THE CHROMOSOMES OF TURKEY EMBRYOS DURING EARLY STAGES OF PARTHENOGENETIC DEVELOPMENT

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ABSTRACT

In the early stages of parthenogenetic development in turkey eggs, many blastoderms are mosaics of haploid, diploid and polyploid cells. The genome composition of these blastoderms can be identified by C-banding. They may be generally described as either $A-Z/2A-ZZ/nA-nZ$ or $A-W/2A-WW/nA-nW$ and are found in a nearly 1:1 ratio. The blastoderms showing the $W$ body ($W^+$) become lethal within two days of incubation. The haploid cell proportion decreases rapidly during the early stage of development, and, as haploid cells decrease, the proportion of polyploid cells appears to increase. At six days of incubation, various kinds of parthenogenetic development can be observed. Their genome compositions are either diploid ($2A-ZZ$) or mosaic ($A-Z/2A-ZZ$). These findings suggest that diploid parthenogenesis occurs by either suppression of meiosis II or chromosome doubling some time after the first cleavage division. The frequent occurrence of mosaic blastoderms indicates that the majority, if not all, of the parthenogenetic embryos initiate their development in haploid ova.

An abortive type of development in unfertilized turkey eggs was first observed by OLSEN and MARSDEN (1953) during the course of fertility checks in a flock of unmated Beltsville Small White (BSW) turkey females. A selective breeding program designed to intensify this trait raised the incidence of parthenogenetic development upon incubation from 16.7% in 1952 to approximately 45% in 1963 (OLSEN 1965). Embryos produced in 1962 were found in 13% of the eggs set, and 8.8% of these embryos reached hatching stage (OLSEN 1965). Anatomical inspection showed that all pouls hatched or nearly hatched were males (POOLE and OLSEN 1957).

Early cytological studies by YAO and OLSEN (1955), and POOLE (1959) were aimed at detecting the ploidy of parthenogens; they found only the diploid number of chromosomes in parthenogenetic embryos and adults. SATO and KOSIN (1960) examined parthenogenetic embryos after six days of incubation and reported that the embryos were mosaics of haploid ($A-Z$) and diploid ($2A-ZZ$) cells. The finding of mosaicism of parthenogenetic embryos in the early stage of

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development suggested that diploid parthenogenesis originated from haploid ova and subsequent chromosome doubling (Sato and Kosin 1960; Darcy et al. 1971). However, heterozygosity reported in histocompatibility loci (Poole et al. 1963) and a down-color gene (Olsen 1966) provides the possibility of an alternative mechanism of diploid parthenogenesis, i.e., suppression of meiosis II.

The present study was undertaken to determine the cell composition of the early stages of parthenogenetic embryos. The proportion of haploid and heteroploid cells in mosaic embryos and the sex-chromosome constitution were of primary concern.

MATERIALS AND METHODS

Turkey stocks and collection of eggs: Two groups of eggs were collected from virgin 1-year-old hens of the high-incidence parthenogenetic lines of BSW turkeys. Eggs were collected in each of the following stages of incubation: premature eggs in the uterus, which were estimated to be 20 hr after ovulation, newly laid eggs, eggs at 1 day of incubation and eggs at 2 days of incubation. The only visibly detectable difference observed in the eggs at these stages was the size of the blastodiscs. Thus, the specimens of these eggs were designated as “blastodisc materials.” Premature eggs were obtained by intracutaneous injection of 0.25 unit of arginine-vasosotonin 20 hr after the previous oviposition. The other group of eggs was collected at 6 days of incubation. Various degrees of embryonic development were observed at this stage; thus, the materials were called “embryonic materials.”

Mitotic arrester: From 0.05 to 0.1 ml of colchicine (0.05%) was injected around the embryonic area, and the eggs were incubated for 45 min at 41° before harvesting. No mitotic arrester was used for the blastodisc materials.

Slide preparation: Slides were prepared from these materials after hypotonic treatment with 0.9% sodium citrate for 15 min and subsequent fixation with 1 part acetic acid and 3 parts methanol. After the removal of albumen, the blastodisc materials attached to the whole yolks were treated with the hypotonic solution and subsequently fixed. Then, the blastodiscs were isolated with a dissecting needle and placed in a small amount (0.05 ml) of the fixative. For both materials, specimens were transferred from fixative to 45% acetic acid just prior to slide preparation. After gentle pipetting several times, a drop of cell suspension was placed on slides prewarmed at 45°.

