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ORIGINAL ARTICLES

# The Use of Chimeric Mice in Studying the Effects of Genomic Imprinting

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**Abstract**—This is a review of the data of clonal analysis of developing tissues in parthenogenetic and androgenetic chimeric mice. The time and causes of death of the parthenogenetic and androgenetic cell clones in chimeras are considered. The data obtained suggest that the development of cell clones, derivatives of the mesoderm and endoderm, is determined by the expression of alleles of the imprinted loci of paternal chromosomes, while the formation of cell clones, derivatives of the ectoderm, depends on the expression of other imprinted loci of maternal chromosomes. The death of androgenetic and parthenogenetic (gynogenetic) mammalian embryos is due to the lack of the expression of certain imprinted loci of the maternal and paternal genome, respectively.

*Key words*: chimeric mice, parthenogenetic cell clones, androgenetic cell clones, genomic imprinting

The discovery of the phenomenon of genomic imprinting was preceded by extensive studies of parthenogenesis. It was of interest to biologists for many years that parthenogenesis was not described in any of the more than 4500 mammalian species, while in other classes of vertebrates, parthenogenesis is compatible with adult life.

The problem of parthenogenesis in mammals, i.e., elucidation of the causes of death of parthenogenetic embryos, has a 30-year history, and M. Kaufman was one of the first to study parthenogenesis in mice, He made a significant contribution to the description of death of parthenogenetic embryos and general characteristics of spontaneous and induced parthenogenesis in mammals (Kaufman, 1983). Specifically, it was established that diploid parthenogenetic mouse embryos usually die at the early developmental stages and rarely reach the stages of 25 pairs of somites. Such embryos are characterized by a delayed formation of extraembryonic membranes, and, specifically, underdevelopment of the placenta. In Russia, A.P. Dyban and his coworkers studied spontaneous and induced parthenogenesis in mice (Dyban and Noniashvili, 1986).

Despite many studies, the causes of death of parthenogenetic embryos remained unclear for a long time. The convincing evidence that the death of parthenogenetic (gynogenetic) embryos was due to a nuclear, rather than cytoplasmic, deficiency, as was hypothesized, was obtained only in the 1980s as a result of elegant experiments with transplantation of pronuclei on mouse zygotes. It was shown by M.A.H. Surani and his coworkers (Surani and Barton, 1983; Surani *et al.*, 1984) and by McGrath and Solter (1983, 1984) that the recombination of the male and female pronuclei alone provides for the normal development of mice. However, combination of two male or two female pronuclei taken from different zygotes leads to the arrest of embryogenesis. In the case of androgenesis (combination of two male pronuclei) a small embryo and rather large trophoblast derivatives develop, while in the case of gynogenesis (combination of two female pronuclei), as well as parthenogenesis (diploidization of chromosomes of one female pronucleus), a rather large embryo and very small trophoblast derivatives develop, i.e., the extraembryonic membranes are underdeveloped.

Hence, both chromosome sets, maternal and paternal, are required for the normal development of mammals. The mechanism regulating such functional differences of the parental genomes has been termed genomic imprinting (Surani *et al.*, 1984).

The autosome genes of mammals are said to be imprinted and are inherited from the mother or father in a repressed or "silent" state. Unlike most genes that are expressed in the diploid somatic cells in a biallelic manner, the expression of imprinted genes is expressed in a monoallelic manner: one of two parental alleles (maternal or paternal) is in a repressed or inactive state.

Note that the effects and mechanisms of genomic imprinting, identification of imprinted genes, and their role in the regulation of development have mostly been studied on mice and humans. At present, more than 30 imprinted loci have been identified (Morison and Reeve, 1998). These may constitute less than half of the actual number of imprinted loci in any higher (placental) mammal (Barlow, 1997).

Many data suggest that genomic imprinting is mostly due to DNA methylation: specific methylation DNA cytosine bases switch off the expression of the

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maternal or paternal allele of the imprinted locus (Surani *et al.*, 1990, 1993; Razin and Cedar, 1994). The data obtained in our laboratory indicate that DNA demethylation may prolong the development of diploid parthenogenetic embryos treated with the demethylating agent 5-azacytidine (Penkov *et al.*, 1996).

