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A repetitive sequence in *Nicotiana plumbaginifolia* contains an element homologous to the hypothetical replication origins of plant viruses

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Abstract. Twelve genomic DNA fragments displaying autonomous replicative activity in yeast have been cloned from *Nicotiana plumbaginifolia*. One of them interacted specifically with nuclear factor(s) from young leaves of this plant, at a region containing 9 copies of tandem repeated sequence. This repeated sequence appeared at many locations in the nuclear genome of this plant, and contained a 21-bp element sharing 70% homology with the hypothetical replication origins of three viruses of *Nicotiana* spp. Two copies of this 21-bp element are overlapped with a 10-residue palindromic sequence. The possible biological roles of this repeated sequence and of the DNA-binding nuclear factor(s) are discussed. The application of this repeated sequence in the study of this plant genome is proposed.

Key words: Autonomously replicating sequence; DNA-binding nuclear factor; *Nicotiana plumbaginifolia*; Replication origin; Tandem repeated sequence.

Introduction

Recent studies of DNA replication have showed that eukaryotic chromosomes initiate replication from multiple sites, called origins (Campell, 1986; Umek *et al.*, 1989; Diffley and Stillman, 1990). Due to the observation that the length of the S phase varies from tissue to tissue of the same organism, it has been proposed that tissues with a shorter S phase utilize more replication origins than those with a longer S phase (Diffley and Stillman, 1990). To examine this hypothesis and to determine what causes the variation in origin usage during the development of eukaryotes, the isolation of replication origins from eukaryotes becomes crucial.

In lower eukaryotes, such as *Saccharomyces cerevisiae,* various autonomously replicating sequences (ARS), which allow bacterial plasmids containing these elements to replicate autonomously in yeast, have been isolated (Struhl *et al.*, 1979; Hsiao and Carbon, 1979; Stinchcomb *et al.*, 1979; Stinchcomb *et al.*, 1980; Tschumper and Carbon, 1980). These elements consist of an 11-base pair (bp) core consensus sequence essential for ARS activity (Broach *et al.*, 1982) and flanking regions auxiliary to the function of ARS (Kearsey, 1984; Eisenberg *et al.*, 1988). Proteins (or genes encoding proteins) bound to the flanking (Eisenberg *et al.*, 1988; Sweder *et al.*, 1988; Halfter *et al.*, 1989; Diffley and Stillman, 1989) and the core sequences (Kuno *et al.*, 1990; Hofman and Gasses, 1991; Schmidt *et al.*, 1991) of yeast ARSs have been identified. Some yeast ARSs were reported to encompass the replication origins of their native chromosomes (Brewer and Fangman, 1987; Huberman *et al.*, 1987). In terms of timing during S phase, differential activation among yeast ARSs has been observed (Ferguson *et al.*, 1991). All of these reports indicate that the availability of clones contain-

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ing replication origins makes it possible to study the initiation of DNA synthesis in yeast at the molecular level.

Fragments capable of replication in yeast have also been cloned from various multicellular higher eukaryotes (Stinchcomb *et al.*, 1980; Uchimiya *et al.*, 1983; Montiel *et al.*, 1984; Overbeeke *et al.*, 1984; Marunouchi *et al.*, 1987; Berlani *et al.*, 1988). Sequence analysis showed that they contained elements similar to those in the core sequence of yeast ARS. There is no evidence, however, to show that they can promote the autonomous replication of plasmids in the cells from which they were isolated (Uchimiya *et al.*, 1986; Jongsma *et al.*, 1987). Nor was protein from their native cells found to interact with these DNA fragments. Therefore, it is still uncertain whether the replication origin in higher eukaryotic genome would have sequence components similar to those in yeast ARS.

In this communication, we describe the isolation from *Nicotiana plumbaginifolia* of twelve genomic DNA fragments capable of replication in yeast. One of these clones interacted specifically with the nuclear factor(s) from young leaves of *N. plumbaginifolia*. The region interacting with plant nuclear factor(s) contained nine copies of tandem repeated sequence, with a repeating unit of 52 bp. From the third repeating unit on, a 21-bp element in each unit was oberved to share about 70% homology with the proposed replication origins of three plant viruses, including cassava latent virus (CLV, Hamilton *et al.*, 1984), tomato golden mosaic virus (TGMV, Hamilton *et al.*, 1984), and beet curly top virus (BCTV, Stenger *et al.*, 1991). The possible roles of this repetitive sequence and the DNA-binding nuclear factor(s) are discussed.