Staining method: The C-banding (Sumner 1972) was employed for detecting sex chromosomes. Saturated barium hydroxide, 8-hydrate was used as denaturing agent.

RESULTS

Cytological events occurring in unfertilized turkey eggs were examined at two separate, relatively early stages of incubation, i.e., in the blastodisc materials and in embryonic materials.

Blastodisc materials

A total of 121 eggs produced by 28 one-year-old virgin hens were examined at this stage. They were composed of 15 premature eggs, 64 newly laid eggs at one day of incubation, and 26 eggs at two days of incubation. The premature eggs were expelled 20 hours after estimated ovulation. Nucleated cells were observed in 110 of the total of 121 eggs. Two morphologically different kinds of cells were identified in these blastoderms: (1) surface blastomeres of regular round nuclei...
and (2) large yolk-laden blastomeres. The former group of cells were more numerous and, in many cases, were mitotically active. Abortive change, such as fragmentation, was developing in the latter group of cells. Chromosome numbers were counted in well-spread metaphase cells. Ploidy of the cells was determined by counting the number of each of the five largest chromosomes. Many of the blastoderms were found to be mosaics of haploid, diploid and heteroploid (mainly polyploid) cells. The $W$ sex chromosome, which is similar in size to the seventh or eighth submetacentric chromosome and is heterochromatic along its whole length, was detected among these blastoderms by C-banding. Both $Z$ and $W$ chromosomes were found in eggs of this group; however, the eggs carried either $Z$ or $W$ chromosomes exclusively and formed mosaics of $A-Z/2A-ZZ/nA-nZ$ or $A-W/2A-WW/nA-nW$ ($n$ indicates the ploidy of polyploid cells) (Figure 1). No eggs carrying both $Z$ and $W$ chromosomes as either diploids ($2A-ZW$) or mosaics were found. The $W$ body, which is shown to be the heterochromatic $W$ chromosome in female turkeys (Bloom 1974), was found in interphase cells of $W$-chromosome-carrying blastoderms. The blastoderms that had a $W$ body in interphase nuclei were designated as "$W^+$"; and the blastoderms that had no $W$ body were designated as "$W^-$." In $W^+$ blastoderms, interphase cells having one, two and more than two $W$ bodies were found, and were assumed to be haploid, diploid and polyploid cells, respectively. Examination of the sex chromosome and the $W$ body in this group of eggs is summarized in Table 1. Among 110 eggs that showed parthenogenetic cell division, 55 were $W^+$ and 49 were $W^-$; the ratio was not significantly different from 1:1 ratio by chi-square test ($x^2=0.346$, d.f.=1, $P>0.50$). Moribund change was observed in $W^+$ eggs at two days of incubation: chromosomes of mitotic cells stuck together and formed a chromatic mass. Diam-

![Figure 1](image_url)

**Figure 1.**—Metaphase cells of parthenogenetic blastoderms following banding. a. Metaphase plate of haploid cell ($A-W$). Note one heterochromatic $W$ chromosome. X1800. b. Metaphase plate of diploid cell ($2A-WW$). Note two heterochromatic $W$ chromosomes. X1800.
TABLE 1

Parthenogenetic development and sex-chromosome composition of unfertilized turkey eggs at the early stage of incubation

<table>
<thead>
<tr>
<th>Stage*</th>
<th>No. eggs examined</th>
<th>No. eggs with nucleated cells</th>
<th>W+</th>
<th>W-</th>
<th>Unidentified</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 hrs.</td>
<td>15</td>
<td>10</td>
<td>5(5)</td>
<td>4(4)</td>
<td>1</td>
</tr>
<tr>
<td>N.L.</td>
<td>64</td>
<td>59</td>
<td>30(26)</td>
<td>26(22)</td>
<td>3</td>
</tr>
<tr>
<td>1D.I.</td>
<td>16</td>
<td>15</td>
<td>9(7)</td>
<td>5(5)</td>
<td>1</td>
</tr>
<tr>
<td>2D.I.</td>
<td>26</td>
<td>26</td>
<td>11(9)</td>
<td>14(12)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>110</td>
<td>55(40)</td>
<td>49(43)</td>
<td>6</td>
</tr>
</tbody>
</table>

*20 hrs.; Twenty hours after ovulation. N.L.: Newly laid. 1D.I.: One day of incubation. 2D.I.: Two days of incubation.

