

1 Genomic imprinting and mammalian reproduction

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8 Abstract

9 Amongst animals, genomic imprinting is a uniquely mammalian phenomenon in which
10 certain genes are monoallelically expressed according to their parent-of-origin. This silencing
11 of certain alleles often involves differential methylation at regulatory regions associated with
12 imprinted genes and must be recapitulated at every generation with the erasure and
13 reapplication of these epigenetic marks in the germline. Imprinted genes encode regulatory
14 proteins that play key roles in fetal growth and development, but they also exert wider effects
15 on mammalian reproduction. Genetic knockout experiments have shown that certain
16 paternally expressed imprinted genes regulate post-natal behavior in offspring as well as
17 reproductive behaviors in males and females. These deficits involve changes in
18 hypothalamic function affecting multiple areas and different neurochemical pathways.
19 Paternally expressed genes are highly expressed in the hypothalamus which regulates
20 growth, metabolism and reproduction and so are well placed to influence all aspects of
21 reproduction from adults to the resultant offspring. Coadaptation between offspring and
22 mother appears to have played an important role in the evolution of some paternally-
23 expressed genes, but the influence of these genes on male reproductive behavior also
24 suggests that they have evolved to regulate their own transmission to successive
25 generations via the male germline.

26

27 Keywords: genomic imprinting; imprinted genes; reproduction; sexual behavior; maternal
28 behavior; hypothalamus; paternally expressed genes; coadaptation; conflict

29 **Introduction**

30 In the early nineteen-eighties it became clear that the parental genomes in mammals were
31 functionally non-equivalent (McGrath and Solter, 1984; Surani et al., 1984), a phenomenon
32 termed 'genomic imprinting'. A subset of autosomal genes is expressed not in accordance
33 with classical Mendelian laws of inheritance, but according to the sex of the parent from
34 which they are inherited. Imprinted genes are thus not expressed biallelically but in non-
35 stochastic, monoallelic fashion from either the maternally donated allele or the paternally
36 donated allele. This parent-of-origin silencing of specific alleles is an important example of
37 stable epigenetic regulation of gene expression and of crucial importance in mammalian
38 development. Silencing at imprinted genes involves both DNA methylation and chromatin
39 modification (Delaval and Feil, 2004) and imprinting research has revealed much about
40 mechanisms of epigenetic regulation.

41 The original experiments by McGrath and Solter (1984) and Surani et al (1984)
42 demonstrated that mouse embryos created with two male pronuclei (androgenetic – 'AG') or
43 two female pronuclei (gynogenetic – 'GG') failed to reach term. The absence or doubling of
44 expression of imprinted genes resulted in lethal phenotypes very early in development. Later
45 research in which such AG or GG embryos were rescued by fusing them with wild-type
46 blastocysts revealed the different developmental roles played by the two parental genomes.
47 AG chimeras were much larger at birth than wild-type embryos while GG chimeras were
48 much smaller (Allen et al., 1995; Barton et al., 1991), suggesting that the paternal and
49 maternal genomes regulate offspring growth in opposite directions. Analysis of the fate of
50 AG and GG cells in brains of these chimeras showed that GG cells segregated almost
51 exclusively to the cortex and striatum, while AG cells were only found in the hypothalamus
52 (Allen et al., 1995; Keverne et al., 1996). The differential fate of these AG and GG cells
53 suggested that paternally and maternally expressed genes regulate the development and
54 function of different brain areas. In recent years imprinted genes have been shown to have
55 important roles in brain function and in behavior (Davies et al., 2008; Isles and Wilkinson,
56 2000; Wilkinson et al., 2007).

