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The organisation, nucleotide sequence, and chromosomal distribution of a satellite DNA from *Allium cepa*

Stephen R. Barnes, Anna M. James, and Gordon Jamieson
Department of Genetics, University of Nottingham, University Park, Nottingham NG7 2RD, England

Abstract. We have investigated the organisation, nucleotide sequence, and chromosomal distribution of a tandemly repeated, satellite DNA from *Allium cepa* (Liliaceae). The satellite, which constitutes about 4% of the *A. cepa* genome, may be resolved from main-band DNA in antibiotic-CsCl density gradients, and has a repeat length of about 375 base pairs (bp). A cloned member of the repeat family hybridises exclusively to chromosome telomeres and has a non-random distribution in interphase nuclei. We present the nucleotide sequences of three repeats, which differ at a large number of positions. In addition to arrays made up of 375-bp repeats, homologous sequences are found in units with a greater repeat length. This divergence between repeats reflects the heterogeneity of the satellite determined using other criteria. Possible constraints on the interchromosomal exchange of repeated sequences are discussed.

Introduction

Satellite DNAs have been described in a wide range of animal genomes, where they appear to be the primary components of constitutive heterochromatin. Their occurrence and sequence organisation in plant genomes are less well established. Just as plant heterochromatin has proved somewhat refractory to many of the cytological techniques developed with animal material, so too have satellite DNAs proved elusive. Characterisation of satellites from the monocots has so far been limited largely to in situ hybridisation analysis (Bennett et al. 1977; Flavell et al. 1981; Deumling and Greilhuber 1982; Jones and Flavell 1982), with analysis of cloned sequences restricted to the Gramineae (e.g., Bedbrook et al. 1980). Several workers have stated that *Allium cepa* lacks any satellite components (Stack and Comings 1979; Ingle et al. 1973). In this paper we describe the organisation and nucleotide sequence of a satellite DNA, isolated from the genome of *A. cepa*, which has a periodicity of 375 base pairs (bp). The satellite displays a considerable degree (about 10%) of sequence divergence between copies within the same genome. The chromosomal location of these sequences and their nuclear distribution at various stages of the mitotic cycle are also described.

Materials and methods

Allium cepa bulbs were obtained commercially. DNA isolation, restriction enzyme digestion, Southern blot analysis, and nick translation were all carried out as described previously (Evans et al. 1983).

Isolation of fragments from agarose gels. Restricted DNA was subjected to electrophoresis, stained with ethidium bromide, and visualised using long-wave UV light. The desired bands were transferred by electrophoresis onto a strip of Whatman DE81 paper, which was then rinsed with 10 mM Tris, 1 mM ethylenediaminetetraacetate (EDTA), 100 mM NaCl, pH 8. DNA was eluted in 10 mM Tris, 1 mM EDTA, 1 M NaCl, pH 8, and concentrated by ethanol precipitation.

DNA cloning. Bam HI-digested *A. cepa* DNA was ligated into the Bam HI site of pAT153 (Twigg and Sherratt 1980) using T4 DNA ligase, and transformed into *Escherichia coli* strain HB101. Transformants were screened (Grunstein and Hogness 1975) with nick-translated 375-bp fragments that had been isolated from a Bam HI digest of *A. cepa* DNA. Positive colonies were used as sources of satellite repeats for subsequent cloning into M13 mp8 or mp9, which was transformed into *E. coli* JM103.

Hoechst 33258-CsCl and actinomycin D-CsCl density gradient centrifugation. Hoechst 33258-CsCl gradients were prepared essentially as described by Manuelidis (1977). A solution of *A. cepa* DNA (30–50 μg/ml) was prepared in 0.17 M NaCl, 3.4 mM KCl, 4 mM Na_2HPO_4, 2.4 mM KH_2PO_4, 0.5% N-lauryl sarkosine, to which Hoechst 33258 (stock solution 100 mg/ml) was added to give a 1:1 (weight:weight) ratio of Hoechst 33258:DNA. Solid caesium chloride (Analar, BDH) was added to produce an initial density of 1.630 g cm^{-3}. Centrifugation was at 40000 rpm for 20 h in the VTi65 or 28 h in the VTi50 rotor (Beckman). Gradients were photographed under ultraviolet (UV) illumination, and fractionated from the bottom by piercing.

Actinomycin D-CsCl gradients were prepared as described by Barnes et al. (1978), and centrifuged in the Beckman VTi65 rotor for 20 h at 40000 rpm.

Antibiotics were removed from DNA-containing fractions by repeated extractions with CsCl-saturated isopropanol; CsCl was removed by dialysis.

