Selective Transcription of Genomic Sequences
Common to both N- and X-Tropic Endogenous Retroviruses
in BALB/c Mouse Tissues

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SUMMARY

Total RNAs from four BALB/c mouse tissues, containing mostly non-dividing
cells (liver and kidney) or variable proportions of dividing cells (uterus and
embryo) were analysed for sequences complementary to 3H-DNA transcripts
synthesized from BALB/c endogenous, N- and X-tropic retroviruses. Extensive
transcription of virogene information was detected in the tissues examined, but
such transcription was found to be mostly limited to the homologous regions of
the two virus genomes. No additivity of hybridization values could be detected
when RNAs from two different tissues were mixed, which suggests that BALB/c
mouse liver, kidney, uterus and embryo transcribe a common set of nucleic acid
sequences of the homologous regions of the N- and X-tropic viral genomes, in
addition to other sequences of the same region that are specific for individual
tissues.

Cells derived from the inbred BALB/c mouse strain contain at least three separate host
range classes (N-, X- and B-tropic) of endogenous type-C virus genomes, integrated within
cellular DNA and these cells release all three of these viruses either spontaneously or after
 treatment with inducing agents (Aaronson et al. 1969; Lowy et al. 1971; Benveniste et al.
1974; Premkumar-Reddy et al. 1978). Although the natural role of the endogenous virus
genomes in uninfected BALB/c cells is still unclear, the transcription of endogenous virus-
specific RNA (Benveniste et al. 1973; Fan & Besmer, 1975) and synthesis of virus gene pro-
ducts (Strand et al. 1977; Stephenson et al. 1976) in such cells have been detected. Our
previous studies have shown that various adult tissues, newborns and early embryos of
uninfected BALB/c mouse contain RNAs which hybridize to 3H-DNA transcripts of an
endogenous, N-tropic BALB/c virus genome (Mukherjee & Mobry, 1975) and the regene-
rating BALB/c liver tissue shows a significant increase in the level of virus-specific RNA
synthesis as compared to normal liver (Vincent et al. 1976). Since portions of the genomic
sequences of the endogenous N-, and X-tropic BALB/c retroviruses are homologous
(Callahan et al. 1974), in the present study, we tried to determine by additive nucleic acid
hybridization whether the virus-specific RNAs in various BALB/c mouse tissues represent
portions of the homologous, non-homologous or both sequences of the N- and X-tropic
virus genomes.

BALB/c (BALB/cJ) mice were obtained from Jackson Laboratory (Bar Harbor, Maine).
Tissues (collected from 11 to 12-week old mice) and 14-day embryos were stored at -65°C
until used for RNA extraction. The N-tropic virus spontaneously released from a BALB/3T3
derived S4Cl8 cell line and xenotropic (X-tropic) virus induced by bromodeoxyuridine
(BrdUrd) from a BALB/3T3 derived cell line and propagated in dog thymus cells (FCf 2 Th),
were gifts from Dr R. E. Benveniste (NIH, Bethesda, Md.). Both viruses were purified by
isopycnic banding on sucrose gradients (Ross et al. 1972).

Total cellular RNA was extracted from various tissues by the method described by Glisin
et al. (1974), with some modifications. Tissues were minced and homogenized in a Potter–Elvehjem homogenizer, in 20 vol. of TNE buffer (0.1 M-NaCl, 0.01 M-tris-HCl, pH 7.4, and 0.001 M-EDTA) containing 1% SDS. The homogenate was extracted twice with water-saturated phenol and phenol-chloroform respectively, and 1 g/ml of CsCl was then added to the homogenate and mixed thoroughly. Four ml of the homogenate was then layered on to 1.2 ml of 5.7 M-CsCl in 0.1 M-EDTA in a cellulose nitrate tube and centrifuged at 134000 g for 12 h at room temperature. After centrifugation the DNA band was removed with a pipette and the tube was inverted. The clear pellet of RNA was then rinsed twice with distilled water, transferred to a clean tube and suspended in distilled water at a concentration of 10 to 15 mg/ml. All RNA preparations were free of DNA (as determined by the use of 3H-labelled DNA in the homogenate) and had a 26o/28o nm absorbance ratio higher than 2.0. The extraction of virus RNA from purified viruses was carried out according to the method of Ross et al. (1972).

