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Prenatal fate of parthenogenetic cells in mouse aggregation chimaeras

A. NAGY[1], A. PÁLDI[2], L. DEZSO[2], L. VARGA[3] and A. MAGYAR[3]

[1]*Department of Behaviour Genetics, L. Eötvös University, H-2131 Göd, Jávorka S. u. 14. Hungary*
[2]*Agricultural Biotechnology Center, H-2100 Gödöllo, Tessedik Sámuel u. 4. Hungary*
[3]*Research Center of Animal Production and Nutrition, H-2053, Herceghalom, Hungary*

Summary

Parthenogenetically activated BCF1 and fertilized BALB/c embryos were aggregated to form chimaeras. The fate of the parthenogenetic component was followed in the conceptus during the second half of gestation. The results indicate an early strong selection against parthenogenetic cells in the extraembryonal part, which is presumably complete by term, and a weaker selective process in the embryo. During early development, parthenogenetic cells have nearly normal developmental potency in the embryo, which allows their balanced contribution in the chimaeras on day 12. Later, this contribution declines significantly resulting in an unbalanced relation to the advantage of the fertilized counterpart. From the results, we suggest that gametic imprinting may play a role not only in the key steps of preimplantation and early postimplantation development, but later in cell and tissue differentiation.

Key words: parthenogenesis, chimaera, parental genomes, mouse embryo, prenatal development, aggregation chimaera, imprinting.

Introduction

Recently, evidence has accumulated to show that there are distinct differences between the paternal and maternal contribution to the embryonic mammalian genome in the control of embryonic development (Surani, Reik, Norris & Barton, 1986*b*). Neither two maternal (parthenogenone and gynogenone) nor two paternal (androgenone) genomes are able to support normal development to term (Surani, Barton & Norris, 1984; McGrath & Solter, 1984). While little is known about the nature of these differences, the paternal genome appears to be more important for the proliferation of the extraembryonic tissues and the maternal genome plays a key role in preimplantation and early postimplantation development (Barton, Surani & Norris, 1984; Barton, Adams, Norris & Surani, 1985).

These phenomena may be relevant to the problem of mammalian parthenogenesis. In the mouse egg, there are several effective ways to induce diploid parthenogenesis and apparently normal preimplantation development. Most of these embryos die during or shortly after implantation and mortality is complete by day 11 (Kaufman, 1983). Study of the postimplantation development of the parthenogenetic conceptus is restricted by this infrequent development beyond implantation and the complete lack of development beyond placentation.

The life and development of the parthenogenetically activated cells can be extended when chimaeras are made between normally fertilized and parthenogenetic embryos (Stevens, Varnum & Eicher, 1977; Stevens, 1978). The spontaneous parthenogenones from the LT/Sv strain were rescued successfully by aggregation with normally fertilized embryos at the 8-cell stage. Contributions of parthenogenetic cells to the chimaeras were low.

Higher rates of parthenogenetic contribution were obtained by injecting parthenogenetic inner cell mass (ICM) cells into the cavity of normally fertilized blastocysts (Surani, Barton & Kaufman, 1977). More recently, complete homozygous gynogenones, produced by microsurgery, were aggregated with normally fertilized embryos at the 8-cell stage. Few of the chimaeras developed to term, but they showed the chimaerism in several tissues (Anderegg & Markert, 1986), and the cells from the homozygous gynogenones were capable of participating in the germ line of the chimaeras.

A certain kind of 'guided' chimaera production was achieved by the blastocyst reconstitution experiments

of Barton *et al.* (1985). These authors introduced parthenogenetic or normal ICM into the cavity of trophectodermal vesicles derived from normal or parthenogenetic embryos, respectively. In contrast to the aggregation chimaeras, none of the reconstituted combinations containing the parthenogenetic counterpart were able to support normal development beyond day 12. Clearly, trophectoderm from normally fertilized embryos alone cannot overcome this developmental arrest. However, the retardation of embryonic development may not be an inherent defect in the embryonic cells, but in the extra-embryonic cells derived from the same progenitors.

