Activation of the Endogenous Proviral Genes in Mouse Cells Is Not Followed by Increased Sensitivity to Deoxyribonuclease I Digestion

(Accepted 31 August 1979)

SUMMARY

The disposition of chromosome proteins about the endogenous proviral DNA of BALB-c mouse has been studied. The sensitivity of the endogenous proviral DNA sequences to deoxyribonuclease I (DNase I) was analysed in BALB-c mouse tissues (liver and spleen) and in the cell line JLS-V9 which does not produce virus. On all of these preparations the endogenous proviral DNA was as sensitive to DNase I digestion as total chromatin. Since the proviral genes in JLS-V9 cells were silent, it was of interest to study possible changes in the chromatin structure following virus induction by iododeoxyuridine. We could not detect any increase in the sensitivity of the endogenous proviral DNA to DNase I digestion following induction. The induction was very efficient, however, since 60% of the cells responded to produce intracellular virus antigens.

Several endogenous proviral genes of retroviruses are integrated in the genome of BALB-c mouse (Chattopadhyay et al. 1974). These proviral genes, which are usually silent, can be induced by halogenated pyrimidines to produce virus in cultured cells (Aaronson et al. 1971; Lowy et al. 1971) and at least three different endogenous viruses, N-tropic, xenotropic and a defective virus, can be readily induced by iododeoxyuridine (IdUrd) from the BALB-c bone marrow cell line JLS-V9 (Besmer et al. 1974; Howk et al. 1978). Treatment with IdUrd results in a five-to tenfold increase in intracellular virus-specific RNA, which is accompanied by the production of virus, indicating that this induction is regulated at least in part at the level of transcription (Besmer et al. 1974; Cabradilla et al. 1976). It is of interest to determine whether the increased expression of these genes was accompanied by alterations in the chromatin structure in the region of the virus gene sequences. The relationship between transcriptional activity of genes and the structure of chromatin is only partially characterized. Weintraub & Groudine (1976) have suggested that pancreatic DNase I is a useful probe for investigating the chromosomal structure of transcriptionally active genes. Thus, active globin sequences in chicken reticulocytes are preferentially digested by DNase I. This same gene, however, is not sensitive to DNase I digestion in other tissues in which globin sequences are not expressed. The DNA sequences of other active genes such as ovalbumin and ribosomal RNA are also sensitive to DNase I digestion (Garel & Axel, 1976; Garel et al. 1977; Mathis & Gorovsky, 1977). Recently, we have demonstrated that integrated proviral genes of Moloney murine leukaemia virus (M-MuLV) in productively infected cells are preferentially digested by DNase I. Thus, limited degradation (15%) of nuclear DNA results in the digestion of as much as 80% of the proviral DNA sequences (Panet & Cedar, 1977).

In the present study the structure of the endogenous proviral genes in BALB-c mouse cells was investigated using DNase I as a probe. The sources of the materials used in this study have been previously described (Panet & Cedar, 1977). Endogenous BALB-c MuLV was induced with IdUrd from the JLS-V9 cell line (Wright et al. 1967), following the
The procedure of Besmer et al. (1974). The virus released from the cells 2 to 5 days after induction was collected and purified. M-MuLV was grown in Swiss mouse cells (clone I) and purified as described by Fan & Paskind (1974). AKR MuLV grown in AKR tissue culture cells was applied by Electronucleonic Laboratories Inc., Md., U.S.A., through the courtesy of Dr J. Gruber of the N.I.H.

The preparation and characterization of single stranded $^3$H-cDNA (sp. act. $7 \times 10^8$ ct/min/µg) has been previously reported (Panet & Cedar, 1977). Degraded DNA was added as primer to improve the representation of $^3$H-cDNA made in the endogenous reactions (Taylor et al. 1976).

The conditions for cell growth and the preparation of DNase I-treated nuclear DNA have been previously described (Panet & Cedar, 1977). In brief, cells were grown as monolayers in roller bottles and harvested by trypsinization. Nuclei were isolated by disrupting cells in a Dounce homogenizer in RSB (0.01 M-tris-HCl, pH 7.4, 0.01 M-NaCl, 3 mM-MgCl₂), containing 0.5% Nonidet P-40. Nuclei washed in RSB buffer were treated with DNase I (10 µg/ml) until 10 to 20% of the nuclear DNA became acid soluble and residual DNA was purified by phenol chloroform extractions. DNA from undigested control nuclei was either sonicated or boiled in 0.3 M-NaOH to obtain DNA with an average size of 300 nucleotides suitable for hybridization experiments.

