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Genetics

Transposition of an *Alu*-containing element induced by DNA–advanced glycosylation endproducts

(mutation/translocation/aging/oncogenesis/Maillard reaction)

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ABSTRACT Advanced glycosylation endproducts react with DNA and cause mutations and DNA transposition in bacteria. To investigate the mutagenic effect of advanced glycosylation in mammalian cells, plasmid DNA containing the *lacI* mutagenesis marker was modified by advanced glycosylation endproducts *in vitro*, transfected into murine lymphoid cells, recovered, and analyzed for mutations, plasmid size changes, and the presence of shared insertion sequences. An 853-bp host-derived DNA sequence, designated INS-1, was identified as an insertion element common to plasmids recovered from multiple independent transfections. Modification of DNA by advanced glycosylation increased by 60-fold the apparent frequency of INS-1 transposition: from 0.025% to 1.5%. The INS-1 element contains a 180-bp region that is homologous to the *Alu* repetitive sequence family. INS-1 was also observed to be present within larger insertional mutations and, in two cases, an apparently truncated version of INS-1 that lacks the *Alu* region was identified. These results demonstrate the experimental induction of DNA transposition involving mammalian chromosomal elements and suggest that advanced glycosylation may play a role in the formation of *Alu*-containing insertions that have been found to disrupt human genes.

Reducing sugars such as glucose react nonenzymatically with protein amino groups to initiate a posttranslational modification process known as advanced glycosylation (1, 2). In this pathway, early glucose-derived Schiff base and Amadori products slowly undergo a series of chemical rearrangement reactions to form advanced glycosylation endproducts (AGEs). AGEs are brown, fluorescent moieties that remain irreversibly bound to tissue proteins and form covalent crosslinks that accumulate with age. Once attached to proteins, AGEs impart on the protein a variety of novel structural and functional activities. AGEs inactivate nitric oxide activity, for example, and serve as signals for endocytosis via distinct AGE "scavenger" receptors (3, 4). The receptor-mediated uptake of AGEs has been shown to stimulate local cytokine release, initiate connective-tissue remodeling processes, and contribute to a variety of pathophysiological alterations associated with diabetes and normal aging (1–5).

Nucleotide bases that contain a primary amino group also participate in advanced glycosylation reactions (6). Glucose, glucose-6-phosphate, and glucose-derived reactive intermediates readily form addition products with DNA that induce strand breakage and cause mutations in model bacteria systems (6, 7). In bacteria, the mutagenic effect of AGE-modified DNA results in part from the activation of host-derived, transposable elements such as IS-1 and $\gamma\delta$ (7, 8). Transposition of $\gamma\delta$ has been demonstrated to occur in plasmids that have been modified by advanced glycosylation *in vitro*, as well as in native plasmids carried by bacterial strains that accumulate high intracellular levels of glucose-6-phosphate (8, 9).

The induction of DNA transposition by AGEs in bacteria prompted us to search for similar inducible phenomena in eukaryotic cells. Until recently, evidence for the existence of eukaryotic transposable elements has been largely indirect and has been based on structural features shared by widely dispersed repetitive DNA families, such as the short interspersed elements (i.e., *Alu*) and long interspersed elements (10–12). Within the last several years, *Alu*-containing insertions have been found to disrupt a number of human genes, including those for ornithine δ-aminotransferase (13), cholinesterase (14), and the neurofibromatosis NF1 gene (15). Recent studies of the L1 repetitive element also have identified a gene for reverse transcriptase that may mediate retrotransposition in eukaryotic cells (16, 17).

To investigate the role of advanced glycosylation in the transposition of mammalian DNA, we studied the effect of AGE modification on a polyoma-based shuttle vector (pPy35) containing the *lacI* mutagenesis marker. Mutant plasmids were isolated from independent transfections and analyzed for the presence of shared, host-derived insertion sequences. An 853-bp *Alu*-containing sequence, designated INS-1,† was identified as an insertional mutation in independently performed DNA transfections. INS-1 also was found to be present within longer DNA insertions, two of which contained an apparently truncated, non-Alu-containing segment of the INS-1 sequence.

