LINE L1 Retrotransposable Element Is Targeted During the Initial Stages of Apoptotic DNA Fragmentation

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Abstract Using a directional cloning strategy, DNA sequence information was obtained corresponding to the site of early radiation-induced apoptotic DNA fragmentation within the human lymphoblastoid cell line TK6. Data were obtained from 88 distinct clones comprising approximately 65 kbp of sequenced material. Analysis of all cloned material showed that sequences in the 10 bp immediately adjacent to the cleavage sites were enriched in short oligoT tracts. The proportion of repetitive DNA within the entire cloned material was found to be within the normal range. However the distribution of Alu and LINE repetitive DNA were biased to positions at or adjacent to the apoptotic cleavage site. In particular, a non-random distribution of five cleavage sites was found clustered within the second ORF of the LINE L1 that partially overlapped with two binding sites for the nuclear matrix-associated protein SATB1. Three other clones, containing alpha satellite elements, were also linked to a DNA matrix binding function. These data indicate that the site of chromatin loop formation at the nuclear matrix may be a specific target for early DNA fragmentation events during apoptosis. J. Cell. Biochem. 79:486–495, 2000. © 2000 Wiley-Liss, Inc.

Key words: apoptosis; cloning; DNA fragmentation; LINE L1

Apoptosis is a regulated form of cell death triggered in response to therapeutic agents, including ionizing radiation, in addition to physiological stimuli active during the course of development. Two phases may be identified: first where a proapoptotic signal is received and processed, leading to the second, effector phase where the cell is structurally and functionally disabled. The effector process consists of a concerted sequence of events involving the destruction of regulatory and structural proteins by a family of proteases, the caspases, and the repetitive scission of DNA into large (~300 kbp), medium (~50 kbp), and small (180-bp multimers) fragments [Thornberry and Lazebnik, 1998; Khodarev et al., 1998]. The combination of these events creates the biochemical and morphological attributes of apoptosis [Beere et al., 1995; Lagarkova et al.,

1995; Vodenicharov et al., 1996; Marini et al., 1996; Chen et al., 1998].

The similarity in size between high-molecular-weight DNA fragments produced during the early stages of apoptosis and the hierarchical organization of chromatin DNA in loops and supraloop domains has been described [Lagarkova et al., 1995]. This has led to the suggestion that early apoptotic cleavage events occur in structures that determine loop organization, possibly through specific interactions with S/MARs (scaffold or matrix attachment regions). This interpretation receives support from studies using inhibitors of DNA topoisomerase II. Topoisomerase II has been linked to S/MAR locations where VP16-driven cleavage of DNA leads to the release of 50-kbp and larger fragments that parallel the sizes produced during apoptosis [Gromova et al., 1995a]. However, it is apparent that such loops are not cleaved by the action of topoisomerase II itself during the course of apoptosis [Gromova et al., 1995a]. We have recently demonstrated, however, that low concentrations of VP16 lead to both a transient cessation of rep-

lication initiation and an increase in net negative supercoiling of DNA loops [Narayana et al., 1998]. This suggests that substantial alterations in chromatin topology are regulated at the interface between DNA and the nuclear matrix and are mediated by action of topoisomerase II. These changes may alter subsequent endonuclease access or cleavage efficiency during apoptosis by formation of endonuclease-hypersensitive structures. We have also shown that the initial stages of stress-induced DNA fragmentation specifically target sites of DNA synthesis initiation and suggested that such targeting occurs in matrix-associated regions [Khodarev et al., 1998a]. More direct evidence of a link between apoptotic fragmentation at unique sites, possibly involving S/MARs, was presented recently by Stanulla et al. [1997]. Here, apoptotic cleavage was observed at a specific location within the break-point cluster region of the MLL gene that includes a S/MAR.

Several laboratories have reported the cloning and sequencing of random samples of apoptotic DNA fragments [Luokkamaki et al., 1993; Moore et al., 1993; Fan and Smith, 1995]. For the mammalian cells studied, these approaches were performed using only relatively short DNA fragments, derived from mononucleosomes and their oligomers. These fragments are released only during the late stages of DNA fragmentation and may correspond to nonspecific cleavage. In addition, the relative locations of apoptotic cut sites were unknown because of the random ligation of sample DNA into the cloning vectors used. In this report, we used the strategy of directional cloning of DNA ends, corresponding to high-molecular-weight chromatin fragments produced during the early stages of radiation-induced apoptosis in the human lymphoblastoid cell line TK6.

