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THEORETICAL PAPERS AND REVIEWS

Genomic Imprinting in Epigenetic of Mammals

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Abstract—Genomic imprinting is one of the most remarkable and important epigenetic phenomena. A biological ban on parthenogenetic and androgenetic development of mammals is an obvious consequence of genomic imprinting. Genomic imprinting defects may cause malformations, clinical syndromes, and tumor growth in humans and to the large offspring syndrome and an increased mortality after in vitro manipulations with early embryos in mammals. Differential expression of parental alleles during ontogeny implies a mechanism of reversible, selective marking of gene alleles. These relatively stable epigenetic modifications, which do not affect the primary nucleotide sequence of DNA, may be transmitted in somatic cell lines and reproduced in the germ line. The genomic imprinting mechanism may be involved in other epigenetic processes, such as epigenetic inheritance, nonrandom allele segregation, meiotic drive, etc. Artificial modulation of genomic imprinting effects with the use of growth factors and demethylating agents permits partial "normocoping" during the development of parthenogenetic mouse embryos. Targeted changes in the transcriptional activity of imprinted genes provide prerequisites for epigenetic correction of syndromes and diseases caused by genomic imprinting defects.

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INTRODUCTION

The study of the epigenetic control of gene expression during the development of complex organisms is a relatively new field of genetic research [1]. Epigenetics deals with various processes altering gene activity via modifications that are relatively stable in the sequence of cell generation but do not affect the primary DNA nucleotide sequence [2]. Epigenetic control mechanisms, including genomic imprinting, are involved in many processes of normal and pathological development of humans and animals [3].

Genomic imprinting is a reversible, selective modification of gene alleles depending on their parental origin leading to differential expression in the course of development [4].

Genomic imprinting has been intensely studied since the early 1980s, the interest in it, as well as the number of experimental studies and reviews on this problem, constantly increasing [4–10]. The theory of genomic imprinting is generally accepted to date owing to studies by Surani and other researchers [4, 11–14]. There is a Web site on genomic imprinting (http://www.geneimprint.com).

Many authors believe that genomic imprinting is widespread not only in Placentalia, but also in Marsupalia [15, 16], fishes [17], insects [18], and plants [19].

In the mammalian genome, imprinted genes are the genes one of the parental alleles of which is repressed and the other is transcribed [4]. About 80 imprinted loci of the mouse and human genomes are known to date [20].

Allele imprinting is related to the methylation of cytosine bases in CpG dinucleotides of the key regulatory elements of a gene [21–23]. Almost all imprinted genes have CpG-rich differentially methylated regions (DMRs). The methylation of DMRs is usually related to allele repression [24]; however, active alleles of some imprinted genes contain DMRs [25]. Many imprinted genes are arranged in clusters (imprinted domains) in chromosomes. Imprinted genes affect animal growth, development, viability, and behavior; however, the phenotypic effects of many imprinted genes remain unknown.

The expression of imprinted genes may be tissue- and stage-specific; i.e., one of the parental alleles may be differentially expressed only at a certain developmental stage and/or in a certain cellular system, the gene expression being biallelic at other stages and in other cellular systems. The monoallelic expression of an imprinted gene is not absolute; in fact, a wide spectrum of relative expression of imprinted parental alleles is formed, from strictly monoallelic to biallelic [26]. Probably, the morphogenetic role of genomic imprinting in the differentiation of tissue types is to determine the transcription rate of the genes that influence growth via a fine balance between the expressions of the parental alleles. This is confirmed by the data that the methylation profile is specific for different cell lines, tissue types, differentiation levels, and stem cell potencies [27].

GENOMIC IMPRINTING AND EPIGENETIC INHERITANCE

The transmission of the allele transcription activity status to successive generations is termed epigenetic inheritance. Usually, genomic imprinting is distin-

