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The cleavage site of the restriction endonuclease Ava II

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ABSTRACT

We have determined that the type II restriction enzyme Ava II, isolated from Anabaena variabilis, recognizes and cuts the sequence

5' - G↓GTCC - 3'
3' - CCAG↑G - 5'

The eight Ava II sites of pBR322 have been mapped, as well as a unique site for Ava I.

INTRODUCTION

The advent of restriction enzymes as tools for in vitro manipulation of DNA has changed the face of molecular biology. The enzymes are crucial to recombinant DNA techniques, isolation and characterization of unique pieces of DNA, and direct DNA sequencing. Continued advancement of the field relies on bolstering the supply of enzymes with different sequence specificities.

The restriction endonuclease Ava II from Anabaena variabilis was isolated and described by Murray, Hughes, Brown, and Bruce[1]. Their attempt to characterize the cleavage site was hampered because they chose to analyze in bulk all 5' products of enzyme digestion. They suggest that Ava II recognizes and cleaves at $G\{^{A}_{T}\}C\downarrow G\{^{A}_{T}\}C$. Methods for the bulk analysis of the ends produced by restriction enzyme cleavage of DNA have been recently reviewed[2]. These methods never examine a unique restriction site. In some instances the data from these techniques can be misleading, and in other cases (those in which enzymes cut remotely from their restrction sequences) it may be impossible to deduce the site using these techniques. Methods which alleviate these difficulties by analyzing isolated cleavage sites have

been suggested[3-5].

The complete nucleotide sequence of the phage ϕx174[6] and the plasmid pBR322[7] are a boon to the process of solving restriction enzyme cleavage sites. Not only can restriction sites be easily mapped by the analysis of double digestion patterns, but intelligent choices of restriction fragments for examining these sites can be made. In this paper we analyze eight isolated sites for the restriction enzyme Ava II which are found on the plasmid pBR322 and conclude that Ava II cleaves the sequence $G\downarrow G^{A}_{T}CC$.

MATERIALS AND METHODS

Enzymes: Ava I and Ava II were obtained from Bethesda Research Laboratories, Inc., Rockville, Md. 20850; and Ava II also from New England Biolabs, Inc., Beverly, Ma. 01915. Alu I, Hin fl, Hae III, Hpa II, and polynucleotide kinase were gifts from U. Siebenlist, D. Hourcade, P. Farabaugh, R. Tizard, and W. McClure, respectively.

DNA: Plasmid pBR322 was prepared by chloramphenicol amplification[8] in host E. coli RR1. Sequencing was performed using the method of Maxam and Gilbert[9].

RESULTS

When incubated with pBR322 DNA, Ava II generates eight fragments (Fig. 1). We localized these eight sites by analyzing patterns produced by double digestion with Ava II and various other enzymes (Alu I, Hin fl, Hae III, and Hpa II) (Fig. 1). Ava II sites are correlated with the pBR322 restriction map for the other enzymes[10] by locating the eight Ava II sites as shown in Fig. 2. For example, from Fig. 1, we see that the fifth largest Hpa II band is absent from the Hpa II - Ava II double digest; it must, therefore, contain an Ava II site. The largest Hae III fragment appears intact, but the sixth largest fragment is absent from the Hae III -Ava II double digest so it must contain the same Ava II site. By correlating these observations with the known pBR322 restriction map[10] (Fig. 2), we can locate this Ava II site in the small region in which these Hpa II and Hae III fragments overlap. More precise mapping was

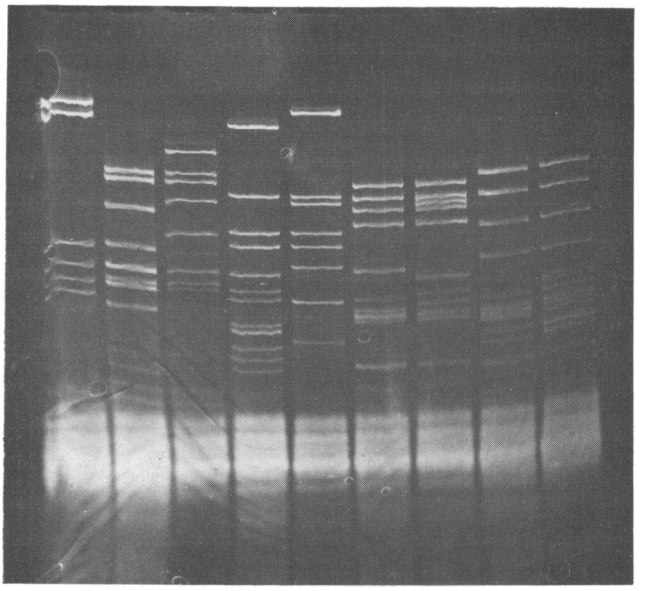

Figure 1. This gel (4% Acrylamide, 0.133% Bis-acrylamide, 50 mM Tris-borate pH 8.3, 1 mM EDTA) displays a variety of restriction digests of pBR322. Tracks A,C,E,G and I are single digests by, respectively, Ava II, Alu I, Hin fl, Hae III and Hpa II. Tracks B,D,F and H are double digests by Ava II and, respectively, Alu I, Hin fl, Hae III and Hpa II. The DNA was stained with ethidium bromide and visualized with ultraviolet irradiation.(13) The densely stained material in all tracks near the bottom of the gel is RNA which was not removed from this particular DNA sample.

achieved by using other enzymes and by accounting for new bands which appear in double digests. The final assignment for all eight Ava II sites is consistent with all single and double digestion patterns we have observed.