DNA reassociation, thermal elution, and dot hybridisation. *Allium cepa* DNA was sonicated to an average length of 400 bp, denatured by boiling for 10 min, and reassociated in 0.12 M orthophosphate buffer (PB) pH 6.8 at 60° C to a Cot of 1000. Reassociated DNA was loaded onto water-jacketed columns of hydroxyapatite (Biorad) equilibrated with 0.12 M PB at 60° C. Single-stranded DNA was washed off with 0.12 M PB. A similar amount of native, sonicated *A. cepa* DNA was loaded onto an adjacent hydroxyapatite

column; the waterjacket temperature was raised by 3°–5° C increments, and DNA denatured by this rise in temperature was eluted from each column with 0.12 M PB. The amount of DNA eluted with each rise in temperature was measured (using OD_{260}); aliquots from each fraction were ethanol precipitated, redissolved in 5 µl 1 M NaCl, 0.1 M NaOH, 10 mM EDTA. Samples were then heated at 100° C for 5 min and then spotted onto nitrocellulose (BA85, Schleicher and Schuell). After drying at room temperature, the filter was baked in vacuo at 80° C for 2 h. Filters were prehybridised in 4 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate), 0.1% sodium dodecyl sulphate (SDS), 0.1% ficoll, 0.1% polyvinylpyrollidone, 0.1% bovine serum albumin at 65° C for several hours prior to overnight hybridisation with ^{32}P-labelled cloned satellite probe in the same solution. Filters were rinsed twice in 4 × SSC, 0.1% SDS at 65° C (20 min each), and then twice in 2 × SSC (room temperature), before autoradiography using preflashed X-ray film. Autoradiographs were quantified using a Helena scanning densitometer.

DNA sequencing. Sequencing was performed by the dideoxynucleotide chain terminator method of Sanger et al. (1977) using a 15-bp oligonucleotide primer and α-^{35}S-dATP (NEN). The products of the sequencing reactions were separated using buffer gradient gels (Biggin et al. 1983).

Cytological techniques. Root tip squashes from commercially obtained *A. cepa* bulbs were prepared as described by Jamieson et al. (1985). Roots were pretreated with colchicine, fixed in acetic/ethanol (1/3), softened in a pectinase-cellulase mixture and squashed in 45% acetic acid on slides coated with Denhardt's solution (Brahic and Haase 1978). Coverslips were removed by a modification of the dry ice method (Conger and Fairchild 1953). Suitable squashes were identified using phase-contrast microscopy.

In situ hybridisation was based on the method of Pardue and Gall (1975). Slides were incubated for 2 h at 37° C in 100 µg/ml preboiled pancreatic RNase in 2 × SSC; they were then rinsed in 2 × SSC, dehydrated through an ethanol series, and air dried.

Chromosomal DNA was denatured by placing the slides in 3 × SSC/50% deionised formamide at 70° C for 30–40 s. Slides were put through an ice-cold ethanol series, and air dried.

Tritiated plasmid (pAc 8009) (specific activity 1.6×10^6 cpm/µg) dissolved in the hybridisation solution (3 × SSC/50% deionised formamide, 200 µg/ml sheared herring sperm DNA) was denatured in boiling water for 10 min and cooled rapidly on ice. A 9-µl sample of probe solution (150000–200000 cpm) was placed on each slide under a siliconised coverslip and incubated at 39°–40° C for 14–16 h in a Petri dish containing filter paper soaked in 3 × SSC/50% formamide. Coverslips were removed by rinsing the slides in an excess of 2 × SSC. Slides were then washed three times in 2 × SSC at 37° C (5 min each), once in 1 × SSC at 60° C (15 min), twice in 2 × SSC at 37° C (30 min each), five times in 2 × SSC at room temperature (10 min each), and finally dehydrated through an ethanol series.

Autoradiography was carried out as described by Rogers (1979). Slides were dipped in a 50% solution of Ilford K2 nuclear emulsion and exposed for 7–28 days at 4° C. Autoradiographs were developed in Kodak D19 for 4 min at 15° C, rinsed in distilled water and fixed in 30% sodium thiosulphate. After being stained with Giemsa Gurr R66 the slides were rinsed and mounted in DPX. Photography was carried out using Ilford FP4 film.

Results

Density gradient centrifugation of A. cepa DNA

Previous workers have failed to identify satellite components in *A. cepa* DNA by centrifugation to equilibrium in neutral CsCl gradients. We have made use of the sequence-specific DNA-binding compounds, Hoechst 33258 and actinomycin D, to resolve satellite DNAs. Actinomycin D binds specifically to cytosine residues in the conformation 5′GpC 3′ (Sobell 1973), and reduces the buoyant density of molecules containing such sequences. The distribution of the *A. cepa* DNA in such a gradient is clearly skewed, but the resolution is not sufficient to permit the identification of individual satellite bands (data not shown, but see Fig. 3).

