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Chromosome Instability Associated with Human Alphoid DNA Transfected into the Chinese Hamster Genome

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Repetitive DNA sequences have been implicated in the mediation of DNA rearrangement in mammalian cells. We have tested this hypothesis by using a dihydrofolate reductase (DHFR) expression vector into which candidate sequences were inserted. DHFR$^-$ Chinese hamster ovary (CHO) cells were transfected with this vector, the amplification of which was then selected for by methotrexate (MTX) exposure. Cells transfected with the vector alone (and resistant to 0.02 or 1.0 μM MTX) or with a poly(dG-dT) insert (and resistant to 0.05 or 1.0 μM MTX) showed little change in chromosome aberrations or sister chromatid exchange frequencies. In contrast, transfection of DHFR$^-$ CHO cells with a vector containing either of two distinct 0.34-kilobase human alphoid DNA segments (and selection to 0.05 to 10.0 μM MTX) showed an approximately 50% increase in chromosome number and marked changes in chromosome structure, including one or two dicentric or ring forms per cell. The sister chromatid exchange frequency also increased, to more than double the frequency of that in cells transfected without insert or those containing poly(dG-dT). In situ hybridization of one 0.34-kilobase insert in some cells suggested clustering of homologous sequences in structurally abnormal recipient CHO cell chromosomes. The approach described provides an introduction to a unique means for a coordinate molecular and cytological study of dynamic changes in chromosome structure.

Repetitive DNA in mammalian genomes represents a high proportion of total nuclear DNA (for reviews, see references 20 and 29). The DNA of chromosomal regions such as centromeres and telomeres, as well as that of constitutive heterochromatin, consists to a large degree of repetitive sequences which might somehow relate to functional characteristics, e.g., chromosome replication, pairing, and stability (2, 19). In addition, repeated DNA appears to be frequently associated with DNA reorganization (6, 19, 28, 30), which in turn underlies some chromosomal rearrangements.

Dihydrofolate reductase (DHFR)-based plasmid vectors (15–17), which can be transfected into DHFR-deficient cells and selectively amplified by methotrexate (MTX), provide one means of deliberately perturbing chromosomal DNA content and structural behavior. Previously it was reported that in DHFR$^-$ Chinese hamster ovary (DUKX) (34) cells transfected with a plasmid expressing DHFR, there was an increase in homogeneously staining regions and dicentric chromosomes at relatively high levels of MTX and of vector amplification (17). There was little associated change in sister chromatid exchange (SCE) frequency. This effect of the parent vector on chromosomes serves as a base line from which to observe the impact of different repeat sequence subunits which can be inserted into these plasmids and then coamplified with the *dhfr* gene. We have initiated a study of the effect of such DNA sequences on chromosome structure, as a function of their state of amplification. Specifically, it was possible to test for effects of two such sequences, the synthetic polymer poly(dG-dT) (22) and 0.34-kilobase (kb) *Eco*RI centromeric alphoid segments (40), on chromosome number, chromosome rearrangement, and SCE formation.

Poly(dG-dT) was selected because homologous segments exist in genomic DNAs from most eucaryotes (9) and because there are reports suggesting its role in enhancement of transcription (10) as well as recombination (32). In addition, since poly(dG-dT) forms Z-DNA under certain conditions (22), it has been reported to be involved in the definition of chromosomal domains through alterations in chromatin structure (14, 23).

Human alphoid DNA was chosen as a biologically important repeat family. It consists of related centromere sequences present at $>10^5$ copies, accounting for several percent of the human genome (5, 37). Alphoid DNA has a consensus monomer length of 167 base pairs (bp) and has a slight AT bias, with regions of high sequence conservation (75 to 80%) between monomers (35, 36). Different higher-molecular-weight periodicities of the repeat are present at centromeres of different human chromosomes (13, 36–38). It is reasonable to expect that alphoid DNA somehow plays a role in centromeric function (39).

We report here that amplification in DUKX cells, of DHFR-containing plasmids with poly(dG-dT) inserts induce small increases in chromosome aberrations. In contrast, transfected plasmids containing alphoid DNA cause chromosome instability at MTX resistance levels at which the parent vector, lacking insert, had little impact. These effects include increased chromosome aberrations, SCE, and aneuploidy.

MATERIALS AND METHODS

The DUKX cell line (34) was kindly provided by R. Kaufman. These cells were maintained in alpha modified Eagle medium (alpha medium) supplemented with adenosine, deoxyadenosine, and thymidine (each at 10 μg/ml) and with 10% fetal bovine serum (Whittaker MA Bioproducts). Approximately 10^6 DUKX cells were treated with 25 μg of plasmid DNA as a calcium phosphate precipitate, followed by glycerol shock, as described elsewhere (15). After 2 days of growth in alpha medium, the cells were subcultured into mildly selective medium (alpha medium without nucleosides and containing 10% dialyzed fetal bovine serum). After 10 to 14 days, approximately 200 colonies were observed, pooled,

