
SHORT PAPER

Partial deletion of the Y chromosome removes the effect of paternal genome imprinting on periovum sensitivity to hyaluronidase in mice

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Summary

Cumulus-oocyte complexes from the two consomic strains of mice, B10.BR and B10.BR-Ydel (with a partial deletion of the Y chromosome) and the BALB/c strain, as well as reciprocal F1 and F2 hybrids, were tested for their susceptibility to attack by hyaluronidase. The cumulus cell dispersal was significantly more rapid in the eggs of B10.BR females and of F1 and F2 hybrid females sired by the B10.BR males than in those of BALB/c females and hybrids sired by BALB/c males. These results confirm the earlier data of Bander *et al.* (1989) which were interpreted as evidence of paternal imprinting of the C57BL genome. In contrast, cumulus cell dispersal in the eggs of females sired by B10.BR-Ydel males was significantly slower than in females sired by B10.BR males, and did not differ from that in the BALB/c strain. The results suggest that the partial deletion of the Y chromosome abolished the effect of paternal genome imprinting which is observed in the B10.BR strain.

Introduction

Failure to complete development of parthenogenetic embryos and gynogenetic or androgenetic embryos produced by pronuclear transplantation in mice, has led to the conclusion that the maternal and paternal genomes are not equivalent and both are necessary for normal development to occur (Surani *et al.* 1987; Peterson & Sapienza, 1993). In mice with a Robertsonian translocation, specific regions in chromosomes 2, 6, 7, 11, 12 and 17 have been identified that require to be transmitted either from the mother or the father to ensure normal embryonic development (Beechey & Cattanach, 1994). This is due to the fact that some genes are inactive when transmitted by the mother and some when transmitted by the father.

The imprinting process may involve a number of gene products that act as heterochromatinizing complexes. It seems likely that at least one imprinting gene is located on a sex chromosome because of the gamete-of-origin dependent behaviour of this phenomenon (Peterson & Sapienza, 1993).

Bander *et al.* (1989), studying eggs from the C57BL/6 and BALB/c strains, as well as reciprocal F1 and F2 hybrids, showed paternal inheritance of the rapidity of cumulus cell dispersal by hyaluronidase. The authors interpreted these results as a genomic

imprinting in C57BL/6 males, resulting in higher sensitivity of egg investments produced by their daughters.

The present paper describes the phenotypes of eggs from females from reciprocal F1 and F2 crosses between BALB/c and two consomic strains, B10.BR having a similar genetic background to the C57BL strain used by Bander *et al.* (1989) and B10.BR-Ydel with the large deletion in the Y chromosome. The aim of these experiments was to study the possible involvement of the Y chromosome in paternal imprinting.

Materials and methods

(i) Mice

Eggs were obtained from adult female mice, aged 10–12 weeks, bred at the Jagiellonian University, Kraków, Poland. Mice from two consomic strains i.e. B10.BR/SgSn and B10.BR-Ydel, and from the BALB/cAnN inbred strain were crossed reciprocally to produce F1 and F2 hybrids. The B10.BR-Ydel strain is characterized by a large deletion in the Y chromosome (Moriwaki *et al.* 1989; Styrna *et al.* 1988). This mutation was found during chromosome analysis of B10.BR/SgSn males from the Jackson Laboratory (Bar Harbor, ME, USA). The consomic

Table 1. Mean time to disperse cumulus cells with hyaluronidase in B10.BR, B10.BR-Ydel, and BALB/c mice and their reciprocal F1 and F2 hybrids

Stock	n	Mean ± s.e. (min)	Stock	n	Mean ± s.e. (min)
Inbred: B10.BR (B)	21	11.1 ± 0.48	Inbred: BALB/c (C)	30	15.2 ± 0.40*
F1 C × B	20	11.0 ± 0.34	F1 B × C	22	16.7 ± 0.46*
F2 (C × B) × (C × B)	20	11.0 ± 0.65	F2 (B × C) × (B × C)	25	15.6 ± 0.95*
F2 (B × C) × (C × B)	20	10.9 ± 0.40	F2 (C × B) × (B × C)	21	16.5 ± 0.45*
F1 BY × B	21	10.9 ± 0.45	—	—	—
Inbred: B10.BR-Ydel (BY)**	21	16.6 ± 0.57*	—	—	—
F1 BY × BY	20	14.8 ± 0.65*	—	—	—
F1 C × BY	20	16.7 ± 0.17*	F1 BY × C	20	14.9 ± 0.52*
F2 (C × BY) × (C × BY)	21	16.5 ± 0.53*	F2 (BY × C) × (BY × C)	20	16.3 ± 0.46*
F2 (BY × C) × (C × BY)	20	16.5 ± 0.16*	F2 (C × BY) × (BY × C)	20	16.4 ± 0.33*

n, number of clutches.

* Significantly different from females sired by B males ($P < 0.001$).

