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The pattern of X-chromosome inactivation in the embryonic and extra-embryonic tissues of post-implantation digynic triploid LT/Sv strain mouse embryos

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Summary

Spontaneously cycling LT/Sv strain female mice were mated to hemizygous Rb(X.2)2Ad males in order to facilitate the distinction of the paternal X chromosome, and the pregnant females were autopsied at about midday on the tenth day of gestation. Out of a total of 222 analysable embryos recovered, 165 (74·3%) were diploid and 57 (25·7%) were triploid. Of the triploids, 26 had an XXY and 31 an XXX sex chromosome constitution. Both embryonic and extra-embryonic tissue samples from the triploids were analysed cytogenetically by G-banding and by the Kanda technique to investigate their X-inactivation pattern. The yolk sac samples were separated enzymatically into their endodermally-derived and mesodermally-derived components, and these were similarly analysed, as were similar samples from a selection of control XmXp diploid embryos. In the case of the XmXmY digynic triploid embryos, a single darkly-staining Xm chromosome was observed in 485 (82·9%) out of 585, 304 (73·3%) out of 415, and 165 (44·7%) out of 369 metaphases from the embryonic, yolk sac mesodermally-derived and yolk sac endodermally-derived tissues, respectively. The absence of a darkly staining X-chromosome in the other metaphase spreads could either indicate that both X-chromosomes present were active, or that the Kanda technique had failed to differentially stain the inactive X-chromosome(s) present. In the case of the XmXmXp digynic triploid embryos, virtually all of the tissues analysed comprised two distinct cell lineages, namely those with two darkly-staining X-chromosomes, and those with a single darkly staining X-chromosome. Four X-inactivation patterns were consequently observed in this group, namely, (XmXp)Xm, (XmXm)Xp, (Xm)XmXp and XmXm(Xp) in which the inactive X is enclosed in parentheses. The incidence of these various classes varied among the tissues analysed. There was, however, a clear pattern of non-random selective paternal X-inactivation in yolk sac endodermally-derived samples which possessed two inactive X-chromosomes. This finding contrasts with the situation observed in the yolk sac mesodermally-derived and embryonic samples which possessed two inactive X-chromosomes, where the ratio of (XmXm)Xp:Xm(XmXp) was 1:1·20 and 1:1·03, respectively, being clear evidence that random X-inactivation had occurred in these tissues.

1. Introduction

Spontaneous triploidy occurs only rarely in mammals, and it is believed that most triploid embryos fail to survive beyond the early post-implantation period (Beatty, 1957; Dyban & Baranov; 1987). In man it is estimated that between 1% and 3% of all conceptuses are triploid, and that these probably account for 15–20% of all first trimester spontaneous abortions with a numerical chromosomal abnormality (Carr, 1971*a*, *b*; Niebuhr, 1974; Boué *et al.* 1975; Beatty, 1978; Lin *et al.* 1985). While a small number survive into the second trimester of pregnancy, only a very few develop to term. The majority of these infants die within the first week (Niebuhr, 1974).

In the LT/Sv strain of mice spontaneous triploidy is relatively commonly encountered, since a significant proportion of their eggs are ovulated as primary oocytes rather than as secondary oocytes. Following monospermic fertilization, the primary oocytes give rise to digynic triploid embryos, whereas the secondary oocytes result in normal diploid embryos (Kaufman & Howlett, 1986; O'Neill & Kaufman, 1987). In young female LT/Sv mice (six weeks old), up to 50% of the embryos isolated on the tenth day of gestation were found to be triploid (Kaufman & Speirs, 1987; Speirs

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& Kaufman, 1990). These digynic triploid embryos are smaller than their normal diploid littermates, and in most cases exhibit abnormalities of the neural tube and heart. The abnormal nature of their morphological features observed during the tenth and eleventh days of gestation, suggests that these embryos are unlikely to survive much beyond this stage.