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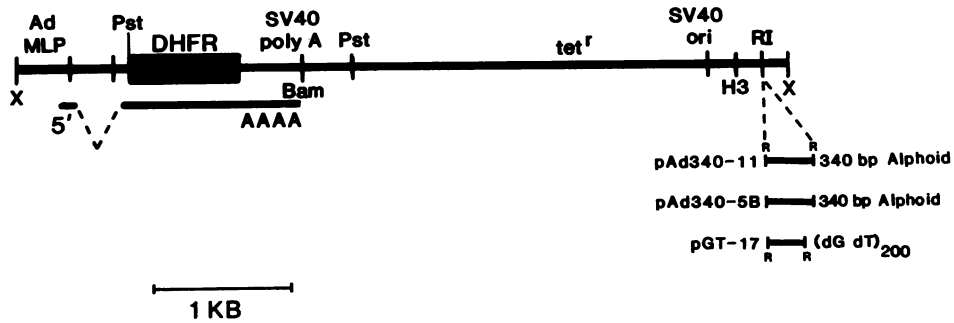

FIG. 1. Plasmid constructs used in the present experiments. Abbreviations: X, *Xho*I; Pst, *Pst*I; Bam, *Bam*HI; H3, *Hin*dIII; RI, *Eco*RI; Ad MLP, adenovirus major late promoter; tet^r, tetracycline resistance; SV40 ori, simian virus 40 origin of replication; SV40 poly A, simian virus 40 poly(A) addition site.

and then subjected to MTX (Sigma Chemical Co.) selection. Individual clones were isolated at 0.02 or 0.05 μM MTX. Clones resistant to higher levels of MTX were then isolated stepwise from these cells with increasing concentrations of MTX up to 10 μM.

The transformants Ad, AdII, and AdIII were isolated in three independent experiments in which DUKX cells were transfected with plasmid pAdD26SVpA3 (Fig. 1) (lacking any additional insert), kindly provided by R. Kaufman (16). These experiments constituted controls for the subsequent transfections. Transformants GT17, Ad340, and Ad3405B are the results of transfection with plasmids pGT17, pAd340-11, and pAd340-5B, respectively. The plasmids were all derived from pAdD26SVpA3 (Fig. 1). Cell lines Ad340 and Ad340II were isolated from separate experiments by using the same plasmid (pAd340-11) with a 0.34-kb insert; Ad3405B contains a second 0.34-kb insert. pGT17 contains approximately 200 bp of a synthetic polymer of (dG-dT) (Pharmacia, Inc.) cloned with *Eco*RI linkers (New England BioLabs, Inc.) and ligated to the unique *Eco*RI site of pAdD26SVpA3. pAd340-11 contains a 345-bp *Eco*RI fragment of the human alpha satellite cloned from gel-purified *Eco*RI-digested human placental DNA. Similarly, pAd340-5B contains a distinct, 345-bp *Eco*RI alphoid subunit cloned in the same way as pAd340-11.

High-molecular-weight genomic DNA was isolated from cultured cells as described previously (1). The genomic DNA was digested with appropriate enzymes (New England BioLabs) in the recommended buffers, then size fractionated by electrophoresis in 0.7% agarose gels (Sigma). The DNA was transferred to nitrocellulose (31) and baked for 2 h at 80°C under vacuum. DNA probes were labeled with ^{32}P by using oligonucleotide primers (Pharmacia) and DNA polymerase I (large fragment, Klenow enzyme; Boehringer Mannheim Biochemicals) (8) and then hybridized to filters as previously described (21).

The dideoxynucleotide method of Sanger et al. (26) was used to sequence the alphoid DNA inserts of pAd340-11 and pAd340-5B. The inserts were first subcloned into pBS18 (Bluescribe; Stratagene, Inc.). Both strands of DNA were sequenced by using the T3 and T7 primers of pBS18. The Sequenase (U.S. Biochemicals) reagents and procedures (33) were used as recommended by the manufacturer.

Cells were arrested in metaphase with 0.1 μg of Colcemid (Sigma Chemical Co.) per ml. At 2 h later the cells were harvested by trypsinization. They were then swollen in 0.9% sodium citrate or 0.075 M KCl and fixed with methanol-acetic acid (3:1). For SCE analysis, 10 μM bromodeoxyuridine was added, in the absence of MTX, for two cell cycles at 37°C (24 h for DUKX, Ad, AdII, AdIII, and GT17, and 30 to 48 h for Ad340, Ad340II, and Ad3405B). An additional SCE experiment was performed with DUKX cells at 33°C. Two cell cycles at this temperature was approximately 48 h.

Staining protocols included that for SCE (24) and Giemsa-trypsin banding (27). Both radioactive and nonradioactive in situ hybridization were carried out as described by Harper et al. (11) as modified by Donlon et al. (7) for radioactive in situ hybridization and as modified by Pinkel et al. (25) for nonradioactive in situ hybridization. Alphoid DNA in pAd340-11 or pAd340-5B was prepared for radioactive in situ hybridization by *Eco*RI digestion, gel electrophoresis, and NACS column (Bethesda Research Laboratories, Inc.) purification followed by oligonucleotide random-primed labeling (8) with using [^{3}H]dATP, [^{3}H]dCTP, and [^{3}H]dTTP. Probe specific activities were greater than 10^7/μg. Slides dipped in NTB-2 emulsion (Eastman Kodak Co.) were then exposed for several days at 4°C and developed with Dektol (Eastman Kodak Co.)-water (1:1). Alphoid DNA from pAd340-11 was prepared for nonradioactive in situ hybridization by nick-translated labeling with biotin-dUTP (Bethesda Research Laboratories) as specified by the manufacturer. Biotin incorporation as determined by addition of a trace amount of [^{3}H]dATP was 11%. These hybridizations were detected by applying fluorescein-avidin DCS (Vector Laboratories, Inc.) and biotinylated goat anti-avidin antibody (Vector) as described by Pinkel et al. (25).

