ISOLATION AND SOME PROPERTIES OF DEOXYRIBONUCLEIC ACID FROM CULTURED CELLS AND ROOT OF CARROT

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Introduction

It is widely known that somatic embryogenesis occurred rapidly in the suspension cultures of carrot cells[8,18,28]. The cells continue to grow actively in a less organized or undifferentiated state in a medium containing 2, 4-D, but transferring the cells to an auxin-free medium to develop the somatic embryos. Although the suspension cultures of carrot cells possess the superior competence and can provide a material for the investigation of developmental process in somatic embryogenesis, they have not been used extensively in detailed investigations of nucleic acids.

It is commonplace that higher plant tissues, with some notable exceptions, are a low nuclear/cellular volume ratio. By this reason, isolation of highly purified DNA from many higher plants may be complicated for the elimination of such contaminants as RNA, protein and polysaccharides. Although some procedures[30] have proven very effective in the isolation of DNA from plant tissues, the results are not always ideal. Therefore it is necessary to develop a extraction and purification procedure of DNA from a plant material which is to be used.

One of principal problems for the isolation of native DNA is that of nucleases. Several expedients have been adopted to overcome this difficulty. Degradation by DNase is prevented by deproteinization with chloroform[7], by the presence of chelating reagent as EDTA[17], and by the action of sodium lauryl sulfate[16], but the most effective reagent for inhibiting the lytic activity of the enzyme seems to be diethyl pyrocarbonate[21,25]. Even after the enzymatic digestions of RNA and protein in the purification step, DNA preparations are often contaminated with low molecular weight RNA and protein

both of which are resistant to the enzymatic degradation. They are precipitated with DNA by the addition of ethanol. Spooling up of DNA precipitate by isopropanol[17] or use of sucrose density gradient centrifugation[20] can separate DNA from the contaminants. However, gel chromatography applied to the removal of colored materials[26,27] seems to be effective for this purpose. Polysaccharides can be tenacious contaminants of plant nucleic acid preparations. Separation against 2-methoxyethanol[12] or precipitation by isopropanol can remove polysaccharides, however, the procedures are not simple. When DNA is banded in CsCl density gradients, these materials float and form a pellicle at the top of the gradients[1].

In this paper we describe an isolation procedure together with many of above features, and some of properties of DNA from cultured cells and intact tap roots of carrot have been undertaken.

Materials and Methods

Plant Materials: Seeds of carrot (*Daucus carota* L., cv. Red Core Chantenay) were surface sterilized chemically, rinsed with steril water and allowed to germinate on 0.6% agar (Difco, Bacto-Agar) containing 2% sucrose for a week at 27°C under constant fluorescent illumination (Toshiba, Type FLA 40 S/M, ca. 1500 lux). These seedlings were conducted under asceptic conditions.

Tissue Culture: The hypocotyl segments of the germinated seeds were grown on MS medium[19] which was modified by the concentrations of NH_4NO_3 and KNO_3 to 10.3 mM and 24.7 mM, respectively. Other organic addenda were supplemented as follows: thiamine-HCl (3 mg/l), nicotinic acid (5 mg/l), pyridoxin-HCl (0.5 mg/l), sucrose (20 g/l), and casein hydrolysate (Difco, certified grade, 500 mg/l), and henceforth the above formula of medium was referred to the basal medium (BM). As a single hormonal source, 2, 4-D at 0.9×10^{-6} M was added in place of IAA plus kinetin. The pH of the medium was adjusted to 5.8 with NaOH before autoclaving. After the cultures on the solid medium were maintained for a month under the conditions stated above, calli developed were separated from the original explant. Pieces of callus tissues were transferred to fresh liquid medium instead of solid one and incubated in a reciprocal shaker agitated continuously at 120 rpm. The suspension cultures were successively subcultured at an interval of 13 to 15 days. At the end of the subculture, the cells were firstly passed through a 177 μm metal screen and then collected on a 44 μm screen to obtain uniform cell clumps as an inoculum for the test culture. These cells were washed twice with BM by repeated centrifugation at 80 g

for 3 min. Aliquots of cell suspensions were divided into two groups for the inoculation onto the two different media: *i. e.* the BM with or without 2, 4-D, the latter was being suitable for the induction of embryogenesis. The cells were usually inoculated at a cell density of 1.0 μl packed cell volume per ml. Both of test cultures were performed under the same conditions as described above.