Figure 1 shows the results of Hoechst 33258-CsCl centrifugation of *A. cepa* DNA. With this dye, which preferen-

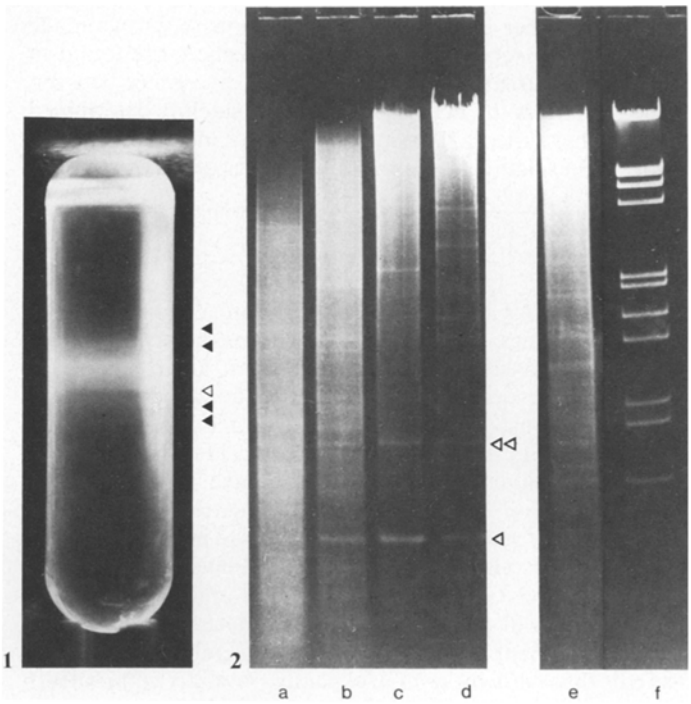

Fig. 1. Hoechst 33258-CsCl gradient of *Allium cepa* DNA. The gradient was prepared as described and the DNA visualised by fluorescence under ultraviolet light. Several satellite bands are visible above and below the main band (*closed arrowheads*). Other bands are clearly distinguishable, despite being of similar density to main band DNA (*open arrowhead*)

Fig. 2a–f. Restriction enzyme digestion of *Allium cepa* DNA. Equal amounts (approximately 5 µg) of *A. cepa* DNA were digested to completion with various restriction enzymes, and subjected to electrophoresis in a 1.2% agarose gel. DNA was visualised by fluorescence after staining with ethidium bromide. Digestion was with Alu I (**a**), Hae III (**b**), Eco RV (**c**), Bam HI (**d**), and Hind III (**e**); **f** contains a marker DNA-bacteriophage λ DNA, digested with Eco RI and Hind III. Satellite monomer (*single arrowhead*) and dimer (*double arrowhead*) bands are clearly visible in several digests

