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Mouse Embryos: Impact of the Parental Origin of the Monosomic X Chromosome

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About 12–17% of the embryos obtained by mating mice carrying the In(X)1H or *Paf* mutations are of the 39,X (X0) genotype. Depending on the mutant mice used for mating, the monosomic X chromosome can be inherited from the paternal (X^P) or the maternal (X^M) parent. The X^P0 embryos display developmental retardation at gastrulation and early organogenesis. X^P0 embryos also display poor development of the ectoplacental cone, which is significantly smaller in size and contains fewer trophoblasts than XX siblings. In contrast, X^M0 embryos develop normally and are indistinguishable from XX littermates. In both types of X0 embryos, an X-linked *lacZ* transgene is expressed in nearly all cells in both the embryonic and the extraembryonic tissues, suggesting that X inactivation does not occur when only one X is present. Of particular significance is the maintenance of an active X^P chromosome in the extraembryonic tissues where normally the paternal X chromosome is preferentially inactivated in XX embryos. The differential impact of the inheritance of X chromosomes from different parents on the development of the X0 embryos raises the possibility that the X^P is less capable than the X^M in providing the appropriate dosage of X-linked activity that is necessary to support normal development of the embryo and the ectoplacental cone. Alternatively, the development of the X^P0 embryo may be compromised by the lack of activity of one or several X-linked genes which are expressed only from the maternal X chromosome. Without the activity of these genes, embryonic development may be curtailed even though all other loci on the X^P chromosome are actively transcribed.

INTRODUCTION

In humans, the 45,X genotype is associated with the pathogenesis of Turner syndrome. Up to 99% of 45,X conceptuses are estimated to die *in utero* (Hecht and Macfarlane, 1969; Hook and Warburton, 1983). After birth, individuals with Turner syndrome have short stature, gonadal dysgenesis, and anatomical anomalies including cardiovascular and renal malformations (Lippe, 1991). X0 mice that have inherited the paternal X chromosome (X^P0) show marginally reduced birth weight, slow postnatal growth (Burgoyne *et al.*, 1983a), reduced fecundity, and a short reproductive life span (Cattanach, 1962; Lyon and Hawker, 1973). More dramatically, some embryos with only the paternal X chromosome (X^P0 or X^PY^{*x}) do not survive when they develop in an XY^{*x} mother with one intact X chromosome and a structurally abnormal sex chromosome consisting of an X-derived centromere but without the pseudoautosomal region (Hunt, 1991). This loss of X^P0 embryos may be a combined effect of maternal monosomy and the lower embryonic viability. X^P0 embryos display growth retardation at the gastrulation and early organogenesis stages (Burgoyne *et al.*, 1983b). In contrast, X^M0 embryos are not growth retarded during postimplantation development (Tada *et al.*, 1993; Thornhill and Burgoyne, 1993) and survive to term (Lane and Davisson, 1990). However, adult XY^{*x} females where the X is maternal in origin, like X^P0 mice, have a shortened reproductive life span and reduced litter size (Hunt, 1991).

These differences in the impact of X monosomy on the development of X^P0 and X^M0 embryos suggest that the X chromosomes inherited from different parents are not equivalent in their genetic activity. The parent-specific effect could be the result of genomic imprinting, an epigenetic process whereby the whole or regions of homologous chromosomes, or alleles of the same genes, behave differ-

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ently according to the parental origin (Surani, 1991; Gold and Pedersen, 1994). Some examples of the imprinting effect are the preferential inactivation of the paternally derived X chromosome in the extraembryonic tissues (e.g., extraembryonic ectoderm, ectoplacental cone) and the primitive endoderm (Harper *et al.*, 1982; Takagi and Sasaki, 1982; Sugawara *et al.*, 1985), the generally lower transcriptional activity of paternal X-linked genes during preimplantation development (reviewed by Jamieson *et al.*, 1996), the inactivation of the X^P chromosome ahead of the X^M chromosome revealed by the activity of an X-linked *lacZ* transgene (Tam *et al.*, 1994a), and the replication patterns of the X chromosomes (Takagi and Sasaki, 1982). We hypothesized that differences in development between X^P0 and X^M0 embryos might also be due to imprinted X-chromosome activity resulting in a deficit of X-linked gene dosage in the X^P0 embryos. This deficit might be due to the obligatory inactivation of the X^P chromosome in the extraembryonic tissues and a lower activity of the X^P chromosome in the embryonic tissues.

While previous studies have shown that trophectoderm derivatives in 9.5 to 12.5 day pc X^P0 embryos show some X-linked PGK1 activity (Frels and Chapman, 1979; Papaioannou and West, 1981), this does not necessarily infer that the X^P chromosome is active in all cells in the extraembryonic tissues and at all stages of development. In order to examine the activity of the X chromosome in X^P0 and X^M0 embryos we assayed the expression at the cellular level of an X-linked *lacZ* transgene that is subject to X-chromosome inactivation (Tan *et al.*, 1993; Tam *et al.*, 1994a,b). This has allowed simultaneous analysis of the morphology and the X-chromosome activity of embryonic and extraembryonic tissues at gastrulation and early organogenesis.

MATERIALS AND METHODS

Transgenic H253 mice (original background: {C57BL/6 × DBA/2}F1) used in this study carry on their X chromosome (X*) a *lacZ* transgene under the control of the 5′ regulatory elements of the mouse gene encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase (Tan *et al.*, 1993; Tam *et al.*, 1994a,b). The mice were maintained as a close-bred colony on the {C57BL/6 × DBA/2} hybrid background.

X^P*0 embryos are produced using In(X)1H mice with an inversion of the X chromosome with breakpoints at bands A1 and F (original background: {C3H × 101}F1). The In(X)1H mice were also bred onto the H253 background and used in matings to produce embryos that can be genotyped by *lacZ* staining in addition to cytogenetic analysis (Figs. 1A and 1B). Due to nondisjunction at meiosis I during oogenesis (Evans and Phillips, 1975) oocytes without any X chromosome are produced. H253 XY mice (X*Y) were mated to females heterozygous for the X inversion, In(X)1H/X and In(X)1H/X, to produce X^P*0 offspring (Figs. 1A and 1B).

X^M*0 embryos are produced using *Patchy fur* (*Paf*) mice (background: C3H/HeSnJ, subline bc^{3J}) that carry an X-linked mutation close to the XY pseudoautosomal boundary. This mutation results in nondisjunction at meiosis I during spermatogenesis so that sperms without any sex chromosome are produced (Lane and Davisson, 1990). The *Paf* male mice were mated with X*/X* females to produce litters containing X^M*0 embryos (Fig. 1C).

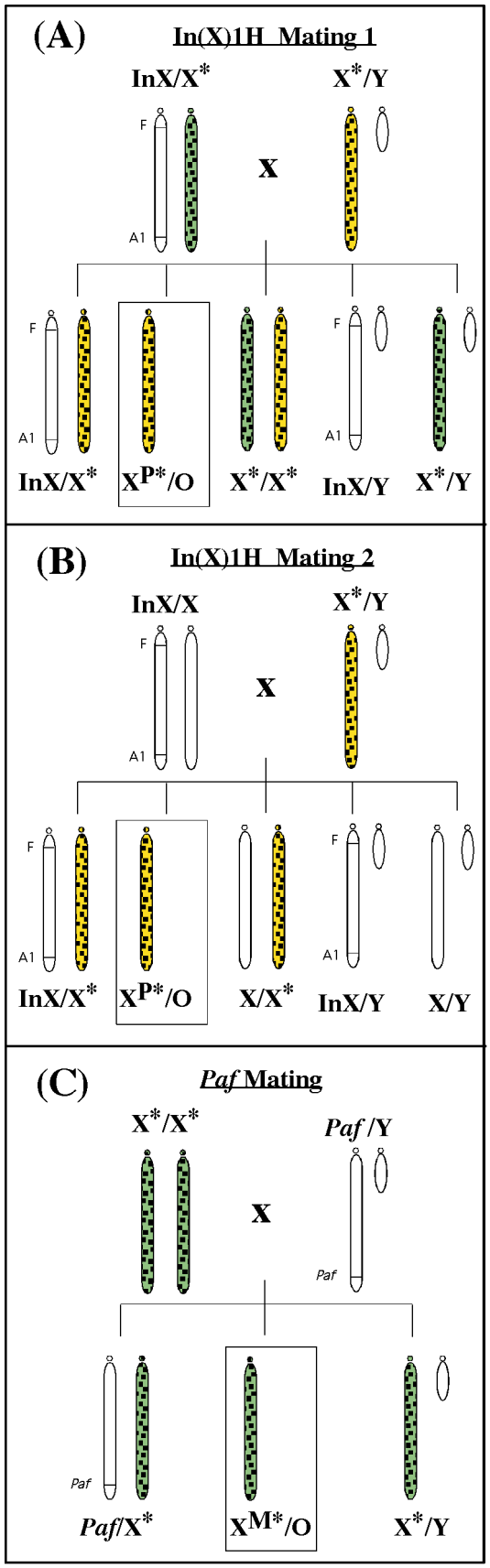
Embryos at 7.5 to 9.5 days pc were collected from pregnant mice and their developmental stages were determined according to Downs and Davies (1993) and Theiler (1989). Developmental stages attained by embryos of different genotypes were scored and between-genotype data were tested statistically using the K–S two-sample nonparametric test.

