Evidence of Lysogeny in Derivatives of *Escherichia coli*

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ABSTRACT

FRAMPTON, E. W. (The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston), AND B. R. BRINKLEY. Evidence of lysogeny in derivatives of *Escherichia coli*. J. Bacteriol. 90:446-452. 1965.—Exposure of cultures of several thymine-requiring derivatives of *Escherichia coli* strain 15 to radiation [X rays or ultraviolet (UV)] resulted in an increased synthesis of ribonucleic acid (RNA) as measured by the incorporation of radioactive uridine. In addition to radiation, mitomycin C (5 or 25 $\mu\text{g/ml}$) or growth in the absence of thymine also stimulated RNA synthesis in cultures in which the bulk of RNA synthesis was terminated by a shift-down in growth conditions. RNA purified from X-irradiated bacteria was resolved on columns of methylated serum albumin and yielded an elution profile which appeared similar to RNA synthesized by phage-infected bacteria. Electron microscopic examination of cultures induced to lyse by exposure to UV radiation, or by growth in the presence of 5-bromouracil before exposure to radiation (X rays or UV), showed the presence of mostly incomplete phage particles.

Exposure of cultures of *Escherichia coli* strain 15 to ultraviolet (UV) radiation was reported to induce colicin formation (Ryan, Fried, and Mukai, 1955; Mukai, 1960). Mukai (1960) recognized the possibility that colicin activity in *E. coli* 15 could be caused by the induction of a defective phage, but evidence was lacking that a defective phage was induced in these bacteria.

During a study on the effects of radiation on ribonucleic acid (RNA) synthesis, it was found that exposure of several derivatives of *E. coli* 15 to radiation (X rays and UV) stimulates RNA synthesis. In contrast, X-irradiated cultures of *E. coli* B/r demonstrated an inhibited synthesis of RNA (Frampton, 1964). Initially it was thought that the stimulated RNA synthesis observed in induced cultures of *E. coli* 15 derivatives represented messenger RNA required for colicin production. However, similarities in the type of RNA synthesized by these cultures and by phage-infected bacteria led to an electron microscopic examination of induced cultures for evidence of phage particles. This paper describes some of the properties of the RNA synthesized by X-irradiated cultures and presents electron micrographs of the phage particles found in lysates of several derivative strains of *E. coli* 15.

MATERIALS AND METHODS

Bacteria and growth conditions. Four thymine-requiring strains descended from *E. coli* strain 15 were employed in this study. Two of the strains used, *E. coli* 15T⁻ and *E. coli* TAU, are subject to lysis after exposure to UV radiation, whereas *E. coli* 15T⁻ (555-7) and *E. coli* TAU-bar are not. *E. coli* 15T⁻ was grown with aeration at 37°C in medium C (Roberts et al., 1955) supplemented with thymine or thymidine (4 to 20 $\mu\text{g/ml}$). *E. coli* TAU (strain 15T⁻A-U⁻), a polyauxotroph (Kanazir et al., 1959), was supplemented with thymine (20 $\mu\text{g/ml}$), arginine (38 $\mu\text{g/ml}$), and uracil (20 $\mu\text{g/ml}$). *E. coli* 15T⁻ (555-7) obtained from R. S. Weatherwax and selected for its resistance to lysis after exposure to UV radiation (Weatherwax and Landman, 1960) was supplemented with thymine or thymidine (4 $\mu\text{g/ml}$), methionine (30 $\mu\text{g/ml}$), tryptophan (14 $\mu\text{g/ml}$), and arginine (38 $\mu\text{g/ml}$). *E. coli* TAU-bar, obtained from P. C. Hanawalt (1963), was supplemented with thymine (20 $\mu\text{g/ml}$), uracil (20 $\mu\text{g/ml}$), proline (20 $\mu\text{g/ml}$), plus methionine, arginine, and tryptophan at the same levels used for *E. coli* 15T⁻ (555-7). Enriched media used in shift-down experiments contained 1% (w/v) Casamino Acids (Difco) in addition to the regular supplements. All liquid media contained 0.2% (w/v) glucose. Membrane filters (Schleicher and Schuell type B-6, 150 mm) were used for media changes and harvesting procedures.

Irradiation of bacteria. X irradiation of bacterial suspensions was performed as previously described (Frampton, 1964). UV irradiation, with stirring,

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was carried out on 50-ml samples of either unconcentrated or fivefold concentrated washed bacterial suspensions in a sterile petri dish 95 cm from two General Electric germicidal lamps (output 7.5 ergs per mm² per sec). Irradiated bacteria were resuspended at one-half harvesting concentration and incubated at 37 C during the postirradiation period.

