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Assessment of Preferential Cleavage of an Actively Transcribed Retroviral Hybrid Gene in Murine Cells by Deoxyribonuclease I, Bleomycin, Neocarzinostatin, or Ionizing Radiation†

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ABSTRACT: Preferential cleavage induced by bleomycin, neocarzinostatin, or ionizing radiation in a transcribed cellular gene was evaluated through comparisons with deoxyribonuclease I. The glucocorticoid-inducible LTL gene (a hybrid viral gene derived from mouse mammary tumor virus DNA) previously described [Zaret, K. S., & Yamamoto, K. R. (1984) *Cell (Cambridge, Mass.) 38*, 29–38] served as the specific DNA target. A Southern blot analysis was used to specifically assess cleavage of the LTL gene in nuclei isolated from cells either treated or untreated with the synthetic glucocorticoid dexamethasone. Hypersensitivity of the gene to bleomycin or neocarzinostatin, which paralleled deoxyribonuclease I hypersensitivity, was evident only in nuclei isolated from dexamethasone-treated cells. Like deoxyribonuclease I, sites of dexamethasone-inducible drug hypersensitivity were coincident with the binding region for the glucocorticoid receptor found within the regulatory sequences of the LTL gene. In contrast, no hypersensitivity to ionizing radiation was evident. Although bleomycin and neocarzinostatin showed qualitatively similar preferences for the transcribed LTL gene, quantitative evaluations of damage to total cellular DNA by filter elution showed that the relative specificity of bleomycin for the hypersensitive region was much less than that of either deoxyribonuclease I or neocarzinostatin.

Active or potentially active chromatin regions have been shown to be preferential targets for deoxyribonuclease I (DNase I)[1] catalyzed cleavage (Weintrab & Groudine, 1970; Garel & Axel, 1976). In particular, hypersensitivity to this endonuclease has been associated with regions involved in the control of transcription and replication [for a review, see Eissenberg et al. (1985)]. The precise mechanisms which render the DNA within these regions more vulnerable to the action of endonucleases are not completely elucidated. However, the accepted view is that the increased susceptibility of active chromatin to enzymatic cleavage is a consequence of a more "relaxed" chromatin conformation (Eissenberg et al., 1985).

The cytotoxic antibiotics bleomycin (BLM) and neocarzinostatin (NCS) are pharmacological equivalents of endonucleases since they also have the intrinsic ability to cleave DNA (Suzuki et al., 1969; Beerman & Goldberg, 1974). Like many endonucleases, these drugs are sequence selective (Sugiura & Suzuki, 1982; Kross et al., 1982; Takeshita et al., 1981) and can cleave chromatin nonrandomly. Apparently, conformational factors contribute to this nonrandom activity on chromatin since drug-induced cleavage was shown to be predominantly confined to the nucleosomal linker (Kuo & Hsu, 1978; Kuo & Samy, 1978; Beerman et al., 1982). In addition, specific chromatin domains may also represent sites of preferential cleavage. Notably for BLM, differential activity on transcribing chromatin has been observed in at least one system (Kuo, 1981), while indirect evidence suggests that NCS shows no such preference (Hatayama & Yukioka, 1982).

Since preferential cleavage of specific chromatin domains, as exemplified by DNase I, may be an important determinant for drug action in vivo, we sought to further assess this possibility by evaluating scission activity on a well-characterized gene target. Accordingly, we chose to study drug activity in

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[1] Abbreviations: DNase I, deoxyribonuclease I; BLM, bleomycin; NCS, neocarzinostatin; kbp, kilobase pair; MMTV-LTR, mouse mammary tumor virus long terminal repeat; HSVtK, herpes simplex virus thymidine kinase; krad, kilorad; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; DMEM, Dulbecco's modified Eagle's medium; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; PBS, phosphate-buffered saline; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

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a murine cell system (L1.2-2 cells), originally developed by Zaret and Yamamoto (1984), which contains several integrated copies of a hybrid viral gene derived from the insertion of the herpes simplex virus thymidine kinase (HSVtK) gene into mouse mammary tumor proviral DNA. Consequently, in these L1.2-2 cells, transcription of the HSVtK gene is dependent upon glucocorticoids. The induction of transcription by glucocorticoids such as dexamethasone is facilitated through the binding of the activated glucocorticoid receptor to a specific site within the adjacent mouse mammary tumor virus long terminal repeat (MMTV-LTR) [reviewed in Yamamoto (1985)].

Using Southern blotting as a method to specifically assess DNase I catalyzed cleavage, Zaret and Yamamoto (1984) were able to show that the activation of transcription by dexamethasone produced changes in DNase I susceptibility. In particular, a region of increased sensitivity within the MMTV-LTR was noted. This region of hypersensitivity was found to be coincident with the chromatin binding region for the activated glucocorticoid receptor. An overall increase in sensitivity of the HSVtK structural gene to DNase I was also observed following dexamethasone treatment.

