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Inheritance of a meiosis I error expressed in mouse oocytes and modulated by a maternal factor

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Summary

NMRI/Han mice ovulate significant numbers of diploid oocytes after gonadotrophin stimulation, most of them arrested at metaphase I. In contrast, females from other mouse strains ovulate only oocytes having completed meiosis I. We investigated the heritability of the trait Dipl I by analysing F_1 hybrid females from crosses between the sensitive NMRI/Han stock and two mouse strains (C3H/HeHan and BALB/c) considered as non-sensitive, and females from backcrosses to NMRI/Han males (only crosses with C3H/HeHan). The results show (1) that the trait Dipl I is inheritable; (2) that an X-chromosomal (location proximally from the X–Y pairing region) or autosomal recessive mode of inheritance is excluded; and (3) that the expression of the trait, measured as frequency of diploidy, is modulated by a maternally transmitted factor.

1. Introduction

Many mutations of meiosis have been described in lower organisms (Baker *et al.* 1976 for review) and serve as a basis for the molecular analysis of controlling elements of meiosis (Thomas & Botstein, 1986). In yeast, several sporulation-defective mutants cause meiotic arrest and the formation of diploid ascospores. Similar abnormalities have been observed in disjunction-defective mutants of Drosophila, in both males and females (Baker *et al.* 1976). In mammals, such defects are more or less unknown, and mutations causing errors at meiosis I or II are hardly detectable because of (for example) the strong selection against chromosomal imbalance during early prenatal development, low number of oocytes/embryos and rather long generation times.

In random-bred NMRI/Han mice, a low percentage (about 1%) of spontaneously ovulated oocytes is diploid instead of being haploid, most of them arrested at metaphase I. Few are blocked at a transitional stage between metaphase I and metaphase II (Hansmann & El-Nahass, 1979). If ovulation is induced in females of that stock by injecting high doses of gonadotrophins, the rate of diploid oocytes increases constantly to 2–4% (Bartels, Jenderny & Hansmann, 1984; Beermann & Hansmann, 1986). The other mouse strains tested did not show this phenomenon, either after spontaneous or after induced ovulation (Hansmann & El-Nahass, 1979). In a first experiment, it was shown that the trait Dipl I is transmissible to F_1 hybrid females derived from crosses of NMRI/Han and C57BL/6J. Yet the frequency of diploid oocytes appeared to be controlled maternally (Bartels *et al.* 1984).

To analyse the inheritance of the defect causing premature arrest of the first meiotic division in oocytes at metaphase I in more detail, and above all to substantiate the predicted maternal factor, we investigated F_1 females from crosses of NMRI/Han with C3H/HeHan or BALB/c, respectively, and females from various back crosses of F_1 hybrids to NMRI/Han or C3H/HeHan. Accordingly, an autosomal recessive and an X-chromosomal (proximally from the X–Y pairing region) inheritance were excluded. The suspected maternal controlling element was proved in four further independent crosses.

2. Materials and methods

Housing and husbandry

Adult mice of the random-bred stock NMRI/Han and of the two inbred strains C3H/HeHan and BALB/c were obtained from the Zentralinstitut für Versuchstierzucht, Hannover, and housed in the same animal room under controlled day–night cycles, darkness lasting for 12 h. They were kept in Makrolon cages (type II) and received Altromin pellets R 1424 and tap water ad libitum. F_1 hybrid lines and

backcrosses were established by mating randomly chosen animals of the appropriate strains overnight (two females were paired with one male). The following morning, plugged females were separated and their offspring reared after weaning up to an age of 8–12 weeks. They were then either used for further breeding or subjected to the experimental procedures.

Experimental procedure

Females aged 8–12 weeks were stimulated for ovulation with 10 i.u. PMS (pregnant mare serum; Vemie, Kempen) followed 48 h later by 10 i.u. HCG (human chorionic gonadotrophin; Organon, München). The animals were killed 15–16 h after the HCG injection, and ovulated oocytes were collected from the ampulla. Cytologic preparation of oocytes followed our standard procedure (Tarkowski, 1966; Röhrborn & Hansmann, 1971) and metaphases were analysed after orcein staining for diploid (mostly due to meiotic arrest at metaphase I) or normal haploid chromosomal complements.