Differential expression of the genes of imprinted loci in development may be tissue- and stage-specific, i.e., one gene may behave as imprinted (inactive) in a certain cell system and at a certain developmental stage, but the same gene may be normally expressed in other cell systems or in the same cell system but at another developmental stage. Solter (1998) stressed that this fact should always be taken into consideration when discussing the possible effects and mechanisms of genomic imprinting.

In order to study the developmental potentials of various parthenogenetic and androgenetic cell clones, chimeric mice are used. Chimeras consist of two or more genotypically different parental components, and they are widely used in the developmental genetics of mammals to determine the site and effect of gene activity (McLaren, 1976; Konyukhov *et al.*, 1988). Analysis of the development of chimeras has shown that the differential expression of parental alleles of the imprinted loci is essential for the normal structure of the organism.

## PARTHENOGENETIC CHIMERAS

Chimeric mice consisting of normal and parthenogenetic cells are called parthenogenetic chimeras and designated as PG ⇌ N or A(PG) ⇌ B, where A and B are lines of laboratory animals and N and PG are normal and parthenogenetic components, respectively. Parthenogenetic chimeras were first obtained in mice. Such chimeras are capable of development to term and to sexual maturity, and parthenogenetic cell clones are included in practically all tissues and organs, including the gonads, and produce intact gametes (Stevens *et al.*, 1977; Surani *et al.*, 1977; Stevens, 1978).

Although the first information about parthenogenetic chimeric mice was published at the end of the 1970s, the clonal analysis of such chimeras was carried out in the second half of the 1980s and in the beginning of the 1990s, with special reference to the problem of genomic imprinting.

Nagy *et al.* (1987) studied parthenogenetic chimeric embryos (C57BL/6 × CBA)F1)PG ⇌ BALB/c] at the age of 12 and 19 days and three adult chimeras. The content of parthenogenetic cells in the tissues steadily decreased during development. Selection against the parthenogenetic cells in the yolk sac began earlier and proceeded at a higher rate than in the embryonic tissues, so that, by day 19 of embryogenesis, the parthenogenetic component was practically absent in the yolk sac. Clarke *et al.* (1988) used transgenic *Tg-MβG-1* mice carrying about 1000 tandem copies of a plasmid with the murine β-globin gene to produce parthenogenetic chimeras. Initially, the parthenogenetic cells were included in the inner cell mass (ICM) and trophectoderm, but by day 6.5 the parthenogenetic cell clones were fully eliminated from the trophectoderm and, later, were not found in its derivatives. At the same time, the parthenogenetic cell clones were present in all ICM derivatives of chimeric embryos at the age of 6.5–7.5 days, and the percentage of parthenogenetic cell clones in the embryonic ectoderm and normal cells was similar. These authors proposed that the chimeras with a large number of parthenogenetic cells in the trophoblast died before day 6.5, as follows from the lower survival rate of parthenogenetic chimeras, as compared to the normal embryos. In the chimeras with a lower content of parthenogenetic cells in the trophoblast, the parthenogenetic cell clones were replaced by normal cells by day 6.5. In all likelihood, the parthenogenetic cells were eliminated from the yolk sac endoderm in a similar way between days 7.5 and 9.5–11.5.

Studies of postnatal development of the parthenogenetic chimeras show a negative correlation between the contribution of the parthenogenetic component in the chimera and its weight at term (Paldi *et al.*, 1989). The number of parthenogenetic cells in the chimeras obtained by these authors (C57BL/6 × CBA)F1(PG) ⇌ (NMRI × BALB/c)F1 and (C57BL/6 × CBA)F1(PG) × BALB/c did not exceed 50%, and the chimeras with a high parthenogenetic component often died during the perinatal period. Parthenogenetic cells were not found in all tissues and organs; specifically, they were absent in the kidneys, spleen, stomach, and blood. At the same time, there was no strict selection against parthenogenetic cells during the postnatal period in the gonads, hair cover, and retinal pigment epithelium.

