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# Novel class of rDNA repeat units in somatic hybrids between *Nicotiana* and *Atropa*

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**Summary.** Behavior of ribosomal RNA genes in the process of somatic hybridization was analyzed using hybrids *Nicotiana tabacum* + *Atropa belladonna*. Blot-hybridization of parental species DNAs to $^{32}$P-rDNA specific probes revealed two classes of ribosomal repeats in both tobacco and nightshade; their length was 11.2 kb, 10.4 kb (tobacco) and 9.4 kb, 10.2 kb (nightshade). For analysis of hybrids, labelled $^{32}$P rDNA specific probes were hybridized to DNA of parental species and somatic hybrids digested with restriction endonucleases EcoR1, EcoRV and BamH1. A new class of ribosomal DNA repeat, absent in parental species, was found in hybrid line NtAb-1. Possible mechanisms of appearence of a new rDNA class in the process of somatic cell fusion are discussed.

**Key words:** *Nicotiana* – *Atropa* – Somatic hybrids – rDNA – Restriction endonucleases

## Introduction

Somatic hybridization techniques allow one to unite genomes of phylogenetically remote species in one cell. Analysis of multiple molecular forms of enzymes (Dudits et al. 1980; Evans et al. 1981; Gleba et al. 1983), as well as specific DNA sequences (Uchimiya et al. 1983; Saul and Potrykus 1984; Sala et al. 1985; Tabaeizadeh et al. 1986) of somatic hybrids usually reveal the presence in hybrids of genetic markers of both parents. In addition, new molecular forms of enzymes absent in parental material could be visualized in some cases (Wetter 1977; Gleba and Hoffmann 1978). This phenomenon is probably due to a changed organization and/or expression of parental genetic material in the hybrid cells. The question of genetic organization of somatic hybrids remains unexplored. Although some data has been accumulated concerning the stability of plastid DNA as well as the high recombination rate of mitochondrial DNA in the process of somatic hybridization, there is little information available about the fate of nuclear DNA and its organization in hybrids.

We have analyzed the inheritance of ribosomal RNA genes in hybrids between *Nicotiana* and *Atropa*, where in one of the clones studied, a novel rDNA repeat unit has been found that was absent in the parents.

## Materials and methods

### *Construction of Nicotiana tabacum + Atropa belladonna somatic hybrids*

Somatic hybrids of *N. tabacum* + *A. belladonna* were obtained by fusion of leaf mesophyll protoplasts (belladonna) and callus protoplasts (tobacco). Isolation and characterization of the clone NtAb-1 is described elsewhere (Gleba et al. 1983).

### *DNA and RNA isolation and DNA analysis*

DNA was isolated using a procedure described by Shure et al. (1983) from 5–10 g of tissue frozen in $N_2$ by means of two phenol extraction, precipitated with 2.5 vol. of ethanol, re-dissolved and purificated twice on a CsCl/EtBr gradient. After extraction of EtBr with isoamylalcohol, the DNA was extensively dialysed against Tris · HCl (10 m*M*)/EDTA (1 m*M*) (pH 7.5) buffer.

Electrophoresis, Southern blotting, nick-translation, hybridization, autoradiography and photography were performed as described by Maniatis et al. (1982). Restriction

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enzyme cleavage was done according to the supplier's instructions.

rRNA isolated from maize seedlings was separated into 18S rRNA and 26S rRNA and $^{32}P$ labelled according to Oono and Sugiura (1980). The plasmid pUC222 containing 3'-fragment of the Citrus lemon 26S rRNA gene was kindly provided by I. Fodor and V. Kolosha (Institute of Biochemistry and Physiology of Microorganisms, Puschino-na-Oke).

## Results

Analysis was performed using the cell line NtAb-1. To study the rDNA organization in the intertribal somatic hybrid, *Nicotiana+Atropa,* restriction endonucleases EcoR1, EcoRV, and BamH1 were used. Ribosomal repeats of the two parents were mapped. $^{32}P$ labelled maize 18S and 26S rRNAs and the pUR222 plasmid, containing a 500 bp fragment of the 26S rRNA gene of lemon, were used as probes. Figure 1 shows that the *Atropa* genome contains two classes of rDNA repeats, 9.4 and 10.2 kb long. *Nicotiana* rDNA is also organized as two repeats: 11.2 kb and 10.4kb (Fig. 2). Differences between the ribosomal gene repeats are due to a variable untranscribed spacer, as has been demonstrated for the range of plant species (Gerlach and Bedbrook 1979; Delseny et al. 1979; Yakura et al. 1983, 1984).