† The number shown in the parentheses is the number of eggs whose sex-chromosome composition was identified as A-W/2A-WW/nA-nW in W+ blastoderms and A-Z/2A-ZZ/nA-nZ in W- blastoderms.

eters of the blastodiscs were measured before fixation for 16 of the two-day-old eggs. Nine of them were W+, and seven were W-; the means of blastodisc sizes were 3.33 mm and 4.64 mm, respectively. The difference was significant at 1% level by student's t-test (t=3.24, d.f.=14).

The number of haploid, diploid and polyploid cells was counted for each blastoderm. A total of at least 30 metaphase cells was counted for each egg. The averages were 98 and 62 for the newly laid eggs at one and two days of incubation, respectively. Then, the percentage of haploid and polyploid cells was calculated for each blastoderm. The frequency distribution of haploid cells is illustrated in Figure 2. It is noticed that in newly laid eggs, the distribution is bimodal: the major peak contains highly haploid blastoderms (mode at 80–90%) and the minor peak, highly diploid blastoderms (mode at 0–10%). No minor peak was detected in eggs at one and two days of incubation; this may have resulted from the small sample size. Means for the haploid and polyploid cell proportion were calculated at each stage of incubation (means for newly laid eggs represent blastoderms of the major peak). The means for premature eggs were derived from the combined total cell count of eight eggs. The changes of mean haploid and polyploid cell proportions are illustrated in Figures 3 and 4, with their 95% confidence intervals, respectively. It is clear that the mean proportion of haploid cells decreased rapidly during the early stage of incubation; whereas, the mean proportion of polyploid cells appeared to increase during this stage. Similar spectra in the change of proportion of haploid and polyploid cells were observed in W+ and W- blastoderms.

Embryonic materials

Eggs were collected every day and held in a cooler for one to four days prior to setting in an incubator. A total of 624 eggs were collected from 19 one-year-old virgin hens of the high-incidence line of BSW turkey stocks. At six days of incubation, a total of 262 eggs (42%) showed visibly detectable development of various kinds, which were classified in the following four categories:
1. Normal-type embryos: These embryos were phenotypically normal and further classified by their size compared with the embryos showing standard development after normal fertilization. Thus, they were designated as one-day equivalent embryos, two-day equivalent embryos, and so on.

2. Unorganized embryonic tissues: This category included embryos showing abnormal development, such as extreme growth retardation or malformation.

3. Blood islets: This category included an abortive type of development that had blood rings or blood vessels, but no embryo formation.
FIGURE 3.—Change in haploid cell proportions in parthenogenetic blastoderms during the early stage of incubation. The duration of incubation is shown by estimated hours after ovulation.

4. Embryonic membranes: This category included the development of the sheet of spreading tissues covering the surface of the yolk, but no embryos. Eggs showing no visibly detectable developments were classified as "undeveloped."

Chromosome composition of these embryos, except embryonic membranes, was examined in the same way as blastoderm materials. Results are summarized in Table 2. All of the embryos or embryonic tissues examined for the karyotype carried Z chromosomes. No embryos carrying W chromosomes were found. Among 118 examined, 13 (11%) were complete diploid (2A-ZZ) and 105 (89%) were haploid-diploid mosaics (A-Z/2A-ZZ). The haploid cell proportion was calculated for each specimen. The average numbers of 63-126 cells were counted for each developmental class at this stage. The mean percentage for each developmental class was summarized in Table 3. A higher rate of haploid cell proportion in less organized development was observed. One-way analysis of variance showed that the difference of means among the developmental classes was significant.
Figure 4.—Change of polyploid cell proportions in parthenogenetic blastoderms during the early stage of incubation. The duration of incubation is shown by estimated hours after ovulation.

Table 2

Parthenogenetic development in unfertilized turkey eggs at 6 days of incubation

<table>
<thead>
<tr>
<th>Developmental class</th>
<th>No. encountered</th>
<th>No. analyzed</th>
<th>24-ZZ</th>
<th>A-Z/24-ZZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryos: 5-day eq.</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>4-day eq.</td>
<td>65</td>
<td>53</td>
<td>9(1)</td>
<td>44(4)</td>
</tr>
<tr>
<td>3-day eq.</td>
<td>22</td>
<td>19</td>
<td>0</td>
<td>19(5)</td>
</tr>
<tr>
<td>2-day eq.</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>3(1)</td>
</tr>
<tr>
<td><strong>Embryo total</strong></td>
<td><strong>96</strong></td>
<td><strong>79</strong></td>
<td><strong>10(1)</strong></td>
<td><strong>69(10)</strong></td>
</tr>
<tr>
<td>Unorganized embryonic tissues</td>
<td>20</td>
<td>16</td>
<td>3</td>
<td>13(3)</td>
</tr>
<tr>
<td>Blood islets</td>
<td>68</td>
<td>23</td>
<td>0</td>
<td>23(9)</td>
</tr>
<tr>
<td>Membranes</td>
<td>78</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Development total</strong></td>
<td><strong>262</strong></td>
<td><strong>118</strong></td>
<td><strong>13(1)</strong></td>
<td><strong>105(22)</strong></td>
</tr>
<tr>
<td>Undeveloped</td>
<td>362</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Grand total</strong></td>
<td><strong>624</strong></td>
<td><strong>118</strong></td>
<td><strong>13(1)</strong></td>
<td><strong>105(22)</strong></td>
</tr>
</tbody>
</table>

