IL-3A virus infection of a *Chlorella*-like green alga induces a DNA restriction endonuclease with novel sequence specificity

Yuannan Xia, Dwight E. Burbank, Lothar Uher¹, Dietmar Rabussay¹ and James L. Van Etten

Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722 and ¹Bethesda Research Laboratories, Molecular Biology Research and Development, Life Technologies, Inc., Gaithersburg, MD 20877, USA

Received March 24, 1987; Revised and Accepted June 24, 1987

ABSTRACT

A type II restriction endonuclease, named *QviJI*, was isolated from a eukaryotic *Chlorella*-like green alga infected with the dsDNA containing virus IL-3A. *QviJI* is the first restriction endonuclease to recognize the sequence PuGCPy; *QviJI* cleaves DNA between the G and C. Methylation of the cytosine in PuGCPy sequences prevents cleavage by *QviJI*. *QviJI* cleaved DNA into smaller but defined fragments in the presence of ATP. This "star" activity was stimulated by dithiothreitol and/or S-adenosylmethionine but did not occur under conditions which favor "star" activity of other restriction endonucleases.

INTRODUCTION

We have isolated and partially characterized 30 large polyhedral, dsDNA containing, plaque forming viruses which infect a unicellular, eukaryotic *Chlorella*-like green alga strain NC64A (1, 2, 3, 4). One unusual feature of these viruses, whose genomes are about 300 kbp in size and contain various amounts of methylated bases [0.1 to 47% 5-methyldeoxycytosine (⁵m₅dC) and 0 to 37% N⁶-methyldeoxyadenosine (⁶m₆dA) (3, 5)], is that infection with at least some of them results in the appearance of DNA methyltransferases and type II DNA restriction endonucleases (6, 7, 8). In fact the virus infected alga is the first source of type II DNA restriction endonucleases from a eukaryotic system. The restriction endonucleases *QviAI* and *QviBI* appear after infection with the viruses PBCV-1 and NC-1A, respectively. *QviAI*, like the bacterial restriction endonuclease *MboI*, recognizes the sequence GATC, cleaves DNA 5' to the G, and does not cleave G^mATC sequences (7). *QviBI* is similar to the bacterial restriction endonuclease *HinfI* because it recognizes the sequence GANTC, cleaves DNA between the G and A, and does not cleave G^mANTC sequences (8).

This report describes a different restriction endonuclease,

named CviJI, which appears after infection of the alga with another of these viruses, IL-3A. CviJI is the first restriction endonuclease to recognize the sequence PuGCPy; cleavage occurs between the G and C.

MATERIALS AND METHODS

Growth and infection of the alga.

The production and purification of IL-3A and the other viruses, and the growth of Chlorella strain NC64A on MBBM medium have been described (1, 3, 9). Chlorella cells (1 to 2×10^7 cells/ml) were infected with IL-3A at a multiplicity of infection of 10 and cells were collected by centrifugation at 6 to 7 hr post infection and used immediately or frozen at -80°C .

Enzyme extracts.

Five to 10×10^{10} infected cells were suspended in 15 ml of buffer A [0.01 M Tris-HCl (pH 7.9), 0.01 M 2-mercaptoethanol and 50 $\mu\text{g/ml}$ phenylmethylsulfonyl fluoride] plus 10 gm of 0.3 mm glass beads and homogenized for 80 sec at 4000 rpm in a Bronwill MSK mechanical homogenizer with cooling from a CO_2 tank. The homogenate was centrifuged at $10,000 \times g$ for 20 min^2 and protein was precipitated from the supernatant at 70% saturation with $(\text{NH}_4)_2\text{SO}_4$. The precipitate was dissolved in buffer A and treated with dextran T500 and polyethylene glycol 6000 as described (7, 10). The resulting upper aqueous phase was adjusted to 20% (v/v) glycerol and the sample was dialyzed overnight at 4°C against three changes of buffer B [20 mM Tris-HCl (pH 7.5), 0.5 mM sodium EDTA, 7 mM 2-mercaptoethanol and 10% glycerol]. The dialysate was diluted 5 fold with buffer B and applied to a heparin sepharose (Pharmacia) column; the column was eluted with a 0 to 1.2 M gradient of NaCl in buffer B. CviJI activity was pooled and adjusted to 0.1 M NaCl with buffer C [10 mM KPO_4 (pH 7.4), 0.5 mM EDTA, 7 mM 2-mercaptoethanol, 10% glycerol]. The sample was applied to a phosphocellulose (Whatman P11) column and eluted with a 0.2 to 1.2 M gradient of KCl in buffer C. Pooled CviJI activity was adjusted to 0.1 M KCl with buffer C and applied to a hydroxylapatite (Bio-Gel HTP, Biorad) column. CviJI was eluted with a 0.2 to 1.2 M KPO_4 gradient; the pooled active fractions were dialyzed against buffer B containing 50 mM KCl and 50% glycerol and then stored at -20°C .