In the present study, BLM, NCS, and ionizing radiation are similarly evaluated and are qualitatively compared to DNase I for their ability to preferentially interact with this DNA target. Ionizing radiation was included in this study as an example of an agent which in general is thought to interact nonspecifically with chromatin. Using this system, we show that both BLM and NCS are similar to DNase I in that they do act upon transcribing chromatin selectively, whereas X-rays do not. However, quantitative evaluation of total DNA strand scission showed that the extent of damage to bulk chromatin required to detect a preference for active chromatin varied with each agent.

Materials and Methods

Drugs and Reagents. Bleomycin and neocarzinostatin were generously supplied by Bristol-Myers, Syracuse, NY. Deoxyribonuclease I (DNase I; no. LS-06330-DPFF, Cooper Biomedical, Melvern, PA), purchased as a solid, was reconstituted in DNase I buffer [10 mM tris(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl), pH 7.4, 10 mM NaCl, and 3 mM $MgCl_2$] in 50% glycerol at a concentration of 1 mg/mL. Dexamethasone (Sigma Chemical Co., St. Louis, MO) was dissolved in ethanol at a concentration of 1 mM. Working stocks of all reagents were divided into small aliquots and stored at −20 °C. Except where noted, serial dilutions of bleomycin and neocarzinostatin were made in H_2O, and dilutions of DNase I were made in DNase I buffer.

Cells and Culture Methods. The transfected L1.2-2 cell line, which contains approximately six integrated copies of the LTR–HSVtK–LTR (LTL) DNA fusion product from plasmid pLTL-1 (see Figure 1), was kindly provided by Dr. K. Yamamoto. The construction of pLTL-1 (also provided to us by Dr. Yamamoto), the transfection of pLTL-1 into mouse L cells, and the isolation of the resultant L1.2-2 cell line have been described previously (Zaret & Yamamoto, 1984). L1.2-2 cells were grown as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM; GIBCO, no. 430-2100, Grand Island, NY) supplemented with 10% bovine calf serum (Hyclone no. A-2151-L, Logan, UT) and 10 mM *N*-(2-hydroxyethyl)-piperazine-*N'*-2-ethanesulfonic acid (HEPES; Hoechst, La Jolla, CA). Stock cultures were routinely passaged 1–2 times per week and maintained at 37 °C in humidified 5% CO_2 incubators. Cells to be used for the preparation of nuclei were plated at a density of $2.5 \times 10^6/150$ cm^2 flask and were uniformly labeled with [2-^{14}C]thymidine ([^{14}C]dT, specific activity 56 mCi/mmol; Moravek Biochemicals, Brea, CA). Except where noted, the label was added to the medium at a final concentration of 2.5 nCi/mL at the time of plating.

Isolation and Treatment of Nuclei. Near-confluent cell cultures, pretreated 1 h either with 0.1 μM dexamethasone or with solvent, were harvested by trypsinization and resuspended in phosphate-buffered saline (PBS, GIBCO) at 4 °C. Nuclei were isolated at 4 °C as described previously (Feinstein et al., 1982). Nuclear pellets were resuspended in DNase I buffer containing 1 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid (EGTA) such that the DNA concentration was between 500 and 1500 μg/mL. The amount of DNA within a preparation was estimated by measuring the optical density at 260 nm in 0.1 N NaOH (1 OD_{260} unit is equal to 35 μg/mL). The specific activity (cpm per microgram of DNA) of a preparation was determined concurrently by scintillation counting, thereby providing a method of quantitation for the amount of DNA to be used in subsequent manipulations. Nuclei isolated from dexamethasone-treated cells were prepared with solutions containing 0.1 μM dexamethasone.

Immediately after isolation, nuclei were treated with either drugs, DNase I, or X-rays as detailed in the figure legends. In a typical reaction, 100 μg of nuclei was suspended in a total of 300 μL of DNase I buffer containing 1 mM EGTA. Where appropriate, dexamethasone was present during treatment at a final concentration of 1×10^{-7} M. Nuclear digestions with neocarzinostatin, bleomycin, or DNase I were done at 37 °C under reduced lighting. Reactions were terminated by adding ethylenediaminetetraacetic acid (EDTA) to 25 mM and cooling to 4 °C. X irradiation was done on ice, EDTA being added after X irradiation was completed for all samples.

DNA Isolation, Restriction Digests, and Southern Blotting. Subsequent to treatment, nuclear suspensions were lysed by adding a 0.1 volume of a 10 times concentrated lysis buffer (100 mM Tris-HCl, 50 mM EDTA, and 5% Sarkosyl, pH 7.4) and incubated at 65 °C for 30 min. The nuclear lysates were put at 37 °C and subsequently treated consecutively with 40 μg/mL ribonuclease A (Boehringer-Mannheim Biochemicals, Indianapolis, IN) and 100 μg/mL proteinase K (Boehringer-Mannheim Biochemicals). Following enzymatic treatment, the DNA was further cleaned by multiple extractions at room temperature with phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1). The DNA was recovered by ethanol precipitation; 8.0 μg of DNA was then digested to completion with 10 units of restriction endonuclease *Nco*I using the conditions outlined by the manufacturer (Boehringer-Mannheim Biochemicals).