Various explanations have been proposed which might account for the limited viability of triploid mammalian embryos. For example, they may die because of gene dosage imbalance and/or their extra-embryonic tissues may be incapable of differentiating sufficiently to support their development to term. With regard to these two possibilities, it is now well established that X-chromosome inactivation, which provides a mechanism for gene dosage compensation (Lyon, 1961, 1972), plays a central role in the differentiation of the extra-embryonic tissues of rodents (Monk & Harper, 1979; Monk, 1981), though whether a similar situation occurs in man has yet to be established. The situation in rodents is of particular interest, in that Takagi and colleagues (Takagi & Sasaki, 1975; Wake *et al.* 1976) have demonstrated that in certain of their extra-embryonic tissues, the inactive X-chromosome tends to be of paternal origin (Lyon & Rastan, 1984) whereas in the embryonic tissues the inactive X-chromosome may be of either maternal or paternal origin. More recently, Chapman and his co-workers (West *et al.* 1977; Frels *et al.* 1980) confirmed that the paternal X-chromosome was not expressed in either the trophoblast or primitive endoderm lineages. We have extended this study by specifically analysing, using a differential staining technique (Kanda, 1973), the pattern of X-chromosome inactivation in the embryonic and extra-embryonic tissues of digynic triploid embryos isolated from LT/Sv strain mice. We wished to investigate whether abnormalities of this system might, in part, account for the reduced size and viability, as well as the morphological anomalies seen in these digynic triploid embryos, as indicated above. Male mice carrying a Robertsonian translocation between the X-chromosome and chromosome 2 (Rb(X.2)2Ad, Adler *et al.* 1989) were employed in this study, and enabled us to identify the parental origin of the inactive X-chromosome in all of the triploids analysed.

2. Materials and methods

Six- to eight-week old randomly cycling female LT/Sv strain mice (MRC, Carshalton) were caged with Rb(X.2)2Ad males (supplied by Dr M. F. Lyon). Early each morning the females were checked for the presence of a vaginal plug, and this was taken as evidence of mating. The morning of finding a vaginal plug was considered to be the first day of pregnancy.

Pregnant females were autopsied during the morning on the tenth day of gestation. The decidual swellings were isolated from the uterine horns and transferred into phosphate buffered saline (PBS). The embryos were separated from their extra-embryonic tissues (visceral yolk sac + amnion) and the developmental stage achieved, their crown-rump length and, where appropriate, the presence of any morphological abnormalities were noted at this time. The embryos, where size permitted, were generally divided into two similarly-sized portions which were subsequently transferred, along with the visceral yolk sac, into tissue culture medium containing colcemid (1 ml of a 0·1% solution of colcemid in 100 ml of TC199) and incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air for 2–3 h.

After this initial incubation period, the visceral yolk sac was separated into its two component parts, namely an inner mesodermally-derived layer and an outer endodermally-derived layer, using an enzyme separation technique described by Levak-Svajger, Svajger & Skreb (1969) for the separation of rat yolk sac, and subsequently modified by Dr John West (personal communication) for use with mouse extra-embryonic membranes. The visceral yolk sac samples were placed in 1 ml of the enzyme solution which contained 2·5 g of pancreatin and 0·5 g of trypsin in 100 ml of PBS, and retained in this solution for 2–3 h at 4 °C. The visceral yolk sac samples were then transferred to individual solid watchglasses containing 2–3 ml of M2 medium (Whittingham & Wales, 1969) and the separation of the two layers was completed mechanically using watchmakers' forceps. The mesodermally-derived and endodermally-derived components were in all cases readily distinguished, principally because blood vessels were only associated with the mesodermally-derived samples.

Four samples were thus obtained from each conceptus, namely two portions of embryonic tissue, the visceral yolk sac endodermally-derived sample, and the visceral yolk sac mesodermally-derived sample. One of the embryonic tissue samples was processed for Giemsa banding, in order to establish the sex chromosome constitution of the individual embryos. The remaining three samples were processed separately using a modification of the Kanda technique (Kanda, 1973) which differentially stains the inactive X-chromosome. The samples were retained in a 0·5% hypotonic solution of KCl at room temperature for 7 min, then transferred into a hot solution of hypotonic KCl pre-heated to 50 °C and maintained at this temperature for 12–13 min. The timing of exposure to the hot hypotonic solution was found to be critical.