Enzyme assays.

Unless noted otherwise, CviJI was assayed at 37°C for 2 hr in 20 μl reaction mixtures containing core buffer [50 mM Tris-HCl

(pH 8.0), 10 mM $MgCl_2$, 50 mM NaCl], 1 μ g substrate DNA and 1 to 3 μ l of enzyme extract². The reaction products were electrophoresed either on 2% agarose gels in 90 mM Tris-HCl, 90 mM boric acid, 2 mM EDTA, pH 8.0 (11) or on 7.5 to 15% linear gradient polyacrylamide gels in 40 mM Tris-acetate, pH 8.3, 20 mM sodium acetate, 2 mM EDTA as described (7, 12). One unit of enzyme is the amount required to completely digest 1 μ g of unmethylated lambda DNA in 1 hr at 37°C in 20 μ l of assay buffer.

Determination of restriction endonuclease cleavage site by DNA sequencing.

Dideoxy chain termination sequencing reactions were conducted on alkali-denatured plasmid pBR322 DNA preannealed with 5'-end-labelled pBR322 SalI site sequencing primer (New England Biolabs). The 15 base sequence 5'-AGTCATGCCCGCGC-3' complementary to the pBR322 region at position 700 to 714 served as a primer for sequencing the DNA in a counterclockwise direction. The oligonucleotide primer was labeled at the 5'-end by the method of McGraw (13) in 10 μ l reaction mixtures containing: 70 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 120 μ C (24 pmol) γ -³²P ATP, 100 ng (20 pmol) of primer DNA and 5 units of T4 polynucleotide kinase (Bethesda Research Labs). The mixture was incubated at 37°C for 60 min and the reaction stopped by adding 20 μ l formamide dye mix (8). The sample was electrophoresed on a 25% polyacrylamide gel at 47 volts/cm (1800 volts total) and the gel was autoradiographed for 2 min on Kodak XAR-5 film. The oligonucleotide primer, identified by autoradiography, was cut out and purified over a NACS prepac column (Bethesda Research Labs). The specific activity of the DNA was about 10⁵ cpm/pmol.

The pBR322 DNA (5 μ g) was denatured with alkali at 30°C for 5 min in 50 μ l of 0.2 M NaOH and 0.2mM sodium EDTA (14). The solution was neutralized with 5 μ l of 2 M ammonium acetate (pH 4.5) and the DNA was precipitated with ethanol, washed, dried and suspended in 20 μ l of water. Five μ l of this solution was mixed with 10 ng of the 5'-³²P-SalI site sequencing primer and the solution was adjusted to 12.5 mM Tris-HCl (pH 8), 12.5 mM $MgCl_2$, and 37 mM NaCl in a total volume of 16 μ l. The mixture was incubated at 35°C for 1 hr, cooled on ice, and 2 μ l of 0.1 M dithiothreitol and 2 units (2 μ l) of the large fragment of DNA polymerase I (Klenow fragment) were added.

Dideoxynucleotide sequencing reactions were carried out as described in the Bethesda Research Lab M13 Cloning and Sequencing

Manual, 1986 edition. DNA primed synthesis products for restriction endonuclease digestions were prepared in the same way as the "A reaction" except that dideoxyATP was omitted. After the chase reaction with unlabeled dATP, the mixture (mixture AA) comprising 14 μ l was heated for 15 min at 65°C. Mixture AA was added to 2 μ l of 10 x core buffer and incubated with 0.4 units CviJI (total volume of 20 μ l) at 37°C for 15 min for partial digestion. The reaction was stopped with 40 μ l of the formamide-dye mixture. The samples were electrophoresed on 8% polyacrylamide/7 M urea sequencing gels adjacent to the products of a set of the dideoxynucleotide DNA sequencing reactions and treated as described previously (8).

