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Segregation of Recessive Phenotypes in Somatic Cell Hybrids: Role of Mitotic Recombination, Gene Inactivation, and Chromosome Nondisjunction

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Somatic cell hybrids heterozygous at the emetine resistance locus (emt^r/emt^+) or the chromate resistance locus (chr^r/chr^+) are known to segregate the recessive drug resistance phenotype at high frequency. We have examined mechanisms of segregation in Chinese hamster cell hybrids heterozygous at these two loci, both of which map to the long arm of Chinese hamster chromosome 2. To follow the fate of chromosomal arms through the segregation process, our hybrids were also heterozygous at the *mtx* (methotrexate resistance) locus on the short arm of chromosome 2 and carried cytogenetically marked chromosomes with either a short-arm deletion ($2p^-$) or a long-arm addition ($2q^+$). Karyotype and phenotype analysis of emetine- or chromate-resistant segregants from such hybrids allowed us to distinguish four potential segregation mechanisms: (i) loss of the emt^+- or chr^+-bearing chromosome; (ii) mitotic recombination between the centromere and the *emt* or *chr* loci, giving rise to homozygous resistant segregants; (iii) inactivation of the emt^+ or chr^+ alleles; and (iv) loss of the emt^+- or chr^+-bearing chromosome with duplication of the homologous chromosome carrying the emt^r or chr^r allele. Of 48 independent segregants examined, only 9 (20%) arose by simple chromosome loss. Two segregants (4%) were consistent with a gene inactivation mechanism, but because of their rarity, other mechanisms such as mutation or submicroscopic deletion could not be excluded. Twenty-one segregants (44%) arose by either mitotic recombination or chromosome loss and duplication; the two mechanisms were not distinguishable in that experiment. Finally, in hybrids allowing these two mechanisms to be distinguished, 15 segregants (31%) arose by chromosome loss and duplication, and none arose by mitotic recombination.

Recombination of genomes with subsequent reassortment of the genes is fundamental to many genetic studies. In mammalian cell cultures no natural system of recombination and reassortment exists, and therefore genetic studies rely on the segregation of genetic markers in experimentally produced somatic cell hybrids. Such hybrids, when heterozygous for a recessive marker, generate segregants displaying the recessive phenotype at a frequency considerably higher than that associated with new mutation (4, 6, 9, 10, 16, 34).

The mechanisms of segregation in hybrid cells are poorly understood. Whereas most marker segregation in interspecific (human-rodent) hybrids results from the extensive loss of chromosomes which occurs in these cells (25), intraspecific hybrids show much less extensive chromosome loss (17, 36). Despite this, our studies (6) have shown that chromosome loss is, in fact, the primary mechanism of segregation at the X-linked *hprt* (hypoxanthine phosphoribosyl transferase) locus in Chinese hamster ovary (CHO) cell hybrids. On the other hand, we have recently reported (34) that chromosome loss accounts for only 25% of segregants at the autosomal *emt* (emetine resistance) locus (8), suggesting the existence of other segregation mechanisms.

Re-expression of the recessive emetine resistance phenotype in emt^r/emt^+ hybrids could occur by at least three mechanisms other than simple chromosome loss. First, mitotic recombination between the *emt* locus and the centromere would result in a proportion of hybrid cells homozygous for the recessive marker and therefore displaying the emetine resistance pheno-

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type. Second, gene inactivation could, if it occurs in such hybrids, extinguish the expression of the wild-type gene, allowing phenotypic expression of the mutant allele. Third, loss of the chromosome carrying the wild-type allele, accompanied by duplication of its homolog carrying the recessive allele, would allow for the generation of emetine-resistant segregants with no apparent chromosome loss. A schematic diagram depicting each of these possible segregation mechanisms is shown in Fig. 1.

In the past, attempts to determine the nature of segregation have been hampered by the lack of appropriate syntenic markers necessary to follow the behavior of individual chromosomes through the segregation process. Thus, Tarrant and Holliday (29) have attempted to detect intragenic recombination at the *hprt* locus in Chinese hamster cells without success, and Rosenstraus and Chasin (24) have sought intergenic recombination between the *hprt* and *g6pd* (glucose 6-phosphate dehydrogenase) loci on the X chromosome, also without positive results. In this laboratory we have observed a polarity of linkage disruption between the two linked markers *emt* and *chr* (chromate resistance) and have suggested both mitotic recombination and gene inactivation as possible explanations (3). We were, however, unable to define the precise segregation mechanism.

In an attempt to develop a system of linked markers useful for segregation analysis, we have recently mapped the *emt* gene to the long arm of chromosome 2 in Chinese hamster cells (34) and have demonstrated linkage of the *chr* locus to the *emt* locus in these cells (3). In the accompanying paper (35) we used microcell-mediated gene transfer to map the *mtx* (methotrexate resistance) locus to the short arm of chromosome 2 and to refine the map position of the *chr* locus to the long arm near the *emt* gene.

