Site-Specific X-Chromosome Rearrangements from Hybrid Dysgenesis in *Drosophila melanogaster*

Abstract. *When the germ line of* Drosophila *males is destabilized by a syndrome known as hybrid dysgenesis, X-chromosome rearrangements are found in up to 10 percent of the gametes produced. Some of these aberrations are simple inversions, but many are complex multibreak rearrangements. Furthermore, most of the breakpoints fall into a few highly localized positions on the chromosome. These positions are mostly at points of intercalary heterochromatin and may vary from one strain to the next. The results suggest that they may represent points of insertion of mobile DNA sequences.*

Hybrid dysgenesis is the name given to a set of chromosome-cytoplasm interactions in *Drosophila* (*1*). These interactions cause various germ line abnormalities including sterility, high mutability, and male recombination (*2–5*). In addition, high frequencies of chromosomal rearrangements in the absence of known mutagens have been reported by Voelker (*6*) and Yamaguchi and Mukai (*7*).

Subsequent work (*8, 9*) has shown that the production of such rearrangements follows the characteristic rules that apparently define the syndrome of hybrid dysgenesis. That is, the rearrangements were produced by offspring from females of a maternally contributing strain (M strain 1) and males from a paternally contributing strain (P strain 1), but their production by the reciprocal hybrids or nonhybrids was greatly reduced or absent (*8, 9*). Chromosome aberrations have also turned up in connection with hybrid dysgenesis–induced mutability (*10*).

We report that X-chromosome inversions and complex multibreak rearrangements can occur in fully 10 percent of the X chromosome in sperm of dysgenic hybrids. Cytological analysis of these and other rearrangements induced by hybrid dysgenesis reveals that most of the breakpoints occur at a few precise hot spots, thus confirming an earlier suggestion (*7*). Other points (''warm spots'') also tend to recur, but with less regularity. Two of the hot spots are at constrictions of the polytene chromosomes, which are thought to be regions of intercalary heterochromatin (*11, 12*) and may be involved with pairing (*13*). Breakage at one of the hot spots appears to cause male lethality, and one warm spot is associated with the locus of a visible recessive mutation known to be destabilized by hybrid dysgenesis. By comparing the X chromosomes of two different P strains, we found that both had hot spots and warm spots but that their positions differed markedly between the two strains. These results invite interpretations in terms of models of hybrid dysgenesis based on mobile sequences of middle-repetitive DNA.

High frequencies of inversions were first observed as an interesting nuisance in an experiment designed to map the chromosomal component of hybrid dysgenesis (the P factor) on the X chromosome (*14*). In a two-generation crossing procedure (see legend to Fig. 1) dysgenic males were produced whose X chromosome, but not autosomes, originated from π_2, a P strain already described (*15*). These males were then mated to females homozygous for *w* (white eyes), *m* (miniature wings), and *f* (forked bristles)—genes that occupy widely separated positions on the X chromosome (*16*) (Fig. 1A). Thus inversions produced by the dysgenic males were detected by the suppression of crossing-over in their inversion-carrying daughters, which were heterozygous for *w*, *m*, and *f*. The X chromosome of the subline of π_2 used in our experiment carries a small inversion near its tip, lowering the frequency of recombination between *w* and *m* in all these females. However, it did not prevent detection of newly arising inversions. Five other sublines of π_2 examined all lack inversions.

Two features of the mating scheme leading to the dysgenic males should be noted. (i) All crosses were performed individually so that the generation in which any inversion occurred could usually be ascertained from the pattern of clustering. For example, if all daughters of a given dysgenic male carried the same inversion, we would infer that the inversion was produced by the male's father. (ii) The parents of the dysgenic males were not themselves expected to be dysgenic by the usual rules of hybrid dysgenesis (*17*).

An average of three daughters from each of 44 dysgenic males was tested, and 13 of 129 carried inversions or complex rearrangements. This frequency is approximately 50 times greater than that observed by Yamaguchi and Mukai (*7*). The experiment was repeated with an inversion-free subline of π_2 and yielded a similar rate (12 of 143). Several lines of evidence point to this extraordinary frequency as resulting from hybrid dysgenesis. (i) Despite the presence of the one previously existing inversion, there is no comparable frequency of rearrangement production in the π_2 strain itself. Summing over the five π_2 sublines tested since 1975, we see that the intrastrain frequency of inversion production is about one in 600 generations as opposed

