Molecular evolution of imprinted genes: no evidence for antagonistic coevolution

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SUMMARY

Genomically imprinted genes are those for which expression is dependent on the sex of the parent from which they are derived. Numerous theories have been proposed for the evolution of genomic imprinting; one theory is that it is an intra-individual manifestation of classical parent–offspring conflict. This theory is unique in predicting that an arms race may develop between maternally and paternally derived genes for the control of foetal growth demands. Such antagonistic coevolution may be mediated through changes in the structure of the proteins concerned. Comparable coevolution is the most likely explanation for the rapid changes seen in antigenic components of parasites and antigen recognition components of immune systems. We have examined the evolution of insulin-like growth factor (Igf2) and its antagonistic receptor (Igf2r) and find that, in contrast to immune genes, at the sites of mutual binding they are highly conserved. In addition, we have analysed the rate of molecular evolution of seven imprinted genes (including Igf2 and Igf2r), sequenced in both mouse and rat, and find that this is the same as that of non-imprinted receptors and significantly lower than that of immune genes (controlling for differences in mutation rate). Contrary to the expectations of the conflict hypothesis, we hence find no evidence for antagonistic coevolution of imprinted genes mediated by changes in sequence.

1. INTRODUCTION

The spread of selfish elements within a population creates the context for the spread of genes that act within the same individual as the selfish element and that oppose the selfish effect. Segregation distorter (SD) in Drosophila melanogaster is a case in point. This meiotic drive gene spreads in a population, not because it increases the fitness of its bearers, but because when a male is heterozygous at the SD locus, SD kills sperm from the same male not bearing the SD allele (i.e. those with the wild-type allele). The spread of such ‘parasitic’ chromosomal elements provides the conditions for the invasion of unlinked suppressors of SD’s distorting potential. Given that within one individual there are genes with opposing effects (one acting to distort, one acting to suppress distortion), we may say that a ‘conflict of interests’ exists. Such a state of conflict can potentially give rise to extended bouts of antagonistic coevolution between distorter and suppressor (reviewed in Hurst et al. 1996a), with distorters evolving to escape suppression and suppressors evolving to counteract the new form of distorter (cf. host–parasite coevolution, predator–prey coevolution, etc.).

A few examples of intragenomic antagonistic coevolutionary arms races have been discussed. The evolution of a multicopy gene on D. melanogaster’s X chromosome that is suppressed by a multicopy gene on the Y chromosome has been proposed to be an instance of an arms race between a meiotically driving X and its Y-linked suppressor (Hurst 1992). The model is born out by both linkage analysis and segregation analysis (employing data from Palumbo et al. (1994)), which shows that, in the absence of the suppressor, X-linked drive is found, and the intensity of drive is proportional to the copy number of the gene (Hurst 1996b). It has also been proposed that many genes involved in maternal–foetal interactions may evolve in coevolutionary arms races (Haig 1993). Foetal genes, for example, may attempt to increase sugar levels in maternal blood, although mothers may prefer to reduce these levels (Haig 1993). Escalated antagonistic coevolution is proposed as the reason for high levels of foetally secreted products (e.g. placental lactogens) and for maternal counteracting products (Haig 1993). Similarly, it has been postulated (Haig 1993) that some multicopy gene arrays putatively involved in maternal–foetal coevolution (e.g. those of human chorionic gonadotrophin) may have arisen under selection for increased dosage of the gene products concerned.

Antagonistic coevolution need not be mediated only by changes in dosage, as in the above examples. Changes in gene sequence may also be effective. Imagine, for example, a system in which the suppressor acts by binding to the protein product of the distorter.

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A novel form of the distorter that can both distort and evade (even to a slight degree) the binding by the suppressor is likely to spread and create the context for the invasion of a novel form of suppressor that can bind more effectively the new form of distorter protein. Such coevolution may be detected by finding high rates of non-synonymous substitutions (controlling for the mutation rate).