** Maintained by backcrossing B × BY.

strain to B10.BR/SgSn was developed from the mutant male and has been maintained since 1977 in the National Institute of Genetics, Mishima, Japan, and since 1987 in the present author's Department. To avoid divergence between the B10.BR/SgSn and B10.BR-Ydel strains, the latter is being maintained by backcrossing B10.BR-Ydel males to B10.BR/SgSn females. The scheme of appropriate crosses is given below:

- ♀ B10.BR/SgSn × ♂ B10.BR-Ydel;
 N1 ♀ B10.BR/SgSn × ♂ (B10.BR × B10.BR-Ydel)
 F1;
 N2 ♀ B10.BR/SgSn × ♂ N1;
 N3 ♀ B10.BR/SgSn × ♂ N2; etc.

In describing the hybrids the maternal parent is given first. The B10.BR/SgSn strain is abbreviated to B, B10.BR-Ydel to BY, and BALB/c to C. For experimental purposes also females arising from crosses of B10.BR/SgSn females with B10.BR-Ydel males were used. These females are called BY females.

(ii) Measurements of eggs

Ovulation was induced by injections of 8 i.u. pregnant mare gonadotrophin (PMSG, BIOWET, Poland) followed by 8 i.u. of human chorionic gonadotrophin (hCG, BIOMED, Poland). Eggs in cumulus were removed from the oviducts 18 h after hCG and transferred to plastic dishes with 0.5 ml of phosphate-buffered saline (PBS) containing 12 i.u. bovine testicular hyaluronidase (Sigma Chemical Co., St. Louis, USA). The dishes were kept at 37 °C on warm microscope plate. Each clutch of eggs was observed every minute until all the eggs were free of cumulus. Observations were made on clutches of at least 6 eggs from the same oviduct. The median dissolution time, for each clutch, was used to calculate the mean and standard error for each genotype. The data were compared by a Kruskal–Wallis U test (Zar, 1984).

Results

The mean times of dispersal of the cumulus cells of eggs from B, BY, and C inbred strains and their F1 and F2 crosses are shown in Table 1.

In the inbred strains the cumulus cell dispersal was significantly more rapid in the eggs of B females than in those of C females. The eggs from (C × B) F1 and (BY × B) F1 females closely resembled the phenotype of their paternal strain as did those of the reciprocal (B × C) F1 females, respectively. The mean hyaluronidase time for the F2 females, having the same father but different mothers, closely resembled those of their paternal and grand-paternal stocks.

In contrast, cumulus cell dispersal in the eggs of females sired by mutant BY males, with partial deletion of the Y chromosome, was significantly slower than in females sired by B males ($P < 0.001$) and the mean value for these crosses was similar to that of the C strain. The differences in timing between the results of crosses and intercrosses sired by BY and C males are not significant (Kruskal–Wallis U test) and may be due to differences in the genetical background of these strains.

Discussion

The experiments performed on B10.BR and BALB/c strains and their reciprocal F1 and F2 hybrids described here confirmed the results obtained by Bander *et al.* (1988, 1989). Those authors interpreted the paternal inheritance of cumulus cell dispersal as a result of imprinting of the paternal genome, X-chromosome linked and cytoplasmic effects being excluded.

In the present experiments, using males with partial deletion of the Y chromosome, the involvement of the Y chromosome in the paternal imprinting was demonstrated. As is clearly shown in Table 1, the cumulus cell dispersal in the eggs of all females sired

by B10.BR-Ydel males was significantly slower than in those of females sired by B10.BR males. Because the B10.BR-Ydel strain and B10.BR have the same genetic background, this difference must be ascribed to the Y chromosome.

According to the model of Peterson & Sapienza (1993) 'when allele from imprintable locus passes through gametogenesis of one sex, its ability to be expressed is unaffected. However, when this allele passes through gametogenesis of the opposite sex, it becomes inactivated and is unable to be expressed. This inactivated allele is thus termed 'imprinted'. The gene that controls this process of inactivation is called an 'imprinting' gene or 'imprintor'. It is possible that such an imprintor resides in that part of the Y chromosome which is missing in the B10.BR-Ydel strain and thus cannot control the imprintable loci in autosomes. This alteration is then transferred through the paternal gametes to the daughters and affects the time of dispersal of the cumulus cells of their ova.

B10.BR-Ydel mutant males were characterized previously by Moriwaki *et al.* (1988) and Styrna *et al.* (1988, 1991 *a, b*). Phenotypically, the Y-del mutation used in this work is similar to the Y-del mutation described by Conway *et al.* (1994). The latter has a deletion on the long arm of the Y chromosome where the Y 353/B multiple-copy gene is situated, necessary for the normal development of the sperm head. It may be that the 'imprintor gene' influencing the trait described here is on the long arm of the mouse Y chromosome. The 'imprintor gene' is needed for the paternal imprinting to be propagated. When it is deleted, this is no longer possible which results in the low susceptibility of cumulus-oocyte complexes to hyaluronidase.

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