Proposed Mechanism of Inheritance and Expression of the Human Fragile-X Syndrome of Mental Retardation

Charles D. Laird

Departments of Zoology and Genetics, University of Washington NJ-15, Seattle, Washington 98195

Manuscript received July 10, 1987
Accepted July 31, 1987

ABSTRACT

A mechanism is proposed for the inheritance and expression of the fragile-*X*-linked syndrome of mental retardation in humans. Two independent events are required for expression of the syndrome: the fragile-*X* mutation, and *X* chromosome inactivation in pre-oogonial cells. The fragile-*X* mutation at site *X*q27 has little or no effect until the chromosome is inactivated in a female as part of the process of dosage compensation. At a stage where the inactivated *X* chromosome would normally be reactivated in preparation for oogenesis, the mutation results in a local block to the reactivation process. This block to reactivation leads to mental retardation in progeny by reducing the level of products from the unreactivated *X*q27 region in male cells, and, for a heterozygous female, in somatic cells in which the normal *X* chromosome has been inactivated. Published data relevant to this proposed mechanism are discussed.

THE *X* chromosome fragile site *X*q27 is one of several dozen heritable fragile sites that are observed in human chromosomes. Fragile sites are specific chromosome regions that exhibit an increased frequency of gaps and breaks after induction in cell culture (SUTHERLAND and HECHT 1985). A common basis of the cytogenetic properties of fragile sites is proposed elsewhere (LAIRD *et al.* 1987). An additional phenotype has been noted for fragile-*X* site *X*q27: it is associated with a major clinical syndrome that includes mental retardation, and occurs in more than 1 in 2000 live births (SUTHERLAND and HECHT 1985; BROWN *et al.* 1986). The fragile-*X* chromosome is thus responsible for the most common form of inherited mental retardation.

The general features of the inheritance and expression of the fragile-*X* syndrome are known from detailed analyses of over 200 pedigrees (SHERMAN *et al.* 1985). The pattern of inheritance is that of an *X* chromosome-linked mutation: most males with the fragile *X* chromosome express the syndrome of mental retardation, and they inherit this chromosome from their mothers but not from their fathers. There is, however, an unusual and perplexing lack of expression of the syndrome in some males and in their daughters: males have been identified who do not express the syndrome of mental retardation even though they can be shown by pedigree or DNA analysis to carry a fragile-*X* chromosome (CAMERINO *et al.* 1983; SHERMAN *et al.* 1985). These mentally normal carrier males have progeny and are thus called "transmitting males" (SHERMAN *et al.* 1985). (Mentally retarded carrier males do not reproduce and thus do not transmit their fragile-*X* chromosome.) Daughters of transmitting males are obligate carriers of the fragile-*X* chromosome, but they, like their fathers, are mentally normal. Mental retardation is first expressed among the grandchildren of transmitting males. Thus, *affected* individuals have always inherited the fragile-*X* chromosome from their mothers. These features of inheritance and expression are illustrated in the stylized pedigree in Figure 1. Transmitting male I-2 transmits a fragile-*X* chromosome through his daughters (II-2, II-5) to his grandchildren. His daughters are mentally normal, but affected grandsons (III-7, III-9) and affected granddaughters (III-2, III-10) often are observed. Affected and normal granddaughters sometimes have affected progeny (generation IV). In addition to this unusual pattern of inheritance from a transmsitting male, the expression of the syndrome is variable: not all females heterozygous for the fragile-*X* chromosome are affected (incomplete penetrance); among affected females, the severity of the syndrome varies, and females are less severely affected than males (variable expressivity).

The well-documented examples of transmitting males, the observations that their daughters are mentally normal and that affected males do not reproduce, and the corollary that only females have affected children, have led to suggestions that the fragile-*X* syndrome is a two-step process: a mutated chromosome must be passed through the ovary of a female before full expression of the syndrome is observed (SUTHERLAND 1985; PEMBREY, WINTER and DAVIES 1985; SHERMAN *et al.* 1985). SUTHERLAND (1985) suggested that "activation" of the mutation occurs in

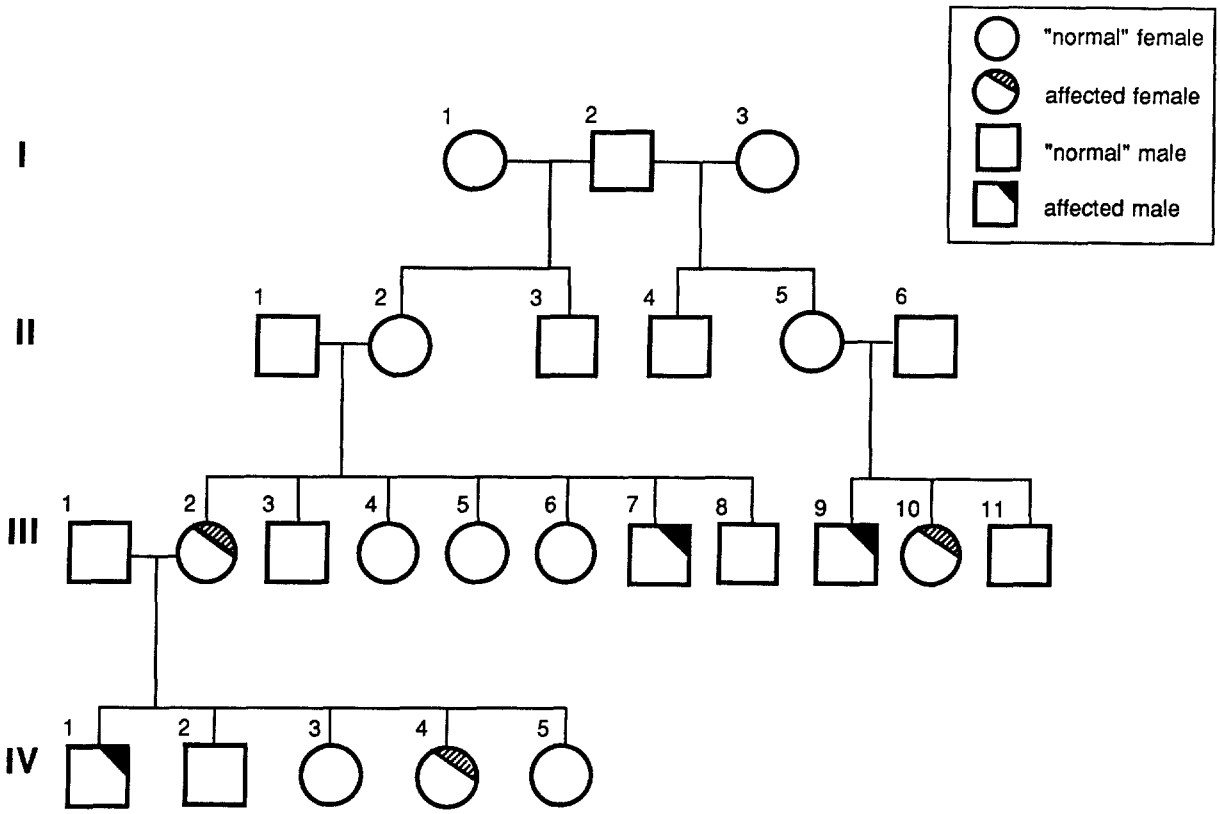

FIGURE 1.—Pattern of inheritance of the fragile-*X* syndrome of mental retardation (stylized pedigree). A mentally normal male (I-2) transmits a fragile-*X* chromosome through his daughters to his grandchildren. None of his daughters is affected, but both affected grandsons and granddaughters often are observed. Affected (and normal) granddaughters sometimes have affected progeny; affected males do not reproduce. The transmitting male I-2 can be identified as the carrier of the fragile-*X* chromosome in this stylized pedigree because both sets of grandchildren have affected individuals. Transmitting males can also be identified by analysis of DNA restriction fragment length polymorphisms (CAMERINO *et al.* 1983). The general pattern of inheritance illustrated here was inferred from analyses of over 200 pedigrees (SHERMAN *et al.* 1985).

the egg. More specifically, PEMBREY, WINTER and DAVIES (1985) proposed that recombination during oogenesis between the fragile-*X* and a normal *X* chromosome was necessary for expression of the syndrome. Genetic analysis using two flanking polymorphic DNA markers, however, clearly demonstrated that homologous recombination in the carrier mother is not a prerequisite for expression of the syndrome in her progeny (OBERLE *et al.* 1986). Another class of models has invoked autosomal genes in the expression of the fragile-*X* syndrome (STEINBACH 1986; ISRAEL 1987). These models are difficult to fit to the observed penetrances of the syndrome (SHERMAN 1986), and they also do not easily accommodate the demonstration that cytogenetic expression of fragile site *X*q27 can occur, in human-hamster hybrid cells, in the absence of any particular human autosome (LEDBETTER, LEDBETTER and NUSSBAUM 1986).