Purification and resolution of RNA. Cell suspensions (250 ml) were harvested, washed, and resuspended in 10 ml of TM buffer (10⁻² M tris(hydroxymethyl)aminomethane, 10⁻² M MgCl₂, pH 7.4). Bentonite (1 mg/ml) was added, and the cells were disrupted in a French pressure cell. After clarification of cell extracts at 20,000 × *g* for 15 min, the RNA was purified with phenol and sodium dodecyl sulfate and precipitated with alcohol, as described by DiGirolamo, Henshaw, and Hiatt (1964). No effort was made to remove the DNA from these preparations. Purified nucleic acid preparations (10 A₂₆₀ units) were applied to columns (9 cm × 2.54 cm²) of methylated serum albumin-coated kieselguhr (MAK) by use of the single-layer modification described by Sueoka and Cheng (1962). A linear gradient (500 ml) of NaCl (0.4 to 1.0 M) in phosphate buffer (pH 6.7) was applied with enough air pressure to give a flow rate of 1.5 ml/min, and 5-ml fractions were collected.

Measurement of radioactivity. Samples (3 ml) were removed from the cultures at various times and added to an equal volume of cold 10% (w/v) trichloroacetic acid. After 30 min, the acid-insoluble material was collected on Millipore filters (0.65 μ pore size, 25 mm in diameter). The washed and dried filters were placed in vials containing a solution of phosphors in toluene and counted in a Packard Tri-Carb scintillation counter. The fractions from the MAK column were treated the same way after the addition of bovine serum albumin (0.5 mg) to each tube.

Induction of lysis in lysis-resistant strains. Cultures were grown in supplemented minimal media to about 10⁸ to 2 × 10⁸ cells per milliliter, washed free from thymine, and incubated for 40 min in media containing 5-bromouracil (2 μg/ml). The cells were then harvested and washed free from 5-bromouracil before exposure to radiation. *E. coli* 15T⁻ (555-7) was X-irradiated (10 kr) and *E. coli* TAU-bar was exposed to UV radiation (400 ergs/mm²) before being returned to thymine-supplemented media and incubated for 2 hr. Absorbancy of these cultures was measured at 420 mμ, and lysis was observed within 60 min after irradiation.

Preparation of material for electron microscopy. Lysed cultures were centrifuged at 3,000 × *g* for 15 min, which removed unlysed cells, cell debris, and adsorbed or trapped phage particles. The upper half of the supernatant material was removed with a pipette and centrifuged at 96,572 × *g* for 2 hr in a Spinco model L-2 preparative ultracentrifuge with a type 50 rotor. Both pellets were gently suspended in unsupplemented growth

FIG. 1. Stimulation of uridine-2-C¹⁴ (4 mμc per 8 μg per ml) incorporation in cultures of *Escherichia coli* 15T⁻ (555-7). The following conditions are shown: (a) stimulation in X-irradiated (60 kr) log-phase cells (16 μg/ml), (b) response to varying doses of X rays after a shift-down, (c) effect of mitomycin C (5 or 25 μg/ml) after a shift-down, and (d) effect of thymineless growth after a shift-down.

FIG. 2. Resolution of purified RNA on columns of MAK. Uridine-H³ (0.4 μc per 2 μg per ml) was added 15 min after the shift-down and incubation was continued for 15 min. RNA was extracted from: (a) unirradiated *Escherichia coli* 15T⁻ (555-7), (b) *E. coli* 15T⁻ (555-7) exposed to 60 kr of X rays, (c) unirradiated *E. coli* 15 TAU, and (d) *E. coli* 15 TAU exposed to 60 kr of X rays.

FIG. 3-8. Electron micrographs of cell lysates. (3) Phage in P_1 fraction from *Escherichia coli* 15T⁻. $\times 770,000$. (4) Dispersed subunits of structures shown in Fig. 5 surrounding almost intact phage in P_1 fraction from *E. coli* 15T⁻. $\times 220,000$. (5) Globular bodies A, B, C, in P_1 fraction from *E. coli* 15T⁻ showing subunits. $\times 220,000$. (6, 7, and 8) Complete and incomplete particles in P_2 fraction from *E. coli* TAU. $\times 280,000$.

