Detrimental effects of two active X chromosomes on early mouse development

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Summary
Matings between female mice carrying Searle's translocation, T(X;16)16H, and normal males give rise to chromosomally unbalanced zygotes with two complete sets of autosomes, one normal X chromosome and one X16 translocation chromosome (XnX16 embryos). Since X chromosome inactivation does not occur in these embryos, probably due to the lack of the inactivation center on X16, XnX16 embryos are functionally disomic for the proximal 63% of the X chromosome and trisomic for the distal segment of chromosome 16. Developmental abnormalities found in XnX16 embryos include: (1) growth retardation detected as early as stage 9, (2) continual loss of embryonic ectoderm cells either by death or by expulsion into the proamniotic cavity, (3) underdevelopment of the ectoplacental cone throughout the course of development, (4) very limited, if any, mesoderm formation, (5) failure in early organogenesis including the embryo, amnion, chorion and yolk sac. Death occurred at 10 days p.c. Since the combination of XO and trisomy 16 does not severely affect early mouse development, it is likely that regulatory mechanisms essential for early embryogenesis do not function correctly in XnX16 embryos due to activity of the extra X chromosome segment of X16.

Key words: X-chromosome inactivation, mouse development, ectoplacental cone, mesoderm, Searle's translocation, inactivation center, functional disomy X.

Introduction
In early embryogenesis of female mammals, one of two X chromosomes is genetically inactivated (Lyon, 1961), which results in compensation of X chromosome dosage difference between male and female. The complete lack of any known mutation that disturbs X-inactivation suggests that it is vital to mammals and its failure leads to death of affected individuals as shown in Drosophila (Belote and Lucchesi, 1980; Lucchesi and Skripsky, 1981). However, no direct observation for this view has ever been reported in any species of mammals.

One way to inquire into the significance and function of X chromosome inactivation and X chromosome itself would be to examine development of mouse embryos in which one entire X chromosome and an additional X chromosome segment are active (partial functional disomy X). It is believed that X-inactivation is controlled by a cis-acting X chromosomal site called X chromosome controlling element (Xce) or inactivation center (Cattanach, 1975), loss of which probably renders the chromosome unresponsive to inactivation signals. We set out to examine developmental effects of partial functional disomy X making use of Searle's X-autosome translocation, T(X;16)16H (Searle, 1962; Lyon et al. 1964). In this translocation, the proximal X chromosome segment, corresponding to 63% of the entire X, has lost Xce, and gained a distal chromosome 16 segment in exchange (X16). The other translocation chromosome (16X) consists of the proximal chromosome 16 segment and the distal X chromosome segment carrying Xce (Fig. 1). Matings of female translocation heterozygotes with chromosomally normal males regularly give rise to unbalanced zygotes with two complete sets of autosomes, one normal X chromosome from father and X16 from mother. These karyotypically unbalanced XnX16 embryos consistently showed only slight growth retardation at 8.5 days p.c. Partial mono- or partial trisomy for chromosome 16 or both are not directly responsible for the severe developmental arrest observed in XnX16 embryos. It seems likely that regulatory mechanisms essential to early embryo-
genesis do not function correctly in XnX16 embryos due to activity of the extra X chromosome segment of X16. We may conclude that functional disomy for the entire X chromosome causes much severer developmental abnormality than that found in XnX16 embryos and X-inactivation is indeed indispensable for normal embryogenesis.

Materials and methods

T(X;16)16H translocation

The breakpoint in the X chromosome is at D band and in chromosome 16 is at B5 band. Thus the X16 chromosome roughly corresponds in length to the intact X (Xn) and 16X to intact chromosome 16 (Fig. 1). Chromosome 16X is assumed to carry the inactivation center or Xce (Rastan, 1983), since this chromosome can be inactivated (Takagi, 1980; McMahon and Monk, 1983).