MATERIALS AND METHODS

Shuttle Vector and Transfection Procedures. The 10.8-kb polyoma-based pPy35 shuttle vector was derived from pPyib (18) by excision of the *gpt* gene and replacement with the gene conferring neomycin resistance (pNEO; Pharmacia) (19). The position of the gene conferring neomycin resistance in resistant plasmid clones was confirmed by restriction enzyme mapping. Plasmid DNA was purified by CsCl density gradient centrifugation and modified by advanced glycosylation prior to transfection. For AGE modification, 5 μg of pPy35 DNA dissolved in 2.5–5 μl of 50 mM Hepes buffer (pH 8.0) containing 1 mM EDTA was added to 5–7.5 μl of AGE-reactive intermediate (AGE-RI), which was prepared by preincubating 1 M glucose-6-phosphate with 10 mM lysine for 4 days at 37°C in the dark (20). The DNA/AGE-RI mixture was then incubated for 1 hr at 37°C. Control DNA was incubated under the same buffer and temperature conditions

Abbreviations: AGE, advanced glycosylation endproduct; AGE-RI, AGE-reactive intermediate.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. Z12123).

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but without added AGE-RI. For transfection, 1.7 × 10^6 X63Ag8.653 cells (21) were resuspended in 1 ml of Opti-MEM (GIBCO) containing 25 μl of Lipofectin (GIBCO) and 5 μg of control or AGE-modified DNA. After incubation for 3 hr at 37°C and in 5% CO_2, the cells were diluted by the addition of 5 ml of RPMI containing 10% (vol/vol) fetal bovine serum. On the following day, the cells were washed and resuspended in RPMI containing 10% fetal bovine serum. G418 (600 μg/ml) was added after an additional 3–4 days of incubation. Episomal DNA was recovered by alkaline/SDS purification when lymphoid cell number reached 5 × 10^6 (2–3 weeks of culture) (19).

Mutation and Insertion Sequence Analysis. Recovered plasmids were transfected into the *Escherichia coli* strain MC1061 F′Δ150kan and scored for *lacI*$^-$ colonies by α complementation (22). Plasmid DNA size was determined in a subset of mutants chosen at random from all the independent transfections performed. DNA was analyzed by agarose gel electrophoresis after digestion with *Eco*RI and *Pvu* II (yielding 8–15 restriction fragments per plasmid), conditions sufficient to detect size changes ≥100 bp in each fragment. Insertions were identified, and DNA was sequenced by the dideoxynucleotide technique (23) using double-stranded template, Sequenase version 2.0 (United States Biochemical), deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate, and primers synthesized for *lacI* and insertion elements according to accumulated sequence information.

Common insertion elements were identified by screening plasmid insertions with DNA amplification primers designed from unique DNA sequences. The DNA amplification primers were INS-1 (set 1), 5′-AATTTGGAGGGCTGGAGA (nucleotides 141–158) and 5′-TCACTCTCTAGCTGATGC (nucleotides 398–381); INS-1 (set 2), 5′-AGAGTAGAGGCTCAAACCT (nucleotides 461–479) and 5′-AACATCTTTGCCCAGAG (nucleotides 742–726); INS-2, 5′-CAAGGTCTCATGAAGTCC (nucleotides 154–171) and 5′-TGCAGTTCAGGTAGAGA (nucleotides 471–455); INS-3, 5′-TAACAGGCAACCATGGCT (nucleotides 189–206) and 5′-CAACTCTCAGTGCTCTCT (nucleotides 467–450); and β-lactamase, 5′-CTTACTGTCATGCCATCC (nucleotides 3802–3819 of pBR322 sequence) and 5′-AAGATCAGTTGGGTGCAC (nucleotides 4051–4034 of pBR322 sequence). For DNA amplification, 10 ng of DNA was added to 45 μl of a reaction mixture containing 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 μM $MgCl_2$, 0.001% gelatin, 0.2 mM dATP, 0.2 mM dCTP, 0.2 mM dGTP, 0.2 mM dTTP, each of the oligonucleotide primers at 1 μM, and 1.2 units of *Taq* polymerase (Perkin–Elmer/Cetus). After an initial 2-min denaturation step, each cycle consisted of 1 min of denaturation at 92°C, 1 min of annealing at 55°C, and 1 min of polymerization at 72°C. DNA was amplified for 25 cycles, and 10 μl was analyzed in 1.6% agarose gels. Each insertion-specific amplification reaction yielded a single DNA band of predicted size that stained with the same intensity as the amplified product obtained with primers for the pPy35 β-lactamase gene.

