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Growth and Behaviour

Epigenetic and Genetic Factors Involved in Hybrid Dysgenesis

WEI SHI

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Abstract
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In mammals, the most frequently observed hybrid dysgenesis effects are growth disturbances and male sterility. Profound defects in placental development have been described and our work on hybrids in genus Mus has demonstrated putative hybrid dysgenesis effects that lead to defects in lipid homeostasis and maternal behavior. Interestingly, mammalian interspecies hybrids exhibit strong parent-of-origin effects in that offspring of reciprocal matings, even though genetically identical, frequently exhibit reciprocal phenotypes. Recent studies have provided strong link between epigenetic regulation and growth, behavior and placental development. Widespread disruption of genomic imprinting has been described in hybrids between closely related species of the genus Peromyscus. The studies presented in this thesis aim to investigate the effects of disrupted epigenetics states on altered growth, female infanticide and placental dysplasia observed in Mus hybrids. We showed that loss-of-imprinting (LOI) of a paternally expressed gene, *Peg1*, was correlated with increased body weight of F1 hybrids. Furthermore, we investigated whether LOI of *Peg1* in F1 females would interfere with maternal behavior. A subset of F1 females indeed exhibited highly abnormal maternal behavior in that they rapidly attacked and killed the pups. By microarray hybridization, a large number of differentially expressed genes in the infanticidal females as compared to normally behaving females were identified. In addtion to *Peg1* LOI, we studied allelic expression of numerous imprinted genes in adult Mus interspecies hybrids. In contrast to the study from Peromyscus, patterns of LOI were not consistent with a direct influence of altered expression levels of imprinted genes on growth. Finally, we investigated the allelic interaction between an X-linked locus and a paternally expressed gene, *Peg3*, in placental defects in Mus hybrids. This study further strengthened the notion that divergent genetic and epigenetic mechanisms may be involved in hybrid dysgenesis in diverse groups of mammals.

Keywords: Interspecies hybridization, hybrid dysgenesis effect, speciation, genomic imprinting, epigenetics

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List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I **Shi W**, Lefebvre L, Yu Y, Otto S, Krella A, Orth A, Fundele R. Loss-of-imprinting of Peg1 in mouse interspecies hybrids is correlated with altered growth. *Genesis* 2004, 39:65-72.

II **Shi W**, Singh U, Yu Y, Fassen J, Broad K, Keverne EB, Fundele R. Female infanticide associated with interspecific hybridization in genus Mus. Manuscript.

III **Shi W**, Krella A, Orth A, Yu Y, Fundele R. Widespread disruption of genomic imprinting in adult interspecies mouse (Mus) hybrids. Submitted.

IV Zechner U, **Shi W**, Hemberger M, Himmelbauer H, Otto S, Orth A, Kalscheuer V, Fischer U, Elango R, Reis A, Vogel W, Ropers H, Rüschendorf F, Fundele R. Divergent genetic and epigenetic post-zygotic isolation mechanisms in Mus and Peromyscus. *Journal of Evolutionary Biology* 2004, 17:453-460.

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Abbreviations

AS	Angelman syndrome
BC	Backcross
BWS	Beckwith-Wiedemann syndrome
CNS	Central nervous system
DMR	Differentially methylated region
Dnmt	DNA methyltransferase
F1	Filial 1
IHPD	Interspecific hybrid placental dysplasia
LOI	Loss of imprinting
MMU	*Mus musculus*
MS	F1 from female MMU and male MSP
MSM	Offspring from female MS F1 and male MMU
MSP	*Mus spretus*
MSS	Offspring from female MS F1 and male MSP
Peg1	Paternally expressed gene-1
Peg3	Paternally expressed gene-3
PMA	*Peromyscus maniculatus*
PPO	*Peromyscus polionotus*
PWS	Prader-Willi syndrome
QTL	Quantitative trait locus
RFLV	Restriction fragment length variant
SCNT	Somatic cell nuclear transfer
SM	F1 from female MSP and male MMU
SRS	Silver-Russell syndrome
UPD	Uniparental disomy

Introduction

What are species?

Species are crucial in biological issues. However, biologists have failed to agree on a single species concept. In the 1940's, Ernst Mayr developed the biological species concept that was subsequently widely adopted. This concept states that species are groups of actually or potentially interbreeding natural populations that are reproductively isolated from other groups. Thus, gene flow between different populations should be difficult or impossible, and this eventually leads to speciation (Dobzhansky, 1951; Mayr, 1963). By the late 1970s, several new species concepts were devised, such as the recognition species concept, which is also based on reproductive isolation mechanisms, assuming that members of one species will have the ability to recognize potential mates from the same species but will ignore potential mates from similar but different species. There are the morphological species concept, which identifies species by resemblances, and the phylogenetic species concept, stating that a species is a member of a population that shares a recent common ancestor. We apply the biological species concept in our study.

Mechanisms of reproductive isolation

New species arise as reproductive isolation evolves between divergent populations. If gene flow remains possible, speciation cannot occur. Hence, speciation can be regarded as the evolution of reproductive barriers or barriers to gene flow. The biological barriers that minimize gene flow between the populations are classified into two groups, i.e., pre- and post-zygotic isolation. Prezygotic isolation reduces the frequency at which gametes combine to form a zygote. These barriers can be (1) Ecological: difference in habitat, leading to spatial separation of subpopulations and resulting in allopatric speciation. (2) Temporal: difference in active time, giving rise to allochronic speciation. (3) Behavioral: potential mates meet, but simply do not mate. (4) Gametic: the egg cannot be fertilized by the sperm. When hybridization is costly, selection favors the evolution of prezygotic isolating mechanisms that reduce heterospecific matings. However, when the prezygotic barriers are overcome, postzygotic barriers become apparent, such as hybrid

inviability due to difficulties of finding an appropriate ecological niche or developmental problems, or hybrid fertility resulted from incapability of displaying successful courtship or developmental abnormalities (Gray, 1971; Coyne and Orr, 2004). The resulting phenotypes of interspecies hybridization are called hybrid dysgenesis effects.

Hybrid dysgenesis effects

Interspecies hybridization in mammals

Hybrids between species of the same genus are known as interspecies hybrids. In most cases interspecies hybridization takes place, intentionally and unintentionally, in captivity. Well-known examples of intentionally produced hybrids are the mule and the hinny, which result from matings between female horse and male donkey and between male horse and female donkey, respectively. Because of their improved stamina and intelligence over their parental species, mules and hinnies have been produced by humans for millennia. The following are more examples of interspecies hybrids:

- Cross between *Mus musculus* (MMU) and *M. spretus* (MSP) in genus Mus (model system of our laboratory) (Bonhomme et al., 1978).
- Zeedonk: cross between a zebra and a donkey (Fig. 1).
- Wolfdog: cross between a domestic dog and a wolf.
- Liger: cross between a male lion and a female tiger (Fig. 2).
- Wolphin: cross between a false killer whale and a bottlenose dolphin.

Figure 1. ‘‘Zeedonk’’, a zebra/donkey hybrid (Figure courtesy of http://en.wikipedia.org).

The study of mammalian hybrids, beautifully compiled in the book “Mammalian Hybrids” by A.P. Gray has disclosed several visible effects of hy-

bridization, of which sterility (mostly male) and inviability are the most prominent. Other visible effects are altered growth and abnormal placental development (Gray, 1971; Rogers and Dawson, 1970; Zechner et al., 1996). Interestingly, both growth effects and placental hybrid dysgenesis exhibit parent-of-origin effects, that is, reciprocal phenotypes are observed in reciprocal matings. For example, F1 hybrids obtained from MMU × MSP (female always shown first) are always smaller than F1 hybrids obtained in the reciprocal MSP × MMU cross. Similar phenomena were also observed in hybridization between other mammalian species, such as hybrids between female lions and male tigers, named "ligers". Ligers grow much larger than their parents (Fig. 2). The reciprocal hybrids, named "tigon", tend towards reduced growth as compared to parental species. In the hybrids of many genera, it appears that strong growth enhancement in one hybrid and moderate to negligible growth reduction in the reciprocal hybrid is quite common. However, hybridization between the two closely related rodent species *Peromyscus maniculatus* (PMA) and *P. polionotus* (PPO) results in very strong growth effects in both matings. In contrast to this, no pronounced effects on growth were reported in hybridization in equids and cervids.

Figure 2. On their hind legs, ligers stand approximately 12 feet tall. At the extreme, male ligers may weigh up to 500 kg. In contrast, males of the parental species, tiger and lion, rarely attain weights of more than 250 kg. (Figure courtesy of http://www.greenapple.com/~jorp/amzanim/cross02a.htm).

For all three mammalian groups in which this has been assessed to date, equids, murids, and peromyscids, profound defects in placental development have been described (Rogers and Dawson, 1970; Allen et al. 1993: Zechner et al. 1996). In the rodent groups Peromyscus and Mus, very similar placental phenotypes were demonstrated (Rogers and Dawson, 1970; Zechner et al., 1996). Depending on the direction of the crosses and backcrosses (BCs), in both instances the placental phenotype manifested itself in increased or decreased growth of the placenta and specifically the spongiotrophoblast layer (Fig. 3). In addition, our own work has further demonstrated other two

putative hybrid dysgenesis effects observed in a subset of F1 hybrids between MMU and MSP, obesity (Rizvi et al., in preparation) and maternal behavior (Shi et al., in preparation).

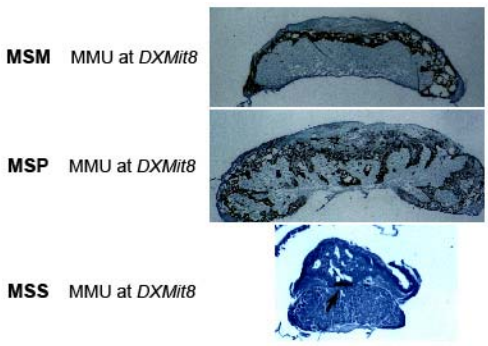

Figure 3. Reciprocal phenotype in hybrid placentas between MMU and MSP, from top to bottom: normal, hyper- and hypoplasitic placentas. The most severely affected tissue is the spongiotrophoblast, which is the darkly stained area indicated by arrow (Figure provided by Dr. Reinald Fundele).

