. Clark a,*

a Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3202, USA
b Marjorie B. Kovler Oncology Laboratory, University of Chicago, Chicago, IL 60637, USA
c Department of Botany, University of Hong Kong, Hong Kong

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Abstract

A smooth colony strain, resistant to phages λ and P22, was isolated from sewage and identified as *Escherichia coli* (strain H). Four temperate phages plaquing on strain H were isolated from sewage. The archetype, HK620, does not plaque on strains C and K12 of *E. coli* nor on the LT2 strain of *Salmonella enterica*. Bacterial mutants resistant to a clear plaque mutant of HK620 produce rough colonies. Some are also galactose-negative, a few are histidine auxotrophs, and most show sensitivity to λ. HK620 can transduce a wide variety of auxotrophic mutants of *E. coli* H to prototrophy. It can recombine with λ but its virions resemble those of P22. © 1998 Published by Elsevier Science B.V.

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1. Introduction

Temperate bacteriophage P22 grows on strain LT2 of *Salmonella enterica*, serovar typhimurium and can transduce the host genes tested at a certain frequency, a phenomenon called general transduction. P22 does not plaque on strains C and K12 (henceforth called K) of *Escherichia coli*, but, with appropriate genetic manipulations, P22 has been successfully hybridized with the *E. coli* phage λ [1]. P22 is therefore now regarded as a member of the λ family of temperate phages, also referred to as the lambdoid phages [2].

We have searched for general transducing, P22-like *E. coli* phages in their natural habitats using *E. coli* strains C and K as the indicators. Over 50 phages of independent origin that were assignable to the λ phage family were tested but none could mediate general transduction ([3,4]; unpublished results). Since the typhimurium strain LT2 is a smooth colony-former (i.e., its cells are coated with lipopolysaccharide [LPS] which includes an O-antigen moiety) and strains C and K are rough (i.e., their cells are coated with LPS which lacks an O-antigen moiety), we speculated that P22-like general transducing *E. coli* phages may be obtainable if smooth colony *E. coli* are used as indicator. Our conjecture has been justified by the isolation of strain H (for Hong Kong, not for its flagellar antigen) of *E. coli*, which has proved to be a satisfactory host for P22-like general...
transducers. This communication deals with the characterization of *E. coli* H and its temperate phage, HK620.

2. Materials and methods

2.1. Media and bacterial identification

The complete, broth based media used were tryptone broth (TB), tryptone yeast extract broth (TYB), and nutrient broth (NB). For bacterial colony counts broth based media containing 2% agar were used. For phage assays two agar concentrations of TBA were used: 1% agar for the bottom agar layer and 0.5% for the top agar layer. Fermentation tests were carried out on MacConkey agar base containing 1% of the requisite carbohydrate. Ingredients for preparing the above were purchased from Difco Company. Further details on the media have been published previously [3].

The minimal medium used was the one described by Vogel and Bonner [5] which was supplemented with the required metabolites at concentrations recommended by Miller [6].

Antibiotics were used at the following concentrations (μg ml⁻¹): ampicillin, 50; streptomycin, 10; and tetracycline, 10.

2.2. Bacteriological and bacteriophage methods

Routine methods for preparing bacterial cultures, bacteriophage lysates, and the agar overlay method of phage assay have been described [3,4].

Bacteriological diagnostic tests for indole production, hydrogen sulfide production, the methyl red test, and motility and fermentation tests were carried out as described [7].

Additional diagnostic tests for species identification were carried out by the Clinical Microbiology Laboratory of the University of Hong Kong.

For electron microscopy concentrated suspensions of phage (10¹⁸ ml⁻¹) were obtained by equilibrium density gradient centrifugation in CsCl solution as described [8].

*N-Methyl-N′-nitro-N-nitrosoguanidine* (MNNG) was used for mutagenesis. It was dissolved in 0.1 M citrate buffer (pH 5.6) at a concentration of 30 μg ml⁻¹ and the treatment of cells was usually for 30 min at room temperature. Prophage mutagenesis was carried out by treating lysogenic bacteria with MNNG. Mutant phage were isolated by incubating treated cells in TYB under aeration for 2 h at 37°C. The suspension of released phage was treated with chloroform, centrifuged to remove cell debris and plated to obtain isolated plaques.