DNAs.

Host nuclear and virus DNAs were isolated as described previously (5). Unmethylated pBR322 plasmid DNA was propagated in dam⁻, dcm⁻ E. coli strain GM2163 (15) as described (5). Plasmid pUC19 was propagated in E. coli strain JM83. SV40 DNA (Form I) isolated from BSC-1 monkey cells, ØX174 DNA (Form I), and adenovirus type 2 DNA isolated from HeLa cells were from Bethesda Research Labs.

Other procedures.

The DNAs were treated with methylases (New England Biolabs) or with restriction endonucleases as suggested by the suppliers.

RESULTS

Properties of IL-3A.

A one step growth curve of IL-3A revealed that the virus was first released about 5 hr after infection and virus release was complete at about 10 to 11 hr after infection. Like infection by the virus PBCV-1 (16), IL-3A infection resulted in the degradation of the host nuclear DNA beginning between 1 and 2 hr after infection; IL-3A DNA synthesis also started at about 1 hr after infection (unpublished results).

Restriction endonuclease activity.

Enzyme extracts were prepared from uninfected Chlorella cells and cells at 7 hr after IL-3A infection and assayed for DNA restriction endonuclease activity. Enzyme activity (named CviJI) was detected in extracts from the virus infected cells but not from healthy cells. A single peak of restriction endonuclease activity appeared after chromatography on heparin sepharose, phosphocellulose, and hydroxylapatite columns. CviJI activity

Figure 1. Ability of *Qvi*JI to digest unmethylated pBR322 plasmid DNA. Lane 1 contains untreated pBR322 DNA, lane 2 contains pBR322 DNA after a 2 hr incubation with *Qvi*JI, lane 3 is the same as lane 2 only the enzyme reaction was incubated for 16 hr, lane 4 is the same as lane 3 but incubated an additional 2 hr with a second aliquot of *Qvi*JI, lane 5 is the same as lane 2 but $MgCl_2$ was omitted from the assay, lane 6 is the same as lane 2 except that *Qvi*JI was heated to $65^\circ C$ for 10 min prior to adding it to the DNA. *Qvi*JI was incubated in core buffer for 4 hr, 8 hr and 16 hr (lanes 7, 8, and 9, respectively) at $37^\circ C$ before adding pBR322 DNA and incubated an additional 2 hr.

eluted at 0.6 to 0.8 M NaCl from heparin sepharose, at 0.7 to 0.9 M KCl from phosphocellulose, and at 0.5 to 0.7 M KPO_4 from hydroxylapatite columns (data not shown). The purification steps increased the *Qvi*JI specific activity greater than 100-fold. The specific activity of the purified enzyme was estimated at about 10^5 units/mg of protein and about 2000 units of activity were obtained per liter (2×10^{10} cells) of infected cells.

As shown in Fig. 1 (lane 2) *Qvi*JI cleaved unmethylated pBR322 DNA into many discrete fragments. The size of the DNA fragments did not change upon exhaustive incubation with *Qvi*JI

Figure 2. Ability of *CviJI* to degrade various DNAs. (A) Untreated pBR322 DNA (lane 1), pBR322 DNA treated with *TaqI*, *MboI*, *HinfI*, and *CviJI* (lanes 2 to 5, respectively). (B) Lane 1 contains pBR322 DNA digested with *MboI* as standards, lanes 2 to 6 contain ØX174, SV40, adenovirus, IL-3A, and host *Chlorella* nuclear DNAs, respectively, after digestion with *CviJI*. Lane 7 contains untreated host nuclear DNA.

(Fig. 1, lanes 3 and 4). Thus *CviJI* was essentially free of nonspecific nucleases and cleaved pBR322 DNA at discrete sites.