The data obtained showed enrichment of target apoptotic sequences by repetitive DNA, including LINE L1, Alu, MER, and alpha-satellites. Specifically, a clustering of apoptotic cleavage sites was found within the second open reading frame of the LINE L1, a site also shown to be a binding site for SATB1, a lymphocyte-specific nuclear matrix protein [de Belle et al., 1998]. It has been shown that matrix-binding sites have the potential to become single stranded under negative superhelical strain, potentially making them accessible to endonuclease activity [Kohwi-Shigematsu and Kohwi, 1990; Benham et al., 1997]. These data lead to the following three suggestions. First, LINE L1 retrotransposable elements may be involved in the formation of loop structures, providing the framework for periodic DNA interactions with S/MARs. Second, the earliest apoptotic fragmentation events may be initiated at nuclease-sensitive base-unpaired tracts within S/MARs. Third, these same regions may provide the physical and functional link between high-order chromatin structure and cleavage/rejoining events that produce both translocations and truncated variants of repetitive LINE sequences.

METHODS

Cell Culture

The human B-lymphoblastoid cell line TK6 was maintained at 37°C in 5% CO_2 in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 50 U/ml penicillin, 50 mg/ml streptomycin, and 5×10^{-5} M 2-mercaptoethanol. To induce apoptosis, cells were grown to a concentration of 5×10^5 cells/ml, washed, and resuspended in fresh complete media at a final concentration of 10^6 cells/ml. Cells were then irradiated with gamma rays at a dose rate of 1.3 Gy/min using a dual-head ^{137}Cs Gammacell 40 irradiator (Nordion International, Inc., Kanata, Ontario, Canada) and were collected at various times after irradiation. Cells were routinely checked for Mycoplasma contamination using a polymerase chain reaction (PCR)-based Mycoplasma detection kit according to the manufacturer's instructions (ATCC, Rockville, MD).

Analysis of Apoptosis

For rapid measurement of total apoptosis, apoptotic nuclei or cells were stained with either 1µg/ml ethidium bromide or trypan blue. Apoptotic cells were scored as those exhibiting nuclear contraction and fragmentation typical of the process. In our experience, the proportion of trypan blue–positive cells and those showing the characteristic nuclear condensation using ethidium bromide staining gave a similar estimate of the frequency of apoptosis in irradiated TK6 cell cultures.

Field-Inversion Gel Electrophoresis Analysis of High-Molecular-Weight DNA Fragments

For separation of high-molecular-weight DNA, cells were immobilized in 1.5% low melt-

ing agarose; the plugs produced were then treated with 0.5 mg/ml proteinase K, 10% sarkosyl, 25 mM EDTA, 10 mM Tris, pH 9.5 at 50°C for 24 h. The plugs were then washed three times in 10 mM EDTA, 1 mM Tris-HCl, pH 8 and separation of high-molecular-weight DNA performed in 1% agarose in 0.5× TBE buffer at 100 V, 25 mA at 4°C using a Power Inverter PPI-200 (MJ Research, Watertown, MA).

Cell Preparation and Ligation to Vector

Cells were washed on ice in a 0.075% NP40– phosphate-buffered saline (PBS) solution and twice in PBS only. They were fixed in 70% ethanol and heat inactivated at 65°C for 5 min. The suspension was stored at −20°C for up to 1 month and washed again in PBS before use. Cells were further cleaved by proteinase K, 100 μg/ml for 4 h at 37°C, washed twice in PBS and twice in 1× T4 DNA ligase incubation buffer (GIBCO BRL, Gaithersburg, MD). The final cell concentration was 10^7 cells/100 μl. Before use, the pBluescript SK+ plasmid was cleaved by SmaI, dephosphorylated by BAP, and additionally cleaved by HindIII (see Fig. 1 for diagram). The 34-mer produced was re-
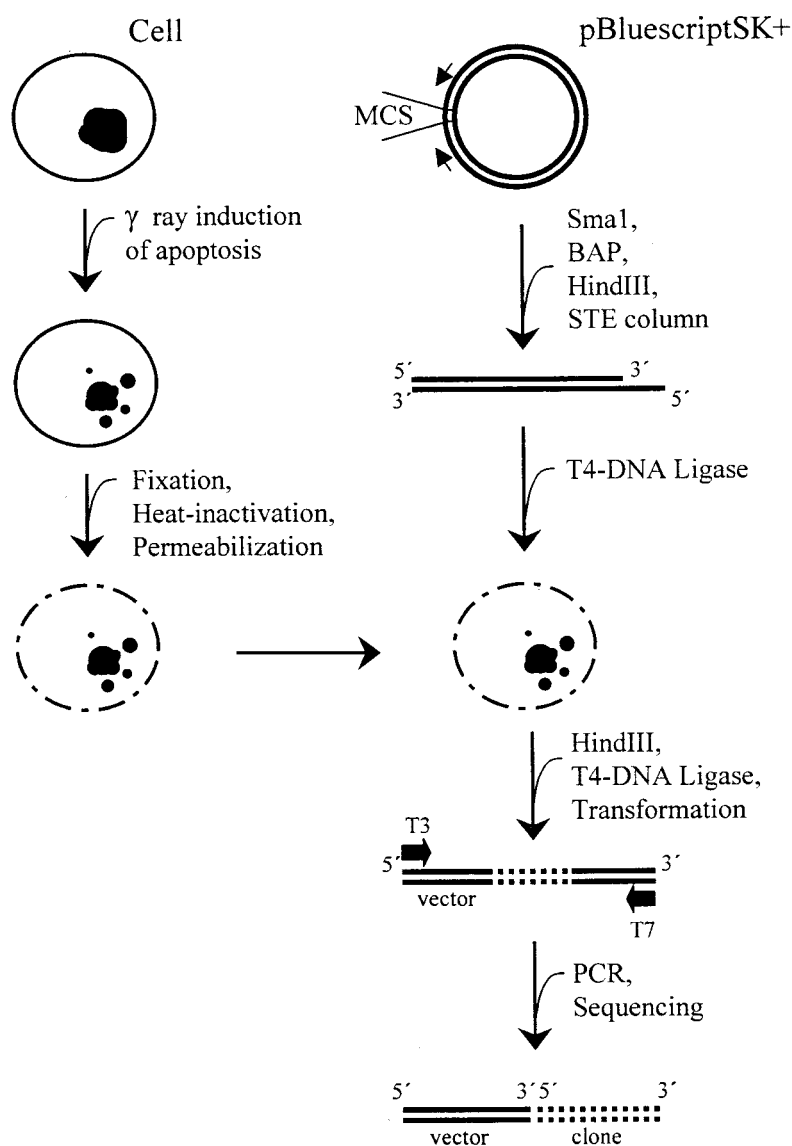

