Chromosome analysis of early postimplantation presumptive haploid parthenogenetic mouse embryos

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SUMMARY

The chromosome constitution of early postimplantation presumptive haploid parthenogenetic mouse embryos was examined. All the embryos isolated were at the egg-cylinder stage and seven contained dividing cells. In two of the apparently healthy embryos only haploid mitoses were seen, whereas in five others an approximately equal proportion of haploid and diploid mitoses was observed. Out of 52 cells in which unequivocal counts could be made, only one contained more than the euploid number of chromosomes (mouse, n = 20). Possible reasons for the poorer development of haploid compared to diploid parthenogenetic embryos are discussed.

INTRODUCTION

The aim of the present study was to investigate whether parthenogenetically activated haploid mouse embryos contain actively dividing haploid cells in the early postimplantation period.

Previous attempts to study the chromosome constitution of presumptive haploid postimplantation embryos have always been unsuccessful, primarily because of technical difficulties associated with their extremely poor development. Tarkowski and his colleagues (Tarkowski, Witkowska & Nowicka, 1970; Witkowska, 1973) obtained reasonable rates of parthenogenetic development to the egg-cylinder stage, but their experimental approach did not allow the original ploidy of these embryos to be determined. Recently a novel experimental approach (Kaufman, Barton & Surani, 1977) has allowed a high proportion of parthenogenetically activated diploid embryos to reach the egg-cylinder stage, and a more modest proportion to develop to the forelimb-bud stage, equivalent to about day 9.5–10.5 of normal (fertilized) development. This experimental approach was used in the present investigation to obtain presumptive haploid egg-cylinder stage embryos.

Embryos which undergo immediate cleavage (Braden & Austin, 1954) were studied. These result when parthenogenetically activated eggs divide into two equal sized blastomeres instead of extruding a second polar body. Each blasto-

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mere therefore contains one of the products of the second meiotic division. These are genetically dissimilar as a result of crossing over events at the first meiotic division, and consequently give rise to two distinct clones of cells within the cleaving embryo. This has accordingly been termed mosaic haploid development (Kaufman & Sachs, 1976). Some of the theoretical reasons which might account for the observed developmental advantage of immediate cleavage embryos over the single pronuclear type, where the nucleus of each cell is genetically identical, have been considered elsewhere (Kaufman & Sachs, 1976).

In a preliminary study immediate cleavage-derived egg cylinders were examined histologically. Many of the cells in mitosis appeared to have far fewer chromosomes than are usually seen in comparable diploid material. However, because of the obvious difficulties involved in attempting to extrapolate cytogenetic data from histological material, air-dried preparations from disaggregated egg cylinders were examined in the present study.

**MATERIALS AND METHODS**

Cumulus masses containing recently ovulated oocytes were isolated from the oviducts of 8- to 12-week-old (C57BL × CBA) F₁ hybrid female mice at 18–19 h after the HCG injection for superovulation, and released into modified Krebs–Ringer bicarbonate embryo culture medium lacking both calcium and magnesium salts (Kaufman et al. 1977; Surani & Kaufman, 1977). About 5–6 h later the cumulus cells were removed with hyaluronidase after a 5–10 min incubation period in standard embryo culture medium (Whittingham, 1971) containing 100 i.u./ml of this enzyme. This treatment facilitated examination of the eggs and enabled the activation frequency and types of parthenogenones induced to be determined.

Only the eggs which underwent immediate cleavage following activation were further studied. Eggs of this type were transferred to standard embryo culture medium and incubated for a further 90 h. A proportion developed to the blastocyst stage, and was subsequently transferred to a single uterine horn of day-3 pseudopregnant recipients previously mated to proven sterile vasectomized males (day of finding vaginal plug = day 1 of pseudopregnancy). Directly after the transfer procedure had been carried out recipients were bilaterally ovariectomized and thereafter maintained on exogenous steroid hormones. After an initial hormone-free period of 2 days recipients were maintained for 2 days on 1 mg progesterone daily, followed by three daily injections of 20 ng oestradiol and 1·6 mg progesterone daily per female.

Recipients were killed on the ninth day after the embryo transfer procedure, after 3 days of oestrogen injections. All uterine horns containing implantation sites were removed and the decidua placed in phosphate buffered saline. Embryos were dissected out under a dissecting microscope using watchmaker's forceps, care being taken to isolate the egg-cylinder (embryonic) region from the
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Table 1. The pathways of development of parthenogenetic eggs at 5–6 h after in vitro activation

<table>
<thead>
<tr>
<th>Total number of eggs examined</th>
<th>1 pronucleus + 2nd polar body</th>
<th>2 pronuclei without 2nd polar body</th>
<th>1 pronucleus without 2nd polar body</th>
<th>Immediate cleavage</th>
<th>Overall activation frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1375</td>
<td>205 (20.4)*</td>
<td>449 (44.7)</td>
<td>15 (1.5)</td>
<td>335 (33.4)</td>
<td>73.0</td>
</tr>
</tbody>
</table>

* The percentage of the total number of eggs activated is given in parentheses.

eroplacental cone region. Only the egg-cylinder region was further examined.

Air-dried preparations of the disaggregated egg cylinders were made by the technique described by Wroblewska & Dyban (1969). With the exception of the initial hypotonic citrate stage, where 30 min treatment was found to be optimal, all other stages were considerably shortened. Thus 2–3 min fixation in 3:1 ethanol-acetic acid mixture, and about 10 min pre-staining in 2 % orcein in 50 % acetic acid was found to be adequate. Complete disaggregation usually took place after 10–20 min in the 50 % lactic-acetic acid solution. The slides were subsequently stained with 2 % orcein and the air-dried material examined by phase-contrast microscopy.