Materials and Methods

Medium and Solution

1x DNA-binding buffer contained 40 mM Tris. HCl pH 7.5, 20% glycerol, 100 mM KCl, 0.2 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. Filter elution buffer contained 20 mM Tris. HCl pH 7.5, 0.2% sodium dodecylsulfate, and 0.3 M sodium acetate. SD medium for plates contained 0.67% bacto-yeast nitrogen base without amino acids, 2% dextrose, and 2% bacto-agar.

Bacterial and Yeast Strins

Escherichia coli HB101 (supE44, hadS20, (rB-mB-), recA13, ara-14, proA2, LacY1, galK2, rpsL20, xyl1-5 mtl-1) was used for propagating plasmids. *E. coli* JM105 (-lac pro, thi, strA, endA, sbcB15, hsdR4, F′ traD36, proAB, LacIqZ-M15) was used as host for bacteriophage M13 sequencing vectors. *Saccharomyces cerevisiae* BWG1-7A (MATa, adel-100, leu2-3, leu2-112, ura3-52, his4-519) was used to select tobacco DNA which could promote the autonomous replication of YIp5 in yeast.

Isolation of DNA Fragments from ***N. plumbaginifolia*** *Displaying ARS Activity In Yeast*

Genomic DNA with high molecular weight was prepared from the leaves of *N. plumbaginifolia* (Richards, 1989), partially digested with EcoRI, and fractionated by 10%-40% sucrose gradient ultracentrifugation at 26,000 rpm (Hitatchi RPS40T rotor) for 24 hours. Fractions containing DNA with sizes ranging from 2 kilobase pairs (kb) to 12 kb were pooled together, precipitated with ethanol, and then resuspended in sterile water. This DNA was ligated with EcoRI-cut and dephosphorylated YIp5 (Struhl *et al.*, 1979), transformed into yeast BWG1-7A by the LiCl method (Sherman *et al.*, 1986), and then selected for Ura+ yeast transformants on plates containing SD medium supplemented with adenine sulfate, leucine, and histidine (Sherman *et al.*, 1986). Total DNA was prepared from each Ura+ transformed yeast (Treco, 1989) and then transformed into *E. coli* HB101 to select AmpR colonies. Plasmids were prepared from AmpR HB101 by the alkaline lysis method (Sambrook *et al.*, 1989), and inserts in the plasmids were analyzed by restriction enzyme digestion followed by gel electrophoresis to construct the restriction map of cloned plant DNA.

Filter-Binding Assay

Nuclear extracts were prepared from young leaves of *N. plumbaginifolia* as described (Giuliano *et al.*, 1988), adjusted to 0.1 mg/ml and stored at -70°C. Filter-binding assay was used to examine the interaction between nuclear extract of *N. plumbaginifolia* and cloned DNA (Papoulas, 1989). Each 50-μl reaction mixture, containing 1x DNA-protein binding buffer, 10 μg poly(dI-dC), and 0.5 μg plant nuclear extract or 10 μg bovine serum albumin (BSA), was preincubated at room temperature for 15 minutes and then 2 ng end-

labeled 32P-DNA probe was added. Reaction mixture was continuously incubated at 30°C for 30 minutes and then passed through a nitrocellulose filter disc (25 mm diameter). Filter was washed four times with 1 ml 1x DNA-protein binding buffer. The bound probe was eluted from the filter by soaking the filter in elution buffer at 30°C for 2 h with occasional shaking, precipitated by ethanol, and then analyzed by electrophoresis followed by autoradiography of the dried gel. For competition experiments, a 30-fold molar excess of cold, specific or non-specific DNA fragment was added at the begining of preincubation.

Nested Deletion of NPARS71H3a

To generate nested deletions from both ends of NPARS71H3a, fragment excised from the original clone pNPARS71 by HindIII digestion was subcloned into the HindIII site of YIp5 to get clones with two different orientations, pNPARS71H3a.1 and pNPARS71H3a.2. Each subclone was linearized with BamHI and SphI, treated with exoIII for various periods to generate a series of nested deletions, polished with S1 nuclease and Klenow fragment, religated to itself, and then transformed into HB101 to select AmpR colonies. Plasmids were miniprepared from the bacterial transformants and analyzed by appropriate restriction enzymes to determine the sizes of deleted fragments. Plasmids with appropriate deleted inserts were transformed into yeast BWG1-7A for ARS activity assay.

DNA Sequencing

Intact or deletion fragments of NPARS71H3a were subcloned from YIp5 into M13. Single-stranded DNA templates were prepared from M13 subclones for sequence determination using the dideoxy method and 35S-dATP (Sanger *et al.*, 1977).

