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Induction of Sister Chromatid Exchanges at Common Fragile Sites

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SUMMARY

Experiments were performed to gain further insight into chromosome structure and behavior at common fragile sites by testing the hypothesis that gaps at these sites predispose to intrachromosomal recombination as measured by sister chromatid exchanges (SCEs). Human lymphocytes were concurrently treated with aphidicolin, for determination of fragile site expression, and with 5-bromodeoxyuridine, for SCE analysis. Aphidicolin induced chromosome gaps nonrandomly, with the great majority of gaps occurring at common fragile sites. On average, 66% of gaps were accompanied by an SCE at the site of the lesion. Analysis of two specific common fragile sites at 3p14 and 16q23 showed the same pattern; that is, on average 70% of gaps at these sites were accompanied by an SCE. These results show that common fragile sites are hot spots not only for chromosomal lesions such as gaps but also for SCE formation.

INTRODUCTION

Fragile sites are regions on chromosomes that are particularly prone to break or form gaps under certain cell culture conditions or following treatment with specific chemical agents. Most fragile sites, including the fragile X, are relatively rare. Others, termed "common" (Glover et al. 1984) or "constitutive" (Daniel et al. 1984; Yunis and Soreng 1985), appear to occur on chromosomes from all individuals and represent a heritable part of chromosome structure at

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these sites. The common fragile sites are only weakly induced by conditions of thymidylate stress, which induce the fragile X and other folate-sensitive fragile sites (Glover et al. 1984; Daniel et al. 1984). Conversely, aphidicolin, a specific inhibitor of DNA polymerase-α, strongly induces the common fragile sites but only weakly induces the fragile X. The expression of all fragile sites is enhanced in particular cell types by posttreatment with caffeine or theophylline (Yunis and Soreng 1985; Glover et al. 1986; Ledbetter et al. 1986*a*). Thus, the common and rare fragile sites share some similarities but also exhibit some differences in their modes of induction. All fragile sites appear the same at the cytological level. Aside from the fragile X, which is associated with one form of mental retardation, the biological role or significance of fragile sites is unknown. However, on the basis of correlation of breakpoints, suggestions have been made that fragile sites may play a role generating nonrandom chromosome rearrangements (Hecht and Glover 1984; Hecht and Sutherland 1984; LeBeau and Rowley 1984; Yunis and Soreng 1985).

Beyond the basic observation that fragile sites form chromosome gaps or occasional breaks, nothing is yet known about the chromosome or DNA structure at any fragile site. It is not known whether fragile-site expression results from single- or double-stranded DNA breaks that may lead to chromosome deletions, rearrangements, or recombination or solely involves a site-specific breakdown in chromosome condensation. In the studies presented in the present paper, we show that the common fragile sites are hot spots for DNA recombination as measured by sister chromatid exchange (SCE). This finding (1) suggests that the common fragile sites often, if not always, result in DNA strand breakage at some point during expression and (2) may help to further explain the mechanism of chromosome gap formation and breakage at fragile sites.

MATERIAL AND METHODS

Cell Culture

Experiments were performed with cultured lymphocytes from whole blood obtained from two adult volunteers. Experiments 1 and 2 were performed with cells from the same male subject; experiment 3 was performed with cells from an unrelated female subject. Cells were cultured by means of conventional microculture technique in RPMI 1640 medium (Irvine Scientific) containing 10% fetal bovine serum, 4 mM glutamine, and antibiotics. Cultures were incubated at 37 C in the dark for a total of 96 h.

Chemical Treatments

Cells were treated with 5-bromodeoxyuridine (BrdUrd) for SCE analysis (Perry and Wolff 1974) and with aphidicolin for induction of fragile-site expression (Glover et al. 1984). BrdUrd at 20 μM was added to cultures either 48 (experiment 1) or 40 h (experiments 2, 3) prior to harvest. Aphidicolin at 0.2 or 0.4 μM was added to cultures either at 24 h prior to harvest or at the time of BrdUrd addition to treat cells for one or two cell cycles, respectively.

Slide Treatments and Scoring

Slides prepared from control cultures not stained for SCE analysis were GTG-banded for analysis of fragile sites only. For analysis of SCEs, BrdUrd-treated slides were stained with 1 μg Hoechst 33258/ml for 5 min, mounted in phosphate-buffered saline, and illuminated with a sun lamp (GE model RSM) on a 60 C hot plate for 25 min. The slides were then stained in 4% Giemsa for 3 min.