Recovery and BrdU labelling of embryos

Spontaneously ovulating T16H/+ females were caged with karyotypically normal males hemizygous for Tabby, and were checked daily for vaginal plugs early in the morning. The day when the vaginal plug was found was taken as day 0 of pregnancy. Embryos were usually recovered from decidual swellings at 9 to 10 a.m. from day 6 to 10 of pregnancy. They will be designated as embryos at 6.5-10.5 days post coitum (p.c.). The staging system proposed by Theiler (1972) was used whenever applicable, because developmental variation between litters was evident in early postimplantation stages. Stages of XnX16 embryos were determined from those of normally grown littersmates.

Recovered embryos were photographed and incubated in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum and 100 μg ml⁻¹ 5-bromo-2-deoxyuridine (BrdU) at 37°C in an atmosphere of 5% CO₂ in air. The duration of incubation was 6 h (6.5-day embryos) to 9 h (9.5-day embryos) including the last hour in the presence of 1 μg ml⁻¹ Colcemid. After hypotonic treatment with 1% Sodium citrate, embryos were fixed with 3:1 methanol:acetic acid. Chromosome slides were prepared according to a modification of the air-drying method described earlier (Wroblewska and Dyban, 1969). Slides were stained with freshly prepared acridine orange and examined under a fluorescence microscope.

Histological examination of embryos

Embryos isolated from decidual swellings were fixed with Bouin's fixative, stained with hematoxylin, embedded in paraffin wax and 7 μm sections were cut and stained with hematoxylin and eosin (HE). In certain cases, the entire implantation sites were sectioned with surrounding uterine tissue. In order to ascertain identification of XnX16 embryos, embryos freed of decidual tissue and Reichert's membrane were cut into the extraembryonic and the embryonic region with the aid of fine glass needles, and the former was used for histological examination and the latter for karyotyping as described above. For detailed histological examination, isolated embryos were fixed with 2.5% glutaraldehyde in cacodylate buffer, postfixed with 1% osmium tetroxide, dehydrated with acetone and embedded in Epon 812. Sections cut at 1-2 μm were stained with 1% toluidine blue.

Staining with lectins

FITC-labelled concanavalin A (Con A), soybean agglutinin (SBA), peanut agglutinin (PNA) and wheat germ agglutinin (WGA) were purchased from Vector Laboratories, Burlingame, CA. Dewaxed tissue sections were incubated in PBS for 1 h and then in a dilute solution of a lectin (100 μg ml⁻¹ in PBS) for 1 h at room temperature in a moist chamber. After incubation, sections were washed throughly in PBS, mounted in fresh PBS and observed under a fluorescence microscope.

Culture of XnX16 embryonic ectoderm in vitro

The embryonic region was removed from 8.5-day presumptive XnX16 embryos after treatment with 2.5% pancreatin with the aid of tungsten needles. The embryonic ectoderm deprived of the embryonic visceral endoderm was cultured in a 30 mm tissue culture dish containing Eagle's minimum essential medium containing 10% fetal calf serum.

Results

Identification of XnX16 embryos

In order to detect morphological traits adequate for the identification of XnX16 embryos, karyotypes were studied in 351 embryos from 86 females heterozygous for T16H mated with chromosomally normal males at 6.5 to 9.5 days p.c. In contradiction to our previous study (Takagi, 1980), the frequency of XnX16 embryos relative to the total embryos produced by alternate or adjacent 1 disjunction remained about 20% during this period of pregnancy (Table 1). The previous conclusion that XnX16 embryos were lost by 8.5 days p.c. was based on a smaller number of embryos and could have been premature.

Developmental variation was evident among litters recovered at day 6 of pregnancy. It was not possible to
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Table 1. Karyotypes of embryos recovered from T16H heterozygous females mated with chromosomally normal males

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Genetic imbalance</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Total</th>
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<tr>
<td>2:2 normal disjunction</td>
<td></td>
<td>20</td>
<td>15</td>
<td>27</td>
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</tr>
<tr>
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<td></td>
<td></td>
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<td>73</td>
</tr>
<tr>
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<td>26</td>
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<td>19</td>
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<td>65</td>
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<tr>
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<td>MsXd;Tsl6d</td>
<td>21</td>
<td>15</td>
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<td>3</td>
<td>62</td>
</tr>
<tr>
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<td>102</td>
<td>86</td>
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<tr>
<td>2:2 adjacent 2 disjunction</td>
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<td>16</td>
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<tr>
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<td>5</td>
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<td>Tsl6</td>
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<td>128</td>
<td>22</td>
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Abbreviations: Ms, monosomy; Ts, trisomy; d, segment distal to the break point; p, segment proximal to the break point.