For analysis of chromosome aberrations, 100 metaphase spreads were scored for each cell line. All types of chromosome aberrations were scored. Only the frequency of dicentric and ring forms is presented here, since these were the aberration type most consistently observed (>90% of all aberrations).

RESULTS

Inserts from the plasmids pAd340-11 and pAd340-5B (Fig. 1) detect 0.34-kb ladders when hybridized to *Eco*RI-digested human DNA (Fig. 2). Each of the two alphoid inserts was mapped by in situ hybridization to distinct subsets of human chromosomes. The first (pAd340-11) hybridized primarily to chromosomes 1 and 5, whereas the second (pAd340-5B) hybridized more strongly to chromosome 10, with a secondary focus at chromosome 1 (data not shown). The DNA sequences of the inserts from pAd340-11 and pAd340-5B differ by nearly 20% (Fig. 3). If the 167-bp alphoid consensus sequence (35) is aligned below the fragments, one can observe regions of extensive sequence similarity with an overall match of 79% for both pAd340-11 and pAd340-5B.

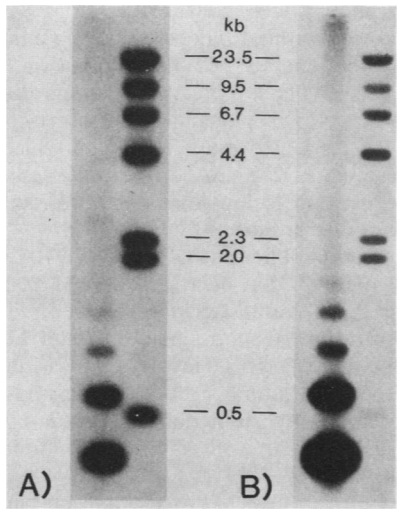

FIG. 2. Hybridization of 0.34-kb alphoid inserts to *Eco*RI-digested GM3798 DNA (from a normal human male). The left lane is GM3798 DNA. The right lane is the λ/*Hin*dIII molecular size standard. (A) is *Eco*RI insert from pAd340-5B. (B) *Eco*RI insert from pAd340-11.

Southern blot hybridization of the $(dG\text{-}dT)_{200}$ insert of pGT17 gave a uniform smear of hybridization to *Eco*RI-digested human DNA (data not shown), similar to that reported previously (9).

Amplification of plasmid sequences in DUKX cells following transfection was determined by blotting of isolated genomic DNA (Fig. 4 and 5). Figure 4 shows the fate of transfected pAdD26SVpA3 (no insert) in genomic blots from cell lines Ad (Fig. 4A; probe: *Xho*I-*Hin*dIII fragment which surrounds the *Eco*RI cloning site) and AdII (Fig. 4B; probe: *Pst*I-*Bam*HI *dhfr* fragment) resistant to 1.0 μM MTX (lane 2)

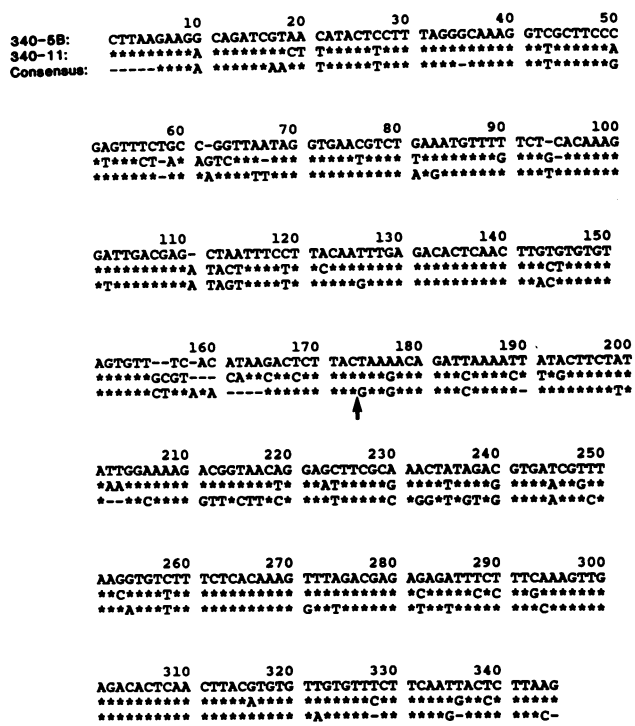

FIG. 3. DNA sequences of *Eco*RI inserts from pAd340-11 and pAd340-5B. Asterisks denote matches to the uppermost sequence. Insertions and deletions indicated by the dashes were made to align the alphoid consensus (35) sequence. The arrow indicates the junction of the two, approximately 170-bp monomers.