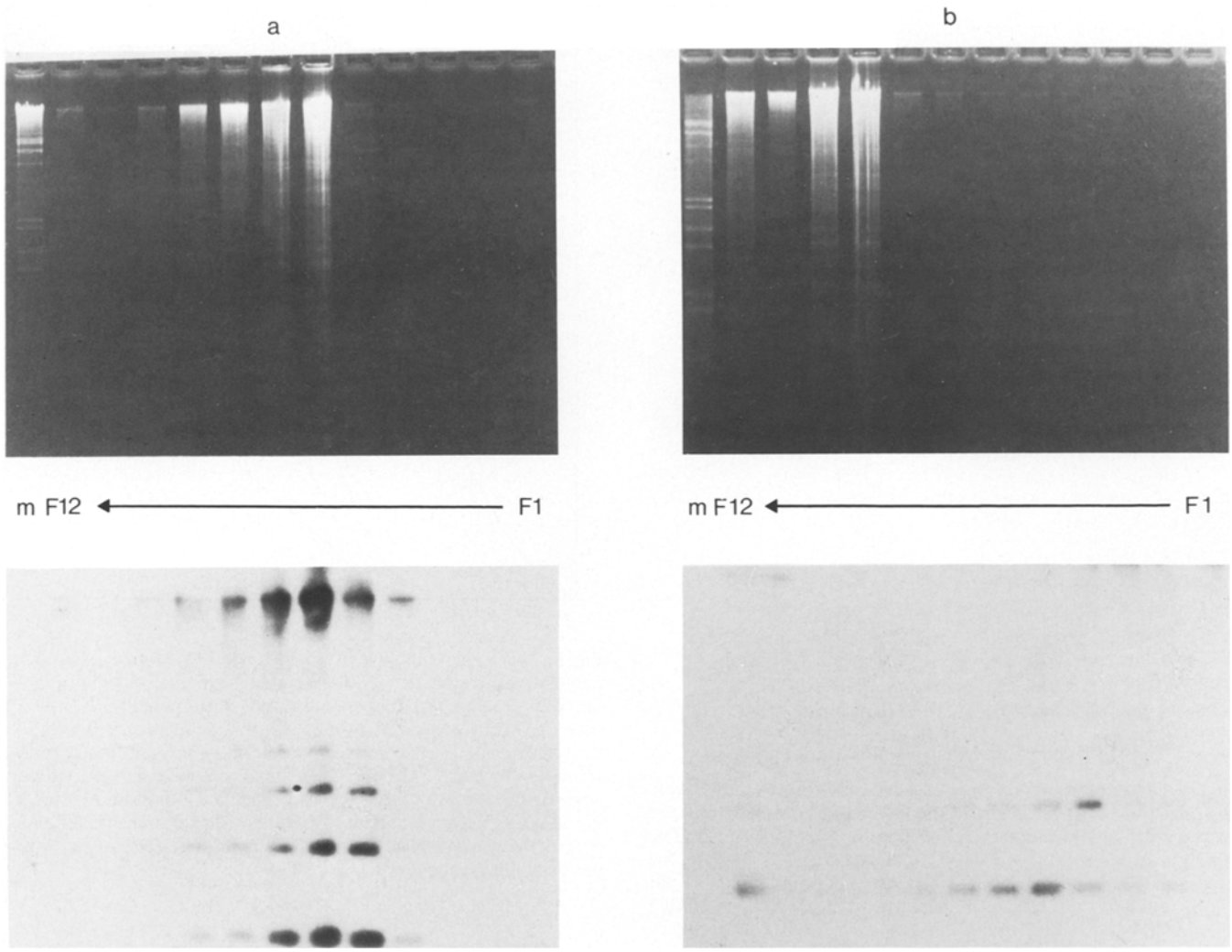

Fig. 3a, b. Restriction enzyme analysis of density gradient fractions. DNA from fractions of Hoechst 33258 (**a**) and actinomycin D (**b**) gradients was digested with Bam HI and subjected to agarose gel electrophoresis (*upper half* of figure) and Southern blotting. The nitrocellulose-bound DNA was probed with nick-translated pAc8009 (a clone that includes a Bam HI-generated dimer of the satellite). Autoradiographs are shown in the *lower half* of the figure. *m* marker DNA (see Fig. 2). *F1* → *F12* fractions 1 to 12 of the density gradients

tially binds to AT-rich sequences, the DNA may be visualised by its fluorescence under illumination with UV light. It is clear that there are several "satellite" components (arrowed) above, below, and within the main band, which may include cytoplasmic DNAs (although the DNA extraction procedure used minimises contamination by mitochondria and chloroplasts).

Identification of satellite components by restriction enzyme digestion

When *A. cepa* DNA is digested with various restriction enzymes, certain prominent bands can be seen that are common to several digests. In particular, a strong band is seen at around 370 bp in digests with Bam HI, Eco RV and Hae III (Fig. 2, lanes b–d). In some of these digests, a dimer-length band is also seen, suggesting that these sequences may be arranged in tandem. To determine whether they represent one of the satellite components seen by density gradient centrifugation, representative 370-bp fragments were cloned from Bam HI-digested *A. cepa* DNA into pAT153. The cloned DNA was nick translated and used to probe Southern blots of Bam HI-digested fractions from antibiotic-CsCl gradients such as that shown in Figure 1. The results are shown in Figure 3. Hybridisation is clearly to a "ladder" of fragments, based upon a 370-bp monomer. The homologous sequences are resolved, to differing extents, from the main band of DNA in each of these gradients. Fractions 4–6 of the actinomycin D gradient contain the satellite DNA. In the actinomycin D gradient there is some hybridisation to DNA in fraction 12, suggesting that some homologous sequences may have a density that differs from bulk satellite; we have no clear explanation for this finding.

The organisation of satellite sequences in A. cepa

To investigate the higher-order repeat organisation of this satellite component in the *A. cepa* genome, the gel shown in Figure 2 was blotted onto nitrocellulose, and probed with nick-translated cloned satellite sequences. The results are shown in Figure 4. In all digests hybridisation occurs to

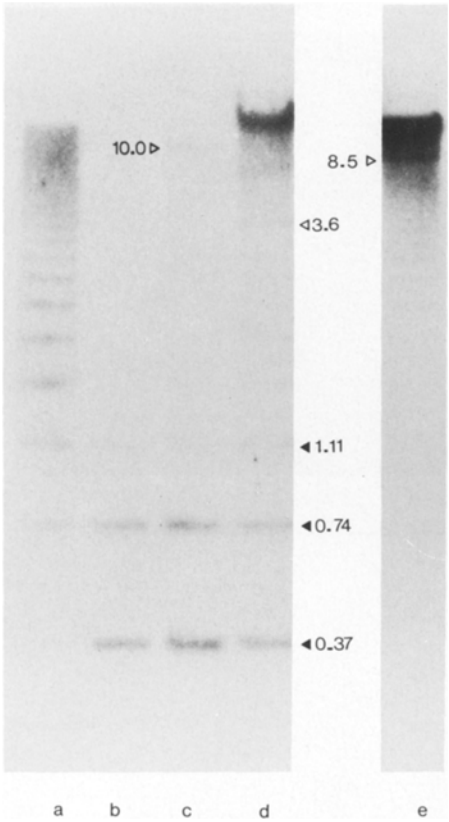

Fig. 4a–e. Southern blot analysis of satellite sequences. DNA from the gel shown in Figure 2 was transferred to nitrocellulose and probed with nick-translated cloned satellite DNA (see legend to Fig. 3). DNA was digested with Alu I (**a**), Hae III (**b**), Eco RV (**c**), Bam HI (**d**), and Hind III (**e**). The first three bands in the 370-bp satellite 'ladder' are indicated (*closed arrowheads*). Other prominent bands of hybridisation are identified by *open arrowheads*. Sizes are in kilobase pairs