Isolation of DNA from Chromatin: At the end of culture, the harvested cells were used for preparing DNA from chromatin. The preparation of chromatin was performed in essentially the same manner as described by HUANG and BONNER[9]. The cells were collected by low speed centrifugation and washed with water. Then, the cells were blotted dry with filter paper, frozen and stored at -40°C until necessary. The frozen cells were pulverized in a chilled motar with pestle and then blended in a motor-driven Potter glass homogenizer with six-fold volumes of grinding medium (0.3 M sucrose, 0.05 M tris-HCl buffer, pH 8.0, 0.01 M β-mercaptoethanol, 0.001 M $MgCl_2$). The homogenate was subsequently filtered through a triple layer of gauze and Miracloth (Chicopee Mills, Inc.). The filterate was centrifuged at 4000 g for 30 min and the gelatinous pellet was scraped from the starchy materials and washed with 0.05 M tris-HCl buffer, pH 8.0, containing 0.3 M sucrose and 0.01 M β-mercaptoethanol, followed by centrifugation at 10,000 g for 10 min. The pellet was suspended in the same buffer and layered on 1.7 M sucrose solution in tris-HCl buffer, pH 8.0. The upper one-third of the tube was gently stirred to form a rough gradient, and the tubes were centrifuged in a Hitachi RPS-25 rotor at 22,000 rpm for 120 min. Then, DNA was prepared from the pellet with the following procedure. The pellet was dissolved in the extraction medium consisting of 0.05 M NaCl, 0.01 M EDTA, 0.1 M tris-HCl buffer, pH 8.0, and 2% sodium lauryl sulfate. Then 25 μl/ml diethyl pyrocarbonate was added to the mixture. After stirring at 37°C for 5 min, the salt concentration was raised to 0.5 M NaCl. The mixture was stirred again at 37°C for 5 min followed by centrifugation at 8,000 g for 5 min at room temperature. The supernatant was shaken with an equal volume of chloroform-isoamylalcohol (24 : 1) for 10 min at 4°C, and aquaous phase was separated by centrifugation at 6,000 g for 10 min. Nucleic acids were prepcipitated by the addition of 2 volumes of cold 95% ethanol and collected by centrifugation at 800 g for 5 min. The pellet was washed with 95% ethanol and dissolved in 0.1 × SSC (1 × SSC: 0.15 M NaCl, 0.015 M trisodium citrate, pH 7.0) and stored overnight at 4°C. Some cloudy materials were removed by centrifugation at 12,000 g for 10 min and then the salt concentration increased to 1 × SSC with 10 × SSC. The DNA was

purified by digestion with pancreatic RNase A (50 μg/ml, Sigma, heat-treated at 80°C for 10 min) for 90 min at 37°C, followed by treatment with Pronase E (50 μg/ml, Kahen Chem. Co., self-digested at 37°C for 120 min) for 60 min at 37°C. After the enzymatic digestion, the mixture was then shaken with an equal volume of chloroform-isoamylalcohol. The aquaous phase was separated and the DNA was precipitated by the addition of cold 95% ethanol. The precipitate was collected by centrifugation and dissolved in 0.1 × SSC. The solution was made up to 1 × SSC with 10 × SSC and stored at 4°C.

Isolation of Total DNA: Total DNA was extracted by the following method. The cultured cells were blotted dry with filter paper, frozen and pulverized in a chilled motar with pestle followed by blending in a Potter glass homogenizer with 5 volumes of 0.1 M tris-HCl buffer, pH 8.0, containing 0.02 M EDTA and 0.05 M NaCl. Tap roots were peeled and homogenized by grinding pan with an equal volume of the medium, and subsequently blended by Polytron (Kinematica) at 60% of maximum power for 30 sec. After the addition of 25 μl diethyl pyrocarbonate per ml homogenate, the salt concentration was adjusted to 0.5 M NaCl followed by stirring at 37°C for 10 min. The mixtures were centrifuged at 8,000 g for 5 min at room temperature and the supernatants were treated with chloroform-isoamyl-alcohol (24 : 1). Further purification of DNA was involved in digestion with RNase and Pronase, and subjected to Sepharose 4 B column chromatography.

Sepharose 4 B Column Chromatography: Two ml of DNA solution in 0.1 × SSC was loaded on a (1.5 × 18 cm) column of Sepharose 4 B and eluted with 0.1 × SSC. DNA was collected in the exclusion fraction of Sepharose 4 B. The solution was adjusted to 1 × SSC with 10 × SSC.