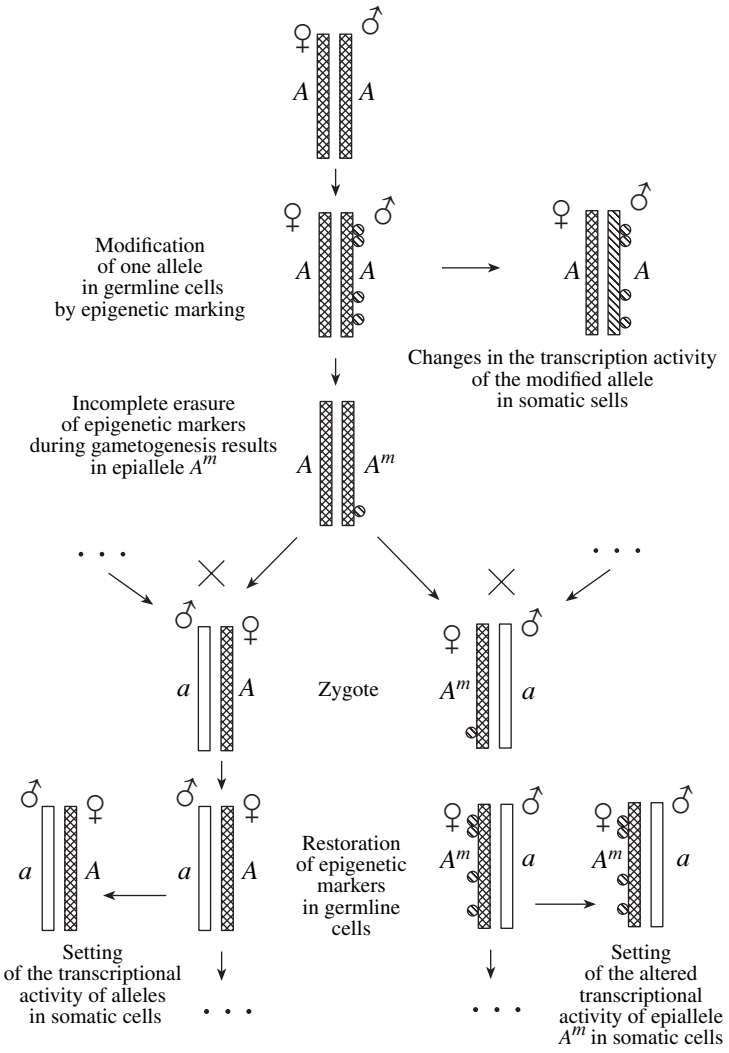

Fig. 1. Epigenetic inheritance.

guished from epigenetic inheritance, because the effects of genomic imprinting are expressed only in somatic cells; in the germ line, the epigenetic differences between the two parental alleles are "erased" altogether during meiosis and emerge again afterwards, depending on the sex of the parent [28]. Nevertheless, some genes are recognized as imprinted in a succession of generations, which may be regarded as an example of epigenetic inheritance. Latham [29] defines genomic imprinting as a form of epigenetic inheritance and demonstrates that the expression of genomic imprinting varies in different laboratory strains of mice.

The inheritance of somatic epigenetic modifications is prohibited by the "Weissmann barrier," the isolation of the genome of germline cells from the effect of the soma. Moreover, epigenetic modifications that appear in the genome of germline cells are eliminated or reprogrammed during gametogenesis and early embryogenesis to ensure the totipotency and pluripotency of stem cell lines. Thus, to avoid contradiction to classic embryological, genetic, and evolutionary theories, epigenetic inheritance should meet two necessary conditions: (1) epigenetic modifications appear in germline cells and (2) there are restored after their erasure during gametogenesis.

An example of epigenetic inheritance is the inheritance of the expressive status of the A^{vy} (*agouti viable yellow*) allele of the *agouti* locus in mice reported by Morgan et al. [30]. Mutant gene $Axin^{Fu}$, which causes the shortening and deformation of the tail in mice, exhibits epigenetic inheritance in the male lineage [31, 32]. Epigenetic inheritance is observed in some cases of transgenesis. Transgenes are methylated, and its completely or partly repressed status is inherited in successive generations, sometimes depending on the parental origin of the transgene allele [33–37].

Note that de novo formation of the previous pattern of epigenetic markers after their elimination during gametogenesis is a problem entailed by both genomic imprinting and epigenetic inheritance (Fig. 1). After

fertilization, the resultant zygote combines two haploid parental sets of chromosomes that have characteristic genetic markers (imprints) resulting from the methylation of cytosine bases in DMRs during gametogenesis. These imprints are then reprogrammed in somatic cells in the course of individual development. In germline cells, these imprints are preserved; however, they are erased in the course of spermatogenesis or oogenesis and are formed de novo by the moment of gamete maturation. The mechanism for recognition of the DMRs of imprinted genes is unknown. Mann [38] considers the following possibilities of this recognition of a gene that is to be imprinted de novo.

(1) The differential expression or activity of transacting factors, e.g., isozymes of DNA methyltransferase, may affect the type of the epigenetic modification of the allele [39]. Probably, there are DMR-binding protein modifiers that prevent DMR methylation "by default" or, conversely, marking DMRs for their recognition by DNA methyltransferases.

(2) Different forms and quantitative ratios of histones in the male and female germ lines may determine different chromatin structures in DMRs, which affects the methylation profile. The dynamic nature of chromatin structure is determined by the modification of histones and association of nonhistone proteins with specific DNA regions. Histones may be modified by methylation, acetylation, ubiquitinylation, phosphorylation, and ADP-ribosylation [40]. Acetylation, the best studied modification of histones, is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs) [41]. Acetylation decreases the affinity of histone protein H4 to DNA, which is followed by chromatin relaxation, whereas deacetylation is related to H1 activation and chromatin condensation [42–45]. Some HATs that are part of the DNA–histone complex may remain bound with DNA throughout the cell cycle; in addition, HATs and HDACs may specifically bind with methylated regions of DNA and, together with methylases, maintain or restore the methylation pattern during replication [46]. Models of imprint reprogramming based on the close relationship between DNA methylation, histone modification, and changes in chromatin structure have been considered in detail by Li [8].

(3) Imprints determined by methylation may be partly preserved in the male germ line (the so-called residual methylation) [47].

