Variable Content of a (GC) Rich Satellite DNA in Tumorous and Normal Cultures of *Crepis capillaris*

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(Z. Naturforsch. **28 c**, 564–567 [1973]; received May 25, 1973)

Satellite DNA, crown-gall tumors

Analyses of DNA from tumorous and non tumorous cultures of *Crepis capillaris* by analytical equilibrium centrifugation in a neutral CsCl gradient revealed significant differences in regard to the content of a heavy component satellite, which was more highly represented in tumorous cultures. DNA of plant leaves showed a unimodal distribution of molecules. The choice of suitable controls served to demonstrate that the component satellite was not exclusive of tumorous tissues. The higher satellite content of tumorous cultures is probable related to their higher growth rate.

The mechanism of the tumoral transformation by infection with *Agrobacterium tumefaciens* will not be fully understood until we know, 1. the basis of the induction process, that is, what type of information is transferred from the bacterium to the plant, and 2. the genetic or epigenetic basis of the differences between normal and transformed cells. The possible causes of these differences and the reasons for the use of plant material in the problem of tumorgenesis have been analysed and discussed by Melchers [1].

Evidence has been obtained through molecular hybridization studies [2–5] that during the induction process a part of the genome of the bacterium is transferred to the host cell, as occurs in the case of animal virus tumors. Probably the incorporation of these sequences gives rise to the changes which characterize the tumor condition, but these new sequences cannot be considered as the only reason for the properties of the transformed cells: otherwise it would be difficult to explain how "habituation" (spontaneous change to tumorous growth) occurs, and how, as in animal tumors, the reversion of transformed to normal cells is not associated with the loss of the virus genome [6].

A modification at the level of the host genome induced by the incorporation of bacterial sequences is postulated by Guillé and Grisvard [7]. In an analytical study of crown-gall and normal DNA by means of isopycnic centrifugations in Ag^+-Cs_2SO_4 gradients the authors found a reiterative fraction (satellite) in the tumor DNA which was not present in the DNA of healthy tissues. In the DNA of tobacco analysed in neutral gradients Schilperoort [8] found a higher content of a dense satellite in tumorous tissues than in normal ones, but he suggested this satellite originated from cytoplasmatic organelles.

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We present in this paper the results of a comparative analytical study of DNAs of crown-gall and normal tissues of *Crepis capillaris* grown *in vitro*. This material is represented by different strains and lines which have been well characterized with respect to their growth and chromosome properties [9].

Methods

For each material at least two methods of DNA extraction were carried out in parallel. DNA was prepared according to the techniques of Kirby [10] and Marmur [11], with some modifications. Chloroform or phenol deproteinisation was followed by ethanol precipitation and purification of the crude DNA preparation by ribonuclease and pronase digestion. The final precipitations of DNA were made with isopropanol. In the cases where the crude extract showed a pronounced pigmentation an initial purification was made by molecular sieve chromatography with agarose [12]. DNA was extracted from whole tissue and from nuclear fractions obtained through differential centrifugation [13]. All buffers utilized had been previously sterilized.

The spectra for ultraviolet absorbancy of the final DNA solutions were characterized by the following ratios: $A_{260}/A_{230} = 1.8 - 2.3$; $A_{260}/A_{280} = 1.8 - 2.0$. Molecular weights of some tested samples were estimated from the sedimentation coefficients of DNA (20 μg/ml) dissolved in SSC (0.15 M NaCl + 0.015 M trisodium citrate), using the relationship established by Doty *et al.* [14]. Values of $4 \cdot 10^6$ to $9 \cdot 10^6$ were found.

Analytical equilibrium ultracentrifugation in a CsCl gradient (using SSC pH = 7 as solvent) was performed in a Spinco Model E ultracentrifuge equiped with UV optics. DNA samples (5 – 10 μg)

were centrifuged for 20 hours at 44,000 or 48,000 rpm, at 20 °C. The buoyant densities were calculated in accordance with the procedure described by Mandel *et al.*[15] using as marker DNA from *Micrococcus lysodeikticus* $p = 1.731\ g \cdot cm^{-3}$. The density increase of the DNA samples after thermal and alkaline denaturation ($0.014\ g \cdot cm^{-3}$) indicated the native state of the isolated DNA.

Results

DNA extracted from developed leaves of haploid and diploid *Crepis capillaris* plants (7 extractions, 10 runs) always banded in the density gradient as a single band at a buoyant density of $1.696\ g \cdot cm^{-3}$ (Fig. 1 a). Initial analyses of the DNAs from rapidly growing tumor cultures (strains TA, TD and TG) revealed the presence of an additional minor fraction (satellite) banding at a density of $1.708\ g \cdot cm^{-3}$ (Fig. 1 d – f). The satellite was also present in DNA extracted from nuclear fractions. This apparent correlation between the tumoral condition and the presence of the satellite component should be confirmed by studying other tumor strains and controls. The results of the analysis of the tumors are summarized in Table I.