Size separation of the resultant DNA fragments was accomplished by electrophoresis at 20 V through 1.0% agarose for 24–27 h using TAE (50 mM Tris-HCl, pH 8.3, 20 mM sodium acetate, and 2 mM EDTA) as the running buffer. Following electrophoresis, the DNA was transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, NH) by the method of Southern (1975), and the resultant blots were hybridized with either plasmid pLTL-1 or the 1.5 kbp *Nco*I–*Bam*HI restriction fragment from pLTL-1 (see Figure 1). Both probes were labeled to high specific activity [$(1–3) \times 10^8$ cpm/μg] by nick translation (Maniatis et al., 1982) using thymidine [α-^{32}P]triphosphate (800 Ci/mmol; Amersham, Arlington Heights, IL). The 1.5 kbp *Nco*I–*Bam*HI fragment from pLTL-1, which consists solely of HSVtK gene sequences (see Figure 1), served as an indirect end-labeling probe (Wu, 1980).

Hybridizations were done at 65 °C in 4× SSC (1× = 0.15 M NaCl and 0.015 mM sodium citrate, pH 7.0), 2× Denhardt's [1× = 0.02% each of ficoll, poly(vinylpyrrolidone), and bovine serum albumin in H_2O], 0.1% SDS, 0.1% sodium pyrophosphate, 10% dextran sulfate, and 150 μg/mL denatured salmon sperm DNA for 16–24 h. Following hybridization, the membranes were washed several times at 65 °C in 0.5× SSC, 0.2% SDS, and 0.1% sodium pyrophosphate, dried at room temperature, and exposed to Kodak XAR-5 film for autoradiography. Multiple autoradiographic exposures were examined for all experiments. Where appropriate, autoradiograms were scanned by using a Helena Quick Scan (no. 1050) densitometer.

Filter Elution Assays. Total DNA cleavage was quantitated by a filter elution assay similar to that described previously (Kohn et al., 1976). Labeled nuclei were prepared from L1.2-2 cells as described above except that the [^{14}C]dT concentration used to label the cells was 12.5 nCi/mL. Following isolation, the nuclei were treated with either DNase I, BLM, NCS, or ionizing radiation as described above, diluted in PBS to a final concentration of 5 × 10^5/mL, and subsequently applied to 2.0-μm pore size polycarbonate filters (Nucleopore, no. 110611, Pleasanton, CA). Approximately 5 × 10^5 nuclei were applied to each filter. The nuclei were washed with 10 mL of cold PBS and lysed in situ for 30 min with a solution containing 2% SDS, 0.02 M EDTA, and 0.25 mg/mL proteinase K, pH 9.7. To assay for double-stranded breaks, the filters were washed with 5 mL of 0.02 M EDTA, pH 9.7, and the DNA was subsequently eluted with 30 mL of 2% tetrapropylammonium hydroxide (Fisher, Rochester, NY), 0.02 M EDTA, and 2% SDS, pH 9.7. Fractions were collected at 5-min intervals using a flow rate of 0.2 mL/min, and the extent of DNA damage, as measured by the fraction of radioactivity retained on the filter for each time point, was quantitated.

Results

The L1.2-2 cell line described by Zaret and Yamamoto (1984) was used to evaluate scission activity on a transcribed cellular gene. Using a Southern blot strategy similar to that described by Zaret and Yamamoto, we assessed specific cleavage of the heterologous LTL gene, induced by either BLM, NCS, or ionizing radiation, in nuclei isolated from cells treated or not treated with dexamethasone. Hypersensitive sites appearing on Southern blots were mapped through the use of indirect end-labeling probes (Wu, 1980) and markers derived from plasmid pLTL-1 (see Figure 1).

For comparative purposes, our results with DNase I, which are similar to those described by Zaret and Yamamoto, are presented in Figure 2A. Digestion of nuclei isolated from dexamethasone-treated cells with increasing concentrations of DNase I results in a dose-dependent decrease in signal intensity associated with HSVtK gene hybridization. This diminution of signal intensity is a direct consequence of HSVtK gene cleavage. A dexamethasone-inducible hypersensitive site (designated as I), as evidenced by the appearance of a new band (2.1 kbp) on the autoradiogram, can be observed at DNase I concentrations of 4 μg/mL and above. This site first appears at DNase I concentrations below those which are required to cause significant digestion of the HSVtK gene. The localization of this hypersensitive site has been found to coincide with the binding region for the glucocorticoid receptor within the MMTV-LTR (see Figure 2A).

Other preferred sites of DNase I action which also are inducible by dexamethasone treatment are seen in Figure 2A. These sites are observed at DNase I concentrations of 6 μg/mL and above, where significant digestion of the HSVtK gene is evident. However, the location of these sites cannot be determined conclusively with the probing strategy used in this experiment since DNase I cleavage at these sites produces fragments smaller in size than that of the full-length HSVtK gene. More definitive localization of these preferential sites would require the use of truncated HSVtK fragments as probes.