Regarding the possible biological functions of genomic imprinting, protection against retroviral infections is often mentioned [5]. It is noteworthy that epigenetic inheritance is associated with the incorporation of heterologous DNA into the genome via retrotransposition or transgenesis. The mammalian genome "protects" itself against the new allele by repressing it via methylation. Apparently, the same molecular mechanism is responsible for the restoration of the pattern of these modifications in the germ line in the case of genomic imprinting and epigenetic inheritance.

GENOMIC IMPRINTING, MEIOTIC DRIVE, AND NONRANDOM ALLELE SEGREGATION

Mammalian oogenesis begins as early as during the embryonic development. The first-order oocyte pool is constant and is not replenished by mitoses; oocyte development is arrested at the diplotene stage of the prophase of the first meiotic division (MI) and often remains in this state for years, until meiosis is resumed after sexual maturation. In most mammals, ovulation occurs at the metaphase stage of the second meiotic division (MII) and complete meiosis only after fertilization. A characteristic feature of meiosis in mammalian oogenesis is that only one haploid ovum, rather than four functional gametes, is formed. In contrast to spermatogenesis, divisions MI and MII of oogenesis are asymmetric, each of them producing a polar body that is not a functional sexual product. This asymmetry of female meiosis underlies the mechanisms leading to nonrandom allele segregation and, hence, distorting the equiprobable Mendelian ratio in allele inheritance [48]. In 1936, Sturtevant and Beadle [49] demonstrated that asymmetric meiosis where certain alleles are preferentially included only into the functional product of oogenesis (oocyte) might explain some cases of deviations from the expected Mendelian frequency of inheritance.

Kaufman [50] coined the term nonrandom segregation to describe the unequal or nonrandom distribution of alleles or chromosomes because of changes in the mechanism of chromosome segregation. Other terms, insignificantly differing in meaning, are also used: preferential segregation [51], polarized segregation [52], abnormal segregation [53], and nonrandom disjunction [54].

Nonrandom segregation is a consequence of an epigenetic mechanism known as meiotic drive, when a heterozygote does not produce two types of gametes with equal frequencies [55–58]. It is generally accepted that meiotic drive, which occurs during gametogenesis, should be differentiated from the cases of deviation from the Mendelian ratio between alleles in a population results from the death of gametes or embryos, selective fertilization, etc. Asymmetric meiosis in oogenesis may be determined by nonrandom allele segregation in either the first or the second meiotic division (Fig. 2).

Nonrandom segregation distorts the Mendelian frequencies of allele transmission to the offspring, which is called transmission ratio distortion (TRD). The term was used to describe the unequal distribution of alleles in the *t*-haplotype system in mice, which afterwards proved to be originally Mendelian but secondarily affected by male gamete dysfunction [58–61]. TRD is defined as a statistically significant deviation from the expected Mendelian frequency of inheritance irrespective of the cause; i.e., TRD itself does not indicate a nonrandom allele segregation [48].

The TRD of locus *Om* (*Ovum mutant*) is an example of a true meiotic drive in mice. Pardo-Manuel de Vil-

lena et al. [48] found that the unequal segregation of the alleles of locus *Om* occurred during MII (after fertilization) and depended on the spermatozoon genotype.

At first glance, the relationship of genomic imprinting with nonrandom allele segregation during meiosis seems the least obvious. Pardo-Manuel de Villena et al. have hypothesized that the main function of genomic imprinting is to ensure that homologous chromosomes be paired during meiosis. Thus, functional heterozygosity in the locus affecting the attachment of chromosomes to the division spindle should be the necessary condition for allele segregation during meiosis. Therefore, imprinting defects leads to TRD in imprinted loci as a result of the disturbance of random segregation [48]. Experimental data obtained by Croteau et al. [62] have led them to the assumption that, conversely, stochastic errors of genomic imprinting appear at any developmental stage and lead to TRD because of embryonic death. It is possible that the contribution of genomic imprinting to is determined, in different situations, by either secondary factors (gamete dysfunction or embryonic death) or meiotic drive.

Two distorter genes have been mapped to the mouse X chromosome, *Dcsx1* and *Dcsx2* [63]. The *Dcsx1* distorter is located in the chromosomal region also containing locus *Ihpd* influencing the development of the placenta [64, 65]. The *Dcsx2* distorter is located near the imprinted gene *Xist* responsible for the preferential inactivation of the paternal X chromosome in nonembryonic tissues. Naumova et al. [66] found the X chromosome region that is the most prone to TRD, which was called *DMS1*. This distorter is assumed to be an imprinted locus necessary for the viability of male embryos.

GENOMIC IMPRINTING DEFECTS

Imprinted genes account for a small proportion of the mammalian genome but play an exceptionally important role in embryogenesis, in particular, the formation of visceral structures and the nervous system. Genomic imprinting defects lead to various malformations and clinical syndromes in humans and animals. Both mutations and stable epigenetic modifications in somatic cells may disturb the expression of imprinted genes [67]. Malformations and syndromes caused by genomic imprinting defects are being intensely studied in model objects (mice) and humans [68].

