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Inducible Expression and Cytogenetic Effects of the *Eco*RI Restriction Endonuclease in Chinese Hamster Ovary Cells

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The cytogenetic endpoints sister chromatid exchange (SCE) and chromosome aberrations are widely used as indicators of DNA damage induced by mutagenic carcinogens. Chromosome aberrations appear to result directly from DNA double-strand breaks, but the lesion(s) giving rise to SCE formation remains unknown. Most compounds that induce SCEs induce a spectrum of lesions in DNA. To investigate the role of double-strand breakage in SCE formation, we constructed a plasmid that gives rise to one specific lesion, a staggered-end ("cohesive") DNA double-strand break. This plasmid, designated pMENs, contains a selectable marker, *neo*, which is a bacterial gene for neomycin resistance, and the coding sequence for the bacterial restriction endonuclease *Eco*RI attached to the mouse metallothionein gene promoter. *Eco*RI recognizes G↓AATTC sequences in DNA and makes DNA double-strand breaks with four nucleotides overhanging as staggered ends. Cells transfected with pMENS were resistant to the antibiotic G418 and contained an integrated copy of the *Eco*RI gene, detectable by DNA filter hybridization. The addition of the heavy metal $CdSO_4$ resulted in the intracellular production of *Eco*RI, as measured by an anti-*Eco*RI antibody. Cytogenetic analysis after the addition of $CdSO_4$ indicated a dramatic increase in the frequency of chromosome aberrations but very little effect on SCE frequency. Although there was some intercellular heterogeneity, these results confirm that DNA double-strand breaks do result in chromosome aberrations but that these breaks are not sufficient to give rise to SCE formation.

Most mutagenic carcinogens induce sister chromatid exchanges (SCEs), chromosome aberrations, or both in eucaryotic cells. Although there is evidence that DNA double-strand breaks lead to the formation of chromosome aberrations (3, 6, 19–21, 34), the precise lesion(s) and the mechanisms involved in the formation of SCEs are unknown. The relationship between a particular DNA lesion and its biological effect is often obscured by the fact that most mutagenic carcinogens induce a spectrum of lesions, the proportions of which vary in different cell types. For example, ionizing radiations induce single- and double-strand breaks and make numerous base modifications (31), UV light generates cyclobutane and (6-4) pyrimidine-pyrimidone dimers plus a host of minor photoproducts (24), and alkylating agents alkylate both purines and pyrimidines in different ratios depending on the compound used (29). Ideally, to define the range of cytogenetic effects due to particular lesions, a specific class of lesions would be induced in cellular DNA in vivo, and the cytogenetic endpoint in question would be examined.

Bacterial restriction endonucleases recognize specific, rather short sequences of DNA as binding sites. Binding is followed by either blunt-end or cohesive-end DNA cleavage at the recognition site itself or elsewhere, depending on the enzyme. A restriction endonuclease makes a double-strand break and few or no other perturbations in DNA. In the past, when commercially available restriction enzymes were introduced into permeabilized Chinese hamster cells, increased frequencies of SCEs (18) and chromosome aberrations (3, 20, 22, 33) were observed. These studies, however, were hampered by (i) the necessity to permeabilize cells to permit access of the endonuclease yet keep the cells viable for cytogenetic assays, (ii) the lack of control over how much, if any, enzyme went into the cell, and (iii) the inability to know whether a lack of cytogenetic effects indicated that little or no enzyme had entered the cell or that the enzyme did not elicit a cytogenetic response.

To overcome these problems and to permit more strict control of endonuclease expression within a cell, we constructed a plasmid containing a selectable marker gene and the bacterial *Eco*RI restriction endonuclease gene attached to the mouse metallothionein gene promoter. Cells successfully transfected with this plasmid can be isolated by selective pressure and induced to produce the *Eco*RI restriction enzyme by the addition of a heavy metal. *Eco*RI recognizes G↓AATTC sequences in DNA and makes cohesive double-strand breaks with four nucleotides overhanging as staggered ends. In the present report we describe the construction of this plasmid and show that inducible expression of *Eco*RI results in increased numbers of chromosome aberrations but not SCEs.

MATERIALS AND METHODS

Cell culture. Chinese hamster ovary (CHO-K1 [27]) cells were cultured as monolayers in McCoy 5A medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, penicillin (50 U/ml), and streptomycin (50 μg/ml). Cells were maintained in exponential growth at 37°C in an atmosphere of 5% CO_2 in air.