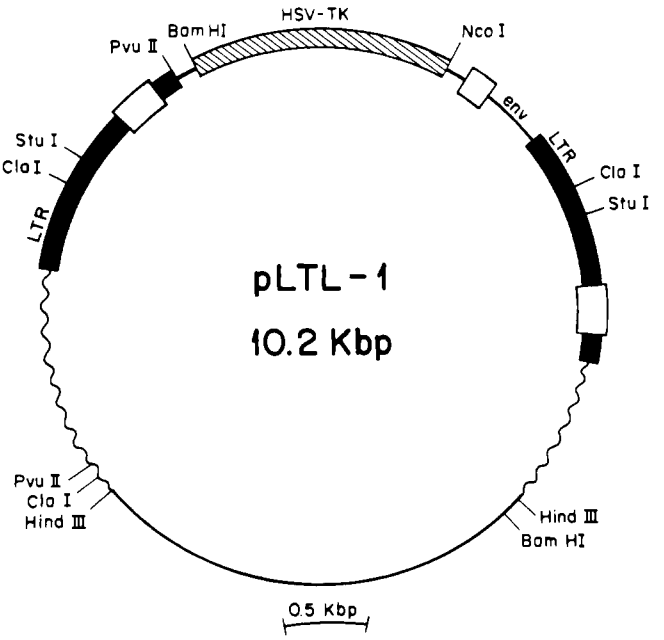
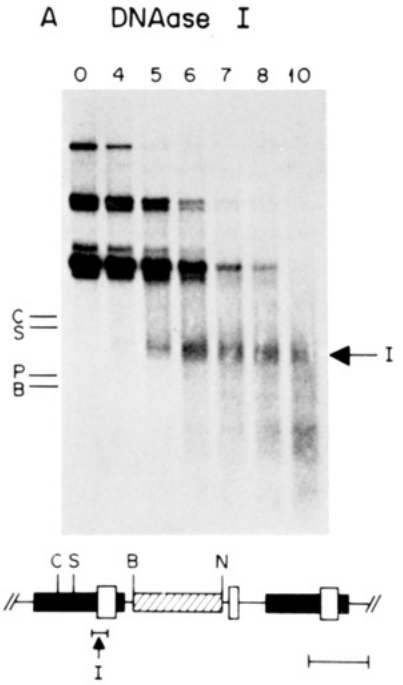


FIGURE 1: In the heterologous pLTL-1 plasmid, the bulk of the coding sequences for the mouse mammary tumor virus are replaced by a 1.5 kbp herpes simplex virus thymidine kinase gene (hatched box) such that this gene is bounded on each side by the retroviral LTRs (thick lines). The glucocorticoid receptor binding regions associated with the mouse mammary provirus are denoted by the open boxes. The wavy lines denote the flanking rat DNA derived from the original proviral clone. Recognition sites for the restriction endonucleases *Nco*I, *Bam*HI, *Cla*I, *Stu*I, *Pvu*II, and *Hin*dIII are also shown. The curved bar below the figure indicates 0.5 kbp. Construction of pLTL-1 has been described elsewhere (Zaret & Yamamoto, 1984).

In contrast to DNase I, hypersensitivity to ionizing radiation could not be demonstrated even at doses as high as 30 krad (Figure 2B). Furthermore, measurable loss of the HSVtK-hybridizing sequences is not apparent.

The response of nuclei to NCS is qualitatively similar to that observed with DNase I (Figure 3A). Increasing concentrations of NCS produce a dose-dependent cleavage of HSVtK gene sequences. In addition, the sequences which are most susceptible to DNase I catalyzed cleavage are similarly sensitive to the action of NCS. A NCS-hypersensitive site coincident with the binding region for the activated glucocorticoid receptor is barely detectable at drug concentrations of 10 μg/mL and is clearly evident at concentrations of 50 μg/mL and above. However, the region of NCS hypersensitivity extends over a slightly larger range than that observed for DNase I. Notably, cleavage within this region first appears at drug concentrations below those required to cause significant degradation of the HSVtK gene.

As seen in Figure 3B, BLM treatment of nuclei gives similar results to those observed for DNase I and NCS. Increasing concentrations of BLM lead to increased HSVtK gene degradation. Furthermore, the sensitivities of the individual gene segments to the action of this drug are qualitatively similar to those observed with NCS and DNase I. Two BLM-hypersensitive sites, designated as I, are evident at BLM concentrations of 10 μg/mL (seen in longer exposures) and above. BLM cleavage at these sites first occurs at concentrations