In 1991, Willadsen [69] reported on the so-called large offspring syndrome (LOS) when describing malformations in newborn calves obtained by embryo cloning. The main manifestation of LOS is an accelerated embryo growth and an increased body weight upon birth. Usually, the weight is increased by 8–50%; however, the weight of some newborn lambs is five times larger than normal [70]. Prolonged pregnancy [71], dystocia (abnormal parturition) [72], respiration or sucking disorders, and sudden perinatal death [70, 73] are also often observed. A frequent fetal death in the first half of pregnancy is also often associated with LOS [74, 75]. Placental abnormalities, including polyhydramniosis, are sometimes observed [72, 76, 77], a large size of the fetus being not correlated with an increased placenta [77, 78]. The newborns may have hypothermia, hypoglycemia, acidosis, and hypoxia [73]. Calves with LOS also exhibit an increased muscular mass and changes in muscle fiber structure [79], cerebellar dysplasia [80], and structural abnormalities of the skeleton, including those of facial bones [70]. There are data on LOS in cloned mice (Fig. 3) [81]. LOS is thought to be caused by disturbance of the expression of the *Igf2r* gene [82]. It is assumed that LOS is related to disturbances in the expression of imprinted genes determined by both manipulations with gametes or early embryos [83–85] and inadequate conditions of in vitro culturing [86]. Note that LOS is found only in a few animals obtained with the use of in vitro culturing.

As soon as the genomic imprinting theory was put forward, it was assumed that some hereditary diseases and syndromes in humans were related to the impairment of this process [87]. To date, many such pathologies are known, and they have been combined into a separate class, genomic imprinting diseases [88–90]. Table 1 shows the best known genomic imprinting diseases and the corresponding defects of imprinted loci.

The interest in genomic imprinting considerably increased in connection with the use of assisted reproduction technologies (ARTs) involving in vitro manipulations with gametes and early human embryos. Potential factors impairing the expression of imprinted genes related to ARTs are considered in [67]. The health of children that were conceived with the use of ARTs has been intensely discussed by the medical scientific community since the late 1980s [100]. According to Schieve et al. [101], the proportion of newborns with a low or very low weight at birth is 2.6 times higher among infants that have been conceived with the use of ARTs (excluding multiple pregnancies) than among those conceived naturally. Independent studies performed in the United States, United Kingdom, and France showed that the relative risk of Beckwith–Wiedemann syndrome was significantly increased by a factor of three to six if ARTs had been used [84, 85, 102]. In almost all cases, this syndrome was caused by defective imprinting in the differentially methylated KvDMR region of the maternal allele of the *KCNQ1B* gene. Data on Angelman syndrome in two children [83] suggest the disease may have been related to the use of ARTs; Orstavik et al. [103] reported one more infant with Angelman syndrome that was conceived by means of intracytoplasmic sperm injection (ICSI). The disease in these three children was caused by a complete or partial loss of the methylation of maternal allele *SNRPN*. A group of Danish researchers reported on five cases of retinoblastoma in infants conceived with the use of ARTs [104]. According to Maher et al. [105], ARTs

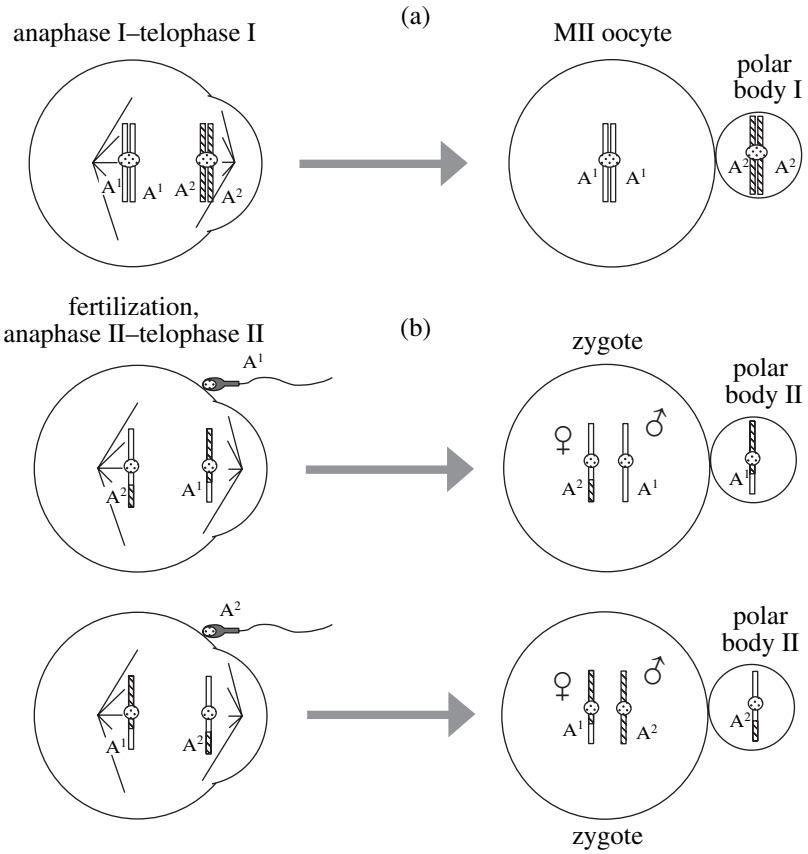

Fig. 2. Meiotic drive. (a) In the absence of recombination between homologous chromosomes, allele A^1 is preferentially included into the oocyte during the first meiotic division; (b) if both alleles are present in the mature oocyte, their segregation during the second meiotic division depends on the spermatozoon genotype.

may cause many other, unknown genomic imprinting defects causing long-term negative consequences.