Fig. 1. Diagram of the cloning strategy used to isolate early apoptotic cleavage sites within irradiated TK6 lymphoblastoid cells. The technique uses intact early apoptotic nuclei, and cloned material is generated from the free DNA ends produced by apoptotic scission.

moved using STE Select-D columns (5′ Prime-3′ Prime, Inc., Boulder, CO) according to manufacturer's instructions. Subsequently, 1 µg of asymmetrically cleaved, dephosphorylated pBluescript SK+ was added to 100 µl of each sample with 60 U of T4 ligase and incubated overnight at +14°C. After ligation with vector, each sample was adjusted to 200 µl with 0.5 M NaCl/0.5% sodium dodecyl sulfate and incubated 6 h with 140 µg/ml of proteinase K (sequence grade, Boehringer Mannheim, Indianapolis, IN) followed by two cycles of deproteinization in chloroform/isoamyl alcohol mixture (24:1). DNA was precipitated by EtOH and redissolved in 50 µl TRIS EDTA (TE) pH8.00.

Cloning of Genomic Sequences Adjacent to Apoptotic Cuts

After ligation of the targeting vector to the apoptotic cut sites, DNA samples were extensively cleaved by HindIII to introduce double-stranded DNA breaks with HindIII complementary 5′ ends distal to the DNA cleaved during apoptosis. Samples were heat inactivated at 70°C for 5 min and diluted to a DNA concentration of 10 µg/ml. Finally, the 3′ terminus of the apoptotic fragments was ligated to the multicloning site of the vector, thereby capturing the fragment within the plasmid. Ligation was performed with 10 U/ml of T4 ligase at 14°C overnight. Samples were heated at 65°C and used to transform DH5α cells (GIBCO BRL) according to manufacturer instructions. White colonies were picked, and PCR amplification was performed using T3/T7 primers and the products were identified by gel electrophoresis. The strategy of experiments is shown in Figure 1. Clones containing genomic inserts of more than 150 bp were selected for further characterization and sequencing. Samples with equivocal sequence data were repeated and/or sequenced in both directions. Sequence data were analyzed using nonredundant GenBank databases with BLAST (www.ncbi.nlm.nih.gov/BLAST). Interspersed repeats were also identified using RepeatMasker2 (http://ftp.genome.washington.edu/RM/Repeat Masker.html).

RESULTS

Apoptosis and Stress-Induced DNA Fragmentation

Trypan blue exclusion was found to be a rapid and accurate indicator of apoptosis in the TK6 cell system, giving data comparable to a morphological assessment using ethidium bromide staining. The data show a detectable increase in apoptosis after 2 Gy (Fig. 2). The media were not changed after irradiation; thus, control cells eventually died of overcrowding. Within this particular experimental system, there was a clear difference between cells irradiated with 0–4 Gy and those irradiated with higher doses. The higher doses gave a rapid increase in apoptosis at shorter periods after irradiation. This feature was studied by field inversion gel electrophoresis and, as seen in Figure 3, after 8 Gy, 50-kbp fragmentation was clearly observed 4 h after irradiation. Both of these data were used in the final selection of 5 h after 6 Gy for the test dose in this system as appropriate conditions to isolate the earliest identifiable cleavage events of apoptosis.