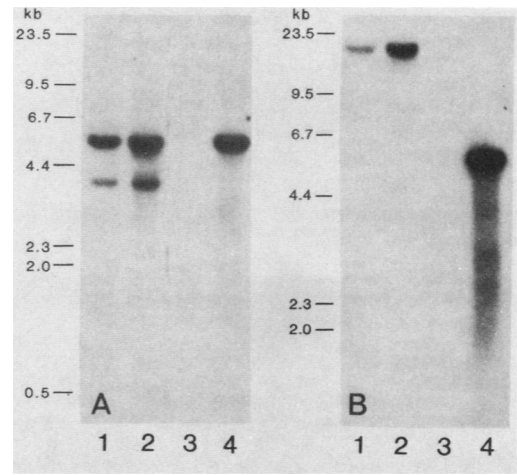

FIG. 4. Integrated DNA in DUKX cell lines transfected with pAdD26SVpA3 [vector lacking alphoid or $(dG\text{-}dT)_n$ insert]. Each lane contains 3 μg of *Eco*RI-digested genomic DNA. (A) DNA from a 1.0 μM MTX-resistant cell line (Ad-1.0; lane 2) stepwise selected from a 0.05 μM MTX-resistant cell line (Ad-0.05; lane 1). Lane 3 in both panels contains 3 μg of DUKX DNA. Lane 4 in both panels contains 0.13 ng of *Eco*RI-cut pAdD26SVpA3 mixed with 3 μg of *Eco*RI-digested DUKX DNA. This lane gives a hybridization intensity equivalent to 25 copies of pAdD26SVpA3. (B) DNA from a different transfection with pAD26SVpA3. AdII-1.0 (lane 2) is a 1.0 μM MTX-resistant line selected from AdII-0.05 (lane 1), a 0.05 μM MTX-resistant cell line. The probe is a ^{32}P-labeled *Xho*I-*Hin*dIII (panel A) or *Pst*I-*Bam*HI *dhfr* fragment (panel B) of pAdD26SVpA3.

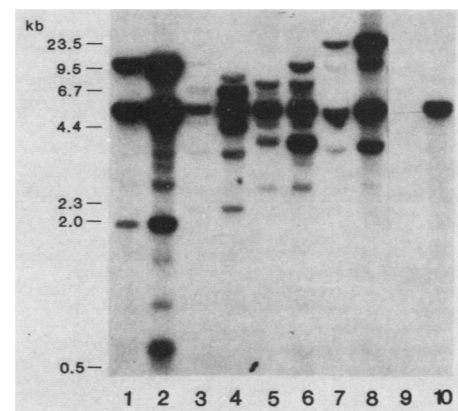

FIG. 5. Amplification of transfected insert-containing sequences in DUKX cell line DNAs. Each lane contains 3 μg of *Eco*RI-digested genomic DNA. The probe is the *Xho*I-*Hin*dIII fragment of pAd3405B. Lanes 1 and 2: GT17; pGT-17 MTX-resistant transformants (resistant to 0.02 μM MTX [lane 1] or 1.0 μM MTX [lane 2]). Lanes 3 and 4: Ad340; pAd340-11 MTX-resistant transformants (resistant to 0.02 μM MTX [lane 3] or 1.0 μM MTX [lane 4]). Lanes 5 and 6: Ad340I1; repeat transformants with pAd340-11 (resistant to 0.05 μM MTX [lane 5] or 1.0 μM MTX [lane 6]). Lanes 7 and 8: Ad3405B; pAd340-5B MTX-resistant transformants (resistant to 0.05 μM MTX [lane 7] or 1.0 μM MTX [lane 8]). Lane 9 is DUKX DNA. Lane 10 is a reconstruction containing 0.13 ng of *Eco*RI-digested pAd340-5B equivalent to 25 copies.

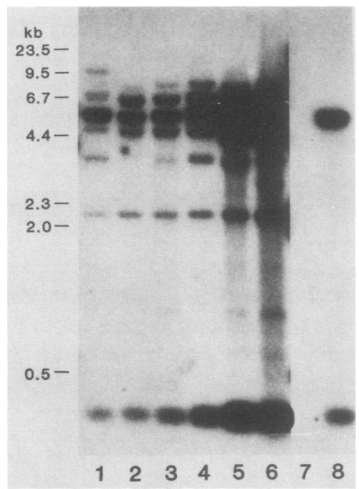

FIG. 6. Amplification of alphoid DNA in pAd340-11-transfected DUKX cell lines. Each lane contains 3 μg of *Eco*RI-digested genomic DNA. The probe is the *Xho*I-*Hin*dIII fragment of pAd340-11. MTX resistance: lane 1, 0.02 μM; lane 2, 0.05 μM; lane 3, 0.10 μM; lane 4, 0.50 μM; lane 5, 1.0 μM; lane 6, 10.0 μM. Lane 7 contains DUKX DNA. Lane 8 contains 0.13 ng of *Eco*RI-digested pAd340-11 mixed with 3 μg of *Eco*RI-digested DUKX DNA. This is equivalent to 25 copies of pAd340-11.

stepwise selected from the 0.05 μM MTX-resistant lines (lane 1). In both Ad cell lines there was integration of a 5.3-kb plasmid monomer into DUKX DNA, followed by a subsequent amplification and possible rearrangement event involving surrounding hamster DNA (M. W. Heartlein, unpublished results). The presence of a higher-molecular-weight band in DNA from AdII cells signifies a change in the hamster or vector DNA or both. In subsequent experiments, the vector DNA was found to undergo deletion of much of the simian virus 40 origin region defined by the 600-bp *Xho*I-*Hin*dIII fragment (including the *Eco*RI site).

