

Influence of germline modifications of homologous chromosomes on mouse development

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INTRODUCTION

The genetic information contributed by eggs and spermatozoa to development of embryos to term has until recently been assumed to be equivalent. However, evidence now suggests that in the mouse and perhaps in all mammals the parental genomes have complementary roles during embryogenesis and both are essential for development to term (Surani, Barton & Norris, 1984; McGrath & Solter, 1984a; Cattanach & Kirk, 1985; Surani, 1986). Functional differences between the parental genomes presumably arise as a consequence of specific modifications of homologous chromosomes in the germline, when they are segregated and exposed to different epigenetic factors during oogenesis and spermatogenesis. These 'imprinted' modifications of parental chromosomes are then brought together following fertilization and subsequently propagated to influence events throughout development. It is also essential that at some stage of ontogeny the specific modifications are reversed and the whole process repeated in the formation of new germ cells.

In this article we demonstrate the influence of the parental origin of chromosomes on mouse embryogenesis. In the initial experiments, the possible role of extragenetic components from spermatozoa introduced into eggs is considered. Next, studies are described to examine if the information with respect to the parental origin of chromosomes is needed only transiently or whether this information is conserved and propagated during development. The influence of the parental origin of chromosomes on development together with the effects on spatial organization of embryonic tissues will also be discussed. Finally, we propose mechanisms involved in modifying chromosomes, the nature and propagation of such modifications and their influence on gene expression, which is crucial for development from fertilization to term.

Key words: chromosomal determinants, nuclear transplantation, mouse embryo, extraembryonic tissues, paternal and maternal chromosomes, chromosomal imprinting, androgenones, gynogenones, parthenogenones.

CYTOPLASMIC FACTORS IN ACTIVATED AND FERTILIZED EGGS

At the time of fertilization, eggs contain a stockpile of maternal RNAs and proteins that are especially needed for the initial cleavage divisions (Howlett, 1986). There has been speculation that apart from the paternal genome, the spermatozoon may introduce extragenetic components of importance for development. However, the evidence does not support this notion.

Examination of artificially activated eggs lacking both genetic and extragenetic contributions from spermatozoa showed that diploid parthenogenones could develop normally to the blastocyst stage and in some instances embryos advanced to the 25-somite stage with forelimbs but always with greatly reduced extra-embryonic tissues (Kaufman, Barton & Surani, 1977). It was surprising that these embryos could progress so far and yet fail to reach term. It had previously been proposed that either some extragenetic contribution from spermatozoa was essential for full development or that the expression of recessive lethal alleles in the homozygous maternal genome was responsible for the demise of parthenogenones (Graham, 1974). Subsequent studies, however, proved that neither of these explanations was correct (see below).

Our first series of experiments was designed to test if gross cytoplasmic differences existed between fertilized and activated eggs and especially if extragenetic components from spermatozoa played a vital role in development to term (Surani & Barton, 1983). Thus, digynic triploid eggs were prepared by fertilization and suppression of the second polar body extrusion. It was observed that while removal of one of the female pronuclei from the eggs allowed development to proceed to term, removal of the male pronucleus led to the development of the resulting digynic (gynogenetic) eggs only as far as the retarded 25-somite embryo with poor extraembryonic tissues. In addition, it was demonstrated that transplantation of a male and a female pronucleus from a fertilized egg into an enucleated activated egg resulted in development to term, whereas the reciprocal transfer of a diploid parthenogenetic genome into an enucleated fertilized egg did not (Mann & Lovell-Badge, 1984). Therefore there appeared to be no functional differences in the cytoplasm of activated and fertilized eggs. In further experiments, haploid activated eggs were used as recipient eggs into which either a male or a female pronucleus from fertilized donor eggs was introduced (Surani *et al.* 1984). Development proceeded to term only when a male donor pronucleus was introduced, but transfer of a female pronucleus resulted in development resembling that obtained with gynogenetic or parthenogenetic eggs. These findings were reinforced by studies in which fertilized eggs were used to produce biparental (heterozygous) eggs containing either two male or two female pronuclei. In neither case did development proceed to term and viable offspring were only seen when reconstituted eggs contained a male and a female pronucleus (Barton, Surani & Norris, 1984; McGrath & Solter, 1984a).

The combined results led to a number of important conclusions. (1) There are no functional differences between the cytoplasm of activated and fertilized eggs.