An arms race of this variety is the most likely explanation for the rapid evolution of antigenic components of parasites and antigen recognition components of immune systems (Hughes & Nei 1988; Hughes et al. 1990; Hughes 1991, 1993, 1997; Kuma et al. 1995; Endo et al. 1996). A parasite with a slightly new antigen unrecognized by the immune system will spread in the population. This in turn will provide the conditions for the spread of a novel immune component capable of recognizing the new antigen. In turn this provides conditions for the spread of a new form of the antigen, etc. Hence continual host–parasite coevolution results in rapid sequence evolution of immune components and parasite antigens. Several genes potentially involved in maternal–foetal interactions are also rapidly evolving (e.g. placental lactogens in ruminants (Wallis 1993) and rodents (discussed in Hurst 1994), growth hormone (Wallis 1994), growth hormone releasing factor (data in Wolfe & Sharp 1993), prolactin (Wallis 1981) and PEM (Maiti et al. 1996)). In some cases the rapid evolution of maternal–foetal interaction genes may be associated with recent development of a new function (e.g. human chorionic gonadotrophin; Haig 1993).

Here we analyse the pattern of molecular evolution of genomically imprinted genes as these are putatively involved in a form of intragenomic conflict (Haig & Westoby 1989; Haig & Graham 1991; Moore & Haig 1991; Haig 1992; Mochizuki et al. 1996). In at least some instances protein–protein antagonistic interactions are involved (discussed in Haig & Graham 1991).

2. GENOMIC IMPRINTING

Although it is usually assumed that the parental derivation of a gene is irrelevant to the functioning of that gene, this is not always true. Some genes are only active if inherited from the mother (paternally imprinted), whereas others are the reverse (for review see Efstratiadis 1994; Ohlsson et al. 1995; Reik & Surani 1997). Imprinting of murine insulin-like growth factor 2 \( (\text{Igf}^2) \) and the \( \text{Igf}^2 \) receptor \( (\text{Igf}^2_\text{R}) \); alias the cation-independent mannose-6-phosphate receptor) is the best-described example (Efstratiadis 1994; Ohlsson et al. 1995; Reik & Surani 1997). Early in murine embryogenesis the paternally inherited copy of \( \text{Igf}^2 \) is expressed while the maternally inherited copy remains silent. Conversely, the maternally inherited copy of an antagonist to \( \text{Igf}^2 \) (i.e. \( \text{Igf}^2_\text{R} \)) is expressed while the paternal copy of this remains silent.

This antagonism has been interpreted as the outcome of a genetic conflict (Haig & Westoby 1989; Haig & Graham 1991; Moore & Haig 1991; Haig 1992; Mochizuki et al. 1996). These authors' arguments stem from their observations that if a mother has more than one mate, paternally derived genes in any given foetus will not necessarily be related to genes in fellow brood members or subsequent offspring of that same mother. In contrast, they note, a maternally derived gene in any given foetus has a constant high probability \( (p = 0.5) \) that its sibs will contain a clonal copy of it. This tripartition asymmetry in relatedness between (i) paternally derived foetal genes, (ii) maternally derived foetal genes and (iii) genes in the mother (and hence in the foetus's sibs) creates, they argue, a three-way conflict of interest over how much nutrition the foetus should demand from the mother. It would be optimal for the mother to divide resources more or less equally and so maximize her net fitness, not the fitness of any given progeny. In contrast, if the paternally derived genes in any given foetus are unrelated to those in other progeny, then any decrement in maternal fitness resulting in reduced offspring-production is irrelevant to these paternally derived genes. Paternally derived genes in a given foetus might thus prefer an allocation in excess of that preferred by the mother. The optimal amount of resources that the maternally derived genes in the foetus should require will be intermediate between the optima for the paternally derived genes (a large amount) and the amount the mother should be prepared to provide (a smaller amount).

It is then argued that growth factor genes that are paternally inherited should be expressed, leading to increased nutrient demand from the mother. The same genes when maternally inherited should be inactive. In contrast, growth factor suppressor genes should be active if maternally derived, so allowing some resources to be directed to their close relatives (both present and future ones). The same genes should be inactive if paternally derived.