Fundele *et al.* (1989) reported a marked elimination of parthenogenetic cell clones in the skeletal muscle of parthenogenetic chimeras, as compared to other mesoderm derivatives. It was noted that, in the endoderm derivatives of the control chimeras (nonparthenogenetic), unlike in other organs, the ratio of genotypically different components varied widely. At the same time, among the endoderm derivatives of parthenogenetic chimeras, the parthenogenetic component was less represented in the liver and pancreas than in the thymus, lungs, and, especially, duodenum. According to Nagy *et al.* (1989), a more significant contribution of parthenogenetic cell clones is observed in the tissues the cells which proliferate at a low rate or start differentiation relatively early, such as brain, cerebellum, and cardiac muscle tissues. The initial ratio of cell clones in such tissues could change only as a result of the early death of the parthenogenetic cell clones. These authors believe that the absence of changes in the hair cover and retinal pigment at the age below six or twelve months epithelium pigmentation is sufficient evidence for the absence of significant differences in the death of parthenogenetic and normal cells in these tissues. However, no quantitative data have been given on the con-

tent of parthenogenetic cell clones in the above-mentioned tissues. The oocytes are also included in this group of tissues, as shown as a result of crosses of the obtained chimeras with normal mice. Note that the cell clones of this group of tissues are separated at the early developmental stages. In the second group of organs (uterus, lungs, appendix, kidneys, spleen, and stomach), the parthenogenetic cells are also capable of surviving and, apparently, the parthenogenetic cell clones do not markedly affect the functions of these organs and do not have selective advantages over the normal cells, due to their lower proliferation rate. As concerns the third group of organs (skeletal muscle, liver, adrenals, blood, urinary bladder, and salivary glands), it is unclear whether there is selection against the parthenogenetic cell clones during formation of these tissues or whether they are gradually eliminated during postnatal development. The second suggestion is supported by the fact that the content of the parthenogenetic component in the blood, skeletal muscle, and liver of newborn chimeras is very high (Paldi *et al.*, 1989). In addition, most tissues of this group are capable of self-renewal, as a result of which the parthenogenetic cell clones may be gradually eliminated.

Subsequent studies have made it possible to determine the patterns of distribution and elimination of the parthenogenetic cells in the chimera tissues. The initial distribution (allocation) of parthenogenetic cells in the preimplantation embryo appears to be accidental (Clarke *et al.*, 1988; Thomson and Solter, 1988), but later, they are progressively eliminated, first from the trophoblast, then from the yolk sac endoderm, and finally from the yolk sac mesoderm. By the mid-gestation period, the parthenogenetic cell clones are practically absent in all extraembryonic tissues (Nagy *et al.*, 1987; Surani *et al.*, 1988; Thomson and Solter, 1989).

In the embryonic tissues, the parthenogenetic cells continue to normally proliferate until the mid-gestation period, and then their contribution to the chimera decreases. Note that parthenogenetic cells of endodermal and mesodermal origin are eliminated more intensely than those of ectodermal origin. A high degree of elimination of the parthenogenetic cell clones is observed between days 13 and 16, and, specifically in the skeletal muscles, between days 13 and 15, when the fusion of myoblasts begins (day 14). However, no noticeable elimination is observed in the pancreas until day 15, and elimination of the parthenogenetic cells in this organ appears to be terminated only by term. The contribution of parthenogenetic cell clones to the pancreas is much lower than the total content of parthenogenetic cells in the chimera. In the liver, unlike the muscles and pancreas, elimination of parthenogenetic cell clones is lasting and continuous. The contribution of parthenogenetic cell clones is insignificant in the colon, kidneys, and urinary bladder. The percentage of parthenogenetic cells in the colon and urinary bladder decreases during the perinatal period. A high percentage of parthenogenetic cells are usually observed in the brain. The rate of elimination of parthenogenetic cell clones in the nervous tissues is the lowest and the percentage of parthenogenetic cells in the adult brain is high. Therefore, the distribution of parthenogenetic cell clones in the tissues of adult chimeras is nonuniform, since their contribution to the formation of different tissues and organs is different (Nagy *et al.*, 1989; Fundele *et al.*, 1989, 1990, 1991). When the tissues of chimeras have a high content of parthenogenetic cells, their growth and development is usually suppressed (Paldi *et al.*, 1989).

Allen *et al.* (1994) used the parthenogenetic embryonic stem cells to obtain parthenogenetic chimeric mice. Although a high percentage of parthenogenetic cell clones was observed in many tissues and organs of such chimeras (blood, heart, liver, spleen, lungs, and brain), no significant suppression of growth was recorded, which is common for the PG ⇌ N chimeras. These authors believe that the absence of growth suppression in such parthenogenetic chimeras may be due to disturbed normal imprinting (epigenetic modification) of one or more imprinted genes involved in growth regulation.