At least 50 metaphases/treatment were scored for the frequencies of chromosome gaps, breaks, or other aberrations and for the number and location of SCEs. With BrdUrd-treated cultures, only second-division cells were scored for both SCEs and chromosomal lesions.

RESULTS

As shown elsewhere (Glover et al. 1984), aphidicolin is highly effective in inducing chromosome gaps. Because slides treated for SCE analysis were not banded, the exact location of all gaps could not be determined in these experiments. However, scoring of banded control slides showed that in 769 cells scored, 600 (43%) of 1,409 gaps occurred at six common fragile sites (viz., 2q31, 3p14, 6q26, 7q32, 16q23, and Xp22) that are among the most sensitive to aphidicolin, and 1,167 (83%) of 1,409 lesions were present at the 50 common fragile sites listed in the Human Gene Mapping 8 (HGM 8) conference report (Berger et al. 1986). These data, consistent with earlier published results (Glover et al. 1984), show that, at the relatively low concentrations used, aphidicolin induces chromosomal lesions nonrandomly in the genome. There is nothing to suggest that gaps in unbanded metaphases stained and scored for SCE analysis occurred in other locations. Thus, the majority of gaps in all cells scored were at a small number of specific sites in the genome—or, by definition, at common fragile sites.

At the concentrations used, aphidicolin only mildly increased total SCE frequency, as shown in table 1. A similar result has been reported with Chinese hamster cells (Ishii and Bender 1980). A large proportion of SCEs accounting for the increased frequencies produced in our experiments were at sites of chromosome gaps and therefore largely at common fragile sites.

An analysis of total chromosome gaps induced by aphidicolin in second-division cells shows that 53%–73% (average 66%) of all gaps were accompanied by an SCE at the site of the lesion (table 1). This effect was consistent for all three experiments and all aphidicolin treatments. As discussed above, the majority of these gaps occurred at common fragile sites.

In two experiments, gaps and SCEs occurring specifically at the common fragile sites 3p14 (FRA3B) and 16q23 (FRA16D) (Berger et al. 1986) were also tabulated. These two fragile sites were chosen because they express in high frequencies and can be easily and unambiguously scored even in unbanded chromosome preparations. Additionally, SCEs occurring at or near these sites in cells not expressing the fragile sites were tabulated as an indication of whether these fragile sites also predispose to SCE formation in cells not having

TABLE 1

INDUCTION OF SCEs AND CHROMOSOME GAPS BY APHIDICOLIN

BrdUrd Status[a]	Aphidicolin (μM)		Cells Scored			Total SCE/Cell[b]	Gaps with SCE/Total Gaps[c] (%)	
	One Cycle	Two Cycles	Experiment 1	Experiment 2	Experiment 3		Experiment 1	Experiment 2
−	...	...	55	56	60	ND	ND/0	ND/1
+	...	...	75	107	102	5.1 ± 0.05	0/1	0/1
+	0.2	...	44	100	111	6.7 ± 1.0	16/30 (53)	53/85 (62)
+	0.4	...	ND	102	113	11.6 ± 1.8	ND	261/388 (67)
+	...	0.2	108	101	ND	7.4 ± 0.02	66/115 (57)	78/139 (56)
+	...	0.4	ND	92	ND	12.7	ND	311/467 (67)

NOTE.—ND = Not determined.

[a] A plus sign (+) denotes presence of BrdUrd; a minus sign (−) denotes absence of BrdUrd.

[b] Average ± SD from three replicate experiments.

[c] No. of chromosome or chromatid gaps that also had an SCE at the site/total gaps.

TABLE 2

SCE AND CHROMOSOME GAPS AT FRAGILE SITES 3p14 AND 16q23

AMPHIDICOLIN (μM)		CELLS SCORED[a]	SCE/CELL AT fra SITE[b]	GAPS WITH SCE/TOTAL Gaps (%)
One Cycle	Two Cycles			
		A. 3p14		
...	...	284	0.10 ± 0.06	0/0
0.2	...	255	0.27 ± 0.07	29/42 (69)
0.4	...	215	0.33 ± 0.11	59/82 (72)
...	0.2	209	0.31 ± 0.17	29/43 (67)
...	0.4	92	0.37	22/35 (63)
		B. 16q23		
...	...	209	0.03 ± 0.01	0/0
0.2	...	211	0.10 ± 0.05	13/19 (68)
0.4	...	215	0.22 ± 0.15	30/37 (81)
...	0.2	101	0.14	9/10 (90)
...	0.4	92	0.22	13/24 (54)

