

Pattern and frequency of nocodazole induced meiotic nondisjunction in oocytes of mice carrying the 'tobacco mouse' metacentric Rb(16.17)7Bnr

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Summary

Oocytes from (C3H/HeH × 101/H)_F₁ and Rb(16.17)7Bnr homozygous females were exposed to a range of doses of nocodazole *in vitro*. The spindle poison caused a dose dependent increase in metaphase I (MI) arrest and hyperploidy. A concentration of 0.03 µg/ml was found to induce a maximum hyperploidy frequency of 3.1% and 11.6% respectively without a high level of MI arrest. Between 0.03 and 0.05 µg/ml MI arrest increased substantially and reached a frequency of approximately 90%. In a further experiment oocytes from Rb7 homozygous, heterozygous and 3H1 females were exposed to 0.03 µg/ml nocodazole 4, 6 or 8 h after the onset of maturation. The phase at which the spindle was inhibited resulted in a specific pattern of nondisjunction which in turn was dependent on whether the female carried an Rb metacentric. 3H1 oocytes gave a normally distributed pattern of increase in aneuploid frequency (over the spontaneous value) centering around a 6 h application. This was thought to be due to the interaction of chromosomes with the microtubules of the spindle during attachment and/or alignment. In contrast both Rb homozygotes and heterozygotes gave the same biphasic response, with a high frequency of aneuploidy in the oocytes when nocodazole was applied 4 and 8 h after the onset of maturation. In Rb homozygotes we demonstrated that the Rb bivalent underwent nondisjunction more frequently than the average acrocentric, when nocodazole was administered early. It can be assumed that the Rb trivalent in Rb heterozygotes showed a similar response. This early Rb specific effect, in combination with a delayed-version of the acrocentric effect found in the 3H1 mice was thought to generate the biphasic pattern. We discuss the implications of (a) the different meiotic behaviours of metacentrics and acrocentrics and (b) the meiotic delay in Rb mice.

1. Introduction

Meiotic nondisjunction is a disturbance in the normal process of chromosome segregation at anaphase. As a result, chromosomally unbalanced gametes are produced, which may subsequently fertilize and form aneuploid embryos. Most numerical chromosomal aberrations are incompatible with embryonic and foetal development. Consequently, a large proportion of foetal wastage in humans is due to such abnormalities (Boué, Boué & Gropp, 1985). Aneuploid

concepts that do survive to term constitute approximately 0.5% of the newborn population (Hassold & Jacobs, 1984) with the majority of individuals exhibiting physical and/or mental impairment.

There are several distinct factors associated with aneuploidy in humans. The frequency of nondisjunction is highly dependent on the particular chromosome involved (Hassold & Sherman, 1993; Jacobs, 1992) and on the sex and the age of the individual. The differential effect in the two sexes is expressed in terms of substantially higher numbers of numerically abnormal oocytes than sperm, with frequencies of 18–19% and 3–4% respectively (Pellestor, 1991; Martin, Ko & Rademaker, 1991). The use of DNA polymorphisms, allowing the determination of parental origin of trisomy, has confirmed the substantial contribution by the female, in particular due to malsegregation during the first meiotic division (Antonarkis, Peterson, McInnis *et*

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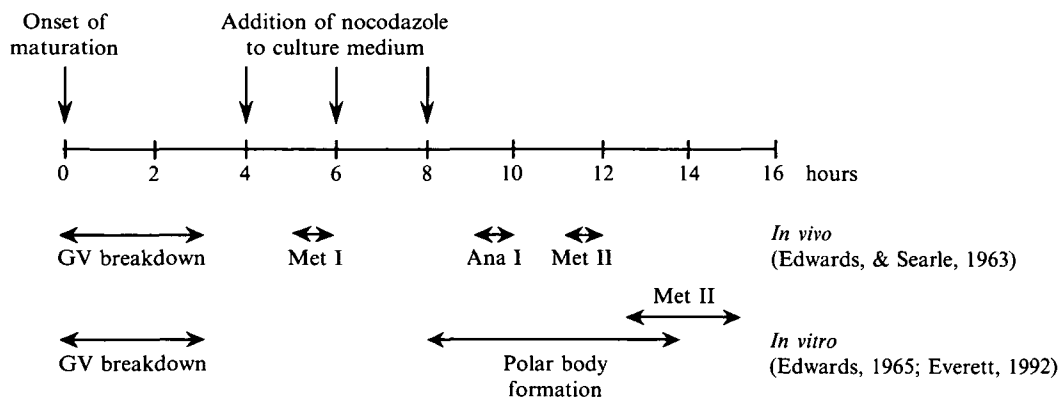


Fig. 1. Approximate timing for the onset of nuclear events during oocyte maturation *in vivo* and *in vitro* for 3H1 mice.

al., 1992; Hassold & Sherman, 1993). The age effect is also primarily a female phenomenon (Bond & Chandley, 1983; Eichenlaub-Ritter, 1993). It has been suggested that as many as 60% of all oocytes from women aged 40 or more may be chromosomally unbalanced (Hassold & Jacobs, 1984).

