SHORT COMMUNICATION

Denaturation Map of the ColE1-Km Plasmid pCR11

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The denaturation map of *Eco*RI-digested pCR11, a ColE1-Km plasmid, is described. The 2.0 kilobase ColE1-derived segment contains an adenine+thymine rich site in the colicin immunity gene region. In the 7.2 kilobase kanamycin resistance region, the transposon Tn903 consists of an adenine+thymine rich 0.98 kilobase *kan* gene region flanked by a guanine+cytosine rich 1.09 kilobase inverted duplication.

INTRODUCTION

Covey *et al.* (1976) constructed the 9.2 kilobase (kb) plasmid pCR11 from DNA of two different genetic origins. A 7.2 kb region is derived from an *Eco*RI-digestion fragment of the *Escherichia coli* antibiotic resistance plasmid R6-5 and confers kanamycin resistance (Km). The remaining 2 kb is part of the 6.4 kb *E. coli* colicinogenic plasmid E1 (ColE1) and contains the origin of replication. The ColE1-Km plasmids pCR11 and the related plasmid pCR1 have been used as cloning vectors in genetic engineering studies (for example, Backman *et al.*, 1976; Humphries *et al.*, 1978). They are also of interest because they carry the kanamycin resistance transposon Tn903 (Plasmid Reference Centre). These plasmids have been mapped by restriction endonucleases and by electron microscope methods (Armstrong *et al.*, 1977; Coggins & McCluskey, 1979).

Another method of characterizing DNA by electron microscopy is provided by denaturation mapping (Inman, 1966). Conditions which partially denature the DNA do so preferentially in regions with a high adenine+thymine (A+T) content. If A+T rich regions are non-uniformly distributed along the molecule, a characteristic pattern of single-stranded denatured regions will be observed. A denaturation map, which indicates the frequency with which particular sites are denatured, can be made by combining results for a population of molecules. In this paper, the application of this technique to the study of pCR11 is described and the results obtained are analysed in light of the known structural and genetic features of this plasmid.

METHODS

Denaturation mapping was carried out by a modification of the method of Inman (1967). *Eco*RI-digested pCR1 or pCR11, prepared as previously described (Coggins & McCluskey, 1979), in 100 mM-NaCl/10 mM-EDTA/10 % (v/v) formaldehyde, pH 6.5, was heated at 57 °C for 10 min, chilled on ice and adjusted to 50 % (v/v) formamide and 100 µg cytochrome *c* ml⁻¹ before spreading on distilled water. Samples were picked up on collodion-coated grids, stained with uranyl acetate (Davis *et al.*, 1971), shadowed with platinum/palladium and examined with a Philips EM300 electron microscope.

The position and extent of denatured regions in 50 molecules were measured on micrographs using a map measurer. Measured lengths were converted into percentages of the total molecular length for each molecule. A weight-average denaturation map (Inman, 1967) was plotted by summing the denatured sites in 0.5 % length increments (46 base pairs).

Fig. 1. (a) *EcoRI*-digested pCR11 molecules denatured at 57 °C. Arrows indicate major denatured regions. Bar marker represents 0.5 μm.

(b) Denaturation map (above) and physical map (below) of *EcoRI*-digested pCR11. The *ColE1* region is shown stippled, with the position of the origin of replication (*ori*) marked by a bar. The inverted duplication (*i*) in the kanamycin resistance region is shown cross-hatched and flanks the *kan* gene region. The position of the *HindIII* site is shown by the bar labelled H. The scale shows the molecular size in kilobases (kb).

RESULTS AND DISCUSSION

Determination of orientation of data

Two *EcoRI*-digested pCR11 molecules which had been partially denatured at 57 °C are shown in Fig. 1(a). One end of each molecule is extensively denatured (small arrows) and one or two large denatured regions (large arrows) are observed 50 to 65 % along the molecule. (Small loops are also present, particularly near the end of the molecule furthest

from the extensively denatured end.) A similar asymmetric distribution of the major denaturation loops was found in other molecules, allowing them to be orientated unambiguously for measurement. The data so produced were combined to form a denaturation map (Fig. 1*b*), the average total amount of denaturation per molecule being $19.6 \pm 3.4\%$.

The physical map of pCR11, as determined by previous studies in this laboratory (Coggins & McCluskey, 1979), is shown beneath the denaturation map in Fig. 1*b*). The orientation of these two maps with respect to each other was determined by examining the partial denaturation pattern of the related plasmid pCR1 under the same conditions. This plasmid differs from pCR11 in having a larger ColE1 region, and the extra 3.9 kb of DNA which it contains is inserted at 2.0 kb on the map of pCR11 (Coggins & McCluskey, 1979). Partially denatured *Eco*RI-digested pCR1 molecules were also observed to have one end which was extensively denatured (data not shown). The major internal denatured region (frequently also a pair of loops) was the same distance from the other end as it was in pCR11. It must therefore lie in the region containing the kanamycin resistance gene (*kan*) which is common to both plasmids. Between these recognizable features was another region which contained two large denaturation loops and it was concluded that these must lie in the extra ColE1 DNA present only in pCR1.

