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Structure and function of an AT-rich, interspersed repetitive sequence from *Chironomus thummi*: solenoidal DNA, 142 bp palindrome-frame and homologies with the sequence for site-specific recombination of bacterial transposons

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ABSTRACT

Chironomus thummi thummi contains a repetitive AT-rich 118 bp sequence mainly in the centromere regions and elsewhere in the genome (1). A large cluster of repeats is regularly present in the non-transcribed spacer of rDNA. Dimers and multimers of the repeat migrate slower in small pore gels than would be expected from their size. The results indicate a solenoidal structure with a coil girth of appr. 350 bp. This structure is most probably due to a highly periodic positioning of di-nucleotides of the type purine - purine or pyrimidine-pyrimidine with distances of appr. 10 bases. In a cluster of 118 bp repeats, regions of dyad-symmetry are positioned such that a 142 ± 2 bp palindrome-frame is generated. Evidence is presented favouring the assumption that the repeat functions primarily in sister chromatid exchange.

INTRODUCTION

Among Chironomides the two subspecies Chironomus thummi thummi and Chironomus thummi piger have received special interest since they represent a system which allows the first steps in species separation to be studied. This separation process is accompanied by a geometric increase of DNA in polytene chromosome bands mainly at the centromeres and neighbouring regions of the Cironomus thummi thummi genome (2, 3). The local increase of DNA is in some way coupled with an increase in the amount of repetitive DNA sequences (4, 5) which is also visible in an increase of the amount of C - banding DNA (6). A comparable situation is found in Drosophila sibling species where it has been emphasized that apparent differences in the amount of highly repetitive DNA sequences accompanies species separation (7, 8). The variation in the highly repetitive portion of the genome might also include regions carrying information for proteins (9).

In the two Ch.thummi subspecies a centromeric cluster of an AT-rich repetitive 118 bp sequence has been characterized (1). This cluster has been magnified and dislocated in Ch. th. thummi and is also found at those sites where the DNA content in bands has increased (1). The fact that this sequence

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has also entered the non-transcribed spacer of rDNA led us assume that the *repetitive sequence may function in promoting sister chromatid exchange* (SCE) (10). A high frequency of SCE would provide the redundant gene cluster with a greater evolutionary flexibility (11). Evidence has been accumulated favouring this hypothesis (12, 13 and this report).

MATERIAL AND METHODS

1. DNA

Cloned rDNA of *Ch. th thummi* pCtt 1505 and pCtt 1507 was kindly provided by E. R. Schmidt (17). The plasmid DNA was further purified from contaminating RNA by ethanol precipitation in the presence of 2.5 M ammonium acetate. If desired, the rDNA insert was separated from the vector pBR 328 by EcoRI restriction of the clone followed by preparative gel electrophoresis and electroelution of the DNA.

2. DNA Restriction and Gel Electrophoresis

Enzyme incubations were performed as described by the producer (Boehringer). Restriction fragments were separated on 3 mm horizontal slab gels in a tris - phosphate - EDTA buffer system (14). Agarose gels were prepared according to (15, 16). Polyacrylamide gels (1 : 20 ratio N,N'methylenbisacrylamide : acrylamide) were reinforced with 0.5% agarose. ØX174 - HaeIII and λ- HindIII fragments were used as size markers and the gels analysed by ethidium bromide staining. Negative prints are shown.

RESULTS

The restriction map of the cloned rDNA of *Ch. th. thummi* pCtt 1507 is shown in figure 1. This clone contains appr. 22 x 118 bp repeats (ClaI repeats) which has been sequenced (17). The map is identical with that of pCtt 1505 except that clone pCtt 1507 exhibits stability in the number of ClaI repeats after replication in *E. coli* unlike pCtt 1505 which is highly unstable in the number of ClaI repeats (17). Sequence data have shown that the stable clone 1507 contains a base substitution at the right end of the ClaI repeat cluster (18) and several uncharacterized mutations in the vector pBR 328 including the spread of ClaI repeats into the vector (Israelewski, unpubl.). It was therefore decided to separate the rDNA insert from the vector by EcoRI restriction and preparative gel electrophoresis.

