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Full Papers

Mapping of restriction enzyme cuts by a new two-dimensional procedure*

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Summary. A new procedure has been worked out to establish restriction maps. The method is fast, does not in general require labeled DNA and has been applied to map the linear palindromic rDNA of *Physarum* with the restriction enzyme *Bst*EII.
Key words. Physarum; ribosomal DNA; restriction; method, two-dimensional; gel electrophoresis.

The establishment of a restriction map for a given piece of DNA is a rather tedious procedure. With unlabelled material, recourse is often made to several digests, whereby elution of fragments from gels is followed by a subsequent cleavage. If DNA pieces are radioactively labelled at both ends, cut once and the 2 fragments separated, restriction maps can be constructed from partial digests[13]. Here we describe a new 2-dimensional procedure. The method entails separating partial digests on a gel, transferring them to a solid support, eluting and digesting them to completion with the same enzyme and separating the fragments in a second gel. The transfer of DNA fragments from one gel to another is simple and quick, and has recently been described in detail in a very different context[7]. This novel procedure has been successfully applied to establish a *Bst*EII restriction map of *Physarum* ribosomal DNA. This rDNA is a linear palindrome of 60 kb size, not integrated in large chromosomes[11, 15].

Material and methods. rDNA was purified from *Physarum polycephalum* plasmodia (strain M_3 CVIII) as previously described[4]. Restriction enzymes *Bam*HI, *Eco*RI and *Hin*dIII were obtained from Bethesda Research Labs; *Bst*EII was obtained from Boehringer Mannheim. Digestion conditions were those used in the procedures described by the manufacturers. Electrophoresis of DNA restriction fragments was performed in agarose gels in the tris-acetate buffer of Hayward and Smith[10]. Borate buffer is not suitable[7]. The gels were stained with ethidium bromide (0.5 µg/ml) for 30 min, and photographed under UV illumination using a Kodak No. 23A Wratten filter.

Two-dimensional electrophoresis and recovery of DNA fragments were performed according to Chen and

Thomas[6,7]. Briefly, the agarose strips cut from the gel of the first electrophoresis and each containing one specific partial digest, were dissolved with 7 M $NaClO_4$, 10 mM tris-HCl (pH 7.5) and 1 mM EDTA, while lying on a glass fiber filter in a set-up similar to the one used to transfer DNA from gels to nitrocellulose filters[14]. The DNA, while adhering to the glass fibers, was washed free of agarose with perchlorate and then freed of the perchlorate with 95% ethanol[7]. The glass fiber strips were eluted with 10–15 µl of 1 mM tris-HCl (pH 7.5), 0.1 mM EDTA and the partial DNA digest incubated with the same restriction enzyme as used before. The products were analyzed in a second gel electrophoresis. Purified cDm103 DNA[9] containing *Drosophila* ribosomal sequences was a gift from Dr T.E. Gilroy, and was labelled with ^{32}P by nick translation[12]. Transfer of DNA segments to nitrocellulose filters and the subsequent hybridization was according to Southern[14].

Results and discussion. Digestion of *Physarum polycephalum* rDNA with restriction enzyme *Bst*EII produced fragments of 8 size classes: 5.9, 5.7, 5.2, 4.8, 4.4, 3.2, 2.45 and 0.72 kb, respectively (fig. 1). The amount of 5.7 kb segments appeared to be only one half of the 5.9 kb fragment. Since the rDNA molecule is a giant palindrome[11,15] the 5.7 kb fragment is assigned to the center of the molecule. The 4.4 kb band is broad, indicating a distribution of lengths. Therefore, it is most likely that those fragments come from the ends of the molecule which are known to be of variable (± 300 bp) lengths[5]. To determine the location of the other 6 fragments, a 2-dimensional analysis was performed (fig. 2). rDNA was first partially digested with *Bst*EII and fractionated on a gel. Each fraction was then re-digested to completion with *Bst*EII, and again separated in a gel slab. In this fashion, one can analyze the constituents of the partial fragments and deduce the linkage relation between the restriction fragments. For example, the 11.6 kb partial fragment was separated into the 5.9 kb and the 5.7 kb fragments, therefore the 5.9 and the 5.7 kb fragments must be linked. Furthermore, the 9.1 kb fragment is made of the 5.9 and the 3.2 kb fragments, and the 8.0 kb fragment is made of the 3.2 kb and the 4.8 kb fragments. This allows one to construct a sequential arrangement: 5.7–5.9–3.2–4.8 kb.

