

Tracing Ancestors and Relatives of *Escherichia coli* B, and the Derivation of B Strains REL606 and BL21(DE3)

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Antecedents of *Escherichia coli* B have been traced through publications, inferences, and personal communication to a strain from the Institut Pasteur in Paris used by d'Herelle in his studies of bacteriophages as early as 1918 (a strain not in the current collection). This strain appears to have passed from d'Herelle to Bordet in 1920, and from Bordet to at least three other laboratories by 1925. The strain that Gratia received from Bordet was apparently passed to Bronfenbrenner by 1924 and from him to Luria around 1941. Delbrück and Luria published the first paper calling this strain B in 1942. Its choice as the common host for phages T1–T7 by the phage group that developed around Delbrück, Luria, and Hershey in the 1940s led to widespread use of B along with *E. coli* K-12, chosen about the same time for biochemical and genetic studies by Tatum and Lederberg. Not all currently available strains related to B are descended from the B of Delbrück and Luria; at least three strains with somewhat different characteristics were derived independently by Hershey directly from the Bronfenbrenner strain, and a strain that appears to have passed from Bordet to Wollman is in the current Collection of the Institut Pasteur. The succession of manipulations and strains that led from the B of Delbrück and Luria to REL606 and BL21 (DE3) is given, established in part through evidence from their recently determined complete genome sequences.

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Introduction

The K-12 and B laboratory strains of *Escherichia coli* and their derivatives have been widely used and have had enormous impact on basic biology, medicine, and biotechnology. The origin of K-12 is straightforward; it was isolated from the stool of a convalescent diphtheria patient in Palo Alto, CA, in 1922.^{1–3} Famously, the *E. coli* strain named B by

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Abbreviations used: CIP, Collection of the Institut Pasteur; ATCC, American Type Culture Collection.

Delbrück and Luria in 1942⁴ was chosen by the phage group that developed around Delbrück, Luria, and Hershey in the 1940s as the host for their common studies of the virulent phages T1–T7.^{5,6} However, the earlier history of B is well established.

This article traces what we can document from the literature and what we infer about the succession of

strains that led first to *E. coli* B and subsequently to its descendants REL606⁷ and BL21(DE3),⁸ whose genome sequences have now been determined.⁹ We make the case that *E. coli* B is the clonal descendant of a *Bacillus coli* strain from the Institut Pasteur in Paris used by d'Herelle in his early studies of bacteriophages. Thus, the strain chosen as a host by

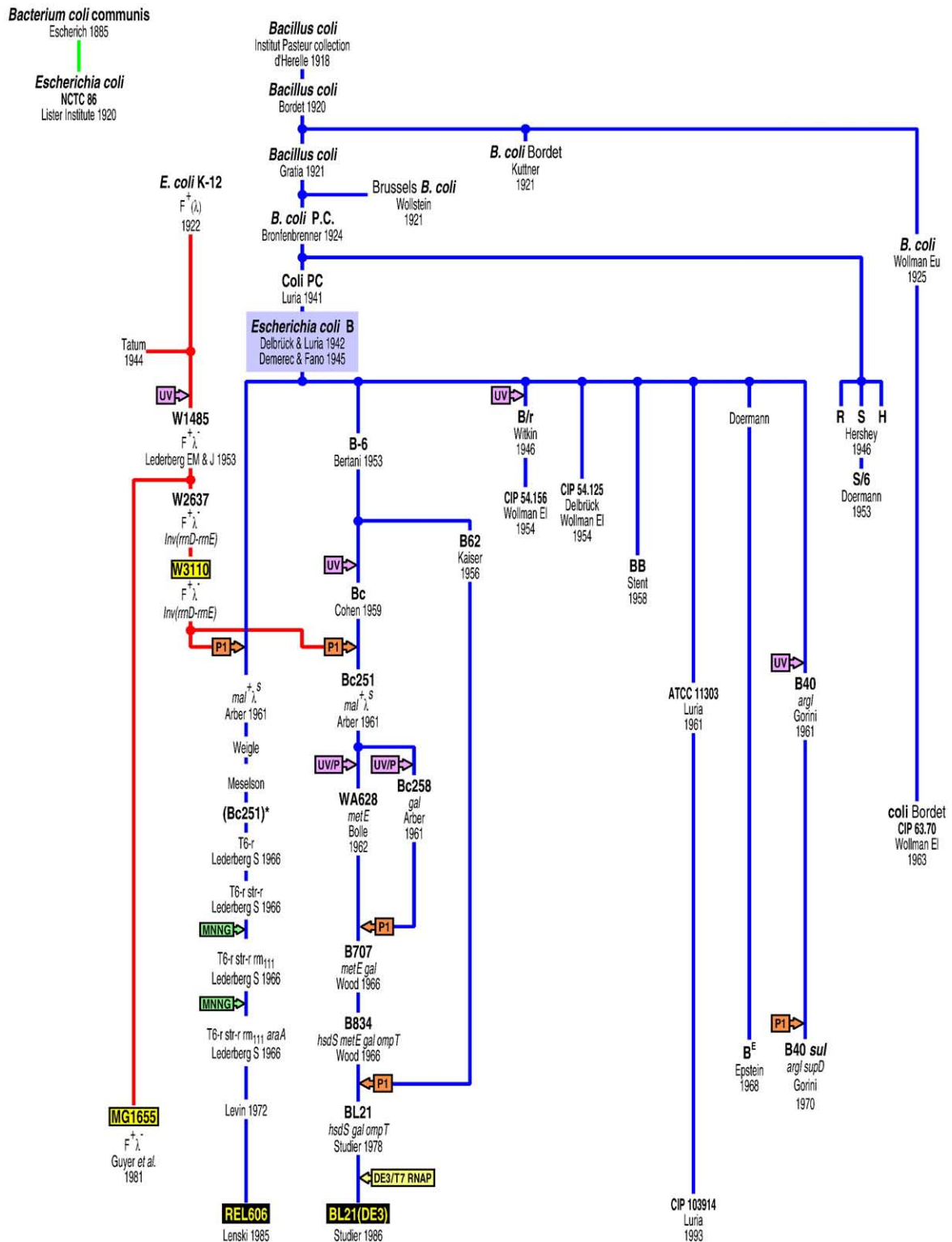


Fig. 1 (legend on next page)

phage workers in the 1940s was apparently descended from the first *E. coli* strain used in laboratory studies of bacteriophages, soon after their discovery in 1917.¹⁰ Relationships among the *E. coli* strains to be discussed are laid out in Fig. 1.