Utilizing this set of three genetic markers,

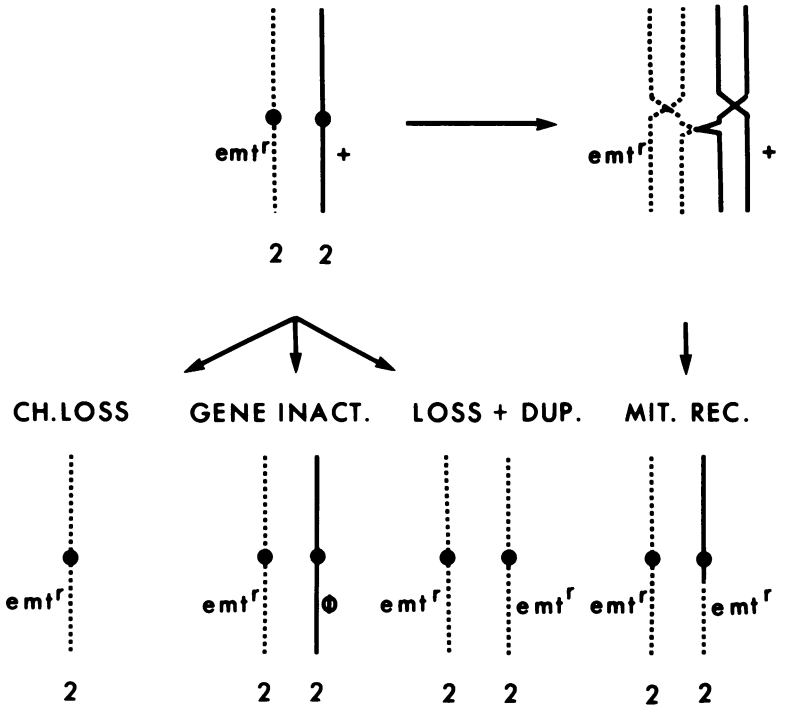

FIG. 1. *Schematic diagram of the four possible segregation mechanisms examined in this study. The chromosomes at the top left represent the two chromosomes 2 in a CHO × CHO hybrid heterozygous at the emt locus (CHO cells have only one normal chromosome 2). Emetine-resistant segregants arising by chromosome loss can be distinguished by the presence of a single chromosome 2 in segregant cells. The other three mechanisms of segregation cannot be distinguished from one another in the absence of other genetic or cytogenetic markers on chromosome 2. Gene inactivation (ϕ = inactive allele) would yield emetine-resistant segregants with no chromosomal change. Loss of the emt$^+$-bearing homolog with duplication of the emt^r-bearing homolog would also produce emetine-resistant segregants with no apparent chromosome change. Mitotic recombination at the four-chromatid stage (upper right chromosomes) followed by segregation of the first and third chromatids to the same cell yields homozygous emetine-resistant segregants, also with no visible alteration in the chromosomes.*

coupled with a pair of cytogenetic markers on Chinese hamster chromosome 2, we have been able to carry out a much more elaborate study of segregation than was previously possible. In particular, the combination of genetic and cytogenetic markers has permitted the isolation of independent segregants whose phenotype and karyotype depend specifically upon the mechanism which gave rise to the segregant. The results of this study indicate that the major mechanism of segregation in our hybrids was loss of the chromosome (or chromosomes) carrying the wild-type allele(s), accompanied by duplication of the homologous chromosome carrying the recessive mutant allele. The small proportion of segregants not accounted for by either simple chromosome loss or chromosome loss and duplication apparently resulted from gene inactivation, small deletion, or new mutation. Mitotic recombination was not observed.

MATERIALS AND METHODS

Cell lines and culture conditions. All cultures were derived from either the CHO (Chinese hamster ovary) or V79 (Chinese hamster lung) lines. Cells were maintained in monolayer cultures in alpha medium with 10% fetal calf serum at 37°C in 5% CO_2 as described elsewhere (31). The origins and descriptions of the various sublines used in this study are provided in Table 1. Our protocols for the isolation of emetine-, thioguanine-, ouabain-, and chromate-resistant mutants have been described elsewhere (2, 3). All emetine mutants used were from the same complementation class as the CHO *emt*^{r1} mutant described by Gupta and Siminovitch (8).

Selection of hybrids. Hybrid cell lines were isolated after polyethylene glycol-induced fusion by means of one of two selection procedures. Hybrids A, B, C, D, G, and H were selected in HAT + OUA medium (10 μg of hypoxanthine per ml + 1 μM methotrexate + 10 μg of thymidine per ml + 3 mM ouabain) as described previously (3). In each case, one parental cell line was resistant to HAT medium and sensitive to ouabain (cell lines LR73, LRE3, LRC3, LRC4, and Mtx^{RIII}C4), and the second parent was sensitive to HAT medium and resistant to ouabain (cell lines EO5T1, EOTC5, H1+5, and VEOT3). Hybrids E and F were derived from fusions of a ouabain-resistant cell line (LRC3O3) with a methotrexate-resistant cell line (Mtx^{RIII} or Mtx^{RIII}E1), and hybrids were selected in medium lacking nucleosides and containing 3 mM ouabain + 1 μM methotrexate.

Selection of segregants. All hybrids were heterozygous for either emetine resistance (*emt*^{r}/*emt*$^+$) or chromate resistance (*chr*^{r}/*chr*$^+$) or both. Segregants reexpressing the drug resistance phenotype were selected by plating hybrids at less than 2×10^5 cells per dish in the appropriate drug (50 μM chromate or 0.20 μM emetine). To maximize the probability that each of the segregants studied was of independent origin, the hybrid culture was first divided into several independent cultures, each initiated from about 100 cells. After a few days of growth, cells from each culture were plated in the selective medium. Only one segregant was examined from each independent culture.