I propose here a mechanism that explains the unusual pattern of inheritance and expression of the fragile-*X* syndrome. The specific mechanism is one in which the fragile-*X* mutation acts as a block to a normal chromosome process that occurs in the ovary. The basic postulates are as follows:

(i) the fragile-*X* mutation is a potential *cis*-acting, local block to the process of *X* chromosome reactivation that occurs in a female prior to oogenesis;
(ii) a cycle of *X* chromosome inactivation and incomplete reactivation in a female results in a local, heritable "imprinting" of a fragile-*X* chromosome;
(iii) the fragile-*X* syndrome results from transcriptional inhibition that accompanies this local chromosome imprinting;
(iv) transmitting males and some heterozygous females are unaffected because their fragile-*X* chromosomes were not imprinted in a previous generation;
(v) variable expression in females with an imprinted fragile-*X* chromosome results from random inactivation of *X* chromosomes in somatic cells.

DISCUSSION

Each postulate will now be discussed more fully, and will be related to relevant data excepting those from pedigree analyses. Pedigree data, which are

quantitatively consistent with the mechanism presented here, will be discussed more completely at the end of this section.

(i) The fragile-X mutation is a potential *cis*-acting, local block to the process of X chromosome reactivation that occurs in a female prior to oogenesis: X chromosome inactivation in female mammals is part of the process of dosage compensation, which ensures that most cells have only one active X chromosome. The choice of which X chromosome is inactivated is usually random in individual embryonic cells early in development; once inactivated, an X chromosome will be stably propagated as such throughout subsequent cell lineages, expressing this inactivation by transcriptional quiescence and a pattern of late DNA replication (LYON 1961). X chromosomes in pre-oogonial cells participate in this inactivation process (GARTLER, ANDINA and GANT 1975). Just prior to oogenesis, however, both X chromosomes are active in oogonial cells of normal females, indicating that the inactive X has been reactivated (GARTLER *et al.* 1972). This critical reactivation step apparently occurs only in preparation for oogenesis. The alteration in DNA that I propose for the fragile-X site Xq27 imposes a local block to this reactivation process (Figure 2).

(ii) A cycle of X chromosome inactivation and incomplete reactivation in a female results in a local, heritable "imprinting" of a fragile X chromosome: The fragile-X mutation has little or no effect until the fragile-X chromosome is altered, or "imprinted," in females by a cycle of X chromosome inactivation and reactivation. Once the fragile-X chromosome goes through a cycle of inactivation and incomplete reactivation, its imprinted state is stably transmitted to subsequent generations.

Chromosomal "imprinting" refers to a nonmutational alteration to a chromosome that predetermines its function—or lack of function—at some time later in development (CROUSE 1960); this terminology has been applied to mammalian X chromosomes that have been inactivated as part of the dosage compensation mechanism (CHANDRA and BROWN 1975). Maternal chromosome imprinting as used here thus refers to a non-mutational alteration of the fragile-X chromosome that occurs in the mother, and predetermines partial chromosomal dysfunction in progeny who inherit an imprinted fragile-X. Support for a cycle of inactivation and incomplete reactivation as the postulated basis of maternal imprinting of a mutated fragile-X chromosome will come from an analysis of pedigree data, to be discussed below. In particular, it will be shown that the pedigree data are consistent with a random process in females that occurs on average in 50% of oocytes or oocyte-precursor cells. This is the same average percentage of pre-oogonial and oogonial cells of a heterozygous female in which a particular X chromosome will experience a cycle of X chromosome inactivation and reactivation.

(iii) The fragile-X syndrome results from transcriptional inhibition that accompanies this local chromosome imprinting: The clinical manifestations of the mental retardation syndrome in individuals that carry a maternally imprinted fragile-X chromosome result from the decrease in gene product(s) encoded at or near the fragile site Xq27. This decrease is a consequence of continued transcriptional repression of this region caused by the incomplete reactivation of the fragile-X chromosome.

The activities of two genes at or near fragile site Xq27 have been measured in attempts to understand the basis of the fragile-X syndrome. Glucose-6-phosphate dehydrogenase (G6PD), the gene which is located just distal to Xq27, has been assayed in cells from affected fragile-X males. MARENI and MIGEON (1981) reported levels of G6PD that were within 30% of normal, thus ruling out a chromosome deletion that includes the G6PD locus. SNYDER *et al.* (1984), however, found G6PD levels reduced by 70% in five fragile-X males. It thus appears that a region of transcriptional inactivity may sometimes extend distally from Xq27 at least as far as G6PD.

Similar results were obtained for a closely linked gene located on the proximal side of Xq27. Assays using selective media to assess activity of hypoxanthine phosphoribosyl transferase (HPRT) revealed that this gene is at least 100 times more likely to be inactive in cells derived from affected males than from normal males (MARENI and MIGEON 1981). Not all cell lines from affected males, or even from the same male, showed high frequencies of cells with an inactive HPRT gene, again suggesting that the extent of inactivation at this region is variable, perhaps even within an individual.

Thus the data, although fragmentary, are consistent with a localized, regional block to transcriptional reactivation of the Xq27 region in a fragile-X chromosome. The conclusion that the block must be localized rather than extending throughout the X chromosome, or even throughout the tip of the X distal to Xq27, is consistent with the observation that at least a major part of the imprinted fragile-X can become early replicating, which is usually correlated with transcriptional reactivation (to be discussed below). In addition, inactivation of the entire tip of the X chromosome distal to the fragile-X site is unlikely because such extensive inactivation would be the functional equivalent of a deletion of the distal tip. Such deletions have not been reported in males (BORGAONKAR 1984), and are presumably lethal. Model (c) in Figure 2 is therefore more likely than model (b). More data on the activity of genes near Xq27, such as those encoding hemophilia factors VIII and IX, and the color vision