(2) Transfer of small amounts of cytoplasm together with donor nuclei cannot account for the differences in development of reconstituted eggs since such transfer occurred with both male and female donor pronuclei. (3) Homozygosity cannot be considered to be the real reason for the failure of parthenogenetic and gynogenetic eggs to develop to term since biparental heterozygous eggs with two different female (or two male) pronuclei failed to reach term. (4) The role of the spermatozoon for successful development to term is likely to be associated with the paternal genome rather than due to introduction of any extragenetic components into eggs. (5) Both a male and a female pronucleus are needed for development to term.

PROPAGATION OF CHROMOSOMAL DETERMINANTS OF DEVELOPMENT

The influence of events prior to fertilization on development of embryos has been considered before (McLaren, 1979). The experiments described here examined if the information with respect to the parental origin of chromosomes is required only transiently to trigger events with consequences later in development. Conversely, the differential information contained in homologous chromosomes may be conserved and propagated throughout development.

We first investigated these alternatives by using male donor pronuclei that had been attenuated by u.v. irradiation under conditions that limited the development of the treated eggs to only 2–4 cleavage divisions (Barton, Surani & Norris, unpublished data). Hence the function of the transplanted male genome would be restricted to the initial cleavage divisions only. We found that the transfer of these paternal pronuclei into recipient diploid parthenogenetic eggs did not result in any detectable improvement in the development of the parthenogenones. By contrast, transfer of nonirradiated male pronuclei into diploid parthenogenetic eggs resulted in embryos with a phenotype similar to that of digynic triploid eggs on day 10 of gestation (Surani & Barton, 1983). In particular, the trophoblast proliferated normally because of the presence of paternal chromosomes. Further, when the nonirradiated male pronucleus was introduced into haploid activated eggs, development proceeded to term (Surani *et al.* 1984). Therefore, transient presence of the paternal genome appears not to be sufficient to ensure normal development.

The next experiment was carried out to test whether the imprinted information with respect to the parental origin of the genomes was heritable through successive DNA replication cycles and survived activation of the embryonic genome at the 2-cell stage (Surani, Barton & Norris, 1986). The male pronucleus was removed from fertilized eggs to produce haploid gynogenetic eggs, some of which developed normally during preimplantation stages to the blastocyst stage following activation of the embryonic genome at the 2-cell stage. These haploid gynogenetic embryos at the 2- to 16-cell stage provided donor nuclei that were transferred back to fertilized eggs from which the female pronucleus had been removed. Some of these reconstituted eggs developed to term. However, when

the donor nuclei were transferred back into eggs in which the female instead of the male pronucleus had been retained, a phenotype virtually identical to that of parthenogenones and gynogenones was observed (Fig. 1).

In reciprocal experiments to test the paternal genome, the female pronucleus was removed from fertilized eggs. Such haploid androgenetic embryos did not usually develop beyond the 4-cell stage although activation of the embryonic genome occurred normally. Nevertheless, transfer of donor nuclei from haploid androgenetic embryos back into eggs from which the male pronucleus had been removed resulted in development to term. By contrast, when the male donor nucleus was transplanted back into eggs that retained the male pronucleus, embryos developed like biparental androgenetic embryos. Thus, nuclei that undergo a number of haploid cleavage divisions and activate the embryonic