Early history of *E. coli*

The earliest description and laboratory isolation of bacteria now identified as *Escherichia coli* were made in 1885 by Theodor Escherich, a pediatrician and bacteriologist who was interested in colonization of the human gut and analyzed the appearance of bacteria in the feces of breastfed infants.¹¹ He named this strain *Bacterium coli communis*. In the early part of the 20th century, microbiologists initiated systematic efforts to classify all known bacterial species based largely on morphological and biochemical characteristics, and the taxonomic classifications *Bacillus coli* and *Escherichia coli* were applied to this species. *Escherichia coli* is now the standard name, but all three names are conserved as identifiers of this species. The Escherich strain was, for some time, the type strain for *E. coli* and was deposited in the UK National Collection of Type Cultures as NCTC strain 86 in 1920 (the year of its founding) by the Lister Institute in London.

Félix d'Herelle, bacteriophage, and *B. coli*

Félix d'Herelle, working at the Institut Pasteur in Paris, discovered a phenomenon he called "bactériolyse" in the stools of patients recovering from dysentery. He recounts this fascinating discovery in his first book *Le bactériophage. Son rôle dans l'immunité*,¹² which is a compilation of his numerous communications published as reports to the French Academy of Sciences and the Belgian Society for Biology between 1917 and 1921. He correctly interpreted the phenomenon of bacterial lysis, caused by an agent that could pass through filters fine enough to retain all known bacteria, as being due to an infectious agent he named "bactériophage" in his first paper on the subject in 1917.¹⁰ D'Herelle extended the observations to other intestinal bacteria, including *B. coli*,^{13,14} and found that bacteriophages

isolated on one host could often be adapted to attack other hosts as well. The bacteriophages d'Herelle isolated and worked with were from human feces, and he also referred to them as *Bacteriophagum intestinale* or *Protobios bacteriophagus*. These discoveries attracted wide interest because of the possibility of using bacteriophages as therapy for bacterial diseases. D'Herelle also concluded, incorrectly, that only a single bacteriophage, which could adapt to any bacterial species, existed, but this idea was soon disputed because different phages had different antigenic specificities.^{15,16}

Scientific papers from the time of d'Herelle (and even now) rarely contain clear descriptions or bibliographic references to the sources of biological materials used. The closest d'Herelle came to identifying the source of *B. coli* used in his work with bacteriophage was in a note on page 198 in his second book, published in 1926,¹⁷ in which he stated that it came from the Collection of the Institut Pasteur (CIP) (quoted in the section on Jules Bordet and André Gratia). Unfortunately, no *E. coli* strains from that period are represented in the current CIP, but it seems likely that the *B. coli* used by d'Herelle was derived from a normal commensal of the human gut, isolated from human feces. We will make the case in the following sections that this strain was the ancestor of the *E. coli* B of Delbrück and Luria.

Jules Bordet and André Gratia

D'Herelle's interpretation of his findings on bacteriophage did not long go unchallenged. His most important and persistent opponents were Jules Bordet, Director of the Institut Pasteur du Brabant (in Bruxelles) and recipient of the Nobel Prize in 1920 for his work in immunology, and André Gratia, who, after studying blood clotting at the Laboratory of Physiology at the Université Libre de Bruxelles, began his work on bacteriophage at the Rockefeller Institute for Medical Research in New York in December 1920.^{18,19} Summers²⁰ states that Gratia was a student of Bordet, although Gratia's son¹⁹ says that Bordet and Gratia studied phages independently. In either case, Gratia's early bacteriophage papers were presented to the Belgian Society of Biology by Bordet, and Gratia joined Bordet's institute before

Fig. 1. Genealogy of *E. coli* B. The figure shows relationships among strains leading to, derived from, or closely related to the *E. coli* B of Delbrück and Luria, as described in the text. Also shown are lines of descent from K-12 to W3110 (the source of DNA for P1 transduction of B and Bc) and MG1655, whose complete genome sequences are known,^{87,88} and the first *E. coli* strain isolated and studied in the laboratory, by Escherich in 1885. Entries contain the name of the strain, the person associated with the strain, and the approximate year the strain was received, constructed, or described in a publication. Note that the Hershey strains R, S, and H; the Doermann strain S/6 (derived from S); and coli Bordet are not descended from the B of Delbrück and Luria but derive from progenitors of B. The strain S. Lederberg referred to as Bc251⁸² was actually a P1 transductant of B itself, not Bc, as described in the text, and is designated (Bc251)* in the figure. P1 transductions and treatments with 1-methyl-3-nitro-1-nitrosoguanidine, UV, or UV followed by penicillin selection (UV/P) are indicated in the figure, as is the integration of DE3 into BL21 to produce BL21(DE3). The *mal*⁺ λ ^S genotype of strains obtained by P1 transduction is retained by their descendants but not annotated in the figure, and the Levin strain, REL606, and BL21(DE3) have the genotype of the strain above them. The accession number, depositor, and year of deposit are given for five strains deposited initially in the UK National Collection of Type Cultures, the CIP, or the ATCC.

the end of 1921. However, the two did not publish jointly.

From their immunological perspective, Bordet and Gratia thought that the lysis phenomenon was more likely to be induced through an interaction of the bacterium with host defenses, which induced a transmissible autolysis of the bacterium catalyzed by its own enzymes.^{21–23} (See van Helvoort²⁴ for an interesting discussion of the contrasting approaches of Bordet and d’Herelle.) Bordet and Ciuca²⁵ also challenged d’Herelle’s priority as the discoverer of bacteriophages, pointing out that a 1915 report by Twort²⁶ describing what appeared to be the same phenomenon had been overlooked. Experiments reported by Gratia and Jaumain²⁷ appeared to show that the observations of Twort and d’Herelle were different manifestations of the same underlying phenomenon, but d’Herelle resisted that interpretation. Opposing views about experiments and interpretation were strongly argued by these investigators and their supporters from the 1920s into the 1940s before the controversy was finally resolved.

