Xce genotype has no impact on the effect of imprinting on *X*-chromosome expression in the mouse yolk sac endoderm

THEODOR BÜCHER[1], INGRID M. LINKE[1], MANFRED DÜNNWALD[1], JOHN D. WEST[2]* AND BRUCE M. CATTANACH[2]

[1] *Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie, Universität München, 8000 München, Goethestr. 33, Federal Republic of Germany* [2] *MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, England*

Summary

The effect of the *Xce* (*X*-chromosome controlling element) genotype on the randomness of *X*-chromosome inactivation in the mouse was studied by monitoring the expression of the *X*-linked locus *Pgk-1*. The main aim was to test whether the *Xce* genotype modified the preferential expression of the maternally derived *X*-chromosome in the yolk sac endoderm. Quantitative electrophoresis of phosphoglycerate kinase (PGK-1) was used to study *Pgk-1* expression in the foetus, yolk sac mesoderm and yolk sac endoderm at $13\frac{1}{2}$ days *post coitum*. The Xce^a/Xce^c genotype caused non-random *X*-chromosome expression in the foetus and yolk sac mesoderm. However, there was no evidence that the *Xce* genotype moderates the preferential expression of the maternally derived *X*-chromosome in the yolk sac endoderm, as reported by Rastan & Cattanach (1983).

1. Introduction

The randomness of *X*-chromosome inactivation in mice is modified by both parental source and genetic factors. A number of studies (reviewed by Cattanach, 1975) have revealed an *X*-linked locus, designated *X*-chromosome controlling element (*Xce*), that influences the choice of *X*-chromosome to be inactivated. Three alleles of *Xce* are known which behave in an apparently *cis*-acting, competitive manner such that *X*-chromosome expression is random in homozygotes but non-random in mice heterozygous for *Xce*. Non-random expression of the *X*-chromosome-linked gene, phosphoglycerate kinase-1 (*Pgk-1*) was seen in mice heterozygous for both *Pgk-1* and *Xce* as early as $7\frac{1}{2}$ days *post coitum* (*p.c.*) by Johnston & Cattanach (1981), which suggests that non-random *X*-chromosome expression is a result of primary non-random *X*-chromosome inactivation. Similarly, Rastan (1982) found cytogenetic evidence for primary non-random *X*-inactivation in *Xce* heterozygotes at $6\frac{1}{2}$ days *p.c.* The three known alleles of *Xce* confer an increasing probability of *X*-chromosome expression in the order $Xce^a < Xce^b < Xce^c$ (Johnston & Cattanach, 1981).

The parental source of the *X* chromosome has two effects on the randomness of *X*-chromosome expression. The first results in a slightly increased probability that the paternally-derived *X*-chromosome (X^p) will be expressed. This has been reported for various *X*-linked genes that affect the coat (e.g. Cattanach & Perez, 1970; Falconer, Isaacson & Gauld, 1982) but the effect is small and requires large numbers of animals to be convincingly demonstrated. The second effect is more easily demonstrated but is restricted to certain extraembryonic tissues. Both cytogenetic and biochemical studies (Takagi & Sasaki, 1975; Takagi, 1978; West *et al.* 1977, 1978; Frels & Chapman, 1980; Frels, Rossant & Chapman, 1979; McMahon, Fosten & Monk, 1981; Papaioannou & West, 1981) have shown that the maternally derived *X*-chromosome (X^m) is preferentially expressed in the derivatives of the trophectoderm (trophoblast and chorionic ectoderm) and primitive endoderm (visceral yolk sac endoderm and parietal endoderm). Expression of the *X*-chromosomes in the primitive ectoderm derivatives (foetus, amnion, visceral yolk sac mesoderm, allantois and chorionic mesoderm), on the other hand, is more nearly random. Observations on early embryos (Takagi, Wake & Sasaki, 1978; Papaioannou *et al.* 1981; Harper, Fosten & Monk, 1982) suggest that the preferential expression of X^m is a result of preferential inactivation of the paternally derived *X* chromosome (X^p) early in development, rather than the result of a secondary phenomenon such as cell selection.