Southern Hybridization

Five micrograms of plant genomic DNA were completely digested with EcoRI or HindIII, analyzed by gel electrophoresis, and then transferred to nitrocellulose filters (Sambrook *et al.*, 1989). Filters were baked at 80°C under vacuum for 2 h, hybridized with probe at 68°C for 1 day and then washed stringently as described by Sambrook *et al.* (1989). The filters were air-dried and then autoradiographed.

Results

Cloning of DNA Fragments from ***N. plumbaginifolia*** *Displaying ARS Activity in Yeast*

Twelve DNA fragments displaying ARS activity in yeast have been isolated from the genome of *N. plumbaginifolia*. Different restriction maps and no cross reaction in Southern blot analysis among the cloned DNA (data not shown) suggests that none of them are identical. Because filter-binding assay revealed that only NPARS71H3a interacted specifically with the nuclear extracts from tobacco leaf (Fig. 1a,b; Fig. 2) we decided to study this clone further.

Deletion Analysis of Clone NPARS71H3a

To define the minimum region of NPARS71H3a with which the vector YIp5 can replicate as an episome in yeast, nested deletions of this fragment in YIp5 were generated by exonuclease III treatment, religated, and then transformed into yeast to test for ARS activity (Fig. 2). Deletion analysis showed that a 160-bp subfragment (NPARS71H3a.25) is long enough to display ARS activity in yeast (Fig. 2). Sequence analysis showed than an 11-bp nucleotide (AAAACATAAAT, nucleotide No. 529-539 in Fig. 3a) in NPARS71H3a.25 was perfectly matched with the consensus sequence of yeast ARS (5′-(T/A)AAA(C/T)ATAAA(A/T)-′3).

Determination of Nuclear Factor(s)-Binding Region in NPARS71H3a

To identify the nuclear factor(s)-binding region in NPARS71H3a, various subfragments of this DNA were subjected to filter-binding assay. Only a 1.4-kb BglII-EcoRI subfragment of NPARS71H3a bound the plant nuclear factor(s) (Fig. 1c 1,2). Further analysis defined the nuclear factor(s)-binding region as a 550 bp subfragment called NPrptl (Fig. 1c 3,4, 1d and Fig. 2). NPrptl contained nine copies of 52-bp tandem repeated sequence (nucleotide No. 1122-1670 in Fig. 3a) and is about 600 bp away from the yeast ARS consensus sequence.

Homology Between an Element in NPrptl and the Hypothetical Replication Origins of Plant Viruses

To predict the possible role of this repetitive sequence in plant genome, sequence comparison was done and presented in figure 3b. From the third to the last repeating unit of NPrptl, each unit contained a

