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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The inheritance of germline-specific epigenetic modifications during development

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SUMMARY

Parental genomes in mammals are programmed in the germline with heritable epigenetic modifications that exert control on the expression of specific (imprinted) genes. DNA methylation is one form of epigenetic modification which shows marked genome-wide variations in the germline and during early development. Certain transgene loci also demonstrate (reversible) germline-specific methylation imprints that are heritable in somatic tissues during development. Recently, four endogenous genes have been identified whose expression is dependent on their parental origin. The mechanism of genomic imprinting and the role of imprinted genes during development is beginning to be analysed. Three of these genes map to the mouse chromosome 7. Human chromosomes 11p13, 11p15, and 15q11–13 are associated with disorders exhibiting parental origin effects in their patterns of inheritance. These regions share syntenic homology with mouse chromosome 7. The relationship between parental imprints, germline-dependent epigenetic inheritance and totipotency is also under investigation using embryonic stem cells derived from the epiblast. These cells are pluripotent or totipotent and evidence indicates the presence of at least the primary parental imprints. However, imprints inherited from the paternal germline in androgenetic cells are apparently more stable than those from the female germline in parthenogenetic cells.

1. INTRODUCTION

At fertilization, each parent contributes a genetically equivalent haploid set of autosomes to the zygote. Although most parental loci function identically during development, there are exceptions to this rule. Expression of some autosomal loci is in fact determined by whether they are of maternal or paternal origin (Cattanach & Beechey 1990). This 'memory' of parental origin of chromosomes controlling gene expression must require heritable epigenetic modifications. It is crucial that the germline must also be able to reverse any previously acquired epigenetic modifications. This whole phenomenon is called genomic imprinting. The non-reciprocal expression of some parental alleles plays a role in development and aberrant expression results in abnormal phenotypes and certain genetic disorders in the human (Solter 1988; Surani & Reik 1922). The molecular mechanism of genomic imprinting is so far scarcely understood. The initial studies were prompted by the identification of some transgene inserts which showed parental origin effects in their epigenetic inheritance and expression (Reik *et al.* 1990; Surani *et al.* 1990). These studies can now be extended to the recently identified endogenous imprinted genes.

Parental origin-specific imprinting probably occurs progressively in which both the germline-specific and post-zygotic events play a role in the process (Surani 1991). The germline-dependent modifications are simplest to envisage as the homologous chromosomes are segregated and subject to distinct influences during spermatogenesis and oogenesis. However, the parental alleles could be subjected to further differential modifications after fertilization both before and after pronuclear fusion. At this time nuclear-cytoplasmic interaction and oocyte cytoplasmic factors may have a significant part to play in epigenetic modifications. (Klose & Reik 1992). Some of the major epigenetic changes could therefore occur during pre-implantation development at a time when the pluripotent–totipotent epiblast cells are established. Thus, the epiblast cells most probably contain the primary parental imprints inherited from sperm and oocytes at

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fertilisation; after implantation and as gastrulation commences, further epigenetic modifications could occur in response to these signals coupled with conversion to more stable parental imprints.

There are two observations that implicate a role for DNA methylation in the events involved in imprinting. The first of these is the genome-wide changes in DNA methylation in the germline and early embryos (Bird 1986; Sanford *et al.* 1987; Monk 1988; Reik *et al.* 1990; Kafri 1992). The second concerns the methylation imprinting of certain transgenic loci (Reik *et al.* 1990; Surani *et al.* 1990). CpG dinucleotide methylation is an epigenetic modification known to be associated in some instances, with gene inactivity. For example, X-linked genes containing CpG rich promoter sequences show methylation of these sites on the inactive-X in females (Grant & Chapman 1988). In addition, a crucial role for DNA methylation during embryogenesis has been shown. Mice with a homozygous mutation inactivating the DNA methyltransferase gene are embryonic lethal after implantation (Li *et al.* 1992). Perhaps this is due to an instability of the primary imprints in the absence of methylation. Nevertheless, it is important to note that there are other forms of epigenetic modifications capable of controlling gene expression that are heritable and affect chromatin structure.

