# Cell Transformation by Different Forms of Polyoma Virus DNA

Lionel Crawford, Renato Dulbecco, Mike Fried, Luc Montagnier, and Michael Stoker

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# *CELL TRANSFORMATION BY DIFFERENT FORMS OF POLYOMA VIRUS DNA**

BY LIONEL CRAWFORD, RENATO DULBECCO, MIKE FRIED, LUC MONTAGNIER, AND MICHAEL STOKER†

INSTITUTE OF VIROLOGY, GLASGOW

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DNA extracted from purified polyoma virus contains closed and open molecules which are separable by velocity sedimentation either in the analytical or in the preparative ultracentrifuge.[1, 2] The closed configuration of the DNA gives rise to the fast band (component I) and the open configuration to a slow band, which is in some cases double (components II and III). Components I and II are able to produce plaques on mouse embryo monolayers, i.e., they possess cytocidal activity[1] but which of the components cause cell transformation[3] could not be determined owing to the lack of an adequate quantitative assay for transformation. A determination of the role of these DNA components in transformation is important, since it may clarify the mechanism of transformation.[4] Such a determination is now made possible by the development of a sensitive quantitative assay for transformation caused by polyoma virus.[5, 6] This method is based on the ability of the transformed cells to form colonies in agar where the vast majority of the untransformed cells are unable to form colonies. Preliminary experiments have demonstrated that this method is also suitable for the study of the transforming ability of polyoma DNA.

*Materials and Methods.—Density gradient centrifugation:* Polyoma DNA extracted from purified virus with phenol[7] was separated into fast and slow components by centrifugation either on sucrose or CsCl density gradients. The DNA was layered on a linear gradient of sucrose (20–5% in 0.1 *M* NaCl, 0.01 *M* EDTA) and centrifuged for 7.5 hr at 35,000 rpm in the SW39 rotor, Spinco model L ultracentrifuge. Alternatively, the DNA was layered on a column of 3 ml of CsCl (Harshaw Chemical Company, optical grade) density 1.50 gm/ml containing 0.015 *M* NaCl and 0.0015 *M* Na citrate, pH 6.9. The tube was then filled with paraffin oil and centrifuged for 4 hr at 35,000 rpm in the SW39 rotor. In both cases drops were collected after puncturing the bottom of the tube.

*Analytical centrifugation:* The band-centrifugation method[8] was used for the analysis of the gradient fractions. Conditions were as follows: 30-mm cell with 4° Kel-F centerpiece, 35,600 rpm, 20°C. The bulk solution was CsCl (density = 1.36 and 1.50 gm/ml in different experiments) containing Tris buffer (0.01 *M*, pH 8.0), and the volume of the lamella of DNA solution layered on the gradient was 10–50 μl. Some fractions were also characterized by equilibrium density gradient centrifugation.[9] Samples in CsCl (density = 1.71 gm/ml) containing Tris buffer (0.1 *M*, pH 8.5) were centrifuged at 35,600 or 31,410 rpm for 3 days at 25°C. Ultraviolet-absorption photographs were taken and scanned with a Joyce-Loebl microdensitometer.

*Transformation assay:* Confluent monolayer cultures of BHK21 hamster cells,[10] which had been seeded 1–2 days previously, were exposed to 0.1 ml of DNA solution after shocking with hypertonic NaCl.[7] After 30 min exposure to the DNA at 29°C, the cells were allowed to recover from shocking for about 18 hr at 37°C in Eagle's medium with 20% calf serum and 10% tryptose phosphate broth. They were then suspended and plated in agar.[5, 6] In order to verify the validity of this assay system under the conditions used, a number of the colonies growing in agar were isolated

