Cytogenetics of Mammalian Embryonic Development

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Preface to the English edition

The Russian version of this monograph, published in 1978, treated the developmental cytogenetics of mammals in a way which had no real equivalent either here in the Soviet Union or abroad. The book summarized numerous original cytogenetic investigations carried out in the Department of Embryology of the Institute of Experimental Medicine, Academy of Medical Sciences of the USSR. It encompassed the numerous key problems, the basic questions, and the major trends in the cytogenetics of mammalian embryogenesis. We tried to make Soviet readers more familiar with relevant work going on abroad, which is sometimes not very readily accessible. Hence our first edition covered most references to this foreign work up to the end of 1976.

We had some serious difficulties in preparing this monograph for the English edition. One major problem was the tremendous growth of experimental work in the field of developmental cytogenetics since 1977. Thorough incorporation of all this new information would have required complete revision of the text, resulting in a quite new, extensive monograph of the handbook type. We have made an effort to avoid this logical outcome, which would have substantially exceeded our mental and physical capabilities!

Another problem was that many comprehensive reviews and extensive monographs (mostly in English) have been published since 1978. We concluded that it would be unnecessary to include detailed accounts of these for the English-speaking reader, who can easily get the original publications.

A further complication results from the language barrier. Many of the theoretical accounts recently produced by our foreign colleagues are in line with basic conclusions already inferred from our own much earlier experimental studies. Unfortunately most of our experiments were published in Russian and thus passed unnoticed by our Western colleagues. Russian scientists, however, are always adequately informed on all scientific work published in English or in any other Western language. This imbalance of information exchange stimulated us to extend the description of experimental results obtained in our own department as well as by other Russian scientists working in developmental cytogenetics.

Trying to avoid major changes in the general structure of the original

monograph, we nonetheless undertook a complete revision of some chapters where there has been substantial new information in recent years. These are Chapters 1 (Haploidy), 6 (Autosomal trisomy), 7 (Monosomy and nullisomy of autosomes), 12 (Influence of genes on early mammalian development), and 13 (Functional activity of chromosomes and mechanisms of early embryonic development). The text of the remaining chapters was updated. The chapter on genetic mosaics and chimaeras which appeared in the Russian edition is completely omitted, as this topic has been thoroughly discussed in several important monographs and major reviews. The English edition of the book does not include the Addendum, which was devoted to the description of standard methods of chromosome preparation at different stages of embryogenesis as well as to the standard karyotypes of common laboratory animals. Both are described satisfactorily in cytogenetic literature published in English.

It should be noted that the Russian edition of the book was prepared not only for narrow specialists in developmental cytogenetics but also for general biologists, and even for clinical geneticists. Hence we tried to avoid unnecessary complications, and some theoretical discussion was deliberately simplified. The English version of these chapters, although somewhat extended, has kept this same style of presentation, which we hope will make the monograph of value to many scientists outside the field of developmental cytogenetics.

Most of the aforementioned remarks concern Chapters 4, 5, 9, and 12. We realize that a good deal of recently published experimental work is not included in this monograph. Most of these works, especially monographs published in the last two or three years, are known to us only by their titles or as short abstracts, which do not allow proper citation in the text. Nevertheless, the list of publications is already overloaded, though still not complete.

We hope that English readers will find this monograph of sufficient use to justify the efforts we have made in preparing this new edition. We hope also that they will forgive any imperfections in presentation resulting from language difficulties, as well as from other obstacles in the way of efficient and rapid exchange of scientific information.

We shall appreciate any kind of constructive criticism and consider the goal of the book achieved if it is of use to the English readers.

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Introduction

The book is devoted to the borderline region of biological sciences related both to genetics and developmental biology. It should be remembered that the term 'cytogenetics' was coined for the science dealing with studies of normal chromosomal complement (karyotype chromosomal transformation, behaviour and interactions in mitoses and meioses) as well as with aberrations of chromosomal structure and number affecting the phenotype of particular cells and the organism as a whole.