Enzymes and plasmids. Plasmid pSV2neo was provided by Paul Berg (Stanford University, Stanford, Calif.), plasmid mMT-I was provided by Richard Palmiter (University of Washington, Seattle), and plasmid yCpGal:1a was provided by Georgina Barns (University of California, Berkeley). Restriction enzymes were purchased from Bethesda Research Laboratories (Gaithersburg, Md.), and G418 (geneticin) was purchased from GIBCO Laboratories (Grand Island, N.Y.). Unless otherwise noted, all other reagents were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Plasmid construction. Plasmid pMENs was constructed

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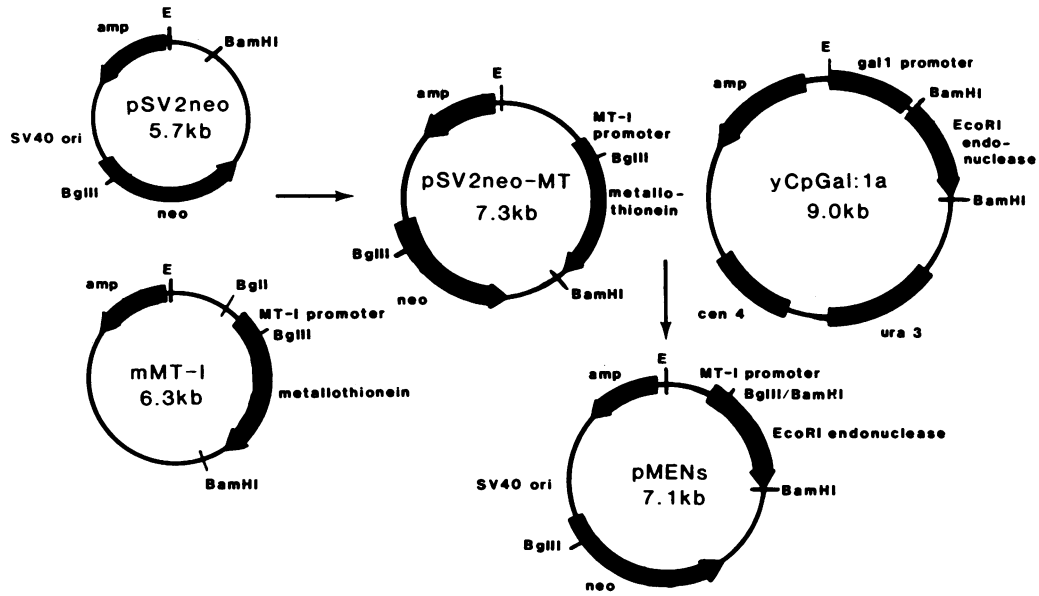

FIG. 1. Structure and method of construction of the pMENs plasmid. Only relevant restriction enzyme sites are shown (E, *Eco*RI). Arrowheads on the major coding regions (■) indicate orientation.

from plasmids pSV2neo, mMT-I, and yCpGal:1a. Large-scale plasmid preparations were purified by cesium chloride-ethidium bromide density centrifugation as described by Maniatis et al. (12). Plasmids pSV2neo and mMT-I were cleaved with *Eco*RI and *Bam*HI (Fig. 1). These enzymes produced a 4.9-kilobase (kb) fragment from pSV2neo containing the gene for ampicillin resistance (*amp*), the simian virus 40 origin of replication, the gene for neomycin resistance (*neo*), and the simian virus 40 bidirectional termination sequences. A similar digestion of mMT-I produced a 2.4-kb fragment (*Bgl*I to *Bgl*II) containing the metal-responsive mouse metallothionein-I (MT-I) gene promoter (30) and the MT-I gene. The 4.9-kb pSV2neo fragment was ligated to the 2.4-kb mMT-I fragment to produce the plasmid pSV2neo-MT. A total *Bam*HI digestion and a partial *Bgl*II digestion of pSV2neo-MT removed the MT-I gene from this plasmid. Plasmid yCpGal:1a was cleaved with *Bam*HI, and the resulting 1.2-kb fragment containing the intact *Eco*RI endonuclease coding sequence was ligated to pSV2neo-MT 3′ to the MT-I gene promoter. The ATG initiation codon is 4 base pairs from the *Bam*HI site at the 5′ end of the *Eco*RI gene (1). The resulting plasmid was designated pMENs. Plasmids were isolated from ampicillin-resistant colonies after transfection of *Escherichia coli* HB101 and screened by restriction mapping to ensure the identity of the resulting constructs.

Cell transfection. The pMENs plasmid was introduced into 10^6 CHO cells by calcium phosphate precipitation (32) without carrier DNA. Cells were allowed 2 days for the expression of the *neo* gene (thus resistance to the antibiotic G418) and were then replated at a density of 3×10^5 cells per 75-cm^2 tissue culture flask in the presence of 800 μg of G418 per ml. We had previously determined this concentration of G418 to be completely toxic to untransfected CHO cells. A number of surviving colonies were clonally selected in G418 and continuously cultured in medium containing G418. Unless specifically stated, cells in all experiments were cultured in medium containing 800 μg of G418 per ml to minimize the possibility of losing the pMENs plasmid.