The 4.4 kb end fragment and the 5.2 kb fragment make up the 9.6 kb partial fragment. The 5.2 kb fragment was part of the 7.7 kb partial fragment. Although we could not detect the 2.45 kb fragment (near the limit of detection), we assigned it to be the other part of the 7.7 kb partial fragment. Therefore we can construct another sequence of 2.45–5.2–4.4 kb. The only other terminally-digested fragment not yet placed in the 2 sequences is the 0.72 kb. (It was beyond the detection limit). It can only be between these 2 sequences. Thus, the complete sequence, from the end to the center, is assigned thus:

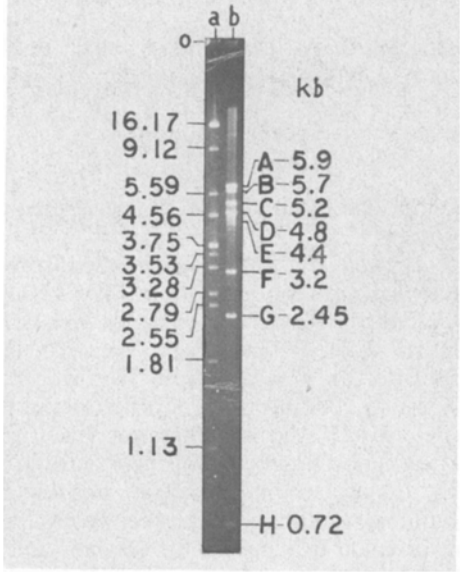

Figure 1. Restriction fragments of *Physarum* rDNA produced by *Bst*EII. rDNA (0.8 µg) was digested to completion with *Bst*EII and electrophoresed in 1% agarose gel (lane b). λ DNA digested with *Bam*HI and *Eco*RI (lane a) was used as molecular weight standard.

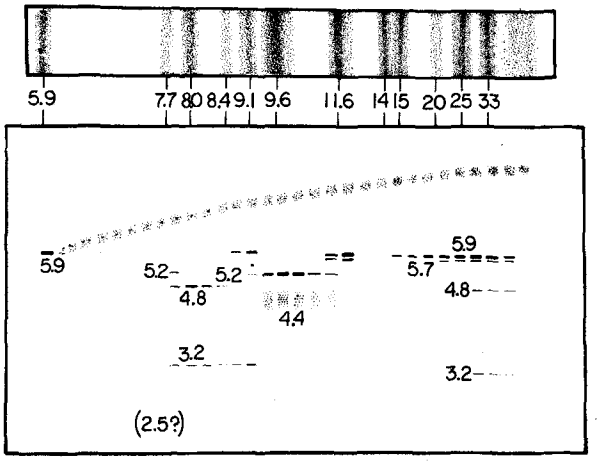

Figure 2. Two-dimensional separation of *Bst*EII fragments of rDNA. rDNA (1 µg) was partially digested with *Bst*EII (1 unit in 20 µl for 7 min at 37 °C), and electrophoresed in a 0.8% agarose gel. Fragments larger than and including the 5.9 kb fragment were blotted on a Whatman GF/A filter and divided into 32 fractions[7]. DNA recovered in each fraction was redigested to completion with *Bst*EII and electrophoresed in a 1.2% gel slab. Schematic presentation shows the gel strip containing the partial digest (top) and the 2-dimensional display of the final termination digest (bottom). The molecular weights of the fragments indicated (in kb) were calibrated against λ DNA digested with *Eco*RI and *Bam*HI. The broad arch-shape distribution is due to the presence of non-ribosomal DNA in the rDNA preparation.

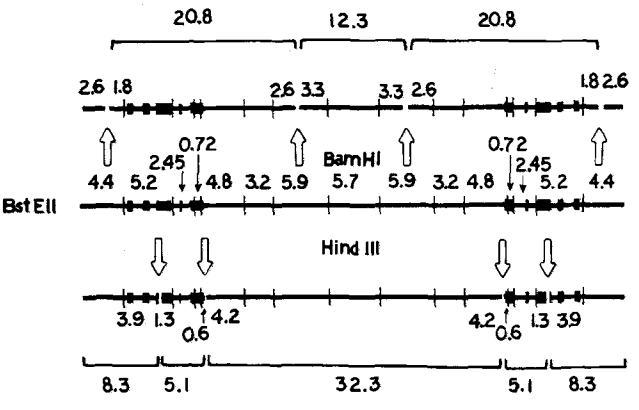

Figure 3. Restriction sites of *Bst*EII (middle line) on rDNA in relation to those of *Bam*HI and *Hind*III[5,11,15]. The thick bars show the sequences coding for rRNA[8].