Distinguish XnX16 embryos based on gross phenotypic differences among litters probably at stage 8. Occasional XnX16 embryos were as big as or bigger than their littermates having a normal or a balanced karyotype. However, growth retardation of XnX16 embryos was striking in remaining litters at stage 9 with the average long axis 53.6% (range, 42%–69%) that of the normally growing littermates (Fig. 2a–e). XnX16 embryos could not, however, be identified with certainty on the growth retardation alone. Embryos with various karyotypic abnormalities other than XnX16 were usually retarded in development. Furthermore, growth retardation was quite common among chromosomally normal or balanced litters. This was further confirmed by a histological study of 6.5-day embryos in situ. 20 out of 65 viable embryos were smaller than their littermates and had an underdeveloped ectoplacental cone (see below). All of these embryos could not be XnX16, in view of the much lower frequency of XnX16 embryos at 6.5 days p.c.

Gross morphology of the 7.5-day XnX16 embryos, on the other hand, was striking enough for their overt identification in most cases. In addition to the increased growth retardation, XnX16 embryos had a small and thin embryonic region, undulated visceral endoderm facing the yolk cavity particularly at the extraembryonic region (Fig. 2f–j). The mean long axis of 7.5-day (stages 10, 11) XnX16 embryos was 54.2% that of normally grown littermates, but the wide range of 30% to 65% shows that the manifestation of the unbalanced karyotype is by no means uniform.

XnX16 embryos are trisomic for the distal segment of chromosome 16 and monosomic for the distal segment of the X chromosome. XO embryos trisomic for chromosome 16, resulting from the adjacent 2 disjunction of T16H quadrivalent, were occasionally obtained in this study. Those embryos were well-balanced, though slightly retarded, at 8.5 and 9.5 days p.c. (Fig. 3) in accord with the observation that embryos trisomic for the entire chromosome 16 survive until immediately before or even shortly beyond birth (Miya-bara et al. 1983), and XO mice are phenotypically normal and fertile female. Thus, the severe developmental anomalies found in XnX16 embryos may not be explained without postulating imbalance associated with X chromosome inactivation.

In order to characterize XnX16 embryos more fully, we carried out combined histological and cytogenetical analyses on 7.5-day embryos. 15 out of 86 embryos studied proved to have the unbalanced karyotype. In addition to marked growth retardation, these embryos were characterized by a very poorly developed, if any, ectoplacental cone irrespective of developmental variation between litters. It was also noted that the dorsal space of the embryo bound by the visceral endoderm was occupied by densely packed cells. It is suggested that the cells destined to form the ectoplacental cone were prevented from invading the decidua cavity and forced to remain in the embryo.

Eight intact 7.5-day embryos presumed to have the unbalanced karyotype were then subject to histological examination. They resembled pregastrulation embryos at stage 9, but their embryonic ectoderm was small and the proamniotic cavity was filled with cell debris.
Furthermore, in occasional embryos, a number of folds were formed by the visceral endoderm in the ventral side of the extraembryonic region. Thus, small size, underdeveloped ectoplacental cone, packed cells at the site of the ectoplacental cavity, poorly developed embryonic ectoderm with a number of dead cells in the proamniotic cavity, lack or shortage of mesoderm and abnormal visceral endoderm appeared features specific to 7.5-day XnX16 embryos.

Morphological abnormalities characteristic to 7.5-day XnX16 embryos were expressed in more exaggerated form in 8.5-day embryos. They were easily recognized under the dissection microscope because of their ruggedly outlined yolk sac and the tiny underdeveloped embryonic region. Later, the outline of the embryo became smooth with the expansion of the extraembryonic region. Now growth of these embryos varied extensively and the average long axis of XnX16 embryos varied from 14% to 60% that of normally grown littermates with the mean value of 38.6% (Fig. 2k-q).