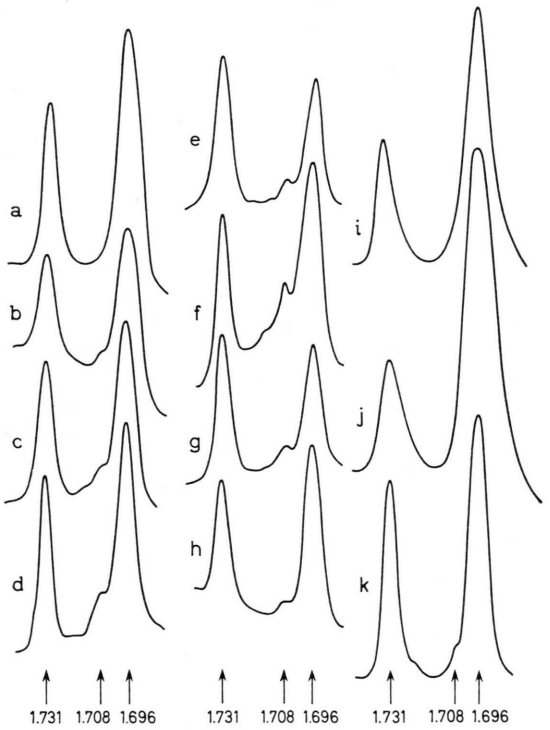

Fig. 1. Microdensitometer tracings of ultraviolet photographs of DNA samples of *Crepis capillaris* from different origin centrifugated to equilibrium (44,000 or 48,000 rpm, 20 hours at 20 °C) in a neutral CsCl gradient. a. Developed leaves. b. Non-tumorous strain R/KB (auxin-heterotrophic). c. Habituated-tumorous strain "Unisat" (auxin-autotrophic). d. Tumorous strain TA (line 1). e. Tumorous strain TD (line 1). f. Tumorous strain TG (line 1). g. Tumorous strain TH (line 2). h. Tumorous strain T_5 (line 1). i. Tumorous strain T_5 (line 2). j. The same sample as i. mechanically sheared before centrifugation. k. DNA from whole seedlings, 7 days old. The peaks at $p = 1.731$ correspond to DNA of *Micrococcus lysodeikticus*.

In the cases where a satellite DNA was present, both components in the CsCl gradient – main band and satellite – were sensitive to deoxyribonuclease. The guanine-cytosine (GC) contents of the main band and the satellite component, estimated on the basis of buoyant density[15], are 36.7 and 48.9, respectively. The proportion of satellite DNA varied from line to line between 4% (recognizable as a light shoulder) and 18% of the total DNA. Degradation of DNA samples without visible satellite band by capillary shear did not result in the recovery of a satellite component (Fig. 1 i, j).

Except for two lines of the strain T_5 (Table I), the presence of the satellite component in the tumorous cultures is general and in many cases it constitutes a considerable proportion of the genome (Fig. 1 d – g). But the exceptions suffice to invalidate the hypothesis that one might recognize *A. tumefaciens*-transformed tissues of *C. capillaris* by the presence of DNA sequences which are not detectable in healthy tissues.

The two lines without satellite DNA were represented by cultures with slow or moderate growth and normal karyotypes. It has been shown[9] that in tumorous cultures of *C. capillaris* rapidly growing lines always contain a high degree of chromosome abnormalities, in contrast to tumorous lines of slow growth rate, which present almost normal karyotypes. An explanation for the appearance of the satellite DNA could be the structural chromosome changes (karyotypes with more heterochromatin?) present in rapidly growing lines, but it is also possible that the satellite DNA is related with the physiological characteristics (higher growth rate) of these lines. A more appropriate control material was necessary to approach this problem.

As controls three types of non-tumorous *C. capillaris* cultures were analysed (Table II), 1. an auxin-heterotrophic strain (R/KB) karyotypically

Table I. Presence of satellite DNA in tumorous cultures of *C. capillaris*.