57 Imprinted genes present a particularly interesting genetic conundrum, as their haploid
58 expression results in the loss of protection from mutation which diploidy confers (Orr, 1995)
59 and so would seem to be a non-adaptive evolutionary step. The debate about how and why
60 such a mechanism has evolved has been ongoing since genomic imprinting was first
61 discovered (Hurst and McVean, 1997; Hurst and McVean, 1998; Moore and Haig, 1991;
62 Moore and Mills, 2008) but the evolution of placentation and viviparity in mammals appears
63 to coincide with the acquisition of genomic imprinting (Kaneko-Ishino et al., 2003). There are
64 approximately 80 imprinted genes in eutherian mammals (Morison et al., 2005), of which
65 only a subset are imprinted in marsupials (Renfree et al., 2008), and none in monotremes
66 (Hore et al., 2007; Killian et al., 2001). The taxonomic distribution of imprinting coincides with
67 the relative placental complexity and the development of viviparity in these different
68 mammalian groups. Eutherians have elaborate placentas and prolonged gestation,
69 marsupials have more rudimentary placentas and give birth to very altricial young, while
70 monotremes are egg-laying mammals in which there is only a brief period of maternal
71 nutrient supply after fertilization (Renfree et al., 2009). Moreover, almost all imprinted genes
72 are expressed in the placenta (Bressan et al., 2009; Coan et al., 2005) and imprinted genes
73 are also strongly expressed in the developing embryo. The importance of imprinted genes in
74 development is indicated by the proteins they encode, which include growth factors,
75 transcription regulators, apoptotic proteins and regulatory non-coding RNAs (Morison et al.,
76 2005). The taxonomic patterns of genomic imprinting show that this regulatory mechanism
77 has evolved in step with modes of reproduction in mammals, while the expression and
78 functions of imprinted genes show that they play important developmental roles in
79 reproduction. However experimental evidence has been also been accumulating that
80 suggests that imprinted genes are involved in mammalian reproduction beyond just fetal
81 development and placental functions. The influence of imprinted genes on brain and
82 behavior extends to post-natal offspring behavior as well as adult reproductive behaviors.
83 These findings raise new questions about the roles that imprinted genes play in behavior
84 and consequently about the selective pressures which drove their evolution.

85

86 **Genomic imprinting mechanisms and reproduction**

87 The majority of imprinted genes are organized into clusters where gene expression is
88 regulated from imprinting control regions (ICRs) associated with these clusters (Williamson
89 et al., 2006). The clustering of imprinted genes and the shared ICRs suggest that imprinting
90 has evolved as a regulatory mechanism to which genes are recruited, rather than evolving
91 independently at each imprinted gene. ICRs contain differentially methylated regions (DMRs)
92 rich in CpG repeats which are methylated on one of the parental alleles and not the other,
93 and which determine the monoallelic expression of genes in the imprinted cluster. While
94 other epigenetic mechanisms are also involved, such as histone modification and non-
95 coding microRNAs, DNA methylation is the main mechanism by which imprinted gene
96 expression is regulated (Delaval and Feil, 2004; Kacem and Feil, 2009). The imprints at
97 each DMR must be recapitulated within the germline of each generation to reflect the gender
98 of the individual and ensure the correct complement of imprinted genes in offspring. This is
99 achieved in the germ line when imprints are erased and re-established in a sex specific
100 manner during gametogenesis in the early embryo. In both males and females, primordial
101 germ cells (PGCs) migrate to the genital ridge where they undergo widespread epigenetic
102 modification including active demethylation of parental imprints (between embryonic days
103 10.5 and 12.5 in the mouse) (Sasaki and Matsui, 2008). After this demethylation of DMRs
104 there is subsequent sexual dimorphism in imprint application. In females, DNA methylation
105 at ICRs occurs after birth as the post-meiotic oocytes grow and mature (Hiura et al., 2006),
106 while in males the paternal imprints are applied in the pre-meiotic prospermatogonia before
107 birth. Moreover, the vast majority of imprinted ICRs are methylated during female gamete
108 formation and only 3 DMRs have been identified which are methylated during
109 spermatogenesis in males (Sasaki and Matsui, 2008). This asymmetry indicates that the
110 mechanism of imprinting is primarily under maternal control and involves active silencing of
111 maternal alleles in the female germ line.