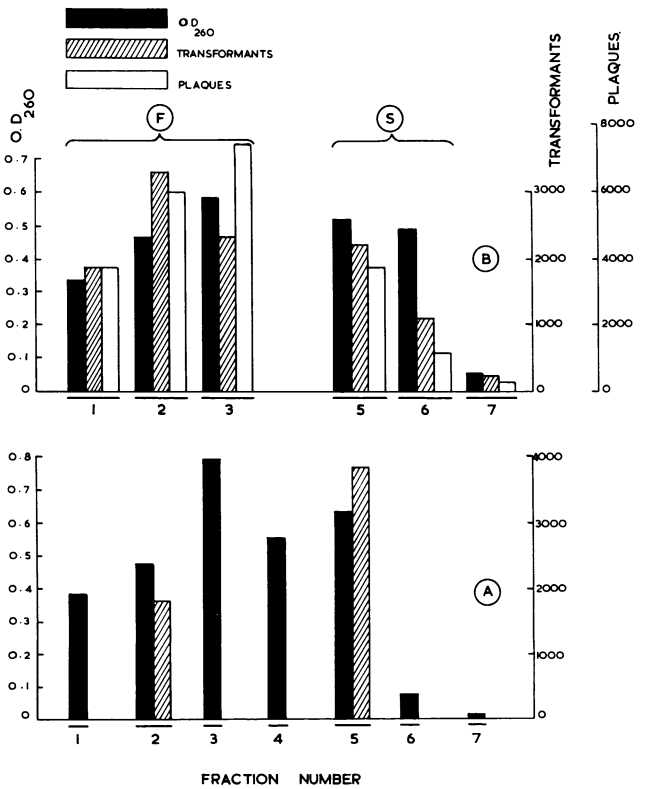


FIG. 1.—Data from first DNA preparation. The fractions were obtained by sedimentation in sucrose density gradients. The bottoms of the gradients are at the left of the figure.

and recultured on glass. It was found that the number of colonies lacking the transformed morphology on replating was in agreement with the number expected from the colony-forming efficiency of untransformed cells, as determined in uninfected cultures. In most of the fractions assayed, this number was negligible.

*Plaque assay:* The assay for cytocidal activity was carried out by plaque formation in mouse embryo monolayers.[7]

*Results.*—Two preparations of polyoma DNA were examined. The first preparation was divided into two parts which were sedimented in two parallel sucrose density gradients in the same rotor. Fractions collected from tube A were used for optical density determinations. Two of the fractions were also studied for transformation and for density distribution; the results are shown in Figure 1*A*. Corresponding fractions from tube B were used for optical density determination and for both transforming and plaque-forming activities, and the results are shown in Figure 1*B*. The molecular species present in the various fractions of tube B were determined by band centrifugation in the analytical ultracentrifuge; the results of these determinations are given in Figure 2. These results show that the fast- and slow-

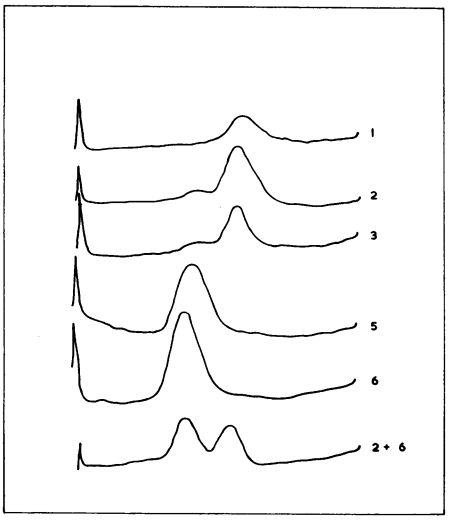


FIG. 2.—Microdensitometer tracings from analytical band centrifugation of fractions from the gradients shown in Fig. 1. Fractions 1, 2, 3, 5, and 6 were centrifuged at 35,600 rpm in CsCl $\rho$ = 1.36; the tracings are from photographs taken 80 min after reaching speed. The mixture of fractions 2 and 6 was centrifuged at 35,600 rpm in CsCl $\rho$ = 1.50; the tracing is from the photograph taken 112 min after reaching speed. The meniscus is at the left of the diagram in each tracing.

moving molecules (rings and rods) were separated in two distinct bands. The fast band was slightly contaminated with slow component, unless the latter was generated during analytical centrifugation. Component III[2] molecules were either absent or were not separated under the conditions used for analytical centrifugation. The second alternative may be more likely since a sample from fraction 5 of tube A showed an additional, lighter, band in equilibrium density centrifugation. Therefore, the fractions of band F contain component I, and those of band S contain component II, perhaps mixed with component III.

With respect to transformation, we wish merely to point out that activity is present both in fractions of band F and in those of band S and in this respect is similar to plaque-forming activity. At this time we do not wish to draw conclusions from the numbers of transformants produced by the various fractions, since the operation of the assay procedure for DNA is not yet known in detail.

