The product of the rep gene of Escherichia coli catalytically separates φX174 duplex DNA strands in advance of their replication, utilizing ATP in the process (Scott, J. F., Eisenberg, S., Bertuch, L. L., and Kornberg, A. 1977) Proc. Natl. Acad. Sci. U. S. A. 74, 193-197). The enzyme has now been purified to near-homogeneity. Relatively large quantities were obtained from ColEI-plasmid-containing cells in which the enzyme level was 7 to 10 times above wild type. The assay for rep protein was based on its essential role, with phage-induced cistron A protein, in enzymatic synthesis of phage φX174 (+) strands, using duplex circular DNA as template. The protein exhibits a molecular weight of 65,000 under denaturing and reducing conditions. The turn-over number of the enzyme is approximately 6000 ATP molecules/min in strand separation as measured by extent of replication, or in an uncoupled reaction using single-stranded DNA effector.

The product of the rep gene of Escherichia coli is required for replication of the duplex replicative form of φX174 phage and for subsequent synthesis of progeny viral single strands. It is not required in conversion of the infecting viral single strand to RF' (1-4). Mutations in the rep locus are not lethal for the host cell, but block RF replication of phages φX174 (1), M13 and fd (5), G4 (6), and P2 (7). These mutations also result in: larger cell size, more DNA per cell, slightly lower ratio of cell to mass, more replicating forks per chromosome, slower fork movement, and a fuller sedimenting nucleoid body containing more DNA (5, 8).

Wild type E. coli contains only about 50 copies of rep protein per cell. Therefore, to obtain pure protein for enzymatic and physical characterization of the enzyme, we selected a plasmid-bearing strain which makes 7 to 10 times more rep protein than wild type. In this paper we also describe the purification of rep protein from this strain (in a yield of 30 mg from 4.6 kg of cells), its enzymatic behavior, and some of its physical properties. The ATPase functions are reported in greater detail in the succeeding paper (9).

Purification of the rep Protein of Escherichia coli

AN ATPase WHICH SEPARATES DUPLEX DNA STRANDS IN ADVANCE OF REPLICATION*

John F. Scott and Arthur Kornberg
From the Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Materials and Methods

Bacterial and Phage Strains

These were generously provided as follows: E. coli HF4704 rep3 [thy (at 37°C), rep3] by Dr. S. Eisenberg of this laboratory; RLM365 [rha, lys, thyA (50 μg/ml), polB, str'], HMS88 [rha, lys, thyA (50 μg/ml), polA, polB, lac, sir'] W3110/ColEI and MV12 (lacY, ΔrepE5, thr, leu, recA/F+/pLC44-7) by Dr. R. McMacken of this laboratory; HF4704 [trd (at 37°C), Str'] and phage φX174 amβ by Dr. R. Sinsheimer (California Institute of Technology); MO676 (recB21, recC22, sbeB15, xthl, endl) by Dr. M. Oishi (Public Health Research Institute of the City of New York); and phage G4 by Dr. N. G. Godson (Yale University). Construction of JFS19 [rha, lys, thyA (50 μg/ml), polB, str'/F/ColEI (pLC44-7) thi-, cys-, rha+, rep+] is described in this paper. The rha+ marker on pLC44 7 was detected by Daniel Oppenheim (Stanford University).

Preparation of Colicin

Colicin was prepared from W3110/ColEI cells as described previously (10) except that the excess signal in the salt wash buffer was saturated for 1 min before removing cells and debris by centrifugation. The ammonium sulfate precipitate containing colicin was dissolved in buffer containing 80% (v/v) glycerol to a concentration of 8 x 10^7 units/ml, and stored at -20°C.