Genetic basis of hybrid dysgenesis

To date the genetic causes of any mammalian hybrid dysgenesis are poorly understood. It is generally assumed that aberrant interactions between independently evolved genes, or rather their products, underlie hybrid dysgenesis effects, as postulated in the Dobzhansky-Muller model (Dobzhansky, 1934; Muller, 1942; also see Fig. 4). In this model, we consider an ancestral species of genotype *AABB*. In one population, an *A* mutation appears and goes to fixation, yielding *aa*BB, which is fertile and viable. In another separate population, a *B* mutation appears and goes to fixation, yielding *AAbb*, which is also fertile and viable. However, when *a* and *b* are brought together in one genome, such as in *AaBb*, the interaction may cause deregulation of downstream effector genes, which in turn causes hybrids incompatibility. Indeed it appears that hybrid sterility and inviability in animals usually evolve as described by this model. To date, several putative hybrid sterility loci (Hst1 - Hst7) in the mouse have been identified, mainly located on chromosome 17 and X (reviewd in Foreijt, 1996). A very strong influence of X-chromosomal loci on placental dysplasia has been demonstrated in hybrids of Mus (Zechner et al. 1996) and Peromyscus (Vrana et al. 2000). For instance, in crosses and backcrosses between MMU and MSP the MSP derived X-chromosome segregated with enlarged placentas (Zechner et al. 1996). Interestingly, many experiments reveal a

disproportionate effect of X-chromosomal genes on hybrid phenotypes. Why this is so is still a puzzle.

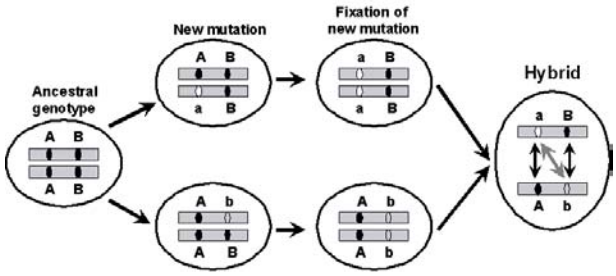

Figure 4. Dobzhansky-Muller model of hybrid incompatibility.

Epigenetics and genomic imprinting

Epigenetics and epigenetic mechanisms

Just as cells inherit genes, they also inherit a set of instructions that tell the genes when to become active, in which tissue and to what extent. Without this ''epigenetic'' instruction manual, multicellular organisms would be impossible.

—— *New Scientist, 1998, 2162: 27*

Epigenetics defines all meiotically or mitotically heritable, but reversible changes in gene expression without alterations in the DNA sequence itself. Epigenetic modifications affect either DNA or proteins that intimately associate with DNA, which in turn affect the local structure and composition of chromatin, thus define and maintain the accessibility and transcriptional competence of the nucleosomal DNA template.

DNA methylation is by far the best studied mechanism among all epigenetic modifications. It takes place at 5'-cytosine residue in a CpG dinucleotide. DNA methylation is widespread among protists, plants, some fungi and animals. However, DNA methylation is absent in both budding and fission yeast and only sparsely present in the nematode *Caenorhabditis elegans* (Lachner and Jenuwein, 2002) and the fruit fly *Drosophila melanogaster* (Lyko et al., 2000). CpG dinucleotides are underrepresented in much of the genome. But CpG islands, which are short regions of 0.5-4 kb in length and with GC content greater than 55%, are rich in CpG content and normally free of methylation. Most CpG islands are found in the proximal region of

almost half of the genes in mammalian genome. DNA methylation within the genome is maintained by a number of cytosine methyltransferases (Dnmts). *Dnmt1* is predominantly involved in maintaining methylation. Targeted disruption of *Dnmt1* resulted in aberrant genome-wide demethylation and mid-gestational lethality of mouse embryos (Li et al., 1992). *Dnmt3a* and *Dnmt3b* have been identified as *de novo* methyltransferases that set up the initial patterns of methylation during embryogenesis. Targeted disruption of *Dnmt3a* and *Dnmt3b* in mice, alone and together, resulted in embryonic lethality (Jaenisch and Bird, 2003). These findings clearly demonstrate that DNA methylation is crucial for appropriate gene expression and normal development.

The nucleosome is the central theme of epigenetic gene regulation. Nucleosome is made up of approximately two turns of DNA wrapped around a histone octamer composed of two copies of each histone H2A, H2B, H3, and H4 and are separated by a short ''linker'' DNA and linker histone H1 (Lund and van Lohuizen, 2004). Distinct modifications of histone N-terminal tails, such as acetylation, methylation, phosphorylation, ubiquitination, ADP-ribosylation and sumolyation, generate synergistic or antagonistic interaction affinities for chromatin-associated proteins, which in turn control dynamic transitions between transcriptionally active and silent states. The combinatorial histone modification thus reveals a hypothesized ''histone code'' that considerably extends the information potential of the genetic code and provides an epigenetic marking system to regulate specific gene expression (Turner, 2000). In general, histone acetylation loosens chromatin packaging and correlates with transcriptional activation, whereas histone deacetylation is associated with repression of transcription. Histone methylation can be a marker for either active or inactive regions of chromatin. Methylation of lysine 9 on the N terminals of histone 3 (H3-K9) is a hallmark of silent DNA and distributes throughout heterochromatic regions such as centromeres and telomeres. In contrast, H3-K4 denotes activity and is found predominantly at promoters of active genes (Lachner and Jenuwein, 2002). DNA methylation and histone modifications can interact with each other through methylcytosine-binding proteins (MBPs) that might recruit histone deacetylases (HDACs) to methylated DNA in regions of transcriptional silencing (Jones and Baylin, 2002).

The role of non-coding RNA, which refers to all RNA transcripts without protein-coding capacity, in processes such as chromatin dynamics and gene silencing has received increasing attention over the past few years (Morey and Avner, 2004). Some of the sense and antisense non-coding RNAs have the capacity to spread in *cis* over long distances. They are also capable to interact with chromatin modifying enzymes. One of the best-characterized regions containing non-coding RNA is the mouse X-inactivation center (Xic). The polycomb-group (PcG) family of proteins function in multimeric complexes and are believed to maintain long-term gene silencing during

development, acting through alterations of local chromatin structure and involving post-translational modification of core histones (Czermin et al., 2002). In *Drosophila melanogaster* and mammals, two members of the PcG family, encoded by enhancer of zeste (E(Z) in D. *melanogaster*; *Ezh2* in mouse) and extra sex combs (*esc* in D. *melanogaster*; *Eed* in mouse), function in the same complex. Both *Eed* and *Ezh2* are important for early embryonic development. *Eed* mutation is responsible for a lethal gastrulation defect with anterior-posterior patterning defects and abnormalities in mesoderm production and localization (Faust et al., 1998). *Ezh2* null mutants also died early and have gastrulation defects (O'Carroll et al., 2001).

Early in the development of female eutherian (placental) mammals, one of the two X chromosomes is transcriptionally inactivated to ensure dosage compensation for X-linked gene products. In mice, X chromosome inactivation is random in the embryonic lineage. By contrast, preferential inactivation of paternal X chromosome occurred in the trophectoderm (Takagi and Sasaki, 1975; West et al., 1977). X-inactivation specific transcript (*Xist*), a non-coding RNA, is expressed only from inactive X chromosome (Xi) and is necessary and sufficient for the initiation of X inactivation (Lee and Jaenisch, 1997). *Xist* expression is controlled by an antisense non-coding RNA *Tsix*. *Xist* up-regulation on the putative Xi and RNA coating of this chromosome will trigger a series of epigenetic modifications, such as H3-K27 and H3-K9 hypermethylation, histone H3/H4 hypoacetylation and polycomb group complex (Morey and Avner, 2004).

Genomic imprinting

Early nuclear transplantation experiments have demonstrated that mouse embryos with only maternal or paternal genomes suffered aberrant growth and lethality (McGrath and Solter 1984; Surani *et al*. 1984). Parthenogenetic (PG) cells contain a complete genome that is exclusively maternally derived, whereas androgenetic (AG) cells contain only paternally derived genome. PG mouse embryos die around mid-gestation, are growth retarded and have poorly developed extra-embryonic tissues, while AG conceptus occasionally reach somite stage of development with well-developed extra-embryonic tissues (McGrath and Solter, 1984; Surani et al., 1984, also see Fig. 5). However, chimeras with PG cells were viable and fertile, though growth-retarded (Fundele et al., 1997). By contrast, chimeras with AG cells were growth-enhanced and exhibited striking overgrowth of the costal cartilage and hypo-ossification of mesoderm-derived bones. These chimeras have an increased contribution of AG cells to mesodermal lineages, notably skeletal muscle (Barton et al., 1991). These results suggested that paternally expressed genes are necessary for the growth of extra-embryonic tissue, whereas maternally expressed genes are important in embryonic development.

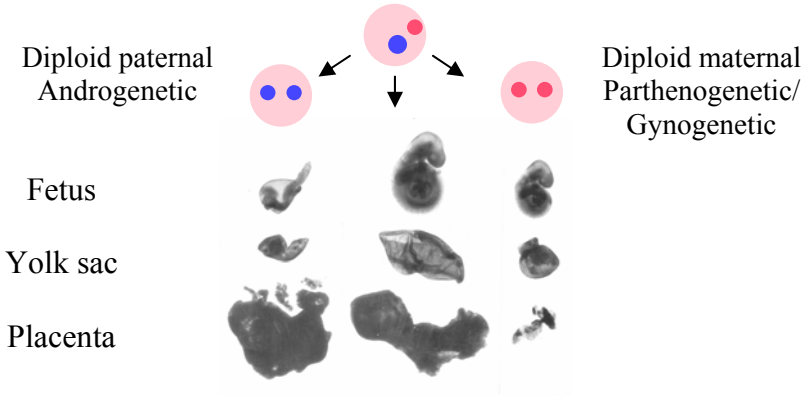

Figure 5. The development of uniparental and wild type embryos at mid-gestation (Figure courtesy of Dr. Azim Surani).

Thus, the maternal and the paternal genomes are both required for normal embryonic and postnatal development. Their functional nonequivalence is due to genomic imprinting, a process by which one allele becomes epigenetically modified and inactivated based on parental origin (McGrath and Solter, 1984; Surani et al., 1984). Genomic imprinting appears to be unique to placental mammals, marsupials and flowering plants. Approximately 70 imprinted genes have been identified in human and mouse (Beechey et al., 2003) and most imprinted genes are involved in fetal growth, placental function and behavior (Fig. 6).