All four samples from each implant were fixed in 3:1 methanol:glacial acetic acid at 4 °C for a minimum of 20 min, although the samples could be left overnight without detrimental effect. Slides were prepared using a modification of the cell spreading technique outlined by Evans *et al.* (1972) and subsequently stained for 5 min in a 3% Giemsa solution.

Slides were randomly coded and analysed blind in order to eliminate observer bias. Analysis of the X-inactivation status of these preparations was made on a sample of chromosome spreads that were either complete, when all of the individual chromosomes could be counted, or in preparations where a darkly staining element(s) could be identified, although the absolute number of chromosomes present could not be established due either to the overlapping of chromosomes, or where chromosome morphology was indistinct. Metaphase spreads in which the Rb(X.2) translocation, though present, was not readily distinguished (except, that is, when a Y-chromosome was observed, i.e. in the XXY triploids), were automatically excluded from the analysis. Since the possibility exists that a low frequency of aneuploid embryos could be generated when normal mice are mated to hemizygous mice bearing the Rb(X.2) translocation (see Adler *et al.* 1989), this factor could have accounted for some of those embryos in which the Rb(X.2) translocation was not observed. Note was also taken of the proportion of metaphase spreads that failed to demonstrate a darkly staining element.

In the following text, the parental origin of the X-chromosome is designated as Xm or Xp, where the chromosome is of maternal or paternal origin, respectively, while the inactive chromosome is enclosed in parenthesis eg (Xm)XmXp.

3. Results

From a total of 27 females autopsied 222 analysable embryos were recovered, 165 (74·3%) of which were found to be diploid and 57 (25·7%) triploid (see Table 1). No aneuploid embryos were recognized. G-banding analysis showed that of the 57 triploid embryos 26 had an XXY sex chromosome constitution, 21 of which yielded an analysable sample of each of the three tissues being examined, namely embryonic tissue, yolk sac mesodermally-derived tissue, and yolk sac endodermally-derived tissue. The remaining 31 triploid embryos had an XXX sex chromosome constitution, and 24 of these yielded an analysable sample of each of the three tissues studied.

The use of the Robertsonian Rb(X.2) translocation allowed the paternally derived X-chromosome to be recognized. Thus, in those cells which possessed a paternally derived X-chromosome, one arm of the metacentric 'marker' chromosome appeared darkly staining, and thus provided a means of establishing its X-inactivation status.

(a) *Control XmXp series*

The incidence of differential staining in the metaphase spreads isolated from the various tissues of XmXp diploid embryos was used as a means of attempting to establish the efficiency of the Kanda staining technique in relation to the various tissues analysed. It was observed that out of 804 metaphase spreads obtained from the embryonic tissues analysed, 365 (45·4%) displayed a single inactive Xm chromosome and 326 (40·5%) a single inactive Xp chromosome, while 113 (14·1%) showed no evidence of the presence of an inactive X-chromosome (see Table 2). In the mesodermally-derived tissue, out of 442 metaphase spreads analysed, 188 (42·5%) displayed evidence of an inactive Xm chromosome and 172

Table 1. *Cytogenetic analysis of digynic triploid and diploid embryos isolated on the 10th day of gestation from LT/Sv females mated with Rb(X.2)2Ad males*

No. of females autopsied	No. of implants	No. of resorptions	No. not analysable	No. embryos analysed	Ploidy of embryos	
					2n (%)	3n (%)
27	243	21	0	222	165 (74·3%)	57 (25·7%)

Table 2. *Differential staining pattern of metaphase spreads isolated from XmXp diploid (control) embryos*

