MOLECULAR AND CELLULAR BIOLOGY, Oct. 1993, p. 6064–6070
0270-7306/93/106064-07$02.00/0

Vol. 13, No. 10

Generation of a New Adenovirus Type 12-Inducible Fragile Site by Insertion of an Artificial U2 Locus in the Human Genome

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Received 7 May 1993/Returned for modification 7 June 1993/Accepted 28 June 1993

Infection with adenovirus type 12 (Ad12) induces four fragile sites in the human genome (H. F. Stich, G. L. van Hoosier, and J. J. Trentin, Exp. Cell Res. 34:400–403, 1964; H. zur Hausen, J. Virol. 1:1174–1185, 1967). The major site, at 17q21-22, contains the U2 gene cluster, which is specifically disrupted by infection in at least a percentage of the cells (D. M. Durnam, J. C. Menninger, S. H. Chandler, P. P. Smith, and J. K. McDougall, Mol. Cell. Biol. 8:1863–1867, 1988). For direct assessment of whether the U2 locus is the target of the Ad12 effect, an artificial locus, constructed in vitro and consisting of tandem arrays of the U2 6-kbp monomer, was transfected into human cells. We report that integration of this artificial locus on the p arm of chromosome 13 creates a new Ad12-inducible fragile site.

Fragile sites are regions of the human genome that are inherently prone to breakage (8, 28). They are classified on the basis of their frequency (common or rare) or mode of induction in vitro and correspond to regions of noncondensed chromatin. Cytogenetically, fragile sites appear as heterochromatic gaps that can, however, evolve into true DNA breaks with recombinogenic potential (4). Cancer breakpoints and oncogenes are frequently located at these sites, as are chromosomal breaks and rearrangements, whether spontaneous or induced (1, 14, 33, 34). Lastly, integration of viral genomes may preferentially occur within these regions (23). The abundance and evolutionary conservation of fragile sites suggest that they may have a biological role, but their structure and function remain largely unknown, as do the general causes of their fragility. Disruption of these sites may, however, contribute to the development of cancer, either directly or by eliciting illegitimate recombination events (33).

Infection of human cells with adenovirus type 12 (Ad12) and other highly oncogenic adenoviruses affects specifically four chromosome regions, commonly referred to as Ad12 modification sites (20, 27, 35). Damage at these sites is manifested as regions of uncoiled DNA and heterochromatic gaps from which breaks may result, suggesting that Ad12 modification sites may be bona fide fragile sites (2, 3). Indeed, three of the Ad12 modification sites colocalize on chromosome 1 with common fragile sites (1, 20, 35). Virus-induced modification is not dependent upon the integration of viral DNA (2) but requires the expression of the Ad12 E1b 55-kDa protein and possibly of other viral gene products (24). These data suggest that viral proteins may interfere in *trans* with chromatin condensation at specific genomic sites, leading to heterochromatic gaps on metaphase chromosomes. Modification sites differ in their sensitivity to the virus. At a low multiplicity of infection, essentially all of the damage is at band 17q21-22. At a higher virus input, sites at 1p36, 1q21, and 1q42-43 and then a variety of other (random) sites are damaged.

A shared feature of Ad12 modification sites is their location at or in close proximity to gene clusters for structural RNAs. The major site at 17q21-22 contains, among other genes, the U2 small nuclear RNA locus, which comprises up to 20 copies of a 6-kbp monomer (6, 15, 30, 32). Similarly, reiterated U1 genes and pseudogenes and 5S rRNA genes have been mapped at the 1p36, 1q21, and 1q42-43 sites, respectively (16, 17, 21, 26). In situ hybridization of a U2 probe to infected-cell chromosomes has indicated that the U2 gene cluster can be specifically disrupted by the virus in at least 8% of the cells (2). In the remainder of the cells, however, U2 sequences remain either proximal or distal to the damaged site. These results and the estimated size of the heterochromatic gap induced by Ad12 infection, 350 to 1,500 kbp, have suggested that the Ad12-sensitive region extends beyond the U2 locus (2). On the other hand, the mapping of structural RNA genes at the other Ad12 modification sites strongly supports a specific role for these loci in virus-induced damage (15).