* Present address and address for reprint requests: Department of Obstetrics and Gynaecology, University of Edinburgh, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, Scotland.

Both of these parental source effects on X-chromosome expression may be manifestations of a more general parental source effect that involves all or part of the genome. Elegant experiments, involving the transfer of pronuclei between mouse eggs have shown that male and female pronuclei are not equivalent (Surani, Barton & Norris, 1984; McGrath & Solter, 1984; Barton, Surani & Norris, 1984). A differential activity of certain chromosome regions of maternal and paternal origin has also recently been found (Cattanach & Kirk, 1985).

The term 'chromosome imprinting' was coined by Crouse (1960) and is used to describe molecular events that occur differently in male and female germ lines and precondition behavioural differences of homologous chromosomes. The molecular events are not yet understood but they need not be restricted to one germ line. In principle, the imprinting differential could arise as a consequence of either qualitative or quantitative molecular differences between male and female germ lines that occur at any time before or during fertilization. The term 'chromosome imprinting' was adopted by Brown and Chandra (Brown & Chandra, 1973; Chandra & Brown, 1975) to describe the preferential expression of X^m seen in certain marsupials, and has also been used to describe the same phenomenon seen in certain extraembryonic tissues of the mouse. The molecular events involved in chromosome imprinting may be responsible for various parental source effects seen in the mouse. In this paper, the term 'chromosome imprinting' is used with reference to the preferential expression of X^m in certain extraembryonic tissues.

It is possible that the non-random X-chromosome expression caused by genetic variation and chromosome imprinting may both be mediated by modifications of the Xce locus. Such modifications may be, respectively, either genetically inherited as allelic variants or caused by the molecular events that create the imprinting differential between male and female germ lines.

Cytogenetic experiments reported by Rastan & Cattanach (1983) indicated that the Xce genotype could in part counterbalance the effect of imprinting on the randomness of X-chromosome inactivation in the yolk sac endoderm. In a cytological study using the long Cattanach's insertion chromosome, Is(In7:X)1Ct, as a marker, these authors found that X^p was dark-staining (inactive) in approximately 90% of yolk sac endoderm cells from Xce^a/Xce^a homozygotes and Xce^c/Xce^a heterozygotes (where Xce^c is of maternal origin). However, this was reduced to 78% in Xce^a/Xce^c yolk sac endoderm cells (where Xce^c is on X^p). Although this observation does not show that the effect of imprinting on X-chromosome expression is mediated via the Xce locus, it does suggest that it can be moderated by genetic variation at Xce. However, no indication of this was obtained from a small scale genetic study involving the analysis of PGK-1

allozymes in the yolk sac endoderm and parietal endoderm from four Pgk-$1^bXce^a/Pgk$-1^aXce^c female conceptuses (West *et al.* 1984). The experiment reported here reinvestigates the purported action of the Xce locus in the yolk sac endoderm using Pgk-1 as a marker for X-chromosome expression. We used quantitative electrophoresis to analyse the phosphoglycerate kinase allozymes present in the foetus, yolk sac mesoderm and yolk sac endoderm. Conceptuses were analysed at $13\frac{1}{2}$ days *post coitum* in order to be comparable with the cytological experiment reported by Rastan & Cattanach (1983).

2. Materials and Methods

Three stocks of mice were used that were homozygous (or in the case of males, hemizygous) at the X-chromosome linked loci Pgk-1 (phosphoglycerate kinase-1) and Xce (X chromosome controlling element). These stocks were respectively the outbred Pgk-1^aXce^c stock, the outbred Pgk-1^bXce^c stock, derived by recombination (Cattanach, Bücher & Andrews, 1982, 1983) and C3H/HeH ♀ × 101/H ♂ F_1 hybrids (Pgk-1^bXce^a). Reciprocal crosses were set up to produce (1) Pgk-$1^bXce^a/Pgk$-1^aXce^c, (2) Pgk-$1^aXce^c/Pgk$-1^bXce^a, (3) Pgk-$1^bXce^c/Pgk$-1^aXce^c and (4) Pgk-$1^aXce^c/Pgk$-1^bXce^c female conceptuses. Each pregnant female was coded so that the genotypes of the conceptuses were unknown at the time of dissection and during the subsequent electrophoretic analysis.