Imprinted genes are often clustered in discrete chromosomal regions, which implicate a long-range mechanism affecting relatively large regions of chromosome. To eliminate any aberrant epigenetic modifications from the previous generation, the existing imprints must be erased and reset at each generation in the germ line. In mice, inherited imprints are largely intact in migrating primordial germ cells (PGCs) (Szabo et al., 2002). The rapid genome-wide demethylation and erasure of imprints in both male and female PGCs occur around the time of entry into the gonadal ridge at e11.5 – e12.5 (Kafri et al., 1992; Hajkova et al., 2002) and is followed by re-establishment of a new set of imprints during gametogenesis.

The epigenetic modification most clearly demonstrated for imprinted genes is the allelic-specific DNA methylation patterns. Most imprinted genes that have been examined contain at least one differentially methylated region (DMR) located in the 5' promoter region or in the gene itself. When *Dnmt1* or an oocyte specific DNA methyltransferase *Dnmt1o* was mutated, the imprinted genes tested so far except *Ascl2* showed either biallelic expression or biallelic repression (Lewis et al., 2004). Allelic-specific acetylation and methylation of specific histone residues have been reported for *Igf2*, *H19*, *Igf2r*,

Snrpn and *U2af1-rs1* (Grandjean et al., 2001; Gregory et al., 2001). Several proteins (DNMTs, MBPs and chromatin insulators) have been identified as *trans*-acting factors involved in the epigenetic regulation of these loci. Non-coding RNAs appear to be especially abundant at imprinted loci. *H19* was the first identified imprinted non-coding RNA. The DMR upstream of *H19* promoter is critical for the maternal expression of *H19* and the paternal expression of the upstream *Igf2*. Silencing of the maternally expressed *Igf2r*, *Slc22a2* and *Slc22a3* genes on the paternal chromosome was regulated by a paternally expressed antisense non-coding RNA *Air* (antisense transcription at the *Igf2r* locus) (Sleutels et al., 2002). PcG proteins also play roles in regulating allelic-specific expression. *Eed* was shown to maintain the silencing of parental chromosomes of some maternally expressed genes (Mager et al., 2003). A recent study indicated that paternal repression in the placenta at the *Kcnq1* domain on mouse chromosome 7 involved repressive histone methylation and *Eed-Ezh2* polycomb group complexes (Umlauf et al., 2004).

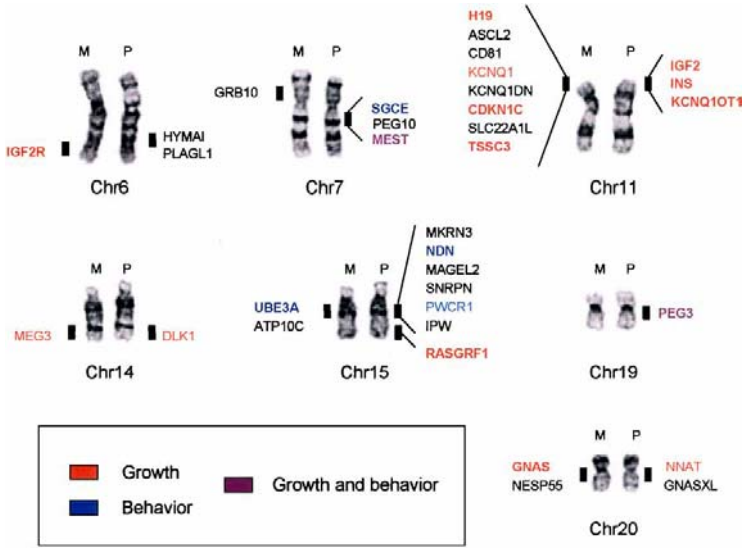

Figure 6. Imprinted genes on human chromosome. Only genes with reported roles in growth and/or behavior are listed. The gene names are colored according to their function. M, maternal; P, paternal. (Figure adapted from Tycko and Morison, 2002).

Functions of imprinted genes

Pre- and post-natal growth Striking growth phenotypes are first revealed by some mouse uniparental disomies (UPDs), that is, mice inherit both copies of a specific chromosome from one parent (Cattanach and Beechey,

1990). Disruption of normal growth patterns is frequent outcome of targeted deletion or over-expression of imprinted genes. Roughly, half of the imprinted genes in mammals are known to control prenatal growth (Beechey et al., 2003). For example, Targeted mutation of the paternally expressed genes *Igf2*, *Peg1*, *Peg3* or *insulin* resulted in intrauterine growth retardation (IUGR), whereas null mutation of the maternally expressed genes *Grb10*, *H19* or *Igf2r*, or over-expression of *Igf2*, resulted in overgrowth of the fetus (Sun et al., 1997; Tycko and Morison, 2002). Interestingly, there is a strong tendency for deletion of paternally expressed genes to inhibit growth, whereas deletion of maternally expressed genes often promotes growth. Some congenital disorders in human that involve a growth phenotype have been shown to result from disruption of imprinting. Beckwith-Wiedemann syndrome (BWS) arises from imprinting disturbances on chromosome 11p, one of the cardinal features of BWS is pre- and post-natal overgrowth.

Although imprinted genes affect growth mainly at prenatal stage, some imprinted genes have also been implicated to play a role in postnatal growth in mice. *Gnasxl* is a paternally expressed gene on mouse distal chromosome 2. Mice with paternally transmitted mutation of *Gnasxl* have poor postnatal growth due to impaired suckling and regulation of energy homeostasis (Plagge et al., 2004). *Rasgrf1* encodes a guanine-nucleotide binding protein and was shown to regulate postnatal growth through synthesis or release of growth hormone (Itier et al., 1998). Prader-Willi syndrome (PWS) in human is characterized by neonatal hypotonia and feeding difficulties. Mice with a PWS imprinting-center (IC) deletion on chromosome 7 and concomitant loss of expression of paternally expressed genes from the region homologous to PWS failed to thrive (Yang et al., 1998).

Placental development In mammals, the majority of imprinted genes are expressed at high levels in the placenta. Interestingly, some of the genes are expressed in an imprinted manner exclusively in placenta, such as *Gatm*, *Obph1*, *Ascl2*, *Dcn*, *Slc22a2*, *Slc22a3* and *Esx1*. Several mouse models with disruption of imprinted genes suggest that genomic imprinting is essential to placental development (Reik et al., 2003). In a screening for genes expressed and regulated between early (e7.5) and late (e17.5) stages of mouse placental development, a non-random distribution of clones with a prevalence of localization on chromosomes subjected to imprinting effects was found. This data indicated a strong correlation between placental growth control and genomic imprinting (Hemberger et al., 2001).

The reciprocally imprinted genes often have opposite effects on placental development, with paternally expressed genes enhancing growth and maternally expressed genes suppressing growth. For instance, mice with mutations of the paternally expressed genes *Igf2*, *Peg1* and *Peg3* have decreased placental size, knockouts of maternally expressed genes *Igf2r*, *Cdkn1c*, *Ipl*, *Grb10* and *Esx1* have increased placental size (Reik et al., 2003). One excep-

tion is the maternally expressed gene *Ascl2*. Placentas of *Ascl2* mutant mice completely lack spongiotrophoblast and have an underdeveloped labyrinthine trophoblast (Guillemot et al., 1995). In addition to these growth effects, imprinted genes also regulate placental function by affecting transport system of the placenta, as indicated by the placental phenotype in mice with targeted mutation of *Slc22a2*, *Slc22a3*, *Slc22a11* or *Ata3* (Reik et al., 2003). The *Igf2* P0 promoter in mice is labryrinthine trophoblast-specific. Deletion of P0 transcript caused a decrease in passive permeability and nutrient transfer across the placenta, leading to subsequent reduction in fetal growth (Constancia et al., 2002).

Behavior A strong influence of imprinted genes on behavioral control in mammals is indicated by various observations. Cells of PG or AG origin are allocated in different regions of brains in chimeric mice. PG cells are found only in cortex and striatum, whereas AG cells contribute extensively to the hypothalamus and pre-optic area (Fig. 7). This spatial specificity probably indicates their differential effects on behavior. However, both uniparental cell types contributed to the main olfactory and vomeronasal systems, and in particular to the receptor neurons themselves (Allen et al., 1995; Keverne et al., 1996a). Altered behavior, that is, increased male aggressivness was also observed in PG ↔ wt chimeras. This aggressiveness was positively correlated with the contribution of PG cells to the chimera (Allen et al., 1995). Aggressive behavior in mice is mediated partially by olfactory cues (Mugford and Nowell, 1970), which coupled with the fact that uniparental cells contribute directly to the olfactory receptor neurones, suggests that imprinted genes may affect the function of olfactory system.

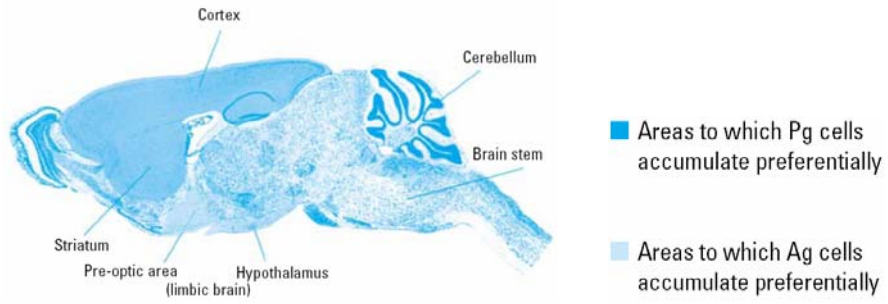

Figure 7. Distribution of uniparental cells in the brain (Figure adapted from Roth and Snell, 1999)

Abnormal behavior has been described in UPDs. For instance, reciprocal phenotypes were found in mice that were disomic for maternally or

paternally inherited copies of distal chromosome 2. Those mice that inherited two copies from their father were hyperkinetic, whereas those that inherited two copies from their mother were hypokinetic and failed to suckle. Both die within a few days of birth (Cattanach and Beechey, 1990). More evidence for a role of genomic imprinting in behavior comes from the studies in human and mice. PWS and Angelman syndrome (AS), two human disorders, result from imprinting errors on chromosome 15q11-q13. PWS is caused by loss of paternal gene expression from this region, whereas AS is caused by loss of maternal contribution. PWS is characterized by mental retardation, learning disabilities, compulsive eating and poor social interaction. AS is characterized by severe cognitive impairment, absent speech, seizures, ataxia, inappropriate laughter, sleep disturbance and mental retardation (Nicholls et al., 1998). *UBE3A* is identified as the locus mutated in AS. The phenotypes of maternal deficiency for *Ube3a* in mice resembled human AS (Jiang et al., 1998). *Rasgrf1* is paternally expressed and the expression is exclusively in neurons of the postnatal and the adult central nervous system (CNS). Mice lacking *Rasgrf1* have impairment in the consolidation of long-term memory and in electrophysiology in the amygdala, a critical part of the neural circuitry involved in emotional response (Brambilla et al., 1997). *Peg1* and *Peg3*, two paternally expressed genes, are expressed throughout brain and at particularly high levels in the hypothalamus. Gene targeting studies revealed that both genes play an important role in nurturing behavior (Lefebvre et al., 1996; Li et al., 1997). Together, these results show that very different types of behavior are under at least partial control of imprinted genes.