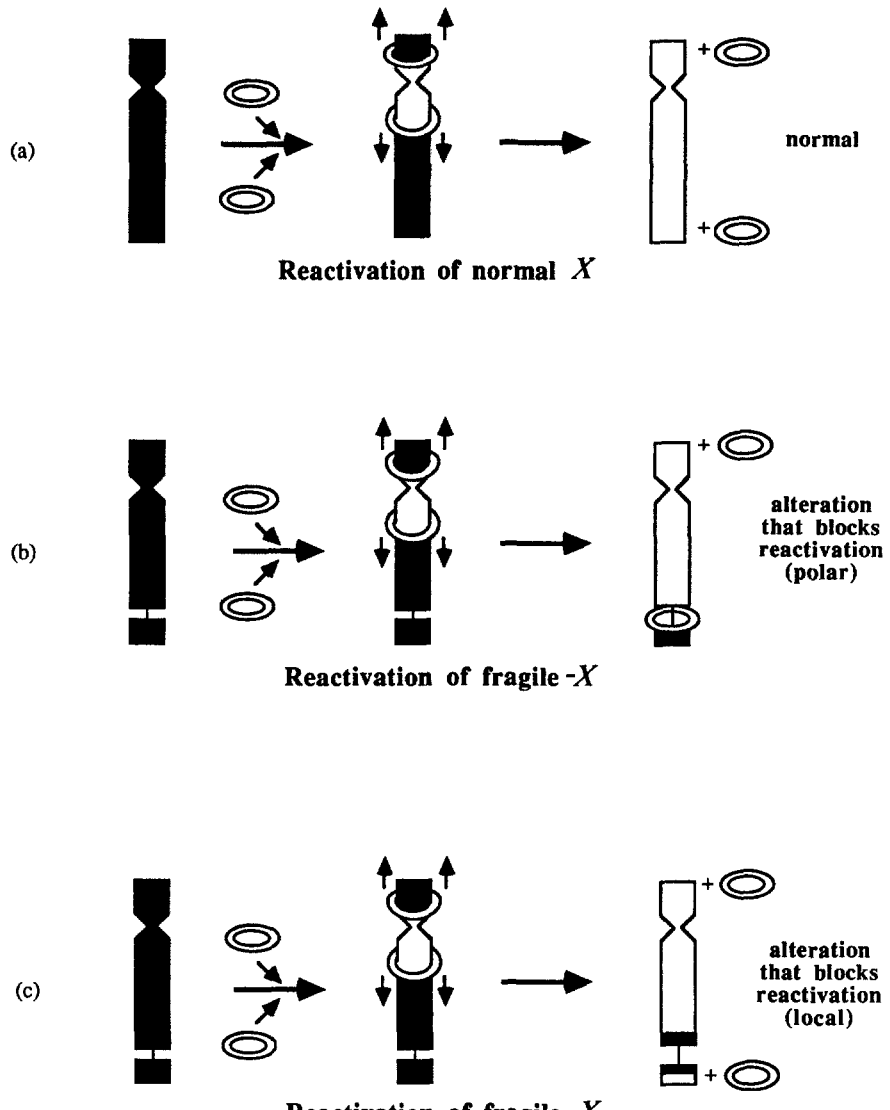

FIGURE 2.—Reactivation, prior to oogenesis, of an inactivated normal *X* chromosome (a), and inactivated fragile-*X*'s (b and c). *X* chromosomes progress from inactivated (*dark*) to reactivated (*light*) states prior to oogenesis. Whereas the normal *X* is indicated as being completely reactivated, the fragile-*X*'s are not. The gapped region on the fragile-*X* chromosome represents an altered site *X*q27 that blocks reactivation either in a polar manner (b), or in a local manner (c). The *X*q27 region of a fragile-*X* chromosome is thus "imprinted" by a cycle of inactivation and incomplete reactivation, and remains inactive over several generations. The angular indentations on the normal and fragile-*X* chromosomes represent the centromere region. Reactivation "rings" are shown binding to one or a few sites near the centromere and proceeding distally in both directions, by analogy with models of inactivation (THERMAN, SARTO and PATAU 1974); however, few of the details of the reactivation process are known. For simplicity, the short arm of the *X* chromosome is shown as being completely inactivated when the rest of the *X* is inactive; in reality, some distal genes on the short arm appear to escape complete inactivation (SHAPIRO, MOHANDAS and WEISS 1979; MIGEON *et al.* 1982). The gap on the inactivated fragile-*X* is proposed to occur only at low frequencies in lymphocytes unless the chromosome has been through at least one cycle of inactivation and incomplete reactivation; this distinction is indicated in the figure by a narrow gap on a mutated chromosome that becomes more pronounced after a cycle of *X* chromosome inactivation/reactivation.

pigments, are needed to delimit the region of inactivation and to assess whether or not all cells from an individual have the same pattern of transcriptional inactivity of affected genes.

Thus, the mechanism presented here is one in which the *X*q27 region remains "heterochromatic" in the sense of being late-replicating and transcriptionally quiescent. When one considers this region in an imprinted fragile-*X* chromosome as being heterochromatic, the transcriptional variability described above is less perplexing. Transcriptional inactivation that varies from cell to cell, and for variable distances, has been described for other organisms in which heterochromatin and euchromatin are juxtaposed, usually as a consequence of chromosome rearrangements [position-effect variegation; see CATTANACH (1974) and SPOFFORD (1976) for reviews]. In the fragile-*X* syndrome, however, this juxtaposition is proposed to result from incomplete reactivation of an *X* chromosome that occurred in an oogonial cell in a previous generation.

(iv) Transmitting males and some heterozygous

females are unaffected because their fragile-X chromosomes were not imprinted by a cycle of maternal inactivation and incomplete reactivation in a previous generation. Individuals who inherit a mutated but not imprinted fragile-*X* chromosome will be mentally normal because their fragile *X* chromosome has never been inactivated in pre-oogonial cells; in such individuals the *X*q27 region will be active in all somatic cells in which the fragile-*X* remains active.

As described in the introduction, mentally normal males can transmit a fragile-*X* chromosome. Such transmitting males usually have grandchildren but not children that express the fragile-*X* syndrome. Some heterozygous females will also be mentally normal because they inherit a mutated but nonimprinted fragile-*X*. Even though these females will imprint a fragile-*X* chromosome in some of their oogonial cells, their somatic cells will have either an active normal *X* chromosome, or an active fragile-*X* chromosome that had not been maternally inactivated in a previous generation; hence such females are expected to be mentally normal because all somatic cells have an *X* chromosome with an active *X*q27 region. This concept will be analyzed quantitatively in the discussion on pedigree analyses. In particular, it will be shown that pedigree and cytogenetic data are consistent with there being two classes of heterozygous females, those with imprinted and those with nonimprinted fragile-*X*'s, and that the penetrance of the fragile-*X* syndrome is expected to be different for these two classes of females and for their progeny.

(v) Variable expression in females with an imprinted fragile *X* results from random inactivation of *X* chromosomes in somatic cells: Among heterozygous females who inherit an imprinted fragile-*X*, about half are classified as affected. This incomplete penetrance for females who have inherited an imprinted fragile-*X* results from the somatic component of *X* chromosome inactivation. Among affected females there is a range in the severity of the syndrome. This variable expressivity is also due to the somatic component of *X* chromosome inactivation: more severely affected females have a higher proportion of cells in which the normal *X* chromosome is inactivated.

Somatic *X* chromosome inactivation has been discussed by others as an explanation for variable expression of the fragile-*X* syndrome in females [see UCHIDA *et al.* (1983) and TUCKERMAN, WEBB and BUNDEY (1985) for review]. I suggest here that somatic *X* chromosome inactivation influences expression of the syndrome only in heterozygous females who have inherited an imprinted fragile-*X* chromosome. The conclusion that about half of the females with an imprinted fragile-*X* are classified as affected is supported by the following considerations and data.

One important distinction between active and inactive *X* chromosomes in female cells is the timing of chromosome replication during the DNA synthesis phase of the cell cycle: the inactive *X* chromosome is late-replicating (LYON 1961, 1972). The timing of replication has been examined in cells of females heterozygous for a fragile-*X* chromosome. In some females, the fragile-*X* chromosome (but not necessarily the *X*q27 region) is predominantly early replicating in lymphocytes. In other females the fragile-*X* is predominantly late replicating, and in some it is randomly early and late replicating (LUBS 1969; KNOLL, CHUDLEY and GERRARD 1984; HOWELL and MCDERMOTT 1982; UCHIDA and JOYCE 1982; UCHIDA *et al.* 1983; PAUL *et al.* 1984; TUCKERMAN, WEBB and BUNDEY 1985; TUCKERMAN, WEBB and THAKE 1986). The general relationship between IQ scores and the pattern of *X* chromosome replication is illustrated by data from a pair of monozygotic twins who were heterozygous for fragile-*X* site *X*q27 (TUCKERMAN, WEBB and BUNDEY 1985). One twin was diagnosed as mentally retarded; her fragile-*X* chromosome was early replicating in 85% of her lymphocytes. The other twin had "higher than normal intelligence"; her fragile-*X* was early replicating in only 30% of her lymphocytes, and late-replicating in the remaining 70%. An especially detailed comparison of IQ scores with the pattern of *X* chromosome replication indicated that, for females whose lymphocytes showed more than 50% early replication of the fragile *X*, there was an inverse correlation between IQ scores and the percentage of lymphocytes with an early replicating fragile-*X*. IQ scores decreased from 88 to 55 as the percentage of cells with early replicating fragile-*X*'s increased from 69% to 95%. Females who had been classified as having normal intelligence had an early replicating fragile-*X* chromosome in less than 50% of their lymphocytes (UCHIDA *et al.* 1983). Although several exceptions to this correlation have been noted (PAUL *et al.* 1984; TUCKERMAN, WEBB and THAKE 1986), a summary of four other data sets reached essentially the same conclusion that there is an inverse correlation of IQ score with percentage of early replicating fragile-*X* chromosomes, for values above 50% (TUCKERMAN, WEBB and BUNDEY 1985). This correlation initially may appear surprising: there is no reason to expect that the stem cell lineage that gives rise to lymphocytes, which are used for cytogenetic analyses, will also lead to the cell populations that are critical for the mental functions assessed by IQ tests. There is, however, a common pool of embryonic cells that is present after *X* chromosome inactivation, and from which different precursor cells are set aside (NESBITT 1971; FIALKOW 1973). Thus the data obtained from analyzing lymphocytes provide a good index for the pattern of *X* chromosome inactivation in other cell populations of each individual.

