Sequence Analysis of the Cloned *Cucumis melo* Highly Repetitive Satellite DNA

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The primary sequence of the prominent *Cucumis melo* satellite DNA is presented. The cloned *Hind*III-repeat unit has a length of 352 bp and a GC-content of 56%. Within this sequence an inverted repeat of 9 bp, including a 286 bp loop, and one direct repeat are shown. Several open reading frames are possible following the sequence in both directions whose significance is not yet clear. The sequence has been compared with other highly repetitive DNA sequences of *Sinapis alba* and *Scilla sibirica*. The genomic satellite DNA exhibits an unusual behaviour in actinomycin D-CsCl gradients in comparison to ribosomal DNA.

Introduction

The role of highly repetitive satellite DNA on the evolution of the eukaryotic genome has been extensively studied and discussed. Species formation is obviously accompanied by a variation in satellite components; but a clear function of these highly repetitive, relatively simple, tandemly arranged DNA sequences for the diversity of species is still not evident [1–3].

For higher plants the family of *Cucurbitaceae* proved to be useful to study satellite DNA [4–6]. Restriction analysis and methylation pattern has been compared for *Cucumis melo* and *Cucumis sativus* satellite DNA [6, 7]. Now we present the DNA sequence of the basic repeat unit of the *C. melo* satellite previously cloned. The sequence obtained has been analyzed by computer for possible reading frames, internal repeats and palindromic structures.

The detailed knowledge of the DNA sequence organization of the *C. melo* satellite will allow to find families of homologous sequences in other *Cucurbitaceae* species as has been already suggested for *C. sativus* satellite DNA [6].

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Materials and Methods

Plant material and DNA isolation

Seeds of the sugar melon *Cucumis melo* var. "Zuckerkugel" were cultivated under sterile conditions on sand at 27 °C in the dark. For DNA isolation from cell nuclei hypocotyls of 5–7 day old seedlings were used and treated as previously described [6, 8]. CsCl and actinomycin D-CsCl gradient centrifugation, restriction enzyme analysis, agarose gel electrophoresis and hybridization with ^{3}H-labeled RNA were performed as previously described [6, 9].

Cloning and sequencing of satellite DNA

The *Hind*III-satellite DNA fragment of *C. melo* cloned in pBR 325 [6] was cut out by *Hind*III digestion of the recombinant plasmid pSat 107, separated from the plasmid DNA on 5–30% sucrose gradients, reintegrated into the *Hind*III site of pBR 322, and *E. coli* K12, RRI was transformed. The recombinant plasmid pSat 312 obtained, containing one satellite repeating unit, was amplified without chloramphenicol treatment and pSat 312 DNA was isolated by ethidium bromide-CsCl gradient centrifugation [10].

For DNA sequencing the Maxam-Gilbert technique was followed [11, 12]. Computer analysis was done using the program of Fristensky *et al.* [13].

Results and Discussion

Sequence analysis

In a previous paper the *Cucumis melo* satellite I DNA was characterized as consisting of a highly redundant, tandemly arranged DNA sequence of 380 bp with a single internal *Hind*III restriction endonuclease site per repeat [6]. This DNA sequence was ligated into the *E. coli* vector pBR 325-*Hind*III site, and *E. coli* K12, RRI was transformed. A recombinant clone was isolated (pSat 107) containing three repeating units of the satellite. In order to sequence the repeating unit, we recloned the *Hind*III-fragment into the *Hind*III site of pBR 322. From the clone pSat 312 with one repeating unit inserted DNA was prepared without previous chloramphenicol amplification of the plasmid and the DNA was sequenced by the chemical method of Maxam & Gilbert [11]. The sequence determined is shown in Fig. 1.