Plasmid Transfer to Construct ColEI-rep Hybrid Strains

Plasmid donor and recipient strains were grown to mid-log phase (A<sub>λs</sub> = 0.5) on a shaker at 37°C. During the last 30 min of growth donor strains were removed from the shaker and kept at 37°C for maximum growth of F-pili. Recipient cells (0.2 ml) and donor cells (0.02 ml) were mixed and rotated slowly (40 rpm) at 37°C for 45 min to permit mating to occur. Addition of colicin (0.2 ml of L + Thy broth [described below] containing 2 units/ml of colicin] followed by a 90-min period on a rotary shaker at 37°C, killed cells without ColEI plasmids. A drop of the mixture was plated on L + Thy + methyl methanesulfonate (400 μg/ml) to counterselect MV12 (repA donor), or on minimal medium M63 (11) containing thymine (2 μg/ml) to counterselect RLM365 or JFS19 donors (lys and high thymine (50 μg/ml) required). Plates were allowed to dry, and streaked to yield single colonies. Clones surviving either of the sequences of two counterselections were presumed to be successful recipients of the ColEI and F plasmide. Such clones were verified to be colicin-resistant and then tested for rep' phenotype by spotting G4 phage (10<sup>5</sup> pfu) on a soft-agar layer containing the clone on an L + Thy plate (see below). HF4704 rep3 served as a positive control; HF4704 rep3 (mapping at 83 min on the revised E. coli map (12) containing

* This work was supported in part by grants from the National Institutes of Health and the National Science Foundation. This is Paper 4 in the series "An Enzyme System for the Replication of Duplex Circular DNA: The Replicative Form of Phage φX174." Paper 4 is Ref. 14. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: RF, replicative form DNA; RF', covalently closed, circular superhelical φX DNA; SS, single-stranded DNA; DBP, E. coli DNA binding protein; holoenzyme, DNA polymerase III holoenzyme; SDS, sodium dodecyl sulfate; rep, rep protein; albumin, bovine serum albumin.
Purification of E. coli rep Protein

ColEl/E. coli hybrids with a his+ fragment of lac- fragment (mapping at 44 and 8 min, respectively) served as negative controls.

Media, Growth and Storage of Cells

Cells used for genetic manipulation were grown in L + thy broth. L broth (13) containing 50 µg/ml of thymine. For solid media, 1.5% agar was added for plates, or 0.7% for soft agar. Colicin plates were prepared fresh before use by adding 25 units of colicin per plate in 4 ml L + thy + 1.5% agar (melted and then cooled to 50°C before adding colicin). JFS19 cells for enzyme preparations were grown in 70-liter batches in a 100-liter New Brunswick Fermacel in media containing: per liter: 10 g of yeast extract (Difco or Ardamine 2), 10 g of Cereoloc, 0.05 g of thymine, 0.01 g of thiamin, 1.85 g of KH₂PO₄, 10 g of K,HPO₄, Cereoloc (80%, w/v) and thiamin were sterilized separately and added to the Permeal medium after sterilization and cooling. Seventy liters of media were inoculated with 6 liters of a 14-h culture of cells grown in four 6-liter Erlenmeyer flasks on a rotary shaker at 37°C. Medium used for inocula was the same, except that the yeast extract was exclusively Difco and colicin (1000 units/liter) was added when cultures were started. Each 2-liter culture was inoculated with 0.25 ml of starter culture, which had been grown to stationary phase in L + thy containing 1 unit/ml of colicin, and had been stored at -20°C after addition of an equal volume of sterile glycerol. The pH in the Fermacel was maintained between 6.4/6.7 and 7.1 by addition of 50% NaOH; the level of foam was controlled by adding the minimum possible amount of SAG 471 antifoam (no more than 3 ml/liter). Cells were harvested at an A₅₇₀ = 8 to 10 by centrifugation in a Sharples continuous flow centrifuge. Yields of cell paste were 300 to 400 g/liter, wet weight. Cells were resuspended in Buffer A = 490 in Buffer A (see below) and frozen by pouring them slowly into liquid nitrogen. The frozen pellets were stored at -20°C in waxed cardboard cartons. HMS83 and MO676 cells were grown and stored in the same manner except that no colicin was added to the inocula.

Chemicals

Sources were as follows: Bio-Rex 70, acrylamide, bisacrylamide, and dithiothreitol, Bio-Rad; Tris, Sigma; bovine albumin (crystaline), Miles; thiamin (Grade B), spermidine-Cl (grade A), and calf thymus DNA (grade A), Calbiochem; DEAE-cellulose DE 52, Whatman; Sephadex G-25 Medium, Pharmacia; ammonium sulfate (ultra-pure), sucrase (enzyme grade), and thymine, Schwarz/Mann; [α-³²P]TP and [³²P]dThdTP, New England Nuclear; [³²P]hThdTP, Amersham or Schwarz/Mann; Cereoloc, Corn Products; Ardamine Z (pH 7.5), Bio-Rex 70 (200 to 400 mesh), Na⁺ was converted to H⁺ form with 500 units of &X CLSA protein; 1.5 µg of DBP; 16 units of hDNAase; 500 units of &X 6X cisc protein; 1.5 µg of DBP, 16 units of hDNAase; and rep protein to be assayed. Incubation was at 30°C for 15 min and treated as above. One unit of rep (reaction) activity is defined as 1 pmol of total nucleotide incorporated/min. Although the values were linear over a 10-fold range with the amount of added rep protein, the absolute values varied up to 4-fold depending on the ciscA protein and holoenzyme preparations. For this reason, each set of assays included pure rep protein (47 × 10⁶ units/mg) as a standard.