The numbers of ovulated oocytes per female were expressed as means ± S.E.M. (except for the C3H/HeHan group). Data concerning the number of both diploid ovulated oocytes and females showing the trait Dipl I were compared by a χ^2 test with Yates correction. A P value $< 0{\cdot}05$ was considered to represent a statistically significant difference.

3. Results

Inheritance of the trait Dipl I

Comparing the frequencies of diploid oocytes from the three parental strains, it became evident that only the NMRI/Han females were sensitive, and ovulated diploid oocytes after gonadotrophin treatment (Table 1). No ovulated diploid oocyte was recorded in either the BALB/c or the C3H/HeHan females. Approximately 50% of all NMRI/Han females ovulated at least one such oocyte (not shown) and altogether 4·0% of the oocytes were arrested at metaphase I. The difference from the non-sensitive strains BALB/c and C3H/HeHan is statistically significant ($P < 0{\cdot}01$). F_1 females from all four crosses ovulated metaphase I-arrested oocytes, though less ($P < 0{\cdot}01$) than females from the parental NMRI/Han stock. Females derived from backcrosses of F_1 females with NMRI/Han males ovulated more diploid oocytes (the difference between F_1 females with NMRI/Han mothers and females from backcrosses with NMRI/Han grandmothers is statistically significant, $P < 0{\cdot}02$) than the respective F_1 females, and respond intermediately between F_1 females and the parental NMRI/Han stock.

Under the assumption of X-linkage, females from non-sensitive C3H/HeHan mothers and F_1 (C3H/HeHan × NMRI/Han) fathers should have ovulated only normal haploid oocytes, since both their

Table 1. *Incidence of ovulated diploid oocytes in mice from the NMRI/Han stock and the C3H/HeHan and BALB/c strains, their F_1 hybrids and two backcrosses (Bc)*

Strain/cross (♀ × ♂)	No. of females	No. of oocytes ovulated per female	No. of oocytes analysed	Percentage of diploid oocytes
NMRI/Han*	92	49·7 ± 21·4	3427	4·0
C3H/HeHan	31†	25·1	490	0·0
BALB/c	19	15·4 ± 9·8	228	0·0
F_1 (NMRI/Han × BALB/c)	57	49·9 ± 18·5	1850	0·7
F_1 (BALB/c × NMRI/Han)	40	56·1 ± 19·4	1596	1·8
F_1 (NMRI/Han × C3H/HeHan)	115	20·4 ± 10·8	1561	2·4
F_1 (C3H/HeHan × NMRI/Han)	73	26·5 ± 13·4	1165	1·3
Bc (F_1(NMRI/Han × C3H/HeHan) × NMRI/Han)	75	19·6 ± 12·5	924	4·3
Bc (F_1(C3H/HeHan × NMRI/Han) × NMRI/Han)	68	24·4 ± 14·9	1137	1·9

* The data of the NMRI/Han females include published results (Bartels *et al.* 1984; Beermann & Hansmann, 1986).
† Exact data of ovulated oocytes were not recorded for every single female.

Table 2. *Incidence of ovulated diploid oocytes in females from backcrosses of NMRI/Han or C3H/HeHan to F_1(C3H/HeHan × NMRI/Han) males*

Backcross (♀ × ♂)	No. of females	No. of oocytes ovulated per female	No. of oocytes analysed	Percentage of diploid oocytes
C3H/HeHan × F_1 (C3H/HeHan × NMRI/Han)	24	43·1 ± 18·0	827	1·3
NMRI/Han × F_1 (C3H/HeHan × NMRI/Han)	28	54·1 ± 23·9	1075	2·7

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Table 3. *Influence of the maternal type on the expression of gonadotrophin-induced diploidy*

Comparison between the crosses	Maternal type	NMRI/Han transmitted sensitivity modulated by	
		Type	Factor
C57BL/6J × NMRI/Han NMRI/Han × C57BL/6J	C57BL/6J*	Increase	2·5
BALB/c × NMRI/Han NMRI/Han × BALB/c	BALB/c	Increase	2·6
C3H/HeHan × NMRI/Han NMRI/Han × C3H/HeHan	C3H/HeHan	Decrease	1·9
(C3H/HeHan × NMRI/Han) × NMRI/Han (NMRI/Han × C3H/HeHan) × NMRI/Han	C3H/HeHan	Decrease	2·3
C3H/HeHan × (C3H/HeHan × NMRI/Han) NMRI/Han × (C3H/HeHan × NMRI/Han)	C3H/HeHan	Decrease	2·1

* From Bartels *et al.* (1984).