Some of the major genome-wide changes in DNA methylation that occur in the germline and early development are worth examining as this provides a background against which we can examine methylation imprinting of transgenes and endogenous genes (Jahner & Jaenisch 1984; Sanford *et al.* 1987; Monk 1988; Kafri 1922). Figure 1 depicts the general genome-wide changes in DNA methylation that have been investigated with respect to both the multi-copy genes as well as single sites within a number of genes. Hence we note dynamic reversible changes in DNA methylation that may have a bearing on parental imprinting of particular loci.

2. PARENTAL IMPRINTING OF TRANSGENES

The initial studies on imprinting were carried out on randomly integrated transgenes which serve as molecular probes for these loci (Reik *et al.* 1990; Surani *et al.* 1990). Two of these transgene inserts are particularly interesting as they show germline-dependent modifications. The MPA434 (Sasaki *et al.* 1992) and RSV-myc (Chaillet *et al.* 1991; Chaillet 1992) both show methylation occurring during oogenesis, i.e. the maternally inherited loci are methylated while the paternal remain hypomethylated (figure 2). There are however some differences between the two transgenes; the paternally inherited RSV-myc transgene undergoes some methylation during early embryogenesis while the *MPA343* transgene remains unmethylated after fertilization. The important point to note is that both transgenes are inherited in their methylated states from the maternal germline and remain so

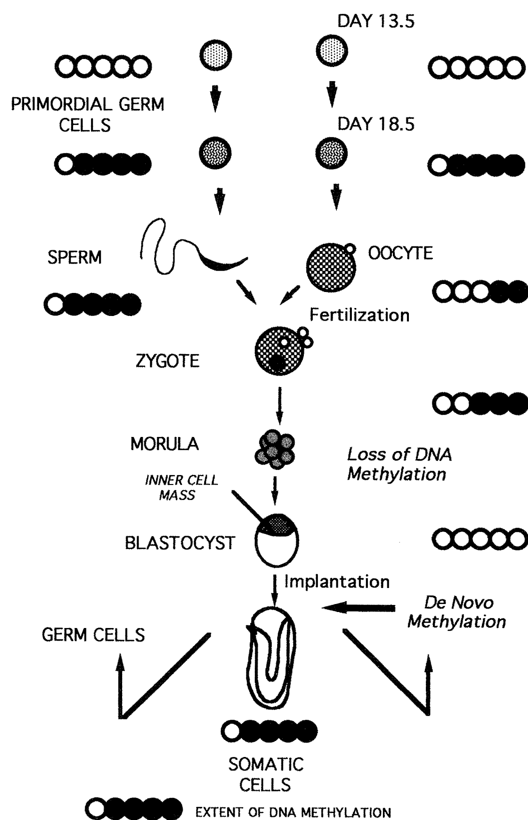

Figure 1. Genome-wide DNA methylation and demethylation events in germ cells and early embryos.

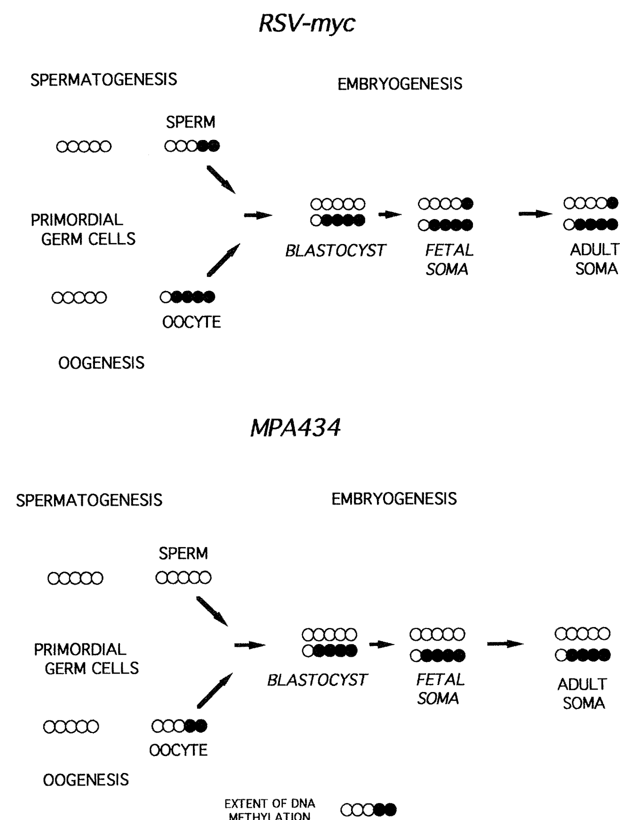

Figure 2. DNA methylation changes in transgene inserts, *RSV-myc* and *MPA434*, in germ cells and early embryos.