3. Results and discussion

3.1. *E. coli* strain H

The wild-type strain H of *E. coli* was recovered upon plating a sewage sample from the suburban locality of Yuen Long in Hong Kong. Ten of the colonies appearing on MacConkey lactose agar were tested for their sensitivity to phage in the chloroform-treated sewage sample. Only one registered well-developed phage plaques of which 2% were turbid-centered, typical of the temperate phage plaques. This strain was retained as TD2158 and will be the founder strain of the *E. coli* H lineage. Plating on TD2158 sewage samples, from the same stream but taken on different days, gave proportions of turbid-centered plaques varying from 1 to 5%.

TD2158 was found to form smooth colonies on nutrient agar of which 1–5% were found to be rough variants. This might indicate that O-antigen formation in this strain is plasmid-borne but unpublished experiments provide no evidence for this although strain H does carry a plasmid. In diagnostic tests TD2158 was found to be indole-positive, H₂S-negative, mannitol-positive, and motile. Further fermentation tests of 10 carbohydrates by the Clinical Microbiology Laboratory supported identification of TD2158 as *E. coli*. We are collaborating with Dr. Richard Wilson of the *E. coli* Reference Center at Pennsylvania State University to determine the O-antigen serotype of TD2158.

TD2158 was found to be inducibly tetracycline-resistant, but streptomycin- and ampicillin-sensitive. The cells are transformable to ampicillin resistance by plasmid pBR322. The latter, however, seems to be unstable in the H cells which tend to lose ampicillin resistance if cultured in the drug-free medium.

The *E. coli* H strain is sensitive to phages Pivir, T4...
and T6, but resistant to λ, T1, T3, T5, and T7; nor is it lysed by the typhimurium phage P22. The bacterium may be harboring one or more temperate prophages as UV-irradiated cultures (300 J m\(^{-2}\)) incubated in broth for 2 h become clear. However, if they have released any infectious phage, we have not yet found a host strain sensitive to such phage.

### 3.2. Temperate phages

Initial plating of a sewage sample on *E. coli* H yielded HK620. An HK620 lysogenic derivative of strain H, TD2509, was treated with MNNG and a derivative was isolated which remained immune to superinfection by HK620 but released no infectious phage. This defective lysogen, TD2512, was used as an indicator to look for heteroimmune phages. Each of three sewage samples gave a heteroimmune phage. Of these three phages, HK626 and HK627 represent two additional immunity specificities; HK628 may be homoimmune with HK626. All four phages are heteroimmune to λ. We have tried to reproduce this phage isolation with H and H(HK620def) with samples taken from the Oakland Sewage Treatment facility and from a pig farm in Davis, California without success. It should be noted that TD2509 and TD2512 were lost in the move to Berkeley from Hong Kong. Consequently, the HK620-defective lysogen now available is TD2836, derived from a lysogen of the temperature-sensitive clear plaque mutant cts3 mentioned in Section 3.3.

All four Hong Kong phages are UV-inducible giving lysate titers of 10\(^{10}\) or more and the titers have remained stable over several months without any stabilizing additives.

Antiserum against HK620 was prepared. It did not inactivate λ or P22. Similarly, anti-λ and anti-P22 sera caused no detectable inactivation of HK620.

### 3.3. Phage-resistant mutants of *E. coli* H

MNNG treatment of prophage yielded clear plaque mutants; HK620c1 forms clear plaques over the temperature range 30–40°C. Five conditional temperature-sensitive (*cts2–cts6*) mutants were also selected. The latter form turbid plaques at 30°C, giving lysogens viable at that temperature, but form clear plaques at 37–40°C at which temperature their lysogens are induced releasing cell-free phage (thermo-induced lysates). Mixed infection of host cells by the six clear plaque mutants was carried out in all pairwise combinations. This was done in spot tests. None of the mixture spots showed any greater turbidity than the unmixed parental spots. Cell-free phage eluted from some of the mixed spots were also plated at 40°C and no turbid plaques were seen. Thus, no evidence of complementation or recombination between the clear plaque mutants emerged.

Ten parallel cultures of *E. coli* H were challenged with phage HK620c1 and the colonies formed by survivors were characterized. Over 90% of the colonies were visibly different from the wild-type in being of the rough type. Some of the colonies also showed additional mutant characteristics such as inability to ferment galactose, inability to grow in media containing bile salts, or auxotrophy for histidine, purine, or nicotinic acid. Irrespective of the above characteristics, a majority of the rough colony formers were resistant to HK620. All these had acquired sensitivity to λ but not to P22. A small proportion of the rough colony formers showed the useful property of being sensitive to both λ and partially sensitive to HK620. The partial sensitivity to HK620 is concluded from the faint and ill-resolved nature of plaques and an efficiency of plating 10% that on wild-type *E. coli* H. Such doubly sensitive strains proved useful for making inter-typic phage crosses.