Cell DNA was annealed with excess $^3$H-cDNA (8000 ct/min) in 25 µl mixtures containing 1 mM-tris-HCl, pH 7.0; 0.1 mM-EDTA. Hybridization reaction mixtures under paraffin oil were treated at 97 °C for 10 min, NaCl was added to 0.4 M final concentration and mixtures were incubated for 48 h at 70 °C. Further incubation did not increase the amount of $^3$H-cDNA hybridized. Samples (20 µl) were assayed for hybridization using S₁ nuclease treatment (Weintraub & Groudine, 1976).

The method of cDNA excess hybridization has been proved to be accurate for measuring the equivalent of proviral genes per haploid genome, since the extent of hybridization per µg of DNA is directly proportional to the amount of proviral DNA sequences (Berns & Jaenisch, 1976; Heilmann et al. 1977). To ensure detection of all of the various virus sequences which undergo induction, several different cDNA probes were employed. The most representative probe was that prepared from the endogenous BALB-c virus induced by IdUrd. In addition, we assayed more specific virus sequences using AKR MuLV and Moloney MuLV probes. The AKR MuLV cDNA is a good probe for detecting the BALB-c N-tropic virus, since these two viruses are indistinguishable by nucleic acid hybridization techniques. This probe also cross hybridizes to a certain extent (57%) with the BALB-c xenotropic virus sequences (Callahan et al. 1975).

We have studied the hybridization of excess AKR MuLV DNA to the DNA of the JLS-V9 cell line and to the DNA of spleens and livers from 8-month-old BALB-c mice. Since these mouse tissues are known to contain proviral sequences (Stephenson et al. 1974) it was of interest to examine the disposition of these genes in vivo. In all three cases DNase I-resistant nuclear DNA and total DNA annealed to $^3$H-cDNA with identical efficiencies (data not shown) indicating that the endogenous proviral DNA sequences are not preferentially digested with DNase I. This is consistent with results previously obtained in NIH Swiss cells (Panet & Cedar, 1977).

We next examined the DNase I sensitivity of endogenous proviruses after their induction by IdUrd. Subconfluent JLS-V9 cultures in roller bottles (covered with aluminium foil to prevent exposure to light) were treated with IdUrd (20 µg/ml) for 18 h and medium was changed. Two days later medium was changed again and on the third day cells were harvested. Control cultures were grown following the same protocol but without IdUrd. As judged by immunofluorescence using antibodies against disrupted viruses, over 60% of the
**Short communications**

**Table 1. Induction of JLS-V9 Cells by IdUrd**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Immunofluorescence*</th>
<th>Enzyme units/10⁶ cells</th>
<th>% hybridization/µg RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>JLS-V9</td>
<td>0/100</td>
<td>0.01</td>
<td>1.8</td>
</tr>
<tr>
<td>IdUrd induced JLS-V9</td>
<td>68/108</td>
<td>8.1</td>
<td>25</td>
</tr>
<tr>
<td>MuLV infected cells (Clone 1)</td>
<td>100/100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Indirect immunofluorescence tests were performed as described by Hilgers et al. (1972). In brief, monolayer cells were trypsinized, washed with phosphate-buffered saline and resuspended to 10⁶ cells/ml. Drops were dried down on glass slides and the cells were fixed with cold acetone. Fixed cells were treated with anti-M-MuLV serum (prepared in goat against disrupted virions). The cells were finally stained with the second antibody, rabbit anti-goat IgG coupled to fluorescein (Hyland Co. Calif., U.S.A.). Stained cultures were analysed in a Zeiss fluorescent microscope. Cells were induced as described in the legend to Fig. 1.

† Reverse transcriptase activity was assayed in the 24-h medium collected before the harvesting of cells. Virus was concentrated and poly(A), olig. (dT) was used as a template-primer for incorporation of ³H-dTMP. Conditions for reverse transcriptase reaction were described previously (Panet & Berliner, 1978). A unit of activity is the amount of enzyme needed for the incorporation of 1 pmol ³H-dTMP per min.