A crucial experiment by Bordet and Ciuca^{22,23} was to inject *B. coli* that they had obtained from d’Herelle’s laboratory into the peritoneum of guinea pigs and to isolate from the leukocytic exudate a lytic agent with the same properties attributed to bacteriophage by d’Herelle: the agent passed through filters, was diffusible, and induced lysis in other bacteria, with the release of an additional quantity of the same agent. Bordet and Ciuca interpreted this result as supporting their idea that leukocytes from the host induced the bacteria to produce a factor that caused them to be lysed by their own enzymes. However, the method of injecting bacteria into the guinea pig peritoneum to isolate transmissible lytic agents appeared to work only sporadically, and others who tried it generally abandoned it in favor of isolating bacteriophages from intestinal sources, as d’Herelle had done, even if they remained undecided about the interpretation of the lytic phenomenon.^{28,29}

D’Herelle apparently felt that the success of the guinea pig experiment in the hands of Bordet and Ciuca may have been due to the particular strain of *B. coli* that they obtained from his laboratory. In a note on page 198 of his second book,¹⁷ d’Herelle says “L’histoire de cette souche est curieuse: elle provient de ma collection et a été donnée à Ciuca, en mon absence (j’étais en Indochine)... L’origine de ce *B. coli* était une des souches de l’Institut Pasteur.” He amplifies on this episode in an unpublished autobiographical memoir in the Archives of the Institut Pasteur (Chapter 7, pp. 497–498), a translation of which is given by Summers²⁰ (pp. 194–195, note 12). Apparently, the *B. coli* that Ciuca obtained was a refrigerated sealed tube of *B. coli* (labeled κ) that d’Herelle was saving for future work because lytic activity could be detected in the culture. This culture might have contained bacteriophages but escaped lysis for any of a number of reasons, including unfavorable metabolic conditions, a limited supply

of some cofactor or receptor needed for adsorption, a DNA restriction barrier, and so on.

Gratia’s publications on his bacteriophage work at Rockefeller^{21,30} describe in considerable detail the culture that Bordet and Ciuca used in their experiment and which he received from them: “The culture employed was derived from a strain given by d’Herelle to Bordet and Ciuca. Before beginning their experiment, Bordet and Ciuca made three successive isolations in order to start, as far as possible, with a culture arising from a single organism, and it was from this source that our culture was derived.” Gratia’s experiments showed that this purified strain, which he referred to as the original culture, was highly sensitive to the lytic agent that Bordet and Ciuca had obtained from the guinea pig experiment. This sensitivity, together with the three successive rounds of what apparently was single-colony isolation before inoculation of the guinea pig, makes it unlikely that the lytic agent isolated by Bordet and Ciuca was due to a bacteriophage present in the culture that they received from d’Herelle’s laboratory. More likely, they encountered phage contamination from some other source, as apparently happened sporadically in other laboratories where guinea pig inoculation was tried.

Distribution of *B. coli* Bordet to others

The *B. coli* strain that Bordet purified after receiving it from d’Herelle was also distributed to others besides Gratia (Fig. 1). While at Rockefeller, Gratia²¹ (pp. 123–124) collaborated with Martha Wollstein, a researcher at Rockefeller, who published her own paper on bacteriophage in 1921²⁸ and used the “Brussels strain of *Bacillus coli*” (p. 470). Ann Kuttner²⁹ (p. 73), working on bacteriophage at Columbia University, used “the *Bact. coli* obtained from Dr. Bordet” as early as 1921. Eugène Wollman received this *B. coli* strain from Bordet for his work on bacteriophage at the Institut Pasteur in Paris, published in 1925³¹ (p. 823). The Bordet strain seems likely to have been maintained there and to be the strain described by Eugène Wollman’s son Elie Wollman in a personal communication to Abedon.³² “At the Paris Institute Pasteur, Elie Wollman worked with an *E. coli* strain called coli Bordet that was considered identical, in terms of phage susceptibility at least, to the *E. coli* B strain of Luria and Delbrück.” The data sheet for a strain deposited in the CIP by Elie Wollman in 1963 (CIP 63.70) carries the notations “strain BAM,” “probably derived from P. Bordet,” “*Escherichia coli* strain,” “phage host,” and “used in bacterial growth and phage propagation experiments.” In light of Elie Wollman’s statement to Abedon, the notations “strain BAM” and “phage host” probably refer to the perceived equivalence to the *E. coli* B of Delbrück and Luria, sometimes abbreviated Bam for B American. P. Bordet would seem to refer to Paul Bordet, the son of Jules Bordet, who succeeded him as Director of the Institut Pasteur in Brussels in

1940. Although considerable time had elapsed, it seems entirely possible that the strain deposited as CIP 63.70 by Elie Wollman in 1963 was descended from the *B. coli* that Eugène Wollman had received from J. Bordet by 1925. DNA sequences sampled from this strain confirm that it is essentially identical with other B strains.³³

Jacques Bronfenbrenner, *B. coli*, *B. coli* PC, and *E. coli* B

Jacques Bronfenbrenner began bacteriophage work upon coming to the Rockefeller Institute for Medical Research from Harvard Medical School in the fall of 1923. It is tempting to think that his interest in bacteriophage work was stimulated by André Gratia's communications of his phage work to the Society for Experimental Biology and Medicine in New York City at the March 16 and April 20, 1921 meetings,^{34,35} and by Ann Kuttner's communications on her phage work at the February 16 and April 20, 1921 meetings.^{36,37} At those same three meetings, Bronfenbrenner communicated his work on anaphylaxis and botulinus toxin at Harvard Medical School.³⁸⁻⁴¹ Whether the papers were presented in person or communicated directly to the *Proceedings*, Bronfenbrenner must certainly have been aware of the phage work in New York, which was stimulated by the works of d'Herelle and of Bordet and Ciuca.

Although Bronfenbrenner arrived at Rockefeller about 2 years after Gratia returned to Brussels, the Bordet strain of *B. coli* that Gratia worked with was presumably still available at Rockefeller through Martha Wollstein (if Bronfenbrenner did not already have it). Bronfenbrenner, like Gratia, collaborated with Wollstein,⁴² and the Bordet strain was also being used at Columbia.²⁹ Bronfenbrenner's first full paper on bacteriophage, describing experiments "conducted during October and November 1923"⁴³ (footnote 19), used "an 18 hour lysed culture of *B. coli*," which could well have been made with the Bordet *B. coli* strain and even with a descendent of some of the "corresponding lytic agent" that Bordet and Ciuca^{22,23} had isolated by injecting the d'Herelle *B. coli* into guinea pig peritoneum and which Gratia^{21,30} had obtained from Bordet. This paper was the first in a series of 12, all headed "Studies on the Bacteriophage of d'Herelle," published by Bronfenbrenner and his Rockefeller collaborators in the *Journal of Experimental Medicine* from 1925 to 1928. This series of papers and Bronfenbrenner's stay at Rockefeller ended in 1928, when he was appointed Chairman of the Department of Bacteriology and Immunology at Washington University in St. Louis. None of these papers reports where the *B. coli* was obtained, although Bronfenbrenner and Korb (1925, p. 491)⁴⁴ did have contact with Gratia, acknowledging "purified lytic filtrates (Phage B.W. and Pet. 2, respectively) sent to us by Gratia."