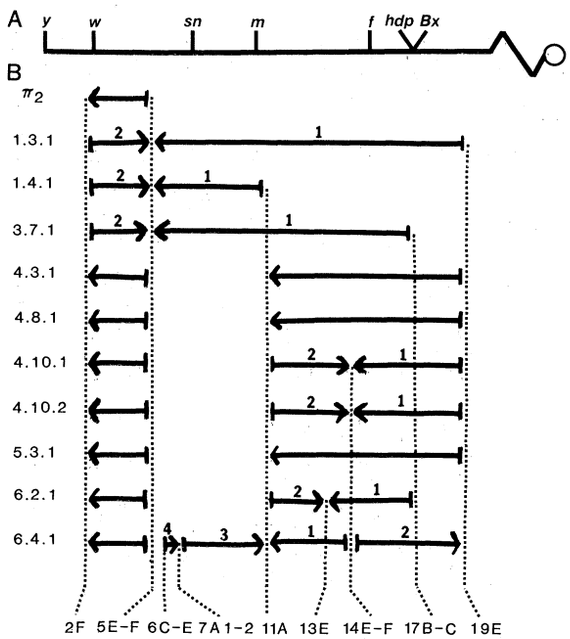

Fig. 1 (A) Map of the X chromosome of *Drosophila*. (B) Configuration of the parent chromosome (π_2) and ten rearrangements [see (*12*) and (*16*) for band designations]. The arrow indicates the end of a segment that is normally proximal, and numbers above segments specify the final order (left to right) of each segment within a rearranged group. Thus, the actual sequence of chromosome 6.4.1, for example, is: 1A — 2E|5E – 2F|5F — 6C-E|14D-E — 11A3|14F — 19E1-2|7B — 11A1-2|6D-E — 7A6|19E — 20. The crosses were as follows. Females from the P strain π_2 and males from *bw;st*, a standard M strain with eye color markers brown (*bw*) and scarlet (*st*) on the second and third chromosomes (*16*), produced nondysgenic males of the genotype π/Y; π/*bw*; π/*st*. These were mated to females from an M stock similar to *bw;st* except that they also carried the compound X chromosome C(1)DX, *yf* (*y* = yellow body) (*16*). Sons from this cross of the genotype π/Y; *bw*/*bw*/ *st*/*st* are the dysgenic males. These crosses were carried out at room temperature (21°C).

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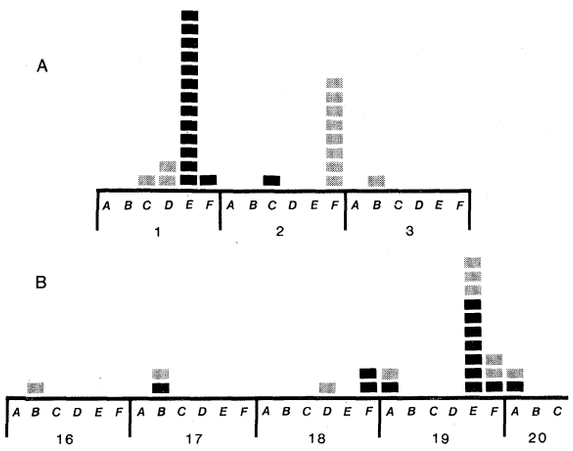

Fig. 2. Distributions of breaks among free duplications and X-Y translocations on the X chromosome of either Madison 75 (solid) or π_2 (shaded). They were detected as duplications of the wild allele of *y* (yellow body) (*16*) in the daughters of compound X females homozygous for *y* mated to dysgenic males with y^+. Each duplication has a break in the distal X (A) and some in the proximal euchromatin (B) as well. Those not represented in (B) are presumed to have another break in either the X or Y centric heterochromatin. No breaks in the middle section of the X were expected because long duplications would lead to hyperploid effects (inviability or sterility) in the bearers.

to one in ten in hybrids. (ii) All but one of the 13 inversion-carrying females had at least one inversion-free sister (excluding the previously existing inversion), indicating that chromosome breakage took place in the germ line of her dysgenic father rather than her nondysgenic grandfather. In the case of the one exception (designated 1.3.1), the time of origin was ambiguous because no sisters of the inversion-bearing female were analyzed. (iii) There was a sibship of three females in which two had identical complex rearrangements (4.10.1 and 4.10.2) and a third had no new inversions. This case illustrates the clustering effect characteristic of premeiotic events, which are a common feature of hybrid dysgenic traits (*8*).