After an examination of the morphology, embryos were dissected into embryonic and extraembryonic parts. For assaying the activity of the X-linked *lacZ* transgene, tissues were fixed for 2–5 min in 4% paraformaldehyde, washed in phosphate-buffered saline, and stained at 37°C with X-gal reagent overnight (Tan *et al.*, 1993). The stained specimens were processed for paraffin embedding and sectioned serially at 8 μm for histological examination. The size of the ectoplacental cone was determined on the section of the extraembryonic tissues along the proximal–distal plane of the egg cylinder. The section showing the maximal longitudinal–sectional area of the ectoplacental cone was selected for morphometric determination of the tissue volume. The base (b) and the height (h) of the ectoplacental cone (see Fig. 6A) were measured using an eyepiece micrometer. By assuming a regular conical shape of the ectoplacental cone, tissue volume was computed according to the formula of Volume $= \pi(b \div 2)^2 h \div 3$. The volumes of the ectoplacental cones of X0 and XX embryos of different developmental stages were compared by regression analysis and ANOVA.

The size of the egg cylinder was determined on the section of the embryonic tissues along the proximal–distal plane. The section showing the maximal longitudinal–sectional area of the embryo was selected for morphometric determination of the tissue volume. Tissue volume of the embryo was estimated by assuming a regular cylindrical shape of the gastrulating embryo or a hemispherical shape of the presomite stage embryo, The base (b) and the height (h) of the cylindrical or cup-shaped embryo (see Fig. 4B) were measured using an eyepiece micrometer. Tissue volume was computed according to the formulae of Volume $= \pi(b \div 2)^2 h$ (cylinder) and Volume $= \{1.33\pi[(b/2 + h)/2]^3\}/2$ (hemisphere). The embryonic volumes of the XX and X0 embryos were compared by regression analysis and ANOVA.

Tissues for genotyping were processed for cytogenetic analysis. Tissues were incubated for 1 h at 37°C in 0.1 μg/ml colcemid, treated with hypotonic solution (0.56% (w/v) aqueous KCl), fixed in 3:1 (v/v) methanol:glacial acetic acid, and disaggregated in 60% (v/v) acetic acid. The cell suspension was dropped onto slides and stained with Giemsa solution to reveal the metaphase chromosomes. Two to four consecutive scores of 39 chromosomes and the absence of the Y chromosome were needed to assign an X0 genotype. In five X^P0 cases, only one metaphase was suitable for analysis. We have not attempted to distinguish between XX and In(X)/X specimens by chromosomal banding since a previous study (Burgoyne *et al.*, 1983b) has shown no difference in embryonic size and development of these two types of female embryos.

For studying the X-linked transgene expression in the embryonic tissues, X-gal histochemical staining was carried out on the dissected embryonic portion of the gastrulating embryo (the egg cylinder) or the embryo proper of the early-organogenesis stage embryos. The genotype of these embryos was determined using the extraembryonic tissues (extraembryonic ectoderm or the yolk sac). Conversely, the embryonic tissues were used for genotyping when the X-linked transgene activity of the extraembryonic tissues was to be studied. Cytogenetic analyses were carried out on embryos



derived from all three matings (Fig. 1). However, for embryos obtained from mating the In(X)1H/X female and H253 male mice (Fig. 1B), the genotype can be determined first from the X-gal staining pattern (see legend to Fig. 1) and the result has been confirmed by cytogenetic analysis.

For detection of *4311* gene expression (Lescisin *et al.*, 1988), extraembryonic tissues were embedded in 2% agar prior to processing in paraffin wax. Wax sections, cut at 7 μm, were mounted onto silanized slides and incubated overnight at 37°C. Following deparaffinization in graded alcohols, sections were brought to water and acid treated (0.2 M HCl) for 20 min before digestion with pronase (125 μg/ml) in Tris buffer. Following rinsing, sections were postfixed in 4% paraformaldehyde (10 min), washed three times in 0.1 M phosphate buffer, and acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine buffer for 10 min before prehybridization (5× SSC, 50% formamide, 5× Denhardt's solution, 100 μg/ml yeast tRNA, 100 μg/ml herring sperm DNA, and 10× dextran sulfate). Hybridization was carried out using *in vitro* transcribed sense (T7 polymerase) and antisense (SP6 polymerase) riboprobes labeled with digoxigenin (Boehringer-Mannheim) from linearized *4311* cDNA. Probes were treated with DNase I, ethanol precipitated with LiCl before adding into hybridization buffer at 2 μg/ml, and allowed to incubate for 16 h at 55°C. Following extensive washing in 5× SSC, 50% formamide, followed by 2× SSC (55°C for 30 min), and 0.1× SSC (55°C for 30 min), riboprobe binding was revealed using anti-digoxigenin antisera (conjugated to alkaline phosphatase) with the chromogenic substrate NBT/BCIP. Sections were counterstained using neutral red, dehydrated in graded alcohols, and coverslipped.

RESULTS

Postimplantation Development of X^P0 Embryos Is Retarded

Altogether 67 X^P0 embryos were found among 393 successfully genotyped embryos from 59 litters of In(X)1H/X mice (Figs. 1A and 1B) and 27 X^M0 embryos were found in

FIG. 1. Matings to produce X^P*0 and X^M*0 embryos. In A, females with the In(X)1H inversion affecting one X chromosome and the *lacZ* transgene on the other X chromosome (InX/X*) are mated with X*Y males. Nondisjunction at meiosis I occurs in the InX/X* females so that oocytes can be produced without an X chromosome. Fertilization with X-bearing sperm leads to the production of X^P*0 embryos. The genotypes can be distinguished by cytogenetic analysis. In B, females heterozygous for the In(X)1H inversion and an X chromosome lacking the *lacZ* transgene (InX/X) are mated with X*Y males. X^P*0 embryos are produced which can be identified by cytogenetic analysis and X-gal staining pattern. InX/X* and X/X* embryos show mosaic staining, X^P*0 embryos show uniform staining, and male embryos are unstained. In C, females homozygous for the *lacZ* transgene (X*/X*) are mated with males carrying the *Patchy fur* (*Paf*) mutation on their X chromosome. Nondisjunction at meiosis I in these males results in sperm without any sex chromosomes and fertilization results in the formation of X^M*0 embryos. Genotypes are determined by cytogenetic analysis. Abbreviations: InX, In(X)1H; X*, X chromosome carrying *lacZ* transgene.