We speculated that the U1, U2, and 5S rRNA gene clusters might be the primary sites of Ad12-induced damage. Following infection, virus-encoded functions might impede chromatin condensation at these loci, and this effect may then expand to neighboring regions. To test this hypothesis, we designed experiments to assess whether the U2 locus on its own represents a specific target for Ad12 infection. Our results indicate that the integration of an artificially constructed U2 gene cluster at a novel location in the human genome results in the expression of a new Ad12-inducible fragile site.

MATERIALS AND METHODS

Cell culture and transfection. The human HT-1080 tumor cell line was obtained from the American Type Culture Collection and grown in alpha minimal essential medium

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plus 7.5% fetal calf serum. The line has a modal chromosome number of 46 but is pseudodiploid. Ad12-transformed human embryonic kidney cells, MH12 (18), were grown in alpha minimal essential medium plus 10% newborn calf serum, and KB cells were grown in Joklik medium plus 5% horse serum. Transfection was carried out on early-passage HT-1080 cells by the calcium phosphate technique (5). The cells were seeded at 2.5×10^5 cells per 60-mm plate and transfected 24 h later with 5 μg of test DNA without carrier DNA. Transfected clones were selected with G418 (400 μg/ml; 25) or with histidinol (0.5 to 1.0 mM) in histidine-deficient medium (9).

Virus and plasmids. Ad12 strain Huie was grown in KB cells, and titers were determined on MH12 cells. For experimental purposes, cells were infected at different multiplicities and harvested after 19 h. Plasmids pTP18 and p12L, containing, respectively, the 6-kbp unit of the U2 gene cluster and its 3.4-kbp *Hin*dIII-*Pvu*II fragment with no *Alu* sequences, were obtained from Alan Weiner (Yale University). pSV2neo (25) was obtained from Paul Berg, pSV2HDK (10a) and pSV2his (9) were obtained from David Johnson (McMaster University), pEVp47 (29), containing the human plekstrin gene, was obtained from Calvin Harley, and pSP72 was obtained from Promega (Madison, Wis.). All plasmids were propagated in *Escherichia coli* DH5α and purified by CsCl density gradient centrifugation. Digestion, modification, and ligation of DNA were carried out by standard protocols and in accordance with instructions from enzyme suppliers.

Slot blot and Southern hybridizations. Undigested genomic DNA was used for slot blot hybridization. For Southern hybridization, appropriately digested genomic DNA was electrophoresed in 0.7% agarose gels, transferred to nitrocellulose membranes, and hybridized by standard procedures. Probes were labelled with ^{32}P by nick translation or random priming.

Cytogenetic analysis and in situ chromosome hybridization. Cells treated with colcemid (0.1 μg/ml) were harvested by trypsinization, incubated in hypotonic 0.075 M KCl, and fixed in methanol-acetic acid (3:1 [vol/vol]). Chromosome spreads were prepared by dropping the cell suspension on ice-cold wet slides. For detection of cytogenetic aberrations by light microscopy, chromosome preparations were stained by silver impregnation, specific for the nucleolus organizer regions (NORs) on the p arms of the D and G chromosomes (10, 31), plus solid Giemsa staining or Giemsa banding. Slides for in situ hybridization were incubated at 60°C overnight and stored desiccated at −70°C until use. Probes, the p12L *Hin*dIII-*Pvu*II fragment, the pSV2his *Pvu*II-*Eco*RI fragment containing the *hisD* gene, or α-satellite probes specific for D chromosomes (Oncor Inc.), were labelled with biotin or digoxigenin by nick translation or random priming. Chromosomes were denatured in prewarmed 70% formamide–2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 2 to 3 min at 70 to 72°C, dehydrated in ice-cold 70 to 100% ethanol, and air dried. Twenty microliters of hybridization cocktail (65% formamide, 10% dextran sulfate, 5× Denhardt's solution, 2× SSC, 100 μg of salmon sperm DNA per ml, and 20 ng of probe [denatured at 70 to 80°C and quenched in an ice-ethanol bath]) was applied to each slide, covered with a coverslip and sealed with rubber cement. Hybridization was done overnight at 37°C in a moist chamber; this step was followed by one wash in 50 or 65% formamide–2× SSC at 43°C for 20 min and two washes in 2× SSC at 37°C for 5 min each. For biotin-labelled probes, the preparations were incubated with fluorescein avidin DCS solution (5 μg/ml in 1% bovine serum albumin–4× SSC; Vector Laboratories) at room temperature for 60 min. Washes and amplification of the signal were done as described by Lawrence et al. (13). When digoxigenin-labelled probes were used, 2 μg of anti-digoxigenin-rhodamine (Boehringer Mannheim) per ml in 1% bovine serum albumin–4× SSC was added for 1 h at room temperature, and the mixture was washed with 4× SSC and then with PN buffer (0.1 M Na phosphate [pH 8.0], 0.1% Nonidet P-40). Chromosomes either were counterstained with 30 μg of propidium iodide (PI) per ml for 30 to 40 min, destained in PN buffer for 3 min, and mounted in antifade solution or were directly mounted in antifade solution containing 0.2 μg of PI per ml. Preparations were examined on a Zeiss Axioskope fluorescence microscope equipped with PI and fluorescein isothiocyanate-PI dual filters and photographed with Kodak Ektachrome 400 color film.