Conceptuses were dissected at $13\frac{1}{2}$ days *p.c.* in the Hepes-buffered medium, M2 (Quinn, Barros & Whittingham, 1982). The yolk sacs were incubated in a mixture of 0·5% trypsin and 2·5% pancreatin (made up in Dulbecco's phosphate buffered saline) for approximately $2\frac{1}{2}$ hrs at 4 °C (Levak-Švajger, Švajger & Škreb, 1969). They were then transferred to M2 medium at 4 °C for at least 30 min before the endoderm was dissected from the yolk sac mesoderm, using watchmakers forceps under a dissecting microscope. Each foetus, yolk sac mesoderm and yolk sac endoderm sample was sonicated in a small volume (200 μl for the foetus and 100 μl for the others) of sample buffer (Bücher *et al.* 1980) using an MSE Soniprep 150. Sonicated samples were centrifuged for 2 mins at 5000 rev/min in a Beckman Microfuge 12 centrifuge and the supernatants were stored in 250 μl plastic tubes at -20 °C for up to 3 weeks before they were sent from Harwell to Munich, on dry ice, by air.

Cellogel electrophoresis, staining and quantitation of PGK-1 allozymes was done as described by Bücher *et al.* (1980) and Rabes *et al.* (1982). The heterozygous female conceptuses were distinguished from their hemizygous male siblings by the two-banded PGK-1AB phenotype produced by the foetus samples. The proportions of cells with an active X^m or an active X^p, in Pgk-$1^a/Pgk$-1^b heterozygous female conceptuses, were estimated by quantitative electrophoresis of the two allozymes of phosphoglycerate kinase-1

(PGK-1). The PGK-1A and PGK-1B allozymes were stained and visualized as fluorescent bands under long wavelength ultraviolet illumination. The bands were scanned with a scanning fluorimeter fitted with a chart recorder and the areas under the peaks were integrated to estimate the relative proportions of the PGK-1A and PGK-1B allozymes.

Statistical tests were done on a Hewlett Packard programmable calculator, programmed by Mr D. G. Papworth of the MRC Radiobiology Unit to compute χ^2, Student's *t*-test and the correlation coefficient, *r*.

3. Results

The two-banded, PGK-1AB phenotype was seen in 76/164 foetuses which indicates that 46% were females. The results from the four crosses analysed are shown in Table 1.

An effect of the *Xce* genotype on *Pgk-1* expression in the foetus and yolk sac mesoderm samples was clearly seen (Table 1) and was similar to that previously demonstrated for foetal (Cattanach & Johnston, 1981) and adult tissues (Cattanach, Bücher & Andrews, 1982, 1983). The percentage PGK-1B is close to 50% in crosses 3 and 4 where the conceptuses are Xce^c/Xce^c but this is reduced to approximately 35% in crosses 1 and 2 where the $Pgk-1^b$ allele is in coupling with Xce^a in Xce^a/Xce^c or Xce^c/Xce^a heterozygotes. Table 2 confirms that the *Xce* genotype has a statistically significant effect on *Pgk-1* expression in both the foetus and the yolk sac mesoderm.

In contrast to the above and at variance with the cytological data of Rastan & Cattanach (1983), no evidence was seen of an effect of *Xce* on non-random *X*-chromosomes expression in the yolk sac endoderm. Only one PGK-1 allozyme was produced by all of the 76 yolk sac endoderm samples and in each case the maternally derived *Pgk-1* allele was exclusively expressed. Significantly, in cross 1 where the conceptuses were $Pgk-1^bXce^a/Pgk-1^aXce^c$ none of the 14 yolk sac endoderm samples produced any PGK-1A allozyme. Thus in this case the paternally derived Xce^c allele in an Xce^a/Xce^c conceptus failed to moderate the preferential expression of the maternally derived $Pgk-1^b$ allele.