CviJI required Mg^{++} (Fig. 1, lane 5) for activity. The enzyme was most active in 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, and 50 mM NaCl. The temperature optimum was 37°C. *CviJI* activity was measured after incubating the enzyme in the assay mixture at 37°C for various intervals prior to adding substrate DNA. The enzyme was still active after a 4 hr incubation but much less active after a 16 hr incubation (Fig. 1, lanes 7 to 9). Heating *CviJI* to 65°C for 10 min reduced activity more than 95% (Fig. 1, lane 6). *CviJI* can be stored at -20°C in buffer B containing 50 mM KCl and 50% glycerol for at least 1 year without loss of activity.

Mapping of *CviJI* cleavage sites.

The *CviJI* cleavage products of pBR322, ØX174, SV40 and adenovirus DNAs were examined on 7.5 to 15% linear gradient polyacrylamide gels (Fig. 2). Plasmid pBR322 DNA was cleaved into at least 20 fragments; however, only 5 of these fragments

Figure 3. Effect of HaeIII methylase modification of pUC19 DNA on cleavage by CviJI. Untreated pUC19 DNA (lane 1), HaeIII digested pUC19 DNA (lane 2), CviJI digested pUC19 DNA (lane 3), HaeIII/CviJI digested pUC19 DNA (lane 4), HaeIII digestion of HaeIII methylase treated pUC19 DNA (lane 5), and CviJI digestion of HaeIII methylase treated pUC19 DNA (lane 6). Note: (i) the identical sizes of the DNA fragments in lanes 3 and 4; (ii) the size of the "A" fragment of pUC19 DNA increased after methylation with HaeIII methylase (compare lanes 3 and 6, arrowheads).

were larger than 140 bp (Fig. 2A, lane 5). Comparing the size of these 5 larger pBR322 DNA fragments with those produced by known restriction endonucleases suggested that CviJI had a unique specificity (17, 18). The number and sizes of the cleavage fragments obtained by CviJI digestion of ϕ X174 and SV40 DNAs supported this conclusion (Fig. 2B).

IL-3A virus DNA is resistant to bacterial restriction endonucleases HindIII, ApaI, AluI, HaeII, and HaeIII (data not shown) as well as CviJI (Fig. 2B, lane 5). The base sequence recognized by each of the bacterial enzymes contains a GC sequence; cytosine methylation in this GC sequence prevents activity of each of the enzymes (18, 19). Therefore, we assumed that the recognition and cleavage site of CviJI contained GC.

Figure 4. Effect of AluI methylase modification of pUC19 DNA on cleavage by CviJI. Untreated pUC19 DNA (lane 1), AluI digested pUC19 DNA (lane 2), CviJI digested pUC19 DNA (lane 3), AluI/CviJI digested pUC19 DNA (lane 4), AluI digested AluI methylase treated pUC19 DNA (lane 5), and CviJI digested AluI methylase treated pUC19 DNA (lane 6). Note: (i) the identical sizes of the DNA fragments in lanes 3 and 4; (ii) the size of the CviJI "B" fragment of pUC19 DNA increased after methylation with AluI methylase (compare lanes 3 and 6, arrowheads).

This assumption led to the successful identification of CviJI cleavage sites on pUC19 DNA. One of the CviJI cleavage sites was located by digesting pUC19 DNA with CviJI and/or HaeIII; HaeIII recognizes the sequence GGCC. As shown in Fig. 3, the CviJI "A" fragment (lane 3) and CviJI/HaeIII "A" fragment (lane 4) are identical in size to the 587 bp HaeIII "A" fragment (lane 2) [located at bases 2088 to 2675 (20)]. Methylation of pUC19 DNA with M.HaeIII prior to CviJI digestion gave a 680 bp fragment in place of the 587 bp fragment (Fig. 3, compare lanes 3 and 6). These results suggest that CviJI recognizes and cleaves GGCC sequences.