RESULTS

Construction and transfection of artificial loci. Our strategy for the introduction and detection of marked U2 and control loci in the human genome involved the in vitro ligation of appropriate fragments in a head-to-tail orientation, the addition of a selectable marker, and transfection into human HT-1080 cells. Clonal cell populations were screened for the presence of single inserts of sizes equal to or larger than that of the native U2 locus, and suitable integrants were mapped to chromosomal sites. For construction of the U2 gene cluster, the U2-containing fragment (corresponding to the monomeric unit of the U2 locus) was subcloned from pTP18 into pSP72 and self-ligated in a head-to-tail orientation as described in Fig. 1a. This protocol introduced two new restriction sites, *Kpn*I and *Eco*RI, which distinguish the artificial cluster from the endogenous cluster at 17q21-22. pSV2neo was then ligated to one end of the U2 array. The two most likely structures generated by this step are shown in Fig. 1a. As a control, head-to-tail arrays containing the *hisD* gene were generated by self-ligating, in the presence of *Xho*I and *Sal*I, the 5.7-kbp *Xho*I-*Sal*I fragment from pSV2HDK, a derivative of pSV2his (Fig. 1b). Ligation products were visualized by ethidium bromide staining after standard gel electrophoresis. All sizes of molecules, from monomer to the limit of gel mobility, were detected. For the U2 construct, Southern hybridization with a pSV2neo probe revealed that this plasmid had been ligated to each rung of the U2 ladder (data not shown). Ligation mixtures were transfected into HT-1080 cells, and colonies were selected for the expression of the *neo* or *hisD* gene.

Mapping of artificial U2 and control loci. Identification of cellular clones that may contain large arrays of the marked U2 gene was initially carried out by slot blot hybridization of genomic DNA with the 3.4-kbp U2 fragment from p12L as a probe (data not shown). Hybridization signals were quantified by densitometry and normalized to the signal for the single-copy plekstrin gene. Of 52 clones, 4 with a two- to fourfold-higher U2 gene content than that in control cells were chosen for Southern analysis. Genomic DNA either was digested with the diagnostic enzyme *Kpn*I or *Eco*RI or was doubly digested with the noncutting enzymes *Bam*HI and *Bgl*II, to eliminate or minimize flanking sequences, and then was hybridized to the p12L U2 probe (Fig. 2A). *Kpn*I releases the 6-kbp monomer from the endogenous U2 locus and fragments of ≈1 and ≈5 kbp, only the latter hybridizing to the U2 probe, from the artificial array (Fig. 1). Only one of the four clones, HT-6B1, had the expected hybridization