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thereafter during development. These transgenes probably undergo re-programming in the germline and become demethylated before the initiation of a new cycle of germline-specific parental imprinting. In all the studies reported so far, there are no definitive examples of preferential methylation of transgenes after paternal transmission; it is not clear whether this maternal specific modification to exogenous sequences has any relevance to the imprinting endogenous genes.

Transgene methylation is also evident after fertilization and during early development and this event is often prone to differences in the genetic background of the embryos. One example of this phenomenon was observed with the *TKZ751* locus as a result of position effect (Allen *et al.* 1990). This transgene is methylated on the BALB/c background but remains unmethylated on the DBA background. Strikingly, *TKZ751* transgene methylation requires a maternally inherited BALB/c modifier, i.e. the modifier itself behaves as an imprinted gene. This modifier, which has not yet been identified, is inherited with the nuclear component and is not present in the oocyte cytoplasm. However, the transgene locus *pHRD*, is subject to methylation in a position-independent manner; *pHRD* remains unmethylated and expressed on DBA/2 and SJL genetic background but is methylated with the loss of expression whenever it is crossed in to C57BL/6 mice. (Engler *et al.* 1991). The modification is not affected by the parental origin of the modifier. The modifier locus in this instance has been mapped to the distal region of chromosome 4 and has been called *Ssm-1*. (Engler *et al.* 1992).

It is not clear at present what factors are responsible for the imprinting of these transgene loci. In most instances it is supposed that imprinting occurs as a result of position-effects; i.e. effects would occur as a result of transgene integration into genes or regions susceptible to imprinting modification (Surani *et al.* 1990). One locus that has been examined in detail is the *MPA434* locus. This locus maps to chromosome 11, an imprinted chromosomal region. However, the endogenous sequences at the site of transgene integration do not show parent of origin-dependent DNA methylation. Nevertheless, some sites showed allele specific methylation that were not affected by their parental origin (Sasaki *et al.* 1992). It seems likely therefore that the imprinting of this transgene locus is due to a combination of transgene sequences and host sequences at the site of integration and may not be a good model for the parental imprinting of endogenous sequences.

As discussed above, two transgenes have so far been described that show position-independent patterns of DNA methylation, *pHRD* and *RSV-myc*. Both these transgenes become methylated independent of their site of integration. This implies presence of DNA sequences within those two different transgenes that act as the targets for methylation. So far it is not known what these sequences are or how the imprinting influence is manifested. The nature of these sequences that act as signals for imprinting would be informative, particularly in the context of imprinting of endogenous genes.

3. IMPRINTING OF ENDOGENOUS GENES

Based on non-complementation tests, genetic evidence shows that a number of chromosomal regions must contain imprinted genes. The mouse chromosome 7 is one of the most important in this respect since both a maternal and a paternal copy of this chromosome is essential for normal development (Searle & Beechey 1990). Three imprinted genes have now been identified on this chromosome. Two closely linked, reciprocally imprinted genes, *H19* and *Igf2*, are detected on the distal region of chromosome 7 (De Chiara *et al.* 1992; Bartolomei *et al.* 1991; Ferguson-Smith *et al.* 1991). The third gene, *snrpn*, is more proximal and located near the albino locus (Barr *et al.* 1992).

The maternal duplication or paternal deficiency of distal chromosome 7 resulting from intercrosses involving the T9H translocation is embryonic lethal at around day 16 of gestation (Searle & Beechey 1990; Cattanach & Beechey 1990). These embryos are growth retarded. The paternal duplication or maternal deficiency is also lethal but at an unknown stage that occurs after day 6 although the precise stage and the phenotype are unknown owing to the lack of adequate early markers. However, cells with the paternal duplication with corresponding maternal deficiency can be rescued in chimeras with normal cells (Ferguson-Smith *et al.* 1991). Paternal disomy cells which were apparently uniformly distributed, induced a growth enhancement reciprocal to that seen in non chimaeric maternal disomy embryos. It appears that neither of the parental duplications show any effects on particular cell lineages in contrast to phenotypes associated with parental duplications of the whole genome.