In other words, cytogenetics is the study of genetic problems by means of cytological methods, primarily through the visualization of chromosomes displaying structural or numerical alterations which make it possible to elucidate their functional activity.

Hence, developmental cytogenetics might be conventionally admitted as a branch of cytogenetics exploring developmental problems in terms of chromosomal structure and functions related to gene action at different stages of ontogenesis. The cytogenetic approach might also be applied to the studies of gametogenesis, fertilization, embryogenesis, and postnatal stages.

This comprehensive definition of developmental cytogenetics enables one to trace the onset of this science back 50 years, to when the first attempts to study developmental peculiarities in mice with chromosomal aberrations were undertaken (Bonnevie 1934, 1940; Snell, Bodemann, and Hollander 1934; Brenneke 1937; Hertwig 1938). Since that time the main goal of this approach has been considered to be the search for the causative correlations between developmental peculiarities and chromosomal alterations which can help to pinpoint the gene loci responsible for the corresponding morphogenetic processes.

However, the absence of proper cytogenetical techniques and shortage of proper embryological information, especially concerning control mechanisms of morphogenetic reactions, made this constructive approach hardly accessible at that time.

Since before the Second World War, serious cytogenetic studies related to embryonic development have been carried out on *Drosophila*. Detailed knowledge of the polytene chromosome map of this species substantiated developmental analysis of X-chromosome deficiencies. The correlation between the size of missing segments and their phenotype manifestations has

been established. The early embryolethals usually corresponded to more widespread deficiencies and vice versa. The few exceptions from this general rule (i.e. early death and minor deficiency) bore witness to the presence of some genes crucial for early development in these particular chromosome regions (Poulson 1940, 1945). These observations led to the hypothesis of step-like action of genes in ontogenesis, according to which stages of embryonic death as well as of other developmental failures correspond in time to the onset of action of genes located in the missing chromosomal segment (Hadorn 1961).

It took many years to adapt the cytogenetic approach for studying embryonic development in mammals. This delay can primarily be attributed to the absence of adequate cytogenetic methods for detailed identification of mammalian chromosomes and their definite regions. For almost 50 years, mammalian chromosomes were studied exclusively in squash preparations, which did not even allow the exact number of chromosomes in humans and other mammalian species to be established with adequate precision.

It is not surprising therefore that after 50 years of research, almost nothing was known for sure on developmental effects of structural and numerical chromosomal aberrations in mammals. Meanwhile, useful information concerning developmental manifestations of gross genome imbalance (i.e. haploidy, triploidy, tetraploidy, and some even more complicated forms) had become available (Beatty 1957).

The onset of modern cytogenetics might be dated from the innovation of new techniques of metaphase chromosome spreading, based on major discoveries of 'hypotonic miracle' combined with other technical improvements (colchicine pretreatment, air-drying, etc.). The next fascinating achievements of cytogenetics were mainly due to the introduction of differential staining methods, which provided precise identification of metaphase chromosomes and their structural rearrangements. A historical essay with very peculiar presentation and intriguing details on this and many other cytogenetic studies in humans and other mammals has already been published (Hsu 1979), so there is no reason to go into further detail here.

It is noteworthy, however, that all methods successfully applied for chromosomal preparations from bone marrow cells or cell cultures are totally unsuitable for chromosome analysis of mammalian embryos.

Therefore, the experimental cytogenetics of mammalian embryogenesis especially its most intriguing part, concerned with oogenesis and preimplantation stages, lags at least 10 years behind clinical cytogenetics and might be dated back to 1966, when a convenient method of chromosomal preparations from oocytes and cleaving eggs was invented (Tarkowski 1966). Several reliable methods of chromosomal analysis of early post-implantation embryos were suggested soon after this (Wroblewska and Dyban 1969; Evans, Burtenshaw, and Ford 1972) and methods of meiotic chromosome

study from both oocytes and spermatocytes were also significantly improved (Evans, Breckon, and Ford 1964; Dyban 1970b; Meredith 1969). Hence metaphase spreads from the oocytes and early mammalian embryos became amenable for detailed analysis from the early 1970s onwards.

