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Genome imprinting and development in the mouse

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Summary

Development in mammals is influenced by genome imprinting which results in differences in the expression of some homologous maternal and paternal alleles. This process, initiated in the germline, can continue following fertilization with interactions between oocyte cytoplasmic factors and the parental genomes involving modifier genes. Further epigenetic modifications may follow to render the 'imprints' heritable through subsequent cell divisions during development. Imprinting of genes can be critical for their dosage affecting embryonic growth, cell proliferation and differentiation.

The cumulative effects of all the imprinted genes are observed in androgenones (AG) and parthenogenones (PG), which reveal complementary phenotypes with respect to embryonic and extraembryonic tissues. The presence of PG cells in chimeras causes growth retardation, while that of AG cells enhanced growth. AG cells apparently have a higher cell proliferation rate and, unlike PG cells, are less prone to selective elimination. However, the PG germ cells are exempt from cell selection. In chimeras, PG cells are more likely to be found in ectodermal derivatives such as epidermis and brain in contrast to AG cells which make pronounced contributions to many mesodermal derivatives such as muscle, kidney, dermis and skeleton. The presence of androgenetic cells in chimeras also results in the disproportionate elongation of the anterior–posterior axis and sometimes in the abnormal development of skeletal elements along the axis. Genetic studies highlight the influence of subsets of imprinted genes, and identify those that are critical for development.

Key words: mouse, androgenone, parthenogenone, chimera, modifier genes, epigenetic modifications.

Introduction

It is paradoxical that in the mouse there is an absolute requirement for both a maternal and a paternal genome for normal development (Surani, 1986; Solter, 1988). This should not be necessary since both parental genomes contribute similar genes. Hence, they must differ in some respect as a result of their parental origins.

Development is initiated with the establishment of the totipotent embryonic genome in the form of a zygote, which consists of the two parental pronuclei and maternally inherited cytoplasmic factors. However, some homologous chromosomes are imprinted with epigenetic information resulting in specific functional differences between parental genomes (Searle and Beechey, 1990; Cattanach, 1986). The resulting chromosomal determinants are relatively stable and vital for development. This contrasts with the imprinting of some transgene loci and dominant mutations which can be affected by changes in the genetic background (Sapienza, 1989; McGowan *et al.* 1989; Reik, 1989; Allen *et al.* 1990; Surani *et al.* 1990; Monk, 1990; Reik *et al.* this volume).

Extensive studies have established that the mouse zygote must be essentially euploid for normal development (Epstein, 1986; Dyban and Baranov, 1987). However, in genetically balanced zygotes, aberrant development occurs if both copies of certain chromosomal regions are derived exclusively from one parent (Cattanach and Beechey, this volume). This evidence demonstrates that the expression of at least some alleles is influenced by their parental origin. Since even tetraploid embryos with equal numbers of parental genomes fail to develop appropriately (Snow, 1973), it is evident that both the relative and the absolute gene dosage levels are critical for mouse development.

If the determinant and invariant form of mosaic development represents one extreme, the highly regulative form of development in mammals represents the other (Alberts *et al.* 1989). In mammals, there is little or no reliance on either the localised cytoplasmic determinants or the invariant cell lineages that characterise development in some non-mammalian species, albeit to varying extents. Mammalian eggs by comparison are exceedingly small, evidently without any localised cytoplasmic determinants or recognisable asymmetry, except for the presence of the polar body. Indeed, development is highly protracted following fertilization and relies on the early activation of the embryonic

genome; in the mouse, this occurs at the 2-cell stage (Johnson, 1981). Furthermore, development is thought to be non-segmental, lineage is not fixed and cell commitment occurs relatively late (Rossant, 1985). This very flexibility and the highly regulative nature of development poses problems. It is possible that the strategy adopted relies on the chromosomal 'imprints' (determinants) to regulate development. Indeed, there is evidence that suggests that the reciprocal activity and functional hemizygosity of imprinted parental alleles are utilised for the co-ordination of embryonic growth, cell proliferation and development of specific cell lineages through the regulation of the dosage of genes involved in these processes.

Genome imprinting must rely at least in part on the introduction of heritable repressed or derepressed chromatin structures (Weintraub, 1985) on homologous parental alleles, chromosomal domains or indeed whole chromosomes. Many instances of selective repression of some parental alleles are observed in plants, insects and animals (this volume). Some aspects of the mechanisms for such fundamental modifications affecting gene expression are likely to be evolutionarily conserved. However, the purpose for which these mechanisms are utilised may be radically different in each instance, ranging from development and sex determination to phenotypic variations. What is common to all such instances is the epigenetic modification through reversible changes in chromatin structures.

Towards elucidating the nature and the mechanism of epigenetic modifications

Recent studies provide us with an overview of the possible mechanisms and the factors that underlie genome imprinting. However, an important caveat is that the imprinting process may be locus-specific with respect to the choice of epigenetic modification employed, as well as the sequence of events and the factors responsible for the structural changes at the locus. A further consideration is that imprinting of certain loci will be invariant on different genetic backgrounds especially when these are critical for development.

Imprinting occurs progressively (Fig. 1). The process can be conveniently divided into three main phases; initiation, establishment, and maintenance or propagation of epigenetic modifications to the later stages of development (and even generations). The initial imprints, probably introduced in the germline, serve as templates for further modifications as a result of interactions between parental genomes and the oocyte cytoplasmic factors during the assembly of the pronuclei. The process may continue during cleavage divisions following the activation of the embryonic genome if some of the imprinted alleles themselves are modifier genes that may act on unlinked loci. Additional changes may occur perhaps immediately after implantation in a lineage-specific manner to reinforce the 'imprints' and render them heritable.