An endogenous reverse transcriptase reaction described by Benveniste et al. (1977) was used to synthesize virus-specific 3H-DNA probes. The reaction mixture contained 0.4 M-tris (pH 7.8), 0.06 M-KCl, 12 mM-magnesium acetate, 2 x 10^-3 M-dithiothreitol, 0.02% (v/v) Triton X-100, 50 μg/ml of actinomycin D, 5 x 10^-3 M-3H-TTP (40 to 60 Ci/mmol), 2 x 10^-3 M each of dATP, dCTP, and dGTP and purified N- or X-tropic viruses (approx. 0.2 to 0.4 mg virus protein/ml). The reaction was incubated for 4 h at 37 °C, deproteinized, adjusted to 0.5 N-KOH, incubated at 37 °C for 12 h and neutralized. The sample was then applied to a Sephadex G-50 column and fractions containing acid-precipitable radioactivity were pooled, dialysed and lyophilized. These 3H-labelled DNA products (3H-cDNA) were 98% single stranded, had a sp. act. of 1.8 x 10^7 ct/min/μg, and represented 70 to 85% of the virus genome at a molar ratio of virus RNA to cDNA of 1:1.

DNA–RNA hybridization reactions contained 0.02 M-tris-HCl (pH 7.4), 0.6 M-NaCl, 0.001 M-EDTA, 0.05% sodium dodecyl sulphate, 3 x 10^4 to 4 x 10^4 ct/min of 3H-labelled virus cDNA, and 0.05 to 8.0 mg of total cellular RNA or virus RNA per ml. Hybridization mixtures were heated to 100 °C for 10 min before incubation at 65 °C. At varying times 0.02 ml portions of reaction mixture were withdrawn and kept at −65 °C until digested with single strand-specific nuclease S1 (Miles Laboratory). Rs1 (product of RNA concentration x reaction time) values were calculated as suggested by Britten et al. (1974) and corrected to a monovalent cation concentration of 0.1 M. The concentration of virus-specific RNA in various tissues was determined by comparing the kinetics of hybridization between virus 3H-cDNA and tissue RNA to that between virus 3H-cDNA and purified virus RNA, using Rs1 value as described by Leong et al. (1972).

For Tm determination of the RNA–DNA hybrids approx. 5000 ct/min of virus-specific 3H-DNA product was incubated separately with saturating levels of virus or tissue RNA, in 0.2 ml reaction mixture for 72 h. The hybridization method was the same as described previously. At the end of incubation at 65 °C, 0.02 ml samples of each reaction mixture were withdrawn, heated at various temperatures for 5 min, chilled rapidly and digested with S1 nuclease. The samples were then assayed for the amount of acid-precipitable hybrid present.

Total RNAs from BALB/c mouse liver, kidney, uterus and embryo were used in this study. These tissues were chosen to represent tissues that contain mostly non-dividing cells (liver and kidney), or variable proportions of dividing cells (uterus and embryo). This selection was carried out because of our previous findings that RNAs from tissues having proliferative activity hybridize the endogenous, N-tropic, BALB/c virus probes to a greater extent than the RNAs from tissues with low or no proliferative activity (Mukherjee & Mobry, 1975; Vincent et al. 1976).

The results presented in Fig. 1(a and b) show that total RNAs isolated from liver, kidney,
uterus and embryo hybridize the N-tropic $^3$H-DNA probe to 30, 38, 46 and 48 % respectively, and the X-tropic probe to 30, 38, 40 and 43 %, respectively. The hybridization values obtained for liver and kidney RNAs with the two virus probes are therefore comparable; however, RNAs from uterus and embryo show approx. 5 % higher hybridization values with N-tropic than with X-tropic virus probe. These hybridization values are considered to be saturation values since such values for kidney, uterus and embryo did not change significantly from an approximate $R_{90}$ t value of $4 \times 10^4$ to $5.4 \times 10^4$. Although hybridization reactions with RNA from liver were not brought to the same $R_{90}$ t value levels as that of other tissues, hybridization values for liver RNA with both N- and X-tropic virus probes remained unchanged from $R_{90}$ t values of $2 \times 10^4$ to $3.4 \times 10^4$, indicating that they are at or near saturation. Thermal stabilities of all hybrids were not examined; however, hybrids between N-tropic virus $^3$H-DNA and N-tropic virus RNA, and between the same $^3$H-DNA and BALB/c liver RNA melted with a sigmoidal curve with $T_m$ values of 81.5 and 82.3, respectively. $T_m$ values for X-tropic virus $^3$H-DNA with homologous virus RNA and BALB/c uterus RNA were 83.1 and 82.8 respectively (data not shown). These results suggest that homology between virus $^3$H-DNAs, and virus-specific tissues RNAs is fairly exact.