a ladder of fragments whose lengths are multiples of around 370 bp. The patterns seen with the different restriction enzymes show varying degrees of digestion into monomer-length fragments, even though the DNA seems to be digested to completion. For example in the Alu I digest only a minority of the satellite sequences are present in the monomer band (Fig. 4a). By contrast, the vast majority of the satellite is cleaved by Eco RV (Fig. 4c) to yield monomers and oligomers of the basic satellite repeat.

In addition to the series of multimers seen in each digest, several other bands are visible. In particular, high-molecular-weight bands can be distinguished in digests with Eco RV (10.0 kb) (Fig. 4c), Bam HI (3.6 kb) (Fig. 4d), and Hind III (8.5 kb) (Fig. 4e). Most of this satellite is not cleaved by Hind III although there is some banding in a 370-bp register.

Quantification of hybridisation such as that seen in Figure 4 permits an estimate of the proportion of the *A. cepa* genome that comprises this satellite. On the basis of ethidium fluorescence, the monomer band constitutes about 1.6% of the DNA in an Eco RV digest; the same band accounts for 36% of the hybridisation to a blot of the same digest. This suggests that satellite sequences make up $1.6/36 \times 100\%$ of the *A. cepa* genome, or 4.4%. This estimate, whilst extremely crude, gives some indication that this sequence is a major component of the genome.

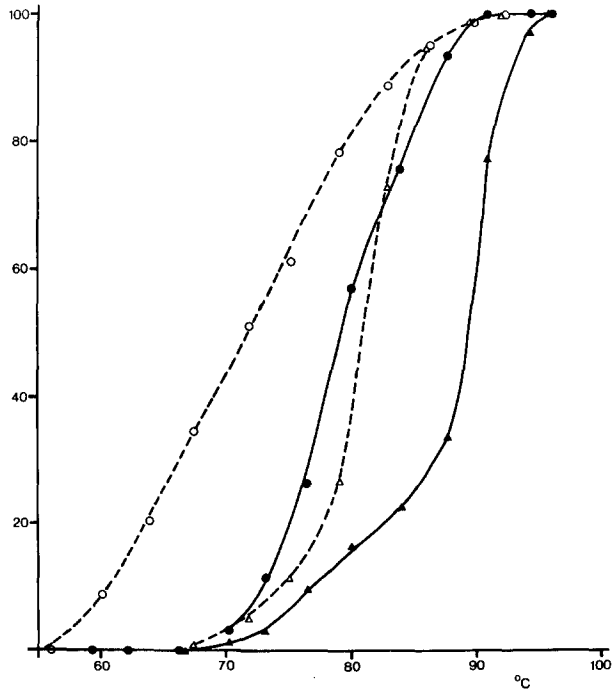

Fig. 5. Thermostability of native and renatured satellite DNA. The cumulative proportion of DNA eluted from hydroxyapatite columns by 0.12 M orthophosphate buffer with increasing temperature (*ordinate*) is plotted against elution temperature (*abscissa*). Bulk native DNA elution was monitored by optical density (*open triangles*), as was the elution of renatured repetitive sequences (*open circles*). The elution of satellite sequences was monitored by probing aliquots from each eluted fraction with radiolabelled satellite probe: (*closed triangles*) native satellite, and (*closed circles*) renatured satellite sequences

Sequence divergence of satellite sequences in A. cepa DNA

To ascertain the degree of heterogeneity of satellite repeats, thermal denaturation studies were carried out with native DNA and DNA that had previously been denatured and allowed to reassociate. The DNA was bound to hydroxyapatite and eluted at increasing temperatures. The proportion of DNA eluting with each increment was determined by optical density; the proportion of satellite eluted was measured by hybridisation of cloned satellite to aliquots from each elution fraction that had been immobilised on nitrocellulose filters. By using this method we could quantify the heterogeneity of *all* homologous satellite repeats *with respect to one another*, rather than with respect to the particular cloned probe used. Figure 5 shows that satellite sequences are relatively thermostable, suggesting a relative preponderance of GC base pairs, when compared with total *A. cepa* DNA. After renaturation they show a decrease in melting temperature (ΔT_m) of 8.5° C, suggesting a heterogeneity of about 8.5% (Bonner et al. 1973). This is slightly greater than the heterogeneity of repeated sequences as a whole (judged from optical density, $\Delta T_m = 7.4°$ C).