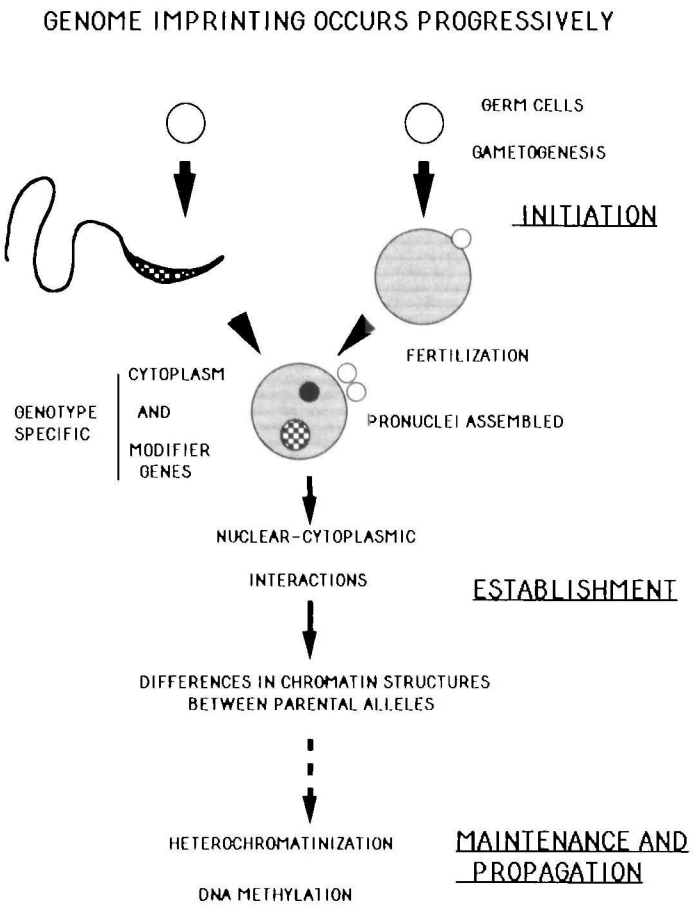

Fig. 1. Summary of factors that influence epigenetic modifications and imprinting of (trans)genes.

Initiation

Based on genetic studies and on certain transgene inserts whose expression is primarily dictated by the parental origin, it is likely that imprinting is initiated in the germline. At present there is virtual ignorance regarding the mechanism and the nature of these primary imprints. Suggestions implicating DNA methylation for this purpose remain unsubstantiated (Sanford *et al.* 1987). Recent proposals attribute germline-specific imprinting to the dose-dependent sex chromosome-linked modifier genes postulated to alter the chromatin structure (Laird, 1990; Sapienza, this volume). Whatever the nature of these imprints, they could serve as templates for further modifications to varying extents following fertilization.

Establishment

There are indications that the imprinting process may be influenced by interactions between parental genomes and oocyte cytoplasmic factors. For example, fertilization of DDK oocytes by sperm from alien males results in aberrant preimplantation development while the reciprocal cross is viable (Renard and Babinet, 1986). Species that are far removed from one another taxonomically differ not only in their genetic characteristics but also in the nature of their oocyte cytoplasm