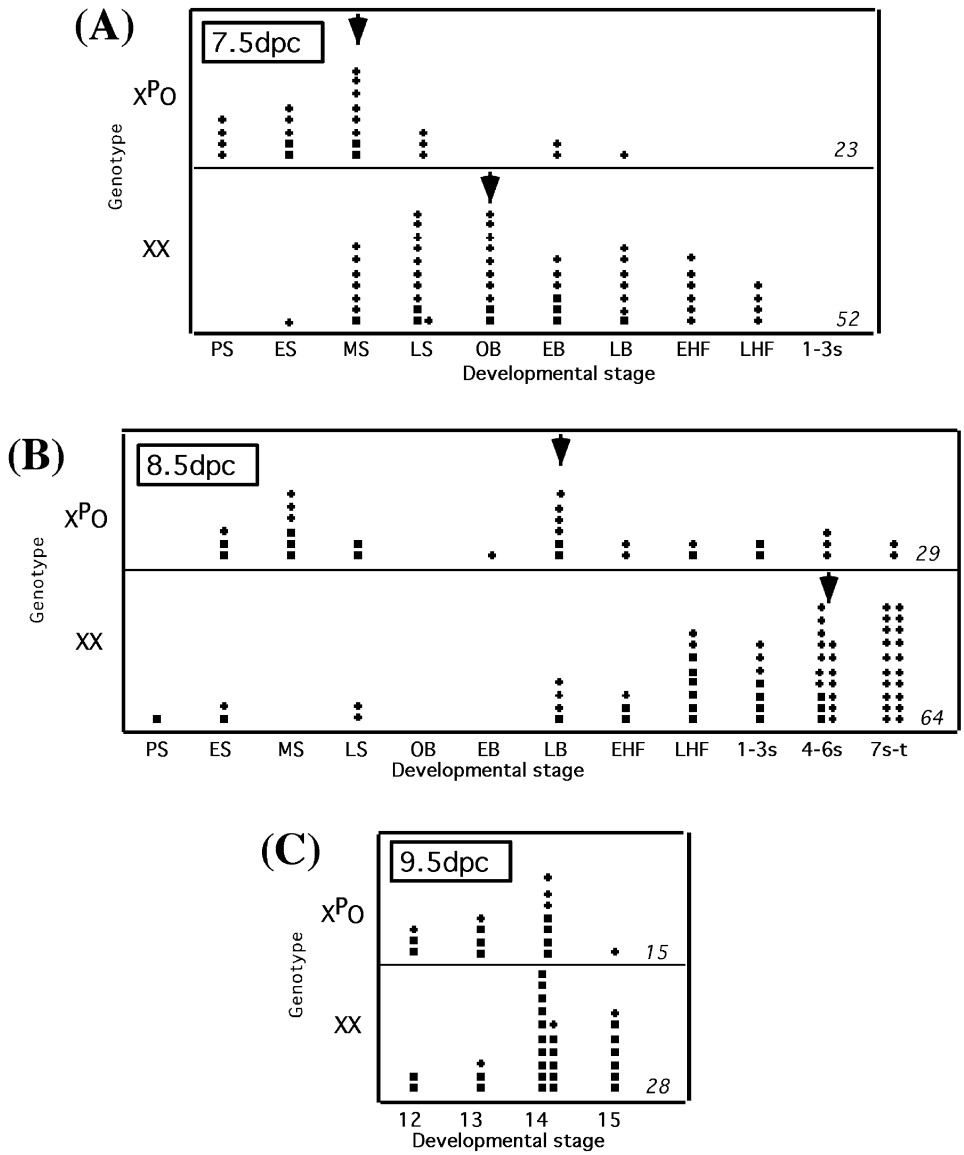


FIG. 2. Developmental stages of X^P0 embryos and littermates. Embryos at (A) 7.5 days, (B) 8.5 days, and (C) 9.5 days pc were collected from pregnant mice from the two In(X)1H matings (■, In(X)1H mating 1 embryo; +, In(X)1H mating 2 embryo) (Figs. 1A and 1B). Developmental stages of the embryos were determined according to the staging systems of Downs and Davies (1993) and Theiler (1989). Embryos were then subject to cytogenetic analysis and X-gal staining for genotype determination. Each embryo is grouped according to its genotype and developmental stage. X^P0 embryos are significantly developmentally retarded compared with XX embryos at 7.5 and 8.5 days pc ($P < 0.001$ for both ages, K–S two-sample nonparametric test). The developmental stage of the median embryo is indicated by the arrow for X^P0 and XX embryos at 7.5 and 8.5 days pc. At 9.5 days pc X^P0 embryos are not different from XX siblings regarding the developmental stages attained when tested on a pooled sample basis. The number of embryos in each genotypic grouping is indicated in italics. There were also 180 male embryos, 19 embryos with an unknown genotype, and 2 embryos with a 41,XXX genotype, that are not included in this figure. Developmental stages: PS, pre-streak; ES, early streak; MS, mid-streak; LS, late streak; OB, no allantoic bud; EB, early allantoic bud; LB, late allantoic bud; EH, early head fold; LHF, late head fold; 1–3s, 1–3 somites; 4–6s, 4–6 somites; 7s–t, 7 somites–turning; Theiler stages 12 - 15.

223 embryos from 26 litters sired by the *Paf* male mice (Fig. 1C). X^P0 and X^M0 embryos therefore constituted 17.0 and 12.1% of the embryos analyzed.

A comparison of the developmental milestone attained by the X^P0 and the XX (including the In(X)1H/X) embryos reveals that the X^P0 embryos are lagging behind their normal counterparts at 7.5 and 8.5 days pc (X0s are significantly retarded at $P < 0.001$ for both ages either on a pooled basis or a within-

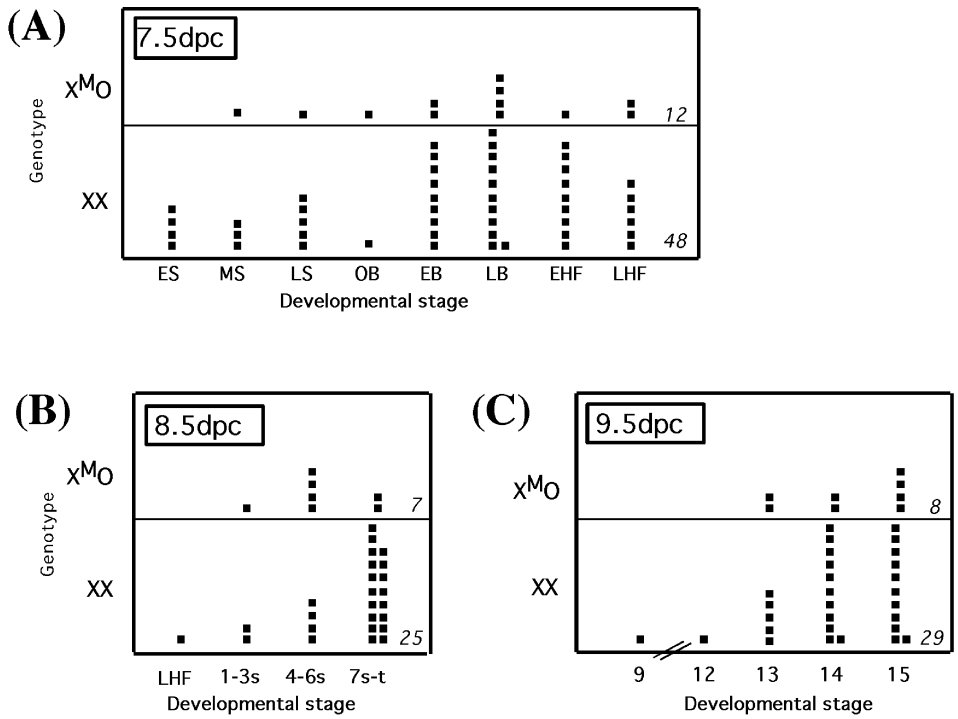


FIG. 3. Developmental stages of X^M0 embryos and littermates. Embryos at (A) 7.5 days, (B) 8.5 days, and (C) 9.5 days pc were collected from pregnant mice from the *Paf* mating (Fig. 1C). Developmental stages of the embryos were determined according to the staging systems of Downs and Davies (1993) and Theiler (1989). The genotype was determined by cytogenetic analysis. Each embryo is grouped according to its genotype and developmental stage. The development of the X^M0 embryos is comparable to that of XX littermates at 7.5 to 9.5 days pc. The number of embryos in each genotypic grouping is indicated in italics. There were 94 male embryos and 12 embryos with an unknown genotype that are not included in this figure. Developmental stages: PS, pre-streak; ES, early streak; MS, mid-streak; LS, late streak; OB, no allantoic bud; EB, early allantoic bud; LB, late allantoic bud; EHF, early head fold; LHF, late head fold; 1–3s, 1–3 somites; 4–6s, 4–6 somites; 7s–t, 7 somites–turning; Theiler stages 9–15.

litter comparison, Figs. 2A and 2B). The developmental stages of these embryos still lag behind XX (includes XX and In(X)1H/X) sibs at both ages even when one 7.5-day litter and three 8.5-day litters that contained an X^P0 embryo identified on a single metaphase score of 39 chromosomes were excluded from the data. The developmental retardation can also be seen in X^P0 embryos when they are analyzed separately for the two types of In(X)1H matings (Figs. 1A and 1B). The development of the X^P0 embryos did not seem to lag behind XX embryos at 9.5 days pc. However, the sample size might have been too small and a different conclusion may be obtained with larger samples. In contrast, the development of X^M0 embryos at 7.5 to 9.5 days pc is similar to their XX siblings (Figs. 3A–3C).

The X^P0 embryos made up 15.2% of embryos at 7.5 days pc and 17.0% at 9.5 days pc and the average litter sizes were 7.13 and 6.7, respectively. These findings suggest that the developmental delay at gastrulation and early organogenesis does not result in loss of the X^P0 embryos.

Histological examination of embryos obtained from the In(X)1H/X mice revealed that some retarded X^P0 embryos were deficient of epiblast cells at gastrulation (Figs. 4C and 4D), although as a group epiblast volume estimated by morphometric measurement was not reduced in X^P0 embryos compared with XX embryos of comparable developmental stages (Fig. 5). The germ layers were disorganized in some X^P0 embryos (Fig. 4D) and the extraembryonic ectoderm displayed a dilated extraembryonic cavity in some (Figs. 4B and 4D). We have not noticed any significant elevation in the incidence of pyknosis or tissue necrosis in the retarded embryos, but a direct test for apoptosis has not been conducted. In the X^P0 embryos, gastrulation is delayed. The X^P0 embryos have only reached the late allantoic bud stage (Fig. 4E) when the 8.5-day XY and XX littermates have developed to the early-somite stage (Figs. 4F and 4G). However, as the X^P0 embryos develop, normal tissue morphology is restored to the germ layers (Fig. 4E) and by the early-somite stage, some X^P0 embryos show normal morphology which is indistinguishable from XX embryos at the same stage (Figs. 4H and 4I).