We examined several of these sites at the nucleotide sequence level by a technique described by McConnell, Searcy and Sutcliffe[4]. This method allows the direct visualization of an isolated sequence which the enzyme cuts. A singly end-labelled restriction fragment (fragment A) generated by an enzyme of known specificity is cut with the enzyme of unknown specificity. The radioactive product of this digestion (fragment B) is displayed by electrophoresis in a slot adjacent to a Maxam-Gilbert sequence ladder of fragment A. Thus fragment B is sized against the partial chemical cleavage products of DNA of exactly the same se-

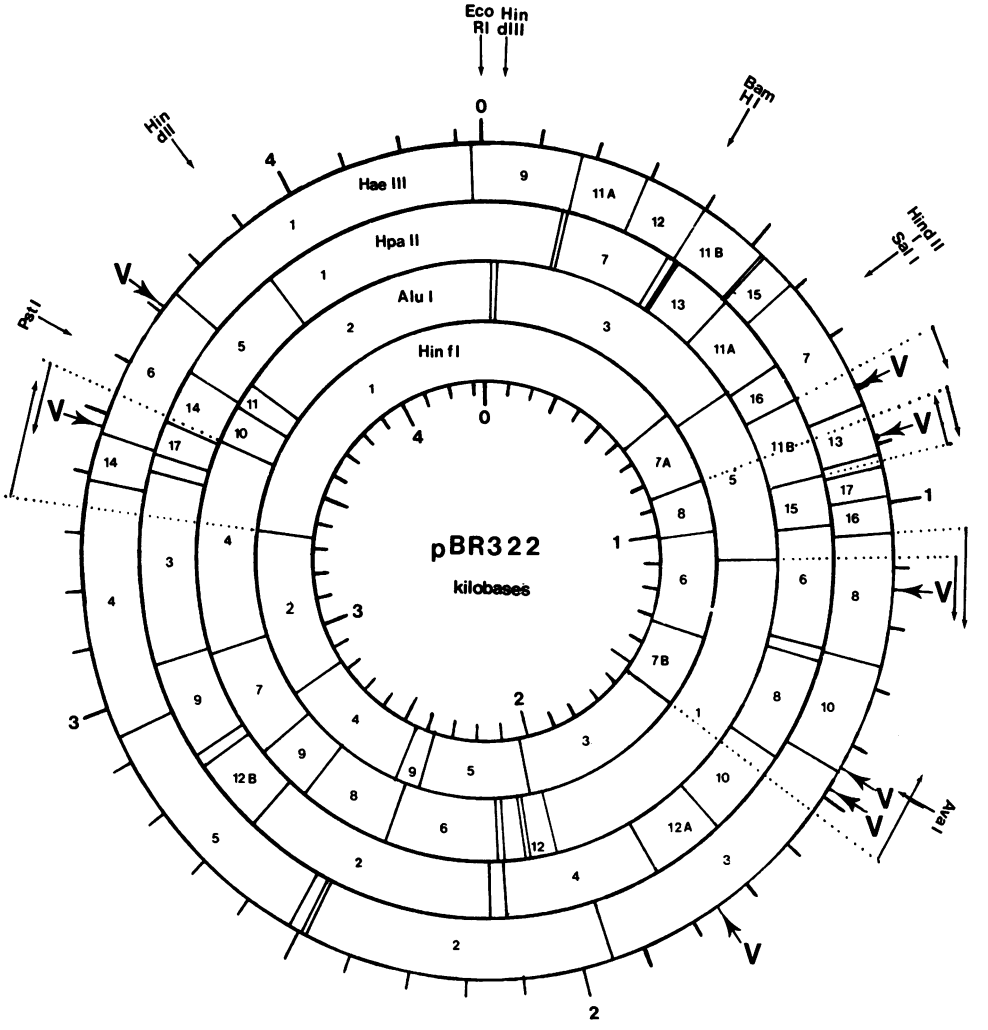

Figure 2. The Ava II sites are located on the pBR322 restriction map by the eight bold V's around its perimeter. The unique Ava I site is shown at coordinate 1.424 kilobases. The fragment produced by cleavage with the enzymes Hae III, Hpa II, Alu I and Hin fI are numbered by size. The arrows represent fragments from which the Ava II (and the single Ava I) cleavage sites were determined directly. The dotted lines from the tails of the arrows indicate the 5'-ends of these fragments.