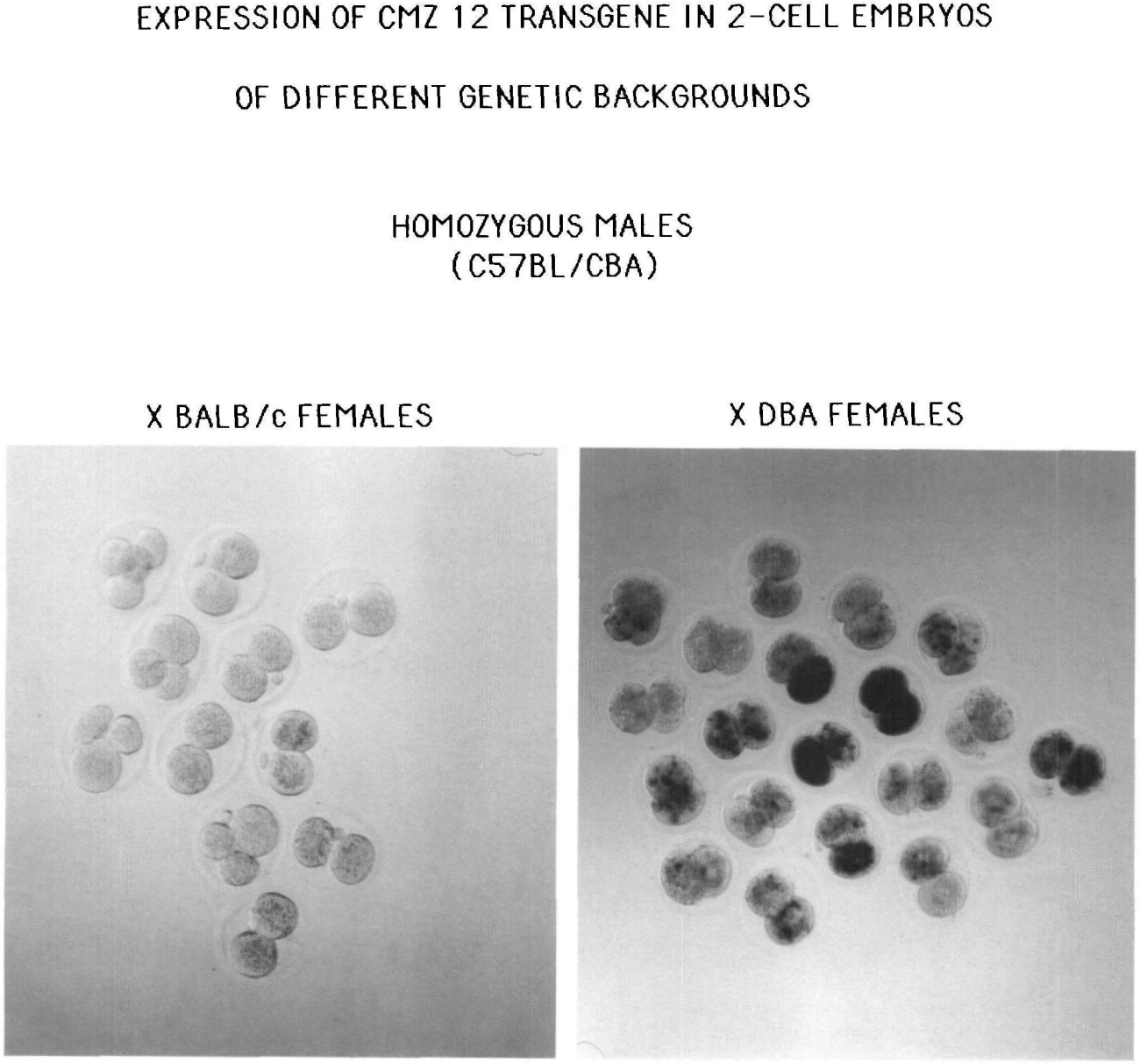

Fig. 2. (A) Expression of CMZ12-LacZ detected histochemically for β-galactosidase in 2-cell mouse embryos of different genetic backgrounds. Expression is enhanced when the transgene is introduced into DBA oocytes but suppressed in BALB/c oocytes.

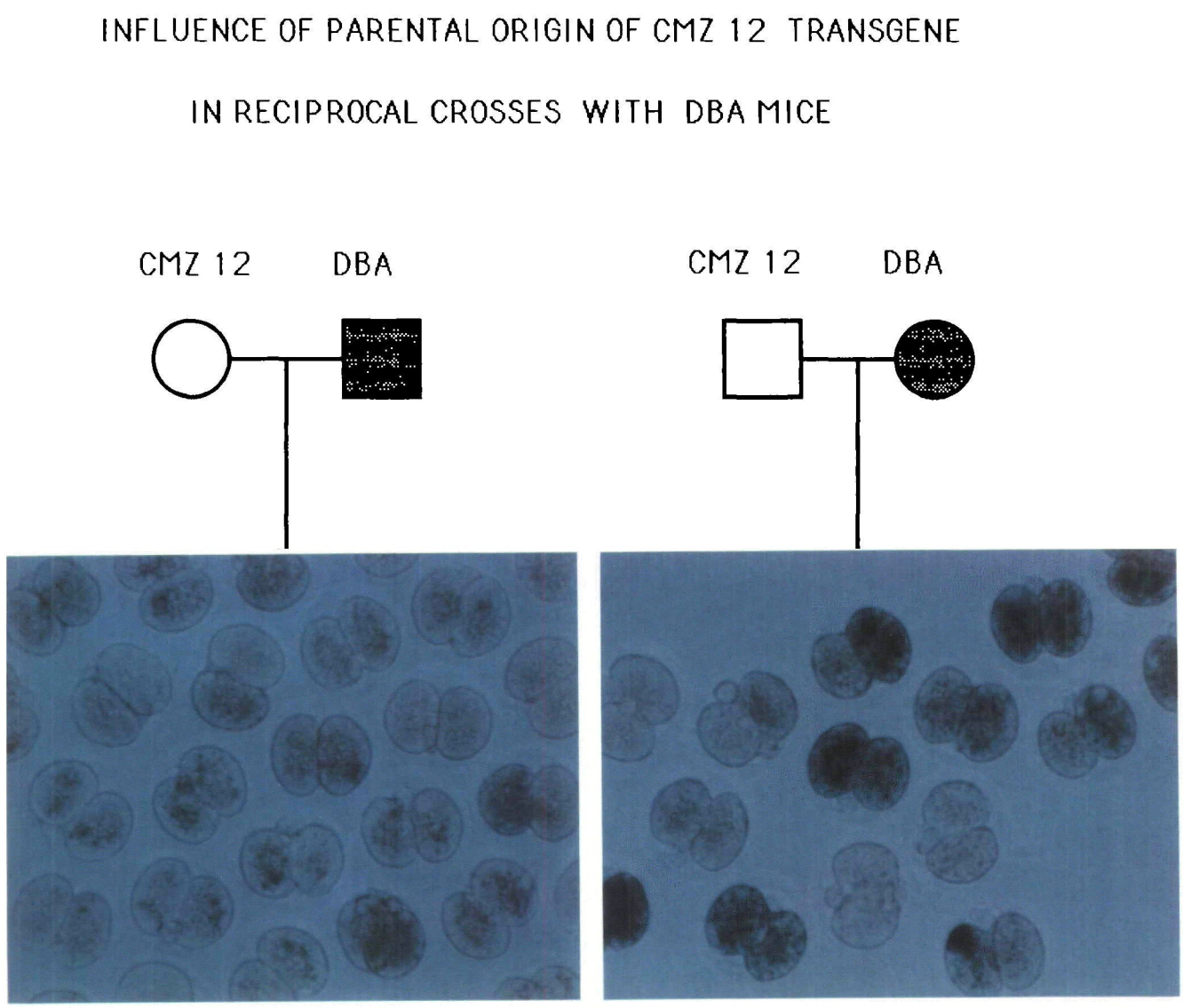

Fig. 2. (B) Parental origin effects on the expression of CMZ12-LacZ in 2-cell embryos. Expression is observed in CMZ12♂×DBA♀ cross but in the reciprocal cross, expression is much reduced.

(Waddington, 1959). Indeed there are marked differences in oocyte cytoplasmic polypeptides even amongst closely related mammalian species, with subtle differences being encountered amongst inbred strains of mice (Norris *et al.* 1985; and unpublished data observations; Babinet *et al.* this volume).

Recent studies on some transgene loci provide further insights into the relationship between nuclear–cytoplasmic interactions and epigenetic modifications (Allen *et al.* 1990). The transgene locus CMZ 12 is expressed as a variegated phenotype at the 2-cell stage (Kothary and Surani, unpublished data) (see Fig. 2A). The strain used in the cross influences the degree of expression. Expression is enhanced when homozygous transgenic (C57BL×CBA) F_1 males fertilize DBA oocytes, but suppressed when BALB/c oocytes are used. Furthermore, in reciprocal crosses between transgenic females and wild-type DBA males, expression is also suppressed, suggesting that enhancement of expression is dependent on maternal inheritance of a DBA/2 allele (Fig. 2B). It is also noteworthy that these differences in expression do not correlate with changes in those methylatable sites tested in the promoter region; other differences in chromatin structure at the locus are not excluded (Kothary *et al.* unpublished data). The epigenetic modifications affecting lacZ expression are not heritable for a prolonged period, since the expression observed in postimplantation embryos, principally in the neural crest cells, is not affected.