RESULTS

When eggs were examined after incubation for 5–6 h in medium lacking calcium and magnesium salts, the activation frequency was 73.0 %, and 33.4 % of the activated eggs were of the immediate cleavage type (Table 1). After a further 90 h incubation period in standard embryo culture medium 112 of the immediate cleavage eggs developed to the blastocyst stage. A total of 96 of these presumptive haploid embryos were transferred to 16 pseudopregnant recipients. Fourteen females contained implants at autopsy, and the implantation rate in the pregnant females was 63.2 %. Out of a total of 48 implants ten egg-cylinder stage embryos were isolated. The embryos ranged in size from early to advanced egg cylinders (Fig. 1 a), being approximately equivalent to fertilized embryos of between 6.5 and 7.5 days gestation. Seven of the embryos were apparently healthy and contained between 1 % and 4 % dividing cells (Table 2). Three additional egg cylinders were obviously unhealthy, containing a high proportion of pyknotic nuclei and no cells in division.

The incidence of haploid and diploid metaphases in the healthy egg cylinders varied considerably between embryos. Two embryos had only haploid metaphases, whereas in the remaining five about half of the cells in division were haploid and the rest diploid. A single tetraploid cell was encountered. Out of
Fig. 1. For legend see opposite.
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Table 2. The ploidy of seven morphologically normal presumptive haploid egg cylinders

<table>
<thead>
<tr>
<th>Embryo</th>
<th>Stage of development</th>
<th>Total cells examined*</th>
<th>Ploidy of cells in mitosis†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Haploid</td>
</tr>
<tr>
<td>1</td>
<td>Advanced egg cylinder</td>
<td>&gt; 1100</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>Advanced egg cylinder</td>
<td>&gt; 1000</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Advanced egg cylinder</td>
<td>&gt; 1000</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>Small egg cylinder</td>
<td>&gt; 400</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Small egg cylinder</td>
<td>350–400</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Small egg cylinder</td>
<td>250–300</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>Small egg cylinder</td>
<td>180–200</td>
<td>3</td>
</tr>
</tbody>
</table>

* These values provide only an approximate estimate of the total cell population in these embryos as many cells were lost during the disaggregation procedure.
† The ploidy of 35 out of a total of 51 haploid cells and 21 out of a total of 57 diploid cells were scored without being able to make an unequivocal count of the chromosome number.

DISCUSSION

The present study is the first to conclusively demonstrate that presumptive haploid embryos may contain a population of actively dividing haploid cells at the egg-cylinder stage. Further, that in those cells in which unequivocal counts could be made, almost all contained the normal haploid (mouse, \( n = 20 \)) or diploid number of chromosomes. In the absence of more sophisticated techniques of chromosome analysis it is, of course, impossible to know whether segments of chromosomes were lost or were involved in rearrangements as was apparent in the chromosomally diploid teratoma cell lines examined by Iles et al. (1975) which were derived from presumptive haploid embryos.

Fig. 1. (a) Transverse section of presumptive haploid advanced egg-cylinder stage embryo approximately equivalent to fertilized embryo of between 6.5 and 7.5 days gestation. (b) Air dried preparation of haploid mitosis from disaggregated egg-cylinder stage embryo. (c) Diploid mitosis from disaggregated haploid–diploid mosaic egg-cylinder stage embryo. (d) Tetraploid mitosis (in early anaphase) from disaggregated haploid–diploid–tetraploid mosaic egg-cylinder stage embryo. (e) Haploid metaphase figure (arrowed) from same embryo as shown in Fig. 1(a). (f) Diploid metaphase figure (arrowed) from presumptive diploid parthenogenetic embryo at the egg-cylinder stage.
Previous studies (Kaufman, unpublished) have indicated that the cleavage rate of haploid embryos is often somewhat slower than that of diploid parthenogenones during the second half of the preimplantation period. In addition, recent observations on the number of cells present in the inner cell mass (ICM) of immediate cleavage-derived haploids, heterozygous diploid parthenogenones and fertilized mouse embryos at the expanded blastocyst stage (Kaufman and Surani, unpublished observations) have indicated that the haploids usually contain fewer cells than diploid parthenogenones, and that the ICMs of the diploids generally contain less than 50% of the number normally present in fertilized blastocysts. Ansell & Snow (1975) have suggested that a critical minimal cell number is required in the ICM for normal embryonic development. Probably fewer haploid than diploid parthenogenones would have achieved this even after several cell divisions. Presumably these relatively ICM-deficient presumptive haploid blastocysts would be capable of evoking a decidual response with, on closer inspection, only minimal evidence of embryonic development. These observations might well explain the lower rate of development to the egg-cylinder stage of the presumptive haploids in the present study (15%) compared to 35% achieved by diploid parthenogenones subjected to similar experimental treatment (Kaufman et al. 1977).

The incidence of apparently completely haploid postimplantation embryos observed in this study, though small, suggests that the haploid state is compatible with a considerable degree of cellular differentiation and apparently normal morphogenesis. A more extensive study will obviously be required to clarify whether the presence of diploid cells conveys any form of developmental advantage to the cells which remain haploid, and to determine whether a relationship exists between the incidence of diploidy and the degree of development which may be achieved in haploid–diploid mosaic embryos.

The relative stability of haploid cells from fungi, higher plants and anuran species (Freed & Mezger-Freed, 1970; Gupta & Carlson, 1972) suggests that the problems associated with the production of a stable haploid mammalian cell line (see Graham, 1974) should not be insurmountable. The two approaches which hold out most promise at the present time, via transplantable teratocarcinomas (Iles et al. 1975) and through the production of cell lines from preimplantation (Hogan & Tilly, 1977) and early postimplantation embryos, are both under active investigation. Attempts are also being made to examine more advanced presumptive haploid parthenogenetic mouse embryos.

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REFERENCES


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