In the present study, we investigated the developmental potency of parthenogenetically activated cells in aggregation chimaeras formed from parthenogenetic and fertilized embryos (P↔F embryos), with special regard to the second half of gestation.

Materials and methods

Animals

Eggs and 8-cell-stage embryos were obtained from females superovulated by *i.p.* injection of 5 i.u. of pregnant mare's serum (Intervet), followed 48 h later by an injection of 5 i.u. of human chorionic gonadotropin. The (C57BL × CBA) F_1/Lati (henceforth called BCF1) females were treated 10 h earlier to synchronize the time of the 8-cell stage used for chimaera production between the parthenogenetically activated BCF1 and normally fertilized BALB/cLati embryos. Fertilized 8-cell embryos were BCF1 × BCF1 F_2 (henceforth called BCF2) and BALB/c obtained on day 3 of pregnancy. (Day 1 designated as the day of the vaginal plug found in mated females.)

Parthenogenetic activation and embryo isolation

To obtain diploid parthenogenetic embryos, BCF1 oocytes were incubated for 6 min in 7% ethanol (Cuthbertson, 1983) in culture medium (Whitten, 1971), then immediately transferred to medium containing 5 $\mu g\,ml^{-1}$ cytochalasin B (Calbiochem). After 4 h the cumulus cell were removed by a short incubation in 300 i.u. ml^{-1} hyaluronidase (bovine testis, type V, Sigma). Eggs containing two pronuclei were cultured at 37°C in humidified 5% O_2, 5% CO_2, 90% N_2 atmosphere up to 8-cell stage.

Preparation of aggregation chimaeras

The zona pellucida was removed by pronase treatment (Mintz, 1971). Two types of aggregates were produced: parthenogenetic (BCF1)↔fertilized (BALB/c) and fertilized (BCF2)↔fertilized (BALB/c). The aggregated pairs of embryos were cultured overnight and those that formed chimaeras and developed into simple blastocysts were transferred to the uterine horns of BCF1 females on day 3 of pseudopregnancy.

Analysis of embryos

Recipients were either sacrificed on days 12 or 19 of pregnancy and the numbers of implants and viable fetuses were determined. The fetuses and extraembryonic tissues (yolk sac and placenta) were typed for GPI.

Statistical analysis

The distributions of individual compositions were compared to each other using Mann–Whitney–Wilcoxon non-parametric U-test.

Results

The frequency of ethanol-induced parthenogenetic activation and suppression of second polar body varied slightly between experiments, but overall 50% of the activated eggs reached the compacted 8-cell stage after about 70 h in culture.

Table 1 summarizes the postimplantation development of the experimentally aggregated embryos. In the control Groups 3 and 4, normally fertilized BCF2 embryos were used in the place of parthenogenetic (BCF1) counterpart of Groups 1 and 2. Overall, relatively high rates of implantation were observed in each group and they did not differ significantly. In contrast, embryonic mortality by day 12 is very high in P↔F aggregates (60%), when compared with F↔F aggregates (23%). There was no difference between the survival rate of P↔F embryos on days 12 and 19 (41% and 45%, respectively). This observation suggests that almost all the P↔F embryos that are normal on day 12 subsequently develop to term.

To characterize the parthenogenetic contribution (in Groups 1 and 2) and fertilized BCF2 contribution

Table 1. *Development of parthenogenetic↔fertilized and fertilized↔fertilized aggregation chimaeras*

Group	Type of aggregates	Number of aggregates produced	Transferred to recipient that became pregnant (total)	Day of autopsy	Implantation (% of aggregates transferred)	Live embryos (% of impants)	Newborn animals (% of implants)
1	P/Fa*	189	114 (142)	12	61 (53%)	25 (41%)	
2	P/Fa	77	65 (88)	19	42 (65%)		19 (45%)†
3	Fb/Fa	73	40 (47)	12	17 (43%)	13 (76%)	
4	Fb/Fa	45	22 (22)	19	11 (50%)		9 (82%)

* P, parthenogenetic BCF1; Fa, fertilized BALB/c; Fb, fertilized BCF2.
† Six newborn animals allowed to reach adulthood for later study.