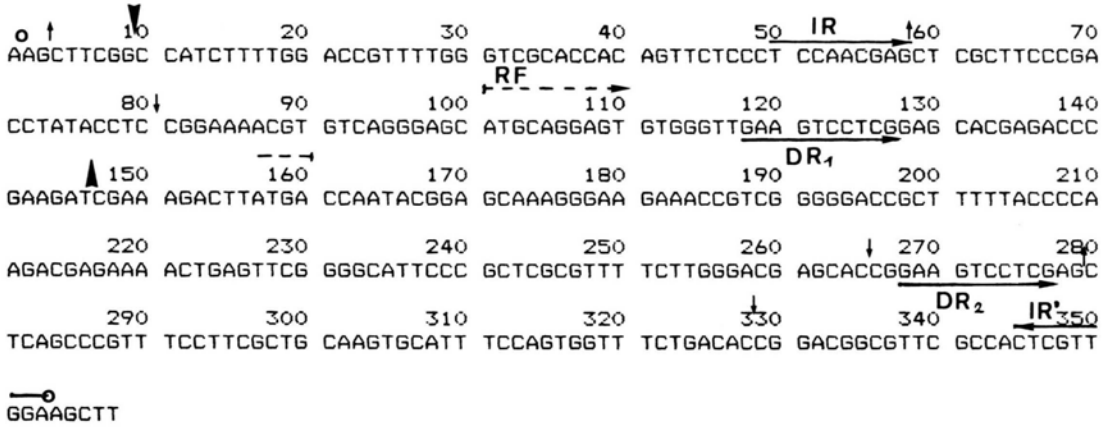

Fig. 1. Primary sequence of the *C. melo* 352 bp-satellite DNA. A single-strand nucleotide sequence (5′−3′) of the cloned satellite monomer is shown. Relevant restriction enzyme recognition sites are indicated (○ *Hind*III; ↑ *Alu*I; ↓ *Hpa*II; ▼ *Hae*III; ▲ *Taq*I). The sequence of the inverted repeat (IR) of 9 bp, one direct repeat (DR_1 and DR_2) of 10 bp, and one possible reading frame (RF) are marked by arrows.

The sequencing data now allow us to correct the length of the repeating unit to precisely 352 bp. The restriction endonuclease sites previously described [6] are confirmed in the sequence and additional sites have been identified by computer analysis. An inverted repeat of 9 bp is detected (see arrows in Fig. 1), leaving a large single-stranded loop-out of 286 bp. Whether this inverted repeat is actually realized as a palindrome in the cell is not yet known. The model of another palindromic structure within the repeating unit proposed previously [6] does not appear to be correct, since no internal sequence homology necessary for the formation of such a construct can be found.

Within the repeating unit of 352 bp two identical sequences of 10 bp in the same orientation are also found. Several open reading frames for small polypeptides are possible, reading the sequence in both orientations (Fig. 1, broken arrows); however, they may not be used *in vivo*, since no transcription products could be detected in RNA preparations from different tissues of seedlings [6]. Similar short open reading frames are detected in the cryptic satellite of *Sinapis alba* [14].

The cloned and sequenced *Hind*III-fragment of the *C. melo* satellite DNA seems to be representative as a repeating unit for a number of reasons: incomplete *Hind*III digestion of purified genomic satellite I DNA, prepared from DNA, separated on actinomycin D-CsCl gradients, run on agarose gels, leaves a ladder of multimers of 352 bp; complete digestion cleaves this DNA to a single band of 352 bp. In the same manner restriction sites for *Alu*I and *Hae*III appear to be conserved in the majority of satellite copies, suggesting that we are dealing with a homogeneous repetitive DNA sequence with a basic repeating unit of 352 bp mostly arranged in tandem. This is in contrast to other highly repetitive DNA sequences of *Sinapis alba* [14], wheat, rye, and barley [15−17], and *Cucumis sativus* (Ganal and Hemleben, unpubl.) where different families of sequences are characteristic. We compared the sequenced satellite of *Sinapis alba* with the *C. melo* satellite DNA and found only small regions of homology of 6 or 7 bp. Another plant satellite sequence, the very GC-rich satellite of *Scilla sibirica* [18], shows no similarities with the *C. melo* satellite sequence.

Currently, we clone and sequence satellite DNA sequences of different species of the *Cucurbitaceae* in order to characterize homologous and species-specific sequence families.