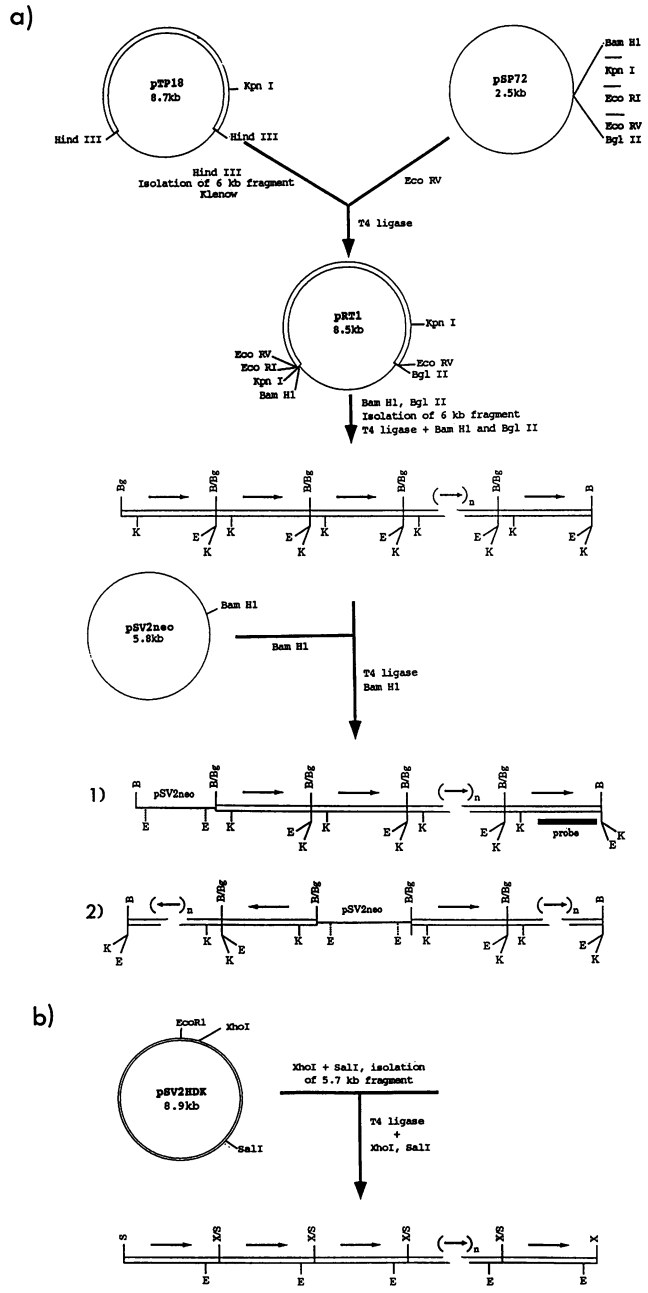


FIG. 1. Construction of artificial loci. (a) The U2-containing fragment (≈6 kbp) was released from pTP18 by *Hin*dIII digestion, gel purified, treated with Klenow polymerase, and ligated to *Eco*RV-digested pSP72. The resulting plasmid, pRT1, was digested with *Bgl*II (Bg) and *Bam*HI (B), and the U2-containing fragment was gel purified and self-ligated in the presence of these enzymes to generate arrays of tandem repeats. pSV2neo, linearized with *Bam*HI, was then ligated, in the presence of the enzyme, to the U2 array. Two possible structures generated by this protocol are shown in diagrams 1 and 2. (b) The 5.7-kbp *Xho*I (X)-*Sal*I (S) fragment from pSV2HDK was gel purified and self-ligated in the presence of *Xho*I and *Sal*I to generate head-to-tail arrays. K, *Kpn*I; E, *Eco*RI.

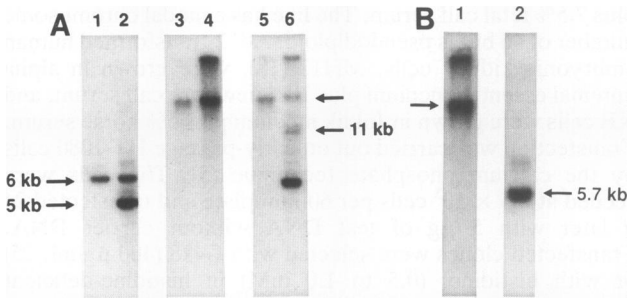
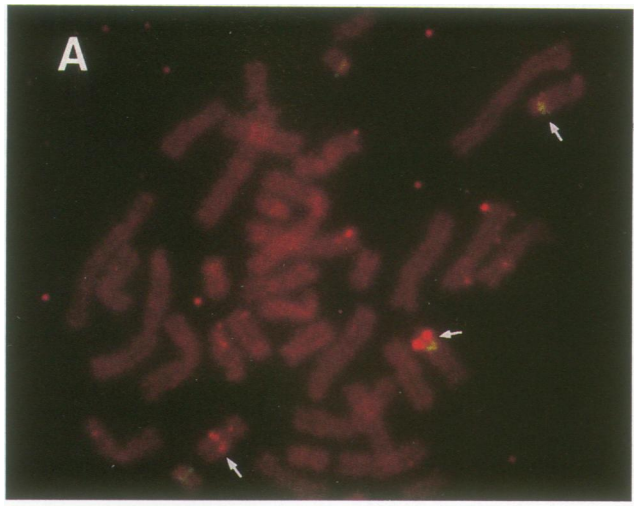