Preparation of DNA-cellulose

SS calf thymus DNA-cellulose was prepared as described previously (21); it was stored as a dry powder at -20°C. Fines were removed from the powder by suspension and settling in Buffer C containing 50 mM NaCl. The column of adsorbant was equilibrated by washing with several bed volumes of Buffer B containing 100 µM NaCl until the A₂₆₀ value reached that of the buffer.

Preparation of Antibody against rep Protein

Antiserum was raised against rep protein in a 6-month-old virgin female White New Zealand rabbit by administration of one 150-µg and two 100-µg injections of the protein with Freund's complete adjuvant, in the foot pads, at 1-week intervals. Four weeks after the first injection, bleedings of approximately 25 ml from the ear vein were begun and taken at 1-week intervals. Each bleeding was preceded by 4 or 3 days earlier by an injection of 10 µg of rep protein intravenously. Serum from three such bleedings were pooled and the IgG fraction was prepared by precipitation with 40% saturation ammonium sulfate, followed by transfer to 20 mM potassium phosphate buffer, pH 7.0, on a G-25 Sephadex column, and passage through a DE52 DEAE-cellulose column equilibrated with the same buffer.

RESULTS

Construction of ColEI-rep Hybrid Strain which Overproduces rep Enzyme—ColEI/E. coli hybrid plasmids from the

Table I

<table>
<thead>
<tr>
<th>Source of Fraction II</th>
<th>Activity</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO676 (no plasmid)</td>
<td>51,000</td>
<td>19,000</td>
</tr>
<tr>
<td>HMS83 (no plasmid)</td>
<td>85,000</td>
<td>13,000</td>
</tr>
<tr>
<td>JFS19 (ColEI-rep hybrid)</td>
<td>587,000</td>
<td>190,000</td>
</tr>
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</table>

Preparation of Bio-Rex 70

Bio-Rex 70 (200 to 400 mesh), Na⁺ was converted to H⁺ form with HCl, washed with water, equilibrated with 20% (v/v) glycerol, titrated with imidazole base, washed with 20% (v/v) glycerol, and equilibrated with respect to conductivity in Buffer C containing 50 mM NaCl.

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Purification of rep protein from ColEl-rep hybrid cells

<table>
<thead>
<tr>
<th>Step</th>
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<th>Replication assay</th>
<th>ATPase assay</th>
<th>Ratio</th>
<th>ATlase replication</th>
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<tbody>
<tr>
<td></td>
<td>mg</td>
<td>Total activity</td>
<td>Specific activity</td>
<td>Total activity</td>
<td>Specific activity</td>
</tr>
<tr>
<td>I Lysozyme &amp; Spermidine</td>
<td>87,000</td>
<td>90</td>
<td>10</td>
<td>450</td>
<td>52</td>
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<tr>
<td>II Ammonium Sulfate</td>
<td>21,000</td>
<td>78</td>
<td>37</td>
<td>260</td>
<td>120</td>
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<tr>
<td>IIIA DEAE-cellulose I</td>
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<td>48</td>
<td>36</td>
<td>170</td>
<td>130</td>
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<tr>
<td>IIb Bio-Rex 70</td>
<td>580</td>
<td>30</td>
<td>520</td>
<td>75</td>
<td>1300</td>
</tr>
<tr>
<td>IV DNA-cellulose</td>
<td>79</td>
<td>18</td>
<td>2300</td>
<td>54</td>
<td>6800</td>
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<tr>
<td>V DEAE-cellulose II</td>
<td>30</td>
<td>14</td>
<td>4700</td>
<td>29</td>
<td>9700</td>
</tr>
</tbody>
</table>