In contrast to the situation in humans, spontaneous aneuploidy has been found to be rare in other mammalian species. For instance, the mouse would be the normal first choice as a mammalian model for studies of nondisjunction, but estimates of maternal meiosis I errors in both laboratory and wild mice with the standard all acrocentric karyotype ($2n = 40$) are as low as 5% or less (Hansmann & El-Nahass, 1979; Hauffe, 1993). However, despite the discrepancy with humans in nondisjunction frequency, there are still a variety of fundamental studies that can be conducted on mice. Most notably, mice of the CBA/Ca strain show the maternal age effect and have been a useful model for studying the origins of such an effect (e.g. Eichenlaub-Ritter & Boll, 1989). Additionally, heterozygotes for Robertsonian (Rb) metacentrics in the mouse, have considerably higher anaphase I nondisjunction frequencies than standard all-acrocentric mice (reviewed in Gropp & Winking, 1981; Redi & Capanna, 1988; Searle, 1988) and have provided valuable information on such features as the relationship between meiotic delay and aneuploidy (e.g. Hansmann, de Boer & Speed, 1988).

The Rb mouse model may also provide insight into the differential involvement of chromosomes in the process of nondisjunction. Homozygotes for an Rb metacentric have a distinct marker chromosome in an otherwise acrocentric karyotype. This permits a study of the degree of synchrony in chromosome-spindle interactions between different chromosomes. For mice and humans the extent of such synchrony is not known, although it is reasonable to suppose that asynchrony may relate to differential predisposition to nondisjunction.

In the present study we use Rb homozygotes to analyse synchrony of the chromosome-spindle interactions between chromosomes in females. Our experimental design has been much influenced by the

studies of Hummler & Hansmann (1985, 1988). Their *in vivo* analysis of the first meiotic division of the female Djungarian hamster revealed a chromosome-dependent interaction with the spindle. Application of the spindle inhibitors, colchicine and Carbendazim (MBC), at an early stage of spindle development yielded a high nondisjunction frequency involving all chromosomes. In contrast, when the spindle poisons were applied at a later stage there was a lower frequency of hyperploid secondary oocytes which preferentially involved the large metacentric and submetacentric chromosomes. Hummler & Hansmann (1988) postulated that spindle-chromosome interactions were asynchronous among the heterogeneous population of chromosomes in hamsters, with the larger (A-D) bivalents segregating last.

In our study of house mice we made use of a homozygous stock carrying the 'tobacco mouse' metacentric, Rb(16.17)7Bnr, formed by the fusion of the acrocentrics 16 and 17 at their centromeres. This metacentric originated in wild mice but was introduced into a (C3H/HeH \times 101/H) F_1 hybrid genetic background (abbreviated to 3H1) (Cattanach & Moseley, 1973). Nocodazole (methyl [5-(2-thienylcarbonyl)-1H-benzimidazole-2-yl]carbamate) was used as the spindle poison; this is a benzimidazole derivative which interferes with the dynamics of the spindle (Jordan, Thrower & Wilson, 1992) and has previously been shown to cause increased frequencies of chromosomal anomalies in mouse oocytes cultured *in vitro* (Eichenlaub-Ritter & Boll, 1989) and 1st division cleavage zygotes after application *in vivo* at the time of fertilization (Generoso, Mitchell, Bishop *et al.*, 1989).

Oocytes can be stimulated to undergo maturation *in vitro* by releasing them mechanically from their follicles. The time course of meiosis is similar to that *in vivo* and is shown in Fig. 1. In our first experiment we applied nocodazole after 8 h culture in order to determine a concentration that caused an increased frequency of nondisjunction without a concomitant rise in metaphase I arrest. Once a suitable concentration had been established the effect of different application times was studied. We chose 4, 6 and 8 h

as suitable times for application of the spindle poison for the second experiment as they represent times between germinal vesicle breakdown and anaphase I in 3H1 mice (Edwards & Searle, 1963; Edwards, 1965; see Fig. 1). A detailed analysis was made of the response of oocytes from mice either homozygous for Rb(16.17)7Bnr or the standard 40-chromosome karyotype (both on a 3H1 genetic background). We also studied heterozygotes for Rb(16.17)7Bnr to gain further insight into the particularly high nondisjunction frequencies suffered by female Rb heterozygotes in the mouse (Gropp & Winking, 1981); a value of 31% has been recorded in the case of this particular metacentric (Redi & Capanna, 1988).