Studies on the TKZ 751 transgene locus provides additional insights into the complex roles of nuclear cytoplasmic interactions in imprinting (Allen *et al.* 1990). Expression in postimplantation embryos of this transgene correlates inversely with DNA methylation of the locus. Expression is high when the transgene is introduced into DBA oocytes but suppressed in BALB/c oocytes. The reciprocal cross between a transgenic female and a BALB/c male does not result in the suppression of transgene expression. Interestingly, when the transgene is introduced into (BALB/c×DBA/2) F_1 embryos both the high and low expression phenotypes are encountered in equal proportions, which suggests segregation of BALB/c and DBA/2 suppressor and enhancer modifiers in different oocytes. However, further evidence demonstrates that these modifiers apparently interact with oocyte cytoplasmic factors to produce variations in phenotype. For example, the TKZ 751 locus in C57BL/6 background is expressed and remains undermethylated provided the transgenic C57BL/6 oocytes are fertilized by DBA or C57BL/6 males (Fig. 3A). However, if the C57BL/6 oocytes are fertilized by BALB/c males, the transgene undergoes methylation and expression is diminished (Allen, unpublished data). For the TKZ 751 locus, these observations provide clear indications for interactions between parental genomes and oocyte cytoplasmic factors for epigenetic changes at the locus. The combined evidence so far suggests that the methylation of the locus requires oocyte cytoplasm either from the BALB/c or C57BL/6 together with a BALB/c genome, which in this instance can be of either maternal or paternal origin (Fig. 3B).

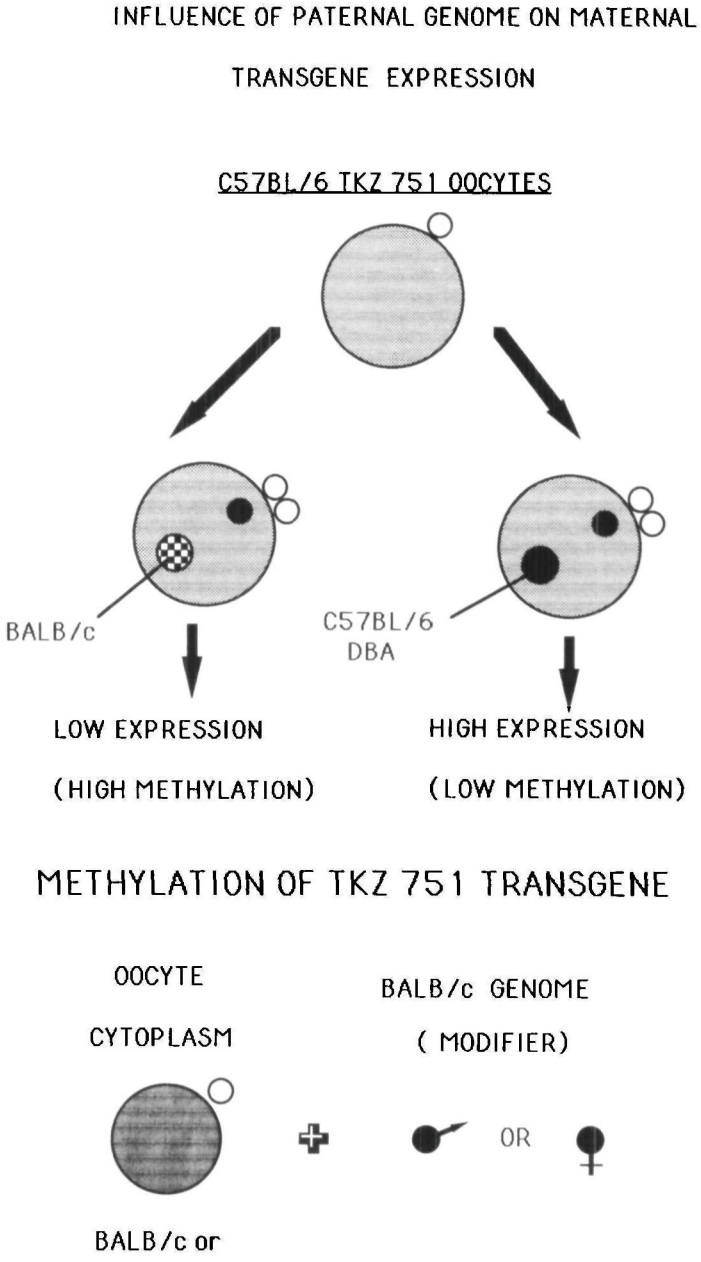

Fig. 3. (A) Influence of paternal genome on methylation and expression of the TKZ 751 locus from C57BL/6 when transmitted from the maternal germline. High expression associated with low DNA methylation is observed with respect to TKZ 751 locus when C57BL/6 oocytes are fertilized by C57BL/6 or DBA sperm. However, when the oocytes are fertilized by BALB/c sperm, there is methylation of the TKZ 751 locus and expression is diminished. (B) Summary of essential conditions for the methylation of the TKZ 751 locus. Oocyte cytoplasm, either of C57BL/6 or BALB/c origin together with a BALB/c genome (either maternal or paternal) are essential for methylation of TKZ 751 locus.