Lipid metabolism Obesity due to defective lipid homeostasis has been described in PWS (Couper et al. 1999). Albright hereditary osteodystrophy (AHO) is a human disorder characterized by obesity, short stature and skeletal defects. AHO arises from dominant inactivation mutations in the *GNAS1* gene encoding the α-subunit of the heterotrimeric G protein Gs (*Gsα*). In mice, *Gsα* is expressed primarily from the maternal allele in renal proximal tubules and adipose tissue but is not imprinted in other tissues (Yu et al., 1998). Heterozygous mice with maternal *Gsα* mutation (*Gsα* -/+) became obese, with increased lipid per cell in white and brown adipose tissue. *Peg1* was shown to be associated with the size of adipocytes. *Peg1* expression was markedly enhanced in white adipose tissue of mice with diet-induced and genetically caused obesity. In transgenic mice with over-expression of *Peg1* in adipose tissue, adipocytes were significantly enlarged (Takahashi et al., 2005). A recent study has found increased adiposity, despite growth retardation, in mice with targeted mutation of a paternally expressed gene, *Dlk1* (Moon et al. 2002).

Adult-onset obesity was also found in several knockout mouse models of imprinted genes. *Igf2* is maternally imprinted in most somatic tissues of mice

with the exception of the choroids plexus and leptomeninges of brain. A 12-Kb deletion of a region 5' of the imprinting control region (ICR) of *Igf2* caused obesity in a small subset (about 5-10%) of heterozygous mice. The onset of the increase in body mass was detected as early as 6.5 weeks and as late as 6 months after birth (Jones et al., 2001). Mice with a targeted mutation of the *Peg3* have higher levels of body fat from two months of age onwards (Curley et al., personal communication).

Testis development To date, no special emphasis has been directed at the role of genomic imprinting in testicular development. We found that the majority of imprinted genes were expressed in adult testis and especially some genes were expressed at specific stages of spermatogenesis (Shi et al., in preparation). It is noteworthy that male patients with PWS often have hypogonadism, which is characterized by smaller genitalia, delayed puberty and sterility. The notion that genomic imprinting is associated with testicular function is also supported by the infertility of male mice with DNA methyltranferase 3-like (*Dnmt3L*) mutation (Hata et al., 2002) and by the defective spermatogenesis in mice with *Dnmt3a* mutation (Kaneda et al., 2004). *Dnmt3L* and *Dnmt3a* are essential for the establishment of parental methylation imprints and appropriate expression of imprinted genes. Thus, in both mutations the *H19* DMR is unmethylated in spermatogonia, whereas normally *H19* is paternally *de novo* methylated in the early pre-meiotic stages of spermatogenesis. Abnormal imprinting status of *H19* has also been implicated in abnormal spermatogenesis in human. Upon examination of semen samples, 24% of oligozoospermic individuals showed abnormal methylation in the *H19* DMR region (Marques et al., 2004).

Evolution of genomic imprinting

As deleterious recessive somatic mutations are hidden in diploids but not in haploids, a multicellular organism usually prefers two sets of chromosome. Why do imprinted genes behave as haploid genes? How can genomic imprinting spread? Numerous hypotheses have been proposed to explain the evolution of genomic imprinting. To date, the most well supported theory is the conflict hypothesis, which is based on a putative tug of war between the sexes (Moore and Haig, 1991). The "imprinting" phenotypes of AG and PG embryos seem to suggest the possibility of the male using imprinting to maximize maternal input to the embryo. Multi-male mating (MMM) by females is relatively common among mammals. According to the conflict hypothesis, it is in the interest of a male to attempt to recover more maternal resources for his developing offspring in relation to offspring in the same mother that were sired by other males. This can be accomplished with a paternal imprint that down-regulates the expression of genes that normally act to slow down the embryonic growth. As a consequence, embryos that are

sired by this male will grow more rapidly than half-siblings sired by other males. Although overgrowth may be beneficial to these offspring, it extracts a heavy reproductive cost from the mother. Consequently, it is in the interest of the mother to counteract this increased level of growth. She can do this with an imprint that down-regulates the relevant growth factor genes themselves. The evolutionary endpoint of this tug of war is the current day situation where genes, such as *Igf2*, that act to increase embryonic growth have inactivated maternal alleles, and genes, such as *Igf2r*, that act to limit growth have inactivated paternal alleles.

There are several major problems with conflict theory. Some genes affect embryonic growth but are not imprinted, such as *Igf1*. Some paternal UPDs may be smaller than normal. *Ascl2* null-mutant mice showed a phenotype that is exact reversal of that predicted by the theory. Thus, other theories have been developed. The first explanation for the existence of imprinting is to prevent parthenogenesis in female mammals. As placental formation is predominantly the responsibility of genes that are only paternally expressed, this will protect the female from malignant trophoblast disease arising in parthenogenetically activated oocytes (Varmuza and Mann, 1994). However, this theory does not explain why there is silencing of maternal alleles, nor does it make any prediction that imprinted genes should affect growth. This theory cannot explain imprinting that occurs in non-placental tissues, such as brain or liver.

Barlow proposed that genome imprinting has evolved from the host defense mechanisms of prokaryotes (Barlow, 1993). Restriction methylases recognize and methylate the specific cutting sites on the host DNA, thus protecting bacterial DNA from cleavage by restriction endonucleases. Foreign DNA is not so protected and can be cleaved and disposed of. In bacteria, methylation offers protection against digestion, whereas in mammals it might have evolved to prevent expression at an inappropriate time of development. This theory does not take into account the fact that many non-imprinted genes are also methylated.

To date, the conflict theory still appears to be the best hypothesis in terms of explaining most of the facts of imprinting. However, it is not necessary that there should be only one unifying theory of imprinting, nor is there one theory that is congruent with all the data.

Are imprinted genes fast evolving?

If imprinted genes are the product of tug-of-war between the sexes, it is expected that these genes are fast evolving. As shown for reproduction-related genes, McVean and Hurst (1997) predicted that intra-genomic conflict would cause imprinted genes to be relatively fast evolving. In their study, they compared the rate of non-synonymous DNA substitution (Ka) to synonymous substitution (Ks). Because Ks does not alter the encoded protein, it

is generally assumed to be nearly neutral with respect to selection. Ka alters the encoded protein and is constrained by selection. When Ka/Ks is high, directional selection in indicated. No significant tendency for Ka/Ks to be higher for imprinted genes was found when the imprinted genes in mouse and rat were compared to non-imprinted genes not involved in mother-fetus interactions. This finding does not support the idea that imprinted genes are fast evolving.

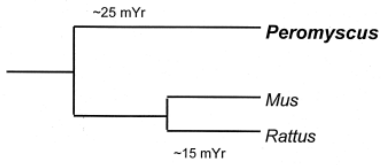

Figure 8. Evolutionary relationship between Peromyscus and Mus.

Of the imprinted genes that have been examined in multiple species, most of them show high degree of conservation (Morison and Reeve, 1998). However, comparative studies indicate that different species have either evolved different sets of imprinted genes following speciation, or have differentially lost imprinting. Several lines of evidence support the idea that imprinting control regions or reading of imprinting marks, rather than imprinted genes *per se*, are fast evolving. *Ascl2* is maternally expressed in Mus, but biallelic in Peromyscus (Vrana et al. 1998). There are several placental lactogen (*Pl*) genes in rodents, in which these genes arise from duplication of prolactin gene. The placental lactogen-1-variant (*pPl1-v*) gene in Peromyscus is paternally expressed throughout fetal development, whereas the *Pl1* gene in both Peromyscus and Mus does not display any preferential expression of the paternal allele. Sequence comparison revealed that duplication of the *pl1* gene occurred after the radiation of rodents, thus, *pPl1-v* represents a case of relatively recent gain-of-imprinting of a gene (Vrana et al., 2001). *Rasgrf1* is imprinted in Mus and Rattus, but not in Peromyscus. A block of direct repeat has been related to the imprinting control of *Rasgrf1*. This repeat element is present in Mus and Rattus and absent in Peromyscus. Since Mus and Rattus are more closely related to each other than to Peromyscus (Fig. 8), the repeat element may have inserted into this region of the genome after Peromyscus diverges (Pearsall et al., 1999). The imprinting status of *ZIM2* is also diverged among mammals. Human *ZIM2* is paternally expressed, mouse *Zim2* is expressed predominantly from the maternal allele in brain. *PEG3* is located upstream of *ZIM2* in the same imprinting domain. The change of imprinting status appears to have resulted from independent insertional events that placed unrelated genes, *Zim1*, between *Zim2* and *Peg3* in mouse (Kim et

al., 2004). This suggests that rearrangements have occurred independently in different mammalian lineages in recent evolutionary time. *IGF2R* is imprinted in marsupials, artiodactyls and rodents, but in human is imprinted only in a minority of cases. Monoallelic expression of mouse *Igf2r* seems to be governed by an imprinted antisense transcript *Air*, which is absent in humans (Smrzka et al. 1995; Killian et al. 2000). Mouse *Grb10* is maternally expressed, whereas human *GRB10* is expressed biallelically in most tissues, except for paternal expression in the fetal brain and maternal-specific expression of one isoform in skeletal muscle. However, the imprinted methylation patterns are conserved in mouse and human, which suggests that the divergent allelic expression is due to differential reading of the imprinting marks (Arnaud et al., 2003).