112 As with other forms of epigenetic regulation, the methylation of imprinted DMRs during
113 gametogenesis is mediated by DNA methyltransferases (DNMTs). DNMT3A mediates *de*
114 *novo* methylation in both male and female gametes, although in males a second
115 methyltransferase, DNMT3B, is also involved in methylation at the *Rasgrf1* imprinted locus
116 (Kato et al., 2007). Furthermore, the methylation of all imprinted DMRs is dependent on
117 DNMT3L, which is non-enzymatic but plays a crucial role in methylation at imprinted loci
118 (Kaneda et al., 2004). DNMT3L appears to form a complex with DNMT3A/DNMT3B and
119 interprets an existing mark (possibly histone-based) which is then converted into gender-
120 specific DNA methylation at DMRs. The importance of appropriate levels of imprinting in
121 mammalian gametogenesis is illustrated by the phenotype of DNMT3L mutant mice.
122 Deleting DNMT3L and thus disrupting methylation at DMRs causes gross reproductive
123 deficits in both sexes such that males are azoospermic and infertile, while females are
124 unable to produce viable offspring (Schaefer et al., 2007). DNMT3L's important role in
125 imprinting mechanisms is also evident from phylogenetic comparisons of the mammalian
126 taxa: while DNMT3L is found in both eutherians and marsupials, there appears to be no
127 ortholog in the monotremes which lack imprinted genes (Renfree et al., 2009; Yokomine et
128 al., 2006).

129

130 **Imprinting and reproduction in mammals**

131 Almost all imprinted genes are expressed in either the embryo, the placenta or both and
132 have been shown to have important regulatory roles in development. Imprinted genes are
133 explicitly involved in mammalian reproduction through their influence on fetal development
134 (Constancia et al., 2002) and placental function (Charalambous et al., 2010), however they
135 also influence reproduction in other ways. Much of the research into the roles and functions
136 of imprinted genes has involved the use of imprinted gene-knockout mice and while such
137 studies have only been conducted in one experimental model, they indicate that imprinted
138 genes are important regulators of reproductive behavior in the mouse and potentially also in
139 other mammals. Due to monoallelic expression of imprinted genes, these knockouts also

140 provide unique opportunities to study imprinted genes independently in mother and offspring
141 (fig. 1). Such knockout mouse studies have demonstrated that imprinted genes have
142 important effects on brain function (Wilkinson et al., 2007) and behavior (Isles and Wilkinson,
143 2000) which include post-natal offspring behavior as well as reproductive behavior in both
144 males and females. While direct effects on reproductive potential of global disruption of
145 imprinting are evident in the infertility of DNMT3L-knockout mice in which appropriate
146 imprinting is not established in the germline, behavioral effects suggest that imprinted genes
147 also regulate different aspects of mammalian reproduction through effects on the brain (table
148 1). Such effects are particularly evident in knockouts of paternally expressed genes, perhaps
149 due to their high levels of expression in the hypothalamus and related structures which
150 mediate reproductive behaviors in mammals.

151 ***Imprinted gene effects in offspring***

152 The first mouse knockouts of the imprinted genes *Igf2*, *Igf2r* and *H19* demonstrated that
153 imprinted genes are involved in placentation and embryonic development (Barlow et al.,
154 1991; Bartolomei et al., 1991; DeChiara et al., 1991), and subsequent studies also showed
155 imprinted gene effects on post-natal behavior in offspring. One of the first such studies
156 involved the paternally expressed gene *Peg1/Mest* on mouse chromosome 6. Although a
157 paternally inherited mutation in this gene results in reduced weight at birth, this deficit is
158 subsequently exacerbated by reduced post-natal growth rate relative to wild-type littermates
159 (Lefebvre et al., 1998). This suggested that imprinted genes not only regulate resource
160 extraction during gestation, but could also influence offspring physiology or behavior post-
161 natally.

162 More detailed evidence for such post-natal effects came from studies of the paternally
163 expressed gene *Peg3*, which encodes a large zinc-finger protein on mouse proximal
164 chromosome 7 (Kuroiwa et al., 1996). Paternally-inherited deletions of *Peg3* cause deficits
165 not only before birth but also post-natally, affecting growth, suckling and thermoregulation
166 (Curley et al., 2004). *Peg3* mutant animals have small placentas and low birth weight which
167 they fail to make up after birth. These pups remain smaller than their wild-type littermates

168 throughout post-natal nursing due to a suckling deficit which reduces their ability to take on
169 milk. The *Peg3* mutation also affects thermoregulation as mutant pups are unable to
170 maintain body temperature in response to maternal separation. Metabolism, appetite and
171 thermoregulation are all mediated by the hypothalamus, an area where there is high *Peg3*
172 expression during development (Li et al., 1999). The *Peg3* protein is involved in p53-
173 mediated apoptosis (Deng and Wu, 2000) and these mutant animals show alterations in
174 postnatal hypothalamic apoptosis (Broad et al., 2009), which suggests that aberrant
175 neuronal pruning during development may disrupt normal functioning of the hypothalamus.
176 Further disruption to hypothalamically-mediated behavior is seen in adolescence when *Peg3*
177 females enter puberty later than their wild-type littermates (Curley et al., 2005).