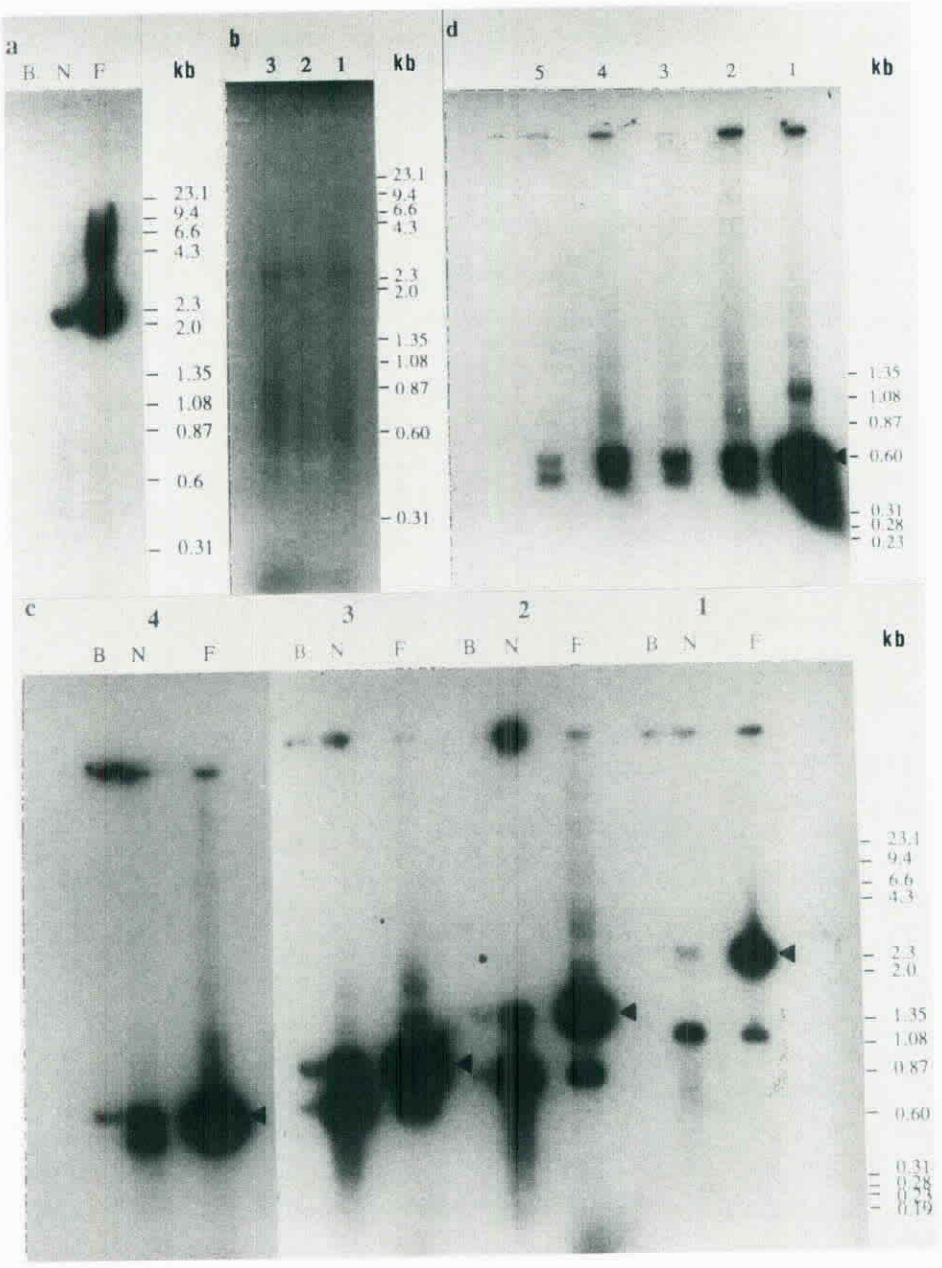

Fig. 1. Filter binding assay of NPARS71H3a.1 and its subfragments for binding tobacco nuclear factor(s). Various end-labeled subfragments of NPARS71H3a.1 were incubated with either tobacco nuclear extract (N) or BSA (B), in the absence or presence of competitor, filtered through nitrocellulose filter, recovered from filter, analyzed by gel electrophoresis, and then autoradiographed. **a)** The probe was 2.4 kb NPARS71H3a.1. F, probe alone was loaded on the gel. **b)** The same probe as that in a was incubated with plant nuclear extract without competitor (lane 1), with excess specific, cold NPARS71H3a.1 (lane 2), or with excess non-specific, cold NPARS31 (lane 3). **c)** Subfragments of NPARS71H3a.1 were used as probes for assay. These probes were 2.4 kb NPARS71H3a.1 (probe 1), 1.4 kb BglII-EcoRI fragment (probe 2), 900 bp BglII-NcoI fragment (probe 3), and 550 bp NPrpt1 (probe 4). *F*, *N*, and *B* have the same meaning as that described above. **d)** Competition assay of probe NPrpt1. Lane 1, probe alone was loaded to the gel; Lane 2-4, probes were incubated with plant nuclear extract without competitor (lane 2), with specific, cold NPrpt1 (lane 3), or with non-specific, cold NPARS31 (lane 4). Lane 5, probe was incubated with BSA. The lower band in each lane of **c** and **d** was probably due to the impurity from the end labeling of each probe.