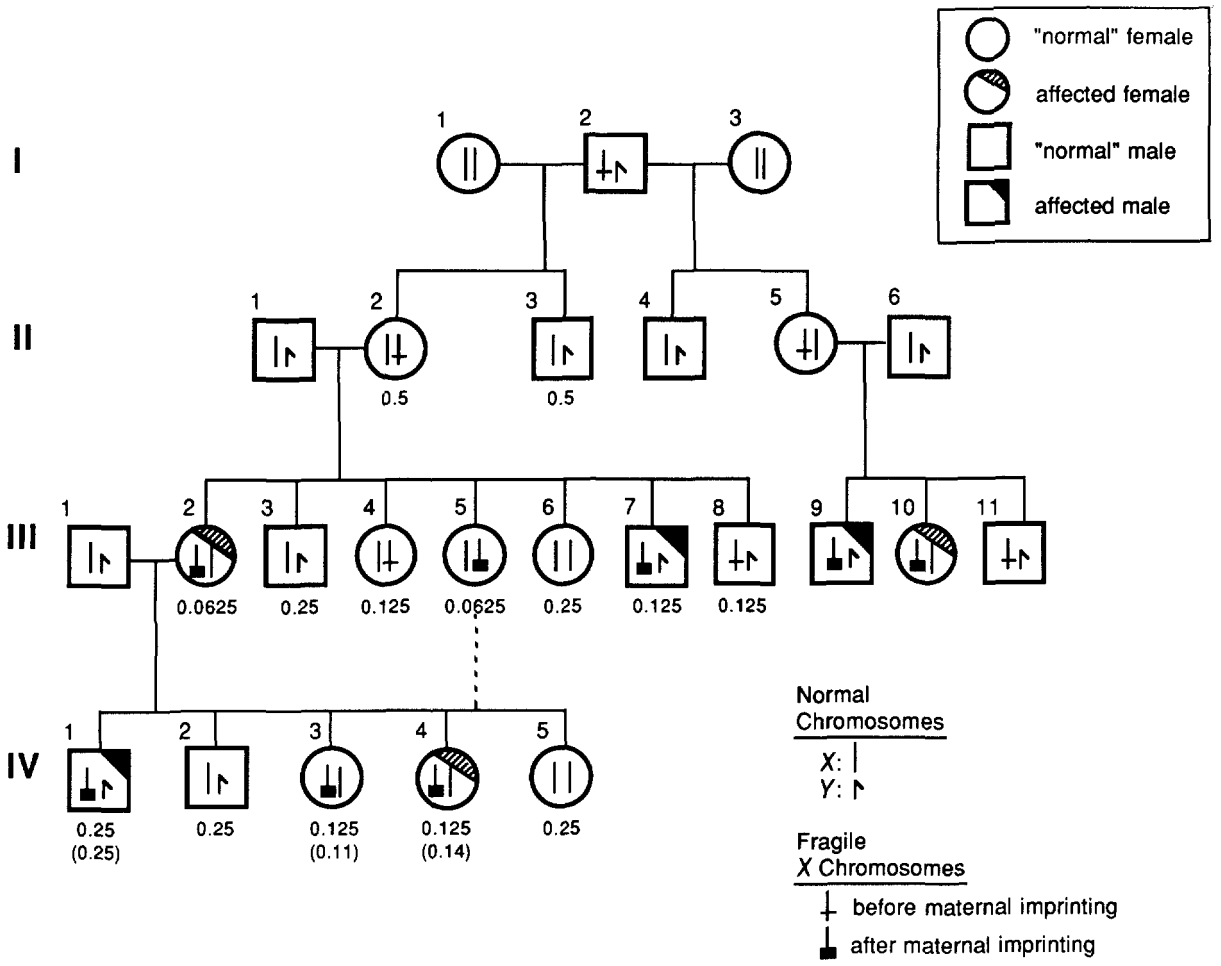

FIGURE 3.—Mechanism of inheritance of the fragile-*X* syndrome. Pedigree from Figure 1 is now completed to illustrate the proposed mechanism of inheritance and expression of the fragile-*X* syndrome. The sex chromosome composition (*X* and *Y* normal chromosomes, and the fragile-*X*), the state of the fragile-*X* (imprinted or nonimprinted), and the mental condition (normal or affected) of the individuals are indicated. A mutated fragile-*X* first appears in this stylized pedigree from a mentally normal carrier male (transmitting male, I-2). It was passed to a female (II-2) who was mentally normal but who sometimes imprinted the mutated fragile-*X* before transmitting it to progeny in generation III. Females III-2 and III-5 will have a similar distribution of progeny, as indicated by the solid and dashed lines, respectively, connecting their symbols to generation IV. The expected frequencies of individuals III-2 through III-8, and IV-1 through IV-5 are shown below each individual (see Table 1 for calculations). The observed values for affected individuals in generation IV, taken from SHERMAN *et al.* (1985) are shown in parentheses.

To account for these data, previous authors (TUCKERMAN, WEBB and BUNDEY 1985) suggested a connection between variable expression of the syndrome in females and "Lyonization" of the *X* chromosome, *i.e.*, the phenotypic effects of inactivating one of the *X* chromosomes in each female cell (LYON 1961, 1972). If a sufficient percentage of somatic cells have a normal *X* chromosome that is early replicating and therefore active, then gene products from normal *X* chromosomes in these cells apparently will lead to mental function in the normal range. The data described above indicate that heterozygous females classified as unaffected usually have 50% or more of their somatic cells with an active normal *X* chromosome. Since *X* chromosome inactivation is usually random (LYON 1961, 1972), this 50% value divides heterozygous females into two equal classes, those with more than, and those with less than, 50% somatic inactivation of the fragile-*X* chromosome. As will be seen in the next section on pedigree analyses, these considerations lead to expected penetrances of the fragile-*X* syndrome that agree well with observed values.

Pedigree analyses

General features: This proposed mechanism of fragile-*X* inheritance and expression is applied to the general aspects of fragile-*X* pedigrees in Figure 3. The sex chromosomes and the two developmental components, namely maternal chromosome imprinting (imprinted or nonimprinted) and somatic expression (affected or normal), are noted for each individual. The presence of mentally normal carrier males (I-2, III-8, etc.) and females (II-2, III-4, etc.), and the inheritance only from mothers of an imprinted fragile-*X* chromosome (generation III) are well accommodated by the mechanism. The fragile-*X* chromosome

TABLE 1

Expected frequencies of carrier progeny from mothers carrying fragile-X

Progeny description	Example from Figure 3	Genetic: P (chromosome inheritance) P(M)	Genetic: P (chromosome inheritance) P(P)	Developmental: P (observed state of fr-X from oocyte) (I or NI)	Developmental: P (observed intelligence) (normal or affected)	Expected frequency
From imprinted (I) mother	III-2	A	B	C	D	E
affected male (I)	IV-1	0.5	0.5	1.0	1.0	0.25
normal carrier female (I)	IV-3	0.5	0.5	1.0	~0.5	~0.125
affected female (I)	IV-4	0.5	0.5	1.0	~0.5	~0.125
From nonimprinted (NI) mother	II-2					
affected female (I)	III-2	0.5	0.5	~0.5	~0.5	~0.0625
normal carrier female (NI)	III-4	0.5	0.5	~0.5	1.0	~0.125
normal carrier female (I)	III-5	0.5	0.5	~0.5	~0.5	~0.0625
affected male (I)	III-7	0.5	0.5	~0.5	1.0	~0.125
normal carrier male (NI)	III-8	0.5	0.5	~0.5	1.0	~0.125

The expected frequencies of the various classes of progeny that inherit a fragile-X from the two classes of carrier mothers (imprinted (I) and nonimprinted (NI)) are presented. The probabilities of chromosomal inheritance are given in columns A and B for maternal and paternal chromosomes, respectively. The developmental component in column C reflects the probability of the observed state of the fragile-X (imprinted or nonimprinted), given its state in the mother. Mentally normal carrier women who have a nonimprinted fragile-X chromosome are assumed to have a probability of 0.5 of inactivating the fragile-X chromosome, and a probability of 0.5 of inactivating the normal X, prior to oogenesis (see text). The probability of a zygote with a fragile-X having an imprinted rather than nonimprinted fragile-X, given that the mother has an imprinted fragile-X, is 1.0 because of the apparent stability of the imprinted state. The next column (D) is the probability of the observed level of intelligence (normal or affected), given the state of the fragile-X. If the individual has a nonimprinted fragile-X, then he or she will have normal intelligence ($P = 1.0$). If the individual has an imprinted fragile-X, then, if male, he will be affected ($P = 1.0$); if female, she will be mentally normal or affected with equal probabilities (0.5), as determined by the proportion of somatic cells that have inactivated the imprinted fragile-X. (As stated in the text, a female likely will be mentally normal if she has inactivated an imprinted fragile-X in 50% or more of her cells.) The product of the probabilities (column E) is the expected frequency of the various carrier progeny. The tilde (~) in front of some numbers designates values that are affected by the process of random chromosome inactivation.

of a normal carrier (transmitting) male represents either a newly arisen mutation at Xq27 or inheritance, from a mother, of a fragile-X that had, by chance, never been imprinted by a cycle of inactivation/incomplete reactivation in oogonial cells. Only females transmit an imprinted fragile-X chromosome because males with imprinted fragile-X's do not have progeny; males carrying mutated but non-imprinted fragile-X's transmit them without imprinting because males, having only one X chromosome, do not inactivate the X chromosome for dosage compensation (LYON 1961).

