Two maternally derived X chromosomes contribute to parthenogenetic inviability

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Summary

In certain extraembryonic tissues of normal female mouse conceptuses, X-chromosome-dosage compensation is achieved by preferential inactivation of the paternally derived X. Diploid parthenogenones have two maternally derived X chromosomes, hence this mechanism cannot operate. To examine whether this contributes to the inviability of parthenogenones, XO and XX parthenogenetic eggs were constructed by pronuclear transplantation and their development assessed after transfer to pseudopregnant recipients. In one series of experiments, the frequency of postimplantation development of XO parthenogenones was much higher than that of their XX counterparts. This result is consistent with the possibility that two maternally derived X chromosomes can contribute to parthenogenetic inviability at or very soon after implantation. However, both XO and XX parthenogenones showed similar developmental abnormalities at the postimplantation stage, demonstrating that parthenogenetic inviability is ultimately determined by the possession of two sets of maternally derived autosomes.

Key words: X chromosome, maternally derived X chromosome, parthenogenones, mouse embryo, dosage compensation, preferential inactivation, inviability.

Introduction

Diploid parthenogenetic 1-cell mouse eggs possess two maternally derived haploid pronuclei. This is also the case in diploid gynogenetic 1-cell eggs (fertilized eggs in which there is no contribution of paternal chromosomes). Both these types of eggs can undergo a high frequency of development to the blastocyst stage and the majority undergoes implantation. However, only a few develop to postimplantation stages, reaching at most about the 25-somite forelimb-bud stage (Kaufman & Gardner, 1974; Kaufman et al. 1977; Surani & Barton, 1983). Their abnormalities result from the presence of two maternally derived genomes (Mann & Lovell-Badge, 1984; Surani et al. 1984; McGrath & Solter, 1984), and parthenogenones and gynogenones are therefore potentially subject to all deleterious effects involving maternal duplication/paternal deficiency of certain chromosome regions (Searle & Beechey, 1978). In these regions, there appears to be a differential expression of maternal and paternal gene homologues, determined by differential gene modifications or 'imprinting' presumably imparted during gametogenesis (Cattanach & Kirk, 1985). These modifications could involve DNA methylation (Swain et al. 1987; Reik et al. 1987; Sapienza et al. 1987).

It is not known at how many loci imprinting is important for development, or the time at which each becomes critical. The only mechanism involving imprinting known to occur around the time of implantation is the preferential inactivation of the paternally derived X chromosome, Xp, in the trophoderm and primitive endoderm of XX blastocysts (Takagi et al. 1978; Frels et al. 1979; Frels & Chapman, 1980; Papaioannou & West, 1981; Harper et al. 1982). This mechanism cannot operate in parthenogenones or gynogenones which have two maternally derived X chromosomes, Xm, and therefore may determine the characteristically poor development of trophoblast in postimplantation-stage parthenogenones and gynogenones (Endo & Takagi, 1981; Surani et al. 1984, 1987). This poor development of trophoblast has been shown to impair significantly the development
of the embryo-proper (Barton et al. 1985).

To investigate whether two X\textsuperscript{M} chromosomes contribute to the inviability of gynogenones, we previously studied the developmental potential of X\textsuperscript{M}O gynogenetic eggs (Mann & Lovell-Badge, 1987). In these eggs, X-dosage-related functions should be essentially normal, as X\textsuperscript{M}O fertilized eggs are viable. Thus, any developmental abnormalities in X\textsuperscript{M}O gynogenones could be attributed to the lack of paternally derived autosomes, A\textsuperscript{p}. XO gynogenetic eggs showed the same mortality at or very soon after implantation as did their XX counterparts, showing that a lack of A\textsuperscript{p} chromosomes was sufficient to cause gynogenetic inviability at this stage. Nevertheless, as suggested, these results could not preclude the possibility that the presence of two X\textsuperscript{M} chromosomes might also have a deleterious effect. Furthermore, postimplantation development of XO or XX gynogenones was not obtained. Therefore it was not possible to determine whether normal X dosage could result in an improved development of gynogenetic trophoblast.

We have now extended these studies to XO and XX parthenogenetic eggs. Postimplantation development was obtained from some XO and XX eggs, allowing for a comparison of their developmental potential during embryonic development.

