The Friend Virus Genome: Partial Characterization of a Complete DNA Copy

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SUMMARY

A complementary DNA probe has been prepared from the Friend murine erythroleukaemia virus complex released by Friend cells (FV cDNA[^]) and Friend cells induced to differentiate (FV cDNA[^]). Molecular hybridization analysis shows that: (a) FV cDNA[^] is close to being a complete copy of the virus genome and the distribution of sequences is uniform with respect to their distribution in the Friend virus genome. (b) Hybridization of 70S RNA from the cloned helper virus to the total FVc DNA[^] probe demonstrates that a large proportion of the cDNA is specific to the transforming spleen focus forming virus. (c) Hybridization of the probe to normal and transformed cell DNA shows that there are about seven Friend virus related genes in normal DNA and almost twice this amount in transformed cell DNA. A significant minor proportion (20%) of the cDNA probe anneals only to virus related sequences in the transformed cell DNA. (d) An analysis of the kinetics of annealing of the cDNA to an excess template RNA shows that the minimum base sequence complexity of the Friend virus complex is $4 \times 10^6$. (e) An analysis of the cross hybridization between FV cDNA[^] and 60 to 70S RNA isolated from virus released by uninduced and induced cells shows that the genome of the induced and uninduced Friend virus is almost identical.

INTRODUCTION

Friend virus (FV) induces an erythroleukaemia in susceptible mice and the transformed erythroid cells can be maintained in tissue culture (Friend et al. 1971). The FV complex has been shown to contain two virus components, lymphatic leukaemia virus (LLV) and spleen focus forming virus (SFFV). The SFFV component is defective and requires the presence of the helper component for transforming activity. However, the helper function can be provided by many murine leukaemia viruses, such as Moloney leukaemia virus, which themselves do not produce the typical erythroleukaemia in susceptible mice (Steeves, 1975). The function associated with the erythroid specificity of the FV complex must therefore reside in the SFFV genome. More detailed knowledge of the SFFV genome...
is required for an analysis of the erythroid specificity of the SFFV and its possible role in induced erythropoiesis in Friend cells. These cells can be stimulated to differentiate along the erythroid pathway by addition of DMSO (Friend et al. 1971). An event which may be related to the onset of differentiation is the increase in virus release during DMSO-induced differentiation (Sato et al. 1971; Ikawa et al. 1973; Ostertag et al. 1973; Dube et al. 1975; Ostertag & Pragnell, 1978). However, it has been shown that this is not a property of all differentiating Friend cells (Ostertag et al. 1973; Dube et al. 1975).

The induced virus has 10- to 100-fold increased activity in spleen focus formation and shows a change in host range properties when tested in the mouse. This change in host range could either be due to induction of an endogenous N-tropic virus during differentiation of Friend cells (Dube et al. 1975), or the SFFV component may induce an endogenous B-tropic virus in the recipient mouse which can then act as an efficient helper in N-type mice but not in B-type mice.

In this paper we have examined the FV genome by synthesizing a good representative cDNA copy of the FV genome of Friend virus induced during erythroid differentiation. We have used this FV cDNA to establish: (a) The proportions of SFFV and helper virus sequences in the FV cDNA probe using 70S RNA from cloned helper virus. (b) The number of virus related genes in the host DNA and Friend cell DNA. (c) The base sequence complexities of the virus released by F4-6 Friend cells before and during differentiation induced by DMSO. (d) The cross homology between virus released constitutively and during induced differentiation of Friend cells.

METHODS

Tissue culture. The origin and the maintenance of our erythroleukaemic cell lines have been described (Ostertag et al. 1972; Pragnell et al. 1977). F4-6 is a virus positive subclone of F4 with a high virus titre which released about 10 mg Friend virus/10⁹ cells per day. The virus is capable of forming 5 × 10⁵ to 10⁶ XC plaques/10⁶ cells/12 h. 10⁸ to 10⁹ Spleen focus forming units of virus are released by 10⁶ F4-6 cells/12 h. F4-6 was used as the source of the virus for virus cDNA preparations. All cells were kept in logarithmic growth at a density of 1 × 10⁶ cells/ml after feeding. This was maintained by removal of about 90% of the cells and medium every second day and addition of new medium to the original volume.

