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Abstract

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SUSCEPTIBILITY OF ROUS SARCOMA VIRUS-SPECIFIC SEQUENCES INTEGRATED INTO SR-C3H/He MOUSE ASCITES SARCOMA CELL CHROMATIN TO DNase I AND DNase II

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Abstract. The susceptibility of Rous sarcoma virus (RSV) genomes integrated in mouse ascites sarcoma cells (SR-C3H/He cells) to DNase I and DNase II was investigated. Approximately half of the viral sequences were sensitive to DNase I and DNase II when 17 % and 7.4 % of the chromatin DNA was rendered acid soluble, respectively. The results suggest that newly acquired exogenous proviral sequences are integrated into both transcriptionally active and inactive regions of chromatin in cells lacking related endogenous viral sequences.

Key words : proviral sequences, mouse ascites sarcoma cells, chromatin, deoxyribonucleases.

Many lines of evidence indicate that certain nucleases can recognize actively transcribed regions of chromatin. Transcriptionally active globin and ovalbumin genes are more sensitive than bulk cellular DNA to DNase I (1, 2). A brief digestion of chromatin with DNase II releases template active DNA as acid insoluble fragments rich in mRNA-coding sequences (3, 4). The state of proviral genomes integrated in the host chromatin has been studied by application of these findings. A preferential digestion of actively transcribed viral sequences by DNase I has been reported with adeno virus (5), and Moloney murine leukemia virus (M-MuLv) (6), integrated in rodent cells. Recent experiments, however, have shown that the preferential digestion of active genes is not a consequence of the transcription process *per se* (1, 7, 8).

We investigated the state of proviral sequences of the avian sarcoma virus integrated in nonproductively transformed rodent cells employing both DNase I and DNase II as a probe. The cell we used was a mouse ascites sarcoma cell (SR-C3H/He), originally induced by the Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV) (9). The SR-C3H/He cell contains the whole genome of SR-RSV, and viral genes are partially expressed in the cell : An immunodetectable

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level of group specific antigen is expressed (10), though the activity of viral reverse transcriptase is not detectable (unpublished observation).

MATERIALS AND METHODS

DNase digestion. Nuclei were prepared from SR-C3H/He cells 10 days after an intraperitoneal inoculation as described previously (11). The nuclei suspended in 0.25 M sucrose, 10 mM Tris-HCl (pH 7.8) and 5 mM $MgCl_2$ (0.5 mgDNA/ml) were digested with 50 u/ml DNase I (Worthington, 2,465 u/mg) at 30 ℃ for 2 min. The reaction was stopped by adding EDTA to a final concentration of 10 mM, after which the suspension was centrifuged (27,500 x g, 20 min) to obtain supernatant (S-) and pellet (P-) fractions.

Chromatin (0.5 mgDNA/ml), prepared from the isolated nuclei by washing them once with a solution containing 24 mM EDTA (pH 8.0) and 75 mM NaCl, and then three times with 10 mM Tris-HCl (pH 7.8), was digested with 100 u/ml DNase II (Worthington, 45,000 u/mg) at 30 ℃ for 5 min in 25 mM sodium acetate (pH 6.5). The reaction was terminated by adjusting the pH to 7.9 with Tris base. Following this, the suspension was fractionated by centrifugation (27,500 x g, 20 min) into supernatant (S-) and pellet (P-) fractions.

The amount of DNA was determined by the absorption of acid-soluble material at 260 nm, after hydrolysis of the DNA in 5 % perchloric acid at 95 ℃ for 15 min. The DNA solubilized during nuclease treatment was estimated by measuring $A_{260\ nm}$ after precipitation of nuclease-resistant DNA with 5 % cold perchloric acid.

For hybridization experiments, DNA was prepared as follows. The nuclease-treated S-fraction was concentrated to one-tenth of its original volume in a Diaflo cell (Amicon) equipped with a Diaflo membrane (UM 10) and then treated with 0.5 mg/ml proteinase K at 37 ℃ for 12 h. The P-fraction was treated directly with proteinase K omitting the concentration procedure. Following an addition of 1 M sodium perchlorate, DNA was isolated by phenol-chloroform extraction followed by RNase A treatment (Sigma, Type I-A, 20 μg/ml), reextraction with chloroform-isoamylalcohol, and precipitation with 2 vol. of ethanol. Total DNA was prepared directly from nuclei and chromatin.

Preparation of [^{3}H]cDNA. [^{3}H]-labeled DNA complementary to the genome of the Bratislava 77 strain of avian sarcoma virus (B77 ASV, originally provided by Dr. P.K. Vogt) was prepared as follows. The purified virus (12) (3 mg protein/ml) was incubated with 4.2 μM [^{3}H]dTTP (60 Ci/mmol, from the Radiochemical Centre, Amersham) in the presence of 0.4 mM each of unlabeled dATP, dGTP and dCTP, 100 μg/ml actinomycin D, 0.02 % Nonidet P-40, 10 mM dithiothreitol, 50 mM NaCl, 8 mM $MgCl_2$ and 50 mM Tris-HCl (pH 8.3) at 37 ℃ for 2 h. The reaction was terminated by adding 0.2 % SDS, and the mixture was treated with proteinase K (330 μg/ml) at 37 ℃ for 30 min. The single stranded [^{3}H]cDNA was precipitated with ethanol following phenol-chloroform extraction and hydrolysis of the RNA moiety by 0.2 N NaOH.