Apoptotic Sequence Classification

According to the protocol described, 95 clones were established containing 7 sequence-paired samples. Six of these were classified as exact duplicates according to the cut-site location, the DNA sequence available for analysis, and
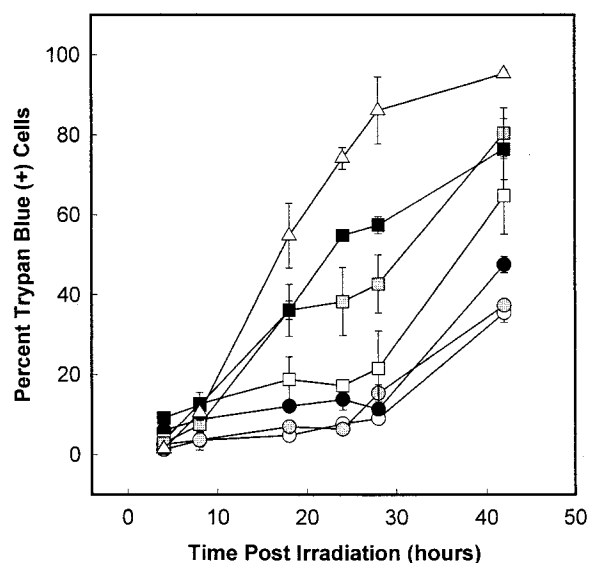

Fig. 2. Apoptotic dose response. TK6 cells were irradiated with the following doses of gamma irradiation: open circles, 0 Gy; shaded circles, 1 Gy; filled circles, 2 Gy; open squares, 4 Gy; shaded squares, 6 Gy; filled squares, 8 Gy; and open triangles, 10 Gy. Analysis was performed using trypan blue exclusion assay; equivalent results were obtained using ethidium bromide staining to reveal apoptotic nuclear morphology. Increased apoptosis after 30 h is caused by serum starvation.
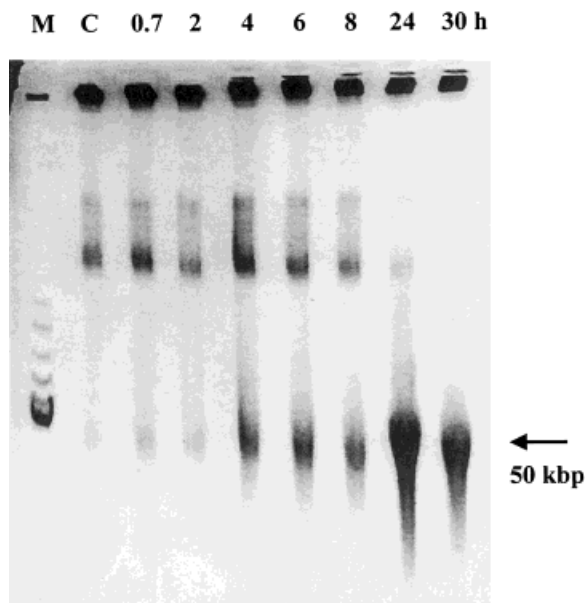
Fig. 3. Apoptotic dose response. Field-inversion gel electrophoresis performed to show the early DNA fragmentation after inducing apoptosis with a single dose of 8 Gy. Evidence of apoptotic fragmentation was seen 4 h after irradiation. M = molecular weight markers; C = no irradiation.

vector insert size. Two samples shared the same cut-site location, but were derived from different inserts as shown by insert size, and thus are examples of two separate events cloning the same cut site. Two samples were excluded as containing unreadable sequences despite attempts at sequencing fresh material derived from frozen stocks. This left a total of 88 unique samples for study containing 65.3 kbp of sequenced material (average, 734 bp: range, 568–922 bp). These were analyzed further using the BLAST program to determine any sequence-dependent associations with material recorded in the databases. Matches were considered if they showed a significance greater than a bit score of 50 using the Karlin-Altschul scoring system [Karlin and Altschul, 1990]. As an example, this level of significance corresponds to a 29/30 base homology from the 2A11 clone—substantially less for longer sequences. With this low threshold for analysis, the majority showed some degree of homology with sequence elements stored in the database. Of these, the most easily identified were sequences matched to repetitive DNA including LINE, SINE (Alu) MER, and alpha satellite DNA. Three clones matched to alpha satellite DNA showed the greatest significance

match, with bit scores of between 700 and 999, corresponding to approximately 95–98% sequence homology over 600 base pairs or greater. All three matches to alpha satellite DNA contained the apoptotic cut site within the alpha satellite sequence itself. For further analysis, a 600-bp segment of sequenced material from each clone was used, starting at the apoptotic cut site, to determine the frequency of repetitive DNA. It was found that 15.2% of the sequenced material was matched to LINE sequences, and 6.0% were Alu derived. These numbers are in good agreement with published estimates for the proportion of these classes of repetitive DNA within the genome [Kariya et al., 1987; Moran et al., 1999].