Comparison of the results for reciprocal crosses 1 and 2 (Table 1) revealed a small difference in the precentage of PGK-1B produced by the foetus. This was consistent with the parental source effect, favouring expression of the paternally derived *X*-chromosome, reported for various *X*-linked genes that affect the coat (Cattanach & Perez, 1970; Falconer, Isaacson & Gauld, 1982) but the effect was small and not statistically significant (Table 2). For the yolk sac mesoderm, the trend was in the opposite direction, towards preferential expression of the maternally derived *Pgk-1* allele. Moreover, comparison of results for reciprocal crosses 3 and 4 (Tables 1 and 2) showed a statistically significant opposite trend for both the foetus and yolk sac mesoderm samples. Overall the results provide no clear evidence of a consistent parental source effect.

The percentage of PGK-1B in the yolk sac mesoderm was not significantly different from that in the foetus in three of the four crosses and was significantly positively correlated in all four crosses (Table 3). The difference in means (Table 1) was only a few percent (the maximum difference is 3·92% in cross 2) but in each case the yolk sac mesoderm mean fell between the means for the foetus and yolk sac endoderm samples. This could reflect a minor contamination of the yolk sac mesoderm with yolk sac endoderm. This is also suggested by the high proportion (48/76) of individual conceptuses that had a percentage of PGK-1B in the yolk sac mesoderm that was between the percentages found in the foetus and yolk sac endoderm. This proportion of conceptuses (48/76 = 63%) is significantly higher ($\chi^2 = 4·75$; $P < 0·05$) than the 50% expected if the yolk sac mesoderm is never contaminated.

4. Discussion

The failure of Xce^c to moderate the preferential expression of the maternally derived $Pgk-1^b$ allele in any of the 14 $Pgk-1^bXce^a/Pgk-1^aXce^c$ yolk sac endoderms is in keeping with the results obtained for four $12\frac{1}{2}$ day conceptuses of this genotype (West *et al.* 1984). This result, however, stands in sharp contrast to the results of the cytogenetic analysis of

Table 1. *Percentage PGK-1B in conceptuses from different crosses at* $13\frac{1}{2}$ *days* post coitum

| | | | Mean % PGK-1B ± s.e. | | |
Cross	Genotype of female conceptuses $(X^m/X^p)^a$	No. of heterozygous female conceptuses[b]	Foetus	Yolk sac mesoderm	Yolk sac endoderm
1	$Pgk-1^bXce^a/Pgk-1^aXce^c$	14	34·64 ± 2·74	35·76 ± 2·70	100 ± 0
2	$Pgk-1^aXce^c/Pgk-1^bXce^a$	28	38·62 ± 1·41	34·70 ± 1·18	0 ± 0
3	$Pgk-1^bXce^c/Pgk-1^aXce^c$	18	51·12 ± 1·92	51·21 ± 1·90	100 ± 0
4	$Pgk-1^aXce^c/Pgk-1^bXce^c$	16	44·78 ± 1·83	43·15 ± 2·52	0 ± 0

[a] X^m = maternally derived *X*-chromosome; X^p = paternally derived *X*-chromosome. [b] The sex ratios for each group were: (1) 14 ♀:24 ♂; (2) 28 ♀:20 ♂; (3) 18 ♀:17 ♂; (4) 16 ♀:27 ♂.

Table 2. *Statistical comparison of % PGK-1B in conceptuses from different crosses*

Comparison	Significance			
	Foetus		Yolk sac mesoderm	
Different *Xce* genotypes				
Crosses 1 and 3	$t = 5.071$	$P < 0.001$	$t = 4.812$	$P < 0.001$
Crosses 2 and 4	$t = 2.654$	$0.01 < P < 0.02$	$t = 3.445$	$0.001 < P < 0.002$
Reciprocal crosses				
Crosses 1 and 2	$t = 1.433$	NS[a]	$t = 0.417$	NS
Crosses 3 and 4	$t = 2.376$	$0.02 < P < 0.05$	$t = 2.587$	$0.01 < P < 0.02$

[a] NS, Not significant.