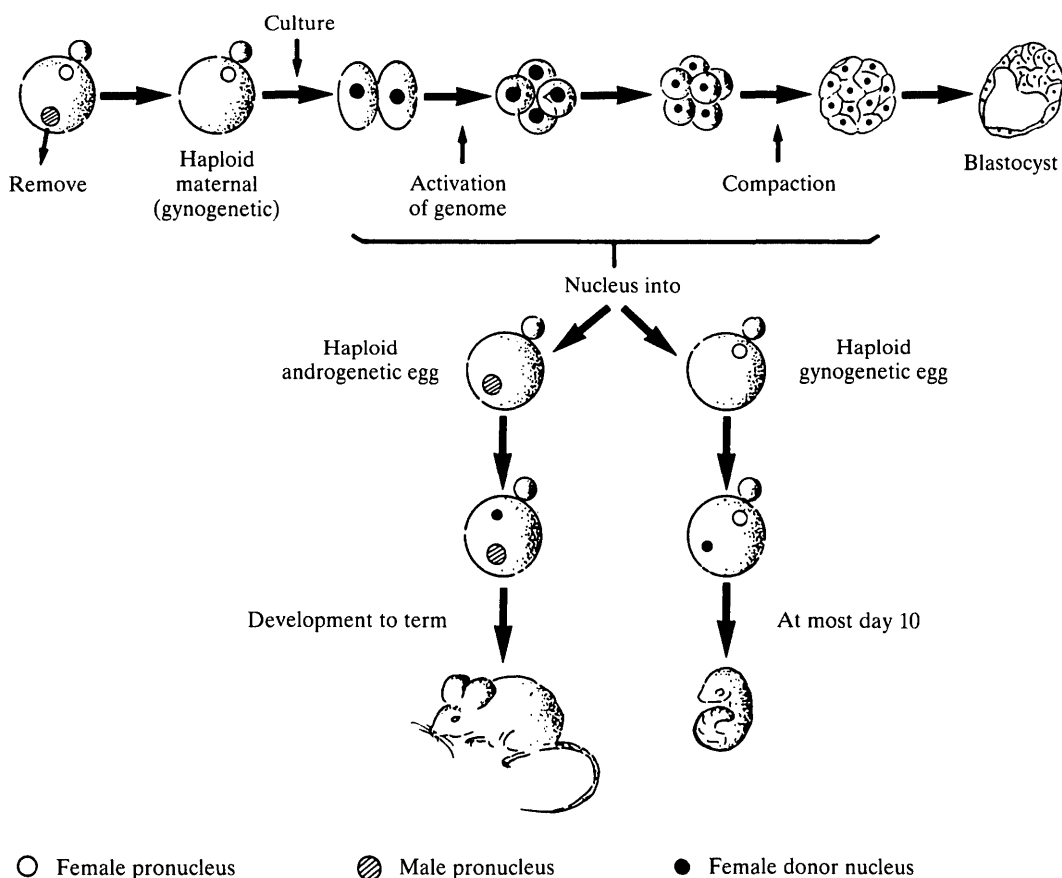


Fig. 1. Propagation of chromosomal modifications. Development to term occurred after transfer of 2- to 16-cell haploid maternal nuclei to haploid recipient eggs in which the male pronucleus was retained but not if the female pronucleus was retained. Hence, the chromosomal determinants from the maternal germline survived activation of the embryonic genome at the 2-cell stage as well as the necessary reprogramming of the donor nucleus transplanted back to a fertilized egg. Paternal chromosomes were also shown to retain their specific chromosomal determinants.

genome do not lose the potential of acting as predicted by their germline origin when brought together with a resident pronucleus in a zygote.

The results from these studies showed that the transient presence of the paternal genome was not sufficient for full development. The imprinted information was being propagated along with the nucleus and survived activation of the embryonic genome and appeared to be highly conserved through a number of DNA replication cycles. It was not even extinguished during the probable reprogramming of the advanced and transcriptionally active donor nucleus during its sojourn in the recipient egg cytoplasm. These results strongly suggested programming of homologous chromosomes in the germline with subsequent propagation of the imprinted information during development.

DEVELOPMENT OF EMBRYONIC CELLS WITH EITHER MATERNAL OR PATERNAL CHROMOSOMES

Since both paternal and maternal chromosomes are essential for development to term, embryonic cells that contain chromosomes of only one parental origin may have a specifically restricted developmental potential. Their study could therefore reveal how the information in homologous chromosomes differs and what influence this has on development of embryonic cells and on the spatial organization of the foetus.

Obvious differences exist in the ability of the parental chromosomes to direct preimplantation development since over 60 % of biparental eggs with two maternal sets of chromosomes developed to the blastocyst stage but only around 20 % of those with two paternal genomes did so (Surani *et al.* 1986). One quarter of the androgenetic eggs contain two Y-chromosomes and thus may not cleave more than twice (Morris, 1968). However, the remaining eggs with either XX or XY genetic constitution should be capable of developing into blastocysts. The reasons for the differences in development are unknown.