Frequencies of affected individuals in sibships, and predicted penetrances: There are also subtleties of the pedigrees that are quantitatively in accordance with the mechanism presented here. The expected frequencies of affected individuals in generation IV show good agreement with published frequencies (in parentheses, Figure 3), which were derived from analysis of 206 pedigrees (SHERMAN *et al.* 1985). For example, the observed frequencies of affected (IV-4) and mentally normal (IV-3) carrier daughters [0.14 and 0.11, respectively, penetrance = 0.55, Table 7 of SHERMAN *et al.* (1985)], are close to the expected frequency of 0.125 for each class (penetrance = 0.5).

The assumptions used to calculate the expected frequencies are presented more explicitly in Table 1. For completeness, the probabilities of the observed chromosomal inheritance are given, *i.e.*, the particular combination of the maternal (column A) and paternal (column B) chromosomes, followed by the probabilities for the two developmental phenomena of maternal imprinting (column C), and somatic X chromosome inactivation (column D). The expected frequencies of carrier progeny (column E) from imprinted (I) mothers are given in the upper part of Table 1, and are, as mentioned above, compared in Figure 3 with observed frequencies.

It is more difficult to compare directly the expected with observed frequencies of affected progeny from heterozygous mothers, such as II-2, who have a nonimprinted fragile-X chromosome (Table 1, lower part). Published pedigree analyses (SHERMAN *et al.* 1985) have not distinguished progeny data from two classes of mentally normal heterozygous women—those with imprinted fragile-X chromosomes (III-5), and those with nonimprinted ones (III-4). However, the expected frequencies of affected progeny from these two classes of mothers span the observed values, as would be expected if the pedigrees do in fact include both classes of mothers (Table 2). Good agreement between observed and expected values is obtained if one assumes that the pedigree data from

TABLE 2

Expected and observed frequencies of carrier progeny from mentally normal carrier mothers

Progeny description	Expected from imprinted mother	Expected from nonimprinted mothers	Expected from equal proportions I and NI mothers	Observed
Affected female (I)	0.125	0.0625	0.09	0.08
Normal carrier female (NI)	0.0	0.125	0.06 } 0.15	0.17
Normal carrier female (I)	0.125	0.0625	0.09 }	
Affected male (I)	0.25	0.125	0.19	0.19
Normal carrier male (NI)	0.0	0.125	0.06	0.06

Mentally normal carrier mothers will have inherited either imprinted or nonimprinted fragile-*X* chromosomes. The expected frequencies of their carrier progeny are different (compare sibships in generations III and IV, Figure 3). These expected frequencies, calculated in Table 1, are compared with observed values that were calculated from a mixture of the two types of sibships (SHERMAN *et al.* 1985). If one assumes an equal proportion of sibships coming from mothers who have imprinted and nonimprinted fragile-*X* chromosomes (see text), then the expected and observed values are in good agreement (last two columns of the table). The expected penetrances referred to in the text are the expected frequencies of affected individuals divided by the expected frequencies of mentally normal carriers plus affected individuals. For example, expected male penetrance = 0.19/(0.06 + 0.19) = 0.76.

mentally normal heterozygous mothers reflect equal proportions of mothers with imprinted and non-imprinted fragile-*X*'s (Table 2). The expected penetrances for males and females from heterozygous mothers, given this proposed mechanism and the assumption just expressed for the distribution of heterozygous mothers with imprinted and non-imprinted fragile-*X*'s, are 76% and 38%, respectively (see legend to Table 2). These values compare favorably with reported values of 78% and 36% (SHERMAN *et al.* 1985).

The above suggestion that there are two populations of mentally normal carrier mothers is supported by cytogenetic data. JACOBS and SHERMAN (1985) reported that about 60% of mentally normal, heterozygous females have less than 1% fragile-*X* positive cells. I suggest that mentally normal heterozygous females who exhibit a low frequency of cytological expression of the fragile-*X* have a nonimprinted fragile-*X* chromosome; those with a high frequency of expression have an imprinted fragile-*X*. Although the frequency of cytological expression of the fragile-*X* chromosome will depend on cell culture conditions, low frequency expression will generally be less than a few percent. For example, in the pedigree analysis reported by CAMERINO *et al.* (1983), in which DNA restriction fragment length polymorphisms were used to identify a transmitting male, the average cytological expression of the fragile-*X* was 1.2% in daughters of the transmitting male. Within the context of the mechanism presented here, these daughters inherited a nonimprinted fragile-*X* chromosome from their father. Three granddaughters, two of whom were classified as mentally normal, had fragile-*X* chromosomes with an average of 30% cytological expression, and would therefore have inherited imprinted fragile-*X* chromosomes. The basis of the difference in the frequency of cytological expression between nonimprinted and imprinted fragile-*X* chromosomes is discussed elsewhere (LAIRD *et al.* 1987).

Penetrances expected among brothers and grandsons of transmitting males: Grandsons of a transmitting male who have a fragile-*X* chromosome will have inherited it from a transmitting mother (III-3,7,8, are such grandsons of transmitting male I-2; their transmitting mother is II-2, Figure 3). Half of these grandsons carrying a fragile-*X* chromosome will be affected, assuming 50% inactivation of the fragile-*X* in oogonial cells of the mother (compare III-7 with III-8). Thus the expected penetrance of the fragile-*X* syndrome in grandsons is 0.5. The expected penetrance in brothers of transmitting males is more difficult to estimate because the fragile-*X* chromosomes of some transmitting males may represent new mutations present in their mothers' gametes. Such males would have no affected brothers if the mutation occurs in meiotic cells because no further opportunity for maternal imprinting would exist in that mother after *X* chromosome reactivation (apparent penetrance would be 0.0). Pedigrees with transmitting males who represent new mutations, however, are probably not represented among published pedigrees in proportion to their expected frequency: the delay of two generations in the appearance of affected individuals would make ascertainment more difficult. If we assume that all reported transmitting males have inherited their fragile-*X* from a transmitting mother (*i.e.*, that none represent new mutations), then the penetrance among their brothers should be the same as that calculated above for grandsons, or 0.5. How do these expected penetrances compare with reported values?

SHERMAN *et al.* (1985) obtained markedly different estimates of penetrances of the syndrome among brothers, compared with grandsons, of mentally normal transmitting males [referred to as the "Sherman paradox" (OPITZ 1986)]. Penetrances of 0.18 and 0.74 were reported for brothers and grandsons, respectively. [The latter value for grandsons is considered to be higher than the actual value because of ascer-

tainment bias (SHERMAN *et al.* 1985); correcting for this bias would thus bring the 0.74 value closer to the predicted penetrance of 0.5.] More recent data, however, lead to a much higher estimate (0.72, uncorrected for ascertainment bias) for the penetrance among brothers of transmitting males (FROSTER-ISKENIUS *et al.* 1986). This value is very close to the penetrance of 0.74 for grandsons, and is four times higher than the previous estimate of 0.18 (SHERMAN *et al.* 1985). SHERMAN (1986) has suggested that the data from older generations (SHERMAN *et al.* 1985) may have been less reliable than data from younger generations (FROSTER-ISKENIUS *et al.* 1986).

Further data and pedigree analyses will be necessary to assess the importance of the uncertainties raised above, namely the discrepancy between different data sets, the correction for ascertainment, and the fraction of transmitting males who represent new mutations. To summarize the predictions from the current proposal, a penetrance of 0.5, corrected for ascertainment bias, is expected for grandsons of transmitting males. A penetrance of 0.5, corrected for ascertainment bias, is expected for brothers of transmitting males assuming no new mutations are represented among the transmitting males studied. A lower penetrance would be reported for this latter class if new mutations are represented in the pedigrees.

Transmitting males in sibships with affected males: Individual III-8 represents a male who inherited a mutated but nonimprinted fragile-*X* chromosome. Such males are expected at a frequency of 0.125 among progeny of mothers who inherited a nonimprinted fragile-*X* chromosome (II-2), but are not expected to occur among progeny of mothers who inherited an imprinted fragile-*X* (III-2, III-5; Table 1). These transmitting males (SHERMAN *et al.* 1985; OBERLE *et al.* 1986) begin again the cycle started in the pedigree by I-2. The recent discovery of cytogenetic methods to detect transmitting males (LEDBETTER, LEDBETTER and NUSSBAUM 1986), coupled with analyses of DNA polymorphisms near the fragile-*X* site (CAMERINO *et al.* 1983), will permit tests of the above prediction.