(in Groups 3 and 4) to the embryo and the different extraembryonal tissues, the semiquantitative GPI typing was used. BCF1 and BCF2 carry the GPI-B (B), while BALB/c carries the GPI-A (A) isoenzyme. We established five categories concerning the electrophoretic patterns of our samples: 1-(B, no A), 2-(B > A), 3-(B = A), 4-(B < A), 5-(no B, A). Separately it was determined that these categories approximately represent the following contribution of parthenogenetic BCF1 or normally fertilized BCF2 embryos in P↔F and F↔F conceptus, respectively: 1-(90–100 %), 2-(65–90 %), 3-(35–65 %), 4-(10–35 %) and 5-(0–10 %). These categories were used to show the distribution of individual compositions (henceforth called DC pattern) of the aggregates studied.

Day 12 embryo

The DC pattern of live P↔F embryos shows an even distribution, which suggests a balanced relationship between the parthenogenetic and fertilized lines (Fig. 1A). On the other hand, DC pattern of F↔F embryos is unbalanced (Fig. 1B), showing the dominance of the BCF2 genotype. At least 11 out of 19 P↔F and 6 out of 13 F↔F aggregates proved to be chimaeric by analysis of the GPI isozymes.

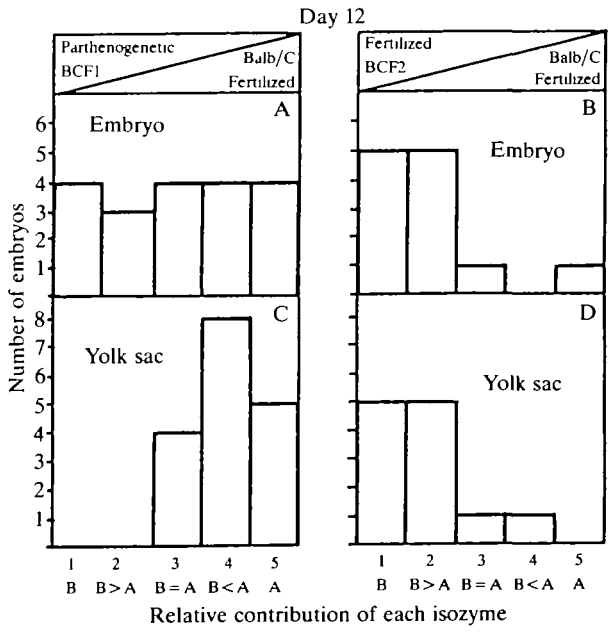

Fig. 1. Composition distribution of parthenogenetic (BCF1)↔fertilized (BALB/c) and fertilized (BCF2)↔fertilized (BALB/c) chimaera embryos (A,B), yolk sacs (C,D) on day 12 of gestation. The relative ratio of the two cell lineages was determined by electrophoretic typing of glucose phosphate isomerase isoenzymes. Note the even distribution of parthenogenetic contribution to the embryo and the strong shift towards the fertilized BALB/c counterpart in the yolk sac. In contrast, the BCF2 (representing similar genotype with the parthenogenetic BCF1) dominates the BALB/c in the fertilized ↔fertilized chimaeras.

Day 12 yolk sac

The DC pattern of the yolk sac of P↔F chimaeras (Fig. 1C) was dramatically different from that of the F↔F yolk sac (Fig. 1D) or from that of the P↔F embryos. The large shift in the distribution to the direction of the normally fertilized BALB/c cells clearly indicates a selection against parthenogenetic cells in this tissue.