A small proportion of embryos with either maternal or paternal chromosomes developed beyond implantation and thereupon displayed striking complementary phenotypes (Surani *et al.* 1984, 1986; Barton *et al.* 1984). When examined on day 10 of gestation, embryos with a diploid set of maternal chromosomes developed to form small 25-somite embryos which were equivalent to the stage of control embryos but the development of extraembryonic tissues, especially the trophoblast, was extremely sparse. The poor development of the visceral yolk sac (mesoderm+endoderm) and the trophoblast may provide one explanation for the retardation of these embryos since they become increasingly reliant on these tissues for nutrition (Surani *et al.* 1984). Conversely, embryos with duplication of the paternal chromosomes never reached beyond the 6- to 8-somite stage and their overall shape was reminiscent of normal day 8 embryos. However, in this instance, the volume of the trophoblast was similar to that of day 10 control embryos (Barton *et al.* 1984). These studies suggested that the maternal chromosomes are essentially programmed to direct development of the embryo proper, whereas

paternal chromosomal modifications predominantly affect the proliferation of the extraembryonic membranes (Surani *et al.* 1984). However, these differences in function of parental chromosomes during development may be preferential rather than mutually exclusive.

The developmental potential of the embryo and the extraembryonic tissues was tested further by reconstitution of blastocysts (Gardner, 1978). Inner cell masses were isolated and trophectoderm vesicles were prepared from fertilized and from parthenogenetic/gynogenetic embryos. Blastocysts were then reconstructed with complementary tissues of different genotypes. In particular, we introduced inner cell masses from parthenogenetic/gynogenetic embryos into trophectoderm vesicles from normal blastocysts to overcome the drastic deficiency of the trophoblast tissue (Barton, Adams, Norris & Surani, 1985). These reconstructed blastocysts developed consistently better than unoperated parthenogenones/gynogenones, partly because the trophoblast development was now normal. Hence, the parthenogenones/gynogenones regularly reached the 40-somite stage on day 12 of gestation. However, there were no indications that such embryos could reach term. In reciprocal reconstructions in which normal inner cell masses were introduced into trophectoderm vesicles from parthenogenetic/gynogenetic blastocysts, development was very poor and resembled that of unoperated gynogenetic/parthenogenetic embryos, probably because the trophoblast failed to proliferate despite the presence of the normal inner cell mass.

A more elaborate form of reconstruction, such as enveloping parthenogenetic ectoderm cells in normal endoderm and trophectoderm, may allow a further improvement in development of parthenogenones. However, if the lack of paternal chromosomes affects other aspects of embryogenesis, then maximum development of parthenogenones/gynogenones may have been attained in these experiments. More recombination experiments are necessary, especially those involving androgenetic cells, to elucidate the respective contributions of the genomes to later postimplantation development.

In alternative experiments, the developmental potential of embryonic cells was assessed by aggregation of preimplantation embryos with different genotypes (McLaren, 1976). The major advantage of this approach is that in such chimaeras all the cells have an equal chance to contribute to the tissues of the developing foetus (Fig. 2). Lack of contribution or excessive contribution by cells of a particular genotype to a defined region of the foetus could provide further insight into the relevance of the programming of homologous chromosomes in the germline for development. In such studies combination of parthenogenetic and fertilized embryos resulted in birth of fertile chimaeric adult mice in which parthenogenetic cells made an extensive contribution to all organ systems, including the germ cells (Surani, Barton & Kaufman, 1977; Stevens, 1978). Hence, the lack of paternal chromosomes in parthenogenetic cells does not preclude them from extensive proliferation and differentiation provided they are in close association with normal cells in chimaeras. In similar studies on androgenetic embryos, however, we have so far failed to detect adults that contain androgenetic

cells. Hence, these results suggest a major distinction in developmental potential of androgenetic and parthenogenetic cells since only cells with maternal chromosomes seem capable of participating in development to term of chimaeras; apparently the cells that contain paternal chromosomes cannot do so. Further experiments were carried out in which androgenetic and parthenogenetic cells were combined (Surani, Barton & Norris, unpublished data). However, no development to term has been detected with these embryos. The results demonstrate that even when chromosomes from both parental sources are present in chimaeras, they fail to reach term unless both sets of chromosomes are present inside individual cells. However, the presence of some normal cells in foetuses provides sufficient information for at least the embryonic cells with duplicated maternal chromosomes to participate in normal development to term in chimaeras.