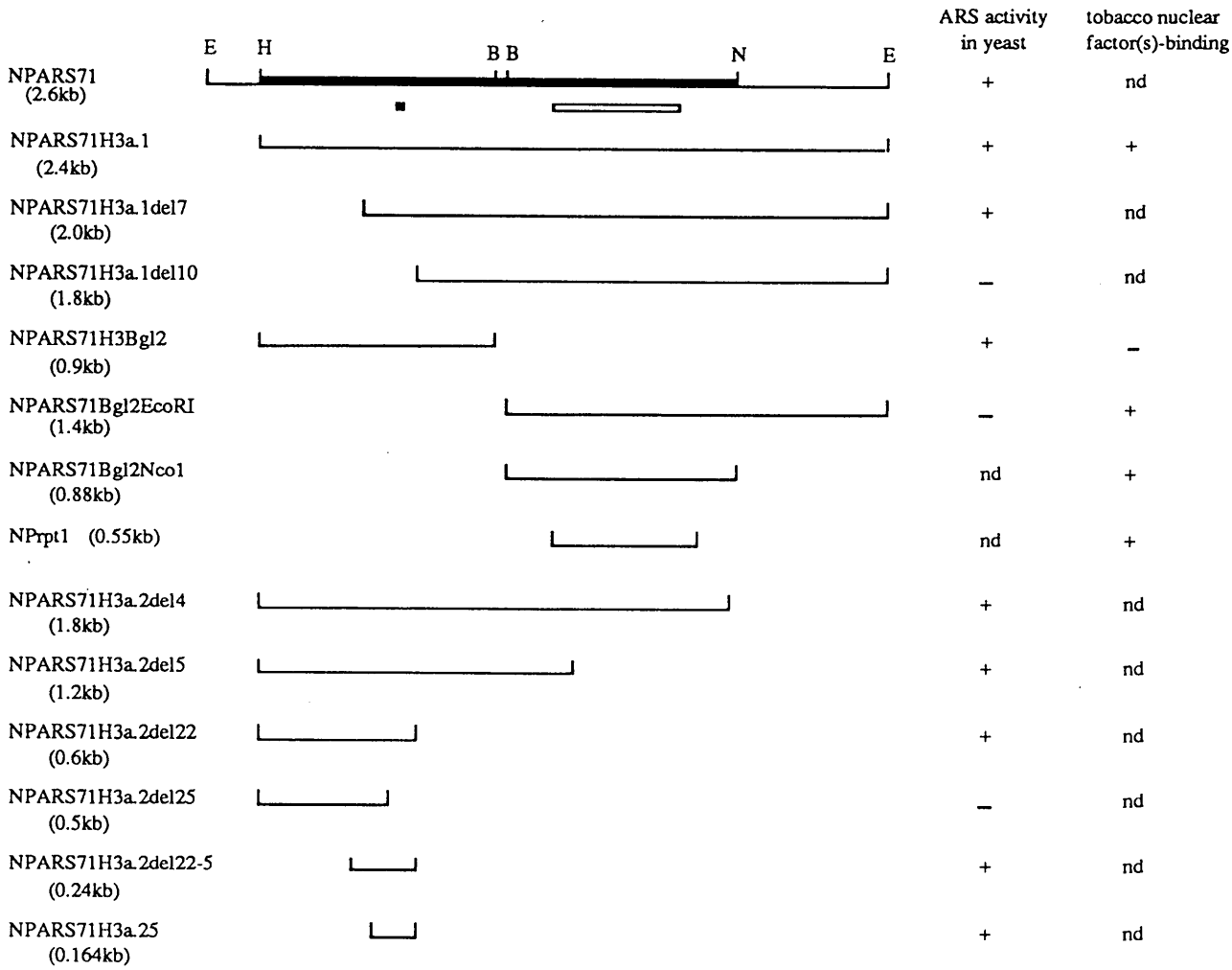

Fig. 2. Deletion analysis of NPARS71. The colony size of transformed yeast was taken to be the criteria for determining the ARS activity in yeast. +, >0.1 mm; -, no visible colony; nd, not determined. Filter-binding assay was used to determine the nuclear factor(s)-binding capability. B, BglII; E, EcoRI; H, HindIII; N, NcoI. Nucleotide sequence in the region of NPARS71, marked with a heavy line, is shown in figure 3a. Solid box, location of yeast ARS consensus sequence; empty box, location of repetitive sequence.

21-bp element of which thirteen to fifteen residues were identical to those in the hypothetical replication origins in three plant viruses, including CLV, TGMV, and BCTV (Fig. 3b). Furthermore, a 10-residue palindromic sequence, CCATATAGGG, was observed to overlap with this 21-bp element in the third and fourth repeating units of NPrpt1 (Fig. 3b).