Speciation genes and imprinted genes

There are two well-documented patterns in speciation. One is Haldane's rule (Haldane, 1922), which states that when hybrid crosses produce sterile or inviable offspring, the sex that exhibits this is most likely the heterogametic sex (the sex with two different sex chromosomes, e.g. X and Y in male rodents, while in birds and butterflies the female is heterogametic with Z and W). Another pattern is that genes affecting reproductive isolation are typically found on the X chromosome (Coyne and Orr, 2004). The current belief about the ''large X effect'' is that advantageous mutations are more likely to accumulate on the X since it is hemizygous in males, so half of the time recessive advantageous mutations will be expressed. However, similar mutations on autosomes will be less likely to be expressed because an advantageous mutation would have to be dominant to be ''visible'' for selection. Thus, divergent populations (incipient species) will tend to accumulate different mutations on their respective X chromosome. Imprinted genes are expressed only from paternal or maternal alleles in some or all tissues. Thus, this hemizygosity of imprinted genes might be subjected to genetic divergence.

Strikingly, it appears that the genes involved in fertility, reproduction and cognition enrich on X chromosome. The density of sex- and reproduction-related genes on the human X chromosome is two-fold higher than that on autosomes (Saifi and Chandra, 1999). A large number of mental retardation genes are known to be X-linked (Chiurazzi et al., 2001; Zechner et al., 2001). The clinical description of X-linked mental retardation (XLMR) traits provides circumstantial evidence that mutations in the same genes affect not only mental performance, but also fertility. For instance, fragile X syndrome is the most common cause of familial mental retardation and it is associated with macroorchidism in males and premature ovarian failure in carrier females (Lubs et al., 1999). Genes involved in speciation are most likely reproduction and behavior-related genes. The high density of these genes on X

chromosome is consistent with ‘‘large X effect’’. Similar patterns can be found in a few human disorders that are associated with imprinted genes. For example, patients with PWS have mental retardation as well as hypogonadotrophic hypogonadism.

Many attempts have been made to identify genes that keep species isolated. These genes are named speciation genes, which defines the genes that lower fitness when moved into another species. Speciation genes are found to lie close together in pea aphids, a common crop pest that appears to be splitting into two species that infect different plants (Hawthorne and Via, 2001). The two new species look identical, but they show little interest in meeting. It was found that genes that increase performance and the tendency to find mates on one plant while decreasing performance on the other plant lie close together within several small chromosomal regions, which might cause rapid evolution and speciation. This feature is reminiscent of imprinted genes, which are clustered on several chromosomal regions. Often the explanation for the clustering is that it is easier to generate a new imprint by extending a locally available imprinted gene. However, it is likely that the tight linkage of imprinted genes might also facilitate rapid co-adaptation of the organisms.

Genomic imprinting and placental mammals

Genomic imprinting arose in early mammals some more than 135 million years ago at the time of divergence of placental from egg-laying mammals such as fishes, reptiles and amphibians (Killian et al., 2000; Killian et al., 2001). Non-placental monotremes (egg-laying mammals) express imprinted genes, which however appear not to be imprinted. For instance, *IGF2* is imprinted in marsupials, artiodactyls, rodents and primates, but not in monotremes or birds. Imprinting has evolved independently in seed plants, where the endosperm has a similar nutrient-providing role as the placenta.

Unlike the basic body plan of the embryo, the structure of the placenta is highly divergent across mammalian species. However, the placental trophoblast-derived structure fulfills two basic functions in every mammalian species (Fig. 9). Firstly, it generates a large surface area for nutrient exchange, consisting of an epithelial barrier and underlying fetal blood vessels. Secondly, trophoblast cells interact closely with the uterus and produce growth factors, cytokines and hormones that target maternal physiological systems, resulting in provision of more blood flow and nutrient delivery to the fetal-placenta unit. Metabolically, the placenta is very expensive for the mother to maintain, yet it is invaluable. Not only that it feeds the embryos, but also it protects embryos from the mother's immune system. Placental hormones also increase maternal food intake, prime the brain for maternal care (Bridges et al. 1997) and the mammary gland for milk production, and silence female sexual interest in males.

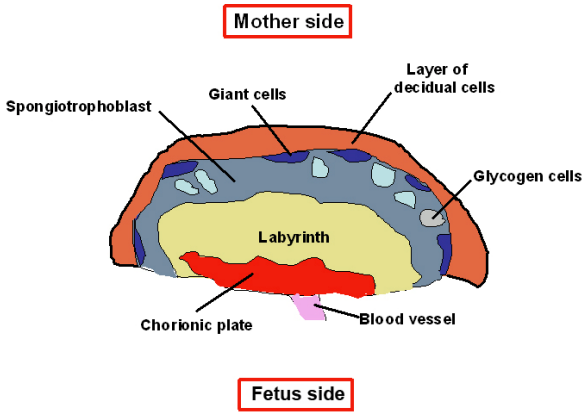

Figure 9. Mouse placenta.

An important characteristic of placental mammals is their vivpiarous reproduction, in which the embryos are developed within their mother. With viviparity, conflict can arise between mothers and developing embryos and between maternal and paternal genomes within individual embryos through the placenta that acts as an interface between the mother and the fetus. This conflict is suggested to cause the co-evolution of placental structure and genomic imprinting (Wilkins and Haig, 2003). As mentioned previously, a substantial number of imprinted genes are known to play roles in placental development and function. A number of imprinted genes are only imprinted in placenta. Imprinted X-inactivation occurs exclusively on the paternally derived X chromosomes in eutherian extra-embryonic tissues. However, little is known how placental genes arose evolutionarily. Are they genes taken over by trophoblast to function in trophoblast development or did new genes evolve to execute trophoblast function? *Ascl2* belongs to a basic helix-loop-helix (bHLH) class and is crucial for trophoblast development. *Ascl2* is a homolog of the *Drosophila* neurogenic *achaete-scute* genes. A related mammalian gene, *Mash1*, is in fact involved in neural development. It is likely that *Ascl2* arose as a recent duplication and was diverted to function in trophoblast development.

Igf2 is imprinted in both marsupials and placental mammals. However, there is significant difference in placental structure among these species. Marsupials have a chorio-vitelline placenta that allows only limited diffusion between maternal and fetal blood. Maternal resources are delivered in marsupials mainly through lactation rather than development *in utero*. Placental mammals develop chorio-allantoic placentas that make prolonged pregnancies and a remarkable liaison between the mother and the unborn infants

possible. If placental structure is indeed co-evolved with genomic imprinting, the imprinting patterns between placental mammals and marsupials would be expected to be somehow different. Thus, comparative study of genomic imprinting in different species for more genes is needed. Allelic expression of *Igf2* in two live-bearing, matrotrophic poeciliid fish species was tested in a recent study. Poeciliidae constitutes a large family of ray-finned fishes, where most species give live birth. Furthermore, placentation has evolved independently and relatively recently (< 1 million year ago in some clades) in several different lineages of poeciliids. However, *Igf2* was found to be expressed biallelically throughout embryonic development even when an enormous dedication of maternal resources is present in these two fish species (Lawton et al., 2005).

Is epigenetics involved in hybrid dysgenesis?

Recent investigations in mammalian hybrid dysgenesis support the idea that epigenetic mechanisms are strongly involved in some of these phenotypes. In an interspecies marsupial hybrid, retroelement amplification as a response to genome hypomethylation was described (O'Neil et al., 1998). However, there was no conclusive evidence that the genome-wide demethylation observed was associated with any hybrid dysgenesis effects and a subsequent study found no evidence of large-scale demethylation events in hybrids between placental mammals (Roemer et al., 1999; Robinson et al., 2000). Loss of imprinting (LOI) of several imprinted genes has been described in hybrids between PMA and PPO (Vrana et al., 1998). Furthermore, it was found that deleterious interactions between *Peg3* and the loci on X-chromosome underlied placenta hyperplasia in Peromyscus hybrids and that the degree of *Peg3* LOI was positively correlated with placental hyperplasia (Vrana et al. 2000). This was the first experimental evidence that disruption of epigenetic states, as manifested by LOI, contribute to hybrid disgenesis effects and thus to speciation. We have reported stochastic LOI of *Peg1* in Mus hybrids and that biallelic expression of this gene was associated with increased growth (Shi et al., 2004). In addition, several imprinted genes exhibited altered expression levels in Mus hybrid placentas and intriguingly the same genes showed expression changes in the enlarged placentas of cloned mice (Singh et al. 2004). It is noteworthy that some of the defects observed in mouse interspecies hybrids, such as placental dysplasia, abnormal growth, and obesity, are faithfully recapitulated in mice derived from somatic cell nuclear transfer (SCNT), in which these defects are exclusively due to disruption of epigenetic states (Tamashiro et al., 2002; Fairburn et al., 2002; Dean et al., 2003).

From the described developmental processes and organs targeted by imprinting defects and by interspecies hybridization, some of the tissues or

processes that are sites of expression of imprinted genes are also targets for postzygotic hybrid phenotypes. Additionally, the complementary phenotypes frequently observed in reciprocal interspecies hybrids also suggest that imprinted genes are involved in the hybrid dysgenesis effects (Table 1).

Table 1. Developmental processes and organs targeted by imprinting defects and by interspecies hybridization

	Functions of imprinted genes	**Hybrid dysgenesis**
Growth	Targeted mutation of imprinted genes affects pre- and post-natal growth	Reduced/increased growth in reciprocal mammalian hybrids
Placenta	Most imprinted genes are expressed in the placenta. Targeted mutation of imprinted genes often has placental phenotypes	Abnormal placentation in hybrids in genera Equus, Mus and Peromyscus
Behavior	Abnormal behaviors were found in imprinting-related human disorders and in knockout mice of imprinted genes	Behavior is the most important prezygotic barrier. Abnormal maternal behavior was found in a subset of F1 hybrids
Lipid metabolism	Obesity was found in mice with defective imprinted genes	A subset of F1 hybrids exhibited obese phenotype
Fertility	Most imprinted genes are expressed in testis, some are expressed in specific stages of spermatogenesis	Male sterility was found in most mammalian hybrids

As described above, stochastic LOI of *Peg1* was observed in a subset of F1 females, and female mice that carry a targeted *Peg1* allele did not exhibit postnatal maternal behavior (Lefebvre et al., 1998), it is of interest to determine whether LOI of *Peg1* in the brain of F1 hybrids would also interfere with normal maternal behavior. We therefore subjected virgin F1 females to maternal behavior testing.