178 The *GNAS* locus on mouse distal chromosome 2 encodes multiple transcripts with different
179 parent-of-origin expression (Peters et al., 1999), including paternally expressed *Gnasxl* and
180 *Nespas*, maternally expressed *Nesp*, and maternally expressed *Gnas*, which is only
181 imprinted in adipose and endocrine tissues (Peters and Williamson, 2007). The relationship
182 between these different transcripts is complex, however a lack of the protein XL α s, the
183 *Gnasxl* product, causes deficits in post-natal behavior that bear some similarities to those
184 seen in *Peg3* mutant pups (Plagge et al., 2004). Mice inheriting a paternal deletion of *Gnasxl*
185 suffer post-natal growth retardation caused by metabolic deficits and an inability to suckle
186 properly resulting in death no later than post-natal day 9. *Gnasxl* is expressed in the
187 hypothalamus and pituitary that regulate energy homeostasis and in pontine and medullary
188 nuclei which innervate the facial and jaw muscles involved in suckling. *Gnasxl* appears to be
189 directly involved in the neural and endocrine control of post-natal suckling behavior and the
190 similar phenotypes seen in the *Peg1*, *Peg3* and *Gnasxl* mutants suggest that these
191 paternally expressed genes are all involved in the ability of mouse pups to thrive post-
192 nally.

193 ***Imprinted gene effects in females***

194 As well as affecting offspring, deletion of paternally expressed *Peg1/Mest* also affects
195 female maternal care (Lefebvre et al., 1998). Pups born to mutant mothers suffer elevated

196 post-natal mortality, even if they are wild-type offspring with a normal paternal *Peg1/Mest*
197 allele. The deletion affects multiple maternal behaviors and mutant females do not engage in
198 placentophagia after birth and exhibit reduced levels of pup investigation, pup retrieval to the
199 nest, nest building and post-natal nursing.

200 Similarly, the *Peg3* deletion also results in an adult phenotype which includes effects on
201 maternal care. Li et al (1999) noted very high levels of pre-weaning mortality in the offspring
202 of heterozygous *Peg3* mutant females despite these pups being wild-type, having been
203 fathered by wild-type males with normal *Peg3* expression. The elevated mortality thus could
204 not have been due to direct genetic effects in the wild-type offspring but rather to the
205 maternal responses of the females. Behavioral phenotyping of the *Peg3* mutant mothers
206 revealed a wide-ranging deficit affecting nest building, retrieval of scattered pups to the nest
207 and milk letdown. More detailed characterization of maternal behavior showed that overall
208 nursing levels are lower and that licking and grooming of pups is also reduced in these
209 females (Champagne et al., 2009). Licking and grooming is an important modulator of
210 offspring behavioral phenotypes (Francis et al., 1999) and it causes stable epigenetic
211 changes in gene expression (Weaver et al., 2004). The offspring and even grand-offspring of
212 these *Peg3* mutant mothers also display lower levels of maternal care, despite being wild-
213 type themselves (Curley et al., 2008). *Peg3* thus appears to exert an influence on offspring
214 behavioral phenotypes through its effects on maternal care. The *Peg3* mutation appears to
215 involve disruption of the oxytocin circuitry in the hypothalamus where *Peg3* is expressed.
216 Oxytocin is an important regulatory neuropeptide in maternal behavior and milk production
217 and the maternal effects of the knockout appear to be mediated by a reduction in the
218 number of oxytocinergic neurons in the paraventricular nucleus (Li et al., 1992) and a
219 reduction in oxytocin receptor density in the medial pre-optic area (Champagne et al., 2009).
220 *Peg3*'s apoptotic function suggests that developmental disruption in the hypothalamus leads
221 to these functional changes and then to the maternal behavior deficits. Indeed, *Peg3* mutant
222 mice have elevated levels of apoptosis in the medial preoptic area at postnatal days 4 and 6.
223 No changes are seen in paraventricular apoptosis at these time points, however this does

224 not discount the possibility of perturbations during embryonic development (Broad et al.,
225 2009).