From over 14 theories for the evolution of genomic imprinting (reviewed in Haig & Trivers 1995; Hurst 1997) the conflict theory is unique in predicting that (i) genes expressed on the paternally derived genome should be growth enhancers while those expressed on the maternal genome should be growth suppressors (Haig & Westoby 1989; Haig & Graham 1991; Moore & Haig 1991; Haig 1992; Mochizuki et al. 1996), and (ii) maternally and paternally expressed imprinted genes may antagonistically coevolve. The first proposition appears, in general, to be correct, i.e. there is a statistically significant covariance between the direction of imprinting and the direction of growth effects (Hurst et al. 1996b). One more recent data point (Plass et al. 1996) is also consistent with this conclusion. A few possible exceptions (e.g. \( \text{Mash}^2 \) (Guillemot et al. 1995) and \( \text{Gnas} \) (Williamson et al. 1996)) do not disturb this general conclusion but require explanation. Analysis of uniparental disomies also provides supportive evidence (Moore & Haig 1991), although there again exist exceptions (Lyon 1993).

Evidence of antagonistic coevolution is less clear. It has been suggested that both the small size of introns of imprinted genes (Hurst et al. 1996b) and the excess of intronless imprinted genes (Hurst et al. 1996b) are consistent with a dosage-mediated arms race (the
former being putatively the result of selection for fast transcription, the latter a result of selection for high copy number mediated by activation of retrotransposed sequences). However, direct evidence of rapid transcription is lacking in most cases and other reasons for small intronic dimensions are possible (Haig 1996; McVean et al. 1996). An excess of intronless genes may also be expected if it is easier for genes to move to imprinted domains than for an imprint to be initiated around a particular gene (Hurst et al. 1996b).

Systematic analysis of rates of evolution of imprinted genes has yet to be performed. Here we report three analyses. We examine the position of substitutions in (i) Igf2 and (ii) Igf2r to see whether at their sites of mutual binding they have either higher or lower rates of non-synonymous substitutions, and (iii) we collate all available gene sequences of imprinted genes in both mouse and rat and compare their rate of evolution to sets of genes that are considered to be undergoing antagonistic coevolution and those that are not expected to do so.

3. METHODS

Sequences were sought by searching EMBL by gene name through SRS. Alignment of sequences was carried out using PILEUP from GCG (1994). This was done both with nucleic acid and protein sequence to ensure correspondence between the two. For analysis of the intragenic location of substitutions into Igf2, we used sequences from nine species of mammal: Bos taurus (Z68151), Caria porcellus (S59899), Equus caballus (U11241), Homo sapiens (M29645), Mustela vison (S63459), Mus musculus (M36331 and M36332), Otis aries (M89788), Rattus norvegicus (M29879 and M29880) and Sus scrofa (X56094). The location of binding sites has been described previously (Sakano et al. 1991; Hashimoto et al. 1995). The sequences were analysed using PROTPARS and DNAPARS from PHYLIP (Felsenstein 1995), which provides phylogenetic independent assessment of the position of substitutions. Two most parsimonious trees were found, the one of greater maximum likelihood was employed.

For analysis of substitutions into Igf2r we used sequences in Mus musculus (U04710) and Rattus norvegicus (U59809). The number of substitutions between the two was averaged in overlapping blocks of 240 base pairs through the gene (i.e. using a sliding window). The location of binding sites has previously been determined (Szabanyi & Rotwein 1994; Schmidt et al. 1995).

The set of seven imprinted genes examined in mice and rats included Igf2 and Igf2r (accession numbers as above), Ins1 (Mus: X04725, Rattus: J00747), Ins2 (Mus: X04724, Rattus: J00748), Snyp (Mus: X62648, Rattus: M29929), Gisp (Mus: L20899, Rattus: X67241) and Mas (Mus: X67735, Rattus: J03823). We compared imprinted genes with two control sets: immune genes and non-immune, non-imprinted receptors. The two control sets were assembled from previous analyses (Wolfe & Sharp 1993). To enable comparison with this previously described data, the extent of synonymous and non-synonymous divergence in imprinted genes was assayed as by the method of Li et al. (1985).

Genes were included as putatively immune-related if (i) their activity was immune-specific, and/or (ii) they had known immune-related function (e.g. a role in disease resistance). This method to prescribe a gene as being within an immune system gene was deliberately chosen to be conservative (hence if imprinted genes do not evolve as fast as immune genes it is not because the immune genes selected are an unusually fast evolving collection). It is indeed quite possible that genes with no interaction with parasite antigens are present in our immune gene collection.