Tissue sample analysed	No. of metaphases scored	No. of metaphases with no darkly staining X-chromosome (%)	No. of metaphases with a single darkly staining X-chromosome (%)	
			Xm	Xp
Embryo	804	113 (14·1)	365 (45·4)	326 (40·5)
Yolk sac mesoderm	442	82 (18·6)	188 (42·5)	172 (38·9)
Yolk sac endoderm	508	167 (32·9)	50 (9·8)	291 (57·3)

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Table 3. *Differential staining of metaphase spreads isolated from XmXmY triploid embryos*

Tissue sample analysed	No. of metaphases scored	No. of metaphases with no darkly staining X-chromosome (%)	No. of metaphases with a single darkly staining X-chromosome (%)
Embryo	585	100 (17·1)	485 (82·9)
Yolk sac mesoderm	415	111 (26·7)	304 (73·3)
Yolk sac endoderm	369	204 (55·3)	165 (44·7)

(38·9%) a single inactive Xp chromosome, while 82 (18·6%) had no darkly staining X-chromosome present. In the endodermally-derived tissue, out of 508 metaphase spreads analysed, 50 (9·8%) showed evidence of an inactive Xm chromosome, 291 (57·3%) showed evidence of an inactive Xp chromosome, while 167 (32·9%) had no darkly staining X-chromosome present. In the latter group, the absence of a darkly staining X-chromosome could either mean that both X-chromosomes present were active, or that the Kanda technique had failed to differentially stain the inactive X-chromosome. In addition, the efficiency of the Kanda staining technique appeared to vary between the different tissues analysed, being least effective in the case of the endodermally-derived tissues. The reason for this is unclear.

(b) *XmXmY digynic triploid embryos*

Each of the three tissues analysed from XmXmY triploid embryos contained two distinct cell lineages. Individual mitoses from one cell lineage possessed a single darkly staining X-chromosome, that is, they contained one inactive X-chromosome, whereas the other contained no darkly staining X-chromosome, which could mean either that both X-chromosomes were active, or that the Kanda technique had failed to differentially stain the inactive X-chromosome.

Analysis of yolk sac endodermally-derived samples revealed that out of 369 metaphases analysed, 165 (44·7%) possessed a single darkly staining X-chromosome (see Fig. 1 *a*), while the remainder displayed no such element (see Table 3). Similarly, in yolk sac mesodermally-derived and embryonic tissue samples, a single darkly staining X-chromosome was observed in 304 (73·3%) out of 415, and 485 (82·9%) out of 585 metaphases analysed, respectively. Thus, the source of the tissue appeared to affect the relative proportions of the two cell lineages present, namely those that had either an (Xm)XmY or XmXmY sex chromosome constitution. It was evident that a higher proportion of metaphase plates with no darkly staining X-chromosome was observed in yolk sac endodermally-derived samples than in either embryonic samples or yolk sac mesodermally-derived samples.

(c) *XmXmXp digynic triploid embryos*

In the XmXmXp triploid embryos, all of the three classes of tissues analysed comprised three distinct cell lineages, namely those with two darkly staining X-chromosomes, those with one darkly staining X-chromosome, and a third group in which there was no darkly staining X-chromosome present. In the latter group, either all three X-chromosomes were active, or the Kanda technique had failed to differentially stain any inactive X-chromosomes present. Consequently, there were four possible X-chromosome inactivation patterns observed in this group, in addition to the preparations in which no evidence of X-inactivation was apparent:

1. Two inactive X-chromosomes present, one of which is maternally-derived and the other paternally-derived (see Fig. 1 *b*).

2. Two inactive X-chromosomes present, both of which are maternally-derived (see Fig. 1 *c*).

3. A single inactive X-chromosome of maternal origin present (this observation is uncommon, out of 213 metaphases with a single inactive X-chromosome present, only 34 (16·0%) were of this type).