Diploid parthenogenetic embryos of mouse hybrids of first-generation (CBA × C57BL/6)F1 or (C57BL/6 × CBA)F1 are often used to produce parthenogenetic chimeras, since the diploid parthenogenetic embryos of such hybrids develop rather steadily *in vitro* during the preimplantation period and reach the somite stages after transplantation into the uterus of pseudopregnant females. At the same time, the use of mouse embryos of inbred lines for production of parthenogenetic chimeras may be preferable in some cases, since the genetic background can modulate the effects of genomic imprinting (Allen and Mooslehner, 1992; Penkov and Platonov, 1992; Latham, 1994; Chaillet *et al.*, 1995; Penkov *et al.*, 1996). It has been shown in our laboratory that the diploid parthenogenetic mouse embryos of diverse inbred lines have different developmental potentials. Specifically, parthenogenetic C57BL/6 embryos develop *in vitro* much better during the preimplantation period than parthenogenetic CBA embryos. However, parthenogenetic CBA embryos develop much better than parthenogenetic C57BL/6 embryos during the early postimplantation period (Penkov and Platonov, 1992; Penkov *et al.*, 1996).

Taking into account the aforesaid, we obtained 12 parthenogenetic C57BL/6(PG) ⇌ BALB/c chimeric mice (Isaev *et al.*, 1997, 1999). Chimeras were produced by aggregation of 8-cell morulas, one of which was parthenogenetic (Fig. 1). We studied the distribution of parthenogenetic cell clones in the retinal pigment epithelium and choroid, as well as in the brain, kidneys, and liver. In none of these chimeras did the percentage of pigmented regions of hair cover exceed 35%. In most chimeras, the pigmented regions were present in the anterior and posterior parts, while the middle part of the body was, as a rule, unpigmented, perhaps