Forty eight genes were found that matched the criteria. These cover a wide diversity of components of the immune system genes and can be subclassified as follows:

1. The immunoglobulin family and the proteins that bind to them (immunoglobulin heavy chain-binding protein, immunoglobulin heavy chain, C (δ, ε, γ1, γ2β, γ3) immunoglobulin light chain, C (κ, λ1), V2L1, IgE high affinity receptor (α and β) and Blast-1 antigen).

2. Components of complement (complement C3 and C-reactive protein).

3. CD antigens and other immune-related antigenic components (CD2, CD4, CD43, CD45, CD8 (α and β) antigens, RT6.1 alloantigen and thy1-antigen).

4. Interleukins, their regulators and their receptors (interleukin 1α, 1 receptor antagonist, interleukin 2, interleukin 3, interleukin 4, interleukin 6 and interleukin 6 receptor).

5. Lymphosub components (lymphosub membrane glycoprotein A, B, and lymphosub acid phosphatase).

6. MHC components (MHC1a invariant gamma chain).

7. Interferon-related genes (interferon regulatory factor 1, TIS7 (interferon related protein) and interferon gamma).

8. Inflammation-specific genes (kallikrein-binding protein, plasma kallikrein and serum amyloid protein).

9. T-cell and immune competent receptors: (T cell receptors Cα, Cβ, Vβ, Tnfr).

10. Platelet related factors (PDGF-inducible KC gene and transferin receptor).

11. Genes implicated in the control of opportunistic infections or with known resistance effects (thymosin alpha, thymosin beta and Mx1).

The receptors were chosen if they had no immune function and were not known to be imprinted (given that 0.1% of genes are expected to be imprinted (Hayashizaki et al. 1994), the chance that an imprinted gene is in the set of receptors is quite slim). The 29 receptors were as follows: nicotinic acetylcholine receptor epsilon, nicotinic acetylcholine receptor gamma, androgen receptor, beta 2 adrenergic receptor, dopamine receptor (D2A subtype), GABA-alpha receptor delta subunit, GABA-alpha receptor gamma-3 subunit, ANF receptor, glucocorticoid receptor, growth hormone receptor, insulin receptor, luteinizing hormone receptor, muscarinic acetylcholine receptor M1, oestrogen receptor, alpha-PDGFR receptor, prolactin receptor, retinoic X receptor beta, sorotonin receptor, substance K receptor and substance P receptor.

4. THE LOCATION OF SUBSTITUTIONS IN IGF2 AND IGF2R

If Igf2 is being antagonistically suppressed by Igf2r, as the conflict hypothesis supposes, then a novel version of Igf2 capable of not being bound by Igf2r should be able to spread (much as the novel parasite antigen should spread), for the same reason that paternally expressed allele of Igf2 can spread, i.e. it increases resource demands from the mother. This should provide the conditions for the spread of a novel version of Igf2r that can bind the new form of Igf2. We have examined the sequences of Igf2 and Igf2r to look for evidence of such antagonistic coevolution. Given that we know the binding site of Igf2 to Igf2r (and vice versa) we can ask whether at these sites the number of
non-synonymous substitutions is higher than in the rest of the gene as would be expected were the two antagonistically coevolving.

Comparison of these nine mammalian sequences, allowing for phylogenetic non-independence, reveals a low number of non-synonymous substitutions into the sequence. None of the non-synonymous substitutions affect the binding site with Igf2r (figure 1). Note, however, that the mutation rate of this gene is remarkably low (\(K_s\), synonymous substitutions, in the mouse–rat comparison is 4.8 per 100 sites, compared with an average for 363 genes of 22.4 (Wolfe & Sharp 1993), and of the 363 only two have a lower rate (AGP/EBP transcription factor and Y box binding protein 1)). None the less, we can ask how many substitutions would be expected in the Igf2r binding domains were these regions under directional selection.