Figure 1 shows the results of examination of the polytene chromosomes of ten of the rearrangements and of the parent chromosome. Dysgenesis-induced sterility caused the other three to be lost before they could be analyzed. Many of the rearrangements are not simple inversions but complex rearrangements involving three or more breakpoints. These multiply broken chromosomes could have come about as a sequence of simple inversions with one or more common endpoints. If this is so, this series of events must have taken place during the development of the germ line of a single individual in the case of 4.10.1 and 4.10.2, which form a cluster. In all other cases, we can say that it happened in no more than two (or in one case, three) generations, because cytological analysis took place in the first (in one case, the second) generation following detection of the inversion. Even the most complex rearrangement (6.4.1) can be produced from the parent chromosome by a series of only three inversions. All others require fewer inversions.

The other important feature shown in Fig. 1 is the repeated and independent occurrence of certain breakpoints. In this case, of the 26 new breaks, only three are unique. Regions 11A and 19E of the polytene chromosomes are the most common, accounting for more than half of all newly induced breakpoints in Fig. 1. These two regions are well-known "constrictions" of the polytene X chromosome; they are thought to be underreplicated and may represent intercalary heterochromatin (*12*). They have also been shown to be involved in the control of meiotic pairing (*13*). Two other locations on the X chromosome, 3C5 and 7A–E, are also pairing sites (*13*), but no breaks were observed in 3C5 and only one was observed in 7A–E. Nor were any breakpoints observed in region 12D–E, which is another major constriction.

All but three of the 13 rearrangements were found to be lethal in the hemizygous state. These three correspond to the only chromosome in Fig. 1 that lacks the 19E breakpoint, suggesting that this point or an associated position effect is the cause of lethality. Males carrying two of the nonlethal chromosomes (3.7.1 and 6.2.1) exhibit the raised wing phenotype of *hdp* (heldup wings) (*16*), which maps at or near the 17C breakpoint shared by these two chromosomes. Females homozygous for either of these as well as their common heterozygote also have raised wings. This observation shows the precision with which these breakpoints recur. It should be mentioned that mutants of this phenotype have frequently been seen among progeny of dysgenic flies (*2*) as have the dominant *Bx* (Beadex wings) (*16*) mutants (*9*). *Bx* maps very close to *hdp*, and mutational analyses indicate a functional relation between the two loci (*18*). However, the other most common mutation associated with hybrid dysgenesis is *sn* (singed bristles) (*16*) at region 7D1–2 where no breakpoints were found.

To determine whether or not the positions of these breakage points were specific to π_2 or varied from one P strain to the next, we examined a series of rearrangements produced by dysgenic males from either π_2 or another P strain designated Madison 75. The rearrangements were detected by the method of Painter and Muller [(*19*); see also (*8*, *9*) and the legend to Fig. 2], which screens for duplications of the tip of the X chromosome joined to either the proximal euchromatin of the X or the centric heterochromatin of the X or Y chromosome. Internal hot spots are not expected to show up in this experiment. As might be expected from the results described above, most of the distal breaks on the π_2 chromosome occurred at the hot spot 2F (Fig. 2A). However, the distribution of distal breaks from Madison 75 differed sharply from π_2, with most of the breaks at region 1E. Most of these duplications also had breaks in the proximal euchromatin of the X chromosome as shown in Fig. 2B. The two distributions are similar with breakage in both strains that occur preferentially at region 19E, a hot spot. Thus the positions of at least some breakage regions can vary from strain to strain. Another case of spontaneous X-chromosome rearrangements (*20*), with crosses unlikely to involve hybrid dysgenesis, revealed hot spots at three other locations: 6E1–2, 7C, and 11A. Only the last corresponds to one of the π_2 hot spots.

One of the duplications from π_2 is of particular interest because its breakpoints (3B and 17B) are identical to those of an inversion in the parental chromosome produced by an ancestral dysgenic hybrid. Another case in which a dysgenesis-induced inversion gave rise to the corresponding deletion was recently observed by Simmons and Lim (*10*).

The apparent relation we observed between breakage regions and meiotic pairing sites brings to mind Sved's (*21*) model of hybrid dysgenesis in terms of the spatial organization of chromosomes. Although our observations were not specifically predicted under Sved's model, they might easily be accommodated by it.

Another possibility is that hybrid dysgenesis is the result of interactions involving mobile DNA sequences analogous to insertion elements, plasmids,

and episomes in prokaryotes (*9, 22, 23*). Such sequences are known to exist in *Drosophila* (*24, 25*), comprising most of the moderately repetitive DNA—about 17 percent of the *Drosophila* genome. Key properties of these elements, designated "nomadic sequences," are their presence at multiple locations in the genome and the strain variations in these positions. The P factor, defined in terms of the induction of hybrid dysgenic traits, has been shown to possess these properties (*17, 26*), and our results show that breakage sites possess them as well. We suggest that the breakage regions represent points of insertion of one of the nomadic sequences and that the P factor itself is also a nomadic sequence, perhaps the same one. The relation between hybrid dysgenesis and nomadic sequences is also indicated by recent indirect evidence suggesting that nomadic sequences also exist as free circles in the nucleoplasm (*27*). If these circles can replicate there independently of their chromosomal counterparts, they could provide an ideal basis for the unusual mixture of chromosomal and extrachromosomal inheritance followed by cytotype—the other component of hybrid dysgenesis (*17*). One might expect these sequences to be found preferentially in regions of intercalary heterochromatin where they are less likely to be selected against, thus explaining the correspondence between constrictions and the hot spots.