Nucleotide sequence of A. cepa satellite

Figure 6 shows the nucleotide sequences of three typical satellite repeating units. As might be expected from the results presented above and from the heterogeneity in restriction sites seen in Figure 4, these monomers are not

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Clone                                                       50                                                        100
8013  TTTTACAGAA AAAATCCACG TGACGAAAAA ACGAAGGGTA AAAAACTTCC ATGACCCGAT TGAGGATC G GATAGGATTT TTTGGGTACG AAACTAACCA
8062  TTTTACAGAA AAAATCCACG TGACGAAAAA ACGAAGGGTA AAAAACTTAC TTGACCCGAT TGAGGTTCCA GATAGGATTT TTTGGGTATG AAACTAACCA
8009  TTTTACAGAA AAAATCCACG TGACGAAAAA ACGAAGGGTA AAAAACTTTC ACGACCCGAT TGAGGTT  G GATAGGCTTT TTTGGGTATG AACGTGACTA
                                                         *  **                * ***     *               *     ** *  *

                                                            150                                                       200
8013  GTGTCGGCTG AAAGCTGTCA TA  GCTGTA GAA CTAATC GAT TCTTCA TTTCGACAGG TTA TGGCTC C GTAACTCT AAACGGGTCC AAGAGTTATG
8062    TGACAGCTG AAACG GTCA TA  GCTGTA GA  CTCACA GATA CTTCG TTTTGACTGG TTA TGGCTC C GTAAATCT AAACGAG CC AAGAGTTATG
8009  GTGACTGCTG AAAGCTTTCA TTACGCTGTA GAAGCTCATG AGTACCTTCG TTTTGACTAG TTAGTGGCTC CCGTAAATCT GGAGGATGCC TAGAGTTATG
       *  *  *        ****    ***        **  * ** ** **    *     *   **   *         *   *     ** * ***   *

                                                            250                                                       300
8013  GCCCTCCGAA GAATC GATA TCCACATGGC GCAA   CTA CGGTGCA GG TCGCAACATC CG TCCAAAA TAACGAAATA GT GTCGT    ACGACTTTTA
8062  GCCCTCCGAA GAGTC GATA TCCACGGGT  GCAACATCTA CGGTGCAAGG T GCAACATC CG TCCAAAA TGACGAAACA GTTTGCGTAG ACGACCTTTA
8009  GCCCTCCGAA GAATCGGATA TCCACAAGGT GCAACATCTA CGGTGGAAGG T GCAACATC CGGTCCAAAA TAACGAAACA GT GCCGT    ACGACCTTTA
                    *  *          ** **     ***        * *   *          *          *     *    ***   **        *

                                                            350                                           394
8013  GAAATG GTG CACAGATTTG AAAC    GTT GCAGTACCAT  GGTTCACCA CTCTCCAGCG ATGGTAAATC ATAGACC GG CCACGGGGAT CCCG
8062  GAAATGAGTG CATGGATTTG AAACACCTCT GCAGTACCAT CGGTGCACCA CTCTCCAGCG ATGGTAAATC GTACGCCTGG CCACGGGGAT CCCG
8009  GAAATGAGTG CATGGATTTG AAACA    TT GCAGTACCAT  GGTGCACAA CTCTCCAGCG ATGGTAAATC ATAGACCA G CCACGGGAAT CCCG
             *        **          *****         *    *    *                     *  **  **
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Fig. 6. Nucleotide sequences of three repeats of the *Allium cepa* satellite DNA. DNA sequences were determined by the Sanger et al. (1977) dideoxynucleotide method, and are aligned to give the greatest number of matches. Positions at which the repeats differ are identified by *asterisks*

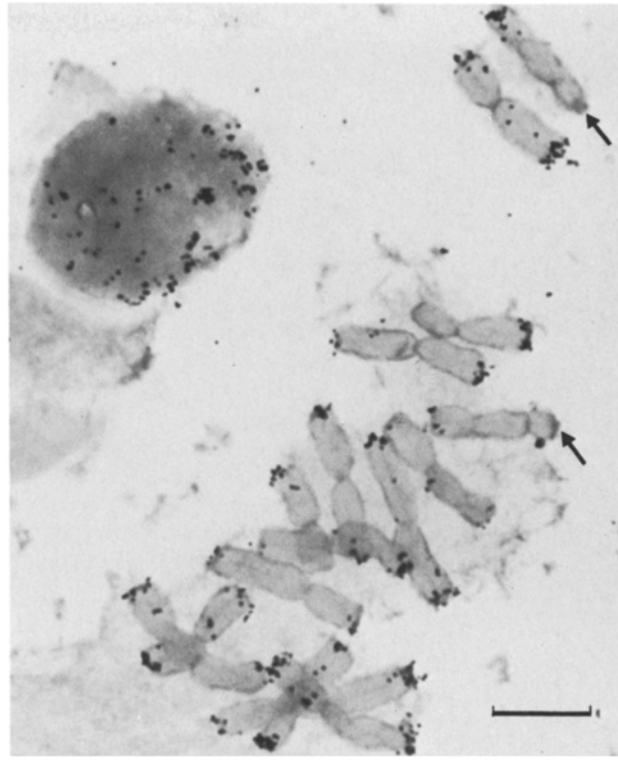

Fig. 7. Hybridisation of the cloned satellite sequence to *Allium cepa* chromosomes. *Arrows* indicate the telomeres of the short arms of homologous pair 8, to which there is no significant hybridisation of the satellite probe. In this chromosome spread the telomeres of the short arms of homologous pair 7 are unlabelled; after prolonged exposure, these telomeres show limited hybridisation to this probe. Exposure was 28 days. Bar represents 10 μm