Characterization of clones containing *Eco*RI. Cellular digestion of DNA with restriction enzymes and filter hybridization analysis were carried out as described by Murnane et al. (17). Cytoplasmic RNA was prepared by the method of Favaloro et al. (7) with vanadyl-ribonucleoside complexes to inactivate RNase. RNA samples were suspended in 10 mM Tris (pH 7.5)–1 mM EDTA buffer and serially diluted, and 0.5 volume of a mixture containing 24% formaldehyde, 0.28 M EDTA, and 0.28 M phosphate buffer (pH 6.8) was added to each sample. The samples were heated at 60°C for 10 min, and 3 volumes of 20× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate) was added to each. The samples were then filtered through a nitrocellulose filter.

Nucleic acid hybridization and probe preparation. After the filters were baked at 80°C for 2 h under a vacuum, they were wetted in 6× SSC and placed in sealable plastic bags in prehybridization solution containing 50% formamide (Bethesda Research Laboratories), 5× SSC, 5× Denhardt solution (1 g of Ficoll per liter, 1 g of polyvinylpyrrolidone per liter, 1 g of bovine serum albumin per liter), 250 μg of denatured sonicated salmon sperm DNA per ml, 20 mM phosphate buffer (pH 6.5), and 1% glycine. The filters were incubated in this solution at 42°C for at least 4 h with constant agitation. The prehybridization solution was removed, and a similar hybridization solution containing 10% dextran sulfate (Pharmacia Fine Chemicals, Piscataway, N.J.), 0.1% sodium dodecyl sulfate, and 10 ng of denatured DNA probe (2×10^8 to 8×10^8 cpm/μg) nick translated with [α-^{32}P]dCTP (3,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) per ml was added. Hybridization was carried out at 42°C for 18 h. The nitrocellulose paper was washed three times for 30 min in 2× SSC containing 0.5% sodium dodecyl sulfate at ambient temperature and then was washed twice for 30 min in 0.1× SSC containing 0.2% sodium dodecyl sulfate at 50°C. The filter paper was rinsed in 0.1× SSC and dried at ambient temperature; this was followed by wrapping in plastic wrap and exposure to X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.) with Lanex intensifying screens (Kodak) for 12 to 48 h.

Immunolocalization of the *Eco*RI antigen. The flasks used for cytogenetic analysis (see below) also contained 12-mm

glass cover slips that were used for immunolocalization of the *Eco*RI protein in CHO cells with a rabbit *Eco*RI antibody (provided by Steven Yanofsky and Patricia Greene, University of California, San Francisco). Whenever metaphase cells were harvested for cytogenetic analysis, a cover slip with cells growing on it was also removed from the flask. The cover slips were rinsed twice with phosphate-buffered saline (PBS), fixed in 2% paraformaldehyde, and rinsed with PBS, then with 50 mM NH_4Cl, and again with PBS. Cells were permeabilized with 0.1% Triton X-100 and rinsed with PBS. The rabbit antibody to *Eco*RI was diluted in PBS and 3% normal rabbit serum to determine the optimal dilution condition, and cover slips were incubated for 1 h. Cover slips were then incubated for 1 h with the secondary antibody, goat anti-rabbit immunoglobulin G labeled with rhodamine (50 μg/ml), and viewed by epifluorescence.

Cytogenetic analysis. Exponentially growing cells from the parental CHO cell line (CHO6) and the transfected cell line (CHO10) were cultured in bromodeoxyuridine (BrdUrd; 10^{-5} M final concentration); 2 h before harvest, Colcemid (10^{-7} M final concentration) was added to arrest cells in metaphase. Because the $CdSO_4$ used to stimulate the MT gene promoter and thus induce the cascade of events leading to production of active *Eco*RI causes some delay in the progression of cells through the cell cycle, multiple harvest times were used. Cells were harvested after gently shaking the flasks to dislodge the metaphase cells, and chromosome preparations were made by treating cells for 2 min with 0.075 M KCl, fixing them with two changes of methanol-acetic acid (3:1), and dropping them onto glass microscope slides. Metaphase chromosomes containing BrdUrd were stained by a slight modification (16) of the fluorescence-plus-Giemsa method of Perry and Wolff (26). In this way, cells having undergone one, two, or three cell cycles can be readily distinguished by their metaphase staining pattern (4). Cells having undergone one cell cycle have both chromatids unifilarly substituted with BrdUrd, and chromosomes stain darkly. Chromosome aberrations were analyzed in first-division metaphase spreads. Cells having undergone two cell cycles have one chromatid unifilarly substituted with BrdUrd and the sister chromatid bifilarly substituted with BrdUrd. Bifilarly substituted chromatids stain lightly, whereas unifilarly substituted chromatids stain darkly, and SCEs are evident as reciprocal exchanges between the darkly and lightly stained chromatids. The frequency of SCEs was determined in second-division cells. In cells having undergone three cell cycles, approximately 75% of their chromatin is bifilarly substituted with BrdUrd and therefore stains lightly, and approximately 25% of their chromatin is unifilarly substituted and stains darkly.

Chromosome aberration frequency was determined in 100 first-division cells, and the frequency of SCEs was determined in 100 second-division cells (50 cells from two replicate experiments).