In spite of all these technical achievements, a substantial gap still exists between experimental cytogenetics of mammalian development and developmental cytogenetics of human beings. There is a good deal of conclusive evidence on the major role of chromosomal aberrations in spontaneous abortions, inborn abnormalities, and well-known chromosomal diseases. At the same time, however, we still remain almost totally ignorant of the developmental effects triggered by chromosomal abnormalities in early human embryos.

The basic information on cytogenetic aspects of gametogenesis, fertilization, pre-implantation, and early post-implantation development can be gained from experiments with laboratory animals. Human embryos of equivalent stages are still rather rare and they are poorly studied both morphologically and cytogenetically.

Unfortunately, only a few laboratories are dealing with experimental cytogenetics of mammalian development at present. Our monograph summarizes the results of experimental studies carried out since 1966 on the developmental cytogenetics of laboratory mice and rats (plus a few studies in golden hamsters) in the Department of Embryology at the Institute of Experimental Medicine, Academy of Medical Sciences of the USSR (Leningrad). Comprehensive analysis of all, or most, available experimental works on cytogenetics of mammalian embryogenesis is also included.

Chapters 1–3 deal with peculiarities of mammalian embryogenesis induced by numerical deviations at the whole genome level (haploidy, triploidy, and tetraploidy, respectively). Chapters 4 and 5 summarize methodological prerequisites elaborated for the deliberate production of mouse embryos with numerical and structural aberrations of particular chromosomes. Developmental peculiarities of mammalian embryos (predominantly of mice) with trisomy, monosomy, or nullisomy of individual chromosomes as well as imbalance of gonosomes (X, Y chromosomes) are discussed in Chapters 6, 7, and 8, respectively. Chapter 9 is devoted to structural chromosomal aberrations. Relevant data on human cytogenetics are only briefly outlined in each chapter as there are many original publications, fundamental reviews, monographs, and textbooks on human cytogenetics, some of which are referred to in the text. Comparative analysis of the effects of chromosomal anomalies on embryos of humans and laboratory animals is presented in Chapter 11. Chapter 10 covers all issues concerned with a relatively new branch of developmental cytogenetics (behavioural peculiarities in mammals with chromosomal anomalies), which was initially launched in our collaborative studies with staff members of the Laboratory of Behavioural Genetics at the

Moscow State University. Although shortage of positive knowledge in current developmental cytogenetics significantly hampers theoretical considerations and even makes fundamental generalizations and theories premature, we found it possible to sum up all basic factors and offer some suggestions as to genetic (chromosomal) and epigenetic factors interacting in early mammalian embryos (Chapter 13). Our considerations at the chromosomal level are substantiated by available information on gene action in early mammalian embryos, reviewed in Chapter 12.

In conclusion, it should be stressed that in updating and revising the monograph we have been quite aware that developmental cytogenetics of mammalian species is still at the stage of fact-gathering. It is to be expected, therefore, that some of our generalizations or suggestions will actually turn out to be erroneous and will be refuted by new experimental evidence. Nevertheless, we have deliberately refused to confine ourselves to delivery of pure facts as we could not resist the temptation to make some modest theoretical generalizations and suggest some theoretical, hypothetical mechanisms. We hope the latter are ready for experimental verification or rejection and may stimulate further searches in this area.

1

Haploidy (androgenesis, gynogenesis, parthenogenesis)

1.1 Mechanisms of origin

Embryos with a haploid set of chromosomes may arise in the course of parthenogenesis, gynogenesis, or androgenesis. Let us dwell on each of these mechanisms in detail.