Comparison with the $R_{90}$ t value for purified virus RNA indicates that although RNAs from the four tissues that were examined hybridized to variable proportions of virus $^3$H-cDNA probes, relative amounts of N- and X-tropic virus-specific RNA contained in total RNA of these tissues were approximately the same (Table 1).

Table 1 shows that purified N- and X-tropic virus RNAs hybridized approx. 83 % of the respective virus $^3$H-cDNA probes. Hybridization of N- and X-tropic virus producer cell RNAs to the respective virus $^3$H-cDNA probes gave slightly lower values – 78 and 81 %, respectively. Reciprocal hybridization with virus RNAs showed approx. 61 % homology between N- and X-tropic virus genomes. RNA extracted from dog thymus cells producing endogenous X-tropic BALB/c viruses (ML-8155) hybridized 59.0 % of the N-tropic virus probe and RNA from $S_2$Cl$_2$ cells producing endogenous N-tropic viruses hybridized approx. 58 % of the X-tropic virus probe. These results suggest that 58 to 61 % genomic sequence homology exists between N- and X-tropic endogenous BALB/c viruses. This value corresponds well to the value reported by Callahan et al. (1974).

As can be seen in Table 1, the hybridization values for both virus probes with RNAs
Table 1. Comparison of hybridization levels of various RNAs to specific virus $^3$H-cDNA probes and the relative content of virus-specific RNA in normal tissues

<table>
<thead>
<tr>
<th>RNA</th>
<th>N-tropic virus $^3$H-cDNA</th>
<th>X-tropic virus $^3$H-cDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hybridization (%) $R_{0t}$</td>
<td>Hybridization (%) $R_{0t}$</td>
</tr>
<tr>
<td>N-tropic virus</td>
<td>83 $4 \times 10^{-2}$</td>
<td>61 $4.5 \times 10^{-2}$</td>
</tr>
<tr>
<td>X-tropic virus</td>
<td>61 $-8$</td>
<td>58 $-8$</td>
</tr>
<tr>
<td>N-tropic virus producer</td>
<td>78 $-8$</td>
<td>58 $-8$</td>
</tr>
<tr>
<td>cell (S$_{25}$I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-tropic virus producer</td>
<td>59 $-8$</td>
<td>58 $-8$</td>
</tr>
<tr>
<td>cell (ML-8155)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>30 $4 \times 10^{-6}$</td>
<td>30 $4 \times 10^{-6}$</td>
</tr>
<tr>
<td>Embryo</td>
<td>48 $4 \times 10^{-6}$</td>
<td>43 $4 \times 10^{-6}$</td>
</tr>
<tr>
<td>Uterus</td>
<td>46 $3.7 \times 10^{-6}$</td>
<td>40 $3.5 \times 10^{-6}$</td>
</tr>
<tr>
<td>Kidney</td>
<td>38 $4 \times 10^{-6}$</td>
<td>38 $4 \times 10^{-6}$</td>
</tr>
<tr>
<td>Liver + embryo</td>
<td>44 $-8$</td>
<td>41 $-8$</td>
</tr>
<tr>
<td>Liver + uterus</td>
<td>43 $-8$</td>
<td>33 $-8$</td>
</tr>
<tr>
<td>Liver + kidney</td>
<td>38 $-8$</td>
<td>38 $-8$</td>
</tr>
<tr>
<td>Embryo + uterus</td>
<td>50 $-8$</td>
<td>43 $-8$</td>
</tr>
<tr>
<td>N-tropic virus + liver</td>
<td>59 $-8$</td>
<td>59 $-8$</td>
</tr>
<tr>
<td>embryo</td>
<td>59 $-8$</td>
<td>59 $-8$</td>
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<tr>
<td>uterus</td>
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<tr>
<td>kidney</td>
<td>59 $-8$</td>
<td>59 $-8$</td>
</tr>
<tr>
<td>X-tropic virus + liver</td>
<td>58 $-8$</td>
<td>58 $-8$</td>
</tr>
<tr>
<td>embryo</td>
<td>63 $-8$</td>
<td>52 $-8$</td>
</tr>
<tr>
<td>uterus</td>
<td>60 $-8$</td>
<td>52 $-8$</td>
</tr>
<tr>
<td>kidney</td>
<td>59 $-8$</td>
<td>52 $-8$</td>
</tr>
</tbody>
</table>