RESULTS

Plasmid DNA (pPy35) was modified by advanced glycosylation *in vitro* and transfected into the murine lymphoid cell line X63Ag8.653 as described in *Materials and Methods*. After selection for growth in neomycin, murine cells were harvested, and the episomal DNA was purified and assayed for *lacI*$^-$ mutations by α complementation in an *E. coli* host (Table 1). The mutation rate increased from 0.1% for control, unmodified plasmids to 28% for AGE-modified plasmids. Incubation of plasmid DNA with higher concentrations of AGE-RI diminished the transfection efficiency and plasmid recovery. Overall, these results were consistent with previous studies of AGE-mediated mutagenesis in bacteria (6, 7).

Plasmid DNA was then isolated and analyzed for size differences by gel electrophoresis and restriction enzyme analysis. The majority of plasmid mutations were found to be associated with large (>100 bp) net insertion or deletion of DNA. Of interest, the mutant population obtained after DNA modification showed an increase in the proportion of plasmids with DNA insertions and a corresponding decrease in the proportion of plasmids with DNA deletions or without an apparent DNA size change (Table 1).

In bacteria, AGE-induced mutations result in part from the insertion of host-derived transposable elements (8). The possibility that DNA transposition had likewise occurred in transfected murine cells was suggested by the appearance of common restriction digest patterns in mutant, insertion-containing plasmids that were isolated from independent transfections (e.g., Fig. 1, lanes f and k and o and p). Restriction enzyme mapping also confirmed that each DNA insertion disrupted the plasmid *lacI* gene (data not shown).

Three partially sequenced DNA insertions, designated INS-1 (0.8 kb), INS-2 (1.5 kb), and INS-3 (4.1 kb), were chosen for further analysis. Southern hybridization revealed that each of these elements hybridized with host X63Ag8.653 DNA (data not shown). The murine genomic origin of these inserted elements was confirmed by DNA sequencing, which showed that the INS-1, INS-2, and INS-3 sequences had regions homologous with murine *Alu*, middle repetitive, and microsatellite sequence families, respectively.

The possibility was considered that the DNA insertions present in mutant plasmids might have originated from the transposition of specific, host-derived DNA elements. Oligonucleotide primers specific for sequenced regions of the INS-1, INS-2, and INS-3 insertions were used in DNA amplification reactions to screen for related elements in 92 insertion-containing mutant plasmids chosen from 15 independent transfection experiments (Table 2). The INS-1 primers were found to amplify a common DNA sequence in 13 of the insertion-containing plasmids isolated from 9 independent transfections. Multiple clones positive for the INS-1

Table 1. Mutation analysis of AGE-modified plasmid pPy35 recovered from murine lymphoid cells

			Mutation rate,§ %		Mutant plasmids with a size change,‖ % (fraction)		
AGE,* mM	Transfections†	Plasmids analyzed‡	Mean	Range	Insertion >100 bp	Deletion <100 bp	No size change
0	6	11,472	0.1	0.03–0.3	33 (4/12)	50 (6/12)	17 (2/12)
5	5	18,456	5.2	0.2–14	38 (24/64)	50 (32/64)	13 (8/64)
6.5	6	5,574	14.4	1.4–26	75 (44/59)	22 (13/59)	3 (2/59)
7.5	6	2,893	28.0	4.0–68	61 (36/59)	37 (22/59)	2 (1/59)

*Expressed as lysine equivalents.
†Number of independent transfections analyzed at each corresponding concentration of AGE.
‡Total number of plasmids pooled for study from each condition of DNA modification.
§Calculated from the ratio of the number of mutant plasmids to the number of total plasmids recovered for each DNA modification condition.
‖Determined in a subset of mutant clones chosen randomly from each transfection, with the exception of the control (0 mM AGE) condition, in which all the recovered mutants were studied by restriction analysis.

