The cleavage site of the Rsal isoschizomer, Cvill, is GITAC

Yuannan Xia, Kenneth E. Narva and James L. Van Etten

Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722, USA
Submitted October 5, 1987

Infection of the green alga, Chlorella NC64A, with the dsDNA virus NY-2A results in the synthesis of a restriction endonuclease Cvill. Chlorella cells infected with NY-2A (m.o.i. of 10) were collected by centrifugation at 12 hr p.i. (1). Enzyme extracts were prepared and chromatographed on phosphocellulose as described for the restriction endonuclease CviJI (2). Cvill, eluted at 0.65 M KCl was rechromatographed on phosphocellulose, dialyzed against buffer B (2) containing 50 mM KCl and stored at -20°C. Cvill was assayed at 25°C in freshly made 6 mM Tris-HCl (pH 8.0), 8 mM MgCl2, 50 mM NaCl, 6 mM 2-mercaptoethanol, and 100 µg/ml bovine serum albumin.

Cvill and Rsal cleaved pUC19 DNA (A) as well as many Chlorella virus DNAs and phages øX174, lambda, and Xp12 DNAs into the same size fragments. Neither enzyme cleaved DNA from the Chlorella viruses NY-2A or CA-4A. Thus Cvill, like Rsal, cleaved GTAC and GTA'T sequences but not GTmAC sequences.

The cleavage sites of Cvill and Rsal were compared as follows (B). M13mp19 DNA was used as a template in a primer extension reaction (3) to generate radiolabeled synthesis products which extended past the GTAC site in the polylinker region. Synthesis reactions were phenol extracted (-Klenow) or heated to 65°C (+Klenow), digested with either Cvill or Rsal, and electrophoresed on a sequencing gel next to a set of similarly -primed dideoxy sequencing reactions. Rsal cleaves between the T and A (4) whereas Cvill cleaves between the G and T [(-) Klenow]. End-filling of the cleavage products [(+) Klenow] confirmed that Cvill generated a 2-base 5’ protruding end. Bacterial alkaline phosphatase treatment inhibited ligation of Cvill restriction fragments suggesting that Cvill generates 5’-phosphate termini. Thus the recognition sequence and cleavage site for Cvill is 5’GTAC 3’; Cvill is the first restriction endonuclease to cleave at this position.

REFERENCES