Multiple Copies of NPrpt1 in the Genome of ***N. plumbaginifolia***

Genomic Southern blot revealed that NPrpt1 hybridized with many genomic DNA fragments (Fig. 4a) but not with chloroplast DNA (data not shown) from *N. plumbaginifolia,* suggesting that the repeated sequence in NPrpt1 may be one of the major repetitive sequences in the nuclear genome of *N. plumbaginifolia.* In contrast, probe NPARS71H3a.25 interacted only with two different genomic fragments from the same organism (Fig. 4b). Preliminary data showed that from zero to several-thousand copies of NPrtp1 also appeared in the genomes of various species of *Nicotiana* (Kuo *et al.,* unpublished data), indicating that NPrpt1 could be a candidate for studies of the evolutionary relationship among the species of *Nicotiana* genus.

a

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  +1  AAGCTTTTCC TCCAATTTCT CCTCATTTAT CCCTCTCTCG AGCAAGATTT TTTTATGGGC
 +61  CATATCGTCC AGATATCGGG CTGCCGCTGA AGCAGACATG AAGCGCGTAT CATCATATAG
+121  TTCATCATCC GCTTCTTGAA TTTCCGGTTG TGATCCGCGT TCGCATTTGT GAATTAAATC
+181  ACCTACAAAA AGTCATCACA AAATTGAATC GGACAATTGA ATCATGTTAA GCAATCCAAA
+241  ACATTGCATT TGAGCCAAAA AAAAGTGGGT TGCACGGGCC CGGGAAGCCA CAGTTGCAAA
+301  CACACTGCCT CAGAATTTCT GGGCAGTTTC CAGTTCTGCG GAAATTACAC GCTTAGGCGT
+361  CCGCTCTGCG AAAATTTTCC GCTTAAGGCT TTCATTTTTT TTTATTTTTT GTTTTCCGCT
+421  TCTGCGAACT TTTGGCTTAA GGCGTTGCCT TAAGCGGTTC TAGGCCTTCT TCTTCGACTG
+481  CTAATTACCA CAATTTTATT TTTCAATTAT AACCAAATTT TGGCATCCAA AACATAAATT
+541  ATGCAAGTTA TCAAGTATAT GATCTAGTGT CACGACCCCA AATGGCCCGC AATGGCGCCG
+601  GTCGGAGACT GCGGTAGCCC GGCCATAATG TCCATAAACC GGATAAGAGG CATGAAGGAA
+661  GTAGTAACTC AGAGCGTATA CAATACAGAT ATCATAAACA GGAAATGCCA ATACTGAATA
+721  TGTACAAAAC CCCTAGGAAC TGGTGACACA AGTCTGAGCC TCTAAGACAT GGATCAATTC
+781  TGATACAAAA CAAGTCCAGA ACTGGAAATA CTGAAAAAGA AATACAAGTA GAGAGAAGGA
+841  GGGTTGATGG CTGCGAACGC CGTGCAGCTA CCTCAACGCT CTGAACTGCT GAGATCTGAC
+901  CTCCGCAAGA CCTACTGTGC CTGTGACTAA TCAAGATCTG CACACATGGT GCAGGAGTAA
+961  TATGAGTACT CGACCCAGTG AGTAATAATA ATAAATAAAG TCTGAAGATA TAAAATCACG
+1021 ATAAATACAC AAAGTAGGCT AAACGACAGG ATATCACCAT AACTGAACTA TACAACTGAA
+1081 TTAACAGTAT ATAAATACGA ACGATGAAAT GCAGTGCAAC TGAAATGATC CATAACATCT
+1141 CACCGGATCT ACCCACAAAG GGCCCACGGC GGTCAAAGTA CAATCCCAGT CTCACCGGAT
+1201 CTACCCACAT AGGGCCCAAG GCGGACGAAT ACAATCCAAA GTCTTACCGG ATCTACCCAT
+1261 ATAGGGCCCA CGGCGGACAA ATACAATCCA AAGTCTCATC GGATCTACCC ATATAGGGCC
+1321 CACAGCGGAC AAATACAATC CAAAGTCTCA CCGGATCTAC CCACATAGGG CCCACGGCGG
+1381 ACAAATACAA TCCAAAGTCT CACCGGATCT ACCCACATAG GGCCCACGGT GGACACATCA
+1441 CAATATCACA GTCACACCGG ATCTACCCAC ATAGGGCCCA CGGCGGACGA ATAACAATAT
+1501 CAAAGTCTCA CCGGATCTAC CCACATAGGG CCCACGGCGG ACAAATATCA ATAGCAGAGT
+1561 CTCACCGGAT CTACCCACAT AGGGCCCACG GCGGACAGCC ATCTCGTGGC AAATAATCAA
+1621 TATCAGGCCA GTCCATGTTA CCTGACCGAT TAAATCATCG CTCCGGTCCG CAGCCCGATT
+1681 CCACCCAAAA TCAGTCCAGA AAATGTAACA TAAGCCCTTG GGCATTTATA AATCATGATC
+1741 CTCAGCCGAT ATGTATTTAA AAGATAACAT ATGAGGTTTG ACGCTTAGAA AATGTGGCTG
+1801 GGTTTCTAAT ACAGTGTTCT AAACCATGG
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b