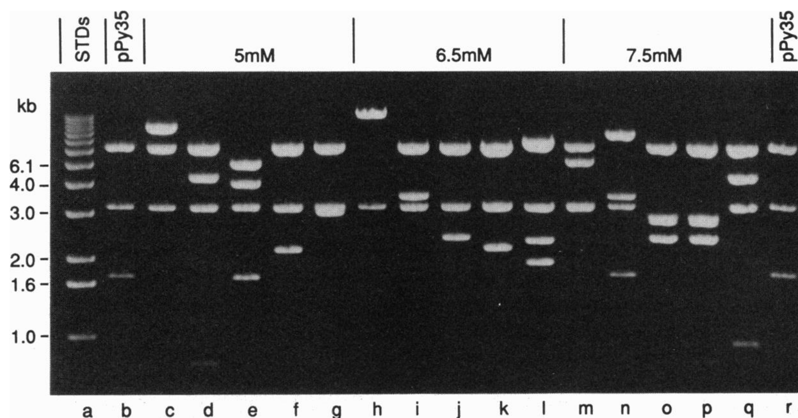

FIG. 1. *Eco*RI restriction enzyme analysis of representative insertion-containing mutant plasmids, each isolated from an independent transfection after different AGE reaction conditions (5–7.5 mM). The *lacI* gene is contained within the 1.7-kb fragment shown in lanes b and r. STDs, 1-kb size markers.

DNA amplification product were observed in two transfections, a result that most likely reflects the presence of plasmid siblings within the plasmid pool recovered from these experiments (22). Mutant plasmids isolated from 2 of 3 total control DNA transfections also contained the INS-1 amplified sequence. In contrast, DNA amplification analyses with the INS-2 and INS-3 primers revealed no additional examples of INS-2 sequence and two additional examples of INS-3 sequence.

The presence of the INS-1 insertion element was verified in the 13 mutants by DNA amplification of a second INS-1 sequence lying 3′ to the region amplified in the first set of screening experiments (Table 2 and Fig. 2). The identity of the INS-1 element in multiple, independent transfections was confirmed by DNA sequencing of the INS-1 insertion in four clones, each isolated from an independent transfection. Three insertions were found to be identical in structure to the INS-1 element that was originally isolated (Fig. 2), and one insertion was found to contain 110 bp of novel (nonplasmid)

Table 2. DNA amplification analysis of insertion-containing mutant plasmids for the sequences designated INS-1, INS-2, and INS-3

AGE, mM	Transfection	Positive elements/ insertions analyzed INS-1	INS-2	INS-3	INS-1 mutation rate, %
0	1	1/2	0/2	0/2	0.025
	2	0/1	0/1	0/1	
	3	1/1	0/1	0/1	
5	1	0/4	0/4	0/4	0.34
	2	1/1	0/1	1/1	
	3	1/7	0/7	0/7	
6.5	1	1/3	0/3	0/3	1.6
	2	0/2	0/2	0/2	
	3	4/14	0/14	1/14	
	4	0/11	0/11	0/11	
	5	1/10	1/10	1/10	
7.5	1	2/5	0/5	0/5	1.4
	2	0/8	0/8	0/8	
	3	1/13	0/13	0/13	
	4	0/10	0/10	0/10	

For analysis, a subset of insertion-containing mutant plasmids was chosen at random from the independent transfections shown, with the exception of the control (0 mM AGE) condition, in which all the recovered insertion-containing mutant plasmids were analyzed. The INS-1 mutation rate was calculated for each of the transfection conditions by the following formula: (INS-1 insertions/total insertions) × (insertions/100 mutants) × (total mutants/total plasmids).

sequence joined 5′ to nucleotide 1 of INS-1. Of significance, the incidence of plasmid mutations attributed to transposition of the INS-1 element was calculated to increase 60-fold after AGE modification (0.025% to 1.4–1.6%) (Table 2).

The complete DNA sequence and notable features of the 853-bp INS-1 element are shown in Fig. 2. The INS-1 element contains a 180-bp region bearing 78% homology to the B1 repetitive sequence superfamily, the murine equivalent of the human *Alu* sequence. Of interest, regions of homology also were found between INS-1 and two recently described insertion sequences causing mutations in human genes. Within INS-1 is one of the two direct repeats (AAAAAANATT-TTTT) that flanks a 342-bp *Alu*-type insertion found to disrupt the cholinesterase gene in a Japanese family (14). Also present is a short (36-bp) region outside the B1 element that is partially homologous (75%) to an *Alu*-containing insertion identified in a patient with neurofibromatosis (15). The four sequenced examples of complete INS-1 elements were found to be located within a 392- to 683-bp size deletion in *lacI* that varied in position between nucleotides 309 and 992 of the *lac* sequence. Examination of the INS-1 sequence and the plasmid *lacI* flanking regions revealed no apparent sequence homologies or repetitive regions.