Purity Test of DNA with Adsorption to Hydroxyapatite: As a method for assessing the purity of DNA, separation of contaminants from DNA was carried out with hydroxyapatite (HA). Hydroxyapatite (Sigma, type I) was previously washed with 0.12 M phosphate buffer (PB: equimolar solution of NaH_2PO_4 and Na_2HPO_4, pH 6.8) and 0.4 M PB at 60°C. DNA in 0.12 M PB was mixed with HA in 0.12 M PB and incubated at 60°C for 10 min. The precipitates obtained by centrifugation at 800 g for 5 min were washed with the same buffer at 60°C for 10 min. The Supernatants were collected and saved. The final precipitates were subsequently suspended in 0.4 M PB and incubated at 60°C for 10 min followed by washing with the buffer. The supernatants were collected. After fractionation absorbance at 260 nm of these two fractions were determined.

Cesium Chloride Density Gradients: DNA samples in 1 × SSC containing 0.1% sodium *N*-lauroyl sarcosinate were adjusted to 1.700 g/cm^3 with solid

CsCl and centrifuged at 18°C for 52 hr at 40,000 rpm in a Hitachi RP 65 TA fixed angle rotor. The gradient was pumped out from the bottom of the tube after centrifugation, and was continuously recorded the absorbance at 254 nm (Altex, model 105 B). The region of gradient from a density of 1.720 g/cm^3 to the light side of the peak was collected, mixed with 3 volumes of H_2O and 10 volumes of ethanol and recovered by centrifugation at 10,000 *g* for 10 min after storage at −20°C overnight. For the determination of buoyant density of DNA, six-drop fractions were collected from the bottom of the tube and the reflactive index of every fifth fraction was measured. Each fraction was diluted to one ml with H_2O and was determined the absorbance at 260 nm. As a marker of density *Micrococcus lysodeikticus* DNA (1.731 g/cm^3) was used.

Ultraviolet Absorption and Determination of Tm: Ultraviolet absorption spectra were determined with a Hitachi 356 spectrophotometer. For the measurement of Tm, DNA samples were dialyzed against to 0.1×SSC or 1×SSC to adjust the salt concentration exactly. The determination was carried out by the procedure of MANDEL and MARMUR[14] in the spectrophotometer equipped with thermospacer holder. The rate of temperature increase was about 1.5 to 2.0°C/min. All specimens of DNA used for the determination of Tm had been purified by banding on CsCl density gradients.

DNA Estimation: The procedure has been used to estimate DNA according to GILES and MYERS[6]. That is to add 2 ml of 4% diphenylamine in glacial acetic acid to 2 ml of test solution of DNA in 10% perchloric acid followed by 0.1 ml of aquaous 1.6 mg/ml acetaldehyde; after incubation at 30°C overnight the optical density difference at 595–700 nm is read against the 595–700 nm blank. DNA was defined by the spectrophotometer on the basis of 50 μg DNA per 1 OD_{260} unit.

Determination of G+C Content: The G+C content of DNA was obtained from the linear relation of MANDEL *et al*[15]. by using CsCl density gradient analysis:

$$(GC) = \frac{\rho - 1.660\ \text{g/cm}^3}{0.098}$$

where (GC)=mole fraction of guanine plus cytosine in native DNA. The G+C content of DNA could be also estimated from the Tm and M, the molar concentrations of the cation, by the relationship:

$$(GC) = 2.44(Tm - 81.5 - 16.6 \log M)$$

This equation was proposed by SHILDKRAUT *et al*[24].

Results

The investigation of DNA isolation procedure has been undertaken from chromatin of the cells cultured in BM with 2, 4-D, while the cultured cells and root tissues were used for the assessment of the isolation procedure of the total DNA.

Extraaction of DNA from Chromatin: DNA extracted from chromatin fraction of the cultured cells was purified by digestion with RNase and Pronase. The fibrous materials that recovered by the precipitation with ethanol revealed a typical absorption spectrum for nucleic acid with the maximum absorbance at 258 nm and the minimum at 233 nm (Fig. 1 A). The ratio of absorbance at 233 nm/258 nm/280 nm of the sample from cells cultured in BM with 2, 4-D was 0.44/1.00/0.51. DNA from cells cultured in BM had essentially the same grade of purity.