Replication and ATPase assays were Assays B and C ("Materials and Methods"). Replication assay values were multiplied by 2.5 to correct the Step VI specific activity to that obtained in an optimum reaction I. Extract: Frozen cell suspension (900 to 1500 g) was thawed at 2°C or less (all subsequent operations were at 4°C or less; centrifugations were in a Beckman JA-14 rotor at 0°C) and diluted with Buffer A to $A_{260} = 200 (2 	imes 10^5$ cells/ul). After the pH was adjusted to 8.0 with Tris base, a solution (1/5 volume) containing 0.18 M spermidine·Cl, 50 mM DTT, 50 mM EDTA, and 1.5 M ammonium sulfate (to produce a more tightly compacted pellet of cell debris after centrifugation) and lysozyme dissolved in Buffer A was added (200 μg/ml final concentration). The mixture was stirred, transferred to centrifuge bottles, and kept on ice for 45 min. After warming in a 37°C bath for 4 min with swirling and mixing by inversion at 1-min intervals, the bottles were chilled on ice, and centrifuged for 1 h at 13,000 rpm. Supernatants were decanted and pooled (about 1200 ml/900 to 1200 g cell suspension). Fraction I represents five such preparations pooled after the next step. II. Ammonium Sulfate: Solid ammonium sulfate (220 g/liter) was added slowly to Fraction I. The mixture was stirred 20 min and then centrifuged at 13,000 rpm for 30 min. The pellet was washed by suspension (using a glass tissue homogenizer) in 0.1 of the Fraction I volume of Buffer B containing 0.1 M NaCl and 0.24 g of ammonium sulfate added to each milliliter of buffer and centrifugation at 13,000 rpm for 30 min, frozen in liquid nitrogen, and stored at −70°C for several weeks without loss of activity. Five such pellets representing a total of 4650 g cells were thawed, each dissolved in 0.1 of its respective Fraction I volume of Buffer C, and pooled (Fraction II, 710 ml). III, DEAE-cellulose and BioRex 70: Fraction II was diluted with Buffer D to a conductivity equivalent to Buffer C containing 93 m~ ammonium sulfate (equivalent to 200 mivf NaCl), applied to a 1250-ml DEAE-cellulose column (9 x 23 cm) equilibrated in Buffer C + 400 μM NaCl eluate (Fig. 1A). Peak fractions were pooled and dialyzed for 8 to 10 h against a volume of Buffer B calculated to dilute the pool to the conductivity of Buffer B + 25 mM NaCl. A precipitate, formed during dialysis was removed by centrifugation; its removal from the supernatant (Fraction IV) is essential to eliminate two contaminants which would otherwise persist after Step V. V, DEAE-cellulose: Fraction IV was loaded on a 30-ml DEAE-cellulose column (2.5 x 6.5 cm) equilibrated with Buffer B + 25 mM NaCl. The column was washed with 30 ml of Buffer B + 25 mM NaCl and 90 ml of Buffer B + 50 mM NaCl, then eluted in 15-ml fractions with 790 ml of Buffer B + 75 mM NaCl. The bulk of the activity was eluted as a sharp concentrated peak with the 75 mM NaCl eluant front (Fig. 1C). Pooled peak fractions (Fraction V) frozen in liquid nitrogen and stored at −70°C were stable at least 8 months; however, losses were incurred on freezing and thawing. Fraction V at 0.1 mg of protein/ml or greater was relatively stable at 0°C. Preliminary experiments indicate that the enzyme is stabilized by the presence of 90 to 60% glycerol.7

Of five cya+ plasmids tested, only pLC44-7, known to carry both $i v^+$ and $c y a^+$ markers, complemented the chromosomal $r e p^-$ deficiency. The plating efficiency of G4 phase on HP704 rep3/F'/pLC44-7 was the same as on rep+ HP704 in a standard plaque assay (data not shown). The pLC44-7 plasmid was then transferred from the MV12/F+ host by F-mediated transduction to RLM365, a polA+, lac+, rep+ derivative of HSM83 (see "Materials and Methods") in order to place the plasmid in a polA- host which is capable of supporting ColEl replication and also lyses well. The strain JFS19, so derived, which is a derivative of HMS83 (see "Materials and Methods"). Rep protein activity was measured (see "Materials and Methods"). Rep protein activity was 7- to 10-fold greater in extracts of JFS19, the plasmid-carrying strain, than in extracts from HSM83 or MO676 (Table I).