Day 12 placenta

Since the placenta consists of both embryonic and maternal components, GPI-B will always be present as the foster mothers were BCF1. Therefore, in this experiment, direct observation of the contribution of parthenogenetic or BCF2 cells to the placenta was not possible. However, indirect information was obtained from the A isoenzyme from the fertilized BALB/c embryo. While the BALB/c contribution normally varies in the placentas of F↔F, in the P↔F aggregates strong and constant placental contributions of BALB/c were observed. This finding clearly indicates the dominance of the normally fertilized BALB/c component in the embryonal part of the P↔F placenta on day 12.

Day 19 embryo

The DC pattern of day 19 P↔F newborn animals (Fig. 2A) differs significantly ($P < 0{\cdot}01$) from that of the day 19 F↔F animals (Fig. 2B). The shift to the direction of the normally fertilized BALB/c cells is very similar to that observed in the case of day-12 yolk sacs. This also means that the contribution of fertilized cells is significantly larger ($P < 0{\cdot}01$) on day 19 than on day 12. The selective processes, which affected the extraembryonal tissues before day 12, extended to the fetus in the last 7 days of gestation. These processes were also reflected in the decrease in the frequency of chimaeras observed (3 out of 13) and in the birthweights. The weight of the day-19 animals having detectable parthenogenetic contribution (category 3, 4 in Fig. 2A) was only about 80 % of that of the F↔F chimaeras.

Day 19 yolk sac

In the yolk sac, the parthenogenetic cells were almost completely absent (Fig. 2C). By contrast, the DC pattern of F↔F newborn animals shows the dominance of BCF2 cells (Fig. 2D).

Discussion

For a long time, the inability of mammalian parthenogenones to develop normally has presented an intriguing problem to experimental embryologists.

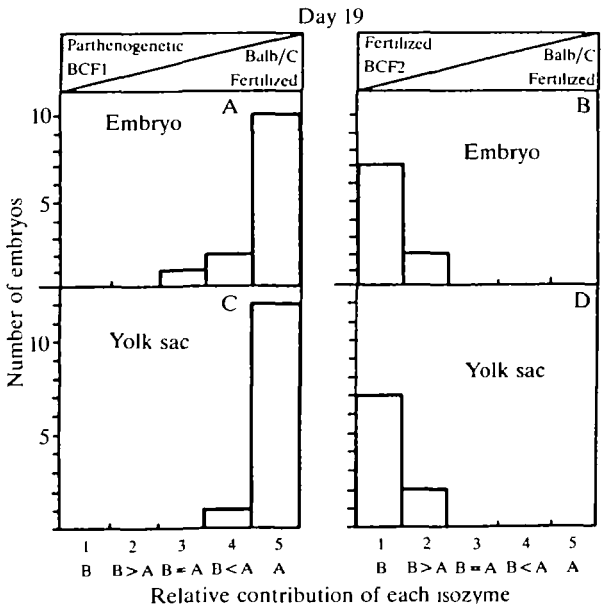

Fig. 2. Composition distribution of parthenogenetic (BCF1)↔fertilized (BALB/c) and fertilized (BCF2)↔ fertilized (BALB/c) chimaera embryos (A,B) and yolk sacs (C,D) on day 19 of gestation. In the parthenogenetic↔fertilized chimaeras a dominance of fertilized BALB/c component has developed in the fetus. The parthenogenetic cells are almost completely absent from the yolk sac.

Parthenogenetic development of embryos to give live offspring occurs naturally in many non-mammalian species (White, 1978) and can also be induced experimentally in non-mammalian vertebrates and invertebrates (Nagy, Rajki, Bakos & Csányi, 1979). Although the parthenogenetically activated diploid mammalian embryos develop normally through preimplantation they usually fail to develop beyond implantation, rarely reaching the limb-bud stage (Kaufman, Barton & Surani, 1977). Two approaches have been made to this problem.