Additional studies of the INS-2 and INS-3 elements showed that these insertions contained a template for the second primer set that was used to confirm the presence of the INS-1 element (the sense primer at nucleotides 461–479 and the antisense primer at nucleotides 742–726; Fig. 2). Complete DNA sequencing of the INS-2 and INS-3 elements revealed the presence of an INS-1 partial sequence that was composed of nucleotides 329–853 (Fig. 3). Apparent truncation of the INS-1 sequence occurred immediately 3′ to the *Alu* homologous segment. Fig. 3 also shows the partial structures of two additional lac^- clones, designated 1A9 and 2A3. The 1A9 clone was found to contain two insertions, a complete INS-1 and an ≈8-kb element, that were separated by 617 bp of intervening *lacI* sequence. The large 2A3 clone was observed to contain an INS-1 element linked at its 5′ end with ≈4 kb of an additional DNA insertion.

DISCUSSION

Previous studies have shown that the modification of DNA by AGEs induces DNA transposition in prokaryotes (8). Transposition is mediated in part by the mobilization of the transposable elements IS-1 and $\gamma\delta$ and affects plasmid DNA modified *in vitro*, as well as plasmid DNA modified *in vivo* by high levels of reactive sugars (8, 9). In the present study, AGE-modified DNA was demonstrated to undergo a high

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                                                       ChE                               1
  1 AGCTCACAAC TTTAAATCTA GCTCTAAATT ATCTGACACC TCTGGCCTCC ACAGGCACCT GTGCTCACAT GTACTTACCT ACACACATAC ACATGATTTA
101 AAATTAACCT AAATAAAAAA CATTTTTTAA AAATAAAAGT AATTTGGAGG GCTGGAGAGA TAGCTCAGTG GTTAAGAGCA CCACCTGCTC TTTCAGAGGT
                                                       Alu
201 CCTGAGTTCA ATTCCCAGCA ACCACATGGT GGCTCACAGA CATCAGTAGA GAGATCTGGT GCCCTCTTCT GGCCTGCAGG ATTCATGCAG GCAGAATGTG
                                  NF
301 TATCCATAAT AAGTAAATCT AAAAAAAAAT GCCAGTACTA CTTAGGAGAT AGAGGTAGGA GGACCAGAAG TTCAAGGCCA GCATCAGCTA GAGATGAGT
                                                                 2                                 1
401 ATGTGGCCAG CCTGGCATCA TTAGATCCTG AAAAGAAAAG GAAGGGAGGG AGGGAGGAAG AGAGTAGAGG CTCAAACCTT TCACTTATCA GTTGTGAAGG
501 TTAGAGTAAA AGAGAAAGTT AATCAAATTG TGCTTTGAGC CCTTATTAAA TGAACTTACT GTATGTGTTT GAATCAAAAA GAAAAGTTAC TATTGGAAAT
601 GAATGCTGAG TGGCTGTAAA TTGAGCCTGG TGGGTGGCAT CTGGCAACAC CATAGCCAGG TCCAAAAACT TGCCAAGTAA AACCAATAAA ATGACGTTTA
701 GAGATATCTT TCACCAACAA TATTACTCTG GGCAAAGATG TTTCCTCCTC ATTAAGATAA TTCAGAAAGT ATTCAGGGTT CAGAGAAAAT CCCCACAGAA
                                            2
801 AGGTCCTTTA ACACAGCCTT TGTGGCCCCT AGACTATGAG GTAAGGCCAG ACA
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FIG. 2. DNA sequence of the insertion element INS-1. The two sets of amplification primers used to screen insertion-containing mutants are indicated by "1" and "2." Also shown are the 180-bp region with 78% homology to the murine B1 repetitive element, or *Alu* homolog (Alu), the sequence common to the two direct repeats present at the ends of a 342-bp *Alu* insertion identified in the cholinesterase gene of a family afflicted with acholinesterasemia (ChE) (14), and a 36-bp region with 75% homology to an *Alu*-containing insertion described in a patient with neurofibromatosis (NF) (15).

frequency of insertional mutagenesis in mammalian cells. A large number of these DNA insertions were found to contain a shared, 853-bp host-derived element. This element, termed INS-1, contains a 180-bp region that is homologous to the B1 repetitive sequence family, the murine *Alu* equivalent.