Conformational specificity of the C. melo satellite I DNA

The GC-content of the *C. melo* satellite I DNA calculated from the results of CsCl density gradient centrifugation ($\varrho = 1.706$ g/ml corresponding to about 46% GC; 9) cannot be confirmed by the sequencing data (56% GC). To demonstrate the previously observed unusual behaviour of the

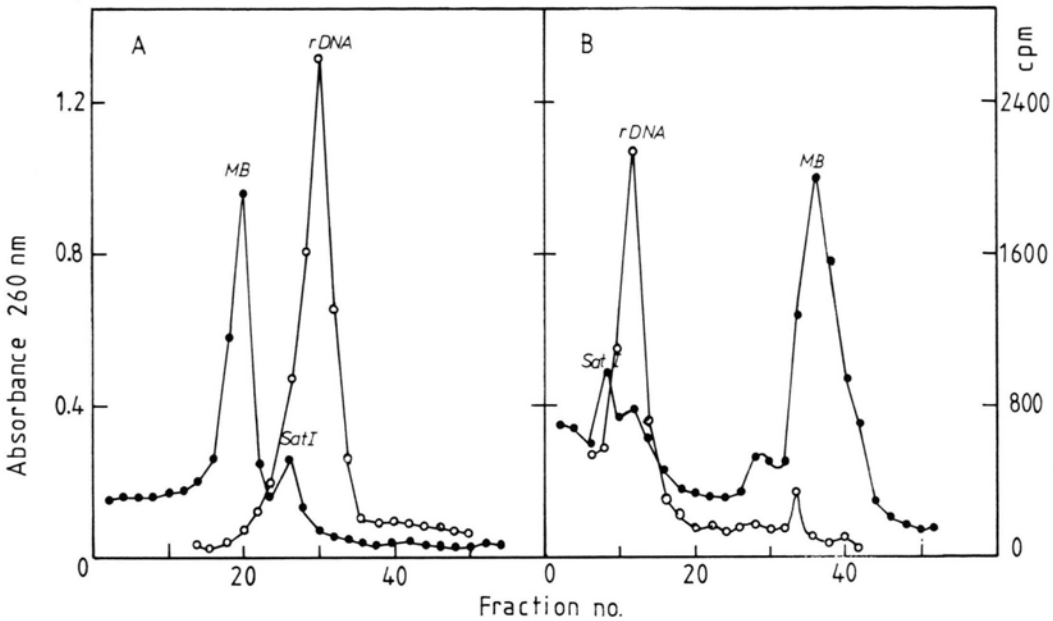

Fig. 2. Fractionation of *C. melo* total nuclear DNA on CsCl (A) and actinomycin D-CsCl (B) gradients. *A*: DNA isolated from cell nuclei of hypocotyls of dark-grown seedlings was separated on a CsCl buoyant density gradient (mainband (MB): $\varrho = 1.693$ g/ml; satellite I (Sat I): $\varrho = 1.706$ g/ml; ribosomal DNA (rDNA): $\varrho = 1.710$ g/ml). *B*: DNA containing fractions from a CsCl gradient were rerun on an actinomycin D-CsCl gradient. Ribosomal DNA was located in the gradients by hybridization of the DNA fractions bound to filters with ^{3}H-labelled 18 S and 25 S rRNA [6]. ●——● absorbance at 260 nm; ○——○ ^{3}H-radioactivity.

melon highly repetitive satellite DNA in actinomycin D complexed CsCl gradients [6] total nuclear DNA from hypocotyl tissues of dark grown seedlings was separated on CsCl gradients (Fig. 2A). The DNA containing fractions of a parallel gradient were combined, and the DNA rerun on an actinomycin D-CsCl gradient (Fig. 2B). In CsCl the satellite fraction shows a density of 1.706 g/ml, whereas ribosomal DNA detected by hybridization against ^{3}H-labelled 18 S plus 25 S rRNA bands at a density of 1.71 g/ml indicating a higher GC-content than the satellite DNA.

In CsCl gradients, containing actinomycin D which binds specificly to GC-rich DNA, the banding pattern seems to contradict this observation: here satellite sequences band at a lower density than the ribosomal DNA (Fig. 2B) indicating that this satellite has more actinomycin D molecules bound relatively to the ribosomal DNA than its GC-content and its buoyant density in CsCl would suggest. The different behaviour may be due to conformational specificity or to the relatively high degree of methylation of this highly repetitive DNA [6]. In this connection, the nucleosomal packaging into a transcriptionally inactive heterochromatin structure is under further investigation. Interestingly, these satellite sequences often occur as DNA repeats of 170–180 bp or multimers of this number [14, 19] – a length which represents one nucleosomal unit in higher plants [8].

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