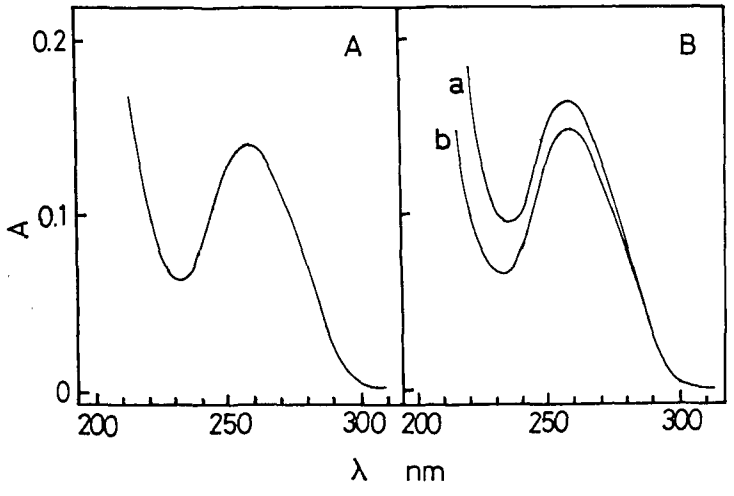

Fig. 1. Absorption spectra of DNA preparations from carrot. A. DNA extracted from chromatin of cells cultured in basal medium with 2,4-D. The DNA was purified by RNase and Pronase. B. Total DNA from roots purified by (a) RNase, Pronase and Sepharose 4B, and (b) further subjected to the treatment of cesium chloride density gradient centrifugation.

Extraction of Total DNA: Total DNA preparations purified by RNase and Pronase treatments were contaminated with undegradated RNA and protein, both of which formed powderly precipitates by the addition of ethanol. These contaminants, when DNA was finally pruified by banding on CsCl density gradients, overlapped the band of DNA and lowered its

purity grade, in considering the fact that small macromolecules should take a considerable time to migrate through the gradients. Additionally DNA preparation derived from tap roots was tenatiously contaminated with brown materials. The elution of these lysates from the Sepharose 4 B column gave

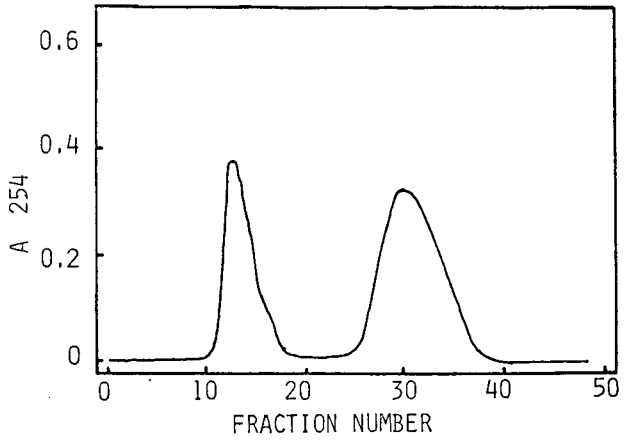

Fig. 2. Molecular sieving on Sepharose 4B of DNA solution from total tissues of carrot root. A 254 was continuously recorded.

TABLE 1. Hydroxyapatite fractionation of DNA preparations. The extracted DNAs were purified by the following two series. One of them consisted of digestion by RNase and Pronase, and the other included additional purification with Sepharose 4B. DNAs were subsequently fractionated from undegradated RNA and protein with hydroxyapatite by the manner described in Materials and Methods. After fractionation A 260 was determined

DNA source	purification steps of DNA	
	RNase and Pronase (%)*	RNase, Pronase and Sepharose 4B (%)*
Cultured cells**		
Total DNA	68.6	92.6
Chromatin DNA	96.7	—
Root		
Total DNA	33.8	92.2

* Percentage of 0.4 M phosphate buffer-eluted fraction to recovery.
** Cells cultured in basal medium supplemented with 2,4-D.

rise to two peaks; the first peak which contained DNA was separated from the second consisted of some low molecular species (Fig. 2). A trace of brown materials eluted in the exclusion peak, but the majority of the pigments were greatly retarded. In molecular sieving with agarose, when a large amount of DNA was loaded to the gel, it was often observed that a portion of DNA resulted in a slight delay of elution from the column because of its higher viscosity. For the removal of these small macromolecules, however, at least 4 OD_{260} units of DNA yielded in a good resolution with 1.6×17 cm column. After purification by agarose gel chromatography the ratios of absorbance at 233 nm/258 nm/280 nm of the exclusion peak (DNA) from the cultured cells was 0.48/1.00/0.50. On the other hand, the ratios from tap roots was 0.60/1.00/0.50 (Fig. 1 B-a), implied that the further purification was necessary. The DNA from tap roots also showed the ratio of 0.45/1.00/0.51 (Fig. 1 B-b) by the final purification step with CsCl density