To determine the relationship, if any, between the site of apoptotic scission and the distribution of repetitive DNA, the location of the Alu and LINE sequences to the cut site was examined. From the 15 clones containing Alu repeats, 8 of 15 (53.3%) were Alu tracts originating at the apoptotic cut site or within the first 10 base pairs (Fig. 4). This analysis was performed by directly comparing each cloned insert with the predominant Alu -Sx form, but a similar distribution was found with other Alu family members [Mighell et al., 1997]. Therefore, the total content of the Alu sequence within the cloned material was consistent with their distribution within the genome as a whole, but their location within each clone was biased toward the proximal or cut-site location of each insert. A similar analysis of the LINE L1 repetitive DNA showed 11 of 15 (73.3%) clones contained the apoptotic cut site within the 10 bp of a LINE L1 repeat and 10 of 15 (66.6%) showed apoptotic scission directly within the LINE itself (Table I). Five LINE L1 clones contain unidentified sequences in the 5′ region with lengths ranging from 5 to 242 nucleotides. These truncated variants of the L1 LINE are therefore adjacent to the introduced apoptotic double-strand break, separated by a distance comparable to a 1–1.5 nucleosomal repeat.

Cleavage Target Specificity

We used two approaches to characterize the presence of any sequence dependency at the cleavage target site. First, we examined the nucleotide frequency in the first 10 nucleotides of all 88 clones. Second, for clones matching the L1P family only, we determined their location
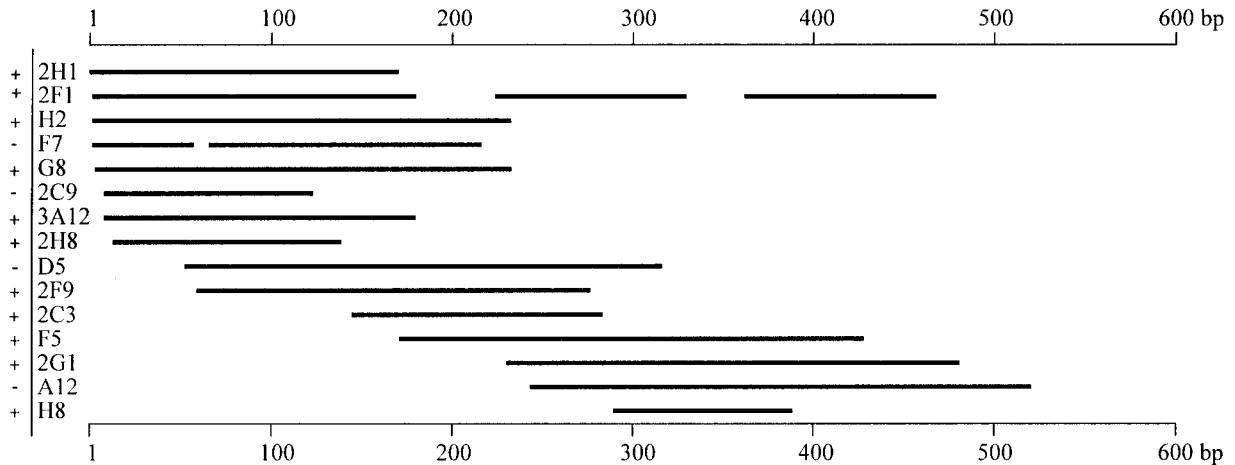
Fig. 4. Relationship between Alu sequences within the first 600 bp of cloned material. Only those Alu matches with bit scores greater than 50 are shown. On the left, a (+) designation indicates the complementary strand and position 1 is the location of apoptotic cleavage. A match coincident with position 1 indicates that apoptotic cleavage occurred within the Alu repeat; some clones, such as 2F1, contain multiple Alu repeat sequences. Although the frequency of Alu repeats (6%) is consistent with their normal distribution in the genome, a strong bias exists for the cut site to occur either within, or immediately adjacent to, the Alu sequence repeat.

compared to a full-size (L1.15) element. The total nucleotide content of the entire sequenced material, comprising approximately 63 kbp, was consistent with that found in bulk DNA (29% A, 30% T, 21% C, and 20% G). However, in the DNA sequenced adjacent to the cut site itself, there was a substantial increase in T content, indicating an asymmetrical distribution of T and A in each DNA strand at the site of cleavage (Fig. 5). The asymmetrical distribution of T within this region was found to be statistically significant ($P = 0.001$; χ^2 statistic) when compared to randomly selected 10-bp tracts at other positions within the cloned material (0 of 10 tested). A bias toward an asymmetrical distribution of T and A in the opposite strands of DNA has been reported previously as a feature of L1 elements [Smit et al., 1995]. We subsequently looked in more detail at the distribution of cleavage sites within L1 elements. Eight clones with the strongest associations with L1 sequences were mapped to the full-size L1.15. It was found that of these, five cleavage sites were clustered within a 299-bp region in the second open reading frame (ORF) (Fig. 6). In this region, the T content of the + strand in our clones (which mostly corresponds to the complementary strand of L1) was equal to 40.7%. Three other clones with high BLAST scores were located either 1,100 bp 5′ to the cleavage cluster (3A10) or in the 3 Ultranslated Region (UTR) with cut sites separated by 4 bp

(2C7 and C2). If apoptotic cuts were inserted at random into the L1 element, then the probability of a cut appearing in any one specific 299-bp segment is 299/6,000, or approximately 0.05. The probability that five such cuts will be inserted in the same 299 bp segment is therefore $(0.05)^5$ or 1:3 × 10^6. These data support the possibility that apoptotic cleavage events within the L1 second ORF do not occur at random. Analysis of the distribution of apoptotic cut sites within the much smaller Alu repeat did not reveal any similar bias.