226 Recent studies of the paternally expressed gene *Mage12* have demonstrated that imprinted
227 genes can influence female fertility and reproductive physiology as well as maternal care.
228 The first knockout study of *Mage12* showed that these mutant mice have aberrant circadian
229 rhythms due to abnormalities in the suprachiasmatic nucleus of the hypothalamus (Kozlov et
230 al., 2007). Further phenotyping has shown they have a more wide-ranging deficit in
231 hypothalamic function, with significant effects on female reproduction (Mercer and Wevrick,
232 2009). *Mage12* mutant females enter puberty late, despite similar post-weaning body weights
233 in mutant and normal females. These females also have extended and irregular estrous
234 cycles and enter reproductive decline at an early age, becoming infertile after 24 weeks
235 when corpora lutea are no longer seen in the ovaries, even though mature follicles are
236 present. *Mage12* mutant females were also slower to mate after pairing with males, produced
237 smaller litters and had significantly higher litter mortality rates which were not connected to
238 litter genotype.

239 ***Imprinted gene effects in males***

240 The effects on female behavior described above all involve paternally expressed genes. All
241 are strongly expressed in the hypothalamus in females and males, and thus it is not
242 surprising that the phenotypes of several paternally expressed gene knockouts involve
243 effects on male reproductive behavior too. As well as disrupting female maternal behavior, a
244 paternal deletion of *Peg3* also causes reproductive deficits in males. *Peg3* mutant males are
245 fertile but are unable to improve copulatory ability with sexual experience, unlike wild-type
246 animals. Sexual experience typically results in shorter latencies and increased frequencies
247 of sexual behaviors such as mounting and intromission in wild-type males but these
248 behavioral changes are not seen in *Peg3* mutant males whose behavior once sexually
249 experienced does not differ from that of virgin animals (Swaney et al., 2007). Wild-type male
250 mice also develop preferences for the odors of receptive estrous females once sexually
251 experienced, potentially enabling them to focus reproductive effort towards receptive

252 females. *Peg3* mutant males show no such changes in female-odor sensitivity (Swaney et
253 al., 2008). While such a deficit may appear relatively minor, olfaction is the primary sensory
254 modality in rodents as illustrated by the gross deficits in reproductive behavior which result
255 from ablation of olfactory membranes (Keller et al., 2009). The *Peg3* mutation appears to
256 disrupt plasticity in the main and accessory olfactory systems and in hypothalamic regions
257 which regulate sexual behavior, where significant increases in female odor-elicited neural
258 activity are seen in sexually experienced wild-type males but not in *Peg3* mutant males
259 (Swaney et al., 2007; Swaney et al., 2008).

260 Although *Peg1* mutant mice have not been reported to suffer deficits in male reproductive
261 behavior, studies in humans and mice suggest that this gene is involved in male fertility. The
262 human homolog *PEG1* and its paternally expressed anti-sense transcript are strongly
263 expressed in human testes and studies of male infertility have shown that hypermethylation
264 of the *PEG1* DMR and consequent silencing are strongly associated with different classes of
265 male infertility (Hammoud et al., 2009; Poplinski et al., 2009). In mice, pre-natal
266 administration of the endocrine disruptor vinclozolin produces male offspring with
267 significantly lower sperm counts with adults. Sperm from these males have greatly increased
268 methylation at both the *Peg1* and *Peg3* DMRs suggesting that normal methylation patterns
269 at these imprinted loci are necessary for male fertility (Stouder and Paoloni-Giacobino,
270 2009).

271 The *Mage/2* mutants also have deficits in male reproductive behavior (Mercer and Wevrick,
272 2009), with some phenotypic similarities between mutant males and mutant females. Mutant
273 males also become infertile by 24 weeks and take several days to mate after being paired
274 with females. However the male phenotype also involves an olfactory deficit which includes
275 a lack of preference for opposite-sex odors which is not seen in the female mutants. Basal
276 forebrain neurochemistry animals is also affected by *Mage/2* deletion which causes
277 reductions in levels of both serotonin and dopamine in the hypothalamus, as well as reduced
278 amygdala and nucleus accumbens volumes (Mercer et al., 2009). In line with the
279 reproductive deficits, *Mage/2*-knockout males also have reduced serum testosterone levels.