The loss of imprints resulting in biallelic expression of some imprinted loci may cause malignant tumors [106]. The difference in imprinting patterns is one of the factors of the malignant transformation of stem cells transplanted into recipient tissues [107, 108].

Genomic imprinting defects have been observed in experiments on animal cloning. Therapeutic cloning technologies are an intensely developing field of modern medicine in which understanding the epigenetic mechanisms of gene effects plays an important role. It has become clear that epigenetic programming should be taken into account for a normal development of a somatic cell or nucleus used for cloning [109, 110]. The frequency of successful animal cloning remains very low; many animals that have grown up to the adult state have malformations undoubtedly related to the impossibility to reprogram the original somatic nucleus.

PARTHENOGENETIC DEVELOPMENT OF MOUSE EMBRYOS AS A MODEL SYSTEM FOR THE STUDY OF GENOMIC IMPRINTING IN MAMMALIAN EMBRYOGENESIS

The death of diploid parthenogenetic or androgenetic mammalian embryos is determined by the absence of the expression of imprinted loci of the maternal or paternal genomes, which leads to gene activity imbalance and abnormal development of tissues and organs [3]. The possibility to induce artificially the parthenogenetic development of mammalian oocytes gave rise to numerous studies on genomic imprinting with the use of experimental parthenogenetic embryos (PEs) as a model system. Studies on artificial parthenogenesis in mammals have been performed since the 1930s [111]; however, it was not until the early 1980s that effective methods of the activation [112] and obtaining PEs at somite developmental stages were found [113]. At present, various laboratory strains of the house mouse (*Mus musculus*) and their hybrids are the most popular object off these studies.

Despite the obvious advantages of this model system, the early death of PEs restricts its use. Parthenogenetic embryos usually die soon after implantation and the start of organogenesis, at stages of no more than 25 somite pairs [114]. Therefore, to overcome these limitations and obtain more advance development of PEs is necessary for the study of imprinted genes controlling individual stages of mouse embryogenesis. Integrated culturing methods applicable to pre- and postimplantation diploid parthenogenetic mouse embryos developed or improved recently allow their

growth and development to be experimentally altered in various ways [115].

We used PEs of inbred CBA and C57BL/6 mice as a model system for studying the effects of genomic imprinting, because the potencies of the development of PEs and parthenogenetic cell clones in a chimeric organism may be substantially different in these two strains. Penkov and Platonov [116] found that CBA and C57BL/6 mice and their reciprocal hybrids considerably differed from one another with respect to the capacity for the development of PEs in the pre- and postimplantation periods (Table 2). Parthenogenetic ova of C57BL/6 mice developed in vitro until the blastocyst stage in 89.7% of cases; however, all of them died soon after the implantation to the uterus. In contrast, only 14.7% of parthenogenetic ova of CBA mice reached the blastocyst stage; however, after the blastocysts were transferred to the uterus of pseudopregnant females, 26% of implanted embryos developed to somite stages. The ova of (CBA × C57BL/6)F_1 and (C57BL/6 × CBA)F_1 hybrid females exhibited intermediate frequencies of the formation of parthenogenetic blastocysts (72.6 and 75%, respectively) compared to the two original inbred strains.

We found that 30.4% of (CBA × C57BL/6)F_1 hybrid embryos developed until somite stages (Table 2). In (C57BL/6 × CBA)F_1 mice, such embryos were fewer (16%) [117]. This suggests that it is important from which strain (CBA or C57BL/6) the X chromosome that underwent inactivation has originated. Apparently, strains CBA and C57BL/6 are homozygous for different alleles of two genes responsible for chromosome imprinting. One of these genes may control the preimplantation development of embryos, affecting the capacity for blastocyst formation, whereas the other gene is likely to control the inactivation of one of the X chromosomes, thereby promoting embryonic development until somite stages.

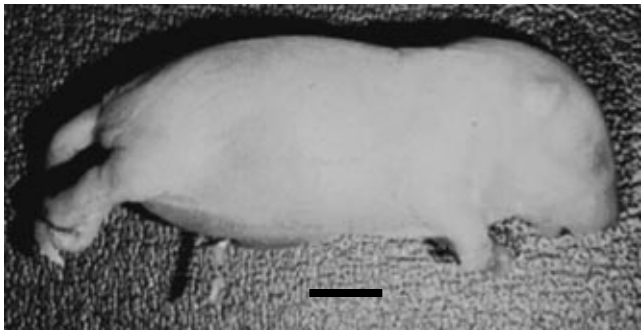

Fig. 3. Large offspring syndrome in cloned mice. Scale, 1 mm (reproduced from [81] with modifications).