The Single X Chromosome Is Active in the X0 Embryos during Early Postimplantation Development

X-chromosome activity as revealed by X-gal staining shows that the X^P* chromosome is active in most of the

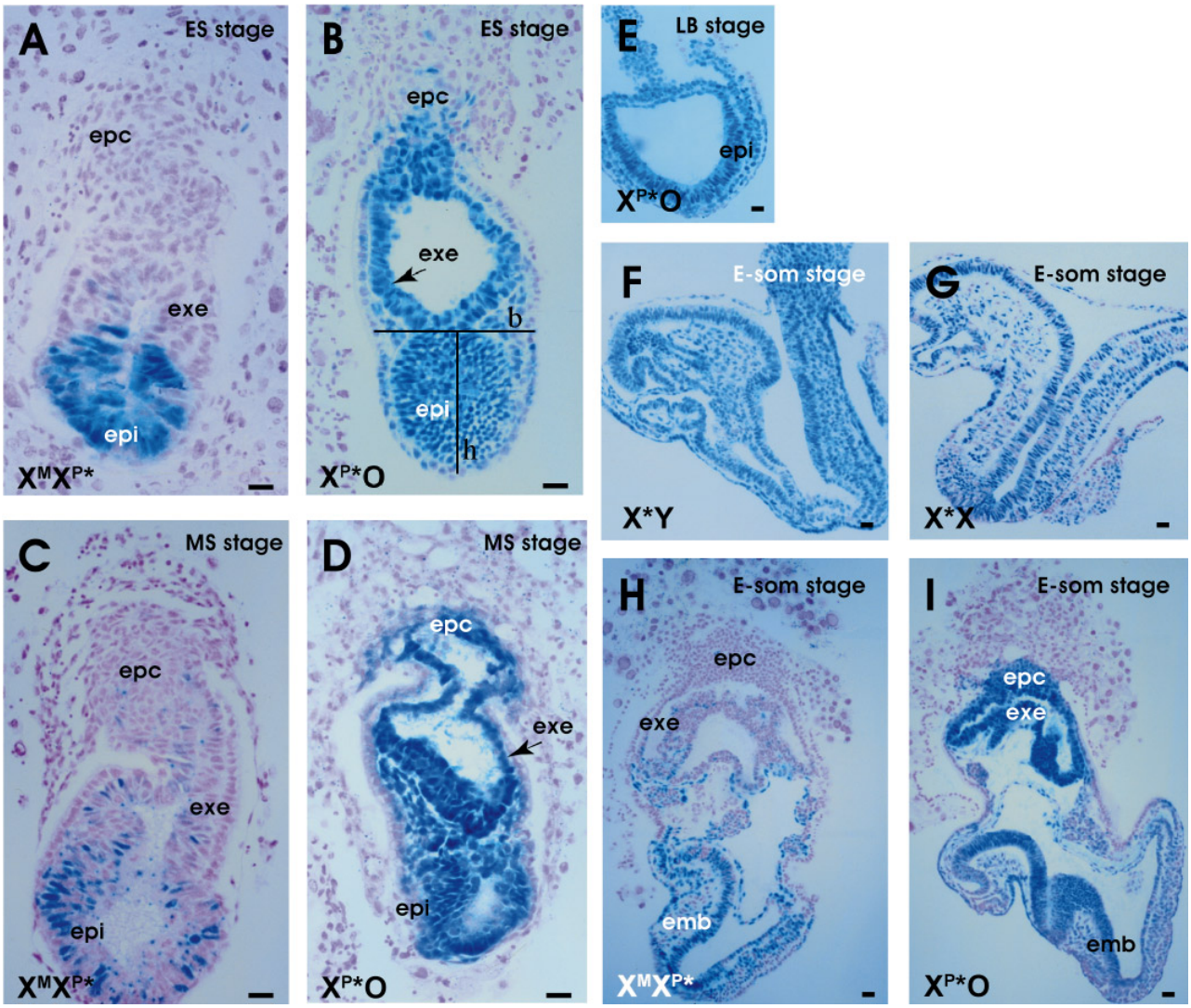
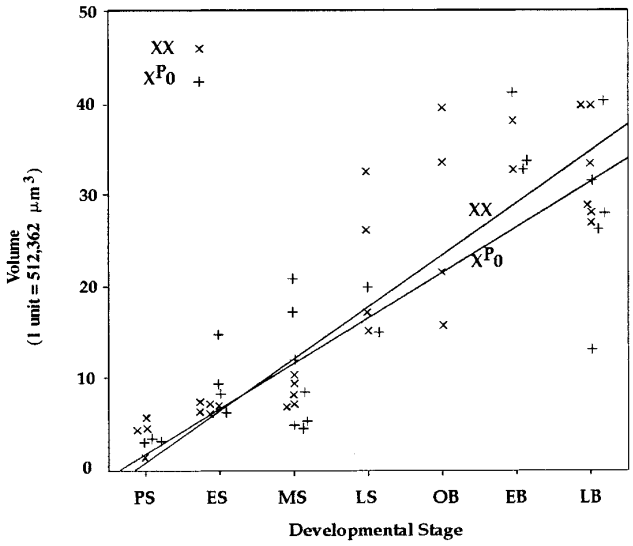


FIG. 4. Development and X-chromosome activity of X^P0 embryos at gastrulation and early organogenesis. In all sections X-gal histochemistry shows cells expressing the X-linked *lacZ* transgene as blue while nonexpressing cells are pink due to the nuclear Fast Red counterstain. Embryos (A–D and H, I) are from In(X)1H mating 2, and (E–G) are from In(X)1H mating 1. Staging is according to morphological characteristics and Downs and Davies (1993). X^P*0 embryos at 7.5 days pc (B, D) are developmentally delayed (early streak and mid-streak stages) being of comparable stage to XX embryos from 6.5 day litters (A, C). This is also the case at 8.5 days pc when the X^P*0 embryo (E) is at the late allantoic bud stage while the normal X*Y (F) and X*X (G) littermates are at the early somite stage. Another X^P*0 embryo at 8.5 days pc (I) shows catch-up in development being at a similar early somite stage as an XX embryo at 8.5 days pc (H). X-gal staining reveals that the X^P* chromosome is active with blue staining in most of the cells of the developmentally delayed embryos (B, D, E). The embryonic portion of the X^MX^P* embryos (A, C, G, H) shows a mosaic pattern of expression of the X-linked *lacZ* transgene due to random X-chromosome inactivation. The X*Y embryo (F) shows ubiquitous expression of the X-linked transgene as expected. Preferential inactivation of the X^P* chromosome is present in the extraembryonic ectoderm (exe) and ectoplacental cone (epc) of the X^MX^P* embryos (A, C, H) with cells staining pink due to the nuclear Fast Red counterstain. In contrast, expression of the X-linked transgene is seen in the extraembryonic ectoderm and ectoplacental cone of the X^P*0 embryos (B, D, I). A paucity of trophoblast cells is seen in the ectoplacental cones of the X^P*0 embryos (B, D, I) when compared with XX embryos of the same developmental stage (A, C, H). Abbreviations: ES, early streak; MS, mid-streak; LB, late allantoic bud; E-som, early (1–6) somites; epc, ectoplacental cone; emb, embryo; epi, epiblast; exe, extraembryonic ectoderm. Scale bar, 20 μm.

cells of the developmentally delayed embryos (early streak stage, Fig. 4B; mid-streak stage, Fig. 4D; late allantoic bud stage, Fig. 4E). The X*Y embryos show normal ubiquitous expression of the X-linked transgene (Fig. 4F) and the X*X embryos show a mosaic pattern of expression (Figs. 4A, 4C, 4G, and 4H) because of random X inactivation.



ANOVA table for embryonic volumes: XX and X^P0

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Stage	5	6213.384	1242.677	40.377	<.0001
Genotype	1	10.885	10.885	.354	.5556
Stage * Genotype	5	137.401	27.480	.893	.4956
Residual	38	1169.513	30.777		

FIG. 5. Embryonic volumes of gastrulating X^P0 and XX mouse embryos. The embryonic portion of the X^P0 conceptuses are not significantly different from those of XX embryos in the In(X)1H litters at the stages of gastrulation by ANOVA (XX, $n = 30$; X^P0, $n = 24$). Volume = $\pi(b \div 2)^2 h$ (cylinder) and Volume = $\{1.33\pi[(b/2 + h)/2]^3\}/2$ (hemisphere). Developmental stages: PS, pre-streak; ES, early streak; MS, mid-streak; LS, late streak; OB, no allantoic bud; EB, early allantoic bud; LB, late allantoic bud.

Cells in the extraembryonic ectoderm and ectoplacental cone of the transgenic X*Y (Fig. 6A) and X*X* embryos (which are homozygous for the transgene, Fig. 6B) show positive X-gal staining as expected. Preferential X^P* inactivation is revealed in these tissues in heterozygous female transgenic embryos. In X^MX^P* female embryos, cells in the extraembryonic ectoderm and ectoplacental cone do not express the transgene (Figs. 4H, 6C, 9A, and 9C), while in X^M*X^P female embryos there is strong and widespread expression of the transgene (Fig. 6D). In the X^P*0 embryos, the X-linked transgene is expressed widely in the extraembryonic ectoderm and the ectoplacental cone (Figs. 4I, 6E, and 9B). In the X^M*0 embryo, as expected, the maternal X chromosome is active in the extraembryonic tissues (Fig. 6F) and the embryonic cells (data not shown). Our results therefore show no evidence of widespread inactivation of the only X chromosome in the embryonic and extraembryonic cells of X^P*0 or X^M*0 embryos. Most interestingly, preferential inactivation of the X^P* chromosome does not occur in the X^P*0 embryos, suggesting that in the absence of another sex chromosome the paternal X chromosome behaves like the maternal X chromosome in the XY and XX embryos.