Under directional selection the number of non-synonymous substitutions per site should be greater than the number of synonymous ones (controlling for the number of potential synonymous and non-synonymous mutations). On average, for every synonymous substitution there are approximately twice as many sites within each codon that could have non-synonymous substitutions. Within the binding domains there are seven synonymous substitutions. We would then expect around 14 non-synonymous substitutions in the binding domain, but find none. There is thus a

\[ \frac{K_a}{K_s} \]

Figure 1. The position of non-synonymous (solid bars) and synonymous (open bars) substitutions in mature Igf2 in nine species of mammal. Note both the low number of substitutions and the absence of substitutions in Igf2 receptor determinants.

\[ \frac{K_a}{K_s} \]

Figure 2. The ratio of the number of non-synonymous to the number of synonymous substitutions (\(K_a/K_s\) in sliding bands of 240 nucleotides across Igf2r compared in mouse and rat. Note that at the position of binding to Igf2 the ratio is very low, hence indicating stabilizing selection, rather than antagonistic coevolution.
significant dearth of non-synonymous substitutions under the null hypothesis that these receptor domains are under directional selection ($\chi^2 = 14, p < 0.001, \nu = 1$) (for the null hypothesis we presume that $K_s$ non-synonymous should, at a minimum be equal to $K_a$). More conservatively, if we were to suppose that there was only one non-imprinted mutation for every synonymous one, there would still be a significant lack of non-synonymous substitutions. We hence find no evidence that selection is favouring changes in $\text{Igf2}$ at the positions where it binds to $\text{Igf2r}$. This dearth of non-synonymous substitutions is consistent with stabilizing selection.

The sliding window comparison of $\text{Igf2r}$ in mouse and rat reveals intragenic variation in substitution rates (figure 2). Around the position of binding of $\text{Igf2}$ to $\text{Igf2r}$ the ratio of the number of non-synonymous substitutions to synonymous ones is very low (figure 2), hence indicating stabilizing selection, rather than antagonistic coevolution. We can contrast this with, for example, the evolution of class I MHC molecules, for here the opposite pattern is reported, i.e. extensive non-synonymous substitutions in the antigen binding cleft (Hughes et al. 1990). Likewise, it is the immunogenic regions of parasite antigens that show high rates of non-synonymous substitution (Hughes 1991).

5. Rates of non-synonymous substitution in imprinted and non-imprinted genes

Do imprinted genes as a class evolve faster or slower than comparable genes that are not imprinted? Do they evolve as fast as genes that are likely to be involved in antagonistic coevolutionary arms races? To examine this issue we have analysed the rate of evolution of seven genes that are known to be imprinted (assumed to be proportional to $K_s$), $\text{Igf2}$ and $\text{Igf2r}$, we collate the two samples (i.e. imprinted and immune, or imprinted and receptors) and rank all values of $K_s$ and $K_a$. For each gene we then subtract the rank of $K_s$ from the rank of $K_a$. We then apply Mann–Whitney $U$-tests to ask whether the two sets of rank differences are different. We find that imprinted genes are not different from receptors ($p > 0.05$, one tailed) but are significantly different from immune genes ($p < 0.01$, one tailed).

We can also ask whether the low mutation rate found in $\text{Igf2}$ is a general property of imprinted genes. We find that it is not and imprinted genes as a class have the same mutation rates as receptors (Mann–Whitney $U$-test, comparing $K_s$ for imprinted genes and receptors gives $p = 1.0$; mean $K_s$ for imprinted genes $= 19.87 \pm 4.38$; mean $K_s$ for receptors $= 21.02 \pm 1.23$). However, comparing an amalgamated set of receptors and imprinted genes ($n = 27$) with immune system genes (mean $K_s = 26.04 \pm 0.91$) we find that immune genes do have a significantly higher synonymous substitution rate (Mann–Whitney $U$-test, $p < 0.01$). The value of $K_a$ for a sample of 238 non-imprinted randomly selected autosomal genes is $22.9 \pm 0.45$ (McVean & Hurst 1997). This set of randomly chosen genes is also significantly different from the immune set (Mann–Whitney $U$-test, $p < 0.01$), but not from imprinted genes (Mann–Whitney $U$-test, $p > 0.05$). We conclude that imprinted genes have ‘normal’ synonymous substitution rates but immune system genes have especially high ones. This is consistent with adaptive enhancement of the mutation rate of immune genes when engaged in host–parasite coevolution (Haraguchi & Sasaki 1996) (note, however, that we have made no control for the effect of doublet mutations, which may account in part for the covariance of $K_s$ with $K_a$).