Thus, it was found that mouse strains CBA and C57BL/6 and their reciprocal hybrids considerably differ in the capacity for the in vitro development of preimplantation and early postimplantation PEs. These experiments demonstrated that the genotypic environment could modulate the effects of genomic imprinting and determined differences between inbred mouse strains in the capacity for the development of diploid PEs.

Experiments on chimeric mouse PEs demonstrated that the expression of the alleles of imprinted loci of paternal chromosomes strongly affected the development of cell clones derived from the mesoderm and endoderm, whereas the formation of cell clones derived from the ectoderm more depend to a higher degree on the expression of the alleles of imprinted loci of maternal chromosomes. Therefore, the derivatives of the mesoderm and endoderm were more markedly underdeveloped in the PEs, and cell clones of ectodermal origin developed better [118].

Isaev et al. [119, 120] found that parthenogenetic cell clones (PGCCs) of ectodermal origin (epidermal melanocytes, retinal epithelial cells, and brain cells) were preferentially preserved in C57BL/6(PG) ⟷ BALB/c

Table 1. Genomic imprinting diseases (from [67])

Disease	Chromosome	Imprinted genes	Imprinting defects
Beckwith–Wiedemann syndrome	11	*IGF2, H19*	Paternal isodisomy of the 11p15.5 cluster or suppressed expression of the maternal *H19* allele because of methylation [91]
Wilms's tumor	11	*IGF2, IGF2AS, ç19*	Imprinting defects resulting in the biallelic expression of *IGF2* [92]
Hemihyperplasia	11	*IGF2*	Paternal isodisomy of the 11p15.5 cluster [93]
Hepatoblastoma	11	*P57äIP2*	Suppressed expression of the maternal *KIP2* allele [94]
Pituitary adenoma	14	*MEG3*	Suppressed expression of the maternal *MEG3* allele [95]
Angelman syndrome	15	*SNURF-SNRPN, UBE3A*	Deletion of the 15q11-q13 cluster of the material chromosome, disomy of paternal chromosome 15; a point mutation at the active allele or an imprinting center mutation [96, 97]
Pradera–Villi syndrome	15	*SNURF-SNRPN, PAR1, PAR5, PAR-SN*	Microdeletion of the 15q11.2-q13 cluster of the paternal chromosome or isodisomy of maternal chromosome 15 [98, 99]

Table 2. Development of diploid PEs in vitro until the blastocyst stage (96 h of culturing) and after transplantation into the uterus of pseudopregnant females

Ova of mice	Number of diploidized cells	Number of blastocysts (%)	Number of transplanted embryos	Number of implantations (%)	Number of embryos at the somite stage (%)
CBA	416	61(14.7)	153	105(68.7)	47(44.7)
C57BL/6	564	506(89.7)	157	89(56.7)	0
(CBA × C57BL/6)F_1	241	175(72.6)	169	102(60.3)	31(30.4)
(C57BL/6 × CBA)F_1	332	249(75.0)	–	–	–

chimeric mice. In 14-day-old C57BL/6(PG) ⟷ BALB/c embryos, PGCCs were found not only in the brain, but also in the kidneys and liver. By the 18th day of development of these chimeric embryos, PGCCs were usually absent in both kidneys and liver. The elimination of parthenogenetic cells from the liver could continue after the birth of the chimeras; therefore, PGCCs were absent in the liver of adult C57BL/6(PG) ⟷ BALB/c chimeras. These data indicate an intense elimination during the development of parthenogenetic C57BL/6 cells in tissues of the endodermal and mesodermal origin [121].

Genomic imprinting effects are expressed differently at the cellular level in PEs of C57BL/6 and CBA inbred mice. CBA PGCCs of the endodermal, mesodermal, and ectodermal origins are rapidly eliminated during early embryogenesis; therefore, CBA(PG) ⟷ BALB/c embryos die more often than C57BL/6(PG) ⟷ BALB/c ones. In live 14-day-old CBA(PG) ⟷ BALB/c embryos, in which, apparently, the contribution of the parthenogenetic component was originally small, CBA PGCCs were absent altogether. In contrast, C57BL/6 PGCCs were gradually eliminated from the tissues and organs of C57BL/6(PG) ⟷ BALB/c embryos during the entire embryogenesis, and this was mainly accounted for by the death of PGCCs of endodermal and mesodermal origins [121].

MODULATION OF GENOMIC IMPRINTING EFFECTS

Cytokines, a diverse group of signal proteins serving as humoral regulators, are important for communication processes in multicellular organisms. Cytokines can modulate the functional activities of individual cells and tissues under normal and pathological conditions [122], thereby participating in normocopying, i.e., phenotype correction in cells with a pathological genotype [123]. Polypeptide growth factors belong to the large family of group II cytokines and play the key role in various processes involved in mammalian early embryogenesis; e.g., they stimulate the proliferation and differentiation of individual cell populations [124, 125].