Measurements of the length of the EcoRI fragment based on its mobility on gel electrophoresis gives rise to different values which are apparently dependent on the pore size of the gel. Higher percentage gels slow down dis-

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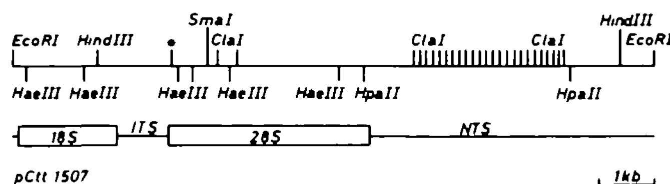

Fig. 1 Restriction map of a cloned Ch. th. thummi rDNA cistron pCtt 1507 (according to 10. 17). Of the HpaII sites only the two in the NTS are shown. The asterix indicates a ClaI site that is present in most of the genomic rDNA cistrons, but is absent in cloned rDNA.

proportionately the migration of the fragment. In 0.5%, 1.0%, 1.5% and 2.0% agarose gels lengths of 11.0 kb, 12.9 kb, 15.5 kb and 40 kb were measured respectively. When HaeIII restricted DNA is electrophoresed it is only the NTS carrying fragment which migrates anomalously. The same is true for HpaII restriction fragments. (fig. 2A). After ClaI digestion it is seen that the two flanking segments of the ClaI repeat cluster migrate correctly in 2.0% agarose gels. Comparisons of the gel dependent shift in the sizes of the EcoRI, HaeIII and HpaII fragments indicate that the shift is more pronounced if the ClaI repeat cluster is flanked by large DNA segments which alone do not show the shift (fig. 2B). In the case of the HpaII fragment there additionally appears a faint band without shift (arrows, fig 2A, clearly seen only in 1.5% and 2.0% agarose gels). Since no HpaII fragment greater than 2.6 kb should exist besides the NTS-HpaII-fragment (3.7 kb) it is possible that in few plasmids one HpaII site is modified (at the left of 28S) resulting in the 3.7 kb band.

For analysis of the gel-type dependent shift in the sizes of monomers and multimers of the ClaI repeat the clone pCtt 1507 was digested incompletely with ClaI and the DNA was run on polyacrylamide (PAA) gels. The shift is measured as percentage deviation from the real value. Three typical curves are shown in figure 3A. In àll three PAA gels (2.0% - 2.5% - 3.0%) the monomer size was measured to be 116 bp corresponding to a deviation of -2% relative to the 118 bp fragment of ØX174-HaeIII. With 2.0% gels the shift of dimers and multimers increases up to +20% for six ClaI repeats. Further increasing of the number of the repeats does not contribute to a further increase of the shift. With 2.5% PAA gels the curve shows systematic steps resulting in kinks of the curve at intervals corresponding appr. to multiples of three ClaI repeats. With 3.0% PAA gels the shift increases dramatically up to nine repeats; further multimers cannot be measured in this type of gel.

In each case care was taken to avoid partial denaturation of the DNA which alters its mobility on electrophoresis. If the gel heated up due to a