In order to fully assess the fate of cells in chimaeras, especially that of androgenetic cells, we have carried out further preliminary investigations (Surani, Barton & Norris, unpublished data). Following aggregation of normal and androgenetic blastomeres, androgenetic cells were detected mainly in the yolk sac and the trophoblast but not in the embryo itself. This finding is consistent with the failure to produce chimaeric adults carrying androgenetic cells. By contrast, in similar studies, parthenogenetic cells were detected in the embryo and the yolk sac but not in the trophoblast.

The preferential localization of parthenogenetic and androgenetic cells in the embryo and extraembryonic tissues could be attributed to the specific information contained in parental chromosomes due to modifications of homologous chromosomes in the germline. The distribution of cells could be influenced in a number of ways. Differences in the cell surface properties associated with the genotype may determine allocation of cells to particular regions of the embryo. Allocation could take place during the formation of blastocysts when the inner cell mass and trophectoderm cells first become established. Alternatively, both cell types could become randomly incorporated into the inner cell mass and trophectoderm lineages. After that, androgenetic and parthenogenetic cells may be selected against in the embryonic and extraembryonic compartments, respectively, due to their limited response to the proliferation and differentiation demands of the appropriate embryonic tissues. An important phase in this selection process could be that of size regulation between 5.5 and 6.5 days of gestation (Lewis & Rossant, 1982), when a finely tuned and acute response to proliferative signals is necessary. Parthenogenetic and androgenetic embryos therefore provide a model system that illustrates the role of maternally and paternally derived information in differentiation and proliferation of distinct embryonic tissues.

DISTINCTION BETWEEN EMBRYONIC AND EXTRAEMBRYONIC TISSUES

The information with respect to the parental origin of chromosomes clearly has a marked effect on the developmental potential and distribution of embryonic cells