(1) Many causes of embryonic lethality have been proposed for mammalian parthenogenones including extreme homozygosity (Graham, 1974) and extragenetic factors contributed by the sperm (Graham, 1971). More recently, specific imprinting of the genome during gametogenesis has been suggested. Consequently, the presence of both a male and a female pronucleus is essential for an activated egg to develop normally to term (Surani, Barton & Norris, 1984; McGrath & Solter, 1984). Further researches revealed that the maternal genome has an important role in the preimplantation and early postimplantation development and the paternal genome is necessary for the normal proliferation of the extraembryonal tissues (Barton *et al.* 1984, 1985). These studies provided very important information about the failure of parthenogenetic, gynogenetic and androgenetic development in the first half of gestation. The last period remained obscure, due to the complete lack of development beyond placentation.

(2) In other studies, successful attempts were made to rescue parthenogenetically activated cells by aggregation with fertilized embryos (Stevens *et al.* 1977; Surani *et al.* 1977; Stevens, 1978). More recently, rescue of microsurgically produced homozygous uniparental mouse embryos (gynogenones) in aggregation chimaeras was reported, emphasizing the practical importance of possible production of inbred lines using this method (Anderegg & Markert, 1986). These studies demonstrate that parthenogenetic or gynogenetic cells are capable of differentiating into most somatic tissues and the germ line in the presence of cells derived from normally fertilized embryos.

In this study, we combined the above approaches by using aggregation chimaeras to characterize the behaviour of parthenogenetically activated cells in the fetus and extraembryonal tissues during the latter half of gestation.

A dramatic shift was observed in the parthenogenetic contribution to the fetus between days 12 and 19 of gestation. This may be explained in two ways: either the embryos with a large parthenogenetic contribution on day 12 die by day 19, or the day-12 embryos remain alive but there is large selection against the parthenogenetic cells by day 19. The similar rate of survival of P↔F embryos on days 12 and 19 supports the second possibility. It is possible that day-12 embryos with large parthenogenetic contributions develop to term, when the contribution of parthenogenetic cells is significantly reduced.

In the yolk sac, the parthenogenetic cells diminish even faster. The selective processes start earlier than in the embryo, so on day 12 they result in a significant shift in the contributions of cells from the parthenogenone in favour of cells from the fertilized counterpart. The parthenogenetic cells are virtually absent from this tissue by day 19.

The constant and large contribution of fertilized (BALB/c) cells to the placenta suggests that a similar selective process exists and that in this extraembryonic tissue it is timed at least as early as in the case of the yolk sac. However Gpi-1a/Gpi-1a foster mother will give more direct information on this tissue. The above suggestions are supported by the finding of Surani, Barton & Norris (1987) who reported that parthenogenetic cells were detected in the chimaeric embryo and in its yolk sac but not in the trophoblast. This absence of parthenogenetic cells might indicate that the selective process against parthenogenetic cells is complete at the time the trophoblast was examined for the GPI isozymes. From the studies of Robertsonian translocations (Cattanach & Kirk,

1985) and from the functional difference of the parental genomes in early development, various categories of genes of maternal and paternal origin are implicated in the control of normal embryonic development (Surani *et al.* 1986*b*). These developmentally important maternal and paternal genes have complementary functions at preimplantation and early postimplantation periods (Surani *et al.* 1987). Our results indicate that the lack of complementarily preprogrammed paternal genome in a parthenogenetic embryo continues to affect development during the second period of gestation. These effects in some way modify the developmental potency of cells lacking paternally derived genome. The disomy/nullisomy studies of chromosome 11 (Cattanach & Kirk, 1985) are a good example of this kind of effect. Paternal disomy/maternal nullisomy causes higher birthweight of newborn animals and maternal disomy/paternal nullisomy resulted in smaller pups. Recently, it was suggested that the differences between parental chromosomes essential for normal development are heritable at least in the first few divisions (Surani, Barton & Norris, 1986*a*). Considering the results presented, we suggest that the phenomenon of gametic imprinting involves genes that not only play a role preferentially in early developmental steps, but also those regulating or affecting the final differentiation of embryonic tissues.

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References

ANDEREGG, C. & MARKERT, C. L. (1986). Successful rescue of microsurgically produced homozygous uniparental mouse embryos via production of aggregation chimeras. *Proc. natn. Acad. Sci. U.S.A.* **83**, 6509–6513.