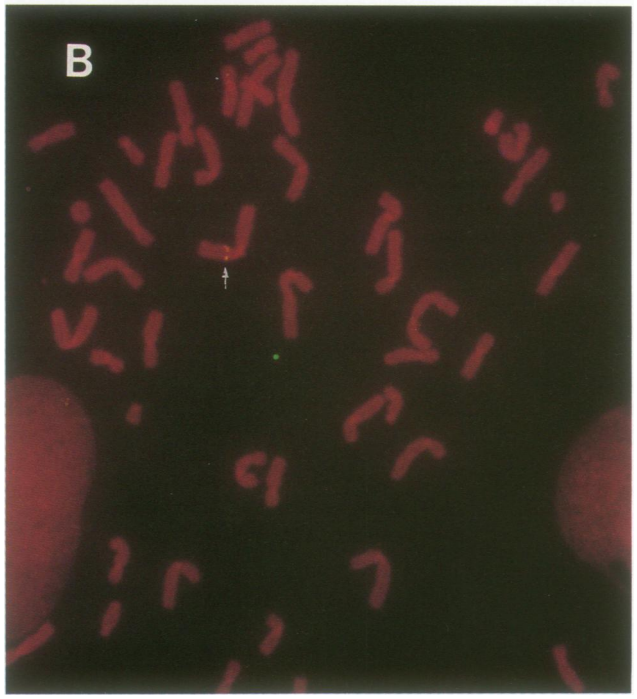
FIG. 2. Southern analysis of transfected clones. (A) Genomic DNAs from HT-1080 control cells (lanes 1, 3, and 5) and from HT-6B1 cells transfected with the U2 construct (lanes 2, 4, and 6) were digested with *Kpn*I (lanes 1 and 2), with *Bam*HI plus *Bgl*II (lanes 3 and 4), or with *Eco*RI (lanes 5 and 6) and then hybridized to the U2 probe. (B) DNA from HT-3A4 cells transfected with the *hisD* construct was digested with *Bgl*II plus *Hin*dIII (lane 1) or with *Eco*RI (lane 2) and then hybridized to the pSV2HDK probe. The sizes of the bands were calculated relative to molecular weight markers (not shown), and copy numbers were quantified by PhosphorImager analysis. kb, kilobase pairs.

pattern and a high copy number for the marked U2 gene (Fig. 2A, lane 2). On the basis of the relative intensities of the 6- and 5-kbp bands, HT-6B1 cells contain about three times as many copies of the artificial U2 monomer as of the native one, in agreement with the results of slot blot hybridization. *Eco*RI does not cleave the endogenous U2 gene but releases the monomeric unit from the artificial construct. In addition, cleavage with *Eco*RI should generate bands of ≈7 and/or ≈11 kbp, depending upon the orientation of pSV2neo relative to the U2 repeats (Fig. 1). Hybridization of HT-6B1 DNA with the U2 probe revealed a prominent band of ≈6 kbp, as well as a unique band of ≈11 kbp (Fig. 2A, lane 6), which was also detected upon hybridization with the pSV2neo probe (data not shown). Double digestion with *Bam*HI and *Bgl*II yielded a single band migrating at the limit of gel mobility, like that from the endogenous U2 locus, a result compatible with the integration of a large construct at one or at most a few sites (Fig. 2A, lane 4).

Fluorescence in situ hybridization to metaphase chromosomes was used to map the artificial U2 locus in HT-6B1 cells. Specific hybridization of the U2 probe was detected on the q arm of chromosome 17, as expected, and on the p arm of a single D chromosome (data not shown). In 95% of the metaphases, the latter hybridization was unequivocal, involved both chromatids, and was stronger than that on 17q, in agreement with the results of Southern hybridization. Control hybridizations to HT-1080 chromosomes yielded only 17q signals. For identification of the D chromosome containing the artificial U2 locus, double hybridization with differentially labelled U2 and α-satellite probes specific for chromosomes 13 and 21, 14 and 22, or 15 was performed; cohybridization to the same chromosome was obtained only with the combination of the U2 and centromeric probes for chromosomes 13 and 21 (Fig. 3A). Thus, HT-6B1 cells contain a marked U2 array, larger than the native one, integrated at a single site on the short arm of chromosome 13.