All cell lines transfected with an insert-containing pAdD26SVpA3 exhibited increased copy numbers with 1.0 μM MTX, which reflected MTX selection (Fig. 5). Additionally, there was evidence (Fig. 5) for integration of two or more tandem copies of plasmid in all insert-containing cell lines given the presence of a 5.3-kb band which comigrated with linearized pAdD26SVpA3. The multiple bands present in cell lines resistant to low levels of MTX could be due to multiple insertions of plasmid DNA.

The effects of amplification of alphoid DNA were studied in detail by the isolation of a series of MTX-resistant cell lines. All of these were stepwise selected with increasing MTX concentrations from a single 0.02 μM MTX-resistant clone (Ad340-0.02). As the level of MTX resistance was increased, the copy number of both plasmid and 0.34-kb insert, detected by hybridization to the *Xho*I-*Hin*dIII fragment of pAd340-11 in *Eco*RI-digested genomic DNA, was concomitantly increased (Fig. 6). Also visible here was the appearance in the 10 μM MTX-resistant cell line of a DNA fragment which was presumably due to rearrangement of DNA sequences during amplification. The alphoid DNA copy number, which ultimately exceeded 100, was estimated by densitometric scanning of the 0.34-kb bands (Fig. 6, lanes 1 to 6), with the alphoid reconstruction (Fig. 6, lane 8) as a copy number standard.

The impact of DNA amplification and rearrangement on chromosome structure was determined by cytogenetic analysis on cell lines transfected with plasmid with or without insert. Table 1 lists the dicentric and ring frequency, SCE frequency, and chromosome number for all cell lines studied. These data reveal that only with amplified alphoid DNA transformants was there a large increase in both chromosome aberrations (most importantly, dicentric and ring forms) and SCE frequency. Associated with these increases in chromosome interchange was the appearance of aneuploidy, with a chromosome number ranging from 25 to 34 per cell. It should be stressed that this increase in chromosome number ceased at relatively low (10 per genome) alphoid

TABLE 1. Chromosome aberrations and SCE in plasmid transformants with increased MTX resistance

Cell line	MTX resistance (μM)	Plasmid	Insert	No. of dicentric and ring chromosomes/cell ± SE (n = 100)	No. of SCEs/chromosome ± SE (n = 20)	No. of chromosomes ± SE (n = 25)
DUKX		None	None	0.01 ± 0.01	0.60 ± 0.04	20.8 ± 0.80
Ad	0.05	pAdD26SVpA3	None	0.13 ± 0.04	0.51 ± 0.04	19.8 ± 0.24
Ad	1.0	pAdD26SVpA3	None	0.04 ± 0.02	0.58 ± 0.03	19.8 ± 0.11
AdII	0.05	pAdD26SVpA3	None	0.05 ± 0.02	0.49 ± 0.04	20.0 ± 1.10
AdII	1.0	pAdD26SVpA3	None	0.03 ± 0.02	0.54 ± 0.04	19.8 ± 0.09
AdIII	0.05	pAdD26SVpA3	None	0.07 ± 0.02	0.64 ± 0.06	21.6 ± 1.50
Ad340	0.02	pAd340-11	Alphoid	1.20 ± 0.11	0.49 ± 0.04	32.9 ± 0.69
Ad340	1.0	pAd340-11	Alphoid	1.76 ± 0.13	1.23 ± 0.11	34.0 ± 0.32
Ad340II	0.05	pAd340-11	Alphoid	0.83 ± 0.09	0.79 ± 0.08	28.8 ± 1.70
Ad340II	1.0	pAd340-11	Alphoid	0.95 ± 0.10	1.10 ± 0.07	27.1 ± 1.60
Ad3405B	0.05	pAd340-5B	Alphoid	0.86 ± 0.09	0.71 ± 0.07	25.1 ± 1.00
Ad3405B	1.0	pAd340-5B	Alphoid	1.08 ± 0.10	1.06 ± 0.07	27.4 ± 1.80
GT17	0.02	pGT-17	$(dG\text{-}dT)_{200}$	0.06 ± 0.02	0.48 ± 0.04	19.8 ± 0.09
GT17	1.0	pGT-17	$(dG\text{-}dT)_{200}$	0.18 ± 0.04	0.60 ± 0.03	20.8 ± 0.80