Use of the EcoRV enzyme, which has only one recognition site per rDNA unit of *Atropa* and, therefore, eliminates a full length ribosomal repeat, shows that the NtAb-1 hybrid possesses only one of two *Atropa* repeats, as well as underamplified rDNA of tobacco. In addition, a novel rDNA repeat of 12.5 kb can be seen (Fig. 3). The same results are obtained when an 18S rRNA probe and pUR222 probe are used with restriction endonuclease EcoR1 (Fig. 4). In these cases we can see the fragments containing parts of 18S RNA and 26S RNA coding sequences, as well as the full sequence of the non-transcribed spacer.

Digestion of DNA with BamH1 also supports the appearance of a new rDNA repeat unit in the hybrid (Fig. 4). The BamH1 analysis reveals two hybridization bands for each rDNA unit of *Atropa* when pUC222 is used as a probe. This is also seen in the analysis of another *Nicotiana+Atropa* somatic hybrid, NcAb-6. In this hybrid line only the smaller of the two *Atropa* rDNA units is present and no *Nicotiana* rDNA units

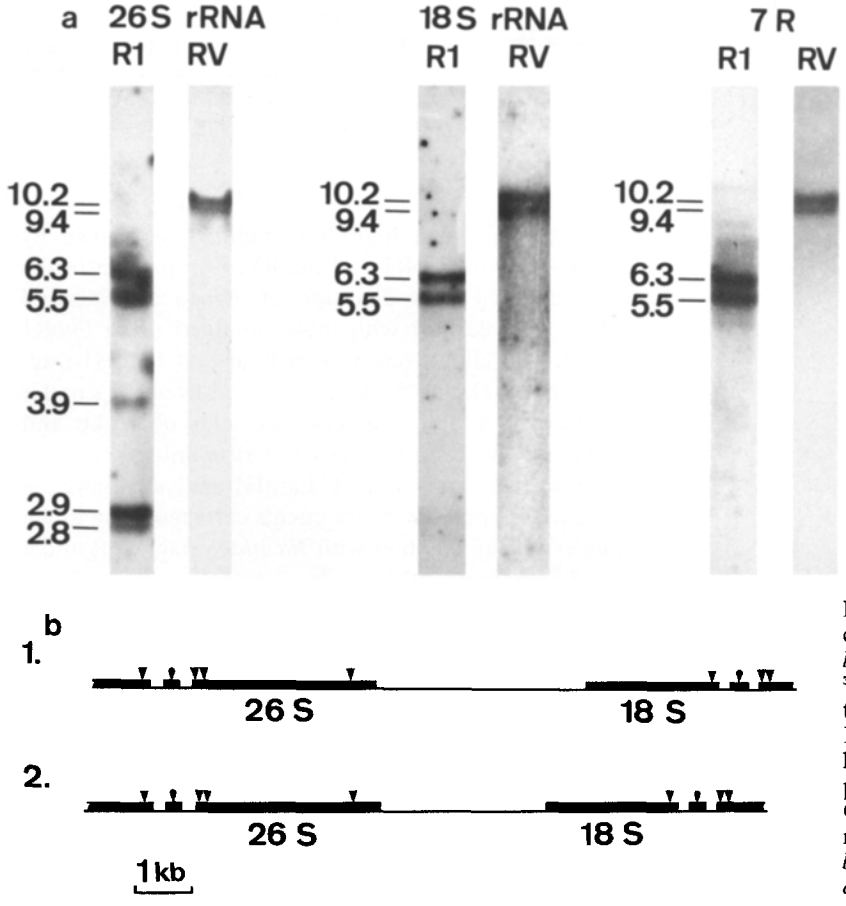


**Fig. 1. a** Autoradiograph of restriction nuclease digests of total nuclear DNA of *Atropa belladonna* hybridized to $^{32}P$-26S rRNA, $^{32}P$-18S rRNA, and $^{32}P$-7R$^{+}$ DNA. R1 = restriction nuclease EcoR1; RV = restriction nuclease EcoRV. *Numerals* indicate molecular sizes in kb. $^{+}$ In all figures *7R* indicates the plasmid pUC222, containing 500bp 3'-fragments of the Citrus lemon 26S rRNA gene. **b** The proposed restriction enzyme map of rDNA of *Atropa belladonna.* Restriction enzyme sites are *indicated* as: ▼ = EcoR1, ▾ = EcoRV