Most 9.5-day XnX16 conceptuses were round in shape sometimes with a structure resembling an embryo proper (Fig. 2r-w). The size and shape of putative XnX16 embryos remained unchanged at 10.5 days p.c.

**Developmental defects in XnX16 embryos**

Because of higher resolution, plastic embedding and ultramicrotomy rather than a paraffin embedding method was applied to the detailed histological study of XnX16 embryos identified by gross morphology.

**6-day embryos at Stage 9**

Although we failed to find a clear histological feature specific to 6.5-day XnX16 embryos in HE-stained sections, we noticed that the proamniotic cavity of occasional retarded embryos was filled with clumps of cells. They could be XnX16 embryos, because the presence of such cells in the proamniotic cavity is one of the most consistent characteristics of XnX16 embryos in later stages. Compared with a normally grown embryo (Fig. 4B), one of such putative XnX16 embryos shown in Fig. 4A had an underdeveloped ectoplacental cone and free cells in the proamniotic cavity.

**7-day embryos at stage 10**

In putative XnX16 embryos at stage 10, the embryonic ectoderm obviously everted or evaginated into the proamniotic cavity near the ventral end (Figs 4C, 10B). This phenomenon was observed in three consecutive
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Fig. 3. Photomicrographs of XO embryos trisomic for chromosome 16 at 8.5 (c,f) and 9.5 (h) days p.c. with normally grown littermates. Embryo proper enclosed by the amnion is evident in these karyotypically unbalanced conceptuses. Bar=0.1 mm.

Embryos. Mitotic cells were abundant in the evaginated tissue. Ectoplacental cone was underdeveloped in comparison with normally grown littermates (Fig. 3D). Furthermore, the extraembryonic visceral endoderm tended to be thicker than expected.

7-day embryos at stage 11

Histological findings made in HE-stained preparation were confirmed in 10 embryos examined. Folds formed by the visceral endoderm were conspicuous in the embryo shown in Fig. 5B, but not in the embryo shown in Fig. 5A. In the latter, however, the extraembryonic visceral endoderm was thickened considerably. In both embryos, embryonic ectoderm was disproportionately small and mesodermal component was still almost absent. It is possible that the lack of mesodermal cells that usually line the extraembryonic visceral endoderm forming yolk sac led the free inner surfaces of the visceral endoderm to stick together (cf. Fig. 5E). A packed cell mass at the dorsal end and underdevelopment of the ectoplacental cone were evident in Fig. 5A,B,D.

8-day embryos at stages 12–13

A total of 10 putative X^nX^16 embryos were studied. At a first glance, the basic structure of a well-developed embryo shown in Fig. 6A resembled that of normal embryos at stage 11 (Fig. 5C). The poorly developed embryonic region still retained the morphology of the embryonic ectoderm and mesoderm cells were sparse both in the embryonic and extraembryonic region. In the cavity lined with the embryonic ectoderm, dead cells were abundant. Observation of serial sections revealed clear differences between X^nX^16 at stage 13 and normal embryos at stage 11. In the latter, three cavities are present: most ventral of these is the amniotic cavity lined with embryonic ectoderm; in the middle is the exocoelom lined with mesoderm; and finally the ectoplacental cavity lined with extraembryonic ectoderm. One of the most remarkable features in X^nX^16 embryos at stage 13 was that embryonic ectoderm was still continuous with extraembryonic ectoderm in a complicated way (Fig. 6B). The cavity apparently corresponding to the exocoelom was lined with a thin single cell layer. Mesoderm was produced much less extensively in X^nX^16 embryos grown poorly.