TABLE 1. *Origin and description of cell lines used to construct the hybrids utilized in this study*

Cell line	Origin and description (reference)[a]	Chromosome 2[b]
EO5T1	CHO *pro*$^-$ *emt*^{r} *oua*^{R} *thg*^{r} (2)	2/Z2
EOTC5	*chr*^{r} derivative of EO5T1	
H1+5	CHO *pro*$^-$ *leuS* (Ts) *oua*^{R} *thg*^{r} *aza*^{r} *ama*^{R} (32)	
LR73	Temperature-sensitive revertant of L73, a derivative of *ts*H1 [LeuS (Ts)] selected for "reversion" of the characteristic CHO cell morphology to a more fibroblast appearance (19)	2q$^+$/Z2
LRE3	*emt*^{r} derivative of LR73	
LRC3	*chr*^{r} derivative of LR73	
LRC4	*chr*^{r} derivative of LR73	
LRC3O3	*oua*^{R} derivative of LRC3	
Mtx^{RIII}	Derivative of CHO pro$^-$ selected in two steps for resistance to methotrexate (6, 34)	2p$^-$/Z2
Mtx^{RIII}E1	*emt*^{r} derivative of Mtx^{RIII}	
Mtx^{RIII}C4	*chr*^{r} derivative of Mtx^{RIII}	
VEOT3	V79/V6 *emt*^{r} *oua*^{R} *thg*^{r} (2)	2/2

[a] Recessive genetic markers are *emt*^{r} (emetine resistance), *thg*^{r} (thioguanine resistance, HAT sensitive), *aza*^{r} (azaadenine resistance), *chr*^{r} (chromate resistance), *leuS* (Ts) (temperature sensitive leucyl transfer ribonucleic acid synthetase), and *pro*$^-$ (proline auxotrophy, a characteristic of all wild-type CHO lines). Dominant markers are *oua*^{R} (ouabain resistance), *ama*^{R} (α-amanitin resistance), and *mtx*^{R} (methotrepate resistance). All of these genetic markers are described and referenced in a review by Siminovitch (27).

[b] The marker chromosomes Z2, 2q$^+$, and 2p$^-$ are described in the text and are pictured in Fig. 2.

After 8 to 12 days in selective medium, colonies were picked and grown for a further 7 days in nonselective medium before phenotype testing. Resistance to emetine, chromate, and methotrexate was determined by plating 500 cells of each segregant into each of four wells of a 24-well plastic dish (Linbro) containing (i) normal medium, (ii) 0.25 μM emetine, (iii) 50 μM chromate, or (iv) 1 μM methotrexate. Cells were scored as resistant or sensitive (wild type) to each compound based on their ability to form colonies at these drug concentrations. Control experiments have shown that this is an accurate method of distinguishing mutant and wild-type phenotypes (3).

Karyotyping and cytogenetic nomenclature. Hybrid and segregant cell lines were harvested and karyotyped by standard techniques (33). All chromosomes were analyzed, but the results presented are restricted to chromosome 2 since the markers of interest (*emt*^{r}, *chr*^{r}, and *mtx*^{RIII}) are located on this chromosome (34, 35).

For normal Chinese hamster chromosomes we followed the standard Chinese hamster karyotype nomenclature of Ray and Mohandas (22), in which chromosomes are numbered from 1 to 10 in descending order of size, and the X chromosome is not numbered. CHO has several chromosomal rearrangements relative to diploid cells, and these marker chromosomes are labeled Z2, Z3, ... Z13 as described by Deaven and Petersen (5) and ourselves (36). Short arms of chromosomes are designated p and long arms are designated q, with + and − signs used to indicate additions or deletions of chromosomal material.

Cytogenetic markers on chromosome 2. Most CHO cell lines, including EO5T1, EOTC5, and H1+5 (Table 1), contain one normal chromosome 2 plus a chromosome called Z2 which has an interstitial deletion of about two-thirds of the long arm (5, 36). In the LR73 line (20) and its derivatives, including LRE3, LRC3, LRC4, and LRC3O3 (Table 1), the normal 2 is replaced by a marker chromosome which has an extra-long q arm (2q$^+$) that appears to be due to tandem duplication or insertion. Cell line Mtx^{RIII} (7) and its derivatives, including Mtx^{RIII}E1 and Mtx^{RIII}C4 (Table 1), have, in place of a normal 2, a marker chromosome with a reduced p arm (2p$^-$), apparently the result of reciprocal translocation between 2p and 5q (35). Derivatives of V79, including VEOT3 (Table 1), contain two normal copies of chromosome 2. A normal chromosome 2 and each of these cytogenetic markers are illustrated in Fig. 2.

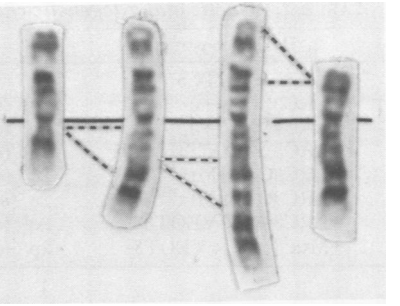

FIG. 2. *Cytogenetic markers of Chinese hamster chromosome 2. Left to right: Z2; normal 2; 2q$^+$; 2p$^-$. The dotted lines indicate the long arm interstitial deletion of the Z2, the long arm insertion or tandem duplication of the 2q$^+$ marker, and the short arm deletion of the 2p$^-$ marker, the latter resulting from a balanced reciprocal translocation with a chromosome 5. The emt and chr loci are on 2q above the upper edge of the insertion in the 2q$^+$ markers. The mtx locus is on 2p below the break point in the 2p$^-$ markers (34).*