6. Discussion

We postulated that if the conflict hypothesis (Haig & Westoby 1989; Haig & Graham 1991; Moore & Haig 1991; Haig 1992) for the evolution of genomic imprinting was correct, then antagonistic coevolution between maternally and paternally expressed genes may be expected, and hence we might find rapid sequence evolution of imprinted genes. We find that $\text{Igf2r}$ is under stabilizing selection at the position at which it binds to $\text{Igf2}$. This is the opposite of what we expected and has two potential explanations: (i) mutations in $\text{Igf2}$ are so rare that none has occurred in the nine species examined and hence antagonistic coevolution is never initiated, or (ii) mutations in $\text{Igf2}$ at the binding site do occur but are under stabilizing rather than antagonistic coevolution. Were the first hypothesis correct, then the finding of stabilizing selection acting on $\text{Igf2r}$ would be consistent with any hypothesis for the evolution of imprinting.

Given the dearth of non-synonymous substitutions into $\text{Igf2}$ at the $\text{Igf2r}$ binding sites compared with the number of synonymous mutations (0.7), we can reject the first hypothesis. It should be noted, however, that $\text{Igf2}$ has a very low mutation rate. Given that imprinted
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Figure 3. The number of synonymous ($K_s$) and non-synonymous ($K_a$) substitutions (per 100 nucleotides) in imprinted genes (filled squares), receptors (open triangles) and immune system genes (open circles). The three lines are the lines of the regression of $K_a$ on $K_s$ (the black line is for imprinted genes, the grey line for receptors and the dotted line for immune genes). Note that the regression lines for receptors and imprinted genes are almost identical whereas that for immune genes is significantly steeper. Hence, controlling for their mutation rate ($K_s$), imprinted genes evolve at around the same rate as receptors and significantly slower than immune genes. The slopes of the regression line of $K_a$ on $K_s$ for imprinted, immune and receptor genes are: $0.125 \pm 0.05$, $0.384 \pm 0.11$ and $0.082 \pm 0.048$ respectively.

As a class have normal mutation rates it is unclear whether special explanation is required.

The first explanation would also predict that imprinted genes of higher mutation rates might be involved in antagonistic coevolution. We can find no evidence for this either. The ratios of $K_a/K_s$ also indicates that stabilizing selection acts on imprinted genes with about the same intensity as it does on any randomly selected group of genes. The mean $K_a/K_s$ of imprinted genes is $0.129 \pm 0.03$, which compares with a ratio of 0.14 for a set of 363 genes in the same comparison (Wolfe & Sharp 1993).

Assuming that fast evolution may be expected in systems of conflict (Hurst 1996a), were we to have found the opposite result this could have been taken as being supportive of the conflict hypothesis. The finding should hence be taken as evidence against this hypothesis, but it would be premature to argue that the conflict hypothesis is wrong. Given that imprinted genes may serve other functions (aside from a role in embryonic growth regulation) it may be that, for example in Igf2, directional selection promoting an escape from suppression could favour a change in gene sequence, but the same change may be disadvantageous under other circumstances. This is not, however, a wholly convincing argument given both that the mutual binding sites in Igf2 and Igf2r are not binding sites for anything else (see figures 1 and 2) and that numerous placentally expressed factors, and those secreted in maternal circulation, do seem to be relatively rapidly evolving (see above) (note, however, that it is unclear to what extent this may be due to adoption of new function and to what extent these genes have other functions).

The major reason to suppose that the conflict hypothesis may be correct is that it is consistent with the observed covariance (Hurst et al. 1996b) between the direction of imprinting and the direction of growth effects (noting the provisos given above). In this regard it is unique among the numerous theories of imprinting (Haig & Trivers 1995; Hurst 1997). There remains the possibility that a non-conflict-based explanation may be found that is consistent with the covariance between imprint direction and growth effect. By virtue of not being a conflict-based hypothesis, such a theory would also be consistent with the absence of antagonistic coevolution. In the absence of such an hypothesis, however, it seems most advisable to suppose that the conflict hypothesis is the best available, but that analysis of sequence evolution does not provide any support for it. It would then be especially helpful to have a testable explanation of the apparent exceptions.
to the rule that paternally expressed genes are growth promoters and maternally expressed ones are growth suppressors.

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