* The number shown in parentheses indicates the number of eggs that are mosaics with any kind of polyploid cells.
TABLE 3

Haploid cell proportion in parthenogenetic development at 6 days of incubation

<table>
<thead>
<tr>
<th>Developmental class</th>
<th>No. eggs counted</th>
<th>Mean percentage of haploid cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryos: 5-day eq.</td>
<td>3</td>
<td>5.1</td>
</tr>
<tr>
<td>4-day eq.</td>
<td>53</td>
<td>5.7</td>
</tr>
<tr>
<td>3-day eq.</td>
<td>19</td>
<td>11.3</td>
</tr>
<tr>
<td>2-day eq.</td>
<td>4</td>
<td>6.3</td>
</tr>
<tr>
<td>Unorganized embryonic tissues</td>
<td>16</td>
<td>10.1</td>
</tr>
<tr>
<td>Blood islets</td>
<td>23</td>
<td>21.8</td>
</tr>
</tbody>
</table>

\( (F=7.77, \ d.f.=5/111, P<0.01) \). The significance detected is presumed to be caused mainly by higher haploid cell proportion in the class of blood islets.

DISCUSSION

The mechanism that ensures diploid parthenogenesis has been discussed by several authors. According to Beatty (1967), diploid parthenogenesis can arise as a result of either a specific disturbance of female meiosis or the first cleavage division. He categorized diploid parthenogenesis into the following three major types: suppression of the first polar body (Type 1PB), suppression of the second polar body (Type 2PB) and suppression of the first cleavage of a developing haploid egg (Type 1CL). Additional routes restoring diploid parthenogenesis were pointed out by D'Amato (1977). They are: (a) premeiotic chromosome doubling, (b) fusion of haploid ovum with one derivative of polar body I, (c) fusion of polar body II with one derivative of polar body I, and (d) chromosome doubling of developing haploid eggs some time after the first cleavage division. Under the condition of a heterogametic female sex-determining system (ZZ-ZW) and pre-reductional maturation division, Beatty's Type 1PB parthenogenesis and D'Amato's routes (a), (b) and (c) would produce female parthenogens. On the other hand, Beatty's Type 2PB and 1CL, and D'Amato's route (d) would produce ZZ and WW in 1:1 ratio. The parthenogens that arise from Beatty's Type 1CL or D'Amato's routes (d) originate from single ova and must be isozygous. On the other hand, in Beatty's Type 2PB parthenogenesis, some degree of heterozygosity of parthenogens is expected by preceding crossing over between the centromere and any loci in which the dam was heterozygous.

In this study, nucleated cells were found in more than 90% of the unfertilized eggs in blastodisc materials. Such a high incidence was also observed in unfertilized sectioned blastodiscs of unselected BSW turkey eggs (Haney and Olsen 1958). These results suggest that the onset of cleavage in unfertilized eggs is a fairly common phenomenon in turkeys, regardless of the incidence of parthenogenetic development in later stage. The developmental potential of unfertilized haploid egg cells of chicken is also suggested in the turkey-chicken hybrid study (Harada and Buss 1981). The karyotypes identified in these blastodiscs were either A-Z/2A-ZZ or A-W/2A-WW, and no 2A-ZW were observed. The abortive
change in $W^+$ blastoderms indicates that $A-W/2A-WW$ are lethal within two days of incubation; this explains why only $A-Z/2A-ZZ$ karyotypes are found in six-day-old embryos and the incidence of parthenogenetic development in later stages never exceeded 50%. From these observations, Beatty’s Type 1PB and D’Amato’s routes (a), (b) and (c) are refuted as possible mechanisms for turkey parthenogenesis. The frequent occurrence of mosaicism suggests that the majority of the eggs, if not all, initiate parthenogenetic development in haploid ova. Also, the finding of only diploid embryos after normal fertilization of eggs from the “high incidence” turkeys supports the idea that eggs in which development occurs without fertilization are haploid (Darcy et al. 1971). However, there is a possibility of another mechanism in turkey parthenogenesis that may account for the existence of a small proportion of completely diploid blastoderms. Complete diploidy could arise either by nuclear fusion immediately after the first cleavage division or by suppression of meiosis II. The embryos originating from these two different routes could be identified by test matings in which genetic markers are used; thus, the heterozygous parthenogens reported (Olsen 1966) could have arisen by the later mechanism.