The effect on growth observed in these studies is undoubtedly due to the parental imprinting of the *Igf2* gene; this gene is an embryonal mitogen with an important role during embryogenesis (De Chiara *et al.* 1990). The role of *H19* gene is unknown at present with no evidence to suggest that it encodes a protein in the embryo (Bartolomei & Tilghman 1992). Four mammalian species have been studied regarding the DNA sequences of *H19* but so far no common open reading frame is evident. The *H19* RNA is apparently located in 28*S* particle and not with the translational apparatus (Bartolomei & Tilghman, 1992).

The embryos with maternal duplication and paternal deficiency of chromosome 7 (maternal disomic embryos; MatDi7) provide a powerful experimental system with which to explore the mechanism of imprinting. We have demonstrated clearly that these embryos have twice the levels of H19 compared to the controls since these embryos contain two active maternal alleles (H. Sasaki & M. A. Surani, unpublished results). By contrast the levels of Igf2 transcripts in MatDi7 embryos are negligible (Ferguson-Smith *et al.* 1991). Excess levels of H19 by itself would be detrimental because attempts to produce *H19* gain of function transgenic embryos resulted in embryonic lethality at around day 15 of gestation (Brunkow & Tilghman 1991). The other important points to note regarding the imprinting of *H19* and *Igf2* are that the

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genes are very closely linked, reciprocally imprinted and display remarkably similar spatial and temporal patterns of expression during development (Bartolomei & Tilghman 1992; Sasaki *et al.* 1992). Taken together, these findings may suggest a functional relationship between the two genes regarding the mechanism resulting in their imprinting and their role in embryogenesis.

4. MECHANISM OF IMPRINTING OF ENDOGENOUS GENES

Little is known about the identity of the heritable epigenetic modifications resulting in the parent of origin specific regulation of endogenous imprinted genes. The temporal sequence of events resulting in a stable and heritable imprint must also be considered. Indeed it is not clear if each endogenous (imprinted) gene is imprinted by the same mechanism. In light of the transgene data, DNA methylation analyses may be informative and can be carried out relatively easily on endogenous imprinted genes by comparing the methylation status of the two parental alleles in normal and MatDi 7 embryos; the latter isolated prior to their death at day 16 of gestation.

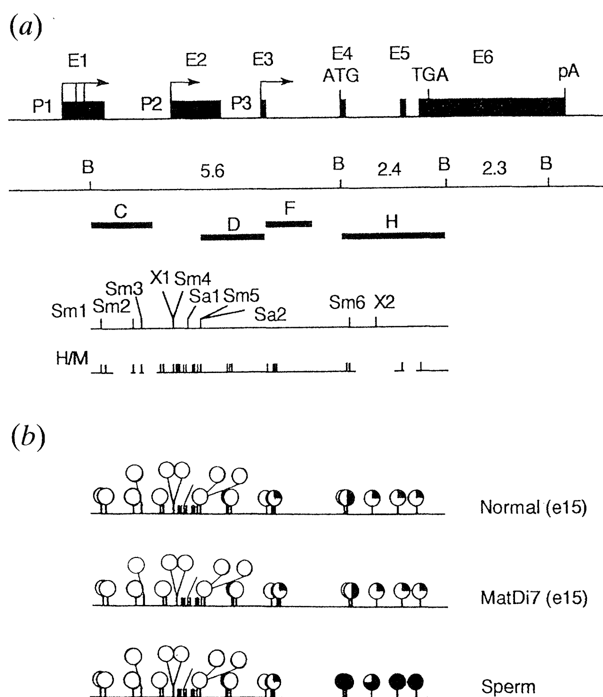

Figure 3. An overall structure and restriction map of the mouse *Igf2* gene (*a*) and summary of methylation analysis (*b*). The positions of six exons (E1–6), three promoters (P1–3) and transcription initiation site (arrows) are indicated. ATG, TGA and pA denote initiation codon, termination codon and major polyadenylation site, respectively. Solid lines indicate the probes which were used in the methylation assays. The methylation-sensitive enzymes are: Sm, *Sma*1; X, *Xho*1; Sa, *Sal*1; H/M, *Hpa*II/*Msp*1. (*b*) The methylation-sensitive enzyme sites are shown by an open circle if unmethylated or a filled one if methylated. Other circles denote intermediate levels of methylation. The methylation status of sites without circles was not determined.