Bronfenbrenner is acknowledged by Delbrück and Luria⁴ as the source of the strain that they named

E. coli B, and they refer to a 1939 paper by Kalmanson and Bronfenbrenner⁴⁵ describing purification of a phage for which the newly named *E. coli* B was the host. This phage, called PC by Kalmanson and Bronfenbrenner, was called γ by Delbrück and Luria and ultimately named T2 by agreement of the phage group.⁵ T2 seems likely to have been isolated from feces around 1927 in Bronfenbrenner's laboratory, since he stated in a 1933 communication that the purified P.C. phage (i.e., T2) had been used for 6 years in his laboratory,⁴⁶ and his laboratory had been isolating phages from fecal material since at least 1925.⁴⁴ Kalmanson and Bronfenbrenner⁴⁵ called the host for this phage *B. coli* P.C. and stated (p. 206) that this strain "was known to be free of spontaneous lytic activity for a period of 15 years," implying that the strain was being maintained and used by Bronfenbrenner's laboratory at least as early as 1924, about the time he came to Rockefeller. Bronfenbrenner referred to P.C. phage or PC-coli phage and its *B. coli* host in 1932⁴⁷ and 1933,⁴⁶ and his collaborator Muckenfuss stated in 1928 that *B. coli* was the host for "P.C. coliphage."⁴⁸ Kalmanson and Bronfenbrenner⁴⁹ stated that the polyvalent phage (PC) had been routinely carried on *B. coli*, and Hershey *et al.*⁵⁰ described the host for PC as "a non-motile intermediate coliform species referred to as 'coli-PC.'"

As we have seen, the passage of *B. coli* from d'Herelle to Bordet to Gratia is firmly documented in the literature, and a fairly strong case can be made that the *B. coli* P.C. of Kalmanson and Bronfenbrenner⁴⁵ (usually referred to in Bronfenbrenner's papers simply as *B. coli*) was in continuous use in Bronfenbrenner's laboratory since 1924, about the time he arrived at Rockefeller. Although entirely consistent with what we can document and infer, the evidence for transmission of Gratia's *B. coli* to Bronfenbrenner is circumstantial, since Bronfenbrenner said nothing in his publications about the source of the *B. coli* he used repeatedly. Our best hope of verifying the circumstantial case seemed to be to ask some of the early workers whether they remembered a possible connection between Bronfenbrenner and Gratia. Wacław Szybalski (personal communication to P.D., 2007) met both Gratia and Bronfenbrenner several times in the late 1940s and remembered the response to a question he had asked of Bronfenbrenner about the derivation of the *E. coli* B strain: "I must have got[ten] it in New York from André Gratia, but it must not have been called B at that time." Thus, our inferences are apparently correct: *E. coli* B is clonally derived from the *B. coli* of d'Herelle through Ciuca and Bordet to Gratia and then Bronfenbrenner (Fig. 1), a conclusion also greatly strengthened by the apparent identity of the coli Bordet strain of the Institut Pasteur to other B strains at the DNA sequence level.³³

The progression from the Bronfenbrenner and Kalmanson designation *B. coli* P.C. in 1939 to the Delbrück and Luria designation *E. coli* B in 1942 can be traced through a series of papers all having Luria

as an author. First, Luria and Exner in 1941⁵¹ used phage P28 (later known as α and finally T1) and host “Coli PC” (along with other phages and hosts) and acknowledged Bronfenbrenner (among others) for supplying bacteriophages and bacteria. Then Luria and Anderson in 1942⁵² used phages P28 and PC with “*Escherichia coli*, strain PC” [and referred to Delbrück and Luria (“to be published”) for the latent period of phage PC, obviously referring to their 1942 paper that was submitted about 3 months later]. Finally, Delbrück and Luria in 1942⁴ used the same two phages and named the host strain *E. coli* B. During this period, Delbrück referred, in his 1942 review article,⁵³ to three different sets of *E. coli* bacteria and their phages as B₁ and P₁ (referring to the strains used by Ellis and Delbrück in 1939),⁵⁴ B₂ and P₂ (referring to the strains used by Delbrück in 1940),⁵⁵ and B₃, P₃, and P₄ [referring to the strains to be called *E. coli* B, α , and γ in the “to be published” Delbrück and Luria (1942) paper]. Their subsequent papers used the name *E. coli* B exclusively (e.g., Luria and Delbrück in 1943⁵⁶ and Luria *et al.* in 1943),⁵⁷ a designation codified by Demerec and Fano in 1945.⁵ Cohen and Anderson⁵⁸ and Hershey⁵⁹ state specifically that the *E. coli* B of Delbrück and Luria⁴ is identical with or derived from strain PC of Bronfenbrenner.

The Phage Group, *E. coli* B, and its variants

As recounted by Delbrück in his 1946 review,⁶ several groups of phage workers decided to concentrate their efforts on a series of seven virulent phages, designated T1 through T7, that all infect *E. coli* B. They also developed a consistent nomenclature for host and phage variants to facilitate exchange of information.^{5,59–62} This led to a blossoming of phage studies at the molecular level, which ultimately resolved earlier controversies and—together with parallel work on bacterial genetics, lysogeny, and regulation in K-12, and host restriction and modification between K-12 and B—created a deep understanding of replication, gene expression, and regulatory mechanisms in these closely related strains. This explosion of research and understanding was fueled by the isolation of many variants of both K-12 and B. We briefly summarize the origin of some B strains referred to in the literature or available in culture collections, whose relationships are shown in Fig. 1.

Hershey⁵⁹ described three B strains that he derived from the coli PC of Bronfenbrenner independently of the B of Delbrück and Luria. Strain R (for rough) differed from B by being sensitive to a “killer substance” to which B is resistant, and Hershey preferred it for mutational studies because of this additional marker. Strain S (for smooth) was used for growing and titering phage stocks because it grew to higher densities and gave a whiter lawn on plates. Strain H was selected for giving sterile lysates of phage T2H and was found to adsorb this

phage better than did R. Strains R, S, and H may represent separate single-colony isolates from coli PC, although this is not stated specifically in Hershey’s paper. We have similarly not found a statement by Luria or Delbrück on whether their B strain was a single-colony isolate or simply a subculture of coli PC. In describing these strain differences, Hershey called “attention to the difficulty of preserving uniform biological materials” and stated, “In general, however, the T system of phages act[s] identically on the bacterial strains mentioned, so that the casual reader may consider the terms R, S, H, B, and ‘sensitive bacterium’ as synonymous.” Hershey’s R, S, and H strains were used by other workers, as were distinctive B strains originating in other laboratories, but the exact strain was not always carefully identified. Most papers simply refer to B or *E. coli* B with no further identification.