*Ectoplacental Cone Development of X^M*0 and X^P*0 Embryos*

Consistent with the normal development of the X^M*0 embryos, the extraembryonic tissues also appeared normally developed (Fig. 6F) and the sizes of the ectoplacental cones were similar to those of XX embryos in the *Paf* litters (Fig. 7). This suggests that maternal X monosomy has no impact on the differentiation of the extraembryonic tissues. In contrast, it was noted during the histological analysis of the X^P0 embryos that not only is the development of the embryo delayed but also that the ectoplacental cone appears smaller. The reduction in the size of the ectoplacental cone was confirmed by morphometric measurement of tissue volume on sectioned specimens (Fig. 8). The X^P0 ectoplacental cone is consistently smaller than the XX counterparts of the same developmental stage throughout gastrulation and early organogenesis. Morphometric analysis of the ectoplacental tissues of more advanced embryos was not performed because of the less-regular shape of the structure (Figs. 4H, 4I, 9C, and 9D). Histological examination shows that there is no apparent difference in the packing density of the trophoblasts (XX embryo, Fig. 9A, compared with X^P0 embryo, Fig. 9B), suggesting that the size deficit is due to a paucity of trophoblast cells in the X^P0 ectoplacental cones (Figs. 4B, 4D, and 9B) as seen when compared with XX counterparts (Figs. 4A, 4C, and 9A). At early-somite stages the ectoplacental cones of the X^P0 embryos are still small for the developmental stage (Figs. 4H and 4I). The tissue layers in the early placenta are also less well organized, but chorioallantoic fusion has occurred (Figs. 9C and 9D). Morphometric measurement of egg cylinder tissue volume on sectioned specimens indicates that egg cylinder size is not reduced in X^P0 embryos compared with XX embryos of comparable developmental stage (Fig. 5) when there is reduction in their ectoplacental cone size (Fig. 8).

To determine the impact of the reduced size of the ectoplacental cone in the X^P0 embryos on subsequent trophoblast differentiation, we have examined these embryos for expression of the spongiotrophoblast marker *4311* (Lescisin *et al.*, 1988). At 9.5 days pc, expression of *4311* is present in spongiotrophobast cells of the X^P0 embryo as it is in the XX embryo (Figs. 9E and 9F), suggesting that the ectoplacental cone tissues have differentiated to trophoblasts in the appropriate layers of the developing placenta.

DISCUSSION

Developmental Delay of the X^P0 Embryos

Results of our study have confirmed a previous finding of Burgoyne *et al.* (1983b) that there is delay in the development of the X^P0 embryos during gastrulation and early organogenesis. Analyses of the litter size and the number of

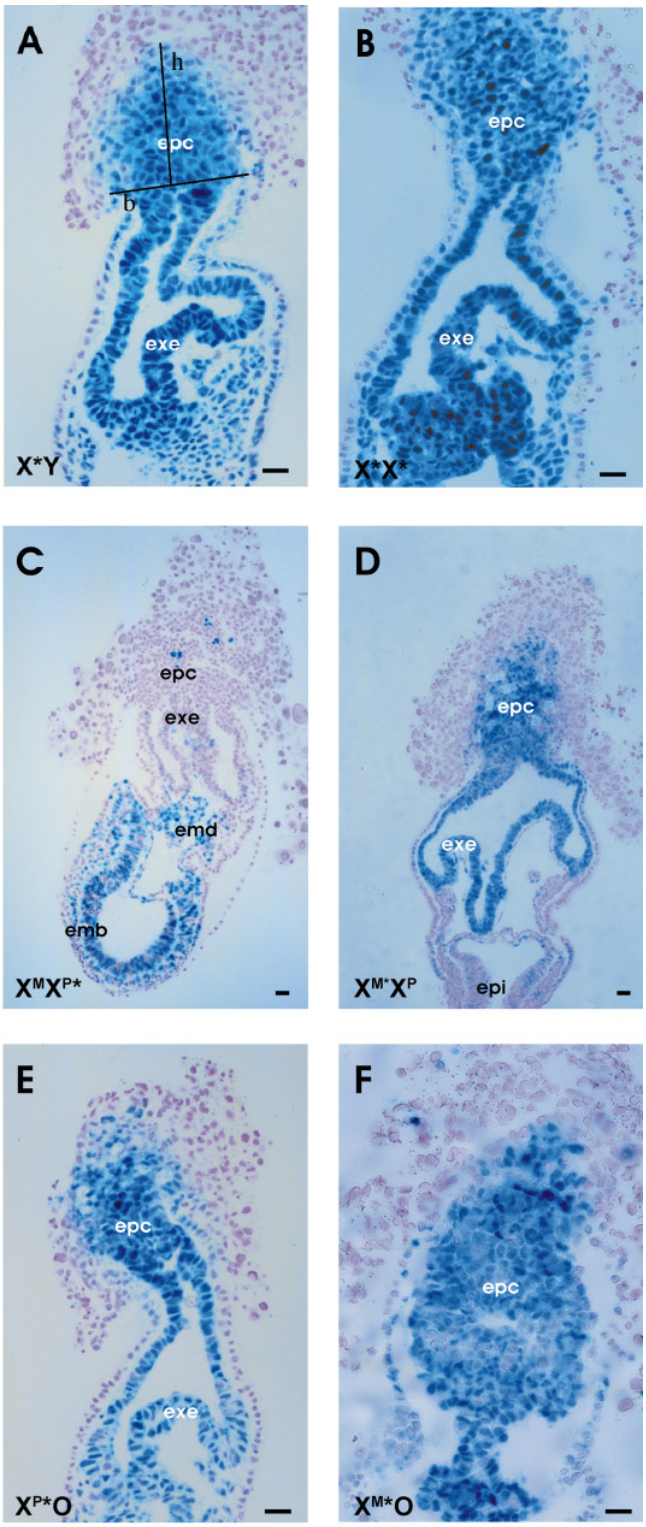


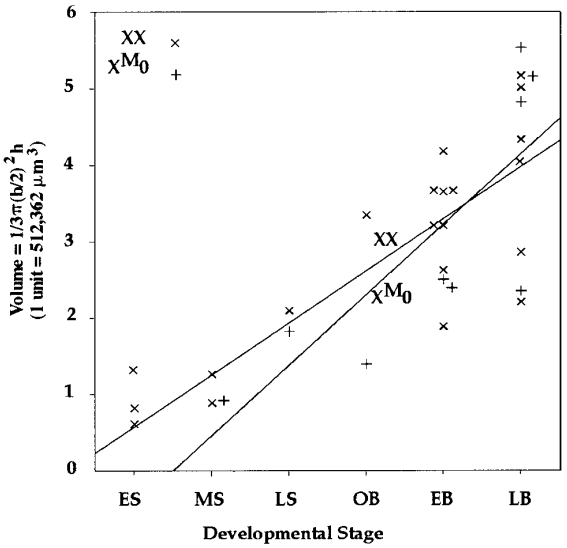
FIG. 6. X-chromosome activity in extraembryonic tissues in X*Y, X*X*, X^MX^P*, X^M*X^P, X^P*0, and X^M*0 embryos. Embryos (A, B) are from In(X)1H mating 1, embryos (C, E) are from In(X)1H mating 2, and embryos (D, F) are from the *Paf* mating. The extraembryonic ectoderm and ectoplacental cone of X*Y (A), X*X* (B), and X^M*0 (F) embryos show ubiquitous X-linked transgene expression as expected. Preferential inactivation of the paternal X chromosome is revealed in the extraembryonic ectoderm and ectoplacental cone in X^MX^P* (C) and X^M*X^P (D) embryos. In the X^P*0 embryo (E), the ectoplacental cone and the extraembryonic ectoderm show widespread activity of the X^P*. In all sections X-gal histochemistry shows cells expressing the X-linked *lacZ* transgene as blue while nonexpressing cells are pink due to the nuclear Fast Red counterstain. Developmental stages: (A, B, E) late streak, (C) early head fold, (D, F) late allantoic bud Abbreviations: epc, ectoplacental cone; emb, embryo; emd, extraembryonic mesoderm; exe, extraembryonic ectoderm. Scale bar, 20 μm.

X^P0 embryos per litter show that there is no selective loss of the retarded embryos during gestation which might account for the less obvious delay in development at the later gestational ages. One other study of X^P0 embryos has shown no size or developmental differences between these embryos and XX siblings at day 10 of gestation (Omoe and Endo, 1993). This may be due to strain-specific differences; however, these embryos were not studied at earlier time points when differences may be present.

The demonstration in our study of differential impact of X monosomy in the X0 embryos associated with the parental origin of the single X chromosome agrees with the findings by Thornhill and Burgoyne (1993). The X^P0 embryos are developmentally retarded at gastrulation and early organogenesis, but the morphology and development of the X^M0 embryos are essentially indistinguishable from XX sibs. For both types of X0 embryos in the present study, development is occurring in an XX or In(X)1H/X uterine environment rather than an X0 environment. This avoids the possible complicating factors of maternal X monosomy, including poor oocyte quality and abnormal intrauterine environment, that contribute to the manifestation of embryonic retardation (Burgoyne and Biggers, 1976; Hunt, 1991; Banzai *et al.*, 1995). Genetic background, however, may vary slightly between the two types of X0 embryos of this study and this may influence the survival and development of the X0 embryos (Hunt, 1991). The X^P*0 embryos produced by mating the heterozygous In(X)1H/X* and In(X)1H/X females with X*Y males will have a mixed background of (C57BL/6 × DBA/2)F1 and (C3H × 101)F1. The X^M*0 embryos produced by mating *Paf* and H253 mice will be of a mixed (C57BL/6 × DBA/2)F1 and (C3H/HeSnJ) background. These are the most closely related genetic backgrounds that could be obtained in our study, given the constraints in the choice of mutant and transgenic lines required to produce X^P*0 and X^M*0 embryos.