Table 3. *Statistical comparison of % PGK-1B in foetuses and yolk sac mesoderms*

Cross	*t*-test		Correlation coefficient (*r*)	
1	$t = 0.289$	NS[a]	$r = +0.802$	$P < 0.001$
2	$t = 2.130$	$0.02 < P < 0.05$	$r = +0.517$	$P < 0.01$
3	$t = 0.033$	NS	$r = +0.735$	$P < 0.001$
4	$t = 0.524$	NS	$r = +0.663$	$P < 0.01$

[a] NS, not significant.

X-chromosome activity in the yolk sac endoderm and mesoderm of $13\frac{1}{2}$ day conceptuses, reported by Rastan & Cattanach 1983).

Rastan & Cattanach (1983) used a modified Kanda technique to stain the allocyclic or heterochromatic (inactive) *X*-chromosome and they distinguished X^m from X^p using the long marker *X*-chromosome produced by Cattanach's insertion, Is(In7;X)1Ct. This technique has given equivalent results to biochemical methods in many other genetical studies of *X*-chromosome expression so it is not clear why in this case there is disagreement. We estimate that our electrophoresis technique should allow us to detect a minor PGK-1 allozyme that represents 1% of the total PGK-1 activity (Bücher, unpublished). So, our analysis is clearly sensitive enough to detect an effect of *Xce* as large as that reported by Rastan & Cattanach (1983).

Rastan & Cattanach (1983) found that the percentage of yolk sac endoderm cells with X^p active was significantly higher for X^m Xce^a/X^p Xce^c conceptuses (22·0%) than for either the reciprocal, X^m Xce^c/X^p Xce^a heterozygotes (7·3%) or either Xce/Xce homozygotes (7·2 and 10·2%). However, it is not clear whether the 7–10% of cells with an active X^p in the control crosses represents the pattern of *X*-chromosome expression in a minor population of yolk sac endoderm cells or whether the frequency is above zero for technical reasons.

In earlier biochemical studies, using a less sensitive assay for PGK-1, a few yolk sac endoderm samples produced a minor PGK-1 allozyme that represented expression of the *Pgk-1* allele on X^p but it is not known whether this enzyme activity was derived from the yolk sac endoderm or from contamination (e.g. West *et al.* 1977; Papaioannou & West, 1981). Our present results, however, are clear-cut. No minor PGK-1 allozyme, representing X^p expression, was seen in any of the 76 yolk sac endoderm samples analysed at $13\frac{1}{2}$ days *p.c.* (Table 1). Exclusive expression of the maternally derived *Pgk-1* allele was also found at $12\frac{1}{2}$ days *p.c.* in all 18 yolk sac endoderm samples analysed by McMahon, Fosten & Monk (1981, 1983) and West *et al.* (1984).

An apparent effect of *Xce* on the *X*-chromosome expression in the yolk sac endoderm could be generated if yolk sac endoderm samples were contaminated with yolk sac mesoderm cells (or enzyme). Using the figures shown in Table 1, a 10% contamination would contribute 4·9% of cells with an active X^p in cross 3 and 6·4% in cross 1. The same amount of cellular contamination could have more impact on the cytogenetic analysis if, for example, the mitotic activity was relatively high in the yolk sac mesoderm or the PGK-1 activity was relatively low. When the yolk sac endoderm is dissected from the yolk sac mesoderm it is possible that some remains attached to the mesoderm. (This may explain the very small differences in the percentage of PGK-1B between the yolk sac mesoderm and foetus samples that was discussed in the Results section.) However, the most likely cellular contamination of the yolk sac endoderm is blood from the yolk sac mesoderm and this is unlikely to be mitotically active at $13\frac{1}{2}$ days *p.c.*

Another possible explanation for the discrepancy between the present genetic results and those of the cytogenetic study reported by Rastan & Cattanach (1983) is that the expression of *Pgk-1* does not reflect the pattern of active and inactive X-chromosomes.