FIG. 9-11. Electron micrographs of cell lysates. (9) Full and empty heads in P_2 fraction from *Escherichia coli* 15T⁻ (555-7). $\times 280,000$. (10) Tails with contracted sheaths and head in P_2 fraction from *E. coli* 15T⁻ (555-7). $\times 280,000$. (11) Cluster of tails showing contracted sheaths in P_2 fraction from *E. coli* TAU-bar. $\times 280,000$.

TABLE 1. Growth requirements and response to induction of various derivatives of *Escherichia coli* strain 15*

Strain	Nutritional requirements	Lysis inducible by UV alone	Lysis inducible by 5-BU+ irradiation	Complete phage in lysates	Incomplete phage in lysates	Plaques on <i>E. coli</i> B, C, 15T	RNA synthesis increased by induction
15T ⁻	<i>thy</i>	+		+	+	0	+
TAU	<i>thy, arg, ura</i>	+		+	+	0	+
15T ⁻ (555-7)	<i>thy, arg, met, try</i>	0	+ (X ray)	0	+	0	+
TAU-bar	<i>thy, arg, ura, met, try, pro</i>	0	+ (UV)	0	+	0	+

* Abbreviations: *thy* = thymine; *arg* = arginine; *ura* = uracil; *met* = methionine; *try* = tryptophan; *pro* = proline; 5-BU = 5-bromouracil.

media. The low-speed pellet was designated as fraction P₁, and the high-speed pellet as fraction P₂. Suspensions of the P₁ and P₂ fractions were negatively stained with potassium phosphotungstate according to the procedure of Brenner and Horne (1959). A Hitachi HU 11A electron microscope was used to examine the material.

Chemicals. Mitomycin C and 5-bromouracil were obtained from Calbiochem. Uridine-2-C¹⁴ (28 mc/mmole) and uridine-H³ (3 to 4 c/mmole) were products of New England Nuclear Corp., Boston, Mass. Adenine-2-C¹⁴ (1.7 mc/mmole) was obtained from Volk Radiochemical Co., Chicago, Ill. Sodium dodecyl sulfate, obtained from L. Light and Co. Ltd., Colnbrook, England, was recrystallized from hot alcohol. Crystalline bovine serum albumin from Nutritional Biochemicals Corp., Cleveland, Ohio, was used to prepare the methylated serum albumin.

RESULTS

RNA synthesis in induced bacteria. A variety of procedures stimulated RNA synthesis in cultures of *E. coli* 15T⁻ (555-7). Postirradiation RNA synthesis was stimulated in bacteria taken from cultures in logarithmic growth and exposed to X rays (Fig. 1a). In cultures in which the bulk of RNA synthesis was terminated by transferring the cells from growth in enriched media to growth in minimal media and defined as a shift-down in growth conditions (Kjeldgaard, Maaløe, and Schaechter, 1958), RNA synthesis increased after exposure to increasing doses of X rays (Fig. 1b). By use of this shift-down technique to assay for the effects of various treatments on the synthesis of messenger RNA (Hayashi and Spiegelman, 1961), it was found that the addition of mitomycin C (5 or 25 µg/ml) or thymineless growth led to an early stimulation of RNA synthesis (Fig. 1c, d). RNA synthesis was also increased in cultures of *E. coli* 15T⁻, *E. coli* TAU, and *E. coli* TAU-bar, which were exposed to UV radiation (200 to 400 erg/mm²) before growth under shift-down conditions. Adenine-2-C¹⁴ incorporation was stimulated in cultures exposed to X rays or UV

radiation, and thus eliminates the possibility that the observations were specific for uridine.

Resolution of purified RNA. Partial characterization of the RNA synthesized by X-irradiated cultures of *E. coli* 15T (555-7) and *E. coli* TAU made by resolution of purified RNA on columns of MAK is shown in Fig. 2. Elution profiles revealed that X-ray exposure resulted in an increased incorporation of radioactivity into heterogeneous RNA components (Fig. 2b, d). The material eluting after the 23S RNA peak was particularly pronounced in the RNA from irradiated *E. coli* TAU (Fig. 2d).