9-day embryos at stages 14–15

In spite of apparent morphological variability, the basic structure of 7 putative X^nX^16 embryos examined at these stages was relatively uniform. The spherical embryo consisted of two cavities, one was lined with the embryonic and extraembryonic ectoderm layer and the other with a thin mesodermal layer (Fig. 7D). Findings in 8-day embryos suggested that the former corresponds to the proamniotic cavity and the latter to the abnormal exocoelom or yolk sac with scanty mesodermal cells. In some embryos, the boundary between these cavities was complicated. The structure resembling an embryo proper in Fig. 7j was a part of such proamniotic cavity lined with a cell layer made up of the embryonic and the extraembryonic ectoderm (Figs 7C, 8G). The atrophic embryonic region was identified because of its morphology resembling the embryonic ectoderm of early postimplantation stages and accompanying dead cells (Fig. 10D). No mesodermal component was found in the smaller embryo shown in Fig. 7A. There was no sign of maternal blood circulation at the ectoplacental region in this case.

10-day embryos at stages 16–17

Putative X^nX^16 embryos were identified because of the general morphology common to embryos at previous stages. A histological study in 3 embryos showed that the basic structure was exactly the same as that of X^nX^16 embryos at 9.5 days p.c., but signs of degeneration were evident (Fig. 8A,B). The yolk cavity was filled with dead endoderm cells (Fig. 8D). Degeneration was also found in embryonic ectoderm and adjoining visceral
endoderm (Fig. 8C,E). Embryonic ectoderm was less affected in other regions (Fig. 8F,G). A large clump of dead or dying cells was found at the dorsal end of the embryo (Fig. 8B). This embryo looked healthier in other regions.

Expression of lectin receptors in X"X" embryos
Abnormal development of X"X" embryos prevented us from identifying various tissues for certain on the basis of morphology alone. Distribution of lectin receptors may help interpreting the abnormal development of X"X" embryos. Deparaffinized sections of normally grown embryos at stage 11 and X"X16 embryos at stage 13 were stained with either of FITC-labelled Con A, SBA, PNA and WGA.

Con A receptors were expressed on various tissues of normal embryos. Fluorescence was strongest on the free surface of the extraembryonic visceral endoderm facing yolk cavity, followed by the ectoplacental cone and cells around it, the inner surface of the embryonic ectoderm facing the amniotic cavity and the mesoderm including the allantois. Con A binding was similar in X"X16 embryo. However, these embryos did not have tissues or groups of cells having a mode of reaction
corresponding to the mesoderm of normal embryos (data not shown).

As reported earlier, PNA receptors were expressed on various tissues of normal embryos (Hamada et al. 1983). Only the extraembryonic ectoderm expressed PNA receptors on both the inner and the outer surfaces facing the exocoelom and the ectlaplacental cavity. In XnX embryos, the cell layer adjoining the embryonic ectoderm (data not shown) showed a similar pattern of expression. This seems to support the finding that the embryonic ectoderm and the extraembryonic ectoderm failed to separate in XnX embryos.

Expression of WGA receptors was also in agreement with the above finding. WGA reacted with the ectlaplacental cone in normal embryos and cells packed at the dorsal end of XnX embryos showed a similar pattern of expression. Expression of Con A and PNA receptors was consistent with this observation. Only cell surface
of the extraembryonic visceral endoderm facing the yolk cavity expressed SBA receptors in normal and X<sup>n</sup>X<sup>16</sup> embryos.

**Proliferative potential of embryonic ectoderm from X<sup>n</sup>X<sup>16</sup> embryos in vitro**

In order to examine the proliferative capability of X<sup>n</sup>X<sup>16</sup> embryonic ectoderm cells, the embryonic region was dissected from five putative X<sup>n</sup>X<sup>16</sup> embryos at stages 12–13 and cultured in MEM supplemented with 10% fetal calf serum. Within 24 h a sheet of cells with epithelial characters spread around the explant (Fig. 9). Outward spreading of endodermal cells continued without appreciable increase in cell number for the next two days followed by gradual deterioration. The embryonic ectoderm cell clump at the center flattened and each cell spread on the substratum within 48 h, but the cells apparently began to die off 2 to 3 days later without undergoing active cell division. No healthy cells were left 7 days after the initiation of culture.