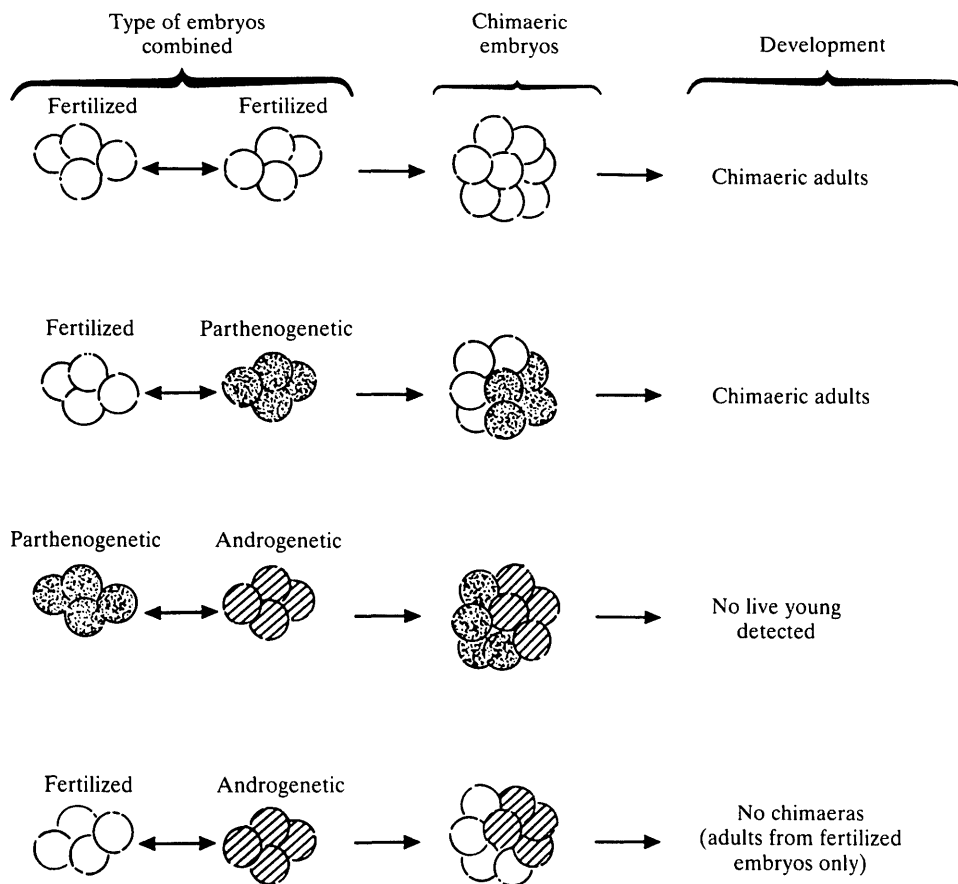


Fig. 2. Fate of aggregation chimaeras examined at term. Fertilized \leftrightarrow parthenogenetic aggregates resulted in chimaeric adults but fertilized \leftrightarrow androgenetic aggregates did not. Hence, maternal chromosomes appear to be essential for participation of embryonic cells through development. No live young were obtained when parthenogenetic \leftrightarrow androgenetic aggregates were tested. Chromosomes from both parental sources are therefore required inside individual cells for development to term.

as described above. It is of interest to note that the tissues of the two foetal compartments also differ substantially in other major respects.

Only the paternal X-chromosome is inactivated in extraembryonic tissues, whereas the inactivation mechanism does not discriminate between the two X-homologues in the embryo (West, Frels, Chapman & Papaionnou, 1977; Harper, Fosten & Monk, 1982). It has been suggested that the maternal X-chromosome bears an imprint for resistance to inactivation (Lyon & Rastan, 1984). This imprint is either not recognized in the ectoderm or lost prior to X-inactivation in this lineage.

Major differences between tissues exist also in the extent to which the DNA is methylated at CpG residues. Thus, a variety of repetitive and unique sequences are undermethylated in extraembryonic tissues, but highly methylated in the embryo (Chapman *et al.* 1984). More interesting still is that these DNA sequences

are undermethylated in the yolk sac endoderm, whereas they are methylated in the adjacent mesoderm layer which is derived from the primitive ectoderm after gastrulation (Sanford, Chapman & Rossant, 1985). However, the timing of *de novo* methylation events and whether they are influenced by the parental origin of chromosomes remains to be established.

The cellular counterparts of retroviral transforming genes, termed cellular oncogenes, have been implicated in growth control and differentiation of nonmalignant cells (Bishop & Varmus, 1982). Some of these *c-onc* genes show a striking tissue and stage specificity of expression in the mouse embryo, most notably *c-fos* and *c-fms*, which are expressed at high levels only in the extraembryonic tissues (Muller, Verma & Adamson, 1983). However, the function of these genes and their relation to paternally derived chromosomal determinants, if any, remains to be established.

The classical transplantation antigens encoded by class I MHC genes also show an interesting spatial distribution of expression with respect to the two compartments. These genes are transcriptionally inactive in the trophoblast but are expressed shortly after implantation in the embryo and are thought to provide a basic framework for cell recognition and morphogenesis (Goodfellow *et al.* 1976; Tanaka *et al.* 1983). It will be relevant to determine whether class I gene expression is absent in androgenetic embryos and whether this could result in failure of these cells to participate in the development of embryonic ectoderm derivatives.

MECHANISM OF CHROMOSOMAL IMPRINTING AND GENE EXPRESSION

The studies described here indicate that programming of homologous chromosomes in the germline has profound consequences throughout development. The mechanism to link these temporally separate events requires three main features: modification of chromosomal regions in the germline, propagation of these modifications and their recognition at particular stages and in specific tissues during development, and activation of appropriate genes resulting from such recognition.

The primary modifications probably occur while the homologous chromosomes are spatially segregated during gametogenesis. While the precise nature of the modification is unknown, differences in methylation of DNA in the germline may serve as a signal to establish a specific chromatin configuration of the parental DNA after fertilization when protamines are replaced by histones (Rodman, Pruslin & Allfrey, 1982). There is evidence to show that some dispersed repetitive DNA sequences in oocytes are undermethylated while the same sequences are hypermethylated in sperm DNA (Sanford *et al.* 1984; Ponzetto-Zimmerman & Wolgemuth, 1984). Further, there are marked methylation events during the formation of primordial germ cells with global undermethylation of DNA followed by relatively high methylation of the paternal but not the maternal DNA (M. Monk personal communication). Recently, *de novo* methylation of specific genes during spermatogenesis has been demonstrated, with point sites of undermethylation in sperm corresponding to hypersensitive chromatin regions in somatic cells

(Groudine & Conkin, 1985). It is of interest that both the patterns of methylation of DNA at CpG residues and the chromatin structure established by histones and other DNA-binding proteins are stable over many cell generations and both are implicated in the regulation of transcription (Jahner & Jaenisch, 1984; Weintraub, 1985). Thus, modifications based on methylation and chromatin structure once introduced into chromosomes during gametogenesis would be replicated until required for activation of specific genes.