Parthenogenesis is defined as development of an egg containing only the female genome without the intervention of the male gamete (Beatty 1957; Kaufman 1978a). Gynogenesis or pseudogamy means the development of an egg fertilized spontaneously or experimentally by a genetically inactive spermatozoon (Beatty 1957; Graham 1974a). Only the female genome remains active both in gynogenetic and parthenogenetic embryos, so they might be considered as genetically similar. Non-genetic contribution of some unknown extrachromosomal factors by the fertilizing sperm should not be neglected however, and this is the main difference between gynogenesis and parthenogenesis. The development of an egg containing only a male genome, the female genome being completely extruded or genetically inactivated, is called androgenesis.

Parthenogenetic, gynogenetic, and androgenetic embryos may be haploid, diploid, or chromosomal mosaics, composed of cells of different ploidy as well as of aneuploid cell clones that may be revealed only in the course of detailed cytogenetical studies. Therefore the data on developmental peculiarities of androgenetic, gynogenetic, and parthenogenetic embryos should be taken with caution, if they are not confirmed by solid cytogenetic analysis.

Let us consider first the data on the developmental profiles of gynogenetic and androgenetic embryos in mammals. Cytogenetic studies of parthenogenetic embryos with special emphasis on developmental characteristics of haploid and diploid parthenogenetic embryos (parthenogenones) and the comparison of these genomes with the developmental capabilities of haploid or diploid androgenetic and gynogenetic embryos, will be considered thereafter.

1.2 Gynogenesis and androgenesis

These routes of abnormal development, known both in rats and in mice, are suggested to arise from ovum overripeness (Beatty 1957; Marston and Chang 1964). There are some indications of a very low incidence of androgenesis compared to gynogenesis in murine rodents (Beatty 1957; Kaufman 1983d). According to some recent data, however, spontaneous androgenesis in human embryogenesis is much more common.

1.2.1 Spontaneous androgenesis in humans

This route of embryonic development has been recently identified in studies of the rather common pathological syndrome known as hydatidiform mole. This syndrome is characterized by hydropic degeneration of chorionic villi, hyperplasia or dysplasia of the trophoblast, accompanied by the early death of the embryo or the fetus. Cytogenetic studies of these moles distinguish between partial and complete (true) diploid moles (Vassilakos, Riotton, and Kajii 1977; Szulman and Surti 1978, 1984). According to chromosomal analysis the partial moles were found to be triploid, while complete moles were diploid. The triploid specimens are characterized by the typical hydatidiform swelling and cystic degeneration of chorionic villi in only some patches of chorion and placenta. Many groups of chorionic villi seem quite normal. Focal trophoblastic hyperplasia is usually confined to the syncytiotrophoblast layer; which nonetheless shows no propensity to become malignant and transform into chorionepithelioma. These partial hydatidiform moles are distinguished by rather late embryonic death (after the ninth week) and are usually aborted during the third or fourth trimester. In some more rare cases the triploid embryos may reach term. They then display a definite syndrome of malformation (Makino, Sasaki, and Takishima 1964; Carr 1971a, b; Niebuhr 1974; Kajii and Niikawa 1977; see also Chapter 2).

True or complete hydatidiform moles are characterized by severe hydropic degeneration of all placental villi, giving a 'bunch of grapes' appearance. Chorionic villi do not contain capillaries, their connective stroma is swollen with many cistern-like cavities, lined by mesenchymal connective tissue. Trophoblastic hyperplasia and aplasia, predisposing to malignant transformation, are also very common features of true hydatidiform moles. The embryos of true moles die very early, before embryo–placental circulation is established. Hence, no embryonic remnants or nucleated blood cells can be seen in these moles. Detailed cytogenetical studies based on chromosomal polymorphism of both parents as well as on different methods of chromosome banding have unequivocally proved the paternal origin of both chromosomal sets in true hydatidiform moles. This means that the diploid complement of each cell of true moles contains exclusively paternal chromosomes (Szulman and Surti 1978; Kajii and Ohama 1977; Jacobs,