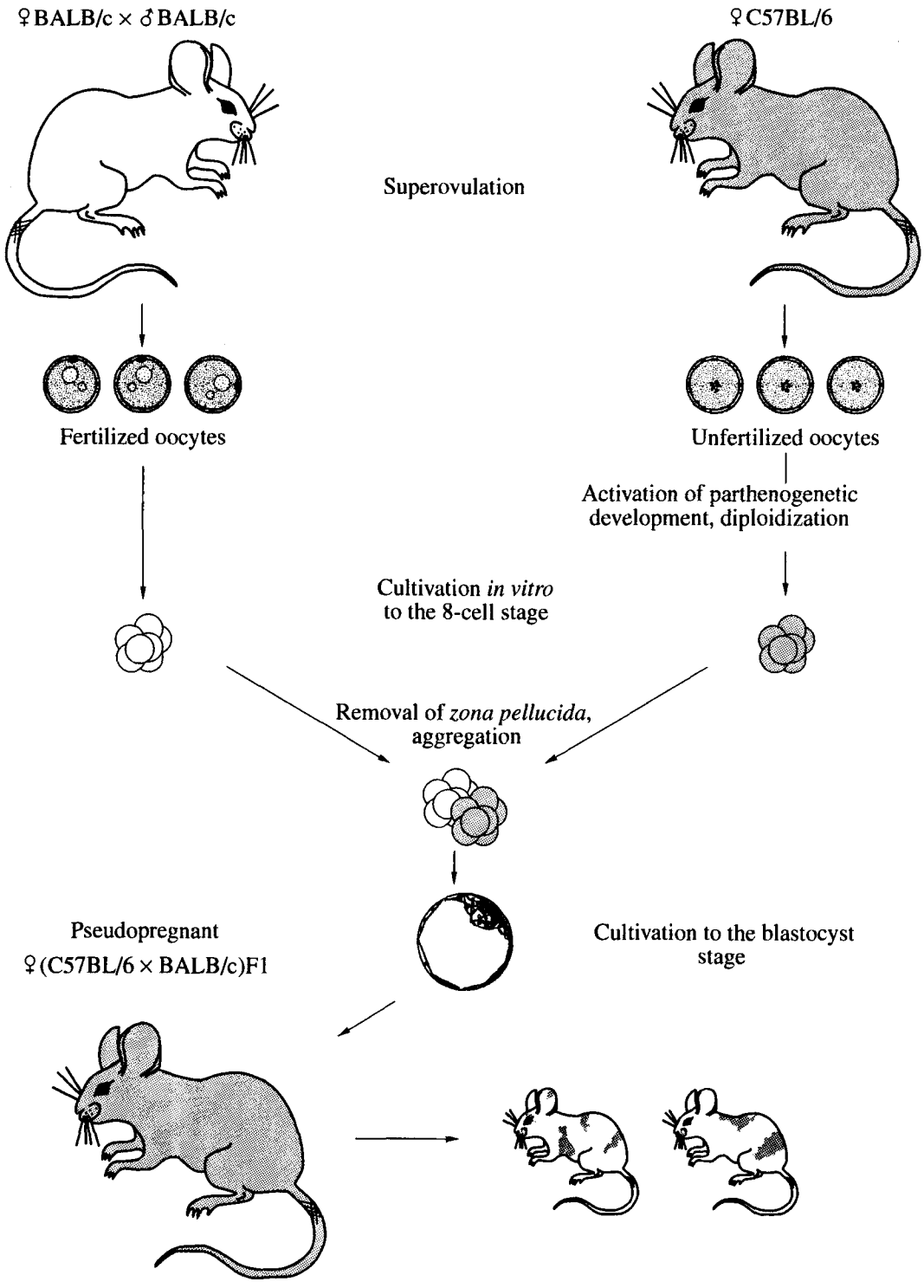


**Fig. 1.** Scheme of production of the parthenogenetic chimeric mice C57BL/6(PG) ⇌ BALB/c.

because of the death of parthenogenetic melanoblasts in this area (Fig. 2). The absence of pigmentation of the hair cover on the ventral part of the body and on distal parts of the limbs in most studied parthenogenetic chimeras suggests a lowered rate of proliferation and migration of the parthenogenetic melanoblasts than normal. The observed disturbance of bilateral distribution of pigmented regions of the hair cover may be due to the accidental death of individual parthenogenetic clones of melanoblasts (Isaev *et al.*, 1997).

Asymmetry in bilateral distribution of the parthenogenetic cell clones was also noted in the retinal pigment epithelium and choroid of chimeras. A high correlation


**Fig. 2.** Distribution of pigmented regions of hair cover in the craniocaudal direction in 12 parthenogenetic C57BL/6(PG) ⇌ BALB/c chimeras. Abscissa: ordinal numbers of body zones; ordinate: number of chimeric animals in which pigmented regions were present in the same zones; zones 6–9 and 15–19 correspond to the regions of fore- and hind-limbs.

was shown between the contributions of parthenogenetic cell clones to the retinal pigment epithelium of the right and left eye and epidermal melanoblasts in the hair cover of the corresponding body half of the chimeras. These data suggest a correlation between the processes leading to characteristic bilateral distribution of parthenogenetic cell clones in the retinal pigment epithelium and of parthenogenetic epidermal melanoblasts in the hair cover of chimeric mice. Electrophoretic analysis of glucose phosphate isomerase isozymes did not reveal parthenogenetic cells in the liver and kidneys of chimeric mice. These data suggest an intense elimination of parthenogenetic C57BL/6 cells in tissues of endodermal and mesodermal origin. In the adult chimeras C57BL/6(PG) ⇌ BALB/c, parthenogenetic cell clones of ectodermal origin are predominantly conserved (Isaev *et al.*, 1999).

A spontaneous parthenogenetic chimera has been described in humans and it has a phenotypic similarity with some hereditary defects (Strain *et al.*, 1995). Parthenogenetic bovine chimeras have been recently obtained experimentally (Boediono *et al.*, 1999), which may be significant for husbandry.

## ANDROGENETIC CHIMERAS

In order to ascertain the differential role of parental genomes in development, not only have parthenogenetic chimeras been created, but also androgenetic (AG ⇌ N) (Barton *et al.*, 1991), and even chimeras consisting of parthenogenetic and androgenetic cells (Surani *et al.*, 1987). Androgenetic embryos were created by transplantation of the second male pronucleus in the fertilized egg with simultaneous removal of the female pronucleus. This is sufficiently time-consuming work and the rate of survival of such chimeric embryos was low. Therefore, these investigations were not repeated, but their informative role is great. They confirm the different role of the maternal and paternal genomes in mammalian development.

