Failure to Detect Homology between the DNA of the Shope Fibroma Virus and the DNA of the Sensitive Cell

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SUMMARY

The nucleic acid of the Shope fibroma virus, a virus which induces tumours in the rabbit, does not show any homology with the DNA of the cell of this animal. The relationship of this fact to the oncogenic mechanism of this virus is not clear.

INTRODUCTION

Shope fibroma virus, a DNA virus of the pox group, develops in the cytoplasm. It induces benign tumours in the adult rabbit and malignant tumours in the new-born rabbit (Harel & Constantin, 1954). It is interesting to compare Shope fibroma virus with other DNA oncogenic viruses such as simian virus 40 (SV40) and polyoma and with RNA viruses such as Rous sarcoma virus (RSV). In the natural host, Shope fibroma virus, like RSV, can give rise to virus producing tumours. Unlike SV40, polyoma and adenoviruses, it does not cause malignant tumours in animals other than its natural host. In vitro, Shope fibroma virus like SV40 and polyoma, suppresses contact inhibition and induces synthesis of cellular DNA. It is different from SV40 and from RSV because cellular modifications appear without previous cellular division (Hinze & Walker, 1964; Tompkins, Walker & Hinze, 1969).

Are homologous sequences between virus DNA and host DNA responsible for the possible appearance of cell transformation by this virus?

Cells transformed by SV40 or polyoma virus contain at least one molecule of virus DNA (Westphal & Dulbecco, 1968). Reich, Black & Weissman (1966) established that SV40 DNA showed a homology with the DNA of different mammalian cells, especially that of the hamster. Winocour (1965) found a complementary property between polyoma virus DNA and RNA synthesized in vitro, using mouse cell DNA as template and also between papilloma virus DNA and RNA similarly synthesized from rabbit cell DNA. Winocour (1967, 1968) also showed that polyoma virus particles contained fragments of cellular DNA. As this has not been reported for other oncogenic viruses, especially adenovirus type 12 (zur Hausen, 1969), it cannot be considered a distinctive property of oncogenic viruses. More recently, Aloni et al. (1969) claimed that SV40 DNA contained a pure virus DNA which can hybridize with monkey and mouse cell DNA.

The aim of our experiments was to see whether a structural homology could be demonstrated between the DNA of Shope fibroma virus and that of RK 13 cells by a DNA–DNA hybridization method.
METHODS

Virus. Shope fibroma virus, OA strain.

Cells. RK13 cells (Beale, Christofinis & Furminger, 1963) were grown in Eagle's basic medium (BME), in Earle's salt supplemented with 10% calf serum. Medium for infected cells contained 2% horse serum.

Purification of the virus. The virus was isolated and purified either from infected RK13 cell cultures or from tumours in adult rabbits, according to the method of Joklik (1962) adapted by Jacquemont, Precosta & Gautheron (1969) with added RNase and DNase treatment (50 µg/ml. of each enzyme for 15 min. at 37°C) to reduce contamination by the cellular nucleic acids.

Extraction of the DNAs. RK13 cells were labelled with 0.1 µc/ml. of [3H]thymidine (10 c/mole) for 72 hr. The extraction was performed according to Marmur's method (1961) modified by using ribonuclease and pronase. For the virus DNA, RK13 cells infected with 10 f.f.u./cell (Israel & Sachs, 1964) were labelled with 0.5 µc/ml. of [3H]thymidine (18 c/mole) between the 5th and the 24th hr after infection. The virus DNA was extracted by successive treatments with 2-mercaptoethanol (25 µg/ml.) sodium dodecyl sulphate (0.3% for 1 hr) pronase (500 µg/ml., overnight at 37°C) and phenol; it was dialysed against 0.15 M-sodium chloride + 0.015 M-sodium citrate (Jacquemont et al. 1969). This DNA was chromatographed on a Kieselguhr methylated albumin column (MAK) according to the method of Mandell & Hershey (1960). Calf thymus DNA was extracted according to Kirby's phenol method (1959), and the DNA of Escherichia coli by Marmur's method (1961).