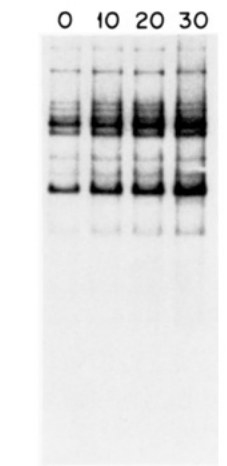
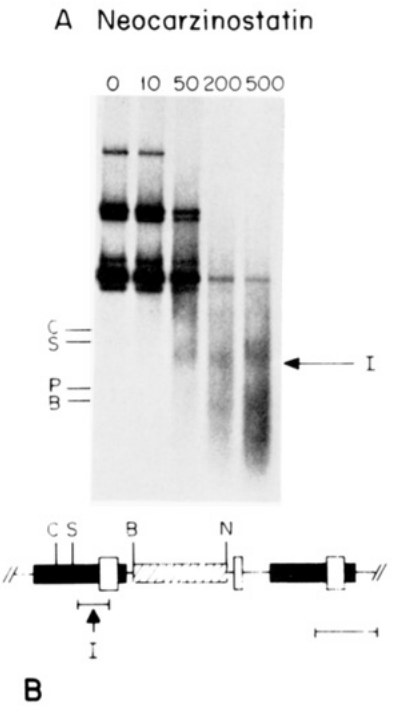


FIGURE 2: Nuclei isolated from dexamethasone-treated L1.2-2 cells were treated either with (A) increasing concentrations of DNase I or with (B) increasing doses of ionizing radiation as described under Materials and Methods. Subsequently, the DNA was isolated, restricted with *Nco*I, fractionated on agarose gels, and transferred to nitrocellulose. The resultant Southern blots were hybridized either with (A) the 1.5 kbp *Nco*I–*Bam*HI fragment from pLTL-1 or with (B) pLTL-1. (A) The DNase I concentrations (in micrograms per milliliter) used to treat nuclei are given at the top of the figure. The band on the autoradiogram identified by the arrow (labeled as I for inducible) represents a site of DNase I hypersensitivity which is induced by dexamethasone treatment. The relative migrations of the fragments produced following the digestion of *Nco*I-restricted pLTL-1 with either *Bam*HI, *Pvu*II, *Stu*I or *Cla*I are denoted on the autoradiogram as B, P, S, and C, respectively. The molecular sizes (in kilobase pairs) of B, P, S, and C are 1.57, 1.70, 2.50, and 2.75, respectively. The locations of the respective restriction sites for B, S, and C within the LTL element are shown on the map below the autoradiogram. The locations of the unique *Nco*I (N) site and the inducible (I) hypersensitive site are also shown on the map. Transcription is initiated in the left-hand LTR and proceeds rightward. The bar in the lower right-hand corner denotes 1.0 kbp. (B) Nuclear suspensions were treated with the radiation doses (kilorads) indicated at the top of the figure. The use of the entire pLTL-1 plasmid as a probe rather than its 1.5 kbp *Nco*I–*Bam*HI derivative results in the appearance of additional bands.



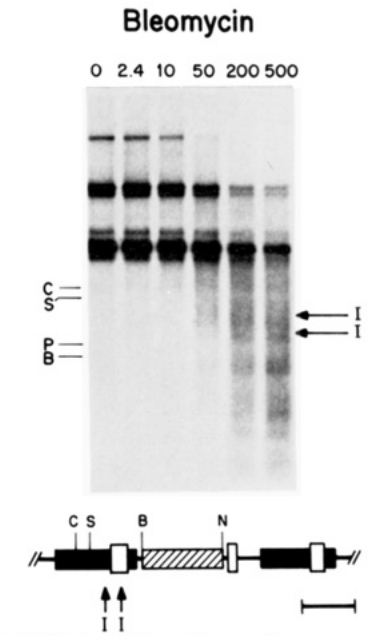


FIGURE 3: Nuclei isolated from dexamethasone-treated cells were treated with increasing concentrations of either (A) neocarzinostatin or (B) bleomycin as described under Materials and Methods. Nuclear suspensions were treated with the drug concentrations (micrograms per milliliter) indicated at the top of each figure. The blotting and subsequent probing of the nuclear DNA with the 1.5 kbp *Nco*I–*Bam*HI fragment from pLTL-1 are described under Materials and Methods and in the legend to Figure 2. The migration of the restriction fragments B, P, S, and C and the location of their respective restriction sites within the LTL element are also shown. The designations B, P, S, C, and N are described in Figure 2. The bands indicated by the arrow (labeled as I) represent sites of either (A) NCS hypersensitivity or (B) BLM hypersensitivity which are induced by dexamethasone treatment. The location of I within the LTL element is shown in the map below each autoradiogram. The bar found in the lower right-hand corner in each figure indicates 1.0 kbp.

below those which are required to cause significant HSVtK gene degradation. Notably, these regions of BLM hypersensitivity are somewhat more defined than those observed with NCS or DNase I. Both BLM hypersensitive sites appear as discrete bands on the autoradiogram (Figure 3B), mapping within the region previously associated with DNase I and NCS