Modifier genes act on unlinked loci giving rise to variable penetrance and expressivity (Fisher, 1931;

Haldane, 1941; Spofford, 1961; Agulnik and Ruvinsky, 1988). The term 'modifier' probably encompasses a disparate group of genes whose potential role in epigenetic modifications is discussed elsewhere in this volume (Reik *et al.*; Sapienza). Suffice it to say that modifiers can alter chromatin structure either directly or following interactions with cytoplasmic factors. The cloning of one class of mammalian modifier genes defined on the basis of sequence homology to two known *Drosophila* modifiers, provides a basis for studies on the role of these proteins in chromosomal imprinting (Singh *et al.* 1990).

Maintenance and propagation

We have seen in the case of the CMZ 12 locus that the epigenetic modifications that accompany variable expression are transient while in the case of TKZ 751 locus they are heritable. Heterochromatinization and DNA methylation are well-known forms of stable epigenetic modifications. There is evidence from *Drosophila* that a variety of modifier genes code for components that act in a dose-dependent manner to induce heterochromatic structures (Locke *et al.* 1988; Tartof and Bremer, this volume). There are also genome-wide changes in DNA methylation during preimplantation development and a major *de novo* methylation event, which acts principally on the epiblast cells (Monk, 1988). We can envisage that, in the case of the TKZ 751 locus, the epigenetic modifications become established during early stages of development and they become irreversibly heritable in the soma at a later stage. Somewhat surprisingly, some epigenetic modifications may be transmitted to subsequent generations, which indicates that the modifications are completed probably before the germline is established (Allen *et al.* 1990; Reik *et al.* this volume). However, in most instances, epigenetic modifications associated with imprinting must be reversed in the germline to restore the genomic totipotency and to introduce the new germline 'imprints' depending on the sex of the individual. The preferential inactivation of the paternal X-chromosome in extraembryonic tissues also progresses through a series of steps (Monk, 1988).

Developmental consequences of genome imprinting

Differences in development as a result of the parental origin of the genomes are evident from the outset (Solter, 1988; Surani *et al.* 1990). For instance, androgenetic embryos can develop poorly during early stages whereas there is apparently normal development of parthenogenetic and gynogenetic embryos (Fig. 4). It appears that the major epigenetic modifications of parental genomes are accomplished at an early stage since nuclei in gynogenetic and androgenetic embryos are already functionally distinct by the 2-cell stage (Surani *et al.* 1986). We do not know the relative extents to which these major differences are dictated by the imprinting initiation events in the germlines, and by the nuclear–cytoplasmic interactions following fertilization or by early differential gene expression. However, the establishment of these differences does not rely on continued interactions between parental pronuclei in the zygote; the differences occur even when the parental genomes are left to develop in separate zygotes (Surani *et al.* 1986; Barra and Renard, 1988). These observations require critical assessment of the role of parental origin of modifier genes and their interactions with oocyte cytoplasmic factors for the imprinting of other endogenous genes.

Development of embryonic and extraembryonic cell lineages

Extensive studies have been carried out to determine the fate of androgenetic (AG) and parthenogenetic/gynogenetic (PG) embryos and cells during postimplantation development. Mid-gestation embryos with different genetic constitution show reciprocal phenotypes with respect to the embryos and extraembryonic tissues, suggesting that the expression of some maternal and paternal alleles affect different lineages (Surani *et al.* 1986, 1990; Solter, 1988). AG conceptuses have relatively well-developed extraembryonic tissues, especially the trophoblast, but the embryo develops maximally to only the 6- to 8-somite stage. PG conceptuses have better development of embryos, which can reach the 25-somite stage, but they tend to be small and with poor extraembryonic tissues. Attempts to rescue PG embryos with the PG-derived inner cell mass or epiblast tissue reconstituted with normal trophectoderm and primary endoderm still produces small PG embryos, which fail to progress beyond about the 40-somite stage on day 13 of gestation (Barton *et al.* 1985; Gardner *et al.* 1990). These studies demonstrate that the influence of genome imprinting is complex and is likely to affect other aspects of development.

A clear example of the influence of genome imprinting is observed on cellular interactions involving the inductive stimulus from the inner cell mass (ICM) on the proliferation of the polar trophectoderm (Gardner *et al.* 1973). Blastocyst reconstitution experiments illustrate that the inductive stimulus is produced both by the normal and parthenogenetic ICM, but the polar trophectoderm fails to proliferate unless the cells contain paternal chromosomes (Barton *et al.* 1985). This may explain the failure of trophoblast development in PG conceptuses.

It is noteworthy that the PG and AG phenotypes are not affected by changes in genetic background (unpublished data observations). Indeed all the attempts so far have failed to alter this very stable epigenetic information, and its phenotypic effects on development. It is of interest that the androgenetic conceptuses in the human resemble those observed in the mouse (Bagshawe and Lawler, 1982), which suggests that imprinting may have similar effects on development in the human.