Another CviJI cleavage site was determined by digesting pUC19 DNA with CviJI and AluI. As shown in Fig. 4 (lanes 3 and

4), DNA digested with both enzymes or with CviJI alone produced identical fragments. DNA digested with either AluI or CviJI alone (lanes 2 and 3) produced some overlapping fragments. For example, there are regions of overlap between the 679 bp AluI "A" fragment (located at bases 2050 to 43) and CviJI "A" fragment (about 580 bp) and the 521 bp AluI "B" fragment (located at bases 1366 to 1887) and CviJI "B" fragment (about 370 bp). Methylation of the DNA with M.AluI followed by CviJI digestion gave a 400 bp fragment in place of a 370 bp fragment (Fig. 4, compare lanes 3 and 6). This implied that M.AluI, which methylates cytosines in AGCT sequences, protects one end of the 370 bp CviJI "B" fragment from digestion by CviJI and we conclude that CviJI also cleaves the sequence AGCT. To determine the location of the AGCT site on the 370 bp CviJI "B" fragment the DNA was double digested with either CviJI/MboII or CviJI/BglI. pUC19 DNA contains two MboII sites, located at bases 1456 and 1547, and one BglI site, located at base 1813. The CviJI "B" fragment contained 2 MboII sites and no BglI site (data not shown) and so the CviJI "B" fragment is located between bases 1366 and 1740 and contains an AGCT site at base 1366.

The ends of the 400 bp CviJI "B" fragment of M.AluI methylated pUC19 DNA should then be near positions 1340 and 1740. These regions were examined and the two sequences AGCC at 1337 and GGCT at 1729 were identified. This allowed us to propose that CviJI recognized (A/G)GC(C/T) sequences.

A computer search was conducted to determine the number and locations of (A/G)GC(C/T) sequences in several DNAs. The computer derived number of CviJI cleavage sites were 72 for pBR322 DNA, 69 for ØX174 DNA, 88 for SV40 DNA, and 692 for lambda DNA. Fragments of 587, 363, 258, and 199 bases were the four largest fragments predicted from the location of the CviJI cleavage sites in pBR322 DNA. The predicted sizes of these fragments matched the experimental data (Fig. 2A, lane 5). Likewise, the predicted sizes of pUC19 and ØX174 fragments agreed perfectly with the experimental determined sizes.

Determination of CviJI cleavage site.

The cleavage sites were confirmed by sequencing a stretch of pBR322 DNA and determining the position of the fragments generated by CviJI. Plasmid pBR322 DNA contains all four subsets of the sequence (A/G)GC(C/T), i.e. AGCT, AGCC, GGCC, and GGCT, within 120 bp of the SalI site (at position 651). A 5'-end

Figure 5. *Cvi*JI cleavage sites of pBR322 DNA. A stretch of pBR322 DNA was sequenced in counterclockwise direction by the dideoxy sequencing method using a 15-base 5'-end labeled pBR322 *Sal*I site sequencing primer annealed to linearized alkali-denatured pBR322 DNA at position 700 to 714 (lanes A,C,G,T). 32 P-labeled pBR322 DNA prepared analogously to the sample in the "A" lane (except that dideoxy ATP was omitted) was partially digested with *Cvi*JI (lanes N). In gel 1 the bromophenol blue marker migrated 30 cm; gel 2 is the same as gel 1 but bromophenol blue migrated 50 cm. The bases recognized by *Cvi*JI are indicated by the bars. The bands in lanes N which coincided with the G where cleavage occurs are indicated by an arrowhead.

labeled pBR322 *Sal*I site primer was extended through and beyond the four suspected *Cvi*JI sites. The extended oligonucleotides were partially digested with *Cvi*JI under conditions which yielded all possible complete and partial products. Four products with increasing sizes co-migrated with bands in the G channel of the sequencing reactions (Fig. 5). Thus *Cvi*JI cleaved DNA between G and C in all four sequences AGCT, GGCT, AGCC, and GGCC and created restriction fragments with blunt ends. The intensity of the G band at the AGCC site was less than at the other 3 sites which may reflect reduced activity at this site. Two additional observations indicate that *Cvi*JI produces fragments with blunt ends. (i) Conditions for ligating blunt-ended fragments were

Figure 6. Effect of ATP on *CviJI* specificity. Lane 1 contains untreated pUC19 DNA, lane 2 is pUC19 DNA after a 2 hr incubation with *CviJI* under standard conditions, lane 3 is the same as lane 2 except that the assay mixture contained 2 mM ATP and 20 mM dithiothreitol, lane 4 is the same as lane 3 except that the reaction time was 16 hr, and lane 5 is the same as lane 4 but with an additional 2 hr incubation after adding a second aliquot of *CviJI*.

required to ligate *CviJI* restriction fragments (data not shown). (ii) No end filling was observed with *CviJI* restriction fragments on the sequencing gel (Fig. 5, lane N). This indicates that *CviJI* creates blunt or 5' recessed ends (7). Thus, the *CviJI* recognition and cleavage site is (A/G)G↓C(C/T).