DISCUSSION

The predominant finding in this study was the distribution bias of early apoptotic cleavage events within or adjacent to sequences of repetitive DNA. Others have also concluded that apoptotic fragmentation occurs within regions of the genome linked to repetitive DNA sequences [Luokkamaki et al., 1993; Moore et al., 1993; Fan and Smith, 1995]. All of these studies, however, have used DNA isolated as oligonucleosomal fragments, where the orientation and context of the earliest DNA breaks are lost. In this study, using directional cloning coupled with the ability to specifically tag the DNA ends produced during apoptosis, we have been able to analyze early apoptotic cleavage events, rather than those linked to extensive internucleosomal degradation. Using the null hypothesis that early (nonnucleosomal) cleavage

TABLE I. Association of LINE DNA Found Within the 88 Cloned Fragments from TK6 Apoptotic Cut Sites[a]

Clone	Score	Type of L1	L1 DNA match (no. 1 is cut site)	Orientation
2A11	1167	L1M4	1-446	−
2C7	3219	L1P3	1-506	−
2D4	1635	L1P	242-565	−
2 E2	2995	L1P	1-650	−
3A10	4177	L1P	1-703	+
3B2	1268	L1PA15	1-189	+
3B6	2208	L1ME2	1-696	−
C2	4053	L1PA3	5-486	+
F1	348	L1MC5	137-305	+
F11	276	L1M3C	58-153	−
G3	3757	L1P	1-713	−
G7	577	L1PBb	41-297	+
2G11	1756	L1M4	1-585	−
2H12	3912	L1P	1-570	−
72	4003	L1P	1-672	+

[a]All clones containing LINE DNA are shown. The site of apoptotic cleavage is always at position 1. Thus, the majority of clones were cut within the LINE sequence but some, F11 for example, were not. Also shown is the orientation of the alignment (+/−). The types of L1 repeats and their significance were determined using Repeat Masker 2 software (http://ftp.genome.washington.edu/RM/Repeat Masker.html; see also [Smit, 1996]).

Fig. 5. Analysis of base sequence distribution within the terminal 10-bp sequence at the apoptotic cut site for all 88 clones. The frequency is the number of bases from the 88 clones that occur at that position. Position 1 is the site of apoptotic cleavage. Plots shown are A (dotted line), T (solid line), G (dot-dash line), and C (broken line). A statistically significant increase in thymine was observed within this tract when compared to 10 randomly selected 10-bp tracts at other locations within the 88 clones ($P = 0.001$; χ^2 statistic).