RESULTS

Rationale. The parental cell lines used in the formation of hybrids contain either a normal 2, a 2q$^+$, or a 2p$^-$ chromosome as described above. In each of the CHO-derived cell lines the other homolog is called Z2 and consists of a chromosome 2 with an interstitial deletion of part of the long arm. Since the *emt* and *chr* genes map onto chromosome 2 in the region opposite the Z2 deletion (34, 35), all CHO lines are considered to be hemizygous at these two loci. Hybrid lines constructed by fusing an emetine-resistant or chromate-resistant CHO line with a wild-type CHO line are therefore heterozygous with the genotype *emt*^{r}/*emt*$^+$ or *chr*^{r}/*chr*$^+$. Re-expression of one of these recessive phenotypes resulting in an emetine- or chromate-resistant segregant requires the loss or inactivation of a single wild-type allele. As indicated earlier, four mechanisms are considered possibly to account for such an event. These are chromosome loss, gene inactivation, loss of one chromosome and duplication of its homolog (loss and duplication), and mitotic recombination.

As can be seen from Fig. 1, without cytogenetic markers and without genetic markers in addition to the segregating marker under selection it is possible to distinguish only one mechanism (chromosome loss) from the other three mechanisms. Our earlier experiments (34) used such hybrids and allowed us to determine that about 20 to 25% of *emt*^{r} segregants arise through chromosome loss.

To distinguish which of the three remaining mechanisms might account for the other 75 to 80% of segregants, we have utilized hybrids constructed with lines carrying cytogenetically and genetically marked chromosomes 2. These are described in Table 2 and schematically illustrated in Fig. 3 and 4. As is readily apparent from these figures, the expected karyotype and phenotype of the segregants is dependent upon the particular mechanism of segregation. Thus, an examination of the karyotypes and phenotypes of a number of independent segregants has allowed us to determine which of the three mechanisms occur and to estimate their relative frequencies of occurrence.

TABLE 2. *Genetic and cytogenetic characteristics of hybrids utilized in segregation study*

Hybrid	Hybrid construction	Genetic and cytogenetic description
A	LR73 × EOTC5	$2q^+$ (emt^+ chr^+)/2(emt^r chr^r)
B	LRE3 × H1+5	$2q^+$ (emt^r chr^+)/2(emt^+ chr^+)
C	LRC4 × H1+5	$2q^+$ (emt^+ chr^r)/2(emt^+ chr^+)
D	LRC3 × EO5T1	$2q^+$ (emt^+ chr^r)/2(emt^r chr^+)
E	LRC3O3 × Mtx^{RIII}	$2q^+$ (emt^+ chr^r mtx^+)/$2p^-$(emt^+ chr^+ mtx^{RIII})
F	LRC3O3 × Mtx^{RIII}E1	$2q^+$(emt^+ chr^r mtx^+)/$2p^-$(emt^r chr^+ mtx^{RIII})
G	LRC3O3 × VEOT3	$2q^+$ (emt^+ chr^r)/2(emt^r chr^+)/2(emt^r chr^+)
H	Mtx^{RIII}C4 × VEOT3	$2p^-$ (emt^+ chr^r mtx^{RIII})/2(emt^r chr^+ mtx^+)/2(emt^r chr^+ mtx^+)

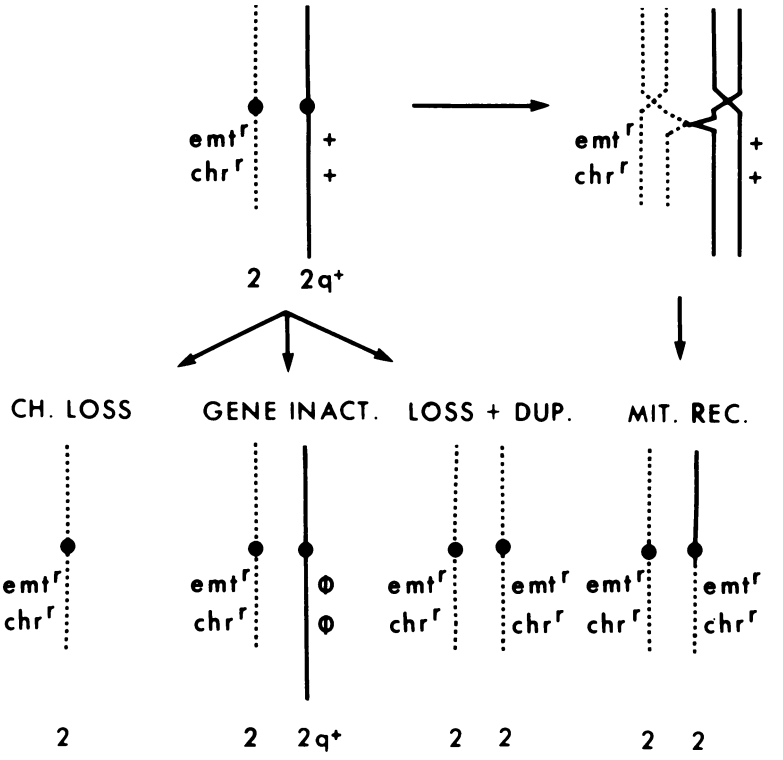

FIG. 3. *Expected genotype and cytogenetic configuration of hybrid A segregants for different mechanisms of segregation. Chromosome loss, 2/0; gene inactivation, 2/2q+ (inactivated alleles are represented by ϕ); chromosome loss and duplication, 2/2; mitotic recombination, 2/2. A crossover between nonsister chromatids with one parental chromatid and one recombinant chromatid traveling to the same pole at mitosis will result in a daughter cell homozygous for everything distal to the crossover, including emt^r, chr^r, and the cytogenetic marker. Any combination of chromatids other than the one indicated will not result in a drug-resistant segregant.*

