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Genome Analysis and Reverse Transcriptase Activity of Human Teratocarcinoma-derived Retroviruses

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SUMMARY

Five of five human teratocarcinomas cultured in vitro could be induced to synthesize retrovirus-like particles, albeit in extremely low amounts. The accumulation and purification of the human teratocarcinoma-derived retrovirus (HTDV) from one of these cell lines allowed characterization of its genomic material as high mol. wt. (60S) RNA. Partial purification of HTDV-associated RNA-dependent DNA polymerase (reverse transcriptase) has also been achieved. HTDVs are easily distinguishable from the exogenous human T-lymphotropic viruses and human immunodeficiency viruses by morphological, biological and immunological criteria.

INTRODUCTION

The first isolation of a human retrovirus, now classified as a member of the group of human T-lymphotropic viruses (HTLV), was described in 1980 (Poiesz et al., 1980; for review, see Wong-Staal & Gallo, 1985). HTLV-I and -II are suspected to play an aetiological role in the development of acute T-lymphocytic lymphomas and rare forms of hairy cell leukaemia, respectively. Infection by HTLV-III (Popovic et al., 1984), also designated lymphadenopathy-associated virus (Barré-Sinoussi et al., 1983) or acquired immune deficiency syndrome (AIDS)-associated retrovirus (Levy et al., 1984) may lead to the development of AIDS. The AIDS viruses are now called human immunodeficiency viruses; at least two strains (HIV-1 and -2) can be distinguished by serological and genomic differences.

In another approach to search for human retroviruses, we had previously reported the presence in human sera of antibodies recognizing the envelope antigens of animal retroviruses (Kurth et al., 1977; Kurth & Mikschy, 1978). Subsequent data indicated that the antibodies may have been of a heterophil nature as they were directed against the carbohydrate side chains of the retroviral envelope antigens. These antibodies were, therefore, very probably formed in response to non-viral carbohydrate-containing antigens (Barbacid et al., 1980; Snyder & Fleissner, 1980; Löwer et al., 1981). However, sera from untreated patients with teratocarcinomas (TC) showed unusually high antiviral antibody titres which could not be completely absorbed with carbohydrate (Kurth et al., 1980; Harzmann et al., 1982).

These at first inexplicable findings were intriguing. Retrovirus-like particles can be observed budding from the syncytiotrophoblast layer of animal and human placentas (Kalter et al., 1973; Dirksen & Levy, 1977) and differentiating TC may contain trophoblastic elements. Therefore, we decided to study in vitro tissues or tumours of embryonic and placental origin for the expression of retroviruses or retrovirus markers. Whereas explants from placentas did not grow for any useful period of time, three differentiating TC could be adapted to grow in tissue culture while retaining their pluripotency to differentiate (Löwer, 1983; Löwer et al., 1984). Early electron microscopical studies (Boller et al., 1983) revealed the very rare presence of retrovirus-like particles in these as well as in two other previously explanted (Fogh & Trempe, 1975) human TC lines, whereas other human cell lines or strains originating from tumours of the reproductive organs (e.g. choriocarcinomas, embryonic carcinomas or seminomas) remained virus-negative.
Rapid characterization of the HTDV was impossible due to the extremely low rate of virus synthesis. All attempts to increase virus production or to rescue virus markers (e.g. by cloning of TC cells, by infection of new host cells with HTDV or by infection of TC cells with animal retroviruses or vesicular stomatitis virus to isolate recombinants or phenotypically mixed particles) were unsuccessful (Boller et al., 1983; Löwer et al., 1984). HTDV production could be increased however by treatment of TC cells with chemicals known to induce synthesis of chromosomally integrated retroviruses (see below).

Based on the assumption that HTDV are probably produced by the trophoblastic cells in the differentiating TC cultures, growth conditions were modified to increase the proportion of trophoblastic cells, as measured by the concentration of beta human chorionic gonadotropin (β-HCG) in the tissue culture medium (Löwer et al., 1984). Induction of retrovirus synthesis in such cultures either by pyrimidine analogues (e.g. iododeoxyuridine, IUdR) or by inhibitors of DNA methylation (e.g. 5-azacytidine) finally allowed the density gradient banding of small amounts of HTDV from large volumes of short-term harvests from mass cultures.

Biochemical investigations reported here establish the retroviral nature of HTDV by showing that (i) their genome is RNA and has a size of 60S to 65S, (ii) they possess an endogenous reverse transcriptase activity as well as a novel inhibitory activity for the corresponding exogenous enzyme assay and (iii) their reverse transcriptase can be purified and characterized from preparations of sucrose density-banded HTDV.