RESULTS

The plasmid pMENs contains a selectable marker for Neo^r (cellular resistance to G418) and the *Eco*RI restriction endonuclease gene attached to an inducible metallothionein gene promoter (Fig. 1). After transfection by calcium phosphate precipitation, a number of G418-resistant CHO cells were obtained. The transfection frequency with pMENs was considerably lower than what we generally obtain with other plasmids in this laboratory. Twenty-two colonies were cloned and screened by DNA dot-blot hybridization for the presence of *Eco*RI DNA. In addition, clones were cultured for one cell cycle (16 to 20 h) with 0.3 μM $CdSO_4$ and analyzed for cytogenetic damage as measured by chromosome aberration frequency. Four cell clones showed both a positive DNA dot-blot hybridization and an increased frequency of chromosome aberrations after exposure to the heavy metal. One cell line, designated CHO10, was selected for detailed investigation.

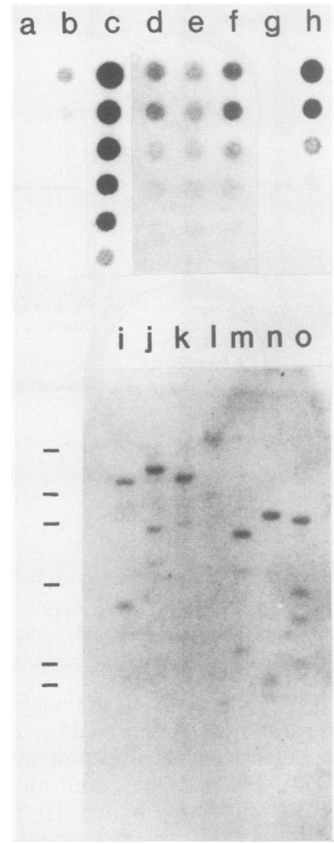

FIG. 2. RNA dot blots from CHO6 (lane a) and CHO10 (lane b) cells hybridized with the 1.2-kb *Bam*HI fragment containing the *Eco*RI coding sequences from yCpGal:1a. In lane c is yCpGal:1a hybridized with the same *Bam*HI fragment as in lane b. Also shown are RNA dot blots from CHO10 cells hybridized with the 1.2-kb *Eco*RI coding sequences after culture for 48 h alone (lane d) or with 0.1 μM $CdSO_4$ (lane e) or 0.3 μM $CdSO_4$ (lane f) and RNA dot blots from CHO6 (lane g) and CHO10 (lane h) cells hybridized with ^{32}P-labeled pSV2neo. Below the first row, each sample represents a 50% dilution of RNA from the sample above it. Lanes i through o show Southern blots of DNA from CHO10 cells digested with *Bgl*II (lane i), *Hin*dIII (lane j), *Eco*RI (lane k), no enzyme (lane l), *Pst*I (lane m), *Pvu*II (lane n), or *Sal*I (lane o) and hybridized with the 1.2-kb *Bam*HI fragment containing the *Eco*RI coding sequences from yCpGal:1a. Markers to the left of lane i correspond to the λ *Hin*dIII standards of 23.10, 9.42, 6.56, 4.36, 2.32, and 2.03 kb in descending order.

The expression of the major fragments of the pMENs plasmid was confirmed by RNA dot-blot hybridization (Fig. 2, lanes a through h). RNA dot blots showed that *Eco*RI RNA was constitutively expressed in clone CHO10 (lanes b and d), but there was surprisingly very little increase in the RNA level after 48 h of culture in the presence of either 0.1 μM $CdSO_4$ (lane e) or 0.3 μM $CdSO_4$ (lane f). The same results were found when blot analysis was performed at various times (17, 28, and 36 h) after the addition of $CdSO_4$