Cloning of the helper virus. F4-6 virus was cloned in SC1 mouse fibroblast cells by two successive end point dilutions (Hartley & Rowe, 1975). During the first step 5/10 of the wells with SC1 cells were uninfected, in the second step 1/10 wells. The virus which was isolated was already free of spleen focus forming activity at the first cloning and contained exclusively 35S and no 30 to 32S RNA (our unpublished data). The F4-6 helper virus released from SC1 cells, clone 643/22 derived from the second cloning, was used in these studies.

Virus assays. The assay conditions have been described previously (Ostertag et al. 1974). Spleen focus formation was measured 10 days after injection of cellular supernatant into DBA/2J or BALB/c mice. Reverse transcriptase assays were performed as follows: 50 µl of filtered tissue culture supernatant (0.25 µm pore size filter) was mixed with 50 µl of 0.3% NP40 (Shell) in 0.05 M-Tris HCl, pH 8.2; 0.02 M-KCl; 0.002 M-DTT; 0.01% Triton X-100; 10⁻⁵ M-dTTP; 2 µCi ³H-dTTP, sp. act. 30 Ci/mmol (Radiochemical Centre, Amersham, Bucks, England); 5 mM-Mg-acetate; 0.5 mM-Mn acetate. This mixture was incubated 1 h at 37 °C and then applied to Whatman DE81 paper and washed six times (4 min) in 5% Na₂HPO₄. The filters were then rinsed with distilled H₂O, ethanol (twice), ether and dried. Radioactivity was determined by liquid scintillation spectrometry.
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**Virus isolation.** The procedure used for large scale isolation of virus from the supernatant of differentiating Friend cells is based on the general method used by Duesberg (Duesberg, 1968; Dube et al. 1975).

**Complementary DNA preparation.** Virus particles released by F4-6 cells were harvested every 12 h after medium renewal. The DMSO induced virus was collected 24, 36 and 48 h after addition of DMSO. The virus pellets were resuspended in virus buffer (10 mM-tris pH 8.3, 0.5 mM-DTT) and added to a reaction mixture containing the following: 50 mM-tris-HCl, pH 8.3, 3 mM-DTT, 0.015 % NP40, 1.5 mM-manganese acetate, 75 μg/ml actinomycin D, virus, measured as protein, 5 to 10 mg/ml. All four deoxynucleotides were used at a concentration of 0.2 mM; dATP, dGTP, dTTP, dCTP. 3H-dCTP (sp. act. 9 Ci/mmol) was used as a source of radioactivity. The sp. act. of the cDNA probes are given in the figure legends. The reaction mixture was incubated for 2 h at 37 °C and the reaction was terminated by addition of 1/10 vol. 0.1 M-EDTA and Sarkosyl 37 (CIBA-Geigy) to a final concentration of 1 %. The 3H-dCTP-labelled cDNA was deproteinized by phenol-chloroform-SDS extraction and precipitated with salt and 2 vol. ethanol together with 20 μg sonicated and denatured *Escherichia coli* DNA. The cDNA precipitate was suspended in 0.2 M-NaCl, treated with 0.3 M-NaOH at 37 °C for 4 h, neutralized, reprecipitated and desalted by molecular sieving. The cDNA was 95% single-stranded and 100% trichloracetic acid precipitable. To obtain maximum yield of cDNA we have found it necessary to perform pilot incubations to determine the optimum concentration of virus required.

**Isolation of virus 70S RNA.** Virus RNA was isolated from purified, pelleted virus (Dube et al. 1975). RNA (60 to 70S) was isolated by sucrose gradient fractionation using 32P-labelled 60 to 70S RNA as marker in the gradients.

**32P-labelling of virus 70S RNA.** F4-6 cells stimulated for one day with 1.3 % DMSO were labelled with carrier-free 32P-inorganic phosphate at 0.5 mCi/ml (32P-inorganic phosphate, Radiochemical Centre, Bucks, England) in phosphate depleted medium. Virus released over a period of three successive periods of 12 h was collected, purified and 32P-70S RNA was extracted as above. The 60 to 70S RNA was largely free of internal nicks as measured by polyacrylamide gel electrophoresis under denaturing conditions (Dube et al. 1976; Ostertag & Pragnell, 1978). The sp. act. of the virus RNA was 106 ct/min/μg. For the isolation of 32P-60 to 70S RNA from virus released by unstimulated cells, identical conditions were used to those described above, except that the cells were not exposed to DMSO.