Segregation in CHO × CHO hybrids with markers on the long arm of chromosome 2. The first four hybrids examined, A to D (Table 2), contained one normal chromosome 2 and one chromosome $2q^+$ carrying different combinations of genetic markers. For example, in hybrid A the normal 2 carried emt^r and chr^r, and the $2q^+$ carried the wild-type alleles, emt^+ and chr^+. Figure 3 illustrates the expected chromosomal constitution for emetine- or chromate-resistant segregants of hybrid A under each of the four proposed models of segregation. Chromosome loss will yield segregants that have lost the $2q^+$ chromosome carrying the wild-type alleles and will thus contain a single chromosome 2. Gene inactivation will result in segregants which have maintained the hybrid chromosome constitution of $2/2q^+$. Loss of the $2q^+$ chromosome with duplication of the normal chromosome 2 will give rise to homozygous drug-resistant segre-

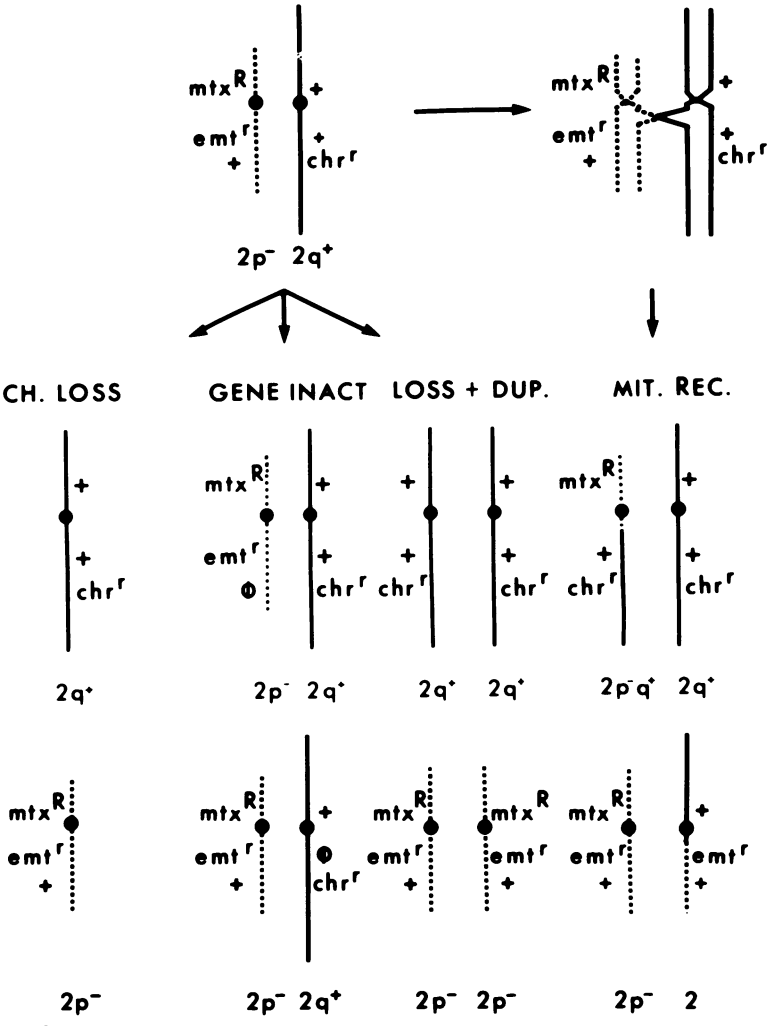

FIG. 4. *Expected genotype and cytogenetic configuration of hybrid F segregants for different mechanisms of segregation. The expectations are different for chromate-resistant segregants (middle set of chromosomes) and for emetine-resistant segregants (lower set of chromosomes).*

gants with a 2/2 constitution. Mitotic recombination between chromosomes 2 and $2q^+$ as described in Fig. 3 will also give rise to segregants containing two copies of the normal chromosome 2. Hybrids B, C, and D contain chromosomes which are structurally similar to those in hybrid A but which carry a different combination of genetic markers so that the expected karyotype associated with each segregation mechanism is also different.

The expected karyotype for each segregation mechanism is presented in Table 3 together with the number of segregants observed with each of these karyotypes. Among 27 independent segregants from hybrids A to D, 4 arose by chromosome loss whereas 21 were consistent with either mitotic recombination or chromosome loss and duplication. (These two mechanisms cannot be distinguished in these hybrids.) One segregant was classified as the result of gene inactivation; however, since this class of segregants was rare it could, in fact, have arisen by a new mutation or small deletion. In a few segregants described in Table 3, secondary alterations involving chromosome 2 were observed, but in only one case did this result in a segregant which could not be classified according to one of the postulated segregation mechanisms.

Segregation in CHO × CHO hybrids with markers on both arms of chromosome 2. To distinguish mitotic recombination from chromosome loss and duplication, the presence of

TABLE 3. *Classification of segregants from hybrids A to D according to mechanism of segregation as determined by karyotype analysis*

Hybrid	Segregant selection	Total no. of segregants	Chromosome loss		Gene inactivation		Mitotic recombination, loss and duplication	
			Expected karyotype	No. of segregants	Expected karyotype	No. of segregants	Expected karyotype	No. of segregants
A	EMT	3	2/0	0	$2/2q^+$	0	2/2	3
	CHR	4	2/0	0	$2/2q^+$	0	2/2	4
B	EMT	3	$2q^+/0$	0	$2/2q^+$	0	$2q^+/2q^+$	3
C	CHR	4	$2q^+/0$	2	$2/2q^+$	0	$2q^+/2q^+$	2[a]
D	EMT	5	2/0	0	$2/2q^+$	0	2/2	5
	CHR	8[b]	$2q^+/0$	2[c]	$2/2q^+$	1	$2q^+/2q^+$	4[d]

[a] Both of these segregants exhibited some cells containing $2q^+/0$.

