6 Molecular Genetics of Genomic Imprinting

Robert Feil, Yuji Goto, and David Umlauf
Centre National de la Recherche Scientifique, Montpellier, France

1 Genomic Imprinting 191
1.1 Embryological Evidence 192
1.2 Imprinted Chromosomal Domains 194

2 Imprinted Genes 196

3 Molecular Mechanisms 198
3.1 Imprinting-control Regions 198
3.2 Reading the Imprint 200

4 Imprinting and Disease 201

5 Evolution of Imprinting 203

Bibliography 204
Books and Reviews 204
Primary Literature 204

Keywords

Androgenetic Embryo
An embryo with two paternal genomes, and no maternal genome, produced by nuclear transplantation.

Chromatin
DNA packaged around nucleosomes. The degree of packaging differs between active (euchromatic) and inactive (heterochromatic) chromosomal regions.

Genomics and Genetics. Edited by Robert A. Meyers.
Copyright © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim
ISBN: 978-3-527-31609-0

DNA Methylation
Attachment of methyl (CH_3) groups to the bases of DNA. In mammals, DNA methylation occurs at cytosines that are followed by guanines (at CpG dinucleotides).

Epigenetic Modification
Any heritable, but reversible, alteration of DNA or associated nucleosomes above the level of the DNA sequence. This additional layer of information may indicate the parental origin of the chromosome.

Genomic Imprinting
A parent-of-origin-dependent mechanism whereby certain gene loci become expressed from only the maternal or only the paternal chromosome.

Histone Modification
The histones in nucleosomes can be altered by covalent modifications. At imprinting-control regions, these modifications are different between the parental alleles.

Imprinting and Behavior
Some imprinted domains are associated with behavioral phenotypes, and genetic disruption of certain imprinted genes gives aberrant behavior.

Imprinting and Cancer
The epigenetic maintenance of imprinting is frequently deregulated in cancer. Since imprinted genes are important in cell proliferation and differentiation, such deregulation is probably involved in the process of tumorigenesis.

Imprinting and Growth
Many imprinted genes influence fetal growth and development. Imprinted genes that enhance growth are mostly expressed from the paternal allele. Several other imprinted genes, which reduce growth, are expressed from the maternal allele.

Imprinting-control Regions
DNA sequence elements that are essential for imprinted gene expression. They are modified by DNA methylation and epigenetic modifications on the chromatin.

Nucleosome
The basic structural unit of chromatin, consisting of ~150 bp of DNA wrapped around an octamer of histone proteins (two each of four different histones).

Nutrient Transfer
Imprinted genes are important for the development of the extraembryonic membranes. These are essential for nutrient transfer to the developing embryo.

Parthenogenesis
The derivation of offspring from eggs only. Parthenogenesis is viable in some animal groups, such as in bird species, but is embryonic lethal in mammals because of the functional nonequivalence of the maternal and the paternal genome.

Uniparental Disomy
Inheritance of a particular chromosome in two copies from one parent, with absence of the chromosome from the other parent.

Genomic imprinting is a developmental mechanism in mammals and other organisms leading to repression or expression of genes depending on whether they are inherited from the mother or the father. The imprinted expression of genes is regulated by various epigenetic alterations, including DNA methylation and covalent modifications at histones. A large number of imprinted genes have been identified in placental mammals. Mostly clustered in the genome, these play important roles in embryonic and extraembryonic development, and in behavior. In humans, genetic and epigenetic alterations at imprinted genes are involved in different disease syndromes and in cancer.

1 Genomic Imprinting

Gene expression is not determined solely by the DNA code itself. It depends also on different epigenetic features. The term *epigenetic* is used to refer to mechanisms that do not involve changes in the DNA sequence and that are heritable from one cell generation to the next. Unlike heritable changes due to mutation or directed gene rearrangement (such as in the immunoglobulin genes), epigenetic modifications are reversible and can be removed from genes and chromosomes without leaving behind any permanent change to the genetic material. The main epigenetic modifications by which gene expression can be altered are DNA methylation and modifications to the chromatin. A well-known epigenetic mechanism is X-chromosome inactivation. In mammalian X-chromosome inactivation, sequential epigenetic modifications lead to the (random) transcriptional repression of one of the two X-chromosomes in all the somatic cells of females.

In this article, we consider a particular class of epigenetic imprints, those that mark the parental origin of genomes, chromosomes, and genes. Genes regulated by such "genomic imprinting" are expressed depending on whether they are on the maternally or on the paternally derived chromosome. Some imprinted genes are expressed only from the paternal chromosome, whereas others are exclusively expressed from the maternal chromosome. During the last fifteen to twenty years, imprinting has evolved from the initial observations in mouse embryos to a rapidly expanding field with importance

for mammalian development and genetics, and human disease. A large number of imprinted genes have now been identified. In addition, molecular studies have unraveled the underlying molecular mechanisms. Imprinting is not unique to mammals but is known to occur in seed plants and invertebrate species as well. This article, however, focuses on the regulation and role of autosomal imprinted genes in mammals.

Following the discovery of genomic imprinting, and the identification of the first imprinted genes in mammals, researchers in the field hypothesized that the epigenetic marks that regulate parent-of-origin-dependent expression are established in either the female or the male germ line and (after fertilization) are maintained throughout development. This epigenetic information needs to be removed upon passage of the imprinted gene through the germ line in the developing fetus, however, so that new imprints can be established. Recent studies on DNA methylation and other epigenetic modifications showed that, indeed, there are three distinct phases in imprinting: establishment of the imprint in the male or female germ line, somatic maintenance of the imprint after fertilization, and its erasure upon (re-)passage through the germ line (Fig. 1).

1.1 Embryological Evidence

Embryological studies in the mouse provided the first evidence that, in mammals, both a maternal and a paternal genome are required for the production of viable offspring. Significantly, it was found that monoparental embryos, carrying either two maternal or two paternal genomes, cannot develop to term. Such monoparental embryos were obtained by nuclear transplantation, immediately following the fertilization of the egg by the sperm. By replacing the female pronucleus (female genome) with a male pronucleus (male genome), for example, it was possible to produce androgenetic embryos (which have two paternal genomes). Conversely, embryos with two maternal genomes (gynogenotes) were made by replacing the male pronucleus with a female pronucleus. Embryos with two maternal genomes were derived by activation of unfertilized eggs (parthenogenesis) as well. Intriguingly, both gynogenetic (parthenogenetic) and androgenetic embryos survived only for a few days after implantation in the uterus and were found to have major developmental abnormalities.

Gynogenetic (parthenogenetic) and androgenetic embryos have different developmental phenotypes (Fig. 2). After

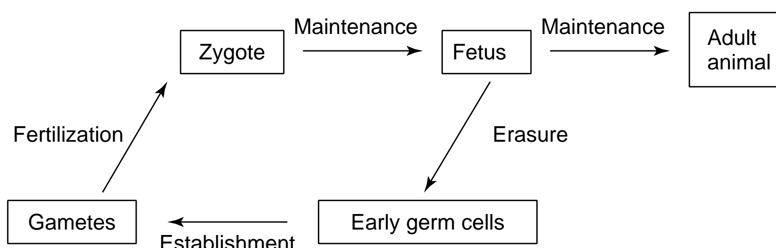

Fig. 1 Ontogeny of genomic imprinting: germ line establishment, somatic maintenance, and erasure.