Krietsch *et al.* (1982) reported that the *Pgk-1* alleles on X^m and X^p are probably activated asynchronously. These results suggest that in the embryo the maternally derived *Pgk-1* allele is activated at $3\frac{1}{2}$ days *p.c.* but the paternally derived *Pgk-1* allele is not expressed before $5\frac{1}{2}$ days *p.c.* (See also Papaioannou *et al.* 1981.) Other cytogenetic and genetic experiments (reviewed in Table II of West, 1982) indicate that both X^m and X^p are active in early embryos before $5\frac{1}{2}$ days *p.c.* There is, therefore, a period when both X-chromosomes are active but only one *Pgk-1* allele (on X^m) is expressed. A similar situation could explain our failure to detect expression of the paternally derived *Pgk-1* allele in the yolk sac endoderm. In other words, some cells in *Pgk-1^{b}Xce^a/Pgk-1^{a}Xce^c* yolk sac endoderms may have an active X^p but the *Pgk-1^a* allele on that chromosome may still be transcriptionally inactive. However the cytogenetic experiments (Rastan & Cattanach, 1983) predict that such cells would have an inactive X^m so we would need to postulate that they failed to express either of the *Pgk-1* alleles (or that they only expressed the *Pgk-1* allele on the *inactive* X^m). It is known that the paternally-derived *Pgk-1* allele is expressed in the yolk sac endoderm in X^pO mice (Papaioannou & West, 1981). It, therefore, seems unlikely that it would remain silent if X^p is active in a proportion of yolk sac endoderm cells in certain XX conceptuses. Nevertheless this possibility cannot be completely discounted until suitable genetic variants of other X-linked loci become available to reinvestigate the problem.

The cytogenetic analysis reported by Rastan and Cattanach (1983) revealed the expected effect of the *Xce* genotype on X-chromosome activity in the yolk sac mesoderm of X^m *Xce^c*/X^p *Xce^a* conceptuses (67·2% of cells had X^m, which carried *Xce^c*, active). However, there was no such effect in the reciprocal cross where only 47·3% of X^m *Xce^a*/X^p *Xce^c* yolk sac mesoderm cells had an active X^p (carrying *Xce^c*). In our present experiments the expected effects of the *Xce* genotype (Johnston & Cattanach, 1981; Cattanach, Bücher & Andrews, 1982, 1983) were seen in the foetus and yolk sac mesoderm samples from all four crosses (Tables 1 and 2).

Our results shed little light on whether there is a parental-source effect on the randomness of X-chromosome expression in the foetus or yolk sac mesoderm (Tables 1 and 2). Preferential expression of the maternally derived X-chromosome was suggested by reciprocal crosses 3 and 4 but not by crosses 1 and 2. This is the opposite to that reported previously for X-chromosome expression in the adult coat where the paternally derived X-chromosome tends to have a slightly higher probability of being expressed (Kindred,

1961; Cattanach & Perez, 1979; Falconer, Isaacson & Gauld, 1982). This situation is confused by several observations, other than crosses 3 and 4 reported here, that indicate that X^m rather than X^p may be preferentially expressed. For example, the results of Johnston & Cattanach (1981) indicate a trend towards preferential expression of X^m for the $7\frac{1}{2}$ day embryo, $13\frac{1}{2}$ day foetus and adult kidney but in the opposite direction for adult liver. Forrester & Ansell (1985) have also reported a tendency for the preferential expression of the maternally derived *Pgk-1* allele in samples of adult blood. Moreover, Kindred (1961) reported that a patroclinous effect on the expression of the X-linked gene tabby (*Ta*) was converted to a matroclinous effect by changing the genetic background. Clearly our present results do nothing to resolve this issue and larger scale studies will be needed.