Among the growth factors whose effects we studied, the following are of special interest: (1) insulin-like growth factor 2 (IGF2), the key component controlling embryonic growth [126, 127], whose synthesis is blocked in PEs because of the imprinting of maternal allele *Igf2* at postimplantation stages and (2) transforming growth factor α (TGFα) activating embryonic growth and playing an important role in the development of trophoblast derivatives [128, 129], whose formation is strongly suppressed in PEs.

The synthesis of IGF2 is substantially decreased or absent altogether because of the imprinting of the *Igf2* gene in maternal chromosomes of PEs. This leads to growth inhibition [11] and the death of PEs before the formation of the mesoderm [130]. Therefore, we prolonged the development of PEs by sequentially adding two exogenous growth factors of different types into the nutrition medium in vitro. One of the growth factors was FGF2 belonging to the large family of fibroblast growth factors, which play an important role in the key developmental processes [131] and increase the number of PEs at somite stages by a factor of two [132]. The other factor was IGF2, a representative of insulin-like growth factors, which is the key component controlling embryonic growth [126, 127]. An in vitro culture of PEs was treated with IGF2 at the stage of 18–20 somites. The experiments showed that PEs treated with IGF2 at somite stages develop in vitro considerably longer than the embryos cultured in the absence of IGF2. After the IGF2 treatment, a greater number of PEs developed until the stage of 30–45 somites, some of them reaching the stage of 50 somites (Table 3). In these embryos, the iris was pigmented and the body length was as large as 4.0 mm. However, the IGF2 treatment did not allow us to obtain PEs with more than 50 somites, whereas normal (fertilized) embryos developed in vitro until the stage of 55–56 somites under similar conditions. Thus, we found that replenishment of IGF2, which was not synthesized in PEs, considerably improved the embryonic development [115, 133].

One of transforming growth factors, TGFα, is known to activate embryonic growth and participate in the development of trophoblast derivatives, including the placenta and extraembryonic membranes [134]. Therefore, we considered it important to study its effect on the development of extraembryonic tissues in the PEs of mice in which these structures were considerably underdeveloped. The study of the TGFα effect on the postimplantation development of PEs showed that the addition of 10 ng/ml of TGFα to the nutrition medium increased the number of somite embryos

Table 3. In vitro development of postimplantation mouse PEs treated with growth factors

FGF2, ng/ml	IGF2, μg/ml	Number of PEs					
		cultured	developed to the following somite stages				
			≥30 (24 h)	≥35 (36 h)	≥40 (48 h)	≥45 (48 h)	≥50 (64 h)
–	–	22	13	8	5	2	–
2.5	–	21	14	10	6	1	–
–	2.5	20	18*	17**	14**	6	2
2.5	2.5	23	20	19**	16**	7	1

Note: FGF2 and IGF2 were added to the culture medium during the preimplantation period at the morula stage and during the *in vitro* cultivation of 18- to 21-somite PEs, respectively, for 64 h. The time of cultivation (hours) is indicated in parentheses.
* $P < 0.05$; ** $P < 0.01$.

developed in utero until the stage of 30–45 somites to 23%, the placenta being well-developed in 37% of PEs.

The PEs with 42–45 pairs of somites had a well-developed placenta 4.0–4.2 mm in diameter, which approximately corresponds to the size of the placenta of normal (developing from fertilized ova) 11-day-old mouse embryos. The improved development of the derivatives of the trophoblast and embryonic tissues in PEs treated with TGFα may have been determined by the activation of some imprinted genes, including the maternal allele of the *Igf2* gene controlling the synthesis of growth factor IGF2, a potent stimulator of embryonic tissue growth [127]. Therefore, we studied the ability of TGFα to participate in the reactivation of imprinted genes controlling the formation of embryonic tissues and the trophoblast. For this purpose, we performed in situ hybridization in whole mounts with the use of a dioxygenin-labeled antisense RNA probe and estimated the possibility o activate the imprinted maternal allele of the *Igf2* locus in mouse PEs preliminarily treated with TGFα during the preimplantation period in vitro. Control PEs that had developed until the stage of 23–25 somites did not express the *Igf2* gene. In contrast, we observed *Igf2* expression in most of the whole mounts of PEs at the most advanced stages (37–42 somites) treated with TGFα in vitro.

These data indicate that the main modulating influence of TGFα on genomic imprinting effects begins at the blastula stage in two original cell lines: the internal cellular mass and the trophoectoderm. At later stages of embryogenesis, TGFα, participating in the formation of cell clones in various tissues and organs, affects the expression of some imprinted genes; e.g., it activates the expression of imprinted the *Igf2* gene.

The mouse *Igf2* and *H19* genes are closely linked and reciprocally imprinted in chromosome 7 [135]; therefore, we analyzed the expression of these genes using reverse-transcriptase polymerase chain reaction (RT–PCR). We found that TGFα caused the derepression of the imprinted *Igf2* gene in PE tissues and the placenta (Fig. 4). Differential analysis of *Igf2* expression with the use of primers specific for individual promoters showed that promoters P1–P3 characteristic of embryonic tissues were reactivated in TGFα-treated mouse PEs but not in placenta specimens [126]. In the placenta, promoter P0 specific for this organ is activated [136].