The results reported in this communication were obtained with HTDV-3 of the TC line GH and confirmed with HTDV-1 of the Tera-1 line (for nomenclature, see also Löwer et al., 1984).

**METHODS**

*Cells and electron microscopy.* Human tumour material, kindly provided by Dr R. Harzmann, Department of Urology, University Clinics Tübingen, F.R.G. was immediately put into culture by mincing the tissues into 1 mm cubes with and without subsequent trypsinization (Löwer, 1983; Löwer et al., 1984). Cells were grown in Dulbecco's modified Eagle's medium containing 20% foetal calf serum, kanamycin and neomycin and were transferred either by trypsinization or physical detachment (Kurth et al., 1980; Löwer et al., 1984). Two TC lines (Tera-1 and Tera-2) were kindly supplied by Dr J. Fogh (Sloan-Kettering Memorial Cancer Center, Rye, N.Y., U.S.A.). Electron microscopical techniques were described previously (Frank et al., 1978; Boller et al., 1983).

**HTDV preparation for RNA-dependent DNA polymerase (RDDP) assays.** TC cultures were induced with 3 µM-5-azacytidine or 10 µg/ml IUdR, plus 1 µM-dexamethasone (DXM) and 1% DMSO (Löwer et al., 1984). After 24 h, the medium was changed to 1 µM-DXM and 1% DMSO. Starting 3 days later, supernatants were harvested every 12 h for approximately 10 days. Cells and debris were pelleted by low and intermediate speed centrifugations and supernatants were stored at −70 °C. Usually 401 batches were harvested and banded by continuous flow centrifugation over 60% sucrose (model RK ultracentrifuge; Electro-Nucleonics, Fairfield, N.J., U.S.A.). Fractions (50 ml) of the self-generated sucrose gradient were diluted to 100 ml with 10 mM-Tris HCl pH 7.2, 5 mM-dCTP, 0.015 mM-[Me-3H]dTTP (sp. act. 10 Ci/mmol), 0.015 mM-[8-3H]dGTP (sp. act. 10 Ci/mmol) at 37 °C for 30 min. The RDDP activity of disrupted viruses with synthetic template/primers was tested in 50 mM-Tris HCl pH 8.2, 80 mM-KCl, 10 mM-MgCl$_2$, 2 mM-dithiothreitol (DTT), 4 mM-phosphoenolpyruvate, 10 mg/ml pyruvate kinase, 0.1% NP40, 0.1 mg/ml actinomycin D, 2 mM-dATP, 2 mM-dCTP, 0.015 µM-[Me-3H]dTTP (sp. act. 10 Ci/mmol), 0.015 mM-[8-3H]dGTP (sp. act. 10 Ci/mmol) at 37 °C for 30 min. The RDDP activity of disrupted viruses with synthetic template/primers was tested in 50 mM-Tris–HCl pH 8.2, 80 mM-KCl, 1 mM-DTT, 0.1% NP40, 10 µg/ml (rA)$_n$-(dT)$_{10}$, or (dA)$_n$-(dT)$_{10}$, 0.015 µM-[Me-3H]dTTP (sp. act. 10 Ci/mmol). One µM-MnCl$_2$ for HTDV, Friend murine leukaemia virus (Fr-MLV), simian sarcoma-associated virus (SSAV), baboon endogenous virus (BEV) or 10 mM-MgCl$_2$ for transformation-defective Schmidt-Ruppin Rous sarcoma virus (RSV) subgroup D yielded optimal incorporation of radioactive dTTP.

**HTDV RNA and simultaneous detection assay.** The product of the endogenous RDDP reaction was isolated by ethanol precipitation after adjusting the reaction mix to 4 mM-guanidinium thiocyanate, 25 mM-sodium citrate pH 7-0, 0-5% N-lauroyl sarcosine, 0-1 M-2-mercaptoethanol and extracting it in phenol/chloroform/isooamyl alcohol (25:24:1). The precipitate was sedimented (2500 g, 20 min, 0 °C), dissolved and analysed in caesium sulphate gradients (starting density 1.46 g/ml; 110000 g, 40 h, 15°C). Fractions (250 µl) of the self-generated gradient were collected, precipitated with 10% TCA and filtered through glass fibre filters (Whatman GF/C). Radioactivity was assessed in a Beckman LS 1800 scintillation counter. Under the conditions described, RNA banded at a density of 1.65 g/ml and DNA at 1.45 g/ml.