Big halos of spindle-shaped and epithelial cells developed around embryonic ectoderm fragments dissected from normally grown littermates within 24 h. These cells were mitotically very active and groups of pulsating cells were found frequently. Apparently, the capacity of outgrowth did not depend on the size of explants. Thus, it is very likely that X<sup>n</sup>X<sup>16</sup> embryonic ectoderm either did not have proliferative potential in vitro or had lost it by 8.5 days p.c. The yolk sac was also cultured in vitro. Cells grew out vigorously from normal yolk sac and stayed mitotically active for at least 2 weeks. No cell outgrowth was found, on the other hand, in the case of X<sup>n</sup>X<sup>16</sup> embryos for several days after explantation, which probably reflects the shortage of mesoderm in the explanted tissue. The X<sup>n</sup>X<sup>16</sup> sex chromosome constitution was ascertained in cultured extraembryonic halves of 3 embryos used for this study. Karyotype could not be determined in remaining 2 embryos because of the lack of good metaphase spreads.

**Discussion**

In the previous study, we concluded that X-inactivation does not occur in X<sup>n</sup>X<sup>16</sup> embryos, because we could never find an X chromosome showing out-of-phase replication (Takagi, 1980). During the course of this study, we examined a total of 516 X<sup>n</sup>X<sup>16</sup> metaphase cells labelled with BrdU mainly from 6.5-day embryos, and in no case could we detect an asynchronously replicating X chromosome. There is no a priori reason to suppose complete selection against X<sup>16</sup>-inactive cells, if they ever occurred, since such cells are genetically balanced or trisomic only for the distal region of chromosome 16. Inactivation of X<sup>n</sup>, on the other hand, makes X<sup>n</sup>X<sup>16</sup> cells partially nullisomic for the X chromosome and trisomic for the distal segment of chromosome 16, an imbalance probably severe enough for rigorous selection against such cells. Continual death of the embryonic ectoderm cells in X<sup>n</sup>X<sup>16</sup> embryos (Fig. 10) may favor selection against such X<sup>n</sup>-inactive cells. However, our study in normal embryos indicated that X-inactivation in the embryonic ectoderm had finished within 24 h (Takagi et al. 1982).
Given the same situation in X<sup>n</sup>X<sup>16</sup> embryos, we have to conclude that, after X-inactivation, selection did not occur rapidly in every cell and cell death continued over a long period of time from 6 to 10 days p.c.: X<sup>n</sup>X<sup>16</sup> cells with a late-replicating X chromosome would have been detected during that period. It is very likely that X-inactivation has not occurred in X<sup>n</sup>X<sup>16</sup> embryos, because X<sup>16</sup> chromosome had lost the Xce or the inactivation center as a result of T16H translocation. Current models of X-inactivation (Gartler and Riggs, 1983) predict that such cells behave as if they had only one X chromosome. It follows from what has been discussed above that most developmental abnormalities found in X<sup>n</sup>X<sup>16</sup> embryos are attributable to partial functional disomy X.

Developmental abnormalities found in X<sup>n</sup>X<sup>16</sup> embryos are: (1) growth retardation detected as early as stage 9, (2) continual loss of embryonic ectoderm cells either by death or by expulsion into the proamniotic cavity, (3) underdevelopment of the ectoplacental cone throughout the course of development, (4) very limited, if any, mesoderm formation, (5) failure in early organogenesis including the embryo, amnion, chorion and yolk sac. It is hard for any mutation affecting early development to identify the specific cell type in which the gene first acts (McLaren, 1976), and the defective gene product on the basis of phenotype (Magnuson, 1986), primarily because of the number and complexity of steps involving close interdependence between different cell types in developing embryos. Functional disomy X studied here is no exception. The difficulty is much greater in this case, because genes on 63% of the X chromosome rather than a single gene have to be taken into account.
Developmental processes are often a result of interactions between different cell types as seen in the classical example of embryonic induction in Amphibians. It is conceivable that the normal course of development is perturbed critically in mice by the absence or shortage of one of definitive germ layers. The absence of mesodermal components seems to explain various aspects of abnormal development in XnX16 embryos.