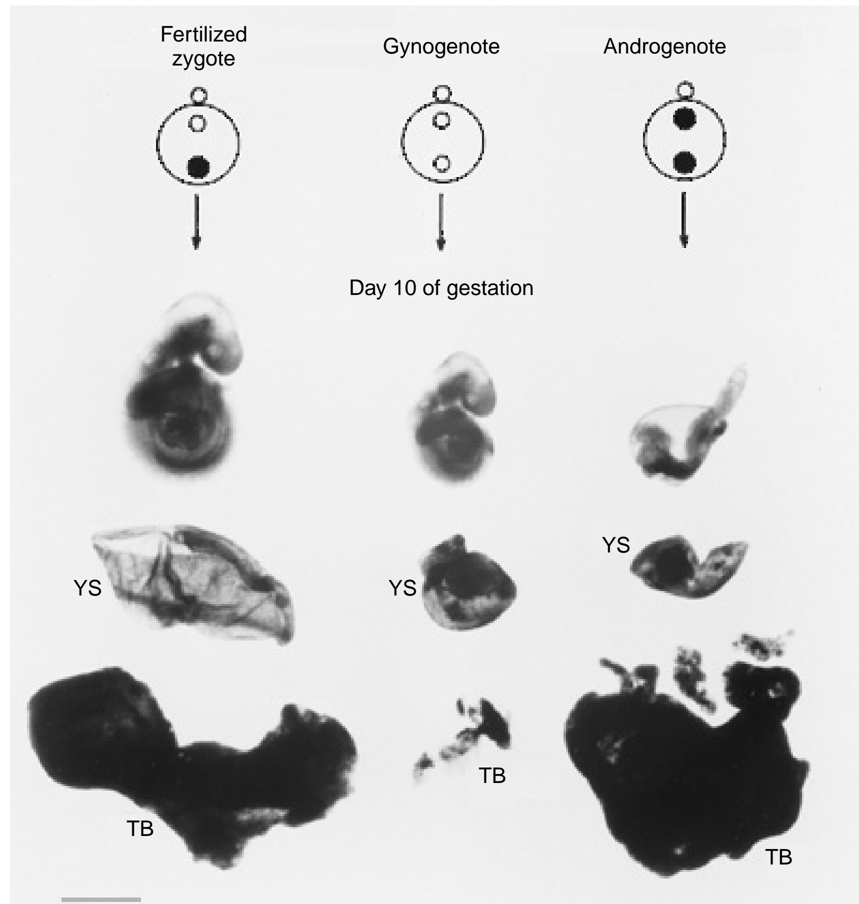

Fig. 2 Normal, androgenetic, and gynogenetic mouse embryos and their extraembryonic membranes at day 10 of gestation. Shown are the embryo, the yolk sac (YS), and the trophoblast (TB).

implantation into recipient females, parthenogenetic conceptuses develop to only about day 10 of gestation, with an apparently normal but small embryo. Development of the extraembryonic membranes (yolk sac and trophoblast), in contrast, is severely deficient, and these are the tissues that are important for nutrient transfer to the embryo. The phenotype of androgenetic conceptuses is opposite to that of parthenogenetic conceptuses. Whereas the extraembryonic tissues are normal in the androgenetic conceptuses, the embryo proper is retarded and progresses rarely beyond the four- to six-somite stage. The investigations on monoparental embryos established that both the parental genomes are required for normal mammalian development. They also provided evidence for the existence of genetic loci at which expression depends on the parental origin of the gene. In parthenogenetic and androgenetic embryos, individual imprinted genes are either expressed from both the

gene copies (double gene dose) or are not expressed at all. Cumulatively, the aberrant levels of expression of imprinted genes are responsible for the striking phenotypes of the two types of monoparental embryos.

1.2 Imprinted Chromosomal Domains

The embryological evidence from the monoparental embryos was reinforced by genetic studies demonstrating that specific chromosomal domains are subject to imprinting. Particularly, mice that were heterozygous for chromosomal translocations were intercrossed to obtain embryos and offspring with uniparental disomy for individual chromosomes (or chromosomal regions). Since during meiosis there is sometimes nondisjunction at the chromosome with the translocation, some of the resulting gametes comprise two copies of the translocated chromosome, whereas others contain none. Embryos that arise from two of such opposite gametes will have two copies of all the chromosomes, but for the translocated chromosome, both the copies will be paternal or maternal. By using different translocation lines, such uniparental disomic embryos were generated for almost all autosomal chromosomes. Phenotypic analyses unraveled the role of subsets of imprinted genes that reside in two paternal or two maternal copies in the different uniparental disomies. These studies also revealed that the maternal and paternal copies of individual chromosomal regions have frequently opposite roles in development and after birth (Fig. 3).

One of the imprinted domains is on the distal portion of mouse chromosome 7. When present in two maternal copies (maternal disomy), it leads to reduced growth and fetal death, whereas paternal disomy of this distal region is associated with enhanced growth and embryonic death. Some 10 imprinted genes have been mapped to this region. Several of these are part of the insulin-like growth factor/insulin signaling pathway (IGF/INS pathway). Being key players in the regulation of fetal growth and development, they contribute to the opposite growth phenotypes in the maternal and paternal distal-7 disomies. The corresponding chromosomal region in humans, chromosome 11p15.5, is involved in the Beckwith–Wiedemann syndrome (BWS), a human growth disorder that can be caused by paternal disomy of this imprinted region.

Another chromosomal domain with opposite phenotypes in paternal and maternal disomies is the proximal portion of chromosome 11. Mice with paternal disomy of this region are larger than their normal littermates, whereas maternal disomy mice are smaller. This indicates that there are imprinted genes in this region, of which aberrant levels of expression in the maternal and paternal disomies cause their abnormal growth. So far, two imprinted genes have been identified in this domain, *U2af1-rs1* and *Grb10*. The latter could be responsible for the phenotypes of the maternal and paternal disomies. Its main embryonic transcript is expressed from the maternal allele only, and it encodes a protein with a negative effect on the growth-regulating IGF/INS pathway.

Disomy phenotypes at a few other imprinted domains involve abnormal postnatal behavior. Paternal disomy for distal mouse chromosome 2, for instance, gives offspring that are hyperactive, whereas maternal disomy is associated with reduced activity after birth. Such behavioral phenotypes emphasize that imprinted genes can affect behavior. A small number of