2. Materials and Methods

(i) Mice

In this study, the Rb(16.17)7Bnr homozygotes (abbreviated to Rb7/Rb7) were derived from the homozygous line set up by Cattnach & Moseley (1973) and the heterozygotes (Rb7/+) were generated by crossing Rb(16.17)7Bnr homozygotes with 3H1 mice. All individuals were either obtained directly from the MRC Radiobiology Unit, Chilton, Didcot, U.K. or bred within the Zoology Department, University of Oxford from matings between mice which originated from the MRC Unit. They were kept under standard laboratory conditions (22–24°C; 14:10 h light:dark) with food and water provided *ad libitum*.

(ii) Experiment 1

Ovaries were removed from hormonally untreated 6–12 week-old 3H1 females after cervical dislocation and placed in a solid watchglass with prewarmed (37°C) M2 medium (Quinn, Barros & Whittingham, 1982). Oocytes were released from large antral follicles by pricking with two fine needles. Oocytes at the germinal vesicle (GV) stage were then isolated from cumulus cells by gentle sucking with a mouth-operated micropipette and were washed twice in M16 medium (Whittingham, 1971). The oocytes were left to mature for 8 h, i.e. close to the beginning of polar body formation (Fig. 1). A late application of the spindle inhibitor was expected to cause the least nondisjunction; it was important to establish a concentration of nocodazole that would work at this time. After 8 h of culture, oocytes that had undergone GV breakdown were transferred to M16 medium containing a specific concentration of nocodazole. Nocodazole was diluted from stocks at 0.001–1.00 mg/ml DMSO so that a minimal volume of DMSO (5–30 µl) was introduced into the culture medium. The chemical was administered at various doses ranging from 0.001 to 1.0 µg/ml. The oocytes were cultured overnight and fixed the following day, giving a total time in culture of 20–22 h. Once the

optimum range of concentrations was established for 3H1 mice, a similar study was carried out for Rb7 homozygotes over a narrow range of concentrations. Oocytes were fixed according to the method of Tarkowski (1966). The slides with oocytes were then post-fixed for 45 min in 3:1 (methanol:acetic acid (v/v)) and hydrolysed in 0.5 M-HCl for a further 45 min, washed in water and air dried. The following day the chromosome preparations were C-banded by a modified method of Sumner (1972) using a 15 s exposure to Ba(OH)₂ at 40°C. Chromosome spreads were determined to be arrested at metaphase I (MI) or to have continued through to metaphase II (MII). For the MII oocytes the number of chromosome arms were scored and the per cent hyperploidy was calculated and used as a measure of nondisjunction (note that this measure disregards the hypoploid contribution to nondisjunction: see Cattnach & Moseley, 1973). Females yielding fewer than five scorable oocytes were excluded from the analysis as were MII spreads with 15 or fewer chromosomes. Diploid oocytes were those with greater than 30 chromosome arms in an MII configuration.

(iii) Experiment 2

Oocytes were obtained as described above. After 4, 6 or 8 h of culture, oocytes that had undergone germinal vesicle breakdown were transferred to M16 medium containing 0.03 µg/ml nocodazole, a concentration which (according to Experiment 1) induced hyperploidy without causing a concomitant increase in MI arrest. The oocytes were replaced in the incubator, cultured overnight and fixed the following morning, processed and scored as described above.

(iv) Statistics

The statistical program, Multisat (Biosoft, Cambridge) was used on an Apple Macintosh computer to calculate χ^2 with Yate's correction and Fisher's exact tests.

3. Results

(i) Experiment 1: The effect of nocodazole concentration on the frequency of MI arrested, hyperploid and diploid oocytes

The dose response of oocytes to nocodazole was determined by the addition of the spindle poison to the culture medium 8 h after their release from the follicles, a time at which all oocytes would have reached MI but not yet undergone anaphase I (see Fig. 1). It was anticipated that the effect of nocodazole during Experiment 2 would be an additive one in that all subsequent stages of meiosis would be disrupted after application (e.g. the effect after the addition of nocodazole at 4 h would be the same as that at 6 h plus the effect specific to the 4–6 h period). The application at 8 h for the dose response in Experiment

Table 1. The frequency of MI arrest, hyperploidy and diploidy in oocytes in response to a range of nocodazole concentrations applied following 8 h of culture