Determination of the purity of the virus DNA by equilibrium density gradient centrifugation. The unlabelled virus DNA was denatured at 100°C for 10 min. in a solution of 1/10 dilution the 0.15 M-sodium chloride + 0.015 M-sodium citrate. Then CsCl was added to a final concentration of 0.5 M and the DNA was incubated at 60°C for 12 hr, when 4 µg. of Micrococcus lysodeikticus DNA marker were added and density increased to 1.700 g/ml, with CsCl. The mixture was centrifuged for 24 hr at 40,000 rev./min. at 25°C in a Spinco centrifuge (model E). The DNA of the tritiated virus was determined by the same procedure in a preparative ultracentrifuge, the DNA of the RK13 cells being labelled with 14C (0.02 µc/ml. at 45 mc/mole for 72 hr). The mixture was centrifuged for 60 hr at 28,000 rev./min. in an SW 50 rotor.

Hybridization procedure. For DNA-DNA hybridizations, different unlabelled DNAs were previously denatured in a 1/10 dilution of the 0.15 M-sodium chloride + 0.015 M-sodium citrate either by heat (100°C for 10 min.) followed by rapid cooling to 0°C, or by NaOH and neutralization by HCl after 20 min. The quantity of DNA fixed was determined by ultraviolet spectroscopy after elution by hydrolysis in 0.2 N-HCl at 100°C for 10 min. The native DNA was 14% fixed, while the DNA denatured by heat or by NaOH was practically 100% fixed in a 0 to 100 µg. scale. Increasing quantities of these different DNAs were placed on Schleicher and Schuell B6 filters (Gillespie & Spiegelman, 1965). After drying, the filters were pre-incubated in 1 ml. of 0.04% bovine serum albumin in 3 x SSC (three times concentrated) for 6 hr at 60°C (Denhardt, 1966). Then 0.1 ml. of increasing quantities of labelled DNA were added after fragmentation by ultrasonic treatment (10 times for 1 min.) each time with a Branson apparatus of 20 kcyc./sec. at full power, then denatured by heat for the time necessary to obtain a minimum background. Simultaneously, the sedimentation coefficient of DNA decreased from 20 to 6 s. The filters were incubated for 24 hr at 60°C and then washed with 200 ml. of 3 mm-tris pH 9.4 (Warnaar & Cohen, 1966). The filters were dried and the radioactivity was determined by liquid scintillation.
RESULTS

To obtain proper hybridizations, a virus DNA free of cellular DNA must be used. While denatured DNA of higher organisms retains the density of single-stranded DNA after reannealing at 60° in equilibrium density gradient centrifugation in CsCl, virus DNA reverts to its original density after the same treatment (Jungwirth & Dawid, 1967). This procedure would have revealed any possible cellular DNA contaminant. The buoyant density of virus DNA was 1.6996 ± 0.0002 g./ml. (≈ 1.700 g./ml. from cellular DNA)

Fig. 1. Equilibrium density gradient centrifugation in CsCl of Shope fibroma virus DNA and RK 13 DNA in the analytical ultracentrifuge. The graph shows the densitometer tracing of u.v. photographs taken after 48 hr of centrifugation at 40,000 rev./min. and 25° in the Spinco model E centrifuge. Each centrifuge cell contains 4 µg. of Micrococcus lysodeikticus DNA marker of density 1.731. (a) Native RK 13 DNA (1.700); (b) reannealed RK 13 DNA (1.712); (c) native Shope fibroma virus (1.699); (d) reannealed Shope fibroma virus DNA (1.701).

Fig. 2. Hybridization between tritiated Shope fibroma virus DNA (0.5 µg. at 12,300 counts/min. µg.) and increasing amounts of Shope fibroma virus DNA, ●; E. coli DNA, △; calf thymus DNA, ■; RK 13 DNA, ○; immobilized on nitrocellulose filters. Fractions of Shope fibroma virus DNA hybridizing with: Shope fibroma virus DNA 70%, (1); RK 13 DNA 0.45% (2); calf thymus DNA 0.4%, (1); E. coli DNA 0.3% (2); no DNA 0.55% (2).
Number of experiments given in parentheses.
It is interesting to compare on the one hand the buoyant density of Shope fibroma virus (an oncogenic virus) with the density of other poxviruses, and on the other hand, the buoyant densities of different types of oncogenic or non-oncogenic adenoviruses (Piña & Green, 1965).