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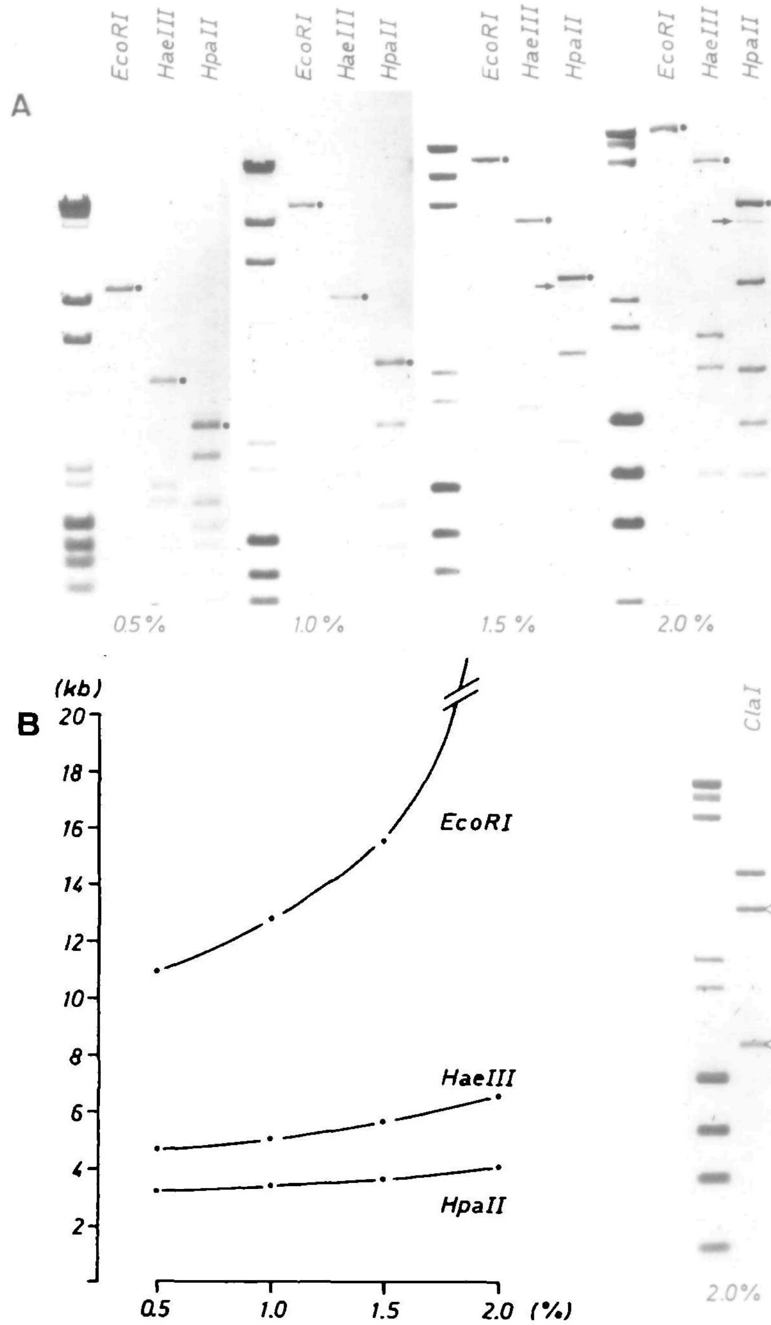

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high current, the curves shown above are not exactly reproducible. However, partial denaturing of the ClaI repeat DNA under controlled conditions (2M urea in the sample application buffer) leads to a systematic enlargement of the steps in the curve for 2.5% PAA gels, especially at higher numbers of ClaI repeats (fig. 3B).

It has been noticed that clone pCtt 1505 is not stable during replication in *E. coli* leading to a decrease in the number of ClaI repeats (17). The presented data of a secondary structure of the ClaI repeat and the possible interference of a recombination system of *E. coli* with the ClaI repeat sequence (17 and discussion) prompted us to re-evaluate the elimination process of ClaI repeats in *E. coli*. Using a single colony isolate for the DNA preparation, the restriction with HaeIII results in a ladder of NTS fragments with size intervals of 120 bp (fig. 4A). It is interesting that certain fragment sizes are prefered during the elimination process in *E. coli*. In the lower molecular weight region it is seen that these fragments have distances of 3 x 120 bp or multiples of that (arrows). If *E. coli* is cultivated successively four times overnight, three prominent HaeIII fragments accumulate which have also size intervals of 360 bp (3 x ClaI repeat, fig. 4B). Thus, it is suggested that recombination in the cloned rDNA occurs at a defined site in the ClaI sequences modulated by the structural feature of the DNA.

DISCUSSION

Structure and function are two aspects of living matter (19). Considering the results suggesting a defined secondary structure of the ClaI repeat DNA of *Ch. th. thummi* it may be anticipated that one can also find a function. Thus, sequence data (17) were analysed.

I. 10 bases periodicity and bent helical DNA structure

Trifonov (20) has predicted that the DNA axis is curved if some dinucleotides of the type purine-purine or pyrimidine-pyrimidine have the tendency to be repeated with a period of about 10 bases. In a long DNA fragment this would lead to a solenoidal DNA structure which is stable in a DNA molecule free of protein. In the ClaI repeat sequence an almost perfect 10 bases

Fig. 2 A) Agarose gel electrophoresis of the rDNA insert of pCtt 1507 after restriction with the enzymes indicated at the top of each lane. The NTS containing fragments (•) migrate anomalously in 1.0% - 2.0% agarose gels. On elimination of the ClaI repeat DNA by digestion with ClaI the two flanking fragments (◁) of the repeat cluster migrate correctly in 2.0% agarose gels. Arrow: see text. B) Plot of the measured size of the NTS containing fragments as a function of the percentage of agarose.

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