[a] Total pooled data from three experiments.
[b] SCE occurring at or near 3p14 or 16q23 as determined from unbanded preparations. The two arms of chromosome 3 could not be differentiated in all cases in these preparations.

a gap at the site. As shown in table 2, 54%–90% (average 70%) of all gaps at the 3p14 and 16q23 fragile sites were accompanied by an SCE directly at the site. In addition, the frequency of SCEs occurring at or near 3p14 and 16q23 without the concurrent presence of a chromosome gap also increased, from untreated control values of 0.10 and 0.03 SCE/cell to 0.37 and 0.22 SCE/cell with 0.4-μM aphidicolin treatment for two cell cycles. Under these same conditions, the total SCE per cell was only approximately doubled (table 1). Examples of SCEs at FRA3B and FRA16D are shown in figure 1.

DISCUSSION

This study was designed to gain further insight into chromosome structure and behavior at fragile sites by testing the hypothesis that gaps at these sites predispose to intrachromosomal recombination as measured by SCEs. The data show that the majority of fragile sites expressed as a chromosome or chromatid gap are accompanied by an SCE at the same site. Although there was a greater scoring bias because preparations were unbanded, an increase in SCEs at or near the location of fragile sites not expressing as a gap was also observed. Previous studies have shown that numerous other agents induce chromosome gaps and breaks as well as SCEs—but have shown little correlation between the sites of the two events (Sono and Sakaguchi 1978). An exception is the observation that chromatid breaks induced by mitomycin C in both Fanconi anemia and normal cells are often accompanied by SCEs at the same sites (Latt et al. 1975). Our data clearly show that, under conditions that cause

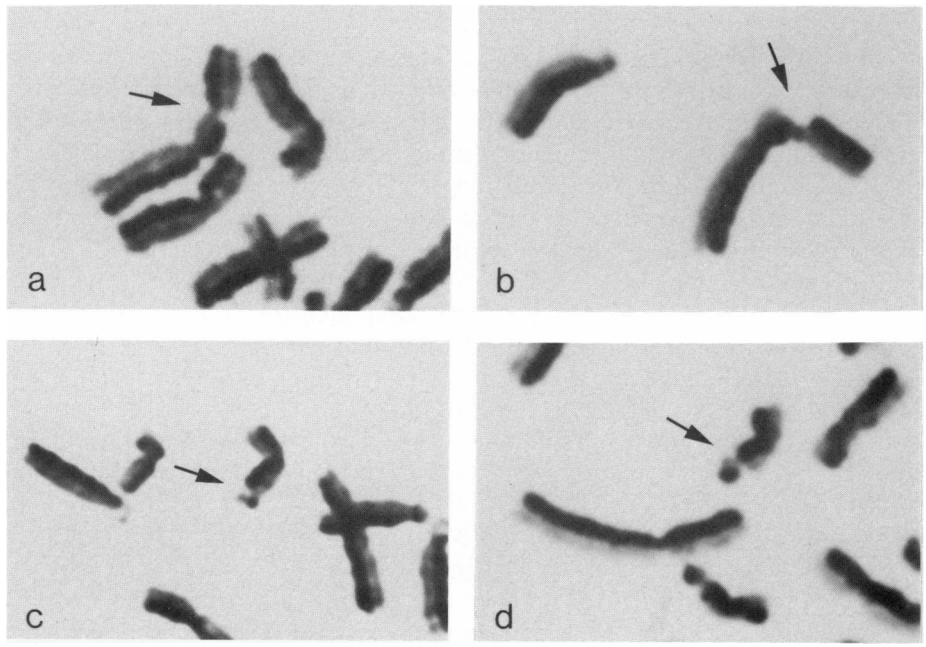

FIG. 1.—Differentially stained metaphase chromosomes with an SCE at a fragile site. *a* and *b*, 3p14; *c* and *d*, 16q23.

fragile-site expression, common fragile sites are hot spots not only for chromosomal lesions such as gaps but also for SCE formation.

In the present experiments, SCEs were scored following induction of common fragile sites. An obvious question is how this may relate to the fragile X and other rare fragile sites. As discussed above, common and rare fragile sites such as the fragile X share some similarities but also exhibit some differences. Ledbetter et al. (1986*b*) have recently suggested that the fragile X is a previously unrecognized common fragile site present on normal X chromosomes. This suggestion is based on the finding that, in hybrid cells, the X chromosomes of normal males express the fragile X. If this suggestion is correct, a high frequency of SCE at the fragile X site, as at other fragile sites, would be predicted. Available data on the SCE frequency at the fragile X site are more limited than those presented in the present paper for common fragile sites but support the suggestion that, in terms of SCE response, the fragile X behaves as do the common fragile sites.