Strain	Line	No. of extractions	Growth	Karyotype	Sat. DNA ($p = 1.708$)
TA	1	5	rapid	A [1], 5, 6 chr.	10% or more
	2	1	rapid	A, 5 chr.	5–10%
	3	5	rapid	A, 5 (6) chr.	5–10%
	4	1	rapid	A, 5, 4 chr.	5–10%
TD	1	6	rapid	A, 6 (5), 12 chr.	10% or more
	2	2	rapid	A, 6, 5 chr.	10% or more
	3	1	rapid	A, 5 chr.	5–10%
TG	1	9	rapid	A, 5 chr.	10% or more
	2	1	rapid	A, 6, 5 chr.	5–10%
	3	1	moderate	A+N [1], 6 chr.	5–10%
	4	1	rapid	A, 5, 4 chr.	5–10%
T_5	1	2	rapid	A+N, 6 chr.	less than 5%
	2	2	slow	N	—
	3	2	slow	N(+A), 6 (5, 4) chr.	—
TH	1	3	rapid	A, 7 chr.	10% or more
	2	1	moderate	N+A, 6 chr.	5–10%
	3	1	moderate	A, 6, 12 chr.	5–10%

[1] A, aberrant; N, normal.

normal, in optimal growth conditions and in conditions of reduced growth due to a non optimal hormonal composition of the medium (Fig. 1 b); 2. an auxin-heterotrophic strain (R/FB) karyologically abnormal; 3. an habituated auxin-autotrophic strain ("Unisat", a chromosome mutant growing rapidly on medium with and without hormones, mutation and habituation not absolutely correlated) (Fig. 1 c). The origin and properties of these strains have been described [16]. From the results obtained, the presence of the satellite DNA seems to be primarily related with the growth rate of the cultures.

If the satellite DNA in *C. capillaris* is characteristic of tissues with high growth intensity, one would also expect to find it in growth regions of *in vivo* material. For this purpose DNA of sterile, oneweek old seedlings was analysed. A band profile of this DNA is represented in Fig. 1 k; it shows a shoulder (representing 2 – 4% of total DNA) at the heavy side of the DNA peak.

Discussion

It would be of great interest to find a criterion which permits the unequivocal recognition of *Agrobacterium*-transformed cells, especially since the auxin-autotrophy of transformed tissues is not exclusive of these but can be acquired spontaneously through "habituation". For this purpose the presented analyses were performed, along with parallel analyses in regard to special metabolic

Table II. Presence of satellite DNA in non-tumorous cultures of *C. capillaris*.

Strain	Medium [1]	No. of extractions	Growth	Karyotype	Sat. DNA ($p = 1.708$)
R/KB	D_2w	5	rapid	N	less than 5%
	Dd_2	1	slow	N	—
	Dk_2	2	slow	N	—
R/FB	D_2w	1	rapid	multiple transloc.	less than 5%
	D_2w	2	moderate	one transloc.	—
Unisat	D_2w	5	rapid	chrom. mutation	~5%

[1] The same basic medium as for tumors [9], but supplemented with hormones. D_2w: 6 mg/l NAA + 0.64 mg/l kinetin; DD_2: 48 mg/l NAA + 0.64 mg/l kinetin; Dk_2: 6 mg/l NAA + 30 mg/l kinetin.

products which Morel and coworkers [17] had found in crown-gall cells [18].

By comparing the DNA profiles of developed plants and rapidly growing crown-gall cultures of *Crepis capillaris*, at first we believed to have an indication that, at least in this species, tumor cells transformed by *A. tumefaciens* could be recognized by the presence of a satellite DNA which is not detectable in plant DNA. However, the study of three types of controls has shown that this satellite component cannot be used as an indicator for transformation to tumor growth: 1. Slowly growing auxin-autotrophic transformed (by *A. tumefaciens)* cultures contain no detectable satellite DNA; 2. satellite DNA in small but clearly detectable amounts is present in rapidly growing auxin-heterotrophic callus cultures and 3. in fast growing habituated auxin-autotrophic material. The possibility of a differential extraction of satellite DNA (raipdly growing tissues are in general softer) cannot be excluded, but it seems improbable because the results were reproducible, even when different methods of DNA extraction were used.

Heavy nuclear satellites of a density similar to that described for *C. capillaris* have been frequently found in plants [19, 20] (other heavier DNA satellites have been recently questioned [21]). In the investigated cases these minor components showed redundancy and a relatively high degree of hybridization with ribosomal RNA.

In *C. capillaris* the presence of the (GC) rich satellite DNA band and their proportion with respect to the total genome seems to be more closely related to the growth intensity of the tissues than with their karyological properties. The differences in the satellite could be due to a different degree of amplification of specific DNA sequences, which would be under-replicated or not amplified in fully developed or slowly growing tissues. An intraspecific variation of DNA satellites has been also described for animal cells [22, 23]. It remains to be proved if such DNA component found by us to occur in relatively larger amounts in tumorous tissues could have played a role in tumor induction, as it seems to be the case for certain reiterative sequences (sometimes recognizable as satellites) in animal virus tumors [22, 24, 25].

We thank Professor G. Melchers for the criticism of the manuscript and Dr. W. Keller for the revision of the English text.

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