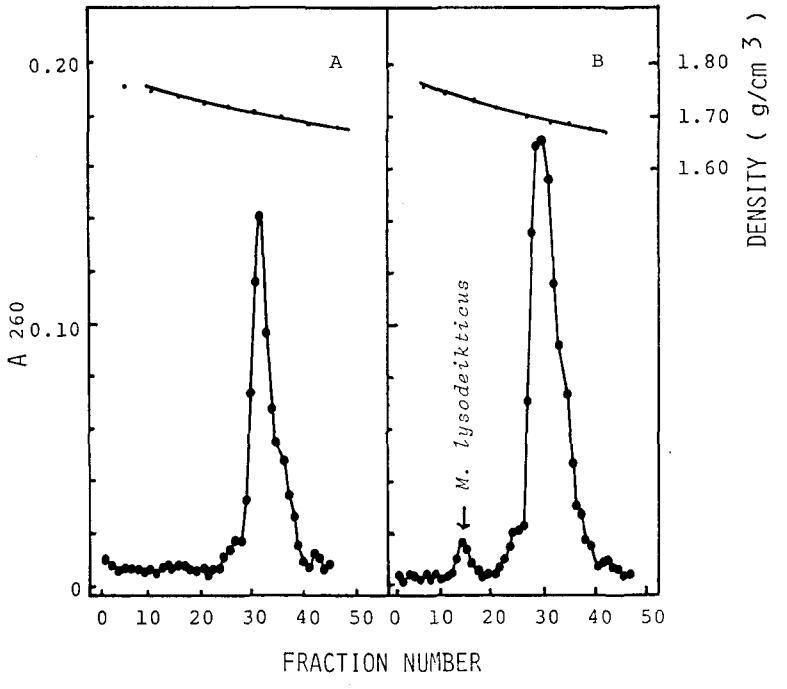

Fig. 3. Cesium chloride density gradient centrifugation of DNA from chromatin of cultured cells of carrot. A. Cells cultured in basal medium with 2,4-D. B. Cells were cultured in basal medium. Centrifugation was performed in a fixed angle rotor for 52 hr at 40,000 rpm, and fractionated from the bottom of gradients. Density marker (arrow) was *M. lysodeikticus* DNA (1.731 g/cm).

gradient centrifugation. The yields of DNA from 10 gr fresh weight of the cultured cells and root tissues were about 12 mg and 0.16 mg, respectively.

Purity Test with Hydroxyapatite (HA): The fraction of 97% of DNA from chromatin of the cultured cells was adsorbed to HA in 0.12 M PB and eluted in 0.4 M PB by the determination at OD_{260}. Adsorptions of RNA and protein to HA in 0.12 M PB at 60°C were below 10% and 6%, respectively. The values above 92% were obtained for total DNAs after the purification by agarose column (Table 1). Thus the undegraded RNA and protein were eliminated by this purification step. Furthermore this results implied that DNAs were isolated in the native state since denatured DNA was not adsorbed to HA in 0.12 M PB at 60°C[2].

Density Gradient Centrifugation of DNAs in CsCl: The DNA from chromatin of cultured cells (Fig. 3 A, 3 B, 4 A) and total DNA from root tissues (Fig. 4 B) were used for determination of the buoyant density. Carrot DNAs were centrifuged in CsCl density gradients as described in the Materials