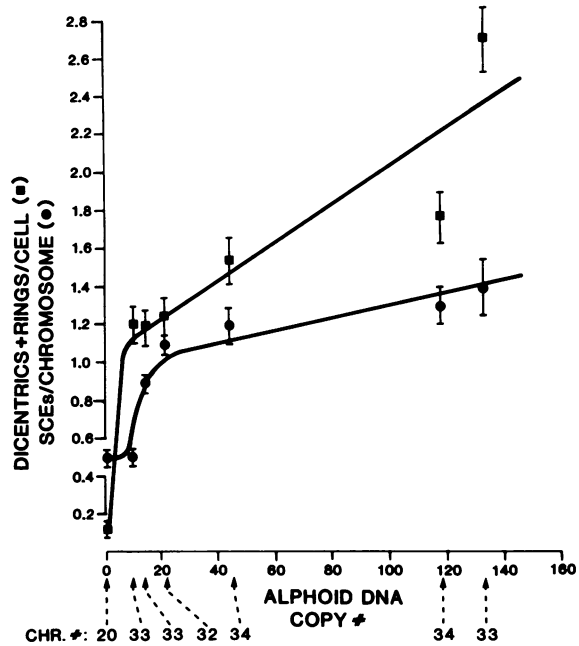

FIG. 7. Amplification of alphoid DNA and effect on chromosome aberrations and SCEs. Bars indicate standard errors of the mean. The alphoid DNA copy number was estimated by densitometric scanning of autoradiographs with a ^{32}P-labeled 345-bp alphoid reconstruction (*Eco*RI-cut pAd340-11) as a copy number standard.

copy numbers, beyond which chromosome aberrations and SCE frequency continued to increase. The other cell lines containing amplified plasmid without insert or plasmid with a $(dG\text{-}dT)_{200}$ insert showed increased aberration frequencies compared with the parent cell line, DUKX, although these dicentric and ring frequencies were at least 10 times lower than those observed in the alphoid DNA transfectants.

Because the alphoid transfectants exhibited longer population doubling times, it was important to determine whether lengthening the cell cycle by reducing the growth temperature increased the SCE frequency independent of the presence of alphoid DNA. The SCE frequency in the parent cell line, DUKX, was found by performing the experiment at either 37°C (12-h cell cycle) or 33°C (24-h cell cycle). No significant increase in SCE frequency was observed by a doubling of the cell cycle time (the SCE frequency was 0.60 ± 0.03 SCE and 0.68 ± 0.03 chromosome at 37 and 33°C, respectively [mean and standard error for 25 cells]).

Development of a series of cell lines with a gradient of coamplified plasmid and alphoid inserts permitted comparison of the alphoid DNA copy number in each cell line with the cytogenetic characteristics of the given cell line (Fig. 7). There was a dose effect of alphoid DNA copy number on SCE as well as on production of dicentric and ring chromosomes. The increase in dicentric and ring forms was at least 1 order of magnitude, with a large increase at relatively small amounts of integrated alphoid DNA followed by a less steep increase with higher copy numbers. The effect of alphoid DNA on SCE frequency paralleled that on dicentric and ring production, except that the SCE increase was less pronounced, i.e., a factor of 2 to 3, in cell lines with resistance to higher levels of MTX.

Regions of cytological change were compared with the location of plasmid incorporation to determine whether the effect of alphoid DNA amplification on SCE and aberration

TABLE 2. Localization of SCEs and autoradiographic grains to Ad340-1.0 chromosomes

SCEs or grains	Dicentric and ring chromosomes		Other chromosomes	
	No. of SCEs or grains/no. of chromosomes	No. per chromosome	No. of SCEs or grains/no. of chromosomes	No. per chromosome
Grains[a]	330/37	8.9	197/65	3.0
SCEs[b]	121/43	2.8	888/781	1.1

[a] In situ hybridization with a ^{3}H-labeled 345-bp alphoid insert from pAd340-11. Grains over chromosomes were scored in 23 metaphases with a total of 766 chromosomes. One dicentric chromosome had no associated grains.
[b] A total of 24 metaphases were used.

frequency was limited to chromosomal regions containing transfected DNA. In situ hybridization to metaphase chromosomes was performed on the cell line Ad340-1.0, a 1.0 μM MTX-resistant cell line, with a ^{3}H-labeled 345-bp alphoid insert purified from pAd340-11 as a probe and on the cell line Ad340-10, a 10.0 μM MTX-resistant cell line, with biotinylated pAd340-11 as a probe. Localization of grains to chromosomes in Ad340-1.0 cells is shown in Table 2. A large proportion of grains was shown to reside over the dicentric and ring forms present in each cell, whereas there were additional hybridizations to other apparently normal chromosomes. Figure 8 shows a particularly striking metaphase, with clusters of grains over a dicentric and a ring chromosome. Nonisotopic in situ hybridization is shown in Fig. 9. Alphoid DNA amplification in the regions of the centromeres in a multicentric and dicentric chromosome is demonstrated.