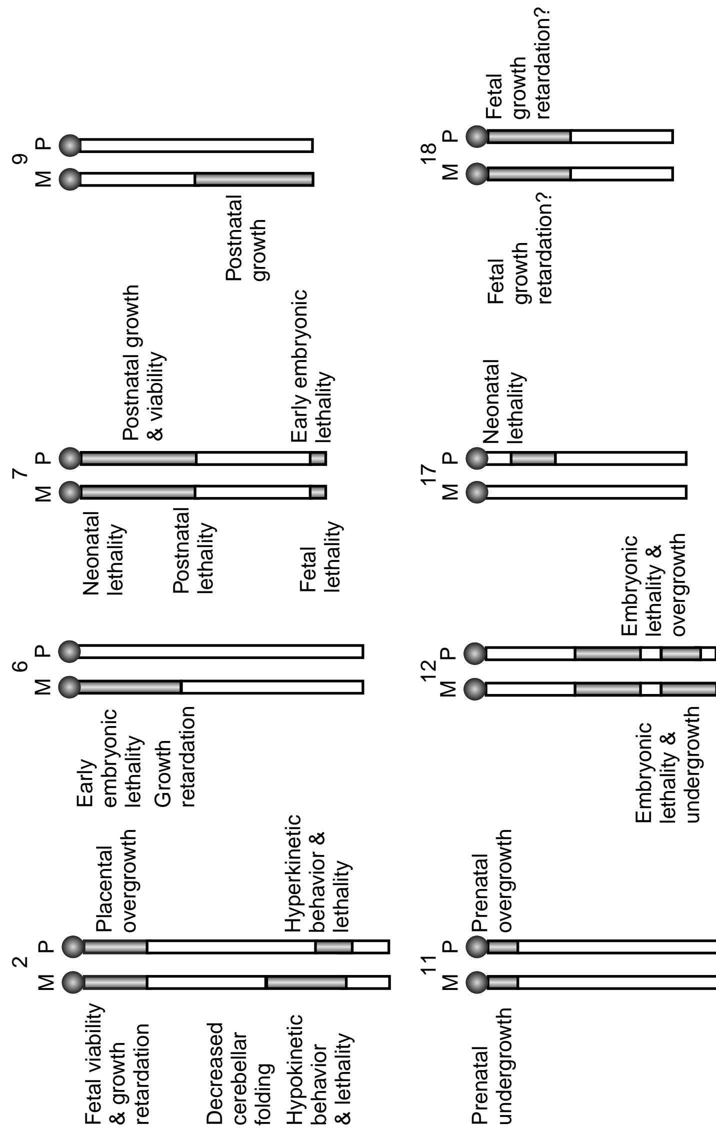

Fig. 3 Imprinted chromosomal domains in the mouse with the associated developmental phenotypes in maternal (indicated to the left) and paternal (to the right) disomies.

imprinted genes were discovered on distal mouse chromosome 2. Two of these have neuroendocrine functions (*Gnas* and *Gnas-xl*) and are involved in the behavioral phenotypes of the maternal and paternal disomy mice.

In total, 12 chromosomal regions with imprinting phenotypes have been identified on 8 different autosomal chromosomes (Fig. 3). The large majority of the known imprinted genes maps to these chromosomal regions. Probably, the remainder of the genome comprises few imprinted genes or contains imprinted genes that give rise to minor phenotypes only when present in two maternal or two paternal copies.

2 Imprinted Genes

It is unknown which proportion of mammalian genes is imprinted and estimates vary between about 100 and a 1000 genes. To date, however, some 70 imprinted genes have been detected in the mouse and most of these are imprinted in humans as well. A consistent feature of imprinted genes is that they are organized in clusters in the genome. These clusters are hundreds to several thousands of kilobases in size and are similarly organized in humans and mice. We selected several imprinted clusters as examples, and we describe their roles in development and behavior. A comprehensive presentation of imprinted genes is given elsewhere.

A well-known imprinted cluster is on distal mouse chromosome 7 (Fig. 4) and on the corresponding chromosome 11p15.5 in humans. This cluster comprises 11 imprinted genes. Several of these genes play key roles in fetal growth and development. The insulin-like growth factor-2 gene (*Igf2*), at the proximal side of the cluster, is expressed from the paternal allele only. Transgenic mice inheriting a null *Igf2* allele from the father are much smaller than their littermates; maternal inheritance of the targeted allele does not alter the phenotype. This strong paternal effect on fetal growth is primarily due to the loss of IGF2 in the extraembryonic membranes, which decreases nutrient transfer to the developing fetus. The neighboring insulin-2 gene (*Ins2*), also of the IGF/INS pathway, is located at about 20 kb from *Igf2*. In the yolk sac, it is the paternal chromosome that expresses *Ins2*, whereas the maternal chromosome is repressed. The paternal expression of *Igf2* and *Ins2* is regulated by an ''imprinting-control region'' downstream of *Igf2*, close to a maternally expressed imprinted gene (*H19*) that produces a noncoding RNA. At the distal side of the cluster, the *Cdkn1c* gene (also called *p57Kip2*) codes for a cyclin-dependent kinase inhibitor. This imprinted gene is expressed from the maternal allele only. When *Cdkn1c* expression is ablated by gene targeting in the mouse, offspring are enhanced in size and also display other similarities to the Beckwith–Wiedemann syndrome in humans. Interestingly, a similar growth phenotype arises as a consequence of *Igf2* overexpression in mice. One role of CDKN1C could therefore be to inhibit the growth-promoting action of IGF2. Thus, several genes in the imprinted gene cluster are involved in the regulation of fetal growth and seem part of the same signaling pathway.

Four of the other imprinted genes at the distal 7 cluster display allelic expression in the extraembryonic tissues, in which they are expressed from the maternal allele. One of these, *Ascl2* (also named *Mash2*) encodes a transcription factor that is essential for placental development. Genetic studies

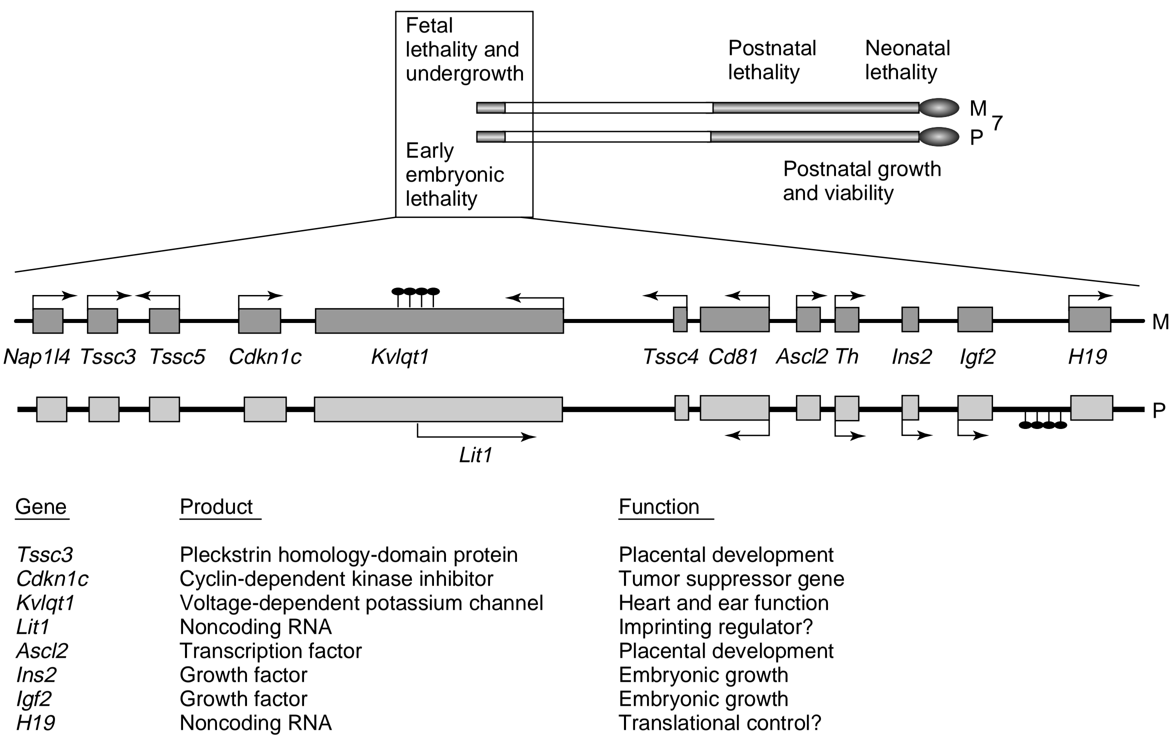

Fig. 4 The imprinted domain on distal mouse chromosome 7 (human chromosome 11q15). Shown are the imprinted genes and their known functions. Lollypops indicate the allele-specific DNA methylation at the two imprinting-control regions.

show that the imprinting of the four extraembryonic genes, and that of *Cdkn1c*, is regulated by a second "imprinting-control region," which is located in the central portion of the cluster.