4. A single inactive X-chromosome of paternal origin present (see Fig. 1 *d*).

The cells with a single inactive X-chromosome were observed more frequently in yolk sac endodermally-derived samples than in the embryonic samples and in the yolk sac mesodermally-derived samples. Thus, out of 168 metaphase plates scored which possessed a single inactive X-chromosome in yolk sac endodermally-derived samples, 3 (1·8%) had a single inactive Xm, whereas 165 (98·2%) had a single inactive Xp, compared with the situation observed in embryonic tissue where 25 (83·3%) out of a total of 30 metaphase plates scored had a single inactive Xm, and 5 (16·6%) had a single inactive Xp, and in the yolk sac mesodermally-derived samples, where 6 (40·0%) out of a total of 15 metaphase plates scored had a single inactive Xm, and 9 (60·0%) had a single inactive Xp present (see Table 4). There was also a clear pattern of non-random X-inactivation in yolk sac endodermally-derived cells which possessed two darkly staining X-chromosomes. Thus, 162 (94·7%) out of 171 of the cells which possessed two darkly staining X-chromosomes had an Xm(XmXp) inactivation pattern. Had random X-inactivation occurred in the cells which possessed one inactive X-chromosome, on theoretical grounds one would have expected to encounter an (Xm)XmXp pattern twice as often as an XmXm(Xp) pattern of X-inactivation. In fact, the ratio of

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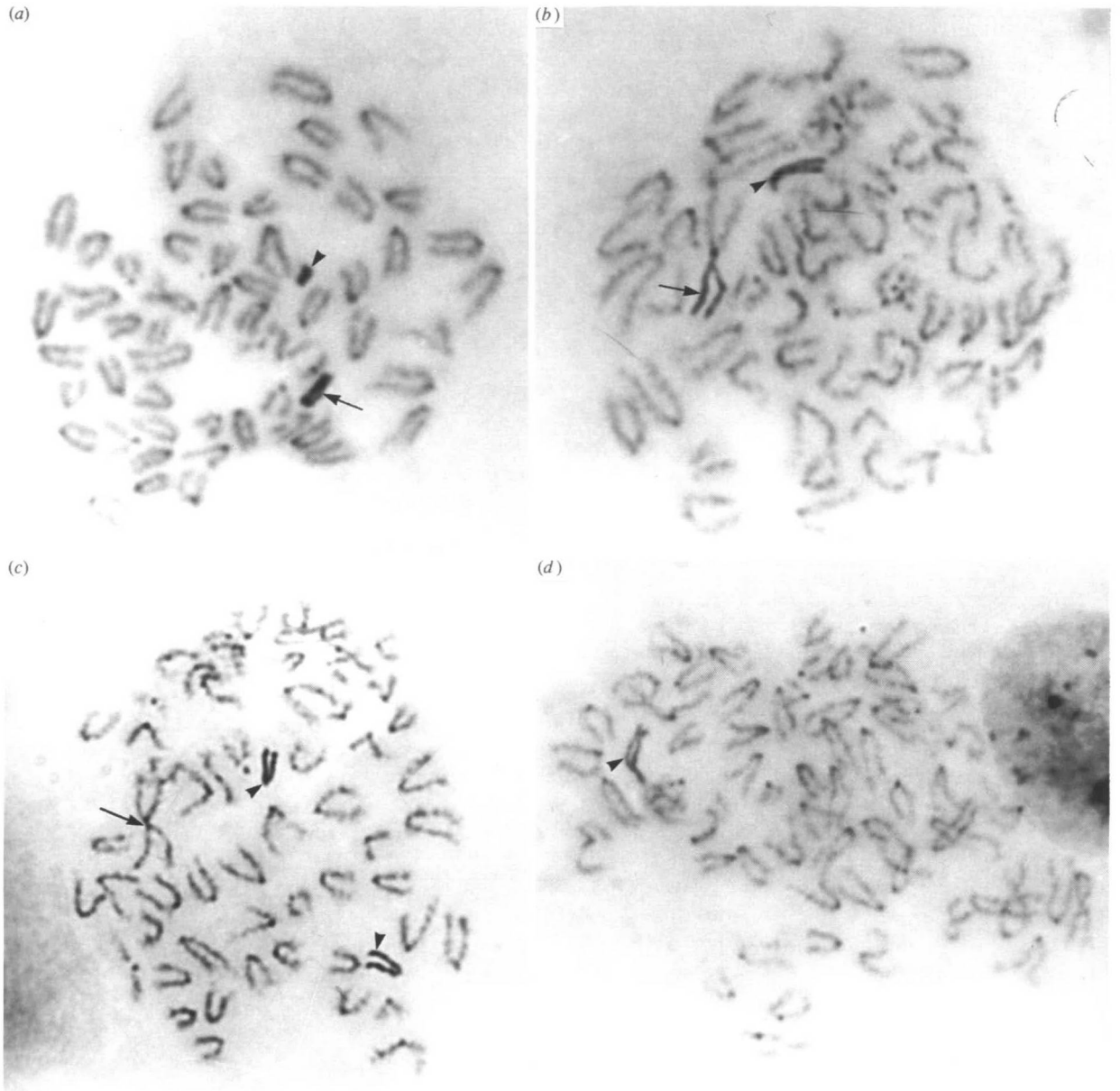