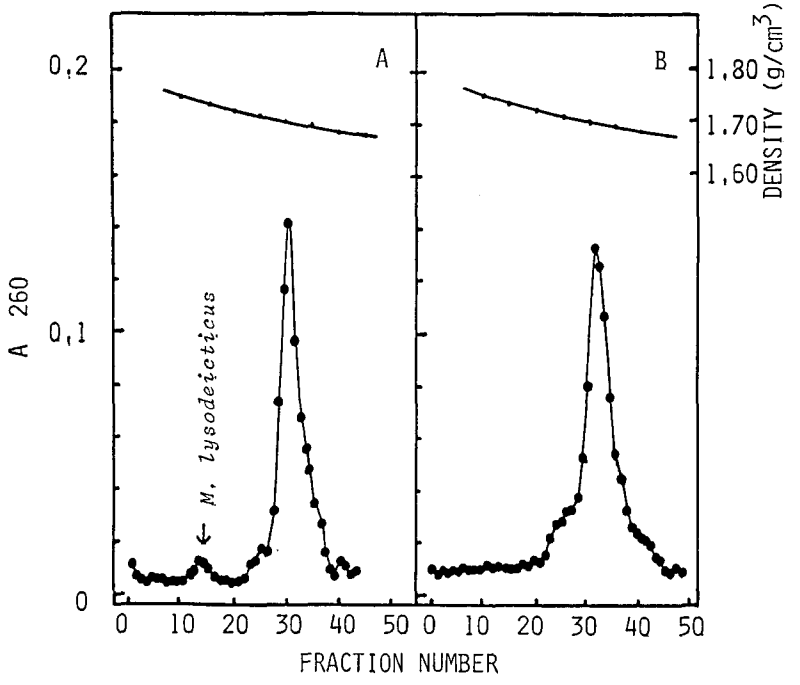

Fig. 4. Cesium chloride density gradient centrifugation of DNA from carrot. A. DNA from chromation of cells cultured in basal medium with 2,4-D. B. Total DNA from roots. Detailes are the same as Figure 3.

and Methods revealed a main band with density of 1.692 to 1.693 g/cm^3 and a satellite component at 1.702 to 1.708 g/cm^3. The main band was slightly asymmetric and some UV-absorbing materials were present on the less dense side of the main band. The guanine plus cytosine contents of DNA calculated from the buoyant densites were 32.7 to 33.7% for the main band and 42.9

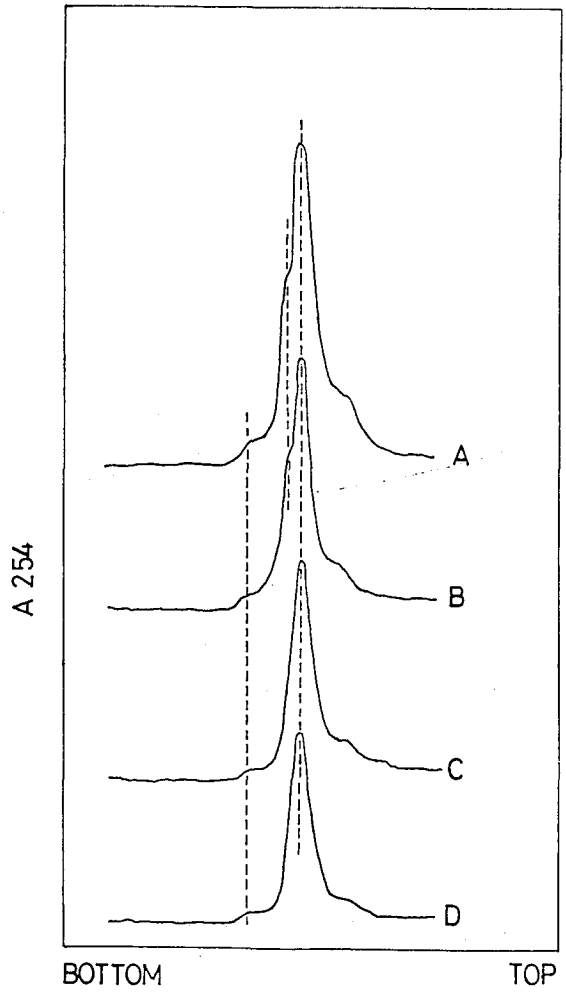

Fig. 5. Cesium chloride density gradient centrifugation of DNA from cultured cells. A. B. Total DNA. C. D. DNA isolated from chromatin. A. C. DNA from cells cultured in basal medium with 2,4-D. B. D. DNA from cells cultured in basal medium. Centrifugation was carried out in the same manner as Figure 3. Absorbance at 245 nm was continuously recorded with a effluent monitor.