The methods of maintaining bacterial strains and transferring them between laboratories can be a substantial source of variability. Hershey *et al.*⁵⁰ described good practice when they stated, “Both the phages and the bacterial culture are pure lines stored in the desiccated state, the culture being recovered from this source at weekly intervals” (where desiccated state refers to lyophilized samples used for preserving strains since at least 1914).⁶³ However, until reliable storage of frozen cultures in ultralow-temperature freezers became readily available in working laboratories, strains were typically maintained either by serial subculturing on agar slants (where a streaked culture is grown overnight and then stored in the refrigerator) or by long-term storage in sealed agar stabs at room temperature. Cultures on slants do not survive long-term storage, so subcultures on fresh slants were typically made every month or two. Variants arise with either method, including transposition by IS elements or deletions that affect regions not essential under the growth and storage conditions.^{33,64} Unsuspected mutations may become fixed in a population through single-colony isolation, which is necessary in constructing a new strain, but may also be done simply to ensure a pure strain within a laboratory or upon transfer of a strain between laboratories.

Strains that have been well preserved from the early days of *E. coli* research are useful for assessing variation and verifying relationships. In addition to the coli Bordet (CIP 63.70) already discussed, we obtained the earliest deposits of B strains we could identify in the CIP, both deposited by Elie Wollman in 1954: CIP 54.125 is identified as M. Delbrück strain B, and CIP 54.156 is identified as B/r, a widely used radiation-resistant mutant of *E. coli* B isolated by Evelyn Witkin in 1946.⁶⁵ We also obtained CIP 103914, the *E. coli* B deposited in the American Type Culture Collection (ATCC) by Luria in 1961 (ATCC 11303) and acquired by CIP from ATCC in 1993. In addition, the B strains BB, B^E, B40 *sul*, and S/6 were provided by D. Belin from the collection of the University of Geneva. These strains were maintained over the years by A. Bolle by monthly streaking onto fresh nutrient agar slants, overnight

incubation at 37 °C, and storage in the refrigerator. Strain BB (for B Berkeley) is a clone of B isolated in Stent's laboratory⁶⁶ that was used as a good host for growing T-even phage stocks. The B^E strain⁶⁷ was isolated by R. Epstein (personal communication to F. W.S., 2008) from a single colony on a mostly dead agar slant of B he had obtained from Doermann; it was designated B^E in case it might differ from B itself. B40 *sul* was used as a good host for growing phage amber mutants. The *supD* gene was introduced by P1 transduction (apparently from a derivative of K-12 strain CR63) into B40, an *argI* amber mutant of B that had been isolated by Gorini after UV irradiation and penicillin selection.^{68–71} Strain S/6 is a T6-resistant derivative of strain S of Hershey.⁷² Analyses of these strains have helped to define the likely genome sequence of the B strain of Delbrück and Luria, differences between the sequenced strains REL606 and BL21(DE3), and relationships among B strains generally.³³

BL21(DE3) and REL606

The lines of descent from B to BL21(DE3) and REL606, whose genome sequences have been determined,⁹ are also shown in Fig. 1. The derivation of BL21(DE3) is well documented in the literature, supplemented by personal communications. B-6 is the designation Bertani gave to the B he received from Luria or Demerec, a strain he lyophilized in 1963 (personal communication to R.E.L., 2006; see also Bertani and Weigle⁷³). B62 is the designation given by Kaiser to a single-colony isolate of B he obtained from Bertani in 1956 (personal communication to F.W.S., 2007). In 1963, Studier made a single-colony isolate from a culture grown from a sealed agar stab of B62 in Kaiser's laboratory and maintained it in a sealed agar stab at room temperature until 1978, when a culture grown directly from the stab was frozen. B62 that was grown from that frozen stock was used to prepare DNA for sequence comparisons to establish the sequence of B at positions where the genome sequences of BL21(DE3) and REL606 had diverged.^{9,33}

Bertani gave B-6 to Denise Cohen, who cured it of an apparently defective P2 λ -like prophage by UV irradiation to 0.1% survival and identified a cured colony by susceptibility to a recombinant hybrid phage that had the same immunity as the defective prophage.⁷⁴ The cured strain is identified in Cohen's paper and Fig. 1 as Bc, but apparently, the laboratory designation was B-15, and this strain also was lyophilized in Bertani's laboratory in 1963.

The B of Delbrück and Luria is resistant to phage λ , but Arber and Lataste-Dorolle⁷⁵ obtained sensitive strains from B and its derivatives BB, B/r, and Bc by P1 transduction from K-12 strain W3110 and by selecting for a Mal⁺ phenotype. The transductant of Bc designated Bc251 was a progenitor of BL21(DE3). The Mal⁺ phenotype and λ sensitivity were later found to be temperature-sensitive.⁷⁶

Strain WA628 is a Met⁻ mutant of Bc251,⁷⁷ and Bc258 is a nonreverting Gal⁻ mutant of Bc251,⁷⁵ both obtained by UV irradiation and penicillin selection. B707 is a Gal⁻ derivative of WA628 obtained by P1 transduction from Bc258.⁷⁸ B834 is a spontaneous mutant of B707 that is defective in B restriction and modification,⁷⁸ found also to have acquired a deletion of the gene for the outer membrane protease OmpT.⁷⁹ The methionine requirement of B834(DE3) (and therefore B707) can also be satisfied by vitamin B₁₂, demonstrating that the mutation is in *metE*.⁸⁰

B707 and B834 were obtained from Arber by Studier in 1973 and maintained in sealed agar stabs until 1978, when cultures grown from the stabs were frozen. At this same time, P1 grown on B62 was used to transduce B834 to Met⁺, thereby producing BL21. The first description of BL21 also reported construction of the lysogen BL21(DE3), whose lambdaoid prophage carries an inducible gene for T7 RNA polymerase inserted into its *int* gene.⁸ To provide the *int* function needed for DE3 to integrate, the temperature-inducible lysis-defective λ mutant cI857, S7 was first integrated into BL21 and then displaced by superinfection with the heteroimmune DE3. The original isolates of BL21 and BL21(DE3) were stored as frozen stocks, and cultures that were distributed to other laboratories or commercial suppliers were generally one or two subcultures removed from the original isolates, as was the sample used for genome sequencing. BL21(DE3) is a minimum of 11 successive single-colony isolations removed from the B of Delbrück and Luria: a minimum of 5 needed to isolate the genetic modifications that led to B834 (Fig. 1) and an additional 6 that were made in producing BL21(DE3) from B834. BL21 (DE3) is widely used for the production of proteins from cloned genes under the control of a promoter for T7 RNA polymerase in appropriate plasmid vectors,⁸¹ and BL21 itself is widely used for expressing genes from other promoters.