The pCR11 denaturation map can now be compared directly with the physical map. *Eco*RI cut the plasmid at one of the two junctions between the ColE1 and Km regions and therefore the linear map is divided into these two sections and the denaturation pattern corresponding to each can be considered separately.

The ColE1 region

The ColE1 region lies from 0 to 2.0 kb and its major feature is the terminal denatured region extending from the *Eco*RI site to an average of 638 ± 187 base pairs into the molecule. An A + T rich region near the *Eco*RI site has also been described by Tanaka *et al.* (1975) in a ColE1-derived chimaeric plasmid denatured by high pH. Inselberg (1977) mapped the colicin immunity gene in this region of the plasmid.

The origin of replication at 1.2 kb from the *Eco*RI site (Lovett *et al.*, 1974) lies outside the terminal denatured region but a denaturation loop occurred at a low frequency in this position. The DNA sequence around the origin of replication in ColE1 has been determined by Tomizawa *et al.* (1977). From their data, the guanine + cytosine (G + C) contents of consecutive groups of 50 base pairs around the origin of replication were calculated to be 68, 54, 52, (origin), 52, 44 and 64 % (from left to right in Fig. 1*b*), so a short A + T rich region is present near the origin.

Another low frequency denaturation site was observed near the junction with the kanamycin resistance region.

The kanamycin resistance region

The remainder of the pCR11 molecule (2.0 to 9.2 kb) is derived from plasmid R6-5 and confers kanamycin resistance. This is due to the Tn903 transposon which is made up of a 0.98 kb *kan* gene region flanked by a 1.09 kb inverted duplication (Coggins & McCluskey, 1979). The corresponding section of the denaturation map shows a pattern which reflects the structural features of the transposon. The major internal denaturation region is correlated with the position of the *kan* gene segment. The observation that it frequently consists of a pair of denaturation loops is reflected in the map as two peaks surrounding a slightly less A + T rich region. The amount of DNA required to code for the enzyme responsible for antibiotic resistance, kanamycin phosphotransferase, is about 750 base pairs (Coggins & McCluskey, 1979). Although the exact position of this sequence within the 980 base pair *kan* gene region is not known, the denaturation data suggests that the outermost sequences, which are probably non-coding, have a higher A + T content.

The two segments of the denaturation map which correspond to the inverted duplication show two important characteristics. First, as would be expected, they show a symmetrical pattern on each side of the *kan* region, and second, their A + T content is markedly different from that of the gene. At the junctions between these two regions the height of the peaks decreases sharply so that the inner third of each inverted duplication is associated with only a low frequency of denaturation. The resolution of the map does not show whether this is due to error in measurement of the extent of the A + T rich gene region or is an intrinsic property of the inverted duplication. Each repeated sequence has a central region which is resistant to denaturation at 57 °C and must have a relatively low A + T content. Towards the junctions of the inverted duplication with the rest of the plasmid the amount of denaturation rises slightly, but overall the duplication appears to have a relatively high G + C content. Previous experiments (Coggins & McCluskey, 1979) have shown that each 1.09 kb repeat sequence contains at least two, and probably three, sites for *Sma*I restriction endonuclease (which cuts the sequence 5'-CCCGGG-3') whereas a random DNA sequence would be expected to be cut only once in 4.1 kb. This is additional evidence that the inverted duplication has G + C rich regions. Recently Oka *et al.* (1978) have sequenced 250 base pairs of Tn903. The G + C contents for consecutive groups of 50 base pairs, starting at the junction of the inverted duplication with the plasmid (calculated from their data), are 48, 44, 60, 58 and 54 %.

The non-transposon regions of the kanamycin-resistance segment have unknown functions. One part (2.0 to 3.9 kb) is relatively G + C rich and the other (6.9 to 9.2 kb) contains A + T rich regions.

CONCLUSION

This study showed that there is a strong correlation between the denaturation map of pCR11 and its structural and genetic map, revealing that G + C content has diverged with the evolution of different functions. Rapid DNA sequencing methods have recently enabled regions of particular interest to be studied in detail, but at present these methods are most readily used for the study of hundreds of base pairs. For molecules larger than a few kilobases, denaturation mapping can quickly provide an overall indication of molecular differentiation.

Note added in proof. B. Y. Tong & S. J. Battersby [*Nucleic Acids Research* (1979) **6**, 1073–1079] have shown that the beginnings and ends of nearly all genes in SV40 and ϕ X174 are A + T rich.

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