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<td>Transduction by HK620 and three other phages(^a)</td>
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\(^a\)Phage lysates were prepared by UV irradiation of TD2158 cells lysogenic for respective prophage. Revertants were found at less than 2\times10^3 ml\(^{-1}\) in all three cases. The titers of transductants per ml (\(\times 10^3\)) are shown. The genetic markers were *trp*-314, *bio*-302 and *ser*-100.

\(^b\) > 20 stands for colonies in excess of 1000 per plate that were too numerous to count. Dilutions to obtain smaller numbers of colonies per plate were not made because of difficulties in controlling the number of transductants made by infection after plating.
Since resistance to HK620 selects for rough colony phenotype, we conclude this phage to be smooth-specific as is the Salmonella phage P22. Conversely, \( \lambda \) may be designated as rough-specific.

3.4. Auxotrophic mutants and general transduction

Monoauxotrophic mutants of \( E. \ coli \) \( H \) requiring biotin, pyridoxine, threonine, leucine, tryptophan, serine, histidine, or uracil and arabinose fermentation-negative mutants were selected after MNNNG treatment. All have been successfully transduced to wild-type by HK620 and three heteroimmune phages. Table 1 shows the typical data for three markers. Although various markers are transduced at different frequencies, these differences are reproducible. Considering the spectrum of mutants transduced, we conclude all four phages to be general transducers.

We have tested but have not observed the cotransduction of markers \( thr \) and \( leu \), which are 1.4 min apart on the K genetic map. However, \( leu \) and \( ara \), which are 0.3 min apart, were co-transduced at frequencies ranging from 27% to 59%.

The method of phage lysate preparation had no effect on the phage's ability to mediate transduction. UV-induced, thermo-induced, and lytic lysates prepared either by infection of sensitive cells in broth cultures, or by eluting phage from confluent plaque-density agar-overlay plates have all successfully transduced the markers listed in Table 1.

3.5. Morphology of HK620 virions

A uranyl acetate stained electron micrograph is shown in Fig. 1. The virions are unlike those of \( \lambda \) because they lack flexible, elongate tails. On the other hand, they are indistinguishable from those of P22 because they possess six spike-like appendages attached to the head. Measurements of 50 virions have provided an average diameter of HK620 heads as 59 ± 2 nm.

3.6. Genetic recombination between \( \lambda \) and HK620

Spot crosses were made between phages differing in plaque morphology so recombinant frequencies were not determined. Wild-type \( \lambda \) was crossed with
HK620cts and imm$^{HK}$imm$^{lam}$ phage were selected as clear plaques on a K indicator strain. Conversely, λ cI857 was crossed with wild-type HK620 and imm$^{lam}$h$^{HK}$ recombinants were selected as clear plaques on E. coli H. In each of these crosses the frequency of clear plaques on selective indicators exceeded the frequency of spontaneous clear plaques of the turbid plaque parent; hence the excess was attributable to recombination. The presumed recombinants, three from each cross, were purified and their temperature-sensitive immunity specificity was verified by showing the stability of the lysogens at 30°C, but a lethal phenotype at 40°C. All six recombinants were tested for their transducing activity; only phage with the imm$^{lam}$h$^{HK}$ genotype transduced the E. coli H markers. Phage with the other recombinant genotype, imm$^{HK}$h$^{lam}$, did not transduce markers of its host, E. coli K.

3.7. Conclusion

We are now engaged in further genetic characterization of phage HK620 and other smooth-specific phages of the 620 series. Such investigations are likely to illuminate the patterns of speciation in the λ family of temperate phages. It has been known for some time that λ and some related E. coli phages share genetic regions with the Salmonella phage P22 ([2] is a recent review). Since P22 and λ grow on cells of different genera, whereas HK620 and λ grow on cells of the same species, it is quite conceivable that some of those ‘P22-like’ genetic regions may have been contributed by one or the other of the HK620 subgroup of phages. Our investigations may also shed light on why P22-like temperate phages seem not to be isolatable on rough E. coli strains. Furthermore, they may reveal a method of engineering such phage to infect rough strains.

Acknowledgments

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References