Maternal behavior

Maternal behavior in mammals

Fitness in mammals involves not only the production of many young, but also nursing, feeding, and protecting them after birth to enhance their survival and future reproduction. Maternal behavior is a highly conserved set of behavioral patterns that are crucial for reproductive success. Pups of altricial animals such as rodents are almost completely deaf, blind, immobile and incapable of body temperature maintenance at birth. Therefore, their survival is dependent on the initiation and maintenance of maternal behaviors. Maternal behavior in mammals is extremely diverse. At one extreme are the minimally maternal eutherian species such as tree shrews and rabbits that spend only a few minutes each day in contact with their young. At another extreme are species, including many primates, showing maternal behavior throughout their life cycle. Between, there are many species for which maternal care is restricted to the postpartum period, such as rodents (Insel and Young, 2001). The central role of maternal care in early life has been extensively studied in rodents. Maternal behavior in rodents involves a complex set of activities, including nest building, sniffing and exploration of pups, pup retrieval, licking, grooming, nursing and placentophagia (the mother will eat the placenta immediately after giving birth) (Pryce et al., 2001, see also Fig. 10).

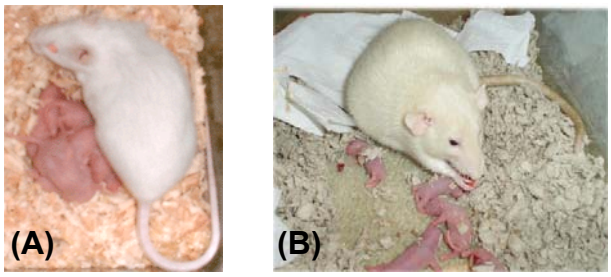

Figure 10. Maternal behavior in rats: (A) crouching; (B) placentophagia.

Rodents are communal animals. Rat dams do not discriminate between her own young and those of others during lactation. However, after weaning, mothers do discriminate between kin and non-kin. The advantages of kin recognition after weaning have been interpreted in the light of inbreeding avoidance. In rat, adult virgin females do not show maternal behavior when first presented with foster pups. However, if virgin female rats are cohabited with young pups, they will eventually display maternal behavior after a period of 4 to 7 days (Rosenblatt, 1967). Laboratory mice are different from rats, in that naive virgin females show a level of maternal responsiveness

somewhat equivalent, at least in home cage tests, to that shown by the puerperal females (Numan, 1988). Under semi-natural conditions, both laboratory and wild mice have been observed to rear their young communally (Lloyd, 1975). When a virgin female mouse is placed in a communal setting with lactating female, the virgin will also care for the young (Sayler et al., 1971). Although virgins do not lactate, they can keep the young warm while the mother is foraging for food. Giving conditions of high population density, where a virgin may not be able to breed on her own, she may increase her inclusive fitness by helping her mother or sisters raise offspring. This suggests that high levels of maternal responsiveness may evolve in virgins of certain mouse populations by kin selection (Lown, 1980).

Mechanisms of maternal behavior

Maternal behavior is virtually absent during pregnancy but appears suddenly at parturition. It is believed that complex interactions between CNS and endocrine system, sensory stimuli from the offspring and environment play an important role in the initiation and maintenance of maternal behavior (Kendrick et al., 1997). Classic lesion studies in rodents have implicated the medial pre-optic area (MPOA) of the hypothalamus, the bed nucleus of the stria terminalis (BNST), and the lateral septum (LS) as regions pivotal for regulation of pup-directed maternal behavior (Numan and Sheehan, 1997; Kalinichev et al., 2000; Sheehan et al., 2000, also see Fig. 11). The MPOA receives input from a variety of brain structure such as olfactory system via the amygdala. Efferent projections lead via the lateral pre-optic area to the ventral tegmental area, and these projections are suggested to connect the MPOA to the basal ganglia, where motor behavior is modified to enhance crouching and retrieving.

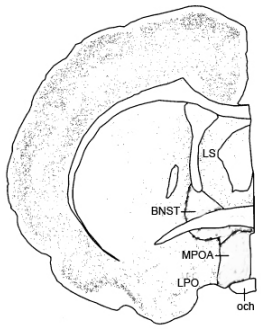

Figure 11. Anatomic illustration of cross section along the rostrocaudal axis of the medial pre-optic area (MPOA) and hypothalamus. BNST: bed nucleus of the stria terminalis (BNST); LPO: lateral pre-optic area; LS: lateral septum; och: optic chiasm. (Figure modified from Kalinichev et al., 2000).

In mammals, olfactory cues are extensively used in many aspects of maternal care. Specifically, olfactory cues from infants play a pivotal role for females in recognition of offspring at parturition, thus facilitating the onset of maternal responsiveness and establishment of mother-infant bond (Calamandrei et al., 1992). Olfactory cues from neonate mice are sufficient for the mother to locate pups in a maze (Smotherman et al., 1974). Anosmia induced either by bulbectomy or by intranasal application of zinc sulphate led to severe impairment of pup-care behavior, with anosmic females either cannibalizing or abandoning their pups (Gandelman et al., 1970).

Several neuroendocrine factors are important for priming the onset of maternal behavior. During pregnancy, females have extensive hormonal alterations that enhance neural activity and contribute to changes in maternal behavior (Kinsley et al., 1999). Progesterone declines during the last day of pregnancy, estrogen and prolactin increased just before birth. A series of experiments have suggested that the maternal behavior is stimulated by the presence of high estrogen levels against a background of low progesterone levels at the end of a normal pregnancy. Pup stimulation might maintain maternal behavior after postpartum. Prolactin and oxytocin have been implicated as central neuroendocrine mediators of maternal care. In rats, prolatin administration facilitated maternal behavior in a steroid-primed non-pregnant rat and treatments that decreased prolactin level inhibited maternal care (Bridges and Ronsheim, 1990). Oxytocin has a pronounced anxiolytic effect (Gimpl and Fahrenholz, 2001) and is required for the milk ejection reflex to occur in nursing pups (Young et al., 1996). In the rat, oxytocin receptor (OT) levels are functionally linked to natural variations in maternal behavior, with some individuals displaying high levels of pup licking/grooming (LG) than the others. Central infusion of a selective OT antagonist on postpartum day 3 reduced LG behavior in high LG mothers (Champagne et al., 2003).

Gene targeting studies have identified a significant number of genes that play important roles in maternal care, such as FBJ osteosarcoma oncogene B (*Fosb*; Brown et al., 1996), dopamine beta hydroxylase (*Dbh*; Thomas & Palmiter, 1997), forkhead box B1 (*Foxb1*; Wehr et al., 1997), prolactin receptor (*Prlr*; Ormandy et al., 1997; Lucas et al., 1998), heterotrimeric G proteins of the Gq/11 family (*Gq/11***;** Wettschureck et al., 2004), testicular orphan nuclear receptor 4 (*Tr4*; Collins et al., 2004), paternally-expressed gene-1 (*Peg1*; Lefebvre et al., 1998), paternally-expressed gene-3 (*Peg3*; Li et al., 1999), and methyl-CpG binding domain 2 (*Mbd2*; Hendrich et al., 2001). Females homozygous for targeted mutations of these genes exhibited a variety of abnormal maternal behaviors. Lack of pup retrieval and crouching over the nest were the only behaviors in common among these knockouts.

Female infanticide

Female infanticide appears to be limited primarily to non-offspring under normal conditions. Female wolverines, for example, will hunt down foreign infants in order to force the mother to abandon choice den sites. In several Old World primates genera the dominant female will kill other female's offspring to reduce population pressure and to provide more resources for her own. In mice, the majority of virgin females do not exhibit infanticide in laboratory strains of mice. However, it was shown that feral MMU virgin females are strikingly different from laboratory strain females in that a high proportion, between 60 and 100%, will exhibit infanticidal behavior when exposed to alien pups (Jakubowski & Terkel, 1982; McCarthy and vom Saal, 1985; McCarthy et al., 1986). As infanticidal behavior in these feral females is positively correlated with intraspecies aggression against both females and males, it is likely that selection against aggressiveness during domestication also led to loss of infanticidal behavior.

Unfortunately, little is known to date about the molecular mechanism of female infanticide. In many mammalian groups, infanticide is much more common in males than in females and it is a reproductive strategy for the males of different species, such as lions and langurs, to terminate the female's investment in pups not carrying the males' genes, thereby allowing them to mate with the female. This sexual dimorphism suggests a role of sex-specific gene expression in the brain. It was indeed shown that 17% of XX mice carrying a *Sry* translocation, which masculinized them, were infanticidal in a behavior tests, whereas 0% of XX littermate females killed pups (Reisert et al., 2002). More information came from studies of mice carrying targeted mutations. A subset of mice with a mutation in the NR1 subunit of *Grin1* (*NMDAR1*), an ionotropic glutamate receptor, performed poorly in maternal tasks. These mothers became aggressive toward their newborns, which lay scattered, displayed bruises and bites, and were sometimes cannibalized (Single et al., 2000). High rates of infanticidal behavior were also observed in estrogen alpha receptor (*Esr1*) mutant females when they were exposed to newborn pups (Ogawa et al., 1996, 1998). *Esr1* is a member of the steroid receptor superfamily of ligand-activated transcription factors. It was suggested that the killing behavior of the *Esr1* mutants is due to elevated levels of testosterone in these females (Ogawa et al., 1998).

Epigenetic basis of maternal behavior

It is noteworthy that methyl-CpG binding domain 2 (*Mbd2*) and two paternally expressed genes *Peg1* and *Peg3* are involved in controlling maternal care. *Peg1* deficient females that inherited the mutant allele from their father were impaired in retrieving pups, nest building skills and placentophagia (Lefebvre et al., 1998). *Peg3* gene knockout also showed similar defects on

pup retrieval and care, but in addition, mutant females were deficient in milk ejection due to a reduced number of oxytocin-producing neurons in the hypothalamous (Li et al., 1999). *Mbd2* is a transcriptional repressor that specifically binds to methylated DNA. Maternal behavior of *Mbd2* null mutant females was defective, although the effects were less pronounced than that of *Peg1* and *Peg3* mutant mice (Hendrich et al., 2001). Furthermore, it has been shown that licking, grooming and arched back nursing among rat dams appeared to mediate the nongenomic transmission of the same maternal behaviors to the next generation (Francis et al., 1999; Weaver et al., 2004). These studies demonstrate the importance of epigenetic regulation in modulating nurturing behavior.