In normal development, mesoderm moves into the extraembryonic region and pushes the junctional region...
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Fig. 9. Phase-contrast micrographs of XnX16 embryonic ectoderm grown in vitro for (A) 24 h, (B) 48 h, (C) 72 h, (D) 120 h. Bar=0.05 mm.

between the embryonic and extraembryonic region into folds at stage 10. The amniotic folds thus formed bulge into the proamniotic cavity and eventually fuse at stage 11. Coalescence of lacunae formed within the amniotic folds leads to the formation of the exocoelom which is lined by mesoderm and separates the extraembryonic ectoderm from the embryonic ectoderm. The failure to form amniotic folds and hence the amnion itself in XnX16 embryos seems to be attributed to deficient mesoderm, which, in turn, accounts for the failure to separate the extraembryonic from the embryonic ectoderm.

Decreasing sizes of the embryonic ectoderm together with the occurrence of cell debris associated with it indicate that the embryonic ectoderm cells die continually in XnX16 embryos. It is tempting to postulate that certain signals from mesodermal tissue(s) are necessary for proliferation of the embryonic ectoderm and for neural induction. With the shortage of such signals, the embryonic ectoderm cells must have been unable to undergo differentiation in vivo and lost proliferative potential in vivo and in vitro. Alternatively, death of XnX16 embryonic ectoderm cells are due to functional disomy X. This is, however, apparently at variance with the fact that certain types of cells are viable with two functional X chromosomes (Epstein, 1969; Gardner and Lyon, 1971; Martin et al. 1978; McBurney and Strutt, 1980). Furthermore, Hockey et al. (1989) showed that clones of C86 embryonal carcinoma could form differentiated cells with two apparently active X chromosomes.

Inadequate nutrition due to the poorly developed ectoplacental cone, and possibly also inadequate differentiation of trophoblasts, may explain the early growth retardation. Blastocyst reconstitution experiments showed that poor development of trophoblast significantly impaired development of the embryo proper (Barton et al. 1985). The large number of cells packed at the dorsal end of the XnX16 embryo seem to correspond to diploid ectoplacental cone cells that failed to invade...
the decidual cavity. It may be speculated that the crowded extraembryonic region put pressure on the growing embryonic ectoderm and compelled it to evaginate into the proamniotic cavity. Alternatively, attenuated cell–cell contact due to cell death or altered cell surface property could explain evagination or expulsion of embryonic ectoderm cells. Putative diploid ectoplacental cone cells retained at the dorsal end of the embryo also suggest alteration in cell surface property or cell motility.

It is evident that neither X chromosome of XnX16 embryos carries any mutant genes that gravely affect embryonic development, e.g. albino deletions (Lewis et al. 1976; Niswander et al. 1988; 1989) and mutations (Bennett and Dunn, 1958; Spiegelman et al. 1976), since Xn and X16 plus 16' were fully expressed in reproductively competent father and mother, respectively. Duplication of the transcriptionally active X chromosome dosage is apparently responsible for the defective cellular differentiation, and, hence, abnormal development of XnX16 embryos, which is consistent with the fact that X-chromosome differentiation occurs at different times in different cell lineages, and is associated with the departure, or differentiation of cells from the stem line (Monk, 1981). In the paucity of data concerning early development of autosomal trisomics and tetrasomics, it is difficult to determine whether the X chromosome is involved in early development and cell differentiation more closely than average autosomes.

In normal female mouse embryos, the paternally derived X chromosome (Xp) is inactivated in the trophectoderm and the primitive endoderm (Takagi and Sasaki, 1975; Takagi et al. 1982). The polar trophectoderm cell lineage fails to develop in mouse embryos carrying an extra maternally derived X chromosome (Xm) such as XmXmXp and XmXmY (Shao and Takagi, unpublished observation). X chromosome inactivation patterns observed in triploid mouse embryos (Endo and Takagi, 1982) predict that two Xm's have remained active in extraembryonic regions of such embryos probably due to imprinting of Xm (Lyon and Rastan, 1984). This, if indeed the case, would probably support the assumption that the X chromosome is involved in early mouse development and two doses of active X chromosome derange regulatory mechanisms. In humans, where XmXmY and possibly XmXmXp are compatible with survival, imprinting, if any, may not last until the time of X-inactivation in extraembryonic tissues.

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