Nocodazole concentration (µg/ml)	Number of mice	Number of oocytes		MI Arrest		Diploid		MII		Scorable MIIs	Hyperploidy	
		Retrieved	Analysed	N	%	N	%	N	%		N	%
3H1												
0.00	11	248	159	2	1.3	—	—	157	98.7	122	—	—
0.001	4	99	66	—	—	—	—	66	100.0	53	—	—
0.01	8	180	99	—	—	—	—	99	100.0	67	2	3.0
0.03	10	221	151	9	6.0	—	—	142	94.0	96	3	3.1
0.05	3	97	59	53	89.8	—	—	6	10.2	3	—	—
0.10	4	83	54	49	90.7	1	1.9	4	7.4	4	—	—
1.00	7	111	60	53	88.3	3	5.0	4	6.7	3	—	—
Rb7/Rb7												
0.00	14	244	144	4	2.8	2	1.4	139	95.9	100	2	2.0
0.01	7	141	78	1	1.3	1	1.3	76	97.4	49	1	2.0
0.03	18	323	176	20	11.4	9	5.1	147	83.5	95	11	11.6
0.05	6	122	57	46	80.7	2	3.5	9	15.8	8	—	—

Table 2. The frequency of MI arrest, hyperploidy and diploidy after the addition of 0.03 µg/ml nocodazole to the oocytes of Rb7/Rb7, Rb7/+ and 3H1 female mice at different times after release from follicles

Time of nocodazole application (hours)	Number of mice	Number of oocytes		MI Arrest		Diploid MII				Scorable MIIs	Hyperploidy		Presegregation	
		Retrieved	Analysed	N	%	N	%	N	%		N	%	N	%
Rb7/Rb7														
Control	14	244	145	4	2.8	2	1.4	139	95.9	101	2	2.0	—	—
4	20	240	151	9	6.0	2	1.3	140	92.7	86	15	17.4***	—	—
6	12	169	121	6	5.0	1	0.8	114	94.2	78	6	7.7	1	1.3
8	18	323	176	20	11.4**	9	5.1	147	83.5	95	11	11.6*	1	1.1
Rb7/+														
Control	11	231	163	7	4.3	1	0.6	155	95.1	109	7	6.4	1	0.9
4	14	191	150	9	6.0	1	0.7	140	93.3	85	19	22.4**	—	—
6	15	193	133	10	7.5	2	1.5	121	91.0	72	9	12.5	—	—
8	13	244	164	17	10.4	2	1.2	145	88.4	101	17	16.8*	—	—
3H1														
Control	11	248	159	2	1.3	—	—	157	98.7	122	—	—	—	—
4	19	244	208	31	14.9***	6	2.9	171	82.2	93	6	6.5*	1	1.1
6	13	245	161	9	5.6	3	1.9	149	92.5	93	11	11.8***	1	1.1
8	10	221	151	9	6.0	—	—	142	94.0	97	3	3.1	1	1.0

Statistics calculated using χ^2 with Yates correction. All comparisons were made with each groups respective controls (oocytes not exposed to nocodazole) *0.01 < P < 0.05; **0.001 < P < 0.01; *** P < 0.001.

1 would therefore potentially yield the minimum effect possible and reveal the best concentration for Experiment 2.

The addition of nocodazole to the culture medium caused a dose-dependent increase in the number of oocytes showing MI arrest and hyperploidy for 3H1 females and Rb7 homozygotes (Table 1). A low frequency of diploid oocytes occurred at all concentrations of nocodazole in Rb7/Rb7 mice, but only at the highest concentrations in the 3H1s.

The oocytes of 3H1 females showed high levels of MI arrest (approximately 90%) at those concentrations above 0.03 µg/ml tested (0.05, 0.10, 1.0 µg/ml; Table 1). Hyperploidy reached a peak of 3.1% at 0.03 µg/ml, a similar response seen for

Rb7/Rb7 homozygotes which showed a maximum frequency of 11.6% hyperploidy at 0.03 µg/ml. The highest concentration of nocodazole tested that induced hyperploidy in oocytes that had progressed to MII without substantial MI arrest was, therefore, 0.03 µg/ml for both 3H1 and Rb7/Rb7 females.