For the simultaneous detection assay, the product of the endogenous RDDP reaction was layered onto a 10 to 30% (w/v) glycerol gradient in 10 mM-Tris–HCl pH 8.2, 100 mM-NaCl, 10 mM-EDTA and centrifuged for 3 h
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Purification of HTDV RDDP activity. We initially purified the RDDP activities of Fr-MLV and HIV-1 by four sequential chromatographic steps on DEAE Trisacryl M and Phospho-Ultrogel A6R (both from LKB), oligo(dT)-cellulose and hydroxylapatite columns (both from Bethesda Research Laboratories; Wondrak et al., 1986). HTDV RDDP activity was purified from sucrose density gradient-banded preparations. Fractions around 1.16 g/ml were diluted and pelleted (90000 g, 45 min), resuspended for 10 min at 4 °C in disruption buffer [0.05 M-Tris-HCl pH 7.5, 0.8 M-KC1, 0.5% NP40, 0.1 mM-PMSF, 0.5 kallikrein-inhibiting units (KIU)/ml trasylol, 0.5 mM-DTT], clarified at 10000 g for 30 min, dialysed overnight at 4 °C against phosphate buffer (0.01 M-potassium phosphate pH 7.8, 0.5% glycerol, 0.2 mM-PMSF, 1 KIU/ml trasylol, 1 mM-DTT) and subjected to DEAE Trisacryl M ion-exchange column chromatography. The column was washed with 0.01 M-potassium phosphate pH 8.0, 0.5% glycerol, 1 mM-DTT and eluted with a linear gradient of 0.01 to 0.5 M-potassium phosphate. Fractions positive for RDDP activity with (rA)n, (dT)10 were pooled, diluted with an equal volume of buffer A (0.05 M-Tris-HCl pH 7.5, 0.02% NP40, 0.3 mg/ml myoglobin, 25% glycerol, 1 mM-DTT, 1 mM-EDTA) and applied to a Phospho-Ultrogel A6R column, which was subsequently washed with buffer A and eluted with a linear 0 to 1.0 M-KCl gradient. Fractions exhibiting polymerase activity with (rA)n, (dT)10 were pooled, dialysed against buffer A and analysed for reactivity with a variety of synthetic template/primers. Care was taken to perform all purification steps rapidly and at 0 to 4 °C. Cellular DNA polymerases alpha, beta and gamma were also purified from diploid cell (MRC-5) lysates and shown to be easily distinguishable from HTDV RDDP by template/primer specificity, ion requirements and kinetic properties.

Activity of 'purified' RDDPs was assayed in 50 mM-Tris-HCl pH 8.2, 50 mM-KCl, 0.01% NP40, 1 mM-DTT and either 6 mM-MgCl2 or 0.4 mM-MnCl2. To distinguish between RDDPs from different retrovirus strains and to detect possible contaminations by cellular DNA polymerases, the following template/primers (0.2 A260/ml) were employed (Sarngadharan et al., 1978): (i) activated DNA prepared by sonication of herring sperm DNA, (dT)n, (rA)12-18, (dA)n, (dT)10, (dC)n, (dG)12-18, all specific for or preferred by DNA-dependent DNA polymerases; (ii) (rA)n, (dT)10, accepted by RDDPs and DNA polymerase gamma; (iii) (rC)n, (dG)12-18 and (rCm)n, (dG)12-18, specific for RDDPs; (iv) (dG)12-18, specific for terminal deoxynucleotidyl transferase. The appropriate labelled deoxynucleoside triphosphates [3H]TTP, [3H]dATP or [3H]dGTP (sp. act. 6 Ci/mmol), were added at a final concentration of 16 μM. Polymerizations were for 60 min at 37 °C and the products were counted for radioactivity as described above. Background filter absorption (< 1000 c.p.m.) by the reaction mixture without added enzyme was subtracted.

RESULTS

Structural features of HTDV

The fine morphological structures of HTDV-3 during budding (a to c) from and uptake (d, e) by TC cells are shown in Fig. 1. Entry of virus via coated pits and coated vesicles into endosomes is thought to be characteristic of specific virus envelope–cell receptor interactions (Goldstein et al., 1979; Marsh & Helenius, 1980). HTDV have rarely been observed to exhibit mature, i.e. condensed, cores or the electron-lucent space between envelope and core that is characteristic of other animal type C retrovirus strains (for structural details, see Boller et al., 1983).