Sporadic cases of mentally normal males who inherited an imprinted fragile *X*: how stable is the imprint? There are occasional unaffected males, such as described in OBERLE *et al.* (1986), who appear to have inherited a fragile-*X* chromosome that was previously imprinted. [A fragile-*X* chromosome sometimes is distinguishable from a normal *X* by DNA polymorphisms (CAMERINO *et al.* 1983; OBERLE *et al.* 1986); prior imprinting is inferred if a mother was mentally subnormal, see postulates (iv) and (v), above.] Such a male presumably received a fragile-*X* that was imprinted in his grandmother, but was altered in his mother in the oocyte (or pre-oocyte) from which he came. A reactivation rate for an imprinted fragile-*X* chromosome of a few percent per generation (instead of the zero percent reactivation assumed above), would not have a major effect on the expected frequencies of affected individuals, but it would explain these few exceptional males. Alternatively, recombination near the fragile-*X* site could partially exchange nonimprinted DNA for imprinted DNA, and thereby reverse or ameliorate the effects of the imprinted region. Males inheriting such recombinant or reactivated fragile-*X* chromosomes may still retain a cytologically observable fragile site if the domain of genes influenced by the failure of chromosome reactivation extends beyond the fragile site itself [see Figure 2c, and discussion of postulate (iv)].

Clustering of affected and transmitting males in sibships: SHERMAN *et al.* (1985) report an unusual clustering of affected individuals in some families. The mechanism proposed here predicts such a phenotypic clustering of progeny from some females. A heterozygous female with a mutated but nonimprinted fragile-*X* may have, by chance, a population of oogonial cells in which the fragile-*X* is inactivated with a frequency very different from the average of 0.5 expected from random inactivation. [Large variations in this frequency are expected because of the small number of embryonic cells—about 20—in which the inactivation process occurs (FIALKOW 1973).] This proportion reflects the frequency of imprinting of the fragile-*X* chromosome in that mother, which determines the probability of that woman having a mentally normal or subnormal child. For example, if 90% of the oogonial cells of a woman have an inactivated fragile-*X*, then the proportion of mentally subnormal progeny would be 0.3375; if only 10% of her oogonial cells had an inactivated fragile-*X*, then the estimate of affected progeny falls to 0.0375, with a corresponding increase in the frequency of unaffected, transmitting sons and daughters. (Substitute 0.9 and 0.1, respectively, for 0.5 where appropriate in column C, Table 1.) Considerable variability in the occurrence of affected children from heterozygous mothers with nonimprinted fragile-*X*'s would therefore be expected, from the mechanism proposed here, for individual pedigrees. This source of variability would explain the clustering of affected individuals observed in some sibships (SHERMAN *et al.* 1985), and it predicts similar clustering of unaffected, transmitting individuals in other sibships. This special feature of the proposed mechanism results from the developmental imprinting of the fragile-*X* in oocytes. This developmental chromosome imprinting thus leads to probabilities of affected or unaffected progeny that depend on the frequency of imprinting by an individual mother, and not simply on Mendelian inheritance of the fragile-*X* chromosome.

Summary of assumptions used in pedigree calculations: The calculations described above are based on the following assumptions and supporting evidence: (i) the fragile-*X* and the normal *X* are inactivated at random in oogonial cells [supported by analysis of *X* chromosome inactivation in lymphocytes of carrier mothers, described above, and evidence for a common source of stem cells, subsequent to inactivation, for human organs and tissues (NESBITT 1971; FIALKOW 1973)]; (ii) there is no preferential loss of a class of gametes or zygotes from these mothers (supported by the close correspondence between observed and expected frequencies of affected males in generation IV [Figure 3 and SHERMAN *et al.* (1985)]; and (iii) that 50% inactivation of the fragile-*X* is the approximate threshold value (either biological or ascertainment threshold) for normal or affected development in heterozygous females that inherit an imprinted fragile-*X* chromosome [consistent with published data correlating IQ scores with the fraction of lymphocytes in which the normal *X* chromosome is inactivated, summarized in UCHIDA *et al.* (1983) and TUCKERMAN, WEBB and BUNDEY (1985)] and with the penetrance of the syndrome among females from affected mothers (generation IV in Figure 3).

Summary of proposed mechanism: The proposed mechanism of inheritance and expression of the fragile-*X* syndrome is summarized in Figure 4 for a female who inherits an imprinted fragile-*X* chromosome. Her gametes will contain the same *X* chromosome alleles, or "states," with which she began life, assuming that reversal of the imprint is rare. A "Punnett square" is shaded to indicate the 50% and 100% penetrances expected for females and males, respectively, who have inherited imprinted fragile-*X* chromosomes from such a mother (Figure 4).

A similar summary of progeny from a mother who inherits a mutated but nonimprinted fragile-*X* requires a rectangle to accommodate the three classes of gametes that she produces from her two original *X* chromosomes (Figure 5d). This "Punnett rectangle" is useful in separating Mendelian inheritance from the developmental components of the syndrome. The random inactivation in pre-oogonial cells that can lead to an imprinted fragile-*X* chromosome, and the somatic inactivation that affects mental ability of the female, must be considered separately from Mendelian segregation of the *X* chromosomes. Nevertheless, the expected frequencies at a population level are highly predictable. Figures 3, 4 and 5, taken together, summarize the components of the inheritance and expression of the fragile-*X* syndrome, based on the mechanism presented here.

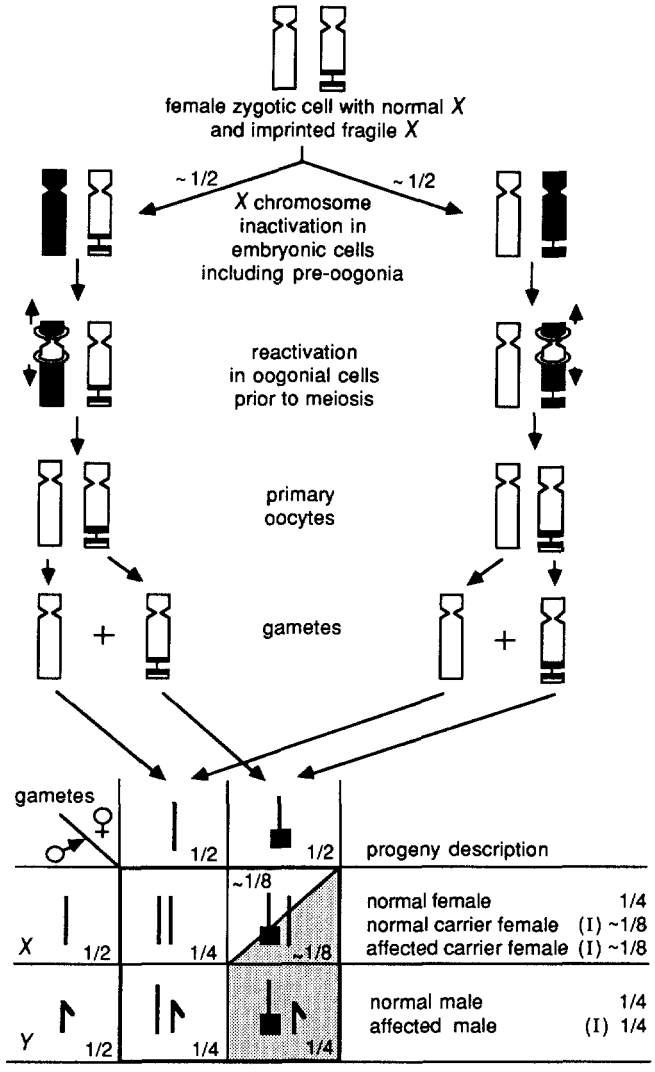

FIGURE 4.—From zygote to gametes and progeny: for a female with an imprinted fragile-*X* chromosome, progeny are classifiable with a "Punnett square." The somatic component of *X* chromosome inactivation that occurs in females includes pre-oogonial cells. Prior to oogenesis, reactivation of the *X* chromosome occurs. A normal *X* chromosome becomes completely reactivated after inactivation; a fragile-*X* chromosome is only partially reactivated because of a block at fragile site Xq27. Even though the initial inactivation is random for the two *X* chromosomes, Mendelian ratios of gametes are produced by this female because the imprinted fragile-*X* that she inherited is usually not altered by additional cycles of inactivation and reactivation. If somatic inactivation in either the female or her daughters is more than 50% for the fragile-*X*, then that individual is likely to have an IQ score in the normal range. If somatic inactivation is less than 50% for the fragile-*X*, then that individual is likely to have a subnormal IQ score (shaded region in Punnett square). Since males have only one *X* chromosome and no process of somatic inactivation, their mental performance is subnormal if they have an imprinted fragile-*X* (*shaded region*). In the "progeny description" column, "I" represents individuals with an imprinted fragile-*X* chromosome. The tilde (~) in front of some ratios indicates the variability introduced by the random inactivation process. Ratios not so marked represent Mendelian ratios determined solely by meiotic segregation.