(ii) Experiment 2: The effect of the time of exposure to nocodazole on the frequency of MI arrested, hyperploidy and diploid oocytes

The results of oocyte exposure to 0.03 µg/ml nocodazole at different times of spindle development for Rb homozygotes, Rb heterozygotes and 3H1s are shown in Table 2. Rb7/Rb7 and Rb7/+ mice showed

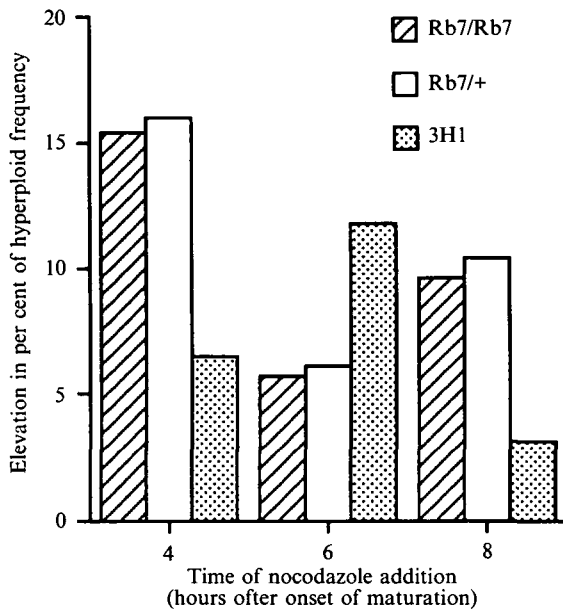


Fig. 2. The increase in per cent of hyperploidy relative to the spontaneous frequency for Rb7 homozygotes, Rb7 heterozygotes and 3H1 female mice after the addition of nocodazole at 4, 6 and 8 h after release from follicles.

low spontaneous levels of MI arrest, with hyperploid frequencies of 2.0 and 6.4% respectively. 3H1 oocytes showed no spontaneous hyperploidy and only 1.3% MI arrest which is within the range previously reported for maturing oocytes (Donahue, 1968). Presegregation and diploidy maintained a low level for all karyotypes with no significant variation according to time of nocodazole application. For each karyotypic group, however, the extent of nocodazole induced MI arrest and hyperploidy was dependent on the time of application.

Rb7 carriers showed an increase in MI arrest only after exposure to nocodazole at 8 h, with a frequency of 11.4% and 10.4% for homozygotes and heterozygotes respectively. This was, however, only a significant increase in the case of Rb7/Rb7 ($\chi^2 = 7.3$, $P < 0.01$). In contrast, the 3H1 mice showed a significant increase to 14.9% MI arrest when nocodazole was applied after 4 h ($\chi^2 = 18.9$, $P < 0.001$).

The pattern of hyperploid oocyte production was similar for Rb7/Rb7 and Rb7/+ mice. There was no significant difference between the two with regard to the increase in hyperploid frequency over spontaneous levels (Fig. 2). There were, however, differences between the Rb mice and the 3H1s. Nocodazole application after 4 h of culture resulted in a significant elevation in hyperploid frequency for all karyotypic groups. Exposure after 6 h gave a significant increase over controls for 3H1s but not Rb carriers. In contrast after exposure at 8 h Rb homozygotes and heterozygotes but not 3H1s, showed significant increases in hyperploidy.

(iii) Chromosome specific effect

The chromosomal complements of the hyperploid oocytes are shown in Table 3. The application of the spindle inhibitor at the early stage not only caused an increased hyperploid frequency in Rb carriers but also a higher incidence of multiple nondisjunction events with up to four bivalents/oocyte being affected. Unfortunately, it is impossible to assess the proportion of nondisjunction events in Rb7/+ mice that are attributable to the trivalent.

The frequency of malsegregated bivalents per hyperploid oocyte for Rb7 homozygotes and 3H1s

Table 3. Chromosomal constitution and origin of the extra chromosome(s) in hyperploid oocytes

Time of nocodazole application (hours)	Number of scorable MIIs	Hyperploid oocytes		Number of chromosome arms present					Malsegregated bivalents/hyperploid oocyte	
		N	%	21	22	23	24	25		
Rb7/Rb7										
Control	101	2	2.0	2 ^a	—	—	—	—	—	1.0
4	86	15	17.4	5 ^a	5 ^d	4 ^c	—	1 ^c	—	1.67
6	78	6	7.7	5 ^e	1 ^b	—	—	—	—	1.0
8	95	11	11.6	9 ^a	2 ^a	—	—	—	—	1.18
Rb7/+										
Control	109	7	6.4	7	—	—	—	—	—	—
4	85	19	22.4	14	1	3	1	—	—	—
6	72	9	12.5	7	2	—	—	—	—	—
8	101	17	16.8	15	2	—	—	—	—	—
3H1										
Control	102	—	—	—	—	—	—	—	—	—
4	93	6	6.5	3	2	1	—	—	—	1.67
6	93	11	11.8	8	2	1	—	—	—	1.36
8	97	3	3.1	8	—	—	—	—	—	1.0

For Rb7/Rb7 homozygotes type of extra chromosome(s): ^a + acrocentric(s) only; ^b + metacentric only; ^c + metacentric + acrocentrics; ^d 3 cells: + acrocentrics only; two cells: + metacentric only. ^e three cells: + acrocentric only, two cells: + metacentric-acrocentric.