Fig 1 *a–d* are representative metaphase spreads from digynic triploid embryonic and extraembryonic tissues stained by the Kanda technique to investigate the pattern of X-chromosome inactivation in these tissues.
(*a*) Metaphase spread from an XmXmY embryo in which a single darkly-staining X-chromosome is present (large arrow). The Y chromosome is also darkly-staining (arrow head). This spread therefore has an (Xm)XmY inactivation pattern.
(*b*) Metaphase spread from an XmXmXp embryo in which two inactive X-chromosomes are present, one of which is one of the maternally-derived X-chromosomes (arrow head), and the other is the paternally-derived marker chromosome (large arrow). This spread therefore has an Xm(XmXp) inactivation pattern.
(*c*) Metaphase spread from an XmXmXp embryo in which two inactive X-chromosomes are present (arrow heads), both of which are the maternally-derived X-chromosomes. This spread therefore has an (XmXm)Xp inactivation pattern. Note that the paternally-derived marker chromosome (large arrow) is not darkly-staining.
(*d*) Metaphase spread from an XmXmXp embryo in which only a single inactive X-chromosome is present (arrow head). This is the paternally-derived marker chromosome, one half of which is darkly-staining. This spread therefore has an XmXm(Xp) inactivation pattern.

(Xm)XmXp:XmXm(Xp) in the single darkly staining X-chromosome cell lineage in the yolk sac endodermally-derived samples, was 1:55. This finding is significantly different from the predicted value, and that observed in the embryonic and yolk sac mesodermally-derived samples, of 5:1 and 0·7:1, respectively, and clearly demonstrates that random X-inactivation was not occurring in this sample. Similarly, had random X-inactivation occurred in yolk sac endodermally-derived cells which possessed two inactive X-chromosomes, on theoretical grounds one would have expected to encounter an (XmXm)Xp pattern of X-inactivation as often as an Xm(XmXp) X-inactivation pattern (i.e. in a ratio of 1:1). The ratio

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Table 4. *Differential staining pattern of metaphase spreads isolated from XmXmXp triploid embryos*

Tissue sample analysed	No. of metaphases scored	No. of metaphases with no darkly staining X-chromosome (%)	No of metaphases with a single darkly staining X-chromosome (%)		No. of metaphases with 2 darkly staining X-chromosomes (%)	
			Xm	Xp	XmXm	XmXp
Embryo	737	31 (4·2)	25 (3·4)	5 (0·6)	307 (41·7)	369 (50·1)
Yolk sac mesoderm	349	36 (10·3)	6 (1·7)	9 (2·6)	147 (42·1)	151 (43·3)
Yolk sac endoderm	445	106 (23·8)	3 (0·7)	165 (37·1)	9 (2·0)	162 (36·4)

Table 5. *Developmental stage and sex chromosome constitution of digynic triploid embryos isolated on the 10th day of gestation*

Embryonic stage	Sex chromosome constitution	
	XXX	XXY
(i) amorphous embryonic mass	2	1
(ii) primitive streak	2	1
(iii) 'unturned'	17	15
(iv) partially 'turned'	4	3
(v) 'turned'	6	6
Total	31	26

observed was in fact 1:18, a finding that was clearly significantly different from the expected value of unity.