**Materials and methods**

Females more than 8 weeks of age were superovulated by intraperitoneal injection of 5 i.u. pregnant mare serum gonadotrophin (Folligon; Intervet) followed about 48 h later with 5 i.u. human chorionic gonadotrophin, hCG (Chorulon; Intervet). Ethanol-induced parthenogenetic activation of cumulus-denuded eggs was carried out 16–17 h after hCG injection (Barton et al. 1985) to produce haploid parthenogenetic 1-cell eggs (one pronucleus, 2nd polar body). The frequency of activation was typically greater than 80%. Eggs were cultured in drops of medium M16 (Whittingham, 1971) under light paraffin oil (British Drug Houses) at 37°C in 5% CO\textsubscript{2} in air. Pronuclei were transfected according to McGrath & Solter (1983) as modified slightly by Mann & Lovell-Badge (1987). The procedure involves fusion of an egg fragment, consisting of a pronucleus surrounded by a small amount of cytoplasm and plasma membrane, with an egg utilizing Sendai virus plasma membrane fusion. Eggs were transferred at the 2-cell stage to the oviducts of recipients on day 4 of pseudopregnancy (day of a vaginal plug after mating to vasectomized males of proven sterility). Phosphoglycerate kinase-1, PGK-1, electrophoresis was carried out according to Bücher et al. (1980). The enzyme was localized using the visible tetrazolium-linked staining procedure of Oelshlegel & Brewer (1972).

**Source of O eggs**

Females [hereafter termed In(X)/X] heterozygous for an X with a large inversion, In(X)1H (Evans & Phillips, 1975), were used as a source of eggs lacking an X chromosome. Karyotypic analyses have shown that they produce eggs of which about 22% are O, 72% carry an X and 6% carry an X dicentric (Phillips & Kaufman, 1974; P. S. Burgoyne, personal communication; Mann & Lovell-Badge, 1987). Unless otherwise stated, In(X)/X females were produced by mating In(X)/Y males (Murdoch Institute colony; original breeding pairs kindly provided by Dr Paul Burgoyne, MRC Mammalian Development Unit, UK) to C57BL/6J females.

**Estimation of the proportion of O and X parthenogenetically activated haploid eggs of In(X)/X females**

The following experiment was carried out in order to assess whether the proportion of O and X eggs produced by In(X)/X females remained similar following parthenogenetic activation. In(X)/X females were superovulated and their eggs parthenogenetically activated. Fertilized eggs were obtained from (C57BL/6J X CBA/CaH)F\textsubscript{1} (hereafter termed B6CF\textsubscript{1}) females superovulated 4–5 h later than In(X)/X females and mated to males carrying the X-linked gene Pgk-1\textsuperscript{p} (coding for the A electrophoretic form of PGK-1). In(X)/X females were homozygous Pgk-1\textsuperscript{p}. Diploid eggs were then constructed by transplanting the parternal pronucleus of fertilized eggs to haploid parthenogenetic eggs of In(X)/X mice. Following micromanipulation, the eggs were cultured overnight to cleave to 2-cells, then transferred to B6CF\textsubscript{1} or ARC Swiss recipients (Animal Resources Centre, Murdoch, Western Australia; foundation stock of ARC Swiss mice were CD-1 mice obtained from Charles River Breeding Labs, USA). These were autopsied at days 13–15 of gestation. Fetuses were sexed by gonad morphology and the PGK-1 allozyme type of female fetuses determined. Female PGK-1 A fetuses would be derived from O haploid parthenogenetic eggs, whereas all other types of fetuses would be derived from X eggs.

**Construction and transfer of diploid XO and XX parthenogenetic eggs**

Two series of experiments were carried out.

**Series I**

This series of experiments were conducted at the MRC Mammalian Development Unit, UK (all other experiments described in this paper were carried out at the Murdoch Institute). In(X)/X females were produced by mating In(X)/Y males (MRC Mammalian Development Unit colony) to MF1 (OLAC, UK) females. In(X)/X and (CBA/CaH X C57BL/6J)F\textsubscript{1} (hereafter termed CBB6F\textsubscript{1}) females were superovulated, killed 16 h after hCG injection, then cumulus-intact eggs parthenogenetically activated by ethanol treatment (Kaufman, 1982; Cuthbertson, 1983). Diploid eggs were then constructed by transplanting the pronucleus of a haploid egg of an In(X)/X mouse to a haploid egg of a CBB6F\textsubscript{1} mouse. The transplantation was done in this fashion as eggs of CBB6F\textsubscript{1} mice were in apparently better condition than those of In(X)/X (MF1 hybrid) mice following ethanol activation. If In(X)/X females produce about 22% O, 72% X and 6% X
Two \(X^M\) chromosomes and parthenogenetic inviability

### Table 1. Development of haploid parthenogenetic eggs of \(In(X)/X\) mice into which a paternal pronucleus was transplanted

<table>
<thead>
<tr>
<th>Transferred*</th>
<th>Implanted</th>
<th>Developed postimplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>101</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>2(\dagger)</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

n.d. = not done.