REL606 was the name given by Lenski *et al.*⁷ to a strain obtained in four steps by Seymour Lederberg⁸² from a strain he received from Meselson and thought was Bc251, the Mal⁺ λ^S derivative of Bc that is a progenitor of BL21(DE3). Although Lederberg's paper states that the strain was Bc251, analyses based on the genome sequences of REL606 and BL21 (DE3)³³ demonstrated that the progenitor of REL606 was actually a different Mal⁺ λ^S transductant made at the same time as Bc251 by Arber and Lataste-Dorolle,⁷⁵ a transductant of B itself. Meselson probably obtained the strain from Weigle, who had obtained it from Arber (personal communication to P.D., 2008); apparently, the strain was misidentified somewhere in the chain of transmission. The discrepancy is indicated by the designation (Bc251)* in the figure. Spontaneous mutations to T6 resistance and then to streptomycin resistance were followed by two rounds of treatment with the mutagen 1-methyl-3-nitro-1-nitrosoguanidine to produce first a strain defective in B restriction and modification, and then Ara⁻.⁸² Bruce Levin⁸³ obtained the Ara⁻ strain from Seymour Lederberg for an early

study on the population dynamics of bacterial competition. Levin, in turn, passed it on to Lenski, who worked in Levin's group from 1982 to 1985 and froze a single-colony isolate in 1985. From that freezer stock, Lenski obtained another single-colony isolate in 1988 for the start of a long-term evolution experiment, designating it REL606. The sample of REL606 that provided genomic DNA for sequencing was a minimum of seven successive single-colony isolations removed from the B of Delbrück and Luria. Sequencing of REL606 and Ara⁺ revertants determined that the Ara⁻ mutation was in *araA*.³³ Lenski *et al.* used REL606 to study evolution-in-action in laboratory-based bacterial populations.⁷ In these controlled environments, changes in genomic and phenotypic characteristics were studied over tens of thousands of generations to gain insight into the dynamics of evolutionary adaptation and its genetic basis.^{84,85}

Concluding Remarks

Tracing the history of *E. coli* B backward from Delbrück and Luria and forward to the present has been a fascinating experience. We have tried as much as possible to document this history in the original research papers of the scientists involved, but we have also benefited from published histories, commentaries, perspectives, and recollections, as well as personal communications, to direct our efforts and to close gaps in published records. Although much of our historical research was initially pursued independently, we soon encountered the paper of Abedon,³² who also explored the origins of *E. coli* B and its T-even phages, and whose paper provided helpful information and perceptive inferences. In addition to the references already named, the 1976 paper of Duckworth⁸⁶ provides interesting context and perspective. The availability of older strains in public culture collections, the likelihood of well-preserved collections of older strains in individual laboratories, and continuing development of ever more economical and accessible high-throughput genome sequencing and analysis offer additional possibilities for interesting bacterial archeology.

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References

- Lederberg, J. (2004). *E. coli* K-12. *Microbiol. Today*, **31**, 116.
- Bachmann, B. J. (1996). Derivations and genotypes of some mutant derivatives of *Escherichia coli*. In *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology (Neidhardt, F. C., ed.), pp. 2460, 2nd edit. ASM Press, Washington, DC.
- Tatum, E. L. & Lederberg, J. (1947). Gene recombination in the bacterium *Escherichia coli*. *J. Bacteriol.* **53**, 673–684.
- Delbrück, M. & Luria, S. E. (1942). Interference between bacterial viruses: I. Interference between two bacterial viruses acting upon the same host, and the mechanism of virus growth. *Arch. Biochem.* **1**, 111–141.
- Demerec, M. & Fano, U. (1945). Bacteriophage-resistant mutants in *Escherichia coli*. *Genetics*, **30**, 119–136.
- Delbrück, M. (1946). Bacterial viruses or bacteriophages. *Biol. Rev.* **21**, 30–40.
- Lenski, R. E., Rose, M. R., Simpson, S. C. & Tadler, S. C. (1991). Long-term experimental evolution in *Escherichia coli*: I. Adaptation and divergence during 2000 generations. *Am. Nat.* **138**, 1315–1341.
- Studier, F. W. & Moffatt, B. A. (1986). Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189**, 113–130.
- Jeong, H., Barbe, V., Lee, C. H., Vallenet, D., Yu, D. S., Choi, S.-H. *et al.* (2009). Genome sequences of *Escherichia coli* B strains REL606 and BL21(DE3). *J. Mol. Biol.* **394**, 644–652.
- d'Herelle, F. (1917). Sur un microbe invisible antagoniste des bacilles dysentériques. *Compt. Rend. Acad. Sci.* **165**, 373–375.
- Escherich, T. (1885). Die Darmbakterien des Neugeborenen und Säuglinge. *Fortschr. Med.* **3**, 515–522.
- d'Herelle, F. (1921). In *Le bactériophage. Son rôle dans l'immunité*. Monographies de l'Institut Pasteur, Masson et Cie, Libraires de l'Académie de Médecine, 120, Boulevard Saint Germain, Paris-VIe, France.
- d'Herelle, F. (1918). Sur le rôle du microbe filtrant bactériophage dans la dysenterie bacillaire. *Compt. Rend. Acad. Sci.* **167**, 970–972.
- d'Herelle, F. (1919). Du rôle du microbe filtrant bactériophage dans la fièvre typhoïde. *Compt. Rend. Acad. Sci.* **168**, 631–634.
- Gratia, A. & Jaumain, D. (1921). Dualité du principe lytique du colibacille et du staphylococque. *Compt. Rend. Soc. Biol.* **84**, 882–884.
- Gratia, A. (1922). Discussion on the bacteriophage (bacteriolysin). *Br. Med. J.* **2**, 23–25.
- d'Herelle, F. (1926). In *Le bactériophage et son comportement*. Monographies de l'Institut Pasteur, Masson et Cie, Libraires de l'Académie de Médecine, 120, Boulevard Saint Germain, Paris-VIe, France.
- Gratia, A. (1931). Sur l'identité du phénomène de Twort et du phénomène de d'Hérelle. *Ann. Inst. Pasteur*, **46**, 1–16.