The ratio of (XmXm)Xp:Xm(XmXp) in both the embryonic tissue samples and the yolk sac mesodermally-derived samples was 1:1·20, and 1:1·03, respectively, being clear evidence that random X-inactivation had occurred in these tissues.

(d) *Relationship between the sex chromosome constitution of the digynic triploids, and their embryonic development at the time of their isolation*

Since a note was always made of the developmental features of the triploid embryos at the time of their recovery, it was possible, to establish whether there was any obvious relationship between the sex chromosome constitution of these embryos and their developmental morphology. To facilitate this analysis, each embryo was allocated to one of five arbitrarily chosen developmental stages (see Table 5). From the results obtained, there appeared to be no very obvious correlation between the sex chromosome constitution of these triploid embryos and the developmental stage achieved at the time of their isolation. Because of the small number of embryos assigned to each of these groups, it was not possible to establish whether any relationship existed between the X-inactivation status of these embryos and their developmental morphology.

4. Discussion

The present study has confirmed that the Kanda technique (Kanda, 1973) is capable of differentially staining the inactive X-chromosome in embryonic and extra-embryonic tissues from LT/Sv strain digynic triploid conceptuses. However, we observed that the proportion of cells that displayed one or more differentially staining X-chromosomes varied depending upon the tissue analysed. Furthermore, the technique consistently yielded lower scores when yolk sac endodermally-derived tissue samples were analysed. We believe that no similar tissue-specific bias in the efficiency of the technique has previously been reported.

This negative finding is important, and should be taken into account when considering the possibility of the existence of a distinct cell lineage which has only active X-chromosome(s) present. Thus, Endo *et al.* (1982), using the Brdu technique to identify the allocyclic X-chromosome, postulated the existence of such a cell lineage in their analysis of the visceral yolk sac of LT-derived XXY digynic triploid embryos. A similar observation was noted in both our XmXp and XmXmXp series. Without additional information, we are unable to distinguish between the two possibilities, namely that all of the X-chromosomes present are active, or that the Kanda technique failed to differentially stain the inactive X-chromosome(s) present in these metaphase preparations. The proportion of

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metaphase spreads with no darkly staining X-chromosome present in our study was in fact substantially greater in the XXY digynic triploids, than in the XX diploid controls. This finding suggests that in the XXY triploids in which no darkly staining X-chromosome was observed, two populations of cells could have been present, namely cells which failed to display differential staining for technical reasons, and cells in which there were only active X-chromosomes present. However, for the reasons indicated above, we are at present unable to distinguish between these two possibilities.

The situation observed in relation to the triploid embryos with an XXX sex-chromosome constitution was particularly interesting in that the XXX triploid embryos possessed two principal cell populations, namely cells with a single inactive X-chromosome, and cells with two inactive X-chromosomes as well as a relatively small population of cells which showed no darkly staining X-chromosome. The cells with a single inactive X-chromosome (generally the Xp) were almost exclusively observed in the yolk sac endodermally-derived samples, while the cells with two inactive X-chromosomes predominated in both the yolk sac mesodermally-derived and embryonic samples.

A similar disparity in the pattern of X-inactivation was also apparent between the embryonic and yolk sac mesoderm-derived samples, and the yolk sac endoderm-derived tissue samples in relation to the parental origin of the inactive X-chromosome(s). In the former two groups, it was observed that the inactivated X-chromosome was just as likely to have been of maternal origin as paternal in origin. However, in the yolk sac endoderm-derived cells, the paternally-derived X-chromosome was almost exclusively found to be inactivated. Thus, when only one inactivated X-chromosome was present, this was almost invariably found to be paternal in origin. When two inactivated X-chromosomes were present, the Xp chromosome was almost invariably inactivated (in association with one or other of the Xm chromosomes), while exclusively maternal X-inactivation was only rarely encountered. These results demonstrate four points:

(i) that in virtually all of the embryonic and yolk sac mesoderm-derived tissues studied, all but one of their X-chromosomes were inactivated, and that the selection was apparently random in relation to their parental origin.