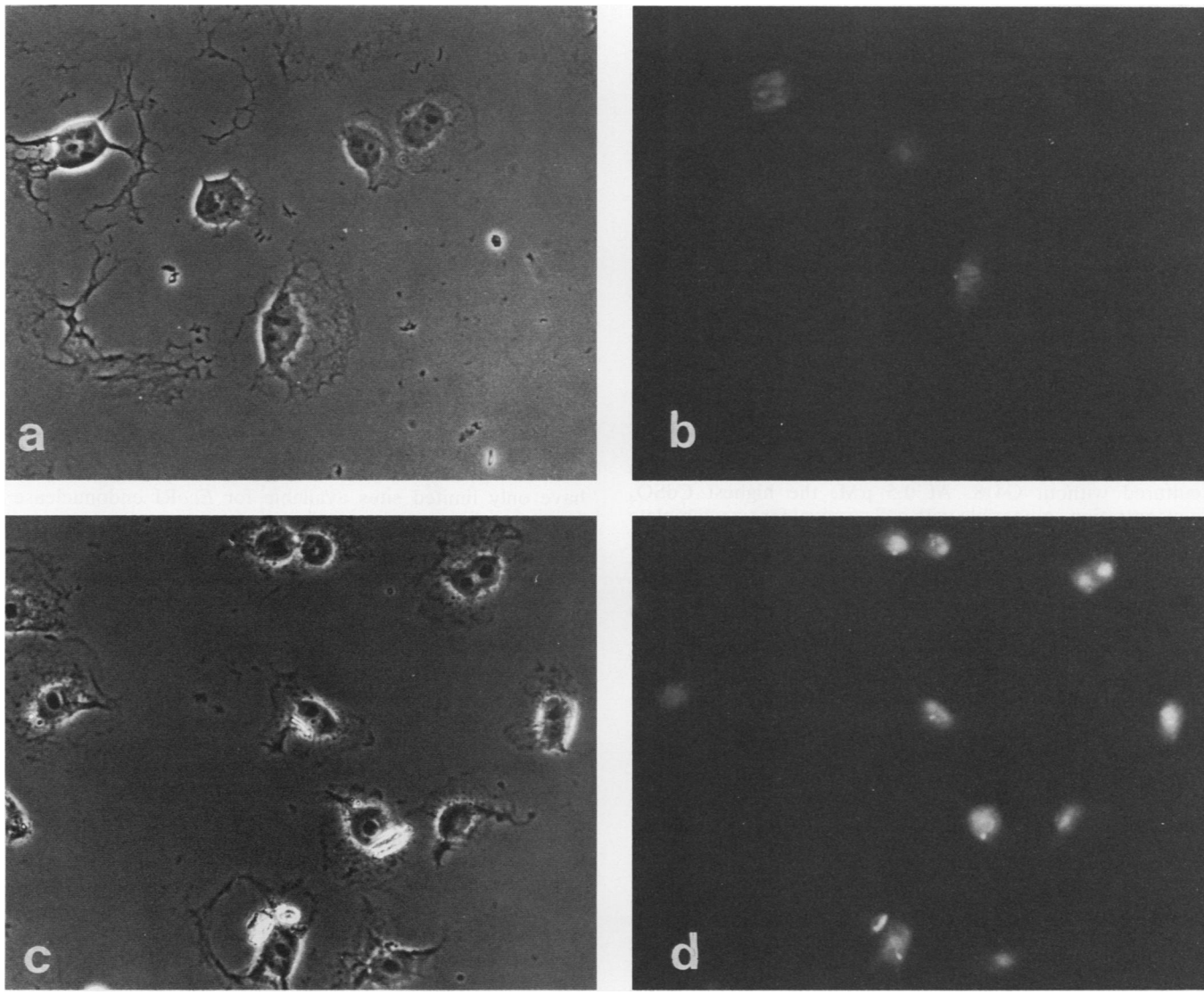

FIG. 3. Immunolocalization of the *Eco*RI antigen. (a) CHO10 cells viewed by phase-contrast microscopy. (b) The same cells as in (a), stained with the rabbit antibody to *Eco*RI and incubated with a secondary antibody, goat anti-rabbit immunoglobulin G labeled with rhodamine. (c) CHO10 cells cultured for 28 h with 0.5 μM $CdSO_4$ and viewed by phase-contrast microscopy. (d) The same cells as in (c) after incubation with rhodamine-labeled antibody. Exposure times were the same in (b) and (d). Note the small amount of residual expression in (b) and the much more intense fluorescence induced by $CdSO_4$ in (d).

(data not shown). The *Eco*RI sequence contained in this clone was characterized by Southern blot analysis with the 1.2-kb insert isolated from yCpGal:1a (Fig. 2, lanes i through o). From the Southern hybridization, there appears to be at least one complete *Eco*RI coding sequence and two partial sequences in CHO10 cells. No hybridization was observed in DNA from the parental CHO6 cells.

We also made an attempt to assay *Eco*RI activity in nuclear extracts (5, 13) and in lysates of whole cells (1). Freshly prepared samples of cell or nuclear extracts were mixed with 2 μg of λ DNA, and the reaction mixtures were incubated at 37°C for 1 h. Gel electrophoresis revealed that in all cases there was complete nonspecific degradation of λ DNA (data not shown).

To confirm that the *Eco*RI gene product was indeed being produced, the presence of intracellular *Eco*RI was determined by using a rabbit antibody against *Eco*RI. Rhodamine immunofluorescence clearly showed the presence of *Eco*RI in CHO10 cells, and the intensity of fluorescence was dramatically enhanced when $CdSO_4$ was added to the medium (Fig. 3). No fluorescence was detected in the parental CHO6 cells after antibody staining. This immunofluorescence assay indicates that the *Eco*RI enzyme is concentrated in the cell nucleus, in particular the nucleolus, with very little of the enzyme being present in the cytoplasm (Fig. 3).