To address the possibility that the integration of any reiterated DNA would generate an Ad12-inducible fragile site, HT-1080 cells were transfected with a control *hisD* construct. Genomic DNA from histidinol-resistant clones was analyzed by Southern hybridization with the pSV2HDK probe following digestion with *Bgl*II and *Hin*dIII, which do not cleave the monomeric unit, or with *Eco*RI, which cuts once within it (Fig. 2B). In 1 clone of 34, HT-3A4, the double digestion released a fragment migrating at the limit of gel





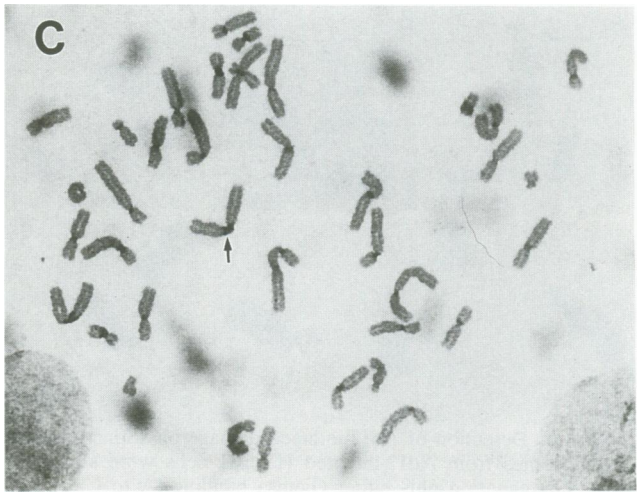


FIG. 3. Chromosomal location of artificial constructs. (A) Two-color fluorescence in situ hybridization of HT-6B1 chromosomes with the digoxigenin-labelled U2 probe (red) and the biotin-labelled α-satellite probe (yellow) specific for chromosomes 13 and 21. Signals on chromosomes 13 and 17 are indicated by arrows; hybridization of the centromeric probe to chromosomes 21 is also visible. (B) Hybridization of the biotin-labelled *hisD* probe (arrow) to HT-3A4 chromosomes. (C) Posthybridization Giemsa banding of the HT-3A4 metaphase chromosomes shown in panel B. The arrow indicates the 1q12 band on chromosome 1.

mobility, suggesting integration of a large construct (Fig. 2B, lane 1). Digestion with *Eco*RI yielded a prominent band of 5.7 kbp, whose intensity corresponded to approximately 40 copies of the monomeric unit, and a larger, lighter band that may contain flanking sequences (Fig. 2B, lane 2). Hybridization of the *hisD* probe to HT-3A4 chromosomes yielded a single signal on chromosome 1 (Fig. 3B). Posthybridization Giemsa banding identified the q arm, on the basis of the presence of the 1q12 variable band (Fig. 3C), and the distance between the hybridization signal and the centromere (13) was used to map the integration site at approximately 1q21-23, at or adjacent to one of the minor Ad12 modification sites.

Susceptibility of the artificial U2 locus to Ad12-induced damage. Initial experiments to determine whether the new U2 locus at 13p was sensitive to Ad12 were carried out on HT-6B1 cells infected with 10 PFU per cell. Giemsa banding together with silver impregnation was used to identify the chromosomes and stain specifically and uniformly the p arms of the D and G chromosomes, where the NORs are located (10, 31). Only damage at 13p was scored in these preparations, since Giemsa banding interferes with the detection of damage at other chromosomal sites. We selected 30 metaphases in which both chromosomes 13 were present and damage on the p arm had occurred; in all cases, only one chromosome of the pair was affected (Fig. 4A to C). The gap or break induced by Ad12 at 13p was flanked in all cases by a NOR, suggesting that the artificial construct had been integrated within this locus. Attempts to detect damage within the U2 locus (whether artificial or endogenous) following in situ hybridization were unsuccessful because of the intensity of the signals and the limit of resolution of the technique.