A role for *Alu* DNA in eukaryotic transposition originally was suggested by structural homology with the highly conserved 7SL RNA sequence and by the widespread genomic dispersion of *Alu* sequences (10, 12, 24). Recent studies also have revealed specific examples of gene disruption by *Alu* DNA (14, 15, 25, 26). The molecular mechanisms responsible for the transposition of the *Alu*-containing INS-1 element remain to be elucidated. Neither flanking repeats nor an open reading frame that might code for a transposase-like protein were found within the INS-1 insertion. The fact that all of the sequenced examples of INS-1 were present as a DNA insertion superimposed over a deletion suggests that DNA recombination may be a necessary feature of INS-1 integration. This is supported by the appearance of clones in which the INS-1 sequence is flanked by additional host-derived sequences and by examples of apparent truncation of the INS-1 element immediately 3′ to the region of *Alu* homology. Additional information concerning the mechanism of INS-1 transposition may be revealed by the cloning and characterization of host, genomic copies of this element and an analysis of the sequence context in which they lie.

A high spontaneous "background" mutation rate has been noted frequently in experiments that have utilized DNA shuttle vectors (18, 22). Plasmids mutated by insertion of the INS-1 sequence also were obtained under control conditions, although at a 60-fold lower frequency than after reaction with AGE-RI. This presents the possibility that at least one class of apparently spontaneous shuttle vector mutations might be attributed to intracellular advanced glycosylation reactions. This would be analogous to the increased frequency of $\gamma\delta$ transposition observed in *E. coli* strains that accumulate high intracellular levels of glucose-6-phosphate, a reactive AGE-precursor sugar (9). To our knowledge, the present study represents the first example of experimentally induced DNA transposition in mammalian cells by a defined, host-derived DNA element. Precisely how DNA damage by advanced glycosylation induces transposition of the INS-1 sequence remains to be determined. DNA transposition might result, for instance, from the activation of a cellular response to remove AGE-mediated DNA crosslinks that cannot be repaired (6–9).

The age-dependent accumulation of AGE products in mammalian tissues and the presence of specific scavenger receptor systems that internalize AGEs suggest that there is a continual intracellular exposure to reactive AGE products (4). Whether AGE-mediated DNA transposition may contribute to the chromosomal aberrations and karyotype instability that underlies oncogenesis deserves further attention (27, 28). Future investigations of the genomic structure, distribution, and inducibility of the INS-1 and other *Alu*-containing elements should yield additional insight into the mechanisms of mammalian DNA transposition.