**Hybridization reactions.** All hybridizations were incubated at 43 °C in hybridization buffer (0.05 M-NaCl, 25 mM-Hepes, 1 mM-EDTA, 50 % formamide, pH 6.7). Hybrid analysis was performed by S-1 nuclease assay (Harrison et al. 1974). The D₀ₜ (where D₀ is the initial concentration of cDNA in moles nucleotide/l and t is the time of annealing in seconds) required for complete hybridization is 20 × D₀t½ (Young et al. 1974). One ng cDNA together with 5 μg carrier yeast RNA was incubated with an increasing amount of template 60 to 70S RNA in sealed siliconized capillaries. The titration points were incubated for sufficient time that the slowest reaction was completed. The hybridizations were carried out to a D₀t of 1. Thus 1 ng FV cDNA in 2 μl hybridization buffer was incubated 8 days at 43 °C giving a D₀t of 1 so that all hybridization was complete.
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Fig. 1. Induction of spleen focus forming and endogenous virus in cell clone F4–6 during DMSO treatment. Cells were maintained in logarithmic growth at a density of $10^5$ to $10^6$/ml for at least 2 weeks prior to stimulation with DMSO. Medium was replaced and the cell density was adjusted every other day. These cells were treated with 1.3% DMSO, and were maintained in logarithmic growth during stimulation. (a) High constitutive release of virus from F4–6 cells. ○—○, Reverse transcriptase activity (RT) in cellular supernatant per 10^6 cells, as described in Methods. The ct/min, not corrected for loss due to quenching, were plotted after substituting the control values obtained by using tissue culture medium for the reaction. The RT scale is plotted as [ being equal to x0% of the constitutive value for F4–6 cells. 10^6 F4–6 cells release Friend virus with reverse transcriptase activity of 15 pmol dTTP incorporated into acid precipitable material, the virus release being measured during 24 h. ○—○, Release of SFFV as assayed in BALB/c mice; ●—●, release of SFFV as assayed in DBA/2 mice. FV cDNA was derived from virus collected after 2 days treatment with DMSO. Cells were 80% benzidine positive at day 4. Up to 20% of the FV cDNA was greater than 25S in size. (b) The change in host range properties of virus released after DMSO induction. The difference in the level of constitutive virus release compared with that in (a) is primarily due to the use of a different serum batch. Full length FV cDNA (up to 80% greater than 25S in size) was obtained from this highly induced virus. The proportion of benzidine positive cells was > 90% at day 4 of stimulation. ○—○, Release of SFFV as assayed in N-type DBA/2 mice (Ostertag et al. 1974; Dube et al. 1975); ○—○, Release of SFFV as assayed in B-type BALB/c mice.

RESULTS

Host range properties and release of Friend virus in cell clone F4–6 before and during DMSO induced differentiation

Virus of subclone F4–6 was used for this study since the levels of virus release in this cell clone are higher than in the parental clone F4. Virus release levels of cell clone F4–6 remained stable for 2 years of tissue culture growth. F4–6 is inducible for haemoglobin synthesis using 1.3% DMSO, but does not show a marked increase in virus release as
Fig. 2. (a) Titration of FV cDNA with FV 60 to 70S RNA. FV cDNA (1 ng, 700 ct/min) was hybridized to completion with increasing amounts of template 60 to 70S RNA. The amount of cDNA in hybrid was measured by S-1 nuclease assay (Harrison et al. 1974). Titration of FV cDNA with 60S RNA from Friend virus released by F4-6 cells stimulated with DMSO (Fig. 1b). The FV cDNA was full length and was isolated from a highly induced preparation (see Fig. 1b). Titration of FV cDNA with 60 to 70S RNA from Friend virus released by F4-6 cells. (b) Protection of FV-60S RNA with FV cDNA. 32P-60S RNA (1 ng, 1000 ct/min) was hybridized to completion with increasing amounts of 3H-FV cDNA. The amount of 32P-60S RNA stabilized by FV cDNA was measured by ribonuclease treatment. The hybridization mixtures were diluted with 1 ml of ×2 SSC and divided into two portions. Ribonuclease (pancreatic ribonuclease 50 µg/ml, T-1 ribonuclease 5 units/ml) was added to one and both were incubated at 37 °C for 30 min. Carrier bovine serum albumin (50 µg/ml) was added to the control and both samples were precipitated with 10% TCA and filtered onto Whatman GF/C filters. The amount of hybrid was determined with respect to the total TCA precipitable 32P ct/min in each hybridization point. Control hybridization values in the absence of virus cDNA (which rarely exceed 5%) have not been subtracted. (––(–), Stabilization of 32P-60S RNA template by FV cDNA. (source of virus, cDNA and 60 to 70S RNA as in Fig. 1b); (––(–), stabilization of 32P-60S RNA template by FV cDNA.