For quantification of the frequency of damage on 13p relative to other sites, HT-6B1 cells were infected with 2, 5, or 10 PFU of Ad12 per cell or mock infected. As an additional control, HT-1080 cells were infected with 10 PFU per cell. Chromosomes were stained by silver impregnation and solid Giemsa staining to detect damage on 13p (Fig. 4D to F) as well as on all other chromosomes. In addition to the known Ad12-specific sites at 17q21-22 and on chromosome 1, a new specific site was detected on the p arm of a single D chromosome (Fig. 4D and E) in each metaphase. No damage on the p arm of D chromosomes was detected in uninfected HT-6B1 cells (Fig. 4F) or infected HT-1080 cells (data not shown), indicating that the Ad12-induced damage was dependent upon the integration of the artificial U2 locus. In infected HT-6B1 cells, the induction of specific and random damage was dose dependent, as shown in Table 1. All sites other than 13p were damaged at similar frequencies in infected HT-1080 and HT-6B1 cells. The U2-containing 13p site was significantly more sensitive than any of the three sites on chromosome 1 but apparently less sensitive than the endogenous site on chromosome 17. When we corrected for the fact that each HT-6B1 metaphase contains a single

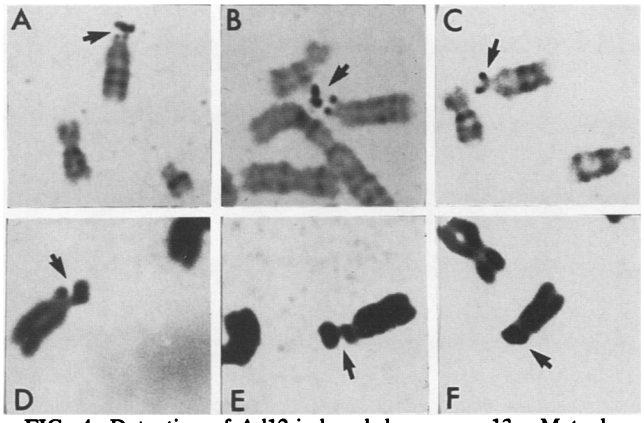


FIG. 4. Detection of Ad12-induced damage on 13p. Metaphase chromosomes from Ad12-infected HT-6B1 cells were stained by silver impregnation plus either Giemsa banding (A to C) or solid Giemsa staining (D to F). Arrows indicate the p arm of chromosome 13. Chromosomal damage and chromatid damage are shown in panels A and B and panels D and E, respectively. Panels C and F contain undamaged chromosomes from the metaphases shown in panels A and D, respectively.

U2-bearing chromosome 13 and two chromosomes 17, we found the frequency of damage at the 13p site to be ≈60% that at the 17q21-22 site. Although we cannot discount the possibility that the novel U2 locus may be inherently less sensitive to Ad12-induced damage than the endogenous locus, detection of damage on 13p was considerably difficult because of its location, and our scoring at this site was deliberately conservative. Both of these factors could easily have resulted in an underestimate of the sensitivity of the novel U2 locus.

Integration of the control *hisD* cluster in HT-3A4 cells occurred at 1q21-23, at or near one of the sites specifically damaged by Ad12 (1q21). The sensitivity of the *hisD* locus was therefore assessed by comparing the frequencies of damage at 1q21 in HT-3A4 versus control HT-1080 cells upon infection with 10 to 60 PFU per cell (Table 2). No significant differences were detected between the two cell types at any multiplicity of infection, indicating that the *hisD* locus did not constitute a specific target for the virus.

DISCUSSION

The results of our experiments indicate that an artificially constructed U2 gene cluster inserted at a novel site in the human genome is revealed as a new fragile site upon Ad12 infection. Sensitivity to the virus appears to be an inherent property of this construct, since damage was not induced at a control locus containing a large artificial tandem repeat of unrelated sequences. Given that the artificial U2 locus was engineered in vitro to be as similar as possible to the endogenous one, our observations in turn suggest that the U2 locus at 17q21-22 might be solely responsible for the Ad12-induced damage at that site.

The experimental strategy adopted in the present work allowed the integration of artificial constructs to occur at random and purposely limited analysis to integrants of sizes similar to or larger than that of the native U2 locus. Since only two suitable clones were obtained by this approach, we were unable to investigate whether the integration of constructs at different chromosomal sites might affect their response to Ad12. An unexpected finding was that both artificial constructs were integrated at or near repeated gene clusters (NORs or U1 pseudogenes). Although the small sample size precludes firm conclusions, these observations raise the intriguing possibility that clusters of this type may be preferred sites of integration, at least for transfected constructs consisting of large tandem arrays. We are presently attempting to generate additional clones by random integration as well as applying methods for targeted integration to address these questions and to assess the effect of differently sized constructs. Our present data, however, indicate that the U2 locus is sensitive to Ad12 in at least two different chromosomal environments (17q21-22 and 13p), strongly supporting the notion that it is the specific target of the virus.