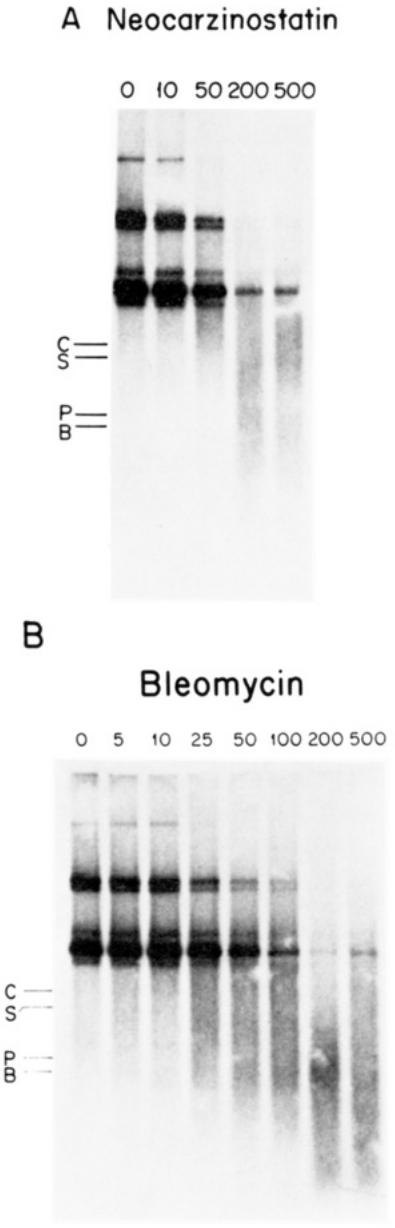


FIGURE 4: Nuclei isolated from L1.2-2 cells not treated with dexamethasone were treated with increasing concentrations of either (A) neocarzinostatin or (B) bleomycin as described under Materials and Methods. Details for the Southern blot analysis are given in the legend to Figure 3.

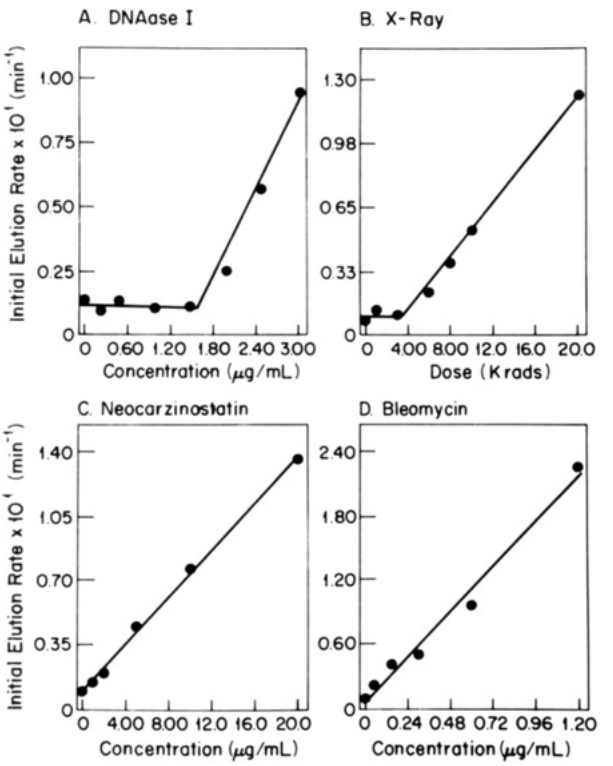


FIGURE 5: Double-stranded cleavage of bulk chromatin was quantitated in nuclei isolated from dexamethasone-treated L1.2-2 cells by filter elution. Following treatment, nuclear suspensions were applied to filters, and the DNA was subsequently eluted as described under Materials and Methods. The initial elution rate of the DNA from the filters is graphed as a function of the concentration or dose of either (A) DNase I, (B) ionizing radiation, (C) neocarzinostatin, or (D) bleomycin.

hypersensitivity. Other well-defined sites of BLM-induced cleavage are also evident in Figure 3B. These sites which appear at BLM concentrations of 50 μg/mL and above may represent sites of preferential cleavage within the HSVtK structural gene.

Since transcriptional activity within the LTL unit is nonexistent in the absence of dexamethasone (Zaret & Yamamoto, 1984), the inducibility of drug hypersensitivity by transcriptional activation can be assessed by comparing scission activity in nuclei isolated from cells either treated or untreated with dexamethasone. As seen in Figure 4A, NCS digestion of nuclei isolated from cells not treated with dexamethasone results in a dose-dependent cleavage of the HSVtK gene. This result is similar to that observed previously with the transcribing HSVtK gene (Figure 3A). However, demonstrable NCS hypersensitivity, especially within the region of the MMTV-LTR, is not readily apparent in the absence of dexamethasone. Therefore, significant NCS hypersensitivity within this region (designated as I in Figure 3A) is induced by dexamethasone treatment. Similarly, BLM hypersensitivity within the HSVtK gene and the flanking control region is not observed in the absence of dexamethasone (Figure 4B). Consequently, transcriptional activation by dexamethasone induces expression of BLM hypersensitivity.