The X^P0 Genotype Has a Significant Impact on Ectoplacental Size and Structure

We have found that a trophectoderm-derived tissue, the ectoplacental cone, is deficient in X^P0 embryos compared



ANOVA table for ectoplacental cone volumes: XX and $X^{M}0$

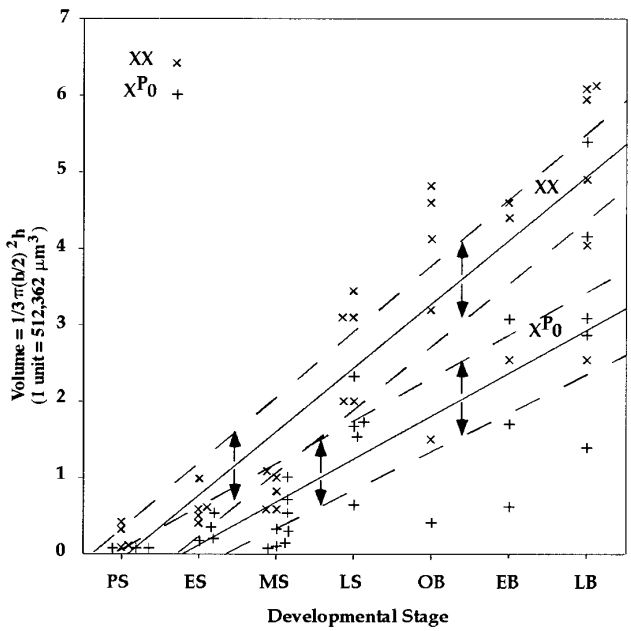
	DF	Sum of Squares	Mean Square	F-Value	P-Value
Stage	4	25.739	6.435	5.098	.0069
Genotype	1	1.269	1.269	1.005	.3301
Stage * Genotype	4	2.683	.671	.531	.7144
Residual	17	21.457	1.262		

FIG. 7. Ectoplacental cone volumes of gastrulating $X^{M}0$ and XX mouse embryos. The ectoplacental cone volumes of the $X^{M}0$ embryos are not significantly different from those of XX embryos at the same developmental stage by ANOVA (XX, $n = 21$; $X^{M}0$, $n = 9$). Developmental stages: ES, early streak; MS, mid-streak; LS, late streak; OB, no allantoic bud; EB, early allantoic bud; LB, late allantoic bud.

with XX or In(X)1H/X sibs. This is most prominent particularly during gastrulation and early organogenesis. Such a deficiency is not seen in $X^{M}0$ embryos compared with their XX siblings. The ectoplacental cone is formed from proliferation of the polar trophectoderm and later differentiates to the spongiotrophoblast (Cross *et al.*, 1994) and labyrinthine trophoblast layers of the mature placenta (Kaufman, 1992; Li *et al.*, 1997). It also contributes to the chorionic ectoderm and secondary trophoblast giant cells (Cross *et al.*, 1994).

The normal expression pattern of the spongiotrophoblast marker *4311* in the $X^{P}0$ embryo suggests that any abnormal factor that affects ectoplacental cone development does not compromise the differentiation of trophoblasts. We have evidence that the expression of an X-linked homeobox gene (*Esx1*) is also unaffected when the extraembryonic yolk sac tissues are analyzed at 13.5 to 15.5 days pc (Tam, Steiner, Li and Behringer, unpublished observation). The *Esx1* gene is expressed in the labyrinthine trophoblast of the mature placenta and normally only the maternal allele is expressed because of preferential inactivation of the paternal X chromosome (Li *et al.*, 1997). The detection of *Esx1* transcripts in $X^{P}0$ extraembryonic tissue suggests that the paternal allele has been activated and may contribute to the compensatory development of the placenta (Burgoyne *et al.*, 1983b).

The size deficit in the ectoplacental cones of the $X^{P}0$ embryos may be due to an abnormality in the proliferation of these cells since there is no apparent difference in the packing density of these trophoblast cells compared with the XX. The abnormality also may affect the organization of the developing placenta. It is not clear if the poor ectoplacental cone development in $X^{P}0$ embryos has any causal effect on the poor development of the $X^{P}0$ embryos at early postimplantation. Defects in implantation and poor trophoblast viability may lead to embryonic death soon after the onset of implantation as seen in some mouse mutants including *Evx1*, A^{y}, *Bld*, t^{w73}, and C^{6H} (Cross *et al.*, 1994;



ANOVA table for ectoplacental cone volumes: XX and $X^{P}0$

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Stage	6	120.307	20.051	26.025	<.0001
Genotype	1	19.838	19.838	25.748	<.0001
Stage * Genotype	6	9.573	1.595	2.071	.0742
Residual	48	36.982	.770		

FIG. 8. Ectoplacental cone volumes of gastrulating $X^{P}0$ and XX mouse embryos. The ectoplacental cones of the $X^{P}0$ embryos are significantly smaller than those of XX embryos in In(X)1H litters at the stages of gastrulation by ANOVA and the 95% confidence bands for the linear regression are shown (arrows indicate the range) (XX, $n = 33$; $X^{P}0$, $n = 29$; $P < 0.0001$ for genotype). Developmental stages: PS, pre-streak; ES, early streak; MS, mid-streak; LS, late streak; OB, no allantoic bud; EB, early allantoic bud; LB, late allantoic bud.

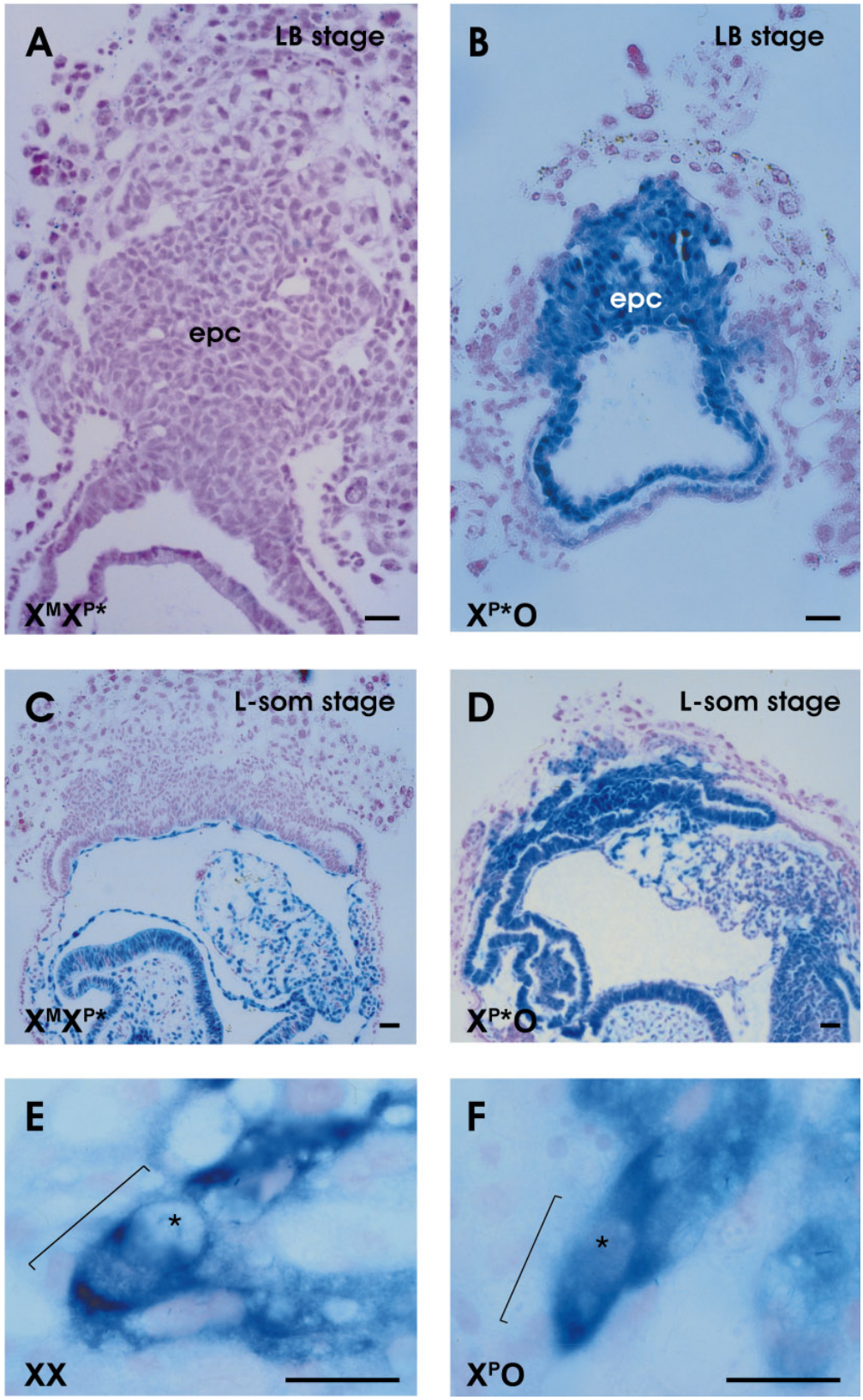


FIG. 9. Extraembryonic development in X^{P}*0 embryos. Embryos (A–F) are from In(X)1H mating 2. In A, a late bud stage $X^{M}X^{P}$* embryo from a 7.5-day litter the ectoplacental cone is well formed compared with that of an X^{P}*0 embryo (B) from an 8.5-day litter where there is

Copp, 1995). The defective development of the embryonic and extraembryonic tissues of the X^P0 embryos may be due to a general effect of the activity of the X chromosome on cell proliferation and differentiation. It is possible that the extraembryonic tissues, particularly the ectoplacental cone, may be more adversely affected by an abnormality in cell proliferation since these are reduced in size in the developmentally delayed embryos, whereas the egg cylinder is not. It is tempting to propose that the growth deficit in the extraembryonic tissues, which normally require maternal rather than paternal X chromosome activity, may underlie the developmental retardation of the X^P0 embryos. However, the initial deficit in ectoplacental cone size (this study) and placental weight (Burgoyne *et al.,* 1983b) is overcompensated and the X^P0 placentae are heavier than the XX placentae at late gestation (Burgoyne *et al.,* 1983b). The underlying genetic and molecular mechanisms leading to these phenomena in the X^P0 conceptus are presently unknown.