to 49.0% for the heavy satellite. The total DNA from root tissues (Fig. 4 B) revealed a slightly high proportion of the heavy satellite component relative to that of DNA from chromation of cultured cells. The main peaks of them showed no significant differences in buoyant density. To characterize the DNAs from cells of carrot, as related to somatic embryogenesis, DNAs isolated from the cells cultured in BM and BM with 2, 4-D were banded on CsCl density gradients and to obtain a better separation of DNA components of close density the profiles were compared with a effluent monitor. As shown in Fig. 5, we could not detect any differences between the profiles of DNA from cells cultured in BM and those of BM with 2, 4-D for six days. Since somatic embryogenesis occurred on the 4th to the 8th day after the transfer of inocula to fresh medium, DNAs from the cells on the 3rd and 12th days' cultures were also analysed by CsCl density gradients. However, they showed essentially the same profile as was shown in Figure 5. The every total DNAs revealed a small shoulder on the more dense side of the main band (Fig. 5 A, 5 B), while this component was not detected in

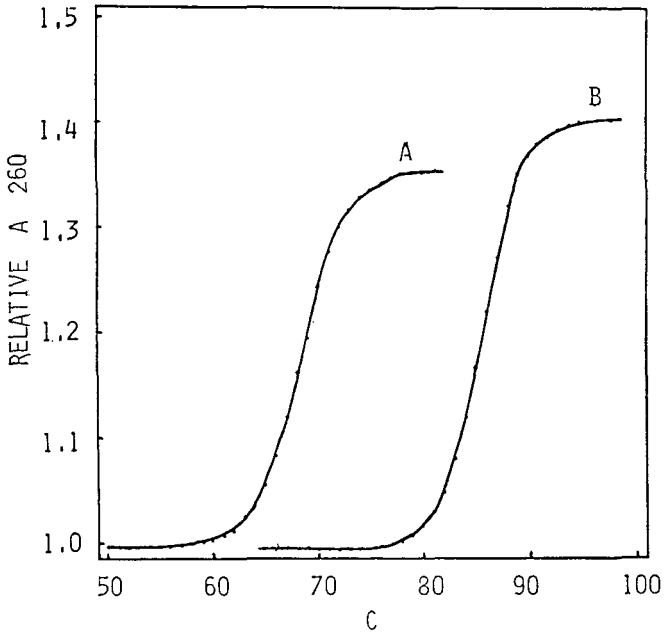

Fig. 6. Thermal denaturation profiles of carrot DNA isolated from chromatin of cells cultured in basal medium with 2, 4-D, which measured from continuous recording of absorbance at 260 nm as a function of temperature. A. DNA in 0.1×SSC. B. DNA in 1×SSC.

DNA from chromatin (Fig. 5 C, 5 D).

Melting Temperature: The thermal denaturation curves of DNA from chromatin of cultured cells in BM with 2, 4-D were shown in Figure 6. The midpoint of transition (Tm) was 68.4°C and hyperchromisity of the DNA was 35.5% in 0.1×SSC. The Tm and hyperchromisity of the DNA in 1×SSC were 85.6°C and 40.3%. The G×C content calculated from the Tm values in 0.1×SSC and 1×SSC were 37.3% and 38.8%, respectively. The Tm of satellite component of the DNA was 71.2°C in 0.1×SSC. The Tm and G+C content of all the DNA examined were summerized in Table 2.

TABLE 2. Tm values and base compositions of DNAs from carrot, determined by thermal denaturation technique

Source of DNA	Tm (C) in 1×SSC	Tm (C) in 0.1×SSC	G+C content* (%)
Cells cultured in basal medium			
Total DNA	—	68.6	37.8
Chromatin DNA	85.4	68.4	37.3
Main band	—	68.6	37.8
Satellite	—	71.2	44.1
Cells cultured in basal medium with 2, 4-D			
Total DNA	—	68.5	37.5
Chromatin DNA	85.6	68.4	37.3
Root			
Total DNA	—	68.7	38.0

* The values were calculated from the Tm in 0.1×SSC according to Shildkraut and Lifson (1965).

Discussion

In the present experiments, the carrot suspension cultures with high embryogenic potential were confirmed to be composed mainly of small but densely cytoplasmic cells with an abundance of nucleic acids, whereas the tissues of the tap root were very poor in them. The level of DNA was virtually roughly seventy five times higher in the cells of the suspension culture than in ones of the tap root. To evaluate the metabolic implication of the DNA in the induction of somatic embryogenesis of carrot plant, an improved methods for DNA isolation and identification were established and the comparative analyses were carried out by means of quite similar procedures using qualitatively different materials such as suspension cultured

cells and root tissue cells. An attempt to refine the purity of DNA as illustrated in Figure 2, by agarose column chromatography was employed effectively for isolation of DNA free from low molecular species and brown colored contaminants. Besides the above methods, highly purified DNAs were obtained by banding on CsCl density gradient centrifugation.