(*a*) *Methylation imprinting of Igf2*

We carried out a detailed analysis of DNA methylation and chromatin structural changes in the *Igf2* gene (Sasaki *et al.* 1992). Two *BamH1* fragments of 5.6 and 2.4 kb were subcloned and used as hybridization probes (figure 3). These fragments contain two strong promoters P2 and P3 and the polypeptide coding exons. P2 contains a CpG island. DNA was isolated from normal and MatDi 7 embryos and was digested with *BamH1* and methylation sensitive enzymes, *HpaII*, *SmaI*, *XhoI* or *SalI*. Any differences in methylation pattern could thus be attributed to the paternal allele absent in MatDi but present in embryos. No detectable differences in methylation were found between control and maternal disomy 7 embryos. The *BamHI* 5.6 kb region was strikingly unmethylated on both alleles suggesting that unlike the imprinting of transgenes, there is no widespread methylation of the maternally repressed *Igf2* gene. However, we cannot exclude the possibility that more subtle differences in DNA methylation exist which are not revealed by analysis using methylation sensitive restriction enzymes alone. The downstream *BamHI* 2.4 kb region however produced a pattern revealing partial methylation in DNA derived from the control and experimental embryos. But again no differences were observed. However, sperm DNA gave a fully methylated pattern in this region.

We have also examined the nuclease sensitivity of the chromatin of the repressed allele in nuclei from the control and MatDi disomic embryos. The 5.6 kb region was found to be highly sensitive to *MspI* in both MatDi7 and control embryos. Similarly DNAaseI hypersensitive sites in MatDi7 and control embryos were examined. Again no differences were found between materials from the two types of embryos. Furthermore prominent DNAase I hypersensitive sites were present in the promoter regions of both alleles. This implies that though one allele is active and the other repressed, both alleles seem equally accessible to transcription factors. Hence, there are no major detectable differences either in DNA methylation or chromatin structure in the active and the repressed loci. This raises the possibility that the chromatin of the repressed maternal allele is potentially active, which was indeed found to be the case when transcription was examined by a sensitive quantitative RT-PCR assay. Low but significant levels of Igf2 transcripts were detected in the MatDi7 embryos. This expression was not attributed to absence of imprinting in a subset of cells as judged by *in situ* hybridization studies for *Igf2* expression in MatDi7 embryos. We conclude that the low level transcription is from most of the cells and not from a subset of cells.

This data raised the possibility that methylation differences may exist elsewhere in the locus. Indeed parent of origin dependent methylation differences were found in a region 3 kb upstream of the first promoter (R. Chaillet, unpublished; Sasaki *et al.* 1992). Multiple *HpaII* sites in this region are slightly more methylated in normal embryos than in MatDi7

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embryos showing that the paternal chromosome with the active *Igf2* gene has higher levels of DNA methylation in this region. However, further studies are needed to elucidate the possible significance of this finding for the regulation of *Igf2* by imprinting.

These results for the *Igf2* gene provide no obvious indications for a role of DNA methylation in the imprinting of this locus. Whatever mechanism causes the repression of the *Igf2* gene, it is essential that this should account for the repression from all the three promoters unless evidence is found for promoter specific imprinting of this gene. In addition, the mechanism must not be able to act in the choroid plexus and leptomeninges of the brain where the gene has been shown to escape imprinting (De Chiara *et al.* 1991). We can also rule out the role of major chromatin structural difference such as heterochromatinization in the repression of this gene. Hence, it is necessary to explore more complex possibilities for the imprinting of the *Igf2* gene. Among these possibilities we have to consider if the presence of the closely linked and reciprocally imprinted *H19* gene is mechanistically relevant for this purpose.

(*b*) *Methylation imprinting of the H19 gene*

H19 gene is approximately 3 kb in size with 5 exons, a defined promoter and two downstream enhancers (Bartolomei & Tilghman 1992). The gene is transcribed by RNA polymerase II spliced and polyadenylated but so far no protein product for this gene has been found in embryos even though it is expressed abundantly. The function of this gene is not known. The promoter lies within a short CpG rich region of approximately 300 b.p. There are other CpG sites which are scattered throughout the locus.