Genomic Imprinting May Play a Role

Several lines of evidence indicate that X-linked genes and their imprinted behavior is important in the regulation of embryonic development. In normal XX embryos, there is preferential X^P inactivation in the trophectoderm of the blastocyst and the extraembryonic ectoderm, ectoplacental cone, and primitive endoderm of postimplantation embryos (Harper *et al.,* 1982; Takagi and Sasaki, 1982; Sugawara *et al.,* 1985). An *Xist* mutation when inherited from the father (Marahrens *et al.,* 1997) and an extra maternally derived X chromosome (Tada *et al.,* 1993) both have deleterious effects on the extraembryonic tissues. X^MX^MY and $X^MX^MX^P$ embryos are retarded in growth at gastrulation and are deficient in extraembryonic tissues compared with X^MX^P and X^MX^PY embryos. Lack of X inactivation, assessed by the absence of asynchronous replication, has been shown in some cells of X^MX^MY embryos. Using the same method, a proportion of cells in $X^MX^MX^P$ embryos show the presence of only an inactivated X^P. These findings have led to the postulate that the abnormal development of these embryos is due to the imprinted nature of X inactivation in the extraembryonic tissues so that two active X^M chromosomes lead to unbalanced X-linked gene dosage.

We have assayed the activity of the X chromosome in X0 embryos by the expression of an X-linked *lacZ* transgene. This transgene is subject to X inactivation and clearly shows preferential inactivation of the paternal X chromosome in the extraembryonic tissues of X^MX^P* and X^M*X^P embryos. The original hypothesis that abnormal development of the X^P0 embryos is due to the complete loss of X-linked gene activity in the X^P0 cell population is not supported by the findings of the transgenic activity. In both types of X0 embryo, the X-linked transgene is expressed in almost all cells in the embryonic and extraembryonic tissues and there is no evidence that X-chromosome activity is lacking at a particular developmental time point. Even in the extraembryonic tissues, the X chromosome is still active irrespective of the parental origin of the chromosome. We do not know, however, if the X chromosome is active at pre- and peri-implantation stages prior to the onset of developmental delay. Support for the concept that the origin of the X^P*0 growth deficiency may be derived from these early stages is provided by the finding that X^P0 embryos are delayed at the blastocyst stage compared with XX and XY sibs (Banzai *et al.,* 1995). The expression of the paternal X-linked transgene supports the concept that the chromosomal imprint that dictates preferential inactivation has been modified in the X0 embryos. The X^P0 embryo therefore presents a situation where the paternal X chromosome can be activated in the extraembryonic tissues of the mouse (Frels and Chapman, 1979; Papaioannou and West, 1981) presumably by overriding the control for preferential inactivation.

Despite the possible active state of the X^P chromosome in both the embryonic and extraembryonic tissues, the X^P0 embryos display developmental delay. A possible explanation is that the X^P chromosome is less active and is unable to deliver the appropriate dosage of X-linked gene activity required to support the differentiation and functioning of the embryonic and extraembryonic cells. In XX embryos, the transcriptional activity of genes on the X^P chromosome is generally lower than that of the X^M chromosome in the cleavage stages (reviewed in Jamieson *et al.,* 1996). In addition, inactivation of the X^P chromosome as determined by cytogenetic analysis (Takagi and Sasaki, 1982) and transgene studies (Tam *et al.,* 1994a) seems always to be ahead of that of the X^M chromosome. This implies that the paternal X chromosome has been imprinted to be the less active chromosome and more prone to inactivation and this could be related to the special chromatin configuration of the X^P chromosome (Jamieson *et al.,* 1996).

Since no endogenous X-linked gene has been assayed for expression at gastrulation and early organogenesis in the X^P0 embryos, we cannot exclude the possibility that specific gene loci that encode critical X-linked gene products may remain transcriptionally inactive. A deficiency of specific activity or molecule may result if the gene loci are

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a paucity of trophoblast cells in the ectoplacental cone. Even when embryonic development has caught up, the tissue layers in the early placenta appear less well organized in the X^P*0 embryo (D) compared with the X^MX^P* embryo (C). Labyrinthine trophoblast cells (brackets and nucleus indicated by asterisks) in XX (E) and X^P0 (F) embryos at 9.5 days pc express *4311* transcripts in the cytoplasm (blue by NBT/BCIP staining). Abbreviations: epc, ectoplacental cone; LB, late allantoic bud; L-som, 7–14 somites. Scale bar, 20μm.

only active from the maternal X chromosome, i.e., imprinted (Surani, 1991). A locus controlling placental growth has been mapped to the proximal part of the mouse X chromosome (Zechner *et al.*, 1996) and two of the genes expressed in the extraembryonic tissues, both of which are in the *paired*-class of homeobox genes, *Pem* (Lin *et al.*, 1994) and *Esx1* (Li *et al.*, 1997), are X-linked. If these genes are imprinted and are only expressed from the maternal X, then the activation of the paternal X chromosome in the conceptus may not be able to provide the required gene activity during the period of developmental delay. Recovery in extraembryonic and embryonic development by midgestation may be the result of erasure of the imprint and/or by then a specific gene product may no longer be critical for development.

While *SHOX/PHOG* from the pseudoautosomal region in humans has been proposed as a candidate for short stature in Turner syndrome (Ellison *et al.*, 1997; Rao *et al.*, 1997), little progress has been made regarding proposals to account for the high rate of embryonic lethality (Ogata and Matsuo, 1995). 45,X abortuses are growth retarded (Hook and Warburton, 1983; Byrne *et al.*, 1985) and some also show marked relative delay in placental development (Boué *et al.*, 1976). Studies on X-chromosome inactivation in the extraembryonic tissues of humans suggest that there is preferential inactivation of X^P in the trophoblast (Harrison and Warburton, 1986; Harrison, 1989; Penaherrera *et al.*, 1997). Differential X-chromosome activity may play a role in the manifestations of the Turner phenotype. Collation of results from molecular studies of parental origin in 45,X conceptuses indicates a predominance of 45,X^M to 45,X^P of 3.9:1 in abortuses and 3.2:1 in liveborns (Hassold *et al.*, 1988, 1992; Jacobs *et al.*, 1990, 1997; Cockwell *et al.*, 1991; Loughlin *et al.*, 1991; Mathur *et al.*, 1991; Lorda-Sanchez *et al.*, 1992). It has been speculated that 45,X which may arise from errors in meiosis or mitosis would present at a ratio of 2:1 (45,X^M:45,X^P) if equal loss at each step is assumed and the poor viability of 45,Y is considered (Mathur *et al.*, 1991). The more marked predominance of 45,X^M in human X monosomy may be related to preferential 45,X^P loss prior to 10 weeks gestation since the parental origin of the X in only one such embryo has been examined and it was paternal (Hassold *et al.*, 1988). It is possible, since placental growth appears to be particularly affected in some 45,X abortuses that, as in the X^P0 mouse, imprinting-related decrease in the activity of the single X^P during preimplantation or the imprinting of a particular X-linked gene important in extraembryonic development may be contributing to the early lethality of the 45,X conceptus, especially the 45,X^P.

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REFERENCES

Banzai, M., Omoe, K., Ishikawa, H., and Endo, A. (1995). Viability, development and incidence of chromosome anomalies of preimplantation embryos from X0 mice. *Cytogenet. Cell Genet.* **70,** 273–277.

Boué, J., Philippe, E., Giroud, A., and Boué, A. (1976). Phenotypic expression of lethal chromosomal anomalies in human abortuses. *Teratology* **14,** 3–20.

Burgoyne, P., Evans, E., and Holland, K. (1983a). X0 monosomy is associated with reduced birth weight and lowered weight gain in the mouse. *J. Reprod. Fertil.* **68,** 381–385.

Burgoyne, P., Tam, P., and Evans, E. (1983b). Retarded development of X0 conceptuses during early pregnancy in the mouse. *J. Reprod. Fertil.* **68,** 387–393.

Burgoyne, P. S., and Biggers, J. D. (1976). The consequences of X-dosage deficiency in the germ line: Impaired development *in vitro* of preimplantation embryos from X0 mice. *Dev. Biol.* **51,** 109–117.

Byrne, J., Warburton, D., Kline, J., Blanc, W., and Stein, Z. (1985). Morphology of early fetal deaths and their chromosomal characteristics. *Teratology* **32,** 297–315.

Cattanach, B. M. (1962). X0 mice. *Genet. Res., Camb.* **3,** 487–490.

Cockwell, A., MacKenzie, M., Youings, S., and Jacobs, P. (1991). A cytogenetic and molecular study of a series of 45,X fetuses and their parents. *J. Med. Genet.* **28,** 151–155.