Electron microscopic examination of lysates. The apparently intact phage particles observed in spontaneously lysed cultures of *E. coli* 15T⁻ (Fig. 3, 4) and in UV-induced cultures of *E. coli* TAU (Fig. 6-8) are not morphologically identical to any of the coliphages described by Bradley (1964). The tail portion differs considerably from phage M-1 which is virulent for *E. coli* TAU (Suit, *personal communication*). The heads are smaller than phage T2, measuring approximately 55 mµ in diameter and 59 mµ long with a tail 100 mµ long and 9 to 10 mµ wide. The head and tail components in some micrographs appear to be separated by a neck region (see arrow, Fig. 8). No tail fibers were detected in any of the preparations. Four spikes appear to be attached to the end plate, although better resolution will be required to establish the precise number. In addition to the complete phage particles, full and empty heads, and tails with contracted sheaths, numerous large globular bodies (Fig. 5A, B, C) were observed in lysates of *E. coli* 15T⁻ and *E. coli* TAU. Subunits (9 to 12 mµ in diameter) of these structures were partially dispersed in some cases (Fig. 4). The nature of the large structures is not known, but it is suggested they may represent partially assembled head material composed of many protein subunits (Epstein et al., 1963). No intact phage particles were detected in lysates of the two strains induced to lyse by exposure to 5-bro-

mouracil prior to being irradiated. However, many full and empty heads, and tail pieces with contracted sheaths were observed in these preparations (Fig. 9–11). Standard phage assay techniques (Adams, 1959) failed to detect plaque-forming capacity in any of the lysates with *E. coli* B, *E. coli* C, and *E. coli* 15T⁻ as indicator strains and confirmed a previous observation (Mukai, 1960).

A summary of the characteristics of the various strains used in this study along with significant results are compiled in Table 1.

DISCUSSION

The major point of interest established in the present paper is that derivatives of *E. coli* strain 15, whether sensitive or insensitive to lysis after exposure to UV radiation, can be induced to liberate particles morphologically resembling bacteriophage. The bacterial strains enumerated in this investigation are of particular interest because they have been utilized in many recent investigations concerned with macromolecular synthesis. The use of any of these strains in experiments requires, therefore, a consideration of how lysogeny might affect the system being studied.

The absence of plaque-forming phage and the presence of apparently incomplete phage particles in lysates suggest that a "defective-lysogenic" state (Arber and Kellenberger, 1958) may be responsible for the colicin activity previously described in various bacterial strains derived from *E. coli* strain 15. The identification of phage particles in lysates, however, does not exclude the possibility that these strains are also colicinogenic. Factors controlling induction appear to be identical in both lysogenic and colicinogenic bacteria (Adams, 1959; Fredericq, 1963). UV radiation and thymineless growth were reported to induce colicin formation in strains derived from *E. coli* 15 (Makai, 1960; Mennigmann, 1964; Luzzati and Chevallier, 1964). Fredericq (1963) suggested that colicins are synthesized by prophage which are so defective that upon induction the only component synthesized is a protein related to a lethal protein located in the tail portion of virulent phage. Obviously the phage particles visualized in electron micrographs shown here are not nearly so defective. This fact may account for the lysis obtained in induced cultures of these strains—a feature not usually observed in colicinogenic bacteria. Epstein et al. (1963) described a variety of defects in phage maturation, and it seems possible that an additional mutation(s) affecting the lytic mechanism may be involved in the lysis-resistant strains *E. coli* 15T⁻ (555-7) and *E. coli* TAU-bar. In subsequent studies, the pres-

ence of incomplete phage particles was observed in UV-induced cultures of these two strains which were lysed with lysozyme without having been exposed to 5-bromouracil (Frampton and Brinkley, unpublished observations). By the negative staining procedure used in this study, there appeared to be more unassembled phage components than complete phage in the lysates of *E. coli* 15T⁻ and *E. coli* TAU. Ratios of intact phage to unassembled components, or of heads to tails, were not calculated.

The radioactivity eluting after the 23S RNA peak (Fig. 2d) is similar to observations made after phage infection (Ishihama et al., 1962; Kano-Sueoka and Spiegelman, 1962). Characterization of this RNA was insufficient, however, for us to conclude that it is phage-specific RNA. Increased RNA synthesis in cultures exposed to less disruptive treatments than X rays suggests that alterations in cell permeability or organization are not major factors in the increased incorporation of RNA precursors. Experiments are in progress to characterize the phage and to determine the properties of the phage nucleic acid.

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ADDENDUM

Subsequent to the completion of the experimental findings presented in this paper, the authors learned of two investigations reporting the presence of defective phage particles in *E. coli* 15. H. D. Mennigmann kindly forwarded to us a copy of a manuscript he submitted for publication describing the presence of a defective phage in lysates of UV-induced cultures of *E. coli* 15T⁻. Sandoval, Reilly, and Tandler (1965) recently reported the likelihood that a defective phage is responsible for colicin activity in induced cultures of a prototrophic strain of *E. coli* 15.