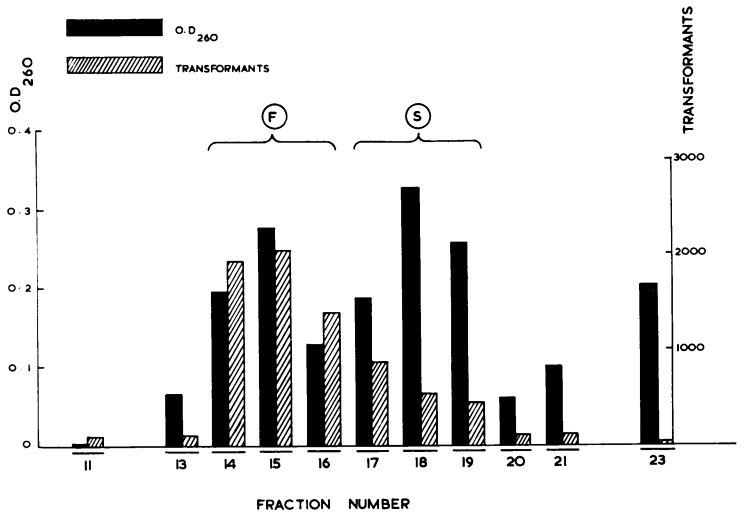


FIG. 3.—Data from second DNA preparation. The fractions were obtained by sedimentation in a CsCl density gradient. The bottom of the gradient is at the left of the figure.

The second preparation of polyma DNA was sedimented in CsCl of density 1.50 gm/ml; fractions were studied both for optical density and for transformation. The results are shown in Figure 3. Two bands were again resolved in this experiment, and again transformation was produced by all the fractions of both bands, although fractions of band F were most active. Fractions outside the two bands produced no transformation. No transformation was obtained with a preparation of DNA from BHK21 cells.

*Conclusions.*—The transforming ability of DNA extracted from polyoma virus is not the exclusive property of any one of the various molecular species of the DNA. Molecules both in the ring form and in the open form are able to transform. Whether molecules of component III produce transformation cannot be established as yet.

These results show that a special type of polyoma DNA molecule is not required *from the start* in order to obtain transformation. They do not exclude the possibility that a special type is required at some later stage. The implication is therefore that the initial stages are similar in the two types of virus-cell interaction which lead either to transformation or to plaque formation.

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* This research was conducted at the Institute of Virology, University of Glasgow, by Lionel Crawford and Michael Stoker (Medical Research Council Experimental Virus Research Unit), Renato Dulbecco (Visiting Professor of the Royal Society from Salk Institute for Biological Sciences, San Diego, California), Mike Fried (California Institute of Technology, Pasadena, California), and Luc Montagnier (Laboratoire Pasteur de l'Institut du Radium, Paris).

† In alphabetical order.

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# *CELLULAR ASPECTS OF THE MAMMALIAN RADIATION SYNDROME: NUCLEATED CELL DEPLETION IN THE BONE MARROW**

BY THEODORE T. PUCK

DEPARTMENT OF BIOPHYSICS, UNIVERSITY OF COLORADO MEDICAL CENTER, DENVER

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One of the striking features of the acute mammalian radiation syndrome is the wide diversity in the degree of cell depletion manifested in different tissues.[1] To explain this, it has been proposed that the cells of different tissues are intrinsically different in their susceptibility to destructive or removal processes induced or accelerated by ionizing irradiation.[2] Many biochemical functions have been postulated to underlie these large, specific differences in cell radiosensitivity, including a special role of cyanide-sensitive enzyme systems; membrane permeability; uncoupling of oxidative phosphorylation; effects on DNA synthesis; and increased sensitivity of cells during mitosis.[3] However, it has been calculated that somatic cells in different tissues would need to differ by more than a thousandfold in their sensitivity to destruction or removal by irradiation in order to account for the observed differences in tissue sensitivity.[4] No critical experiments exist demonstrating the validity of any of these postulated factors in producing differences *of the required order of magnitude* in the radiosensitivity of cells of the different tissues.

An alternative formulation presupposes that the fundamental radiosensitive function lies only in the ability of cells to reproduce. The differential sensitivity with respect to cell depletion of different tissues is interpreted as reflecting merely their differences in normal rates of cell turnover. This theory has been proposed by several workers for various tissues. For example, Osgood[5] invoked it to explain the changes in the bone marrow of irradiated animals and carried out experiments *in vitro* in which cell numbers of bone marrow suspensions were measured at various times after X irradiation. The results, while consistent with the proposed formulation, did not constitute conclusive evidence that could eliminate the alternative theory. Obviously, both effects might operate.

The theory that reproductive inhibition is the immediate and fundamental cellular action resulting from irradiation received new and substantial support when techniques were introduced which made possible accurate measurement of single cell survival curves for the reproductive function of mammalian cells.[6] The quantitative measurements which then became possible demonstrated: (*a*) The value for the mean lethal reproductive dose ($D^0$) for a representative mammalian