Demonstration of endogenous RDDP activity

According to extensive electron microscopical studies and testing for endogenous RDDP activity, the induction procedure increased HTDV release by a factor of about ten compared to spontaneous release. Fig. 2 shows the typical peak of endogenous RDDP activity from large volumes of TC supernatant centrifuged on continuous flow sucrose density gradients. HTDV banded at 1.16 g/ml, the characteristic density for retroviruses in sucrose.

HTDV are associated with an inhibitor of exogenous RDDP activity

Earlier attempts to demonstrate exogenous RDDP activity in density gradient-purified HTDV preparations yielded inconsistent results (Kurth et al., 1980; Löwer et al., 1984) regardless of which synthetic templates, (rA)n, (dT)10, (rC)n, (dG)12-18 or (rCm)n, (dG)12-18 or divalent cations (Mn2+, Mg2+ and others) were added. During the course of these studies it became obvious that an inhibitor co-purified with HTDV. Aliquots (20 μl) of the 1.16 g/ml HTDV fractions reduced the exogenous RDDP activities of avian and mammalian retroviruses
Fig. 1. Electron micrograph of HTDV-3 induced by IUdR. (a to c) Budding sequence; (d, e) entry via coated pits (d) and coated vesicles into endosomes (e). Bar marker represents 100 nm.

Table 1. Inhibition of exogenous RDDP activity of mammalian and avian retroviruses by an inhibitor associated with density-gradient-banded HTDV*

<table>
<thead>
<tr>
<th>Virus</th>
<th>BSA</th>
<th>HTDV</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>53700</td>
<td>5800</td>
<td>89.2</td>
</tr>
<tr>
<td>Fr-MLV</td>
<td>16400</td>
<td>990</td>
<td>94.1</td>
</tr>
<tr>
<td>SSAV</td>
<td>42600</td>
<td>3500</td>
<td>91.8</td>
</tr>
<tr>
<td>BEV</td>
<td>13300</td>
<td>2300</td>
<td>82.7</td>
</tr>
</tbody>
</table>

* Twenty μl (5 mg/ml) bovine serum albumin (BSA) or 20 μl (5 mg/ml) sucrose density gradient-banded HTDV were added to 60 μl standard RDDP reaction buffer. Incubation after addition of 20 μl test virus was for 30 min at 37 °C.

by 80 to 95% (Table 1). The potent inhibitory activity could be titrated out (data not shown). In increased amounts, the HTDV-associated inhibitor could completely block the RDDP activities of all tested retrovirus strains, including HIV-1.

HTDV particles contain RNA and associated endogenous RDDP

Despite the regular detection in the presence of actinomycin D of endogenous RDDP activity at densities characteristic of intact retrovirus particles (Fig. 2), contaminating cellular DNA and DNA polymerase could have mimicked the reaction. High mol. wt. nucleic acids were isolated
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Fig. 2. Profile of distribution of RDDP activity in linear sucrose density gradients with endogenous (■) and exogenous templates [■, (rA)n, (dT)10; ○, (dA)n, (dT)10]. The density of each fraction (▲) is also shown.

Fig. 3. Demonstration of high mol. wt. RNA as template for endogenous RDDP activity. Density (○) and 3H incorporation (●) are shown. The radioactive product of the endogenous RDDP reaction was analysed in caesium sulphate gradients. (a) Radioactive HTDV cDNA bound to the viral RNA template (1.65 g/ml). (b) RNA template following alkali treatment (1.45 g/ml).

by conventional SDS/phenol extraction in the presence of 4 M-guanidinium thiocyanate. Analysis of the hybrid RNA–cDNA molecules in caesium sulphate density gradients consistently revealed the association of radioactively labelled cDNA with its template (Fig. 3a). At the density of DNA, small peaks were observed. The variable amounts of radioactivity detectable at intermediate densities presumably represented RNA–cDNA hybrids with various lengths of both degraded RNA and cDNA transcripts. As expected, alkali digestion of RNA destroyed the RNA–cDNA hybrids and increased the peak of free cDNA (Fig. 3b). These experiments with HTDV-1 and HTDV-3 demonstrated that sucrose density gradient-banded HTDV particles possess an endogenous RDDP activity that utilizes RNA as a template.