Purification of rep Protein from Strain JFS19 – A procedure was devised which yielded 30 mg of rep protein from 4650 g of strain JFS19 cells, with an overall recovery of 15% (Table II) (Fig. 1). A soluble protein extract was prepared by gentle heat lysis after treating the cells with lysozyme. The cell debris was removed by centrifugation in the presence of spermidine and 150 mM ammonium sulfate in the lysate. The proteins were then fractionated by ammonium sulfate precipitation, and residual nucleic acid fragments were removed by passage of the protein fraction containing the bulk of the rep protein activity through DEAE-cellulose. The proteins were further fractionated by chromatography on Bio-Rex 70. A nearly pure preparation of rep protein was achieved by (i) dialysis of the DNA-cellulose fraction against a buffer containing 25 mM NaCl; (ii) removal of an insoluble precipitate which

Purification of E. coli rep Protein

Purification of rep Protein of Strain HMS83

**Fig. 1.** Successive steps in the chromatographic purification of rep protein. A, Bio-Rex 70. The abscissa is linear with respect to volume; each point represents a single fraction. Fractions were of the following volumes: [1] 380 ml; [2] 3450 ml; [3] 1250 ml; [4 to 8 and 18 to 20] 625 ml; and [9 to 17] 125 ml. The position of the symbols (---) indicates the center of the fraction. Rep protein activity was determined by Assay B ("Materials and Methods"). The fractions in which each of the salt fronts appear are marked with arrows and the appropriate NaCl concentration of the step. B, DNA-cellulose. The abscissa is linear with respect to volume. Fraction volumes were as follows: [1] 225 ml; [2] 300 ml; [3 to 15] 45 ml. The position of the symbols indicates the centers of the fractions and the ordinate is indicative of the total quantity of activity and protein in the fractions. The assays and symbols are as in A. C, DEAE-cellulose. The abscissa is linear with respect to volume. All fractions were 15 ml. The assays and symbols are as in A with the addition of rep ATPase activity (---) determined by Assay C ("Materials and Methods").

**Fig. 2.** SDS-polyacrylamide gel electrophoresis of rep protein. The slab gel contained 6% acrylamide, 0.16% bisacrylamide, 0.1% SDS. The gel was run in a Tris-glycine system containing 0.1% SDS in the upper tray buffer. Samples were treated with 1% (v/v) 2-mercaptoethanol in Tris-Cl, pH 6.8, at room temperature for 10 min and at 100°C for 2 min before loading on the gel. Track A contained 5 µg each of the following markers: phosphorylase a (phos a) (92,500 daltons); albumin (BSA) (66,000 daltons); and 4X F protein (46,000 daltons). Track B contained 5 µg of Fraction V rep protein. Other markers run in parallel tracks not shown were E. coli RNA polymerase BB' and σ subunits (155,000, 135,000, and 90,000 daltons, respectively) and DNA polymerase I (pol I) (110,000 daltons).
inhibition of the uncoupled ATPase activity observed using Assay C (based on ATP hydrolysis by rep in the presence of δX SS DNA effector). This result provides yet another example of the special characteristics of rep protein at the replication fork when compared with the uncoupled ATPase reaction using single-stranded DNA effector (see succeeding paper (9)).

Amino Acid Analysis of rep Protein — Samples of rep protein were subjected to amino acid analysis (Table III). The predominant residues in the protein are glutamic and aspartic acids (including glutamine and asparagine) and leucine. No residue appears to be absent or present in a single copy in the polypeptide, nor are any other striking features apparent. A comparable sample of rep protein was also analyzed by Dr. Marian Koshland at the University of California, Berkeley. The two analyses were in good general agreement, and both included indications of possible modified half-cystine residues present. Such residues have not as yet been positively identified.

DISCUSSION

To provide a better source of rep activity for purification of the enzyme, a bacterial strain was constructed which contained several copies of rep gene in CoElIE. coli hybrid plasmids. This strain provided a 7- to 10-fold increase in production of rep protein compared to the standard strain. Its use, coupled with improved yields from the early steps of the purification procedure, have enabled us to prepare pure rep enzyme in quantities sufficient for future physical studies.

The rep protein has a polypeptide molecular weight of 65,000 when purified from either the overproducing strain (JFS19) or from the standard strain (HMS83) used routinely in this laboratory. Sedimentation of rep in a glycerol gradient (20) yielded an apparent native molecular weight of 85,000 to 90,000. This value while higher than expected for a monomer, falls short of the value for a globular dimer. Cross-linking studies to determine whether rep protein is oligomeric have thus far been equivocal.