[b] One segregant had a karyotype which was either $2q^+/2q^-$ or $2q^+/(2q^+)q^-$ and therefore could not be unambiguously assigned.

[c] Both of these segregants lost the long arm of 2 only. The short arm of 2 was involved in a translocation.

[d] One of these segregants had in addition to $2q^+/2q^+$ a new chromosome consisting of the long arm of Z2 fused to the long arm of the $2q^+$.

genetic or cytogenetic markers is required on both arms of chromosome 2. Since the methotrexate-resistant Mtx^{RIII} cell line contains a $2p^-$ chromosome carrying a deletion of the short arm, any hybrid between this line and LR73 or one of its derivatives will have the appropriate cytogenetic markers to distinguish between these two mechanisms. Two such hybrids were constructed (hybrids E and F, Table 2), and unlike the previous hybrids, a unique cytogenetic result is predicted for each of the four proposed mechanisms of segregation. (See expected karyotypes of both *chr*^{r} and *emt*^{r} segregants of hybrid F in Fig. 4.) The chromosomal and genetic constitution of hybrid F is $2p^-$ (*emt*^{r} *chr*$^+$ *mtx*^{R})/$2q^+$(*emt*$^+$ *chr*^{r} *mtx*$^+$). Chromosome loss will produce chromate-resistant segregants carrying a single $2q^+$ and emetine-resistant segregants carrying a $2p^-$. Gene inactivation (or small deletion or new mutation) will result in either *emt*^{r} or *chr*^{r} segregants with the original hybrid chromosomes $2p^-/2q^+$. Chromosome loss and duplication will produce chromate-resistant segregants which contain $2q^+/2q^+$ and emetine-resistant segregants with $2p^-/2p^-$. Mitotic recombination will result in segregants containing one intact hybrid chromosome and one recombinant chromosome. For chromate-resistant segregants the recombinant chromosome ($2p^-q^+$) would have the short arm of the $2p^-$ and the long arm of the $2q^+$. Emetine-resistant recombinants would carry chromosomes $2p^-/2$, the normal chromosome 2 resulting from recombination of the normal short arm of the $2q^+$ with the normal long arm of the $2p^-$ (Fig. 4).

The expected and observed karyotypes of the 21 segregants selected from hybrids E and F are presented in Table 4. Despite the presence of a few secondary rearrangements involving chromosome 2, all 21 segregants could be classified. It is clear from Table 4 that the major mechanism of segregation in these experiments was chromosome loss and duplication and not mitotic recombination.

Phenotype testing confirmed that segregants were either emetine or chromate resistant and revealed that all segregants retaining the short arm of the $2p^-$ chromosome also retained resistance to methotrexate. Conversely, those segregants which had lost the entire $2p^-$ chromosome also lost their resistance to methotrexate. This is in agreement with evidence mapping the mtx^{RIII} marker to the short arm of the $2p^-$ chromosome (35).

From all of the above experiments, representing karyotype analysis of 48 independent segregants, 36 segregants (75%) had karyotypes consistent with loss of the chromosome carrying the wild-type allele with duplication of the chromosome carrying the drug-resistant mutant allele, 9 (20%) were clearly most compatible with simple chromosome loss or deletion, and 2 segregants (4%) were apparently the result of gene inactivation, small deletion, or new mutation. One segregant had a karyotype which could not be unambiguously assigned.

Segregation in CHO × V79 hybrids. Chromosome loss and duplication could result from one of two different mechanisms. In the simplest case, loss and duplication represents two separate nondisjunction events occurring at different points in time. If hybrids with an extra or missing chromosome 2 have a growth disadvantage, then the second event compensates for the first and restores full growth potential to the cell. Alternatively, chromosome loss and duplication might be viewed as a single event due to some form of homolog pairing followed by aberrant

TABLE 4. *Classification of segregants from hybrids E and F according to mechanism of segregation as determined by karyotype analysis*

Hybrid	Segregant selection	Total no. of segregants	Chromosome loss		Gene inactivation		Mitotic recombination		Loss and duplication	
			Expected karyotype	No. of segregants	Expected karyotype	No. of segregants	Expected karyotype	No. of segregants	Expected karyotype	No. of segregants
E	CHR	8	$2q^+/0$	3[a]	$2q^+/2p^-$	0	$2q^+/2p^-q^+$	0	$2q^+/2q^+$	5[b]
F	CHR	6	$2q^+/0$	2[a]	$2q^+/2p^-$	1	$2q^+/2p^-q^+$	0	$2q^+/2q^+$	3[c]
	EMT	7	$2p^-/0$	0	$2q^+/2p^-$	0	$2p^-/2$	0	$2p^-/2p^-$	7[d]

[a] One *chr*^{r} segregant from each of hybrids E and F lost only the long arm of the $2p^-$ chromosome, but these were classified as chromosome loss events.
[b] One of the segregants also contained an extra Z2. A second contained an addition on the short arm of one $2q^+$ [i.e., $2q^+/(2q^+)p^+$]. A third contained some cells which were $2q^+/0$.
[c] These segregants contained an extra Z2.
[d] One segregant contained an extra Z2. A second contained in addition to $2p^-/2p^-$ an iso($2q^+$)p. Two other segregants contained some cells which were $2p^-/2p^-$ and others exhibiting secondary alterations [additions or deletions on $(2p^-)$p and $(2p^-)$q].

segregation of the four chromatids. To distinguish between these possibilities we examined segregation in CHO × V79 hybrids.