* Into recipients that became pregnant.

\(\dagger\) These females presumably resulted from the loss or absence in the paternal pronucleus of a sex chromosome and have been observed in previous experiments (Mann & Lovell-Badge, 1987; Mann, 1987).

dicentric eggs, then these diploids would be in the expected proportions of 22% XO (viability?), 72% XX (lethal before 12\(\dagger\) days of gestation) and 6% X dicentrics [perimplantation lethal (P. S. Burgoyne, personal communication)]. Eggs were cultured overnight to cleave to 2-cells, then transferred to CBB6F\(1\) recipients which were autopsied at 9-11\(\dagger\) days of gestation. Postimplantation-stage parthenogenetic embryos were dissected from the uterus and XOs and XXs identified by chromosome counts. Metaphase spreads were made from the egg cylinder alone, or together with the yolk sac (Burgoyne et al. 1983). XO embryos possess 39 chromosomes. No other monosomy in mice is known to develop after implantation (Epstein, 1985).

### Results

**Estimation of the proportion of O and X parthenogenetically activated haploid eggs of \(In(X)/X\) females**

In transplanting a paternal pronucleus into haploid parthenogenetic eggs of \(In(X)/X\) mice, fusion of the egg fragment occurred in 119 out of 143 (83%) micromanipulated eggs. All but one cleaved to 2-cells. They were transferred to 12 recipients. One did not possess implantations at autopsy. The frequency of implantation and postimplantation development of these eggs, and the number of fetuses of each type obtained is shown in Table 1. As four female PGK-1 A fetuses (derived from O eggs) were obtained, at least four OY eggs would also have been expected, these being preimplantation lethal. 32 fetuses that must have been derived from X eggs were also obtained. Thus, the ratio of O to X haploid parthenogenetic eggs produced that were able to participate in normal development was 8:32 = 1:4. This ratio is similar to that expected from previous estimates of the proportion of O and X eggs produced by \(In(X)/X\) females, i.e. 22% O and 72% X eggs; 1:3-3. Hence, it is apparent that ethanol activation of eggs of \(In(X)/X\) females does not result in markedly
Table 2. Development of diploid XO and XX parthenogenetic eggs

<table>
<thead>
<tr>
<th>Number of eggs</th>
<th>Developmental stage</th>
<th>Number obtained (Recipient No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferred*</td>
<td>Implanted</td>
<td>Genotype</td>
</tr>
<tr>
<td>Series I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>135 (30 XO, 97 XX)†</td>
<td>XO</td>
<td>25 somites</td>
</tr>
<tr>
<td></td>
<td>XX</td>
<td>8 somites</td>
</tr>
<tr>
<td></td>
<td>?</td>
<td>16 somites</td>
</tr>
<tr>
<td>Series II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>108 (35 XO, 66 XX)†</td>
<td>XO</td>
<td>egg cylinder-like</td>
</tr>
<tr>
<td></td>
<td>108 (35 XO, 66 XX)†</td>
<td>XO</td>
</tr>
<tr>
<td></td>
<td>108 (35 XO, 66 XX)†</td>
<td>XO</td>
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<td>XO</td>
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<tr>
<td></td>
<td>108 (35 XO, 66 XX)†</td>
<td>XX</td>
</tr>
<tr>
<td></td>
<td></td>
<td>?</td>
</tr>
</tbody>
</table>

* Into recipients that possessed implantations at autopsy.
† Expected numbers of XO and XX eggs transferred, calculated from the expected proportions of O and X eggs present (see Materials and methods section).
‡ Recipients 1 and 2 were CBBF, 3–7 ARC Swiss and 8–14 B6CBF.

Different proportions of O and X parthenogenetic eggs compared to fertilized eggs.

Development of diploid XO and XX parthenogenetic eggs

Series I

Results are shown in Table 2. Only three postimplantation-stage embryos were obtained. Two of these, the 25-somite and 8-somite embryos, were present in the same uterine horn of a recipient autopsied at 9½ days.

