A paternally imprinted X chromosome retards the development of the early mouse embryo

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SUMMARY

It has previously been shown that XO mouse fetuses with a paternally derived X chromosome (Xp) are developmentally retarded and consequently smaller than their XX sibs, and that XX fetuses are retarded when compared with their XY sibs. The genetic basis for these early XO-XX and XX-XY differences has not been determined. Here we show that 10.5 day post coitum XO mouse fetuses with a maternal X chromosome, rather than being smaller than their XX sibs, are significantly larger and equivalent in size to their XY sibs. Thus the retardation of XpO fetuses must be due to an effect of their paternally derived X chromosome. The finding that XmO fetuses are larger than XX fetuses and equivalent in size to XY fetuses suggests that the XX-XY difference present at 10.5 days post coitum is largely due to the difference in X chromosome constitution rather than to a Y chromosome effect.

Key words: XO mice, X chromosome imprinting, XX-XY differences, mouse chromosome

INTRODUCTION

In the study of XO development by Burgoyne et al. (1983), XX females, which were heterozygous for a large X inversion, were used as a source of ‘O’ eggs (Evans and Phillips, 1975) and hence of XO fetuses carrying a paternal X chromosome (Xp). XpO fetuses were shown to be retarded in their development and consequently smaller than their XX sibs. The developmental retardation was already apparent at the early egg cylinder stage (the earliest age analysed). The authors argued that this XpO-XX difference could not be due simply to X dosage deficiency since XY fetuses were not retarded (in fact the XY fetuses were more advanced and consequently larger than XX fetuses), and they suggested two alternative explanations: (1) that there may be genes common to the X and Y, expressed in double dose in males and females, for which XO fetuses would be deficient (this led to the concept of ‘pseudoautosomal genes’ shared between the X and the Y - Burgoyne, 1982), or (2) that a paternally derived X chromosome had a retarding effect on early development. In the present paper, we differentiate between these possibilities by comparing XO fetuses with a paternally derived X chromosome with XO fetuses with a maternally derived X.

MATERIALS AND METHODS

Experiment 1

From a cross between a female mouse heterozygous for the X inversion In(X)1H (Evans and Phillips, 1975) and a male carrying the Y chromosome rearrangement Y* (Eicher et al., 1991), an unusual aneuploid male was produced of the genotype In(X)Y*Y*X (where Y* X is a recombinant product of Y*). This male proved to be fertile and amongst the offspring were a number of XO females. Since these XO daughters were produced from several different mothers, it was concluded that the aneuploid male must be regularly producing ‘O’ sperm and that the XO daughters must have a maternally derived X. This male was mated to MF1 (random bred albino) females to produce litters for analysis.

Experiment 2

While experiment 1 was in progress, Lane and Davison (1990) reported a new X-linked mutation (Patchy fur, Paf) associated with the production of XmO offspring by male carriers. In order to generate XmO and XpO fetuses in the same litters and so allow within litter comparisons, XPaf/Y males were mated to XPaf/O females. The paternal X was marked with Pgk-1A and the maternal X with Pgk-1B. In both experiments, the pregnant females were killed at 10.5 days post coitum (dpc), the fetuses were removed, dissected free of fetal membranes and weighed on a Cahn electrobalance (Burgoyne et al., 1983). Genotypes were determined from yolk sac metaphases as described by Evans et al. (1972) except that after treatment with 60% acetic acid, the detached cells were washed with 3:1 methanol:glacial acetic acid fixative before being air-dried on slides. In experiment 2, the X chromosome status of each fetus was established from an analysis of sex chromatin in air-dried amnion cells (Palmer and Burgoyne, 1991), enabling a cross check with the chromosome analysis from yolk sac. Also, a sample from each fetus was used for PGK isozyme analysis as described by Monk (1987), enabling XO fetuses with a paternal X (PGK A) to be distinguished from those with a maternal X (PGK B).
RESULTS

Experiment 1

The results are somewhat complicated by the fact that the male regularly produced offspring of five different genotypes, including In(X)Y* X and XY* X which carry the recombinant chromosomes from the Y* system. However, preliminary analyses showed that In(X)Y* X fetuses did not differ in size from In(X)X fetuses, and that XY* X fetuses did not differ from XY* fetuses. These genotypes have therefore been pooled as ‘XX’ and ‘XY’ classes, respectively (Table 1). The genotypes have been compared by calculating mean weighted within litter differences (see Burgoyne et al., 1983) and significance tested using a two-tailed t-test. The ‘XY’ fetuses are significantly larger than ‘XX’ fetuses, in agreement with previous data on XX-XY differences (Seller and Perkins-Cole, 1987). The XmO fetuses, rather than showing the weight deficiency previously reported for XpO fetuses, are larger than their ‘XX’ litter mates and equivalent in size to ‘XY’ fetuses.