We have commenced DNA methylation analysis of this gene once again by comparing MatDi7 (with the two active *H19* genes) and control embryos (containing the repressed paternal copy of the gene) (A. C. Ferguson-Smith, H. Sasaki & M. A. Surani, unpublished results). The results clearly demonstrate that there are multiple methylatable sites in the promoter region which are methylated on the paternal gene but which are unmethylated on the maternal gene. Examination of the methylation status of these sites in sperm shows that these sites are not methylated. The combined results therefore indicate that methylation occurs after fertilization. There is variable methylation throughout the body of the gene. Further studies have been carried out to examine chromatin structural differences by examining the nuclease accessibility of the promotor and enhancer regions. These studies clearly reveal that there are differences between the active and the repressed loci in the promoter, with the active locus being more accessible (H. Sasaki, A. C. Ferguson-Smith & M. A. Surani, unpublished results).

The methylation of the repressed paternal *H19* gene is therefore more in line with the expectations from imprinting of transgenes and consistent with the inverse correlation between methylation and gene expression (Allen *et al.* 1990; Reik *et al.* 1990; Surani *et al.* 1990; Sasaki *et al.* 1992). However, the results raise some important questions regarding the exact mechanism of imprinting and in particular the nature of the primary imprint. The paternal gene does not appear to inherit its methylation imprint from the sperm but acquires it after fertilization. The precise time when this occurs is unknown at present. However, one possibility is that this methylation occurs after implantation at day 6.5 of gestation when a major *de novo* methylation event is known to occur (Sanford *et al.* 1987; Monk 1990; Li *et al.* 1992). However, the possibility of inheritance of subtle methylation differences by the parental alleles is not excluded nor is it known to what extent the postzygotic methylation differences observed in the case of *H19* will apply in the case of other endogenous imprinted genes. Alternatively, other forms of epigenetic differences between the maternal and paternal copies of the gene may exist from the germline which are recognized subsequently and which result in differential methylation. Further studies are needed to determine the exact nature of this primary imprint.

To address this question though, information on the precise timing of DNA methylation in the promoter region of the paternal allele is required. In particular, it will be highly informative to establish if the *H19* gene is methylated in oocytes. Alternatively, the maternal gene may become methylated during oogenesis but undergoes demethylation during the course of early preimplantation development when genome wide demethylation events are occurring. (see figure 1)

(*c*) *Comparisons between the imprinting of Igf2 and H19*

At the available levels of analysis, there appear to be major differences in the role of DNA methylation in the imprinting of *H19* and *Igf2* (figure 4). One

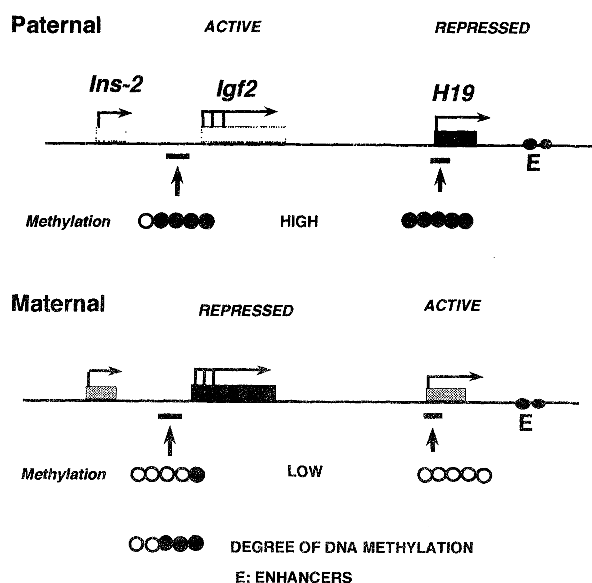

Figure 4. A summary of major parent of origin-dependent methylation difference detected so far at the *Igf2* and *H19* loci.