Series II

22 postimplantation-stage embryos were obtained. Chromosome counts were made in 18 of these; 17 were XO and one was XX. Given that the probability of an embryo being XO or XX is 0·38 and 0·62, respectively, given that they are equally viable (see Materials and methods section), then the probability of obtaining 17 or more XOs out of 18 embryos is \( (0·38)^{17}(0·62) \times 18! / 17!1!+ (0·38)^{18} = 8·3 \times 10^{-7} \). Where more than one embryo was obtained in a recipient, there was no obvious tendency for these to be similar in developmental stage. In all embryos, the development of the trophoblast was very limited, often represented by only a small piece of necrotic tissue. A distinctive Reichert's membrane was often present, as has been observed previously (Surani et al. 1984), that could be easily separated from the decidua. This could be attributed to the sparse development of trophoblast. The extent of development of the yolk sac appeared consistent with that of the embryo proper. Three of the somite-stage parthenogenetic embryos obtained in the Series II experiments are shown in Fig. 1.
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Two series of experiments were conducted in order to determine whether a difference exists in the developmental potential of diploid XO and XX parthenogenetic eggs. In the experiments of series I, the majority of XO and XX parthenogenetic eggs did not develop to postimplantation stages. This result was similar to that obtained with XO and XX gynogenetic eggs (Mann & Lovell-Badge, 1987), and demonstrates that in these experiments the lack of $A^P$ chromosomes was sufficient to cause the inviability of parthenogenones at this stage. However, in the experiments of series II, the frequency of postimplantation development of XO eggs (1 out of an expected 56 transferred) was much greater than in XX eggs (17 out of an expected 27 transferred, 3 out of an expected 53) of embryos transferred to recipients, only three implantation sites were obtained in which postimplantation development was evident. However, only yolk sac vesicles had developed.

**Discussion**

Two series of experiments were conducted in order to determine whether a difference exists in the developmental potential of diploid XO and XX parthenogenetic eggs. In the experiments of series I, the majority of XO and XX parthenogenetic eggs did not develop to postimplantation stages. This result was similar to that obtained with XO and XX gynogenetic eggs (Mann & Lovell-Badge, 1987), and demonstrates that in these experiments the lack of $A^P$ chromosomes was sufficient to cause the inviability of parthenogenones at this stage. However, in the experiments of series II, the frequency of postimplantation development of XO eggs (1 out of an expected 56 transferred) was much greater than in XX eggs (17 out of an expected 53 transferred).
35 transferred). The probability of obtaining this result by chance is extremely low (see Results section). Therefore, it can be safely concluded that in the experiments of series II, XO blastocysts were better able to continue development after implantation than XX blastocysts. Considering that ethanol activation can cause aneuploidy in up to 19% of haploid parthenogenetic eggs (Kaufman, 1982) and that aneuploidy often results in death at implantation (Epstein, 1985), the frequency of postimplantation development of euploid XO eggs was likely to be as high as that obtained following transplantation of pronuclei between synchronous fertilized eggs, i.e., 67% (Mann & Lovell-Badge, 1987).

In the experiments of series II then, the increased frequency of postimplantation development of XO compared to XX parthenogenones must have depended on the absence of a second X M, this being the only difference between them. It is evident therefore that two X M chromosomes can contribute significantly to parthenogenetic inviability, the effect being manifest at implantation. This result is consistent with XX parthenogenones having difficulties in achieving normal X-dosage compensation in the trophectoderm and primitive endoderm, presumably because of a failure of X inactivation in these tissues where in normal conceptuses X p is preferentially inactivated. If this explanation is correct, then the survival of some XX parthenogenones beyond implantation could be due to just enough trophectoderm and primitive endoderm cells managing to inactivate an X to enable further development. Also, the higher frequency of postimplantation development of XX parthenogenetic blastocysts subjected to implantational delay (Kaufman et al., 1977), may result from the longer time available to inactivate an X, or from an increased number of cells with an inactive X resulting from an overall increase in delayed blastocyst cell number (Copp, 1982). These explanations are in keeping with observations of a high proportion of cells with an inactive X in the yolk-sac endoderm (derived from the primitive endoderm) in postimplantation-stage XX parthenogenones (Rastan et al., 1980; Endo & Takagi, 1981).