Presence of 60S RNA

The size of retroviral RNA in its dimeric form ranges from 60S to 70S, depending on the retrovirus strain studied. The product of the endogenous RDDP reaction from sucrose density gradient-banded HTDV particles was centrifuged in a glycerol gradient. Under the conditions used (10 mM-EDTA) ribosomal subunits migrated as 30S and 50S (Hamilton & Ruth, 1969) and HTDV RNA was determined to have a size of 60S to 65S (Fig. 4). Control RNA from purified BEV sedimented at approximately 70S.

Purification of HTDV RDDP activity

Density-banded and pelleted HTDV were disrupted by NP40 treatment and adsorbed to DEAE Trisacryl M ion-exchange columns. Elution with 0.035 M-phosphate identified an
RDDP activity that used (rA)_n.(dT)_{10} as template/primer (Fig. 5a). Peak fractions were pooled and re-chromatographed on Phospho-Ultrogel (Fig. 5b). RDDP activity was eluted at moderate salt concentration (0.16 M-KCl) at which the RDDPs of Fr-MLV and BEV were also recovered, whereas HIV-1 RDDP eluted at 0.05 M-KCl. Further chromatography of HTDV RDDP on oligo(dT)-cellulose or hydroxylapatite columns led to significant loss of enzyme activity, presumably by degradation.

As summarized in Table 2, the RDDP preparations from HTDV-1, Fr-MLV and HIV-1 were free from contaminating cellular DNA polymerases alpha, beta and gamma as well as from terminal deoxynucleotidyl transferase. HTDV RDDP readily accepted (rA)_n.(dT)_{10}, (rC)_m.(dG)_{12-18} and, to a lesser extent (rC)_n.(dG)_{2-18} with a preference for Mn^{2+} for the first two. RDDPs also possessed DNA-dependent DNA polymerase activity, although their rate of synthesis was much lower than with primed RNA templates. However, with (dC)_n.(dG)_{12-18} and Mg^{2+}, all three viral RDDPs exhibited high levels of activity.

**DISCUSSION**

HTDV can be banded at 1.16 g/ml in sucrose, as judged by extensive electron microscopical observations (Boller et al., 1983) and measured by endogenous RDDP activity (Fig. 2). They possess a 60S to 65S RNA (Fig. 4) and a reverse transcriptase that, after removal of an associated inhibitor, also accepts synthetic template/primers (Table 2). By all these criteria, HTDV seem to belong to the retrovirus family.

HTDV can be distinguished from all tested animal retrovirus strains by a variety of morphological (Kurth et al., 1980; Boller et al., 1983), immunological (Löwer et al., 1984), reverse transcriptase (association with an inhibitory activity) and host range (Löwer et al., 1984).
Table 2. Template/primer specificities of RDDPs purified from HTDV-1, Fr-MLV and HIV-1

<table>
<thead>
<tr>
<th>Template/prime</th>
<th>Divalent cation</th>
<th>RDDP activities† of</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>Mg$^{2+}$</td>
<td>HTDV-1</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>500</td>
<td>NT‡</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>0§</td>
<td>NT</td>
</tr>
<tr>
<td>(dT)$<em>n$. (rA)$</em>{12-18}$</td>
<td>Mg$^{2+}$</td>
<td>NT</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>2400</td>
<td>3000</td>
</tr>
<tr>
<td>(dA)$<em>n$. (dT)$</em>{10}$</td>
<td>Mg$^{2+}$</td>
<td>300</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>100</td>
<td>400</td>
</tr>
<tr>
<td>(dC)$<em>n$. (dG)$</em>{12-18}$</td>
<td>Mg$^{2+}$</td>
<td>53200</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>16300</td>
<td>170000</td>
</tr>
<tr>
<td>(rA)$<em>n$. (dT)$</em>{10}$</td>
<td>Mg$^{2+}$</td>
<td>2200</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>110100</td>
<td>1607000</td>
</tr>
<tr>
<td>(rC)$<em>n$. (dG)$</em>{12-18}$</td>
<td>Mg$^{2+}$</td>
<td>3100</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>800</td>
<td>63800</td>
</tr>
<tr>
<td>(rCm)$<em>n$. (dG)$</em>{12-18}$</td>
<td>Mg$^{2+}$</td>
<td>0</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>15000</td>
<td>127400</td>
</tr>
<tr>
<td>(dG)$_{12-18}$</td>
<td>Mg$^{2+}$</td>
<td>500</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>300</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mg$^{2+}$, 6 mM; Mn$^{2+}$, 0–4 mM.
† [3H]dTTP incorporation (c.p.m.).
‡ NT, Not tested.
§ Below background (filter control).