Amongst the imprinted genes that influence the IGF/INS pathway, there is also the IGF2-receptor gene (*Igf2r*) on mouse chromosome 17. *Igf2r* is expressed exclusively from the maternal allele and exerts a negative effect on growth by reducing the levels of active IGF2. Whereas most imprinted mouse genes are imprinted in humans as well, *Igf2r* is one of the exceptions. In humans, this gene is expressed from both the parental alleles.

Some chromosomal domains comprise imprinted genes that are expressed predominantly in the brain. One of these clusters maps to the central portion of mouse chromosome 7 (and to human chromosome 15q11–q13) and comprises a large number of genes that are all expressed from the paternal chromosome only (Fig. 5c). In humans, loss of expression at these genes (*SNRPN, ZNF127, NDN*, and others) leads to the Prader–Willi syndrome, a variable disorder that is partly due to a hypothalamic defect (see below). Biallelic expression of the genes and loss of expression of a neighboring imprinted gene (*UBE3A*) is associated with the clinically distinct Angelman syndrome (AS). The regulation of imprinting in this domain is complex and involves at least two distinct genetic elements.

A minority of imprinted genes are not part of an imprinted gene cluster. One of these is the U2af1-related sequence-1 gene (*U2af1-rs1*) on proximal mouse chromosome 11. This intronless gene is repressed on the maternal chromosome and encodes a brain-specific RNA splicing factor homologous to the splicing factor U2AF. The imprinted *U2af1-rs1* gene has arisen via a retrotransposition event in rodents, and in humans there is no equivalent imprinted gene.

3 Molecular Mechanisms

3.1 Imprinting-control Regions

The expression of imprinted genes is regulated by epigenetic modifications that mark the parental alleles to be active or repressed. These epigenetic modifications are put onto key regulatory elements, depending on the parental origin of the allele, and lead to the allelic gene expression.

At all imprinted loci, there are sequence elements at which DNA methylation is present on one of the two parental alleles only. At many of these "differentially methylated regions" (DMRs), the DNA methylation originates from either the egg or the sperm. After fertilization, the allelic methylation is maintained in the somatic cells. Regions with such a germ line methylation mark are essential in the control of imprinting. They are referred to as *imprinting-control regions.* Most imprinting-control regions are rich in CpG dinucleotides and correspond to CpG islands.

At the imprinted *U2af1-rs1* gene, DNA methylation is present exclusively on the repressed maternal allele (Fig. 5a). This differential DNA methylation becomes established during oogenesis along its CpG island, located at the 5′ side of the gene, and spreads throughout the entire maternal gene during early embryonic development.

The maternally expressed *Igf2r* gene has an imprinting-control region within the second intron that is methylated on the

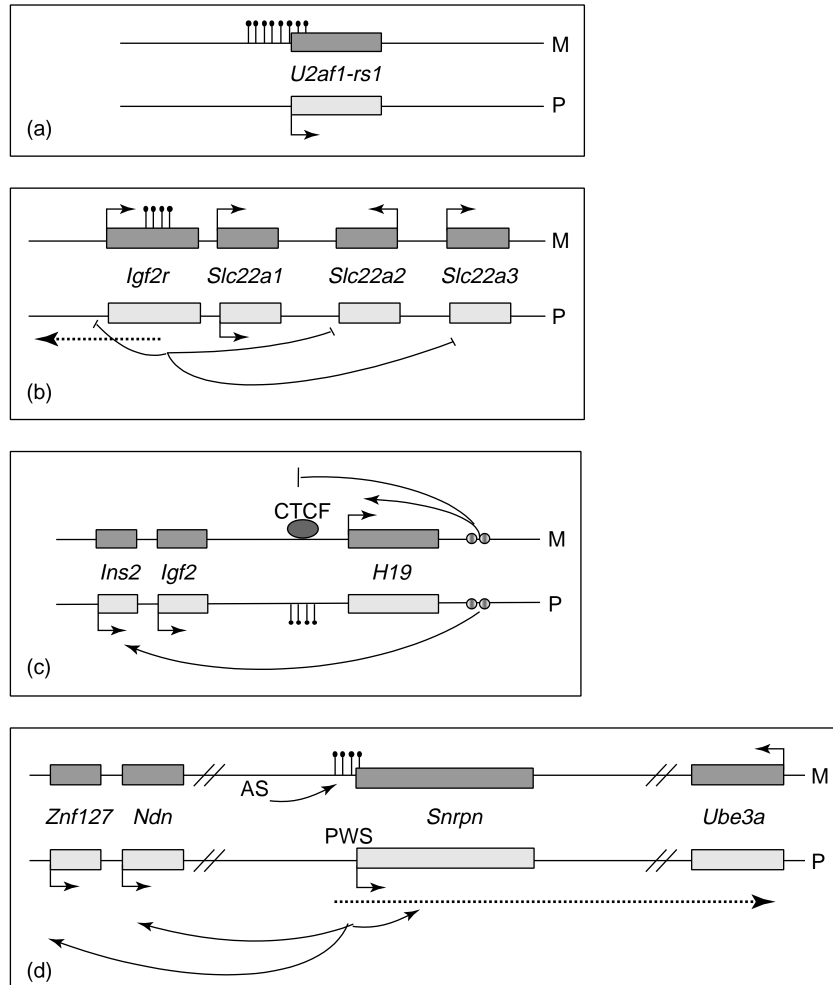

Fig. 5 Reading the imprint. Imprinting-control regions confer allelic gene expression at the (a) *U2af1-rs1* gene, (b) the Igf2r locus, (c) the imprinted cluster containing the *Igf2* gene, and (d) at the PWS/AS region in the mouse. Lollypops indicate the allele-specific DNA methylation at the imprinting-control regions. Antisense transcripts are shown as interrupted lines; circles indicate transcriptional enhancers.

maternal allele (Fig. 5b). This maternal methylation is established during oogenesis and is maintained in all the somatic lineages. The intronic imprinting-control region is essential for the allelic repression at the locus: removal by gene targeting leads to expression from both the parental alleles.

Most imprinting-control regions are methylated on the maternal allele. However, in some, the DNA methylation is found at the paternal allele, and it is the maternal allele that is unmethylated. One of these paternal methylation marks controls the allelic expression of the *Igf2* and *Ins2* genes on distal mouse chromosome 7

(Fig. 5c). This region, a CpG island located upstream of the close-by *H19* gene, acquires its DNA methylation during spermatogenesis. After fertilization, this paternal mark is maintained in all the somatic tissues. Deletion of the control region gives rise to biallelic expression of *Igf2* and *Ins2*.

It is unclear why imprinting-control regions attract DNA methylation in either the female or the male germ line. Several studies suggest, however, that close-by tandemly-repeated sequences might be essential in this choice process.