identical. The sequence of each is around 375 bp long although individual repeats have differences, which lead to some length variation. There are no strong internal direct or inverted repeats, apart from several very AT-rich regions, which also appear to be relatively less variable between repeats. The repeating unit contains 45.8% G+C, compared with 34.6% for the genome as a whole (Kirk et al. 1970). The sequences shown are of Bam HI-generated repeats, and therefore represent a non-random subset of all satellite repeats.

The distribution of satellite sequences on metaphase chromosomes

Tritiated pAc8009 was hybridised in situ to metaphase chromosomes. Autoradiographs developed over a range of exposure times show that most telomeres have DNA with significant homology to the probe sequence (Fig. 7). Only two telomeres, those assocated with the short arms of chromosomes with the major secondary constrictions, do not hybridise with this probe.

There was no consistent hybridisation of the probe to interstitial or centromeric heterochromatin located by C-banding (Vosa 1976).

The distribution of satellite sequences in interphase nuclei

Silver grains on relatively heavily labelled nuclei are clustered and often clearly polarised towards one side of the

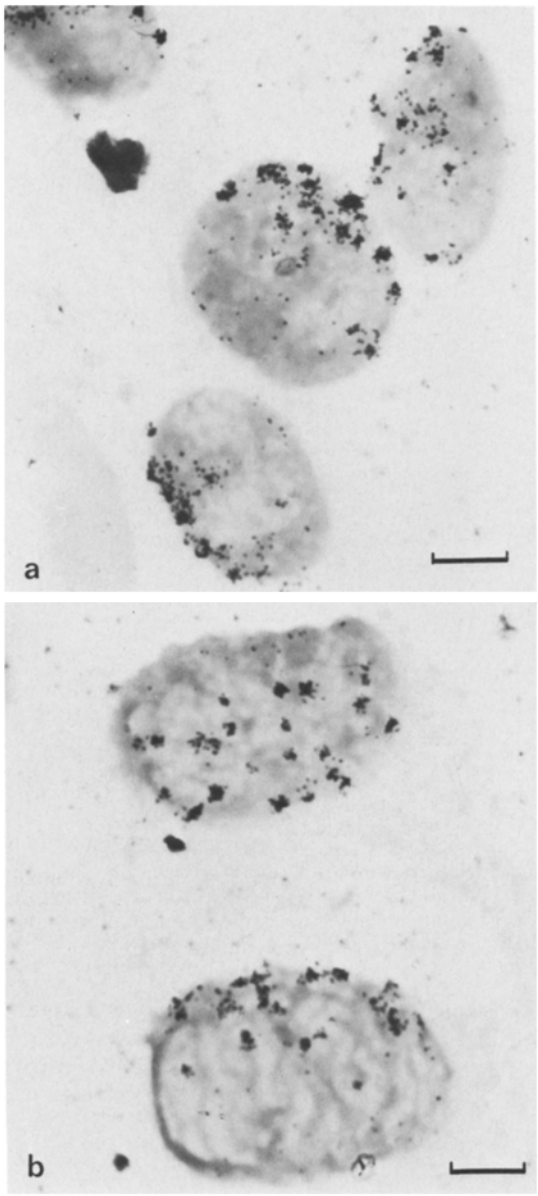

Fig. 8a, b. Hybridisation of cloned satellite sequences to *Allium cepa* nuclei. **a** Interphase nuclei. The clustering of telomeric hybridisation towards one pole of the nucleus is clearly visible. **b** Late telophase nuclei. The telomeres are distributed in similar fashion to those at interphase. Exposure was 28 days. Bar represents 10 μm

nucleus (Fig. 8a, b); these features are shared with the distributions of prominent C-bands and late replicating DNA, described by Stack and Clark (1973) and Fussell (1975). While metaphase chromosomes show 30 regions of hybridisation to the tritiated probe, interphase nuclei show approximately 15 clusters of hybridisation in heavily labelled nuclei (data not shown). This concurs with the results of Fussell (1975), who found a similar number of clusters when labelling late replicating DNA, and may indicate telomere pairing.