markers. By the same parameters, HTDV are also not detectably related to the human retrovirus strains HTLV-I or HIV-1 (Poiesz et al., 1980; Wong-Staal & Gallo, 1985). In addition, sera from HIV-1-infected individuals do not react with HTDV nor do TC patient sera react with HIV-1. Furthermore, the cation requirements of the RDDPs of each virus group are different (Wong-Staal & Gallo, 1985; Rho et al., 1981 and Table 2) and molecularly cloned HTLV-I and HIV-1, kindly provided by R. C. Gallo (Bethesda, Md., U.S.A.), do not hybridize to TC genomic DNA (F. J. Ferdinand, J. Löwer & R. Kurth, unpublished observations).

The electron microscopical appearance of HTDV is indistinguishable from the previously described particles seen budding from syncytiotrophoblasts of human placenta (Kalter et al., 1973; Dirksen & Levy, 1977). As differentiating human TC cultures contain trophoblast-like cells producing β-HCG (Löwer et al., 1984) and as HTDV are synthesized by only a fraction of the cells in culture, possibly trophoblasts, it is tempting to speculate that HTDV are identical to the placenta particles. If so, HTDV would represent a group of human endogenous retroviruses.

Leong et al. (1984) have recently described a similar RDDP-inhibiting activity in preparations of human placental trophoblast lysates. Likewise, Reddy et al. (1983) have semi-purified seminal plasmin from bovine semen which inhibits the exogenous RDDP activities of a number of animal retrovirus strains when added prior to the binding of polymerase to synthetic templates. Competition with polymerase or inhibition of binding of polymerase to templates may explain why the endogenous RDDP activities in HTDV preparations are not detectably impaired as, in this case, the polymerase molecules remain associated with their natural RNA template inside the virus particle. Because of the great interest in inhibitors of HIV-1 RDDP, we are presently trying to purify the HTDV-associated inhibitor biochemically. Preliminary experiments indicate that trivial enzymic activities like contaminating DNases or proteases, co-purifying with or adsorbed to HTDV particles, may be ruled out.

The low rate of HTDV synthesis in human TC cultures resembles embryonic carcinoma cells which can be infected by retroviruses although there is a block in gene expression at the level of transcription and/or RNA maturation (Peries et al., 1977; Teich et al., 1977; Jaenisch et al., 1981; Linney et al., 1984). 1UdR induction of human TC cultures leads to a transient increase in HTDV synthesis (Boiler et al., 1983; Löwer et al., 1984), indicating that induced TC cells may pass through a certain stage of differentiation that allows the release of HTDV. The presently
obtainable rate of HTDV synthesis seriously hampers molecular cloning of HTDV and interpretation of Southern blot hybridization experiments involving human genomic DNA and HTDV cDNA reverse-transcribed by the endogenous RDDP reaction (strong-stop DNA). Our recent immunological identification of an HTDV protein(s) by TC patients' sera and by two of more than 20 tested antisera specific for primate retrovirus antigens may help to circumvent these difficulties.

The question of the biological significance of endogenous retroviruses is still open. Only in the special example of inbred mouse strains have these proviruses been shown to be involved in the pathogenesis of disease. Therefore, HTDV as endogenous viruses cannot easily be suspected of playing a causative role in the development of teratocarcinomas. The demonstration of RDDP activity in HTDV preparations provides direct evidence that the gene for this enzyme is a normal constituent of the human genome. It may be speculated whether this enzyme possesses any physiological function, whether it helps to explain the existence of intron-less pseudogenes or whether it is involved in the causation of certain pathological conditions.

This paper is dedicated to Professor Dr W. Schäfer (Tübingen) on the occasion of his 75th birthday. We would like to thank R. Köhler (Behringwerke AG, Marburg, F.R.G.), A. Berbott, U. Mikschy and C. Tondera for expert technical assistance, Drs R. Löwer and F.-J. Ferdinand for stimulating discussions, H. Bartel and U. Wenzel for photographic help and G. Bolz and C. Gaiser-Kleebach for secretarial help. We are also very grateful to Drs J. Hilfenhaus and R. Mauler (Behringwerke) for allowing us to use their RK centrifuge (Electro-Nucleonics) for the large scale banding of HTDV and we thank Dr H. Frank (Max-Planck-Institut für Entwicklungsbiologie, Tübingen) for providing us with the electron micrographs. Part of this work was supported by a donation from the Elizabeth Homberger Foundation to R.K.

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