ATP influences specificity of *CviJI*.

Blunt end ligation of *CviJI* restriction fragments, followed by recleavage with *CviJI*, produced more fragments than expected. This led to the surprising finding that the size of the *CviJI* restriction fragments was affected by ATP. That is, *CviJI* cleaved pUC19 DNA into smaller but defined fragments when 1 to 2 mM ATP was present in the assay buffer (Fig. 6, compare lanes 2 and 3). The reaction was stimulated by reducing agents such as

Figure 7. Time course of pUC19 DNA digestion by *Cvi*JI under standard and "star" conditions. Lane 1 contains untreated pUC19 DNA, lanes 2 and 8, 3 and 9, 4 and 10, 5 and 11, 6 and 12, and 7 and 13 are DNAs after incubation with *Cvi*JI for 1, 5, 10, 30, 60, and 120 min, respectively. The DNAs in lanes 2 to 7 were incubated with *Cvi*JI under standard conditions and in lanes 8 to 13 under "star" conditions (see legend of Fig. 6). The reactions were stopped by heating at 65 C for 10 min.

dithiothreitol or 2-mercaptoethanol and/or S-adenosylmethionine. Adenosine-5'-O (1-thiotriphosphate) (ATP- α -S), adenosine-5'-O (3-thiotriphosphate) (ATP- γ -S), dATP, and ADP substituted, albeit poorly, for ATP whereas, GTP, CTP, UTP, and non hydrolyzable analogues adenylyl-imido-diphosphate (AMP-PNP) and adenylyl (β , γ -methylene)-diphosphonate (AMP-PCP) did not.

Reaction conditions influence substrate specificity of several bacterial type II restriction endonucleases. Altered activities are referred to as "star (*) activity" (21, 22). By analogy the ATP-requiring specificity of *Cvi*JI is referred to as *Cvi*JI*. However, *Cvi*JI* activity is not observed under conditions which favor "star" activity of other restriction endonucleases; i.e. products of *Cvi*JI* activity were not obtained by adding glycerol [up to 30% (v/v)] or NaCl (up to 0.15 M) to the reaction mixture, varying the pH of the reaction mixture from 6 to 9, or replacing Mg^{++} with Mn^{++} or Zn^{++} (data not shown).

The optimum conditions for *Cvi*JI* activity were the same as for *Cvi*JI except that 1 mM ATP, 20 mM dithiothreitol, and 100 μ M S-adenosylmethionine were added to the core buffer. *Cvi*JI*

produced the same cleavage fragments if pUC19 DNA was incubated with the enzyme for 2 hr, 16 hr, or if the DNA was treated with a second aliquot of enzyme for 2 additional hrs after a 16 hr incubation (Fig. 6, lanes 3 to 5). Thus, like *Cvi*JI, *Cvi*JI* cleaved pUC19 DNA at a limited number of sites. *Cvi*JI* does not cleave IL-3A DNA (data not shown); therefore, the presence of m⁵dC in the sequence PuGCPy inhibits its activity.

Several reasons suggest that *Cvi*JI and *Cvi*JI* activities result from the same protein. First, *Cvi*JI and *Cvi*JI* activities co-eluted from heparin sepharose, phosphocellulose, and hydroxylapatite columns. Second, comparison of the time courses of *Cvi*JI and *Cvi*JI* digestion indicates that *Cvi*JI* cleaves at the same sites as *Cvi*JI as well as at additional sites. As seen in Fig. 7, digestion of pUC19 DNA by *Cvi*JI was still incomplete after a 10 min incubation (lane 4) but was complete after 30 min (lane 5). However, digestion under "star" conditions produced an almost complete *Cvi*JI cleavage pattern within 5 min of incubation (Fig. 7, lane 9). With longer incubation times the pattern shifted towards the complete *Cvi*JI* pedigree (Fig. 7, lanes 10 to 13). If pUC19 DNA was digested first under standard *Cvi*JI conditions and then used as a substrate for *Cvi*JI*, the final cleavage pattern was identical to that of pUC19 DNA cleaved directly by *Cvi*JI* (data not shown). These results indicate that *Cvi*JI and *Cvi*JI* share common cleavage sites and that *Cvi*JI cleavage sites are more susceptible to *Cvi*JI* cleavage than the *Cvi*JI* specific sites.