Experiment 2

Once again the genotypes have been compared by calculating mean weighted within litter differences (Table 2). Overall, the fetuses, irrespective of genotype, are much smaller than in experiment 1. This is, at least in part, a consequence of the maternal XO monosomy since fetuses from Xpaf/Xpaf mothers are significantly larger at the same gestational age (A. R. T. and P. S. B., unpublished data). The XpO fetuses, as in the original 1983 study, are very significantly smaller than their XX litter mates. [Although numbers of somites were not recorded, it was very apparent that the small fetuses were developmentally retarded, as previously documented by Burgoyne et al. (1983)]. As in experiment 1, the XY fetuses are significantly larger than their XX litter mates. The important comparison is between XmO and XpO fetuses, and this shows that XmO fetuses are, on average, almost twice the size (7.2 mg) of their XpO litter mates (3.9 mg). In fact, they are also significantly larger than their XX litter mates and are not significantly different from their XY litter mates. These differences between

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Table 1. A comparison of the weights of XmO fetuses with those of ‘XX’ and ‘XY’ litter mates at 10.5 dpc

<table>
<thead>
<tr>
<th>Genotypes compared</th>
<th>No. of fetuses*</th>
<th>Mean±s.e.m. log wt† (wt in mg)‡</th>
<th>Mean±s.e.m. weighted difference§ diff. (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘XY’ v. ‘XX’</td>
<td>36</td>
<td>1.30±0.04 (20.1)</td>
<td>0.039±0.012</td>
</tr>
<tr>
<td>‘XY’ v. ‘XY’</td>
<td>45</td>
<td>1.26±0.04 (18.2)</td>
<td>0.029±0.017</td>
</tr>
<tr>
<td>XmO v. ‘XX’</td>
<td>14</td>
<td>1.29±0.04 (19.7)</td>
<td>0.1-0.05</td>
</tr>
<tr>
<td>XmO v. ‘XY’</td>
<td>47</td>
<td>1.26±0.04 (18.2)</td>
<td></td>
</tr>
<tr>
<td>‘XX’ v. ‘XY’</td>
<td>11</td>
<td>1.30±0.05 (19.8)</td>
<td>−0.006±0.013</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>1.31±0.04 (20.2)</td>
<td></td>
</tr>
</tbody>
</table>

*The number for each genotype differs between comparisons, because not all litters contain all three genotypes.
†Means of litter means.
‡Antilog of mean weight.
§Standard error calculated using the variance within genotypes within litters.

Table 2. A comparison of the weights of XmO fetuses with those of XpO, XX and XY litter mates at 10.5 dpc

<table>
<thead>
<tr>
<th>Genotypes compared</th>
<th>No. of fetuses*</th>
<th>Mean±s.e.m. log wt† (wt in mg)‡</th>
<th>Mean±s.e.m. weighted difference§ diff. (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX v. XpO</td>
<td>26</td>
<td>0.79±0.04 (6.2)</td>
<td>0.147±0.043</td>
</tr>
<tr>
<td>XY v. XX</td>
<td>15</td>
<td>0.61±0.05 (4.0)</td>
<td></td>
</tr>
<tr>
<td>XY v. XpO</td>
<td>31</td>
<td>0.90±0.03 (7.9)</td>
<td>0.130±0.032</td>
</tr>
<tr>
<td>XX v. XX</td>
<td>35</td>
<td>0.76±0.03 (5.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>XmO v. XpO</td>
<td>13</td>
<td>0.86±0.04 (7.2)</td>
<td>0.226±0.057</td>
</tr>
<tr>
<td>XmO v. XX</td>
<td>16</td>
<td>0.59±0.07 (3.9)</td>
<td>0.005-0.001</td>
</tr>
<tr>
<td>XmO v. XX</td>
<td>13</td>
<td>0.83±0.03 (6.8)</td>
<td>0.078±0.036</td>
</tr>
<tr>
<td>XX v. XX</td>
<td>35</td>
<td>0.77±0.03 (5.9)</td>
<td>0.05-0.025</td>
</tr>
<tr>
<td>XmO v. XY</td>
<td>15</td>
<td>0.82±0.03 (6.5)</td>
<td>−0.060±0.039</td>
</tr>
<tr>
<td>XY v. XX</td>
<td>29</td>
<td>0.90±0.04 (7.9)</td>
<td></td>
</tr>
</tbody>
</table>

*†‡§As for Table 1.
the majority of the Xp O fetuses are larger than the XX mean, while observations falling above or below the XX mean is given. The genotypes compared in Table 2. For each genotype the number of histograms of the log fetal weight data for the four genotypes are apparent in the histograms of the log fetal weight data in Fig. 1. From this figure, it is also clear that the genotypes are apparent in the histograms of the log fetal weight data in Fig. 1. From this figure, it is also clear that the Xp O data are much more variable than those for other genotypes.