(ii) that preferential paternal X-inactivation occurred in the yolk sac endoderm-derived cells of digynic triploid XXX conceptuses, as it does in diploid controls.

(iii) that in the absence of a paternally-derived X-chromosome, in the case of the XXY conceptuses, maternal X-inactivation occurred in the yolk sac endoderm-derived cells at a frequency higher than that observed in diploid XX embryos.

(IV) finally, in the presence of preferential paternal X-inactivation, an additional maternally-derived inactivated X-chromosome was relatively frequently encountered in yolk sac endoderm-derived tissue isolated from XmXmXp individuals. However, if only a single inactivated X-chromosome was present, this was almost exclusively the paternally-derived X-chromosome.

The fact that a significant number of cells demonstrated a maternally-derived inactivated X-chromosome in these triploid tissues, suggests that although paternal X-inactivation predominates, it is not exclusive. A similar situation was also evident in diploid parthenogenones where no paternally-derived X-chromosomes are present (Rastan *et al.* 1980). In addition, Jacobs *et al.* (1979), in their analysis of the late replicating pattern of X-chromosomes in human triploids, noted that there was no correlation with the parental origin of the triploidy.

During the present study, a spectrum of developmental retardation was observed between XXX and XXY conceptuses ranging from those that were present as amorphous embryonic masses, or were arrested at the early primitive streak stage, to the developmentally most advanced embryos recovered, that were 'turned', albeit small, forelimb bud stage embryos. In addition, no obvious relationship was observed between the sex chromosome constitution of these embryos and their developmental stage, or morphological appearance, at the time of their isolation.

Similar findings have been reported by Speirs & Kaufman (1989), who observed, in a substantially larger population of LT/Sv strain embryos than analysed here, no correlation between developmental stage and sex chromosome constitution for XXX and XXY digynic triploid embryos. The reduced size of digynic triploids compared with normal diploids may result from an inability of their extra-embryonic membranes to support the growing embryo/foetus. Clearly an autosomal:X-chromosomal imbalance invariably occurs in all of the embryonic and extra-embryonic tissues of these conceptuses, and presumably contributes to their nutritional deficiency and subsequent inviability. The possibility also exists, that the failure to inactivate the supernumerary X-chromosomes in a proportion of their tissues may additionally contribute to their progressive inviability. The fact that diandric triploid embryos, though smaller than diploid controls at the same developmental stage, are generally morphologically normal (Kaufman *et al.* 1989) is of undoubted interest, and clearly demonstrates that the abnormalities generally seen in the digynic triploids is not a direct consequence of triploidy *per se*.

Since the trophoblast and yolk sac endoderm-derived tissues, which are both located at the embryonic/maternal interface, show selective paternal X-inactivation, it is possible that this phenomenon may serve a primarily protective function. Thus the

embryo, which is almost invariably genetically dissimilar from the maternal host, would always tend to be surrounded by a protective shield of tissue in which, under normal conditions (i.e. in the case of diploid XX or XY embryos), only maternally-derived X-chromosome gene products were produced. This 'protective' mechanism therefore facilitate the continuation of pregnancy into the post-implantation period, despite the presence of the embryo in an immunologically hostile environment. By contrast, in the case of both digynic and diandric triploids, the X:autosome relationship is invariably unbalanced in both the embryonic and extra-embryonic tissues. It would be surprising, therefore, if this did not have a detrimental effect on the development of these conceptuses. Why the digynic but not the diandric triploid embryos should develop neural tube and cardiac abnormalities is, however, far from clear at the present time. Further studies are clearly necessary to investigate this intriguing topic.

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