LITERATURE CITED

- ADAMS, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
- ARBER, W., AND G. KELLENBERGER. 1958. Study of the properties of seven defective-lysogenic strains derived from *Escherichia coli* K12 (λ). *Virology* 5:458–475.
- BRADLEY, D. E. 1964. The structure of some bacteriophages associated with male strains of *Escherichia coli*. *J. Gen. Microbiol.* 35:471–482.

- BRENNER, S., AND R. W. HORNE. 1959. A negative staining method for high resolution electron microscopy of viruses. *Biochim. Biophys. Acta* **34**:103-110.
- DIGIROLAMO, A., E. C. HENSHAW, AND H. H. HIATT. 1964. Messenger ribonucleic acid in rat liver nuclei and cytoplasm. *J. Mol. Biol.* **8**:479-488.
- EPSTEIN, R. H., A. BOLLE, C. M. STEINBERG, E. KELLENBERGER, E. BOY DE LA TOUR, R. CHEVALLEY, R. S. EDGAR, M. SUSMAN, G. H. DENHARDT, AND A. LIELAUSIS. 1963. Physiological studies of conditional lethal mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. **28**:375-392.
- FRAMPTON, E. W. 1964. Synthesis of ribonucleic acid by X-irradiated bacteria. *J. Bacteriol.* **87**:1369-1376.
- FREDERICQ, P. 1963. On the nature of colicinogenic factors: a review. *J. Theoret. Biol.* **4**:159-165.
- HANAWALT, P. C. 1963. Involvement of synthesis of RNA in thymineless death. *Nature* **198**:286.
- HAYASHI, M., AND S. SPIEGELMAN. 1961. The selective synthesis of informational RNA in bacteria. *Proc. Natl. Acad. Sci. U.S.* **47**:1564-1580.
- ISHIHAMA, A., N. MIZUNO, M. TAKAI, E. OTAKA, AND S. OSAWA. 1962. Molecular and metabolic properties of messenger RNA from normal and T2-infected *Escherichia coli*. *J. Mol. Biol.* **5**:251-264.
- KANAZIR, D., H. D. BARNER, J. G. FLAKS, AND S. S. COHEN. 1959. Some physiological and genetic properties of a strain of *Escherichia coli* requiring thymine, arginine, and uracil. *Biochim. Biophys. Acta* **34**:341-353.
- KANO-SUEOKA, T., AND S. SPIEGELMAN. 1962. Evidence for a nonrandom reading of the genome. *Proc. Natl. Acad. Sci. U.S.* **48**:1942-1949.
- KJELDGAARD, N. O., O. MAALØE, AND M. SCHAECHTER. 1958. The transition between different physiological states during balanced growth of *Salmonella typhimurium*. *J. Gen. Microbiol.* **19**:607-616.
- LUZZATI, D., AND M. R. CHEVALLIER. 1964. Induction par carence en thymine de la production de colicine par des bactéries colicinogènes thymine-exigenates. *Ann. Inst. Pasteur* **107**:152.
- MENNIGMANN, H. D. 1964. Induction in *Escherichia coli* 15 of the colicinogenic factor by thymine-less death. *Biochem. Biophys. Res. Commun.* **16**:373-378.
- MUKAI, F. H. 1960. Interrelationship between colicin sensitivity and phage resistance. *J. Gen. Microbiol.* **23**:539-551.
- ROBERTS, R. B., D. B. COWIE, P. H. ABELSON, E. T. BOLTON, AND R. J. BRITTEN. 1955. Studies of biosynthesis in *Escherichia coli*. Carnegie Inst. Wash. Publ. 607.
- RYAN, F. J., P. FRIED, AND F. MUKAI. 1955. A colicin produced by cells that are sensitive to it. *Biochim. Biophys. Acta* **18**:131.
- SANDOVAL, H. K., H. C. REILLY, AND B. TANDLER. 1965. Colicin 15: possibly a defective bacteriophage. *Nature* **205**:522.
- SUEOKA, N., AND T. Y. CHENG. 1962. Fractionation of nucleic acids with the methylated albumin column. *J. Mol. Biol.* **4**:161-172.
- WEATHERWAX, R. S., AND O. E. LANDMAN. 1960. Ultraviolet light-induced mutation and deoxyribonucleic acid synthesis in *Escherichia coli*. *J. Bacteriol.* **80**:528-535.