From the above, it could be predicted that the few androgenones that develop to postimplantation stages (Barton et al., 1984) may be of an X pY constitution, whereas X pX p androgenones may be less viable due to difficulties in achieving normal X-dosage compensation at the time of trophectoderm and primitive endoderm formation.

The abnormal development observed in the postimplantation-stage XO parthenogenones was essentially equivalent to that reported previously in their XX counterparts. However, some retardation of XOs relative to XXs would have been expected, as XO embryos derived from fertilized eggs are slightly retarded in comparison to their XX counterparts at 7½ days of gestation (Burgoyne et al., 1983). Significantly, the trophoblast was very poorly developed (Fig. 1) as has been described previously in X M X M A M A M eggs (Surani et al., 1984) (where A refers to autosomes). Thus, it is apparent that the presence of two X M chromosomes does not contribute significantly to the characteristically poor development of trophoblast, or any other developmental abnormality in postimplantation-stage XX parthenogenones. Rather, as X M O A M A P eggs are viable, and X M O A M A M eggs are not, these abnormalities can be attributed to a lack of A p chromosomes.

Why was the frequency of postimplantation development of X M O A M A M eggs high in the series II experiments, yet low in two previous sets of experiments (Mann & Lovell-Badge, 1987 and series I)? It should first be noted that X M X M A M A M eggs are also variable in terms of the frequency that they will develop to postimplantation stages, as well as in the degree of developmental normality attained. Low frequencies of postimplantation development have been consistently obtained by the authors (Mann & Lovell-Badge, 1984, 1987 and in the present experiments) following transfer of 2-cell eggs to oviducts, and have been also been reported by others following transfer of blastocysts to the uterus (Tarkowski et al., 1970; Witkowska, 1973; Kaufman & Gardner, 1974). In contrast, a frequency of 25% development to somite stages has been reported by Kaufman et al. (1977) after inducing delayed implantation in blastocysts and up to 22% developing to various postimplantation stages by Surani & Barton (1983) and Surani et al. (1984) following transfer of 2-cell eggs to oviducts. Concerning the degree of developmental normality obtained, at midgestation this can vary from disorganized egg-cylinder-like structures to reasonably well-formed embryos that are not retarded at least in terms of somite number.

Therefore, the success or failure of parthenogenetic eggs developing after implantation appears to depend on the penetrance and expressivity of certain loci at which maternal duplication affects development at the immediate postimplantation stage. These loci would appear to be associated with both the X chromosome and autosomes, as the results of the present and previous experiments (Mann & Lovell-Badge, 1987) have demonstrated that the presence of (i) two sets of A M and (ii) two X M chromosomes both have the potential to affect the viability of X M X M A M A M eggs at this stage. The genetic background may be involved, as in the experiments of series II, a different strain combination had been used compared to previous experiments (series I; Mann & Lovell-Badge, 1984, 1987). However, we have only
ever obtained very low frequencies of postimplantation development of \(X^M X^M A^M A^M\) eggs derived from B6CBF\(_1\) mice, whereas others have reported much higher frequencies using the same eggs (Surani & Barton, 1983; Surani et al. 1984). Conditions of the experimental or maternal (recipient) environment, and possibly certain qualities of the egg cytoplasm may also influence the success of parthenogenetic postimplantation development. In the experiments of series I, two out of the three postimplantation-stage parthenogenones were present in one recipient in the same uterine horn. Also, Kaufman has described four very advanced XX parthenogenones obtained in a single recipient (Kaufman, 1981). However, in the experiments of series II, no such pattern was evident.

Given the above, \(X^M O A^M A^M\) eggs would have a greater potential to continue development after implantation than \(X^M X^M A^M A^M\) eggs as they have only to contend with a lack of \(A^P\) chromosomes. Presumably therefore, in the experiments of series II, the environment and/or genetic background usually resulted in a low expressivity of relevant autosomal loci (in XO parthenogenones), but not of X-linked loci (in XX parthenogenones).

Further definition of the causes for the variable development of parthenogenones beyond implantation awaits future study. Nevertheless, in the experiments of series II, there was essentially no variability in the experimental and maternal conditions and in the genetic background of XO and XX parthenogenetic eggs. Furthermore, the estimated frequency of postimplantation development obtained in XOs is at least double that which has been obtained in XXs in any other study, by ourselves or others, following transfer of 2-cell eggs to oviducts. This demonstrates that, at least under some circumstances, the presence of two \(X^M\) chromosomes does have a deleterious effect on the viability of parthenogenones at the immediate postimplantation stage.

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References


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