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obvious explanation may be that repression of maternally and paternally inherited genes may require different mechanisms. This question will be resolved when more is known about the imprinting of other endogenous genes such as *Igf2r* on chromosome 17 which is also repressed when paternally inherited (Barlow *et al.* 1991). It will be of interest to determine whether the maternally repressed *Snrpn* gene will share the absence of modification seen with *Igf2*. As far as *Igf2* and *H19* are concerned, one obvious question which arises is the significance, if any, of their close proximity and reciprocal imprinting. The observation of methylation differences in the case of *H19* and a lack of major differences in *Igf2*, prompts speculation on whether *H19* carries the primary imprint while imprinting of *Igf2* occurs secondarily and reciprocally as a consequence of *H19* imprinting. Further studies are required to resolve this and a number of theories addressing this question are discussed elsewhere (Sasaki *et al.* 1992; Bartolomei & Tilghman 1992).

5. IMPRINTING IN ANDROGENETIC AND PARTHENOGENETIC EMBRYOS

The biparental androgenetic and parthenogenetic or gynogenetic embryos produce characteristic phenotypes after implantation; the former with well developed extrembryonic tissues and the latter with relatively well developed embryos (Solter 1988; Surani *et al.* 1990). These reciprocal phenotypes have been suggested to arise as a result of differential expression of imprinted genes. However, no systematic studies have so far been reported to show if the expression of the recently identified imprinted genes occurs appropriately in these embryos. Such studies are important since it would provide proof that the phenotypes are due to the reciprocal expression of imprinted genes. For example, analysis of the 'appropriate' activity of imprinted genes in androgenetic and parthenogenetic conceptuses should also provide information about any interactive roles between the parental genomes in the regulation of imprinting. Studies arising from experiments on the *TKZ751* transgene locus suggest that methylation imprinting segregates with a modifier. Specifically, the modifier from the BALB/c strain of mice induces hypermethylation of the locus and results in the extinction of expression. Evidence suggests that this modifier must be of maternal origin for it to have an effect. In other words the modifier itself reveals a parent of origin effect (Allen & Mooslehner 1992).

It is possible that similar modifiers are important during postzygotic aspects of the imprinting of endogonous genes. For example, it will be interesting to determine methylation status of the *H19* gene in androgenetic embryos in the absence of a maternal genome, since such methylation occurs postzygotically. Clearly if the imprinting of *H19* is perturbed in these circumstances, it will reveal the existence of postzygotic modifiers in imprinting of endogenous genes, and expose the relationship between imprinting of *H19* and *Igf2*. There is also interest in the fate of such imprints in embryonic stem cells of normal, androgenetic and of parthenogenetic origin. These studies would allow evaluation of the stability of imprints.

6. EMBRYONIC STEM CELLS AND THE NATURE OF PRIMARY IMPRINTS

Embryonic stem cells are derived from the primary epiblast cells which are present in blastocyts. These cells are pluripotent and possibly totipotent and derived at a time when there is a marked decline the levels of DNA methylation (Evans & Kaufman 1981; Monk 1990; Nagy *et al.* 1990; Li *et al.* 1992). Whether the loss of genome-wide methylation during preimplantation development is prerequisite for pluripotency of the epiblast cells remains to be determined. Nevertheless, the dramatic changes in this major epigenetic modification is of interest from the point of view of parental imprints. In the absence of genome wide methylation, embryonic stem (ES) cells may contain the less stable primary imprints. As discussed above, methylation of the *H19* paternal gene occurs postzygotically and only the primary germline specific imprints may be present prior to methylation. It is important to determine the nature and stability of this imprint. Embryonic stem cells are very useful in this regard.

The phenotypic and molecular properties of androgenetic, parthenogenetic and control cells can be compared after passaging the cells *in vitro* to test for the stability of the parental imprints (Mann *et al.* 1990; Mann 1992). In addition, these properties can be compared directly with those of the epiblast cells in the inner cell mass (ICM) from which the ES cells are derived. The most obvious test which has been applied to check for the properties of these cells is by assessing their effects in chimeras. Androgenetic cells derived from ICM induce marked growth enhancement and severe abnormalities of skeletal elements in chimeras (Barton *et al.* 1991; Mann 1992). This is consistent with the propensity of these cells to contribute to the many derivatives of the mesenchymal pluripotent cells such as chondrocytes and skeletal muscle. These very profound phenotypic effects are also observed when androgenetic ES cells are used to produce chimeric embryos (Mann *et al.* 1990). These phenotypic effects are observed even when these cells have undergone considerable passaging *in vitro* and with euploid as well as aneuploid cells. These remarkable similarities in properties between ES and ICM derived epiblast cells indicate that the ES cells probably retain their parental imprints, assuming that appropriate imprinting does indeed occur in androgenetic embryos in the absence of a maternal genome. Although androgenetic cells can contribute to a variety of somatic cell types in chimeric embryos, there is as yet no evidence to show if they can contribute to germ cells.