More is known about the DNA methyltransferases (DNMT) that are involved in the germ line establishment of the methylation marks. The methyltransferases DNMT3A and DNMT3B are essential in this process. In addition, a DNMT-like protein (DNMT3L) is required for the establishment of methylation imprints as well, particularly in the female germ line. Once established, allelic patterns of DNA methylation need to be maintained in the developing embryo. The maintenance methyltransferase, DNMT1, plays an important role in this process and differential chromatin features are likely to be involved as well. At imprinting-control regions, pronounced differences in histone modifications have been detected between the parental alleles. Levels of histone acetylation are low on the allele that comprises methylated DNA, whereas high levels of acetylation are present on the chromatin of the opposite, unmethylated, allele. In addition, there are strong allelic differences in histone methylation at specific lysine residues on histone H3. Whereas methylation of lysine residue 9 of H3 is detected on the parental allele that has DNA methylation, it is on the opposite parental allele (without DNA methylation) that there are high levels of H3 lysine-4 methylation. At several imprinted loci, there is also evidence for allele-specific chromatin compaction, occurring in association with the differential histone modifications.

It is not yet understood how DNA methylation, histone acetylation, and histone methylation are mechanistically linked at imprinting-control regions. However, at several imprinting-control regions, (allelic) DNA methylation was found to be linked to histone deacetylation (the removal of the acetyl group from the histones). This link is brought about by proteins that bind the methylated DNA and attract large protein complexes that comprise histone deacetylases. It is to be explored also to which extent the differential histone modifications are important in the somatic maintenance (and germ line establishment) of the allelic patterns of DNA methylation at imprinted loci. Nonhistone proteins, binding to the unmethylated allele of many imprinting-control regions, are likely to be involved in the maintenance of the allelic DNA methylation as well.

3.2
Reading the Imprint

Imprinting-control regions are comparable in that they all have allele-specific DNA methylation and differential chromatin organization. The way in which this gives rise to imprinted gene expression differs between loci. The simplest scenario, whereby differential methylation and associated chromatin features lead to imprinted gene expression, is that of the *U2af1-rs1* gene on mouse chromosome 11 (Fig. 5a). Here, methylated DNA and compacted chromatin are present across the promoter on the maternal allele. As a consequence, the gene can be transcribed from the paternal allele only.

The imprinting-control region of *Igf2r* regulates allelic expression in a rather different way (Fig. 5b). Here, the maternal methylation covers the promoter of an antisense transcript. As a consequence, this antisense transcript (named *Air*) is produced from the (unmethylated) paternal allele only. Via a yet-unclear mechanism, this paternal antisense transcript represses the paternal *Igf2r* gene and two flanking ion-transporter genes (*Slc22a2* and *Slc22a3*). A similar antisense transcript is produced at the imprinting-control region that regulates the extraembryonic tissue-specific imprinted genes on distal mouse chromosome 7 (Fig. 3).

Another example of how a germ line mark conveys imprinted expression is provided by the *Ins2-Igf2-H19* locus on distal mouse chromosome 7 (Fig. 5c). Here, the imprinting-control region located upstream of the noncoding *H19* gene, is methylated on the paternal allele and acts as a chromatin boundary on the unmethylated maternal allele. In fact, this upstream element has multiple recognition sites for a zinc finger protein called CTCF. The binding of CTCF is prevented by methylation. This chromatin protein is therefore associated with the unmethylated maternal allele only, at which it forms a specialized chromatin structure. This unusual structure insulates the *Igf2* and *Ins2* promoters from their enhancers (located downstream of *H19*). As a consequence, *Igf2* and *Ins2* are repressed on the maternal chromosome. This maternal repression is not exclusively at the transcriptional level but influences posttranscriptional processes as well.

The central portion of mouse chromosome 7 (Fig. 5d) corresponds to the Prader–Willi syndrome (PWS) region and Angelman syndrome (AS) region on human chromosome 15q11–13. The key regulatory element in this domain is the 5′ portion of the *SNRPN* gene and it is methylated on the maternal chromosome. This imprinting-control region is essential for the paternal expression of *SNRPN* and its flanking genes (including *Znf127* and *Ndn*). When the element is deleted on the paternal chromosome, these brain-specific genes are all no longer expressed. Upstream of the *SNRPN* gene, a paternal RNA of several hundreds of kilobases in size is produced as well. This transcript is in antisense orientation to a gene at the far extremity of the imprinted domain. This gene, *UBE3A*, is the only one in the cluster that is repressed on the paternal chromosome. The *SNRPN* imprinting-control region itself is regulated by a second control region, which is located further upstream, and is essential for the acquisition of the allelic DNA methylation at *SNRPN*. Precisely how the allelic expression and repression is brought about along thousands of kilobases remains to be unraveled. It has been observed, however, that there is differential timing of replication in the S-phase between the parental chromosomes along the entire domain. Such a differential replication timing has been detected at other imprinted loci as well. Future work should investigate the role of the differential replication timing and whether it reflects differential chromatin organization along entire imprinted domains.

4
Imprinting and Disease

In many genetic diseases, the clinical manifestations depend on whether the mutation is inherited from the mother or the father. Although imprinting is suspected to be involved, causal genes and molecular mechanisms are yet to

be identified for most of these disorders. Clinical phenotypes can be associated with uniparental disomies as well, similarly as in the mouse. Additionally, imprinting can become deregulated during embryonic development, by epigenetic alterations or by somatic mutations, resulting in loss or biallelic expression of imprinted genes. Such somatic loss of imprinting can result in specific disease phenotypes as well.

Beckwith–Wiedemann syndrome (BWS) is a fetal overgrowth syndrome with a high incidence of embryonal tumors, including Wilms' tumor of the kidney and rhabdomyosarcoma. Genetically, the syndrome is linked to the cluster of growth-related imprinted genes comprising *IGF2* (see Fig. 4). Paternal disomy of this domain is responsible for a proportion of cases and leads to a double dose of *IGF2* expression and loss of expression of *CDKN1C* and other maternally expressed genes in the cluster. BWS can also be caused by genetic mutations at *CDKN1C* and by alterations at the *KVLQT1* gene, where there is one of the two imprinting-control centers of the cluster. The finding that the growth syndrome can be caused by mutations at different places in the imprinted domain supports the idea that its genes are coregulated and involved in the same biological functions. The majority of the BWS cases are sporadic, however, and apparently without genetic mutations. These are mostly caused by epigenetic alterations in the developing embryo. In some of the sporadic cases, for instance, there is aberrant, biallelic methylation at the imprinting-control region at the *H19* gene (Fig. 5c). This results in expression of *IGF2* from both the parental chromosomes during development and therefore in fetal overgrowth.

The neurobehavioral Angelman syndrome (AS) includes mental retardation, ataxia, and hyperactivity and arises from maternal deletion or paternal disomy of the imprinted domain on chromosome 15q11–13. Prader–Willi syndrome (PWS), on the other hand, arises from paternal deletion or maternal disomy of this imprinted domain. This opposite syndrome involves mild mental retardation, obesity due to hyperphagia, and hypogonadism. Cases with small genetic deletions have been identified, and analysis of these patients has revealed that the PWS and AS are caused by distinct regions in the large imprinted domain (Fig. 5d). The smallest identified deletions in PWS remove the imprinting element at the 5′ portion of *SNRPN*. This gives loss of expression of *SNRPN, NDN, ZNF127,* and several other genes in the cluster. The smallest deletions in AS removes the control region that is essential for the establishment of the epigenetic imprint at *SNRPN*. Consequently, there is expression of *SNRPN, NDN*, and *ZNF127* from both the parental alleles and loss of expression of the *UBE3A* gene located at the end of the cluster. The latter seems to be the main cause of the clinical phenotype of AS.