The rep protein was first partially purified on the basis of its ability to complement rep extracts for in vitro replication of δX RF (18). Subsequently, the partially purified rep protein was assayed by its complementation of three other purified proteins and RF I as template in a reconstituted reaction for the extensive net synthesis of δX viral (+) circles (19, 20). This assay was later applied for the entire purification procedure of rep protein. The pure enzyme obtained by this procedure fully complements rep extracts for δX RF replication. Thus, rep protein is the only required component absent from such deficient extracts, and is, therefore, what was cautiously termed the rep-dependent protein (18). That rep protein is in fact the product of the rep gene is further substantiated by overproduction of the enzyme by the E. coli strain bearing extra copies of a fragment of DNA mapping at the rep genetic locus contained in the CoElIE. coli hybrid plasmid.

The rep protein shown previously to be an ATPase dependent on single-stranded DNA (20) is a member of a growing family of such E. coli enzymes. These include: a 75,000-dalton protein (23–25), dnaB protein (26, 27), protein Y required for replication of δX SS to RF (28), a 180,000-dalton DNA unwinding enzyme I (29, 30), rec B-C nuclease (31), type I restriction endonucleases (e.g. EcoK12, EcoB, EcoP1) (32), and T-phage-induced enzymes; the latter include a T4-induced 50,000-dalton protein (33), the T4 gene 44-62 and 45 products needed in replication (34) and the T7 gene 4 protein with primase and strand-separation functions (35, 36). Among these, the 75,000-dalton protein bears the closest resemblance to rep protein in its physical features and ATPase properties. However, the denatured polypeptide size is clearly larger than that of rep protein; the strand-separating activity associated with it has not been shown to be catalytic or interactive with cistron A protein, or to be the product of the rep gene (24, 25). Within the family of single-stranded DNA-dependent ATPases, it is impressive that most are known to effect strand separation of duplex DNA or have a role in DNA replication. Instances of ATPases, as those found in eukaryotic virus cores (37), may prove to have such replicative functions upon further study.

Among general mechanisms that may be considered for coupling energy of ATP hydrolysis to the catalytic separation of DNA strands by rep protein, one is distributive (nonprocessive), and the other is processive. In a distributive mechanism (Fig. 3) a rep-ATP complex binds at the opening of a DNA fork causing the melting of a base pair (I); hydrolysis of ATP (II) enables rep-ADP to dissociate from the fork and DNA binding protein to attach to it (III) thus keeping the strands separated; rep-ATP is regenerated (IV) by a displacement of ADP from rep by ATP.

In a processive mechanism (Fig. 4), rep protein remains bound at the unzipping fork. Once the ternary complex of rep, ATP, and DNA fork is formed (I), hydrolysis of ATP and melting of a base pair ensues (II), followed by attachment of DBP (III), and a replacement of ADP by ATP (IV).

The stoichiometries in both schemes remain vague. Measurements of ATP hydrolysis during replication of δX RF clearly indicate 2 molecules of ATP (or dATP) consumed for each nucleotide polymerized and, by inference, each base pair melted (9). However the number of rep protein subunits involved is not known and the binding of a DBP tetramer is

Table III

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>moles/65,000 g</th>
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<tbody>
<tr>
<td>Alanine</td>
<td>46.2</td>
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<tr>
<td>Arginine</td>
<td>41.9</td>
</tr>
<tr>
<td>Aspartic acid</td>
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<tr>
<td>Glutamic acid</td>
<td>80.7</td>
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<tr>
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<tr>
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<tr>
<td>Valine</td>
<td>20.7</td>
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</table>

M. Koshland, personal communication.
complicated by the fact that it occupies a DNA stretch of about 40 nucleotides (17).

In a choice between these two types of mechanisms, the processive kind seems far more attractive. A reaction which would likely include cistron A and rep proteins. The preference which rep protein exhibits for a cistron A protein-complexed replicating fork as a DNA effector for ATPase action (9) suggests an active role for rep protein in the maintenance of a highly ordered protein-DNA complex. Further studies are needed to collect the crucial facts required to establish the correct mechanism of the ATP-driven unwinding of the replicating fork.

Acknowledgment – We are grateful for the excellent technical assistance of T. Trobough on the large scale preparation of rep protein.

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Purification of the rep protein of Escherichia coli. An ATPase which separates duplex DNA strands in advance of replication.
J F Scott and A Kornberg


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