**Estimation of the fractions of RK13 and Shope fibroma virus DNAs which hybridize**

Constant quantities of [3H]DNA of Shope fibroma virus were hybridized with increasing quantities of different, homologous, heterologous and unlabelled DNAs put on filters (Fig. 2). Shope fibroma virus DNA hybridized at more than 70% with its homologue. The percentages obtained with the DNAs of RK13 cells of calf thymus and E. coli were similar to those obtained with the background.

Another series of experiments consisted in hybridizing a constant quantity of [3H]DNA from RK13 cells with increasing quantities of different homologous and heterologous unlabelled DNAs fixed on the filters (Fig. 3). The RK13 DNA hybridized at 30% with its homologue and at 64% with calf thymus DNA, but only at 1.17% with E. coli DNA and at 1.22% with Shope fibroma virus DNA. When the hybridization was performed at laboratory temperature instead of 60°, the percentage of Shope fibroma virus DNA was 1.43%, as against 1.5% at 60°.
DISCUSSION

If the fraction of Shope fibroma virus DNA hybridizing with RK13 DNA is smaller than that of calf thymus DNA and about the same as that of Escherichia coli DNA, is the fraction of RK13 DNA hybridizing with Shope fibroma virus DNA significant? The percentage of Shope fibroma virus/RK13 hybridization (1.22 %) was not negligible when compared with a background of 0.43 %, even if background determination was of course influenced by uncertainties in the determination of the specific activity. As the DNA of E. coli is known not to hybridize with the DNA of mammalian cells, it was the best available control. Thus, adsorption was eliminated. The difference between 1.22 and 1.17 % obtained with E. coli is not significant. Moreover, there is no difference between a hybridization performed at 60° and one performed at room temperature. Under our experimental conditions, no structural homology was detectable between the Shope fibroma virus genome and that of the rabbit cell, at least to the same extent as that found in the other systems.

To analyse these results, it is advisable to recall previous work on the comparison of the genome of oncogenic viruses and that of different normal cells. For the RNA viruses, Harel et al. (1966a, b) showed that the genome of the Rous sarcoma virus (RSV) and that of avian myeloblastosis were analogous to the genome of chicken cells, infected or not, and to a lesser degree to the genome of mammalian cells. This work, repeated by Wilson & Bauer (1967), was also done with Rauscher virus (Emanoil-Ravicovitch, Larsen & Boiron, 1969; Emanoil-Ravicovitch, Baudelaire & Boiron, 1969). With regard to oncogenic DNA viruses, Aloni et al. (1969) offered two explanations for the results obtained with SV40: the virus DNA molecules may have contained areas of homology with cellular DNA which arose during the evolution of this oncogenic virus or may have incorporated cellular DNA sequences into a fraction of replicating virus DNA molecules, possibly during the lytic interaction of cell + virus.

The absence of hybridization between the Shope fibroma virus DNA and the complete DNA of the rabbit cell could mean a difference between the mechanism of induction of tumours by Shope fibroma virus and the mechanism of induction of tumours by other oncogenic viruses, such as SV40 and polyoma virus. This notion is in agreement with the hypothesis of Kato et al. (1966). Investigations were made in this direction.

Recently, Yoshikawa-Fukada & Ebert (1969) established a homology between the genome of RSV and those of the oncogenic viruses like SV40 and adenovirus 12, but not with non-oncogenic viruses like adenoviruses 2 and 4. Moreover, these hybrids have identical melting points so that a single region of mammalian DNA can be considered as complementary to the different nucleic acids of oncogenic viruses.

If this hypothesis is correct, further investigations on a homology between Shope fibroma virus DNA and SV40 DNA or oncogenic adenovirus DNA would be interesting.

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