280 These hypothalamic and endocrine deficits are of interest given the involvement of the
281 human homolog *MAGEL2* in Prader-Willi syndrome (PWS), a multigenic imprinting disorder
282 involving paternal deletion or maternal duplication at chromosome 15q11-q13 region (Lee et
283 al., 2000). PWS symptoms involve hypothalamic dysfunction affecting appetite and
284 metabolism, as well as hypogonadism and infertility (Eiholzer et al., 2006). Aberrant
285 expression of paternally expressed *NDN* is also involved in the etiology of PWS and mouse
286 knockout studies have shown that paternal deletion of homologous *Necdin* disrupts
287 reproductive hormone circuits. Muscatelli et al (2000) reported that *Necdin*-null mice (both
288 male and female) have fewer oxytocin neurons in the paraventricular nucleus and fewer
289 hypothalamic GnRH neurons. This latter deficit has recently been shown to be due to
290 reduced migration of GnRH neurons to the hypothalamus during development (Miller et al.,
291 2009), and lower numbers of both oxytocin and GnRH neurons have been reported in PWS
292 patients. The *Magel2* and *Necdin* mutant phenotypes suggest that disruption of
293 developmental expression contributes to PWS hypogonadism and provides further evidence
294 that paternally expressed genes influence male reproductive behavior.

295

296 **Reproduction and the evolution of genomic imprinting**

297 There have been many theories proposed to explain the evolution of imprinting, each of
298 which has different strengths and weaknesses regarding mechanisms of imprinting and
299 imprinted gene-related phenotypes. The most widely cited of these is the conflict or kinship
300 theory for the evolution of imprinting (Haig and Graham, 1991; Moore and Haig, 1991). This
301 predicts that the conceptus (placenta and offspring), which inherits approximately half of its
302 genes from its mother and half from its father, may be a site for potential conflict between the
303 two parental genomes. The paternal genome would favor maximal maternal investment in
304 offspring, to the possible detriment of the mother's long term health and any subsequent
305 offspring conceived with other males. Conversely, the maternal genome would favor
306 balanced investment of resources in all offspring across her entire reproductive career. The
307 placenta is an important endocrine organ that interfaces and communicates with the

308 maternal brain to regulate maternal investment and so is functionally capable of influencing
309 maternal behavior and physiology. The conceptus can thus be viewed as a parasite in which
310 imprinted genes influence resource extraction from the mother in opposite directions.
311 Paternally expressed genes are predicted to be growth-enhancing and maternally expressed
312 genes are predicted to be growth-restricting. The first knockout studies showed that
313 paternally expressed *Igf2* is indeed growth enhancing (DeChiara et al., 1991), while
314 maternally expressed *Igf2r* and *H19* limit fetal growth (Lau et al., 1994; Leighton et al.,
315 1995). While mutations at other imprinted loci also cause growth phenotypes which match
316 the predictions of the conflict theory, there are some that do not (Hurst and McVean, 1997).
317 Furthermore, some of the imprinted gene knockout mice also exhibit phenotypes which
318 extend beyond birth and even into adulthood, where conflict between the parental genomes
319 is less apparent. While it can be argued that post-natal, pre-weaning effects of imprinted
320 genes are in line with the predictions of the conflict hypothesis (Isles and Holland, 2005), the
321 adult phenotypes of some imprinted gene mutants are more difficult to reconcile with it.
322 The parent-of-origin transmission of the *Peg3* transgene allowed the effects of the mutation
323 to be compared independently in mutant pups and mutant mothers. The phenotypes are
324 remarkably complementary, with offspring deficits in pre-natal growth, suckling, and
325 thermoregulation matched by reduced maternal food intake, milk letdown and nesting
326 behavior (Curley et al., 2004). These result in similar outcomes for the offspring whether they
327 or the mother carry the mutation. The different peri-natal behaviors are regulated by an
328 endocrine interplay between the fetal hypothalamus, the maternal hypothalamus and the
329 placenta (Keverne and Curley, 2008), all areas of high *Peg3* expression. *Peg3* appears not
330 to have evolved in response to conflict between parental genomes but under selection
331 pressures that favor coadaptation of behaviors between offspring and mother, leading to
332 increased fitness for both. While no other imprinted gene mutants have such clearly
333 dovetailing phenotypes, the effects of deletions of other paternally expressed genes on
334 maternal behavior and post-natal behavior suggest that coadaptation may have played an
335 important role in the evolution of imprinting at multiple loci. Detailed behavioral phenotyping