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CLV        133  GGGGCCAACCG--TATAATATTACCGGATGGCCCC                    165
TGMV B     117  TGGCGGCCATCCGTTTAATATTACCGGATGGCCCCGCGATCGTC           160
BCTV        70  CAGGGGCCATCCGTTATAATATTACCGGATGGCCCCAAAATTTT           123
NPrpt1    1128  ATCCATAACATCTCACCGGATCTACCCACAAAGGGCCCACGGCGGTCAAAGTACA 1182
          1183  AT--CCCAGTCTCACCGGATCTACCCACATAGGGCCCAAGGCGGACGAA-TACA 1233
          1234  AT-CCAAAAGTCTTACCGGATCTACCCATATAGGGCCCACGGCGGACAAA-TACA 1285
          1286  AT-CCAAAAGTCTCAATCGGATCTACCCATATAGGGCCCACAGCGGACAAA-TACA 1335
          1336  AT-CCAAAAGTCTCACCGGATCTACCCACATAGGGCCCACGGCGGACAAA-TACA 1389
          1390  AT-CCAAAAGTCTCACCGGATCTACCCACATAGGGCCCACGGTGGACACATCACA 1442
          1443  ATATCACAAGTCACACCGGATCTACCCACATAGGGCCCACGGCGGACGAATAACA 1496
          1497  ATATCAAAAGTCTCACCGGATCTACCCACATAGGGCCCACGGCGGACAAATATCA 1550
          1551  ATAGCAGAAGTCTCACCGGATCTACCCACATAGGGCCCACGGCGGACA        1597
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Fig. 3. **a)** Nucleotide sequence of the HindIII-NcoI fragment of clone NPARS71. Yeast ARS consensus and tobacco repetitive sequences are underlined with heavy and thin lines, respectively. **b)** Sequence comparison between the NPrpt1 and the hypothetical replication origins of plant viruses CLV, TGMV B, and BCTV. The proposed replication origin in BCTV is italicized and sequences in CLV, TGMV B, and BCTV. The proposed replication origin in BCTV is italicized and sequences in CLV, TGMV B, and NPrpt1 homologous to BCTV are boxed. The 10-residue palindromic sequences are underlined.

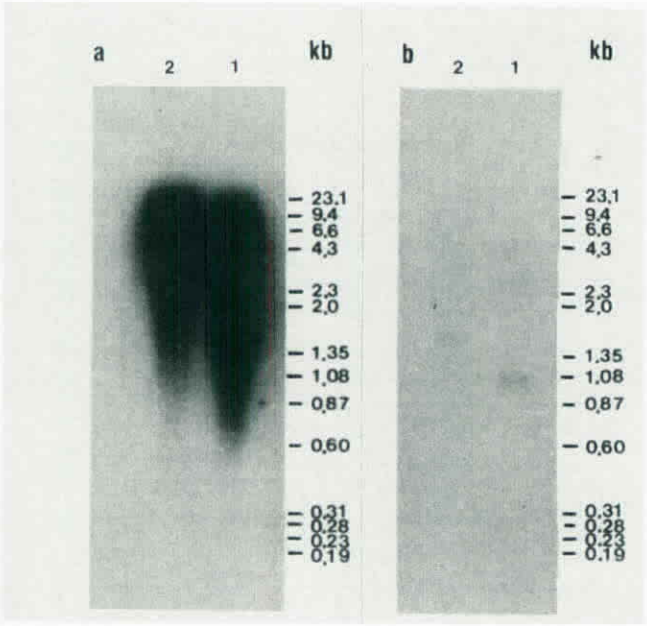

Fig. 4. Southern hybridization of *N. plumbaginifolia*. **a)** Probed with 550 bp NPrpt1 containing the repetitive sequence. **b)** Probed with 160 bp NPARS71H3a.25 encompassing the yeast ARS consensus sequencs. *N. plumbaginifolia* genomic DNA was cut with EcoRI (lane 1) or HindIII (lane 2) prior to analysis by gel electrophoresis.