The positive correlation between the % PGK-1B in the foetus and the yolk sac mesoderm for all four crosses is in keeping with earlier work (McMahon, Fosten & Monk, 1983; West *et al.* 1984). These reports discuss the implications of this correlation for the time of allocation of cells to the foetal and yolk sac mesoderm lineages relative to the time of X-chromosome inactivation.

In conclusion, our experiments confirm that the *Xce* genotype has an effect on the randomness of X-chromosome expression in the foetus and show a similar effect for the yolk sac mesoderm. However, we found no evidence to support the claim that the *Xce* genotype moderates the effect of imprinting on X-chromosome expression in the yolk sac endoderm. Contamination of the yolk sac mesoderm could mimic a weak moderating effect of *Xce* on X-chromosome expression in the yolk sac endoderm but this seems an unlikely explanation for the magnitude of the effect reported by Rastan & Cattanach (1983). Further experiments with genetic variants of other X-linked loci will probably be required to resolve the discrepancy between our results and the results of the earlier cytogenetic study of Rastan & Cattanach (1983).

References

Barton, S. C., Surani, M. A. H. & Norris, M. L. (1984). Role of paternal and maternal genomes in mouse development. *Nature* **211**, 374–376.

Brown, S. W. & Chandra, H. S. (1973). Inactivation system of the mammalian X-chromosome. *Proceedings of the National Academy of Sciences, USA* **70**, 195–199.

Bücher, Th., Bender, W., Fundele, R., Hofner, H. & Linke, I. (1980). Quantitative evaluation of electrophoretic allo- and isozyme patterns for developmental genetics. *FEBS Letters* **115**, 319–324.

Cattanach, B. M. (1975). Control of chromosome inactivation. *Annual Review of Genetics* 9, 1–18.

Cattanach, B. M., Bücher, Th. & Andrews, S. J. (1982). Location of *Xce* in the mouse *X*-chromosome and effects on PGK-1 expression. *Genetical Research* 40, 103–104.

Cattanach, B. M., Bücher, Th. & Andrews, S. J. (1983). PGK-1 expression in *Pgk-1*ᵃ/*Pgk-1*ᵇ feral mice associated with *Xce* allele substitutions. *Genetical Research* 41, 312–313.

Cattanach, B. M. & Kirk, M. (1985). Differential activity of maternally and paternally derived chromosome regions in mice. *Nature* 315, 496–498.

Cattanach, B. M. & Perez, J. N. (1970). Parental influence on *X*-autosome translocation-induced variegation in the mouse. *Genetical Research* 15, 43–53.

Chandra, H. S. & Brown, S. W. (1975). Chromosome imprinting and the mammalian *X*-chromosome. *Nature* 253, 165–168.

Crouse, H. V. (1960). The controlling element in sex chromosome behaviour in *Sciara*. *Genetics* 45, 1429–1443.

Falconer, D. S., Isaacson, J. H. & Gauld, I. K. (1982). Non-random *X* chromosome inactivation in the mouse: difference of reaction to imprinting. *Genetical Research* 39, 237–259.

Forrester, L. M. & Ansell, J. D. (1985). Parental influences on *X*-chromosome expression. *Genetical Research* 45, 95–100.

Frels, W. I. & Chapman, V. M. (1980). Expression of the maternally derived *X*-chromosome in the mural trophoblast of the mouse. *Journal of Embryology and Experimental Morphology* 56, 179–190.

Frels, W. I., Rossant, J. & Chapman, V. M. (1979). Maternal *X*-chromosome expression in mouse chorionic ectoderm. *Developmental Genetics* 1, 123–132.

Harper, M. I., Fosten, M. & Monk, M. (1982). Preferential paternal *X* inactivation in extraembryonic tissues of early mouse embryos. *Journal of Embryology and Experimental Morphology* 67, 127–135.

Johnston, P. G. & Cattanach, B. M. (1981). Controlling elements in the mouse. IV. Evidence of non-random *X*-inactivation. *Genetical Research* 37, 151–160.

Kindred, B. M. (1961). A maternal effect on vibrissa score due to the Tabby gene. *Australian Journal of Biological Sciences* 14, 627–636.