19. Gratia, J.-P. (2000). André Gratia: a forerunner in microbial and viral genetics. *Genetics*, **156**, 471–476.
20. Summers, W. (1999). *Félix d'Hérelle and the Origin of Molecular Biology*. Yale University Press, New Haven.
21. Gratia, A. (1921). Studies on the d'Hérelle phenomenon. *J. Exp. Med.* **34**, 115–126.
22. Bordet, J. & Ciuca, M. (1920). Exsudats leucocytaires et autolyse microbienne transmissible. *Compt. Rend. Soc. Biol.* **83**, 1293–1295.
23. Bordet, J. & Ciuca, M. (1920). Le bactériophage de d'Hérelle, sa production et son interprétation. *Compt. Rend. Soc. Biol.* **83**, 1296–1298.
24. van Helvoort, T. (1992). Bacteriological and physiological research styles in the early controversy on the nature of the bacteriophage phenomenon. *Med. Hist.* **36**, 243–270.
25. Bordet, J. & Ciuca, M. (1921). Remarques sur l'histoire des recherches, concernant la lyse microbienne transmissible. *Compt. Rend. Soc. Biol.* **84**, 745–747.
26. Twort, F. W. (1915). An investigation on the nature of ultra-microscopic viruses. *Lancet*, **2**, 1241–1243.
27. Gratia, A. & Jaumain, D. (1921). Identité du phénomène de Twort et du phénomène de d'Hérelle. *Compt. Rend. Soc. Biol.* **85**, 880–881.
28. Wollstein, M. (1921). Studies on the phenomenon of d'Hérelle with *Bacillus dysenteriae*. *J. Exp. Med.* **35**, 467–476.
29. Kuttner, A. G. (1923). Bacteriophage phenomena. *J. Bacteriol.* **8**, 49–101.
30. Gratia, A. (1922). The Twort-d'Hérelle phenomenon: II. Lysis and microbic variation. *J. Exp. Med.* **35**, 287–302.
31. Wollman, E. (1925). Recherches sur la bactériophagie (phénomène de Twort-d'Hérelle). *Ann. Inst. Pasteur*, **39**, 789–832.
32. Abedon, S. T. (2000). The murky origin of Snow White and her T-even dwarfs. *Genetics*, **155**, 481–486.
33. Studier, F. W., Daegelen, P., Lenski, R. E., Maslov, S. & Kim, J. F. (2009). Understanding the differences between genome sequences of *Escherichia coli* B strains REL606 and BL21(DE3), and comparison of the *E. coli* B and K-12 genomes. *J. Mol. Biol.* **394**, 653–680.
34. Gratia, A. (1921). Studies on the lytic agent of Bordet and Ciuca. *Proc. Soc. Exp. Biol. Med.* **18**, 192–193.
35. Gratia, A. (1921). Preliminary report on a *Staphylococcus* bacteriophage. *Proc. Soc. Exp. Biol. Med.* **18**, 217–219.
36. Kuttner, A. (1921). Preliminary report on a typhoid bacteriophage. *Proc. Soc. Exp. Biol. Med.* **18**, 158–163.
37. Kuttner, A. G. (1921). On the influence of tissue enzymes on the bacteriophage principle. *Proc. Soc. Exp. Biol. Med.* **18**, 222–225.
38. Bronfenbrenner, J. & Schlesinger, M. J. (1921). Concerning anaphylaxis following the administration of diphtheria antitoxin. *Proc. Soc. Exp. Biol. Med.* **18**, 147–148.
39. Bronfenbrenner, J., Schlesinger, M. J. & Orr, P. F. (1921). Concerning toxic byproducts of *Bacillus botulinus*. *Proc. Soc. Exp. Biol. Med.* **18**, 181–182.
40. Bronfenbrenner, J. & Weiss, H. (1921). Serumtherapy of advanced botulism. *Proc. Soc. Exp. Biol. Med.* **18**, 253–254.
41. Bronfenbrenner, J. & Schlesinger, M. J. (1921). The composite nature of botulinus toxin. *Proc. Soc. Exp. Biol. Med.* **18**, 254.
42. Bronfenbrenner, J. J. & Korb, C. (1925). Studies on the bacteriophage of d'Hérelle: II. Effect of alcohol on the bacteriophage of d'Hérelle. *J. Exp. Med.* **42**, 419–429.
43. Bronfenbrenner, J. J. & Korb, C. (1925). Studies on the bacteriophage of d'Hérelle: I. Is the lytic principle volatile? *J. Exp. Med.* **42**, 73–79.
44. Bronfenbrenner, J. J. & Korb, C. (1925). Studies on the bacteriophage of d'Hérelle: III. Some of the factors determining the number and size of plaques of bacterial lysis on agar. *J. Exp. Med.* **42**, 483–497.
45. Kalmanson, G. M. & Bronfenbrenner, J. (1939). Studies on the purification of bacteriophage. *J. Gen. Physiol.* **23**, 203–228.
46. Bronfenbrenner, J. (1933). True polyvalence of pure bacteriophage. *Proc. Soc. Exp. Biol. Med.* **30**, 729–732.
47. Bronfenbrenner, J. (1932). The heat inactivation of bacteriophages. *Proc. Soc. Exp. Biol. Med.* **29**, 802–804.
48. Muckenfuss, R. S. (1928). Studies on the bacteriophage of d'Hérelle: XI. An inquiry into the mode of action of antibacteriophage serum. *J. Exp. Med.* **48**, 709–722.
49. Kalmanson, G. M. & Bronfenbrenner, J. (1942). Evidence of serological heterogeneity of polyvalent "pure line" bacteriophage. *J. Immunol.* **45**, 13–19.
50. Hershey, A. D., Kalmanson, G. & Bronfenbrenner, J. (1943). Quantitative methods in the study of phage-antiphage reaction. *J. Immunol.* **46**, 267–279.
51. Luria, S. E. & Exner, F. M. (1941). The inactivation of bacteriophages by X-rays—influence of the medium. *Proc. Natl Acad. Sci. USA*, **27**, 370–375.
52. Luria, S. E. & Anderson, T. F. (1942). The identification and characterization of bacteriophages with the electron microscope. *Proc. Natl Acad. Sci. USA*, **28**, 127–130.
53. Delbrück, M. (1942). Bacterial viruses (bacteriophages). *Adv. Enzymol.* **2**, 1–32.
54. Ellis, E. L. & Delbrück, M. (1939). The growth of bacteriophage. *J. Gen. Physiol.* **22**, 365–384.
55. Delbrück, M. (1940). The growth of bacteriophage and lysis of the host. *J. Gen. Physiol.* **23**, 643–660.
56. Luria, S. E. & Delbrück, M. (1943). Mutations of bacteria from virus sensitivity to virus resistance. *Genetics*, **28**, 491–511.
57. Luria, S. E., Delbrück, M. & Anderson, T. F. (1943). Electron microscope studies of bacterial viruses. *J. Bacteriol.* **46**, 57–77.
58. Cohen, S. S. & Anderson, T. F. (1946). Chemical studies on host-virus interactions: I. The effect of bacteriophage adsorption on the multiplication of its host, *Escherichia coli* B, with an appendix giving some data on the composition of the bacteriophage, T2. *J. Exp. Med.* **84**, 511–523.
59. Hershey, A. D. (1946). Mutation of bacteriophage with respect to type of plaque. *Genetics*, **31**, 620–640.
60. Benzer, S., Delbrück, M., Dulbecco, R., Hudson, W., Stent, G. S., Watson, J. D. et al. (1950). A syllabus on procedures, facts and interpretations in phage. In *Viruses* (Delbrück, M., ed.), California Institute of Technology, Pasadena.
61. Adams, M. H. (1959). *Bacteriophages*. Interscience Publishers, Inc., New York.
62. Cairns, J., Stent, G. S. & Watson, J. D. (1966). Phage and the Origins of Molecular Biology. Cold Spring Harbor Laboratory of Quantitative Biology, Cold Spring Harbor, NY.
63. Rogers, L. A. (1914). The preparation of dried cultures. *J. Infect. Dis.* **14**, 100–123.
64. Naas, T., Blot, M., Fitch, W. M. & Arber, W. (1994). Insertion sequence-related genetic variation in resting *Escherichia coli* K-12. *Genetics*, **136**, 721–730.
65. Witkin, E. M. (1946). Inherited differences in sensitivity to radiation in *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **32**, 59–68.