DISCUSSION

This report establishes that IL-3A infection of *Chlorella* strain NC64A induces the synthesis of a DNA restriction endonuclease, *Cvi*JI, which recognizes the sequence PuGCPy and cleaves between the G and C; *Cvi*JI does not cleave PuG^mCPy sequences. *Cvi*JI is the first DNA restriction endonuclease to recognize this site (17, 18). *Cvi*JI should cleave DNAs more frequently than any known restriction endonuclease since, it recognizes on a probability basis, the equivalent of a three base sequence.

IL-3A is one of six viruses that we have grouped in class 4 which infect *Chlorella* NC64A (3). Enzyme extracts prepared from *Chlorella* cells infected with each of these viruses (i.e. CA-1A, CA-2A, IL-2A, IL-2B, and IL-3D) cleaved pBR322 DNA into the same

size fragments as CviJI and all exhibited star activity under the conditions described above (unpublished data). In each case the restriction endonuclease cleaves host DNA in vitro but not the virus DNA.

The function of these restriction endonucleases has not been established unequivocally. However, circumstantial evidence suggests that these enzymes are involved in the breakdown of host DNA and might thus facilitate virus replication (7, 8). If this is true, these viral enzymes would serve a function exactly opposite of what is considered the main function of bacterial type II restriction endonucleases, namely the prevention of successful infection by bacterial viruses. In this sense the Chlorella virus-induced restriction endonucleases would function as "facilitating" rather than "restriction" enzymes.

Restriction endonucleases are classified into three types based on their cofactor requirements and other criteria. Type I enzymes, such as EcoK and EcoB, have stringent requirements for ATP, S-adenosylmethionine and Mg^{++} (23, 24, 25). Type II enzymes such as EcoRI and HindIII, require only Mg^{++} for activity (22). Type III enzymes, such as EcoPI and EcoP15, require only ATP and Mg^{++} for activity; however, S-adenosylmethionine stimulates activity (25, 26, 27). In the absence of ATP, CviJI cleaves DNA at all PuGCPy sites and only requires Mg^{++} for activity. Thus, CviJI functions as a typical type II restriction endonuclease. However, CviJI obviously recognizes and cleaves a smaller base sequence when ATP is included in the reaction mixture. Assuming that CviJI and CviJI* activities are associated with the same protein, the CviJI* activity differs from "star" activities of other type II restriction endonucleases (22). First, CviJI* activity does not occur under conditions which favor "star" activity of other restriction endonucleases, e.g. high enzyme concentration, addition of glycerol, variations of pH and ionic strength, or replacement of Mg^{++} with Mn^{++} . Second, CviJI* activity absolutely requires ATP; related compounds such as ADP, dATP, ATP- α -S, ATP- γ -S induce some star activity whereas, GTP, CTP, UTP, AMP-PNP, and AMP-PCP do not. CviJI* activity is stimulated by reducing agents and S-adenosylmethionine; these compounds by themselves do not induce "star" activity. Therefore, CviJI* resembles a type III restriction enzyme in respect to its dependence on Mg^{++} and ATP and its stimulation by S-adenosylmethionine.

The role of ATP in the CviJI⁺ activity is also not clear. It will be interesting to determine if ATP changes the conformation of CviJI as it does with type I and type III restriction endonucleases and if CviJI has ATPase activity like Type I restriction endonucleases. The successful purification of CviJI will help to answer these questions.

ACKNOWLEDGMENTS

We thank Robert Blakesley, Russel Meints, Les Lane, and Myron Brakke for many helpful discussions. This investigation was supported, in part, by Public Health Service grant GM-32441 from the National Institute of General Medicine and grant DE-AC02-82ER12086 from the Department of Energy.