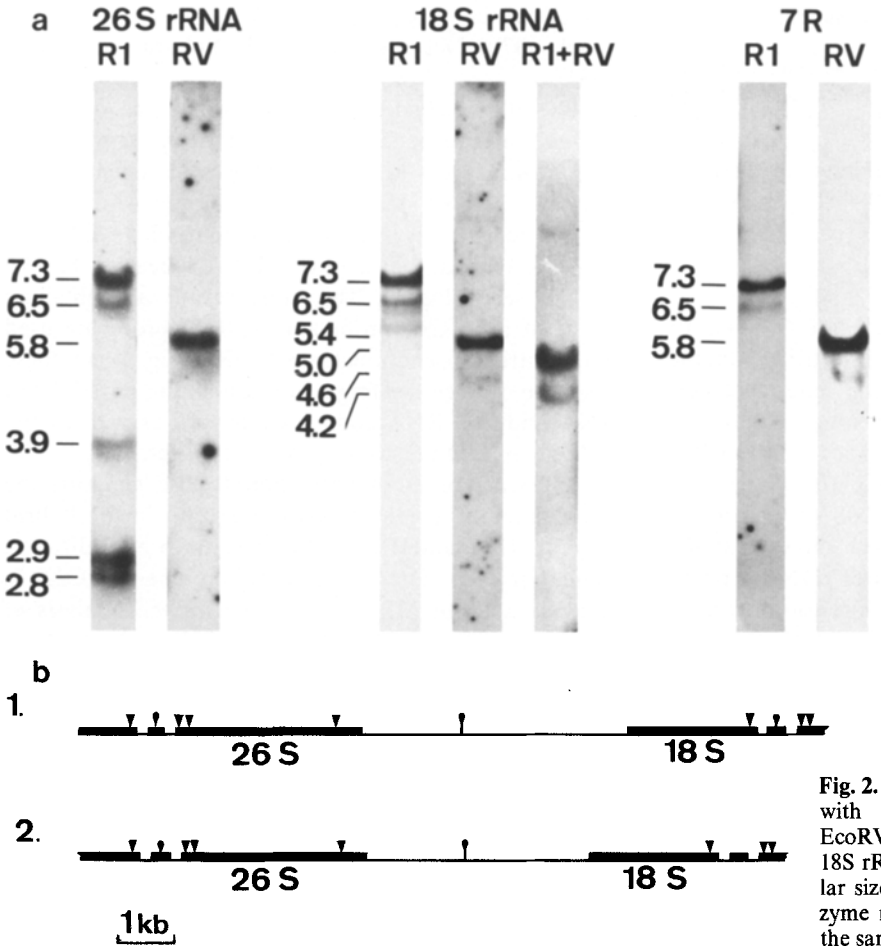


**Fig. 2.** **a** *Nicotiana tabacum* DNA digested with EcoR1 (R1), EcoRV (RV), EcoR1+ EcoRV (R1+RV) and probed with 26S rRNA, 18S rRNA and 7R. *Numerals* indicate molecular sizes in kb. **b** The proposed restriction enzyme map of *Nicotiana tabacum*. *Symbols* are the same as those used in Fig. 1b

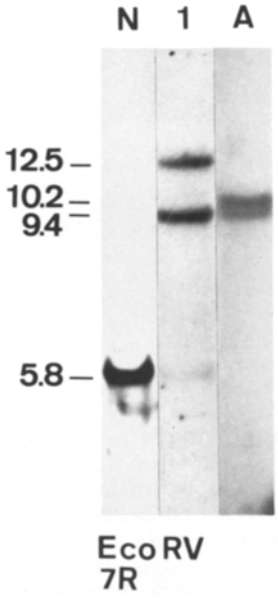


**Fig. 3.** *Nicotiana* (*N*), *Atropa* (*A*) and somatic hybrid NtAb-1 (*1*) DNA digested with *EcoRV* and probed with *7R*. *Numerals* indicate molecular sizes in kb

are detectable in a high copy number, as shown by restriction with EcoR1 and EcoRV. Comparison of the data obtained after digestion of *Atropa* and NcAb-6 DNAs with BamH1 with those obtained using EcoR1 and EcoRV allows one to conclude that BamH1-fragments of 4.0 kb and 5.4 kb are derived from the smaller *Atropa* rDNA unit, whereas fragments of 4.7 kb and 6.3 kb are products of the larger *Atropa* unit.