The Southern blot data indicate that the interactions of DNase I, BLM, and NCS with defined regions of chromatin are nonrandom. Specifically, preferential sites of cleavage exist within discrete segments involved in the control of transcription, and the relative preference for these sites can be altered by transcriptional activity. Conversely, since no sites of preferential cleavage are evident following X irradiation, it would appear that damage resulting from such treatment is random. However, these interpretations are made without consideration of the extent of damage occurring elsewhere within the genome. Therefore, determinations of the relative specificities of cleavage in dexamethasone-treated cells require the quantitative assessment of damage to total cellular DNA. In this study, total DNA cleavage following treatment of nuclei with either DNase I, BLM, NCS, or ionizing radiation was quantitated by filter elution. The results of these filter elution assays are presented in Figure 5.

In these assays, the elution rate of DNA from filters is dependent upon the strand length. Hence, increasing the amount of DNA damage increases the elution rate (Kohn, 1979). When double-stranded scission is expressed in terms of the initial elution rate, a clear linear relationship is evident between the amount of damage and the concentration or dose of the agent used to induce that damage (Figure 5). Such linearity allows expression of the data in rad equivalents (the dose of ionizing radiation required to produce an equivalent amount of damage). Thus, direct quantitative comparisons

Table I: Summary of Thymidine Kinase Gene Damage[a]

	initial appearance of hypersensitivity		first observation of >90% gene loss	
treatment	dose or concn	krad equiv	dose or concn	krad equiv
DNase I	4 μg/mL	23	7 μg/mL	48
neocarzinostatin	10 μg/mL	13	200 μg/mL	192
bleomycin	10 μg/mL	262	500 μg/mL	13000
X-ray	[b]		[b]	

[a] The data obtained from the autoradiograms presented in Figures 2 and 3 are converted into values of kilorad equivalents using the standard curves generated in Figure 5. The values of kilorad equivalents in the table represent the doses of ionizing radiation required to give identical initial elution rates on the radiation elution curve. [b] Not detected at 30 krad.

can be made between different agents using the rad equivalent as a standard representation of the elution rate (see Table I).

Although Southern blot analyses indicate that BLM, NCS, and DNase I act similarly, the data in Table I suggest that there are distinct differences in the relative degrees of specificity between the three agents. The activities of DNase I and NCS are comparable in that both these agents show preference for identical gene segments at concentrations which produce similar amounts of overall chromatin damage (i.e., 13 and 23 krad equiv for NCS and DNase I, respectively). However, the overall sensitivity of the transcribing HSVtK gene to the action of these two agents appears to be different. Almost complete obliteration of the HSVtK gene occurs at a DNase I concentration of 7 μg/mL, a concentration which is equivalent to a dose of 48 krad of ionizing radiation. When compared to DNase I, NCS must produce 4 times as much damage to bulk chromatin before excessive loss of the HSVtK gene is evident.

The discrete regions of hypersensitivity observed following BLM treatment suggest that this drug, like DNase I, also has a strong preference for regions associated with transcriptional control. However, so-called BLM hypersensitivity is not observed until significant damage to bulk chromatin is produced. The amount of damage required is somewhere between 10 and 20 times greater than the amount required for the production of similar effects by NCS and DNase I (i.e., 262 krad equiv for BLM and 13 and 23 krad equiv for NCS and DNase I, respectively). When compared to NCS, almost 100 times as much damage to bulk chromatin must be introduced by BLM to cause an equivalent loss of the HSVtK gene (i.e., compare 192 to 13 000 krad equiv).

Hypersensitivity of specific gene regions to X irradiation is not detected at the doses used in this study (0–30 krad). However, the filter elution data indicate that the failure to detect such activity with ionizing radiation does not necessarily mean that this agent interacts randomly with chromatin. If X-rays have the same level of specificity for the hypersensitive region within the MMTV-LTR as does BLM, then a dosage equivalent to or greater than 260 krad would have been required to produce observable hypersensitivity within this region. Due to technical constraints, we were unable to X irradiate nuclei beyond 30 krad.

Discussion

This report evaluates the sensitivity of transcriptionally active chromatin to DNA strand scission agents using the inducible LTL gene as a specific target. A recent study by Zaret and Yamamoto (1984) clearly showed that transcriptional activation of LTL by dexamethasone was accompanied by changes in its sensitivity to DNase I. An increase in moderate DNase I sensitivity observed throughout the entire LTL element and hypersensitivity within the transcriptional regulatory region were both noted. The moderate DNase I sensitivity of the transcribing HSVtK gene was increased 4–10-fold when compared to its nontranscribing counterpart. This type of sensitivity required dexamethasone for its induction but not its maintenance since its presence was detected for several days following dexamethasone withdrawal. Conversely, the expression of hypersensitivity within the regulatory region was absolutely dependent upon the presence of dexamethasone.