RESULTS

Previous studies have shown that the DNase II-sensitive fraction (S-fraction) is enriched in transcriptionally active chromatin (3, 4, 13), and that pulse-labeled nascent chromatin-bound RNA cofractionates with S chromatin. It was shown that over 60 % of the nascent RNA fractionated with 10 % of the chromatin DNA when the chromatin prepared from hepatoma cells was digested (14), and

that 50 % or more of the nascent RNA fractionated with less than 1 % of the chick reticulocyte chromatin DNA (13). To test preferential release of nascent RNA in the system we used, SR-C3H/He ascites cells were incubated with [^{3}H] uridine (15), and chromatin was then prepared and briefly digested with DNase II for varying times as described under Materials and Methods. Consistent with other reports, about 80 % of the nascent RNA labeled with [^{3}H]uridine was released in the S-fraction when 8 % of the DNA was degraded.

TABLE 1. DISTRIBUTION OF SR-RSV PROVIRAL SEQUENCES IN SR-C3H/HE CELL CHROMATIN FRACTIONATED AFTER TREATMENT WITH DNASE I OR DNASE II.

DNase	Chromatin	DNA amount	Proviral sequence concentration	Sequence amount
DNase II	Unfractionated	1	1	1
	S-fraction	0.13	0.34	0.04
	P-fraction	0.80	0.58	0.46
	Acid soluble	0.074	6.76	0.50
DNase I	Unfractionated	1	1	1
	S-fraction	0.25	1.1	0.28
	P-fraction	0.58	0.57	0.33
	Acid soluble	0.17	2.29	0.39

Values are expressed relative to unfractionated chromatin. Proviral sequence concentrations in acid soluble fractions were deduced from the loss of sequences due to the DNase treatments.

Following DNase II treatment at 30℃ for 5 min, SR-C3H/He chromatin was fractionated by centrifugation into S- and P-fractions. The relative amounts of DNA found in the chromatin fractions are shown in Table 1. The SR-RSV proviral sequences in each fraction were hybridized with excess ^{3}H-labeled cDNA made against B77 ASV which has a sequence homology of about 100 % to SR-RSV (16). As shown in Figure 1A and Table 1, the relative concentration, which is proportional to the slope determined with excess probe, of the proviral sequences was 1 : 0.34 : 0.58 in the untreated chromatin, S-fraction and P-fraction, respectively. The relative amount of proviral DNA in each chromatin fraction was calculated by multiplying the proviral sequence concentration by the amount of DNA in the fraction. About half the virus specific sequences were in a DNase II-sensitive conformation. The other half were resistant to DNase II and remained in the P-fraction.

To test the accessibility of DNase I to integrated SR-RSV genomes, DNA from intact and from DNase I-digested nuclei was hybridized separately with excess cDNA (Fig. 1B). As a considerable amount of chromatin was released from the nuclei after the DNase I digestion, the treated nuclei were centrifuged and DNA was extracted from both the pelleted nuclei (P-) and the released chromatin in the supernatant (S-). The relative amount of proviral sequences was