Krietsch, W. K. G., Fundele, R., Kuntz, G. W. K., Pehlau, M., Burki, K. & Illmensee, K. (1982). The expression of *X*-linked phosphoglycerate kinase in the early mouse embryo. *Differentiation* 23, 141–144.

Levak-Švajger, B., Švajger, A. & Škreb, N. (1969). Separation of germ layers in presomite rat embryos. *Experientia* 25, 1311–1312.

McGrath, J. & Solter, D. (1984). Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* 37, 179–183.

McMahon, A., Fosten, M. & Monk, M. (1981). Random *X*-chromosome inactivation in female primordial germ cells in the mouse. *Journal of Embryology and Experimental Morphology* 64, 251–258.

McMahon, A., Fosten, M. & Monk, M. (1983). *X*-chromosome inactivation mosaicism in the three germ layers and the germ line of the mouse embryo. *Journal of Embryology and Experimental Morphology* 74, 207–220.

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

Papaioannou, V. E., West, J. D., Bücher, Th. & Linke, I. (1981). Nonrandom *X*-chromosome expression early in mouse development. *Developmental Genetics* 2, 305–315.

Quinn, P., Barros, C. & Whittingham, D. G. (1982). Preservation of hamster oocytes to assay the fertilizing capacity of human spermatozoa. *Journal of Reproduction and Fertility* 66, 161–168.

Rabes, H. M., Bücher, Th., Hartmann, A., Linke, I. & Dünnwald, M. (1982). Clonal growth of carcinogen-induced enzyme-deficient preneoplastic cell populations in mouse liver. *Cancer Research* 42, 3220–3227.

Rastan, S. (1982). Primary non-random *X*-inactivation caused by controlling elements in the mouse demonstrated at the cellular level. *Genetical Research* 40, 139–147.

Rastan, S. & Cattanach, B. M. (1983). Interaction between the *Xce* locus and imprinting of the paternal chromosome in mouse yolk-sac endoderm. *Nature* 303, 635–637.

Surani, M. A. H., Barton, S. C. & Norris, M. L. (1984). Development of reconstituted mouse eggs suggest imprinting of genome during gametogenesis. *Nature* 308, 548–550.

Takagi, N. (1978). Preferential inactivation of the paternally derived *X* chromosome in mice. In *Genetic Mosaics and Chimeras in Mammals* (ed. L. B. Russell), pp. 341–360. New York and London: Plenum Press.

Takagi, N. & Sasaki, M. (1975). Preferential inactivation of the paternally derived *X* chromosome in the extra-embryonic membranes of the mouse. *Nature* 256, 640–642.

Takagi, N., Wake, N. & Sasaki, M. (1978). Cytologic evidence for preferential inactivation of the paternally derived *X*-chromosome in *XX* mouse blastocyts. *Cytogenetics and Cell Genetics* 20, 240–248.

West, J. D. (1982). *X*-chromosome expression during mouse embryogenesis. In *Genetic Control of Gamete Production and Function: Proceedings of the Serono Clinical Colloquia on Reproduction 3* (eds. P. G. Crosignani, B. L. Rubin and M. Fraccaro), pp. 49–91. London and New York: Academic Press/Grune and Stratton.

West, J. D., Bücher, Th., Linke, I. M. & Dünnwald, M. (1984). Investigation of variability among mouse aggregation chimaeras and *X*-chromosome inactivation mosaics. *Journal of Embryology and Experimental Morphology* 84, 309–329.

West, J. D., Frels, W. I., Chapman, V. M. & Papaioannou, V. E. (1977). Preferential expression of the maternally derived *X*-chromosome in the mouse yolk sac. *Cell* 12, 873–882.

West, J. D., Papaioannou, V. E., Frels, W. I. & Chapman, V. M. (1978). Preferential expression of the maternally derived *X*-chromosome in extraembryonic tissues of the mouse. In *Genetic Mosaics and Chimeras in Mammals* (ed. L. B. Russell), pp. 361–377. New York and London: Plenum Press.