measured by reverse transcriptase assays of the cellular supernatants during induction (Fig. 1a). There is, however, as in the parent F4 cells, an increase in the release of spleen focus forming virus from the induced cell with a peak at day 2 of induction (Fig. 1b), coinciding with an induction of an N-tropic endogenous helper virus (Fig. 1b). The virus induction takes place before the increase of globin mRNA synthesis (Pragnell et al. 1977). The level of virus induction varies from experiment to experiment depending on the source of the serum and can be as high as 100-fold or as low as 6-fold (Fig. 1a, b). The virus which is induced during differentiation and collected every 12 h was used for most of our experiments.

**Extent of transcription**

Virus cDNA was prepared from lysed virions as described in the Methods. We have used two methods to determine the extent of transcription of virus genome. In the first method, the 3H-labelled virus cDNA probe of virus of DMSO induced cells (cDNA+) was titrated with unlabelled 60 to 70S RNA (Fig. 2).

The lowest ratio of RNA : cDNA at which maximum hybridization of the cDNA will occur when equal amounts of cDNA and its complementary RNA sequences are present. Therefore, the proportion of the total template RNA sequences which are found in the cDNA can be calculated from measurement of this ratio. Thus it can be seen (Fig. 2a) that about 70%, hybridization takes place at a ratio of RNA-cDNA of 1:5:1, and therefore
Fig. 3. Comparison of uninduced and induced Friend virus RNA. FV cDNA\textsuperscript{D+} (1 ng, 700 ct/min) was hybridized to completion (see Methods) with increasing amounts of 60 to 70S RNA. The amount of cDNA in hybrid was measured by S-1 nuclease assay (Harrison et al. 1974). ○—○, FV cDNA\textsuperscript{D+} and 60 to 70S RNA from unstimulated F4-6 cells; ●—●, FV cDNA\textsuperscript{D+} and 60 to 70S RNA from highly induced F4-6 cells (Fig. 1b).

at least 70\% of the virus genome is represented in the sequences present in the cDNA probe, since theoretical kinetics of the hybridization possible cannot be achieved (Young et al. 1974). The specificity of the probe is indicated in that at higher ratios 85\% of the probe is hybridized whereas no significant hybridization takes place with either Escherichia coli RNA or murine ribosomal RNA. Since this type of titration curve does not determine the uniformity of the cDNA transcript, a titration was employed in which the amount of \textsuperscript{32}P-60 to 70S RNA stabilized by the cDNA was measured. It can be seen (Fig. 2b) that 65\% of the \textsuperscript{32}P-RNA was stabilized by the cDNA at a cDNA:RNA ratio of 1:1. Thus not only more than 80\% of the virus genome is represented in the virus cDNA probe, but also the sequences are uniformly represented with respect to those present in the virus 70S RNA. The virus cDNA synthesized (cDNA\textsuperscript{D+}) therefore constitutes an excellent probe for the estimation of Friend virus RNA in the Friend cell.