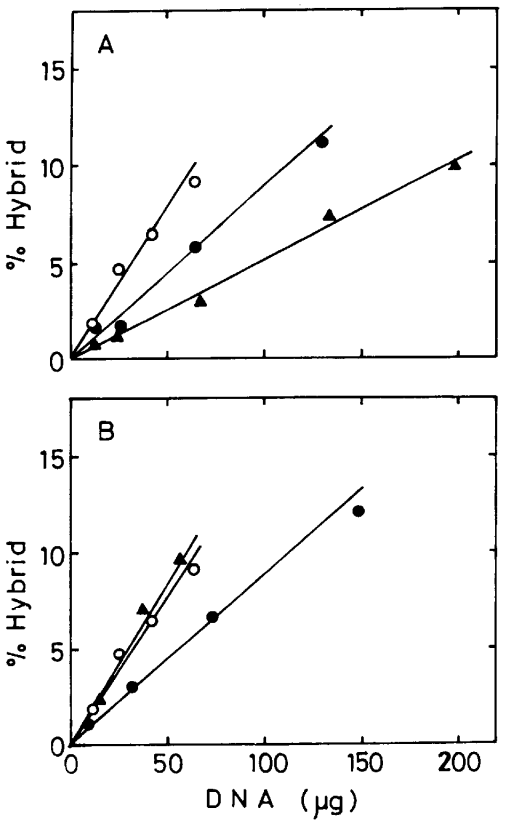

Fig. 1. Hybridization of fractionated cellular DNAs with excess B77 [^{3}H]cDNA. Varying amounts of cellular DNA (8-200 μg) previously sheared to 300-400 base pairs by extensive sonication were incubated with the single stranded [^{3}H]cDNA (75,000 cpm) in a 25 μl reaction mixture of 10 mM Tris-HCl (pH 7.8), 0.3 M NaCl and 1 mM EDTA at 100 ℃ for 10 min followed by prolonged incubation at 60 ℃ for 70 hr. The protected [^{3}H]cDNA was assayed with S1 nuclease treatment (2×10^4 u/ml) (13). (A) Hybridization of B77 [^{3}H]cDNA with total DNA from SR-C3H/He cells (○); DNA from DNase II-treated S-fraction (▲); and P-fraction (●). (B) Hybridization of B77 [^{3}H]cDNA with total DNA from SR-C3H/He cells (○); DNA from DNase I-treated S-fraction (▲); and P-fraction (●). Background hybridization due to self-annealing of the probe (7 %) was subtracted from the data.

calculated as described above (Table 1). Approximately 40 % of the proviral sequences were degraded when 17 % of the bulk DNA was rendered acid soluble. It has been shown that 10 % digestion of isolated nuclei by DNase I preferentially removes globin DNA sequences from nuclei obtained from chick red blood cells, but not from nuclei obtained from fibroblasts or from brain (1), and that 10 % digestion of the oviduct nuclei from laying hens solubilizes over 70 % of the ovalbumin sequences (2), suggesting that DNase I preferentially attacks transcribing genes. Panet and Cedar (6) quantitated DNase I digestion of M-MuLv sequences

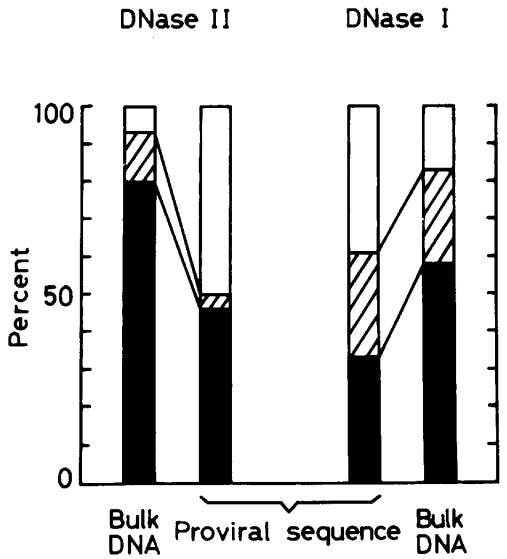

Fig. 2. Comparison of the susceptibility of SR-RSV proviral DNA and bulk DNA to DNase I and DNase II : ☐ , asid soluble ; ▨ , S-fraction ; ■ , P-fraction.

integrated in NIH Swiss mouse cells as a function of the extent of nuclear DNA degradation, showing that when 5 % of the nuclear DNA was rendered acid-soluble, 50 % of the proviral sequences were digested, and that most of the proviral sequences sensitive to DNase I were digested when 15 % to 17 % of the nuclear DNA was solubilized. Taking these previous studies into account, almost all the SR-RSV sequences sensitive to DNase I seem to be rendered acid soluble when 17 % of the SR-C3H/He nuclear DNA was solubilized. It is interesting that the DNase I-resistant proviral sequences were recovered in the S-fraction as chromatin fragments enriched with the proviral sequences. These results are summarized in Fig. 2.

DISCUSSION

The absence of endogenous viral sequences of avian origin is one of the advantages of the system we used. Since DNA sequences complementary to the genome of avian C-type viruses do not exist in mouse DNA, complementarity detected with B77 cDNA is due entirely to SR-RSV genomes integrated in SR-C3H/He chromatin. Our preliminary analysis of reassociation kinetics with the virus prove-DNA used in this experiment showed that only a small number (less than 2 per haploid genome) of the viral copies are integrated in the cell. The present study revealed that approximately 40-50 % of the exogenous proviral sequences integrated in nonproductively transformed SR-C3H/He cells were in

a DNase I and DNase II-sensitive conformation and the remaining half in a resistant conformation. It is noteworthy that most of the DNase II-sensitive proviral sequences were rendered acid soluble and that only a small portion of the proviral genomes were recovered in the S-fraction. This is in contrast to the abundance of actively transcribed sequences in this fraction (3, 4). The proviral sequences in the S-fraction seem to be rapidly degraded to nonhybridizable small oligonucleotides by DNase II which shows that the virus specific sequences are extremely sensitive to DNase II under conditions of limited digestion. Hendrick et al. (13) have also shown that active DNA is preferentially susceptible to breakdown by DNase II.

The evidence that only half the proviral genomes were sensitive to DNase I and DNase II is interesting in light of previous studies on the DNase I sensitivity of integrated RSV (8, 18) and M-MuLv (6, 19) genomes. It appears to be a general rule that newly acquired exogenous proviral sequences are always found in the DNase I sensitive region of the host chromatin if the cell contains genetically transmitted endogenous viral genomes (6, 8, 18, 19), whereas integration occurs into both DNase I sensitive and resistant regions in cell lines lacking related endogenous viral sequences (8). Further studies with other systems are required to verify this generalization.

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