Wilson, Sprenkle, Rosenshem, and Migeon 1980; Wake, Takagi, and Sasaki 1978). These original findings have been recently confirmed by combined cytogenetical and biochemical studies. The latter included some marker enzymes such as phosphoglucomutase -1 (PGM-1) and HLA-haploid types (Jacobs *et al.* 1980). The overwhelming majority of true moles have an XX chromosome constitution and they are believed to result from fertilization of an 'empty egg' by a haploid sperm, with subsequent duplication of its genome to give a 46 XX complement. In some rare cases complete moles were shown to be Y-chromosome positive: these may arise from penetration of an egg by two sperms followed by ejection of the female pronucleus (Ohama, Kajii, Okamoto, Fukuda, Imaizunni, Tsukuhara, Kobagashi, and Hagiwara 1981). The time of maternal haploid chromosomal set elimination, its exact mechanism, as well as the factors predisposing for maternal chromosome loss remain entirely unknown as yet.

Homozygosity for some recessive paternal genes responsible for cell proliferation has been implicated as one of the possible reasons for malignant transformation of true mole cells (Kajii and Ohama 1977). These genes are unlikely to be sex-linked, however, as both XX and XY true moles are prone to malignant transformation. Whether such transformation is actually caused by some malfunction of paternal chromosomes or is due to some other factors apart from androgenesis still remains totally unclear.

Thus, diploid androgenesis in humans is quite compatible with preimplantation and early post-implantation development. All of these embryos are inevitably resorbed during the third to fourth weeks of development, while the chorionic part of the embryonic sac survives for some time after embryonic death. The cells of the chorion often reveal a complex of pathological changes and may often undergo malignant transformation at more advanced stages of embryogenesis. No evidence in favour of the existence of haploid androgenetic embryos in humans has been obtained so far. Diploid androgenesis in humans affects embryonic survival and development somewhat differently than in murine rodents, as no cases of true hydatidiform moles with two sets of paternal chromosomes and omitted female genome have ever been reported in rodents.

The existence of testicular teratomas arising from germ cells has been repeatedly reported in murine rodents (Stevens 1967). They might be considered as an example of androgenesis (Graham 1977). They start as groups of undifferentiated germ cells inside testis tubules, which gradually transform into unorganized ectoderm and endoderm epithelia surrounded by mesenchymal cells. The groups of numerous neuroepithelial cells sometimes resemble nerve tube rudiments but no typical trophectodermal or giant cells appear and no morphogenetic processes equivalent to any normal steps of embryogenesis have ever been found.

Thus, androgenetic germ cells with an XY chromosome set possess all the

properties necessary for control of tissue differentiation, but the full embryonic programme encoded by the male genome can be realized only inside the ooplasm. We will come back to this question later (see Section 1.3.1.2).

1.2.2 Experimental production of gynogenetic and androgenetic embryos in mice

Several experimental procedures are already available for the deliberate production of both gynogenetic and androgenetic embryos in mice. The same techniques with some minor modifications may also be applied to early embryos of other mammals.