quence and, at the same time, that sequence is determined. The position of the cut by the enzyme of unknown specificity is read directly from the sequence autoradiogram, remembering that the chemically generated bands represent pieces of DNA

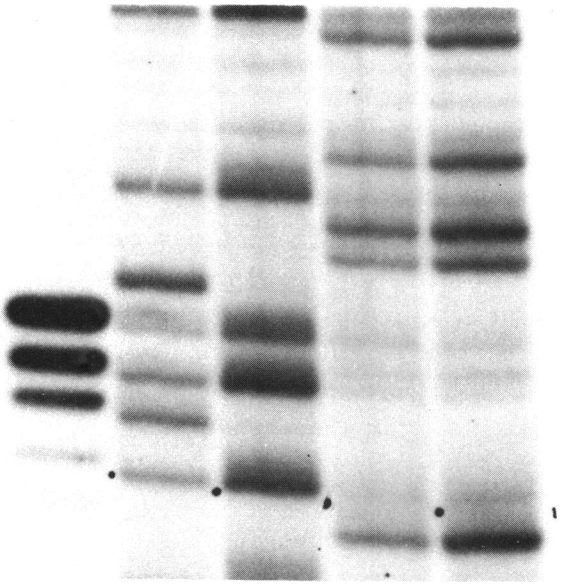

Figure 3. The cleavage site of Ava II can be read directly from this autoradiogram. The ^{32}P-5'-end of the fragment shown was the Hpa II site at coordinate 769 on the map. The lane on the left is this fragment cleaved by Ava II. The DNA was overdigested and exonuclease produced the bands below the darkest band. The other tracks form a Maxam-Gilbert sequence ladder for the fragment. The lanes are A>G, G only, C only and C+T. The sequence reads (in part) CGAGGACCGCTTTC. The band produced by Ava II cleavage comigrates with the second G of GGACC. This observation places the cut between the two G's because the chemical products are released 5' to the chemically modified base and appear in the penultimate position, wherease the enzyme-produced band is full length.

which end immediately 5' to the base destroyed by the sequencing reactions. We studied the fragments indicated in Fig. 2 by this method.

All Ava II 3' ends we examined in this way were at the arrows in the sequence G↓GACC or G↓GTCC. (Example Fig. 3). Two cleavage sites were examined in both orientations by sequencing the complementary strands. The cuts were

5' - G↓GTCC - 3'

3' - CCAG↑G - 5'

The complete sequence of pBR322 was scanned. At all locations where we had mapped an Ava II site, the sequence GG$^{A}_{T}$CC was found. Furthermore, this sequence was found

nowhere else in pBR322. We have found two sequences of the form G{A/T}CG{A/T}G (such as suggested as the Ava II site by Murray et al.[1]) in the pBR322 sequence, but they were not near any of the mapped Ava II sites. Fuchs et al. have scanned the ϕx174 and SV40 sequences by computer. GG$^{A}_{T}$CC appears once near where the unique Ava II site has been mapped, and the SV40 Ava II-fragment sizes are consistent with this cleavage site.

We obtained further evidence that this assignment is correct by labelling the 5' end of seven fragments produced by Ava II with γ-^{32}P-ATP and polynucleotide kinase. The Maxam-Gilbert sequences from these ends were 5'-GTCC or 5'-GACC in all cases.

A second activity from Anabaena variabilis was also examined. Ava I made a unique cut in pBR322 (Fig. 2), which was mapped by double digests (data not shown). The sequence of this Ava I site is

5' - C↓CCGAG - 3'

3' - GGGCT↑C - 5'

This agrees with the cleavage specificity of Ava I determined by Hughes and Murray to be CPyCGPuG.[12]

DISCUSSION

We have examined both the 3' and 5' sides of several Ava II cuts. Our data unambiguously demonstrate that Ava II recognizes and cuts the sequence GG$^{A}_{T}$CC, leaving a three nucleotide 5' extension. Murray et al.[1] report that the short oligonucleotides produced by pancreatic DNase digestion of 5' -^{32}P-labelled Ava II ends have the common sequences GACN and GTCN. Inspection of their data, however, shows that N is predominantly C. We suggest that the very slight heterogeneity at this position reflects "mistakes" made by Ava II. We have routinely noticed that many restriction enzymes (e.g. Hin fI, Hind II) put single-strand nicks in double-stranded DNA at some sequences which closely resemble their generally accepted cleavage site. Presumably the enzyme has a lower affinity for these sites, but can still cut. These nicks are most noticeable when the DNA has been

overdigested with the restriction enzyme.

One of the pBR322 Ava II sites contains a methylated base because it overlaps an EcoRII site (5' -GGTCC*TGG -3'). This particular site is cleaved at about a ten times slower rate than the other Ava II sites on the plasmid.

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