OHYAMA *et al.*[20] have already reported that the DNA isolated from carrot cells consisted of a major band with buoyant density of 1.693 g/cm^3 having a shoulder at 1.698 g/cm^3 and satellite component in the region of 1.706 g/cm^3, suggested that the component of 1.698 g/cm^3 might be originated from chloroplast DNA and the other satellite DNA (1.706 g/cm^3) from mitochondria. From the profiles represented in Figure 5 A and 5 B, two heavy components were also experimentally verified to be located on somewhat higher dense side other than the large major band, while the one of them with a density ranged between 1.702 to 1.708 g/cm^3 was likewise ascertained in the chromatin-DNA (Fig. 5 C, 5 D). Therefore the present results thus far obtained show unequivocally that this component in question was, mainly though not entirely, based on the nuclear DNA, irrespective of the fact that this type of component exclusively contained mitochondrial DNA in the wide variety of plant sources[11,29]. The some UV-absorving material on the less dense side of main band was firstly reported by JAWORSKY and KEY[10] using carrot root tissues. Furthermore it was also observed that a high proportion of the DNA from secondary phloem of the carrot showed a lower buoyant density than the main band[22]. Although they found that the light material was susceptible to DNase digestion, the direct evidence was not yet established whether the material was contaminated polysaccharide or an A+T rich satellite DNA.

The G+C values of main band calculated from buoyant density were at variance with the value calculated from the Tm. This contradiction indicates that the DNA may contain unusual bases such as 5-methylcytosine, in consideration of the facts that the methylation of cytosine could raise the melting point[5] and reduce the buoyant density[13] of DNA. The difference of G+C values of the heavy satellite may implicate that the modification of cytosine is low in heavy satellite component.

In some investigations on wheat embryos[3,4], it suggested that the amplification of rRNA genes occurred during maturation of embryo and the metabolic DNA present at the early stage of germination. On the other hand, SHÄFFER *et al.*[22] demonstrated that the modification of DNA composition on the way of differentiation was found using the technique of CsCl density gradient centrifugation and of reassociation kinetics of DNA[23]. They

detected the heavy components (1.717 and 1.721 g/cm^3) which were absent in the secondary phloem tissues and present in the cultured explant of carrot root. In the present experiment, however, no sure occurrence could be found these heavy satellite compoents in DNA from carrot cell suspension cultures and significant difference of Tm values between them was unable to confirm.

It must be treated with some caution unless the culture consisted of cells with fairly high competence of differentiation that cell suspension cultures are likely to provide an convenient system for the study of cell differentiation from the biochemical point of view. As stated above, the carrot cell suspension culture is virtually well suited for this study, since those sustained high embryogenic potential leading their own embryogenesis with high frequency. Further substantiation of this work resides in the results obtained when non-embryogenic cells, for instance, large vacuolated senescent cells or their aggregated cell clusters were excluded by means of sieving and centrifugation procedures.

In conclusion, the gross amplification or deletion of satellite component of DNA did not occur during the induction of somatic embryogenesis in carrot cell suspension culture. However, the possibility that the modification of genetic information content occurs during the process of somatic embryogenesis cannot be eliminated. Further work other than the centrifugal separation and the analysis of thermal denaturation of DNA should be required.

Summary

DNA from the cultured cells of carrot which were inductive and non-inductive for somatic embryogenesis, as well as DNA from the intact tap root tissues of carrot, were isolated and characterized. DNA from chromatin was purified by the treatment of ribonuclease A and Pronase E, while the purification of total DNA was performed by means of the enzymatic digestion and molecular sieving on agarose gel. DNA was finally purified and analysed by cesium chloride density gradient centrifugation, and the resulting purified DNA was also used for the determination of the melting temperature.

As far as analyzing by these methods, the qualitative differences of DNA were not detected, in relation to induction of somatic embryogenesis.

Total DNA from cultured cells consisted of main band of density at 1.692 to 1.693 g/cm^3 with small shoulder on the more dense side of the peak and of still heavier component in the region of density at 1.702 to 1.708 g/cm^3. The small shoulder just dense side of the main band was not

detected in the DNA from chromatin, while a large portion of the still heavier component was observed in it. Some UV-absorving material was present on the less dense side of main band. The melting temperature of DNA tested was 68.4 to 68.7°C in 15 mM NaCl and 1.5 mM trisodium citrate buffer, pH 7.0.

Acknowledgment

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