Published with the approval of the Director as paper no. 8247, Journal Series, Nebraska Agricultural Research Division.

REFERENCES

1. Van Etten, J.L., Burbank, D.E., Schuster, A.M. and Meints, R.H. (1985) *Virology* 140, 135-143.
2. Meints, R.H., Schuster, A.M. and Van Etten, J.L. (1985) *Plant Mol. Biol. Rept.* 3, 180-187.
3. Schuster, A.M., Burbank, D.E., Meister, B., Skrdla, M.P., Meints, R.H., Hattman, S., Swinton, D. and Van Etten, J.L. (1986) *Virology* 150, 170-177.
4. Van Etten, J.L., Xia, Y. and Meints, R.H. (1987) in *Plant-Microbe Interaction*, Kosuge, T. and Nester, E.W. Eds., Vol II. pp. 307-325, Macmillan Publishing Co., New York.
5. Van Etten, J.L., Schuster, A.M., Girton, L., Burbank, D.E., Swinton, D. and Hattman, S. (1985) *Nucl. Acid. Res.* 13, 3471-3478.
6. Xia, Y. and Van Etten, J.L. (1986) *Mol. Cell Biol.* 6, 1440-1445.
7. Xia, Y., Burbank, D.E., Uher, L., Rabussay, D. and Van Etten, J.L. (1986) *Mol. Cell. Biol.* 6, 1430-1439.
8. Xia, Y., Burbank, D.E. and Van Etten, J.L. (1986) *Nucl. Acid. Res.* 14, 6017-6030.
9. Van Etten, J.L., Burbank, D.E., Xia, Y. and Meints, R.H. (1983) *Virology* 126, 117-125.
10. Schleif, R. (1980) *Methods in Enzymology*, Grossman, L. and Moldave, K. Eds., Vol. 65, pp. 19-23, Academic Press, New York.
11. Peacock, A.C. and Dingman, C.W. (1967) *Biochemistry* 6, 1818-1827.
12. Jeppesen, P.G.N. (1980) *Methods in Enzymology*, Grossman, L. and Moldave, K. Eds., Vol. 65, pp. 305-319, Academic Press, New York.
13. McGraw, R.A. (1984) *Anal. Biochem.* 143, 298-303.
14. Chen, E.Y. and Seeburg, P.H. (1985) *DNA* 4, 165-170.
15. Marinus, M.G., Carraway, M., Frey, A.Z., Brown, L. and Arraj, J.A. (1983) *Mol. Gen. Genet.* 192, 288-289.

16. Van Etten, J.L., Burbank, D.E., Joshi, J. and Meints, R.H. (1984) *Virology* 134, 443-449.
17. Roberts, R.J. (1985) *Nucl. Acid. Res.* 13, r165-r200.
18. Kessler, C., Neumaier, T.S. and Wolf, W. (1985) *Gene* 33, 1-102.
19. McClelland, M. and Nelson, M. (1985) *Nucl. Acid. Res.* 13, r201-r207.
20. Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene* 33, 103-119.
21. Polisky, B., Greene, P., Garfin, D.E., McCarthy, B.J., Goodman, H.M. and Boyer, H.W. (1975) *Proc. Natl. Acad. Sci. USA* 72, 3310-3314.
22. Modrich, P. and Roberts, R.J. (1982) in *Nucleases*, Linn, S.M. and Roberts, R.J. Eds. pp. 109-154, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. Boyer, H., Scibienski, E., Slocum, H. and Roulland-Dussoix, D. (1971) *Virology* 46, 703-710.
24. Bickle, T.A. (1982) in *Nucleases*, Linn, S.M. and Roberts, R.J. Eds. pp. 85-108, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. Yuan, R. and Hamilton, D.L. (1984) in *DNA Methylation - Biochemistry and Biological Significance*, Razin, A., Cedar, H. and Riggs, A.D. Eds, pp. 11-38, Springer-Verlag, New York.
26. Kauc, L. and Piekarowicz, A. (1978) *Eur. J. Biochem.* 92, 417-426.
27. Bachi, B., Reiser, J. and Pirrotta, V. (1979) *J. Mol. Biol.* 128, 143-163.