Surani *et al.* (1987) created chimeras between androgenetic and parthenogenetic mouse embryos. They created seven 10-day-old chimeric embryos (6% of the number of aggregated morulas). The rate of development of the chimeric embryos was far below that of the normal embryos. Clonal analysis showed that, in most cases, almost the entire embryo was made up of parthenogenetic cells, while the trophoblast consisted of androgenetic cells. The yolk sac consisted, to a different extent and sometimes equally, of androgenetic and parthenogenetic cells. In the control nonparthenogenetic chimeras, the distribution of parental components in these tissues was random. The simplest explanation may be that the androgenetic cells have a lower division rate and, therefore, are included predominantly in the trophoblast. However, in the same study, 4-cell androgenetic embryos were aggregated with 2-cell parthenogenetic embryos, and the result was similar to that after the aggregation of morulas. Thus, the maternal and paternal genomes play different roles in the development of the embryonic tissues and extraembryonic membranes. Therefore, the normal development of mammals requires the presence of both maternal and paternal genomes in the zygote and cell clones arising as a result of its division. Hence, the effects of genomic imprinting are expressed already during the primary separation of cell lineages at the early developmental stages. This may be accompanied by selective proliferation and/or elimination of cells in the embryo and extraembryonic tissues.

In the experiments of Mann and Stewart (1991), several androgenetic chimeric embryos survived to term, and the development of one of them continued during the postnatal period. Skeletal defects were found in all androgenetic chimeras and the androgenetic cells were present in the extraembryonic tissues.

Barton *et al.* (1991) compared the development of chimeric mice obtained by injection of androgenetic (AG) or gynogenetic (GG) (analogous to parthenogenetic) ICM cells in the cavity of a normal blastocyst. The clonal analysis of tissues of the 12- to 16-day-old androgenetic chimeras 129/HG(AG) ⇌ (C57BL/6J × CBA/Ca)F1 confirmed the earlier data about the predominant involvement of androgenetic cells in the formation of extraembryonic membranes. Two androgenetic chimeras developed to term. However, one of them soon died, and the other was sacrificed for clonal analysis on day 7. By this time, both chimeras were well behind the normal chimeras in their development and had skeletal defects, specifically, deformation of the limbs and sternum, excessive growth of the ribs, distortion of the vertebral column, and elongation and thinning in the caudal part of the vertebral column. Most tissues of mesodermal origin contained androgenetic components. At the same time, no androgenetic cell

clones were found in the tissues of ectodermal origin. The gynogenetic chimeras 129/HG(GG) ⇌ (C57BL/6J × CBA/Ca)F1 were more viable than the androgenetic ones, and, except for delayed development, had no visible defects. The gynogenetic cell clones were involved in the formation of predominantly neuroectodermal derivatives. The data obtained suggest differential roles of the parental genomes, paternal and maternal, in the formation of mesodermal and ectodermal derivatives, respectively.

Note, in conclusion, that the clonal analysis of forming tissues in parthenogenetic and androgenetic chimeric mice showed different roles of the expressed alleles of the imprinted loci of maternal and paternal genomes in the development of the embryo itself and of its extraembryonic membranes. If the expression of alleles of the imprinted loci of maternal chromosomes plays an important role in the development of the tissues and organs of the embryo, that of alleles of the imprinted loci of paternal chromosomes is essential for the formation of extraembryonic membranes, specifically, amnion and allantois. The development of different cell clones originating from three germ layers is also under the differentiated control of the expressed alleles of the imprinted loci of maternal or paternal chromosomes. If the development of cell clones derived from mesoderm and endoderm is determined by the expression of alleles of the imprinted loci of paternal chromosomes, the development of cell clones derived from ectoderm depends, to a greater extent, on the expression of alleles of other imprinted loci of the maternal chromosomes.

The normal development of mammals requires both chromosome sets: maternal and paternal. A corresponding balance of gene activity essential for normal development appears as a result of differential expression of the genes of various imprinted loci. The death of diploid parthenogenetic (gynogenetic) or androgenetic mammalian embryos is due to the absence of expression of the genes of imprinted loci of the paternal or maternal genomes, which leads to unbalanced gene activity and defective development of tissues and organs.

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