Stage-dependent changes of chromosomal radiosensitivity in primary oocytes of the Chinese hamster

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Chromosomal studies on X-ray-exposed oocytes have shown that structural chromosome aberrations can be induced in female mice (Searle and Beechey, 1974; Caine and Lyon, 1977; Brewen and Payne, 1979). Incidences of the aberration yields rose with dose but fluctuated with time after the exposure. Chromosomal radiosensitivity was shown to be dependent on the interval between irradiation and the time when meiotic chromosomes were studied. So far, the time factor has been analyzed at relatively long intervals, i.e., weeks and days. In these studies, it was found that chromosomes of oocytes within small, multilayered follicles were more prone to be damaged by radiation than those within large, growing follicles. However, there is a report indicating that meiotic chromosomes are highly susceptible

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The aim of the present experiment is to clarify the aspects of alteration of chromosomal radiosensitivity during the spontaneous estrous cycle by assessing mitotic chromosomal lesions induced by X-irradiation at various stages prior to ovulation. Such a study is possible only with animals whose estrous cycles persist regularly.

Virgin female Chinese hamsters, 5–6 mo of age, were used. They were raised in our closed colony, which is kept under constant laboratory conditions: 14 h illumination, from 0500 to 1900 hours; temperature, $23 \pm 2^\circ \text{C}$; and humidity, 50–60%. Under these conditions, mature females can well maintain a stable 4-day estrous cycle. In this colony, the surge of luteinizing hormone (LH) starts at 1400–1500 of the day of proestrus; thereafter, breakdown of germinal vesicle occurs within 2 h. Ovulation takes place at 0330–0430 hours on the day of estrus. Vaginal smears of each animal were taken daily for at least two estrous cycles in order to confirm their regularity and to determine the time of irradiation.

The lower abdomen, including the ovaries, was exposed locally to 200 rad X-irradiation (220 KVP; 20 mA; HVL, 2.06 mm Cu; filter, 1.2 mm Cu + 0.5 mm Al; 40 rad/min). The rest Chromosomal radiosensitivity in oocytes

of the body was shielded by 6-mm lead plates. This dose was selected because higher doses were found to cause difficulty in analyzing aberrations due to the frequent occurrence of extremely damaged chromosomes in highly sensitive stages. Fifteen stages before ovulation were chosen for irradiation, as shown in table I. The phase of oogenesis at each stage was confirmed histologically. The last stage (stage XV, 0130 hours on the day of estrum) was judged to be the end of the first meiotic division, since many of the oocytes passed from telophase to the stage of first-polar-body emission.

The MII oocytes were recovered by squeezing the oviduct ampulla about 5 h following ovulation. Chromosomal preparations were made by the method previously described (Kamiyuchi et al., 1976). Nearly 90% of the collected oocytes were karyotyped successfully. The numbers of females irradiated, the numbers of secondary oocytes analyzed, and the incidence of oocytes with structural chromosome aberrations in each irradiation group are shown in table II. The types of chromosome aberrations observed included breaks, gaps, fragments, deletions and exchanges. Their frequencies per 100 oocytes are also shown in the table. Although the numbers of oocytes analyzed are relatively small, the variable radiosensitivity during the estrous cycle is clearly shown, as expressed in the percentages of oocytes with structural chromosome aberrations. During the period from stage I to stage VI, there was no significant increase in the percentage of abnormal oocytes (2.4–3.9%), although their incidence was slightly higher than that of nonirradiated controls (1.7%). The effect of irradiation seems to be little, if any, indicating that the oocytes within growing follicles are apparently resistant to acute X-irradiation. Two hours later (stage VII), i.e., a few hours before the onset of LH surge, however, the radiosensitivity of the oocytes became appreciable ($0.05 < P < 0.1$, χ^2 with Yates' correction) and reached a maximum

Table I. Stages of X-irradiation during the first meiotic division

Stage of Day Time Hours before Phase of oogenesis irradiation

ovulation

I

Estrum
1530
84.5
Dictyotene
11

Diestrum **I**
0530
70.5
Dictyotene
III

Diestrum I
1730
58.5
Dictyotene
IV

Diestrum II
0530
46.5
Dictyotene
V

Diestrum II
1730
34.5
Dictyotene
VI

Proestrus
0530
22.5
Dictyotene
VII

Proestrus
0930
18.5
Dictyotene
VIII

Proestrus
1130
16.5
Dictyotene
IX

Proestrus
1330
14.5

Dictyotene
X

Proestrus
1530
12.5
Onset of breakdown of germinal vesicle
XI

Proestrus
1730
10.5
Early diakinesis
XII

Proestrus
1930
8.5
Late diakinesis
XIII

Proestrus
2130
6.5
MI
XIV

Proestrus
2330
4.5
Late MI-anaphase I
XV

Estrus
0130
2.5
Telophase I-polar body I emission
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Table II. Incidences of structural chromosome aberrations in MI oocytes of the Chinese hamster after X-irradiation (200 rad)

Stage of irradiation	Number of animals used	Number of oocytes analyzed	Oocytes with		Types of aberrations and their		
			MI	Percent	Break	Gaps	Fragments

