LIBERATION OF AGROBACTERIUM TUMEFACIENS DNA FROM THE CROWN GALL TUMOR CELL DNA BY SHEARING*

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Summary. Unsheared DNA from normal or crown gall tumor tissue of tobacco and the sheared DNA from the normal tissue gave only one band (ρ = 1.694) on CsCl density-gradient centrifugation. CsCl density-gradient centrifugation of sheared DNA from the tumor tissue, however, produced two bands, one corresponding to tobacco DNA (ρ = 1.694) and the other corresponding to A. tumefaciens DNA (ρ = 1.717). These results further strengthen the concept that crown gall tumor formation may involve integration of bacterial genome with the host cell genome.

Introduction. Ledoux and Huart (1969) have shown the integration and replication of Micrococcus lysodeikticus DNA in DNA of barley seedlings by CsCl density gradient centrifugation of sonicated DNA from barley seedlings which had been incubated with M. lysodeikticus DNA. Furthermore, recent work from several laboratories (Schilperoort et al., 1967; Quétier et al., 1969; Milo and Srivastava, 1969) strongly suggests that transformation of a normal plant cell into a tumor cell

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by A. tumefaciens may involve integration of bacterial genome into host genome. Bacterial specific RNA (Milo and Srivastava, 1969) and antigens (Schilperoort et al., 1969) have been detected in the bacteria-free tumor tissues and homology between bacterial DNA and the tumor DNA has been demonstrated (Quétier et al., 1969; Srivastava, 1970). Assuming that the tumor cell contained integrated A. tumefaciens DNA the possibility whether this bacterial DNA could be separated by CsCl density gradient centrifugation of sonicated tumor cell DNA was examined. The normal cell DNA was used as the control. The results of this investigation are described in this report.

Materials and Methods. The cultures of normal tissue and bacteria-free crown gall tumor tissue of tobacco (Nicotiana tabacum L. var. Wisconsin 38) maintained on complete and minus indoleacetic acid and kinetin media respectively (Srivastava, 1968) were used in the present work. To label DNA, small tissue segments were transferred on media low in P (8 mg/l of KH_2PO_4) and containing 4 mCi of $Na_2HP^{32}O_4$ per liter. After two weeks of growth on P^{32} containing media, the tissues were used for the isolation of DNA. The DNA was isolated essentially as described by Milo and Srivastava (1969) except that the tissue was homogenised using pestle and mortar and the SSC (0.15 M NaCl-0.015 M Na citrate, pH 7.0) used in the extraction contained 0.01 M of β-mercaptoethanol. A. tumefaciens (strain 4-32), was grown for 48 hours in nutriet broth plus 0.5% glucose. Bacteria were recovered by centrifugation and used for the isolation of DNA by the procedure of Marmur (1961).

The DNA from the plant tissue or from the bacteria was finally purified by chromatography on methylated albumin coated Hyflosupercel column (Srivastava, 1967). Fractions containing DNA were pooled and dialysed overnight against 1/10 SSC. The P^{32} labeled normal or tumor tissue DNA was sheared by 120 seconds sonic vibration at full

power in a 20 k cyc./sec. Bronwill Biosonic vibrator.

CsCl (3.5 g) was dissolved in 3 ml of DNA solution (in 1/10 SSC) in 5 ml nitrocellulose tubes. The tubes were then topped with 1 ml of paraffin oil and spun in SW-50L rotor at 40,000 rpm for 42 hrs. at 20°C. After centrifugation, 4 drop fractions were collected by bottom puncture of the tubes. The system was standardised by using E. coli DNA (ρ = 1.710) as the standard and by preparing a density standard curve from the refractive index readings of the fractions. Each fraction was diluted with 2.5 ml of water and its A_{260} determined. If DNA centrifuged was labeled than after reading the A_{260}, 50 µg bovine serum albumin and 1 ml of 25% cold trichloracetic acid (TCA) were added to each fraction. After standing overnight at 1°C the precipitates were collected on B_6 membrane filters and washed with cold 5% TCA. The membrane filters were dried and counted for P^{32} using toluene based scintillation fluid.

Results and Discussion. Figure 1 show that the unsheared DNA from the normal tobacco tissue and from A. tumefaciens banded at ρ = 1.694 and ρ = 1.717 respectively as reported for these DNA's by other investigators (Whitfeld and Spencer, 1968; Meyer and Lippincott, 1967). The sheared normal cell DNA produced only one band in CsCl gradient with peak at ρ = 1.694 but no new bands. The unsheared tumor cell DNA gave a sharp band at ρ = 1.694-1.696. The sheared tumor cell DNA, on the other hand, gave two bands, one band corresponding to normal cell DNA (ρ = 1.694) and the other small band corresponding to bacterial DNA (ρ = 1.717). Although the small size of this new band is not unexpected, it would probably still be considered an artifact if detected in only one isolated experiment. This band corresponding with the position of bacterial DNA was, however, observed in all the 6 experiments that were conducted and it was never detected for the normal tissue. The results of this study together with other hybridization

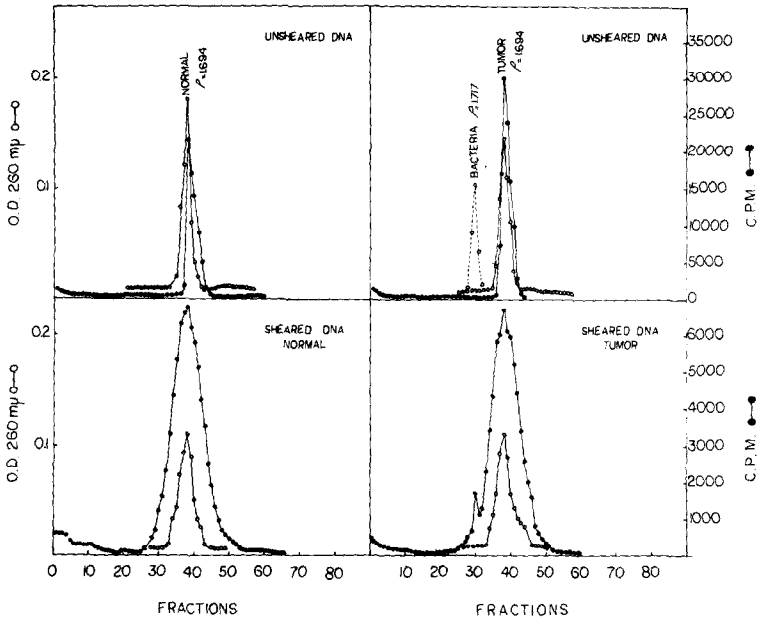

Figure 1. CsCl density-gradient centrifugation of DNA from normal tobacco tissue, tumorous tobacco tissue and the crown gall bacteria.

(Quétier et al., 1969; Milo and Srivastava, 1969; Srivastava, 1970) and immunological data (Schilperoort et al., 1969) suggest that cellular transformation by A. tumefaciens may involve integration of bacterial genome with host genome.

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