DISCUSSION

It is well established that XO mice produce fewer XO offspring than expected, and there has been debate as to whether this is solely due to a deficiency of ‘O’ eggs (the X chromosome tending to remain in the egg, rather than segregating to the polar body, at the first meiotic division), or whether there is also preferential XO loss during pregnancy (Kaufman, 1972; Luthardt, 1976; Brook, 1983). Hunt (1991) has studied the breeding of XY*X females (on a C57BL/6 inbred background), and these females produced markedly fewer XY*X females than expected. In this instance, compelling evidence was provided that the deficiency of XY*X offspring was due to preferential loss during pregnancy. In both the above examples, we are dealing with fetuses carrying a single paternally derived X chromosome borne by X monosomic mothers and Hunt (1991) suggested that a fetal Xp effect, perhaps interacting with some consequence of the maternal X monosomy, may be the underlying cause of XY*X and XO fetal loss. Our present finding that XpO, but not XmO fetuses, are retarded in early development, provides some support for Hunt’s suggestion that a single paternal X may increase the likelihood of fetal failure. However, it is clear that a single fetal Xp is not a sufficient cause in itself. We have collected XpO frequency data throughout pregnancy, and at birth, for outbred mothers heterozygous for the X inversion In(X)1H (Evans and Phillips, 1975), and there was no detectable selection against the XpO fetuses (preimplantation XO:XX ratio = 1:3.15, XO:XX ratio at birth = 1:3.10 - Burgoyne and Evans, unpublished data). Furthermore, we have produced XY*X females on a random bred background, and these females show no marked deficiency of XY*X offspring (Peitz and Burgoyne, unpublished). We therefore view Xp monosomy as a factor which puts fetuses ‘at risk’ (Mahadevaiah et al., 1993) by causing developmental retardation, but only if the mother is affected by the combined detrimental effects of X monosomy (Burgoyne & Biggers, 1976) and inbreeding, are these ‘at risk’ fetuses preferentially eliminated during pregnancy.

Why should a paternally derived X chromosome be less efficient than a maternally derived X chromosome in supporting early development? In the original study of Burgoyne et al. (1983), it was established that the reduced size of XpO fetuses was due to a retardation of development already evident by 7.25 dpc. Thus, a paternally imprinted X has a retarding effect on development at some point prior to 7.25 dpc. This could most simply be explained if the paternal imprint reduces X-linked gene expression, as has been recently reported for the X-linked gene Hprt in preimplantation mouse embryos (Moore and Whittingham, 1992). Variation in the strength of the imprint could explain the variability of the XpO weight data in the present study.

Our finding that XmO fetuses are significantly larger than XX fetuses establishes that a maternally derived X chromosome is more efficient at supporting early development in the mouse, than a maternal together with a paternal X chromosome. Since XY fetuses, like XmO fetuses, have a single maternal X chromosome, we would expect XY fetuses to be larger than XX fetuses simply because of the difference in X chromosome constitution. This effect of X chromosome constitution will be superimposed on any accelerating effect of the Y chromosome on preimplantation development, such as that reported for some strains of Y chromosome by Burgoyne (1992).

What aspect of the difference in X chromosome constitution is responsible for the slight retardation of XX fetuses as compared to XmO and XY fetuses? In view of the retarding effect of the paternally imprinted X on XO development, it is tempting to attribute the retardation of XX fetuses to the presence of a paternally derived X. However, in the first tissues in which X inactivation occurs (in the trophectoderm at 3.5 dpc and primary endoderm at 4.5 dpc), it is the paternal X which is preferentially inactivated (Takei and Sasaki, 1975; West et al., 1977; Harper et al., 1982), so these tissues should be equivalent in XX and XY embryos in having a single Xm chromosome expressed. Once random X inactivation has occurred in the epiblast (ca. 6.5 dpc), XX embryos will differ from XY embryos in having a proportion of their cells (half on average) expressing a paternal rather than a maternal X, but the very fact that X inactivation is random at this time has been taken as evidence that any differential X imprint between the paternal and maternal X chromosomes has been erased (Lyon and Rastan, 1984). If this is so, then there is once again no basis for a reduction in the level of X chromo-
some expression in XX compared to XY embryos. However, it is possible that these two effects of X chromosome imprinting (on the choice of X to be inactivated in early differentiating tissues and on early embryonic growth) are mediated through different X chromosome regions or different forms of imprint.

Rather than a reduction in X chromosome activity in XX embryos being responsible for the XX-XY difference, it is possible that prior to X inactivation, tissues with both X chromosomes active have too much X activity. Takagi and Abe (1990) have presented compelling evidence that a surfeit of X chromosome activity, due to a failure of X inactivation, is very deleterious for the early mouse embryo. It is therefore quite plausible that expression from two X chromosomes prior to X inactivation is suboptimal for development.

Intriguingly, there is evidence from studies of sex chromosome trisomies that an additional maternal X is more deleterious than an additional paternal X in mice (Shao and Takagi, 1990; Takagi, 1991). The available evidence suggests that this is due to the resistance of maternal X chromosomes to inactivation in the extraembryonic membranes, which results in two X chromosomes remaining active in many cells. However, it is possible that the level of X chromosome expression prior to X inactivation is also a factor, with an extra X chromosome contributing more activity than an X0 chromosome. Further work is needed to differentiate between these possibilities.

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REFERENCES


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