CONCLUDING REMARKS

The maternal chromosome imprinting associated with oogenesis, and the somatic inactivation of *X* chromosomes in females, are proposed as developmental, or "epigenetic," components to the fragile-*X*

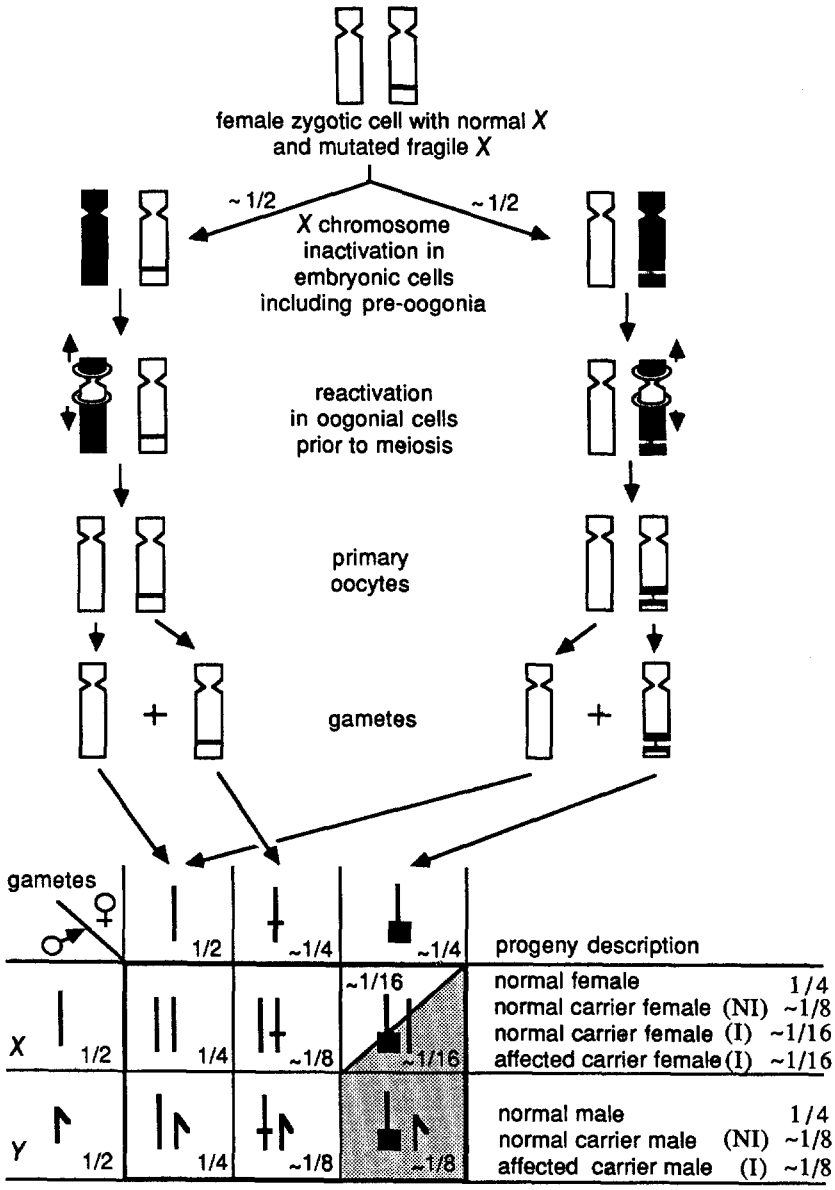

FIGURE 5.—From zygote to gametes and progeny: for a female with a mutated but nonimprinted fragile-*X* chromosome, progeny are classifiable with a "Punnett rectangle." As described in the legend to Figure 4, a fragile-*X* chromosome is only partially reactivated, if it has even been inactivated, because of a block at fragile site Xq27. For a female who inherits a nonimprinted fragile-*X*, however, there is also a class of gametes that represents a mutated fragile-*X* that by chance was not inactivated in the pre-oogonial cell giving rise to that gamete. Thus a rectangle is needed to display her three classes of gametes. In the "progeny description" column, "I" and "NI" represent individuals with imprinted and nonimprinted (but mutated) fragile-*X* chromosomes, respectively. See legend to Figure 4 for further details.

syndrome. These components provide explanations for many perplexing features of inheritance and expression of the fragile-*X* syndrome: (i) transmitting males have inherited a fragile-*X* chromosome that had not been imprinted in a previous generation by a cycle of maternal chromosome inactivation and incomplete reactivation; (ii) transmitting males have unaffected daughters because males do not inactivate their *X* chromosome for dosage compensation; (iii) daughters of transmitting males have transmitting sons because these daughters have an imprinted fragile-*X* chromosome in only about half of their primary oocytes; in progenitor cells of the other half of their primary oocytes, the fragile-*X* remained the active *X* chromosome and thus did not become imprinted; (iv) the two cytological classes of heterozygous females (low and high frequency of cytological expression) reflect those with nonimprinted and imprinted fragile-*X* chromosomes, respectively; (v) affected females generally do not have transmitting sons because an imprinted fragile-*X* chromosome is stably imprinted; (vi) the unusual penetrances of the syndrome in males and females is in part explicable in terms of two classes of mentally normal carrier females, those with imprinted and those with nonimprinted fragile-*X* chromosomes, who have different distributions of progeny classes; (vii) variable expression of the syndrome in females is caused by random somatic inactivation of the *X* chromosome ("Lyonization"); (viii) the clustering of affected individuals in some sibships is a result of the mosaic nature of the ovary. These explanations are based on well-described phenomena in mammalian developmental genetics, except for the proposed block to *X* chromosome reactivation. Such a block is consistent, however, with available transcription and pedigree data, and is subject to experimental test.

Although the formal genetic analysis presented here does not depend on knowledge of either the molecular basis of the fragile-*X* mutation or the mechanism by which the mutation may block the reactivation process, some information and suggestions are available. It is proposed elsewhere that the fragile-*X* mutation at *X*q27 results in late DNA replication at that region, and that this late DNA replication blocks locally the reactivation process (LAIRD *et al.* 1987). No information is yet available on the molecular basis of the putative mutation to late replication, although the apparent high rate of mutation (SHERMAN *et al.* 1985) is consistent with there being an unusual mutational event or structure at *X*q27 in fragile-*X* chromosomes. Thus, changes to DNA other than an altered nucleotide sequence at *X*q27 should be investigated. For example, the imprinting event as well as the mutation itself could result from an inappropriate methylation of DNA that would be propagated to descendant cells (SAGER and KITCHIN 1975; HOLLIDAY and PUGH 1975; RIGGS 1975). If both the mutation and imprinting events are base modifications rather than more conventional mutational changes, then the nucleotide sequence of DNA at the fragile-*X* site could be the same in normal *X* and fragile-*X* chromosomes. The apparent stability of an imprinted fragile-*X* from one generation to another illustrates the difficulty, without molecular characterization of a mutant allele, in distinguishing between chromosome imprinting and more conventional mutations such as changes in base sequence. Analysis of fragile-*X* DNA by nucleotide sequencing should therefore be complemented by experiments to assess, for genes at or near fragile site *X*q27, methylation patterns, the timing of replication, and transcriptional states. It will be important to reexamine the details of DNA replication in imprinted fragile-*X* chromosomes that are primarily early replicating. Does any part of the long arm of the *X* chromosome, at or distal to *X*q27, show the late replication expected for residual, local inactivation?

A general implication of the mechanism proposed here is that there may be other mutations that block gene or chromosomal reactivation. Human disorders that are characterized by a pattern of inheritance similar to that of the fragile-*X* syndrome could reflect additional mutations that interfere with dosage compensation and the cycle of maternal *X* chromosome inactivation/reactivation. Other disorders may affect different inactivation/reactivation cycles, such as the chromosomal inactivation that occurs during spermatogenesis and is apparently reversed at the subsequent embryogenesis; these disorders would exhibit patterns of inheritance different from that of the fragile-*X* syndrome. An understanding of the developmental events involved with inactivation/reactivation cycles may lead to methods that are effective in reversing abnormal states of gene or chromosomal inactivation.

Supported by grants from the National Institutes of Health (General Medical Sciences) and the National Science Foundation (Eukaryotic Genetics). I thank my colleagues and teachers who helped lay foundations that led to the analysis presented here, my current associates R. NELSON, E. JAFFE and G. KARPEN for their challenges, R. KVITEK for the illustrations, and my family for their patience. A. MOTULSKY, H. WEINTRAUB, L. HARTWELL, E. THOMPSON, S. GARTLER, G. ODELL, B. WAKIMOTO, M. GOLDMAN, R. NELSON, G. KARPEN, K. NELSON, J. FELSENSTEIN, E. JAFFE, M. LAMB, L. SANDLER, B. SCHULTZ, M. CHAMPE, V. SCHWEICKART, G. MARTIN, J. SHEPHERD, H. HOEHN, E. YELVERTON, E. BRYANT, E. MAGENIS, R. NUSSBAUM, B. MIGEON, S. SHERMAN, W. T. BROWN and I. HERSKOWITZ provided helpful comments on various versions of the manuscript or on the ideas presented here.