Genomic imprinting and evolution of maternalism

Around 70 million years ago mammals underwent a significant diversification to form the present 109 families and around 4000 species. This rapid expansion, which colonized environmental extremes of land, sea and air, probably owes much to the hallmark features that mammals appropriated during evolution, including viviparity, homeothermy, maternal care and milk provisioning. These traits have a common outcome, namely successful maternalism, and failure of any one of them would severely compromise lifetime reproductive success. In this mother-infant relationship of eutherian mammals, conflicts between mother and offspring emerge. The interest of an offspring is to get as many resources as possible for itself, regardless of the interests of its siblings or mother. The interest of the mother is to ensure that the needs of her offspring are met while retaining the ability to take care of other or future offspring. This particular genetic conflict provides insight into the mammalian brain and its features. Maternal genes are expressed in the cerebral cortex of the brain, but paternal ones build the limbic system (Fig. 7). This may be explained by the fact that the limbic system controls basic instinctual and emotional needs, such as hunger and thirst, aggression, sex and other innate behaviors. *Peg1* is known to control maternal behavior in mice and is expressed in the limbic system. The cerebral cortex, on the other hand, specializes in inhibiting, controlling and moderating instinctual demands from the limbic system. It seems that the mother's genes build the part of the brain that can be nurtured and can exercise restraint, inhibition and conscience, whereas the father's genes construct the part of the brain that is notoriously incorrigible, dominated by instinct, egoism and irrationality, and is concerned with the consumption of resources and with gratification of innate needs and basic biological drives.

The evolution of maternalism is also implicated in the increased brain size through evolution. Throughout mammalian lineages, the ratio of the neocortex and striatum (high proportion of PG cells) to the hypothalamus, MPOA and LS (high proportion of AG cells) has increased (Keverne et al.

1996b, 2001). This increase in brain size places a higher prenatal demand on the mother to transfer oxygen and lipid-rich nutrients to the offspring, and requires an extended postnatal care period so offspring receive sufficient support for the lengthy postnatal period of brain development. Furthermore, the increase in brain size also places higher demand for the fetus to attract more energy from its mother. It has recently shown that these differential behaviors in the mother and the offspring are co-regulated at least in part by *Peg3*. *Peg3* mutant offspring were less competent in suckling, delayed in developing self-thermoregulation and delayed in reproduction. The same mutation in a mother resulted in reduced maternal care, reduced maternal food intake during pregnancy, and impaired milk let-down, which in turn reduced infant growth and delayed weaning and onset of puberty. Thus, targeted mutation of *Peg3* in offspring reared with wild-type mother experienced 32% mortality and wild-type offspring reared with mutant mothers experienced 28% mortality. The combined mutation in mother and offspring was 94% lethal (Curley et al., 2004). These results provide the evidence that the behaviors of mother and offspring evolve because of co-adaptation and can have common genetic basis.

Searching for speciation genes

To map the chromosomal locations of speciation genes, researchers often use pairs of related species that do not normally mate but will do so in the laboratory if given no other choice. The hybrid offspring are then examined for traits that are related to reproductive isolation between the two original species. Such traits might be postzygotic-related, such as sterility of the hybrids, altered growth (Gray, 1971) and placental dysplasia (Rogers and Dawson, 1970; Zechner et al., 1996; Allen et al., 1993) or prezygotic-related, such as altered male sexual behaviors and female mating preferences. At the same time, the genes that are associated with the isolating traits will be searched for.

At present, several putative speciation genes have been identified in *Drosophila*. *Odysseus* (*OdsH*) is a fast-evolving homeobox gene that caused male sterility in the *D. simulans* background when co-introgressed with an X-chromosome region containing *OdsH* from *D. mauritiana* (Ting et al., 1998). An obvious question is that what the normal functions of those speciation genes are since there is no advantage for a gene to cause hybrid dysgesis effects to be selected. A recent study on *OdsH* in *Drosophila* has shown that this gene is largely dispensable for morphology, viability, and fertility, although a subtle fertility effects was observed in *OdsH* knockouts, with young males moderately defective in sperm production (Sun et al., 2004). It is suggested that speciation genes might be dispensable and thus allow species to compete without altering their fundamental genes.

From the above discussions, we have some evidence that imprinted genes may be involved in speciation. However, we do not know how imprinted genes are involved, whether they are the cause of speciation, the cause of hybrid dysgenesis or just downstream effector genes. Based on the conflict theory, a possible situation could be that a paternal multilocus genotype of high resource transfer activity will result in normal offspring only when paired with maternal genotypes of similarly vigorous resource transfer suppression activity. Matings between males and females with imbalanced gene expression are likely to result in hybrid dysgenesis, due to over-demanding of the progeny or incapability of getting adequate maternal resources (Zeh and Zeh, 2000). In fact, we have found differential expression levels of several imprinted genes in diverse rodent species, even though the imprinting status of these genes remained unchanged (Shi et al., unpublished data).

Aims of the Present Studies

Paper I

Previous studies have shown that loss-of-imprinting (LOI) is a regular occurrence in interspecies hybrids of the genus Peromyscus. Furthermore, evidence was presented that LOI of one paternally expressed gene, *Peg3*, is involved in abnormal placental growth in Peromyscus hybrids. Differential growth is observed in hybridization between the closely related rodent species *Mus musculus* (MMU) and *M. spretus* (MSP). Most of these F1 hybrids are genetically identical, thus, their phenotypic variation suggests the involvement of epigenetic deregulation.

One imprinted gene, *Peg1* (also called *Mest*), is strictly paternally expressed. *Peg1* is a growth-promoting gene as evidenced from the growth phenotype of *Peg1*-null mice, which exhibited reduced growth. In this study, we aimed to answer two questions. Firstly, whether LOI and over-expression of *Peg1* are correlated with increased body weight and tissue weight of F1 hybrids? Secondly, in which cell type does *Peg1* LOI occur? To answer these questions, we initially examined the allelic expression of *Peg1* in a variety of tissues isolated from F1 hybrids. Later we assessed the *in situ* expression of LOI by X-gal staining of tissues derived from *Peg1* +/- × MSP F1 mice that carried a maternal *LacZ* knock-in allele of *Peg1*.

Paper II

In addition to its growth effect, *Peg1* is also important in normal postnatal maternal behavior such as retrieval, nest building and crouching, as shown by the finding that these behaviors are severely disturbed in female mice that do not express *Peg1*. Thus, it was considered to be of interest to know whether LOI of *Peg1* in F1 females can interfere with maternal behavior towards foreign pups. First of all, we assessed maternal behavior in virgin MS F1 females by exposing them to newborn pups. Subsequently we examined differentially expressed genes in the brains of abnormally behaving females by microarray hybridization.

Paper III

Mammalian interspecies hybrids exhibit strong parent-of-origin effects in that offspring of reciprocal matings frequently exhibit reciprocal phenotypic patterns, especially in pre- and post-natal growth. In the genus Mus, the F1 with *M. spretus* mothers are larger than those with *M. musculus* mothers. This suggested that imprinted genes could be involved in these growth patterns. A previous study in the distantly related rodent genus Peromyscus had shown that patterns of LOI were indeed consistent with a direct influence of altered expression levels of imprinted genes on growth. In this study, we extended our investigation on *Peg1* to other imprinted genes. Aims of this study were to determine whether differential growth in reciprocal Mus F1 hybrids is also associated with preferential LOI of growth-promoting genes in large hybrids and growth-inhibiting genes in the small hybrids; whether relaxation of imprinting in interspecies hybrids is caused by *cis*- or *trans*-effect; and finally, whether altered DNA methylation patterns are associated with LOI.

Paper IV

Abnormal placentation is another well-documented hybrid dysgenesis effect and interspecific hybridization in the rodent genera Peromyscus and Mus results in similar placental defects. In the Peromyscus interspecies hybrids, abnormal allelic interaction between an X-linked locus and *Peg3* was shown to cause the placental defects. In addition, LOI of *Peg3* was positively correlated with increased placental size. In the Mus interspecies hybrids, a strong role of X-linked loci in placental dysplasia has also been detected.

The placental phenotypes observed in the two genera seem to be identical, and these two genera are separated about 25 million years ago. The aim of this study was to determine whether the same mechanisms, that is, LOI of *Peg3* and/or abnormal interactions between *Peg3* and X-linked loci, are involved in generating placental dysplasia in Mus hybrids.

Results and Discussion

Paper I

Initially, *Peg1* RT-PCR/RFLV analysis was performed in tail biopsies from 108 adult F1 hybrids. The mean weight of the mice with LOI was 26.6 ± 10.5 g, compared to the mean weight of control mice without LOI of 22.4 ± 7.20 g (P = 0.0164). Subsequently, allelic expression of *Peg1* was analyzed in different tissues of F1 hybrids. Overall frequency of LOI was different between tissues in that 38% of brains, 24% of hearts, 28.6% of spleens and 9% of tails exhibited *Peg1* LOI. In addition to the body weight, our analysis also showed that spleens and kidneys exhibiting *Peg1* LOI were significantly heavier than those without LOI. Importantly, *Peg1* LOI was observed in both MS and SM F1 hybrids, thus there was no preferential biallelic expression in the SM cross, which produces large offspring. Allelic expression of *Peg1* was also examined in F1 hybrids at prenatal stage. However, out of 4 litters, only in one litter *Peg1* LOI was clearly correlated with increased fetal and placental weights. Real-time RT-PCR analysis revealed that LOI was associated with increased *Peg1* transcript levels, which supports the growth-promoting role for PEG1.

The in situ expression of *Peg1* LOI by X-gal staining of tissues, which were derived from *Peg1* +/- x MSP F1 mice that carried a maternal *LacZ* knock-in allele of *Peg1*, revealed that both localization and size of blue foci was variable in different organs and sometimes *Peg1* LOI occurred in only a negligible proportion of cells. Thus, it is possible that even LOI detected by RT-PCR/RFLV analysis in the same tissues in two different mice could in fact be caused by LOI in different cell types with corresponding phenotypic differences.

To examine the genetic basis for *Peg1* LOI, the MSP *Peg1* allele was crossed into MMU to backcross generation 5 and allelic expression of *Peg1* was analyzed in brain, kidney and spleen. The expression of *Peg1* in all tissues except one spleen was exclusively from paternal allele. This finding indicates that relaxation of imprinting in the F1 mice is caused by a modifier gene acting in *trans*, not by the presence of heterozygosity at the *Peg1* locus.

In summary, we showed that biallelic expression of an imprinted gene, *Peg1*, occurred in Mus interspecies hybrids and that this LOI was correlated with significant increases in organ and body weight. While there is no evidence that the relatively minor weight increase of animals with LOI are dele-

terious to them, our findings support the potential role of imprinted genes in mammalian speciation.