BARTON, S. C., ADAMS, C. A., NORRIS, M. L. & SURANI, M. A. H. (1985). Development of gynogenetic and parthenogenetic inner cell mass and trophectoderm tissues in reconstituted blastocysts in the mouse. *J. Embryol. exp. Morph.* **90**, 267–285.

BARTON, S. C., SURANI, M. A. H. & NORRIS, M. L. (1984). Role of paternal and maternal genomes in mouse development. *Nature, Lond.* **311**, 374–376.

CATTANACH, B. M. & KIRK, M. (1985). Differential activity of maternally and paternally derived chromosome regions in mice. *Nature, Lond.* **315**, 496–498.

CUTHBERTSON, K. S. R. (1983). Parthenogenetic activation of mouse oocytes *in vitro* with ethanol and benzyl alcohol. *J. exp. Zool.* **226**, 311–314.

GRAHAM, C. F. (1971). Experimental early parthenogenesis in mammals. *Adv. Biosci.* **6**, 87–97.

GRAHAM, C. F. (1974). The production of parthenogenetic mammalian embryos and their use in biological research. *Biol. Rev.* **49**, 399–422.

KAUFMAN, M. H., BARTON, S. C. & SURANI, M. A. H. (1977). Normal postimplantation development of mouse parthenogenetic embryos to the forelimb bud stage. *Nature, Lond.* **265**, 53–55.

KAUFMAN, M. H. (1983). *Early Mammalian Development: Parthenogenetic Studies.* Cambridge University Press.

MCGRATH, J. & SOLTER, D. (1984). Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* **37**, 179–183.

MINTZ, B. (1971). Allophenic mice of multiembryo origin. In *Methods in Mammalian Embryology* (ed. J. C. Daniel), pp. 186–214. San Francisco: W. H. Freeman.

NAGY, A., RAJKI, K., BAKOS, J. & CSÁNYI, V. (1979). Genetic analysis in carp (*Cyprinus carpio*) using gynogenesis. *Heredity* **43**, 35–40.

STEVENS, L. C. (1978). Totipotent cells of parthenogenetic origin in a chimaeric mouse. *Nature, Lond.* **276**, 266–267.

STEVENS, L. C., VARNUM, D. S. & EICHER, E. M. (1977). Viable chimaeras produced from normal and parthenogenetic mouse embryos. *Nature, Lond.* **269**, 515–517.

SURANI, M. A. H. & BARTON, S. C. (1983). Development of gynogenetic eggs in the mouse: implication for parthenogenetic embryos. *Science* **222**, 1034–1036.

SURANI, M. A. H., BARTON, S. C. & KAUFMAN, M. H. (1977). Development to term of chimaeras between diploid parthenogenetic and fertilized embryos. *Nature, Lond.* **270**, 301–601.

SURANI, M. A. H., BARTON, S. C. & NORRIS, M. L. (1984). Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. *Nature, Lond.* **308**, 548–550.

SURANI, M. A. H., BARTON, S. C. & NORRIS, M. L. (1986*a*). Nuclear transplantation in the mouse: Heritable differences between parental genomes after activation of the embryonic genome. *Cell* **45**, 127–136.

SURANI, M. A. H., BARTON, S. C. & NORRIS, M. L. (1987). Influence of parental chromosomes on spatial specificity in androgenetic↔parthenogenetic chimaeras in the mouse. *Nature, Lond* **326**, 395–397.

SURANI, M. A. H., REIK, W., NORRIS, M. L. & BARTON, S. C. (1986*b*). Influence of germline modifications of homologous chromosomes on mouse development. *J. Embryol. exp. Morph.* **97 Supplement**, 123–136.

WHITE, M. J. D. (1978). *Modes of Speciation.* San Francisco: W. H. Freeman.

WHITTEN, W. K. (1971). Nutrient requirements for the culture of preimplantation embryos *in vitro*. *Adv. Biosci.* **6**, 129–139.

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