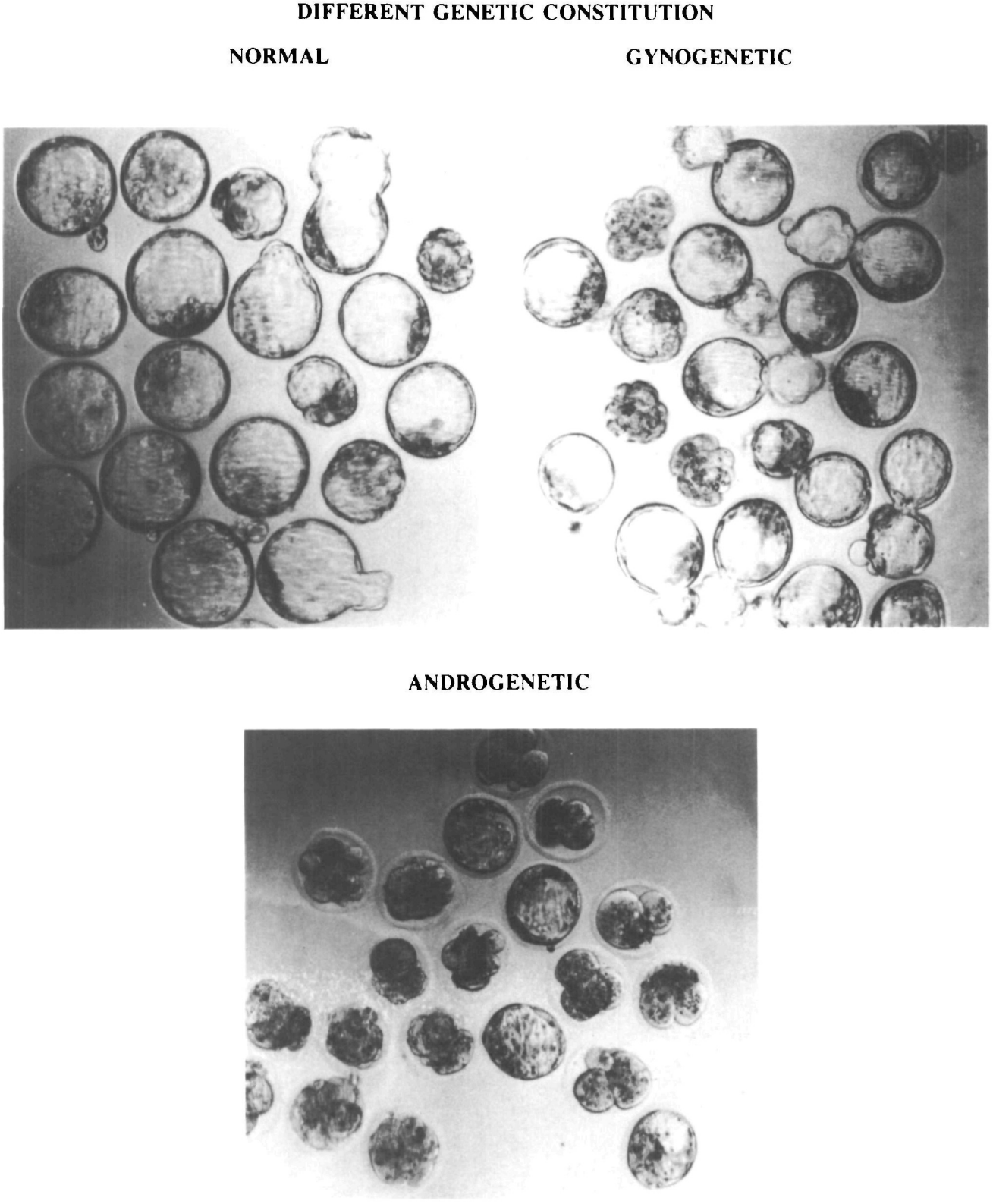

Fig. 4. Preimplantation development to day 5 blastocyst stage *in vitro* of embryos of different genotype.

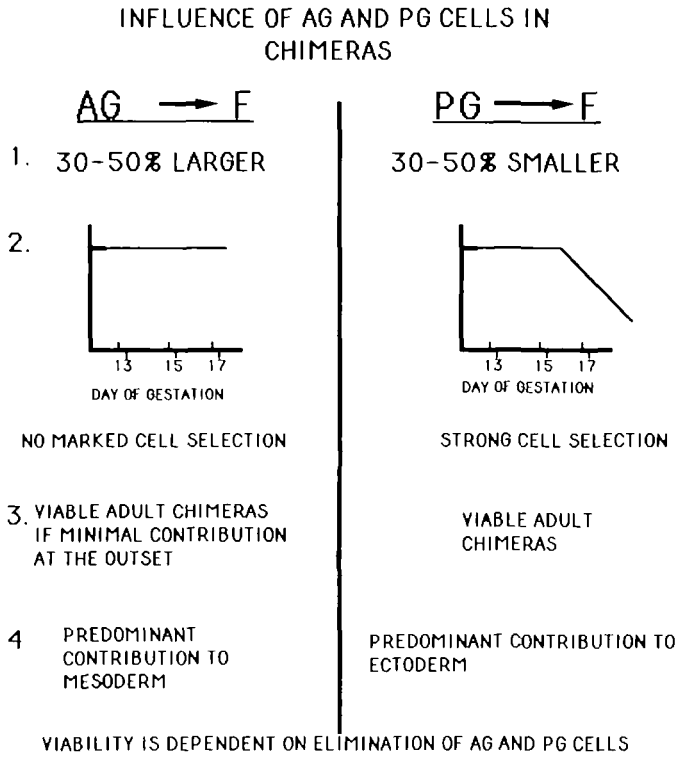

Fig. 5. Summary of the influence of AG and PG cells on development of chimeric fetuses.

Influence of genome imprinting on embryonic viability, growth, cell interactions, and specific lineages

The fate of PG and AG embryonic cells can be examined in greater detail in chimeras with normal fertilized embryos (F). Cell autonomous events will not be affected directly but the presence of normal cells will provide a degree of 'rescue' by overcoming deficiencies (or excesses) of extracellular or diffusible gene products in the experimental cells. These studies could reveal whether different lineages are affected to different degrees. The experimental cells may interact with and influence normal cells to give rise to particular phenotypes (Fig. 5).

Size regulation

Embryonic size is precisely controlled during mammalian development and it appears that imprinted genes play a critical role in this respect. This is demonstrated by the presence of AG or PG cells in chimeric fetuses which have reciprocal influences on the overall size of the conceptus. The presence of AG cells increases the size of the conceptus (by weight) by as much as 30–50% (Surani *et al.* unpublished data) while the presence of PG cells causes a decrease in the size of the conceptus by 30–50% (Nagy *et al.* 1987; Fundele *et al.* 1990). The influence on the size of conceptuses is dependent on the contribution of experimental cells to the chimeras; greater contribution exerts a greater effect. In the case of PG↔F chimeras, the size differences persist at least until birth (Nagy *et al.* 1987; Fundele *et al.* 1990). The changes in the overall size of the conceptuses do not influence the stages of development. However, some overall phenotypic effects are observed. For instance, the anterior–posterior axis is elongated in AG↔F chimeras (Barton *et al.* unpublished data). Detailed analyses of these conceptuses are underway to discover the precise reason for this effect.