In hybrid clone NtAb-1, BamH1 analysis shows the presence of both *Atropa* fragments corresponding to the smaller repeat, together with *Nicotiana* fragments and a new 8.3 kb fragment.

## Discussion

Our analysis of rRNA genes revealed a novel variant of the rDNA repeat unit in a somatic hybrid as compared to its parents, *Atropa* and *Nicotiana*. The cause of the novel rDNA repeat in the NtAb-1 hybrid is not known. There is some evidence that both in vitro culture and hybridization cause variability in rDNA organization

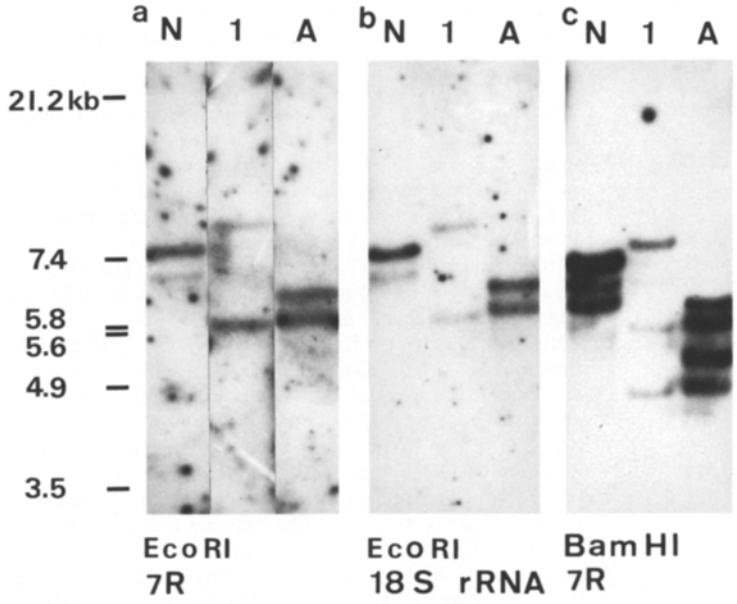


**Fig. 4.** *Nicotiana* (*N*), *Atropa* (*A*), and Nt Ab-1 (*1*) DNA digested with *EcoR1* (**a, b**) and *BamH1* (**c**) and probed with *7R* (**a, c**) and *18S rRNA* (**b**)

in plant cells. Landsmann and Uhrig (1985) revealed a deficiency in the number of rRNA genes in some of the potato somaclones analysed. Variation in rDNA associated with the in vitro culture process has also been demonstrated in regenerated *triticale* plants (Brettell et al. 1986). Lin et al. (1985) showed that a new restriction site was generated in the spacer region of the rDNA repeat and amplified to a great copy number as a result of the cross between maize and *Tripsacum*.

Our results demonstrate for the first time such a nuclear DNA rearrangement following somatic cell hybridization. There are several possible mechanisms to explain the observed appearence of the novel rDNA repeat unit.

a) The repeat unit revealed in the NtAb-1 hybrid line pre-existed in the genome of *A. belladonna* or *N. tabacum* at low copy number (and hence was not detectable by hybridization to the rDNA probe), but was amplified in hybrid cells. Evidence has been obtained to confirm the existance of low-copy-number sequences homologous to rDNA in nucleolus-less mutants of *Xenopus laevis* (Tashiro et al. 1986).

b) The new rDNA repeat unit length variant is caused by insertion of transposable element-like sequences into one or several repeat units followed by amplification to greater copy numbers. Although transposable elements have not been found in either *Nicotiana* or *Atropa,* they are described in some of the higher plants.

c) It is known that nontranscribed spacer regions which separate rRNA genes in many species, including higher plants (Appels and Dvorak 1982; Yakura et al. 1984), consist of a number of short sub-repeats. One can hypothesize that the increase in rDNA unit length in our case is due to amplification of spacer sub-repeats followed by amplification of the resultant rDNA unit of increased size via a mechanism of "concerted evolution" (Zimmer et al. 1980; Arnheim 1983).

d) The appearance of a novel rDNA class in a hybrid can also be the result of interspecific recombination between two rDNA units as is sometimes seen in the case of mitochondrial DNA. However, because of the specific compact organization of rDNA in the chromosome, this possibility is not very probable.

Cloning and sequencing of the new rDNA unit is currently underway to find out which of the above-mentioned mechanisms are involved.

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