We have also synthesized a cDNA probe from virus released by cells replicating in the absence of DMSO using identical conditions (Fig. 2). It can be seen that this cDNA (cDNA\textsuperscript{D-}) constitutes a copy of a much smaller fraction of the genome (Fig. 2a, b) since only 50\% of the \textsuperscript{32}P-60 to 70S RNA is stabilized at a cDNA:RNA ratio of 10:1 (Fig. 2b). A large excess of cold 70S RNA over FV cDNA\textsuperscript{D-} (50 to 200:1) is required to saturate the cDNA\textsuperscript{D-} probe (Fig. 2a) showing that representation of the genome in cDNA\textsuperscript{D-} is non-uniform. Once again the probe is virus specific since negligible hybridization is observed with either E. coli RNA or murine ribosomal RNA. We have measured the size of the two cDNA preparations by formamide gel electrophoresis (Staynov et al. 1972). The gel profiles show that the cDNA\textsuperscript{D+} has a larger average size of sequence lengths. In several instances we were able to demonstrate synthesis of very large cDNA transcripts (25 to 30S) using induced virus. When higher deoxynucleotide concentrations are used
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Fig. 4. Hybridization of Friend helper virus 70S RNA to FV cDNAD+. (a) FV cDNAD+ (1 ng, 700 ct/min) was hybridized to completion with increasing amounts of 70S RNA from cloned helper virus. As a control, the template (FV 60 to 70S RNA) was titrated in the same experiment. Hybrid formation was measured by S-1 nuclease analysis. ○—○, FV 60 to 70S RNA; □—□, LLV helper 70S RNA. (b) 32P-70S RNA from cloned helper virus (1 ng, 1000 ct/min) was hybridized with increasing amounts of 3H-FV cDNAD+. The amount of 32P-70S RNA stabilized by FV cDNAD+ was measured by ribonuclease treatment as described in Fig. 2 (b) □—□, 32P-FV 60 to 70S RNA; ○—○, 32P-LLV 60 to 70S RNA.

(Rothenberg & Baltimore, 1976) in the presence of Mg2+ the proportions of very large cDNA transcripts can be increased to 80% using induced virus (Fig. 1b). Appreciable amounts of full length cDNA were not obtained with induced FV at a low induction ratio (Fig. 1a), although the distribution of sequences was uniform like the cDNA of the virus with a high induction ratio (Fig. 1b). We have not been able to synthesize full length transcripts from uninduced virus. This is possibly due to the different genome subunit distribution of induced and uninduced virus (Ostertag & Pragnell, 1978). The hybridization characteristics of the full length FV cDNA are not significantly different from that of the FV cDNAD+ synthesized in the presence of Mn2+ (data not shown).

Comparison of uninduced and induced 60 to 70S Friend virus RNA

We have already established that a change in host range of the FV complex takes place during induction (Dube et al. 1975), which may have been caused by the induction of an endogenous N tropic helper virus in Friend cells. Alternatively the induced SFFV might have the (increased) capacity to induce N tropic helper virus in the spleen of infected mice. We therefore checked whether the proportional distribution of RNA species in the uninduced and induced FV was appreciably different. FV cDNAD+ was titrated with 60 to 70S RNA from virus released by untreated and DMSO induced F4-6 cells (Fig. 3). The major proportion of the RNA of FV of induced and uninduced F4-6 cells seems to be identical.

Measurement of Friend helper virus related sequences in the FV cDNAD+ probe

FV cDNAD+ was titrated with 70S RNA from cloned helper virus (Fig. 4a). The plateau value of 70% with respect to the plateau of 90% in the template control hybridization shows that at least 20% of the total FV cDNA sequences contain SFFV specific sequences. We have also determined the homology between the LLV 70S genome and the FV cDNA probe by measuring the proportion of 32P-labelled LLV 70S RNA which is stabilized by cDNA excess (Fig. 4b). Measurement of the proportion of LLV 70S RNA stabilized at a
Fig. 5. Rates of hybridization of FV cDNA with excess FV 60 to 70S RNA. FV cDNA and 60 to 70S RNA template: Incubations containing a 25:1 excess of 60 to 70S RNA over FV cDNA were hybridized for appropriate times and hybrid formation was measured by S-I nuclease assay. The rate of hybridization is expressed in terms of Rot (see text). The sp. act. of the cDNA was 700 ct/min/ng. The Rot value for both reactions was $4.4 \times 10^{-2}$. The base sequence complexity is calculated with reference to the Rot for globin mRNA (see text) as follows:

$$\text{base sequence complexity} = \frac{4 \times 10^{-2}}{4 \times 10^{-3}} \times 4 \times 10^9,$$

where the mol. wt. of globin mRNA is taken to be $4 \times 10^5$. ––, FV cDNA$^{\text{p+}}$ and 60 to 70S RNA D$^+$ (source of FV cDNA, 60 to 70S RNA, as in Fig. 1b). ○—○, FV cDNA$^{\text{p-}}$ and 60 to 70S RNA D$^-$.  