Discussion

In this paper we present the detailed structure of a satellite DNA component from *A. cepa*. The satellite may be resolved either on the basis of its buoyant density in antibiotic-CsCl gradients, or as a band produced by restriction enzyme digestion. The organisation and nucleotide sequence of the *A. cepa* satellite show good agreement with the results of similar studies on animal satellite DNAs. The fact that previous workers have failed to identify satellites in many monocots, including *A. cepa*, thus probably indicates differences in the techniques used, rather than a fundamental difference between plant and animal genomes. The satellite repeats are clearly heterogeneous. This is seen in the reassociation-remelting studies, which suggest a sequence divergence of about 8.5%. The rationale behind this experiment allows an estimate of average difference between satellite repeats, since the repeats are reassociated at random with one another, and later identified by homology with a cloned probe. It should be stressed that we are not measuring the degree of divergence from the cloned probe sequence itself. It must also be appreciated that 8.5% represents the *average* heterogeneity between copies; individual pairs of repeats may differ by substantially more than this figure.

The pattern of this satellite's cleavage by restriction enzymes also leads to the conclusion that not all repeats are identical. The results obtained closely mirror those found with animal satellites, in which some restriction sites are found in most repeats (producing a "type A" pattern), whereas others are found in a minority of the repeating units (a "type B" pattern) (Horz and Zachau 1977; Singer 1982). For the *A. cepa* satellite, EcoRV and Hae III give type A patterns of digestion, suggesting that sites for these enzymes were present in ancestral repeats. Bam HI cleaves only a subset of the repeats; the size distribution of Bam HI-digested satellite fragments indicates that these sites are not distributed at random throughout the satellite arrays. The ratio of oligomers to high-molecular-weight material suggests that Bam HI sites are clustered. Alu I sites appear to be distributed fairly randomly throughout the satellite, whereas Hind III sites are apparently rare. It is not clear from these results whether the segments of satellite cleaved by each enzyme (i.e., the Bam HI segment and the Hind III segment) represent arrays on different chromosomes; such an arrangement has been observed in animal genomes (Cooke and McKay 1978; Beauchamp et al. 1979).

An unusual feature of the satellite's organisation is seen in Hind III and Eco RV digests (Fig. 4). In each case prominent bands can be seen, equivalent to approximately 8.5 kb and 10 kb, respectively. Thus there are long stretches of satellite-containing DNA that lack the sites for these endonucleases. Such sequences may represent segments of the satellite that have an altered repeat length. Alternatively, they may be clusters of repeats (all lacking the site concerned) that are embedded in regions of the genome susceptible to those enzymes. Similar results have been found in other plants (Bedbrook et al. 1980); clusters of satellite-like DNA have been observed in single-copy environments in animal genomes (Lam and Carroll 1983).

The heterogeneity in satellite sequence observed by nucleotide sequencing is in good agreement with the other results presented in this paper. Such heterogeneity has been observed in a wide range of satellite DNAs (Miklos and Gill 1981; Miklos 1982; Strachan et al. 1982), and should not be seen as surprising. The observations that changes are non-randomly distributed within the repeating unit are similar to those seen elsewhere. This apparent non-randomness has led several workers to suggest that the satellite

sequences evolve in concert ("concerted evolution"). A variety of mechanisms have been put forward to account for repeated sequence homogeneity, a consequence of non-random change (Smith 1976; Dover 1982). Of these, the unequal exchange model of Smith (1976) provides the simplest and most attractive mechanism for the generation of homogeneity among tandemly organised repeats such as those described here. It is also possible that unequal exchange mechanisms could account for homogeneity between arrays on different chromosomes although the rate of homogenisation would depend upon the frequency of exchange between arrays on non-homologues. In *A. cepa* there are 15 arrays of this satellite.

Our observation that telomeres bearing satellite sequences are associated during interphase suggests that these satellite elements from different arrays might interact with one another for a substantial part of each cell cycle. Both Flavell (1982) and Arnheim (1983) have suggested that the proximity of chromosomal regions may be an important factor in the interchromosomal transmission of repetitive DNA. Clearly, both gene conversion and unequal crossing over require that the DNA helices involved are close to one another. The telomeres of non-homologues, which are closely associated in interphase nuclei, may thus have more homology than different regions from the same chromosome.

The degree of heterogeneity seen in the *A. cepa* satellite may reflect the likelihood that the sequenced repeats were derived from different arrays, if exchange between these arrays is an infrequent occurrence. We are currently testing this hypothesis by sequencing contiguous satellite repeats. The correspondence of satellite 'segments' to different chromosomal arrays has previously been recorded in animal genomes (e.g., Beauchamp et al. 1979). The demonstration here of close association between non-homologous satellite arrays makes it possible that sequences in separate domains within a chromosome (e.g., at opposite telomeres) may have less in common with one another than with sequences at one or more non-homologous telomeres. Fussell (1975) noted that *A. cepa* chromosomes are polarised within the nucleus (centromeres at one pole, telomeres at the other), effectively isolating centromeres from telomeres. This is in accord with the general observation that while some repeat families are primarily telomeric, others are centromeric (Flavell 1982).

Factors influencing the association of non-homologues are unclear although Bennett (1982) has suggested that it may occur on the basis of chromosome arm length. The localisation of particular repeat families to regions in a chromosome complement may be constrained simply by nuclear organisation, rather than any major selective force.

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