In previous studies of segregation at the *emt* locus (34) it was found that in CHO × V79 hybrids of presumptive genotype *emt*$^+$/*emt*^{r}/*emt*^{r} and karyotype 2/Z2/2/2, segregation was not associated with the consistent loss of a chromosome 2, whereas in karyotypically similar hybrids of presumptive genotype *emt*^{r}/*emt*$^+$/*emt*$^+$, the loss of expression of two wild-type alleles was correlated with the loss of a single chromosome 2. Since these events result in segregants with three and two copies of chromosome 2, respectively, it suggests that optimization of growth through reestablishment of a balanced karyotype is not the major factor in determining the final karyotype.

This problem was examined further through the use of cytogenetically marked chromosomes in hybrids G and H (Table 2). These hybrids were constructed by fusing VEOT3 with either LRC3 (hybrid G) or $Mtx^{RIII}C4$ (hybrid H). The CHO parental line contributes one of the marker chromosomes $2q^+$(LRC3) or $2p^-(Mtx^{RIII}C4)$ carrying *emt*$^+$ and *chr*^{r}. The V79 line, VEOT3, contributes two chromosomes 2 and presumably two copies of the *emt*^{r} and *chr*$^+$ alleles to the hybrid. The hybrids therefore have genotype *emt*$^+$ *chr*^{r}/*emt*^{r} *chr*$^+$/*emt*^{r} *chr*$^+$ and karyotype $2q^+/2/2$ (hybrid G) or $2p^-/2/2$ (hybrid H).

Eight emetine-resistant and eight chromate-resistant segregants were examined from hybrids G and H. Among the eight emetine-resistant segregants one had a 0/2/2 constitution (chromosome loss), one retained the parental $2p^-/2/2$ constitution (gene inactivation), and six contained three copies of the normal chromosome 2, demonstrating loss of the marker chromosome (either $2p^-$ or $2q^+$) and duplication of one of the V79 chromosomes.

Among the eight chromate-resistant segregants, in which two copies of the *chr*$^+$ gene had to be lost or inactivated, one had a $2q^+/0/0$ constitution (double chromosome loss), one had a $2q^+/2/0$ constitution (loss + gene inactivation), and six had either $2q^+/2q^+/0$ (hybrid G) or $2p^-/2p^-/0$ (hybrid H), demonstrating loss of both chromosomes 2 from the V79 parent and duplication of only one of the marker chromosomes.

Again, loss and duplication appear to be the primary segregation mechanism, except that when two alleles must be lost only one of the two lost chromosomes is replaced. The fact that most emetine-resistant segregants did not simply lose the *emt*$^+$-bearing chromosome suggests that the duplication of the other homolog may be a coupled event.

DISCUSSION

Several segregation mechanisms have been observed in or attributed to eucaryotic cells. The first, mitotic recombination, has been studied most extensively in the diploid cells of lower eucaryotes (21) and the somatic cells of fruit flies (28). Although there is circumstantial evidence favoring the existence of mitotic recombination in mammalian cells, this evidence is predominantly cytological and is based upon the observation in cultured cells of chromosome configurations which could be recombination intermediates. The frequency of these quadriradial figures is increased in some human diseases (14, 30) and in cells treated with chemicals known to induce recombination in lower eucaryotes (12, 26). The presence of sister chromatid exchanges in cultured cells (15) has often been cited as evidence that the necessary enzymes for breaking and recombining deoxyribonucleic acid molecules are present in these cells. However, sister chromatid exchange is not equivalent to mitotic recombination, since the latter involves exchanges between nonsister chromatids and the mechanism may be quite different.

Genetic studies designed to detect mitotic recombination involving the X chromosome of Chinese hamster cells have not succeeded in demonstrating its occurrence. Thus, Tarrant and Holliday (29) found no evidence for intragenic recombination at the *hprt* locus in heterozygous hybrids, and Rosenstraus and Chasin (24), in an extensive study of linkage disruption between the *hprt* and *g6pd* loci, concluded that mitotic recombination was not involved.

Although our present study could certainly have detected mitotic recombination between homologous regions on chromosome 2 of Chinese hamster, we could find no evidence for such events. Complicating factors possibly precluding recombination in our particular hybrids are discussed later.

Another segregation mechanism considered for certain somatic cell hybrids is gene inactivation wherein re-expression of the recessive phenotype in heterozygous hybrids is due not to physical segregation of the gene, but rather to inactivation of the wild-type allele. Studies by Harris (10, 11) on re-expression of the recessive phenotype in tk^+/tk^- hybrids, and similar studies by Bradley (1) in diploid cells, have suggested a role for epigenetic events acting to switch off the tk^+ (thymidine kinase) gene. Our finding of a few segregants which retained the chromosome constitution of the parental hybrid is consistent with gene inactivation in these cells. The fact that these segregants were rare (frequency less than 10^{-5}), however, means that mutation or a small undetected deletion cannot be ruled out. Clearly, the proof of inactivation must ultimately rely on the demonstration of reactivation of the wild-type allele.