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Tumor Promoter TPA Mimics Irradiation Effects on the Cell Cycle of HeLa Cells

Abstract. *When asynchronous and synchronous HeLa cells were incubated with small doses (10^{-7}M) of tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA), a variety of transient alterations in the replication cycle were detected within 24 hours by the use of independent methods. Especially, a delayed passage through the S phase and influences on the G2 phase resemble x-ray irradiation effects on cell cultures. None of these alterations was observed with the hyperplasiogenic but nonpromoting 4-O-methyl-TPA.*

It is generally accepted that carcinogenesis is usually a multistep process, as illustrated by the two-stage mouse skin model (*1*). Initiation and subsequent promotion are elicited by separate classes of chemicals. Tumor promoters are regarded neither as carcinogenic (*2*) nor as mutagenic (*3*). They markedly increase tumor frequency and shorten the latency period of tumor appearance when administered after an initiator. Despite a number of models (*2*), the mechanism of the promoter action is not well understood, since the effects of the potent tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (*4*) seem to be reversible (*5*) when dealing with cells not previously treated with a carcinogen. We have shown that HeLa cells represent a good system for the study of the TPA action (*6*). These cells respond to a large series of phorbol derivatives, their effectiveness corresponding closely to their promoting ability in mouse skin. We (*7*) and others (*8*) had obtained indications that tumor-promoting TPA might specifically interfere with the replication cycle. This study has now elabo-

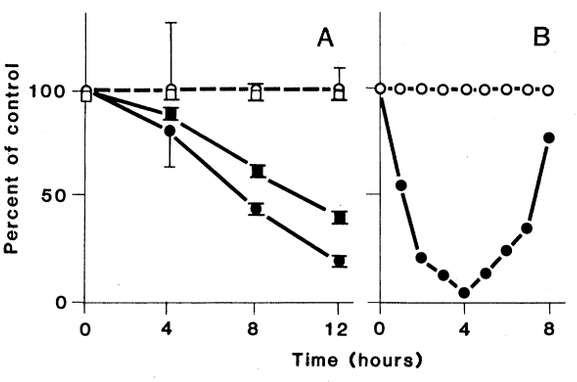

Fig. 1. Response of asynchronous HeLa cells to TPA ($10^{-7}M$) or acetone (0.2 percent) as evaluated by measuring thymidine incorporation rate and labeling index (A), and the mitotic index (B). HeLa cells were cultivated in Eagles minimal essential medium containing Earle's salts supplemented with 10 percent calf serum and were kept in a mixture of humidified air and CO_2 (95:5) at 37°C. Cells were transferred in complete medium to plastic (Falcon) petri dishes (1.45×10^6 cells per dish) 15 hours prior to the experiment. At time 0 TPA or acetone was added. (A) For determination of the incorporation rate and labeling index [*methyl*-^{3}H]thymidine (specific activity, 40 to 60 Ci/mmole; New England Nuclear) (5 μCi/5 ml) was added for 30 minutes prior to the indicated time. The medium was removed, and the cultures were rinsed three times with cold phosphate-buffered saline, fixed in a mixture of ethanol and acetic acid (7:3), and washed twice with cold trichloroacetic acid (5 percent). Some of the dishes were processed for colorimetric determination of DNA (*19*) and scintillation counting. The other dishes were further processed and coated with K2 emulsion (Ilford) for autoradiography. After exposure and development, cells were stained with Giemsa. The labeling index was determined from at least 3×1000 cells per group. ●, ○, Incorporation rate; ■, □, labeling index; ●, ■, TPA; and ○, □, acetone. Bars indicate standard deviation. (B) At the times indicated, photographs were taken (six per group) from undisturbed cultures with the use of an inverted phase-contrast microscope (Leitz Diavert; ×10 lens, ×10 ocular) in order to overcome problems arising from loosely attached mitotic cells. Evaluation was from enlarged prints (13 by 18 cm) for at least 1000 cells per group; ●, TPA; and ○, acetone.

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