events occur at random, it would be expected that a proportion of these events would occur in repetitive DNA by chance. Analysis of the repetitive elements detected within the 88 derived clones showed that 6% and 15% shared homology with the Alu and LINE L1 repeats, respectively. These numbers are comparable to that reported in the literature for the genomic distribution of Alu and LINE elements [Kariya et al., 1987; Moran et al., 1999]. This would seem to be in contrast to other reports, in which apoptotic DNA fragments were reportedly enriched in repetitive DNA [Luokkamaki et al., 1993; Miore et al., 1993; Fan and Smith, 1995]. However, in this study we found that although the percentage of repetitive DNA within the entire cloned material was not elevated above normal levels, the location of the apoptotic cut site was biased toward both Alu and LINE locations. For the Alu repeats, enrichment of Alu elements was found at the cleavage site (proximal) location within the cloned DNA compared to the distal portion
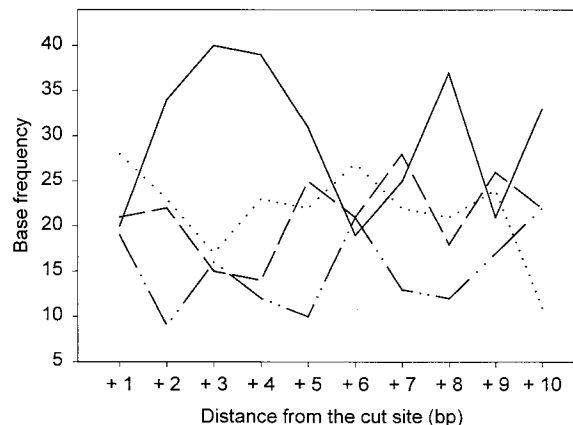
(Fig. 4). In the 15 clones containing Alu sequence matches greater than a BLAST bit score of 50, 58% of the DNA within 100 bp of the cut site were Alu elements; this gradually decreased to 0.8% in the distal 100 bp of the 600-bp inserts (Fig. 4). For the LINE L1 material, approximately three quarters contained an apoptotic scission event within the repeat sequence itself. Thus, although the percentage of these repetitive elements within the sequence library matches that found in the entire genome, they are preferentially found at the site of apoptotic cleavage, confirming the results of others [Luokkamaki et al., 1993; Moore et al., 1993; Fan and Smith, 1995]. In the specific case of those cloned sites containing L1 elements, it was found that a proportion of them was clustered within a 300-bp region of the second ORF (Fig. 6). This indicates that there may be some specific feature of the LINE elements that makes them susceptible to apoptotic fragmentation. It is known that the majority of LINE L1 sequences within the genome are truncated at their 5′ end. Of the 15 clones containing L1 tracts, two thirds contained L1 sequences up to and including the cleavage site, the remaining one third were located adjacent to the 5′ truncated region. A general association between LINE and SINE DNA and apoptosis has been reported before by obtain-
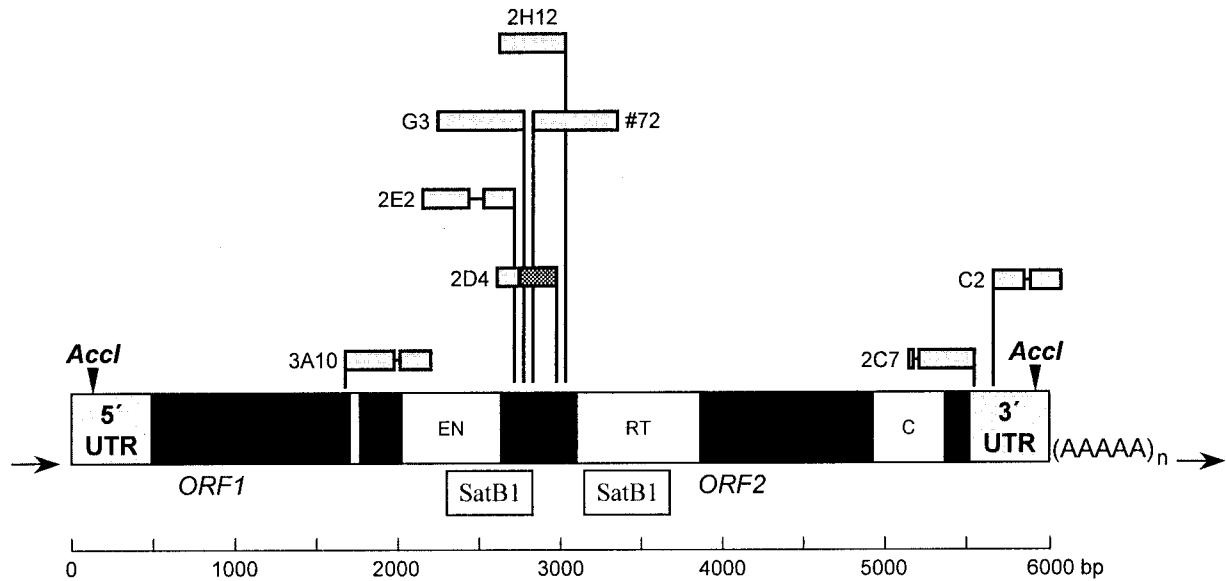
Fig. 6. Representation of the L1.15 LINE, showing sites of apoptotic cleavage. Length of flagged identifiers indicates length of sequence match, hatched regions (2D4 and 2C7) are genomic sequences linked to L1, and gaps in flagged sequences reflect the diversity among the L1P family members in that not all will contain the L1.15 exact sequence. The second open reading frame encodes an endonuclease (EN) and reverse transcriptase (RT) that participate in retrotransposition. The binding sites for the nuclear matrix attachment protein, SatB1, are shown. Other LINE matches from Table I are either not within the L1P family, or fall outside the L1.15 copy used for comparison.

ing sequence data from material released during apoptotic fragmentation in rat chloroma cells [Luokkamaki et al., 1993]. In this latter study, the predominant finding was also an excess of repetitive DNA within the apoptotic fragments. To try to detect any common element of nuclear structure that might be responsible for apoptotic cleavage, the base composition of the first 10 bases adjacent to the cut sites was examined. It was found that, on average, the library of cloned material contained an excess of thymine within the 10 bp adjacent to the cut sites (Fig. 5). The remainder of the cloned material had an AT:GC content comparable to that found in total DNA. Such tracts of AT-rich DNA are known to adopt unusual DNA structures, such as the ability to become single stranded mediated by superhelical tension, and are found within regions of DNA in contact with the nuclear matrix [Khohwi-Shigematsu and Kohwi, 1990; Laemmli et al., 1992; Benham et al., 1997]. These tracts of DNA are able to separate under increased negative supercoil tension, and this base unpairing function is important to maintain DNA-matrix binding [Benham et al., 1997; Kohwi-Shigematsu and Kohwi, 1990].