Wenger et al. (1987) have compared SCE frequency at Xq27 in lymphocytes from fragile X males to that in lymphocytes from normal males. The frequency of SCE at Xq27 showed a significant increase for fragile X male cells grown in folic acid– and thymidine-deficient medium. An analysis of SCEs at sites expressing as a gap or break was not performed. In similar experiments with lymphoblastoid cells from a fragile X male, we have noted numerous expressed fragile X sites accompanied by an SCE at the site. However, quantitation of the

exact frequency of such occurrences has been hampered by our difficulty in accurately scoring SCEs (1) at fragile sites expressed as gaps following protocols using low BrdU concentrations that allowed fragile X expression or (2) between a unifilarly labeled chromatid and an unlabeled chromatid. Neither protocol allowed optimal chromatid differentiation but did allow fragile X expression. Using an alternative, thymidine-block, approach to induce the fragile X, Tommerup (1986) showed apparent site-specific SCE at the fragile site. In one male tested, ~50% of expressed fragile X sites were accompanied by an SCE. Thus, the limited data available suggest that, following treatments causing its induction, the fragile X site is also a hot spot for SCE.

Despite the accumulating data on SCE at fragile sites, no models have been forwarded to explain the mechanism behind this occurrence. An increasing number of studies have implicated inhibition of replication fork progression in both SCE and fragile site formation. However, the exact nature of both cytological phenomena remains unknown. It is clear, however, that DNA strand breakage and reunion occur at sites of SCEs. The observation that common fragile sites—and perhaps the fragile X—frequently are accompanied by SCEs suggests that at some point in most or all cells a DNA strand break occurs during expression of fragile sites.

To account for the findings shown in the present paper, we propose a model that is based on the replication bypass SCE model of Painter (1980) and predicts that an expressed fragile site represents a single-stranded region of DNA at an area where replication fork progression is stalled. This single-stranded region is secondarily prone to intrachromosomal recombination. Painter's model for SCE formation predicts that, following treatments that reduce or block replication fork progression, regions with replicated replicon clusters next to unreplicated DNA in adjacent clusters would be created and be prone to SCE formation. The model is based on the premise that such junctions frequently result in DNA strand breakage and reannealing in parental strands, possibly owing to the action of topoisomerases. Most, if not all, fragile sites are induced by agents that inhibit DNA synthesis by blocking replication fork displacement (Glover 1985). For unknown reasons, certain regions of the genome are more sensitive to this inhibition, thus creating junctions of unreplicated single-stranded DNA and replicated double-stranded DNA. According to the Painter model, these regions are targets for SCE formation. An SCE at a fragile site expressed as a gap in mitotic cells would result if single-strand exchange occurred between parental template strands without subsequent replication or repair synthesis to fill the gap. An SCE at a fragile site not expressed as a gap would result from strand breakage and exchange, followed by subsequent DNA replicative or repair synthesis over single-stranded regions.

This model predicts that fragile sites are unreplicated or single-stranded regions of DNA that are not resolved to double-strand form. An alternative possibility exists—i.e., that replication does proceed over these regions but does so too late in the cell cycle for normal chromosome condensation to occur. Both possibilities predict that fragile sites will be induced by conditions—folate deficiency, 5-fluorodeoxyuridine, aphidicolin, and, possibly, caf-

feine—that specifically block replication fork progression (but not necessarily initiation) and that SCEs will be seen at many expressed sites following treatment. The model does not explain why specific areas of the genome are particularly sensitive to disruption of replication fork progression, but it is conceivable that different DNA sequences vary in their susceptibility to perturbations of either rate-limiting precursors or polymerase activity.

Equal exchange of homologous DNA sequences at fragile sites would have no genetic consequences. On the other hand, unequal exchange could result in amplification of DNA sequences. Thymidylate depletion has been shown to result in various forms of intrachromosomal recombination, including unequal SCE in yeast (Kunz et al. 1986); and both aphidicolin and thymidylate stress induce common fragile sites in human cells (Daniel et al. 1984; Glover et al. 1984; Yunis and Soreng 1985). Speculations that fragile sites represent amplified DNA sequences that are due to overreplication (Schimke et al. 1986) or errors in meiotic recombination (Ledbetter et al. 1986*b*) have been made. The present study's observation of induction of SCEs at fragile sites suggests that, as a result of unequal intrachromosomal recombination, a further possible mechanism exists by means of which fragile site DNA sequences could undergo gene amplification or variation in copy number.

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