Amongst other imprinting disorders are Albright Hereditary Osteodystrophy (AHO), linked to a cluster of imprinted neuroendocrinal genes on human chromosome 20q, and transient neonatal diabetes mellitus (TNDM), linked to chromosome 6q24–25. The latter is mostly sporadic and is caused by aberrant expression of the imprinted gene *ZAC*. This zinc finger protein–encoding gene has a CpG island with maternal DNA methylation. It was discovered recently that in cases of TNDM without genetic defects, this imprinting-control region has lost its methylation.

Epigenetic alterations at imprinting-control regions occur frequently in tumors

as well. This has been observed in Wilms' tumor of the kidney, but also in lung cancer, breast cancer, and various other cancers. In particular, *IGF2* was found to be expressed from both the parental alleles during tumorigenesis and this could confer a proliferative advantage to the cells. In many cases, the biallelic *IGF2* expression is caused by acquisition of DNA methylation at the imprinting-control region upstream of *H19*, similarly as in BWS. This epigenetic alteration occurs early in tumor formation and could be linked to the pathological tendency of tumorigenic cells to acquire methylation at CpG islands.

When early embryos are taken from their natural environment and put into a culture dish, it can lead to aberrant imprinting as well. This was observed in the mouse and in domestic animals. It is unclear, at present, whether loss of imprinting due to embryo culture is mechanistically comparable to that in human imprinting disorders or in tumors. However, culture of embryos and early embryonic cells can also induce aberrant DNA methylation at imprinting-control regions. This results in biallelic, or loss of, imprinted gene expression and can have pronounced phenotypic consequences at later developmental stages. An important issue to be investigated is whether there are culture conditions that do not affect imprinting and would thus be best suitable for *in vitro* culture and manipulation procedures in animals and humans.

5 Evolution of Imprinting

There is a lot of interest in how broadly imprinting is conserved amongst mammals. Also, in species other than the mouse, both the parental genomes are essential for normal development. Parthenogenesis, for instance, leads to embryonic lethality in humans, pigs, and sheep. In the latter (ruminant) species, parthenogenetic conceptuses die shortly after implantation, due to deficient development and functioning of the extraembryonic membranes. These studies indicate that imprinting is conserved amongst different groups of mammals. Indeed, most of the known imprinted mouse genes are also imprinted in humans and, as far as this has been analyzed, in other placental mammals as well.

Evolutionary biologists have proposed several hypotheses to explain why imprinting has arisen in placental mammals and to account for the different imprinting-related phenotypes. In placental mammals, there is continuous transfer of nutrients from the mother animal to the developing offspring, and this determines their development and growth. Possibly, the most attractive theory of imprinting says that paternally inherited genes tend to increase nutrient transfer and thereby the growth of the developing fetus. This would enhance their chances of being propagated to future generations. Maternally derived genes, however, would be best propagated by limiting the growth of the developing fetus. This is because too high a burden of nutrient transfer compromises the reproductive success of the mother animal and hence of all its offspring. During the evolution of placental mammals, there would therefore have been a 'parental tug-of-war' between these opposing maternal and paternal strategies, leading to balanced combinations of expression levels of maternally and paternally derived genes. As outlined with different examples, imprinted genes such as *Igf2*, *Ins2*, and *Igf2r* indeed play important roles in nutrient

transfer and growth, for instance, by promoting or reducing the development of the extraembryonic tissues. Other imprinted genes are important in determining the activity of the newborn animals, which, again, could have an impact on nutrient transfer, but now after birth.

See also Molecular Basis of Genetics.

Bibliography

Books and Reviews

Beechey, C.V., Cattanach, B.M.C., Selley, R.L. (2002) MRC Mammalian Genetics Unit, Harwell, United Kingdom. World Wide Web site – Mouse imprinting data and references (http://www.mgu.har.mrc.ac.uk/imprinting/imptables.html).

Feil, R., Khosla, S. (1999) Genomic imprinting in mammals: an interplay between chromatin and DNA methylation? *Trends Genet.* **15**, 431–435.

Lee, J.T. (2003) Molecular links between X-inactivation and autosomal imprinting: X inactivation as the driving force for the evolution of imprinting? *Curr. Biol.* **13**, R242–R254.

Ohlsson, R., Tycko, B., Sapienza, C. (1998) Mono-allelic expression: 'there can only be one', *Trends Genet.* **14**, 435–438.

Reik, W., Walter, J. (2001) Genomic imprinting: parental influence on the genome, *Nat. Rev. Genet.* **2**, 21–32.

Sleutels, F., Barlow, D.P., Lyle, R. (2000) The uniqueness of the imprinting mechanism, *Curr. Opin. Genet. Dev.* **10**, 229–233.

Surani, M.A. (1998) Imprinting and the initiation of gene silencing in the germ line, *Cell* **93**, 309–312.

Tilghman, S.M. (1999) The sins of the fathers and mothers: genomic imprinting in mammalian development, *Cell* **96**, 185–193.

Wilkins, J.F., Haig, D. (2003) What good is genomic imprinting? *Nat. Rev. Genet.* **4**, 359–368.

Primary Literature

Barlow, D.P., Stöger, R., Herrmann, B.G., Saito, K., Schweifer, N. (1991) The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the *Tme* locus, *Nature* **349**, 84–87.

Bartolomei, M.S., Zemel, S., Tilghman, S.M. (1991) Parental imprinting of the mouse *H19* gene, *Nature* **351**, 153–155.

Bourc'his, D., Xu, G.L., Lin, C.S., Bollman, B., Bestor, T.H. (2001). *Dnmt3l* and the establishment of maternal genomic imprints, *Science* **294**, 2536–2539.

Brandeis, M., Kafri, T., Ariel, M., Chaillet, J.R., McCarrey, J., Razin, A., Cedar, H. (1993) The ontogeny of allele-specific methylation associated with imprinted genes in the mouse, *EMBO J.* **12**, 3669–3677.

Buiting, K., Saitoh, S., Gross, S., Dittrich, B., Schwartz, S., Nicholls, R.D., Horsthemke, B. (1995) Inherited microdeletions in the Angelman and Prader-Willi syndromes define an imprinting centre on human chromosome 15, *Nat. Genet.* **9**, 395–400.

Cattanach, B.M., Kirk, M. (1985) Differential activity of maternally and paternal derived chromosome regions in mice, *Nature* **315**, 496–498.

Cavaillé, J., Seitz, H., Paulsen, M., Ferguson-Smith, A.C., Bachellerie, J.P. (2002) Identification of tandemly-repeated C/D snoRNA genes at the imprinted human 14q32 domain reminiscent of those at the Prader-Willi/Angelman syndrome region, *Hum. Mol. Genet.* **11**, 1527–1538.

Chaillet, J.R., Vogt, T.F., Beier, D.R., Leder, P. (1991) Parental-specific methylation of an imprinted transgene is established during gametogenesis and progressively changes during embryogenesis, *Cell* **66**, 77–83.

Charlier, C., Segers, K., Karim, L., Shay, T., Gyapay, G., Cockett, N., Georges, M. (2001) The callipyge mutation enhances the expression of coregulated imprinted genes in cis without affecting their imprinting status, *Nat. Genet.* **27**, 367–369.

Cockett, N.E., Jackson, S.P., Shay, T.L., Farnir, F., Berghmans, S., Showder, G.D., Nielsen, D.M., Georges, M. (1996) Polar overdominance at the ovine callipyge locus, *Science* **273**, 236–238.

Constancia, M., Hemberger, M., Hughes, J., Dean, W., Ferguson-Smith, A., Fundele, R.,

Stewart, F., Kelsey, G., Fowden, A., Sibley, C., Reik, W. (2002) Placental-specific IGF-II is a major modulator of placental and fetal growth, *Nature* **417**, 945–948.