Table 4. Nondisjunction products and the involvement of Rbs and acrocentrics (As) after the addition of 0.03 µg/ml nocodazole in Rb7/Rb7 homozygotes

Time of nocodazole addition (hours)	Number of scorable oocytes	Hyperploid oocytes								Hyperploid events		
		Total		+ A only		+ Rb only		+ Rb + A		Total number	Involving As	Involving Rbs
		N	%	N	%	N	%	N	%			
Control	101	2	2.0	2	2.0	—	—	—	—	2	2	—
4	86	15	17.4	8	9.3	2	2.3	5	5.8	25	18	7†
6	78	6	7.7	3	3.8	3	3.8	—	—	6	3	3‡
8	95	11	11.6	11	11.6	—	—	—	—	13	13	—

† $P = 0.0002$; ‡ $P = 0.0025$.

Binomial expansion for $P = 1/19$ where P is the probability of Rbs being involved in nondisjunction events.

Table 5. The presence or absence of Rbs in the segregation products of Rb7/+ (considering only oocytes with 20 chromosome arms)

Karyotype of oocyte				
39, X (with Rb)		40, X (without Rb)		Total N
N	%	N	%	
29	54.7	24	45.3	53

$\chi^2 = 0.31$; $P = 0.58$.

show a maximum of 1.67 after nocodazole application at 4 h (Table 3). For 3H1 all these events inevitably concern nondisjunction of acrocentric bivalents. An analysis of the products of Rb7 homozygotes can also be achieved readily and are summarized in Table 4. After nocodazole treatment at 4 h a large proportion of the hyperploid oocytes showed nondisjunction of the Rb bivalents (7/15), which were significantly more likely to undergo nondisjunction ($P < 0.001$) than would be expected if there was equal chance of each of the 19 bivalents being involved. After nocodazole application at 6 h 50% (3/6) of nondisjunction events involved the Rbs, which was also found to be significantly greater than would be expected by chance ($P < 0.01$). Hyperploids scored for the nocodazole treatment at 8 h only contained extra acrocentrics.

In summary, for Rb7/Rb7 mice, nocodazole application at an early phase of spindle development caused high nondisjunction with many multiple events per oocyte which preferentially involved the Rb bivalent. Spindle disruption at the mid phase caused a low nondisjunction frequency involving single bivalents which were Rbs in 50% of cases. At a late phase there was a medium nondisjunction frequency involving just acrocentric bivalents.

(iv) Segregation distortion

To ascertain whether there was segregation distortion in the heterozygous carriers of Rb7, the euploid

products of meiosis were analysed and are shown in Table 5. No significant transmission of the Rb into the oocyte or polar body was found.

4. Discussion

In this study we considered the chemical induction of meiotic errors in the oocytes of Rb carrying mice. From these results three important points emerged. These were that: (1) the spindle poison nocodazole is an efficient inducer of nondisjunction in its own right and causes a dose-dependent increase in hyperploidy and MI arrest, (2) the phase at which the spindle is inhibited results in a specific pattern of nondisjunction which is different for 3H1 and Rb carriers, (3) in Rb homozygotes the Rb bivalent was affected more substantially than the average acrocentric bivalent, when nocodazole was administered early. Also in our study there was no evidence for segregation distortion in female mice carrying the 'tobacco mouse' meta-centric Rb(16.17)7Bnr.

(i) Nocodazole as an efficient inducer of nondisjunction

Nocodazole disrupts meiosis in a similar manner to the other spindle poisons, colchicine and vinblastin, which interfere with the assembly of the spindle microtubules by inhibiting the polymerization of tubulins (Liang & Brinkley, 1985). It was not unexpected, therefore, that the dose response of the oocytes to the application of nocodazole showed a similar profile to that reported for vinblastine sulphate *in vivo* (Russo & Pachierotti, 1988). Nocodazole has previously been shown to cause meiotic arrest and nondisjunction in mouse oocytes (Generoso *et al.*, 1989). Also due to the reversibility of its action, at higher doses it has been used as a temporary meiotic block (Eichenlaub-Ritter & Boll, 1989).

It was anticipated that after nocodazole application all subsequent stages of meiosis would be disrupted (see Results section). The bimodal pattern that emerged for Rb carriers in Experiment 2 (see Fig. 2) was therefore, surprising as it indicated otherwise. We

can only assume that the disruption caused by nocodazole at such a low concentration, as that used in this experiment, was therefore primarily one of initial impact, and that once it became bound, the pool of available tubulin was reduced, but the spindle was otherwise unharmed. The interpretation of the results, therefore is that nocodazole gave maximum effect when applied immediately prior to or during the most dynamic periods of the lifetime of the spindle. Such stages include chromosome attachment at prometaphase, alignment at metaphase and segregation at anaphase. Thus, the phase at which the spindle was disrupted determined the pattern of nondisjunction and MI arrest.