SCE formation in dicentric or ring chromosomes was also examined as a measure of the site specificity of SCE formation due to integrated alphoid DNA (Table 2). The number of SCEs in dicentric and ring chromosomes was higher than that in presumably normal chromosomes. This provides evidence for clustering of SCEs in these abnormal chromo-

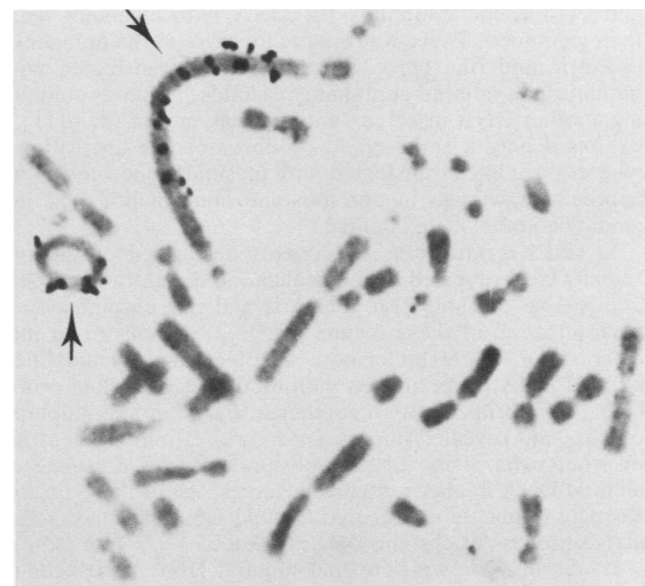

FIG. 8. Incorporation of pAd340-11 into structurally abnormal CHO chromosomes. In situ hybridization to a metaphase from Ad340-1.0 was performed with a ^{3}H-labeled 345-bp insert of pAd340-11. An autoradiograph is shown. The arrows point to a dicentric and a ring chromosome.

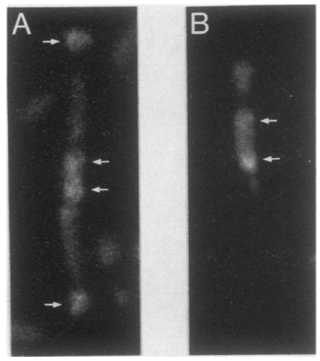

FIG. 9. Hybridization (as indicated by the arrows) with biotinylated pAd340-11 to a multicentric (A) and dicentric (B) chromosome from Ad340-10 cells.

somes. A differentially stained metaphase from Ad340-1.0 with increased SCE frequency, particularly in a ring chromosome, is shown in Fig. 10.

Karyotypic changes were further evident on examination of Giemsa-trypsin-banded chromosomes from three different cell lines (Fig. 11), DUKX, Ad-1.0, and Ad340-1.0. Amplified plasmid transfectants had considerable rearrangements of chromosomal material, with Ad340-1.0, an alphoid-containing transfectant, showing the most extensive of the rearrangements. Evidence of disjunctional errors in Ad340-1.0 were seen in this karyotype, specifically the duplication of at least three chromosomes.

DISCUSSION

The experiments reported here were designed to test the ability of different types of exogenous DNA sequences to cause chromosome rearrangement on amplification. A system was used that permitted transfection of a defined sequence into a Chinese hamster cell line. Cells which had amplified this plasmid could then be isolated. Both molecular and cytogenetic endpoints for DNA rearrangement were then examined. There was a large increase in the number of dicentric and ring chromosomes in cells transfected with human alpha satellite-containing plasmids. Plasmids containing another DNA insert, a synthetic polymer of $(dG\text{-}dT)_{200}$, exhibited only a small effect on chromosome aberrations, whereas cell lines transfected with plasmid alone showed no appreciable change in chromosome abnormalities, at the amplification levels examined.

In situ hybridization experiments performed on alphoid transfectants revealed the colocalization of autoradiographic grains (Fig. 8; Table 2) to dicentric and ring chromosomes. Quantification of these results (Table 2) demonstrated that dicentric or ring forms or both were enriched for amplified alphoid DNA. Experiments with biotinylated alphoid probe (Fig. 9) hybridized to metaphases from another alphoid transfectant revealed fluorescent hybridization to dicentrics in other cells. This further substantiates the presence of alphoid DNA in aberrant chromosomes. All of these results strongly implicate transfected alphoid DNA plasmids in an active process of chromosome breakage.

It was also shown here that alphoid DNA transfectants had an increased frequency of SCE, however, only upon amplification of transfected DNA. We examined the frequency of SCE in aberrant (dicentric and ring) chromosomes which, on the basis of in situ hybridization experiments, were likely to harbor amplified alphoid DNA plasmids (Table 2). With this analysis, it was shown that the SCE frequency was increased in dicentric and ring forms compared with other chromosomes in the cell (Table 2; Fig. 10). Assuming that SCE frequency is somewhat proportional to chromosome length, the larger size of most dicentrics and rings might account for some of the increased SCE frequency observed in these chromosomes, when averaged over all chromosomes and cells.