Dexamethasone-dependent hypersensitivity to BLM and NCS was clearly evident in our studies. Hypersensitivity to both drugs, as well as DNase I, was characterized by a preferential cleavage occurring within a DNA region contiguous with the binding region for the glucocorticoid receptor (Figures 2A and 3A,B). Although the regions of hypersensitivity were identical, the distribution of cleavage within these regions differed for each of the three scission agents. Specifically, hypersensitivity to BLM was characterized by the appearance of two discrete bands in the autoradiogram (Figure 3B), while cleavage within this same region by either NCS or DNase I produced one band (Figures 2A and 3A). The NCS-generated band by comparison was the most diffuse (Figure 3A). Earlier studies with both NCS and BLM have shown that several factors including DNA sequence (Sugiura & Suzuki, 1982; Kross et al., 1982; Takeshita et al., 1981) can influence strand scission. Therefore, the differences in cleavage activity just described could reflect differences in the relative preferences for particular DNA sequences or the availability of such sequences. The inducibility of drug hypersensitivity was confirmed by demonstrating that its presence was detected only in nuclei isolated from dexamethasone-treated cells (compare Figure 3 to Figure 4). Hence, hypersensitivity within the region responsible for transcriptional regulation was the most significant change in sensitivity observed.

A rigorous comparison of preferential cleavage of specific sites also requires knowledge about the extent of damage occurring throughout the entire genome. Using filter elution data, it was shown that NCS and DNase I were similar since demonstrable hypersensitivity to both agents was observed when equivalent amounts of damage were introduced into the genome. However, preferential interactions with BLM were observed at doses which were at least 10–20 times greater. Although it is significant that hypersensitivity is found within the region responsible for transcriptional regulation, the data indicate that BLM is much less specific for such regions than either DNase I or NCS.

Unlike DNase I, only minor increases in the overall sensitivity of the HSVtK gene to drug-induced cleavage were observed following dexamethasone treatment. Densitometric scanning of several autoradiograms from different experiments with NCS and BLM (data not shown) showed that the initial rate of degradation was at best 2 times faster in nuclei from dexamethasone-treated cells. Filter elution measurements showed that DNase I had the greatest relative specificity for the transcribing HSVtK gene (see Table I). When compared to DNase I, at least 4 times as much damage to bulk chromatin had to be introduced by NCS in order to cause an equivalent loss of the HSVtK gene. Apparently, BLM was even less specific for this target than NCS since almost 100-fold more damage had to be introduced in order to see a similar effect.

Preferential cleavage of a transcribed cellular gene by BLM has been described previously. Kuo (1981) has shown that the comparative sensitivities of the ovalbumin and globin genes

to BLM-induced cleavage differed in nuclei isolated from different cell types. In oviduct nuclei, where the ovalbumin gene is active and the globin gene inactive, the ovalbumin gene was more sensitive to BLM action. In the red cell nuclei, the situation was reversed such that the transcribing globin gene was more sensitive to cleavage than was the inactive ovalbumin gene. Therefore, using different systems and DNase I sensitivity as a model, both Kuo and we have shown that BLM can preferentially degrade regions associated with active transcription. We have also observed that hypersensitivity to BLM was characterized not by profound changes in sensitivity within the coding sequences but rather by an increased sensitivity within the region responsible for transcriptional control. In both studies, however, the qualitative changes in BLM sensitivity paralleled the changes in sensitivity to DNase I. Therefore, the altered chromatin structures presumably found within the transcribing regions were similarly recognized by both DNase I and BLM.

In contrast to the data presented here, a previous study (Hatayama & Yukioka, 1982) with NCS, albeit based on indirect evidence, indicated that the drug showed a lack of preference for transcribing chromatin. Using rat liver nuclei and a cDNA probe prepared from poly(A) mRNA, Hatayama and Yukioka showed that the chromatin solubilized from these nuclei following NCS digestion was depleted in sequences representing active genes. However, it is likely that preferential cleavage within very limited regions of a transcribed gene or within regions responsible for transcriptional regulation would not be detected through such a strategy.

In contrast to the drugs, ionizing radiation showed no preferential activity. However, the hypersensitivity to BLM just described would also not have been detected at concentrations equivalent to 30 krad and below (i.e., the dose range studied for ionizing radiation). On the basis of these data, we cannot determine conclusively whether or not ionizing radiation has any potential for preferential interactions with transcribing chromatin. Since ionizing radiation produces single-strand breaks at a greater frequency than double-strand breaks, we are currently reevaluating its potential for preferential interactions using denaturing gels to look at single-strand, rather than double-strand, site preference. In this regard, it is interesting to note that preferential damage by ionizing radiation to transcribing (Chiu & Oleinick, 1982) and replicating (Warters & Childers, 1982) chromatin has been described.

In summary, using nuclei isolated from L1.2-2 cells either treated or untreated with dexamethasone, we were able to assess the potential of the strand scission antibiotics to preferentially cleave transcribing chromatin. Evidence for preferential activity in vitro was provided through comparisons with DNase I. Since transcribing chromatin may be a potentially important target for drug-induced cytotoxicity, we are currently interested in evaluating preferential strand scission and repair in intact L1.2-2 cells.

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