The third and most well-known type of segregation in somatic cell hybrids is chromosome loss resulting from a high frequency of nondisjunction. In interspecific hybrids the chromosomes of one parent often undergo extensive loss, allowing the mapping of the genes of that species (25). Although intraspecific hybrids appear to have more stable karyotypes (17, 36), our recent studies in CHO × CHO hybrids have shown that chromosome loss or deletion accounts for the majority of segregants in $hprt^+/hprt^-$ hybrids and about 25% of segregants in emt^+/emt^r hybrids (6, 34). Our present results confirm the latter figure for the frequency of simple chromosome loss but reveal that among the remaining segregants, most have arisen by loss of the chromosome carrying the wild-type allele and duplication of the homologous chromosome carrying the recessive mutant allele.

Segregation by loss and duplication has been observed previously in *Aspergillus* (13) and in *Saccharomyces* (19). If, as Kafer (13) suggested for *Aspergillus*, such loss and duplication is the result of two successive nondisjunction events, then the difference in segregation between the *hprt* and the *emt* locus may be more apparent than real. The difference may simply reflect the ability of cultured cells to tolerate variation in the number of X chromosomes more easily than variation in the number of autosomes, such that loss of a chromosome 2 requires a second nondisjunction event to restore the growth potential of the cell. Although this possibility seems to offer the most reasonable explanation to account for segregants from CHO × CHO hybrids, it poses some problems in explaining the CHO × V79 data. Specifically, in these hybrids it was observed that segregants contained either three chromosomes 2 or two chromosomes 2, depending upon whether segregation involved one or two wild-type alleles, respectively. If chromosome loss and duplication occur because cells with a particular numerical complement of chromosomes are more viable than others, then both the emetine-resistant and the chromate-resistant segregants of hybrids G and H would be expected to contain the same number of chromosomes 2. The fact that they do not suggests that differences in viability cannot adequately explain loss and duplication, leaving the interesting possibility that the loss and duplication events may be linked.

The general applicability of our conclusions

regarding segregation must be viewed with some caution because of the possibility that the results were influenced by the presence of the cytogenetic markers. In particular, our lack of evidence for mitotic recombination could be attributed to one of at least four possibilities: (i) recombinant chromosomes involving cytogenetic markers may be lethal to the cell; (ii) cytogenetic markers on chromosome 2 may themselves inhibit recombination; (iii) the presence of other rearranged chromosomes in the genome may also prevent recombination; (iv) segregants arising from mitotic recombination occur at a frequency of less than 10^{-5} in our cultures and therefore were not detected against the background frequency of loss and duplication.

The absence of mitotic recombinants among the chromate-resistant segregants of hybrids E and F in which recombination would result in a chromosome constitution of $2q^+/2p^-q^+$ could be explained by lethality of this particular chromosome combination, since it has never been observed. However, emetine-resistant segregants of hybrid F arising through a recombination event would contain a normal chromosome 2 and a $2p^-$, a combination which is known to be viable in other hybrids. Hence, the nonviability of cells carrying recombinant chromosomes is probably not responsible for the failure to detect recombination in these hybrids.

The possibility that the cytogenetic markers on chromosome 2 might interfere with normal recombination along that chromosome cannot be completely excluded since chromosomal rearrangements have been shown to influence recombination in *Drosophila* (18). However, a comparison of the frequency and type of segregants arising from hybrids with and without cytogenetic markers tends to argue against this possibility, since segregants from both types of hybrids arose with the same frequency (about 10^{-4}) and displayed chromosome combinations that could be accounted for by the same combination of mechanisms described above.

With regard to point (iii) above, there is evidence in *Drosophila* that mitotic recombination can be influenced by rearrangements of chromosomes other than those directly involved in the recombination event (23). Since the karyotype of CHO contains multiple chromosomal rearrangements, mitotic recombination may be inhibited in CHO relative to normal diploid cells. Hence, the role of mitotic recombination in normal mammalian cells deserves further study.

Before leaving the discussion of mitotic recombination it should be pointed out that in a recent paper (3) we reported a polarity of linkage disruption between the *emt* and *chr* loci suggestive of mitotic recombination with *chr* distal to *emt* on the long arm of chromosome 2. A similar polarity has not been observed in our studies with cytogenetically marked chromosomes. In a total of 35 segregants selected from hybrids of karyotype $2/2p^-$, $2/2q^+$, or $2p^-/2q^+$ and of genotype $emt^r\ chr^r/emt^+\ chr^+$, only 3 have shown linkage disruption. One of these was selected in emetine and remained sensitive to chromate, whereas the other two were selected in chromate and remained sensitive to emetine. Whether or not the polarity observed previously is a characteristic peculiar to hybrids with normal number 2 chromosomes remains to be determined.

Finally, the existence of chromosome loss and duplication as a mechanism of segregation in somatic cell hybrids raises an important point with regard to the feasibility of using intraspecific cell hybrids for mapping studies. Chromosome loss and duplication allow for the loss of whole chromosomes without any net alteration in karyotype, and hence, in the absence of suitable markers, such chromosome loss will remain undetected. On the other hand, even though segregation analysis of intraspecific cell hybrids may not prove to be useful as an initial mapping tool, having established that segregation of markers on chromosome 2 generally involves loss of an entire chromosome, it should be possible to map other markers to chromosome 2 by testing for cosegregation with the *emt* and *chr* loci. Wasmuth and Chu (32) have recently mapped the gene coding for leucyl transfer ribonucleic acid synthetase to Chinese hamster chromosome 2 by utilizing this approach.

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