1:1 ratio (35%) as compared to the homologous reaction (70%) allows us to estimate the maximal proportion of LLV related sequences in the FV cDNA. Thus the SFFV component constitutes at least 50% of the total FV cDNA probe. The value can be regarded as a minimum since extensive cross homologies between the SFFV and LLV are known to exist (Troxler et al. 1977; see also below). End-point dilution of F4-6 virus in SC-1 cells has shown that the SFFV is present in at least threefold excess over the LLV component (Pragnell et al. 1978). These results therefore support our conclusion that the major proportion of the FV cDNA$^{\text{p+}}$ probe is related to the SFFV.

**Base sequence complexity of the Friend virus genome**

Fingerprint analysis and length measurements of 60 to 70S RNA isolated from Friend cells has shown that the major 30 to 35S RNA subunit of the FV has a mol. wt. of $2.5 \times 10^6$ (Ostertag et al. 1973; Dube et al. 1976). It has been established that the Friend virus complex of DMSO induced Friend cells is composed of two virus components, the murine leukaemia virus (LLV) and the spleen focus forming virus (SFFV), which is thought to be the erythroid specific transforming component of the complex, and possibly an endogenous virus (Dube et al. 1975; Steeves, 1975). It is likely, therefore, that the base sequence complexity of the Friend virus genome is higher than that indicated by the fingerprint data. Another experi-
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Fig. 6. Thermal denaturation profiles of hybrids. A series of capillaries containing a 10:1 excess of 60 to 70S RNA over FV cDNA were incubated at 43 °C such that hybrid formation was essentially complete (see Methods). Individual capillaries were incubated at the raised temperature for 5 min, quenched and immediately flushed out with ice-cold nuclease assay buffer. The proportion of cDNA in hybrid was measured by S-1 nuclease assay. The hybrid values obtained have been adjusted to a 0 to 100 scale. ○—○, FV cDNA D+ and 60 to 70S D+; ■—■, FV cDNA D+ and 60 to 70S D-; FV cDNA and 60 to 70S RNA as in Fig. 4.

An experimental approach to the problem is to measure the complexity of the 60 to 70S RNA by kinetic annealing analysis (Bishop, 1969; Taylor et al. 1974). The rate of annealing of the labelled cDNA to an excess of template RNA will be inversely proportional to the complexity of that RNA. We have measured the $R_{0.5}$ for the annealing of both cDNA D− and cDNA D+ to the respective 60 to 70S RNA preparations (Fig. 5). The data are plotted as a function of $R_0t$, where $R_0$ is the initial concentration of RNA in moles nucleotide/litre and $t$ is the time of annealing in seconds. Hybridizations were performed in standardized conditions (Young et al. 1974; Getz et al. 1975). The base sequence complexity of the 60 to 70S RNA samples is determined by measurement of the $R_{0.5} t$, the $R_0t$ value at which 50% of the cDNA is hybridized (Taylor et al. 1974; Getz et al. 1975). The sequence complexity is determined by comparison to a standard figure for mouse globin 9S RNA measured under identical conditions (Getz et al. 1975). Thus the $R_{0.5} t$ for 60 to 70S RNA D− is $4 \times 10^{-2}$ and for 60 to 60S RNA D+ is $4 \times 10^{-3}$ mol s/l for globin mRNA. The base sequence complexity is therefore about $4 \times 10^6$ daltons for 60 to 70S D− and 60 to 70S D+. These data strongly suggest, therefore, that there is considerable cross homology of base sequences in the two major viral 30 to 35S species of the FV genome.
Fig. 7. DNA excess reassociation kinetics of FV cDNA with: (a) DBA/2 adult liver DNA, (b) Friend cell (F4-6) DNA. DNA was extracted, purified and sonicated to a size of 6 to 9S as described previously (Harrison et al. 1974). Each hybridization mix (0.5 M-NaCl, 0.001 M-EDTA, 0.005 M-Hepes, pH 7.0, 50% formamide) contained 50 μg of DNA and 0.1 ng FV DNA (1150 ct/min). Hybrid formation was analysed by hydroxylapatite fractionation (Harrison et al. 1974). ●—■, DNA renaturation; ◯, cDNA-DNA hybridization.