Cell proliferation

An influence of imprinted genes is observed on cell proliferation. The fate of experimental cells in various tissues in chimeras can be monitored more precisely by the use of isozyme and *in situ* markers (Surani *et al.* 1987, 1988; Clarke *et al.* 1988; Fundele *et al.* 1989, 1990; Nagy *et al.* 1989; Thomson and Solter, 1988). With respect to the PG↔F, PG cells contribute to all the tissues in the conceptus to an equal extent until day 13 (d13) of gestation. Their contribution in most tissues declines markedly thereafter between d13 and d17 so that at term there are less than 10% PG cells left (Fundele *et al.* 1990). Further studies now underway suggest that cell selection is not associated with cell death but rather that PG cells cease to proliferate (Fundele, unpublished data). Recent studies on AG↔F chimeras indicate that AG cells are apparently immune to such cell selection, at least until d15 and to term in some tissues (Barton *et al.* unpublished data). We suspect that the increased size of AG↔F is due to the increased proliferation of both the AG and normal cells, which implies that AG cells also exert a positive effect on the proliferation of normal cells.

Establishment of specific cell lineages

An influence of imprinted genes is observed on the development of specific cell lineages. The significant influence of paternal chromosomes on the proliferation of trophoblast cells has already been discussed above. Some cell types within the embryo itself also appear to be influenced by the imprinted genes, although such effects can be either cell autonomous or occur indirectly through cell interactions. For example, in PG↔F chimeras, PG cells make relatively greater contributions to the ectodermal derivatives such as the brain and epidermis compared to the other somatic tissues, independently of the genetic background of the PG cells (Fundele *et al.* 1990 and unpublished data observations). Furthermore, germ cells appear to be exempt from such selective elimination, and viable oocytes are derived at a normal frequency from PG cells (Nagy *et al.* 1989; Fundele *et al.* 1990). Conversely, the contribution of PG cells to some mesodermal derivatives such as skeletal muscle is minimal with drastic selection being observed between d15 and d17 (Nagy *et al.* 1987; Fundele *et al.* 1989). Preliminary indications are that the PG-derived myoblasts simply stop proliferating since, once formed, they fuse to form myotubes (Fundele, unpublished data). AG cells contribute more to the mesodermal derivatives such as muscle, skeleton, kidney, heart and possibly dermis but less to the ectodermal derivatives (Surani *et al.* unpublished data).

The high contribution is associated with phenotypic abnormalities of skeletal elements. In this regard, it is particularly interesting that embryonic stem cells (ES) derived from AG blastocysts give rise to tumours composed almost entirely of striated muscle when transplanted to ectopic sites in the subcutaneous region (although they can give rise to most somatic tissues in chimeras), while the PG-derived ES cells give rise to tumours consisting of a variety of tissues (Mann *et al.* 1990). Furthermore, these studies show that adult chimeras containing AG embryonic stem cells produce phenotypes with abnormalities of skeletal elements, especially in the ribs. All these studies indicate that imprinted genes may exert their influence on specific cell types, directly through cell autonomous effects and indirectly through cell interactions and short-range paracrine effects.

It is interesting to note that some human embryonal tumours, including nephroblastomas and rhabdomyosarcomas, preferentially retain paternal chromosome 11p (Schroeder *et al.* 1987; Mannens *et al.* 1988; Scrable *et al.* 1989), which shares synteny with the imprinted region of chromosome 7 in the mouse (Searle and Beechey, 1990). A number of proposals have been put forward to suggest that genome imprinting can be a contributory factor in the genesis of these tumours (Ferguson-Smith *et al.* 1990).

Viability of conceptuses

With PG cells being selected against automatically after d13, a proportion of chimeras survive to term in which the average contribution of PG cells is reduced to 10% or less (Fundele *et al.* 1990). It appears that AG cells may not be similarly selected against. We have previously observed an AG↔F chimera on d14 with a large contribution from AG cells to the embryo (Surani *et al.* 1988). We have made further chimeras by injecting either small AG inner cell masses or 1–5 AG epiblast cells into recipient blastocysts in order to keep their contribution in chimeras to a minimum (Surani *et al.* unpublished data). By doing so we have obtained three AG↔F chimeras reaching term. One of these is apparently normal. Of the other two, one died shortly after birth and the second had severe skeletal abnormalities with some skeletal elements and ribs developing more posteriorly. This may partly be the result of the elongation of the anterior–posterior axis in the presence of AG cells in conjunction with aberrant proliferation of mesodermally derived cell types. Clearly the influence of imprinted genes is extremely critical for normal development as judged from the studies on chimeric conceptuses.

Chimeras have recently been obtained with AG-derived ES cells (Mann *et al.* 1990). These chimeras were obtained by introducing between 3 and 5 cells only, which is compatible with survival, although a large proportion of them also die *in utero*. It is not known what proportion of the 'imprints' are retained in these cells especially after culture for a prolonged number of generations.

Search for endogenous imprinted genes

The studies on the influence of the entire maternal and paternal genomes described above reveal the cumulative effects of all the imprinted genes on development. Since specific cell lineages as well as the overall embryonic growth and cell proliferation are apparently affected, this may provide important clues as to the identity of the imprinted genes. This search should be aided by genetic studies which have identified particular imprinted chromosomal regions, some of which have marked effects on development (Cattanach and Beechey, this volume). In particular, proximal Ch 2, proximal Ch 6 and distal Ch 7 are especially interesting because of their marked effects on development. For instance, the maternal duplication of the distal Ch 7 is lethal. The embryos die after midgestation and are substantially smaller than the controls, superficially analogous to parthenogenetic embryos (Searle and Beechey, 1990). The paternal duplication of this region is also lethal apparently at a very early stage, but the precise phenotype is not yet known. Studies are in progress to determine the role of this chromosomal region during development, which will be compared with the effects of AG and PG cells. These studies are beginning to reveal how a subset of imprinted genes affects development (Ferguson-Smith, unpublished data).