Cytogenetic analysis of CHO10 cells not exposed to $CdSO_4$ showed a higher frequency of SCEs in transfected cells compared with those in the parental cell line (Table 1). CHO10 had a background frequency of 0.71 SCEs per chromosome compared with 0.39 SCEs per chromosome in CHO6. This increase in the background frequency of SCEs can be attributed to the presence of 800 μg of G418 per ml in the culture medium. In a separate experiment, when CHO10 cells were cultured for two replication cycles without G418, a background SCE frequency of 0.38 SCEs per chromosome was observed. Adding increasing concentrations of $CdSO_4$

TABLE 1. SCE frequency in parental (CHO6) and transfected (CHO10) cells after exposure to increasing concentrations of $CdSO_4$ for two cell cycles with or without G418[a]

$CdSO_4$ (μM)	SCEs/chromosome		
	CHO6 without G418	CHO10	
		Without G418	With G418
0	0.39	0.38	0.71
0.1	0.39	0.35	0.75
0.3	0.46	0.51[b]	0.80[b]
0.5	0.46	0.62[b]	0.63

[a] For each experiment 50 second-division cells were analyzed in two replicate cultures, for a total of 100 cells per point.
[b] Significantly different from control ($P < 0.005$, Student t test).

for the duration of the culture period caused no significant increase in SCE frequency in CHO6 cells, whereas a small but significant increase in SCE frequency was observed in CHO10 cells (Table 1). This was most noticeable in cells cultured without G418. At 0.5 μM, the highest $CdSO_4$ concentration compatible with cell survival for two complete replication cycles, 4% of cells showed very high levels of SCE (average, 2.03 SCEs per chromosome; range, 1.76 to 2.43 SCEs per chromosome; Fig. 4b). The addition of $CdSO_4$ at various times after the addition of BrdUrd had no effect on SCE frequency (data not shown).

In contrast to the small increase in SCEs, increasing concentrations of $CdSO_4$ had profound effects on the frequency of chromosome aberrations (Table 2). Metaphase spreads were prepared at various times after the addition of $CdSO_4$, and analysis of chromosome aberrations was restricted to first-division cells. It appears that *Eco*RI activity takes at least 26 h to exert its cytogenetic effects. As the incubation time with $CdSO_4$ increased, so did the frequency of aberrations. After 45 h at the highest concentration used, 0.5 μM, 90% of metaphase cells were aberrant. Aberrations ranged from simple chromatid and isochromatid breaks (Fig. 4c) to complex exchange aberrations (Fig. 4d). In cells showing less than 10 aberrations per cell a breakdown of the aberrations is given (Table 2); cells with >10 aberrations were classified as heavily damaged (Fig. 4e); those in which very little chromosome morphology could be recognized, but which were obviously grossly aberrant, were scored as pulverized (Fig. 4f). The above experiments were done with BrdUrd in the culture medium to facilitate discrimination of cells having undergone one, two, or more cell cycles. The same experiments performed without BrdUrd showed almost identical levels of chromosome aberrations (data not shown).

DISCUSSION

The mammalian cell line CHO10 contains the plasmid pMENs stably integrated into chromosomal DNA. This plasmid confers resistance to the antibiotic G418, and in the opposite orientation to the *neo* gene is the bacterial *Eco*RI restriction endonuclease gene linked to the mouse metallothionein gene promoter. RNA dot blots and immunofluorescence with a rhodamine-labeled antibody against the *Eco*RI enzyme indicated that the *Eco*RI gene is constitutively expressed.

We were surprised that after the addition of $CdSO_4$ there was very little increase in transcription as measured by RNA dot blots (Fig. 2), despite enhanced antibody fluorescence and a dramatic increase in cytogenetic damage. We have no explanation for this, but we are currently investigating the possibility that the relatively high constitutive expression of the *Eco*RI gene may be largely due to nontranslatable RNA. Dot-blot analysis indicates that *Eco*RI expression is weak compared with the *neo* gene (Fig. 2). Therefore, it is possible that because cells are continuously cultured in G418, there is readthrough from the strong simian virus 40 promoter, which is attached to the *neo* gene in the opposite orientation. Alternatively, a cellular promoter near the site of integration may produce a high background level of nonsense RNA that masks the specific RNA produced after the addition of $CdSO_4$.

It is of interest that the *Eco*RI endonuclease is concentrated in the cell nucleus. Presumably, the enzyme is assembled in the cytoplasm and diffuses into the nucleus, where it remains owing to some affinity with DNA. The efficiency of *Eco*RI to induce double-strand breaks within a cell depends on the number of specific sites available for cleavage. Because chromosomal DNA is tightly packed and often masked by histones and various nuclear proteins, it may have only limited sites available for *Eco*RI endonuclease binding and cleavage.

Attempts to demonstrate *Eco*RI activity in cell lysates of nuclear extracts of CHO10 cells resulted in a total nonspecific degradation of exogenously supplied λ DNA. This was probably caused by cellular nucleases; attempts to neutralize these nucleases would also compromise *Eco*RI activity and were not pursued.