It may be assume that TGFα can selectively reactivate promoters of the *Igf2* gene, which causes its derepression. When the product of the imprinted gene appears, the effects of genomic imprinting are relatively normalized, and the viability of PEs increases.

Our studies demonstrated that the model conditions of the parthenogenetic development of embryos of inbred mouse strains and hybrid mice permitted considerable modulation of the effects of genomic imprinting with the use of the corresponding peptide growth factors.

Various approaches to normalizing the imbalance of gene activity and modulating the effects of genomic imprinting in parthenogenetic development of mammals are being developed. Kono et al. [137] reported that diploid mouse PEs denoted ng^{wt}/fg^{wt}, which contained one genome from an immature (nongrowing) oocyte (ng^{wt}) and the other one from a mature oocyte that had completed its growth (fg^{wt}), developed until 13.5 days of embryogenesis, i.e., three days longer than control diploid PEs. In that study, the ng^{wt}/fg^{wt} embryos

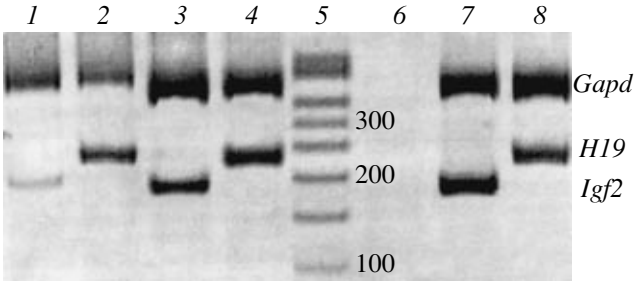

Fig. 4. The results of the PCR analysis of the expression of genes *Igf2, H19*, and *Gapd* (control) in (*1, 2*) the mouse placenta and (*3, 4*) tissues of mouse PEs treated with TGFα and (*7, 8*) in tissues of normal (fertilized) mouse embryos. (*5*) Marker nucleotides.

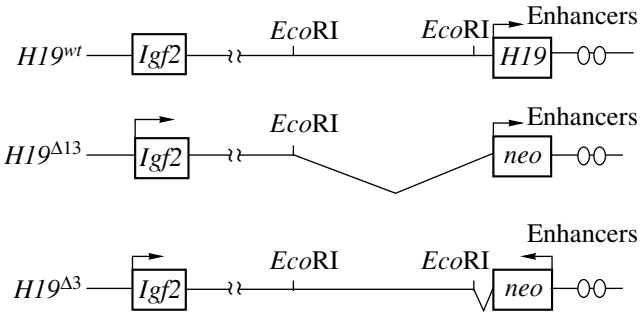

Fig. 5. The diagram of mutant maternal alleles of mice (from [139]).

were obtained by serial transplantation of oocyte nuclei. The results of another work by the same authors [138] where ng^{wt}/fg^{wt} mouse embryos reconstructed by the same method were used demonstrated that disturbance of primary imprinting during oocyte growth could alter the expression of imprinted genes in the course of embryogenesis. Expression of three paternal alleles (*Peg1/Mest*, *Peg3*, and *Snrpn*) and repression of two maternal alleles of imprinted loci (*Igf2r* and $p57^{KIP2}$) was observed in 9.5- and 12.5-day-old embryos. To block the expression of the *H19* gene, Kono et al. obtained mutant mice carrying a 3-kb deletion in the transcribed region of the *H19* gene. These mice were the source for obtaining embryos with genotype $ng^{H19\Delta 3}/fg^{wt}$, which developed until an embryonic age of 17.5 days. Recently, Kono et al. [139, 140] reported striking data on obtaining two viable adult parthenogenetic mice with genotype $ng^{H19\Delta 13}/fg^{wt}$ carrying a 13-kb deletion (Fig. 5) in the region of the *H19* gene. This impressive result was obtained after numerous experiments (457 reconstructed ova), so that the efficiency was as low as 0.5% (two adult mice). We think, however, that these mice should be classified with gynogenetic organisms, because the modified genome was introduced into the ovum from outside.

It should be emphasized that the results of this study are one more proof that the effects of genomic imprinting can be modulated, in particular, by activating the expression of the imprinted *Igf2* gene in parthenogenetic or gynogenetic mice simultaneous with the monoallelic expression of the *H19* gene. It may be hoped that the control of the expression of imprinted genes with the use of exogenous growth factors and other biochemical agents changing the transcription activity of imprinted genes will make it possible to attain normal parthenogenetic development of mammals without the use of complex transgenic technologies and nucleus transplantation. However, the possibility of targeted correction of syndromes and diseases caused by mutations in imprinted loci and epigenetic changes in the functions of imprinted genes seems to be a considerably more important challenge. This becomes increasingly more urgent as ARTs are developed and implemented in medicine, which entails the risk of genomic imprinting diseases.

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