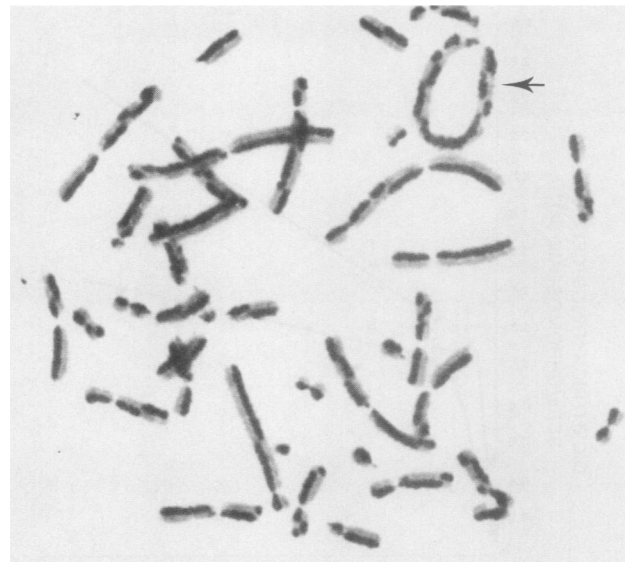

FIG. 10. SCE in chromosomes from an alphoid DNA transfectant Ad340-1.0 (resistant to 1 μM MTX). A ring chromosome is indicated by an arrow.

Since the alphoid DNA transfectants exhibited significantly longer population doubling times than other plasmid transfectants did (see Materials and Methods), it is also possible that some of the SCE increase is due to an effect on DNA replication rate. It has been observed that some agents (e.g., nucleoside analogs, heat) which attenuate replication also increase SCE frequency (18). However, it is not simply lengthening of the cell cycle which induces SCE, as shown here with temperature shift experiments (see Results). Targeting of specific replication enzymes such as topoisomerase II (12), along with induction of DNA breakage, may cause SCE while subsequently prolonging replication. The evidence presented here, however, suggests that alphoid DNA might serve as a hot spot for rearrangement. The reason for the high incidence and "dose" dependence of chromosome structure change with alphoid DNA amplification is presently under investigation.

The special nature of pericentromeric satellites in mouse cells as unstable DNA was recently demonstrated by Butner and Lo (3, 4). It was reported that heterologous selectable DNA containing the thymidine kinase gene transfected into endogenous centromeric satellite DNA was associated with a high degree of rearrangement in the transfected DNA, providing evidence for increased fluidity of centromeric heterochromatin. The present experiments differ in that centromeric DNA was used as the agent, introduced into foreign chromatin, rather than serving as the target for transfection.

It appears surprising that alphoid DNA causes chromosome instability in CHO cells when thousands of copies exist in human DNA without apparent adverse effect. However, we have used alphoid DNA in these experiments because it

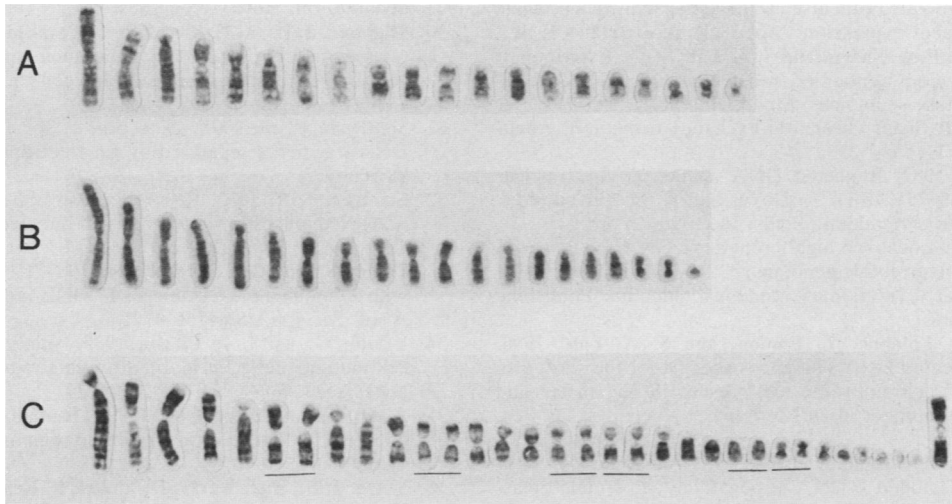

FIG. 11. Giemsa-trypsin-banded karyotypes from the cell lines. (A) DUKX (no transfecting plasmid); (B) Ad-1.0 (transfected with pAdD26SVpA3, no insert, resistant to 1 μM MTX); (C) Ad340-1.0 (transfected with pAd340-11, alphoid insert, after amplification to resistance to 1 μM MTX). Homologous chromosomes are underlined in the Ad340-1.0 karyotype (panel C).

is repetitive while being confined to the pericentromeric regions of human chromosomes. These are loci to which alphoid sequences have stabilized over the course of evolution. A speculative conclusion is that alphoid DNA organization could underlie a functional role in the maintenance of chromosome stability.

In sum, the experiments reported here have demonstrated that cell lines with amplified alphoid DNA-containing plasmids exhibit a frequency of chromosome aberrations at least 10 times higher than aberrations seen in cell lines containing plasmids without insert or a plasmid with a $(dG\text{-}dT)_{200}$ insert. The SCE frequency was significantly increased only in the alphoid DNA cell lines, providing further evidence for the chromosome instability seen with amplification of these plasmids in CHO cells. It is now possible to test whether the genomic instability seen here is a general feature of DNA repeats as such or is unique to centromeric DNA. In either case, detailed molecular analysis of the region undergoing rearrangement should provide new insights into the molecular basis of chromosome structure fluidity.

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