from individual tissues, mixed with reciprocal virus RNAs, did not rise, in most cases, beyond the values obtained with the virus RNA alone. That the hybridization values for most of these reactions did not reach the expected levels (58 to 61%) can be explained by the fact that only 0.5% of the RNA in these reactions was viral and higher $R_{0t}$ values might have been required to achieve the expected values. It appears, therefore, that transcription of virus-specific RNA in the BALB/c tissues examined is mostly limited to the homologous region of the two virus genomes. RNA (5 mg/ml) from BALB/c embryo mixed with X-tropic virus RNA (25 μg/ml) consistently hybridized 63% of the N-tropic virus probe, which is slightly higher than the highest value (61%) obtained with the X-tropic virus RNA alone. However, attaching any significance to this small but consistent difference would be inappropriate without further experimentation.

The results presented in Table 1 also show that, in general, the presence of two tissue RNAs in the same reaction (5 mg/ml each) does not give additive hybridization values. This observation suggests that cells of BALB/c mouse liver, kidney, uterus and embryo transcribe a common set of nucleic acid sequences of the homologous regions of the N- and X-tropic virus genomes, in addition to other sequences of the same region that are specific for individual tissues. Slight additivity (2%) in the hybridization value was consistently detected when a mixture of RNAs from uterus and embryo was hybridized to an N-tropic virus probe but we cannot yet assess the significance of this difference.

The transcription of both N- and X-tropic endogenous retrovirus genomes in uninfected cells, derived from BALB/c mouse bone marrow (JLS-V9), was examined by Fan & Besmer (1975) and by Fan & Mueller-Lantzsch (1976). According to these studies, virus-specific RNA in JLS-V9 cells represented predominantly the X-tropic virus genome and such RNA had little or no sequence homology to $^3$H-cDNA prepared from N-tropic virus. This conclusion is in disagreement with the data reported by Callahan et al. (1974),
Mukherjee & Mobry (1975) and Vincent et al. (1976). Our present study clearly shows that 58 to 61% of genomic sequences of the N- and X-tropic viruses are highly related (Table 1) and a significant level of transcription of this homologous region takes place in uninfected BALB/c tissues (Fig. 1, Table 1). This discrepancy may therefore be due to the possible unevenness of the virus cDNA probe used by Fan & Besmer (1975).

The natural role of the endogenous retrovirus genomes in uninfected BALB/c mouse cells is still unclear. BALB/c mouse tissues and embryos have been found to contain both the major internal virus structural protein (p30) and the major virus envelope glycoprotein (gp70) [Strand et al. 1977]. Our present finding that most of the extensively transcribed virus-specific RNAs in BALB/c tissues represent portions of only the homologous regions of the N- and X-tropic virus genomes suggests that the nucleic acid sequences for p30 and gp70 in these two virus genomes are highly related. The observation that the N- and X-tropic BALB/c endogenous retroviruses share immunologically related p30 antigenic determinants (Benveniste et al. 1974) supports the above conclusion.

The facts that 58 to 61% of the genomic sequences of the BALB/c endogenous N- and X-tropic retroviruses are highly related and portions of only the homologous regions of these two genomes are expressed in normal tissues support the view that these separate host-range classes of endogenous viruses originated from a common ancestor (Callahan et al. 1974) and only the virogenic sequences which might have a natural role in normal tissues were highly conserved.

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Department of Biology, McGill University
1205 Docteur Penfield Avenue, Montreal, Canada
Canada H3A 1B1

Barid B. Mukherjee
Pamela M. Mobry

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