The properties of the U2 locus that confer its fragility remain unknown. Although all four Ad12 modification sites contain gene clusters, it seems unlikely that this feature would be responsible for their fragility. Mere reiteration of genes would explain neither the differential sensitivity among modification sites nor why other gene clusters (e.g., other small nuclear RNAs, α-satellites, NORs, and *hisD*) do not respond to virus infection. Compared with the other Ad12 modification sites, in which substantial divergence between repeated units has occurred, the U2 locus is very homogeneous, consisting of perfect repeats of the 6-kbp monomer (30). This fact has suggested that the U2 repeat may contain a recombinogenic element that has promoted efficient homogenization in situ, a hypothesis supported by the presence of *Alu* sequences within the 6-kbp U2 monomer (7, 19, 30). It is tempting to speculate that a perfect and

TABLE 1. Chromosome damage induced by human Ad12 in HT-6B1 and control cells

Cells	PFU/cell	No. of cells analyzed	No. of aberrations/100 cells				
			17q21-22	13p	Chromosome 1 (3 sites)	Random	Total
HT-6B1		150	0	0	0	6	6
HT-6B1	2	150	53	18	13	35	119
HT-6B1	5	150	73	22	27	35	157
HT-6B1	10	150	102	31	38	68	239
HT-1080	10	150	101	0	37	73	211

TABLE 2. Chromosome damage induced by human Ad12 in HT-3A4 and control cells

Cells	PFU/cell	No. of cells analyzed	No. of aberrations/100 cells				
			17q21-22	1q21-23	1p36 and 1q41-42	Random	Total
HT-1080		100	0	0	0	7	7
HT-1080	10	200	75	10	21	59	165
HT-1080	20	100		30	74		
HT-1080	40	100		33	69		
HT-1080	60	100		54	98		
HT-3A4		100	0	0	0	26[a]	26
HT-3A4	10	200	80	8	27	76	191
HT-3A4	20	100		28	36		
HT-3A4	40	100		36	59		
HT-3A4	60	100		61	83		

[a] The majority (17 of 26) of these aberrations were double minutes or fragments.

closely spaced reiteration of such sequences may play a role in the heightened response of this locus to Ad12. Virus infection does not affect the normally active transcription of U2 genes (2, 12) but significantly enhances *Alu* transcription (22). It seems possible that a region in which active PolII (U2) transcription and PolIII (*Alu*) transcription are in proximity and intercalated constitutes a more sensitive target for Ad12 than regions in which primarily PolII (U1) or PolIII (5S rRNA) transcription is ongoing. Interestingly, we have shown that the transcriptional activation of *Alu* sequences requires adenovirus E1b and E4 proteins (22). Furthermore, we have shown that the E1b 55-kDa protein is necessary but not sufficient for the induction of fragile sites and have postulated the contribution of E4 proteins in this process (24). It has been suggested that fragile sites may correspond to regions of late-replicating DNA that are prevented for completing replication prior to chromatin condensation (11 and references therein). Whether Ad12-inducible fragile sites are caused by a similar mechanism and whether the activation of *Alu* transcription is responsible for the heightened sensitivity of the U2 locus are unknown. Construction and integration of tandem arrays containing the U2 and *Alu* portions of the 6-kbp U2 monomer may answer this question.

ACKNOWLEDGMENTS

We are very grateful to T. Pavelitz and A. Weiner for generously providing the pTP18 and p12L U2-containing plasmids and to A. Weiner for advice on the work and manuscript. We also thank Paul Berg, Calvin Harley, and David Johnson for gifts of plasmids. For their invaluable help with fluorescence in situ hybridization and for their liberal sharing of the Zeiss Axioskope, we are indebted to Cindy Millan, Viola Freeman, and Ron Carter. We also acknowledge the excellent technical assistance of Emmy Rusangawa and Ping Wang.

This work was supported by the National Cancer Institute and the Medical Research Council of Canada. S.B. and J.R.S. are Terry Fox Research Scientists of the NCIC.

ADDENDUM IN PROOF

We have recently obtained a second cell line bearing an artificial U2 locus that is as sensitive to Ad12 infection as the endogenous locus at 17q21-22.

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