It is likely that one of the genes that maps to the distal region of Ch 7 is the insulin-like growth factor II (IGF-II). IGF-II, an embryonal mitogen is expressed during preimplantation development and as early as d7.5 of gestation and in a wide variety of tissues including extraembryonic tissues, as well as in many mesodermally derived tissues such as skeletal muscle, liver and kidney (Beck *et al.* 1987; Heyner *et al.* 1989; Graham *et al.* 1990). Furthermore, expression of IGF-II (or mannose-6-phosphate) receptor also occurs in extraembryonic tissues, as well as in embryonic tissues such as skeletal muscle and heart, at about the same time in development when the ligand is expressed (Senior *et al.* 1990). Such coordinated expression of the ligand and receptor is indicative of autocrine and short-range paracrine action of IGF-II in these tissues (Ohlsson *et al.* 1989). Indeed, we have observed disproportionate growth of some organs such as the heart in some AG↔F chimeras (Surani *et al.* unpublished data), which correlates with the extent of contribution from AG cells. It is also of interest in this regard to note that PG↔F chimeras are consistently smaller than AG↔F chimeras by up to 100% (Surani *et al.* unpublished data).

Inactivation of the IGF-II gene by homologous recombination has recently resulted in smaller offspring when the mutated allele is inherited from the father (De Chiara *et al.* 1990). Expression from the intact maternal allele was tenfold less than in mice with both alleles intact. The maternal duplication of distal Ch 7 also gives rise to fetuses and placentas which are smaller by about 50% (Searle and Beechey, 1990). It is possible that one of the imprinted genes influencing this phenotype is in

fact IGF-II. However, since no live fetuses are produced in this genetic cross, it is reasonable to assume that the distal region of Ch 7 must carry at least one other essential imprinted gene. Furthermore, observations reveal that IGF-II is elevated in the human trophoblastic tumours (hydatidiform moles) (R. Ohlsson, personal communication) as well as in embryonal tumours such as Wilm's tumour in which the paternal chromosome carrying the IGF-II gene is duplicated (Reeve *et al.* 1985; Scott *et al.* 1985). In most cases of the Beckwith Wiedmann syndrome (BWS) with trisomy 11p 15.5, the duplicated region is of paternal descent; the high incidence of embryonal tumours and other phenotypic characteristics such as gigantism and macroglosia may be associated with high expression of IGF-II, which maps to this region (Henry *et al.* 1989).

Since imprinted genes appear to be particularly important for size regulation, cell proliferation, cell interactions and differentiation, it will be of interest to determine if some of the other growth factors and their receptors are subject to imprinting. Furthermore, some of the genes encoding cell surface and adhesion molecules may constitute another important category of imprinted genes because of their importance in cell–cell interactions during development. Indeed, in the rat, expression of a major histocompatibility complex class 1 antigen suggests that the gene is imprinted since the paternal allele was reported to be preferentially expressed in the basal trophoblast cells (Kanbour-Shakir *et al.* 1990).

Conclusion

Imprinted genes have profound effects on development in the mouse and possibly in all mammals. Mammalian development is unique not only because of placentation, but also because of the highly regulative nature of development. It appears that the chromosomal 'imprints' serve as chromosomal 'determinants' of development. Their impact on development is seen even in chimeras since the presence of AG or PG cells exerts phenotypic effects as they interact with normal cells. These effects are due to the cumulative action of all the imprinted genes.

Mammalian development is essentially intolerant of deviations from an euploid state and, additionally, homologous chromosomes which contain imprinted genes must be derived from both parental sources. Imprinting evidently controls gene dosage and duplication of either maternal or paternal imprinted alleles leads to increased or decreased gene dosage. Hence, embryonic growth and cell proliferation are enhanced with the paternal duplication of the genome but the reverse occurs with maternal duplication. The apparent influence of imprinted genes on specific cell lineages is particularly noteworthy. Maternally imprinted genes appear to exert their effects on development of ectodermal derivatives such as brain and epidermis while paternally imprinted genes appear to be crucial for development of mesodermal derivatives especially muscle and skeleton. In addition, the overall body plan can be affected by imprinted genes with effects on the anterior–posterior axis. Hence genes that provide positional information for development in mammals may be imprinted.

Further studies are needed to define the role of imprinted genes on development more precisely. In this regard genetic studies are helpful and reveal that with paternal or maternal duplications of a subset of imprinted genes, a variety of abnormal phenotypes can be obtained. Of particular interest for development are the proximal region of Ch 2 and Ch 6, and the distal region of Ch 7. It appears that one of the genes on Ch 7 is probably IGF-II whose expression is apparently influenced by its parental origin. This embryonal mitogen is widely expressed during development and may particularly affect growth as well as proliferation of many different cell types including skeletal muscle. A systemic analysis of this type may eventually yield precise information on the role of key imprinted genes in development.

Much of our understanding of epigenetic modifications stems from studies on transgene loci. While these provide important insights, it is essential to identify endogenous imprinted genes so that their epigenetic modifications can be examined directly. A variety of epigenetic modifications may be employed that are likely to occur progressively. Imprinting is probably initiated in the germline but there are additional factors that operate following fertilization. The interactions between parental genomes and maternally derived oocyte cytoplasmic factors can influence epigenetic modifications. It appears that the imprinting process may be essentially complete by the 2-cell stage and that the two parental genomes can be imprinted independently of each other. Further modifications of the imprinted templates may continue resulting in heritable modifications, such as DNA methylation or heterochromatization. There are indications that modifier genes may act to bring about changes in chromatin structures as a part of the imprinting process. Their activity is most readily observed as a result of nuclear–cytoplasmic interactions between different inbred strains of mice. There are also indications that modifiers themselves may be subject to parental origin effects. This emphasises the notion that mechanisms exist prior to fertilization that may be involved in the imprinting of these genes amongst others. However, virtually nothing is known about either the mechanism or the epigenetic modifications responsible for the initiation of imprinting events in the germline.

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