The recognition of specific chromosomal modifications may occur by sequence-specific DNA-binding protein molecules (Rogers & Saunders, 1985). These molecules may be qualitatively different at each stage of development and in different embryonic tissues. Since the parental origin of chromosomes has a marked effect even on preimplantation embryos, the distribution of the trans-acting molecules may differ from the earliest stages of the establishment of primary lineages. Therefore, different sets of trans-acting molecules may bind to appropriate accessible cis-acting DNA sequences at specific stages of development on either of the homologous chromosomes, predetermined by the modifications introduced in the germline. However, a particular DNA-binding protein could recognize numerous identical sequences dispersed on different autosomes throughout the genome to achieve coordination of response.

For the control of gene expression, the modification on the parental chromosomes may act in cis. The studies on the proximal region of chromosome 17 involving the T^{hp} deletion mutation show that the maternal but not the paternal complement of this region is essential for normal development (Johnson, 1975; McGrath & Solter, 1984b). Perhaps this region may act in cis to activate a particular allele on the maternal chromosome. However, we further suggest that there may be at least four separate categories of genes (Fig. 3). The first category (c) consists of housekeeping genes and others located on autosomes or chromosomal domains whose functions have already been shown by genetic studies to be independent of their parental origin (Searle & Beechey, 1985). Hence, chromosomes 1, 4, 5, 9, 13, 14 and 15 and particular regions of other chromosomes fit into this category. The next group of regulatory genes (designated *s*), located on chromosomes 2, 6, 7, 8, 11 and 17 (Searle & Beechey, 1985) and perhaps elsewhere, are probably activated only when these chromosomes are maternally derived. These regulatory genes together with the housekeeping genes presumably dictate development of the embryo itself and allow embryonic cells to participate extensively in the formation of all the tissues, organs and germ cells, but not the extraembryonic tissues as judged by the developmental potential of parthenogenetic cells (Surani *et al.* 1977; Stevens, 1978). The next category of regulatory genes (*w*), located on chromosome 7 (Searle & Beechey, 1985) and most probably elsewhere may only be activated if these chromosomes are modified in the paternal germline. These regulatory genes appear very important for the development of the extraembryonic tissues. The final category of genes (*z*) are probably activated when chromosomes from both parental sources are present inside the same cells. The activation of these genes may be determined directly by

specific chromosomal modifications or perhaps they may be triggered indirectly as a consequence of activation of other genes. It is essential to postulate the presence of this category of genes because of the failure of development to term of androgenetic \leftrightarrow parthenogenetic chimaeras despite the presence of chromosomes from both parental sources, albeit in separate cells (Surani, Barton & Norris, unpublished data).

It is not possible at present to predict if parental alleles are differentially expressed in normal embryos. Studies on overall protein synthesis in fertilized, parthenogenetic and androgenetic embryos (Petzoldt *et al.* 1981; Surani, Barton &