X chromosomes were derived from C3H/HeHan. However, they ovulated altogether 1·3% diploid oocytes (Table 2). The other females (NMRI/Han × F_1 (C3H/HeHan × NMRI/Han)) showed as expected diploid oocytes (2·7%) and served as a matched control. Thus an X-chromosomal mode of inheritance can be excluded. The exclusion is, however, valid only for that portion of the X chromosome which does not recombine with the Y. Such a recombination between the X and Y chromosome has been shown recently for the STS-locus (Keitges *et al.* 1985). Thus the theoretical possibility of a pseudoautosomal inheritance exists, although recombination between X and Y is restricted to a minute distal region.

Maternal influence on the frequency of Dipl I

All experiments with females from one reciprocal cross, i.e. those from NMRI/Han × C3H/HeHan and C3H/HeHan × NMRI/Han or NMRI/Han × BALB/c and BALB/c × NMRI/Han, have been performed in parallel under the same conditions, using the same batch of hormones and analysing coded slides. The results within a reciprocal cross are therefore directly comparable. The predicted maternal factor modulating the frequency of diploid oocytes and therewith the expression of the trait (Bartels *et al.* 1984) was effective in females from all four reciprocal crosses (Table 1). The difference between the frequencies of diploid oocytes is statistically significant ($P < 0·05$) within each reciprocal cross. Beyond that the maternal modulation became effective in both directions. The frequency of Dipl I increased with the maternal type from C57BL/6J (Bartels *et al.* 1984) and BALB/c, but decreased in three different crosses with the maternal type from C3H/HeHan (Table 3).

4. Discussion

The cytogenetic data obtained from females of eight different crosses (Tables 1 and 2) together with our earlier results from crosses between NMRI/Han and C57BL/6J (Bartels *et al.* 1984) allow the conclusion that the trait Dipl I is heritable. This is the first demonstration of a genetically determined meiotic error in mammals causing specifically chromosomal imbalance. An autosomal recessive and an X-chromosomal mode of inheritance (proximally from the X–Y pairing region) can be excluded. The possibility of a pseudoautosomal linkage or at least an influence of a pseudoautosomally segregating gene is worth mentioning in the light of recent results obtained with oocytes from XO mice (Beermann *et al.* 1986). After gonadotrophin treatment of F_1 (XO × NMRI/Han) females, a high level of 24·6% diploid oocytes was ovulated from these XO females, whereas their XX littermates only reached 1% of oocytes arrested at metaphase I. This effect is surely accentuated by the influence of the NMRI/Han genome present in our F_1 hybrids. This may explain why a similar study (Brook, 1983) failed to detect such an effect if XO and XX sibs were compared. Furthermore, not only in oocytes but also in spermatocytes a higher risk for disturbances of regular meiosis I may exist. This assumption is accentuated by cytogenetic observations with XO mice

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carrying the *Sxr* mutation. These XO *Sxr* males are sterile and their spermatogenesis is not only defective but produces also many diploid spermatids (Burgoyne & Baker, 1983). The defect is still more pronounced in XO males carrying *Sxr'*, a variant of that mutation. The variant has retained its function for testis induction, but apparently has lost the expression of H–Y antigen determined by the T-cell killing (McLaren *et al.* 1984).

The meiotic arrest at metaphase I is not irreversible in diploid oocytes. The premature block before the first meiotic division can be circumvented and triploid embryos can result after fertilization (A. Babassikas & I. Hansmann, unpublished results). Such digynic triploidy has also been described in the A/He mouse strain, although no information on the heritability of that phenomenon was given (Takagi & Sasaki, 1976). These A/He mice are similar to our NMRI/Han females and express the defect already after spontaneous ovulation, with 4·5% of the resulting zygotes being triploid. The phenomenon is inducible as well, and the frequency increases up to 20% after superovulation. Yet the underlying mechanisms seem to differ between the two strains. In triploid zygotes from A/He females, Takagi & Sasaki (1976) observed two small female pronuclei, and suggested that digyny is due to suppression of the second polar body. They did not mention any observation of a single giant maternal pronucleus which was present in triploid zygotes from our NMRI/Han females after superovulation (unpublished results). However, triploid embryos from both strains do have the potential to develop up to or even beyond early post-implantation stages.