(ii) *Differential pattern of hyperploidy and MI arrest for 3H1 and Rb carriers*

Oocytes of Rb carrying and 3H1 females showed differences in their response to nocodazole application at any given time (see Fig. 2). A similar differential response has been reported in another study (Pachierotti, Tiveron & Bassani, 1993). They injected Rb(3·8)2Rma heterozygous female mice with 0·06 and 0·09 mg/kgbwv vinblastine sulphate and showed an elevation in the frequency of hyperploidy from 22·2% to 33·1 and 35·9% respectively compared with only a slight elevation from 1·8% to 7·1 and 6·9% in the non-Rb carrying controls. It is not known whether the increase in hyperploidy in the heterozygotes specifically involved the trivalents. In the current study Rb heterozygotes showed a similar response to homozygotes suggesting that the Rb effect was *not* specific to the trivalent.

(iii) *Meiotic behaviour of Rb bivalents*

The difference in response between oocytes from the Rb carrying and 3H1 females is highlighted by the results of the analysis of the nondisjunction products from the Rb7 homozygotes. The Rb bivalent was affected more substantially than the average acrocentric bivalent, when nocodazole was administered early, suggesting that Rb bivalents (and presumably trivalents) are interacting with the microtubules of the spindle at an earlier phase on average than the acrocentric bivalents. This is the first clear evidence for a homozygous mouse that chromosome-spindle dynamics differ between chromosomes. The Rbs behaved differently from the population of acrocentrics, although it is not known to what extent there are differences between different acrocentrics. Unfortunately it was not possible to perform a similar analysis on the nondisjunction products of the heterozygotes, but as both Rb homozygotes and heterozygotes demonstrated a similar biphasic pattern of hyperploidy (Fig. 2) it is probable that the same nocodazole sensitive process was disrupted.

A differential response between chromosomes has also been reported by Hummler & Hansmann (1988),

who showed that after the application of MBC at an early phase of spindle development a high (40·6%) nondisjunction frequency involving all chromosomes resulted. However, with a later application a lower frequency (22·5%) of hyperploidy, was achieved, which preferentially involved the larger A–D groups of metacentric and submetacentric chromosomes.

Hummler & Hansmann (1988) interpreted their results as being due to asynchrony in the segregation pattern of chromosomes with the large bivalents segregating last. In the present study the results gave a high specific nondisjunction frequency with early disruption and lower non-specific nondisjunction later. We suggest that it is not likely to be asynchronous segregation but asynchronous interaction of the Rbs with microtubule fibres of the spindle, during attachment and orientation. Segregation, even if it is asynchronous is such a rapid process it is unlikely to be revealed in our study, especially as anaphase does not commence until at least 8 h after the release of oocytes from their follicles.

(iv) *Differential response pattern of 3H1 and Rb mice is due to an alteration in the kinetics of meiotic progression*

Oocytes from mice of different age groups have been reported to show a differential response to the spindle poisons, colchicine and nocodazole (Tease & Fisher, 1986; Mailhes & Yuan, 1987; Eichenlaub-Ritter & Boll, 1989). This effect has been attributed to changes in cell cycle progression rather than a change in sensitivity of the spindle with age (Eichenlaub-Ritter & Boll, 1989). It is possible that such an alteration in the kinetics of meiosis is also the cause of the differential response at any given time point seen between Rb carriers and non-Rb carriers in this study and that of Pachierotti *et al.* (1993).

The question is, what causes such an alteration in the kinetics of meiosis? Whatever the cause, it is not an effect limited to the Rb trivalent; both heterozygotes and homozygotes undergo a similar pattern of meiotic progression. It is therefore, likely that the alteration is the result of the introduction of a dominant factor carried by the original Rb/Rb stock. This factor could be genetic and/or chromosomal.

(v) *Possible genetic causes of an alteration in the kinetics of meiosis*

Genetic differences could arise from two sources. First, some of the original tobacco mouse genes and other DNA sequences may still exist in the centromeric region of Rb7, despite this chromosome having been introduced into laboratory mouse background by many generations of backcrossing. Secondly, although the Rb was introduced into the same 3H1 background as the all-acrocentric 40 chromosome mice used in our study, the Rb/Rb mice will be more genetically homozygous due to maintenance as a homozygous

line. Rb/Rb, Rb/+ and +/+ mice are, therefore, genetically related but not identical. It is possible that a change in gene expression may alter some physiological parameter thereby altering the progression of meiosis and causing nondisjunction. In aged females changes in protein phosphorylation have been proposed as a cause of the loss of cell cycle control (Eichenlaub-Ritter, 1994). Equally, differences in protein synthesis or modification could explain the differential rates of meiotic progression in Rb carrying and non-carrying oocytes.