The *Eco*RI gene is constitutively expressed in CHO10 cells; this suggests that these cells are able to tolerate a certain frequency of *Eco*RI-induced staggered-end double-strand breaks with few adverse effects. CHO10 has a cell doubling time similar to that of its parental CHO line. In addition, the background frequencies of SCEs and chromosome aberrations in the absence of G418 were similar to those observed in the parental line. When $CdSO_4$ was added to the culture medium, a dose-dependent delay in cell cycle proliferation and a dramatic increase in all types of chromosome aberrations was observed in CHO10. There was some heterogeneity in the number of aberrations produced per cell. This cell-to-cell variability may reflect differences in the amount of *Eco*RI produced, differences in the efficiency of breaks produced, cellular modification of those breaks, or the relative ability of cells to repair a cohesive-end double-strand break. Very little effect of $CdSO_4$ was found under similar conditions in the parental cells. A small but significant increase in SCEs was observed in CHO10 cells after the addition of 0.3 μM $CdSO_4$, but not at the highest concentration of $CdSO_4$ used (0.5 μM). This small increase was most noticeable in cells cultured without G418. The fact that G418 alone almost doubled the SCE frequency in cells containing the *neo* gene is of interest. If SCEs are, as we believe, a manifestation of homologous recombination, then the observation that G418 increases this recombination frequency may have important implications for the use of plasmids containing the *neo* gene in studies of recombination in transfected cells.

Incorporation of BrdUrd into chromosomal DNA in place of thymidine is the most commonly used method of sister chromatid differentiation. It is unlikely that BrdUrd interfered with the induction of SCEs, because *Eco*RI readily cleaves BrdUrd-substituted DNA (2) and dramatically induces chromosome aberrations under conditions in which few SCEs are induced. It has been shown, however, that endonucleases recognizing guanine-cytosine stretches for