The inhibition of the first meiotic division in oocytes from our NMRI/Han females is not likely to originate from a primary defect within the oocyte, but seems to be caused by an altered communication between the maturing oocyte and the surrounding somatic cells (Hansmann *et al.* 1985). This assumption has recently been proved in gonadotrophin-stimulated chimeric females generated between NMRI/Han and C57BL/6J, which are considered non-sensitive with respect to the ovulation of diploid oocytes. The results showed that all NMRI/Han-derived ovulated oocytes from these chimaeras (NMRI/Han origin proved by the presence of a chromosomal marker) had completed meiosis I normally (Bartels *et al.* 1984).

Another feature observed now with five different crosses (Table 3) is the apparent maternal influence which modulates the expression of the trait Dipl I by increasing or decreasing the frequency of diploid oocytes. Such maternal effects may originate postnatally, as shown, for example, for the mouse mammary tumour virus, a milk-borne B-type retrovirus (Heston, 1948; Coffin, 1984) or from events during gestation. Finally, maternal effects may be exerted before conception. The latter effects need not originate from cytoplasmic inheritance, since experiments with pronuclear transplantation have suggested imprinting of the nuclear genome during gametogenesis (Surani, Barton & Norris, 1984).

Only a few examples for a cytoplasmic inheritance in mice have been described (McLaren, 1979, for review). One of them is the Mta cell-surface antigen (Mta+ or Mta−) determined by a defective virus or, more likely, by a mitochondrial gene (Fischer Lindahl & Hausmann, 1983). It is interesting that NMRI/Han mice are Mta− in contrast to the old inbred strains like BALB/c, C57BL/6J and C3H, which are Mta+. Mitochondria are strictly maternally inherited organelles with an own genome (Bibb *et al.* 1981; Lansman, Avise & Huettel, 1983; Hecht *et al.* 1984), and altogether 108 point-mutational differences per mt genome were estimated to exist between Mta− and Mta+ mice (Ferris *et al.* 1983).

A role of mitochondrial function in the expression of the trait Dipl I can be deduced from recent experiments with chloramphenicol. When gonadotrophin-primed NMRI/Han mice were treated with this potent inhibitor of mitochondrial peptidyl transferase, both follicular maturation and meiosis I were affected and the frequency for diploidy was significantly increased (Beermann & Hansmann, 1986). During oogenesis, mitochondria synthesize proteins involved in adenosine triphosphate synthesis (Cascio & Wassarman, 1981). A rate-limiting step in steroidogenesis, the conversion of cholesterol to pregnenolone, is located within mitochondria of steroidogenic cells and is regulated hormonally by, for example, luteinizing hormone and corticotrophin (Tanaka & Strauss, 1982; Privalle, Crivello & Jefcoate, 1983). During the formation of the meiosis I spindle in mouse oocytes, mitochondria aggregate specifically in the perinuclear region and subsequently disperse during and after the extrusion of the first polar body (Van Blerkom & Runner, 1984). These authors regarded the mitochondrial rearrangements as being essential for the final phase of oocyte maturation and suggested them to be necessary for locally restricted activities of adenosine triphosphate in the ooplasm.

Mitochondrial migrations have similarly been reported in cattle oocytes by Hyttel, Callesen & Greve (1986), who moreover noticed an abnormal asynchrony between nuclear and cytoplasmic maturation within oocytes after hormonal treatment for superovulation. An asynchrony between nuclear and cytoplasmic maturation was predicted earlier and it was suggested that such an asynchrony would cause a premature arrest of spindle function before (resulting in metaphase I arrest) or during bivalent segregation (Hansmann, Jenderny & Probeck, 1983). Congenic strains are bred currently between C57BL/6J and NMRI, differing only in the origin of their cytoplasm. Their analysis will show whether a cytoplasmically inherited factor is really responsible for the maternally controlled expression of Dipl I in mouse oocytes.

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