336 of other imprinted gene knockouts would help to substantiate the evolutionary significance of
337 coadaptation and clarify whether other imprinted genes may also have evolved under such
338 coadaptive selection pressures. Moreover, data from other species is required to confirm the
339 importance of imprinted genes in mammalian reproduction and whether coadaptation has
340 played a wider role in the evolution of imprinting.

341 Some imprinted genes seem to have evolved to regulate female reproductive behavior
342 despite being paternally expressed. Such effects are not surprising given the high
343 expression of paternally imprinted genes in the hypothalamus which regulates reproductive
344 behavior and maternal care, however any adaptive effects will skip every other generation
345 due to the silencing of paternally expressed genes in the female germline. Most of these
346 genes have also been shown to influence male reproductive behavior to greater or lesser
347 degrees. This is significant, as any effects of paternally expressed genes on male behavior
348 would occur in every generation due to patrilineal transmission of actively expressed alleles
349 at these loci. Any effects on male reproductive success would be magnified by a
350 combination of paternal allele-only expression and reproductive skew, resulting in much
351 faster spread of any paternally expressed alleles that regulate adaptive male reproductive
352 behavior (Keverne, 2009). Paternal expression appears to have allowed the imprinted genes
353 that govern the development and function of the hypothalamus to regulate both male and
354 female reproduction, and so maximize reproductive success in both sexes.

355

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541

542 Figure 1. Non-Mendelian parent-of-origin expression allows imprinted genes to be studied
543 independently in mother and offspring using knockout mice. Selective silencing of one
544 parental allele means that heterozygous transgenic mice have either full mutant or full wild-
545 type (WT) phenotypes depending on the imprinting status of the gene and the parent from
546 which the mutation is inherited. By pairing different combinations of mutant-heterozygous,
547 WT-heterozygous and WT individuals, mutant offspring can be born to wild-type mothers
548 and wild-type offspring born to mutant mothers. a) Hypothetical mating combinations to
549 study a paternally expressed gene knockout: i) crossing a WT father with a mutant-
550 heterozygous mother produces WT and WT-heterozygous offspring; ii) crossing a mutant-
551 heterozygous father with a WT mother produces WT and mutant-heterozygous offspring. b)
552 Hypothetical mating combinations to study a maternally expressed gene knockout: i)
553 crossing a WT father with a mutant-heterozygous mother produces WT and mutant-
554 heterozygous offspring; ii) crossing a WT-father with a WT-heterozygous mother produces
555 WT and mutant-heterozygous offspring.

556

557 Table 1. A summary table of the imprinted genes which have been linked to behavioral and
558 neuroendocrine components of reproduction in mammals.

Gene	Expression	Offspring	Females	Males	References
Peg1	Paternal	Post-natal growth	Maternal behaviors	Male fertility and sperm viability	(Lefebvre et al., 1998; Hammoud et al., 2009; Poplinski et al., 2009)
Peg3	Paternal	Post-natal growth, suckling, thermo-regulation, puberty	Maternal behaviors, milk letdown, hypothalamic oxytocin	Sexual behavior, reproductive olfaction, hypothalamic plasticity	(Li et al., 1992; Curley et al., 2004; Champagne et al., 2009; Swaney et al., 2007; Swaney et al., 2008)
Gnasxl	Paternal	Post-natal growth, suckling	-	-	(Plagge et al., 2004)
Magel2	Paternal	-	Reproductive behavior, fertility, gonadal histology hypothalamic serotonin and dopamine, hypogonadism	Reproductive behavior, fertility, reproductive olfaction, hypothalamic serotonin and dopamine, testosterone levels, hypogonadism	(Mercer and Wevrick, 2009; Mercer et al., 2009)
Necdin	Paternal	-	Hypothalamic oxytocin and GnRH, hypogonadism	Hypothalamic oxytocin and GnRH, hypogonadism	(Muscatelli et al, 2000; Miller et al., 2009)