The proportion of haploid cells in mosaic blastoderms appeared to decrease rapidly during the early stage of incubation. This decrease seems to be attributed mainly to the selection against haploid blastoderms. This idea may be supported by the following observation: the proportion of highly diploid embryos (containing less than 20% of haploid cells) at the newly laid stage is six of 64 eggs (9.4%) sampled and cannot explain the minimum proportion of highly diploid embryos of 15.4% (96 of 624) at six days of incubation. The lower mean haploid cell proportion in six-day-old embryos, as compared with the early stage blastoderms, suggests that haploid cells are eliminated continuously. A declining mean haploid cell proportion with increasing embryonic age was also observed at five to nine days of incubation, using the same turkey stock, by cytofluorometric analysis (Deford et al. 1979). The developmental abnormalities found in the haploid mosaics in turkey parthenogenesis may be compared to the “haploid syndrome” (Gurdon 1960) known in amphibians. Haploid embryos are fully viable in these species; however, they usually show various kind of abnormal development, and embryos usually cannot survive beyond the neurula stage (Hamilton 1963). A higher proportion of haploid cells remaining in blastoderms at six days of incubation may result in a significant increase in the abortive type of development (e.g., 21.8% haploid cells in blood islets). Haploid mosaics were also reported in fertilized four-day-old chicken embryos (Bloom 1970). They were mosaics of haploid (A-Z), diploid (2A-ZZ) and, in one case, triploid (3A-ZZZ) cells; these possibly originated from a haploid cell by gyno- and/or androgenesis. They all showed phenotypic abnormalities, such as growth retardation and malformation.

A remarkable portion of the parthenogenetic blastoderms or embryos were mosaics for various kinds of polyploid cells, although the proportion was much smaller than haploid cells. The increase in proportion of polyploid cells during the early stage of incubation (Figure 4) appears to be related to the mechanism of diploidization. The most frequently occurring ploidy among these polyploid cells
identified in blastoderm materials was even-numbered, i.e., tetraploid (39 of 47) and octaploid (2 out of 47); only one of the 47 cells was triploid. This observation suggests that the mechanism of polyploidization is mitotic division without cytokinesis and/or subsequent nuclear fusion. Frequent occurrence of binucleated cells was reported in pathenogenetic embryos (SATO and KOSIN 1960), and division without cytokinesis may be proposed as a means by which diploidization of haploid cells occasionally occurs. However, BLOOM, BUSS and STROTHER (1970) reported that binucleated red blood cells in turkeys resulted from the effects of an autosomal recessive gene. Hence, the binucleated cells observed by SATO and KOSIN (1960) may have been present because of an independent factor.

Spontaneous parthenogenesis has been reported in ovulated eggs in certain strains of mice (STEVENS, VARNUM and EICHER 1977). Using experimental chimeras composed of normal and parthenogenetic embryos, STEVENS, VARNUM and EICHER showed that at least one of the parthenogenetic embryos resulted from either mitosis of a primary oocyte or lack of a second meiotic division in a secondary oocyte. Enzymatic analysis of ovarian teratoma cells produced from the same strains of mice suggested that parthenogenetic developmental occurs after a normal first meiotic division (EPPIG et al. 1977). The cells found in the teratomas were mostly diploid, suggesting that they originated from second polar body suppression. On the other hand, haploid mosaics were commonly found in induced gyno- or androgenesis of mice (TARKOWSKI 1977), appearing to be typical phenomena when development originates from haploid cells, irrespective of whether they develop in vivo (TARKOWSKI and ROSSANT 1976) or in vitro (TARKOWSKI 1977). The poor survival of diploid YY (TARKOWSKI 1977; HOPPE and ILLMENSEE 1977) and diploid OY (MORRIS 1968) cells found in mice is consistent with the lethality of WW embryos in turkey parthenogenesis.

LITERATURE CITED


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