Paper II

In total, 31 virgin interspecies F1 females were tested for maternal behavior. Ten females showed complete maternal performance, from sniffing, to retrieving all three pups, to nest-building and to crouching over them. In sharp contrast, nine females started to attack and kill the alien pups. This behavior was consistent. After parturition and during lactation infanticidal females exhibited normal maternal behavior towards their own as well as towards alien pups, but after weaning reverted to infanticidal behavior. Virgin female mice from laboratory strains are somewhat exceptional in that they readily accept and care for alien pups, even without having undergone parturition. Thus, the finding that a large proportion of virgin F1 hybrid females consistently exhibited infanticidal behavior whereas other females, sometimes from the same litter, exhibited perfectly normal maternal behavior towards pups, provided us with a model for studying the molecular basis of female infanticide in the mouse.

To investigate global gene expression differences in brains of infanticidal versus normal F1 females, microarray hybridizations were performed. Comparing infanticidal to normal females after exposure to alien pups, 130 ESTs showed differential expression at threshold of 1.414-fold change, with 59 up-regulated and 71 down-regulated in the brains of infanticidal females. In the arrays for the baseline experiment, that is, infanticidal vs. normal females without exposure to pups, only 14 ESTs were differentially expressed. Gene expression patterns were also compared between infanticidal and normal brain after vs. without exposure to alien pups, 39 ESTs in normal females and 14 ESTs in infanticidal females that changed after exposure were identified. As compared to non-exposed females, the majority of the genes were up-regulated in brain of exposed females, which suggests that gene activity was triggered by the stimulation from the pups. Unexpectedly, two times more genes were up-regulated in normal females than in infanticidal females. This indicated that brains of infanticidal females are less active than those of normal females when being exposed to pups.

Functional classification of differentially expressed genes revealed that about half of the genes are involved in signal transduction, transcription regulation, cytoskeleton organization and metabolism. A number of genes were reported to be expressed in olfactory bulb or nasal mucosa. In mice, olfactory cues from young play a pivotal role in enabling females to recognize pups, thus facilitating the onset of maternal responsiveness. Our result suggested the possibilities that the response threshold of maternal recogni-

tion is shifted or that processing of olfactory cues from the pups is disturbed in infanticidal females.

Two genes associated with epigenetic modification, the paternally expressed gene *Igf2*, and *Dnmt3b*, which is involved in DNA *de novo* methylation, exhibited decreased expression by a factor of 1.6 fold. The differential expression of *Igf2* and *Dnmt3a* between infanticidal and normal females provides further evidence for an epigenetic basis of this infanticidal behavior in F1 hybrids.

In conclusion, we describe infanticidal behavior in F1 hybrids between different mouse species and identify a large number of genes that are aberrantly expressed in brains of infanticidal females. In addition, we have obtained some evidence that epigenetic mechanisms are involved in control of maternal behavior in these F1 females.

Paper III

Allelic expression of a large number of imprinted genes was assessed in brain, kidney and skeletal muscle of MS and SM adult hybrid mice. We found that 15 out of 18 genes showed disrupted imprinting patterns in F1 hybrids in at least one tissue analyzed. For some imprinted genes, strong parent-of-origin effects were obvious in reciprocal crosses. The disruption of imprints also showed tissue-specific effects. LOI was not evenly present in all the individuals in a specific cross. When LOI of paternally and maternally expressed genes was compared in MS and SM hybrids, no correlation between direction of cross and the number of either maternally or paternally expressed genes with LOI could be observed. Hence, there was no tendency for LOI of paternally or maternally expressed genes to occur in a specific cross. Real-time RT-PCR revealed that LOI was associated with increased transcript levels.

In muscle and kidney of MS hybrids 5 out of 8 mice showed biallelic expression of *Peg3*. By analyzing backcross mice, it is possible that *Peg3* LOI was cause by a *trans*- rather than a *cis*-effect. Furthermore, we investigated the relationship between DNA methylation and disruption of imprinted expression of *Peg3* and *Snrpn* by bisulfite sequencing. *Peg3* is a paternally expressed gene. In normal mice, *Peg3* promoter and exon 1 region was almost completely unmethylated on the paternal allele and methylated on the maternal allele. However, significant demethylation of maternal allele was observed in the majority of the clones in skeletal muscle with reactivated maternal allele of *Peg3*. Similar results were obtained with *Snrpn*, another paternally expressed gene. Together these results indicate that LOI of some imprinted genes is associated with disturbed DNA methylation patterns.

Our result revealed that LOI in Mus is more chaotic than in Peromyscus. We here propose that abnormal reprogramming after fertilization and during

pre-implantation development is at least in part responsible for hybrid dysgenesis for which a strong epigenetic basis has been demonstrated. It is likely that some steps in these reprogramming cascades are not performed efficiently due to the fact that sperm derived from an alien species has entered the oocyte, which provides the components of the reprogramming mechanisms and this inefficient reprogramming is the ultimate cause for LOI in mammalian interspecies hybrids.

Paper IV

We performed a genome-wide polymorphic marker screen of MSM backcross fetuses. This analysis provided no evidence for genetic linkage between interspecific hybrid placental dysplasia (IHPD) and proximal chromosome 7. Subsequently, RT-PCR/RFLV analysis in *Peg3* heterozygous, hyperplastic MSM placentas showed that *Peg3* expression was strictly paternal in all placentas analyzed. Thus, there is no indication for *Peg3* LOI in MSM placentas.

To determine whether abnormal interactions between MMU-derived *Peg3* and X-chromosomal loci contribute to placental dysplasia, as suggested by findings in Peromyscus hybrids, we set up matings between fertile BC3 (MSMMM) males heterozygous at *Peg3* and MS F1 females and analyzed placental phenotypes of the offspring. Four placentas were obtained that had an MSP-derived X-chromosome and expressed the MSP-derived *Peg3* allele. However, all these placentas exhibited increased weight and abnormal morphology. Together, these results argue against a major role of *Peg3*, or other loci on proximal chromosome 7, in the causation of IHPD in the genus Mus.

To assess whether hybridization in the genus Mus has an effect on fetal body growth, we performed a statistical analysis of e18 embryonic body weights in MSM hybrids. Weights of both male and female hybrids exhibited strong variability. In females, significant linkage was detected for markers on the central and distal X-chromosome only. Linkage of female overgrowth to the X-chromosome suggested that female Mus hybrids display no skewed X-inactivation in favor of the MMU X-chromosome.

In a previous study, in Peromyscus interspecies hybrids, abnormal allelic interaction between an X-linked locus and *Peg3* locus was shown to cause the placental defects and *Peg3* LOI was positively correlated with increased placental size. Our results presented here revealed that interspecies hybridization and the associated hybrid dysgenesis effects in Mus and Peromyscus exhibit both similarities and dissimilarities. Thus, the genetic effects of the X-chromosome on fetal and placental growth are observed in both groups, whereas the epigenetic effects on X-inactivation are unique to Peromyscus. The most striking difference is that in Peromyscus placental dysplasia follows the general Dobzhansky-Muller model of speciation with strong nega-

tive interactions between X-chromosomal loci and *Peg3* or other paternally expressed genes close to *Peg3*. This was not observed in Mus. In conclusion, our results suggest that different molecular mechanisms are involved in the generation of an almost identical hybrid dysgenesis effect.

Conclusions

Peg1 LOI is associated with altered growth in F1 hybrids

We show for the first time that loss-of-imprinting (LOI) of a paternally expressed gene, *Peg1*, occurs in both *M. musculus* × *M. spretus* and *M. spretus* × *M. musculus* interspecies hybrids and that this LOI is correlated with increases in body weight and weight of two of the organs tested, kidney and spleen. In situ expression analysis demonstrates that LOI is stochastic in that it affects different tissues to variable extents and that, even within one tissue, not all cells are similarly affected. This study provides further evidence that epigenetic mechanisms are involved in hybrid dysgenesis effects in mammals.

Female infanticide is a novel hybrid phenotype

Normal maternal behavior can be readily induced in virgin female mice of laboratory strains. However, we observed that a subset of F1 virgin females exhibited highly abnormal maternal behavior in that they rapidly attacked and killed the pups. Interestingly, infanticidal females exhibited normal maternal behavior towards their own as well as towards alien pups after parturition and during lactation, but after weaning reverted to infanticidal behavior. By microarray hybridization, we compared gene expression patterns in brains of infanticidal vs. normal females after and without exposure to alien pups. In addition, we compared gene expression in both infanticidal and normal females with vs. without exposure to pups. These microarray hybridization experiments yielded a large number of differentially expressed genes. Further functional analysis of differentially expressed genes may facilitate the understanding of normal maternal behavior in mammals and the disturbances in this behavior that result in infanticide.

Divergent genetic and epigenetic mechanisms may be involved in hybrid dysgenesis in diverse groups of mammals

We report here that tissue-specific LOI of a large number of imprinted genes occurs in adult Mus interspecies hybrids. However, patterns of LOI are not

consistent with a direct influence of altered expression levels of imprinted genes on growth, as had been shown in the distantly related rodent genus Peromyscus, even though similar growth alterations have been observed in F1 hybrids of both genera. For some genes, *Igf2r* and *Rasgrf1*, we demonstrate that LOI is associated with increased expression levels. For two other genes, *Peg3* and *Snrpn*, reactivation of the normally suppressed maternal allele by LOI, is accompanied by partial demethylation of maternal allele at their potential imprinting control regions. The finding that LOI for two genes, *Peg1* and *Peg3*, in congenic mouse lines leads us propose that abnormal reprogramming after fertilization and during pre-implantation development is at least in part responsible for those hybrid dysgenesis effects for which a strong epigenetic basis has been demonstrated.

Interspecific hybridization in the rodent genera Peromyscus and Mus results in almost identical placental phenotype. In both genera, a strong role of an X-linked locus in placental dysplasia was detected. Here we show that neither LOI of *Peg3* nor abnormal interactions between *Peg3* and an X-linked locus are involved in generating placental dysplasia in Mus hybrids. This result is again in contrast to the study in Peromyscus hybrids. Thus, studies III and IV suggest that even in related groups divergent molecular mechanisms may be involved in the production of phenotypically similar post-zygotic barriers against hybridization.

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I always believe that name is only a tag, it is the soul that makes the person different,
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