Tm determinations

The melting temperatures of the homologous and heterologous hybrids were determined (Fig. 6). There is very little mismatching of sequences in the hybrids, and furthermore, the value of the Tm (68 °C) indicates that the hybrids represent faithful matching of homologous base sequences. The similar Tm value in the heterologous reaction indicates that there is no mismatching of sequences taking place in this reaction.

The number of Friend virus genes in mouse and Friend cell DNA

We have measured the virus gene frequencies in two types of DNA, that isolated from DBA/2 mouse liver and that from Friend cells of DBA/2 origin. The reassociation kinetics obtained by hybridizing 3H-DNA transcripts of virus RNA to cellular DNA can be used to estimate relative gene frequencies by determination of $C_{ot}$ value (the midpoint of the renaturation curve). The $C_{ot}$ value for DBA/2 is $8.5 \times 10^2$ and 50% of the probe is hybridized at $C_{ot}$ of $1.2 \times 10^2$ indicating a gene frequency of about 7 (Fig. 7a). Measurement of these values for Friend cell DNA show that there are 12 Friend virus related virus genomes/haploid genome (Fig. 7b). Almost all of the virus probe can be hybridized to the Friend cell DNA and 20% less to normal mouse DNA indicating that extra Friend virus related information is integrated in the transformed cell DNA.

DISCUSSION

Present evidence suggests that the target cells for Friend virus are cells which belong to the erythroid lineage (Tambourin & Wendling, 1971; Steeves, 1975). In order to study the mechanisms of transformation at the molecular level and its relationship to the differentiation process, one obvious first step is to characterize the Friend virus genome. With this in mind, we have synthesized a cDNA copy of the Friend virus genome using the virus released by the Friend cell. The best source of cDNA is that synthesized by virus released during DMSO induced differentiation (cDNA$^{+}$). cDNA of virus of uninduced cells (cDNA$^{-}$) is usually not a uniform copy of the virus genome. Since 65% of the $^{32}$P-labelled
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Friend virus genome is hybridized at a ratio of 1:1 and 88% is hybridized at a ratio of 5:1, we conclude that the cDNA\textsuperscript{b+} constitutes almost a complete copy of the genome and that the representation of sequences is mostly uniform with respect to the distribution in the Friend virus genome. Under optimal conditions (see section on the extent of transcription and Rothenberg & Baltimore, 1976) virtually full length cDNA can be synthesized from highly induced virus, the proportion of material larger than 25S being increased from 20% to 80%.

There also appears to be a correlation between the efficiency of transcription and the source of the FV (induced or uninduced). The 60 to 70S RNA of both virus preparations usually yields the same proportion of undegraded 30 to 35S RNA (Ostertag & Pragnell, 1978). The presence of DMSO in the medium of the induced cells does not explain the higher efficiency of transcription since the yield in full length cDNA seems directly correlated to the increase in biological titre during induction. We have found recently that a uniform cDNA probe can be prepared from uninduced virus if calf thymus DNA hydrolysate is added to the incubation as primer (Taylor et al. 1976; Pragnell et al. 1978) although the cDNA is composed of small pieces of size 4 to 6S.

Our evidence shows that the major fraction of the FV cDNA\textsuperscript{b+} probe which we are using is composed of DNA related to the spleen focus forming component of the Friend complex. The RNA from virus released constitutively by F4–6 cells is almost indistinguishable from the RNA of the induced spleen focus forming virus complex in all parameters which have been checked; cDNA-RNA heterologous hybrid formation (Fig. 3), complexity of the genome (Fig. 5) and Tm of the heterologous hybrids (Fig. 6). These data exclude the possibility that more than a small proportion is contributed by the endogenous virus induced during differentiation. Indeed they support the contention (see Introduction) that there is no induction of an endogenous virus in Friend cells during differentiation, and that SFFV only is induced. The change in host range could be explained by assuming that SFFV induces N-tropic endogenous virus in the spleens of recipient mice. We have already demonstrated that there is an excess of SFFV over LLV in our virus preparations and we would therefore expect that infection of spleen cells with SFFV only, with no further virus replication, might be a relatively common event. Thus SFFV induced N-tropic endogenous virus would be able to rescue the SFFV\textsuperscript{+} LLV\textsuperscript{−} spleen cells and act as helper virus for the rescued SFFV leading to increased spleen focus formation. The N-tropic endogenous virus will be restricted in its rescue potential by the FV−1 locus of the mouse genome. On the other hand we observe a marked increase in spleen focus formation and the pronounced change in host range during induction without an increase in reverse transcriptase activity (Dube et al. 1975; Ostertag & Pragnell, 1978; Fig. 1). Some of this change in biological properties might be related to a small change which we have observed in the genome composition related to the SFFV (Ostertag & Pragnell, 1978).