Cui, H., Cruz-Correa, M., Giardello, F.M., Hutcheon, D.F., Kafenok, D.R., Brandenburg, S., Wu, Y., He, X., Powe, N.R., Feinberg, A.P. (2003) Loss of IGF2 imprinting: a potential marker of colorectal cancer risk, *Science* **299**, 1753–1755.

Dean, W., Bowden, L., Aitchison, A., Klose, J., Moore, T., Meneses, J.J., Reik, W., Feil, R. (1998) Altered imprinted gene methylation and expression in completely ES cell-derived mouse fetuses: association with aberrant phenotypes, *Development* **125**, 2273–2282.

Debaun, M.R., Niemitz, E.L., Feinberg, A.P. (2003) Association of in vitro fertilization and Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19, *Am. J. Hum. Genet.* **72**, 156–160.

DeChiara, T.M., Robertson, E.J., Efstratiadis, A. (1991) Parental imprinting of the mouse insulin-like growth factor II, *Cell* **64**, 849–859.

Doherty, A.S., Mann, M.R., Tremblay, K.D., Bartolomei, M.S., Schultz, R.M. (2000) Differential effects of culture on imprinted *H19* expression in the pre-implantation embryo, *Biol. Reprod.* **62**, 1526–1535.

Feil, R., Walter, J., Allen, N.D., Reik, W. (1994) Developmental control of allelic methylation in the imprinted mouse *Igf2* and *H19* genes, *Development* **120**, 2933–2943.

Feil, R., Boyano, M.D., Allen, N.D., Kelsey, G. (1997) Parental chromosome-specific chromatin conformation in the imprinted *U2af1-rs1* gene in the mouse, *J. Biol. Chem.* **272**, 20893–20900.

Ferguson-Smith, A.C., Cattanach, B.M., Barton, S.C., Beechey, C.V., Surani, M.A. (1991) Embryological and molecular investigations of parental imprinting on mouse chromosome 7, *Nature* **352**, 609–610.

Ferguson-Smith, A.C., Sasaki, H., Cattanach, B.M., Surani, M.A. (1993) Parent-origin-specific epigenetic modification of the mouse *H19* gene, *Nature* **362**, 751–755.

Fitzpatrick, G.V., Soloway, P.D., Higgins, M.J. (2002) The brain on microarrays, *Nat. Genet.* **32**, 426–431.

Fournier, C., Goto, Y., Ballestar, E., Delaval, K., Hever, A.M., Esteller, M., Feil, R. (2002) Allele-specific histone lysine methylation marks regulatory regions at imprinted mouse genes, *EMBO J.* **23**, 6560–6570.

Gregory, R.I., Randall, T.E., Johnson, C.A., Khosla, S., Hatada, I., O'Neill, L.P., Turner, B.M., Feil, R. (2001) DNA methylation is linked to deacetylation of histone H3, but not H4, on the imprinted genes *Snrpn* and *U2af1-rs1*, *Mol. Cell. Biol.* **21**, 5426–5436.

Gunaratne, P.H., Nakao, M., Ledbetter, D.H., Sutcliffe, J.S., Chinault, A.C. (1995) Tissue-specific and allele-specific replication timing control in the imprinted human Prader-Willi syndrome region, *Genes Dev.* **9**, 808–820.

Hajkova, P., Erhardt, S., Lane, N., Haaf, T., El Maarri, O., Reik, W., Walter, J., Surani, A. (2002) Epigenetic reprogramming in mouse primordial germ cells, *Mech. Dev.* **117**, 15–23.

Hata, K., Okano, M., Lei, H., Li, E. (2002) Dnmt-3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice, *Development* **129**, 1983–1993.

Hatada, I., Nabetani, A., Arai, Y., Ohishi, S., Suzuki, M., Miyabara, S., Nishimune, Y., Mukai, T. (1997) Aberrant methylation of an imprinted gene *U2af1-rs1* (SP2) caused by its own transgene, *J. Biol. Chem.* **272**, 9120–9122.

Howell, C.Y., Bestor, T.H., Ding, F., Latham, K.E., Mertineit, C., Trasler, J.M., Chaillet, J.R. (2001) Genomic imprinting disrupted by a maternal effect mutation in the *Dnmt1* gene, *Cell* **104**, 829–838.

Humpherys, D., Eggan, K., Akutsu, H., Hochedlinger, K., Rideout, W.M., Biniszkiewcz, D., Yanagimachi, R., Jaenisch, R. (2001) Epigenetic instability in ES cells and cloned mice, *Science* **293**, 95–97.

Inoue, K., Kohda, T., Lee, J., Ogonuki, N., Mochida, K., Noguchi, Y., Tanemura, K., Kaneko-Ishino, T., Ishino, F., Ogura, A. (2002) Faithful expression of imprinted genes in cloned mice, *Science* **295**, 297.

Izumikawa, Y., Naritoma, K., Hariyama, K. (1991) Replication asynchrony between homologs 15q11.2: cytogenetic evidence for genomic imprinting, *Hum. Genet.* **87**, 1–5.

Jouvenot, Y., Poirier, F., Jami, J., Paldi, A. (1999) Bi-allelic transcription of *Igf2* and *H19* in individual cells suggests a post-transcriptional contribution to genomic imprinting, *Curr. Biol.* **9**, 1199–1202.

Judson, H., Hayward, B.E., Sheridan, E., Bonthron, D.T. (2002) A global disorder of imprinting in the human female germ line, *Nature* **416**, 539–542.

Khosla, S., Aitchison, A., Gregory, R., Feil, R. (1999) Parental allele-specific chromatin configuration in an insulator/imprinting control element upstream of the mouse *H19* gene, *Mol. Cell. Biol.* **19**, 2556–2566.

Khosla, S., Dean, W., Brown, D., Reik, W., Feil, R. (2001) Culture of pre-implantation mouse embryos affects fetal development and the expression of imprinted genes, *Biol. Reprod.* **64**, 918–926.

Killian, J.K., Byrd, J.C., Jirtle, J.V., Munday, B.L., Stoskopf, M.K., MacDonald, R.G., Jirtle, R.L. (2000) M6P/IGF2R imprinting evolution in mammals, *Mol. Cells* **5**, 707–716.

Kitsberg, D., Selig, S., Brandeis, M., Simon, I., Keshet, I., Driscoll, D.J., Nicholls, R.D., Cedar, H. (1993) Allele-specific replication timing of imprinted gene regions, *Nature* **364**, 459–463.

Knoll, J.H., Cheng, S.D., Lalande, M. (1994) Allele specificity of DNA replication timing in the Angelman/Prader-Willi syndrome imprinted chromosomal region, *Nat. Genet.* **6**, 41–46.

Lee, J., Inoue, K., Ono, R., Ogonuki, N., Kohda, T., Kaneko-Ishino, T., Ogur, S., Ishino, F. (2002) Erasing genomic imprinting memory in mouse clone embryos produced from day 11.5 primordial germ cells, *Development* **129**, 1807–1817.

Li, E., Beard, C., Jaenisch, R. (1993) Role for DNA methylation in genomic imprinting, *Nature* **366**, 362–365.

Mager, J., Montgomery, N.D., de Villena, F.P., Magnuson, T. (2003) Genome imprinting regulated by the mouse Polycomb group protein Eed, *Nat. Genet.* **33**, 502–507.

McGrath, J., Solter, D. (1984) Completion of mouse embryogenesis requires both the maternal and paternal genomes, *Cell* **37**, 179–183.