‡ Hybridization of cytoplasmic RNA from JLS-V9 cells. Cytoplasmic fractions obtained after removal of nuclei were the source of cellular RNA. RNA was purified by phenol–chloroform extractions (Fan & Baltimore, 1973) and hybridized in the presence of 0.1% SDS to AKR MuLV ³H-cDNA in excess as described in the text. Hybridization was carried out using a fixed amount of cDNA and varying quantities of RNA. The results shown in the Table were obtained from the slope of the linear curve from a plot of % hybridization vs RNA.

cells responded to inducer by producing virus antigens and this correlated with a 15-fold increase in virus-specific RNA (Table 1). The number of cells actually releasing virus may be much lower than 60% (Aaronson & Stephenson, 1974; Lonai et al. 1974) but the induced cultures released significant amounts of virus reverse transcriptase (Table 1). Despite this successful induction the proviral genes remained in a chromosomal conformation which was not preferentially sensitive to DNase I (Fig. 1). DNase-digested nuclear DNA from both normal and induced cells hybridized to the same extent to both an AKR MuLV ³H-DNA probe (Fig. 1a), which should be efficient for detecting N-tropic proviral sequences (Callahan et al. 1975), and to a probe prepared from the virus released by IdUrd-induced cells (Fig. 1b). Using kinetic hybridization, rather than cDNA excess annealing, to measure residual proviral DNA, the C₀₉₂ values for normal and induced cell DNA preparations were also found to be identical (data not shown).

The cells used in these experiments had been treated with IdUrd for 18 h and harvested 3 days later at the peak of virus production (Besmer et al. 1974). To rule out the possibility that increased sensitivity of active endogenous genes was either a transient phenomenon or required the continuous presence of IdUrd, cells were also harvested either immediately after removal of the inducer or 1 to 2 days later. In none of these cell preparations did we observe any increased sensitivity of endogenous proviral DNA towards DNase I. Moreover, no preferential digestion of these DNA sequences was noted when JLS-V9 cells were grown for 7 days in the presence of IdUrd and deoxycytidine. These conditions allowed cell multiplication and virus production to continue for a longer time, since deoxycytidine decreases the cytotoxic effect of the inducer IdUrd (Besmer et al. 1974).

DNase I digestion has been shown to be an effective tool for the probing and identification of active regions of chromatin. In those cases examined, genes which were actively transcribed reveal increased sensitivity to digestion by this enzyme (Weintraub & Groudine, Garel et al. 1977). This same phenomenon also seems to be true with regard to virus sequences (Panet & Cedar, 1977; Groudine et al. 1978). In this report we examined the disposition of endogenous virus sequences both in cultured cells and in two mouse tissues. In all cases the...
proviral sequences were found to be relatively refractory to digestion. This lack of sensitivity is consistent with the biochemical evidence indicating that these genes are not actively transcribed.

In the JLS-V9 cell line treatment with IdUrd causes the induction of the endogenous proviral sequences and the production of virus particles. As demonstrated clearly in Table 1, this induction is mediated at the level of RNA synthesis and is occurring in a majority of the cells in culture. Despite this, there seems to be no detectable change in the disposition of the virus sequences in chromatin as probed by their sensitivity to DNase I.

Since the number and variety of endogenous virus sequences is quite complex, the possibility exists that this induction is mediated through changes in a small number of specific virus sequences which might go undetected in our assay systems. Groudine et al. (1978) have, in fact, shown that in the case of exogenously inserted RSV sequences, only 50% of the proviral genes are in a DNase I-sensitive conformation. In order to state that there are no changes in nuclease sensitivity following IdUrd induction we have attempted to probe all of the different virus sequences present in this cell line which contains about 10 proviral copies (Chattophadhyay et al. 1974). We estimate that our essay is sensitive enough to detect changes in as few as 25% of the total virus complement.

It is clear that although DNase I selectively digests many active genes, it is not sensitive to all of the factors which ultimately contribute to gene expression at the level of transcription. Thus the gene for haemoglobin is in a DNase I-sensitive conformation in reticulocytes actively producing haemoglobin, but is digestible by DNase I to the same extent in erythrocytes which have ceased to transcribe the gene (Weintraub & Groudine, 1976).
Recent findings of Lowy (1978) have implied that IdUrd directly affects cellular sequences which in turn induce the proviral genes. This type of gene activation may not be mediated through the same type of chromatin conformational changes which are characteristic for other active genes.

This work was supported by grants from the NIH and the United States-Israel Binational Science Foundation to H. C. and from the Stiftung Volkswagenwerk to A. P. The technical assistance of Miss B. Tal and Miss A. Solage is acknowledged.

Departments of Molecular Biology and Virology
The Hebrew University - Hadassah Medical School
Jerusalem, Israel

REFERENCES


Short communications


(Received 21 February 1979)