Parthenogenetic ES cells by contrast may not retain their phenotypic properties. For example, the presence of parthenogenetic cells derived from the inner cell mass in chimeras produces growth retardation (Solter 1988; Surani *et al.* 1990). Furthermore there is a remarkable lack of contribution by these cells to

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skeletal muscle (Surani *et al.* 1990). However, parthenogenetic ES cells do not cause such growth retardation and they contribute to skeletal muscle (Mann 1992; N. D. Allen, S. C. Barton & M. A. Surani, unpublished results). There is at present little information on precisely why this difference exists and whether the change can be attributed to alterations in the maternal imprints in ES cells. However, a major point of similarity between parthenogenetic ES cells and ICM cells is seen in their ability to contribute to the germline resulting in viable oocyte (Surani *et al.* 1990; Mann 1992).

Further studies are needed to elucidate the properties of the ES cells with regard to expression of imprinted genes. In particular it is important to know how the androgenetic cells are able to retain relatively stable phenotypic properties while the parthenogenetic cells apparently do not. We assume that the primary parental imprints are retained in the pluripotent normal ES cells. The recent derivation of stem cells directly from primordial germ cells (the so called EG cells) should provide a valuable source of material with which to explore the question of primary imprints and to compare their properties with a variety of ES cells of different genotypes (Matsui *et al.* 1992; Resnick *et al.* 1992). The normal ES cells are obtained from the epiblast cells from which both the primordial germ cells and somatic cells are derived.

7. IMPRINTING IN THE HUMAN

There is compelling evidence now to suggest that germline specific epigenetic modifications occur in the human with important consequences for some forms of genetic disorders. This is particularly well characterised for the human chromosomal regions which correspond to the mouse chromosome 7 (Cattanach & Beechey 1990; Bartolomei & Tilghman 1992). The distal end of the mouse chromosome 7 is syntenic with the human chromosome 11q13 and 11q15. The latter is particularly interesting since both *Igf2* and *H19* map to this region and is the potential contributor to the Beckwith–Wiedemann syndrome (Bartolomei & Tilghman 1992). This disorder is a foetal overgrowth syndrome associated with certain embryonal tumours such as rhabdomyosarcomas and Wilms. In sporadic cases of these diseases, paternal isodisomy of 11p15.5 is observed. The role of IGF2 in these instances is predictable since such isodisomy will result in an excess of IGF2.

The other significant human genetic disorders attributed to genetic imprinting are the Prader–Willi and Angelman syndromes which are due to parental origin specific deletions in the 15q11–13 regions and to uniparental disomies with respect to the maternal and paternal regions, respectively (Nicholls *et al.* 1992; Barr *et al.* 1992). A number of cDNA clones have now been obtained from this region which map to the mouse chromosome 7 in the vicinity of the albino locus. Indeed a new imprinted gene has now been identified in this region called SNRNP and is expressed specifically in the brain. The fact that the Prader–Willi and Angelman syndromes manifest as behavioural disorders suggests that this imprinted gene may have an important role in the genesis of Prader–Willi syndrome.

8. CONCLUSIONS

Genomic imprinting is a reversible germline-specific epigenetic modification. The identification of the first imprinted genes will allow an understanding of the mechanism of this process. In particular it is necessary to identify the nature of epigenetic modifications, their mode of inheritance, and their influence in regulating gene expression. Understanding such chromosomal controls of gene expression will provide an important insight into gene regulation in general. These studies should also broaden our understanding of the fundamental concept of totipotency as work on ES cells of different genotypes is extended. The critical steps in the reversal and re-introduction of parental imprints in the germline and their subsequent modifications after fertilization are crucial for normal development and for restoring totipotency. Aberrant germline events, for example in the process requiring erasure of previous epigenetic modifications could have detrimental consequences. These studies will ultimately demonstrate the importance of germline specific epigenetic inheritance in development and disease.

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