66. McFall, E. & Stent, G. S. (1958). Three star mutants of coliphage T2. *J. Gen. Microbiol.* **18**, 346–363.
67. Bolle, A., Epstein, R. H., Salser, W. & Geiduschek, E. P. (1968). Transcription during bacteriophage T4 development: synthesis and relative stability of early and late RNA. *J. Mol. Biol.* **31**, 325–348.
68. Gorini, L., Gundersen, W. & Burger, M. (1961). Genetics of regulation of enzyme synthesis in the arginine biosynthetic pathway of *Escherichia coli*. *Cold Spring Harbor Symp. Quant. Biol.* **26**, 173–182.
69. Gorini, L. (1969). The contrasting role of *strA* and *ram* gene products in ribosomal functioning. *Cold Spring Harbor Symp. Quant. Biol.* **34**, 101–109.
70. Strigini, P. & Gorini, L. (1970). Ribosomal mutations affecting efficiency of amber suppression. *J. Mol. Biol.* **47**, 517–530.
71. Jacoby, G. (1971). Mapping the gene determining ornithine transcarbamylase and its operator in *Escherichia coli* B. *J. Bacteriol.* **108**, 645–651.
72. Doermann, A. H. & Hill, M. B. (1953). Genetic structure of bacteriophage T4 as described by recombination studies of factors influencing plaque morphology. *Genetics*, **38**, 79–90.
73. Bertani, G. & Weigle, J. J. (1953). Host controlled variation in bacterial viruses. *J. Bacteriol.* **65**, 113–121.
74. Cohen, D. (1959). A variant of phage P2 originating in *Escherichia coli*, strain B. *Virology*, **7**, 112–126.
75. Arber, W. & Lataste-Dorolle, C. (1961). Erweiterung des Wirtsbereiches des Bakteriophagen λ auf *Escherichia coli* B. *Pathol. Microbiol.* **24**, 1012–1018.
76. Ronen, A. & Raanan-Ashkenazi, O. (1971). Temperature sensitivity of maltose utilization and lambda resistance in *Escherichia coli* B. *J. Bacteriol.* **106**, 791–796.
77. Kellenberger, G., Zichichi, M. L. & Epstein, H.T. (1962). Heterozygosis and recombination of bacteriophage. *Virology*, **17**, 44–55.
78. Wood, W. B. (1966). Host specificity of DNA produced by *Escherichia coli*: bacterial mutations affecting the restriction and modification of DNA. *J. Mol. Biol.* **16**, 118–133.
79. Grodberg, J. & Dunn, J. J. (1988). *ompT* encodes the *Escherichia coli* outer membrane protease that cleaves T7 RNA polymerase during purification. *J. Bacteriol.* **170**, 1245–1253.
80. Studier, F. W. (2005). Protein production by auto-induction in high density shaking cultures. *Protein Expression Purif.* **41**, 207–234.
81. Studier, F. W., Rosenberg, A. H., Dunn, J. J. & Dubendorff, J. W. (1990). Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* **185**, 60–89.
82. Lederberg, S. (1966). Genetics of host-controlled restriction and modification of deoxyribonucleic acid in *Escherichia coli*. *J. Bacteriol.* **91**, 1029–1036.
83. Levin, B. R. (1972). Coexistence of two asexual strains on a single resource. *Science*, **175**, 1272–1274.
84. Woods, R., Schneider, D., Winkworth, C. L., Riley, M.A. & Lenski, R. E. (2006). Tests of parallel molecular evolution in a long-term experiment with *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **103**, 9107–9112.
85. Blount, Z. D., Borland, C. Z. & Lenski, R. E. (2008). Historical contingency and the evolution of a key innovation in an experimental population of *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **105**, 7899–7906.
86. Duckworth, D. H. (1976). “Who discovered bacteriophage?” *Bacteriol. Rev.* **40**, 793–802.
87. Blattner, F. R., Plunkett, G., III, Bloch, C. A., Perna, N. T., Burland, V., Riley, M. *et al.* (1997). The complete genome sequence of *Escherichia coli* K-12. *Science*, **277**, 1453–1474.
88. Hayashi, K., Morooka, N., Yamamoto, Y., Fujita, K., Isono, K., Choi, S. *et al.* (2006). Highly accurate genome sequences of *Escherichia coli* K-12 strains MG1655 and W3110. *Mol. Syst. Biol.* **2**, 1–5.