(vi) *Chromosomal factors causing an effect on rate of meiotic progression*

In order to see how chromosomal factors could alter the rate of meiosis in Rb carriers, the progression of meiosis in the 3H1 oocytes must be considered. The asynchronous nature of oocyte maturation *in vitro* means that any disturbance at a specific point of the cell cycle will appear normally distributed. The hyperploidy frequency exhibited such a pattern with maximum effect when the nocodazole was applied after 6 h (Fig. 2). Possible nocodazole sensitive events are chromosome attachment, alignment and segregation. Anaphase I does not occur until at least 8 h after the onset of oocyte maturation in 3H1 mice (Fig. 1), the disturbance centring around the 6 h maximum in 3H1 mice is therefore likely to be that of attachment and/or alignment of the chromosomes on the spindle.

The main difference between the response of Rb carrying and 3H1 oocytes to nocodazole is the biphasic distribution of hyperploidy evident in the latter. It is possible that this pattern may be the combined effect of two independent events. The first of these may be the great sensitivity of the Rb bivalents to nocodazole applied after 4 h, the second being the normal association of acrocentrics with the spindle which showed a similar profile to that of the hyperploidy distribution for 3H1 oocytes but with a 2 h delay (see Fig. 2).

We suggest that it is possible that this early Rb bivalent–spindle interaction inhibits attachment of the acrocentrics, and hence introduces a delay in meiotic progression in Rb mice compared with 3H1 individuals.

(vii) *Other considerations*

One question that remains unanswered is whether we are seeing a phenomenon that also occurs *in vivo* or one that is specific to *in vitro* conditions. The development of the oocytes in culture is free-running and not under proper hormonal control and so there is no external restriction on the rate of maturation. *In vivo* the oocyte must progress in synchrony with the follicle cells. *In vivo* conditions may, therefore, cause additional problems for the Rb carrying oocytes. This may explain our lower spontaneous nondisjunction frequency of 12.8% ($2 \times$ hyperploidy value) for the

oocytes of Rb7 heterozygotes matured *in vitro* compared with the previous estimate of 34% *in vivo* (Redi & Capanna, 1988). A similarly low nondisjunction frequency (6.6%) has been recorded for *in vitro* matured oocytes of Rb(9.12)163H heterozygotes (Everett, 1992).

This difference between *in vitro* and *in vivo* maturation of oocytes may explain the lack of segregation distortion. Gropp & Winking (1981) found that female Rb7 heterozygotes preferentially transmitted acrocentrics to their progeny, while we found no segregation distortion in our Rb7/+ mice. It may thus be entirely an *in vivo* specific phenomenon although the effect of different genetic backgrounds cannot be ruled out.

(viii) *Conclusion*

In conclusion, it is apparent that the introduction of Rbs into the mouse karyotype not only introduces heterogeneity in the way of chromosome type (metacentric and acrocentric) but also in the timing of the interaction of chromosomes with the meiotic spindle in oocytes. These results add to those of Hummler & Hansmann (1988) who demonstrated a similar effect in Djungarian hamsters. Differences between chromosomes in the timing of their interaction with the spindle may generate different susceptibilities to nondisjunction. This is of interest from a medical perspective as human chromosomes vary in their nondisjunction frequencies (Hassold & Sherman, 1993; Jacobs, 1992).

The delay in meiotic progression revealed in Rb homozygous females requires further investigation. Studies on wild mice with Rbs (all wild genome) would be worthwhile to untangle the genic and chromosomal effects. We did attempt a second study of the laboratory derived metacentric Rb(9.12)163H but this failed due to high frequencies of MI arrest in our oocyte preparations (Everett, 1992).

The fact that the Rb7 homozygotes did show meiotic delay furthers the value of such mice as biomedical models for nondisjunction. It is widely thought that delay may promote nondisjunction *in vivo*, both in spermatocytes and oocytes (Eichenlaub-Ritter, 1994; Mailhes & Marchetti, 1993; Miller & Adler, 1992; Russo & Pachierotti, 1988). Surprisingly there appears to be no information on the nondisjunction frequency *in vivo* for female mice homozygous for feral-derived metacentrics in a laboratory strain genetic background (despite a lot of information on heterozygotes, as reviewed by Redi & Capanna, 1988). We suggest that it might be possible to construct homozygotes for one or more Rb metacentrics with nondisjunction frequencies close to those suffered by human females.

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