Androgenetic and gynogenetic haploid embryos in mice may be obtained by mechanical removal of one (male or female) pronucleus from the fertilized egg (Modlinski 1975, 1980; Markert and Petters 1977; Hoppe and Illmensee 1977; Surani and Barton 1983) or by bisection of the zygote into halves, each containing a pronucleus, using a dissecting microscope with a glass needle operated by hand (Tarkowski 1977; Tarkowski and Rossant 1976). Other methods for pronucleus removal include extrusion of one pronucleus with the aid of osmotic shock (Opas 1977); or centrifugation of zona-free mouse zygotes in a gradient density, resulting in two parts, a cytoplast and a karyoplast (nucleated fragment). Some of the latter contain both pronuclei, while others possess only a single pronucleus, thus being androgenetic or gynogenetic (Dyban, Waisman, and Golinsky 1983). All the above-mentioned procedures result in one-cell gynogenetic or androgenetic haploids. If cultured in cytochalasin B or D supplemented medium, which specifically blocks cell division, these one-cell haploids do not cleave but their DNA content doubles. This technique has been widely used for the production of homozygous diploid androgenetic and gynogenetic embryos (Hoppe and Illmensee 1977; Markert and Petters 1977; Modlinski 1980; Surani and Barton 1983). An even more efficient procedure for the production of gynogenetic heterozygous diploid embryos has been offered recently (Borsuk 1982). It consists of two successive steps. First, mouse zygotes are treated with cytochalasin B (or D), which blocks emission of the second polar body and results in zygotes with three pronuclei (one male and two female). (This step is now adopted for the efficient production of triploid embryos—see Chapter 2.) Second, the male pronucleus is withdrawn mechanically, the two female pronuclei being left intact. The resulting product will give rise to a heterozygous gynogenetic diploid embryo capable of further development (see Section 1.3.4).

After dispermic fertilization, withdrawal of female pronucleus will result in heterozygous diploid androgenetic embryos. These and many other possible interferences in the ploidy of zygotic genomes are thoroughly discussed elsewhere (Markert and Seidel 1981; Seidel 1983), though many of these theoretically possible routes have not been tested experimentally as yet.

1.2.3 Embryonic development in gynogenetic and androgenetic haploidy and diploidy

The data on developmental capacities of androgenetic and gynogenetic embryos in mice are still rather contradictory. A proper consideration of available facts is significantly hampered by the diversity of procedures used to produce these embryos. Some of these procedures are quite damaging and may significantly reduce the survival capacity of the resulting gynogenetic and androgenetic embryos. For instance, pronucleus expulsion after osmotic shock is especially harmful for the resulting haploid zygotes, which usually show very poor development *in vitro* and are eliminated in a few cleavages. However, if transplanted into the oviducts, some of these zygotes proceed to the morula stage. Of 22 haploid embryos induced by osmotic shock, four embryos recovered from the oviduct on the fifth day were at the morula stage, with cell counts between 12 and 62. Two of them were successfully karyotyped and were found to be gynogenetic in origin (Opas 1977). Thus, expulsion of the female pronucleus may be more damaging for subsequent development than absence of the male one.

Withdrawal of the female pronucleus according to Modlinski's original technique is also highly traumatic. This is probably because of peculiarities of male and female pronuclear origin and the microfilamentous links formed between each pronucleus and the oolemma membrane. Microfilaments of the male pronucleus are formed relatively late and do not seem to come into intimate contact with sub-oolemma regions of the egg, being rather loosely dispersed in the ooplasm. The female pronucleus results from disjunction of the meiotic chromosomes of the ovum. During the second meiotic division the mitotic spindle rotates so that its main axis becomes radially oriented and the group of chromosomes left after emission of the second polar body gives rise to the female pronucleus. This remains connected with the oolemma membrane by numerous filaments. Mitotic fibres of the female pronucleus are still evident after completion of the second meiotic division. The destruction of these links between pronucleus and oolemma membrane during female pronucleus removal results in much more severe damage to the oolemma and sub-oolemma regions than removal of the male pronucleus, which is still rather loosely connected with ooplasm structures. These morphological peculiarities might be responsible, at least in part, for the poor development of androgenetic haploids (removal of female pronucleus) compared to gynogenetic haploids (removal of male pronucleus). Such an explanation might be admitted as quite plausible for haploid embryos obtained by Modlinski's original technique, which did not involve any artificial derangement of microfilaments and microtubuli before pronucleus withdrawal. The more sophisticated microsurgical technique used at present includes as its initial step the incubation of the zygotes in the presence of cytochalasin B and colcemid (or nocadozol), to destroy microfilament