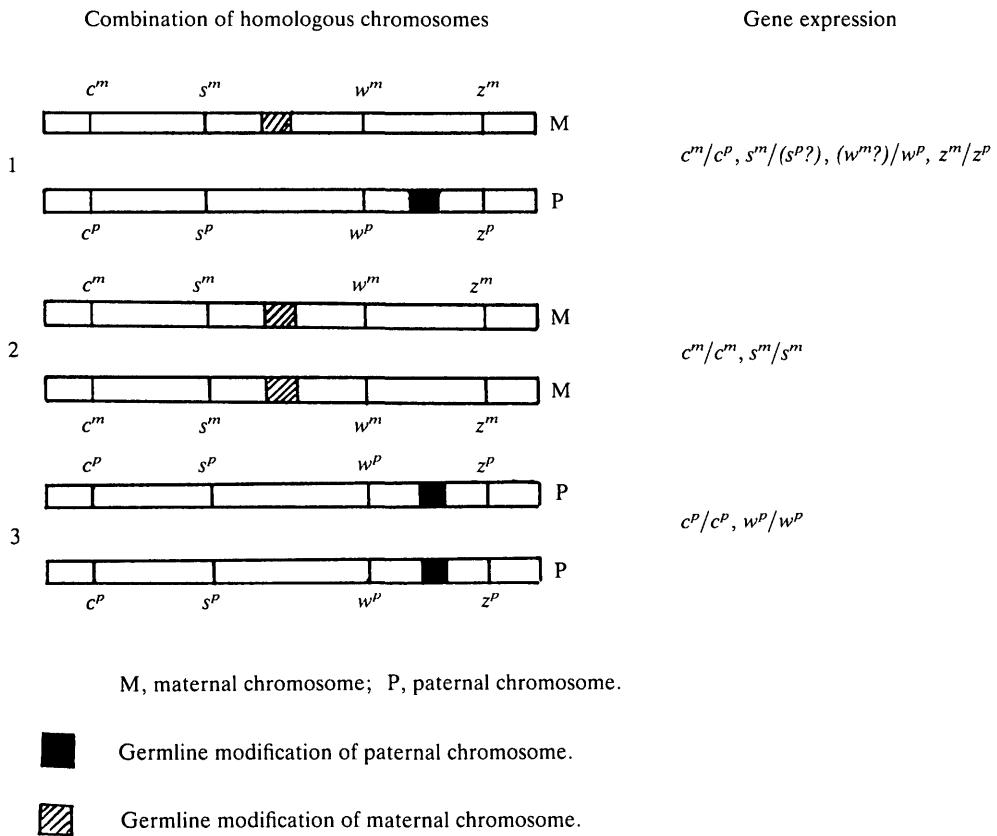


Fig. 3. Influence of germline modifications on gene expression. It is proposed that the housekeeping genes and others (designated *c*) are expressed regardless of their parental origin. Some regulatory genes (designated *s*) are expressed only when specific chromosomal modifications occur in the maternal germline, while other regulatory genes (designated *w*) are expressed when chromosomal modifications are introduced in the paternal germlines. Expression of some genes (designated *z*) occurs only when chromosomes from both parental sources are present inside individual cells. It is proposed that the chromosomal modifications are propagated throughout development. At particular stages and in particular tissues during development, trans-acting molecules bind to the specific DNA sequences as determined by the germline modifications of chromosomes to evoke a coordinated response of several maternal or paternal genes. The chromosomal modifications act in *cis* to induce gene expression.

Norris, unpublished data), specific housekeeping genes (Gilbert & Solter, 1985) cell surface molecules (Sawicki, Magnuson & Epstein, 1981) and genes of the MHC complex (Ozato, Wan & Orrison, 1985), reveal no differential activities of paternal and maternal alleles. But these studies are not comprehensive enough to reach a firm conclusion. However, all the specific genes examined so far could fall into category 'c', whose expression is independent of the parental origin of chromosomes. Indeed, differential activities of some key regulatory genes dependent on their parental origin could account for the opposite phenotypes detected with androgenetic and gynogenetic embryos. Similarly, opposite phenotypes were also encountered when studying the influence of the parental origin of a specific distal region of chromosome 2 and a proximal region of chromosome 11 with respect to shape, size and behaviour of neonates (Cattanach & Kirk, 1985). With reference to the proposed model (Fig. 3), these opposite phenotypes could be explained on the basis of excess or deficiency of particular gene products.

Alternatively, however, both alleles may be eventually activated when modifications on either of the two homologous chromosomes are recognized. Such a case could perhaps be made from the phenotypes of two mutations in the T/t locus; development of t^{w73}/t^{w73} (Spiegelman, Artzt & Bennet, 1976) and T^{Orl}/T^{Orl} (Erickson, Lewis & Slusser, 1978) overtly resembles the development of parthenogenetic and androgenetic embryos, respectively. If a coordinated response of several genes located on different chromosomes occurred as a result of modifications of parental chromosomes, mutation of any one of the key regulatory genes could perhaps produce phenotypes resembling androgenetic and gynogenetic embryos. Such coordination of response could occur by the modifications of several identical DNA sequences throughout the genome favouring binding of specific trans-acting molecules. This type of mechanism is also appropriate for the overall effects of maternally and paternally derived chromosomes on the embryo and extraembryonic tissues. Hence, a combined approach from embryological, genetic and molecular studies should lead to a precise understanding of modifications of homologous chromosomes in the germline and their subsequent influence throughout development in the mouse.

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