LITERATURE CITED

BORGAONKAR, D., 1984 *Chromosomal Variation in Man: A Catalog of Chromosomal Variants*, Ed. 4. Alan R. Liss, New York.

BROWN, W. T., E. C. JENKINS, M. S. KRAWCZUN, K. WISHIEWSKI, R. RUDELLI, I. L. COHEN, G. FISCH, E. WOLF-SCHEIN, C. MIEZEJES and C. DOBKIN, 1986 The fragile-X syndrome. Ann. N.Y. Acad. **477:** 129–150.

CAMERINO, G., M. G. MATTEI, J. F. MATTEI, M. JAYE and J. L. MANDEL, 1983 Close linkage of fragile X-mental retardation syndrome to haemophilia B and transmission through a normal male. Nature **306:** 701–704.

CATTANACH, B. M., 1974 Position effect variegation in the mouse. Genet. Res. **23:** 291–306.

CHANDRA, H., and S. BROWN, 1975 Chromosome imprinting and the mammalian X chromosome. Nature **253:** 165–168.

CROUSE, H. V., 1960 The controlling element in sex chromosome behavior in Sciara. Genetics **45:** 1429–1443.

FIALKOW, P., 1973 Primordial cell pool size and lineage relationships of five human cell types. Ann. Hum. Genet. **37:** 39–48.

FROSTER-ISKENIUS, U., B. C. MCGILLIVRAY, F. J. DILL, J. G. HALL and D. B. HERBST, 1986 Normal male carriers in the fra(X) form of X-linked mental retardation (Martin-Bell syndrome). Am. J. Med. Genet. **23:** 619–631.

GARTLER, S. M., R. ANDINA and N. GANT, 1975 Ontogeny of X-chromosome inactivation in the female germ line. Exp. Cell Res. **91:** 454–457.

GARTLER, S., M. LISKAY, B. CAMPBELL, R. SPARKES and N. GANT, 1972 Evidence for two functional X chromosomes in human oocytes. Cell Diff. **1:** 215–218.

HOLLIDAY, R., and J. PUGH, 1975 DNA modification mechanisms and gene activity during development. Science **187:** 226–232.

HOWELL, R. T., and A. MCDERMOTT, 1982 Replication status of the fragile X chromosome fra(X)(q27) in three heterozygous females. Hum. Genet. **62:** 282–284.

ISRAEL, M., 1987 Autosomal suppressor gene for fragile-X: an hypothesis. Am. J. Med. Genet. **26:** 19–31.

JACOBS, P. A., and S. L. SHERMAN, 1985 The fragile (X): a marker for the Martin-Bell syndrome. Dis. Marker **3:** 9–25.

KNOLL, J. H., A. CHUDLEY and J. GERRARD, 1984 X-linked mental retardation. II. Frequency and replication pattern of fragile (X)(q28) in heterozygotes. Am. J. Hum. Genet. **36:** 640–645.

LAIRD, C., E. JAFFE, G. KARPEN, M. LAMB and R. NELSON, 1987 Fragile sites in human chromosomes as regions of late-replicating DNA. Trends Genet. In press.

LEDBETTER, D. H., S. LEDBETTER and R. NUSSBAUM, 1986 Implications of fragile X expression in normal males for the nature of the mutation. Nature **324:** 161–163.

LUBS, H. A., 1969 A marker X chromosome. Am. J. Hum. Genet. **21:** 231–244.

LYON, M. F., 1961 Gene action in the X chromosome of the mouse (*Mus musculus* L). Nature **190:** 372–373.

LYON, M. F., 1972 X-chromosome inactivation and developmental patterns in mammals. Biol. Rev. **47:** 1–35.

MARENI, C., and B. MIGEON, 1981 Fragile X syndrome: search for phenotypic manifestations at loci for hypoxanthine phosphoribosyltransferase and glucose-6-phosphate dehydrogenase. Am. J. Hum. Genet. **33:** 752–761.

MIGEON, B. R., L. SHAPIRO, R. NORUM, T. MOHANDAS, J. AXELMAN and R. DABORA, 1982 Differential expression of steroid sulphatase locus on active and inactive human X chromosome. Nature **299:** 838–840.

NESBITT, M. N., 1971 X chromosome inactivation mosaicism in the mouse. Dev. Biol. **26:** 252–263.

OBERLE, I., R. HEILIG, J. MOISAN, C. KLOEFPER, M. MATTEI, J. MATTEI, J. BOUE, U. FROSTER-ISKENIUS, P. JACOBS, G. LATHROP, J. LALOUEL and J. MANDEL, 1986 Genetic analysis of the fragile-X mental retardation syndrome with two flanking polymorphic DNA markers. Proc. Natl. Acad. Sci. USA **83:** 1016–1020.

OPITZ, J., 1986 Editorial comment: on the gates of hell and a most unusual gene. Am. J. Med. Genet. **23:** 1–10.

PAUL, J., U. FROSTER-ISKENIUS, W. MOJE and E. SCHWINGER, 1984 Heterozygous female carriers of the marker-X chromosome: IQ estimation and replication status of fra(X)(q). Hum. Genet. **66:** 344–346.

PEMBREY, M. E., R. WINTER and K. DAVIES, 1985 A premutation that generates a defect at crossing over explains the inheritance of fragile-X mental retardation. Am. J. Med. Genet. **21:** 709–717.

RIGGS, A. D., 1975 X inactivation, differentiation, and DNA methylation. Cytogenet. Cell Genet. **14:** 9–25.

SAGER, R., and R. KITCHIN, 1975 Selective silencing of eukaryotic DNA. Science **189:** 426–433.

SHAPIRO, L. J., T. MOHANDAS and R. WEISS, 1979 Non-inactivation of an X-chromosome locus in man. Science **204:** 1224–1226.

SHERMAN, S., 1986 Invited editorial comment: a new genetic model for the fragile X syndrome involving an autosomal suppressor gene—comments on the paper by M. H. Israel. Am. J. Med. Genet. **26:** 33–36.

SHERMAN, S., P. JACOBS, N. MORTON, U. FROSTER-ISKENIUS, P. HOWARD-PEEBLES, K. NIELSEN, M. PARTINGTON, G. SUTHERLAND, G. TURNER and M. WATSON, 1985 Further segregation analysis of the fragile X syndrome with special reference to transmitting males. Hum. Genet. **69:** 289–299.

SNYDER, F., C. LIN, C. HARASYM, H. JAMRO, M. KUSHNIG, J. LOWE and S. O'BRIEN, 1984 Evidence for close association between the fragile X(q27-28) chromosome site and glucose-6-phosphate dehydrogenase (G6PD) but not with hypoxanthine-guanine phosphoribosyltransferase (HPRT). Cytogenet. Cell Genet. **37:** 587.

SPOFFORD, J. B., 1976 Position-effect in Drosophila. pp. 955–1018. In: *The Genetics and Biology of Drosophila*, Vol. 1c, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

STEINBACH, P., 1986 Mental impairment in Martin-Bell syndrome is probably determined by interaction of several genes: simple explanation of phenotypic differences between unaffected and affected males with the same X chromosome. Hum. Genet. **72:** 248–252.

SUTHERLAND, G., 1985 The enigma of the fragile X chromosome. Trends Genet. **1:** 108–112.

SUTHERLAND, G., and F. HECHT, 1985 *Fragile Sites on Human Chromosomes* (Oxford Monographs on Medical Genetics, No. 13). Oxford University Press, New York.

THERMAN, E., G. SARTO and K. PATAU, 1974 Center for Barr body condensation on the proximal part of the human Xq: a hypothesis. Chromosoma **44:** 361–366.

TUCKERMAN, E., T. WEBB, and S. BUNDEY, 1985 Frequency and replication status of the fragile X, fra(X)(q27-28), in a pair of monozygotic twins of markedly differing intelligence. J. Med. Genet. **22:** 85–91.

TUCKERMAN, E., T. WEBB and A. THAKE, 1986 Replication status of fragile X(q27.3) in 13 female heterozygotes. J. Med. Genet. **23:** 407–410.

UCHIDA, I. A., and E. JOYCE, 1982 Activity of the fragile X in heterozygous carriers. Am. J. Hum. Genet. **34:** 286–293.

UCHIDA, I. A., V. FREEMAN, H. JAMRO, M. PARTINGTON and H. SOLTAN, 1983 Additional evidence for fragile X activity in heterozygous carriers. Am. J. Hum. Genet. **35:** 861–868.

Communicating editor: I. HERSKOWITZ