Copp, A. J. (1995). Death before birth: clues from gene knockouts and mutations. *Trends Genet.* **11,** 87–93.

Cross, J. C., Werb, Z., and Fisher, S. J. (1994). Implantation and the placenta: Key pieces of the development puzzle. *Science* **266,** 1508–1518.

Downs, K. M., and Davies, T. (1993). Staging of gastrulating mouse embryos by morphological landmarks in the dissecting microscope. *Development* **118,** 1255–1266.

Ellison, J. W., Wardak, Z., Young, M. F., Robey, P. G., Laig-Webster, M., and Chiong, W. (1997). PHOG, a candidate gene for involvement in the short stature of Turner syndrome. *Hum. Mol. Genet.* **6,** 1341–1347.

Evans, E. P., and Phillips, R. J. S. (1975). Inversion heterozygosity and the origin of X0 daughters of *Bpa*/+ female mice. *Nature* **256,** 40–41.

Frels, W. I., and Chapman, V. (1979). Paternal X chromosome expression in extraembryonic membranes of X0 mice. *J. Exp. Zool.* **210,** 553–559.

Gold, J. D., and Pedersen, R. A. (1994). Mechanisms of genomic imprinting in mammals. *Curr. Top. Dev. Biol.* **29,** 227–280.

Harper, M. I., Fosten, M., and Monk, M. (1982). Preferential paternal X inactivation in extraembryonic tissues of early mouse embryos. *J. Embryol. Exp. Morphol.* **67,** 127–135.

Harrison, K. B. (1989). X-chromosome inactivation in the human cytotrophoblast. *Cytogenet. Cell Genet.* **52,** 37–41.

Harrison, K. B., and Warburton, D. (1986). Preferential X-chromosome activity in human female placental tissues. *Cytogenet. Cell Genet.* **41,** 163–168.

Hassold, T., Benham, F., and Leppert, M. (1988). Cytogenetic and molecular analysis of sex-chromosome monosomy. *Am. J. Hum. Genet.* **42,** 534–541.

Hassold, T., Pettay, D., Robinson, A., and Uchida, I. (1992). Molecular studies of parental origin and mosaicism in 45,X conceptuses. *Hum Genet.* **89,** 647–652.

Hecht, F., and Macfarlane, J. P. (1969). Mosaicism in Turner's syndrome reflects the lethality of X0. *Lancet* **2,** 1197–1198.

Hook, E. B., and Warburton, D. (1983). The distribution of chromosomal genotypes associated with Turner's syndrome: Live birth prevalence rates and evidence for diminished fetal mortality and severity in genotypes associated with structural X abnormalities or mosaicism. *Hum. Genet.* **64,** 24–27.

Hunt, P. A. (1991). Survival of X0 mouse fetuses: Effect of parental origin of the X chromosome or uterine environment. *Development* **111,** 1137–1141.

Jacobs, P. A., Betts, P. R., Cockwell, A. E., Crolla, J. A., Mackenzie, M. J., Robinson, D. O., and Youings, S. A. (1990). A cytogenetic and molecular reappraisal of a series of patients with Turner's syndrome. *Ann. Hum. Genet.* **54,** 209–223.

Jacobs, P., Dalton, P., James R., Mosse, K., Power, M., Robinson, D., and Skuse, D. (1997). Turner syndrome: a cytogenetic and molecular study. *Ann. Hum. Genet.* **61,** 471–483.

Jamieson, R. V., Tam, P. P. L., and Gardiner-Garden, M. (1996). X-chromosome activity: Impact of imprinting and chromatin structure. *Int. J. Dev. Biol.* **40,** 1065–1080.

Kaufman, M. H. (1992). "The Atlas of Mouse Development." Academic Press, London.

Lane, P. W., and Davisson, M. T. (1990). Patchy fur (*Paf*), a semidominant X-linked gene associated with a high level of X–Y nondisjunction in male mice. *J. Hered.* **81,** 43–50.

Lescisin, K. R., Varmuza, S., and Rossant, J. (1988). Isolation and characterization of a novel trophoblast-specific cDNA in the mouse. *Genes Dev.* **2,** 1639–1646.

Li, Y., Lemaire, P., and Behringer, R. R. (1997). *Esx1,* a novel X chromosome-linked homeobox gene expressed in mouse extraembryonic tissues and male germ cells. *Dev. Biol.* **188,** 85–95.

Lin, T.-P., Labosky, P. A., Grabel, L. B., Kozak, C. A., Pitman, J. L., Kleeman, J., and MacLeod, C. L. (1994). The *Pem* homeobox gene is X-linked and exclusively expressed in extraembryonic tissues during early murine development. *Dev. Biol.* **166,** 170–179.

Lippe, B. (1991). Turner syndrome. *Endocrinol. Metab. Clin. North Am.* **20,** 121–152.

Lorda-Sanchez, I., Binkert, F., Maechler, M., and Schinzel, A. (1992). Molecular study of 45,X conceptuses: correlation with clinical findings. *Am. J. Med. Genet.* **42,** 487–490.

Loughlin, S. A. RS. A., Redha, A., McIver, J., Boyd, E., Carothers, A., and Connor, J. M. (1991). Analysis of the origin of Turner's syndrome using polymorphic DNA probes. *J. Med. Genet.* **28,** 156–158.

Lyon, M. F., and Hawker, S. G. (1973). Reproductive life span in irradiated and unirradiated chromosomally X0 mice. *Genet. Res. Camb.* **21,** 185–194.

Marahrens, Y., Panning, B., Dausman, J., Strauss, W., and Jaenisch, R. (1997). *Xist*-deficient mice are defective in dosage compensation but not spermatogenesis. *Genes Dev.* **11,** 156–166.

Mathur, A., Stekol, L., Schatz, D., MacLaren, N. K., Scott, M., and Lippe, B. (1991). The parental origin of the single X chromosome in Turner syndrome: lack of correlation with parental age or clinical phenotype. *Am. J. Hum. Genet.* **48,** 682–686.

Ogata, T., and Matsuo, N. (1995). Turner syndrome and female sex chromosome aberrations: deduction of the principal factors involved in the development of clinical features. *Hum. Genet.* **95,** 607–629.

Omoe, K., and Endo, A. (1993). Growth and development of 39,X mouse embryos at mid-gestation. *Cytogenet. Cell Genet.* **63,** 50–53.

Papaioannou, V. E., and West, J. D. (1981). Relationship between the parental origin of the X chromosomes, embryonic cell lineage and X chromosome expression in mice. *Genet. Res., Camb.* **37,** 183–197.

Penaherrera, M. S., Lau, A. W., Brown, C. J., Wilson, R. D., Barrett, I. J., Kalousek, D. K., and Robinson, W. P. (1997). X chromosome inactivation patterns in human placenta. *Am. J. Hum. Genet.* **61**(Suppl.), A137.

Rao, E., Weiss, B., Fukami, M., Rump, A., Niesler, B., Mertz, A., Muroya, K., Binder, G., Kirsch, S., Winkelmann, M., Nordsiek, G., Heinrich, U., Breuning, M. H., Ranke, M. B., Rosenthal, A., Ogata, T., and Rappold, G. A. (1997). Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. *Nature Genet.* **16,** 54–63.

Sugawara, O., Takagi, N., and Sasaki, M. (1985). Correlation between X-chromosome inactivation and cell differentiation in female preimplantation mouse embryos. *Cytogenet. Cell Genet.* **39,** 210–219.

Surani, M. A. (1991). Genomic imprinting: developmental significance and molecular mechanism. *Curr. Opin. Genet. Dev.* **1,** 241–146.

Tada, T., Takagi, N., and Adler, I. (1993). Parental imprinting on the mouse X chromosome: effects on the early development of X0, XXY and XXX embryos. *Genet. Res., Camb.* **62,** 139–148.

Takagi, O. S., and Sasaki, M. (1982). Regional and temporal changes in the pattern of X-chromosome replication during the early post-implantation development of the female mouse. *Chromosoma* **85,** 275–286.

Tam, P. P. L., Williams, E. A., and Tan, S.-S. (1994a). Expression of an X-linked *HMG-lacZ* transgene in mouse embryos: implication of chromosomal imprinting and lineage-specific X-chromosome activity. *Dev. Genet.* **15,** 491–503.

Tam, P. P. L., Zhou, S. X., and Tan, S.-S. (1994b). X-chromosome activity of the mouse primordial germ cells revealed by the expression of an X-linked *lacZ* transgene. *Development* **120,** 2925–2932.

Tan, S.-S., Williams, E. A., and Tam, P. P. L. (1993). X-chromosome inactivation occurs at different times in different tissues of the post-implantation mouse embryo. *Nat. Genet.* **3,** 170–174.

Theiler, K. (1989). "The House Mouse: Atlas of Embryonic Development." Springer-Verlag, New York.

Thornhill, A. R., and Burgoyne, P. S. (1993). A paternally imprinted X chromosome retards development of the early mouse embryo. *Development* **118,** 171–174.

Zechner, U., Reule, M., Orth, A., Bonhomme, F., Strack, B., Guenet, J.-L., Hameister, H., and Fundele, R. (1996). An X-chromosome linked locus contributes to abnormal placental development in mouse interspecific hybrids. *Nat. Genet.* **12,** 398–403.

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