Discussion

Searching the genomes of *Nicotiana* spp. for sequences homologous to the hypothetical replication origin of TGMV has been suggested (Hamilton *et al.*, 1984), however no positive result has been reported. In this communication, DNA fragment NPrpt1 from *N. plumbaginifolia* encompassing one class of tandem repetitive sequence was observed to contain multiple copies of a 21-bp element which shared about 70% homology with the hypothetical replication origins of three single-stranded DNA plant viruses. Two copies of this 21-bp element are also overlapped with a 10-residue palindromic sequence. Whether this 21-bp element or the 10-residue palindromic sequence (or both) would interact with any protein(s) remains to be verified. Considering 1) the abundance of this 21-bp element in plant nuclear genome, 2) the homology between it and the hypothetical replication origins of three plant viruses of which the common host is *Nicotiana* spp., and 3) the specific interaction of plant nuclear factor(s) with the fragment NPrpt1 containing this highly repetitive sequence, it is possible that this repetitive sequence contains an element(s) involved in the initiation of chromosomal DNA replication in *N. plumbaginifolia*. If this is the case, then the NPrpt1-binding nuclear factor(s) might play a role in the initiation of DNA synthesis in this plant. Alternatively, the conservation of sequence in the hypothetical replication origins among three different viruses implies that this sequence could involve interaction with a protein from their common host, the *Nicotiana* spp. Therefore, the NPrpt1-binding plant nuclear factor(s) might be able to interact with the hypothetical replication origins of these viruses.

On the other hand, several repeated DNA sequences from *N. plumbaginifolia* have been cloned (Marchesi *et al.*, 1989). Some of them stimulated the frequency of direct gene transfer by increasing the integration of foreign DNA into the genome of transformed cells (Marchesi *et al.*, 1989). Therefore, if the repetitive sequence in fragment NPrpt1 (or NPARS71H3a) can enhance the integration of foreign genes into the genome of transgenic plants, the nuclear factor(s) bound to NPrpt1 may be involved in the process of DNA recombination. It is interesting to transform plant protoplasts with plasmids containing NPARS71H3a and then investigate the fate of the plasmids in the transformed cells. In addition, purification and characterization of the nuclear factor(s) bound to NPrpt1 may also provide some information about the role of this highly tandem repetitive sequence in the nuclear genome of *N. plumbaginifolia*.

We have used the fragment NPARS71H3a.25 (160 bp), encompassing the yeast ARS consensus sequence, as a probe for mobility-shift assay (data not shown), however, no DNA-protein complex was detected. Since the ARS-protein complex in yeast was also undectable by mobility-shift assay when long double-stranded DNA probe (140-300 bp) was used in the reaction (Hofmann and Gasser, 1991), the possibily that a plant nuclear factor corresponding to the yeast initiator protein binds to the ARS consensus sequence can not be ruled out. Genomic Southern hybridization, however, showed that only two different genomic fragments interacted with NPARS71H3a.25, suggesting that it is less likely that this fragment contains the replication origin of plant chromosome since it did not appear at multiple locations in the nuclear genome of *N. plumbaginifolia*.

Since highly tandem-repetitive sequence appears at many locations in the genome, it is expected that

large inserts (>200 kb) of many YAC (yeast artificial chromosome) clones in a library may contain various numbers of copies of the same tandem-repetitive sequence. It is also possible that the overlapping relationship among YAC clones in a library can be defined by using this type of repetitive sequence as a probe for fingerprinting. Indeed, mapping of the human genome has been facilitated by fingerprinting of YAC clones using a tandem - repetitive sequence as a probe (Bellanne-Chantelot *et al.*, 1992). Therefore, the fragment NPrptl containing the tandem-repetitive sequence described here may be useful as a probe for fingerprinting YAC clones of *N. plumbaginifolia* which are under construction in this lab.

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皺葉菸草的一段 DNA 重複性序列和某些菸草病毒的複製起源點有同源性

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由皺葉菸草基因組分離出 12 條具有自主性複製能力的 DNA 片段。其中一條和菸葉核蛋白有專一性結合。DNA 序列分析顯示一種長約 52 個鹼基的序列在這段 DNA 中以頭尾相連方式重複出現 9 次。這個重複性序列在皺葉菸草基因組中至少存在數千或數萬個拷貝，可能是此種材料基因組中第一個被報導的高度重複性序列。這個序列包含一小段 DNA 和若干菸草 DNA 病毒的複製起源點有 70%同源性。由於這個重複性序列可能出現於皺葉菸草基因組中許多不同的位置，將來可用它作探針，分析此種材料的酵母菌人造染色體 (yeast artificial chromosome，YAC) 選殖體，定出 YAC 選殖體間所含皺葉菸草 DNA 片段的重疊關係，幫助建立此種植物基因組的物理圖譜。