Milligan, L., Forné, T., Antoine, E., Weber, M., Hémonnot, B., Dandolo, L., Brunel, C., Cathala, G. (2002) Turnover of primary transcripts is a major step in the regulation of mouse *H19* gene expression, *EMBO Rep.* **3**, 774–779.

Muscatelli, F., Abrous, D.N., Massacrier, A., Boccaccio, I., Le Moal, M., Cau, P., Cremer, H. (2000) Disruption of the mouse *Necdin* gene results in hypothalamic and behavioral alterations reminiscent of the human Prader-Willi syndrome, *Hum. Mol. Genet.* **12**, 3101–3110.

Nabetani, A., Hatada, I., Morisaki, H., Oshimura, M., Mukai, T. (1997) Mouse *U2af1-rs1* is a neomorphic imprinted gene, *Mol. Cell. Biol.* **17**, 789–798.

Paulsen, M., El-Maarri, O., Engemann, S., Strodicke, M., Franck, O., Davies, K., Reinhardt, R., Reik, W., Walter, J. (2000) Sequence conservation and variability of imprinting in the Beckwith-Wiedemann syndrome cluster in human and mouse, *Hum. Mol. Genet.* **9**, 1829–1841.

Pant, V., Mariano, P., Kanduri, C., Mattson, A., Lobanenkov, V., Heuchel, R., Ohlsson, R. (2003) The nucleotides responsible for the direct physical contact between the chromatin insulator protein CTCF and the *H19* imprinting control region manifest parent of origin-specific long-distance insulation and methylation-free domains, *Genes Dev.* **17**, 586–590.

Perk, J., Makedonski, K., Lande, L., Cedar, H., Razin, A. (2002) The imprinting mechanism of the Prader-Willi/Angelman regional control center, *EMBO J.* **21**, 5807–5814.

Rainier, S., Johnson, L.A., Dobry, C.J., Ping, A.J., Grundy, P.E., Feinberg, A.P. (1993) Relaxation of imprinted genes in human cancer, *Nature* **362**, 747–749.

Reik, W., Brown, K.W., Schneid, H., Le Bouc, Y., Bickmore, W., Maher, E.R. (1995) Imprinting mutations in the Beckwith-Wiedemann syndrome suggested my altered imprinting patterns in the IGF2-H19 domain, *Hum Mol. Genet.* **4**, 2379–2385.

Reik, W., Collick, A., Norris, M.L., Barton, S.C., Surani, M.A. (1987) Genomic imprinting determines methylation of parental alleles in transgenic mice, *Nature* **328**, 248–251.

Ripoche, M.A., Kress, C., Poirier, F., Dandolo, L. (1997) Deletion of the *H19* transcription unit reveals the existence of a putative imprinting control element, *Genes Dev.* **11**, 1596–1604.

Sasaki, H., Jones, P.A., Chaillet, J.R., Ferguson-Smith, A.C., Barton, S.C., Reik, W., Surani, M.A. (1992) Parental imprinting: potentially active chromatin of the repressed maternal allele of the mouse insulin-like growth factor II (Igf2) gene, *Genes Dev.* **6**, 1843–1856.

Schoenherr, C.J., Levorse, J.M., Tilghman, S.M. (2002). CTCF maintains differential methylation at the *Igf2/H19* locus, *Nat. Genet.* **33**, 66–69.

Simon, I., Tenzen, T., Reubinoff, B.E., Hillman, D., McCarrey, J.R., Cedar, H. (1999) Asynchronous replication of imprinted genes is established in the gametes and maintained during development, *Nature* **401**, 929–932.

Sleutels, F., Zwart, R., Barlow, D.P. (2002) The non-coding Air RNA is required for silencing autosomal imprinted genes, *Nature* **415**, 810–813.

Smith, R.J., Dean, W., Konfortova, G., Kelsey, G. (2003) Identification of novel imprinted genes in a genome-wide screen for maternal methylation, *Genome Res.* **13**, 558–569.

Stöger, R., Kubicka, P., Liu, C.G., Kafri, T., Razin, A., Cedar, A., Barlow, D. (1993). Maternal-specific methylation of the imprinted mouse *Igf2r* locus identifies the expressed locus as carrying the imprinting signal, *Cell* **73**, 61–71.

Strain, L., Warner, J.P., Johnston, T., Bonthron, D.T. (1995) A human parthenogenetic chimaera, *Nat. Genet.* **11**, 111–113.

Surani, M.A.H., Barton, S.C., Norris, M.L. (1984) Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis, *Nature* **308**, 548–550.

Szabo, P., Tang, S.H., Rentsendorj, A., Pfeifer, G.P., Mann, J.R. (2000) Maternal-specific footprints at putative CTCF sites in the *H19* imprinting-control region give evidence for insulator function, *Curr. Biol.* **10**, 607–610.

Swart, R., Sleutels, F., Wutz, A., Schinkel, A.H., Barlow, D.P. (2001) Bidirectional action of the *Igf2r* imprint control element on upstream and downstream imprinted genes, *Genes Dev.* **15**, 2361–2366.

Takagi, N., Sasaki, M. (1975) Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes of the mouse, *Nature* **256**, 640–642.

Tucker K.L., Beard C., Bausmann J., Jackson-Grusby L., Laird, P.W., Lei, H., Li, E., Jaenisch, R. (1996) Germ-line passage is required for establishment of methylation and expression patterns of imprinted but not nonimprinted genes, *Genes Dev.* **10**, 1008–1020.

Varrault, A., Bilanges, B., Mackay, D.J., Basyuk, E., Ahr, B., Fernandez, C., Robinson, D.O., Bockaert, J., Journot, L. (2001) Characterization of the methylation-sensitive promoter of the imprinted ZAC gene supports its role in transient neonatal diabetes mellitus, *J. Biol. Chem.* **276**, 18653–18656.

Webber, A.L., Ingham, R.S., Levorse, J.M., Tilghman, S.M. (1998) Location of enhancers is essential for the imprinting of H19 and Igf2 genes, *Nature* **391**, 711–715.

Weksberg, R., Shen, D.R., Fei, Y.L., Song, Q.L., Squire, J. (1993) Disruption of insulin-like growth factor 2 imprinting in Beckwith-Wiedemann syndrome, *Nat. Genet.* **5**, 143–150.

Xin, Z., Allis, C.D., Wagstaff, J. (2001) Parent-specific complementary patterns of histone H3 lysine 9 and H3 lysine 4 methylation at the Prader-Willi syndrome imprinting center, *Am. J. Hum. Genet.* **69**, 1389–1394.

Yokomine, T., Kuroiwa, A., Tanaka, K., Tsudzuki, M., Matsuda, Y., Sasaki, H. (2001) Sequence polymorphisms, allelic expression status and chromosome localisation of the chicken IGF2 and *MPR1* genes, *Cytogenet. Cell Genet.* **93**, 109–113.

Yoon, B.J., Herman, H., Sikora, A., Smith, L.T., Plass, C., Soloway, P.D. (2002) Regulation of DNA methylation of *Rasgrf1*, *Nat. Genet.* **30**, 92–96.

Young, L.E., Fernandes, K., McEvoy, T.G., Butterwith, S.C., Gutierrez, C.G., Carolan, C., Broadbent, P.J., Robinson, J.J., Wilmut, I., Sinclair, K.D. (2001) Epigenetic change in *IGF2R* is associated with fetal overgrowth after sheep embryo culture, *Nat. Genet.* **27**, 153–154.