By determining the proportion of the FV cDNA which can be saturated by excess LLV 70S RNA we have shown that at least 20% of the FV cDNA is SFFV-specific (Fig. 4a). Less than 50% of the cDNA is LLV related (Fig. 4b). The genetic relatedness between murine RNA tumour viruses is well documented (Haapala & Fischinger, 1973; Callahan et al. 1974, 1975; Chattopadhyay et al. 1974; Gilden, 1975; Gillespie et al. 1975). An estimate of 4 × 10\textsuperscript{6} daltons was made (Fig. 5) for the complexity of the major components and each of the two or three RNA subunits of the complex correspond to at least 2.4 × 10\textsuperscript{6} daltons (Dube et al. 1976). We therefore have to assume that there is extensive cross homology between the two virus components of the complex. A measurement of the proportion of \textsuperscript{32}P-LLV 70S RNA stabilized at a cDNA:RNA ratio of 1:1 enables us to estimate the
maximum of LLV sequences in the total FV cDNA probe. This value of 35% (Fig. 4b) and 70% for the homologous reaction shows that less than 50% is LLV and more than 50% SFFV related. Measurement of the melting temperature of this heterologous hybrid shows that there is 10% mismatching of sequences (data not shown). The analysis of the subunits of the FV complex indicates that only a minor fraction of the total RNA is identical in mobility to non-defective helper virus (Maisel et al. 1973; Ostertag et al. 1973; Dube et al. 1976; and our own unpublished work). We therefore can conclude again that a large proportion of the FV cDNA probe consists of SFFV sequences. This conclusion is further supported by experiments which show that SFFV is in at least threefold excess over LLV in the FV complex of F4-6 cells (Pragnell et al. 1978).

The presence of host-related or endogenous virus sequences in the Friend virus genomes is shown by the fact that there are about seven genomes homologous to the Friend virus genome in the DNA of normal DBA/2 mice (Fig. 7). Ecotropic endogenous viruses show about 30 to 50% homology to the Moloney or Rauscher MuLV virus genomes (Haapala & Fischinger, 1973; Chattopadhyay et al. 1974; Callahan et al. 1975; Gillespie et al. 1975). Our data show in addition that there is a considerable amount of DBA/2 host-related sequences in the Friend virus genome. There are also sequences in the FV cDNA which hybridized only to the transformed Friend cell DNA (Fig. 7). Our results are in agreement with similar values published for Mo-MuLV specific sequences in Mo-MuLV transformed cells (Jaenisch, 1976).

Fractionation of the virus genome of the Friend virus of FV transformed cells into its various components is now possible using the cDNA copy of the complete virus genome and 70S RNA from cloned helper virus (Pragnell et al. 1978).

The difference in the SFFV genome of the SFFV released by the erythroleukaemia cells (Pragnell et al. 1978) or by fibroblasts (Troxler et al. 1977) are as yet unexplained. They might be related to the fact that our FV is replicating in its target cell. A non-target cell might select for host range mutants of SFFV. Alternatively, the mouse-fibroblasts we have used for cloning LLV may contain virus sequences homologous to part of the SFFV genome. Thus the disadvantage of our approach to study the FV is obvious. We cannot completely exclude the presence of a heterogeneous virus population in the erythroleukaemia cell which is derived from the animal. In vitro transformation with cloned SFFV which would obviously combine the advantage of both approaches, the use of cloned SFFV and of erythroid transformed target cells, are not yet possible. Our approach using FV or FV transformed erythroleukaemia cells, however, seems to us to be more advantageous for studying the real biological function of SFFV in erythropoiesis induction and possibly transformation.

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REFERENCES


Friend virus genome characterization


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