Based on our general finding of AT asymmetry, potentially unusual DNA structures at the

apoptotic cut site, and possible influence of Alu and L1 context, an attempt was made to determine any association between the cluster of L1 restricted cleavage site and DNA conformation. Following this approach, we observed an overlap with two binding sites for the nuclear matrix protein SATB1 within the second ORF, at positions 2427–2887 and 3238–3626 of the L1 cleavage cluster [de Belle et al., 1998] (Fig. 6). The SATB1 specificity is characterized by "ATC" sequences, enrichment of A and T, with oligoT tracts and low G content [de Belle et al., 1998]. As discussed above, this sequence profile is consistent with the 5′ sequences of the majority of our clones and links the L1 cleavage cluster with a nuclear matrix binding function. A possible link between apoptotic scission sites and DNA matrix attachment was also observed in additional cloned material. The strongest sequence homology seen was with three clones containing alpha satellite–repetitive DNA. This type of DNA is associated with the centromere of all chromosomes and has been shown to be enriched in DNA matrix-binding sites [Strissel et al., 1996]. In addition, one of the clones matched to alpha satellite DNA contained a DNA matrix-binding site that had been previously defined by experiment [Jackson et al., 1996]. Thus, of the 88 clones

studied, 8 of 88 (9%) were linked to nuclear matrix binding through either SatB1 association, alpha satellite DNA content, or by direct experimental analysis. This association is not a unique finding in that data generated in other systems also support a relationship between repetitive DNA and matrix attachment regions. It was found that the S/MARs detected within the human serpin gene cluster consist of 83–93% repetitive DNA elements, all degenerate copies of retrotransposons including LINEs as the predominant component [Rollini et al., 1999]. The same authors noted enrichment within the serpin S/MARs by several simple AT-rich sequences and H-boxes. In addition, the only documented site-specific cleavage during apoptosis occurs in the MLL gene, at a location containing a strong operationally defined S/MAR [Stanulla et al., 1997]. As already mentioned, one common feature of such simple AT-rich sequence motifs is their increased ability for base unpairing. More specifically, A/T/C sequences are reported to have a high potential for base unpairing under negative superhelical strain [Kohwi-Shigematsu and Kohwi, 1990]. This is consistent with the role of S/MAR-binding regions of chromatin loops serving as topological domain boundaries and regulators of topological energy and gene function [Schubeler et al., 1996; Benham et al., 1997]. A mechanistic link between this observation and apoptotic DNA cleavage may be provided by the potential of base unpaired DNA to adopt nuclease hypersensitive structures, as seen for S1 nuclease [Aoyagi et al., 1998]. Indeed, the role of single-stranded DNA cleavage during the initial stages of apoptotic DNA fragmentation is well documented [Tomei et al., 1993; Walker et al., 1999]. This may also provide the structural link between DNA supercoiling and nuclease sensitivity, which has been documented previously [Khodarev et al., 1998b; Lee et al., 1998; Schoenlein et al., 1999].

The precise types of nucleases that are triggered during the initial stages of chromatin cleavage remain elusive [Khodarev et al., 1998a]. Those that have a documented link with apoptosis, such as DFF-40/CAD nuclease, are primarily involved in internucleosomal fragmentation, rather than the initial stages [Enari et al., 1998; McIlroy et al., 1999]. The use of potential target apoptotic sequences, such as those characterized here, may provide an appropriate tool for the identification of those enzyme(s) responsible for the initial stages of stress-induced DNA fragmentation. It has been previously suggested that L1 and Alu clusters may correspond to morphologically defined human metaphase chromosome bands and participate in the formation of large chromatin domains with different replication patterns [Chen and Manvelidis, 1989; Korenberg and Rykowski, 1998; Khodarev et al., 1998b; Lee et al., 1998; Schoenlein et al., 1999]. Our recent data, in combination with that of others, suggest that L1 may be involved in formation of chromatin domains through specific interactions with S/MARs [de Belle et al., 1998; Rollini et al., 1999]. These data raise specific questions concerning the regulation of apoptotic cleavage at the S/MAR locations containing LINEs and SINEs. In particular, the presence of identifiable sequences or motifs that are targets for stress-induced DNA cleavage may stimulate the search for factors that may alter enzyme accessibility during apoptosis, thus modifying the execution of apoptotic stimuli. Additionally, the structural motifs identified here may be relevant to other biological events whereby nuclease-mediated scission of DNA leads to a biological change. Of these, the most interesting may be translocations leading to a leukemogenic event. As discussed above, the matrix attachment site within MLL is a hotspot for both translocations and apoptotic cleavage [Strissel et al., 1996; Stanulla et al., 1997]. The relevance of the structural motifs found here that enhance nuclease sensitivity may be a unifying feature tying together both apoptotic scission events and aberrant nuclease attack, leading to a leukemogenic translocation.

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