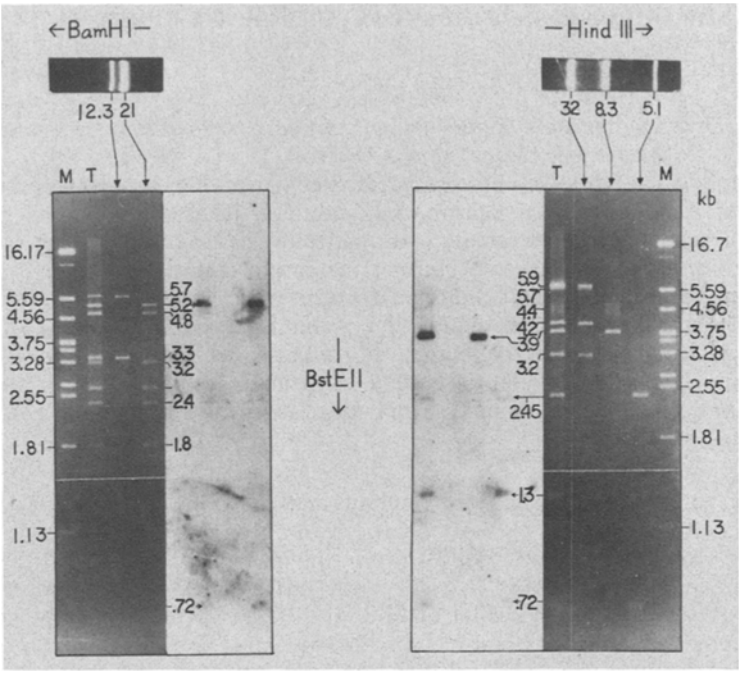

Figure 4. Sequential double digestion of rDNA. rDNA (4 µg) was digested with *Bam*HI or *Hin*dIII and separated in a 1.2% gel (top). Individual fragments were recovered from the gel[6], redigested with *Bst*EII and separated in a 1.2% gel as indicated in the figure. Lanes T in both panels contain double digest (*Bam*HI and *Bst*EII, or *Hin*dIII and *Bst*EII) of total rDNA. Lane M contains *Bam*HI and *Eco*RI double digest of λ DNA as markers. The DNA fragments were transferred to nitrocellulose filters[14] and hybridized with ^{32}P-labelled cDm103 DNA. The resulting autoradiographs are shown next to the gels.

4.4–5.2–2.45–0.7–4.8–3.3–5.9–5.7 kb (fig. 3). The size of the complete rDNA molecule calculated from those fragments is 59.04 kb, comparable to the values of 57–61 kb obtained by other workers[5, 11, 15].

To confirm this *Bst*EII map of rDNA, we performed double digestions with *Bam*HI and *Bst*EII or *Hin*dIII and *Bst*EII, and compared the results with the published *Bam*HI and *Hin*dIII maps (fig. 3). Furthermore, we transferred fragments to nitrocellulose filters[14], and hybridized them with ^{32}P labelled cDm103 DNA[12] which was found to crosshybridize with the coding sequences on the *Physarum* rDNA (T.E. Gilroy and R. Braun, unpublished observation). Our *Bst*EII map predicts hybridization of cDm103 DNA to these *Bst*EII fragments: 5.2 kb, 2.45 kb, 0.72 kb and 4.8 kb. In fact, these fragments, and some of the smaller fragments generated from them, all hybridized to cDm103. The results of these experiments are shown in figure 4 and the protocols are given in the appropriate legend. These results obtained with our 2-dimensional procedure are in excellent agreement with recent results of conventional mapping by Ferris and Vogt[8].

This new two-dimensional method for restriction mapping will be useful also for other pieces of rather large DNA, provided the partials separate reasonably well in agarose. The method requires no labelling of the DNA and is therefore inexpensive. It is also simple and rapid. The limit of detection can of course be lowered by labeling the DNA or by hybridization with labelled probes.

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