Control	38	295	5	1.7	0.3	—	0.3
I	16	127	4	3.1	0.8	-	1.6
II	15	120	3	2.5	-	-	1.7
III	14	125	3	2.4	-	-	1.6
IV	16	131	5	3.8	0.8	-	1.5
V	15	122	4	3.3	0.8	-	0.8
VI	14	102	4	3.9	2.9	-	1.0
VII	15	114	6	5.3	0.9	0.9	0.9
VIII	15	117	14	12.0	3.4	0.9	5.1
IX	15	116	13	11.2	2.6	0.9	5.2
X	15	108	36	33.3	16.7	2.8	13.0
XI	16	117	51	43.6	29.9	10.3	14.5
XII	15	121	41	33.9	18.2	5.0	10.7
XIII	15	122	36	29.5	12.3	6.6	13.1
XIV	16	121	22	18.2	12.4	4.1	7.4
XV	13	94	25	26.6	19.1	9.6	2.1

I The number and types of aberrations in these oocytes could not be determined with accuracy, since their chromosomes were extremely damaged, with multiple breakages, exchanges, etc. Therefore, these particular cases were not included in the estimation of the aberration frequencies.

at early diakinesis (stage XI), when the affected oocytes amounted to 43.6% of the oocytes analyzed. It then decreased quickly over a period of 6 h (18.2% at stage XIV). Within two more hours, at the end of the first meiotic division, it again increased to a remarkable extent (26.6%). It was also found that incidence of oocytes having plural aberrations increased remarkably during the highly sensitive period (stage X-XV), as shown in the analysis of aberration frequencies per 100 oocytes (table II).

To the best of our knowledge, no work has been done to determine the precise timing of dynamic changes of chromosomal radiosensitivity in mammalian oogenesis

during the spontaneous estrous cycle. If the experimental animals were not assured of their regular cycle, the results could not be relied upon. Our closed colony of Chinese hamsters has been improved by selective matings to maintain stable 4-day cycles. Furthermore, as mentioned earlier, the animals used in the present work were chosen by means of daily smears to include only those who maintained regularity.

Thanks to our experimental animals, we were able to observe the interesting phenomenon that meiotic chromosomal radio-sensitivity changes dramatically in oocytes during the period from the onset of the resumption of the first meiotic division to the

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frequencies per 100 oocytes

Deletions Exchanges Total Remarks

1.0

-

1.7

0.8

-

3.1

1.7

-
 3.3
 0.8
 -
 2.4
 1.5
 -
 3.8
 1.6
 -
 3.3
 -
 -
 3.9
 2.6
 0.9
 6.1
 1.7
 0.9
 12.0
 3.4
 -
 12.1
 8.3
 1.9
 42.6
 19.7
 4.3
 78.6
 3.3
 0.8
 38.0
 4.1
 0.8
 36.9
 3.3
 -
 27.3
 4.3
 1.1
 36.2

end of the division, and that it was strikingly high at early diakinesis. A high radiosensitivity has been shown in mice 3 h after the injection of human chorionic gonadotropin following administration of pregnant mare serum (Reichert et al., 1975). (This is the stage in which the oocytes pass from late dictyotene to diakinesis.) From their result it could not be concluded that this particular stage is the most radiosensitive, since in their experiment the irradiation was

restricted only to this single stage. Nevertheless, their results and ours generally coincide well with each other, with respect to the strikingly radiosensitive meiotic stage. It is also known that diakinesis is one of the most radiosensitive phases in spermatogenesis (Walker, 1977). In dominant lethal tests with mice (Russell and Russell, 1956; Edwards and Searle, 1963) and rats (Mandl, 1963), it has long been known that there is the most radiosensitive stage at or near meta-phase of the first meiotic oocyte division. With regard to the time of greatest radio-sensitivity, it is apparent that there is approximately 3 hours of discrepancy between the studies of dominant lethals and those of structural chromosome aberrations, since primary oocytes of these common laboratory rodents require 3–4 hours from early diakinesis to metaphase. A dominant lethal test is under way with our Chinese hamsters in an attempt to discover the cause of this discrepancy.

In order to elucidate causes for the change in meiotic chromosomal radiosensitivity during oogenesis, investigation of the factors involved in the ability of damaged DNA to repair itself would be a promising approach. Unscheduled DNA synthesis has been assessed in mouse oocytes after exposure in vitro to ultraviolet rays, with the finding that the repair capacity, while similar at MI and MII, was considerably lower than at the germinal vesicle stage (Masui and Pedersen, 1975).

As to the risk in human reproduction with respect to the effects of ovarian irradiation, the sensitivity of dictyate oocytes within resting follicles is an important medical concern because of the characteristic length of human oogenesis, in which usually about 20 yr or more elapse before an ovum is fertilized. However, it should be remembered that there may also be a period in women shortly before ovulation when radiosensitivity of oocyte chromosomes increases strikingly. In the present report, nondisjunctional

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outcomes were not mentioned, although they are also of great concern. Owing to the time-consuming method used, the samples of each stage are still limited, and relatively small numbers of oocytes have been analyzed at the moment. Discussion of the induction of aneuploids must be postponed until samples become sufficiently numerous for statistical evaluation.

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