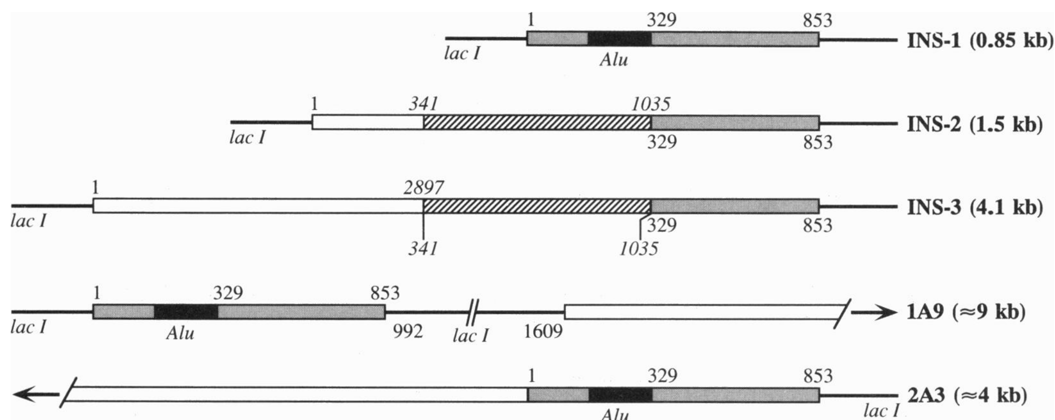

FIG. 3. Summary of insertion sequences isolated from *lac*⁻ plasmid clones. The INS-1 element shown is representative of four insertional mutants isolated from independent transfections. Identical regions are indicated by similar shading, and unique sequence is represented by the open, unshaded segments. *lac* sequence is indicated by the solid line. Also shown are the partially determined structures of the large 1A9 and 2A3 *lac*⁻ clone.

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1. Bucala, R. & Cerami, A. (1992) *Adv. Pharmacol.* **23**, 1–34.
2. Njoroge, F. G. & Monnier, V. M. (1989) *Prog. Clin. Biol. Res.* **304**, 85–107.
3. Bucala, R., Tracey, K. & Cerami, A. (1991) *J. Clin. Invest.* **87**, 432–438.
4. Vlassara, H., Brownlee, M. & Cerami, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5588–5592.
5. Vlassara, H., Brownlee, M., Manogue, K. R., Dinarello, C. A. & Pasagian, A. (1988) *Science* **240**, 1546–1548.
6. Bucala, R., Model, P. & Cerami, A. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 105–109.
7. Bucala, R., Model, P., Russel, M. & Cerami, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8439–8442.
8. Lee, A. T. & Cerami, A. (1991) *Mutat. Res.* **249**, 125–133.
9. Lee, A. T. & Cerami, A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8311–8314.
10. Jagadeeswaran, P., Forget, B. G. & Weissman, S. M. (1981) *Cell* **26**, 141–142.
11. Fanning, T. G. & Singer, M. F. (1987) *Biochim. Biophys. Acta* **910**, 203–212.
12. Deininger, P. L. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington, DC), pp. 619–636.
13. Mitchell, G. A., Labuda, D., Fontaine, G., Saudubray, J. M., Bonnefont, J. P., Lyonnet, S., Brody, L. C., Steel, G., Obie, C. & Valle, D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 815–819.
14. Muratani, K., Hada, T., Yamamoto, Y., Kaneko, T., Shigeto, Y., Ohue, T., Furuyama, J. & Higasino, K. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11315–11319.
15. Wallace, M. R., Andersen, L. B., Saulino, A. M., Gregory, P. E., Glover, T. W. & Collins, F. S. (1991) *Nature (London)* **353**, 864–866.
16. Dombrowski, B. A., Mathias, S. L., Nanthakumar, E., Scott, A. F. & Kazazian, H. H. (1991) *Science* **254**, 1805–1808.
17. Mathias, S. L., Scott, A. F., Kazazian, H. H., Boeke, J. D. & Gabriel, A. (1991) *Science* **254**, 1808–1810.
18. Calos, M. P., Lebkowski, J. S. & Botchan, M. R. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3015–3019.
19. Maniatis, T. E., Fritsch, E. F. & Sambrook, J. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Plainview, NY).
20. Lee, A. T. & Cerami, A. (1987) *Mutat. Res.* **179**, 151–158.
21. Kearney, J. F., Radbruch, A., Liesegang, B. & Rajewsky, K. (1979) *J. Immunol.* **123**, 1548–1550.
22. Lebkowski, J. S., DuBridge, R. B., Antell, E. A., Greisen, K. S. & Calos, M. P. (1984) *Mol. Cell. Biol.* **4**, 1951–1960.
23. Hattori, M. & Sakaki, Y. (1986) *Anal. Biochem.* **152**, 232–238.
24. Ullu, E. & Tschudi, C. (1984) *Nature (London)* **312**, 171–172.
25. Calabretta, B., Robberson, D. L., Barrera-Saldana, H. A., Lambrou, T. P. & Saunders, G. F. (1982) *Nature (London)* **296**, 219–225.
26. Lehrman, M. A., Goldstein, J. L., Russell, D. W. & Brown, M. S. (1987) *Cell* **48**, 827–835.
27. Cairns, J. (1981) *Nature (London)* **289**, 352–357.
28. Nowell, P. C. (1976) *Science* **194**, 23–28.