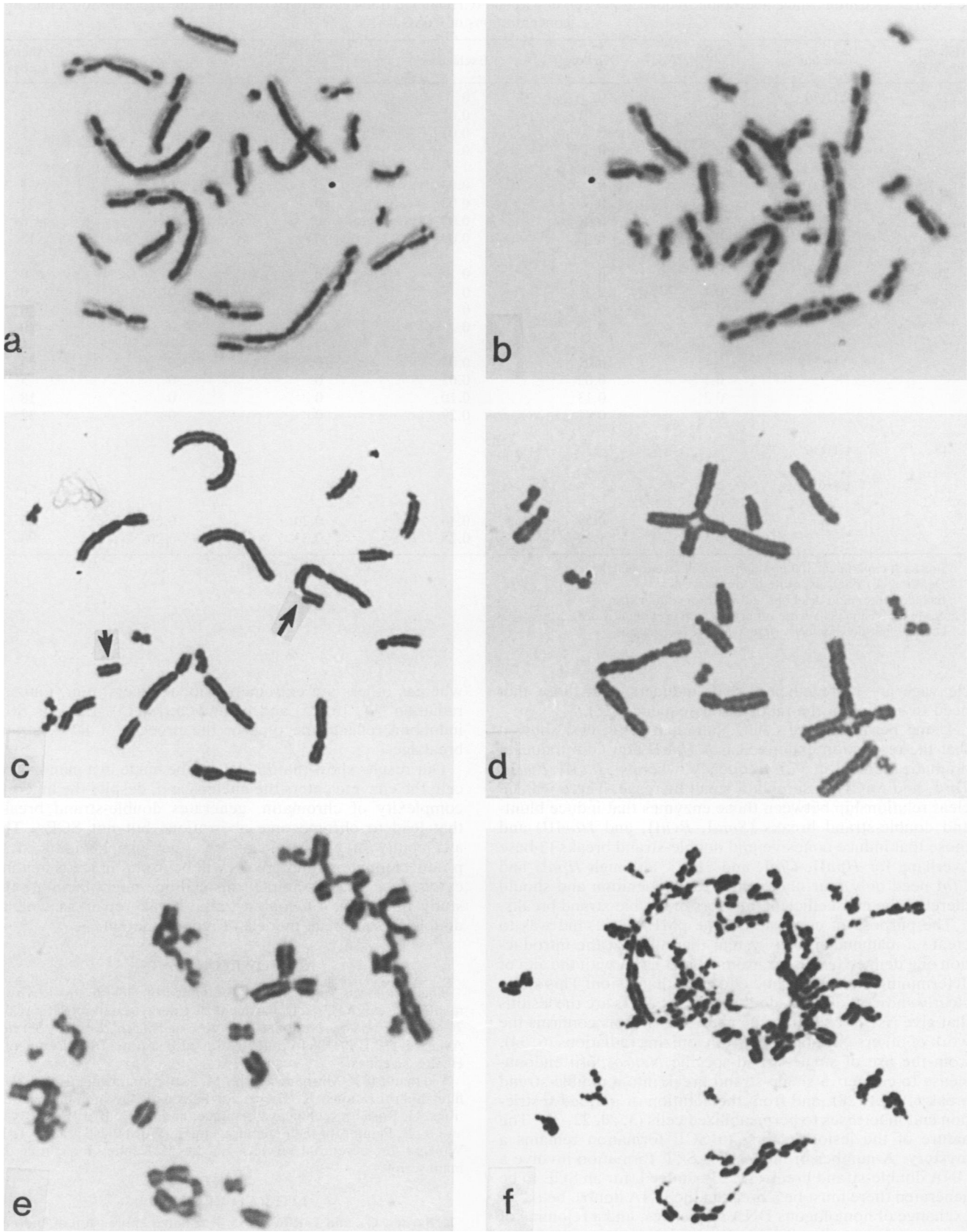

FIG. 4. (a) Background SCEs in a second-division CHO10 cell. (b) Induced SCEs in a CHO10 cell. (c through f) Aberrant metaphase cells: (c) chromatid deletion (arrow), isochromatid deletion (arrowhead), (d) two exchange figures, (e) >10 aberrations; (f) pulverized.

TABLE 2. Chromosome aberration frequency per cell in parental (CHO6) and transfected (CHO10) cells after exposure to increasing concentrations of $CdSO_4$[a]

Harvest time[b] (h)	Cell line	$CdSO_4$ (μM)	Deletions[c]	Exchanges	No. with >10 aberrations	No. pulverized	% Aberrant cells
21	CHO6	0	0	0	0	0	0
		0.1	0.01	0.01	0	0	2
		0.3	0	0.01	0	0	1
		0.5	0	0	0	0	0
	CHO10	0	0	0.03	0	0	3
		0.1	0	0.02	0	0	2
		0.3	0.01	0.05	0	0	6
		0.5	0.05	0.09	0	0	12
26	CHO6	0	0	0	0	0	0
		0.1	0	0	0	0	0
		0.3	0	0	0	0	0
		0.5	0	0	0	0	0
	CHO10	0	0.01	0.04	0	0	3
		0.1	0.02	0.03	0	0	5
		0.3	0.13	0.10	0	0	18
		0.5	0.41	0.29	0	0	32
45	CHO6[d]						
	CHO10	0[d]					
		0.1[d]					
		0.3	0.54[e]	0.40	0.20	0.28	80
		0.5	1.10	0.25	0.33	0.20	90

[a] For each experiment 100 first-division cells were analyzed.
[b] Duration of $CdSO_4$ exposure before harvest.
[c] Includes chromatid deletions and isochromatid breaks.
[d] No first-division cells were observed, with or without $CdSO_4$.
[e] Only 50 first-division cells were available for analysis.

cleavage are more efficient SCE inducers than those that need thymidine in the recognition sequence (18).

Using permeabilized cells, Natarajan et al. (18) showed that the restriction endonucleases *Hpa*II and *Cfo*I induce a dramatic increase in SCE frequency, whereas *Hae*III, *Pvu*II, *Taq*I, and *Sma*I cause only a small increase. There was no clear relationship between those enzymes that induce blunt-end double-strand breaks (*Sma*I, *Pvu*II, and *Hae*III) and those that induce cohesive-end double-strand breaks (2-base overhang for *Hpa*II, *Cfo*I, and *Taq*I), although *Hpa*II and *Cfo*I need only four nucleotides for recognition and should therefore be more efficient inducers of double-strand breaks.

The purpose of constructing the pMENs plasmid was to create a mammalian cell system that allowed the introduction of a defined lesion in chromosomal DNA with the aim of determining the cytogenetic effects of that lesion. This study clearly shows that DNA double-strand breaks are the lesions that give rise to chromosome aberrations. This confirms the work of others extrapolating from ionizing radiations (6, 34), from the use of single-strand-specific *Neurospora* endonuclease to convert a single-strand break into a double-strand break (19, 21, 28), and from the addition of purified restriction endonucleases to permeabilized cells (3, 20, 22, 33). The nature of the lesion leading to SCE formation remains a mystery. A number of models for SCE formation involve a DNA double-strand break (11, 23). Indeed, for an SCE to be generated there must be a break in the DNA double helix, an exchange of homologous DNA sequences, and a rejoining of the two sister chromatids. Some DNA strand-breaking agents are very efficient inducers of SCEs, e.g., fluorescent light illumination of BrdUrd-substituted DNA (9, 10), whereas others are extremely poor inducers, e.g., ionizing radiation (14, 16, 25) and bleomycin (8, 15). Perhaps SCE induction reflects the type or the process of DNA strand breakage.

Our results show that *Eco*RI can be made in a mammalian cell; the enzyme enters the nucleus and, despite the inherent complexity of chromatin, generates double-strand breaks that lead to chromosome aberrations but not SCEs. The availability of a mammalian cell that can be induced to produce double-strand breaks will be useful in areas beyond cytogenetics. For example, this cell line might facilitate the study of models of double-strand-break repair as well as double-strand-break models of recombination.

ACKNOWLEDGMENTS

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